The Design and Synthesis of a New Lead against Tuberculosis

A thesis Submitted to the University of Strathclyde for the Degree of Doctor of Philosophy by

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Abbreviations

Å	Angstrom
ACP	Acyl carrier protein
AcpM	Mycobacterial acyl carrier protein
AG	Arabinogalactan
AIDS	Acquired immune deficiency syndrome
Ala	Alanine
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
2-AT-4C	2-aminothiazole-4-carboxylate
ATR	Attenuated Total Reflection Mode
°C	Celsius
CADD	Computer aided drug design
CAMHB	Cation Adjusted Mueller Hinton Broth
$CDCl_3$	Deuterated chloform
CFU	Colony-forming unit
CHN	carbon, hydrogen and nitrogen elemental analysis
CI	Chemical ionization
CoA	Coenzyme A
^{13}C NMR	Carbon nuclear magnetic resonance
CS	Cycloserine
C-terminus	Carboxyl-terminus
Cys	Cysteine

d	Doublet
Da	Dalton
dd	Doublet of doublet
DMSO-d6	Deuterated Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DOT	Direct observed treatment
ecfabB	*E. Coli* β-ketoacyl-ACP synthase I
ecFabF	*E. Coli* β-ketoacyl-ACP synthase II
ecFabH	*E. Coli* β-ketoacyl-ACP synthase III
E. Coli	*Escherichia coli*
efFabH	*Entercoccus faecalis* β-ketoacyl-ACP synthase III
EI	Electrospray
EMB	Ethambutol
FAB	Fast atom bombardment
FabH	β-ketoacyl-ACP synthase III
FAS-I	Fatty acid synthase I
FAS-II	Fatty acid synthase II
FQ	Fluroquinolones
FT-IR	Fourier transform infrared spectroscopy
GA	Genetic algorithem
Gln	Glutamine
Gly	Glycine
GOLD	Genetic optimization for ligand docking
^{1}H NMR	Proton nuclear magnetic resonance

H-bond	Hydrogen bond
His	Histidine
Hz	Hertz
IC_{50}	Half maximal (50%) inhibitory concentration of a substance
Ile	Isoleucine
InhA	2-*trans*-enoyl-acyl carrier protein reductase
INH	Isoniazid
J	Coupling constant
k	Kilo
KasA	β-ketoacyl-acyl carrier protein synthase A
KasB	β-ketoacyl-acyl carrier protein synthase B
KatG	Mycobacterial catalase-peroxidase enzyme
KBr	Potassium bromide
Lys	Lysine
m	multiplet
m	*Meta*
M. aurum	*Mycobacterium aurum*
MDR-TB	Multi-drug resistant tuberculosis
mg	milligram
$MgSO_4$	Magnesium sulphate
MHz	Megahertz
MIC	Minimum inhibitory concentration
ml	millilitre
μM	Micromolar

mM	millimole
M.P	Melting point
MS	Mass spectroscopy
Mtb	*Mycobacterium tuberculosis*
mtFabD	mycobacterial malonyl-CoA:ACP transcyclase
mtFabH	*Mycobacterium tuberculosis* β-ketoacyl-ACP synthase
m/z	Mass over charge
NA	Not active
NaCl	Sodium chloride
NADH	NADH dehydrogenase
NADPH	Nicotinamide adenine dinucleotide phosphate-oxidase
No.	Number
NT	Not tested
o	*Orthro*
p	*Para*
PAS	*Para* amino salicylic acid
PDB	Protein data bank
PG	Peptidoglycan
Phe	Phenylalanine
pKa	Acid dissociation constant
ppm	Parts per million
Pro	Proline
PZA	Pyrazinamide
q	Quartet

RNA	Ribonucleic acid
rRNA	Ribosomal RNA
s	singlet
SAR	Structure activity relationship
SDS	Sodium dodecyl sulfate
Ser	Serine
SM	Streptomycin
S_N2	Bimolecular nucleophilic substitution
spp	Species
RIF	Rifampicin
t	Triplet
TB	Tuberculosis
Thr	Threonine
TLM	Thiolactomycin
Trp	Tryptophan
Val	Valine
WHO	World health organization
XDR-TB	Extremely drug resistant tuberculosis

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Abstract

It is estimated that one-third of the world's population has been exposed to the tuberculosis bacterium; killing eight million every year. Since the 1960s there have been no new drugs marketed, which is primarily due to the *Mycobacterium tuberculosis* bacilli having a complex cell wall structure, intracellular existence in the host and its ability to stay dormant for extended periods. The continual increase in drug resistance and the lack of new chemotherapeutic agents means the search for a new antimycobacterial agent is of great importance. Our molecular modelling studies identified a thiazole-based scaffold as a potential candidate for optimization, which has a feasible synthetic route and is amenable to library generation to effectively explore local chemical space.

Biological screening of the prepared compounds resulted in the identification of some compounds expressing excellent activity against *M. tuberculosis* with no observed toxicity. The most active compounds will be tested *in vivo* in mouse models infected with the pathogenic bacilli in order to perform further lead optimization to more drug-like compounds.

Chapter 1: Introduction

1.1 Tuberculosis

Discovered by Koch in 1882, tuberculosis (TB) is caused by *M. tuberculosis* (Mtb) and is now the second leading cause of death world–wide after AIDS.[1, 2] It is an ancient disease that has plagued humanity throughout history; indeed Egyptian mummies have been diagnosed to have TB.[3, 4] Two billion people, equivalent to one-third of the world's population, are infected with Mtb and each year eight million new cases are reported, a number that is rising owing to the increase in AIDS cases globally.[2, 5] The World Health Organization (WHO) has reported a clear correlation between TB and AIDS patients in the areas where AIDS is endemic.[5] The appearance of multi-drug resistant strains and the absence of any new drugs on the market in over fifty years have contributed to the re-emergence of the disease.[6, 7]

1.2 *Mycobacterium tuberculosis*

The mycobacterium genus includes pathogenic organisms called the mycobacterium complex, and includes bacteria such as *M. bovis*, *M. lepra, M. afrecanum* and *M. tuberculosis. M. tuberculosis* are gram positive-bacilli that contain a high proportion of lipid in their cell wall.[8, 9]

Members of the genus mycobacterium are widespread in nature, ranging from harmless species that inhabit water and soil to very harmful species that cause serious diseases such as leprosy and tuberculosis. Mycobacterium species are classified into many categories including slow growers and fast growers, with the pathogenic Mtb and *M. leprae* being members of the former.[10, 11]

1.3 The transmission and diagnosis of the disease

People with pulmonary and laryngeal forms of TB transfer the disease in droplets through sneezing and coughing, especially in poorly ventilated environments. The inhaled bacilli reach the alveoli in the lung periphery, where they are ingested by lung macrophages and transferred *via* the lymph system to other organs in the body. The TB bacilli are intracellular organisms with a complex cell wall, which is responsible for making the treatment of the disease very difficult.[2]

Whilst diagnosis of TB is important, the main problem is that its symptoms are non-specific; among these are weight loss, fever, night sweats, fatigue, chest pain and haemoptysis. Chest X-rays can detect its presence, since 80% of the cases are pulmonary, whereas other cases occur in other organs such as the liver and the kidneys. The gold standard in TB diagnosis is by culturing Mtb cells by taking them from sputum, lymph node aspirate and biopsy in suspected lymph nodes.[12, 13] Unfortunately, culture-based tests are difficult to implement in the field due to the requirement of dedicated facilities and staff who are trained and familiar with the safety procedures. Whilst the conventional methods are simple and cheap, they are considered very slow, and the result can take up to fifty six days to achieve.[13, 14]

1.4 Current treatment

The aim of TB treatment is to eradicate the mycobacterial infection as rapidly as possible, to prevent a secondary infection, and to prevent the formation of drug resistant strains. Additionally, the symptoms of the disease vary widely and can cause, in particular, lesions which are difficult to treat, and may lead to the formation of drug resistant strains. As a result, the period of treatment has to be of sufficiently

long duration to destroy all the mycobacteria, and the regimen consists of a number of drugs to prevent resistance.[15-17]

WHO recommendations are patient specific; treatment strategy depends on the severity of the disease and medical history. Before the introduction of chemotherapy for TB treatment, fresh air, good food and sunshine were the only ways of curing the disease. Drug treatment was first administered to humans in 1940 with the introduction of streptomycin. Resistance emerged quickly, and was reduced by incorporation of aminosalicylic acid into the drug regimen. Isoniazid was later introduced and was considered an important step in developing successful primary chemotherapy, and reinforced the case for combination treatment.[15-17]

Today, TB therapy is a two-phase process; an initial phase usually for two months using four drugs, namely rifampicin (RIF), isoniazid (INH), pyrazinamide (PZA), and ethambutol (EMB).[18] The concurrent use of these four drugs is designed to reduce bacterial population as rapidly as possible. The second is a continuation phase, in which both RIF and INH are used in combination for a total period of between six to nine months. Compliance is the most important feature in TB treatment, otherwise resistance will emerge quickly and the treatment will fail.[19]

A new increase in the number of TB cases is related to the appearance of new strains that are resistant to some or all the drugs that are in use, and is called multi-drug resistant TB (MDR-TB). The rise of these resistant strains is due to inconsistent or partial treatment, through poor patient compliance, doctors giving incorrect

medication or in the wrong combination, unreliable medication sources, and the appearance of AIDS. For these reasons, WHO has issued new global guidelines to control the treatment called the Direct Observed Treatment (DOT) system in developing countries, and involves medication being given under the supervision of the health workers. DOT has been enforced in the UK for patients who are thought to be unable to adhere to the regimen independently.[20-22] Worryingly, despite DOT, a new resistant strain has emerged, which is the Extremely Drug Resistant TB (XDR-TB), where the Mtb is resistant to all the fluoroquinilones and at least to one of the injectable second–line drugs (amikacin, capreomycin and kanamycin).[23]

The DOT system involves five elements: political commitment, microscopy services, drug supplies, monitoring systems and direct observation of treatment. WHO guidelines indicate that the first phase of treatment should start with RIF and INH, with either streptomycin (SM) or EMB or both. If PZA is added to the regimen, treatment duration can be reduced to six months. The continuation phase should be for four months using INH and RIF.[24]

Traditionally, TB drugs are generally considered to be either first-line or second line. The first line drugs INH, RIF, EMB, PZA and SM exhibit superior activity with acceptable toxicity, whereas the second line drugs *para*-aminosalicylic acid (PAS), fluoroquinolones (FQ), and cycloserine (CS) have either less activity, more side effects or both.[17]

1.4.1 First line drugs

INH **(1)** was introduced in 1952 and is a pro-drug that is activated *in vivo* by an enzyme that is a combined catalase and peroxidase (KatG).[25] The activated form is believed to inhibit the synthesis of mycolic acids (long fatty acids in the bacterial cell wall) by inhibiting enoyl-acyl carrier protein reductase (InhA) and β-ketoacyl-acyl carrier protein synthase.[25, 26]

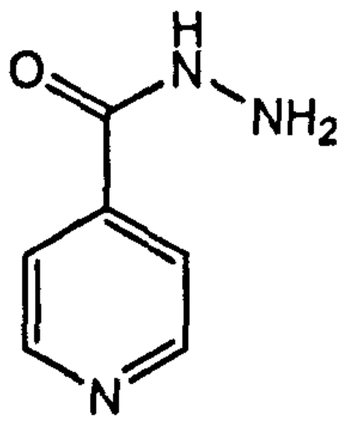
RIF **(2a)**, discovered in 1966, is a semi-synthetic derivative of the rifamycin broad-spectrum antibiotics. RIF acts by targeting the Mtb DNA dependent RNA polymerase β-subunit and inhibiting RNA synthesis. [26-28] To overcome the undesirable metabolism properties of RIF, Rifapentine **(2b)** was developed. It has advantages over RIF including longer half-life, maximum serum concentration and lower maximum inhibitory concentration, but has the disadvantage of being highly protein bound.[27, 29]

SM **(3)**, discovered in 1944, belongs to the aminoglycoside therapeutic group. It is a broad spectrum antibiotic that binds irreversibly to the bacterial 30s ribosomal subunit preventing the initiation of protein synthesis. However, due to adverse side effects and the fact that it requires intramuscular or intravenous administration, SM is no longer a preferred choice of drug and is nowadays used mainly in the treatment of MDR TB.[26]

EMB **(4)** is believed to inhibit the polymerization of the cell wall arabinan component of the integral structural polysaccharide mycolylarabinogalactam and

lipoarabinomannan, through targeting arabinosyl transferase. Its major side effect is neuropathy resulting in visual problems and hypersensitivity reactions [18, 30]

PZA **(5)** is an analogue of nicotinamide, and is a prodrug that has a similar mechanism of action to INH. It undergoes deamidation by the bacterial enzyme pyrazinamidase to form the active form pyrazinoic acid.[26]



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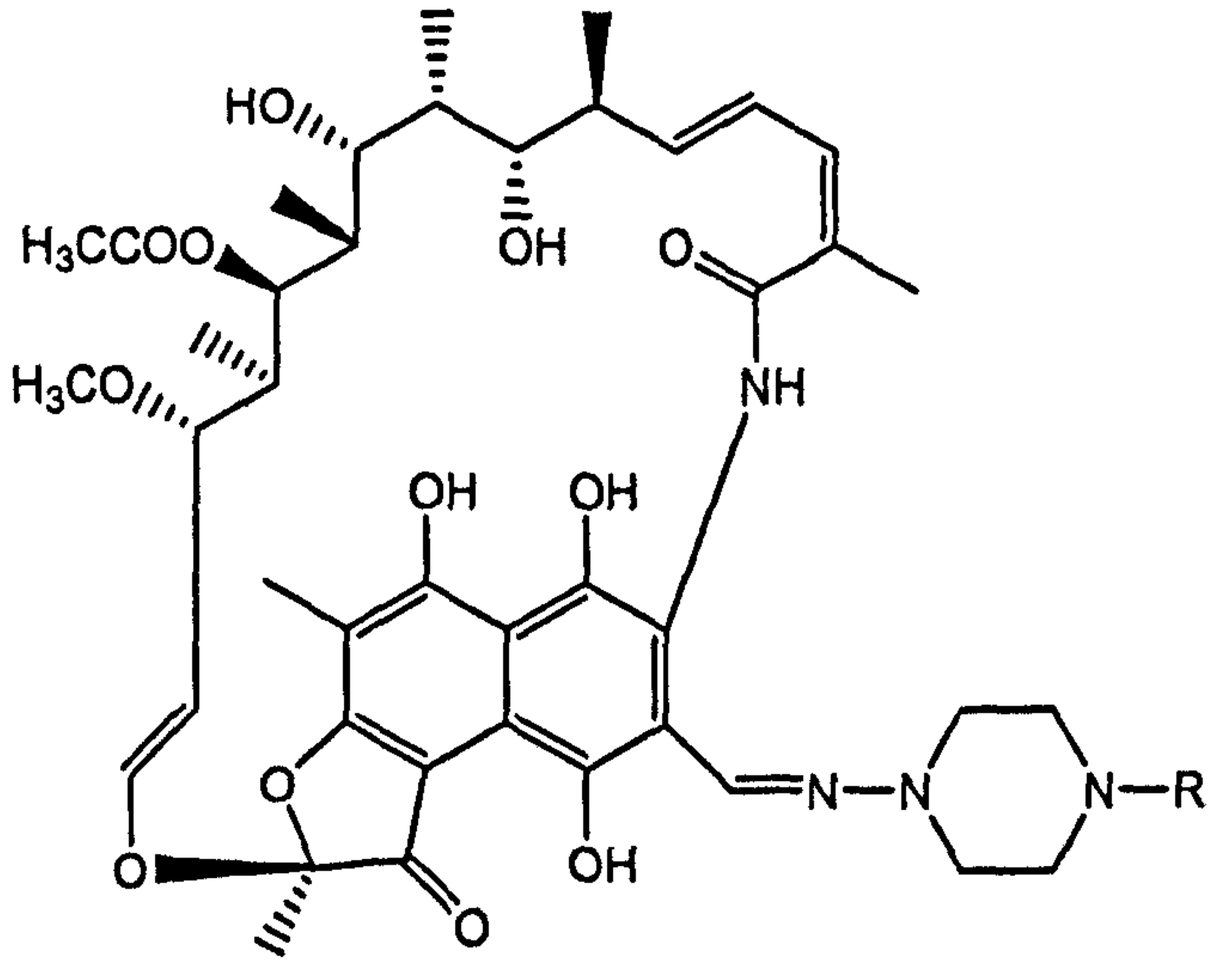

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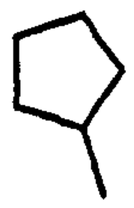
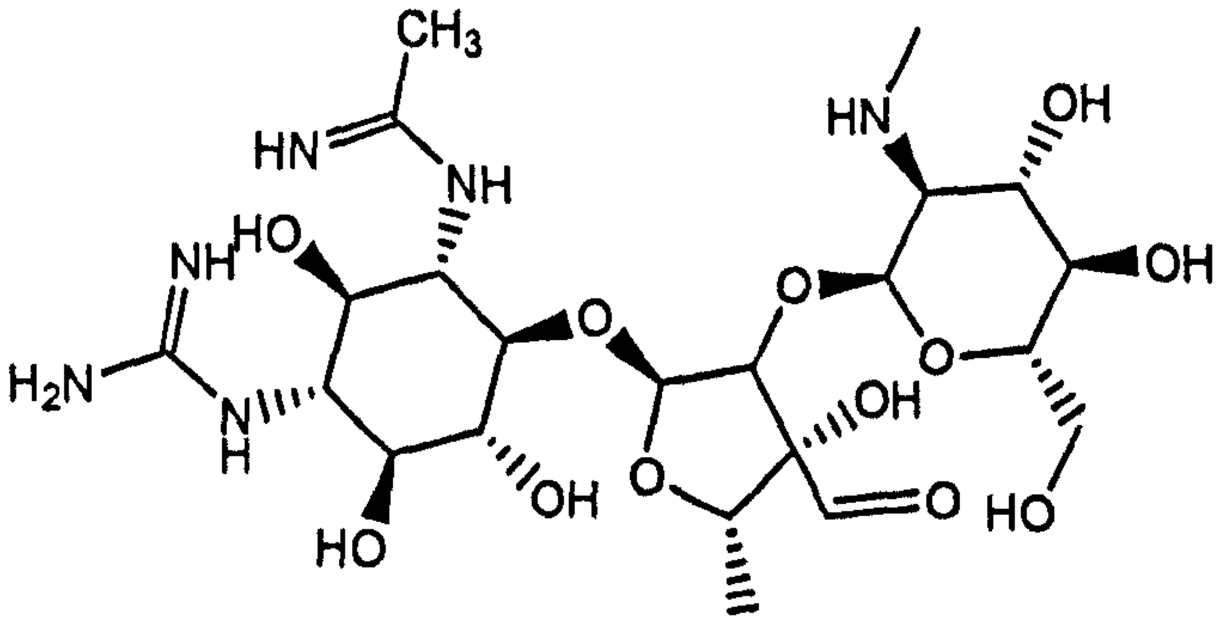
(b) R= 



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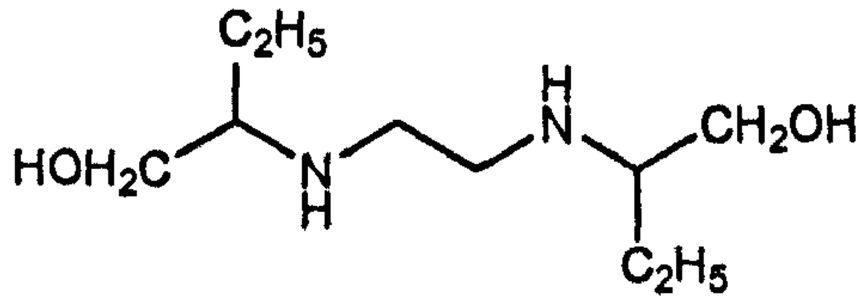

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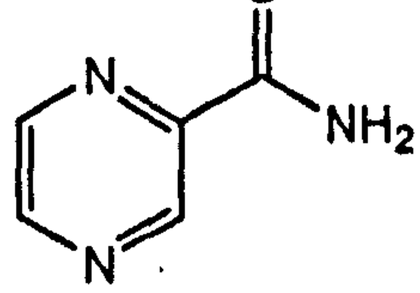
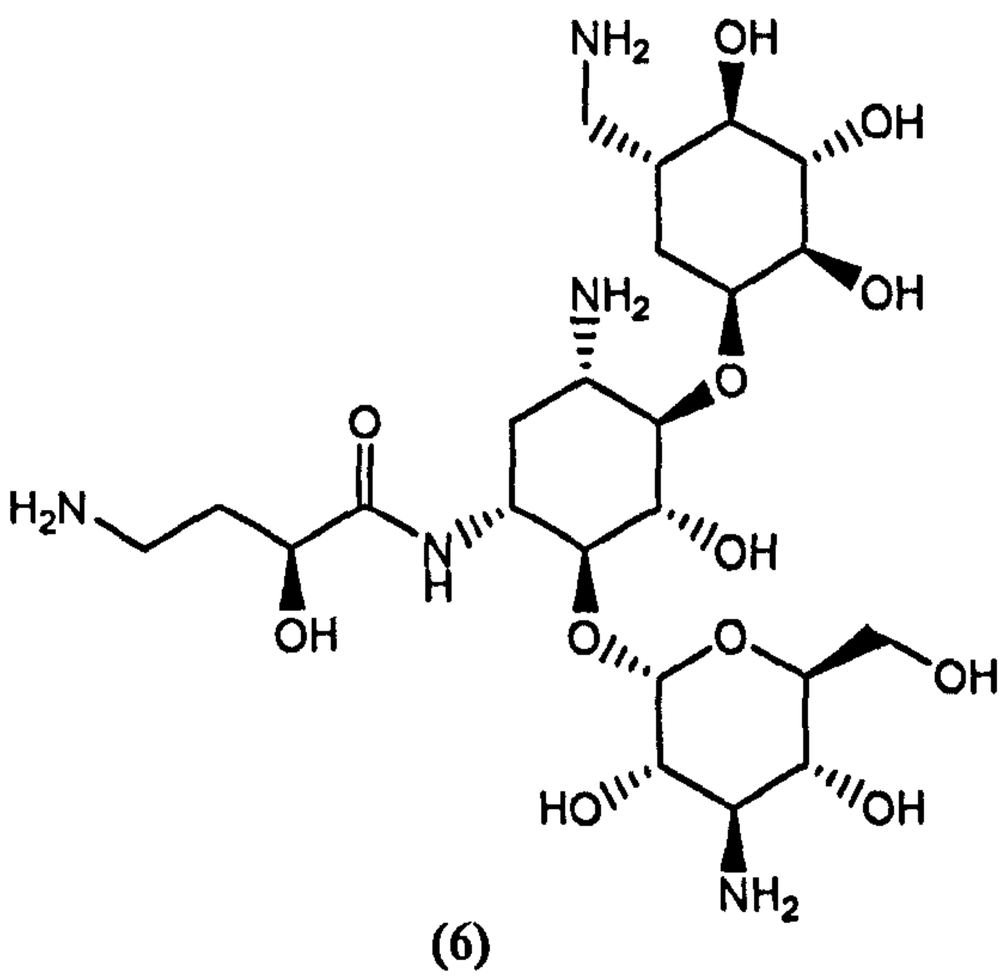


(5)

1.4.2 Second-line drugs

Amikamycin (**6**), kanamycin (**7**), and capreomycin (**8**) inhibit protein synthesis by targeting the 16S rRNA unit,[26, 31] and FQs such as ciprofloxacin (**9**) disturb DNA synthesis by causing lethal breaks in DNA replication.[32] CS (**10**), introduced in 1952, interferes with peptidoglycan synthesis by inhibiting *D*-alanine racemase/synthase,[26] and PAS (**11**) inhibits folic acid synthesis and iron uptake, although its precise mechanism of action is unknown.[26]



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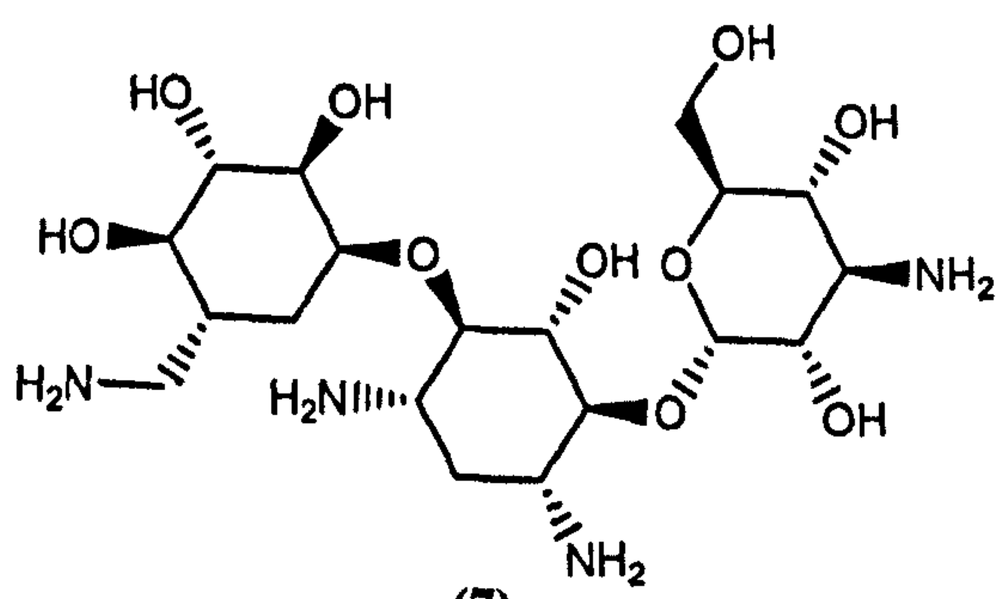

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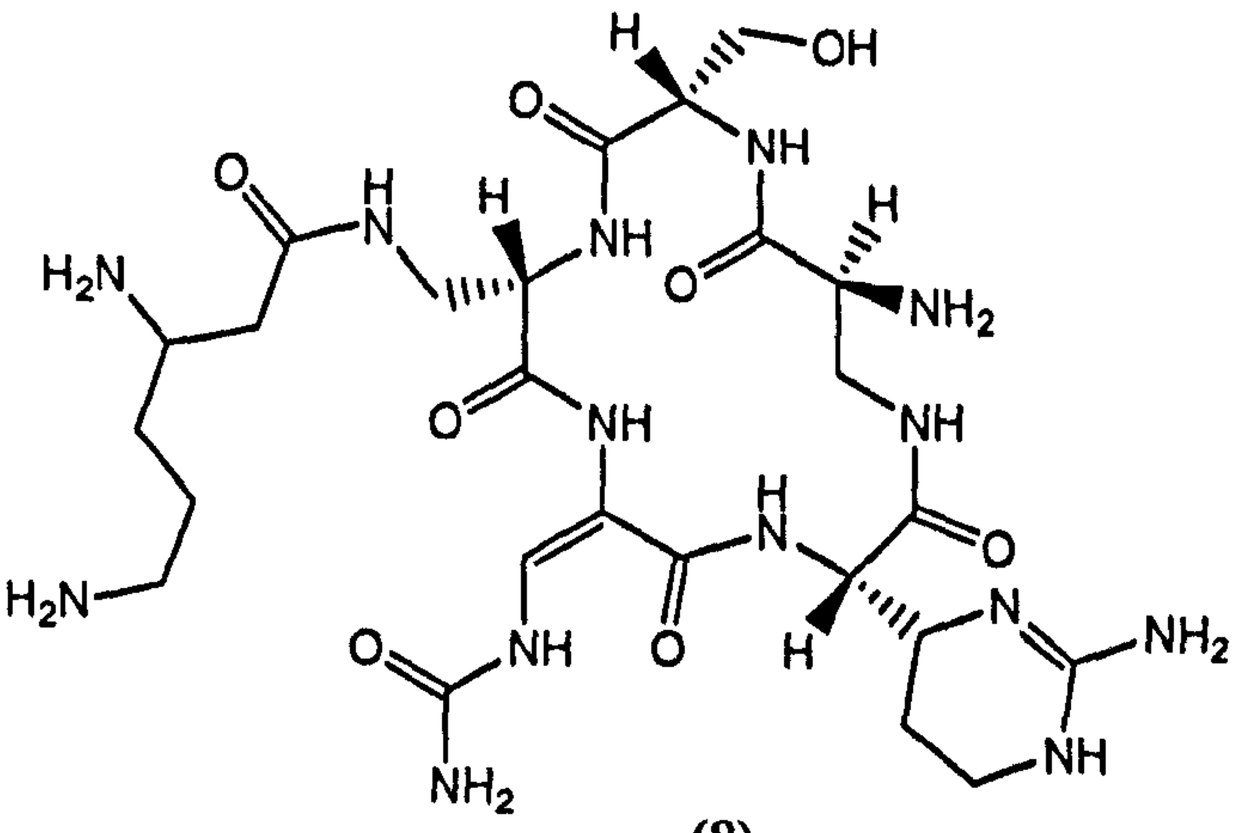

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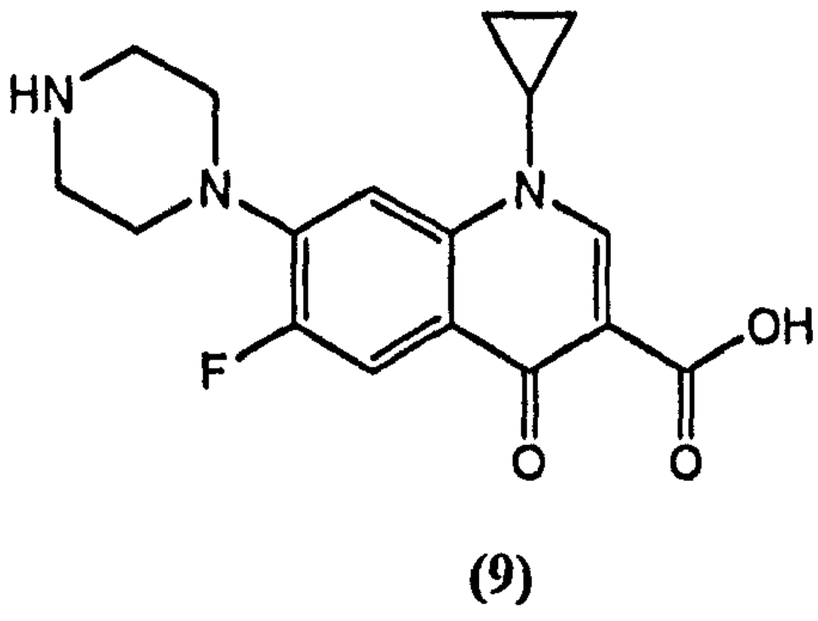

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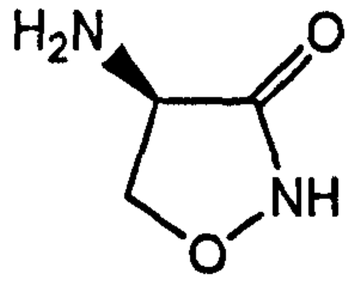

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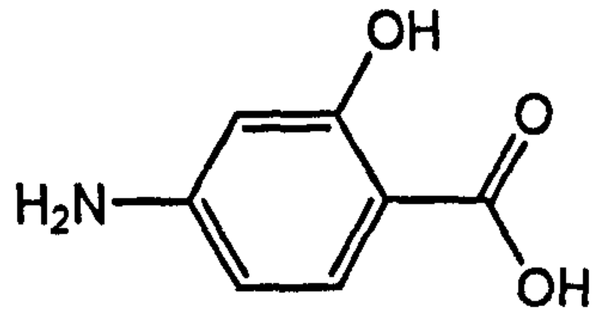

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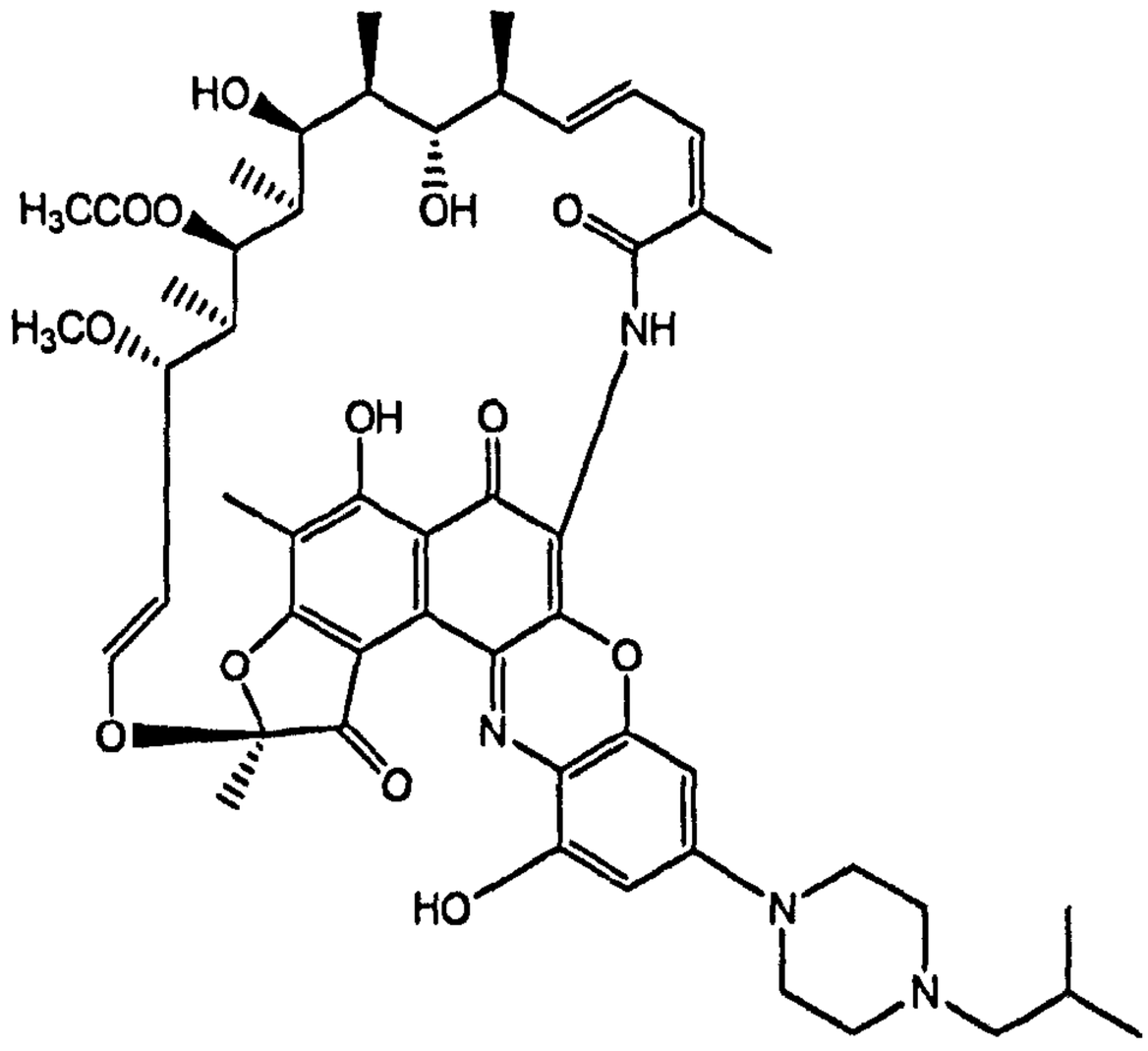
1.5 Future trends in TB treatment

The main problems with current TB therapy are the long treatment regimen (which can be longer than nine months or longer), short intervals of drug administration which are an extra burden on the health officials applying the DOT system, current drugs are old and prone to resistance and no new immunomodulators have been introduced to help the body fight the disease. To this end there has been much research in TB drug development over the last decade, and a number of candidates have been discovered and are at various stages of development. [29]

Rifalazil **(12)** (KRM-1648), an analogue of RIF, is still under clinical trials and is showing promising results by having longer duration of action because of its tendency to deposit in macrophages.[33]



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Fluoroquinolones have further potential to help improve the treatment of TB in the near future through their advantage of improving the efficiency of other TB drugs. For example, moxifloxacin, a development of ciprofloxacin, was found to be the best fluoroquinolone against Mtb, which kills the bacteria by binding to the DNA gyrase and topisomerase IV, an enzyme involved in bacterial replication.[32, 34, 35]

Other groups of antimicrobials being reported in the literature currently are the oxazolidinones, nitroimidazopyrans and thiolactomycins. However, these compounds are still in early stage drug development programmes, and have not yet reach clinical trials.[36-38]

PA-824 is a nitroimidazopyran, and has been adopted by the Global Alliance For TB Drug Development to make it available in the countries where the disease is endemic. It was found *in vitro* to be active against MDR-TB and slow growing Mtb, and is believed to kill the bacteria by inhibiting both protein synthesis and cell wall lipid synthesis.[39, 40] TMC-207 is a diarylquinolone that inhibits the proton pump of the Mtb adenosine triphosphate (ATP) synthase that is its main source of fuel. It has many promising characteristics for a drug-candidate against TB, such as its low molecular weight, high potency against drug-sensitive and drug-resistant TB strains, very long half-life (allowing once-weekly dosing), and has few drug interactions.[41]

1.6 Mycobacteria cell envelope- a target for drug development

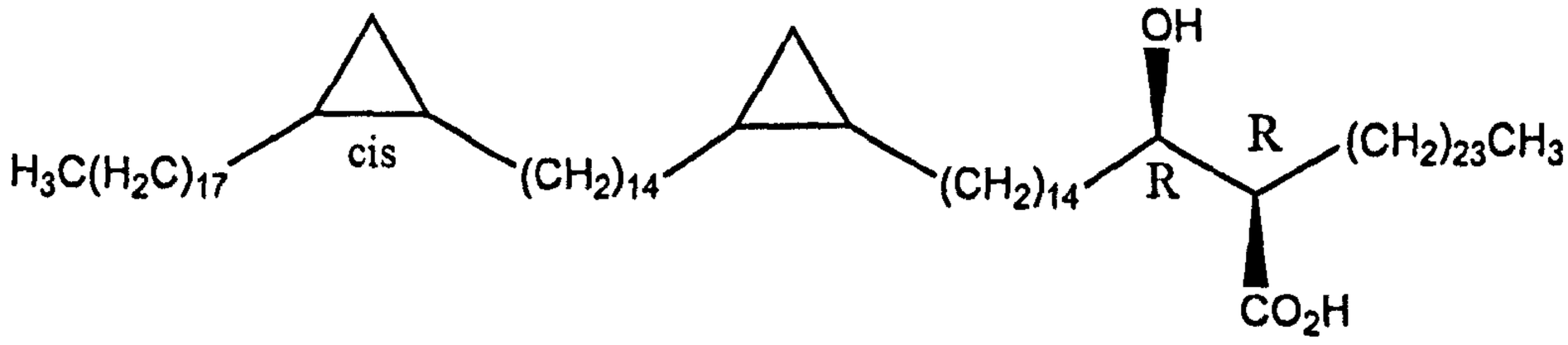
The cell envelope of Mtb is composed mainly of a cell membrane, a polysaccharide rich capsule, and a covalently-linked mycolic acid- arabinogalactan (AG) -

peptidoglycan (PG) complex. At its core, peptidoglycan consists of two sugar components, N-acetylglucosamine and N-acetylmuramic acid, and the amino acids *L*-alanine, *D*-alanine, and *D*-glutamic acid.[42] Arabinogalactan is composed of arabinose and galactan sugars in the furanose form. The galactan sugars form phosphodiester link to the peptidoglycan, and the peptidoglycan complex provides the Mtb envelope with rigidity.[43, 44]

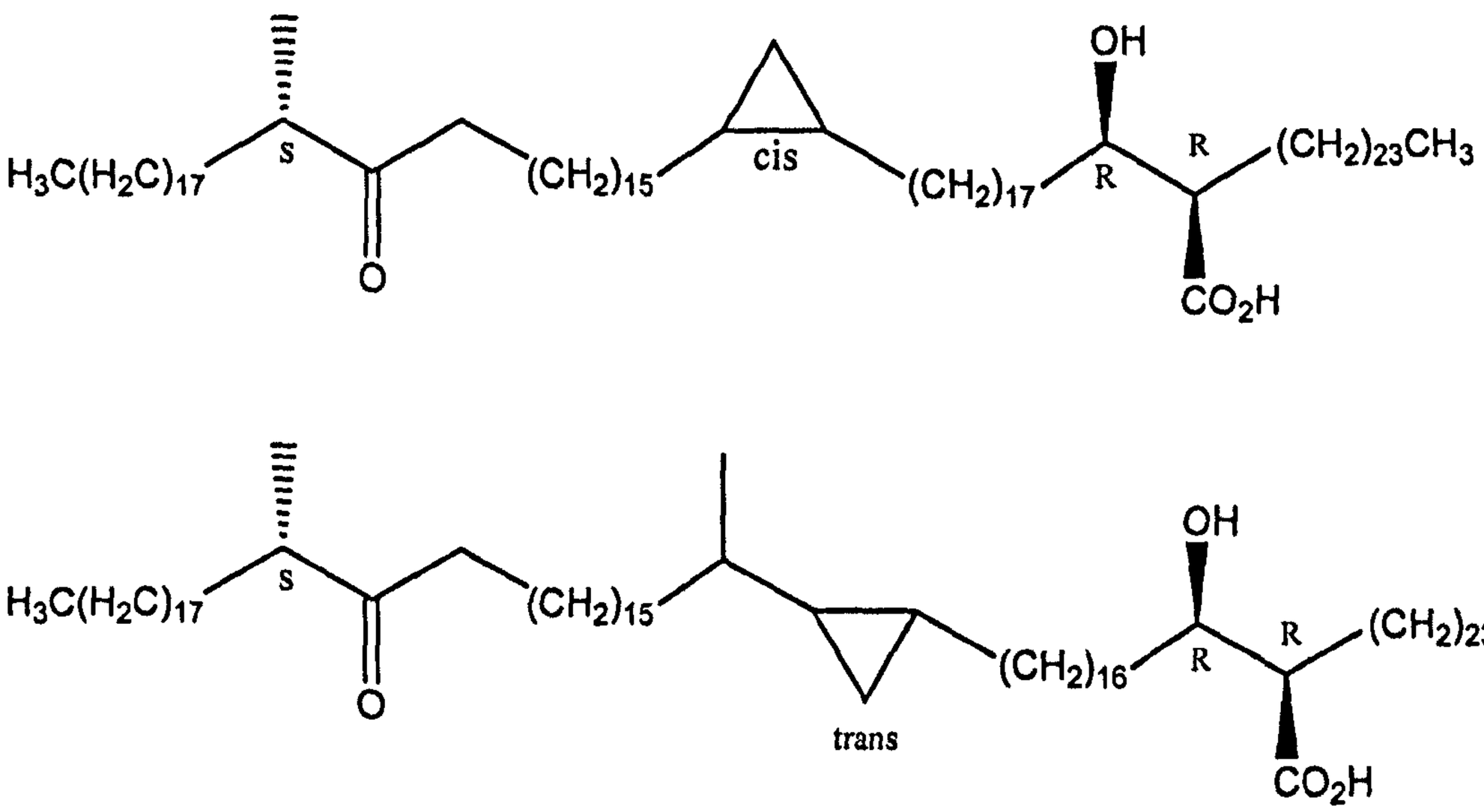
1.6.1 Mycolic acids

Mycolic acids, a series of C_{60}-C_{90} α-alkyl-β-hydroxy fatty acids, are the hydrophobic components in the cell wall whose organization, structure and chemical properties determine its low permeability. The mycolic acids found in mycobacteria differ from other genera such as *Norcardia, Rhodococcus, and Corynebacterium* by having two branches; a C_{24}-C_{26} α- branch and a C_{46}-C_{56} meromycolate branch, both of which are normally fully saturated. There are only two places in the meromycolate chain that are appropriate for functionalities such as double bonds or cyclopropyl rings.[45] These functionalities in the meromycolate chain classify the mycolic acids into three major types; the α-mycolic acids, (*cis, cis*-dicyclopropyl fatty acids which are the most abundant, forming more than 70% of the mycolic acids in the cell wall), the keto and the methoxy-mycolic acids (Figure 1.1).[46, 47]

α-Mycolates



Keto-mycolates



Methoxymycolates

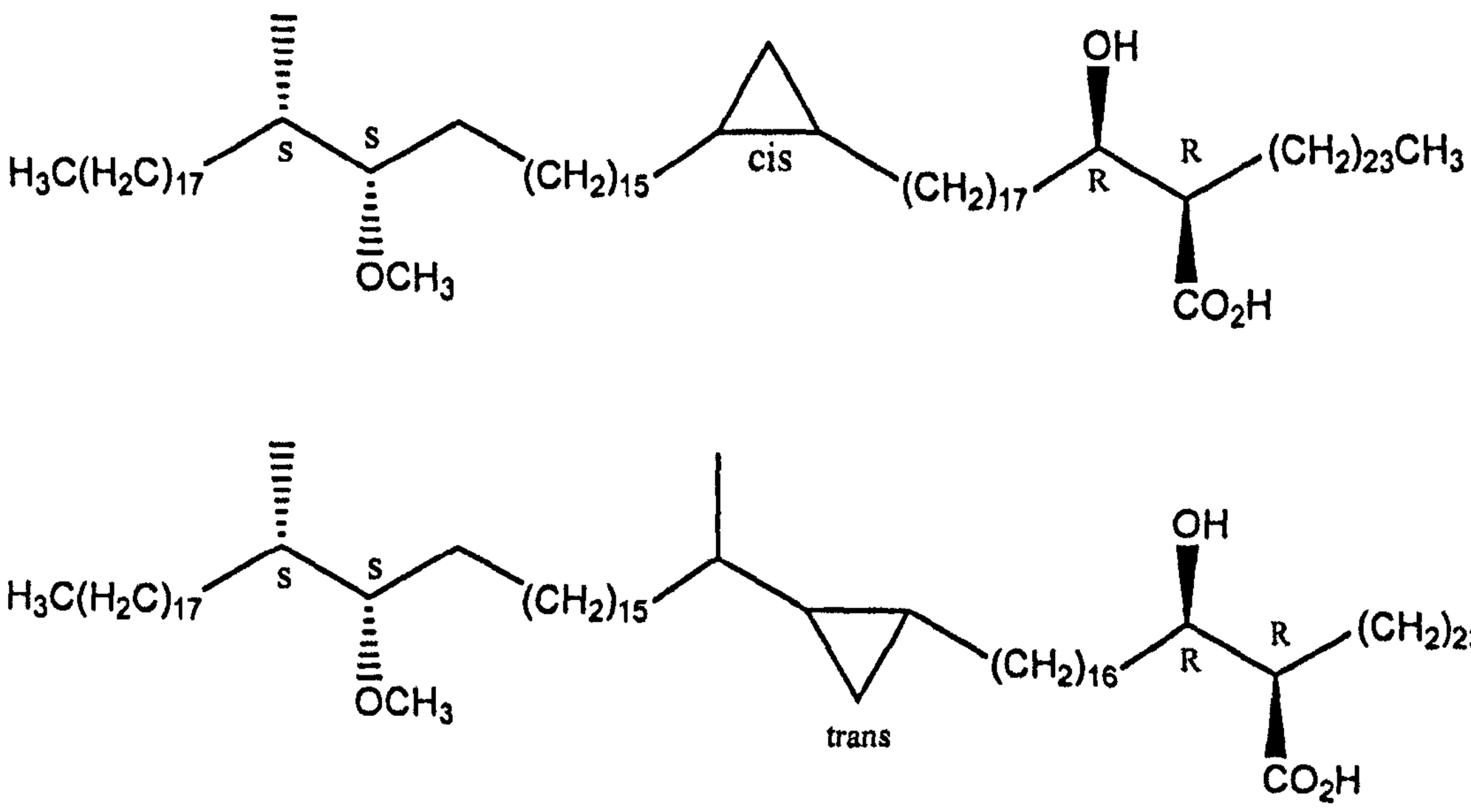


Figure 1.1: The alpha, keto and methoxy-mycolic acids in the Mtb cell wall.

The presence of mycolic acids in Mtb confers many characteristics that are unique to mycobacteria, including resistance to chemical injury and hydration, low permeability to hydrophobic drugs and accommodation to the hostile environment inside the phagocyte. The presence of cyclopropyl groups provides additional

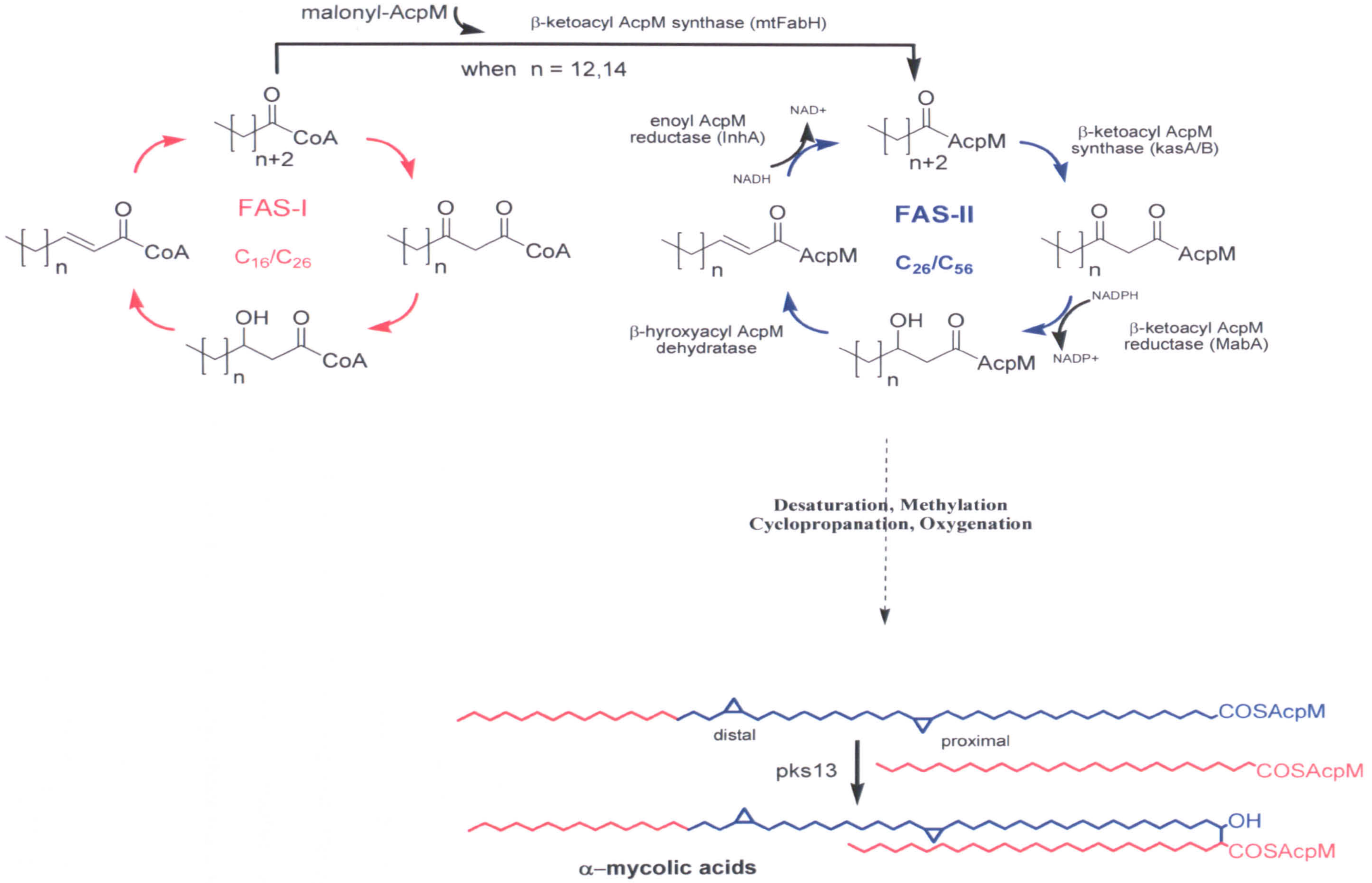


Figure 1.2: The synthesis of mycolic acids in M. tuberculosis

Mycolic acid biosynthesis in mycobacteria requires two fatty acid synthases, FAS-I and FAS-II. FAS-I (also found in eukaryotes and advance prokaryotes) is a multifunctional system composed of a homodimer containing all the necessary functionality for *de novo* fatty acid synthesis. The sequence of fatty acid chain elongation is carried out as in Figure 1.2. In the FAS-I system, acetyl-CoA is condensed with malonyl-CoA to give the corresponding β-ketoacylated enzyme, followed by reduction to the hydroxyl homologue, then dehydrated by the dehydrase subunit before a further reduction to give butyryl-CoA. This sequence is repeated until C_{16} or C_{26} chain lengths are obtained (Figure 1.2).[46]

The FAS-II system contains disaggregated enzymes (also found in other plants and bacteria), and receives the products that have been produced in FAS-I prior to the synthesis of meromycolates and the full mycolic acids (Figure 1.2).[46]

The pivotal link enzyme between FAS-I and FAS-II in Mtb is the β-ketoacyl-acyl carrier protein synthase III (mtFabH), which initiates the chain elongation of (C_8-C_{20})-S-CoA produced *via* FAS-I by condensing with malonyl-S-AcpM to produce the β-ketoacyl-S-AcpM product. Malonyl-S-AcpM itself is prepared from malonyl-CoA *via* the enzyme mtFabD (malonyl-CoA:ACP transcyclase).[46] The process of transferring the products from FAS-I to FAS-II is mediated through the mycobacterial acyl carrier protein (AcpM), a protein with a phosphopantetheine prosthetic group that is covalently linked to the growing acyl chain *via* a terminal sulfhydryl group.[56, 57]

At this stage the substrate is ready for elongation *via* the FAS-II multifunctional system involving four enzymes that perform different reactions to add two carbon atom units in each cycle analogous to FAS-I. Initially, KasA and KasB (β-ketoacyl-ACP synthases) extend long-chain fatty acids of more than C_{14} acyl-AcpM by condensation with malonyl-AcpM. KasA begins this sequence using myristoyl-CoA up to stearoyl-CoA to produce monounsaturated fatty acids that average 40 carbons in length, which are then utilised by KasB to give the full length multi-unsaturated C_{50}–C_{56} meromycolate. MabA is involved in the next reduction step and produces β-hydroxy acyl-AcpM units by catalysing an NADPH-specific carbonyl reduction of long chain C_{16}-C_{56} β-ketoacyl-AcpM substrates. β-hydroxy acyl-ACP dehydrase is the next enzyme in the synthesis and is responsible for conversion of β-hydroxy acyl-AcpM to *trans*-2-enoyl-AcpM. Completion of the synthesis is carried out by InhA (2-*trans*-enoyl-AcpM reductase) which is an NADH dependent reductase and produces the saturated chain ready for further transformations in mycolic acid biosynthesis (Figure 1.2).[58-69]

1.7 β-Ketoacyl-acyl carrier protein synthase III as a drug target

MtFabH belongs to a group of enzymes in the mycobacterial FAS-II system called condensing enzymes. These enzymes are mtFabH, KasA, and KasB, and are responsible for the synthesis of mycolic acids up to 90 carbons in length. The equivalent enzymes in *E. coli* are ecFabH, ecFabF, and ecFabB. ecFabB is essential for the unsaturated fatty acid biosynthesis, while ecFabF is required for thermal modulation of fatty acid composition and is not essential. ecFabH is an equivalent enzyme to mtFabH in Mtb, which is crucial for initiating FAS-II cycle.[70, 71] β-Ketoacyl-Acyl Carrier Protein Synthase III (mtFabH) as a target in mycobacteria has

been studied in parallel with its equivalent ecFabH. *E. coli* is considered the "workhorse" of molecular biology and is used as a model for Mtb as the active sites are highly conserved. mtFabH posses 37.3% identity and 46.8% similarity to ecFabH with identical active site residues.[72] The main difference in fatty acid biosynthesis between the two species is that Mtb produces long chain fatty acids while the fatty acids in *E. coli* are in the range of about 16-18 carbons. Additionally, in Mtb the substrate utilizes AcpM and not ACP.[72]

1.7.1 The structure of FabH

There are many X-ray crystal structures for ecFabH deposited in the Protein Data bank (PDB) such as 1hn9, 1ebl and 1hnj. ecFabH is a homodimer (interface of 2670 $Å^2$ buried accessible surface area) with a slab shape structure (dimensions 45 Å X 55 Å X 80 Å), the lower two thirds of which are constructed mainly of α-β motifs, while the rest is an extended loop region. The dimer is composed of two chains, each composed of 317 amino acids, an N-terminus of 171 residues and a C-terminus of 146 residues, both folded in the same manner and forming a β1-α1-β2-α2-β3-α3-β4-β5 arrangement of eight secondary parts (figure 1.3).[71, 73-75]

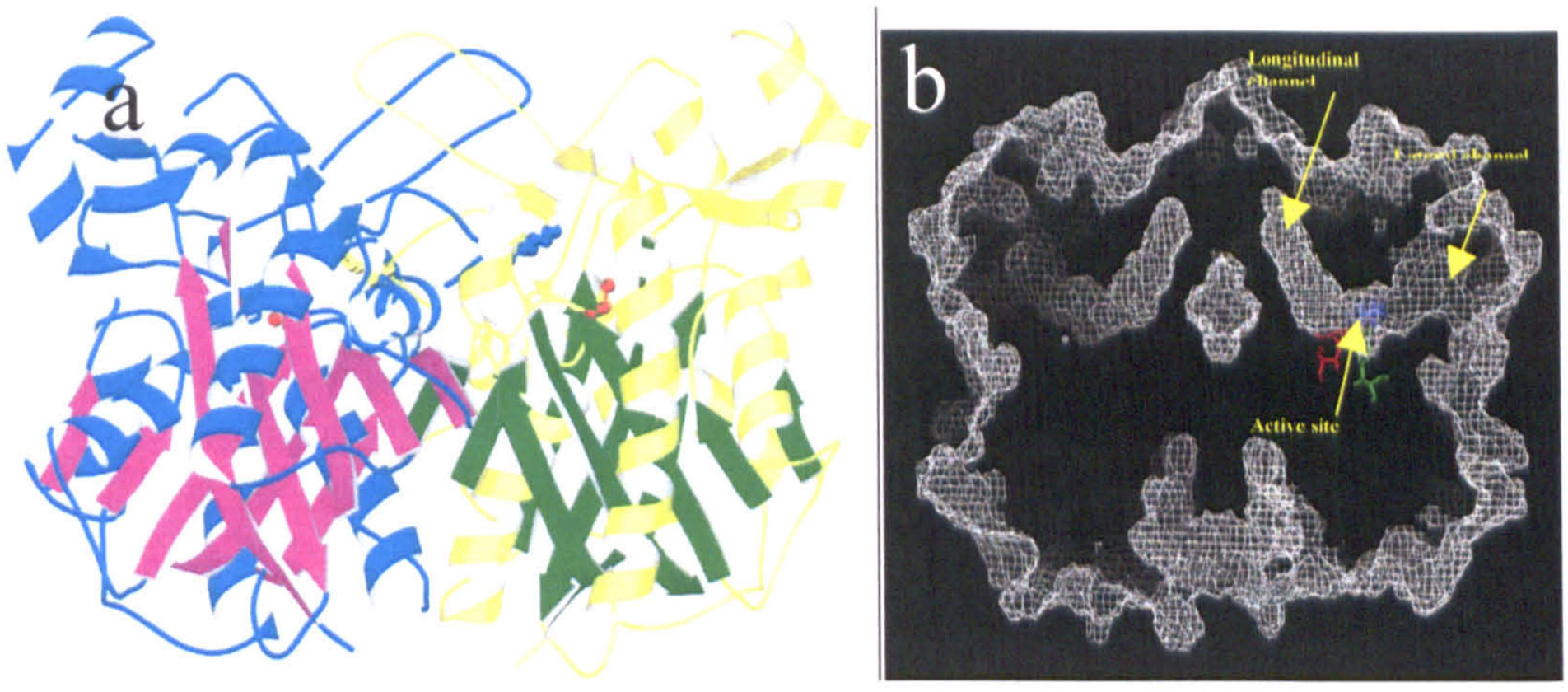


Figure 1.3: (a) Ribbon diagram of the ecFabH dimer. The dimer is viewed perpendicular to the two-fold axis. One monomer is shown in yellow and green; the other is shown in cyan and magenta. Phe87 (blue)and Cys112 (red) are shown as ball-and-stick models. (b) The homodimer structure of mtFabH enzyme showing the channels and the active site

The entrance to the active site can be distinguished from the rest of the protein surface by the presence of mixed hydrophobic and electropositive residues, unlike the rest of the surface, which is composed predominantly of electronegative residues. There are two channels converging at the active site; the lateral channel is a tunnel to the surface of approximately 20 Å in length and 5 Å in diameter, while the longitudinal channel is shorter and tighter, which can only accommodate at maximum a butyl group. Phe87 of one monomer closes the longitudinal channel, and plays an important role in determining substrate specificity. Cys112, His244, and Asn274 are three conserved residues found in the active site of all FabH enzymes and are involved in the condensation process. Many structures have been isolated and crystallized from different bacterial and plant species such as *Steptomyces glaucescens*, *Plasmodium falciparum* and spinach and all demonstrate similarity in structure and function with that of ecFabH.[73, 76-82]

Comparison of mtFabH with ecFabH reveals only a few differences in the backbone arrangement of each enzyme with an average root mean square deviation for the backbone atoms of 1.37 Å (Figure 1.4). mtFabH has extra insertions at residues 1-10, 202, 263, and the last three residues, which all have an important role in protein stability.[81] Both enzymes have the same lateral tunnel of 20 Å in length leading to the active site residues Cys112, His244, and Asn274. The active site of both enzymes have a similar oxyanion hole, which is believed to play an important role in the condensation step that is performed by these enzymes.[72, 81]

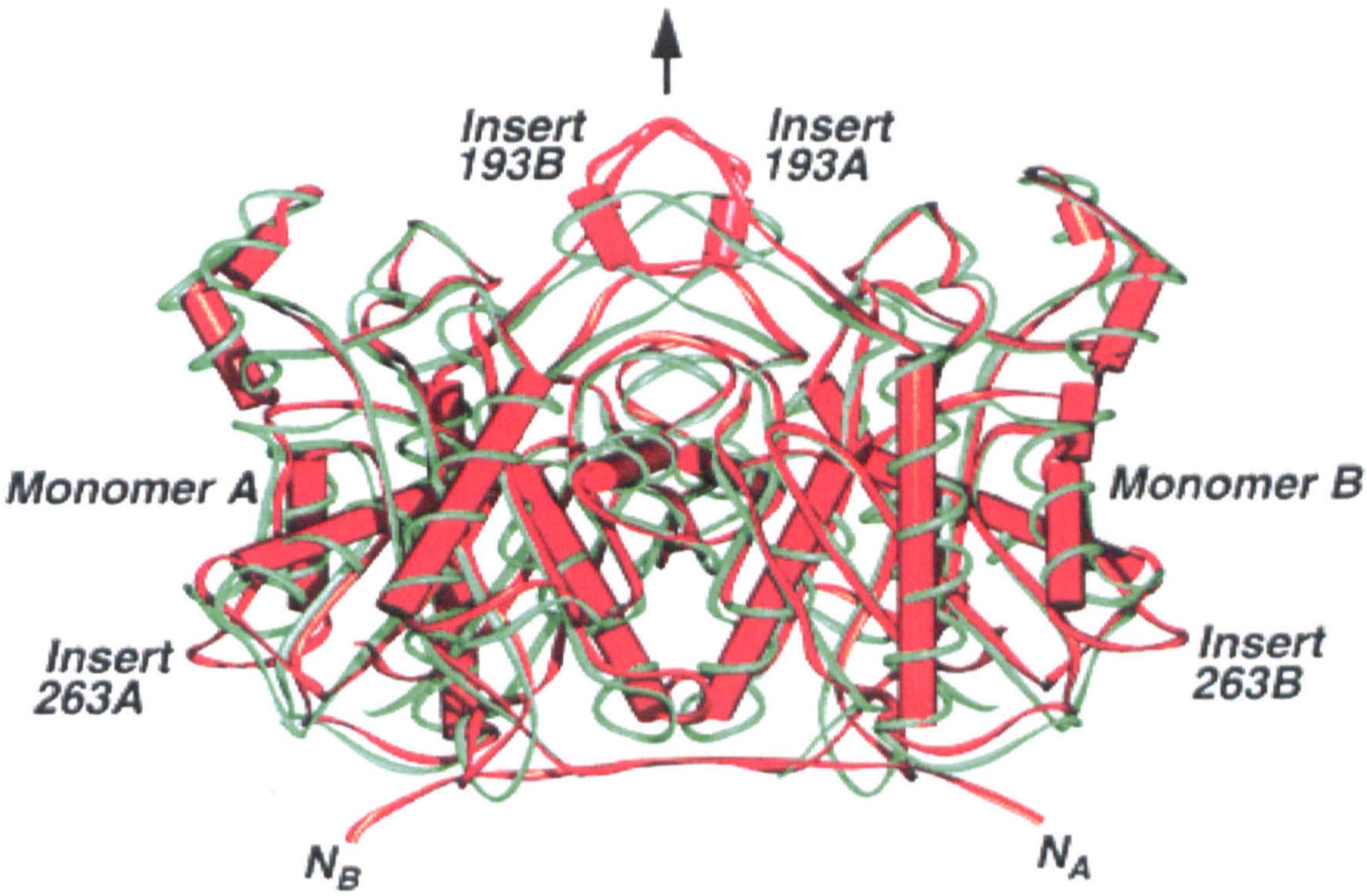


Figure 1.4: Crystal structure of mtFabH (red) and ecFabH (green).

In mtFabH, the side chain of Arg144 is spatially replaced by the side chain of Lys141 and Pro144 and Pro210 are replaced by Arg and Asp residues respectively in ecFabH. The amino acids at each termini form H-bonds and hydrophobic interactions between the monomers, which help to maintain the integrity of the dimer protein; the

H-bonds are formed between the side chain residues Thr9-Gln237, Arg316-Asn5, and Arg316-Ile174 and are mediated through water molecules. A four-residue insertion at the top of the longitudinal channel in mtFabH replaces Ser202 in ecFabH and leads to distortion in the local conformation of the loop and produces intra-molecular stabilizations. Phe87 in ecFabH obstructs the longitudinal channel at approximately 7.6 Å from the active site and restricts access to chains of no more than four carbons, which accounts for the selectivity of ecFabH for the substrate acetyl-CoA over other acyl-CoAs. Conversely, the longitudinal channel in mtFabH has Thr in the equivalent position thus allowing it to accommodate chains up to 20 carbons in length, a limit imposed by an α-helix capping it at the top of the channel. This difference in longitudinal channel length results in a difference in selectivity for substrates between ecFabH and mtFabH.[81] For the latter enzyme, the highest activity is obtained when the CoA carrier protein is conjugated to chain of 14-18 carbons in length, the activity decreasing sharply with chains either two carbons longer or shorter.[72]

The mtFabH homodimer weighs 76.6 ± 2.5 kDa, as determined by size exclusion chromatography, and SDS-polyacrylamide gel electrophoresis separated the monomers at 37 kDa each.[73, 81]

1.7.2 The mechanism of FabH

There are the three major functional residues involved in the FabH catalytic condensation mechanism, namely Cys112, His244, and Asn274. Others have a structural role, for example, Trp42, Arg161 and Arg46 that facilitate the binding of acyl-CoA in the active site by interacting with the CoA residue. Site directed

mutagenesis of any of the catalytic triad residues abolishes the activity of the enzyme completely, inferring the vitality of these residues to the catalytic process. The mechanism of FabH involves three major steps (Figures 1.5 and 1.6); initially, acetylation transfers the acetyl group from CoA to the sulphydryl group of Cys112; after the deacylated CoA has dissociated, malonyl-ACP (acyl carrier protein) enters the lateral channel, and is decarboxylated to form a carbanion; finally the carbanion undergoes claisen condensation with the acetylated Cys112 to produce the product.[73, 81, 83]

The mechanism begins through formation of H-bonds between the carbonyl oxygen of the acetyl group and the backbone NHs of Gly306 and Cys112 to orientate the acetyl group in the active site for nucleophilic attack by Cys112. His244, positioned 3.4 Å from Cys112, is used as a base to abstract a proton from the SH group. The reaction proceeds through the formation of a tetrahedral intermediate, where the carbonyl group forms an oxyanion that is stabilized by Gly306-NH and the positive charge on the protonated His244, the latter completing the reaction through protonation of the CoA leaving group (Figure 1.5). His244 and Asn274 are involved in the condensation process with malonyl ACP; His244 binds to the carboxylic acid moiety of malonyl-ACP (*via* a salt bridge formed through deprotonation), whereas the carbonyl group interacts with the side chain NH_2 of Asn274. The latter may promote decarboxylation by general acid catalysis *via* stabilization of an enol intermediate and immobilization of the carbanion for nucleophilic attack in the condensation step. The claisen condensation step is initiated by nucleophilic attack from the carbanion to the enzyme-bound acetyl group to form a tetrahedral

intermediate (again stabilized by Gly306-NH and the protonated His244) to finally yield the product acetoacyl-ACP (Figure 1.6).[73]

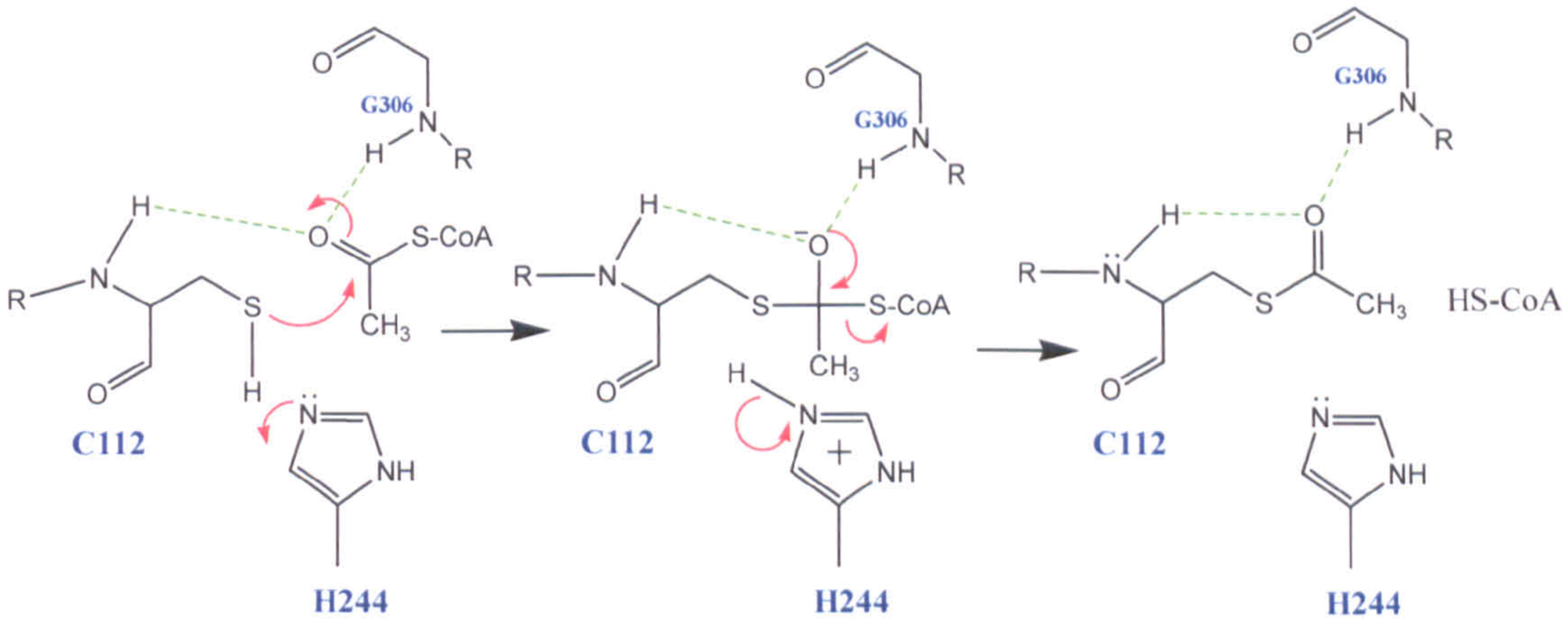


Figure 1.5: schematic diagram of the acetylation reaction mechanism in mtFabH.

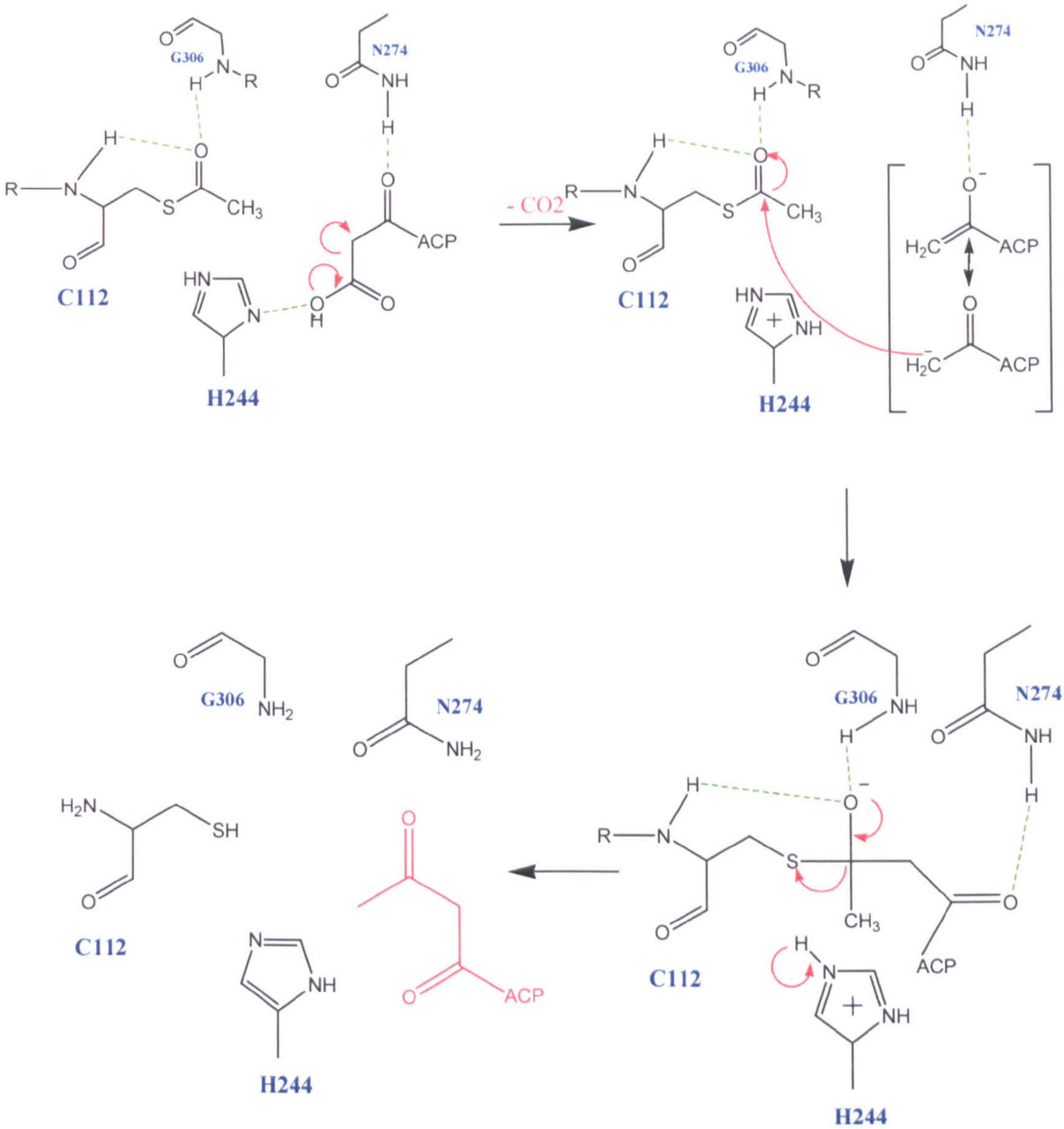


Figure 1.6: Proposed Scheme for the decarboxylation and condensation mechanism in mtFabH.

A key step in the condensation reaction is the ionization of Cys112 in the active site; the electrostatic field associated with an α-helix pointing with its terminus towards the Cys112 lowers the pKa of the thiol group (by 1.6 units) and encourages its ionization.[84]

Brown *et al* has suggested that thiolate ion formation proceeds *via* deprotonation of an active site water molecule by His244. The resultant hydroxide ion then deprotonates the sulphydril and acyl transfer from the CoA ensues (Figure 1.7).[85]

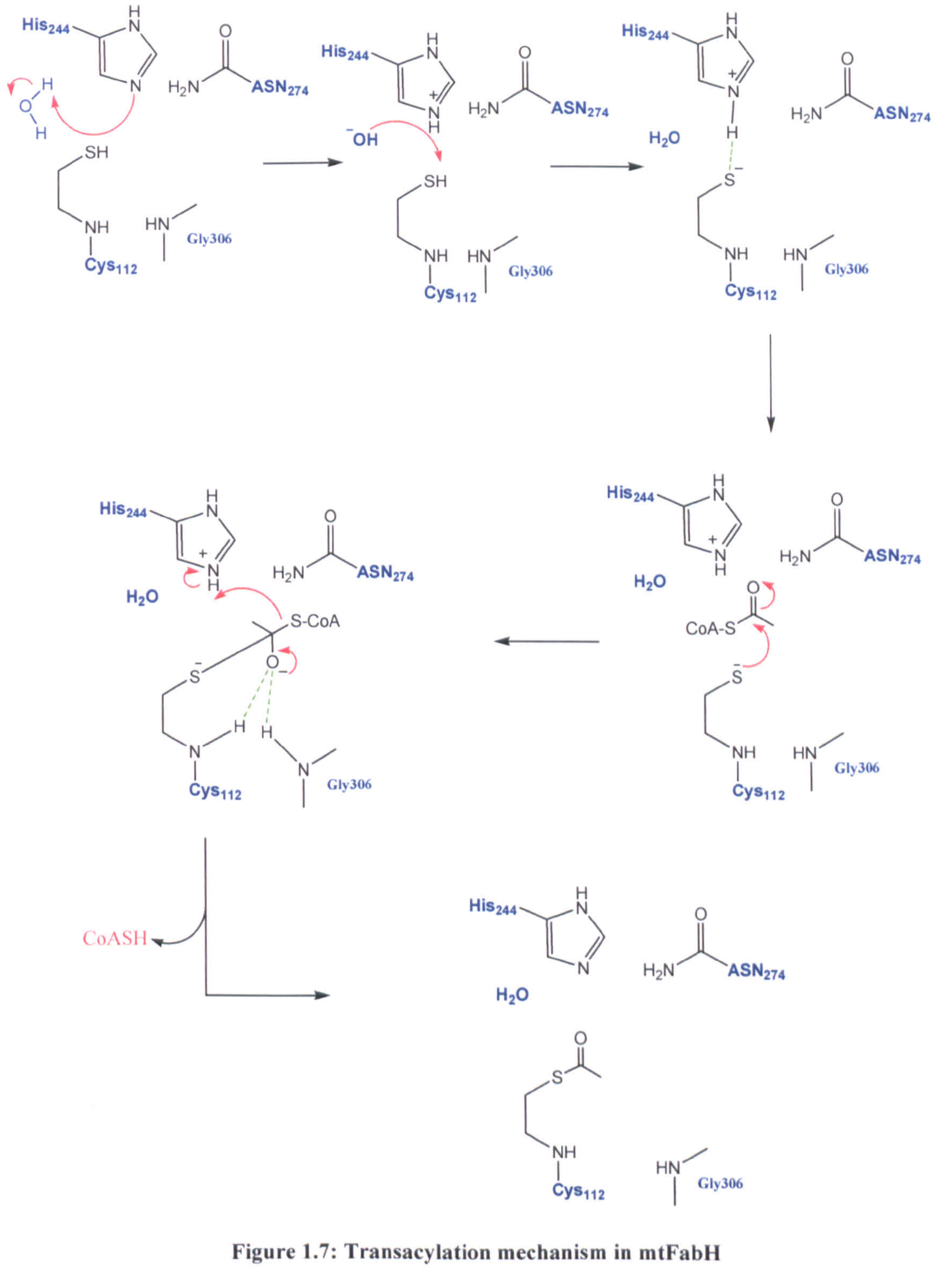


Figure 1.7: Transacylation mechanism in mtFabH

1.8 Biological substrates of FabH

Whilst CoA is the prosthetic group involved in the transacylation step transferring acyl groups to mtFabH, AcpM is the other substrate that binds to the enzyme after the acetylation process has taken place. Unlike ecFabH, in mycobacteria spp. mycobacterial Acyl Carrier Protein (AcpM) is the shuttling unit in the FAS-II system that facilitates movement of the intermediates between the separate enzymes in the complex. The presence of the *acpM* gene in the same operon that encodes the other enzymes in fatty acid synthesis supports its role and function.[86]

AcpM is a small, acidic, and highly conserved protein that is 8.87 kDa in weight. It is an asymmetric monomer composed of α-helices packed in a bundle and is distinguished from other ACPs by possessing an extended and flexible C-terminus of thirty-five amino acids, which is believed to have a role in protein-protein interactions (Figure 1.8).[56] Malonates are attached to ACP *via* a sulfhydryl bond linked to the 4-phosphopantethine prosthetic group, and binding is both specific to deliver the substrate and weak to allow rapid turnover.[57, 75, 76, 87]

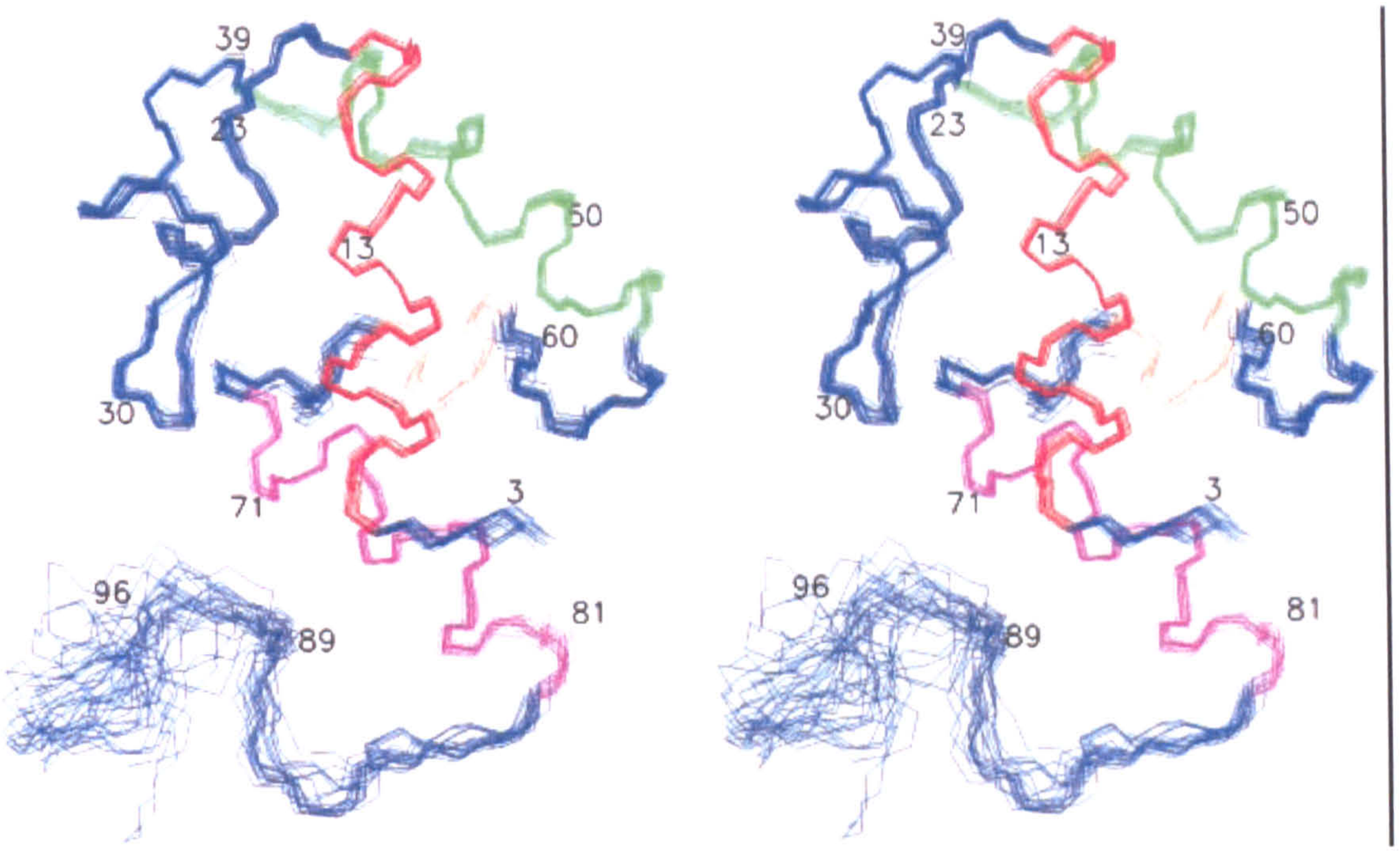


Figure 1.8: Twenty NMR structures of AcpM showing the long chain C-terminus

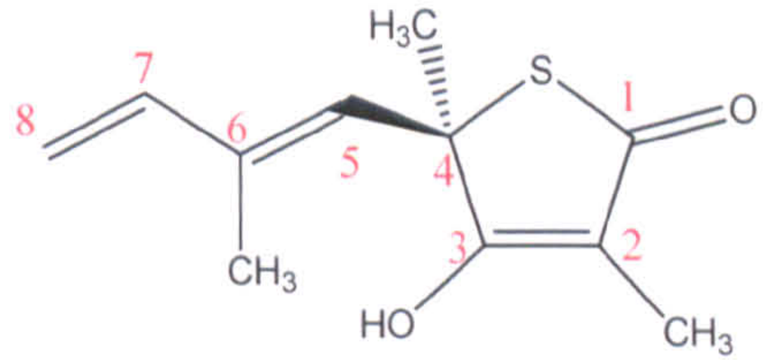
1.9 Inhibitors of FabH enzyme

mtFabH is considered a crucial target for disruption of the growth of Mtb.[46] The strategic position of this enzyme between FAS-I and FAS-II (see figure 1.2) makes it essential for the synthesis of mycolic acids and hence lipids in the mycobacteria cell wall. Moreover, mtFabH has no counterpart in mammalian cells, an advantage that could provide specificity and selectively for drug design.[46]

1.9.1 TLM as a FabH inhibitor

Thiolactomycin (TLM) **(13)**, [(4R)(2E,5E)-2,4,6-trimethyl-3-hydroxy-2,5,7-octatriene-4-thiolide], a natural product that has been isolated from the soil fungus *Nocardi* species, belongs to a small group of antibacterial compounds called thiotetronic acids. It exhibits antibacterial activity against both Gram-negative and Gram-positive bacteria including Mtb. TLM has also shown activity against

Plasmodium falciparum and trypanosomes, the latter being attributed to the presence of a FAS-II system in these eukaryotic unicellular parasites.[36] TLM inhibits fatty acid synthesis in Mtb and *E. coli* by reversible and competitive inhibition with ACP binding to enzymes in the FAS-II system.[88] TLM inhibits both mtFabH and ecFabH, the former more effectively with an IC_{50} of 5.0 μg/ml compared to 16.4 μg/ml for ecFabH.[72]



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Whereas a co-crystal structure of TLM with FabH has not yet been reported, the crystal structure with another condensing enzyme in the FAS-II system, FabB, has been studied in order to understand the interaction of TLM against condensing enzyme targets. FabB was proven to be related to FabH in its structure as both have in their monomer a duplicated helix-sheet-helix motif and the active site in both enzymes is buried and accessed through a channel. Moreover, the mouth of the active site in both enzymes has conserved residues to bind CoA and ACP.[88] FabB is also found to perform a similar role by executing a crucial step in the fatty acid cycle by condensation of malonyl-ACP with acyl-ACP to give a β-ketoacyl-ACP, which is a similar product from FabH.[36] TLM appears to mimic malonyl-ACP in the FabB active site, the second step in the condensation reaction (Figure 1.9).

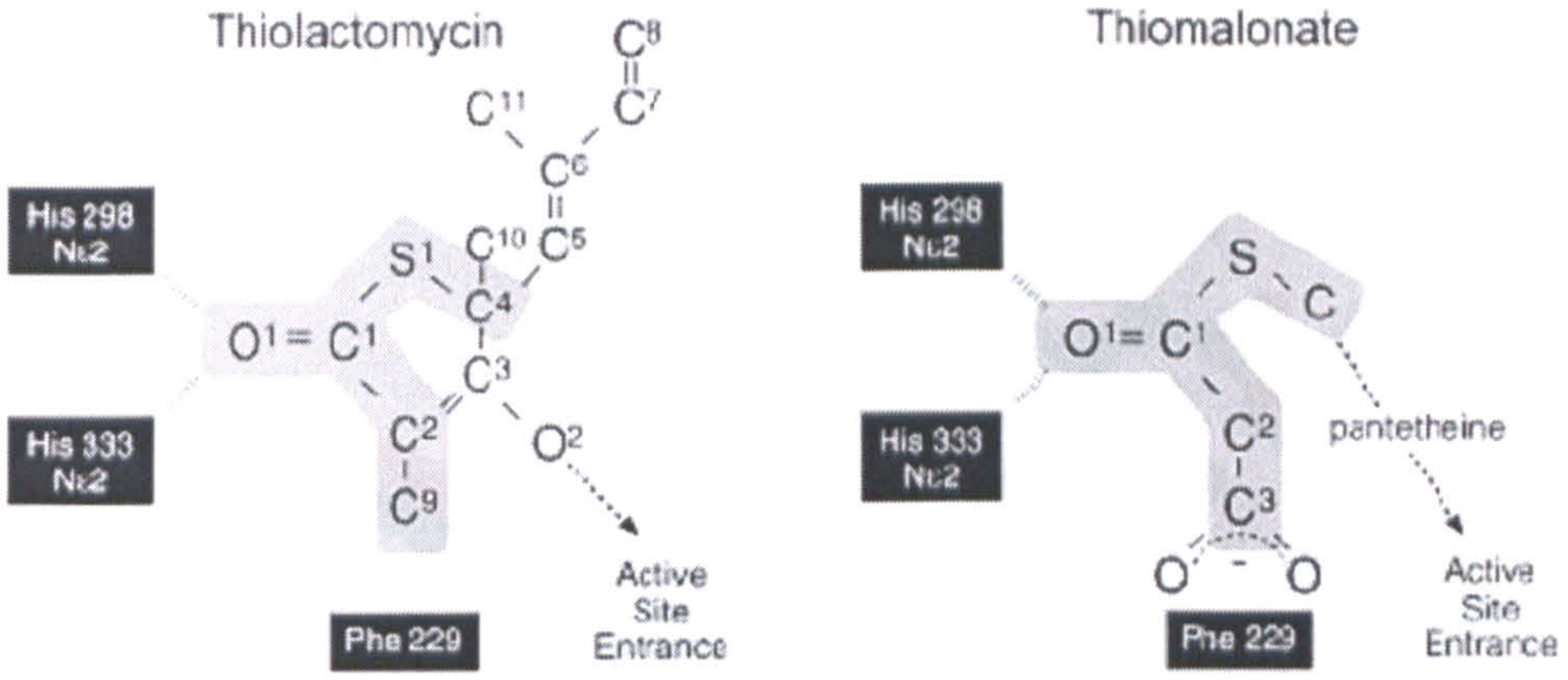


Figure 1.9: TLM mimics thiomalonate in the active site of FabB.[88]

The minor differences between the active site in FabB compared with FabH may be responsible for workers inability to co-crystallize TLM with FabH; the active site residues in FabB are His-His-Cys, compared to Asn-His-Cys of FabH, and the former triad was found to facilitate the strong hydrogen bonding between the two catalytic histidines and the carbonyl group of TLM through water intermediates in the active site (Figure 1.10).[88] Mutation of the active site of FabB by replacing one His by Asn to mimic FabH led to drastic reduction of inhibitory activity by TLM and illustrates the importance of the two His residues. [88]

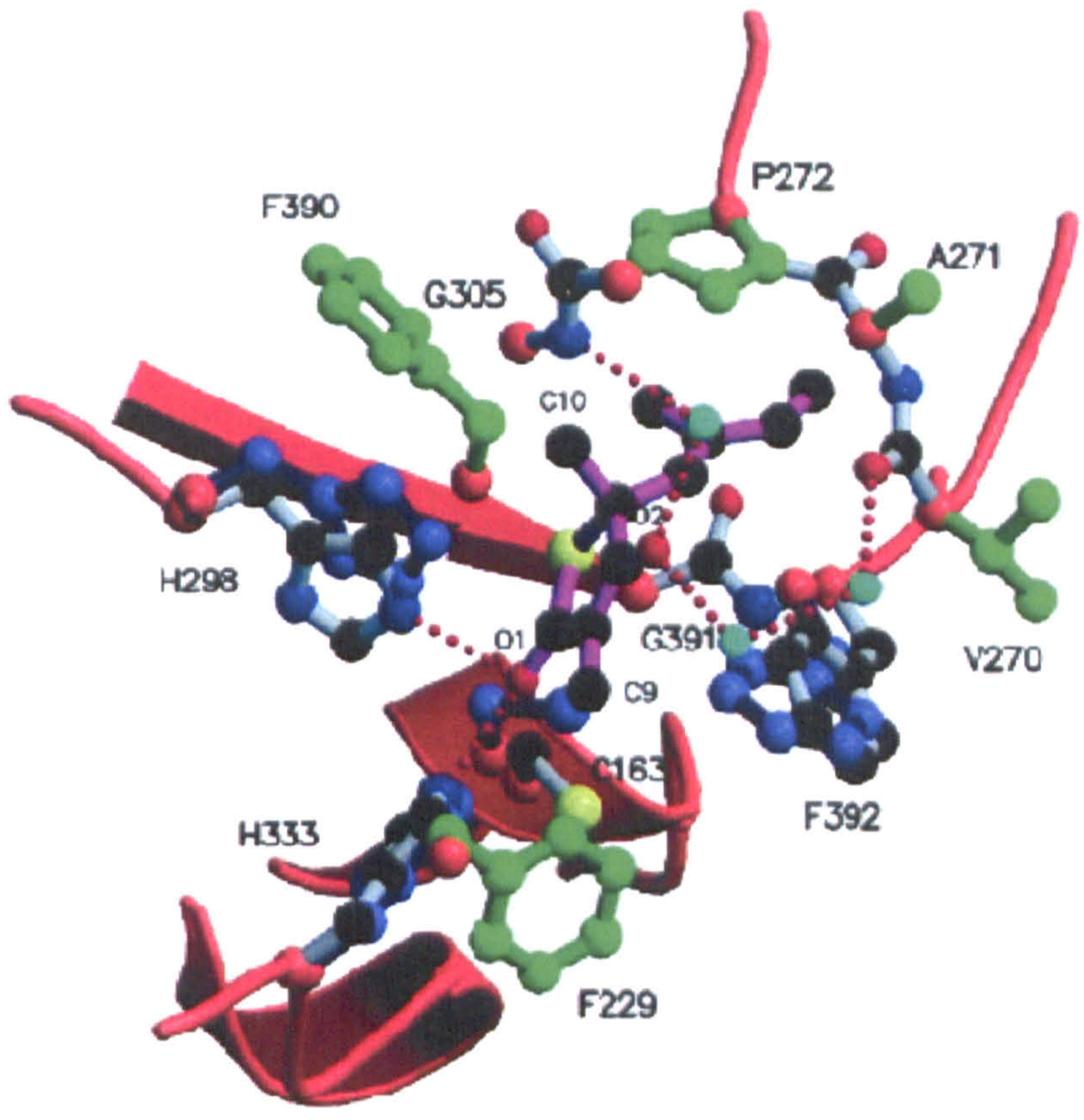
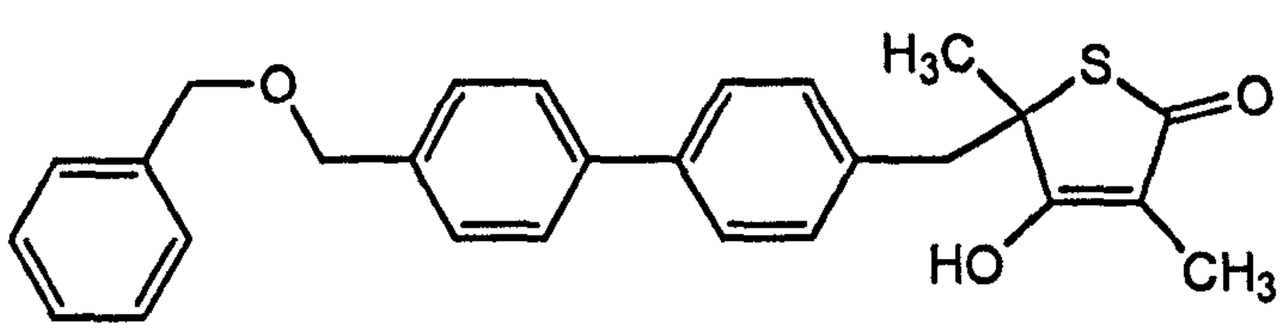


Figure 1.10: The structure of the FabB-TLM binary complex

As TLM is a weak inhibitor of FabH, extensive work has been performed to optimize its activity and produce more potent inhibitors, primarily through modification of position 5 of the thiolactone ring where the isoprenoid unit is attached. SAR studies have shown that the integrity of the thiolactone ring is essential as it resembles the malonyl-ACP substrate for mtFabH. Substitution at the sulfur atom or the carbonyl group present at C_2 decreases or abolishes activity. This latter group forms an H-bond with one or more of the catalytic residues in FabB (figure 1.10), and is expected to perform a similar function in FabH. The methyl group at C_3 and the double bond between C_3 and C_4 are both important for activity, possibly by a hydrophobic effect. Removal or esterification of the hydroxyl group at C_4 reduces activity.[89-91]

Various substitutions at position 5 have produced some of the most potent derivatives of TLM against mtFabH; the biphenyl-based analogue **(14)** has an IC_{50} of 17 μM, (TLM had an IC_{50} 74.9 μM),[92] whilst the phenyl acetylene derivative **(15)** was the most active with an IC_{50} of 4.0 μm.[93]



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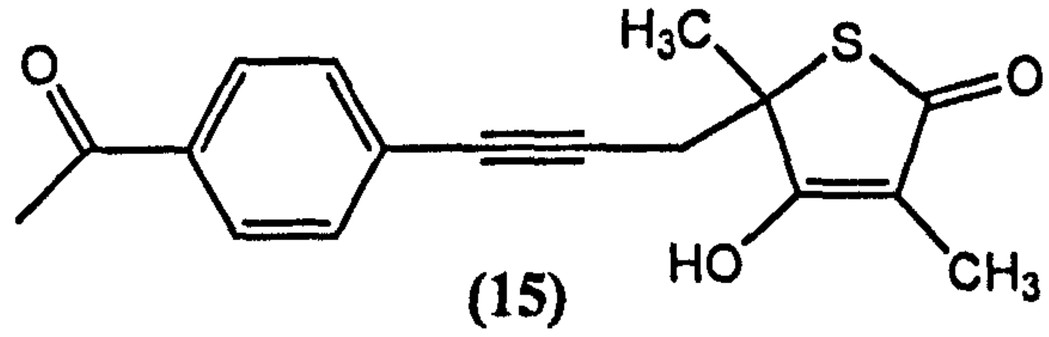


(15)

Douglas *et al* prepared a series of racemic TLM analogues derivatized at position 5 and tested them against whole cell Mtb (table 1.1).[89]

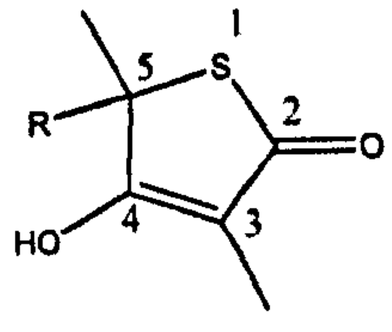
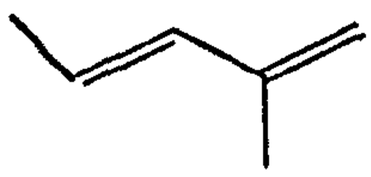
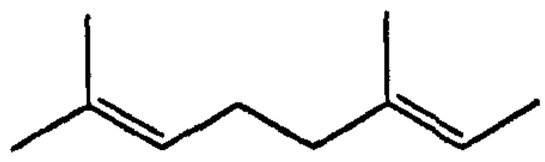
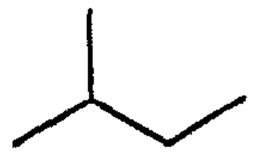
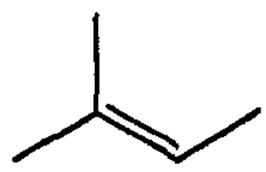
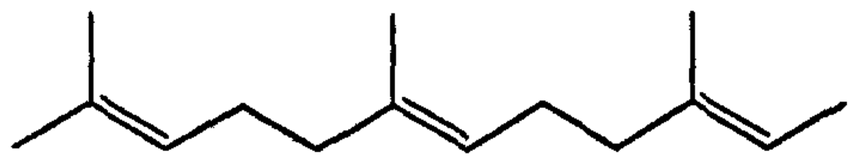
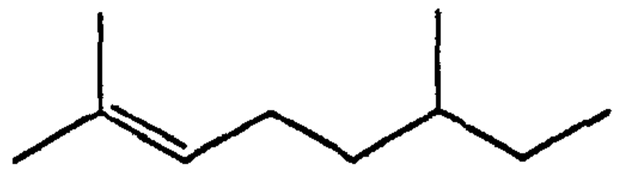
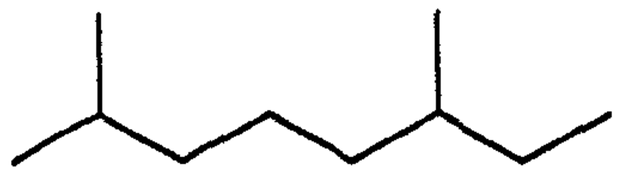
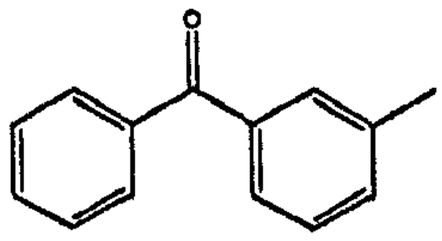
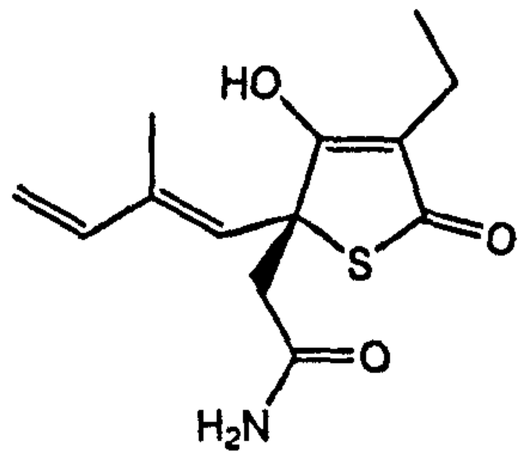
Compound		*In vivo* activity against M. tuberculosis(μM)	
	R group	MIC_{50}	MIC_{90}
TLM		56	125
1		96	200
2		230	> 300
3		> 300	> 300
4		23	29
5		43	56
6		22	29
7		50	128

Table 1.1: Thiolactomycin analogues and their MIC_{50} and MIC_{90} values against *M. tuberculosis*

This study by Douglas *et al* (Table 1.1) revealed two general trends that contributed to the increase of TLM activity against the whole cell. Firstly, increasing the chain length improved activity, whereas branching did not appear to be important. Secondly, conformational restriction which is confirmed by the presence of double bonds in the side chain appeared to reduce activity.[94]

The natural analogue of TLM, TU3010 **(16)** was also found to inhibit the FAS-II system in *S. aureus* with IC_{50} = 41μM compared to IC_{50} = 61μM for TLM.[95]

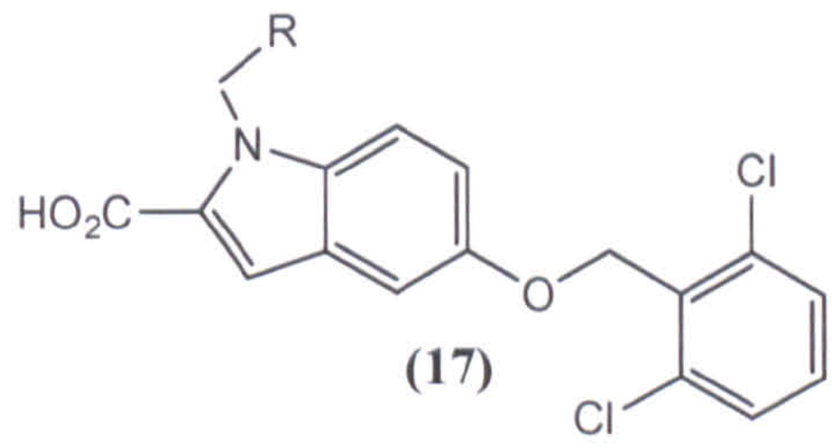


(16)

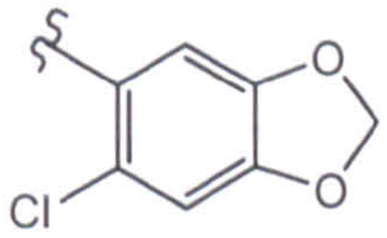
Over-expression of mtFabH showed that it is not the only target for TLM *in vivo;* other enzymes such as the elongating enzymes KasA and KasB are also inhibited.[89] Experiments have revealed that over-expression of KasA shifts the MIC against *M. bovis* from 30 μg/ml to 80 μg/ml, whilst over-expression of KasB raises the MIC to 100 μg/ml, suggesting that both enzymes are targets for TLM.[94] In a separate study to determine the role of KasA and KasB as drug targets, DNA microarrays were used to generate signature profiles of Mtb in response to treatment with INH, TLM, and triclosan. Response profiles to the three drugs and a predictive model based on the expression pattern of twenty-one genes enabled the clarification of INH, TLM, or triclosan treated Mtb in terms of their precise mode of action.[96]

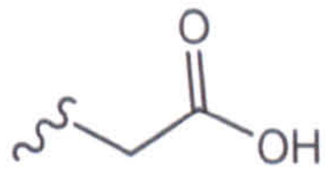
1.9.2 Other inhibitors of FabH

In addition to TLM and its derivatives, there are other classes of compounds that inhibit FabH. The 2,6-dichlorobenzyl derivatives **(17)** are a group of inhibitors that have been co-crystallized with the FabH enzyme. The key elements of binding between the ligand and the enzyme are; (i) the 2,6-dichlorobenzyl group that occupies a hydrophobic region within the longitudinal channel of the active site; (ii) the acidic group that forms an ionic interaction with one of the Arg residues at the top of the active site lateral channel (Figure 1.11). The IC_{50} of the most active compounds **(17a)** and **(17b)** are 0.83μm and 1.70μm respectively against ecFabH.[97]



(17)

(17a) R= 

(17b) R= 

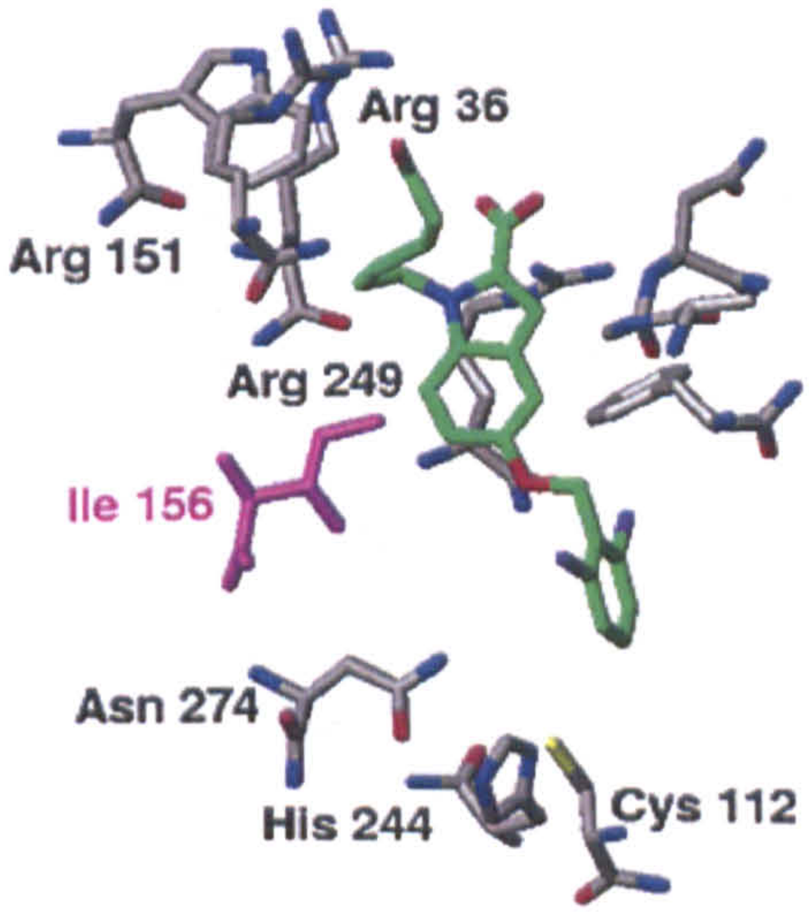
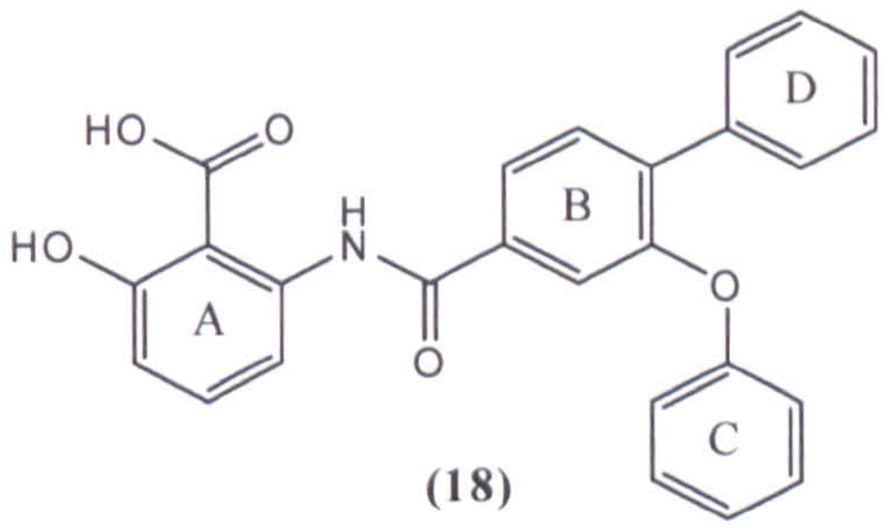


Figure 1.11: Co-crystal structure of 2,6-dichlorobenzyl derivative (17b) with *E. coli* FabH

Ashek *et al* has performed a combined approach of docking and QSAR (quantitative structure activity relationship) studies for hits against *Entercoccus faecalis* (ef)FabH. 2-hydroxy-6-(3-phenoxy-4-phenyl-benzamido) benzoic acid **(18)** was found to fit optimally in the active site, with the phenyl ring (D) strongly interacting with the side chains of Thr43, Val160 and Trp38. His250 (equivalent to His244 in mtFabH) had strong H-bond interactions with the carboxylic acid of ring (A) whilst the phenoxy group of the same compound was adjacent to Ile223 and interacts with the Asg221 *via* an H-bond interaction (figure1.12).[98]



(18)

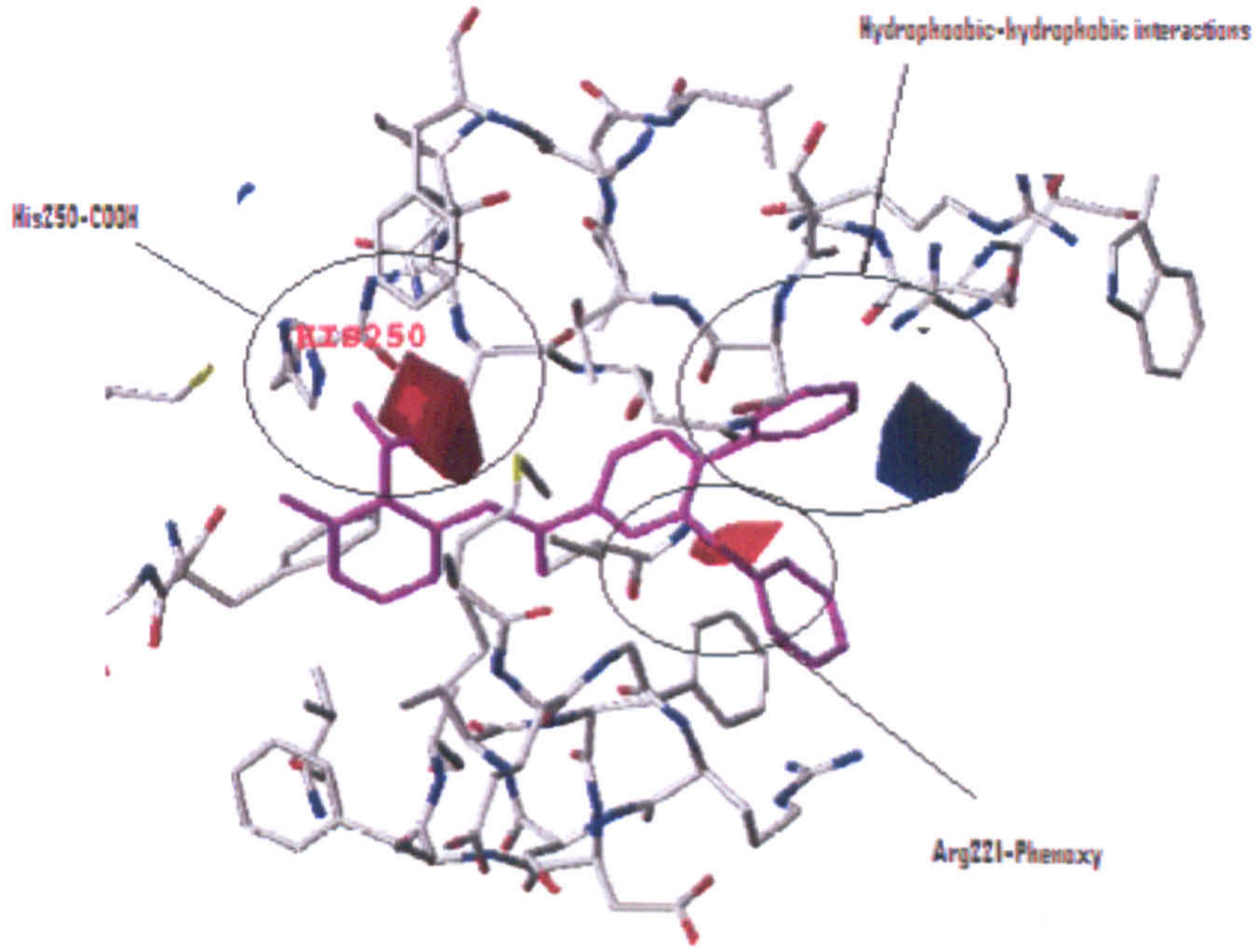
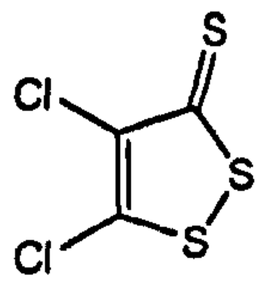
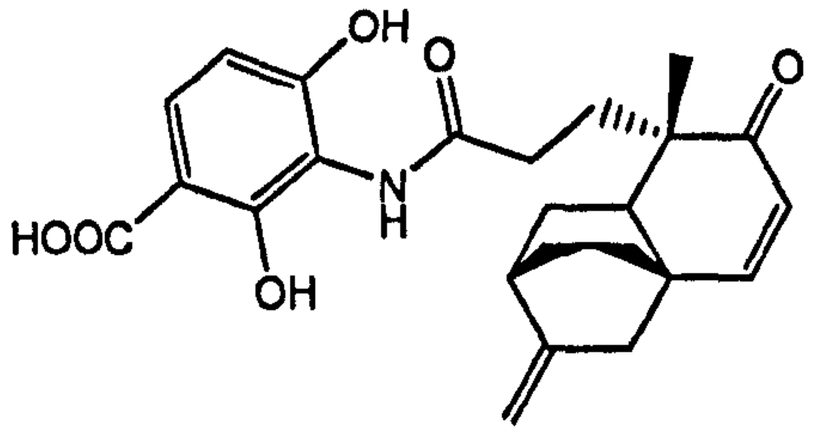


Figure 1.12: Proposed complex between the ecFabB-2-hydroxy-6-(3-phenoxy-4-phenyl-benzamido) benzoic acid (18)

In 2004 He *et al* demonstrated that the 1,2 dithiol-3-ones were inhibitors of both ecFabH and *Staphylococcus aureus* FabH, the dichloro analogue **(19)** being the most active with an IC_{50} value of 1.0 μM.[91, 99] Wang *et al*, investigating natural products as hits, showed that platencin **(20)** inhibited both FabH and FabF with IC_{50} values of 3.91 μg/ml and 1.95 μg/ml respectively.[100]



(19)



(20)

1.10 Aims and objectives

Our aim was to design and synthesise a series of novel compounds based on TLM, and to test them against the enzyme mtFabH and the whole organism Mtb. We were particularly interested in this enzyme because of its pivotal role in the synthesis of mycolic acids in Mtb, and through which inhibition should paralyse the ability of the bacteria to synthesize the cell wall. TLM itself has many disadvantages as a hit candidate; the main one being its multistep synthesis that requires chiral considerations. Additionally, in the absence of a crystal structure of TLM bound to mtFabH, and there being a limit to the amount of inference possible from the related TLM-FabB structure, a ligand based approach through the generation of a wide and diverse set of compounds becomes necessary. This is not possible for TLM itself because of its laborious synthetic preparation, and we required an alternative scaffold amenable to tractable synthesis that could furnish a large number of derivatives to probe and optimize activity.

The specific objectives were as follows:

- To design a novel scaffold for the inhibition of mtFabH.

- To synthesise a large series of compounds based around the scaffold to probe for activity against mtFabH.

- To test the synthesized compounds against mtFabH and in parallel *in vitro* whole cell assays using a variety of mycobacterial strains including *M. aurum* and Mtb.

- To model the synthesised compounds *in silico* using the crystal structure of the target enzyme to develop a structure activity relationship for the synthesized compounds.

- To test the synthesized compounds against normal cell lines to evaluate their cytotoxicity.

Chapter 2: Results and Discussion

The design of a new lead against the target enzyme mtFabH was performed and evaluated using Computer Aided Drug Design (CADD) programs to facilitate the process of choosing a suitable scaffold and building a hypothetical structure activity relationship (SAR). In addition, the literature was investigated to help build a more comprehensive picture concerning the enzyme importance and role. For example, the process by which the enzyme performs its job, and the way it is inhibited has established a clear scenario of how it can be targeted. To this end, a series of compounds was synthesized in the laboratory and evaluated *in vitro* against the whole cell in addition to the enzyme itself. The synthesis of these compounds was guided using these data in parallel with the data obtained from the docking programs.

2.1 Thiazole scaffold selection

The choice of the thiazole ring system as a candidate for the inhibition of mtFabH was based on various criteria: the active site geometry, the binding mode of the natural substrates (CoA, AcpM), the binding pattern of known inhibitors of FabH, and *in silico* docking studies to evaluate the fitness of the designed compounds in the active site.

2.1.1 Active site geometry

Figure 2.1 shows the mtFabH enzyme, a homodimer where each monomer has two channels; the longitudinal channel that is narrow, hydrophobic and capped, and the lateral channel, which is wider and is the entrance for substrates to the active site. The active site itself is positioned at the junction between the two channels where

they form an angle of around 100-110° and includes three key residues; cysteine 112, histidine 244 and asparagine 274.

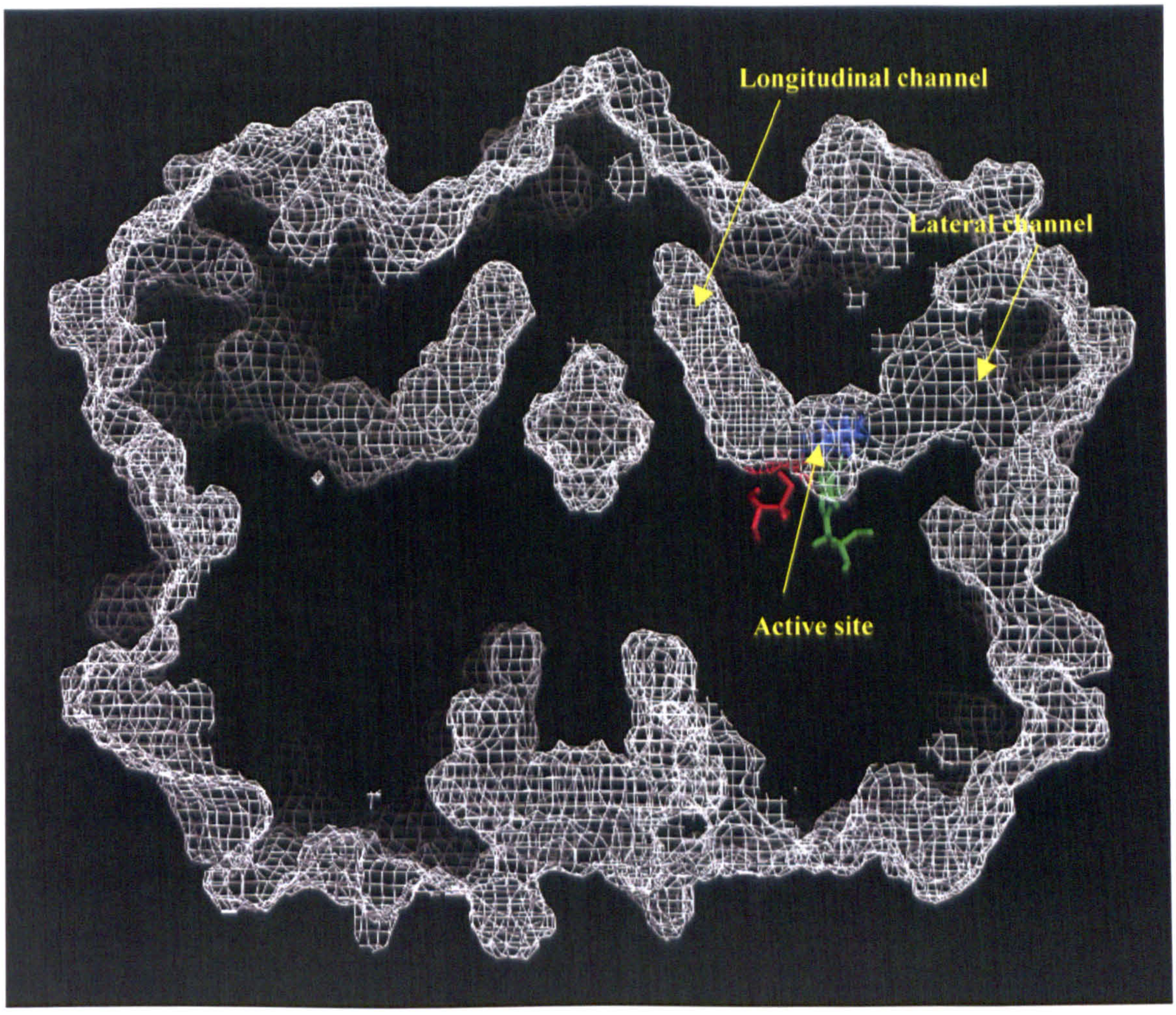


Figure 2.1: The homodimer structure of mtFabH enzyme showing the channels and the active site residues, Cys112-red, His244-green, and Asn274-blue.

The arrangement of the active site residues is shown in figure 2.2, and an appreciation of their spatial arrangement is crucial to understanding the mechanism of the enzyme. The distance between Cys112 and His244 enables deprotonation of the thiol group by the imidazole ring to initiate acylation, whilst the proximity of His244 to Asp274 facilitates decarboxylation of the malonate substrate and subsequent condensation (figures 1.5 and 1.6).

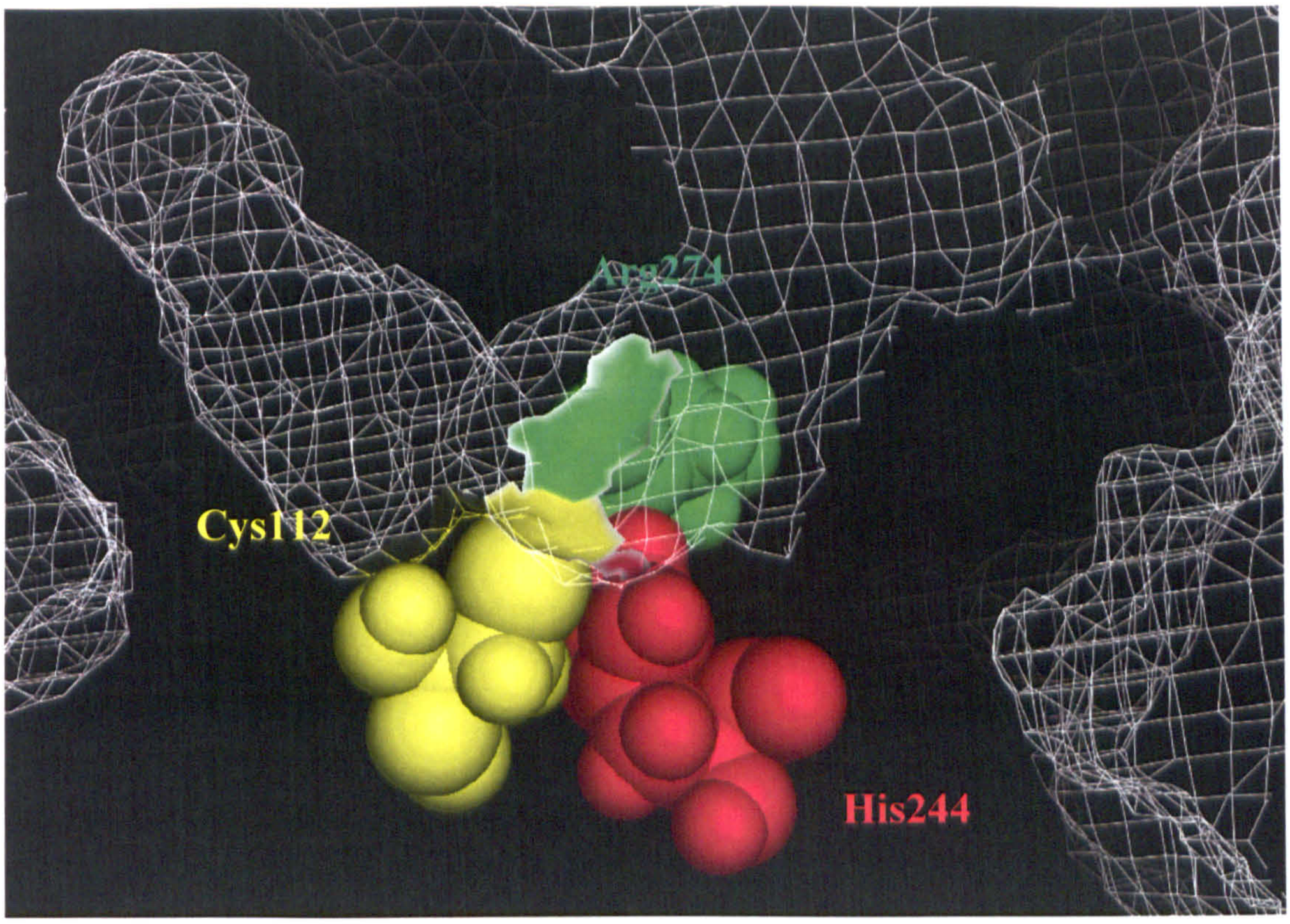


Figure 2.2: Spatial arrangement of the active site residues.

In terms of this active site geometry, a candidate inhibitor has to have features that satisfy the properties of the channels in terms of their angle and size, and to have optimum interactions with residues in the active site to competitively inhibit the substrate.

2.1.2 Substrate binding pattern

Figure 2.3 depicts how the acyl-CoA substrate would be positioned in the active site of mtFabH prior to acylation of the Cys112 residue, with the lipophilic acyl moiety filling the longitudinal channel. The transacylation intermediate of acyl-CoA with the active site (figure 2.4) demonstrates the importance of H-bonding with Cys112 and Gly306, with charge stabilization by the protonated His244 also an important feature. The sulphur atom of acyl-CoA is thought to have a hydrophobic interaction with the equivalent group of Cys112 in the active site. The acyl moiety in the longitudinal

channel provides further predominantly hydrophobic interactions whilst the CoA in the lateral channel interacts with the mouth of the active site at the surface.

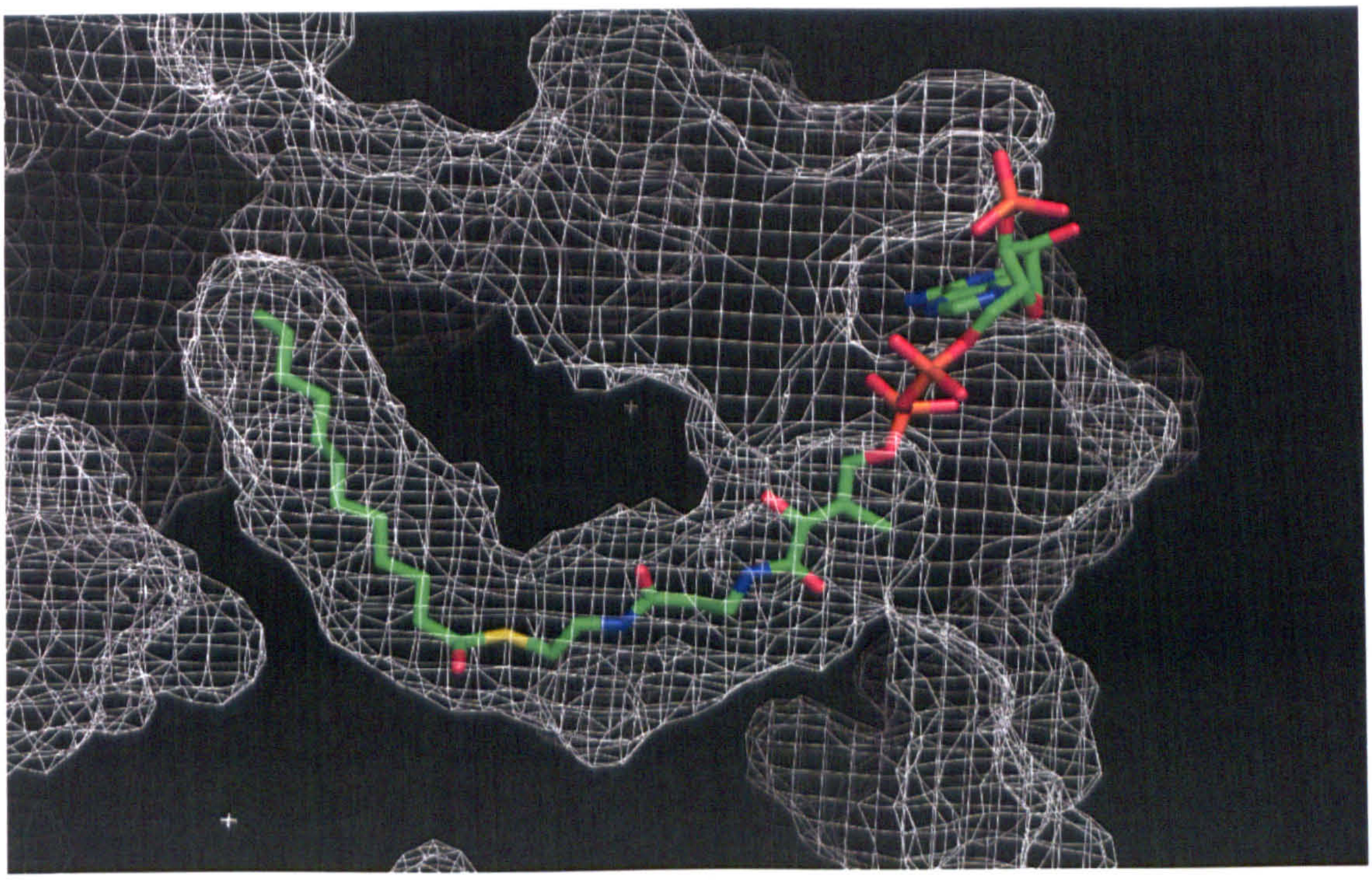


Figure 2.3: X-ray crystal structure of mtFabH: acyl-CoA.[101]

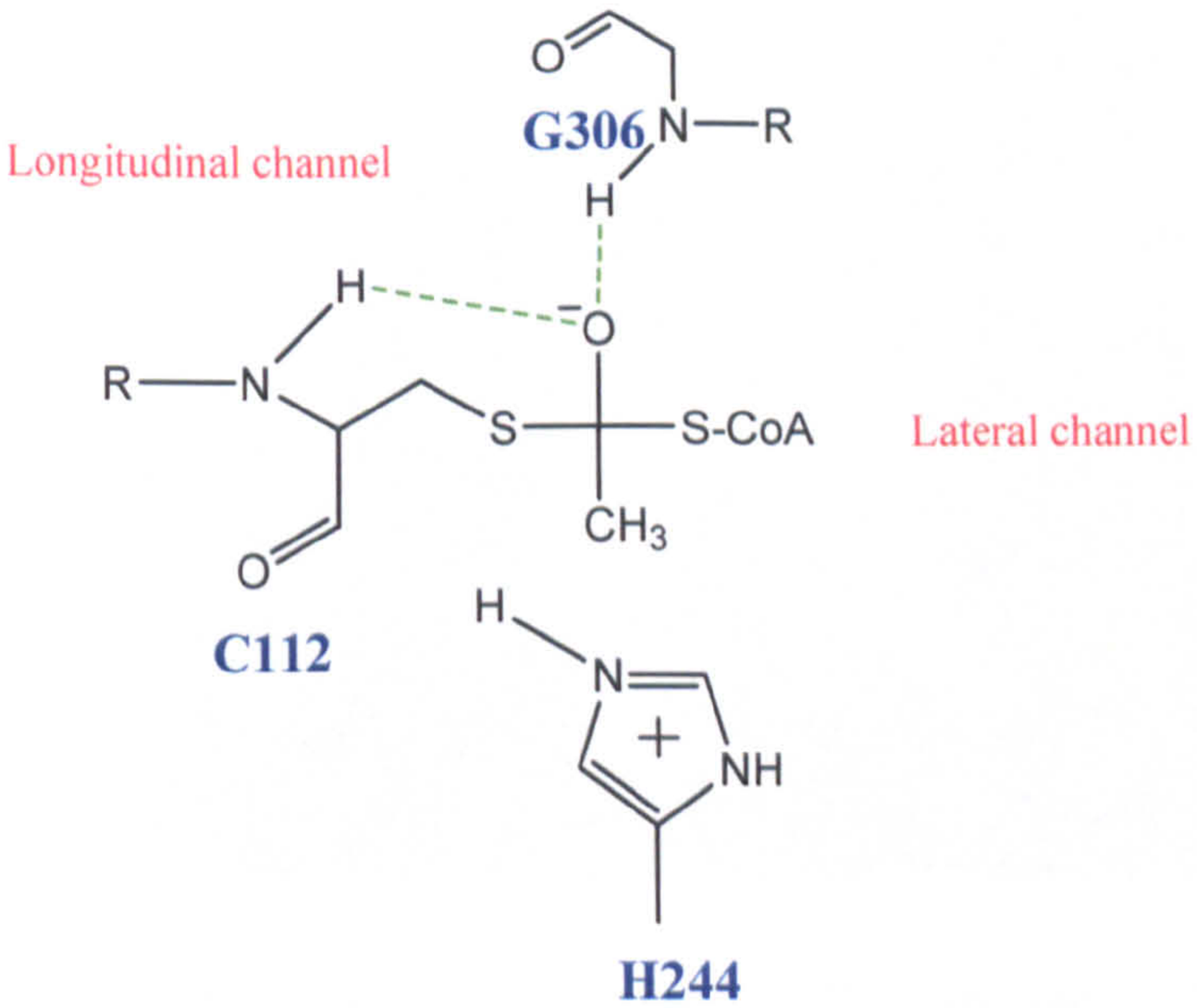


Figure 2.4: Schematic diagram of the nature of acyl-CoA interaction with mtFabH active site residues.

Whilst the binding of the natural acyl-CoA substrate can reveal some important interactions to be taken into consideration for the design of a new lead, studying known inhibitors can also help direct drug design.

2.1.3 Inhibitors binding pattern

The binding of TLM to the active site of mtFabH is still under investigation and no co-crystal structure is yet available, whereas TLM complexed with ecFabB has been solved, and useful parallels can be drawn for inhibitor design (figure 2.5).

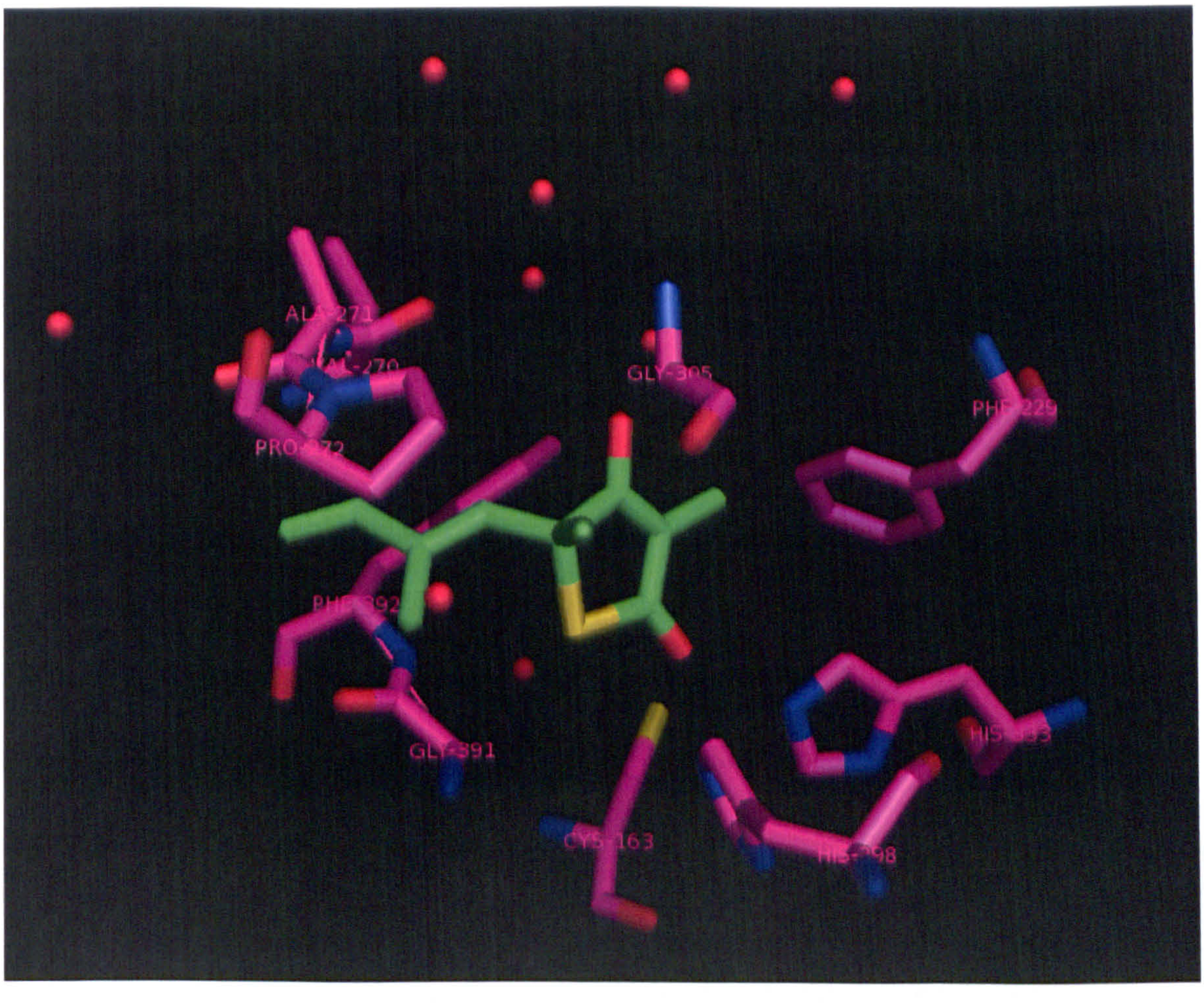


Figure 2.5: The structure of the ecFabB-TLM binary complex.[88]

TLM is a reversible inhibitor of ecFabB by forming a number of non-covalent interactions; the methyl group at carbon 3 of TLM is positioned in a hydrophobic pocket defined by residues Phe229 and Phe392, the isoprenoid moiety is wedged between two peptide bonds; from above by residues Val271 and Phe272, and from below by Gly391 and Phe392, which are important for specificity. The carbonyl oxygen forms two H-bonds with the two histidines in the active site, the hydroxyl group bonds to the carbonyl oxygen of Val270 and the amide nitrogen of Gly305 through a lattice of three water molecules, and the sulphur is adjacent to the active site cysteine, although without any obvious interaction. The strong H-bonding in the active site of ecFabB between the TLM carbonyl group and the histidines is believed to be responsible for the good activity against this enzyme. Based on this analysis, binding of TLM to the active site of mtFabH could be expected to have some similarity with that of ecFabB, and suggests that maintaining an equivalent H-bonding network is very important.

Other inhibitors of FabH related enzymes could also help in the design of a new scaffold for mtFabH itself. For example, 2-hydroxy-6-(3-phenoxy-4-phenyl-benzamido) benzoic acid showed very good activity against efFabH, and revealed an important role for a carboxylic acid moiety in the ligand as it forms specific interactions in the active site (figure 1.12).[98]

In order to gain a better understanding of TLM binding in the active site of mtFabH, we performed a docking study with different algorithms to find suitable binding modes for the ligand by exploiting the space, shape, hydrophobicity and H-bond

donor and acceptor properties of both the active site and the ligand. Using "Affinity Docking" in Insight II, TLM can be positioned in different sites in both channels in many different orientations that have similar affinity, which makes identifying a definitive binding mode for TLM in the active site difficult to ascertain. When TLM derivatives with biphenyl or acetylene groups at position 5 that show greater activity against mtFabH *in vitro* were docked in the active site, once again no preferred orientation could be identified with any confidence.

Similar results were found when using the GOLD (Genetic Optimization for Ligand Docking) software; the thiolactone ring took different poses; both in the longitudinal and lateral channels, and in the active site, and all poses showed no significant hydrogen bonding network with the protein. All details for docking procedures are described in section 3.4.

2.1.4 Thiazole ring choice

By combining our analysis of TLM binding to ecFabB with the 2-hydroxy-6-(3-phenoxy-4-phenyl-benzamido) benzoic acid binding to efFabH, we proposed the thiazole scaffold for developing mtFabH inhibitors. This scaffold was selected for a number of reasons; thiazoles are metabolically stable and feature in a number of drugs; the planar, aromatic ring is achiral and overcomes the need for the chiral synthesis of the TLM scaffold; thiazole derivatives are readily accessible and can be the source of large libraries to probe for activity.

The five membered aromatic ring was chosen despite the active site being able to accommodate six membered rings because a five membered ring would allow more flexibility and movement of to explore the optimum interactions with the surrounding residues in the active site once substituents had been incorporated. 2-aminothiazole-4-carboxylate (2-AT-4C) binding was deliberately chosen to mimic the binding of the substrate by producing a H-bonding network, electrostatic binding, and sulphur-sulphur hydrophobic interactions between ligand and protein. Figure 2.6 shows the expected binding mode of the 2-AT-4C derivatives inside the active site. Side-chains at position 2 and 5 were also chosen to complement the active site geometry between the two channels and potentially exploit further interactions with the protein.

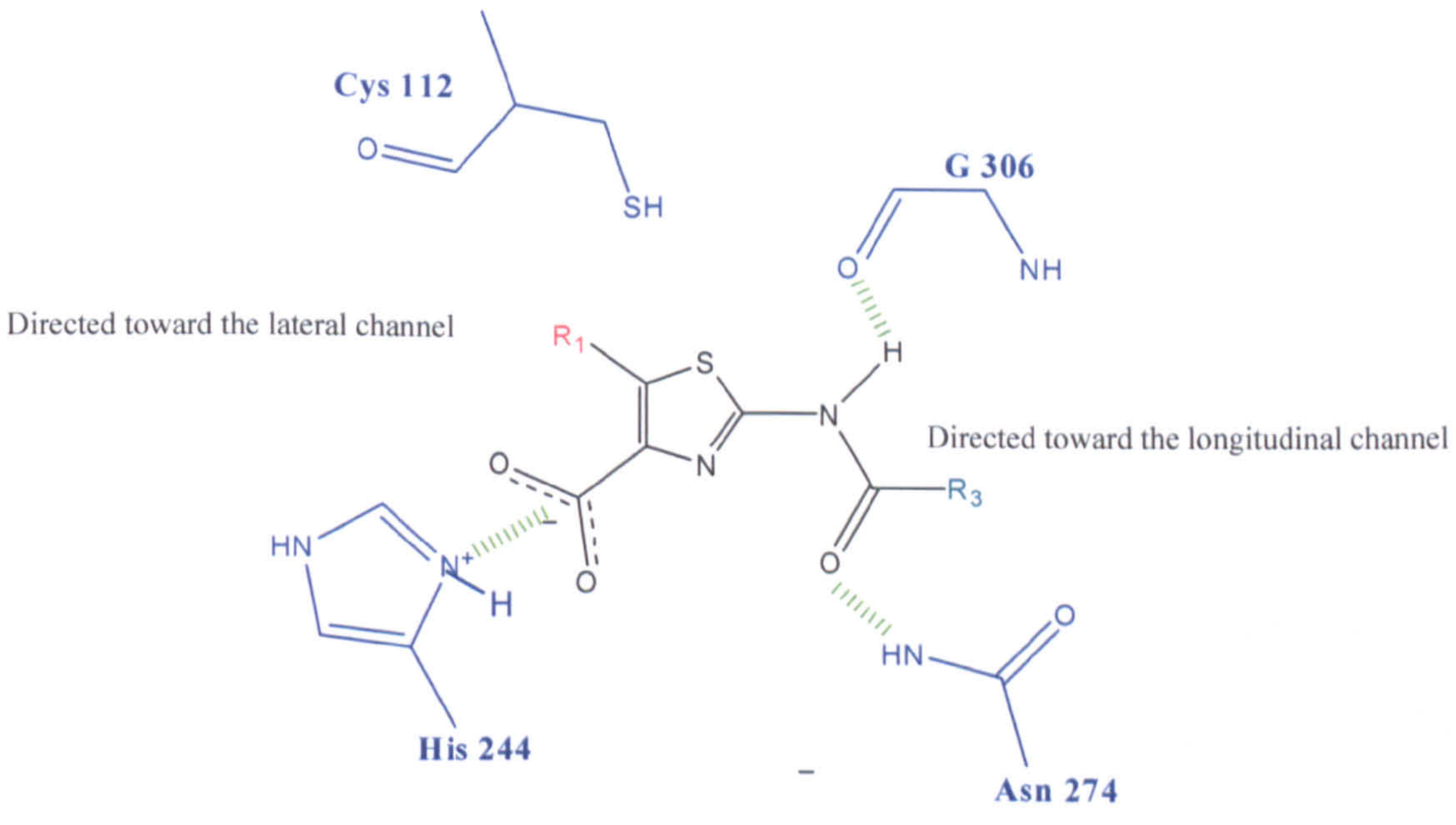
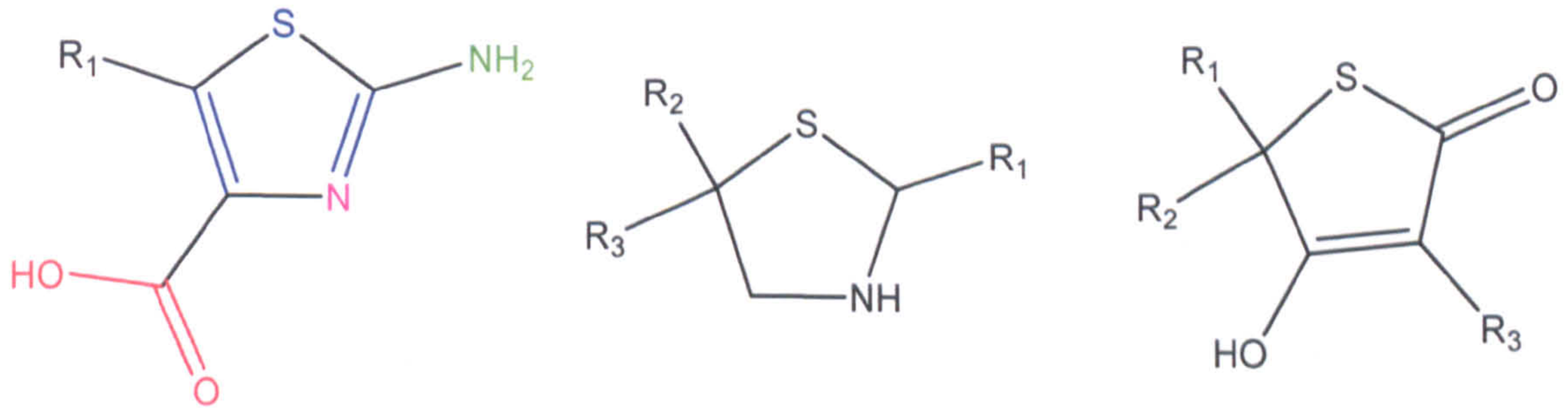


Figure 2.6: The proposed pattern of the binding of thiazole ring scaffold inside the active site of mtFabH.

The docking program GOLD was used to direct the choice of the thiazole ring derivatives and to analyze and predict how these compounds would adapt themselves in the active site. Figures showing the orientation of some of the derivatives that were docked with GOLD inside the active site will be presented later in the chapter.

2.2 Hypothetical structure activity relationship (SAR) of thiazole derivatives

The anatomy of the thiazole ring is shown in figure 2.7. It is a more lipophilic, aromatic system than the saturated counterpart thiazolidine ring and thus greater similarity to the thiophene-2-(5H)-one ring found in TLM. This generates the increased hydrophobicity needed to form hydrophobic interactions with the lipophilic patches that are present in the active site. Moreover, the sulphur atom was retained in the structure in order to provide hydrophobic interactions with the thiol group of the cysteine residue that was shown to interact with the CoA sulphur atom of the substrate. Structurally, the thiazole ring is a flat, five membered ring, which is similar to the flat thiophene-2-(5H)-one ring. The nitrogen atom in the thiazole ring at position 3 is included as a potential H-bond acceptor.



2-amino-1,3-thiazole | 1,3 thiazolidine | thiophene-2-(5H)-one

Figure 2.7: comparison of the 5-membered ring structures under discussion

Position 2 can remain unsubstituted as the free amine, or substituted as an amide derivative. The free amine is a H-bond donor that has the potential to interact with carbonyl groups in the active site, whereas the amide derivatives contain both acceptor (C=O) and donor (NH) groups and have the opportunity of forming peptide-like H-bonds with equivalent amino acids in the active site. The amides derivatized at position 2 were expected to occupy the longitudinal channel in the active site, which is narrow, hydrophobic, and accommodates the acyl chain of the natural substrate. As a result, the groups chosen were mainly long unsaturated hydrocarbon chains, with some exceptions selected to study the possibility of accommodating larger size groups to explore protein movement and flexibility.

The free carboxylic acid or the methyl ester at position 4 (4-carboxylates) is expected to be an important group in the structure through its potential to make specific interactions with His244 and/or Asn274 through electrostatic (as the acid) and/or H-bonding interactions. In order to maximize the possibility of the carboxylic acid group interacting with the active site residues, its placement at position 5 of the thiazole ring (5-carboxylates) was also investigated

Substituents chosen at position 5 of the 4-carboxylate derivatives were expected to occupy the lateral channel, which is larger and has more capacity to accommodate different functional groups that vary in size and hydrophobicity, with the same approach being applied to the 5-carboxylates.

2.3 The design of mtFabH inhibitors

2.3.1 Simple scaffolds

The design of a new lead against mtFabH was initiated by selecting a test library of eight simple compounds compatible with mtFabH binding site. These compounds were chosen to explore the space inside the active site following modification at the 2, 4 and 5 positions to fill the available space in the channels. Using the docking program GOLD, their expected binding was determined. Figures 2.8 and 2.9 show the postulated orientations adopted by compounds **(21)** and **(25)** respectively.

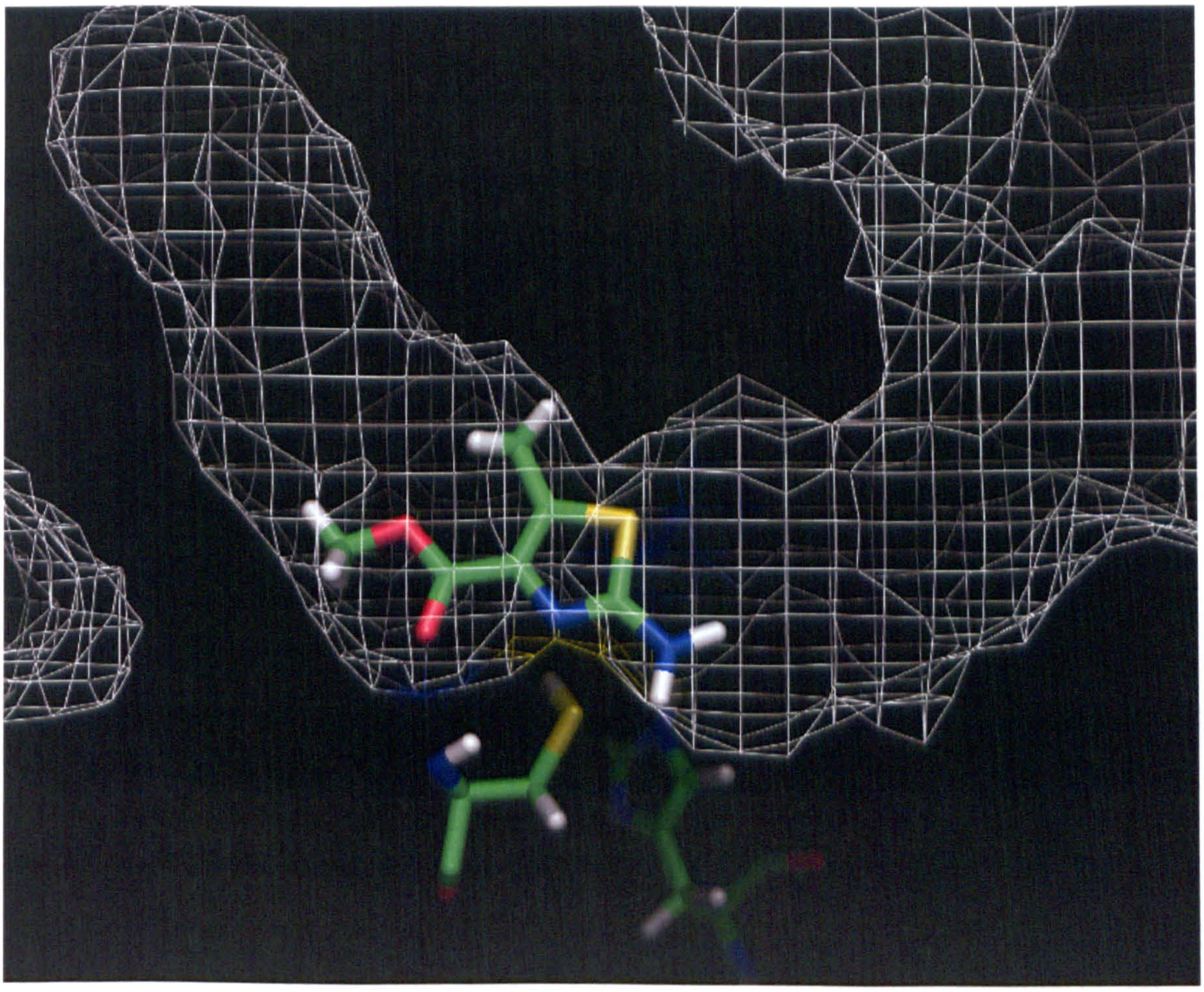


Figure 2.8: Docked methyl 2-amino-5-methylthiazole-4-carboxylate (21) in the active site of mtFabH.

The carbonyl group forms H-bonds with the NH of Cys112, whilst the NH_2 is proximal to the imidazole ring of His244 also forming H-bonds. The pose of compound **(25)** (5-carboxylate thiazole) suggests the orientation of the ethyl ester to the lipophilic longitudinal channel and positioning of the methyl group at a pocket near the base of that channel, while the NH_2 forms H-bonds with Asn274.

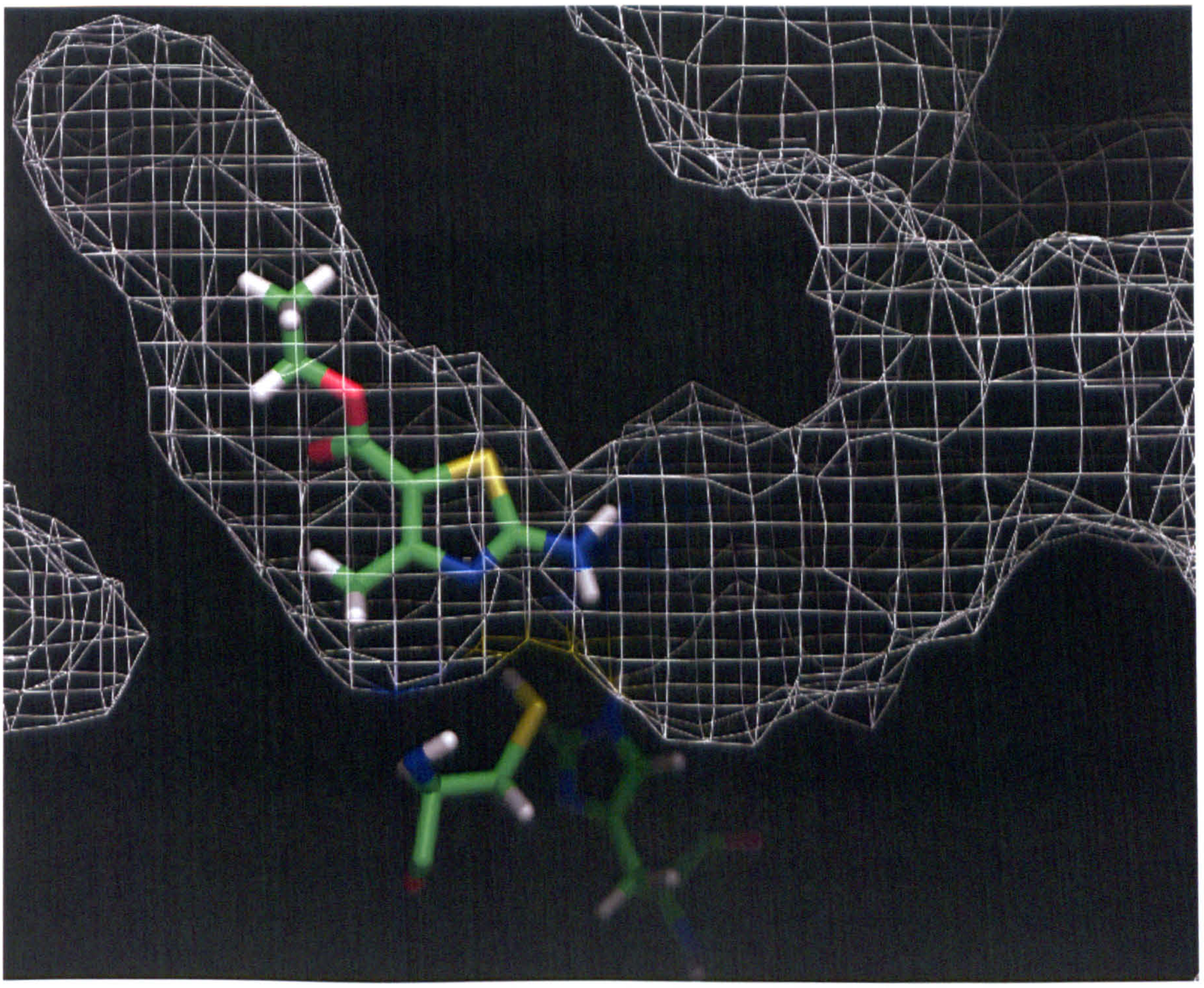


Figure 2.9: Docked ethyl 2-amino-4-methylthiazole-5-carboxylate (25) in the active site of mtFabH.

All compounds synthesized (the initial fragment library and libraries thereafter) were assessed in five systems:

(i) mtFabH: compounds were assayed by collaborators at the University of Birmingham using the procedure by Brown *et al.*[85] This is a complex, laborious and

time consuming procedure as it relies on a number of recombinant enzymes from Mtb, incorporated in a single assay and requires technical expertise primarily because of the preparation and purification time of substrates (AcpM, Acetyl-CoA) and the enzymes. It was difficult to conduct assay experiments with the flexibility required for use in an iterative design program during the time of the project.

(ii) FAS-I and FAS-II: the selectivity of the compounds shown to be active against mtFabH was carried out using a complex assay containing a preparation of FAS-I and FAS-II lysates according to the methods by Kremer *et al* and Brown *et al*.[60, 102]

(iii) *M. aurum*: Assays were performed *in-house* using the procedure described in section 3.3. MIC determination was through visual readout of assay plates, where viable bacteria in wells reduced the yellow tetrazolium redox dye to purple. An example of a plate used in these studies is shown in figure 2.10.

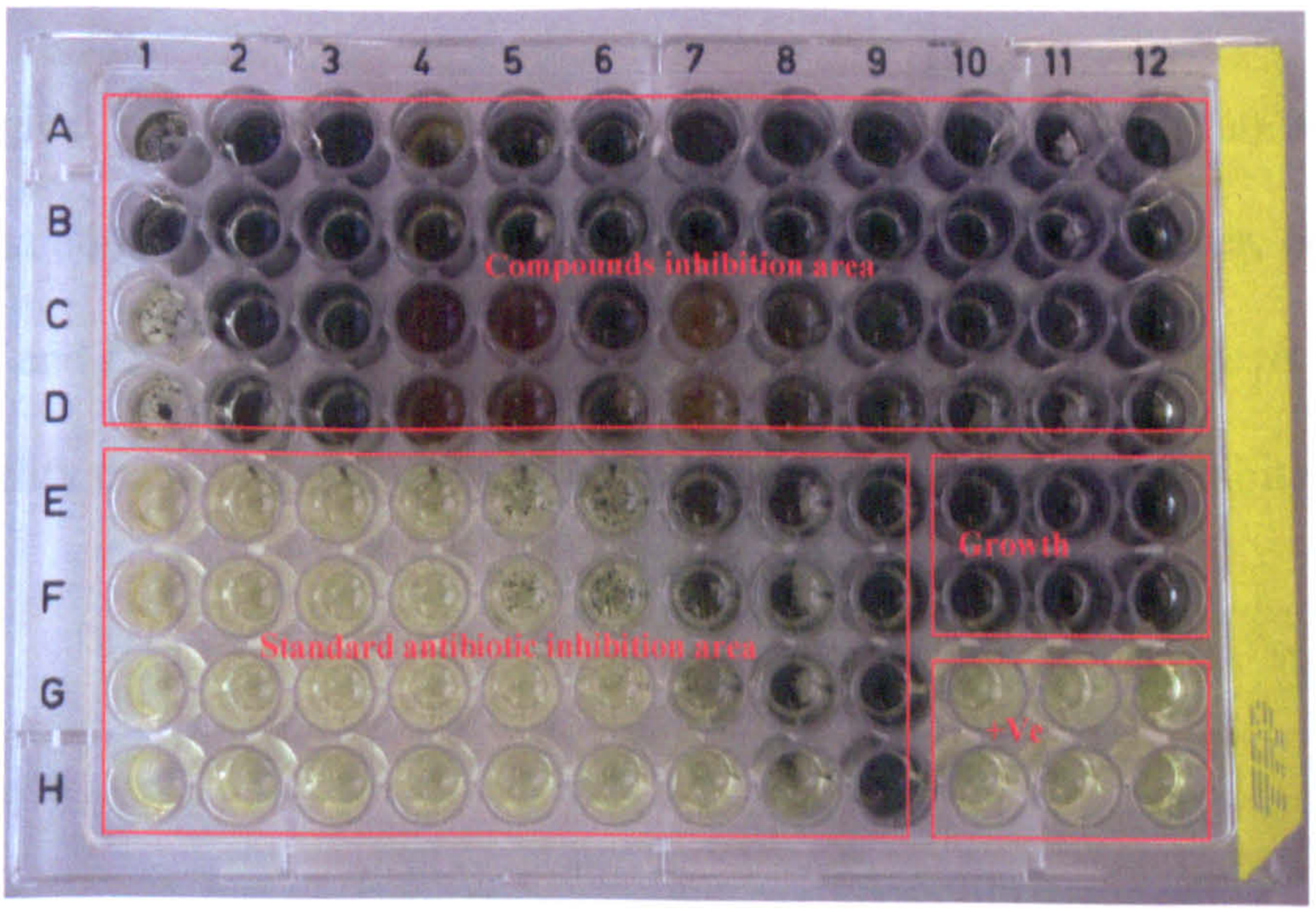


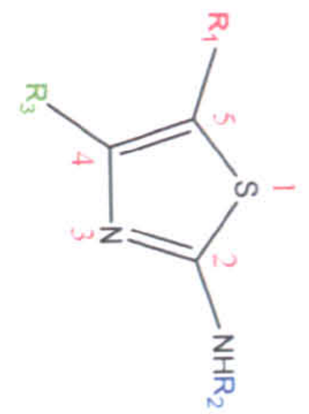
Figure 2.10: A 96 wells plate shows the how the MIC determination of the compounds performed.

(iv) *M. tuberculosis*: assays against this pathogen required a containment level 3 laboratory, which is not available at the University of Strathclyde. Collaborators at the Royal Free Hospital, University College London performed all the assays. In these assays all compounds were screened for their *in vitro* antimycobacterial activity against *M. tuberculosis* H37Rv by broth microdilution assay. Compounds that showed activity in this screening step were tested further by different methods. These were in brief, the agar proportion method, microdilution broth assay, resazurin microdilution assay, and the MP/ Bactec 3D assay.[103]

(v) Toxicity studies were performed in association with Strathclyde Innovation in Drug Research (SIDR). All compounds were tested against mammalian cells in order to investigate and evaluate their toxicity against the host using three normal cells lines, namely L929 (normal mouse fibroblast), H9C2 (cardiac mycoblasts), and HS27 (normal human fibroblast) at 100μM concentrations.[104]

The biological results shown in table 2.1 demonstrate that there is no activity for these initial compounds against either mtFabH or *M. aurum*. However, compounds **(21)**, **(22)**, and **(25)** showed activity against the whole cell *M. tuberculosis* indicating the possibility that a target, or targets, other than mtFabH are being inhibited.

Compound No.	R_1	R_2	R_3	Mtb (μM) MIC	*M. aurum* (μM) MIC	mtFabH (μM) IC_{50}
21	$-CH_3$	H	$-COOCH_3$	93.0	NA	NA
22	$-CH_3$	H	-COOH	0.35	NA	NA
23	$-CH_3$	$-COCH_3$	$-COOCH_3$	NA	NA	NA
24	$-CH_3$	$-COCH_3$	-COOH	NA	NA	NA
25	$-COOCH_2CH_3$	H	$-CH_3$	172.0	NA	NA
26	-COOH	H	$-CH_3$	NA	NA	NA
27	$-COOCH_2CH_3$	$-COCH_3$	$-CH_3$	NA	NA	NA
28	-COOH	$-COCH_3$	$-CH_3$	NA	NA	NA



NA: Not Active

Table 2.1: The structure and the biological data of the simple scaffold compounds. The values in the table are the average of three readings.

2.3.2 2-Bromoacetamido derivatives of the 4-carboxylates and carboxylic acids

Whilst activity against the whole cell for some of our fragments was extremely encouraging, the absence of activity against the target enzyme mtFabH meant finding a strategy to investigate whether mtFabH was a viable target of our thiazole scaffold. Small fragments often have low affinity for protein binding sites, and the complex assay was not sufficiently sensitive to record weak inhibitory activity. Consequently, we proposed to introduce a functionality that could covalently link with enzyme active site. The Cys112 sulphydryl group has the potential to be alkylated through nucleophilic substitution, and the bromoacetamido group in position 2 of the thiazole could potentially provide such a label. Different substituents at position 5 could be introduced ranging from a small methyl group to rigid and flexible bulky groups such as phenyl and benzyl (table 2.2). Bulky groups at position 5 promoted orientation in the active site of the bromoactamido substituent towards Cys112 according to our modeling studies.

In the active site of mtFabH, the docked compound **(35)** has its bromine atom pointing toward the longitudinal channel and proximal to the sulphur of Cys112 to enable nucleophilic attack, the carbonyl of the amide group of the ligand is stabilized by H-bonding with Asn274 and the benzyl group is adjacent to a hydropobic pocket near the entrance of the active site (figure 2.11).

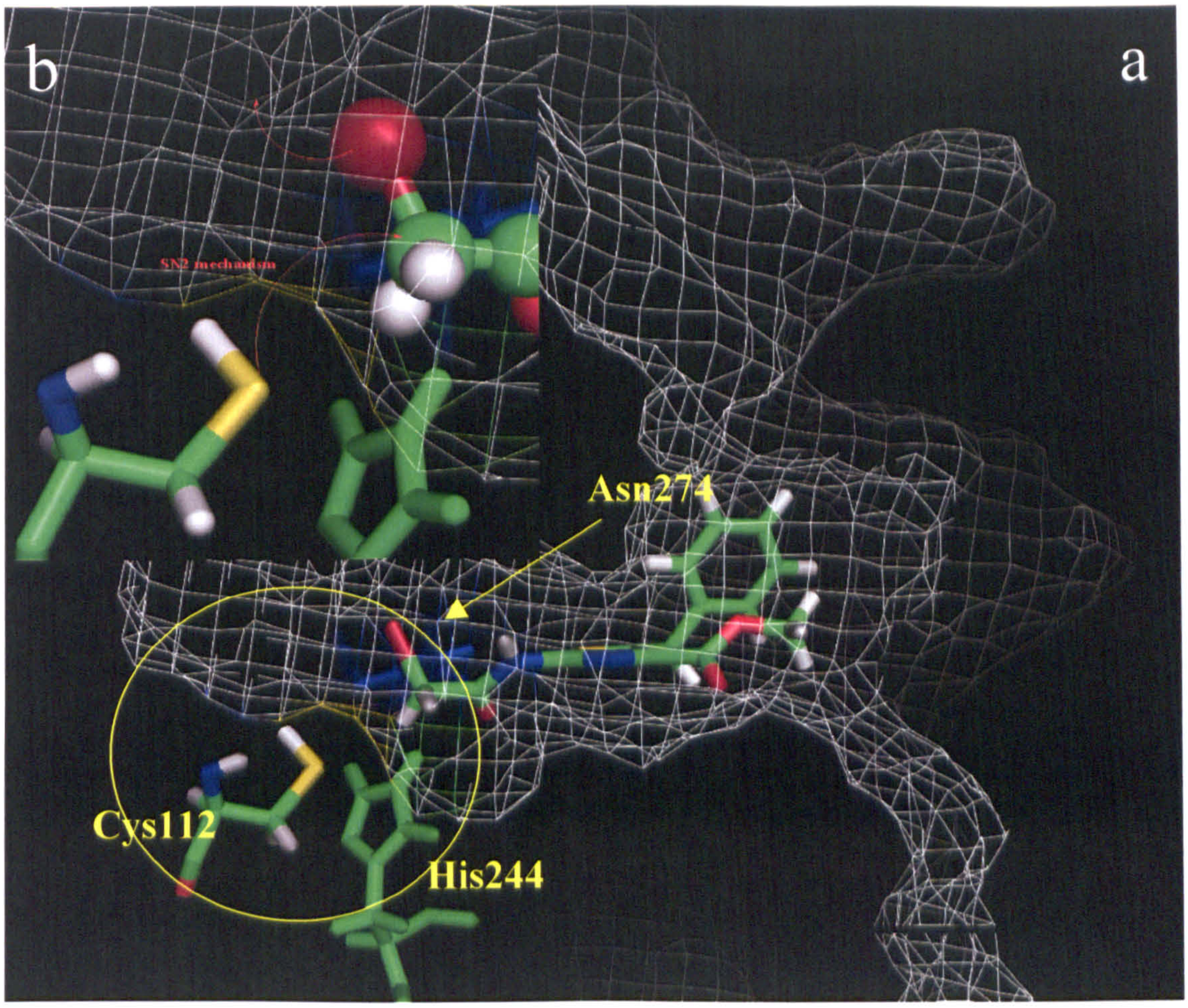


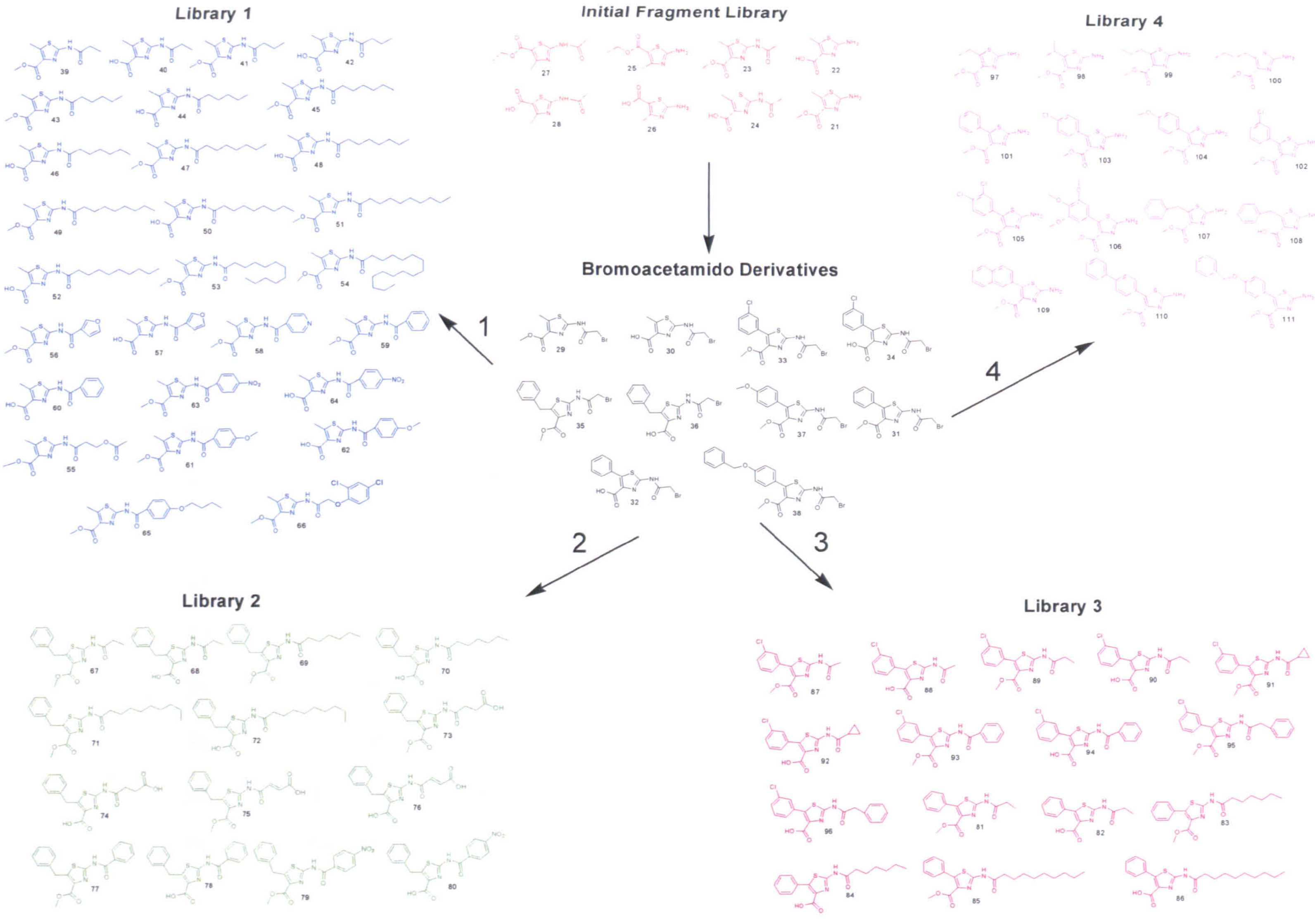
Figure 2.11: (a) Docked methyl 5-benzyl-2-(2-bromoacetamido)thiazole-4-carboxylate (35) in the active site of mtFabH. (b) Enlargement of the active site (yellow circle) to explain the proposed S_N2 mechanism between Cys112 and compound (35)

The bromoacetamido derived compounds proved successful inhibitors of *M. aurum* as methyl esters, whilst their corresponding free acids did not show any activity (table 2.2). This could be explained by the inability of these compounds to form a suitable interaction in the active site, or through their inability to penetrate the bacterium by having inappropriate physicochemical properties. Significantly, the methyl esters of these compounds showed very good activity against the enzyme mtFabH, although with reduced activity for some of the free acid counterparts (table 2.2). Unfortunately, all of the bromoacetamido compounds proved inactive when tested against *M. tuberculosis*. It is not clear whether this phenomenon is a result of

the technique used to assay the compounds, which may not favor the low hydrophobicity of the compounds, or whether these compounds have unfavorable structural properties retarding their penetration of the cell wall or activity whilst in the cell.

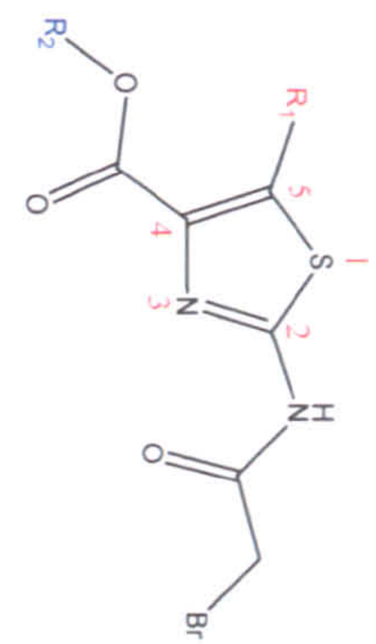
Nevertheless, the data obtained suggest that the bromoacetamido thiazole structure is a suitable scaffold for inhibitors of mtFabH. As the anticipated mechanism of these compounds may involve alkylation of the enzyme, and given that they are alkylating agents and therefore toxic, their selectivity is always going to be questionable. However, we were able to demonstrate that the bromoacetamido derivatives were selective for FAS-II over FAS-I at a concentration of 200μg/ml, which suggests that mammalian enzymes in fatty acid synthesis are unaffected. Unfortunately, preliminary studies on whole mammalian cells (L929, H9C2 and HS27 cell lines) revealed significant toxicity with these particular derivatives (see section 2.4).

Having established that our thiazole fragment was a viable inhibitor of mtFabH, in order to remove the potential for toxicity through alkylation, we prepared groups of libraries based on the adequate replacement of the bromoacetamido group, accurate fulfilment of the active site geometry, and consideration of the data received from the docking studies. The libraries and their inter-relationships are summarized in Scheme 1.



Scheme1: Summary of the libraries and their inter-relationships.

Compound No.	R_1	R_2	FAS-II selectivity	Mtb (μM) MIC	*M. aurum* (μM) MIC	mtFabH (μM) IC_{50}
29	$-CH_3$	$-CH_3$	NT	NA	426.0	NA
30	$-CH_3$	H	NT	NA	NA	NA
31	-Ph	$-CH_3$	Yes	NA	22.5	2.8
32	-Ph	H	NT	NA	NA	NA
33	*m*-chloro-Ph-	$-CH_3$	Yes	NA	20.5	2.4
34	*m*-chloro-Ph-	H	NT	NA	NA	50.0
35	$-CH_2Ph$	$-CH_3$	Yes	NA	43.0	160.0
36	$-CH_2Ph$	H	NT	NA	NA	155.0
37	*p*-OCH_3Ph-	$-CH_3$	Yes	NA	41.5	2.7
38	$-PhOCH_2Ph$	$-CH_3$	Yes	NA	NA	2.2



NA: Not Active
NT: Not tested

Table 2.2: The structure and the biological data of the bormoacetamido derivatives. The values in the table are the average of three readings.

2.3.3 Library 1: 2-Amide derivatives of the 5-methyl 4-carboxylate esters and carboxylic acids

Our docking studies suggested that to promote occupation of the narrow longitudinal channel, long, flexible and hydrophobic alkyl chains that resembled the substrate should be included in position 2 *via* an amide link. The amide **(45)** forms H-bonds with the Cys112 backbone, whilst the 5-ester function H-bonds with Asn274 and the methyl group of the ligand is positioned in a small pocket on the base of the lateral channel with the thiazole sulphur positioned adjacent to Cys112 (figure 2.12).

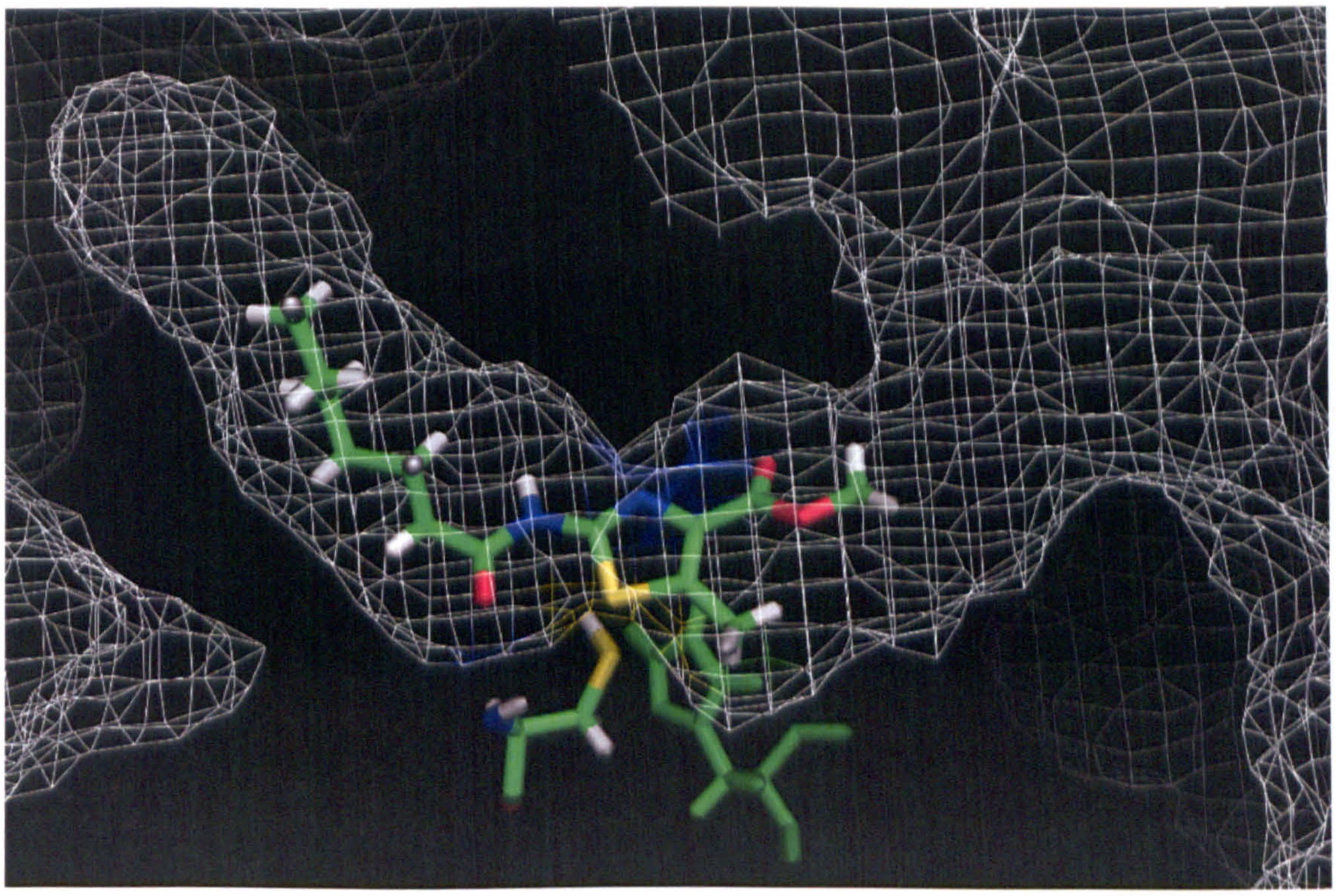


Figure 2.12: Docked methyl 2-heptanamido-5-methylthiazole-4-carboxylate (45) in the active site of mtFabH.

Bulky groups were substituted at position 2 to investigate the effect on orientation in the active site. For example, for **(58)** and **(66)** the bulky group attached at position 2 re-orientates the ligand so that this moiety occupies the lateral channel and pushes

the thiazole ring into the longitudinal channel. In this pose, the amide bond forms hydrogen bond interactions with the NH of Cys112, the methyl group at position 5 is forced in a pocket at the base of the longitudinal channel, whilst the sulphur atom of the thiazole ring is adjacent to sulphydril group of Cys112 (figures 2.13 and 2.14).

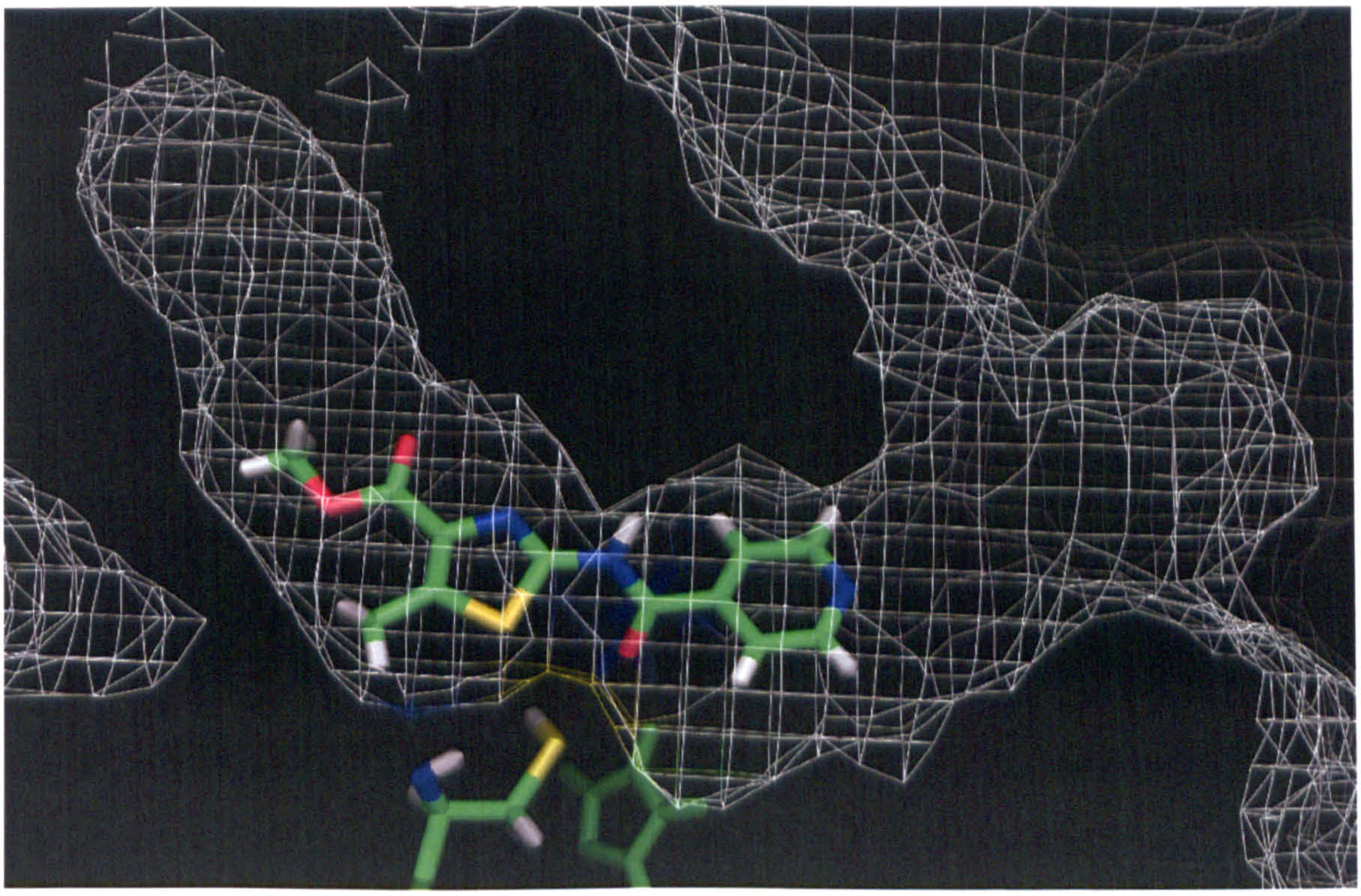


Figure 2.13: Docked methyl 2-(isonicotinamido)-5-methylthiazole-4-carboxylate (58) in the active site of mtFabH.

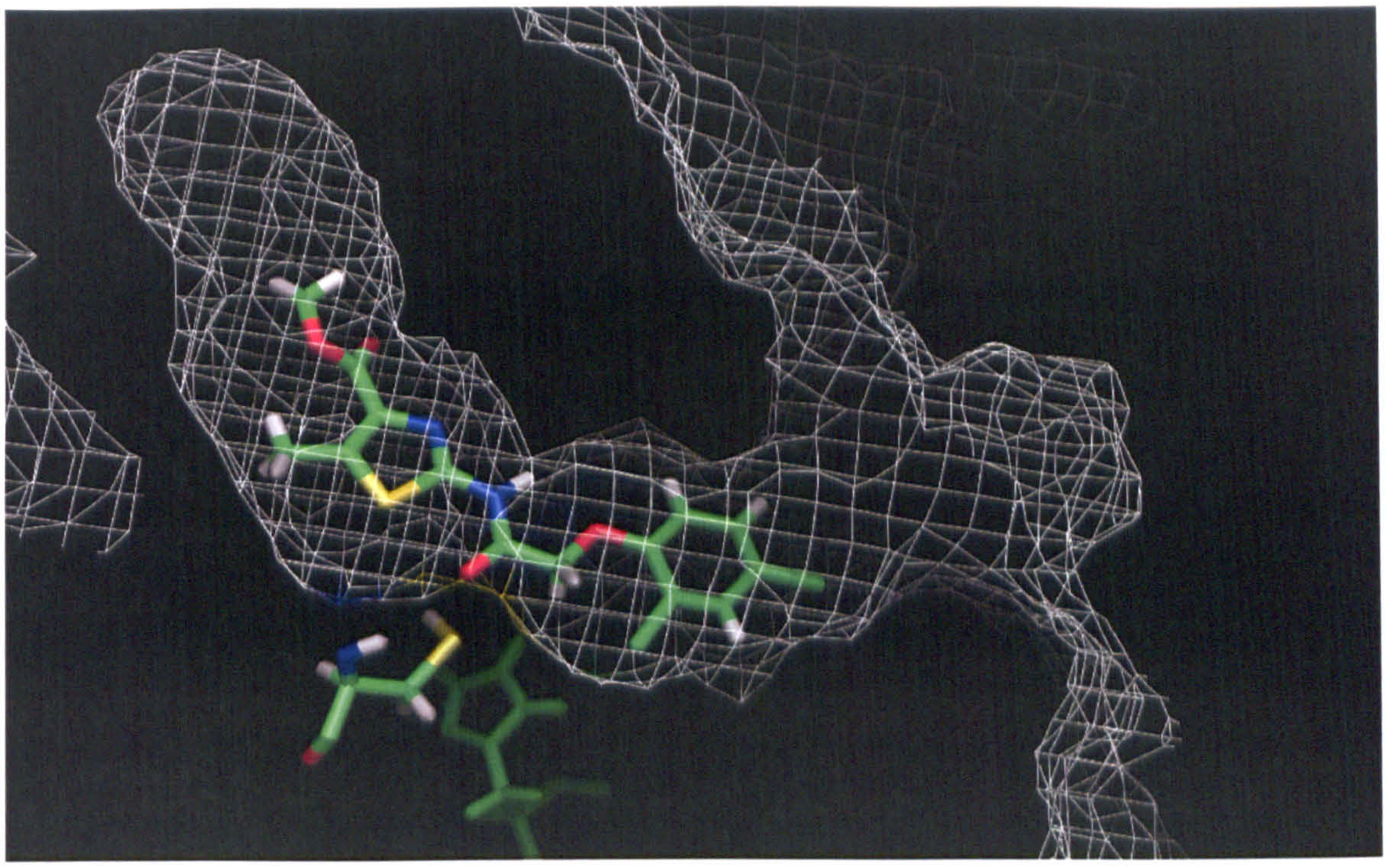


Figure 2.14: Docked methyl 2-(2-(2,4-dichlorophenyl)acetamido)-5-methylthiazole-4-carboxylate (66) in the active site of mtFabH.

A library of seventeen derivatives of **(21)** (5-methyl substituted) was synthesized, of which eleven were hydrolyzed to give the corresponding free acids. The groups chosen at position 2 ranged from small chain **(39)** to long chain **(51)** hydrocarbons, as well as various bulky and aromatic substituents such as **(58)** and **(61)**.

Analysis of the biological data for the compounds in table 2.3 revealed that the activity against *M. aurum* is restricted to the medium and long chain hydrocarbons, whereas none of the bulky or aromatic groups show any activity. Generally, the activity of the free acid derivatives compared to the esters of the hydrocarbon chain derivatized compounds increased against *M. aurum*. Compounds **(43)**, **(52)**, and **(45)** are the only compounds from the library to show activity against *M. tuberculosis*, where the last has an MIC of 0.21μm. Similarly none of the bulky or aromatic groups

show any activity. Unfortunately, only one compound has demonstrated weak activity against the enzyme mtFabH, which is the hydrolyzed form of **(39)** namely **(40)**, although no activity was expressed against the bacteria, which may be due to its inability to enter the cells. Compound **(40)** was docked inside the active site of mtFabH; in this pose, the carbonyl amide function forms a H-bond with the Cys112 backbone, whilst the methylacetamido derivative is oriented to the longitudinal channel and the carboxylic acid group experienced H-bonding from His244 backbone (figure 2.15).

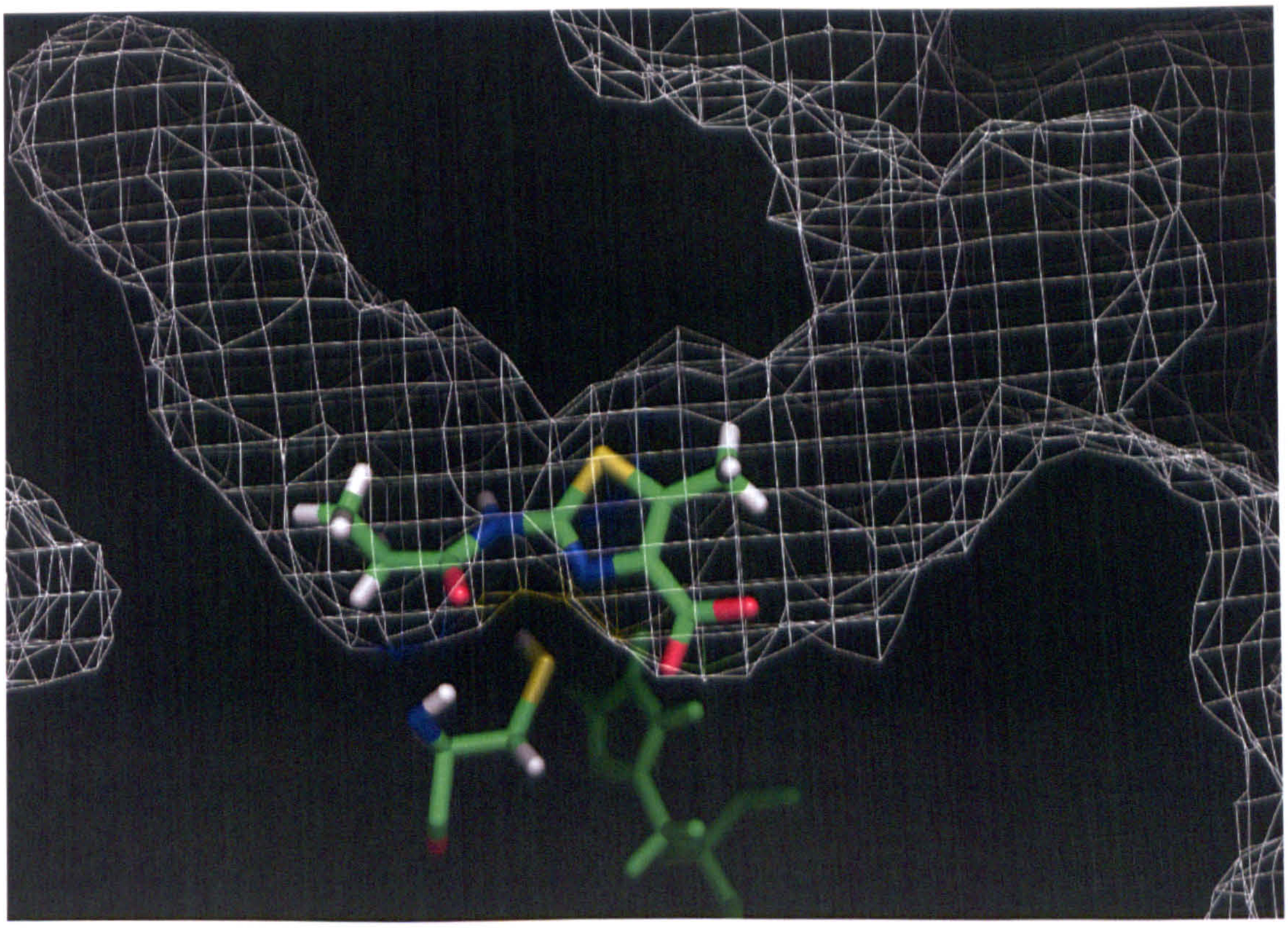
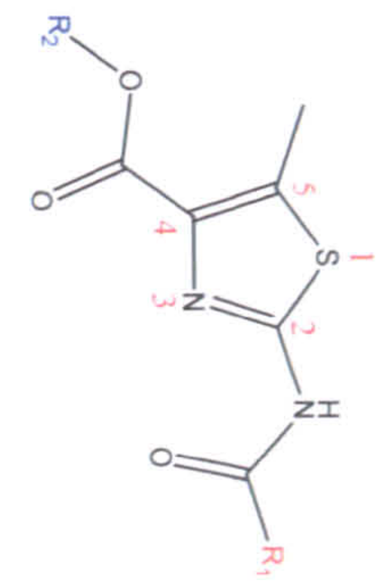


Figure 2.15: Docked 5-methyl-2-propionamidothiazole-4-carboxylic acid (40) in the active site of mtFabH.

Compound No.	R_1	R_2	Mtb (μM) MIC	*M. aurum* (μM) MIC	mtFabH (μM) IC_{50}
39	CH_3CH_2-	CH_3	NA	NA	NA
40	CH_3CH_2-	H	NA	NA	313
41	$CH_3CH_2CH_2$-	CH_3	NA	NA	NA
42	$CH_3CH_2CH_2$-	H	NA	NA	NA
43	$CH_3(CH_2)_3CH_2$-	CH_3	102	173	NA
44	$CH_3(CH_2)_3CH_2$-	H	NA	NA	NA
45	$CH_3(CH_2)_4CH_2$-	CH_3	0.21	113	NA
46	$CH_3(CH_2)_4CH_2$-	H	NA	56	NA
47	$CH_3(CH_2)_5CH_2$-	CH_3	NA	NA	NA
48	$CH_3(CH_2)_5CH_2$-	H	NA	113	NA
49	$CH_3(CH_2)_6CH_2$-	CH_3	NA	NA	NA
50	$CH_3(CH_2)_6CH_2$-	H	NA	27	NA
51	$CH_3(CH_2)_7CH_2$-	CH_3	NA	NA	NA
52	$CH_3(CH_2)_7CH_2$-	H	122	26	NA
53	$CH_3(CH_2)_9CH_2$-	CH_3	NA	NA	NA
54	$CH_3(CH_2)_{13}CH_2$-	CH_3	NA	NA	NA



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2.3.4 Library 2: 2-Amide derivatives of the 5-benzyl 4-carboxylate esters and carboxylic acids

A similar library was produced for the 5-benzyl thiazoles, using both small and large aliphatic and aromatic groups to generate diversity. Further substitutions were chosen to investigate the possibility of increasing the hydrophilic interactions inside the active site, such as alkyl groups with terminal carboxylic acid functionalities, for example compounds **(73)** and **(75)** (table 2.4). All seven compounds prepared were hydrolyzed to the free acids. The rationale for the synthesis of the 5-benzyl thiazoles can be illustrated for compounds **(71)**, **(75)**, and **(80)** when docked into the active site of mtFabH. Figure 2.16 shows compound **(71)** with the hydrocarbon chain oriented in the hydrophobic longitudinal channel, while the carbonyl of the amide group interacts by H-bonding with the NH of Cys112. The derivative with a terminal carboxyl group in the 2 substituent **(75)** is able to form a H-bond with the NH group of Cys112, and the carbonyl amide H-bonds with Asn274 NH_2 group (figure 2.17). The free acid derivative **(80)** shown in figure 2.18 suggests a reorientation with the group in the longitudinal channel, while the more bulky *p*-nitro ring locates in the lateral channel owing to its higher polarity. The free carboxylic acid at position 4 is anchored by H-bonding from the NH of the Cys112 backbone.

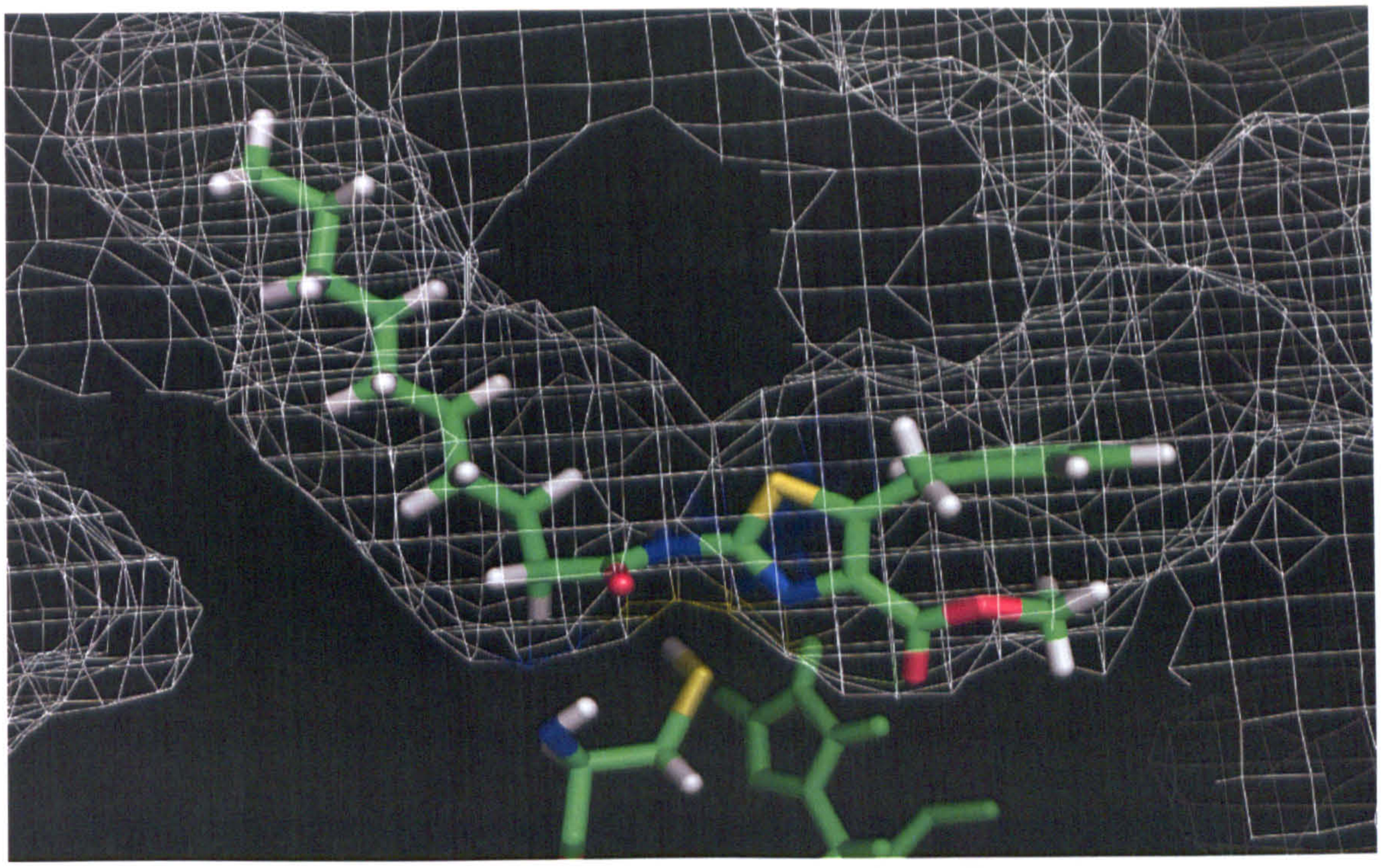


Figure 2.16: Docked methyl 5-benzyl-2-decanamidothiazole-4-carboxylate (71) in the active site of mtFabH.

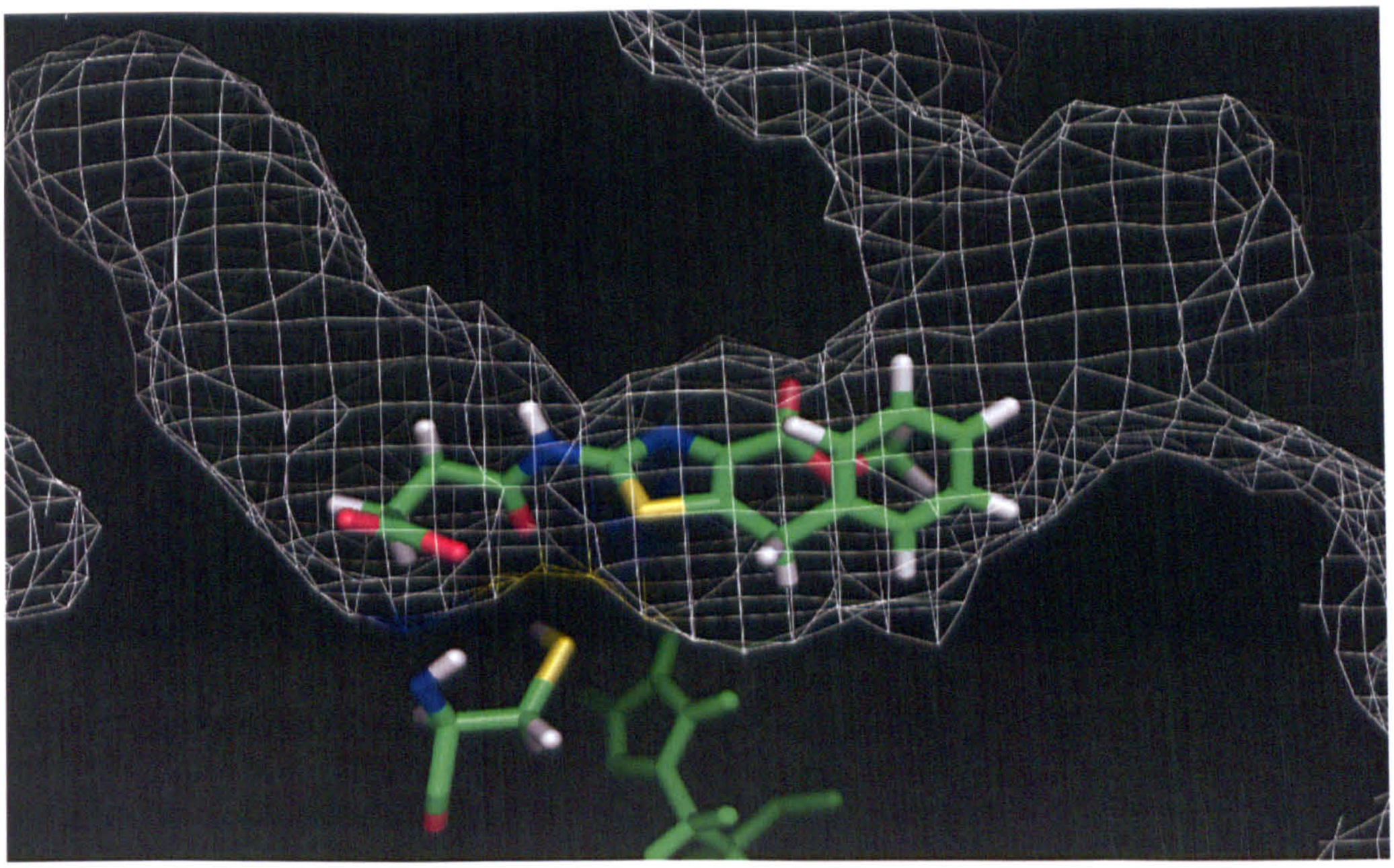


Figure 2.17: Docked 4-(5-benzyl-4-(methoxycarbonyl)thiazol-2-ylamino)-4-oxobutanoic acid (75) in the active site of mtFabH.

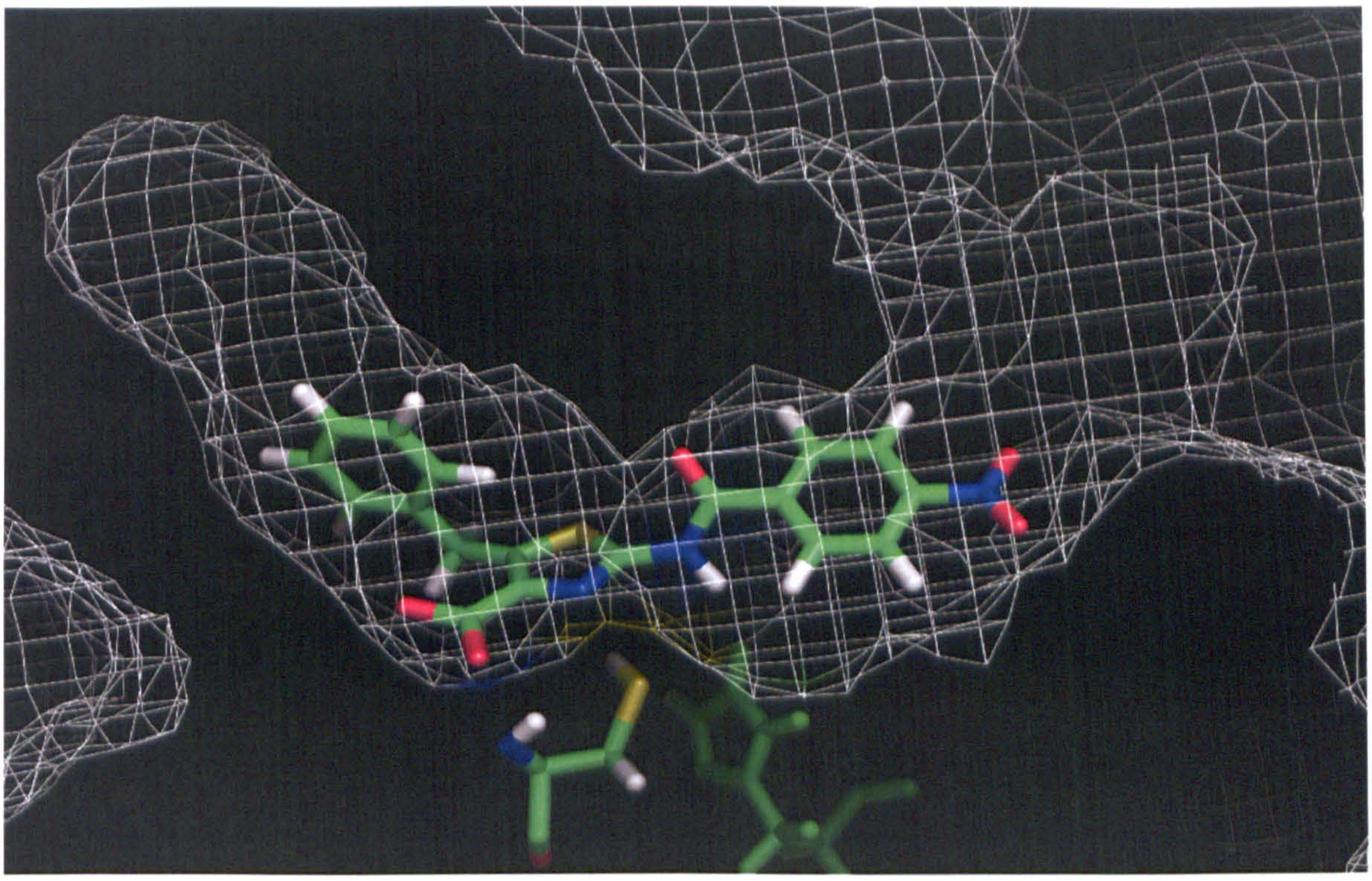


Figure 2.18: Docked 5-benzyl-2-(4-nitrobenzamido)thiazole-4-carboxylic acid (80) in the active site of mtFabH.

The results shown in table 2.4 support the trend seen for the 5-methyl thiazoles, namely only those compounds with medium to long hydrocarbon chains showed activity against the whole cell *M. aurum*. In both series, the free acids did not prove successful as mtFabH inhibitors despite possessing the designed functionalities to interact with the active site residues. The only notable exceptions were compounds **(40)** and **(76)**, which have short amide chains in position 2, although activity itself was poor. Some compounds that had bulky groups at position 2 showed good activity against the whole cell of *M. aurum* when they were hydrolysed, for example **(78)** and **(80)**, which contrasted with the 5 methyl thiazole series where the activity was absent when a bulky substituent was present in position 2 (but present in the long chain derivatives). Unexpectedly, none of the 5-benzyl derivatives expressed any activity against the whole cell *M. tuberculosis* in contrast with the 5-methyl derivatives, of

which some showed very good activity such as **(45)**. The only notable exception in the 5-benzyl library which showed activity against mtFabH was **(76)**. Its postulated binding in the active site is explained in figure 2.19, in which the hydrophilic terminal carboxylic acid is positioned near the active site to form H-bonds with His244, and the carbonyl amide of the ligand H-bonds with Asn274, whilst the bulky hydrophobic moiety (benzyl) prefers the longitudinal channel (lipophilic) despite its limited size.

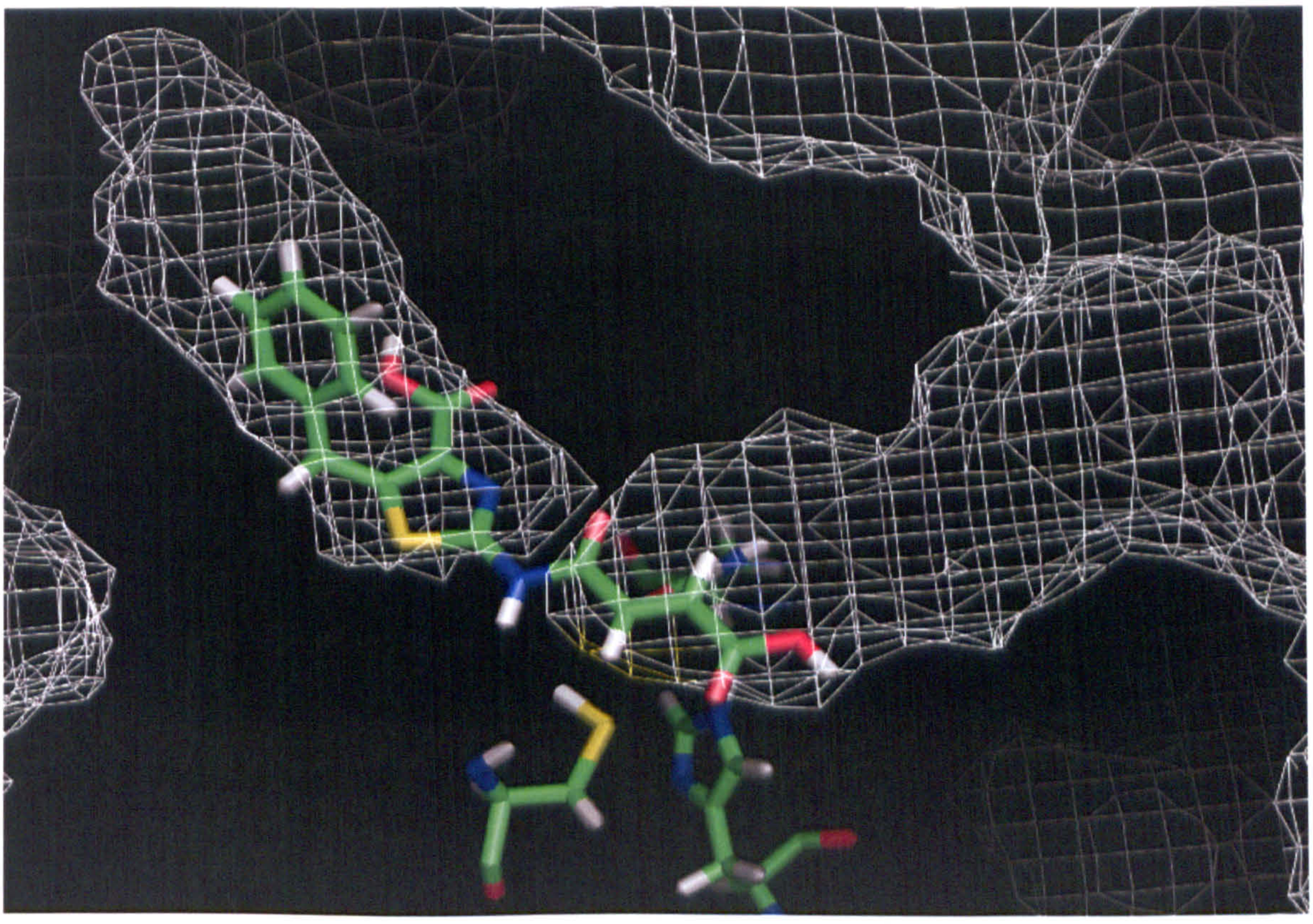
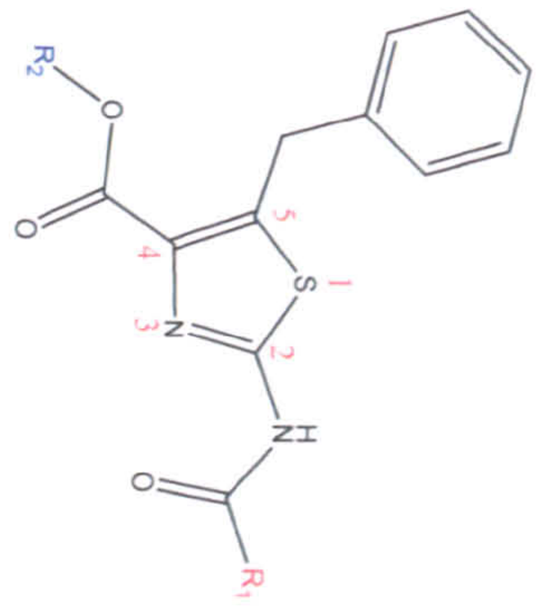


Figure 2.19: Docked 5-benzyl-2-(3-carboxyacrylamido)thiazole-4-carboxylic acid (76) in the active site of mtFabH.

Compound No.	R_1	R_2	Mtb(μM) MIC	*M. aurum* (μM) MIC	mtFabH (μM) IC_{50}
67	CH_3CH_2-	CH_3	NA	NA	NA
68	CH_3CH_2-	H	NA	NA	NA
69	$CH_3(CH_2)_4CH_2$-	CH_3	NA	355	NA
70	$CH_3(CH_2)_4CH_2$-	H	NA	31.2	NA
71	$CH_3(CH_2)_7CH_2$-	CH_3	NA	19.8	NA
72	$CH_3(CH_2)_7CH_2$-	H	NA	4.9	NA
73	$HOOCCH_2CH_2$-	CH_3	NA	NA	NA
74	$HOOCCH_2CH_2$-	H	NA	NA	NA
75	HOOCCHCH-	CH_3	NA	NA	NA
76	HOOCCHCH-	H	NA	NA	325
77	Ph-	CH_3	NA	NA	NA
78	Ph-	H	NA	62.5	NA
79	*p*-NO_2Ph-	CH_3	NA	NA	NA
80	*p*-NO_2Ph-	H	NA	62.5	NA



NA: Not Active

Table 2.4: 5- benzyl derivatives of the 4-carboxylate and carboxylic acids thiazole derivatives. The values in the table are the average of three readings.

2.3.5 Library 3: 2-Amide derivatives of the 5-phenyl and 5-*m*-chlorophenyl-4-carboxylate esters and carboxylic acids

A continuation of the strategy to replace the bromoacetamido group with groups of different sizes and shapes involved preparing a library of compounds with a variety of groups at position 2 with rigid bulky phenyl and *m*-chlorophenyl groups at position 5. The selection of such rigid bulky groups at the latter position was intended to expand chemical diversity over the earlier libraries that have small (methyl) and flexible bulky (benzyl) groups at position 5. Furthermore, our modeling studies showed that they could promote a hydrophobic interaction with the lateral channel hydrophobic patches. The rationale for the selection of *m*-chloro-phenyl as a candidate derivative at position 5 is explained in figure 2.20, where the chlorine atom of the *m*-chloro-phenyl group was identified through docking studies to be surrounded by hydrophobic side chains near the mouth of the active site. Amino acids such as Phe165, Trp42, Ile49, and Thr47 could also provide further stabilizing forces for the ligand to add interaction with the active site and also allow the possibility of increasing the selectivity of this ligand for mtFabH.

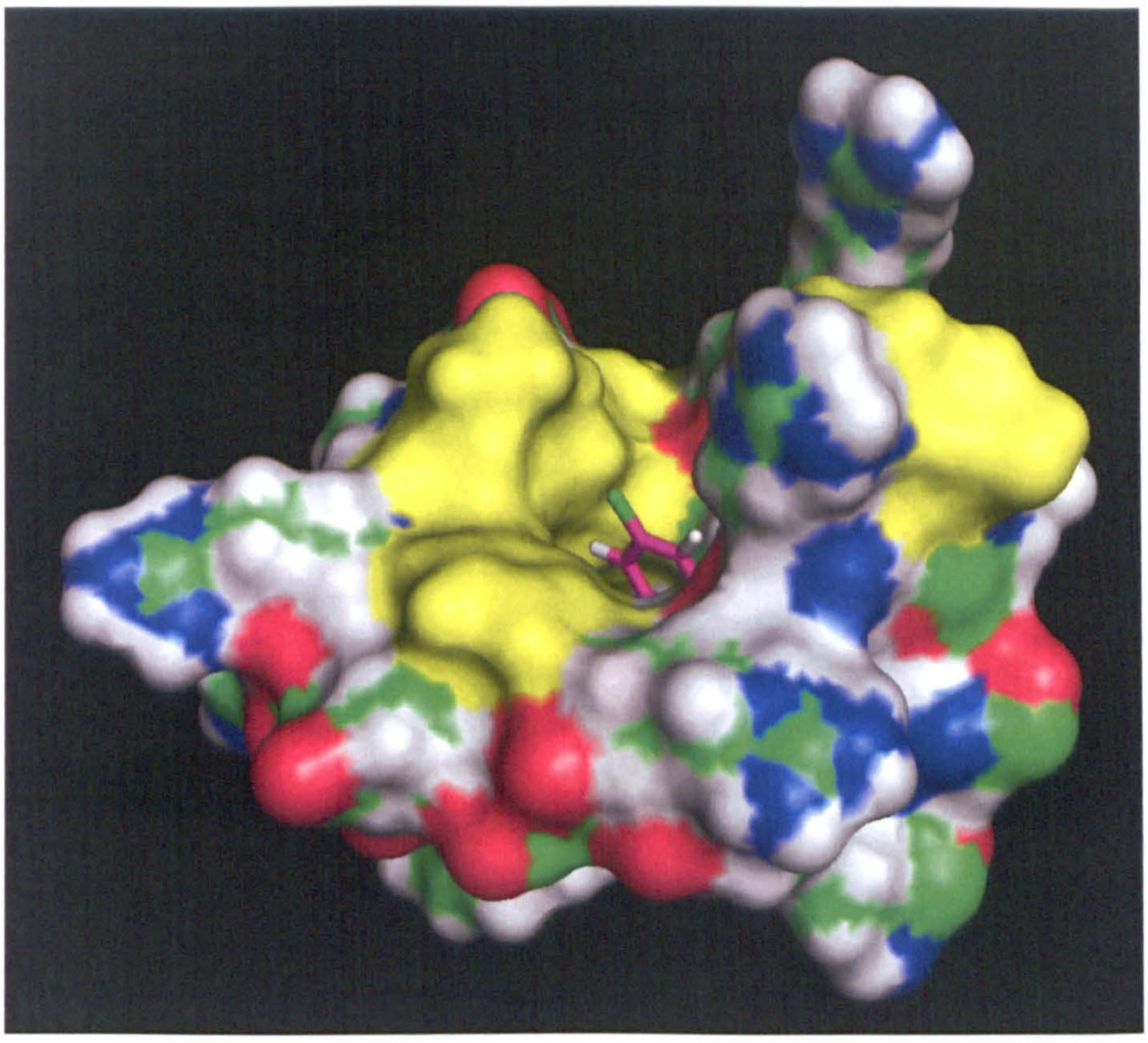


Figure 2.20: The hydrophobic areas around the mouth of the active site (yellow) where the *m*-chloro-phenyl residue expected to interact.

The lipophilic side chains at the entrance of the active site of mtFabH were given the yellow color, whilst the chlorine atom (green) is positioned proximal to the curvature-forming hydrophobic interactions (figure 2.20). This interaction can be seen in more detail in figure 2.21, where the surface of the protein has been removed, and shows the chlorine atom (green) of compound **(33)** completely surrounded by the hydrophobic patches (small balls).

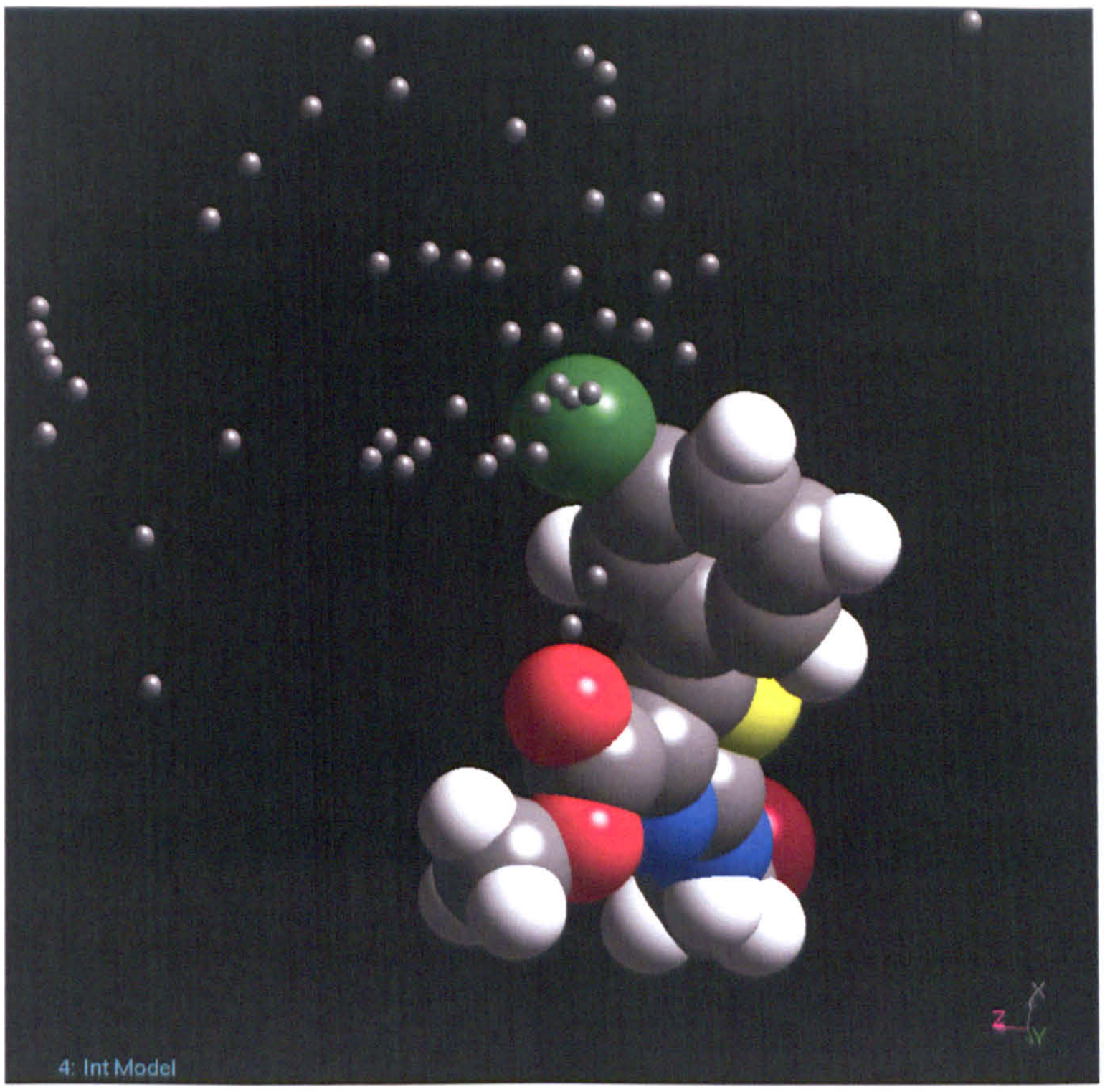
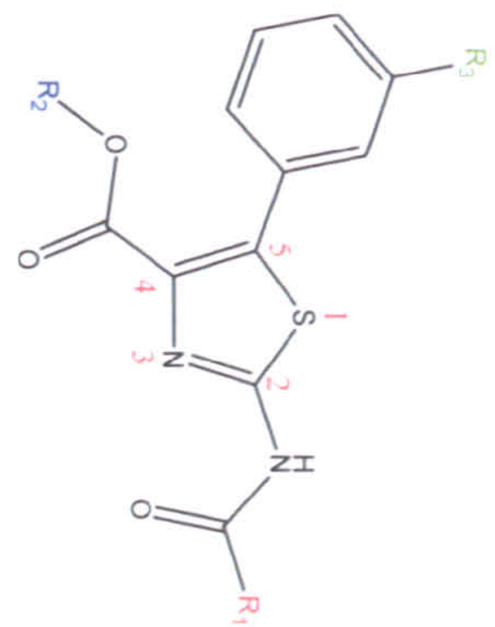


Figure 2.21: The hydrophobic patches around the chlorine atom (green) where the *m*-chlorophenyl residue expected to interact.

Surprisingly, and against our modeling expectations, none of the designed compounds that are *m*-chlorophenyl derivatives showed activity against the enzyme. Similarly, none of these compounds demonstrated any activity against the whole cell *M. aurum*, whilst only compound **(96)** showed moderate activity against the whole cell *M. tuberculosis* (table 2.5).

Both ester and free acid analogues of the 5-phenyl series with medium to long fatty chains at position 2 showed good activity against *M. aurum*. The improved activity of the esters could be attributed to their better ability to penetrate the waxy cell wall.

Compound No.	R_1	R_2	R_3	Mtb (μM) MIC	*M. aurum* (μM) MIC	mtFabH (μM) IC_{50}
81	CH_3CH_2-	CH_3	H	NA	NA	NA
82	CH_3CH_2-	H	H	NA	NA	NA
83	$CH_3(CH_2)_4CH_2$-	CH_3	H	NA	5.5	NA
84	$CH_3(CH_2)_4CH_2$-	H	H	NA	94	NA
85	$CH_3(CH_2)_7CH_2$-	CH_3	H	NA	2.5	NA
86	$CH_3(CH_2)_7CH_2$-	H	H	NA	10.4	NA
87	CH_3-	CH_3	Cl	NA	NA	NA
88	CH_3-	H	Cl	NA	NA	NA
89	CH_3CH_2-	CH_3	Cl	NA	NA	NA
90	CH_3CH_2-	H	Cl	NA	NA	NA
91	Cyclopropyl	CH_3	Cl	NA	NA	NA
92	Cyclopropyl	H	Cl	NA	NA	NA
93	Ph-	CH_3	Cl	NA	NA	NA
94	Ph-	H	Cl	NA	NA	NA
95	Ph CH_2-	CH_3	Cl	NA	NA	NA
96	Ph CH_2-	H	Cl	86	NA	NA



NA: Not Active

Table 2.5: 2-amide derivatives of the 5-phenyl and 5-m-chloro-4-carboxylates and carboxylic acid thiazole derivatives. The values in the table are the average of three readings.

2.3.6 Library 4: Methyl 2-amino-5-derivatives of the 4-carboxylates

Despite our original study demonstrating that the bromoacetamido derivatives inhibited mtFabH, our first three libraries yielded no further inhibitors of the target enzyme. We had achieved moderate activity in the whole cell assays, which was encouraging, but optimizing mtFabH inhibition remained elusive. Our final library sought to investigate whether a free 2-amino group was a pre-requisite for activity.

In this final library, the 2-amino group was retained as the free amine, the 4-carboxylate as the protected methyl ester, and position 5 varied in size and shape. For example, small groups at position 5 such as methyl, ethyl, propyl and isopropyl, medium size groups such as pentyl, phenyl, and benzyl and larger groups such as the biphenyls and substituted phenyls were all introduced to investigate the spatial and physicochemical limits on activity at this position.

The fifteen compounds that were synthesized are listed in table 2.6. Docking studies on compounds with small substituents at position 5, exemplified by the *n*-propyl derivative **(99)**, suggest that they would orient their side chains in the narrow longitudinal channel. The carbonyl group of the ester is predicted to H-bond with the NH backbone of Cys112, whilst the free NH_2 group interacts with the imidazole group of His244 (figure 2.22)

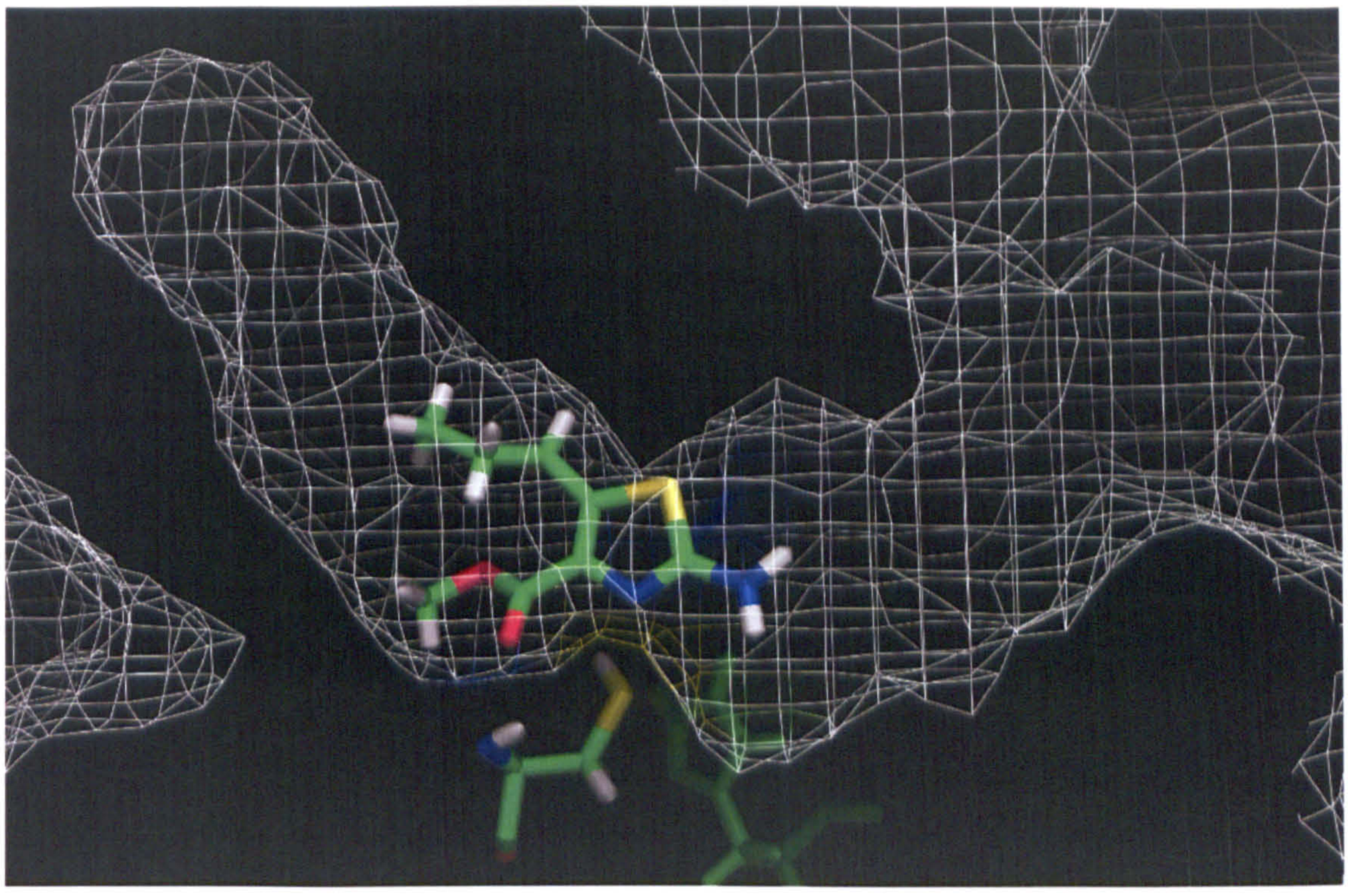


Figure 2.22: Docked methyl 2-amino-5-propylthiazole-4-carboxylate (99) in the active site of mtFabH.

Medium size groups at position 5 such as **(107)** are predicted to reorientate the ligands; the NH_2 group occupies the longitudinal channel to form H-bonds with the NH of Cys112, whilst the benzyl group prefers the wider lateral channel that is able to accommodate the larger benzyl group (figure 2.23).

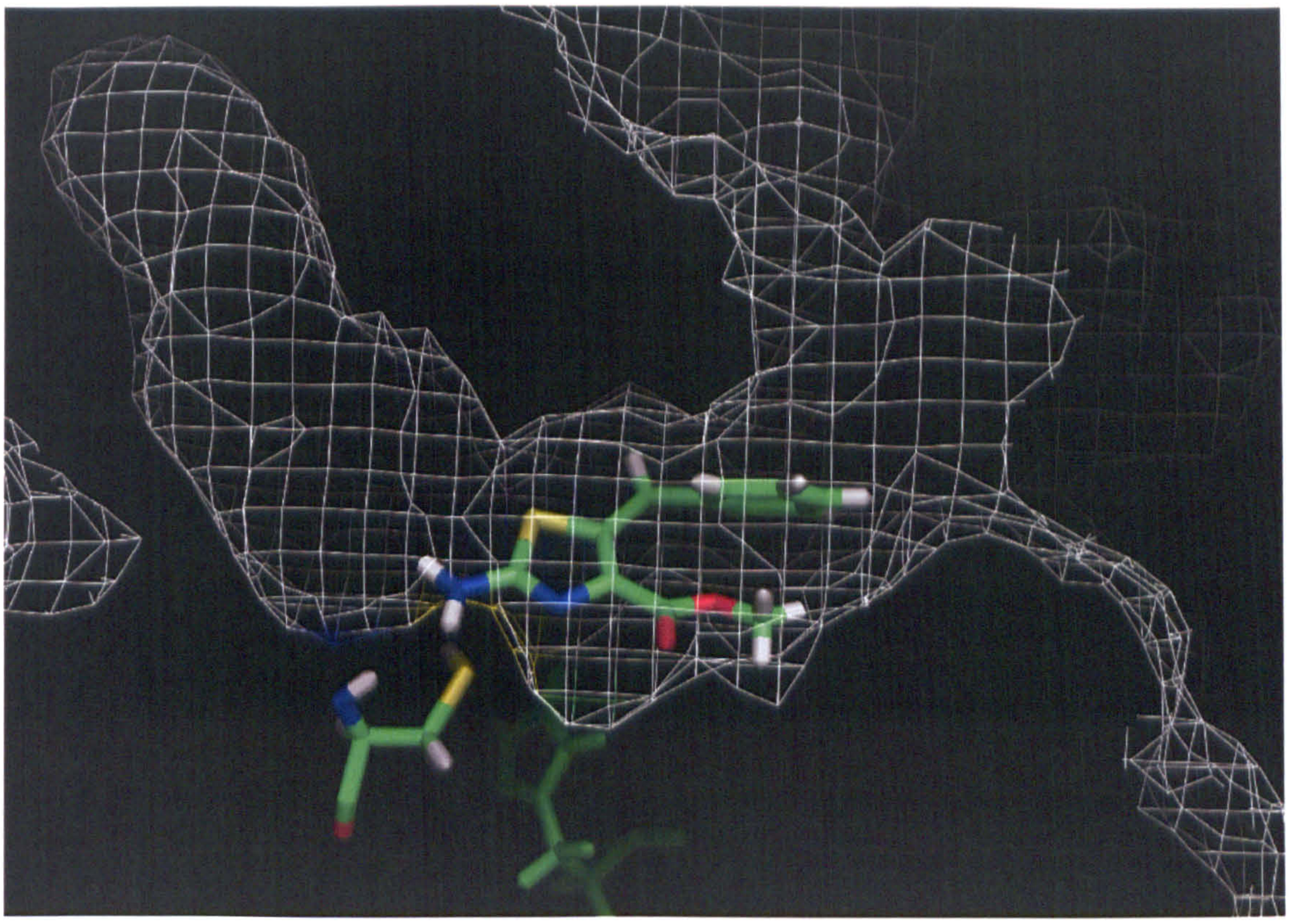


Figure 2.23: Docked methyl 2-amino-5-benzylthiazole-4-carboxylate (107) in the active site of mtFabH.

Larger groups at position 5, such as phenoxy benzyl emphasize this reorientation according to our modelling studies; large groups occupy the lateral channel and pushing the free amine away from the active site toward the longitudinal channel. Figure 2.24 views the postulated orientation adopted by **(111)**; the bulky phenoxy benzyl is in the lateral channel, whilst the thiazole ring occupies the longitudinal channel fortified by H-bonding between the carbonyl ester and the NH group of the cysteine backbone.

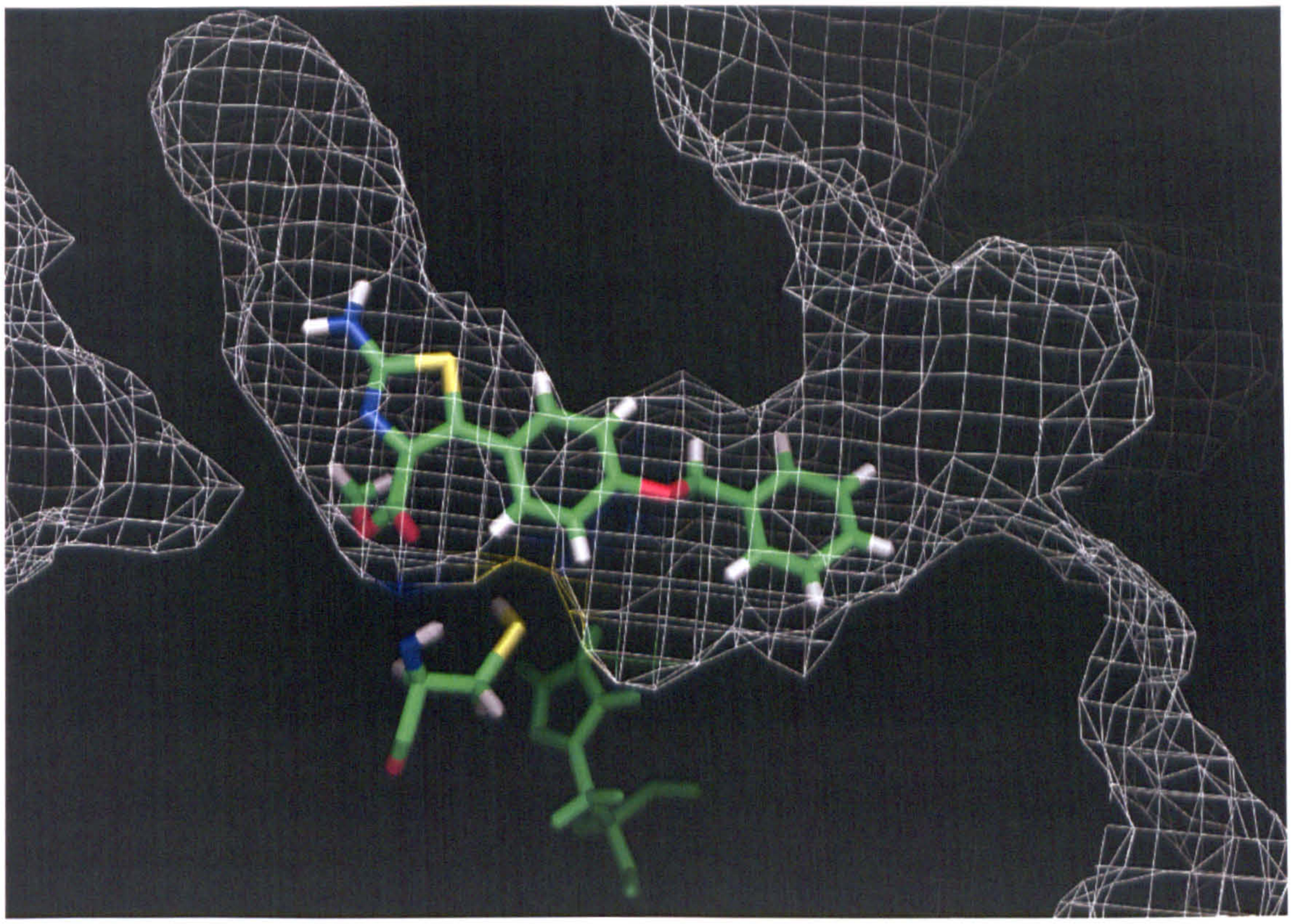
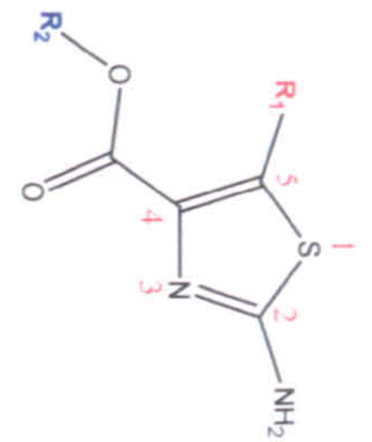


Figure 2.24: Docked methyl 2-amino-5-(4-(benzyloxy)phenyl)thiazole-4-carboxylate (111) in the active site of mtFabH.

The biological results shown in table 2 demonstrate that there is no activity for the free amines against the enzyme mtFabH, although some compounds showed activity against the whole organism, and suggests that activity is expressed through targets other than mtFabH. Compounds **(97)** and **(99)** exhibited moderate activity against *M. tuberculosis*, and **(107)** showed very good activity against the same pathogen. The latter was selected for hydrolysis to investigate whether its corresponding free acid form was more active in the assays.

Surprisingly **(108)** showed no activity against all the targets compared with **(107)**, and is probably related to its inability to access its target.

Compound No.	R_1	R_2	Mtb (μM) MIC	*M. aurum* (μM) MIC	mtFabH (μM) IC_{50}
97	CH_3CH_2-	CH_3	172	NA	NA
98	$(CH_3)_2CH$-	CH_3	NA	NA	NA
99	$CH_3CH_2CH_2$-	CH_3	160	NA	NA
100	$CH_3CHCH_2CH_2$-	CH_3	NA	NA	NA
101	Ph-	CH_3	NA	NA	NA
102	*m*-chloro-Ph-	CH_3	NA	NA	NA
103	*p*-chloro-Ph-	CH_3	NA	NA	NA
104	*p*-OCH_3-Ph-	CH_3	NA	NA	NA
105	*m,p*-dichloro-Ph-	CH_3	NA	NA	NA
106	*m,p,m*-$(OCH_3)_3$-Ph-	CH_3	NA	NA	NA
107	$PhCH_2$-	CH_3	0.24	NA	NA
108	$PhCH_2$-	H	NA	NA	NA
109	Naphthalene-	CH_3	NA	NA	NA
110	Biphenyl-	CH_3	NA	NA	NA
111	$PhCH_2OPh$-	CH_3	NA	NA	NA

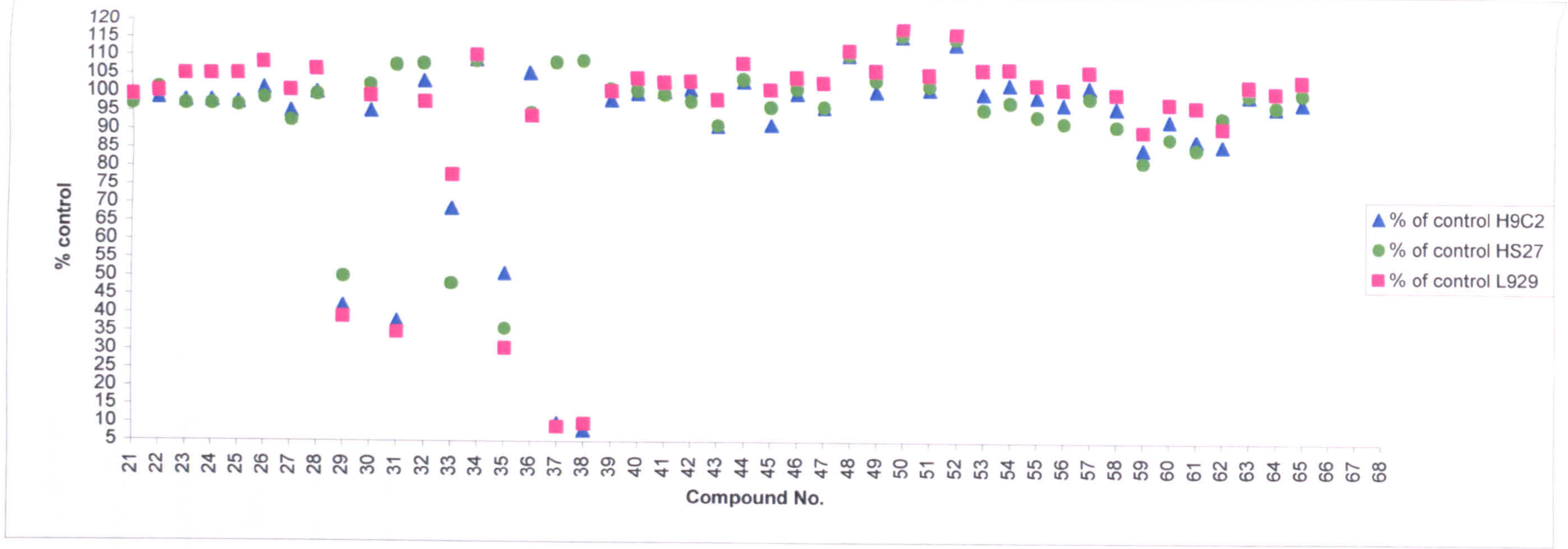


NA: Not Active

Table 2.6: The structure and biological data for the free amines thiazole derivatives. The values in the table are the average of three readings.

2.4 Mammalian cell toxicity study

The data from these studies (figure 2.25) indicate that most of the synthesized compounds are not cytotoxic against normal mouse fibroblast, cardiac myoblasts and normal human fibroblast cell lines except the esters of the bromoacetamido derivatives such as **(31), (35), (37)** and **(38)**. These compounds could be involved in non-specific alkylation reactions within normal cells (e.g. DNA alkylation), which would exclude them being candidates for lead compounds against TB. Perhaps significantly, the free acid derivatives of the bromoacetamido compounds **(30, 32, 34, 36)** are also non-toxic, which may suggest that the ester is a requirement for cellular penetration and masks the polar free acid in a pro-drug capacity.



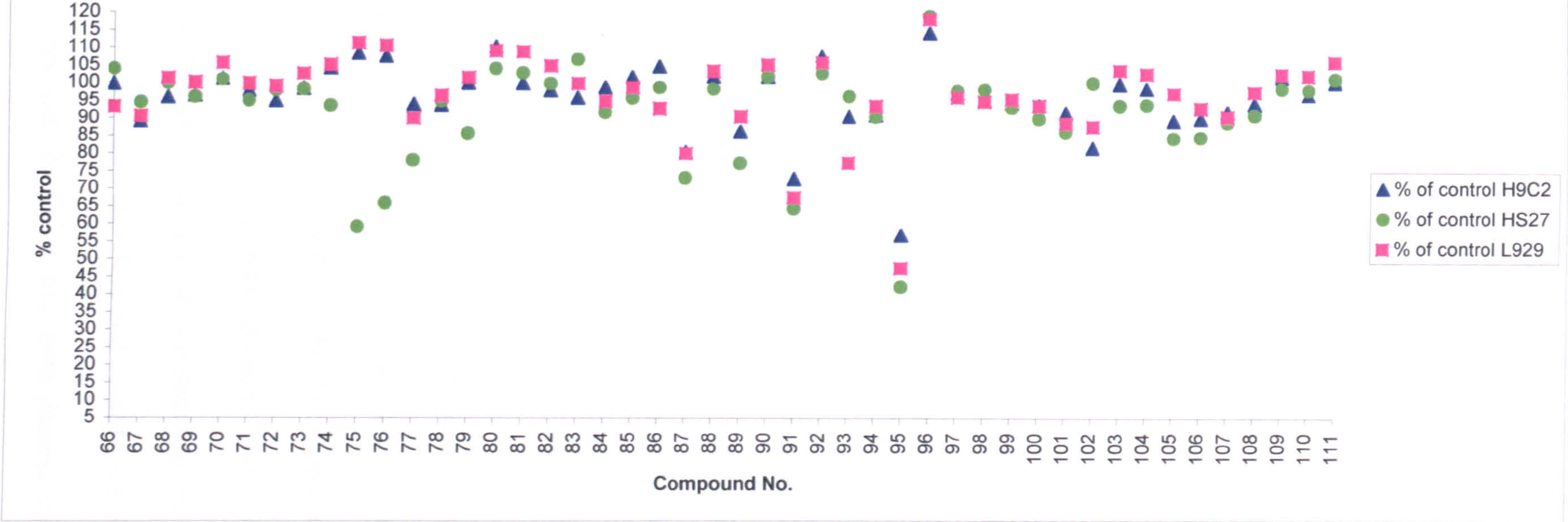


Figure 2.25: Cytotoxicolgical results against host cells L929, H9C2, and HS27

2.5 Summary of SAR

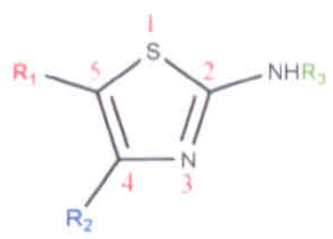
Unfortunately nearly all the compounds from our libraries did not show activity against mtFabH. However, the data obtained from the bromoactamido-derived compounds have provided us with an insight into the pattern of binding. A noticeable trend for the activity against mtFabH by the bromoacetamido compounds is that the ester compounds are much more active than their corresponding free acids, which could be explained by the presence of a specific hydrophobic interaction in the active site that is complementary with the methoxy group.

Although diverse libraries were synthesized to substitute the bromoacetamido derivatives at positions 2 and 5, only two compounds showed very weak activity against the mtFabH. Against the trend observed for the bromoacetamido compounds where the ester form is more active, compound **(40)** is a free acid with small groups at position 2 and 5. Furthermore, compound **(76)**, which has a relatively rigid side chain with terminal carboxylic acid at position 2, also contradicts our prediction of a flexible hydrophobic chain in this position to occupy the longitudinal channel.

Overall, this lack of inhibition could be explained by the location of the active site; it is deeply immersed inside the protein and only accessible through the lateral channel of approximately 20Å long and 5Å wide, which could be thermodynamically unfavourable for flexible and large inhibitor binding. Any enthalpy gain from inhibitor binding to the channel and active site may not be compensated by its entropy loss, which is supported by the presence of the CoA and AcpM co-factor shuttling units that orientate the substrates into the active site; both possess well-

established protein-protein binding sites at the entrance of the channel to augment the enthalpic contribution to the process.

Whilst activity in the whole cell assays is more encouraging, discovering actual structure-activity relationships is difficult. This is particularly apparent for *M. tuberculosis*. The small, low molecular weight and polar free amines such as compounds **(21, 22, 25, 97, 99, 107)** that showed good or moderate anti Mtb activity against target sites other than mtFabH, could penetrate the cell wall *via* the porins that are used by the bacteria for utilizing small polar molecules.[105, 106] Conversely, the larger active molecules possessing straight C_5-C_9 chains **(43,45,52)** also do not inhibit mtFabH, but may penetrate the fatty cell wall through their high lipophilicity. This may account for the activity of **(96)**, which whilst having a polar free acid group, has two lipophilic substituents in positions 2 and 5 (table 2.7).



Compound No.	R_1	R_2	R_3	Mtb (μM) MIC
21	$-CH_3$	$-COOCH_3$	H	**93.0**
22	$-CH_3$	-COOH	H	**0.35**
25	$-COCH_2CH_3$	$-CH_3$	H	**172**
43	$-CH_3$	$-COOCH_3$	$-COCH_2(CH_2)_3CH_3$	**102**
45	$-CH_3$	$-COOCH_3$	$-COCH_2(CH_2)_4CH_3$	**0.21**
52	$-CH_3$	-COOH	$-COCH_2(CH_2)_7CH_3$	**122**
96	*m*-chloro-Ph-	-COOH	$-COCH_2$-Ph	**86**
97	$-CH_2CH_3$	$-COOCH_3$	H	**172**
99	$-CH_2CH_2CH_3$	$-COOCH_3$	H	**160**
107	$-CH_2Ph$	$-COOCH_3$	H	**0.24**

Table 2.7: The structure and the activity of the compounds active against Mtb.

In the case of *M. aurum*, we found that all compounds with short alkyl chains (C_2) at position 2 were inactive regardless of the substituent at position 5. Conversely, all compounds with medium alkyl chains at position 2 (C_6) showed good activity, the highest for the ester form of the 5-phenyl derivative **(85)** and the lowest for the 5-benzyl derivative **(69)**. A similar trend was shown for the long alkyl chains (C_9); all showed activity with the exception of the ester form of the 5-methyl derivative, and the highest activity was demonstrated by the 5-phenyl ester. In general, for the medium and the long alkyl chain forms of both the 5-methyl and 5-benzyl derivatives, the free acids were more active than their ester counterparts, whilst the

situation is reversed for the 5-phenyl derivatives. Bulky groups at position 2 for the 5-methyl derivatives did not show any activity against *M. aurum*, whilst the 2-phenyl and 2-*p*-nitro phenyl of the 5-benzyl and the 2-benzyl of the 5-*m*-chlorophenyl analogous shown activity against the bacteria. In contrast to what was seen for the free 2-amino compounds, where many had showed activity against Mtb, none expressed any activity against *M. aurum* and indicates the need for care when extrapolating activity from one species to another (table 2.8).

The crucial role of lipophilicity for activity against whole cell bacteria (as demonstrated for mammalian cell toxicity) is exemplified by the bromoacetamido series; antibacterial activity against *M. aurum* is present for the esters, but not for the free acid counterparts.

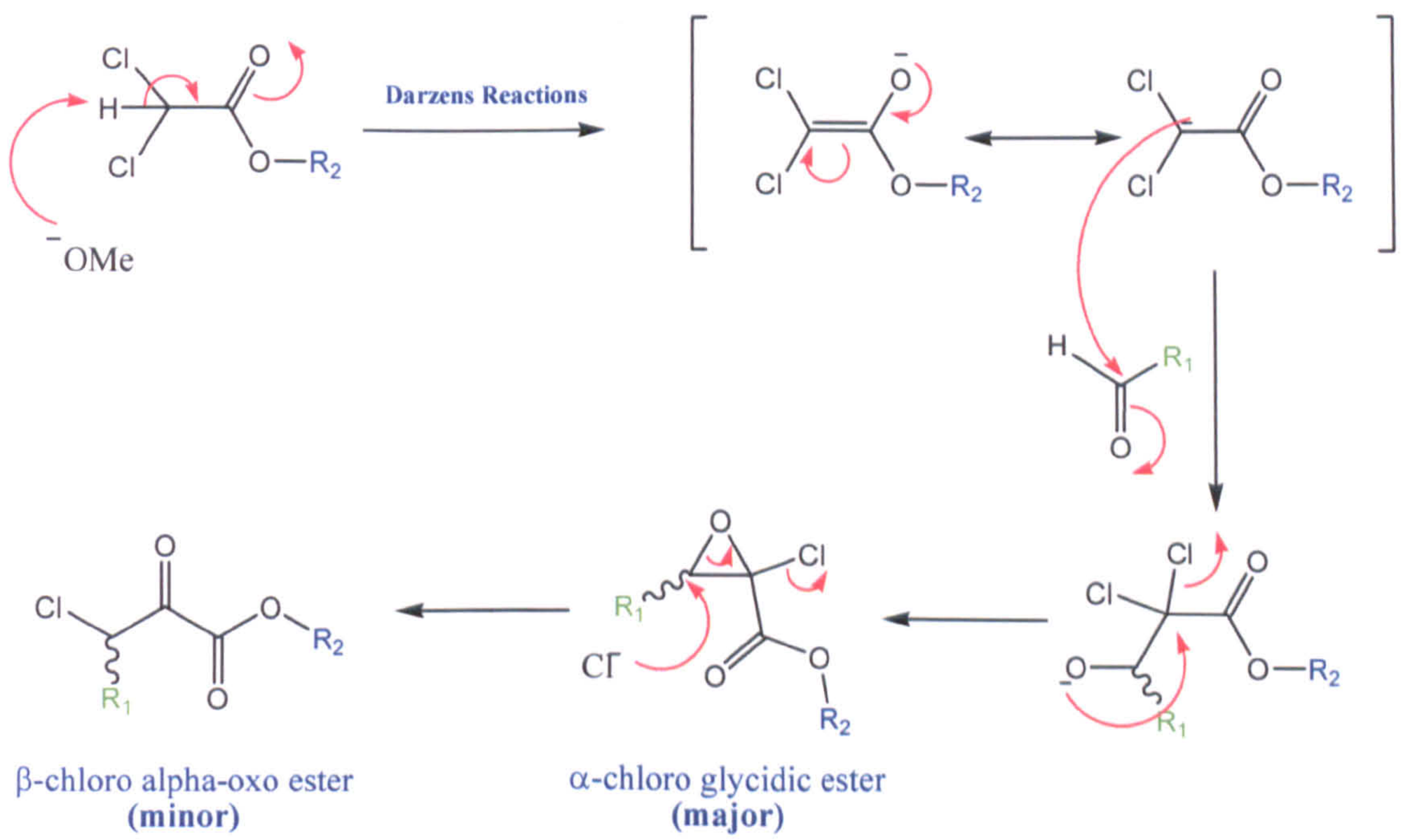
Compound No.	R_1	R_2	R_3	*M. aurum* (μM) MIC
29	$-CH_3$	$-COOCH_3$	$-COCH_2Br$	**426.0**
31	-Ph	$-COOCH_3$	$-COCH_2Br$	**22.5**
33	*m*-chloro-Ph-	$-COOCH_3$	$-COCH_2Br$	**20.5**
35	$-CH_2Ph$	$-COOCH_3$	$-COCH_2Br$	**43.0**
37	*p*-OCH_3Ph-	$-COOCH_3$	$-COCH_2Br$	**41.5**
43	$-CH_3$	$-COOCH_3$	$-COCH_2(CH_2)_3CH_3$	**173**
45	$-CH_3$	$-COOCH_3$	$-COCH_2(CH_2)_4CH_3$	**113**
46	$-CH_3$	-COOH	$-COCH_2(CH_2)_4CH_3$	**56**
48	$-CH_3$	-COOH	$-COCH_2(CH_2)_5CH_3$	**113**
50	$-CH_3$	-COOH	$-COCH_2(CH_2)_6CH_3$	**27**
52	$-CH_3$	-COOH	$-COCH_2(CH_2)_7CH_3$	**26**
69	$-CH_2Ph$	$-COOCH_3$	$-COCH_2(CH_2)_4CH_3$	**355**
70	$-CH_2Ph$	-COOH	$-COCH_2(CH_2)_4CH_3$	**31.2**
71	$-CH_2Ph$	$-COOCH_3$	$-COCH_2(CH_2)_7CH_3$	**19.8**
72	$-CH_2Ph$	-COOH	$-COCH_2(CH_2)_7CH_3$	**4.9**
78	$-CH_2Ph$	-COOH	-Ph	**62.5**
80	$-CH_2Ph$	-COOH	*p*-NO_2Ph-	**62.5**
83	-Ph	$-COOCH_3$	$-COCH_2(CH_2)_4CH_3$	**5.5**
84	-Ph	-COOH	$-COCH_2(CH_2)_4CH_3$	**94**
85	-Ph	$-COOCH_3$	$-COCH_2(CH_2)_7CH_3$	**2.5**
86	-Ph	-COOH	$-COCH_2(CH_2)_7CH_3$	**10.4**

Table 2.8: The structure and the activity of the compounds active against *M. aurum*.

2.6 Synthetic strategy

The synthesis of 2-aminothiazole-4-carboxylates and 5-carboxylates is well established.[107-109] For the former, the reaction involves two steps; formation of the α-halo glycidic esters (and β-halo α-oxo esters form as minor intermediates), followed by ring formation after addition of thiourea (Schemes 2,3 and 4). The mechanism for 5-carboxylate thiazole formation is shown in Scheme 5. Both reactions have to be performed in dry conditions to achieve optimum yields.

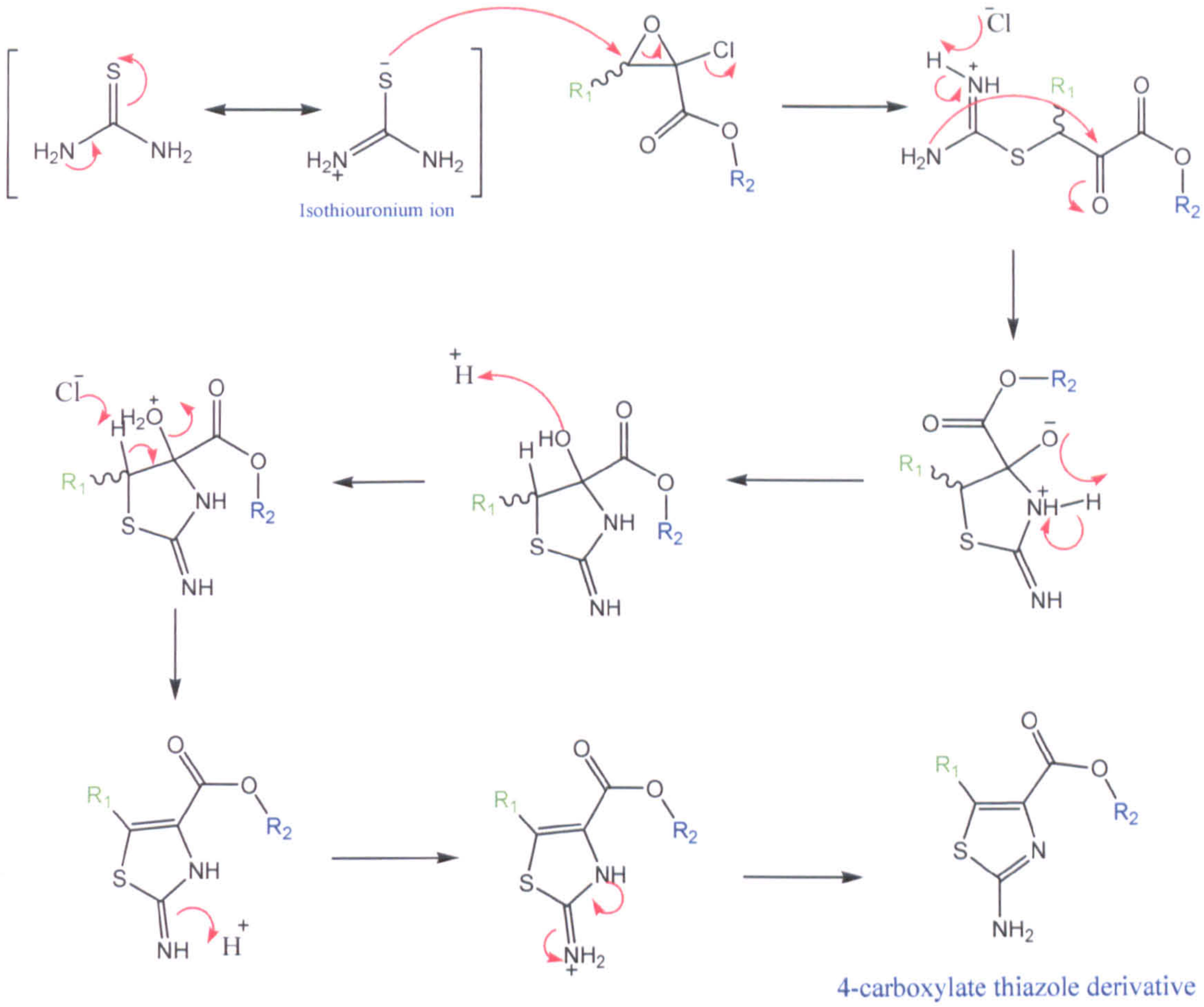
The synthesis of 2 amino-4-carboxylates involves the Darzens reaction between an aldehyde and methyl dichloroacetate at 0°C in diethyl ether in the presence of sodium methoxide. After washing with water, the diethyl ether extract yields the α -halo glycidic ester as the major product and the β-halo α-oxo ester as the minor product (Scheme 2).



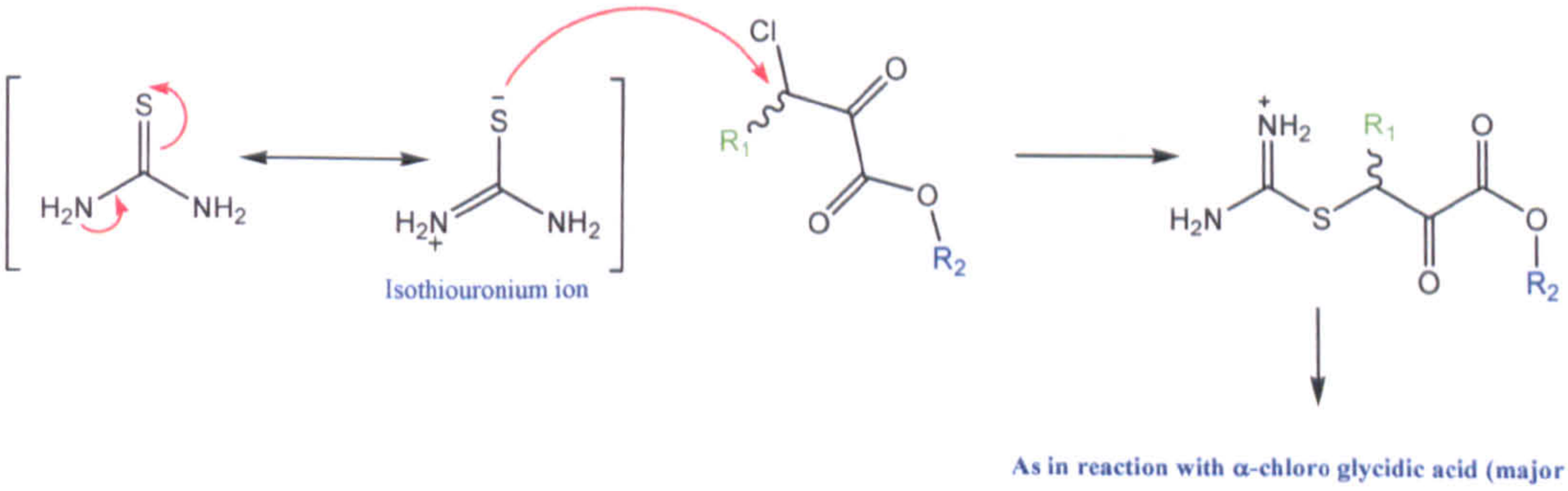
Scheme 2: Mechanism of the formation of α-chloroglycidic acid and β-chloro-oxo ester.

In order to synthesize the thiazole ring, thiourea was refluxed with the major and minor product in methanol. In the case of the major product, the isothiouronium salt is formed and the thiolate ion attacks the epoxide ring of the α -halo glycidic ester releasing a chloride ion, which abstracts one of the hydrogens of the isothiouronium salt to stabilize its charge. The next step of ring formation starts with the S_N2 nucleophilic attack of the amine on the carbonyl group, which is followed by acid-catalyzed dehydration of the thiazolidine ring to form the first double bond. A second acid-catalyzed reaction aromatizes the thiazole ring (Scheme 3).

A similar reaction occurs with the minor product of the mixture, and is illustrated in Scheme 4. The isothiouronium salt reacts with the β-halo α-oxo ester to form a similar intermediate as the major product, then proceeds by the same mechanism shown in Scheme 3.

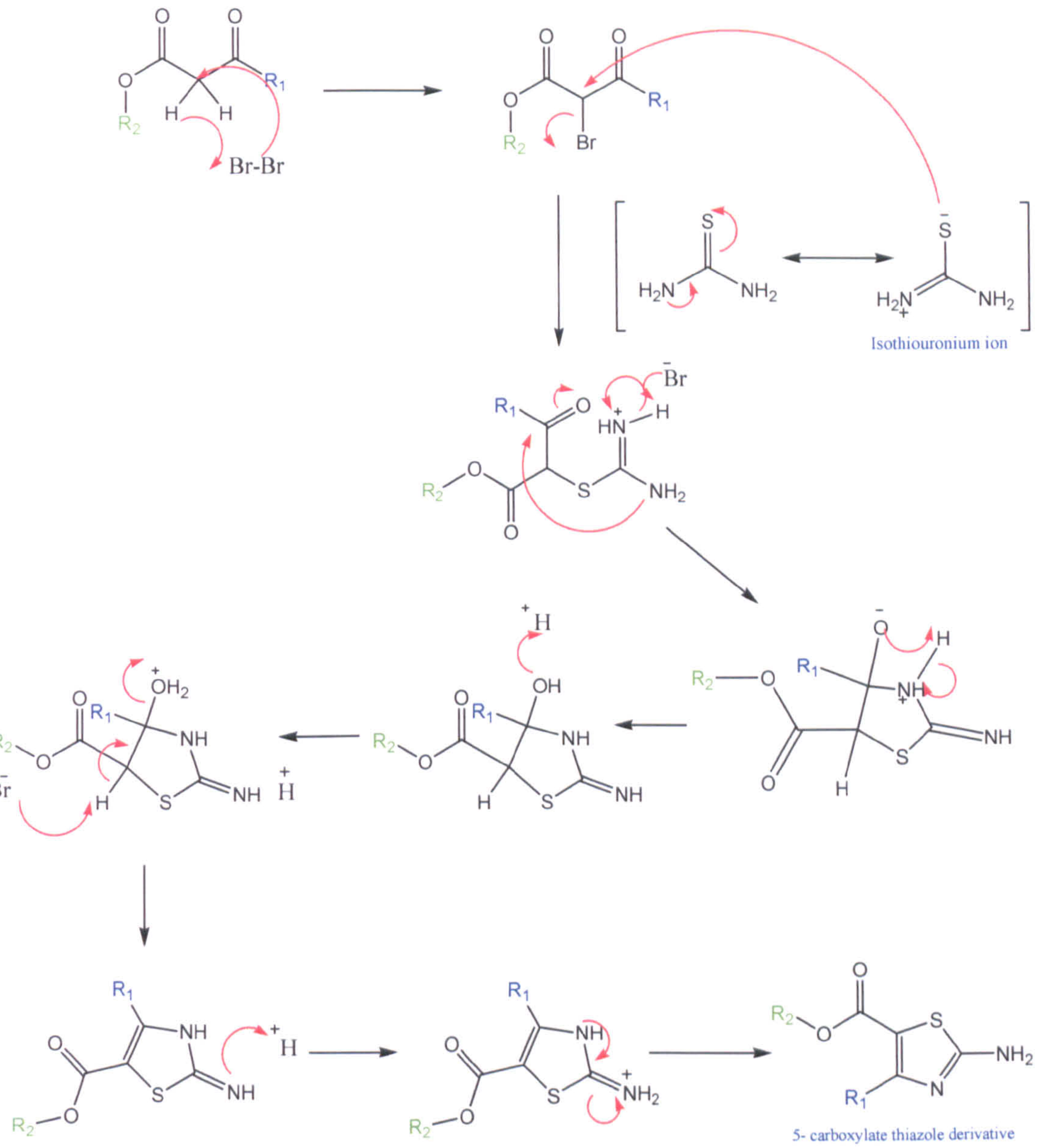


Scheme 3: Mechanism of the formation of 2,5 thiazole-4- carboxylate derivatives from α-chloroglycidic acid (major product).



Scheme 4: Mechanism of the formation of 4-carboxylate derivatives of thiazole ring from β-chloro-oxo ester (minor prouct).

The synthesis of the 5-carboxylate-2-aminothiazole involves addition of bromine dropwise to a suspension of ethyl 3-oxobutanoate in water at 0°C to form the α-halo product, which was extracted with ether and evaporated *in vacuo*, and the product refluxed with thiourea in ethanol. Mechanistically, the thiolate group of the isothiouronium salt attacks the α-halo product and releases the bromide ion, which then deprotonates the conjugated isothiouronium moiety. Ring formation occurred *via* nucleophilic attack of the amine on the carbonyl group, followed by two acid-catalyzed steps of dehydration and aromatization to produce the 2-amino-5-carboxylate thiazole ring (Scheme 5).



Scheme 5: Mechanism of the synthesis 5-carboxy-2-aminothiazole *via* β-oxo esters.

The advantage of this methodology is its versatility; it allows the introduction of substituents at three different sites in a facile manner. The free amine at position 2 can be converted to various amides by reacting with acid chlorides, acid anhydrides and esters *via* nucleophilic acyl substitution. Other functional groups can also be formed, for example, imines could be prepared from aldehydes or ketones and

alkylated amines *via* nucleophilic substitution with alkyl halides. In this work, we focused only on the amide derivatives in order to produce both H-bond acceptor (C=O) and donor (NH) features and different sized side chains to explore interactions with the channels. Position 5 of the 4-carboxylate derivatives can be varied depending on the choice of the reacting aldehyde; aromatic and alkyl aldehydes of different sizes and shapes were inserted at this position with good yields. Methyl esters at position 4 can be easily hydrolyzed to the free carboxylic acid, which has further possibilities for derivatization through coupling reactions (which is not explored in this work).

2.6.1 Establishing the synthetic methodology and the synthesis of the simple scaffolds (21-28)

The formation of the thiazole ring in the simple scaffold compounds was performed by the same procedure of Barton *et al.*[110] Both 4- and 5-caboxylate esters were synthesized and then hydrolyzed to the free acids. Small group substitution of the free amine to the amide was performed by the addition of acetyl chloride in dry conditions in the presence of a tertiary amine to facilitate the product formation (see experimental chapter). Synthesis of the simple derivatives **(21)** and **(25)** and examination of the proton, carbon NMR spectra illustrate the key spectral features of successful ring formation and the essential differences between the isomers. The ^{1}H and ^{13}C NMR of **(21)** and **(25)** are shown in figures 2.26 and 2.27 and figure 2.28 and 2.29 respectively. Analysis of the proton spectrum of **(21)** shows that there is a singlet around δ 2.47 ppm composed of three protons, which corresponds to the (CH_3) directly attached to the thiazole ring at position 5 that is shifted downfield by the presence of the adjacent aromatic ring. The singlet at δ 3.7 ppm corresponds to the methoxy protons (OCH_3), and the singlet at δ 7.0 ppm corresponds to the two protons from NH_2 (figure 2.26).

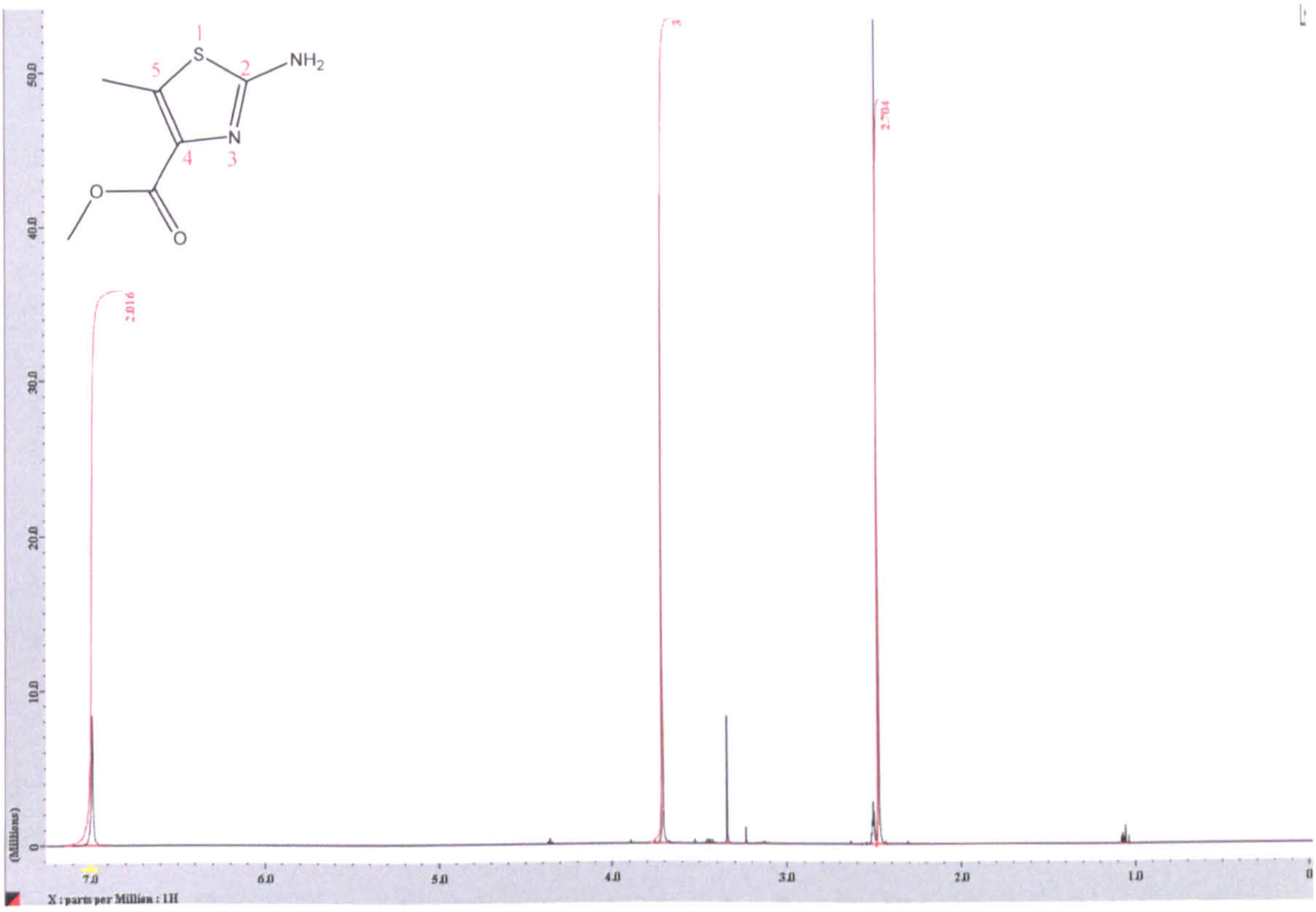


Figure 2.26: ^{1}H spectrum of methyl 2-amino-5-methylthiazole-4-carboxylate (21).

The ^{13}C spectrum of **(21)** (Figure 2.27) has six peaks, one at δ 13 ppm representing the methyl carbon attached directly to the thiazole ring, whilst the carbon of the methoxy carbon appears at δ 52 ppm. The signals at δ 126, 131, and 167 ppm correspond to the quaternary carbons of the ring, confirming thiazole formation, and the signal at δ 160 ppm corresponds to the carbonyl carbon.

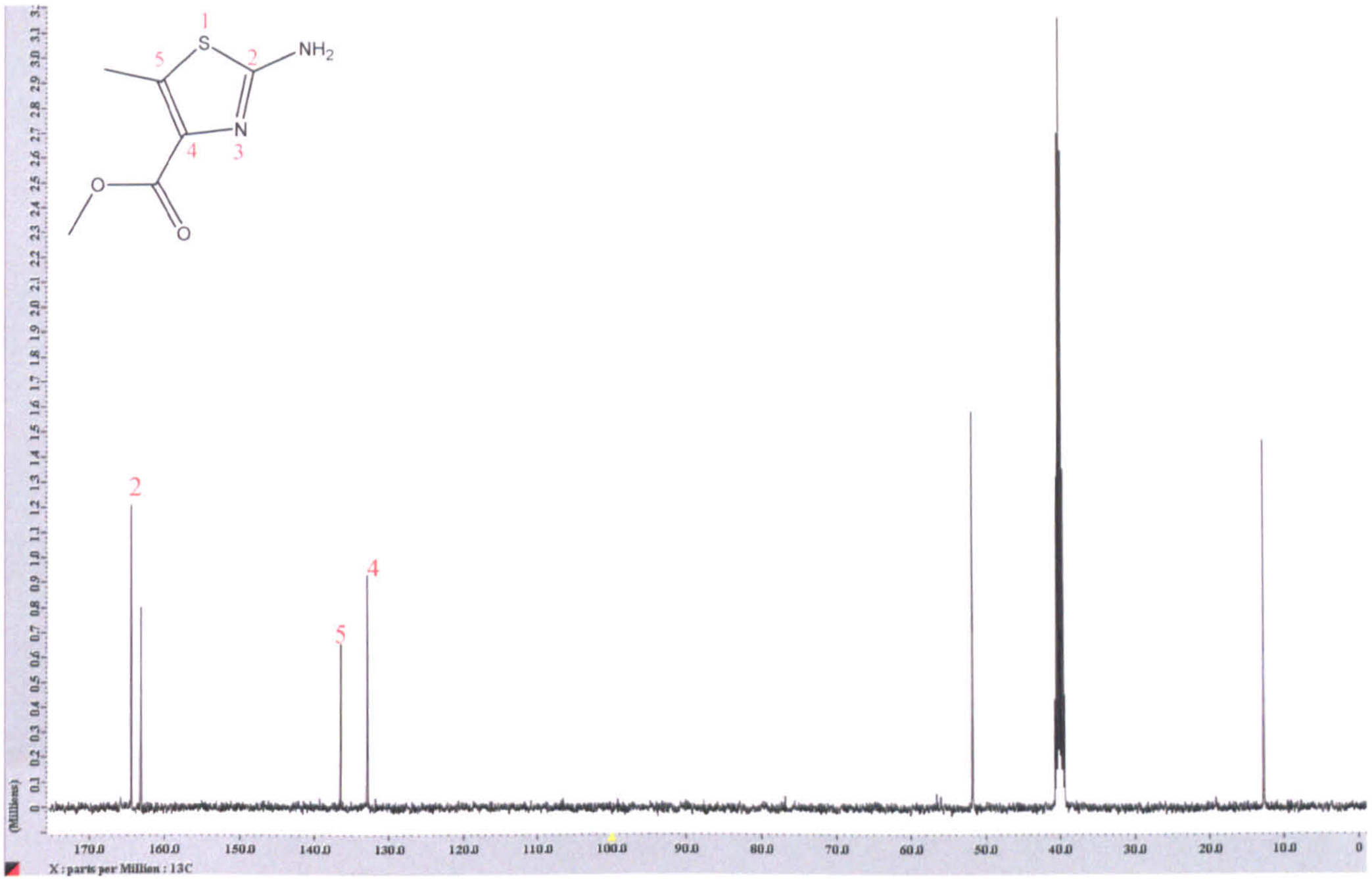


Figure 2.27: ^{13}C spectrum of compound methyl 2-amino-5-methylthiazole-4-carboxylate (21).

In the ^{1}H spectrum of **(25)** (figure 2.28), the triplet at δ 1.2 ppm and quartet at δ 4.15 correspond to the ethyl ester (-OCH_2CH_3), whilst the methyl protons directly attached to the aromatic thiazole ring appear as a singlet at δ 2.4 ppm, and the other singlet at δ 7.7 ppm represents the two protons of the amine group (NH_2). The ^{13}C spectrum (figure 2.29) demonstrates a change in the order of the aromatic carbon signals compared with **(21)** owing to the different isomeric arrangement in the ring, while the signal at δ 163 ppm corresponding to the carbonyl carbon appears at the same position.

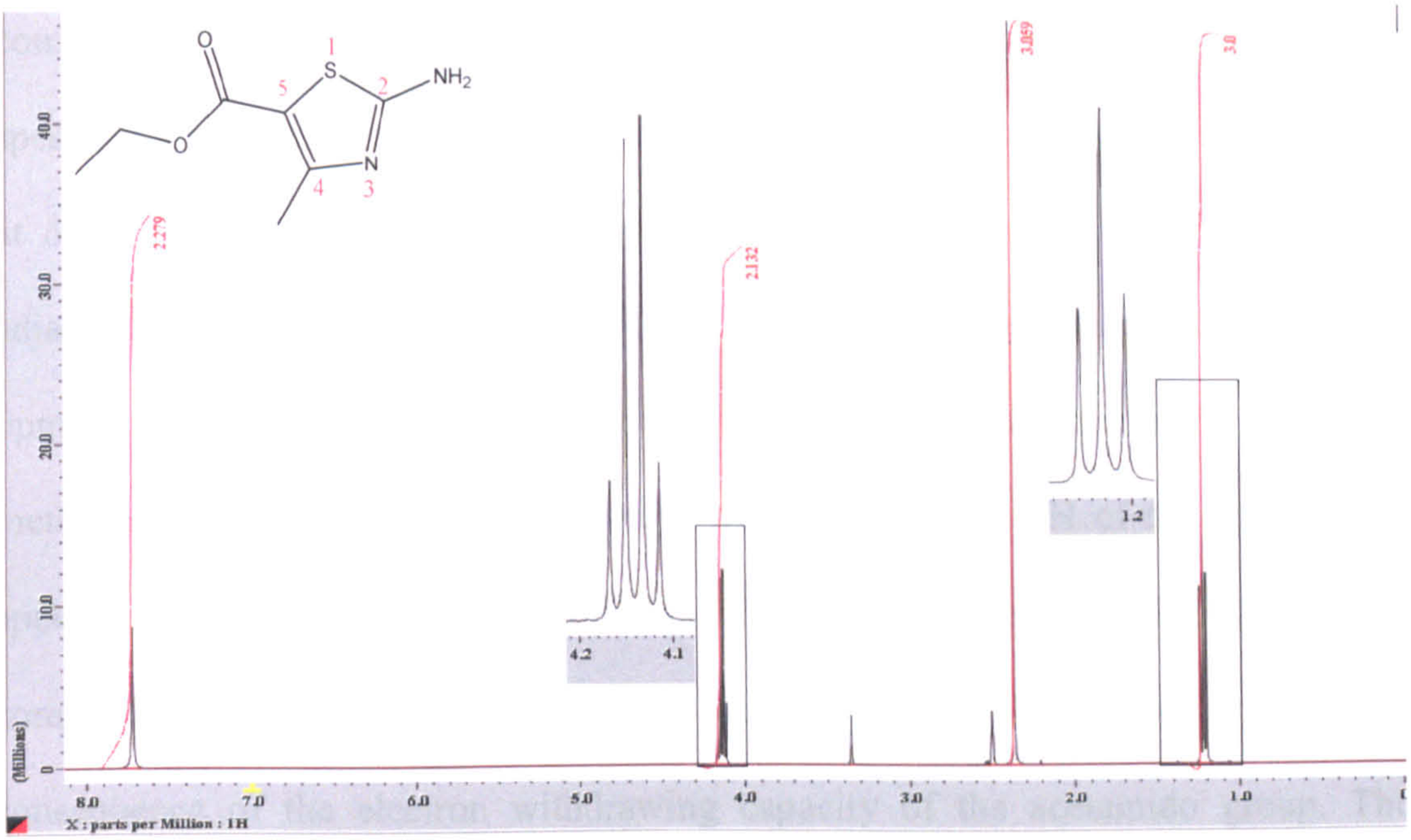


Figure 2.28: ^{1}H spectrum of ethyl 2-amino-4-methylthiazole-5-carboxylate (25).

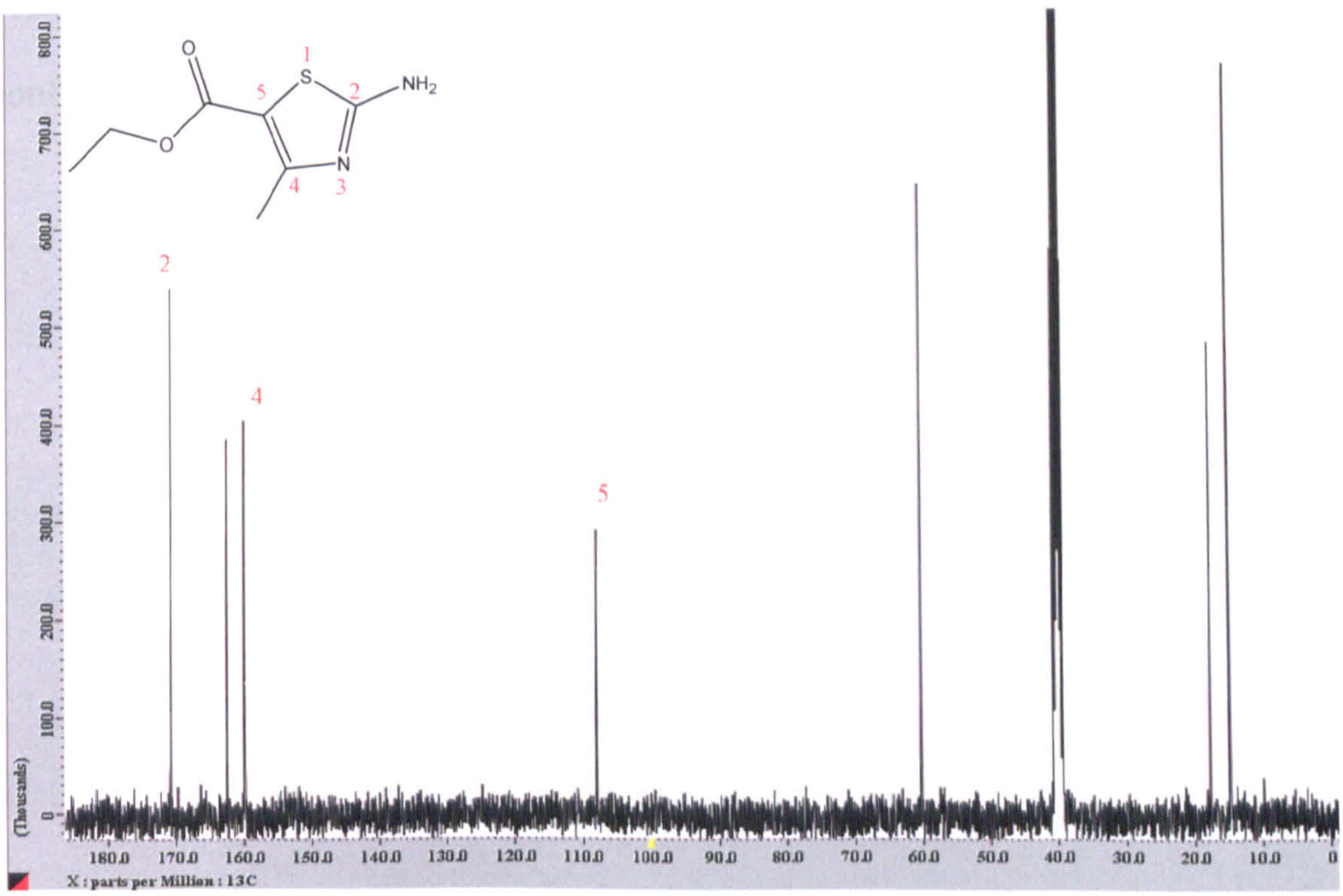


Figure 2.29: ^{13}C spectrum of ethyl 2-amino-4-methylthiazole-5-carboxylate (25).

The successful amidation of compound **(21)** to produce compound **(23)** was confirmed by the NMR spectrum illustrated in figure 2.30. Analysis of the ^{1}H spectrum shows the presence of three singlets in the aliphatic area, the first, a singlet at δ 2.16 ppm composed of three protons that corresponds to the methyl group adjacent to the carbonyl amide group ($NHCOCH_3$). The other two singlets at δ 2.67 ppm and δ 3.86 ppm correspond to the methyl attached to the thiazole ring and the methoxy group of the ester respectively, whilst the proton NH of the amide bond appears at δ 12.3 ppm. The downfield shifts of the 5-methyl and 2-NH signals compared to **(21)** [δ 2.67 ppm versus δ 2.47 and δ 12.3 versus δ 7.0 ppm] is a consequence of the electron withdrawing capacity of the acetamido group. The appearance of extra carbon signal of the ^{13}C spectrum of **(23)** compared to **(21)** is further evidence of successful amidation, whilst the presence of carbonyl absorption peak at 1647 cm^{-1} in the FT-IR spectrum adjacent to the ester 1707 cm^{-1} absorption confirms the formation of the amide bond.

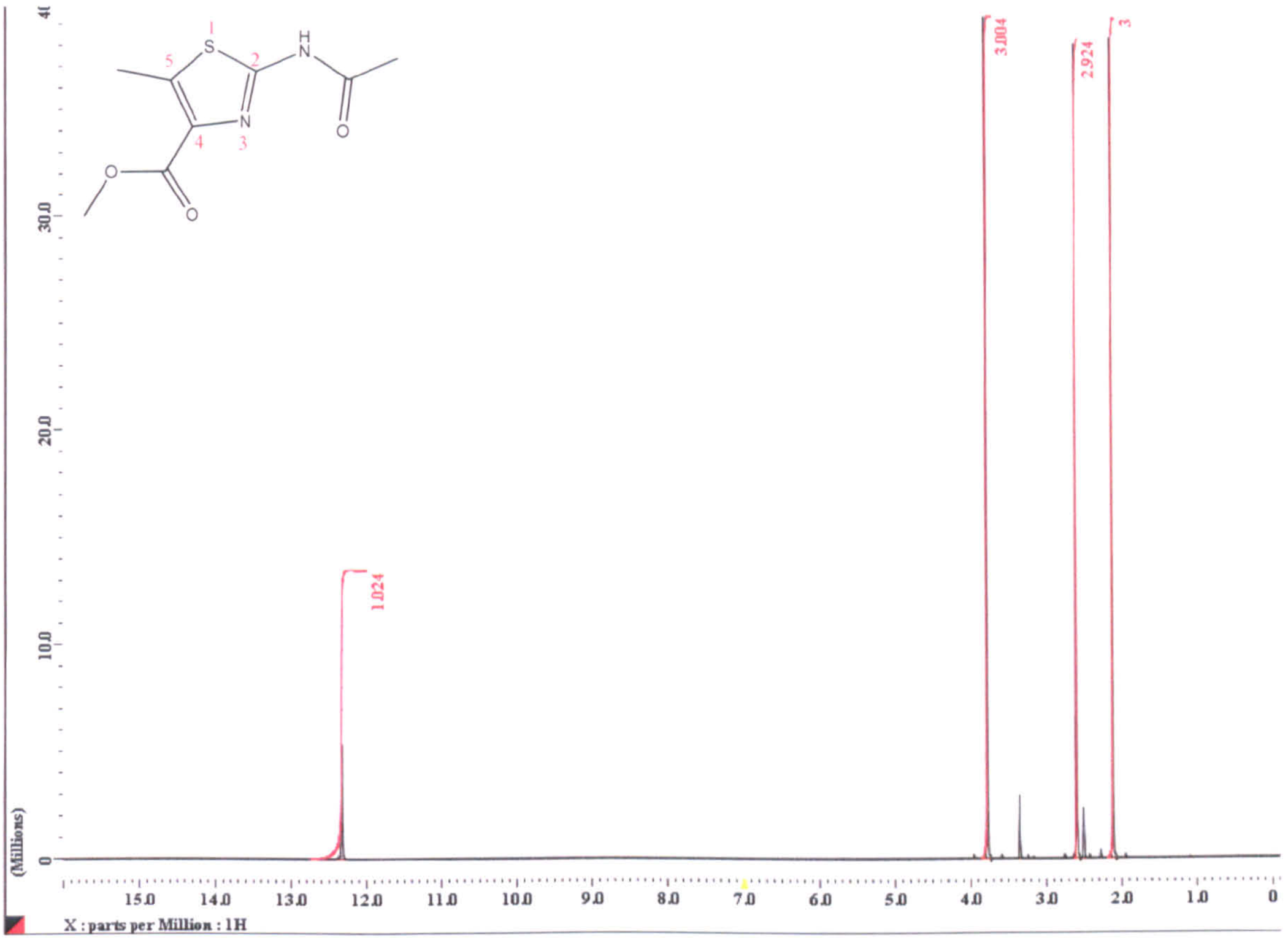


Figure 2.30: 1H spectrum of methyl 2-acetamido-5-methylthiazole-4-carboxylate (23).

Hydrolysis of compound **(23)** to produce **(24)** was achieved using 85mM NaOH solution at 50°C, and was carried out to increase the possibility of forming H-bonding inside the active site. Mild hydrolytic conditions were required to avoid removal of the amide group, and despite this, the yield of the hydrolysis step was poor, which could be due to the retention of the hydrolysed products (which can be zwitterionic in some cases) in the aqueous medium during work-up. The ^{1}H spectrum of compound **(24)** showed that the only differences to the ^{1}H spectrum of compound **(23)** was the disappearance of the signal at δ 3.7 ppm and the appearance of the OH proton signal at δ 12.2 ppm, which proved the hydrolysis of ester. A shift in the carbonyl absorption peak in the FT-IR spectrum from 1707 cm^{-1} to 1661 cm^{-1} also confirmed ester hydrolysis of the free acid.

A large number of compounds were prepared in each library, and the characterization of these compounds can be found in the experimental procedure. In the following sections representative examples from each library are used to illustrate the process by which such characterization were performed.

2.6.2 Synthesis of 2-bromoacetamido 5-derived 4-carboxylate esters and carboxylic acids

The synthesis of 2-bromoacetamido derivatives was performed by reacting bromoacetyl bromide with the free amine derivatives in dry conditions and in the presence of triethylamine. The ^{1}H NMR spectrum of **(35)** is shown in figure 2.31.

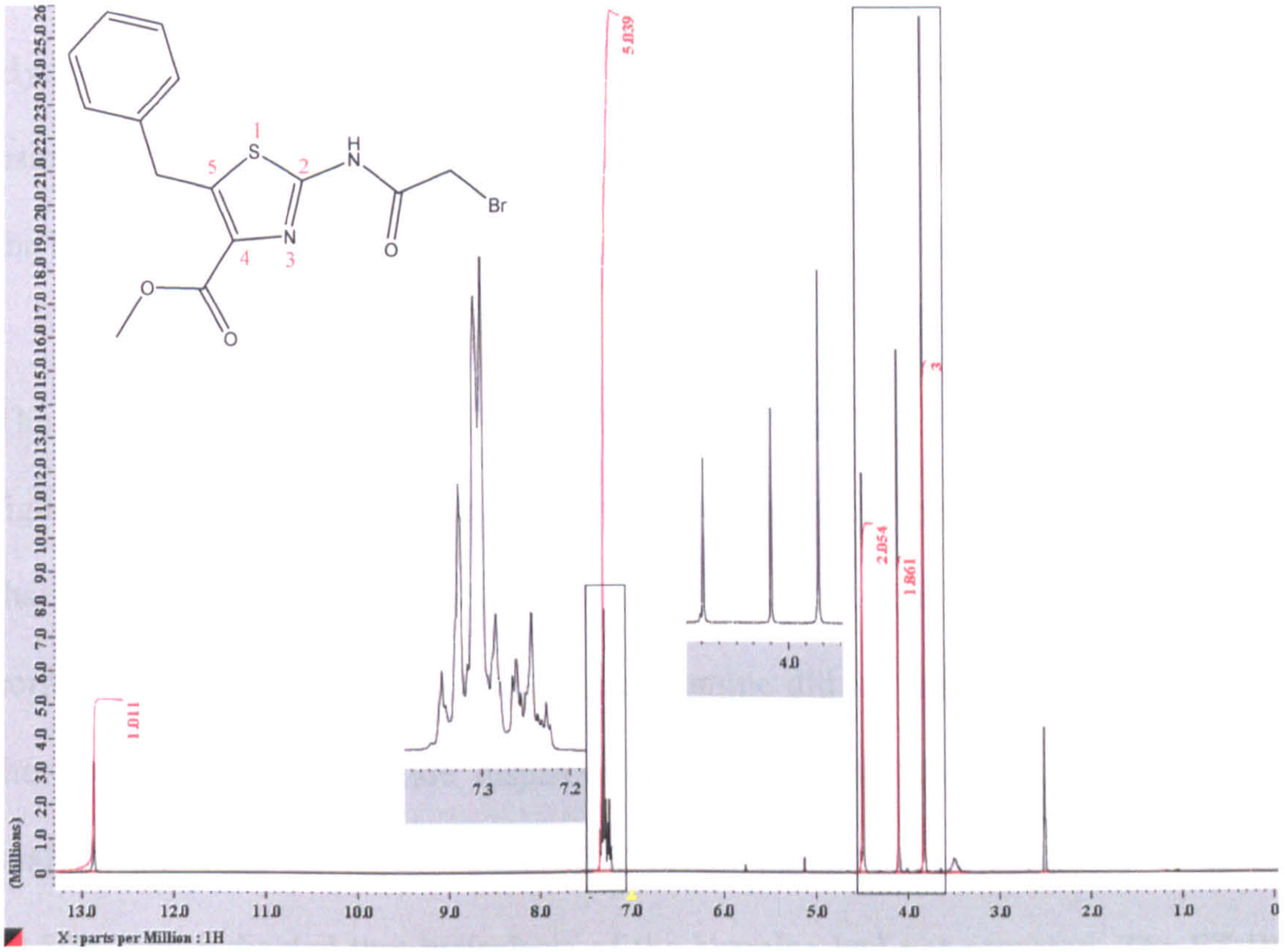


Figure 2.31: ^{1}H spectrum of methyl 5-benzyl-2-(2-bromoacetamido) thiazole-4-carboxylate (35).

As with the other esters prepared previously, the singlet at δ 3.8 ppm corresponds to the three-methoxy protons (OCH_3). The singlet at δ 4.1 ppm corresponds to CH_2 adjacent to the bromine atom, whilst the singlet at δ 4.5 ppm represents the CH_2 of the benzyl group. The phenyl protons appear as a multiplet at δ 7.2-7.4 ppm and a downfield singlet at δ 12.8 ppm represents the NH proton. Further conformation of

the presence of the bromine atom in the structure was performed by mass spectroscopy (MS), where two equally intense peaks of m/z 369 and 371 were seen representing the molecular ion and the M+2 for the isotope ^{81}Br. Elemental analysis has also proved the presence of bromine where the experimental percentage was 21.46% compared with expected value of 21.64%.

Hydrolysis of the bromoacetamido compounds to the free acid was performed by using mild base (NaOH 85mM at 50C°) for 30 minutes to avoid the displacement of the bromine atom and hydrolysis of the amide bond.

The ^1H NMR spectrum of the hydrolyzed form of **(35)**, namely **(36)**, is shown in figure 2.32. The only difference between the two spectra is the absence of the singlet that corresponds to the methoxy group at δ 3.8 ppm. The signal at δ 4.1 ppm corresponding to the CH_2 adjacent to the bromine did not shift, which proved that there was no S_N2 hydroxide displacement mechanism. Moreover, the elemental analysis provided an experimental 22.37% compared to the calculated value of 22.50%, and indicated that hydrolysis of the bromine had not occurred. The FT-IR spectrum also provided evidence that the amide was not a hydrolysed with an absorption peak at 1661 cm^{-1}.

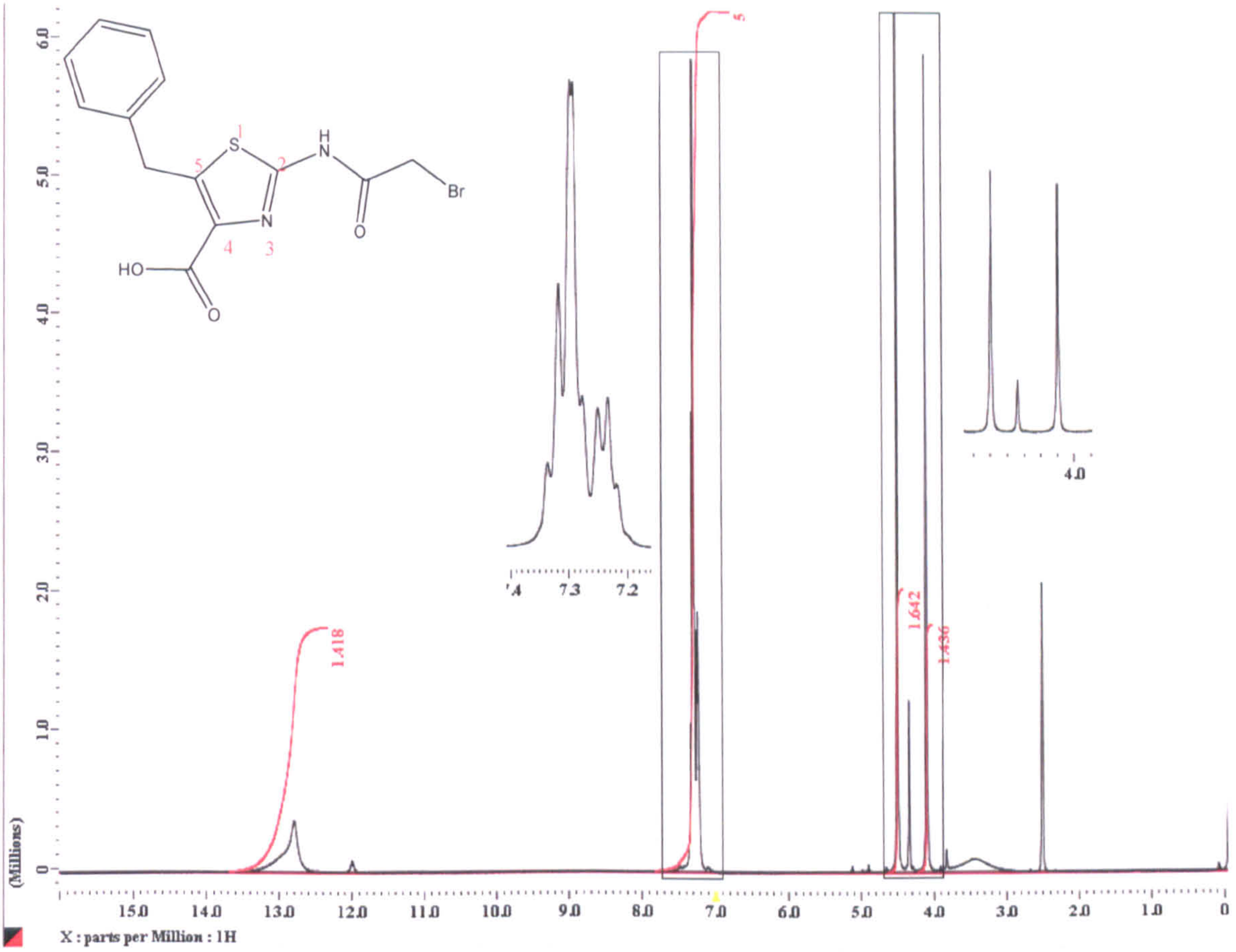


Figure 2.32: ^{1}H spectrum of 5-benzyl-2-(2-bromoacetamido)thiazole-4-carboxylic acid (36).

2.6.3 Library 1: Synthesis of the 2-amide derivatives of the 5-methyl 4-carboxylate esters and carboxylic acids

In this library, compound **(21)** was derivatized at position 2 to produce a series of amide derivatives. A variety of substituents of different sizes and shapes were introduced by the same procedure, which involved the addition of an acid chloride dropwise to the free amine in the presence of a tertiary amine to drive the reaction forward. As an example, the ^{1}H NMR spectrum of **(45)** is shown in figure 2.33. The peaks that correspond to the hexyl side chain appear at δ 0.9-2.4 ppm, the methyl substituent at position 5 appears at δ 2.6 ppm as a singlet, whilst the methoxy protons are located downfield as a singlet at δ 3.7 ppm, and the NH of the amide appears at δ 12.3 ppm.

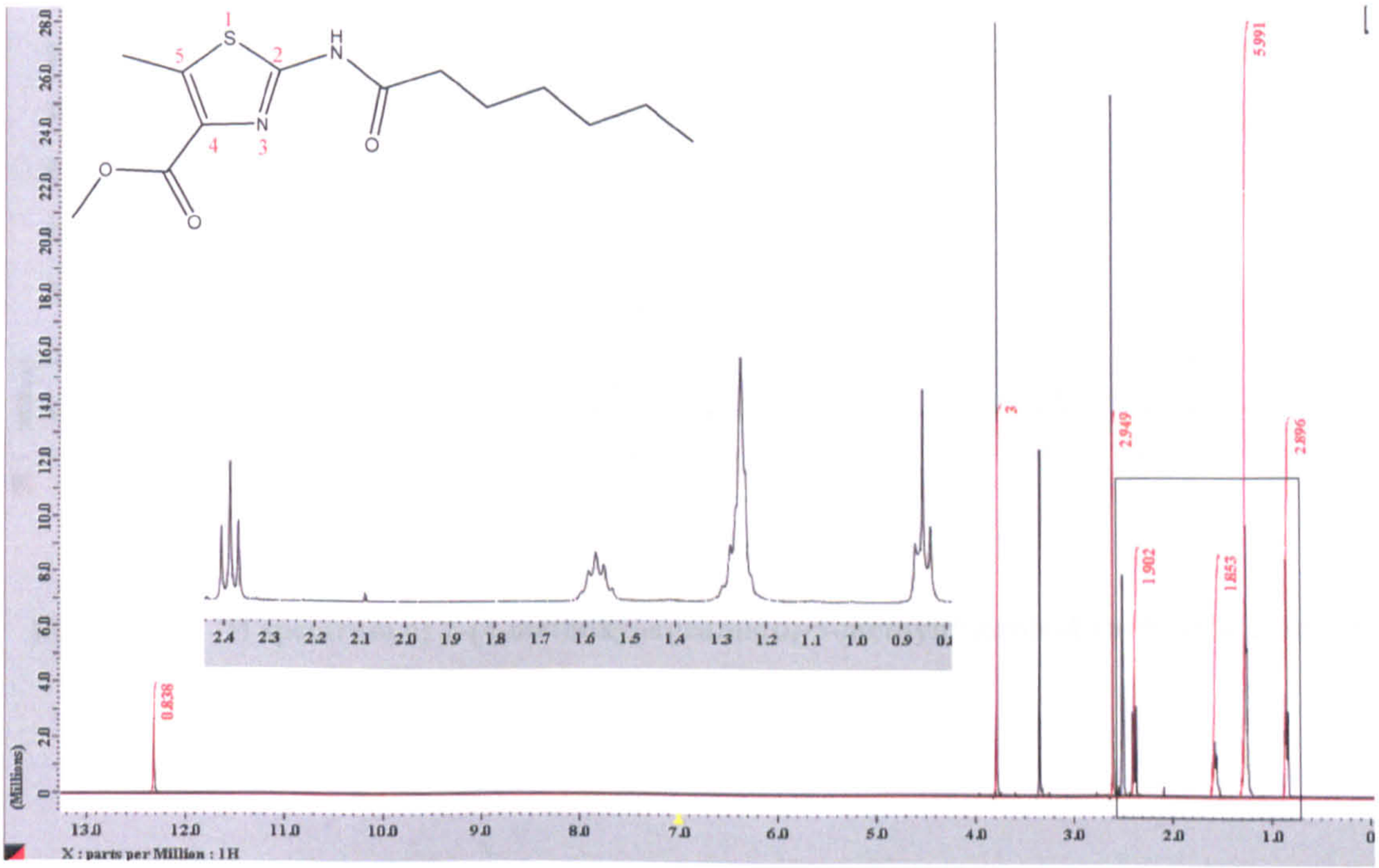


Figure 2.33: ^{1}H spectrum of methyl 2-heptanamido-5-methylthiazole-4-carboxylate (45).

Another example of this library where position 2 is occupied by a bulky aromatic group and the ester has been hydrolysed is shown in figure 2.34. Compound **(62)** was

derivatized with *p*-methoxy benzoyl chloride to form the amide followed by ester hydrolysis. The ^{1}H NMR spectrum of **(62)** shows the methyl peak at position 5 remains at δ 2.6 ppm as a singlet, whilst another singlet corresponding to *p*-methoxy group but appears at δ 3.87 ppm as a singlet. The parent compound **(61)**, which retains the ester, has two methoxy peaks. The two doublets at δ 7.06 ppm and δ 8.11 ppm correspond to *p*-disubstituted aromatic protons.

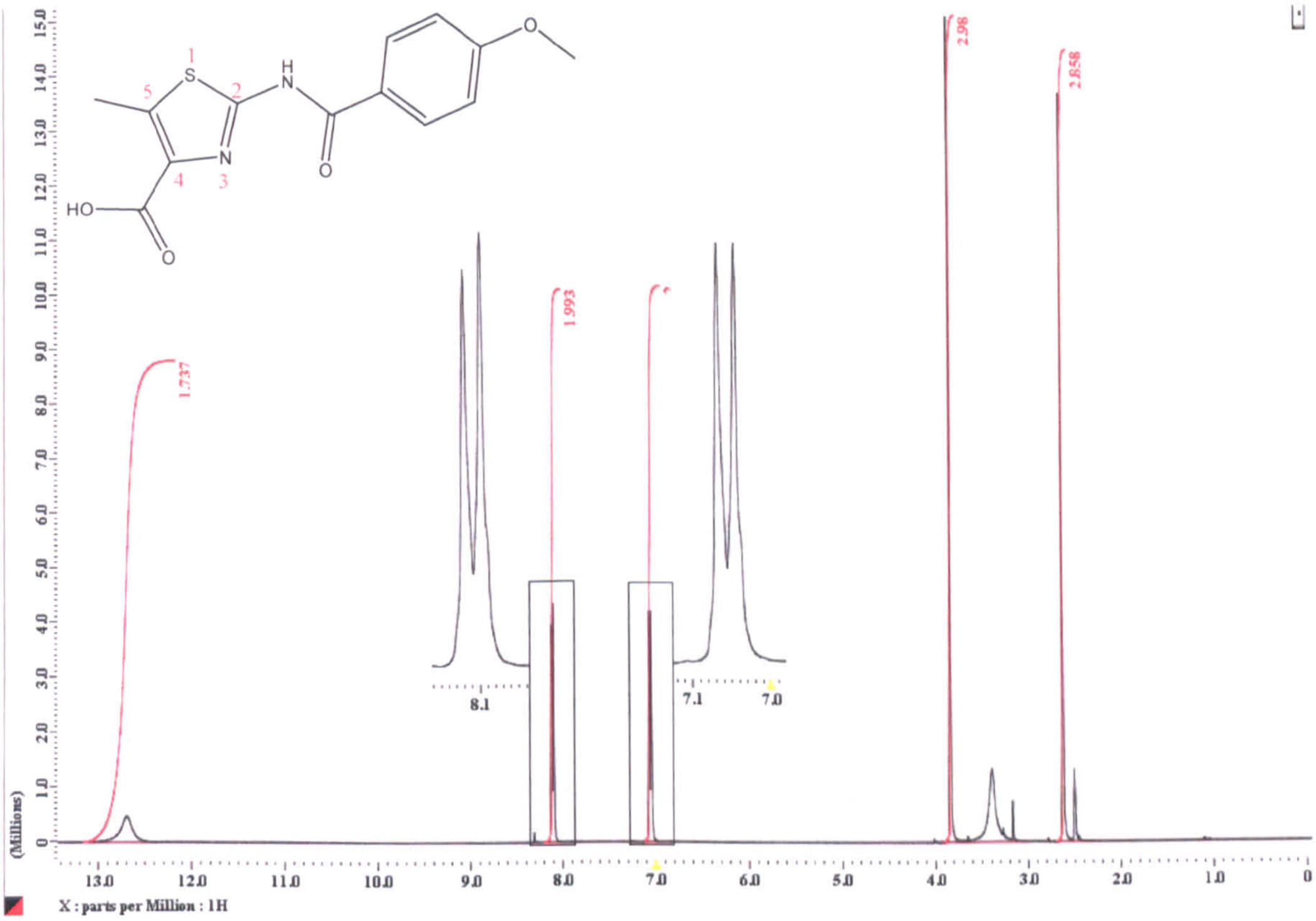


Figure 2.34: ^{1}H spectrum of 2-(4-methoxybenzamido)-5-methylthiazole-4-carboxylic acid (62).

2.6.4 Library 2: Synthesis of 2-amide derivatives of the 5-benzyl 4-carboxylate and carboxylic acids

The same procedure used for library 1 was adopted for the 5-benzyl derivatives; compound **(75)** was synthesized by reacting maleic anhydride with **(107)**. The ^1H NMR spectrum of **(75)** is shown in figure 2.36; the methoxy singlet appears at δ 3.81 ppm, whilst the singlet for CH_2Ph appears downfield at δ 4.48 ppm, and the double bond protons appear downfield at δ 6.4 ppm and δ 6.5 ppm as doublets. The coupling constant of these two protons indicates that they are in the *cis* form with a value of 11.88 Hz, and could be explained by the formation of intra-molecular H-bonding between the terminal carboxylic acid and the carbonyl amide (figure 2.35). The five phenyl protons appear as a multiplet at δ 7.21-7.35 ppm, whilst the carboxylic acid OH proton appears as a broad peak at δ 12.7 ppm. The presence of three carbonyl absorptions in the FT-IR spectrum at 1690, 1701, and 1723 cm^{-1} is due to the three different carbonyl groups in the compound, namely amide, carboxylic acid and ester respectively (figure 2.37). Moreover, the elemental analysis and the MS spectroscopy have confirmed the structure of **(75)** by showing the correct composition of the elements, and an m/z of (M+1) equal to 347.2.

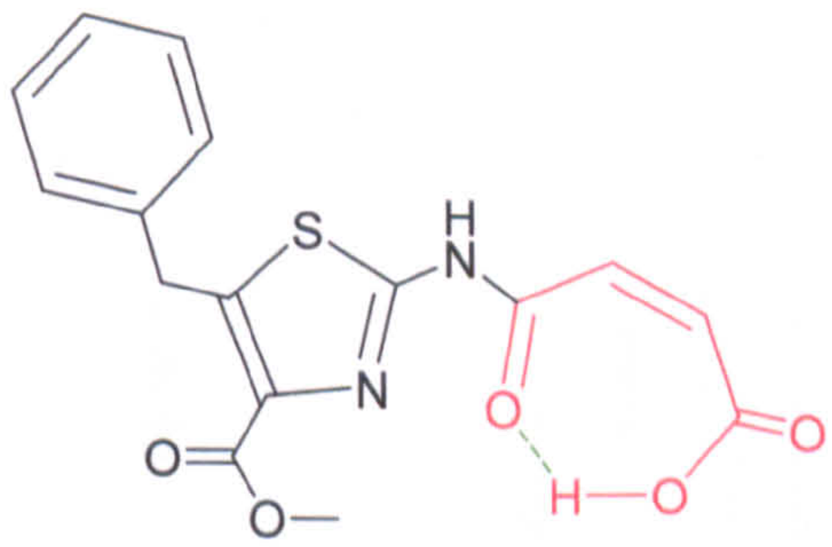


Figure 2.35: Intramolecular H-bonding of (Z)-4-(5-benzyl-4-(methoxycarbonyl)thiazol-2-ylamino)-4-oxobut-2-enoic acid (75).

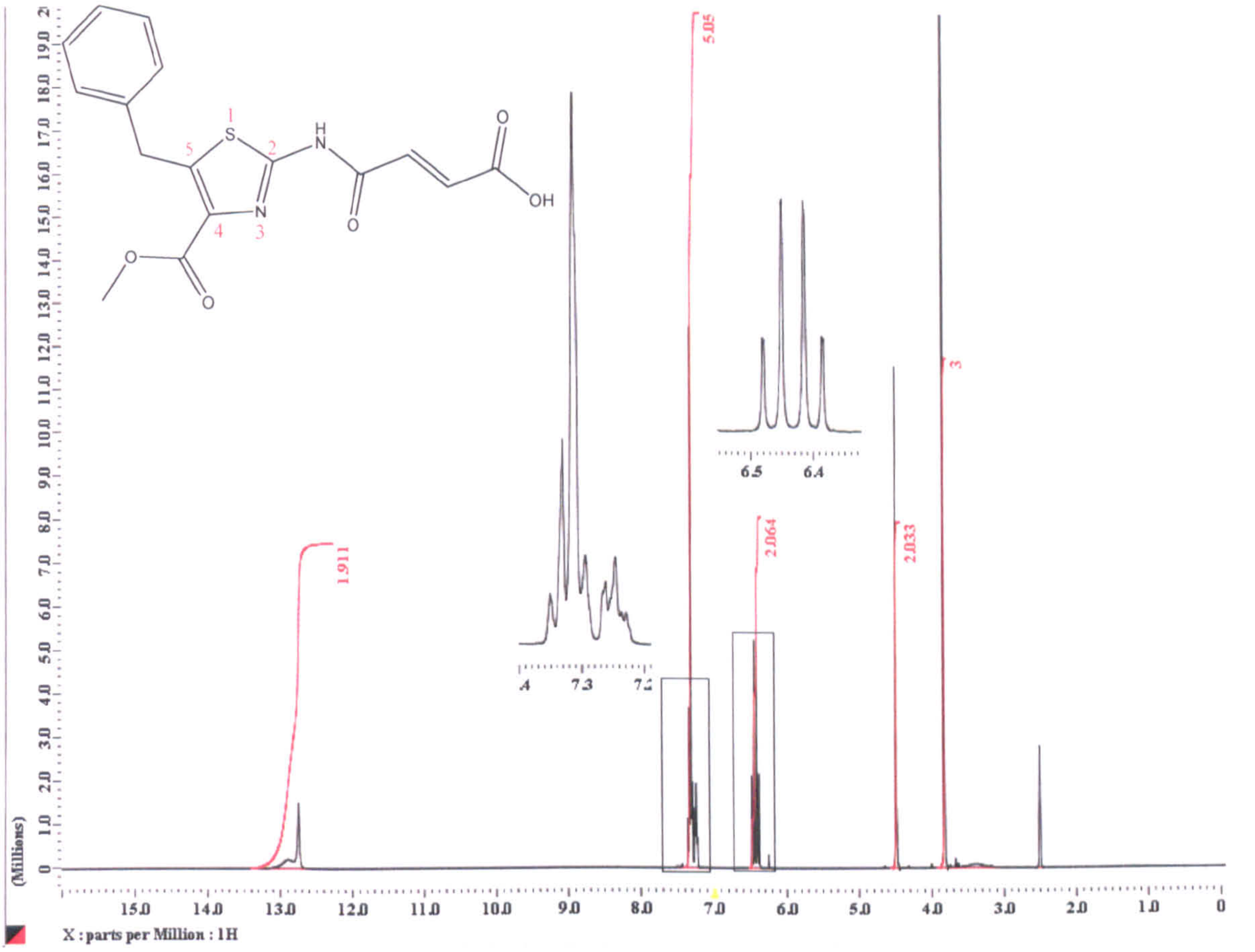


Figure 2.36: ^{1}H spectrum of (Z)-4-(5-benzyl-4-(methoxycarbonyl)thiazol-2-ylamino)-4-oxobut-2-enoic acid (75).

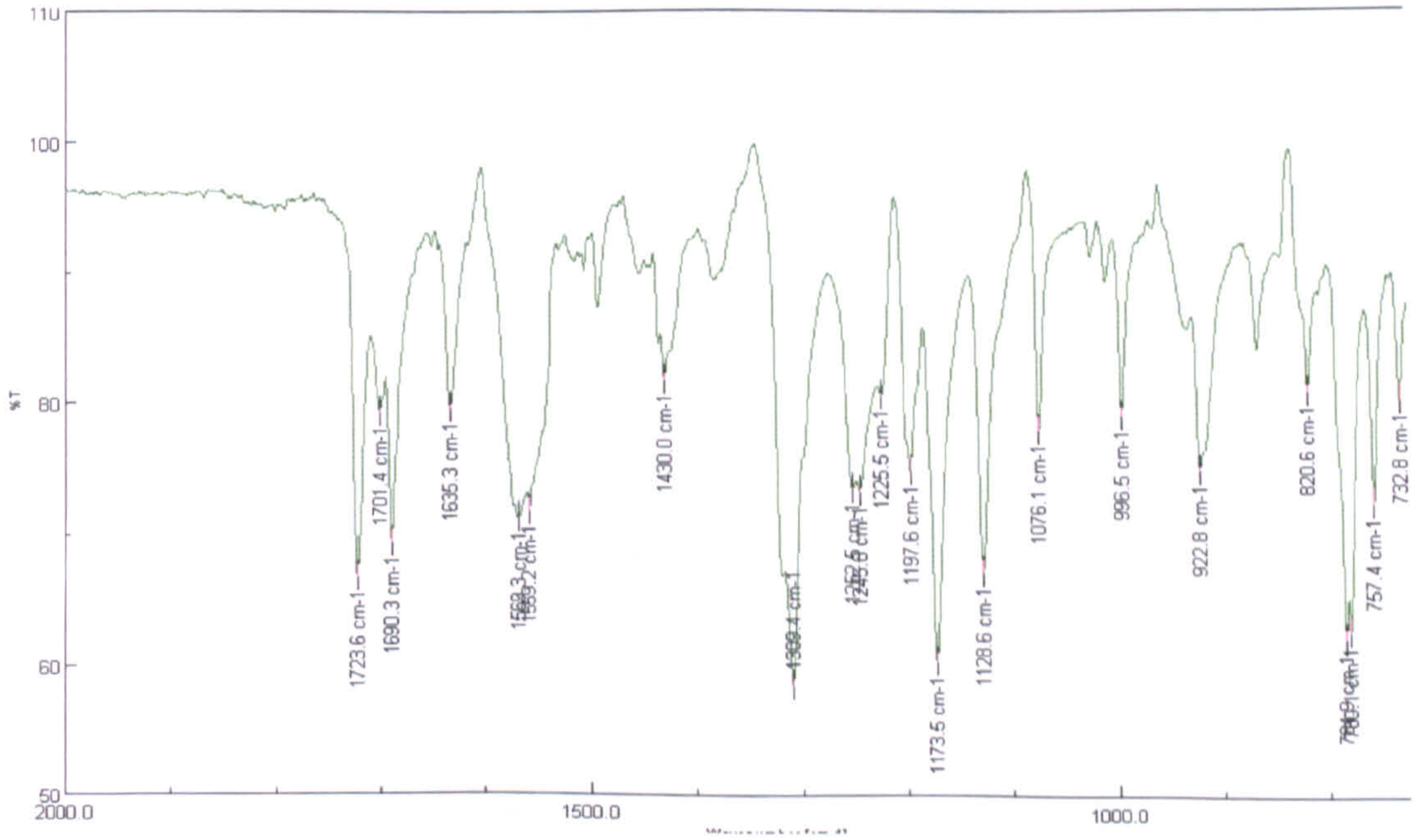


Figure 2.37: FT-IR pectrum of (Z)-4-(5-benzyl-4-(methoxycarbonyl)thiazol-2-ylamino)-4-oxobut-2-enoic acid (75).

2.6.5 Library 3: Synthesis of 2-amide derivatives of the 5-phenyl and 5-*m*-chlorophenyl 4-carboxylate esters and carboxylic acids

The synthesis of this library was performed using the same procedures adopted for libraries 1 and 2. However, the synthesis of this library was less efficient, which we attribute to the reduced nucleophilicity of the free amine group to form the amide bond. This low nucleophilic character is due to the distribution of p-orbital electrons between the two adjacent aromatic rings. To compare productivity, the reaction required warming with the addition of increased amounts of acid chloride, which led to problems during purification. Compound **(91)** is an example of this library, where the cyclopropyl moiety forms the amide side chain and the *m*-chloro benzene is at position 5. Figure 2.38 depicts the ^{1}H NMR spectrum of **(91)**; at δ 0.9 ppm a multiplet represents four protons of the two CH_2's of the cyclopropyl ring, whilst a multiplet of the remaining CH of the cyclopropyl ring appears at δ 1.9 ppm. Three protons as a multiplet and a one proton singlet at δ 7.45 ppm and δ 7.6 ppm respectively correlate to the four protons of the *m*-chloro-benzene at position 5.

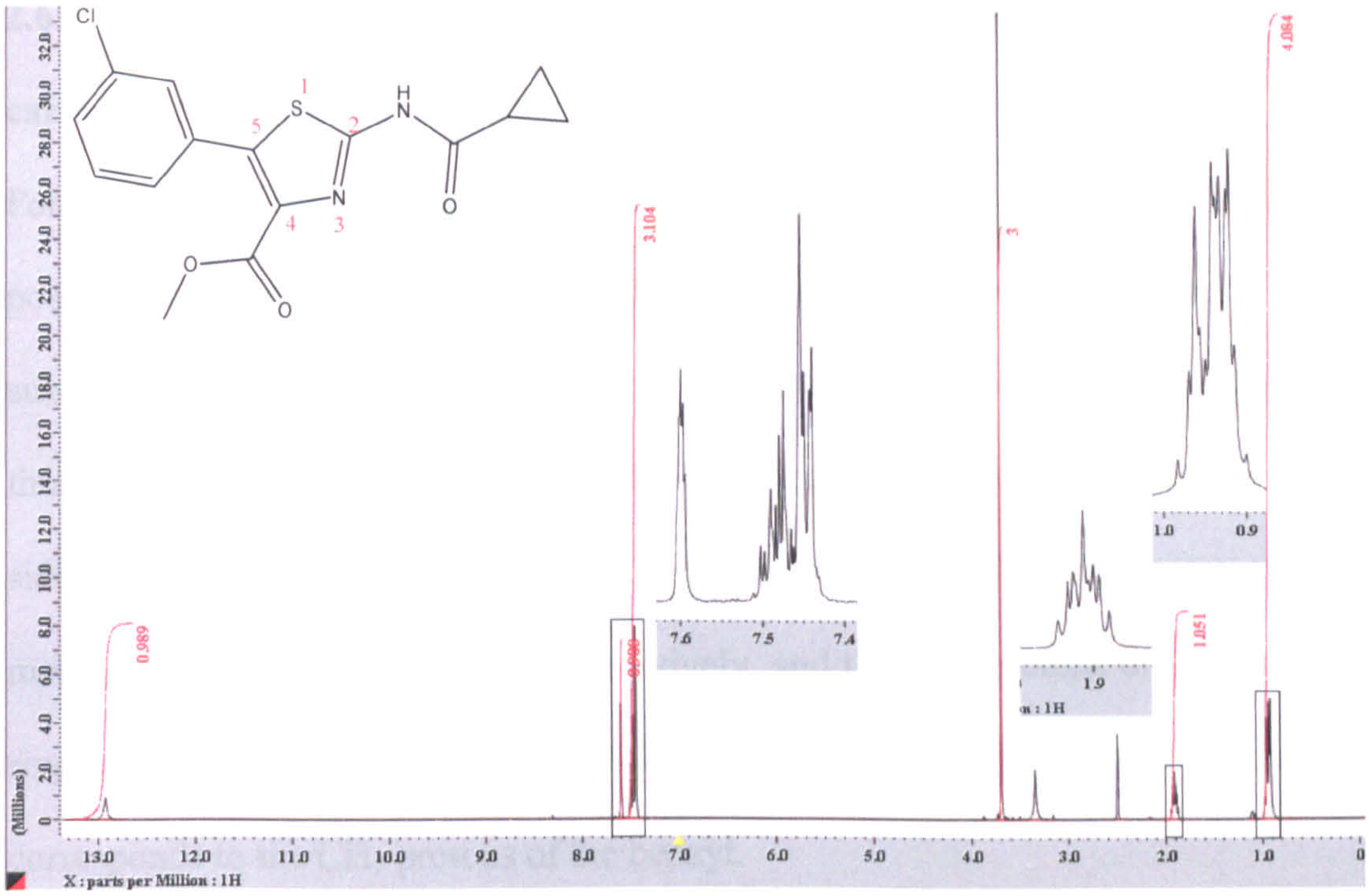


Figure 2.38: ^{1}H spectrum of methyl 5-(3-chlorophenyl)-2-(cyclopropanecarboxamido)thiazole-4-carboxylate (91).

2.6.6 Library 4: Synthesis of methyl 2-amino-5-derivatives of the 4-carboxylates

For the final library, position 2 was left unsubstituted as the free amine, whilst position 5 had different groups ranging from small to large. The nature of the 5-substituted groups was determined by the choice of the aldehyde used to form the thiazole ring (Barton *et al*). The ^{1}H NMR spectrum of **(111)** with a phenoxy benzyl side chain is shown at figure 2.39, with singlets at δ 3.7 and 7.2 ppm representing the methoxy OCH_3 and NH_2 protons respectively, and the nine protons of both aromatic rings appearing in the area between δ 7.0-7.5 ppm. The singlet located at δ 5.2 ppm corresponds to the CH_2 protons of the benzyl.

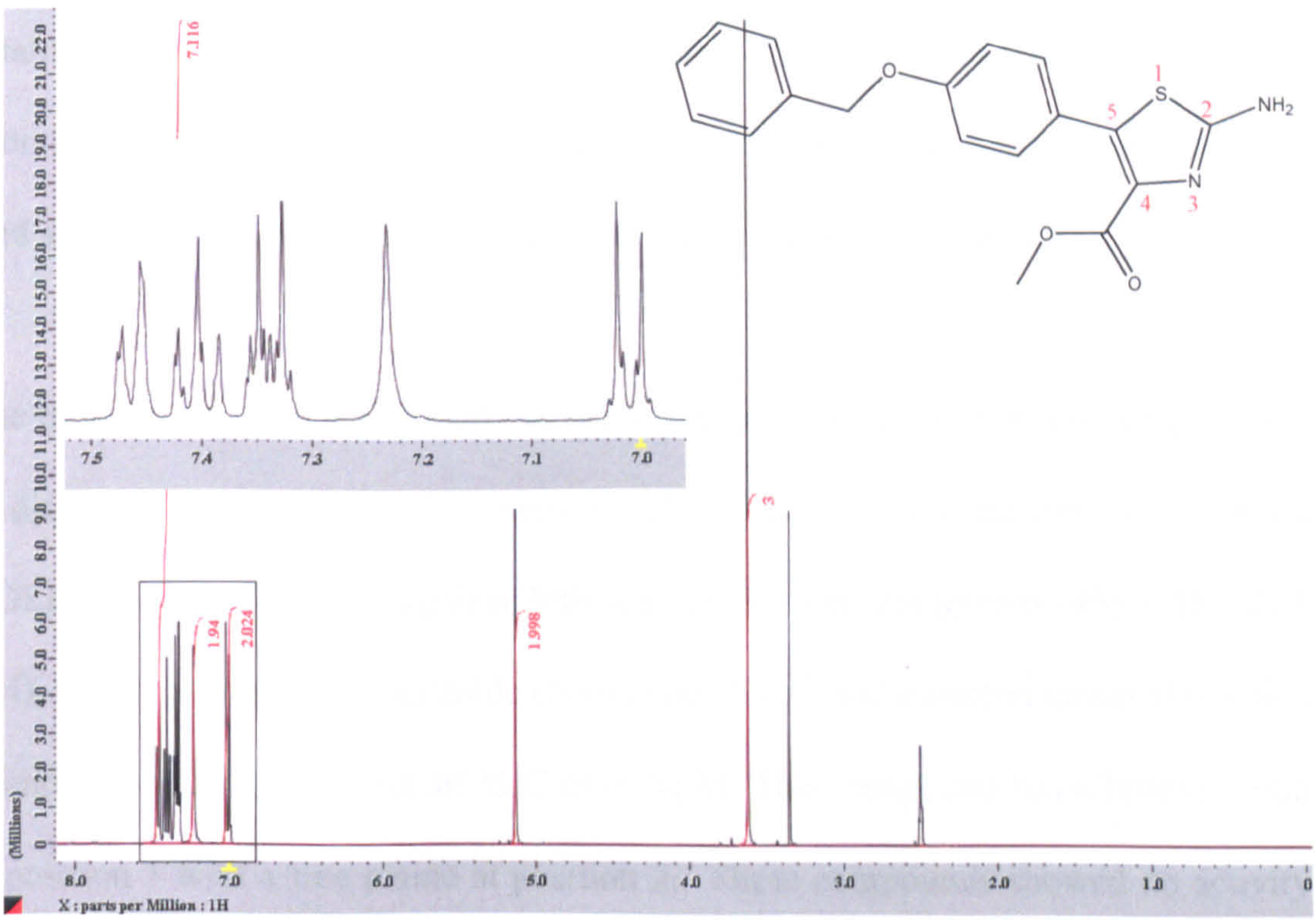


Figure 2.39: ^{1}H spectrum of methyl 2-amino-5-(4-(benzyloxy)phenyl)thiazole-4-carboxylate (111).

2.7 Conclusions and future work

Preliminary molecular modelling studies identified the thiazole ring as a potential lead candidate for inhibiting mtFabH. A series of fragment sized, chemically diverse thiazoles were then synthesized to explore the active site of the enzyme, and tested against mtFabH, *M. aurum* and *M. tuberculosis*. These compounds showed no activity against the enzyme, although compound **(22)** showed very good activity against *M. tuberculosis* with an MIC of 0.35 μM. This led us to investigate the possibility of inhibiting the enzyme by alkylating the active site through a bromoactamido side chain reaction with the sulphydril group at Cys112 residue. Some of the synthesized compounds showed very good activity against the enzyme, but had serious toxicity in mammalian cell lines L929, H9C2 and HS27. Having established mtFabH inhibition was possible with thiazoles, we replaced the bromoacetamido group with other substituents varying in size, property and shape to find a suitable and non-toxic inhibitor of mtFabH and *M. tuberculosis*.

The thiazole ring was substituted extensively at position 2 and 5 by choosing groups of different sizes, shapes and properties with the assistance of the docking software GOLD. Improved activity against Mtb was found with compound **(45)** (MIC 0.21 μM), which possesses heptanamide chain at position 2 and a methyl group at position 5, and compound **(107)** with an MIC of 0.24μM. This compound has a benzyl group at position 5 with a free amine at position 2. These compounds showed no activity against mtFabH, whilst compound **(45)** was weakly active against *M. aurum*.

The esters of the bromoacetamido compounds showed excellent activity against the enzyme compared to the free acids, but had marked toxicity against the mammalian cells, which excludes them as suitable candidates for future drug development against TB.

The best activity against *M. aurum* was demonstrated by compounds substituted with a rigid phenyl group at position 5, and with medium to long acyl side chain (C_6-C_9) at position 2. The esters of these compounds expressed better activity than the free acid forms.

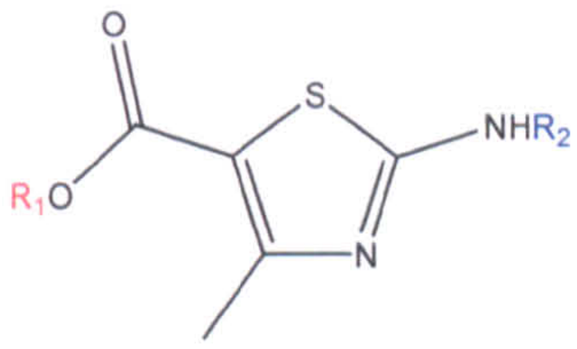
Future work will involve optimizing the three active compounds **(22, 45, 107)** to more drug-like candidates, and trying to establish the target these compounds are inhibiting. Co-crystallization of one of the bromoacetamido derivatives with mtFabH would be the first example of an inhibitor crystallized with this enzyme, and would open the door widely for discovering the secrets of inhibitor binding with this essential enzyme and aid the selection of a non-toxic inhibitor with more accuracy.

Due to the fact that the FabH enzyme is present in all prokaryotic organisms, and some of thiazole derivatives synthesised have shown antimycobacterial activity, these compounds could be tested against other bacterial strains (MRSA, *E. coli*) to investigate the possibility of inhibiting their growth.

The low activity that was expressed by the free acid derivatives compared to their ester counterparts could be attributed to the inability of the free acids ionized at

physiological pH to access the lipophilic cell wall as charged molecules. Consequently, preparing compounds that are pro-drugs as amide or ester derivatives could be a powerful tool to encourage the penetration of pro-drugs these then being activated through hydrolysis by the organism amidases and esterases inside the cell.

A small library of nine compounds that are 5-carboxylate or carboxylic acids was prepared, but due to time constraints, it was not fully finalized and evaluated (Table 2.9). Further derivatives of this group will be prepared in order to investigate the effect on activity of changing the carboxylate from position 4 to 5.



Compound No.	R1	R2
112	$-CH_2CH_3$	$-COCH_2CH_3$
113	H	$-COCH_2CH_3$
114	$-CH_2CH_3$	-CO-Cyclopropyl
115	H	-CO-Cyclopropyl
116	$-CH_2CH_3$	$-COCH_2Br$

Table 2.9: 5-carboxylates and carboxylic acids derivatives of thiazole ring.

Chapter 3: Material and Methods

3.1 General experimental

Melting point determination

Stuart Scientific Melting Point SMP1 apparatus was used in the melting point determination with degrees Celsius (°C) as the unit.

Elemental analysis

Elemental analysis was performed on Perkin Elmer 2400 Analyzer. Standard methods were used to determine C, H, and N simultaneously and halogens separately.

Infrared spectroscopy

Infra red spectra were run on Jasco FT-IR-4200 ATR (Attenuated Total Reflection Mode) and Mattson Genesis Series FT-IR spectrometers with samples compressed with KBr in to disks. Wavenumbers, ν_{max} are expressed as cm^{-1}.

Nuclear magnetic resonance (NMR) spectroscopy

Proton nuclear magnetic resonance (^{1}H NMR) and carbon thirteen (^{13}C NMR) spectra were run on JEOL EX 270 (270MHz), Bruker AMX-400 (400MHz) and JEOL Lambda delta 400 (400MHz) spectrometers. Chemical shifts are stated in parts per million (ppm) and multiplicity indicated as singlet (s), doublet (d), triplet (t), quartet (q), and multiplet (m). Coupling constants (J) are quoted in hertz (Hz) and deuterated solvents used specified for each of the compound.

Mass spectroscopy

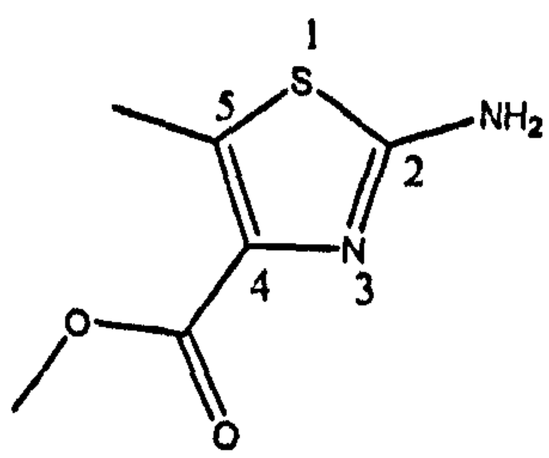
High-resolution mass spectroscopy (ms) was obtained using electron impact (EI), chemical impact (CI), and fast atom bombardment (FAB) ionizations on a JEOL JMS-700 Dual-sector High-resolution Mass Spectrometer. Mass to charge ratio (m/z) and relative abundance are stated for molecular ion radical is $M^{+\cdot}$ (i.e. M plus, dot).

Reagents and solvents

Unless otherwise stated, all reagents and solvents were obtained from commercial sources. Solvents were dried according to standard procedures when deemed necessary.

3.2 Chemical synthesis

3.2.1 Synthesis of methyl 2-amino-5-methylthiazole-4-carboxylate (21) as a general method for the synthesis of 2-amino-5-derivatized 4-carboxylate thiazoles (General procedure A).



Acetaldehyde (1.6g, 36mmol, 1eq) was added to a stirring solution of methyl dichloro acetate (MDA) (5g, 36mmol, 1eq) dissolved in anhydrous ether (150 ml) at 0°C before adding NaOMe (1.65g, 54mmol, 1.5eq) dissolved in anhydrous MeOH (20ml) dropwise over a period of 45 minutes. The mixture was allowed to stir for a

further one hour at 0°C before adding brine (150 ml), extracting the organic phase with ether (150 ml), drying over $MgSO_4$, and reducing *in vacuo*. To the crude residue was added thiourea (1.9 g, 36mmol, 1eq) dissolved in MeOH (100 ml) and the mixture refluxed for four hours before concentrating *in vacuo* and neutralizing with concentrated ammonium hydroxide solution. The mixture was washed with dichloromethane (DCM) (3X, 50ml), dried over anhydrous $MgSO_4$, and purified by re-crystallization using chloroform and methanol (1:3) to give a white powder *methyl 2-amino-5-methylthiazole-4-carboxylate* **(21)** (1.7 g, 38.3%).

M.P: 165-168 °C.

IR: ATR, υ_{max} (cm^{-1}): 3433, NH stretch, amine; 1688, C=O stretch, conjugated ester.

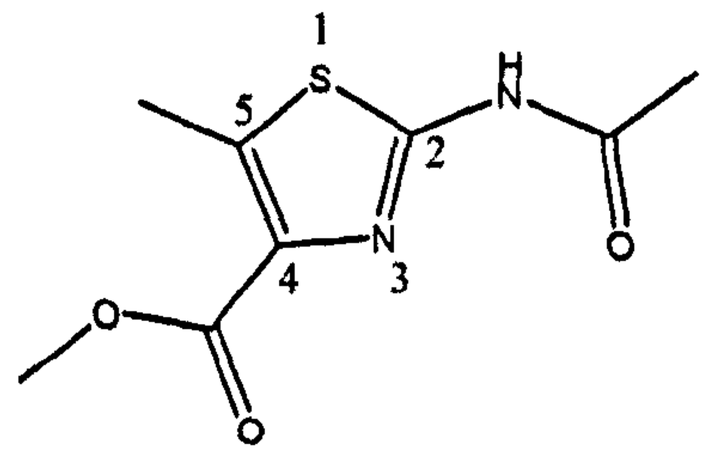
MS: FAB/NOBA, (M+H): 173.8, $C_6H_8N_2O_2S$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

NMR: ^{1}H NMR (270MHz): δ(DMSO-d_6): 2.49 (3H, s, CH_3-Ar); 3.70 (3H, s, OCH_3) ; 6.97 (2H, s, NH_2).

NMR: ^{13}C NMR (270MHz): δ(DMSO-d_6): 12.7 (CH_3Ar); 52.1 (OCH_3); 135.2 (C_4); 137.1 (C_5); 164.3 (CO); 167.5 (C_2).

CHN Analysis: Found: C, 42.08; H, 5.08; N, 15.83; S, 18.51 (Required for $C_6H_8N_2O_2S$: C, 41.85; H, 4.68; N, 16.27; S, 18.62).

3.2.2 Synthesis of methyl 2-acetamido-5-methylthiazole-4-carboxylate (23) as general method for the amidation by acid chlorides at position 2 (General procedure B).



The free amine **(21)** (3.0g, 17.5mmol, 1eq) was added to a stirring solution of anhydrous tetrahydrofuran (THF) (100 ml) and triethylamine (TEA) (3.5g, 35mmol, 2eq) at 0°C before adding acetyl chloride (1.33g, 17.5mmol, 1eq) dropwise over a period of 30 minutes. The mixture was left to stir for a further 30 minutes at 0°C before allowed to warm at room temperature for one hour. THF was reduced *in vacuo* and the crude residue was re-dissolved in a mixture of DCM and water before the pH of the solution was adjusted to 3.0 using 0.1M HCl. The mixture was washed with DCM (3X, 50ml), dried over anhydrous $MgSO_4$, and purified by re-crystallization using chloroform and methanol (1:6) to give a white powder *methyl 2-acetamido-5-methylthiazole-4-carboxylate* (23) (2.3g, 56.4%).

M.P. 233-235 °C.

IR: ATR, υ_{max} (cm^{-1}): 3171, NH stretch, primary amide; 1706, C=O stretch, conjugated ester; 1646, C=O stretch, primary amide.

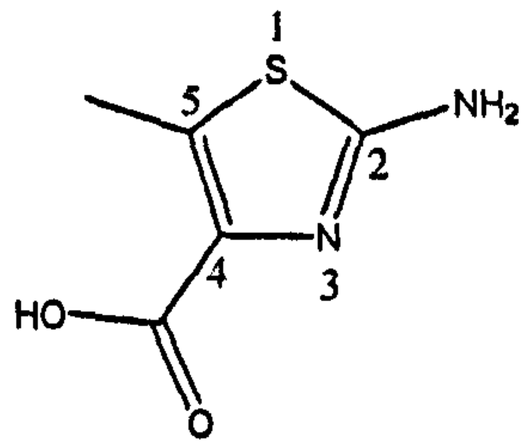
MS: FAB/NOBA, (M+H): 215.4, $C_8H_{10}N_2O_3S$; (m/z 100%): 215.4, $C_8H_{10}N_2O_3S$.

NMR: ^{1}**H NMR** (270MHz): δ($CDCl_3$): 2.16 (3H, s, CH_3CO); 2.67 (3H, s, CH_3Ar); 3.86 (3H, s, OCH_3).

NMR: ^{13}**C NMR** (270MHz): δ($CDCl_3$): 12.8 (**CH_3**Ar); 23.1 (**CH_3**CO); 52.1 (O**CH_3**); 135.0 (C_4); 139.3 (C_5); 155.1 (C_2); 162.9 (**COO**CH_3); 169.0 (**CO**NH).

CHN Analysis: Found: C, 44.90; H, 4.91; N, 13.04; S, 14.85 (Required for $C_8H_{10}N_2O_3S$: C, 44.85; H, 4.70; N, 13.08; S, 14.97).

3.2.3 Synthesis of methyl 2-amino-5-methylthiazole-4-carboxylic acid (22) as a general method for the hydrolysis of 2-amino-5-derivatized 4-carboxylic acid thiazoles (General procedure C).



Compound **(21)** (1.0g, 6.3mmol) was added to a stirring solution of NaOH (150ml, 85mM) at 50-60˚C over a period of 30 minutes before a clear solution formed, cooled and acidified with 1 M HCl to pH 3-4. A precipitate was formed and collected in a Buchner funnel, which was re-crystallized using methanol to give *methyl 2-amino-5-methylthiazole-4-carboxylic acid* (22) (0.8g, 75.0%).

M.P. 320-324 ˚C.

IR: ATR, υ_{max} (cm^{-1}): 3433, NH stretch, amine; 1688, C=O stretch, conjugated ester; 1688, COO stretch, carboxylic acid.

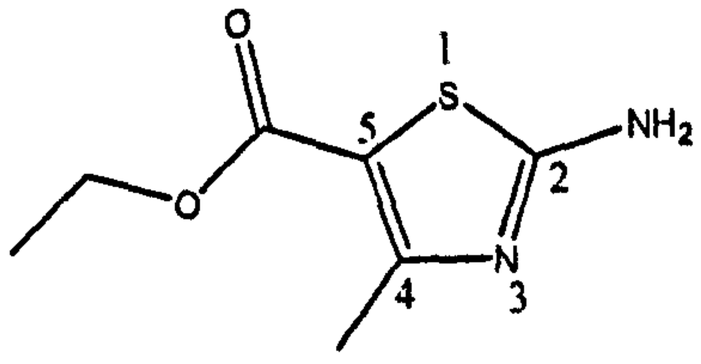
MS: CI/ISO, (M+H): 159.1, $C_5H_6N_2O_2S$; (m/z 100%): 79.0.

NMR: ^{1}**H NMR** (270MHz): δ(DMSO-d_6): 2.49 (3H, s, **C**H_3-Ar); 6.97 (2H, s, **N**H_2).

NMR: ^{13}**C NMR** (270MHz): δ($CDCl_3$): 12.7 (**CH_3**Ar); 135.2 (C_4); 137.1 (C_5); 164.3 (**C**O); 167.5 (C_2).

CHN Analysis: Found: C, 33.71; H, 3.71; N, 15.37; S, 17.17 (Required for $C_5H_6N_2O_2S$: C, 37.97; H, 3.82; N, 17.71; S, 20.27).

3.2.4. Synthesis of ethyl 2-amino-4-methylthiazole-5-carboxylate (25) as a general method for the synthesis of 2-amino-5-carboxylate thiazoles (General procedure D).



Bromine (Br_2) (5g, 27mmol, 1eq) was added to a stirring dispersion of ethyl acetoacetate (3.5g, 27mmol, 1eq) in water at 0°C over a period of 30 minutes. The mixture was allowed to stir for a further one hour at 0°C before extracting the organic phase with ether (100 ml), drying over anhydrous $MgSO_4$, and reducing *in vacuo*. To the crude residue was added thiourea (2g, 27mmol, 1eq) dissolved in EtOH (100 ml) and the mixture refluxed for one hour before ice-water was added and then neutralized with concentrated ammonium hydroxide solution. A precipitate was formed, filtered, and collected through a Buchner funnel and purified by re-crystallization using methanol to give a yellow powder *ethyl 2-amino-4-methylthiazole-5-carboxylate*(*25*) (3.5g, 68.5%).

M.P. 161-163 °C.

IR: ATR, υ_{max} (cm^{-1}): 3366, 3296, asymmetric NH stretch, amine; 1670, C=O stretch, conjugated ester.

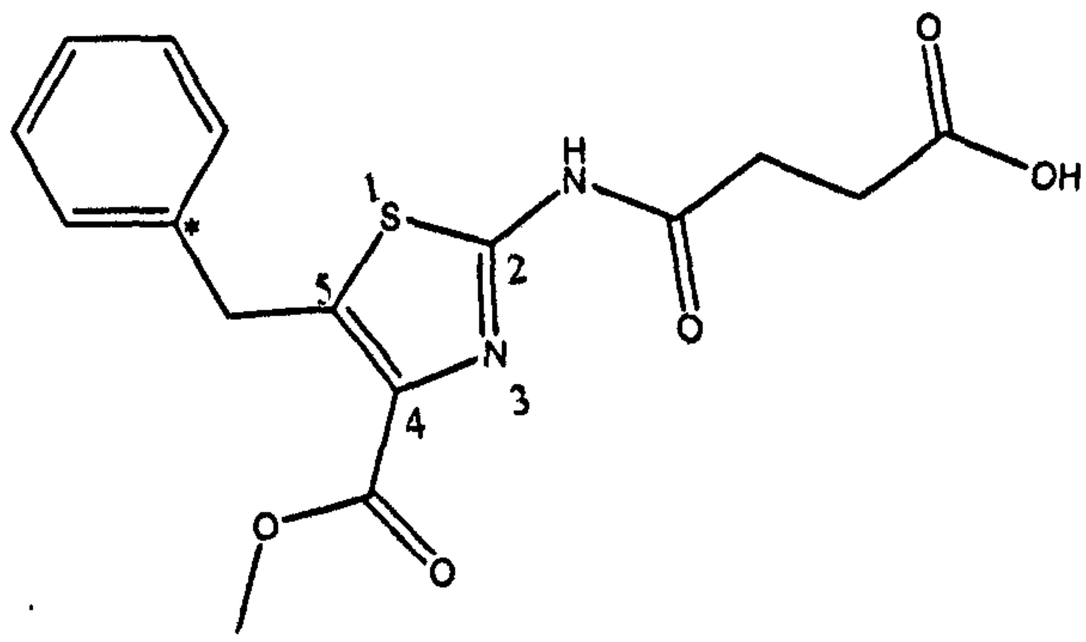
MS: FAB/NOBA, (M+H): 187.7, $C_7H_{10}N_2O_2S$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

NMR: ^{1}H NMR (270MHz): δ(DMSO-d_6): 1.29 (3H, t, J=7.29 Hz, $OCH_2\mathbf{CH_3}$); 2.43 (3H, s, J=7.29 Hz, $\mathbf{CH_3}$-Ar); 4.22 (2H, q, OCH_2CH_3); 4.86 (2H, s, NH_2).

NMR: ^{13}C NMR (270MHz): δ(DMSO-d_6): 14.8 ($\mathbf{CH_3}CH_2CO$); 17.2 ($\mathbf{CH_3}Ar$); 61.6 ($CH_3\mathbf{CH_2}CO$); 115.0 (C_5); 160.2 (C_4); 164.3 ($\mathbf{COO}CH_2CH_3$); 173.1(C_2).

CHN Analysis: Found: C, 45.21; H, 5.28; N, 14.57; S, 17.44 (Required for $C_7H_{10}N_2O_2S$: C, 45.15; H, 5.41; N, 15.04; S, 17.22).

3.2.5 Synthesis of 4-(5-benzyl-4-(methoxycarbonyl)thiazol-2-ylamino)-4-oxobutanoic acid (73) as a general method for the amidation by acid anhydrides at position 2 (General procedure E).



The free amine **(21)** (3.0g, 17.5mmol, 1eq) was added to a stirring solution of anhydrous THF (100 ml) at room temperature before adding succinic anhydride (1.75g, 17.5mmol, 1eq) dissolved in dry THF dropwise over a period of 30 minutes. The mixture was left to stir for a further one hour. THF was reduced *in vacuo* and the crude residue was re-dissolved in a mixture of chloroform and water before the pH of the solution was adjusted to 3.0 using 0.1M HCl. The mixture was washed with chloroform (3X 50ml), dried over anhydrous $MgSO_4$, and purified by re-crystallization using methanol to give pale yellow powder *4-(5-benzyl-4-(methoxycarbonyl)thiazol-2-ylamino)-4-oxobutanoic acid* (73) (2.70g, 45.4%).

M.P. 216-218 °C.

IR: ATR, υ_{max} (cm^{-1}): 3184, NH stretch, primary amide; 1721, C=O stretch, conjugated ester; 1701, carboxylic acid; 1691, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 349.0, $C_{16}H_{16}N_2O_5S$; (m/z 100%): 63.0.

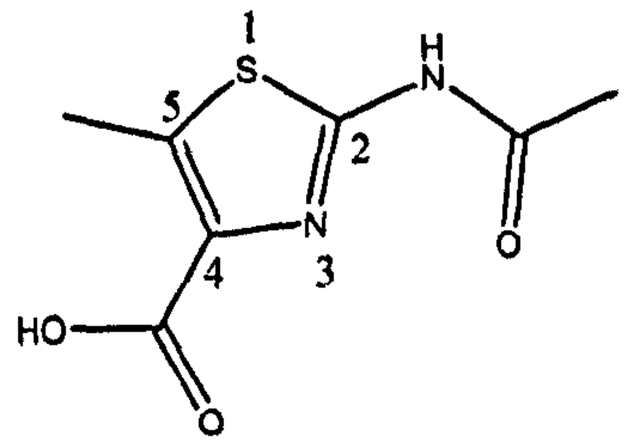
NMR: ^{1}H NMR (270MHz): δ(DMSO-d_6): 2.48-2.5 (2H, m, OCC**H**$_2$CH$_2$COOH); 2.52-2.61 (2H, m, OCCH$_2$C**H**$_2$COOH); 3.80 (3H, s, OC**H**$_3$); 4.45 (2H, s, C**H**$_2$-Ar); 7.20-7.34 (5H, m, Ar); 12.40 (1H, s, O**H**); 12.6 (1H, s, N**H**).

NMR: ^{13}C NMR (400MHz): δ(DMSO-d_6): 28.8 (Ar-CH$_2$-); 30.3 (NHCOCH$_2$CH$_2$COOH); 32.5 (NHCOCH$_2$**C**H$_2$COOH); 52.2 (O**C**H$_3$); 127.2–129.2 (5C, Ar); 135.1 (C_4);140.2 (C_5); 142.4 (C^*); 155.1 (C_2); 163.0 (**C**OOCH$_3$); 171.4 (**C**OOH), 174.0 (**C**ONH).

CHN Analysis: Found: C, 54.98; H, 4.63; N, 7.80; S, 9.17 (Required for $C_{16}H_{16}N_2O_5S$: C, 55.16; H, 4.63; N, 8.04; S, 9.20).

2-Acetamido-5-methylthiazole-4-carboxylic acid (24)

The title compound was obtained as a white powder (0.5g, 53.2%) using general procedure C.



M.P. 275-277 °C.

IR: ATR, υ_{max} (cm^{-1}): 3171, NH stretch, primary amide; 1661, C=O stretch, carboxylic acid; 1646, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 201.2, $C_7H_8N_2O_3S$; (m/z 100%): 201.2, $C_7H_8N_2O_3S$.

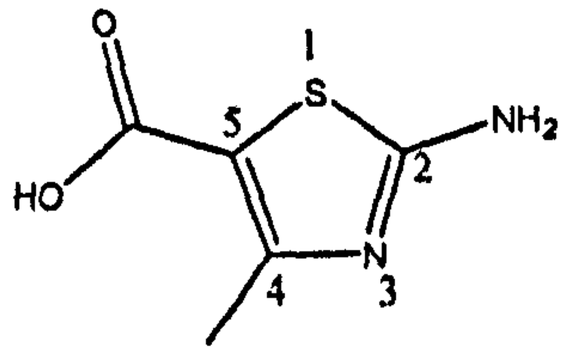
NMR: ^{1}H NMR (400MHz): δ($CDCl_3$): 2.11 (3H, s, CH_3CO); 2.59 (3H, s, CH_3Ar).

NMR: ^{13}C NMR (400MHz): δ($CDCl_3$): 12.6 (CH_3Ar); 22.8 (CH_3CO); 136.6 (C_4); 137.1 (C_5); 153.7 (C_2); 164.1 (COOH); 169.2 (CONH).

CHN Analysis: Found: C, 40.50; H, 4.39; N, 14.20; S, 15.85 (Required for $C_7H_8N_2O_3S$: C, 41.99; H, 4.03; N, 13.99; S, 16.02).

2-Amino-4-methylthiazole-5-carboxylic acid (26)

The title compound was obtained as a pale yellow powder (0.5g, 54.1%) using general procedure C.



M.P. 194-196 °C.

IR: KBr disk, υ_{max} (cm^{-1}): 3257, NH stretch, primary amine; 1668, C=O stretch, carboxylic acid.

MS: CI/ISO, (M+H): 159.14, $C_5H_6N_2O_2S$; (M+H 100%): 79.09.

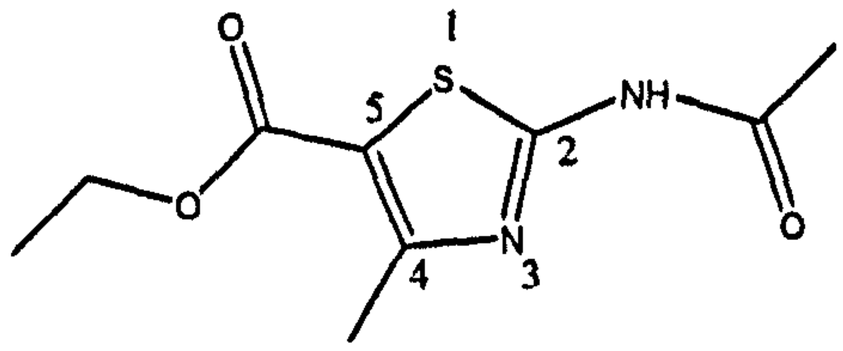
NMR: ^{1}H NMR (400MHz): δ(DMSO-d_6): 2.50 (3H, s, CH_3-Ar); 7.61 (2H, s, NH_2).

NMR: ^{13}C NMR (400MHz): δ(DMSO-d_6): 17.5 (CH_3Ar); 109.2 (C_5); 158.9 (C_4); 164.0 (COOH); 170.4(C_2).

CHN Analysis: Found: C, 38.37; H, 3.55; N, 17.62; S, 20.37 (Required for $C_5H_6N_2O_2S$: C, 37.97; H, 3.82; N, 17.71; S, 20.27).

Ethyl 2-acetamido-4-methylthiazole-5-carboxylate (27)

The title compound was obtained as a brown powder (1.8g, 30.0%) using general procedure **B**.



M.P. 222-225 °C.

IR: ATR, υ_{max} (cm^{-1}): 3168, NH stretch, primary amide; 1706, C=O stretch, conjugated ester; 1661, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 229.37, $C_9H_{12}N_2O_3S$; (m/z 100%): 229.37, $C_9H_{12}N_2O_3S$.

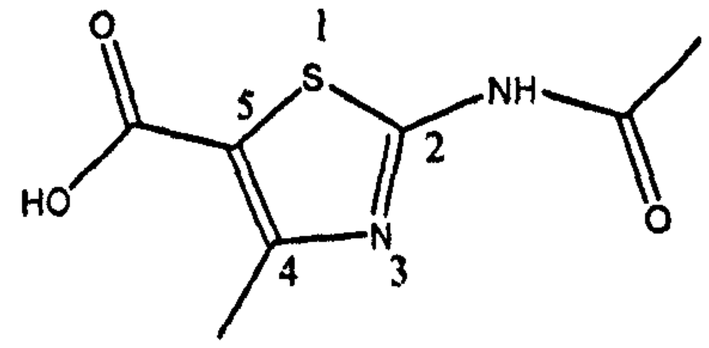
NMR: ^{1}H NMR (400MHz): δ(DMSO-d_6): 1.28 (3H, t, J=7.08 Hz, OCH_2CH_3); 2.1 (3H, s, O=CCH_3) ; 2.50 (3H, s, CH_3-Ar); 4.24 (2H, q, J=7.04 Hz, OCH_2CH_3).

NMR: ^{13}C NMR (400MHz): δ(DMSO-d_6): 14.7 (CH_3CH_2OCO); 17.5 (CH_3Ar); 23.0 ($COCH_3$); 60.9 (CH_3CH_2OCO); 114.3 (C_5); 156.6 (C_4); 160.1 (C_2); 162.6 ($COOCH_2CH_3$); 169.7 (CONH).

CHN Analysis: Found: C, 47.24; H, 5.41; N, 12.32; S, 14.32 (Required for $C_9H_{12}N_2O_3S$: C, 47.35; H, 5.30; N, 12.27; S, 14.05).

2-Acetamido-4-methylthiazole-5-carboxylic acid (28)

The title compound was obtained as an off white powder (0.4g, 42.1%) using general procedure **C**.



M.P. 320-322 °C.

IR: KBr disk, υ_{max} (cm^{-1}): 3169, NH stretch, primary amide; 1696, C=O stretch, carboxylic acid; 1661, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 201.5, $C_7H_8N_2O_3S$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

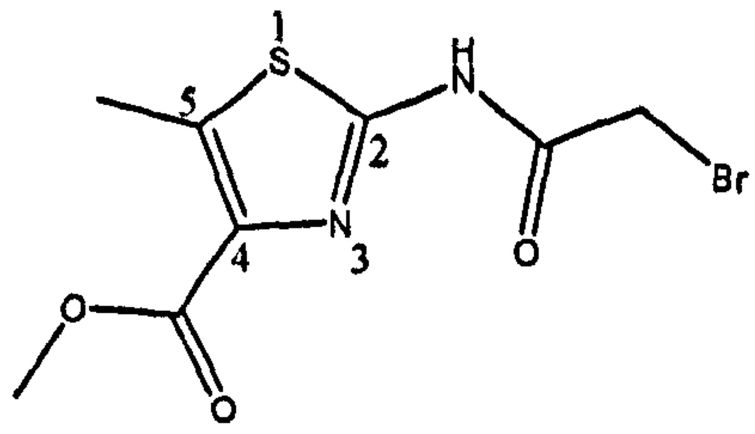
NMR: ^{1}H NMR (400MHz): δ(DMSO-d_6): 2.1 (3H, s, O=C**CH$_3$**) ; 2.50 (3H, s, **CH$_3$**-Ar).

NMR: ^{13}C NMR (400MHz): δ(DMSO-d_6): 17.4 (**C**H$_3$Ar); 23.0 (COCH$_3$); 115.6 (C_5); 155.8 (C_4); 159.7 (C_2); 164.2 (**C**OOH); 169.6 (**C**ONH).

CHN Analysis: Found: C, 41.60; H, 4.31; N, 14.15; S, 15.95 (Required for $C_7H_8N_2O_3S$: C, 41.99; H, 4.03; N, 13.99; S, 16.02).

Methyl 2-(2-bromoacetamido)-5-methylthiazole-4-carboxylate (29)

The title compound was obtained as an off-white powder (2.6g, 53.7%) using general procedure **B**.



M.P. 220-222 °C.

IR: ATR, υ_{max} (cm^{-1}): 3235, NH stretch, primary amide; 1717, C=O stretch, conjugated ester; 1699, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 295.0, $C_8H_9N_2O_3SBr$; (m/z 100%): 78.0.

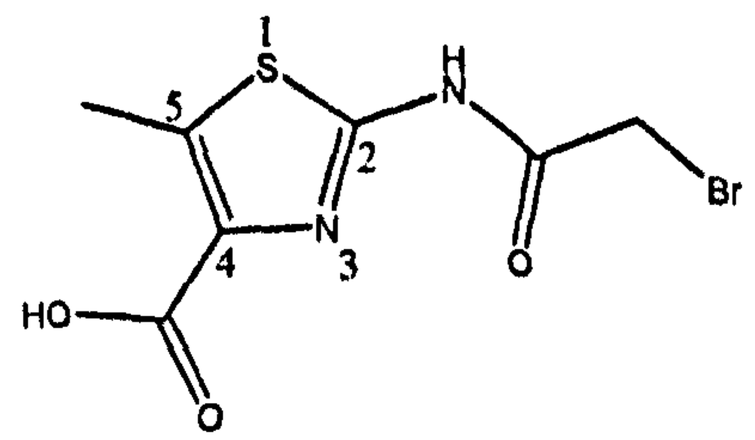
NMR: ^{1}H NMR (400MHz): δ(DMSO-d_6): 2.62 (3H, s, **C**$\mathbf{H_3}$-Ar); 3.79 (3H, s, OC$\mathbf{H_3}$) ; 4.12 (2H, s, $\mathbf{CH_2}$-Br); 12.8 (1H, s, **NH**).

NMR: ^{13}C NMR (400MHz): δ(DMSO-d_6): 12.66 (**C**H3-Ar); 28.7 ($COCH_2Br$); 52.19 (OCH_3); 135.9 (C_4); 138.7 (C_5); 153.7 (C_2); 163.0 (**C**$OOCH_3$); 166.0 (**C**ONH).

CHN Analysis: Found: C, 32.92; H, 2.90; N, 9.36; S, 10.49; Br, 27.17 (Required for $C_8H_9BrN_2O_3S$: C, 32.78; H, 3.09; N, 9.56; S, 10.94; Br, 27.26).

2-(2-Bromoacetamido)-5-methylthiazole-4-carboxylic acid (30)

The title compound was obtained as an off-white powder (0.7g, 73.5%) using general procedure **C**.



M.P. 218-220 °C.

IR: ATR, υ_{max} (cm^{-1}): 3185, NH stretch, primary amide; 1699, C=O stretch, carboxylic acid; 1662, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 279.0, $C_7H_7N_2O_3SBr$; (m/z 100%): 79.0.

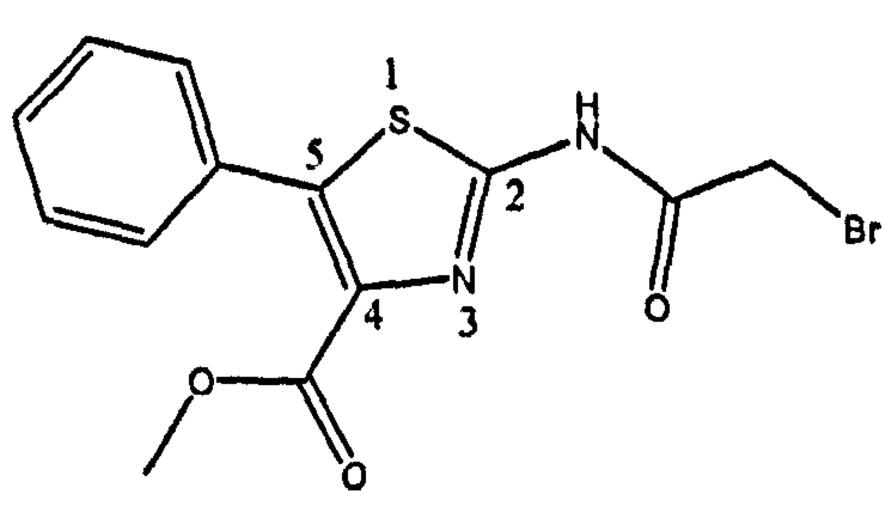
NMR: ^{1}H NMR (400MHz): δ(DMSO-d_6): 2.65 (3H, s, CH_3-Ar); 4.11 (2H, s, CH_2-Br); 12.8 (1H, s, NH).

NMR: ^{13}C NMR (400MHz): δ(DMSO-d_6): 12.77 (CH3-Ar); 28.82 ($COCH_2Br$); 137.0 (C_4); 138.5 (C_5); 153.0 (C_2); 164.0 (COOH); 166.0 (CONH).

CHN Analysis: Found: C, 30.28; H, 1.99; N, 9.98; S, 14.03; Br, 28.55 (Required for $C_7H_7BrN_2O_3S$: C, 30.12; H, 2.53; N, 10.04; S, 11.49; Br, 28.63).

Methyl 2-(2-bromoacetamido)-5-phenylthiazole-4-carboxylate (31)

The title compound was obtained as a brown powder (0.8g, 47.9%) using general procedure B.



M.P. 218-220 °C.

IR: ATR, υ_{max} (cm^{-1}): 3230, NH stretch, primary amide; 1697, C=O stretch, conjugated ester; 1697, C=O stretch, primary amide.

MS: FAB/NOBA, $(M+H)^+$: 357.1, $C_{13}H_{11}BrN_2O_3S$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

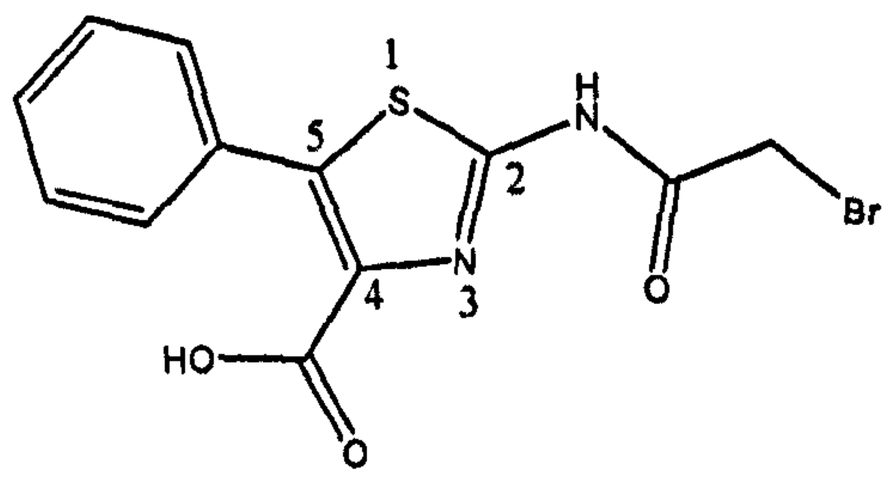
NMR: ^{1}H NMR (270MHz): δ(DMSO-d_6): 3.69 (3H, s, OCH_3) ; 4.16 (2H, s, CH_2-Br); 7.48-7.55 (5H, m, Ar) ; 13.08 (1H, s, NH).

NMR: ^{13}**C NMR** (400MHz): δ(DMSO-d_6): 28.6 ($COCH_2Br$); 52.3 ($COOCH_3$); 128.9-130.5 (6C, Ar); 135.1 (C_4); 139.5 (C_5); 155.6 (C_2); 162.6 ($COOCH_3$); 166.4 (CONH).

CHN Analysis: Found: C, 44.12; H, 3.05; N, 7.96; S, 11.55; Br, 22.84 (Required for $C_{13}H_{11}BrN_2O_3S$: C, 43.96; H, 3.12; N, 7.89; S, 9.03; Br, 22.50).

2-(2-Bromoacetamido)-5-phenylthiazole-4-carboxylic acid (32)

The title compound was obtained as a brown powder (0.1g, 18.6%) using general procedure C.



M.P. 208-210 °C.

IR: ATR, υ_{max} (cm^{-1}): 3216, NH stretch, primary amide; 1675, C=O stretch, carboxylic acid; 1675, C=O stretch, primary amide.

MS: FAB/NOBA, (M+2): 343.0, $C_{12}H_9BrN_2O_3S$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

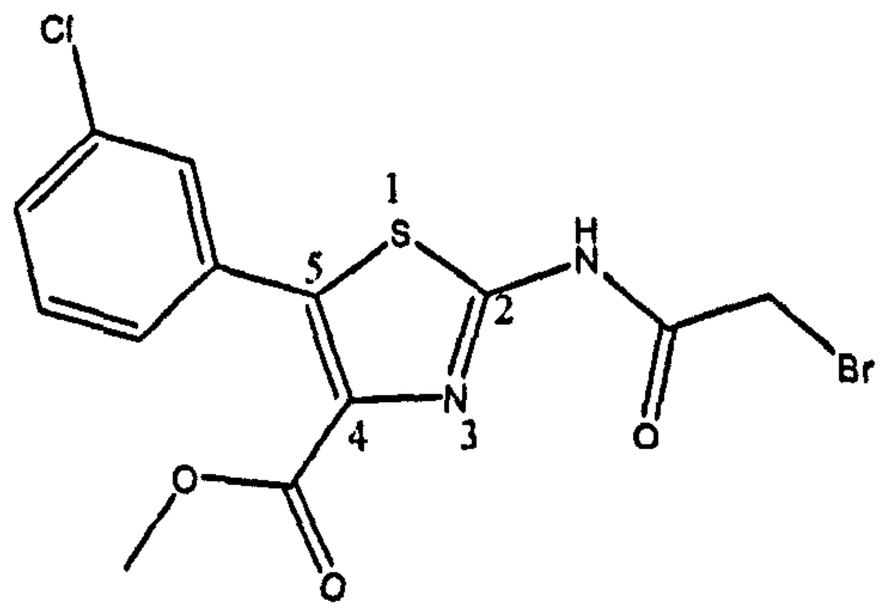
NMR: ^{1}**H NMR** (400MHz): δ(DMSO-d_6): 4.16 (2H, s, CH_2-Br); 7.41-7.50 (5H, m, Ar).

NMR: ^{13}**C NMR** (400MHz): δ(DMSO-d_6): 28.7 ($COCH_2Br$); 128.8-130.3 (6C, Ar); 135.0 (C_4); 138.0 (C_5); 155.0 (C_2); 164.0 (COOH); 167.0 (CONH).

CHN Analysis: Found: C, 42.85; H, 2.35; N, 8.31; S, 9.88; Br, 22.01 (Required for $C_{12}H_9BrN_2O_3S$: C, 42.24; H, 2.66; N, 8.21; S, 9.40; Br, 23.42).

Methyl 2-(2-bromoacetamido)-5-(3-chlorophenyl)thiazole-4-carboxylate (33)

The title compound was obtained as a white powder (1.4g, 33.1%) using general procedure B.



M.P. 218-220 °C.

IR: ATR, υ_{max} (cm^{-1}): 3216, NH stretch, primary amide; 1699, C=O stretch, conjugated ester; 1684, C=O stretch, primary amide.

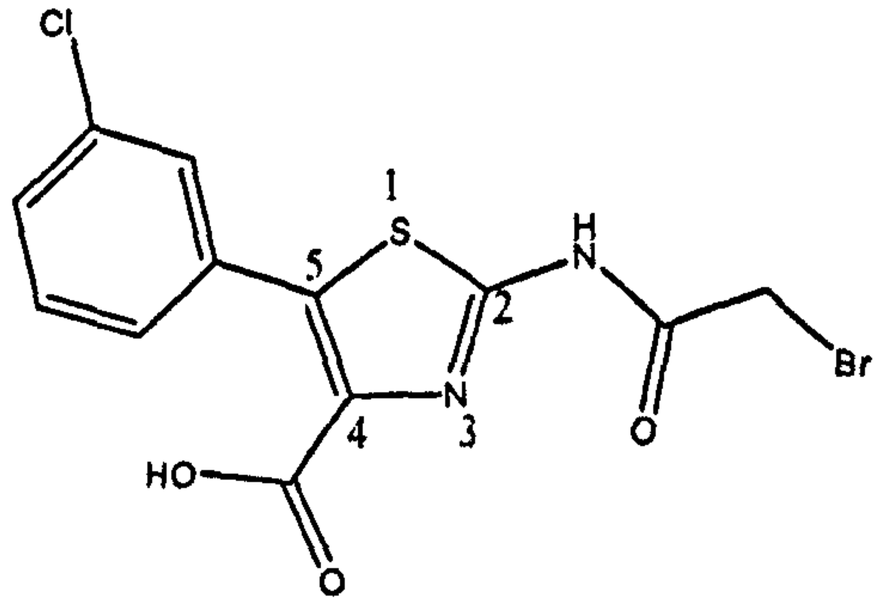
NMR: ^{1}H NMR (400MHz): δ($CDCl_3$): 3.80 (3H, s, OC**H**$_3$) ; 4.11 (2H, s, C**H**$_2$-Br); 7.36-7.39 (3H, m, Ar); 7.47 (1H, s, Ar).

NMR: ^{13}C NMR (400MHz): δ($CDCl_3$): 27.7 ($COCH_2Br$); 52.2 ($COOCH_3$); 128.4-131.7 (6C, Ar); 134.2 (C_4); 139.0 (C_5); 156.0 (C_2); 162.3 ($COOCH_3$); 164.9 (CONH).

CHN Analysis: Found: C, 40.11; H, 2.51; N, 7.45; S, 8.09 (Required for $C_{13}H_{10}N_2O_3SClBr$: C, 40.07; H, 2.59; N, 7.19; S, 8.23).

2-(2-Aromoacetamido)-5-(3-chlorophenyl)thiazole-4-carboxylic acid (34)

The title compound was obtained as a white powder (0.3g, 37.2%) using general procedure C.



M.P. 228-230 °C.

IR: ATR, υ_{max} (cm^{-1}): 3180, NH stretch, primary amide; 1679, C=O stretch, carboxylic acid; 1679, C=O stretch, primary amide.

MS: FAB/NOBA, (M+2): 377.1, $C_{12}H_8N_2O_3S$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

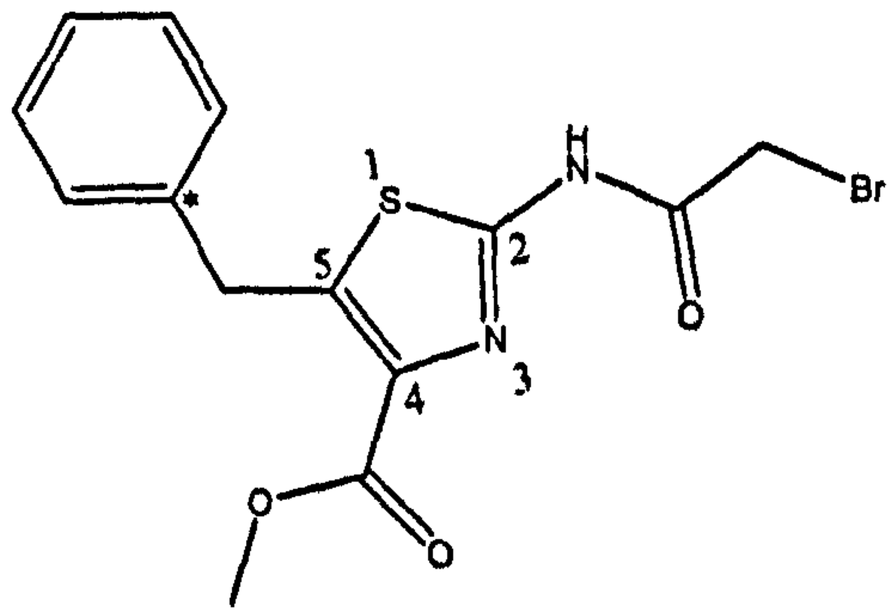
NMR: ^{1}H NMR (400MHz): δ(DMSO-d_6): 4.17 (2H, s, CH_2-Br); 7.46-7.48 (3H, m, Ar); 7.61 (1H, s, Ar).

NMR: ^{13}C NMR (400MHz): δ($CDCl_3$): 28.6 ($COCH_2Br$); 129.1-133.3 (6C, Ar); 136.7 (C_4); 137.2 (C_5); 155.7 (C_2); 163.4 (COOH); 166.4 (CONH).

CHN Analysis: Found: C, 40.57; H, 3.09; N, 7.95; S, 9.24 (Required for $C_{12}H_8N_2O_3S$: C, 38.37; H, 2.15; N, 7.46; S, 8.54).

Methyl 5-benzyl-2-(2-bromoacetamido)thiazole-4-carboxylate (35)

The title compound was obtained as a brown powder (1.5g, 34.5%) using general procedure **B**.



M.P. 218-220 °C.

IR: ATR, υ_{max} (cm^{-1}): 3231, NH stretch, primary amide; 1701, C=O stretch, conjugated ester; 1701, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 371.0, $C_{14}H_{13}N_2O_3SBr$; (m/z 100%): 78.0.

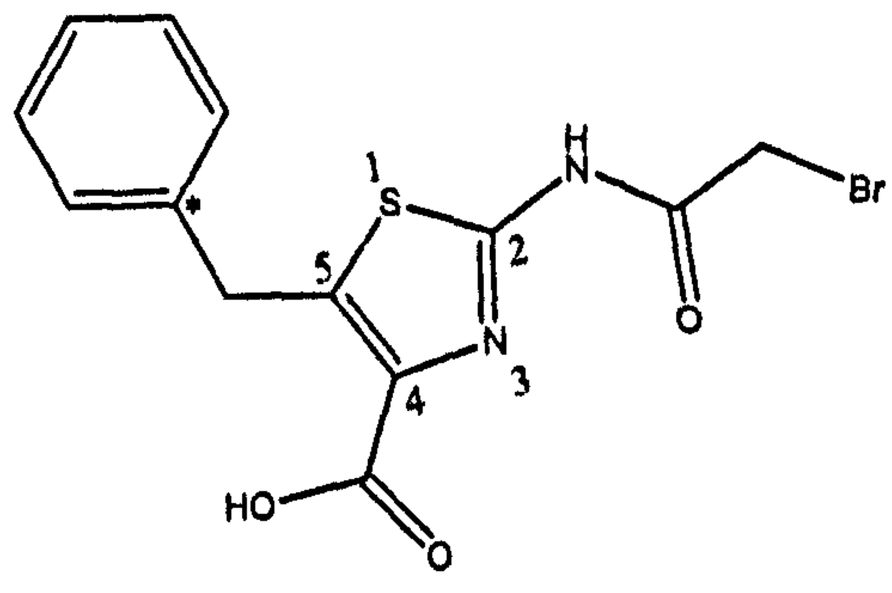
NMR: ^{1}H NMR (270MHz): δ($CDCl_3$): 3.96 (3H, s, OCH_3) ; 4.05 (2H, s, CH_2-Br); 4.54 (2H, s, CH_2-Ar); 7.25-7.32 (5H, m, Ar); 9.90 (1H, s, NH).

NMR: ^{13}C NMR (400MHz): δ(DMSO-d_6): 28.6 ($COCH_2Br$); 32.5 (Ar-CH_2-); 52.3 (OCH_3); 127.3–129.2 (5C, Ar); 135.5 (C_4); 140.5 (C_5); 143.2 (C^*); 154.7 (C_2); 163.5 ($COOCH_3$); 166.0 (CONH).

CHN Analysis: Found: C, 45.58; H, 3.41; N, 7.57; S, 8.75; Br, 21.46 (Required for $C_{14}H_{13}N_2O_3SBr$: C, 45.54; H, 3.55; N, 7.59; S, 8.68; Br, 21.64).

5-Benzyl-2-(2-bromoacetamido)thiazole-4-carboxylic acid (36)

The title compound was obtained as a brown powder (0.3g, 33.3%) using general procedure **C**.



M.P. 220-222 °C.

IR: ATR, υ_{max} (cm^{-1}): 3185, NH stretch, primary amide; 1695, C=O stretch, carboxylic acid; 1661, C=O stretch, primary amide.

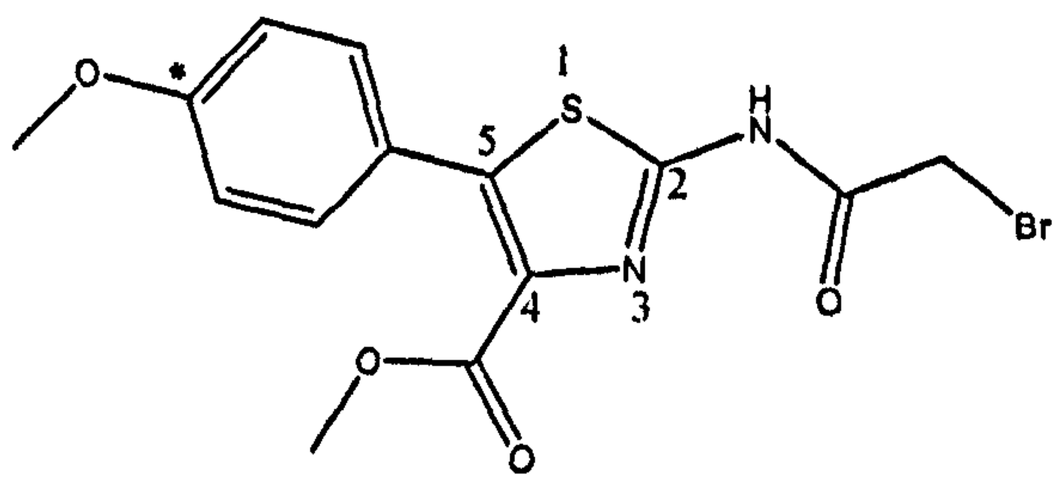
NMR: ^{1}H NMR (400MHz): δ(DMSO-d_6): 4.10 (2H, s, CH_2-Br); 4.49 (2H, s, CH_2-Ar); 7.22-7.32 (5H, m, Ar); 12.80 (1H, s, OH).

NMR: ^{13}**C NMR** (400MHz): δ(DMSO-d_6): 28.7 (COCH$_2$Br); 32.5 (Ar-CH$_2$-); 127.2–129.2 (5C, Ar); 136.8 (C_4);140.3 (C_5); 142.4 (C*); 154.3 (C_2); 164.0 (COOH); 166.0 (CONH).

CHN Analysis: Found: C, 44.50; H, 3.15; N, 7.93; S, 9.46; Br, 22.37 (Required for $C_{13}H_{11}N_2O_3SBr$: C, 43.96; H, 3.12; N, 7.89; S, 9.03; Br, 22.37).

Methyl 2-(2-bromoacetamido)-5-(4-methoxyphenyl)thiazole-4-carboxylate (37)

The title compound was obtained as a white powder (1.7g, 38.0%) using general procedure B.



M.P. 210-212 °C.

IR: ATR, υ_{max} (cm^{-1}): 3261, NH stretch, primary amide; 1705, C=O stretch, conjugated ester; 1684, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 385.1, $C_{14}H_{13}BrN_2O_4S$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

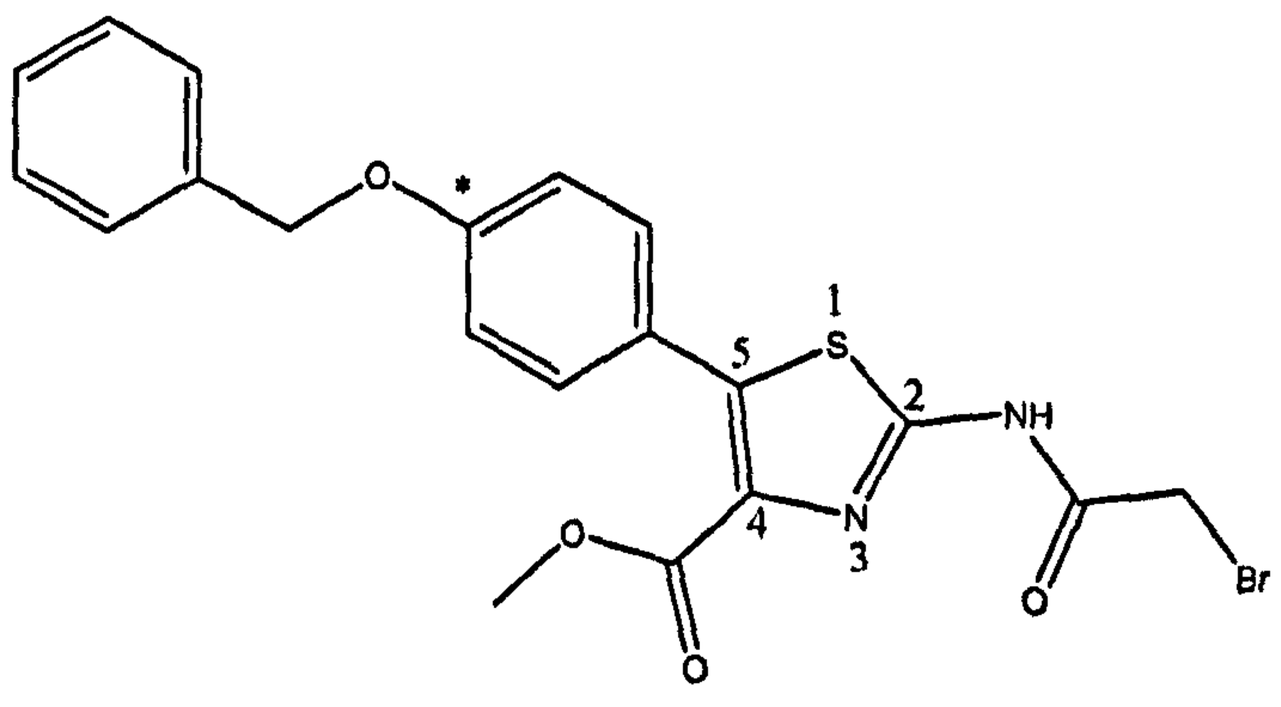
NMR: ^{1}**H NMR** (270MHz): δ(DMSO-d_6): 3.75 (3H, s, OC**H$_3$**) ; 3.80 (3H, s, Ar-OC**H$_3$**); 4.15 (2H, s, C**H$_2$**-Br); 7.00 (2H, d, J=6.48 Hz, Ar); 7.46 (2H, d, J=8.64 Hz, Ar); 12.98 (1H, s, N**H**).

NMR: ^{13}**C NMR** (400MHz): δ(CDCl$_3$): 28.6 (COCH$_2$Br); 52.3 (COOCH$_3$); 128.8-134.2 (5C, Ar); 136.0 (C_4); 139.0 (C_5); 155.8 (C_2); 162.0 (**C***, Ar); 162.1 (COOCH$_3$); 166.4 (**C**ONH).

CHN Analysis: Found: C, 43.40; H, 3.36; N, 7.12; S, 8.01; Br, 21.16 (Required for $C_{14}H_{13}BrN_2O_4S$: C, 43.65; H, 3.40; N, 7.27; S, 8.32; Br, 20.74).

Methyl 5-(4-(benzyloxy)phenyl)-2-(2-bromoacetamido)thiazole-4-carboxylate (38)

The title compound was obtained as a white powder (0.6g, 14.8%) using general procedure B.



M.P. 222-224 °C.

IR: ATR, υ_{max} (cm^{-1}): 3231, NH stretch, primary amide; 1705, C=O stretch, conjugated ester; 1705, C=O stretch, primary amide.

MS: FAB/NOBA, (M+2): 463.1, $C_{20}H_{17}BrN_2O_4S$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

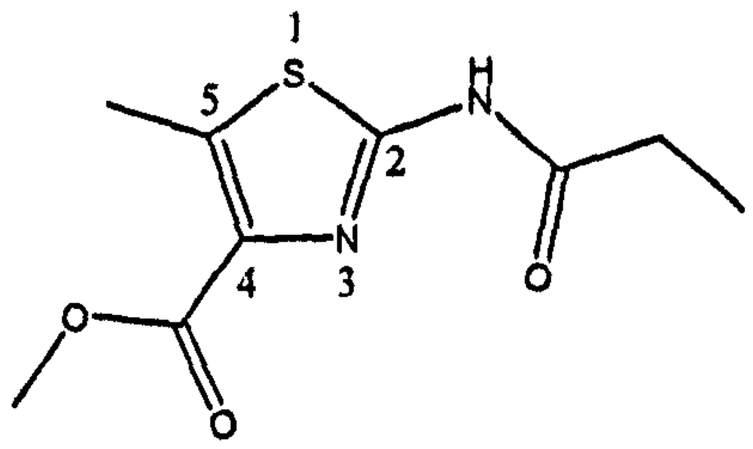
NMR: ^{1}H NMR (270MHz): δ(DMSO-d_6): 3.68 (3H, s, OCH_3) ; 4.15 (2H, s, CH_2-Br); 5.2 (2H, s, OCH_2-Ar); 7.05 (2H, d, Ar); 7.33-7.46 (7H, m, Ar); 12.96 (1H, s, NH).

NMR: ^{13}C NMR (400MHz): δ(DMSO-d_6): 28.6 ($COCH_2Br$); 52.2 ($COOCH_3$); 69.9 (Ar-CH_2OAr); 115.1-132.0 (11C); 137.4 (C_4); 139.9 (C_5); 160.0 (C^*); 160.3 (C_2); 162.7 ($COOCH_3$); 166.3 (CONH).

CHN Analysis: Found: C, 51.30; H, 3.54; N, 5.88; S, 6.72; Br, 18.77 (Required for $C_{20}H_{17}BrN_2O_4S$: C, 52.07; H, 3.71; N, 6.07; S, 6.95; Br, 17.32).

Methyl 5-methyl-2-propionamidothiazole-4-carboxylate (39)

The title compound was obtained as a white powder (2.2g, 56.4%) using general procedure B.



M.P. 229-231 °C.

IR: ATR, υ_{max} (cm^{-1}): 3267, NH stretch, primary amide; 1719, C=O stretch, conjugated ester; 1685, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 229.4, $C_9H_{12}N_2O_3S$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

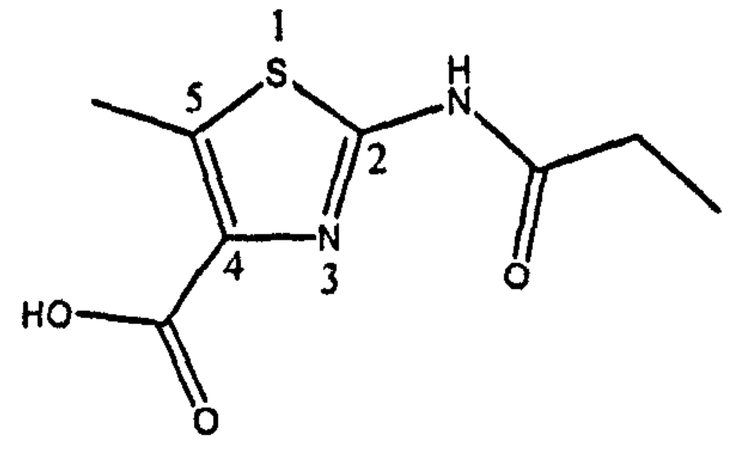
NMR: ^{1}H NMR (270MHz): δ($CDCl_3$): 1.22 (3H, t, J=7.56 Hz,$\mathbf{CH_3}CH_2$-); 2.43 (2H, q, J=7.56 Hz,$CH_3\mathbf{CH_2}$-); 2.7 (3H, s, $\mathbf{CH_3}Ar$); 3.89 (3H, s, OCH_3).

NMR: ^{13}C NMR (270MHz): δ($CDCl_3$): 9.1 ($\mathbf{CH_3}CH_2$-); 12.7 ($\mathbf{CH_3}Ar$); 29.4 ($CH_3\mathbf{CH_2}$-); 52.1 ($O\mathbf{CH_3}$); 135.1 (C_4); 139.4 (C_5); 154.2 (C_2); 162.9 ($\mathbf{C}OOCH_3$); 172.0 ($\mathbf{C}ONH$).

CHN Analysis: Found: C, 47.05; H, 5.32; N, 12.24; S, 13.94 (Required for $C_9H_{12}N_2O_3S$: C, 47.35; H, 5.30; N, 12.27; S, 14.05).

5-Methyl-2-propionamidothiazole-4-carboxylic acid (40)

The title compound was obtained as a white powder (0.4g, 42.4%) using general procedure C.



M.P. 286-288 °C.

IR: ATR, υ_{max} (cm^{-1}): 3188, NH stretch, primary amide; 1690, C=O stretch, carboxylic acid; 1660, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 215.4, $C_8H_{10}N_2O_3S$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

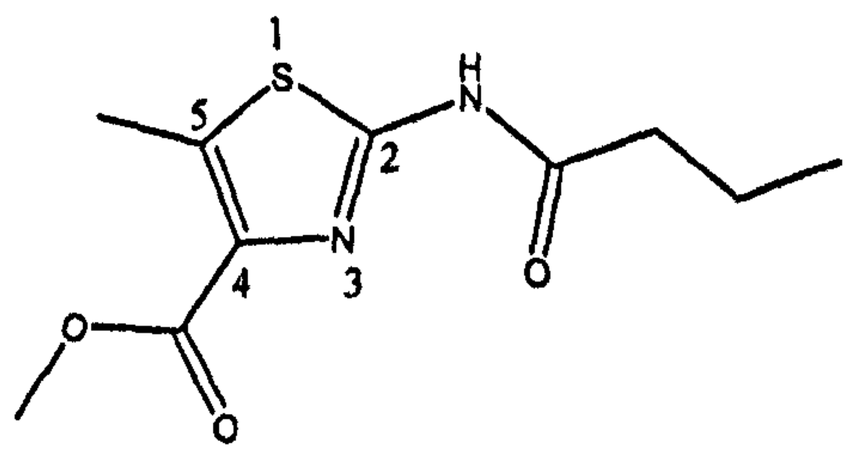
NMR: ^{1}H NMR (270MHz): δ(DMSO-d_6): 1.06 (3H, t, J=7.56 Hz, **C**H_3CH_2-); 2.35 (2H, q, J=7.56 Hz, CH_3**C**H_2-); 2.58 (3H, s, **C**H_3Ar); 12.21 (1H, s, O**H**).

NMR: ^{13}C NMR (270MHz): δ($CDCl_3$): 9.0 (**C**H_3CH_2-); 12.1 (**C**H_3Ar); 28.1 (CH_3**C**H_2-); 136.1 (C_4); 136.4 (C_5); 153.2 (C_2); 163.6 (**C**OOH); 172.3 (**C**ONH).

CHN Analysis: Found: C, 44.70; H, 4.68; N, 13.01; S, 14.88 (Required for $C_8H_{10}N_2O_3S$: C, 44.85; H, 4.70; N, 13.08; S, 14.97).

Methyl 2-butyramido-5-methylthiazole-4-carboxylate (41)

The title compound was obtained as a white powder (1.0g, 24.1%) using general procedure B.



M.P. 147-149 °C.

IR: ATR, υ_{max} (cm^{-1}): 3275, NH stretch, primary amide; 1718, C=O stretch, conjugated ester; 1687, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 243.3, $C_{10}H_{14}N_2O_3S$; (m/z 100%): 265.2, $C_{10}H_{14}N_2O_3S$ + Na.

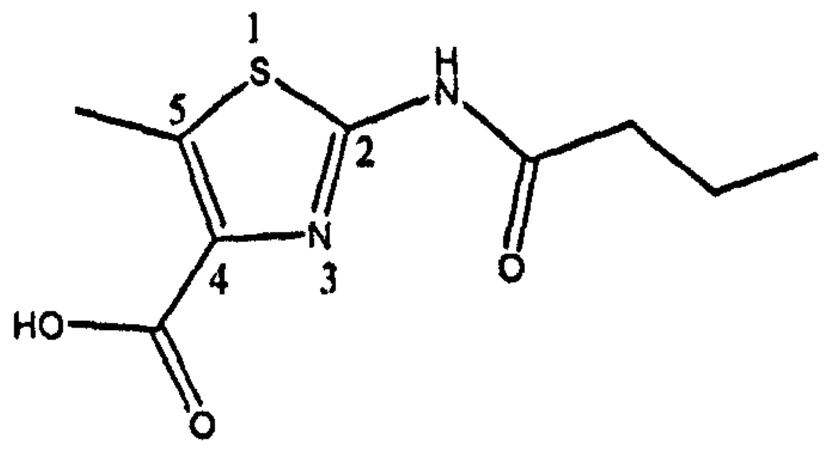
NMR: ^{1}H NMR (270MHz): δ($CDCl_3$): 0.95 (3H, t, J=7.29 Hz, $CH_3CH_2CH_2$-); 1.72 (2H, m, $CH_3CH_2CH_2$-); 2.37 (2H, t, J=7.29 Hz, $CH_3CH_2CH_2$-) ; 2.69 (3H, s, CH_3Ar); 3.88 (3H, s, OCH_3).

NMR: ^{13}C NMR (270MHz): δ($CDCl_3$): 12.3 (CH_3Ar); 13.3 ($CH_3CH_2CH_2$-); 18.1 ($CH_3CH_2CH_2$-); 37.6 ($CH_3CH_2CH_2$-); 51.7 (OCH_3); 134.6 (C_4); 139.0 (C_5); 153.9 (C_2); 162.5 (COOCH$_3$); 171.0 (CONH).

CHN Analysis: Found: C, 49.52; H, 5.98; N, 11.29; S, 13.43 (Required for $C_{10}H_{14}N_2O_3S$: C, 49.57; H, 5.82; N, 11.56; S, 13.23).

2-Butyramido-5-methylthiazole-4-carboxylic acid (42)

The title compound was obtained as a white powder (0.5g, 50.9%) using general procedure C.



M.P. 288-290 °C.

IR: ATR, υ_{max} (cm^{-1}): 3191, NH stretch, primary amide; 1690, C=O stretch, carboxylic acid; 1664, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 229.4, $C_9H_{12}N_2O_3S$; (m/z 100%): 229.4, $C_9H_{12}N_2O_3S$.

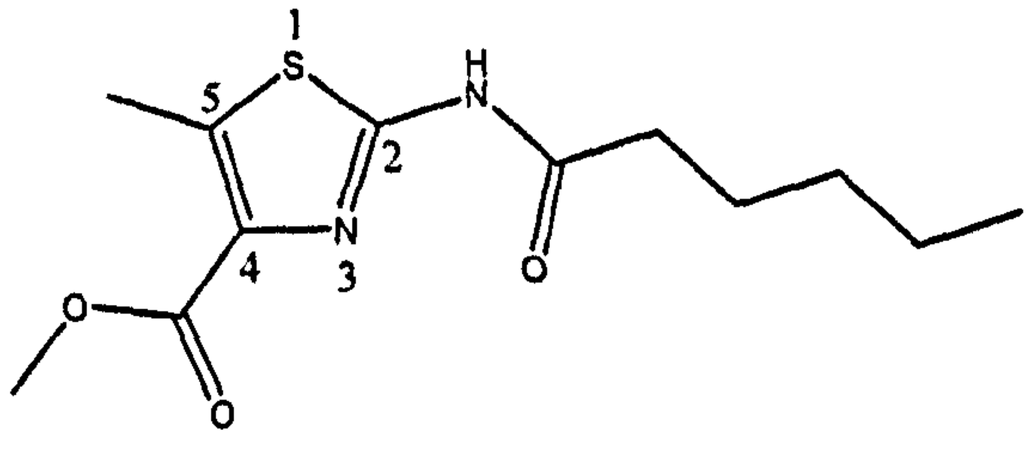
NMR: ^{1}H NMR (270MHz): δ($CDCl_3$): 1.03 (3H, t, J=7.29 Hz, $CH_3CH_2CH_2$-); 1.84 (2H, m, $CH_3CH_2CH_2$-); 2.50 (2H, t, $CH_3CH_2CH_2$-) ; 2.72 (3H, s, CH_3Ar).

NMR: ^{13}**C NMR** (270MHz): δ($CDCl_3$): 11.9 (CH_3Ar); 13.5 ($CH_3CH_2CH_2$-); 18.2 ($CH_3CH_2CH_2$-); 37.6 ($CH_3CH_2CH_2$-); 133.8 (C_4); 138.7 (C_5); 155.8 (C_2); 165.9 (COOH); 171.7 (CONH).

CHN Analysis: Found: C, 47.09; H, 5.19; N, 12.14; S, 13.91 (Required for $C_9H_{12}N_2O_3S$: C, 47.35; H, 5.30; N, 12.27; S, 14.05).

Methyl 2-hexanamido-5-methylthiazole-4-carboxylate (43)

The title compound was obtained as a white powder (1.3g, 27.5%) using general procedure B.



M.P. 90-92 °C.

IR: ATR, υ_{max} (cm^{-1}): 3273, NH stretch, primary amide; 1716, C=O stretch, conjugated ester; 1681, C=O stretch, primary amide.

MS: +EI, (m/z): 270.2, $C_{12}H_{18}N_2O_3S$; (m/z 100%): 172.1, $C_6H_8N_2O_2S$.

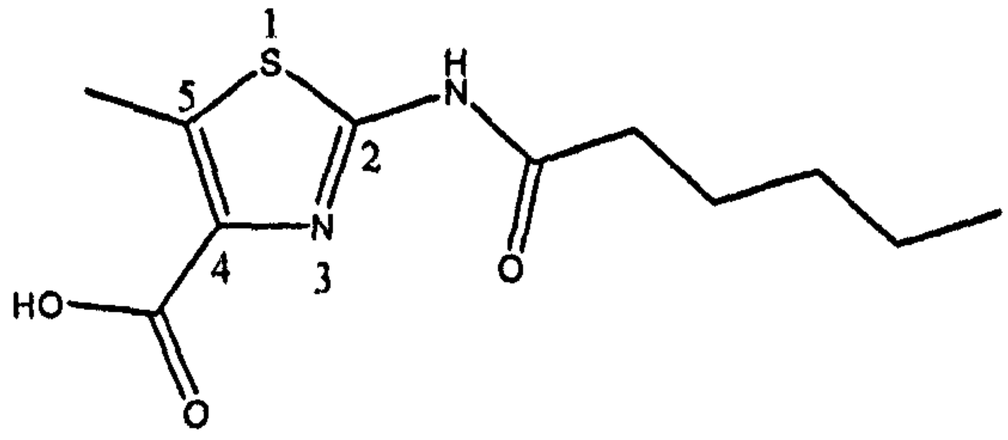
NMR: ^{1}**H NMR** (270MHz): δ($CDCl_3$): 0.86 (3H, t, $CH_3(CH_2)_3CH_2$-); 1.26-1.33 (4H, m, $CH_3(CH_2)_2CH_2CH_2$-);1.68(2H,m,$CH_3(CH_2)_2CH_2CH_2$-); 2.39 (2H, t, J=7.29 Hz, $CH_3(CH_2)_2CH_2CH_2$-) ; 2.69 (3H, s, CH_3Ar); 3.86 (3H, s, OCH_3); 9.80 (2H, s, NH_2).

NMR: ^{13}**C NMR** (270MHz): δ($CDCl_3$): 12.7 (CH_3Ar); 14.0 ($CH_3(CH_2)_3CH_2$-); 22.4 ($CH_3CH_2(CH_2)_2CH_2$-); 24.8 ($CH_3CH_2CH_2CH_2CH_2$-); 31.3 ($CH_3CH_2CH_2CH_2CH_2$-); 36.2 ($CH_3CH_2CH_2CH_2CH_2$-); 52.1 (OCH_3); 135.0 (C_4); 139.3 (C_5); 154.3 (C_2); 162.9 ($COOCH_3$); 171.6 (CONH).

CHN Analysis: Found: C, 53.12; H, 7.01; N, 10.23; S, 11.92 (Required for $C_{12}H_{18}N_2O_3S$: C, 53.31; H, 6.71; N, 10.36; S, 11.86).

2-Hexanamido-5-methylthiazole-4-carboxylic acid (44)

The title compound was obtained as a white powder (0.5g, 55.7%) using general procedure C.



M.P. 244-246 °C.

IR: ATR, υ_{max} (cm^{-1}): 3188, NH stretch, primary amide; 1687, C=O stretch, carboxylic acid; 1663, C=O stretch, primary amide.

MS: +EI, (m/z): 257.2, $C_{11}H_{16}N_2O_3S$; (m/z 100%): 257.2, $C_{11}H_{16}N_2O_3S$.

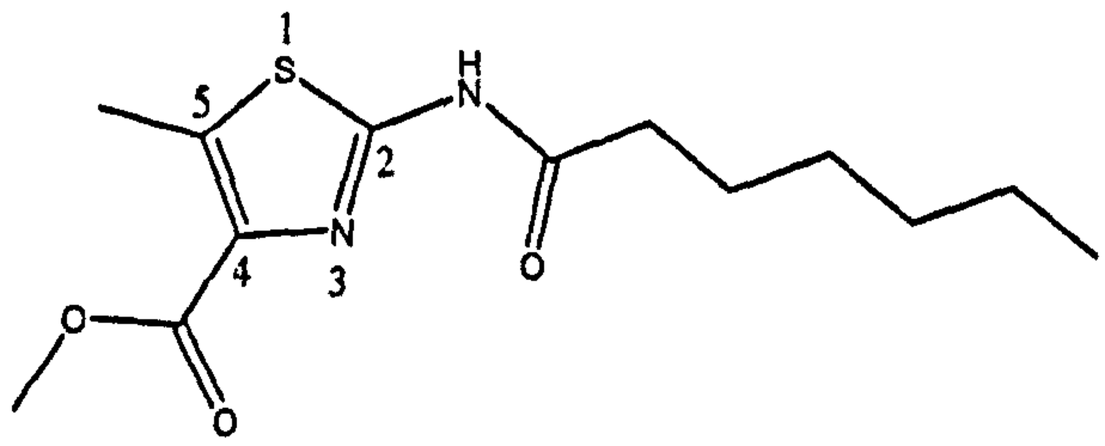
NMR: ^{1}H NMR (270MHz): δ($CDCl_3$): 0.89 (3H, t, $CH_3(CH_2)_3CH_2$-); 1.10-1.40 (4H, m, $CH_3(CH_2)_2CH_2CH_2$-); 1.75 (2H,m,$CH_3(CH_2)_2CH_2CH_2$-); 2.47 (2H, t, J=8.91 Hz,$CH_3(CH_2)_2CH_2CH_2$-) ; 2.64 (3H, s, CH_3Ar); 12.29 (1H, s, NH); 15.12 (1H, s, OH).

NMR: ^{13}C NMR (270MHz): δ($CDCl_3$): 12.3 (CH_3Ar); 14.1 ($CH_3(CH_2)_3CH_2$-); 22.5 ($CH_3CH_2(CH_2)_2CH_2$-); 24.8 ($CH_3CH_2CH_2CH_2CH_2$-); 31.5 ($CH_3CH_2CH_2CH_2CH_2$-); 36.2 ($CH_3CH_2CH_2CH_2CH_2$-); 134.2 (C_4); 139.0 (C_5); 156.2 (C_2); 166.3 (COOH); 172.3 (CONH).

CHN Analysis: Found: C, 51.14; H, 6.37; N, 11.09; S, 12.67 (Required for $C_{11}H_{16}N_2O_3S$: C, 51.54; H, 6.29; N, 10.93; S, 12.51).

Methyl 2-heptanamido-5-methylthiazole-4-carboxylate (45)

The title compound was obtained as a white powder (3.1g, 65.2%) using general procedure B.



M.P. 83-85 °C.

IR: ATR, υ_{max} (cm^{-1}): 3273, NH stretch, primary amide; 1720, C=O stretch, conjugated ester; 1683, C=O stretch, primary amide.

MS: CI/ISO, (M+H): 285.3, $C_{13}H_{20}N_2O_3S$; (M+H 100%): 285.3, $C_{13}H_{20}N_2O_3S$.

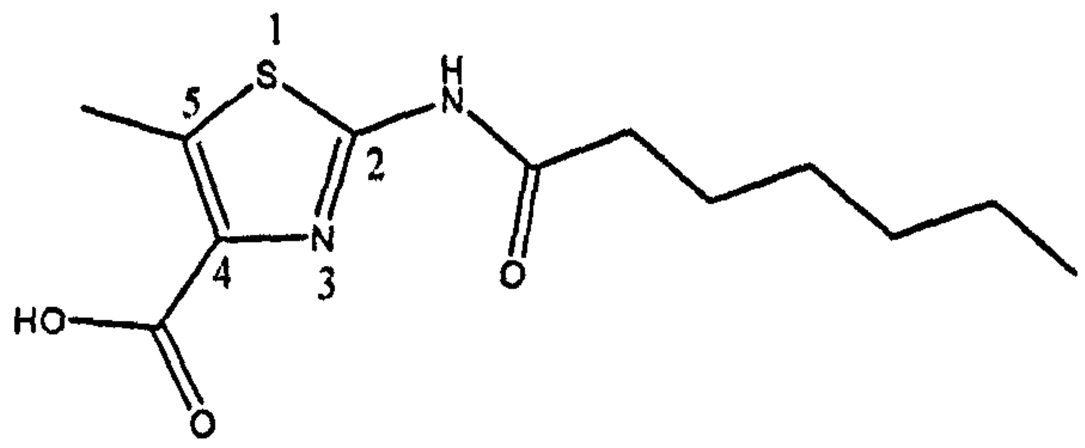
NMR: ^{1}H NMR (270MHz): δ($CDCl_3$): 0.86 (3H, t, J=6.21 Hz,$\mathbf{CH_3}(CH_2)_4CH_2$-); 1.23-1.34 (6H, m, $CH_3(\mathbf{CH_2})_3CH_2CH_2$-);1.64(2H, m, J=7.02 Hz,$CH_3(CH_2)_3\mathbf{CH_2}CH_2$-); 2.41 (2H, t, $CH_3(CH_2)_3CH_2\mathbf{CH_2}$-); 2.68 (3H, s, $\mathbf{CH_3}$Ar); 3.88 (3H, s, O$\mathbf{CH_3}$).

NMR: ^{13}C NMR (270MHz): δ($CDCl_3$): 12.1 ($\mathbf{CH_3}$Ar); 14.1 ($\mathbf{CH_3}(CH_2)_4CH_2$-); 22.6 ($CH_3\mathbf{CH_2}(CH_2)_3CH_2$-); 25.1 ($CH_3CH_2\mathbf{CH_2}(CH_2)_2CH_2$-); 28.9 ($CH_3(CH_2)_2\mathbf{CH_2}CH_2$. CH_2-); 31.5 ($CH_3(CH_2)_2CH_2\mathbf{CH_2}CH_2$-); 36.3 ($CH_3(CH_2)_2CH_2CH_2\mathbf{CH_2}$-); 52.1 ($OCH_3$); 134.9 ($C_4$); 139.2 ($C_5$); 154.5 ($C_2$); 162.9 ($\mathbf{COOCH_3}$); 171.6 ($\mathbf{CONH}$).

CHN Analysis: Found: C, 54.73; H, 7.39; N, 9.88; S, 11.38 (Required for $C_{13}H_{20}N_2O_3S$: C, 54.91; H, 7.09; N, 9.85; S, 11.28).

2-Heptanamido-5-methylthiazole-4-carboxylic acid (46)

The title compound was obtained as a white powder (0.5g, 52.8%) using general procedure C.



M.P. 214-216 °C.

IR: ATR, υ_{max} (cm^{-1}): 3188, NH stretch, primary amide; 1687, C=O stretch, carboxylic acid; 1663, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 271.3, $C_{12}H_{18}N_2O_3S$; (m/z 100%): 271.3, $C_{12}H_{18}N_2O_3S$.

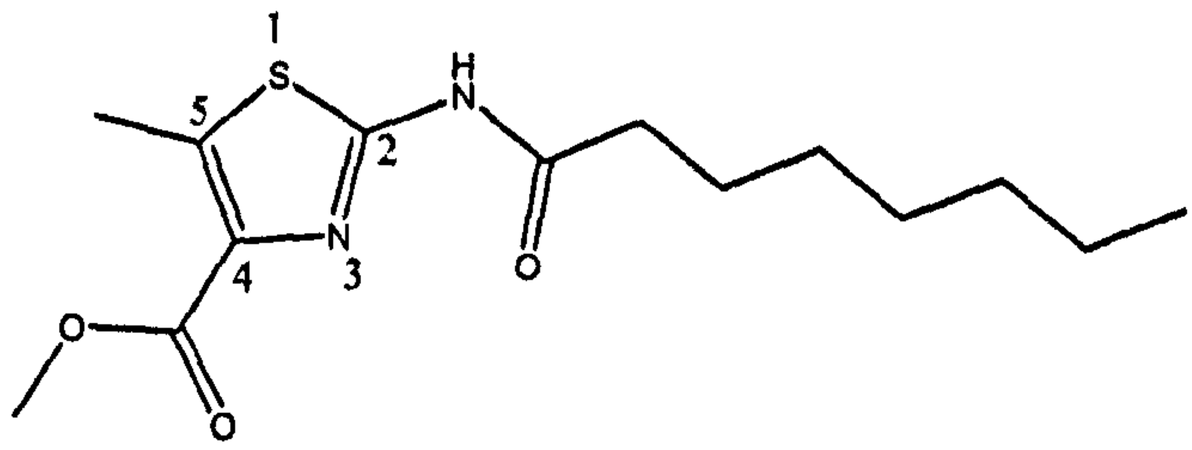
NMR: ^{1}H NMR (270MHz): δ(DMSO-d_6): 0.84 (3H, t, J=6.75 Hz, $CH_3(CH_2)_4CH_2$-); 1.24 (6H, m, J=6.75 Hz, $CH_3(CH_2)_3CH_2CH_2$-); 1.54(2H, m, J=6.75 Hz,$CH_3(CH_2)_3CH_2CH_2$-); 2.38 (2H, t, $CH_3(CH_2)_3CH_2CH_2$-); 2.50 (3H, s, CH_3Ar); 12.20 (1H, s, OH).

NMR: ^{13}C NMR (270MHz): δ($CDCl_3$): 12.1 (CH_3Ar); 13.9 ($CH_3(CH_2)_4CH_2$-); 21.9 ($CH_3CH_2(CH_2)_3CH_2$-); 24.5 ($CH_3CH_2CH_2(CH_2)_2CH_2$-); 28.1 ($CH_3(CH_2)_2CH_2CH_2$-); 30.9 ($CH_3(CH_2)_2CH_2CH_2CH_2$-); 34.8 ($CH_3(CH_2)_2CH_2CH_2CH_2$-); 136.1 (C_4); 136.5 (C_5); 153.1 (C_2); 163.6 (COOH); 171.6 (CONH).

CHN Analysis: Found: C, 53.12; H, 6.49; N, 10.30; S, 11.67 (Required for $C_{12}H_{18}N_2O_3S$: C, 53.31; H, 6.71; N, 10.36; S, 11.86).

Methyl 5-methyl-2-octanamidothiazole-4-carboxylate (47)

The title compound was obtained as a white powder (1.4g, 27.4%) using general procedure B.



M.P. 106-109 °C.

IR: ATR, υ_{max} (cm^{-1}): 3274, NH stretch, primary amide; 1711, C=O stretch, conjugated ester; 1683, C=O stretch, primary amide.

MS: +EI, (m/z): 298.2, $C_{14}H_{22}N_2O_3S$; (m/z 100%): 172.1, $C_6H_8N_2O_2S$.

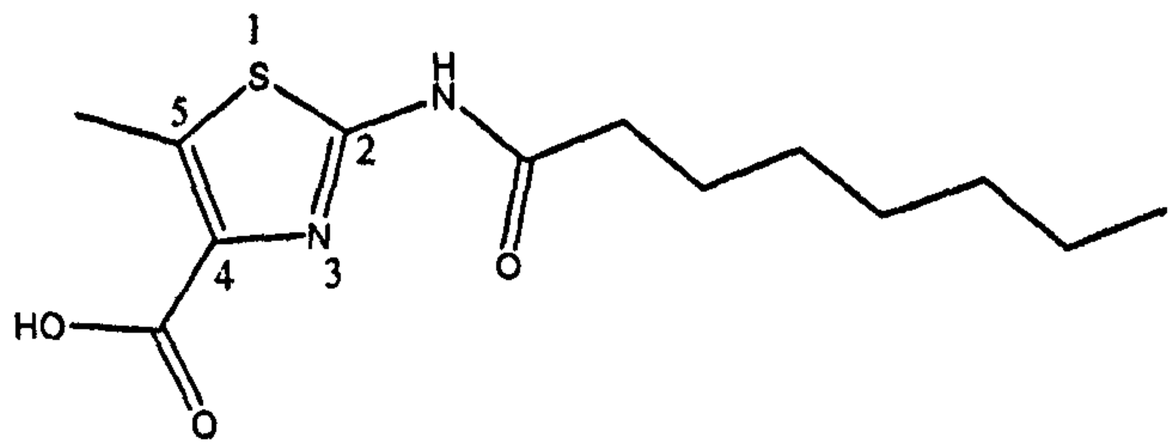
NMR: ^{1}H NMR (270MHz): δ($CDCl_3$): 0.87 (3H, t, $CH_3(CH_2)_5CH_2$-); 1.24-1.26 (8H, m, $CH_3(CH_2)_4CH_2CH_2$-);1.66(2H,m,$CH_3(CH_2)_4CH_2CH_2$-); 2.39 (2H, t, J=7.29 Hz,$CH_3(CH_2)_4CH_2CH_2$-) ; 2.69 (3H, s, CH_3Ar); 3.88 (3H, s, OCH_3).

NMR: ^{13}C NMR (270MHz): δ($CDCl_3$): 12.7 (CH_3Ar); 14.2 ($CH_3(CH_2)_5CH_2$-); 22.7 ($CH_3CH_2(CH_2)_4CH_2$-); 25.1 ($CH_3CH_2CH_2(CH_2)_3CH_2$-); 29.0- 29.2 ($CH_3CH_2CH_2(CH_2)_2$ CH_2CH_2-); 31.7 ($CH_3(CH_2)_3CH_2CH_2CH_2$-); 36.1 ($CH_3(CH_2)_3CH_2CH_2CH_2$-); 52.1 (OCH_3); 135.0 (C_4); 139.4 (C_5); 154.4 (C_2); 162.9 ($COOCH_3$); 171.6 (CONH).

CHN Analysis: Found: C, 56.37; H, 7.61; N, 9.49; S, 10.66 (Required for $C_{14}H_{22}N_2O_3S$: C, 56.35; H, 7.43; N, 9.39; S, 10.75).

5-Methyl-2-octanamidothiazole-4-carboxylic acid (48)

The title compound was obtained as a white powder (0.4g, 41.7%) using general procedure C.



M.P. 164-166 °C.

IR: ATR, υ_{max} (cm^{-1}): 3180, NH stretch, primary amide; 1688, C=O stretch, carboxylic acid; 1663, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 285.2, $C_{13}H_{20}N_2O_3S$; (m/z 100%): 285.2, $C_{13}H_{20}N_2O_3S$.

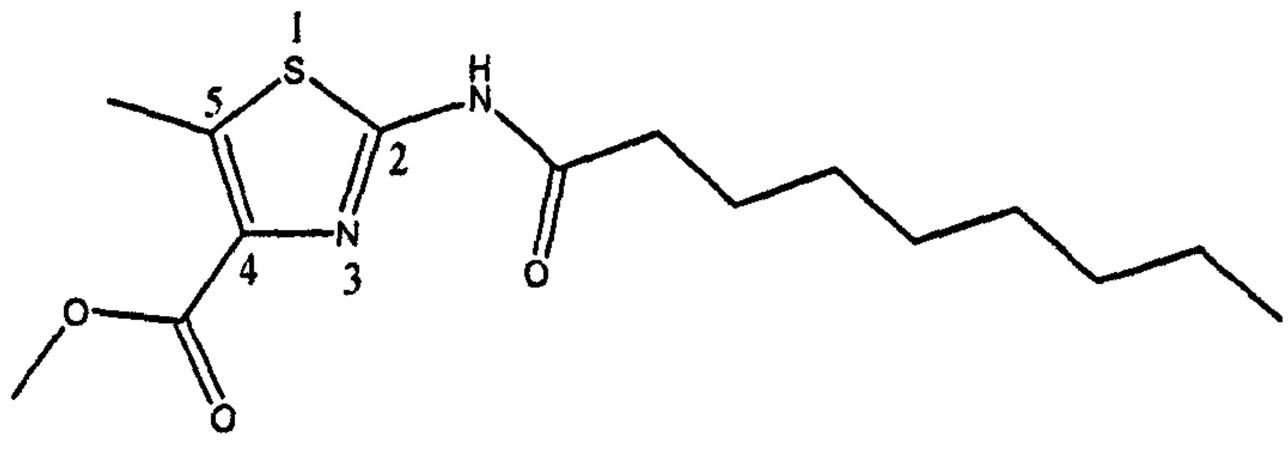
NMR: ^{1}H NMR (270MHz): δ($CDCl_3$): 0.88 (3H, t, $CH_3(CH_2)_5CH_2$-); 1.29-1.34 (8H, m, $CH_3(CH_2)_4CH_2CH_2$-);1.79 (2H,m,$CH_3(CH_2)_4CH_2CH_2$-); 2.49 (2H, t, J=7.29 Hz, $CH_3(CH_2)_4CH_2CH_2$-) ; 2.66 (3H, s, CH_3Ar); 12.44 (1H, s, NH); 15.09 (1H, s, OH).

NMR: ^{13}C NMR (270MHz): δ($CDCl_3$): 11.9 (CH_3Ar); 13.8 ($CH_3(CH_2)_5CH_2$-); 22.4 ($CH_3CH_2(CH_2)_4CH_2$-); 24.7 ($CH_3CH_2CH_2(CH_2)_3CH_2$-); 28.7- 29.0 ($CH_3CH_2CH_2(CH_2)_2$ CH_2CH_2-); 31.5 ($CH_3(CH_2)_3CH_2CH_2CH_2$-); 35.8 ($CH_3(CH_2)_3CH_2CH_2CH_2$-); 133.8 (C_4); 138.5 (C_5); 155.8 (C_2); 165.9 (COOH); 171.8 (CONH).

CHN Analysis: Found: C, 54.51; H, 7.19; N, 9.78; S, 11.50 (Required for $C_{13}H_{20}N_2O_3S$: C, 54.91; H, 7.09; N, 9.85; S, 11.28).

Methyl 5-methyl-2-nonanamidothiazole-4-carboxylate (49)

The title compound was obtained as a white powder (1.8g, 34.8%) using general procedure B.



M.P. 92-101 °C.

IR: ATR, υ_{max} (cm^{-1}): 3275, NH stretch, primary amide; 1718, C=O stretch, conjugated ester; 1685, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 313.2, $C_{15}H_{24}N_2O_3S$; (m/z 100%): 335.2, $C_{15}H_{24}N_2O_3S$ + 23.

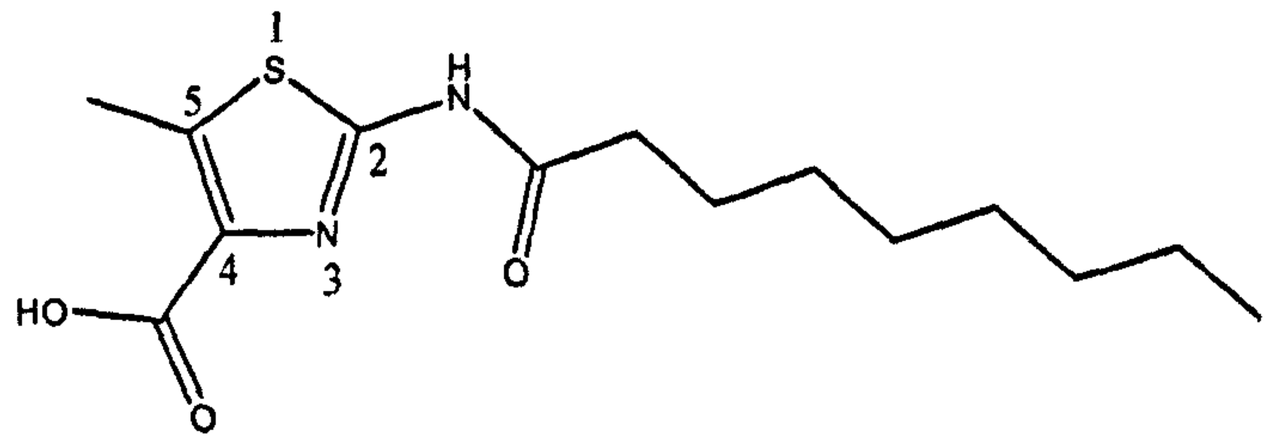
NMR: ^{1}H NMR (270MHz): δ($CDCl_3$): 0.86 (3H, t, $\mathbf{CH_3}(CH_2)_6CH_2$-); 1.24 (10H, m, $CH_3(\mathbf{CH_2})_5CH_2CH_2$-);1.66(2H,m,$CH_3(CH_2)_5\mathbf{CH_2}CH_2$-); 2.39 (2H, t, J=7.29 Hz,$CH_3(CH_2)_5CH_2\mathbf{CH_2}$-) ; 2.69 (3H, s, $\mathbf{CH_3}$Ar); 3.88 (3H, s, O$\mathbf{CH_3}$).

NMR: ^{13}C NMR (270MHz): δ($CDCl_3$): 12.7 ($\mathbf{CH_3}$Ar); 14.2 ($\mathbf{CH_3}(CH_2)_6CH_2$-); 22.8 ($CH_3\mathbf{CH_2}(CH_2)_5CH_2$-); 25.1 ($CH_3CH_2\mathbf{CH_2}(CH_2)_4CH_2$-); 29.2- 29.3 ($CH_3CH_2CH_2(\mathbf{CH_2})_3$ CH_2CH_2-); 31.9 ($CH_3(CH_2)_4CH_2\mathbf{CH_2}CH_2$-); 36.3 ($CH_3(CH_2$.$)_4CH_2CH_2\mathbf{CH_2}$-); 52.1 ($OCH_3$); 135.0 ($C_4$); 139.4 ($C_5$); 154.3 ($C_2$); 162.9 ($\mathbf{C}OOCH_3$); 171.5 ($\mathbf{C}ONH$).

CHN Analysis: Found: C, 57.62; H, 7.78; N, 8.80; S, 10.24 (Required for $C_{15}H_{24}N_2O_3S$: C, 57.66; H, 7.74; N, 8.97; S, 10.26).

5-Methyl-2-nonanamidothiazole-4-carboxylic acid (50)

The title compound was obtained as a white powder (0.3g, 31.4%) using general procedure C.



M.P. 150-152 °C.

IR: ATR, υ_{max} (cm^{-1}): 3188, NH stretch, primary amide; 1695, C=O stretch, carboxylic acid; 1668, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 299.3, $C_{14}H_{22}N_2O_3S$; (m/z 100%): 299.3, $C_{15}H_{24}N_2O_3S$.

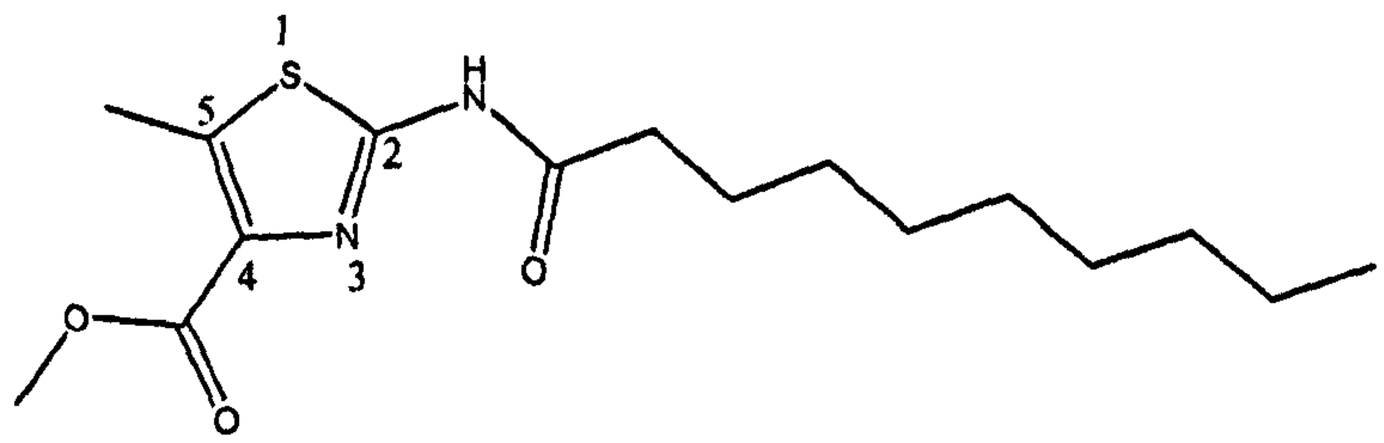
NMR: ^{1}H NMR (270MHz): δ($CDCl_3$): 0.87 (3H, t, CH_3 $(CH_2)_6$ CH_2-); 1.25-1.34 (10H, m,$CH_3(CH_2)_5CH_2CH_2$-);1.77(2H,m,$CH_3(CH_2)_5CH_2CH_2$-);2.51(2H,t,J=7.29 Hz,$CH_3(CH_2)_5CH_2CH_2$-) ; 2.69 (3H, s, CH_3Ar); 12.46 (1H, s, NH); 15.16 (1H, s, OH).

NMR: ^{13}C NMR (270MHz): δ($CDCl_3$): 12.3 (CH_3Ar); 14.2 ($CH_3(CH_2)_6CH_2$-); 22.8 ($CH_3CH_2(CH_2)_5CH_2$-); 25.2 ($CH_3CH_2CH_2(CH_2)_4CH_2$-); 29.3- 29.5 ($CH_3CH_2CH_2(CH_2)_3$ CH_2CH_2-); 32.0 ($CH_3(CH_2)_4CH_2CH_2CH_2$-); 36.3 ($CH_3(CH_2$.$)_4CH_2CH_2CH_2$-); 134.2 (C_4); 139.0 (C_5); 156.2 (C_2); 166.3 (COOH); 172.3 (CONH).

CHN Analysis: Found: C, 56.16; H, 7.56; N, 9.33; S, 10.84 (Required for $C_{14}H_{22}N_2O_3S$: C, 56.35; H, 7.43; N, 9.39; S, 10.75).

Methyl 2-decanamido-5-methylthiazole-4-carboxylate (51)

The title compound was obtained as a white powder (2.4g, 42.8%) using general procedure B.



M.P. 103-106 °C.

IR: ATR, υ_{max} (cm^{-1}): 3275, NH stretch, primary amide; 1718, C=O stretch, conjugated ester; 1684, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 327.3, $C_{16}H_{26}N_2O_3S$; (m/z 100%): 349.2, $C_{16}H_{26}N_2O_3S$ + Na.

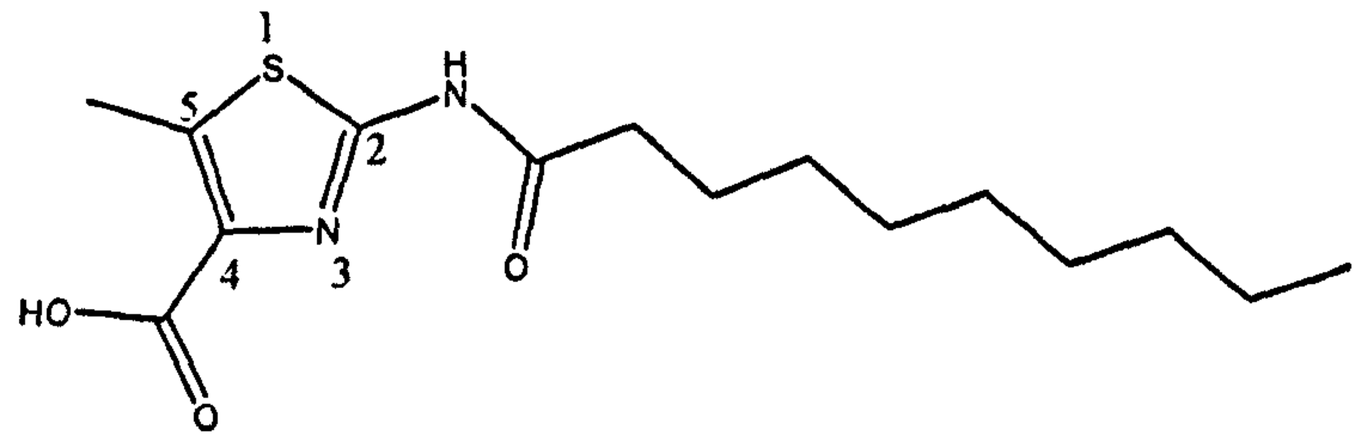
NMR: ^{1}H NMR (270MHz): δ($CDCl_3$): 0.83 (3H, t, $CH_3(CH_2)_7CH_2$-); 1.20 (12H, m, $CH_3(CH_2)_6CH_2CH_2$-);1.63 (2H,m,$CH_3(CH_2)_6CH_2CH_2$-); 2.35 (2H, t, J=7.56 Hz, $CH_3(CH_2)_6CH_2CH_2$-) ; 2.65 (3H, s, CH_3Ar); 3.85 (3H, s, OCH_3); 9.70 (1H, s, NH).

NMR: ^{13}C NMR (270MHz): δ($CDCl_3$): 12.5 (CH_3Ar); 14.5 ($CH_3(CH_2)_7CH_2$-); 22.0 ($CH_3CH_2(CH_2)_6CH_2$-); 25.5 ($CH_3CH_2CH_2(CH_2)_5CH_2$-); 29.0- 30.2 ($CH_3CH_2CH_2(CH_2)_4$ CH_2CH_2-); 31.7 ($CH_3(CH_2)_5CH_2CH_2CH_2$-); 37.5 ($CH_3(CH_2$.$)_5CH_2CH_2CH_2$-); 52.0 (OCH_3); 135.0 (C_4); 139.5 (C_5); 154.0 (C_2); 163.0 ($COOCH_3$); 172.0 (CONH).

CHN Analysis: Found: C, 59.06; H, 8.42; N, 8.51; S, 9.95 (Required for $C_{16}H_{26}N_2O_3S$: C, 58.87; H, 8.03; N, 8.58; S, 9.82).

2-Decanamido-5-methylthiazole-4-carboxylic acid (52)

The title compound was obtained as a white powder (0.25g, 26.1%) using general procedure C.



M.P. 122-124 °C.

IR: ATR, υ_{max} (cm^{-1}): 3180, NH stretch, primary amide; 1694, C=O stretch, carboxylic acid; 1667, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 313.0, $C_{15}H_{24}N_2O_3S$; (m/z 100%): 313.0, $C_{15}H_{24}N_2O_3S$.

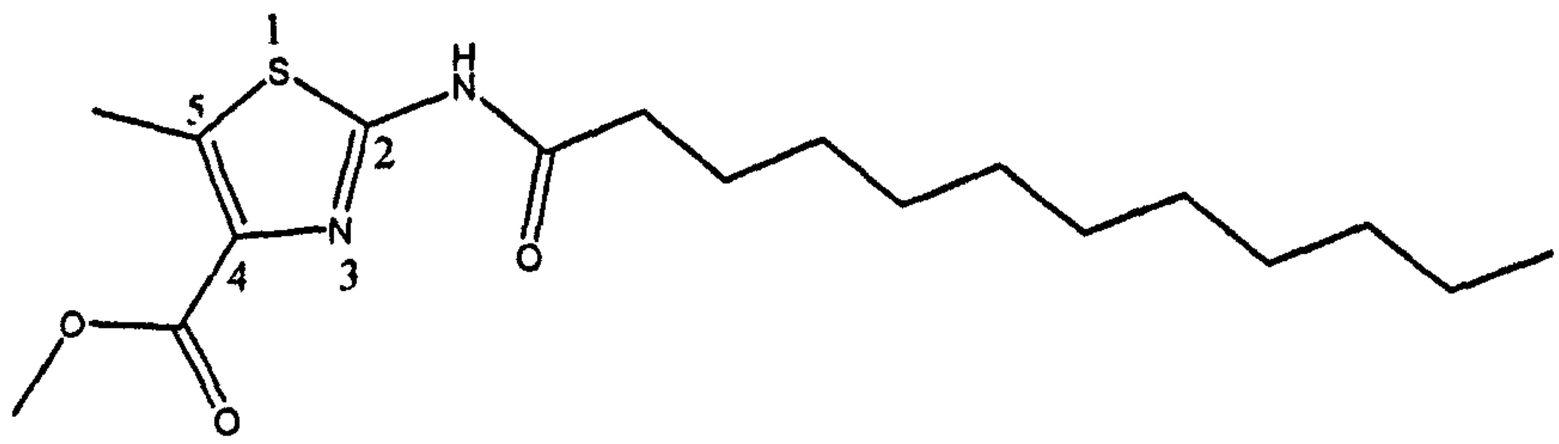
NMR: ^{1}H NMR (270MHz): δ($CDCl_3$): 0.87 (3H, t, $CH_3(CH_2)_7CH_2$-); 1.26 (12H, m, $CH_3(CH_2)_6CH_2CH_2$-);1.76 (2H,m,$CH_3(CH_2)_6CH_2CH_2$-); 2.50 (2H, t, J=7.56 Hz, $CH_3(CH_2)_6CH_2CH_2$-) ; 2.72 (3H, s, CH_3Ar); 12.47 (1H, s, NH); 15.22 (1H, s, OH).

NMR: ^{13}C NMR (400MHz): δ(DMSO-d_6): 12.6 (CH_3Ar); 14.5 ($CH_3(CH_2)_7CH_2$-); 22.6 ($CH_3CH_2(CH_2)_6CH_2$-); 25.1 ($CH_3CH_2CH_2(CH_2)_5CH_2$-); 29.0- 29.4 ($CH_3CH_2CH_2(CH_2)_4$ CH_2CH_2-); 31.8 ($CH_3(CH_2)_5CH_2CH_2CH_2$-); 35.3 ($CH_3(CH_2$-$)_5CH_2CH_2CH_2$-); 136.6 (C_4); 137.0 (C_5); 153.7 (C_2); 164.1 (COOH); 172.2 (CONH).

CHN Analysis: Found: C, 57.68; H, 8.04; N, 8.97; S, 10.41 (Required for $C_{15}H_{24}N_2O_3S$: C, 57.66; H, 7.74; N, 8.97; S, 10.26).

Methyl 2-dodecanamido-5-methylthiazole-4-carboxylate (53)

The title compound was obtained as a white powder (2.8g, 46.7%) using general procedure B.



M.P. 101-103 °C.

IR: ATR, υ_{max} (cm^{-1}): 3275, NH stretch, primary amide; 1719, C=O stretch, conjugated ester; 1684, C=O stretch, primary amide.

MS: +EI, (m/z): 354.3, $C_{18}H_{30}N_2O_3S$; (m/z 100%): 172.1, $C_6H_8N_2O_2S$.

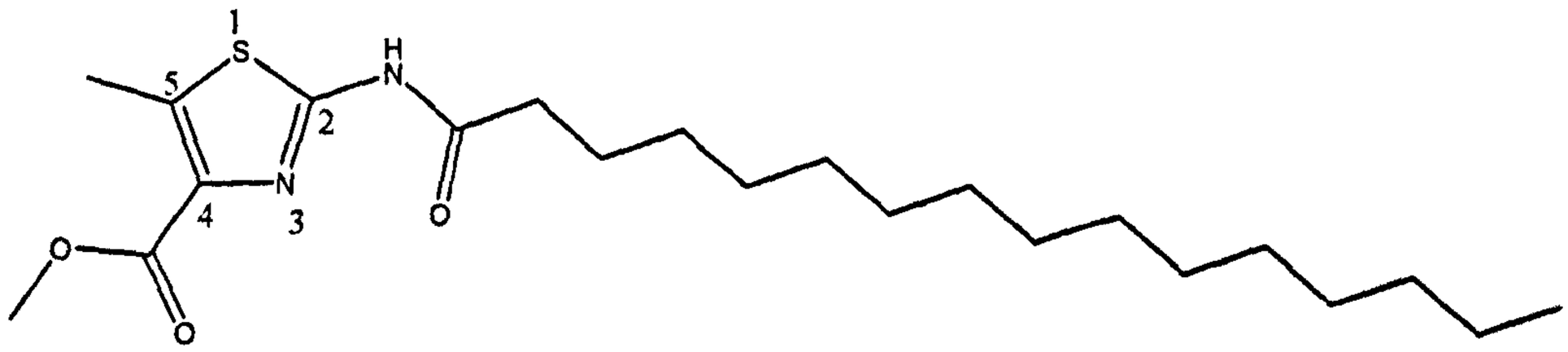
NMR: ^{1}H NMR (270MHz): δ($CDCl_3$): 0.87 (3H, t, J=6.39, $CH_3(CH_2)_9CH_2$-); 1.24 (16H, m, $CH_3(CH_2)_8CH_2CH_2$-);1.67(2H,m,$CH_3(CH_2)_8CH_2CH_2$-); 2.42 (2H, t, J=7.34 Hz, $CH_3(CH_2)_8CH_2CH_2$-) ; 2.69 (3H, s, CH_3Ar); 3.89 (3H, s, OCH_3).

NMR: ^{13}C NMR (270MHz): δ($CDCl_3$): 12.7 (CH_3Ar); 14.3 ($CH_3(CH_2)_9CH_2$-); 22.8 ($CH_3CH_2(CH_2)_8CH_2$-); 25.1 ($CH_3CH_2CH_2(CH_2)_7CH_2$-); 29.2- 29.7 ($CH_3CH_2CH_2(CH_2)_6$ CH_2CH_2-); 32.0 ($CH_3(CH_2)_7CH_2CH_2CH_2$-); 36.4 ($CH_3(CH_2)_7CH_2CH_2CH_2$-); 52.1 (OCH_3); 135.1 (C_4); 139.5 (C_5); 154.1 (C_2); 162.9 ($COOCH_3$); 171.4 (CONH).

CHN Analysis: Found: C, 61.07; H, 8.53; N, 8.18; S, 8.92 (Required for $C_{18}H_{30}N_2O_3S$: C, 60.98; H, 8.53; N, 7.90; S, 9.04).

Methyl 5-methyl-2-palmitamidothiazole-4-carboxylate (54)

The title compound was obtained as a white powder (4.0g, 57.1%) using general procedure B.



M.P. 96-99 °C.

IR: ATR, υ_{max} (cm^{-1}): 3275, NH stretch, primary amide; 1710, C=O stretch, conjugated ester; 1684, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 411.3, $C_{22}H_{38}N_2O_3S$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

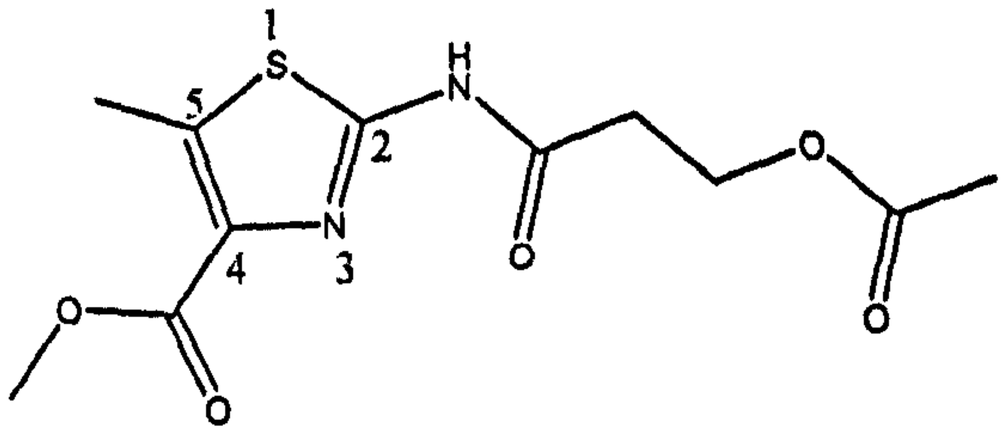
NMR: ^{1}H NMR (270MHz): δ($CDCl_3$): 0.90 (3H, t, J=6.34 Hz, $CH_3(CH_2)_{13}CH_2$-); 1.25 (24H, m, $CH_3(CH_2)_{12}CH_2CH_2$-); 1.65(2H,m,$CH_3(CH_2)_{12}CH_2CH_2$-); 2.41 (2H, t, J=7.68 Hz, $CH_3(CH_2)_{12}CH_2CH_2$-) ; 2.69 (3H, s, CH_3Ar); 3.89 (3H, s, OCH_3).

NMR: ^{13}C NMR (270MHz): δ($CDCl_3$): 13.2 (CH_3Ar); 14.8 ($CH_3(CH_2)_{13}CH_2$-); 23.3 ($CH_3CH_2(CH_2)_{12}CH_2$-); 24.9 ($CH_3CH_2CH_2(CH_2)_{11}CH_2$-); 29.5- 30.3 ($CH_3CH_2CH_2(CH_2)_{10}CH_2CH_2$-); 32.6 ($CH_3(CH_2)_{11}CH_2CH_2CH_2$-); 36.9 ($CH_3(CH_2$-.)$_{11}CH_2CH_2CH_2$-); 52.6 ($OCH_3$); 135.5 ($C_4$); 139.9 ($C_5$); 154.6 ($C_2$); 163.4 ($COOCH_3$); 171.9 (CONH).

CHN Analysis: Found: C, 66.65; H, 10.54; N, 5.37; S, 7.01 (Required for $C_{22}H_{38}N_2O_3S$: C, 64.35; H, 9.33; N, 6.82; S, 7.81).

Methyl 2-(3-acetoxypropanamido)-5-methylthiazole-4-carboxylate (55)

The title compound was obtained as a white powder (2.4g, 49.8%) using general procedure B.



M.P. 163-165 °C.

IR: ATR, υ_{max} (cm^{-1}): 3270, NH stretch, primary amide; 1713, C=O stretch, conjugated ester; 1687, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 287.2, $C_{11}H_{14}N_2O_5S$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

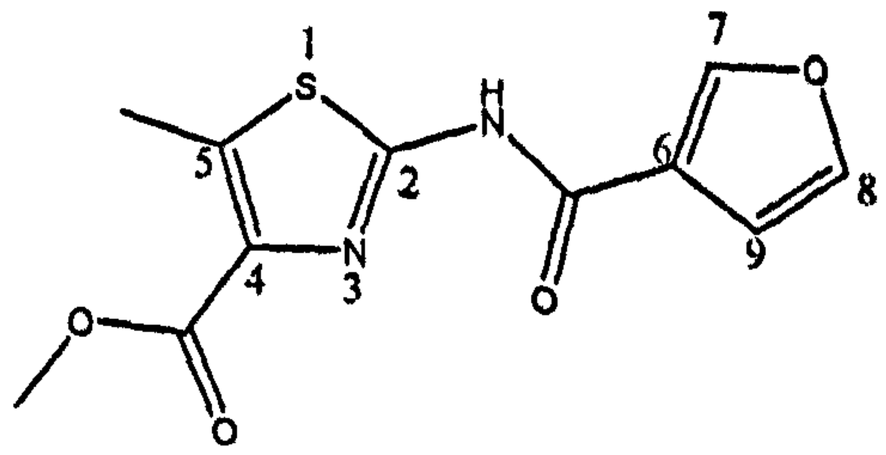
NMR: ^{1}H NMR (270MHz): δ(DMSO-d_6): 2.49 (3H, s, C**H**$_3$Ar); 2.59-2.69 (4H, m, COC**H**$_2$C**H**$_2$CO); 3.58 (3H, s, C**H**$_3$CO) 3.77 (3H, s, OC**H**$_3$).

NMR: ^{13}C NMR (400MHz): δ(DMSO-d_6): 12.5 (**C**H$_3$Ar); 28.5 (**C**H$_3$COO); 30.3 (**C**H$_2$CON); 52.03 (Ar-COO**C**H$_3$); 52.09 (**C**H$_2$OCOCH$_3$); 135.5 (C_4); 137.9 (C_5); 154.0 (C_2); 163.0 (**C**OOCH$_3$); 171.0 (OCOCH$_3$); 173.0 (**C**ONH).

CHN Analysis: Found: C, 46.16; H, 4.90; N, 9.57; S, 11.20 (Required for $C_{11}H_{14}N_2O_5S$: C, 46.15; H, 4.93; N, 9.78; S, 11.20).

Methyl 2-(furan-4-carboxamido)-5-methylthiazole-4-carboxylate (56)

The title compound was obtained as a yellow powder (3.4g, 75.1%) using general procedure B.



M.P. 223-226 °C.

IR: ATR, υ_{max} (cm^{-1}): 3270, NH stretch, primary amide; 1796, C=O strctch, conjugated ester; 1680, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 267.1, $C_{11}H_{10}N_2O_4S$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

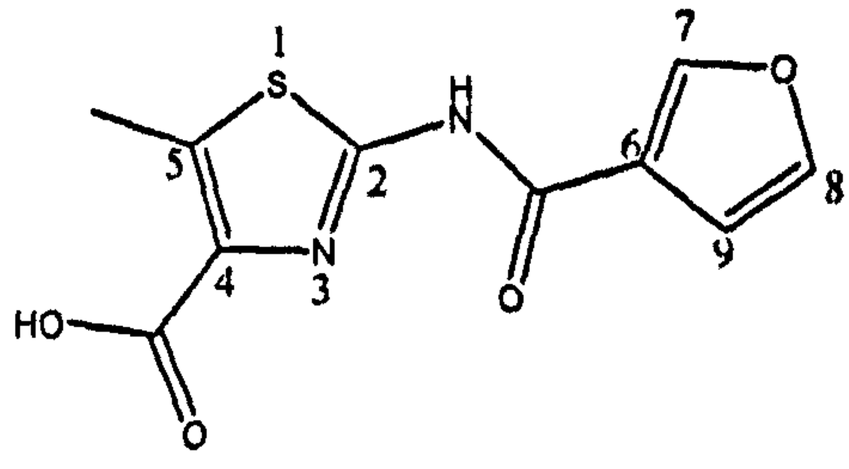
NMR: ^{1}H NMR (270MHz): δ(DMSO-d_6): 2.65 (3H, s, CH_3Ar); 3.80 (3H, s, OCH_3); 7.1 (1H, d, J=2.73 Hz, Furan); 7.80 (1H, d, J=3.46 Hz, Furan); 8.57 (1H, s, Furan).

NMR: ^{13}C NMR (270MHz): δ(DMSO-d_6): 11.4 (CH_3Ar); 50.9 (OCH_3); 109.6 (C_9); 121.1 (C_6); 135.6 (C_4); 138.4 (C_5); 145.3 (C_8); 148.0 (C_7); 154.2 (C_2); 160.8 ($COOCH_3$); 163.0 (CONH).

CHN Analysis: Found: C, 45.27; H, 4.02; N, 11.25; S, 11.70 (Required for $C_{11}H_{10}N_2O_4S$: C, 49.62; H, 3.79; N, 10.52; S, 12.04).

2-(Furan-4-carboxamido)-5-methylthiazole-4-carboxylic acid (57)

The title compound was obtained as a brown powder (0.63g, 66.5%) using general procedure C.



M.P. 320-322 °C.

IR: ATR, υ_{max} (cm^{-1}): 3183, NH stretch, primary amide; 1663, C=O stretch, carboxylic acid; 1663, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 253.2, $C_{10}H_8N_2O_4S$; (m/z 100%): 81.1, $C_2H_6N_2$ + Na.

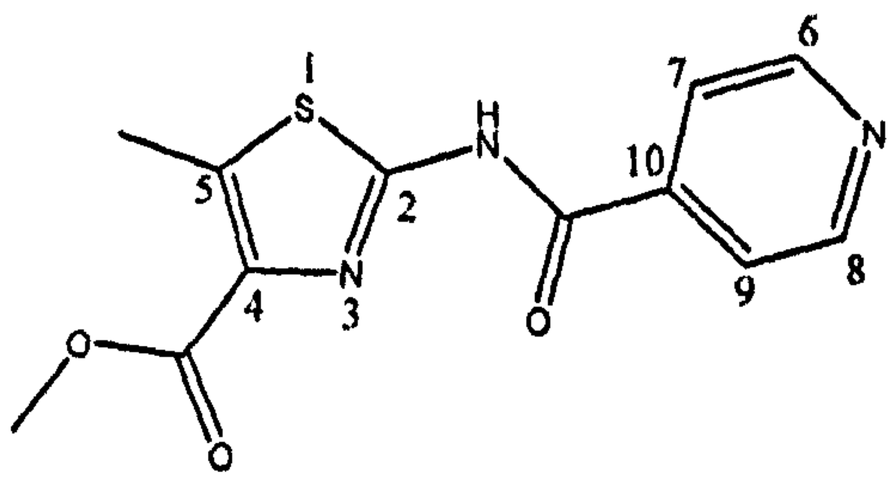
NMR: ^{1}H NMR (400MHz): δ(DMSO-d_6): 2.61 (3H, s, CH_3Ar); 7.1 (1H, d, J=4.4 Hz, Furan); 7.80 (1H, d, J=3.08 Hz, Furan); 8.57 (1H, s, Furan).

NMR: ^{13}**C NMR** (270MHz): δ(DMSO-d_6): 12.6 (**C**H_3Ar); 109.6 (C_9); 121.2 (C_6); 136.9 (C_4); 137.5 (C_5); 145.2 (C_8); 147.9 (C_7); 153.9 (C_2); 160.8 (**C**OOH); 164.1 (**C**ONH).

CHN Analysis: Found: C, 45.90; H, 3.40; N, 10.81; S, 12.43 (Required for $C_{10}H_8N_2O_4S$: C, 47.61; H, 3.20; N, 11.11; S, 12.71).

Methyl 2-(isonicotinamido)-5-methylthiazole-4-carboxylate (58)

The title compound was obtained as a white powder (2.2g, 46.7%) using general procedure B.



M.P. 177-179 °C.

IR: ATR, υ_{max} (cm^{-1}): 3200, NH stretch, primary amide; 1729, C=O stretch, conjugated ester; 1661, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 278.0, $C_{12}H_{11}N_3O_3S$; (m/z 100%): 79, C_5H_5N.

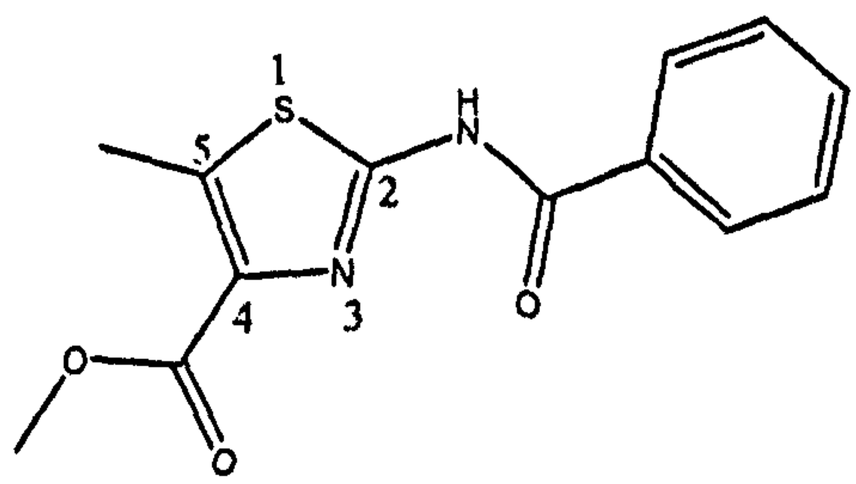
NMR: ^{1}**H NMR** (270MHz): δ(DMSO-d_6): 2.65 (3H, s, C**H**$_3$Ar); 3.80 (3H, s, OC**H**$_3$); 7.98 (2H, d, J=6.11 Hz, Pyridine); 8.80 (2H, d, J=6.11 Hz, Pyridine).

NMR: ^{13}**C NMR** (270MHz): δ(DMSO-d_6): 13.0 (**C**H_3Ar); 50.9 (O**C**H_3); 122.0 (C_7,C_9); 135.9 (C_4); 138.9 (C_5); 139.2 (C_{10}); 151.0 (C_6,C_8); 154.0 (C_2); 162.8 (**C**OO**C**H_3); 164.1 (**C**ONH).

CHN Analysis: Found: C, 51.68; H, 4.20; N, 14.85; S, 11.82 (Required for $C_{12}H_{11}N_3O_3S$: C, 51.98; H, 4.00; N, 15.15; S, 11.56).

Methyl 2-benzamido-5-methylthiazole-4-carboxylate (59)

The title compound was obtained as a white powder (0.4g, 46.7%) using general procedure B.



M.P. 243-245 °C.

IR: ATR, υ_{max} (cm^{-1}): 3160, NH stretch, primary amide; 1709, C=O stretch, conjugated ester; 1661, C=O stretch, primary amide.

MS: CI/ISO, (M+H): 277.2, $C_{13}H_{12}N_2O_3S$; (M+H 100%): 277.2, $C_{13}H_{12}N_2O_3S$.

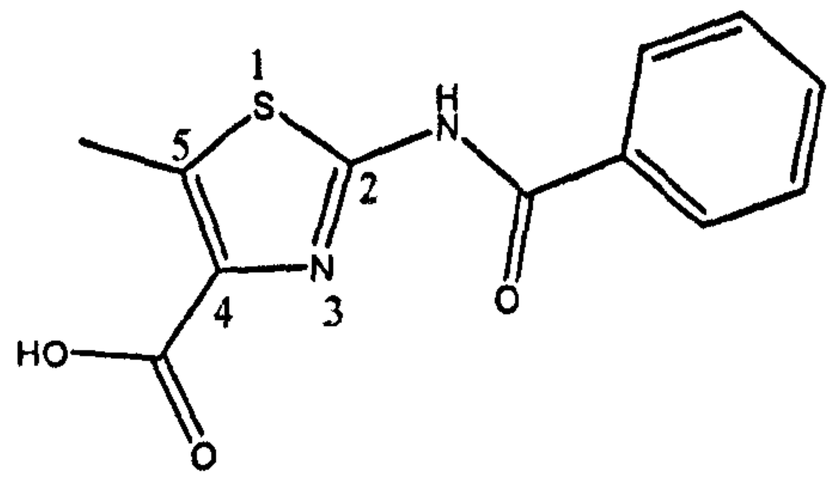
NMR: ^{1}H NMR (270MHz): δ(DMSO-d_6): 2.64 (3H, s, **C**H$_3$Ar); 3.80 (3H, s, OC**H**$_3$); 7.50-7.63 (3H, m, Ar); 8.12 (2H, d, Ar); 12.90 (1H, s, N**H**).

NMR: ^{13}C NMR (270MHz): δ(DMSO-d_6): 12.0 (**C**H$_3$Ar); 51.5 (OCH$_3$); 128.1-132.7 (6C, Ar); 135.2 (C_4); 137.9 (C_5); 154.2 (C_2); 162.5 (**C**OOCH$_3$); 170.0 (**C**ONH).

CHN Analysis: Found: C, 56.24; H, 4.41; N, 10.19; S, 11.42 (Required for $C_{13}H_{12}N_2O_3S$: C, 56.51; H, 4.38; N, 10.14; S, 11.60).

2-Benzamido-5-methylthiazole-4-carboxylic acid (60)

The title compound was obtained as a white powder (0.6g, 63.4%) using general procedure C.



M.P. 318-320 °C.

IR: ATR, υ_{max} (cm^{-1}): 3185, NH stretch, primary amide; 1667, C=O stretch, carboxylic acid; 1667, C=O stretch, primary amide.

MS: CI/ISO, (M+H): 263.2, $C_{12}H_{10}N_2O_3S$; (M+H 100%): 79.1.

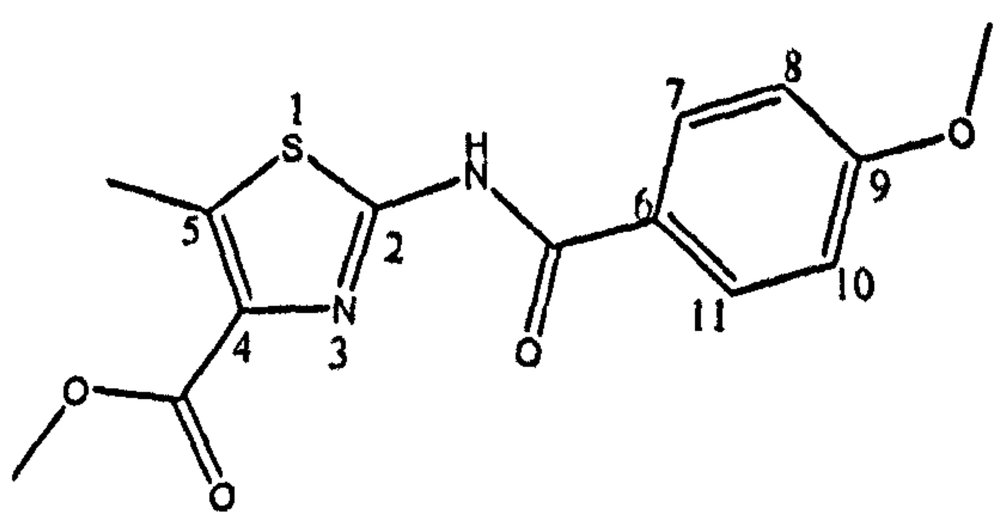
NMR: ^{1}H NMR (270MHz): δ(DMSO-d_6): 2.63 (3H, s, CH_3Ar); 7.50-7.63 (3H, m, Ar); 8.09 (2H, d, Ar).

NMR: ^{13}C NMR (270MHz): δ(DMSO-d_6): 12.1 (CH_3Ar); 128.1-132.7 (Ar); 136.4 (C_4); 137.1 (C_5); 153.6 (C_2); 163.6 (COOH); 170.0 (CONH).

CHN Analysis: Found: C, 53.05; H, 4.06; N, 10.03; S, 12.14 (Required for $C_{12}H_{10}N_2O_3S$: C, 54.95; H, 3.84; N, 10.68; S, 12.23).

Methyl 2-(4-methoxybenzamido)-5-methylthiazole-4-carboxylate (61)

The title compound was obtained as a white powder (1.8g, 39.9%) using general procedure B.



M.P. 189-191 °C.

IR: ATR, υ_{max} (cm^{-1}): 3308, NH stretch, primary amide; 1710, C=O stretch, conjugated ester; 1654, C=O stretch, primary amide.

MS: CI/ISO, (M+H): 307.2, $C_{14}H_{14}N_2O_4S$; (m/z 100%): 307.2, $C_{14}H_{14}N_2O_4S$.

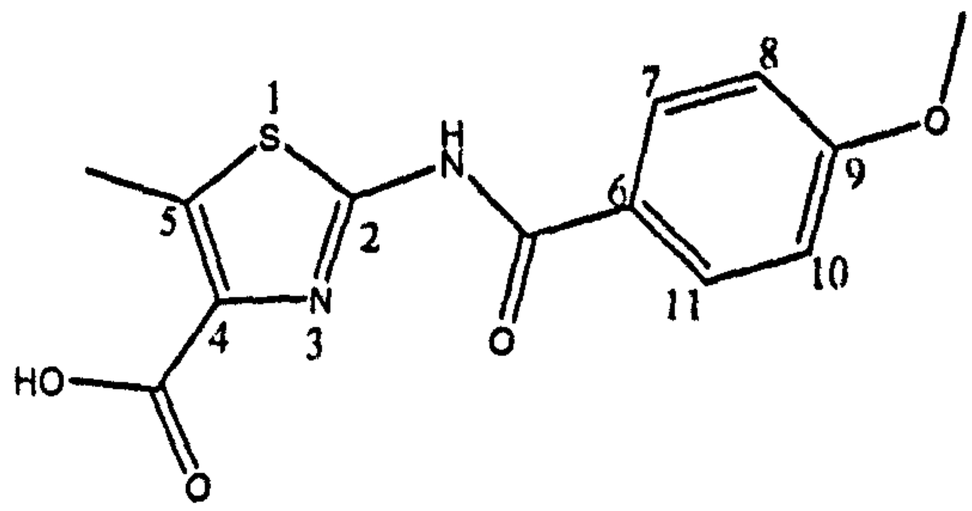
NMR: ^{1}H NMR (270MHz): δ(DMSO-d_6): 2.64 (3H, s, CH_3Ar); 3.80 (3H, s, OCH_3); 3.84 (3H, s, Ar-OCH_3); 7.05 (2H, d, J=8.91 Hz, Ar); 8.13 (2H, d, J=9.18 Hz, Ar).

NMR: ^{13}C NMR (270MHz): δ($CDCl_3$): 12.4 (CH_3Ar); 51.9 (OCH_3); 56.1 (Ar-OCH_3); 114.5 (C_7, C_{11}); 124.4 (C_6); 130.8 (C_8, C_{10}); 136.0 (C_4); 139.0 (C_5); 156.0 (C_2); 163.0 (C_9); 163.4 ($COOCH_3$); 164.5 (CONH).

CHN Analysis: Found: C, 54.59; H, 4.29; N, 9.14; S, 10.32 (Required for $C_{14}H_{14}N_2O_4S$: C, 54.89; H, 4.61; N, 9.14; S, 10.47).

2-(4-Methoxybenzamido)-5-methylthiazole-4-carboxylic acid (62)

The title compound was obtained as off-white powder (0.5g, 52.3%) using general procedure C.



M.P. 316-318 °C.

IR: ATR, υ_{max} (cm^{-1}): 3225, NH stretch, primary amide; 1661, C=O stretch, carboxylic acid; 1661, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 293.2, $C_{13}H_{12}N_2O_4S$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

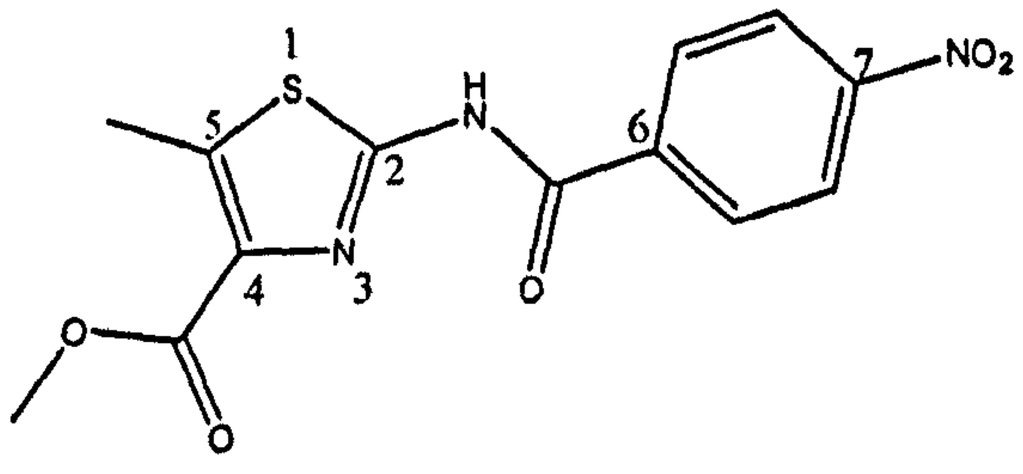
NMR: ^{1}H NMR (400MHz): δ(DMSO-d_6): 2.60 (3H, s, CH_3Ar); 3.87 (3H, s, Ar-OCH_3); 7.06 (2H, d, J=9.2 Hz, Ar); 8.11 (2H, d, J=8.0 Hz, Ar).

NMR: ^{13}**C NMR** (400MHz): δ(DMSO-d_6): 12.6 (**C**H_3Ar); 56.0 (O**C**H_3); 114.4 (C_8, C_{10}); 124.3 (C_6); 130.8 (C_7, C_{11}); 136.9 (C_4); 137.4 (C_5); 154.6 (C_2); 163.3 (C_9); 164.2 (**C**OOCH$_3$); 165.1 (**C**ONH).

CHN Analysis: Found: C, 51.16; H, 4.03; N, 8.92; S, 10.38 (Required for $C_{13}H_{12}N_2O_4S$: C, 53.42; H, 4.14; N, 9.58; S, 10.97).

Methyl 5-methyl-2-(4-nitrobenzamido)thiazole-4-carboxylate (63)

The title compound was obtained as a yellow powder (1.1g, 20.7%) using general procedure B.



M.P. 225-228 ˚C.

IR: ATR, υ_{max} (cm^{-1}): 1714, C=O stretch, conjugated ester; 1669, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 322.2, $C_{13}H_{11}N_3O_5S$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

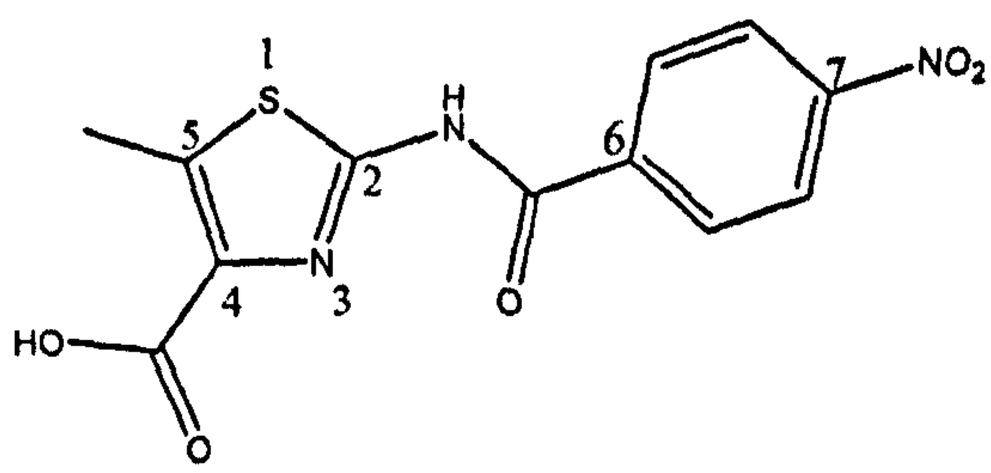
NMR: ^{1}**H NMR** (400MHz): δ(DMSO-d_6): 2.65 (3H, s, C**H**$_3$Ar); 3.77 (3H, s, OC**H**$_3$); 8.33 (4H, dd, Ar).

NMR: ^{13}**C NMR** (500MHz): δ(DMSO-d_6): 12.1 (**C**H_3Ar); 51.5 (O**C**H_3); 123.4-130.5 (4C, Ar); 135.3 (C_4); 137.4 (C_6); 138.2 (C_5); 149.68 (C_7);154.1 (C_2); 162.4 (**C**OOCH$_3$); 163.9 (**C**ONH).

CHN Analysis: Found: C, 46.35; H, 3.90; N, 11.58; S, 8.84 (Required for $C_{13}H_{11}N_3O_5S$: C, 48.59; H, 3.45; N, 13.08; S, 9.98).

5-Methyl-2-(4-nitrobenzamido)thiazole-4-carboxylic acid (64)

The title compound was obtained as a yellow powder (0.2g, 20.8%) using general procedure C.



M.P. 332-336 °C.

IR: ATR, υ_{max} (cm^{-1}): 3182, NH stretch, primary amide; 1664, C=O stretch, carboxylic acid; 1664, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 329.2, $C_{12}H_9N_3O_5S$ + Na; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

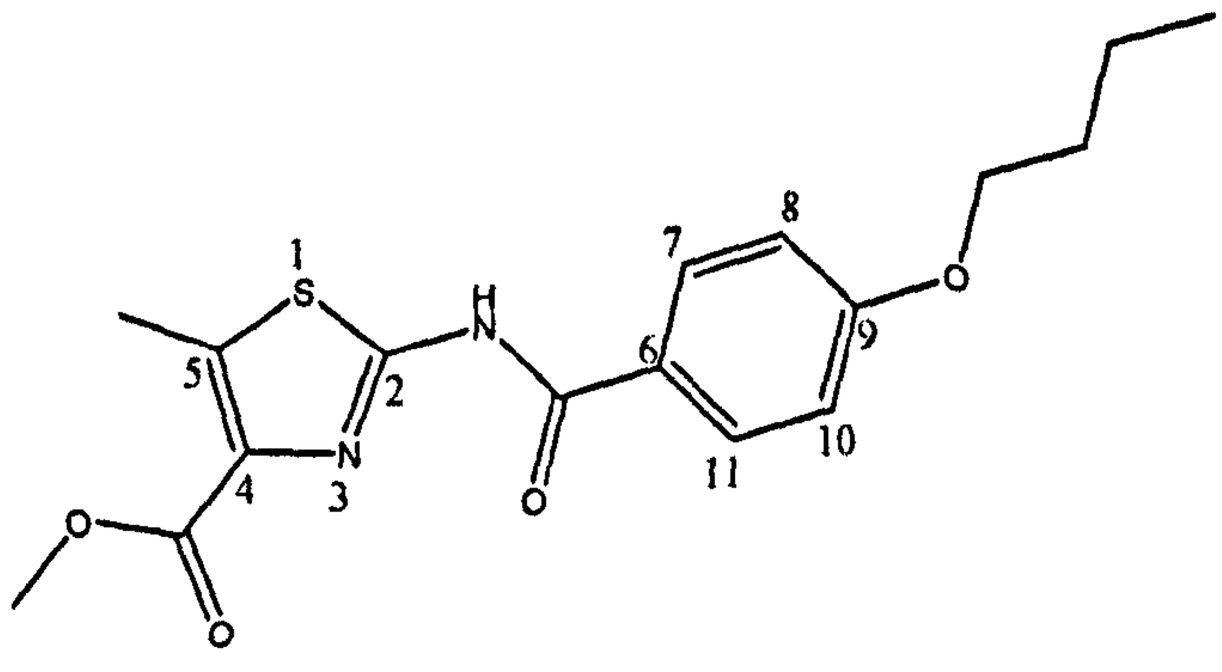
NMR: ^{1}H NMR (400MHz): δ(DMSO-d_6): 2.65 (3H, s, CH_3Ar); 8.32 (4H, dd, Ar).

NMR: ^{13}C NMR (400MHz): δ(DMSO-d_6): 12.6 (CH_3Ar); 136.2 (C_4); 137.8 (C_6); 138.0 (C_5); 150.3 (C_7); 154.2 (C_2); 164.4 (COOH); 166.2 (CONH).

CHN Analysis: Found: C, 46.94; H, 2.95; N, 13.33; S, 10.33 (Required for $C_{12}H_9N_3O_5S$: C, 46.90; H, 2.95; N, 13.67; S, 10.44).

Methyl 2-(4-butoxybenzamido)-5-methylthiazole-4-carboxylate (65)

The title compound was obtained as a white powder (2.1g, 35.1%) using general procedure B.



M.P. 137-139 °C.

IR: ATR, υ_{max} (cm^{-1}): 3283, NH stretch, primary amide; 1707, C=O stretch, conjugated ester; 1673, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 349.0, $C_{17}H_{20}N_2O_4S$; (m/z 100%): 177.0, $C_{11}H_{13}O_2$.

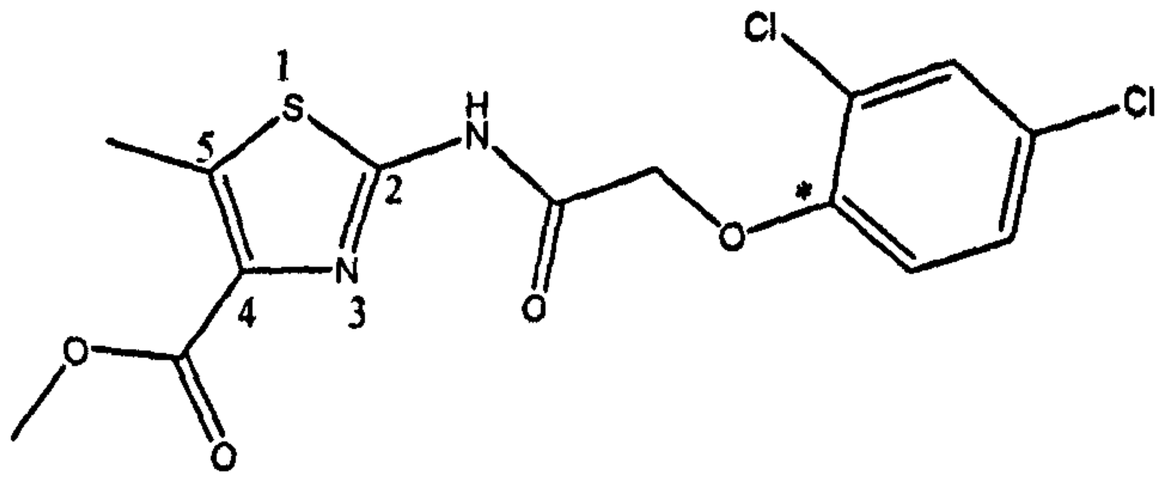
NMR: ^{1}H NMR (270MHz): δ($CDCl_3$): 0.98 (3H, t, J=7.56 Hz, $CH_3(CH_2)_2CH_2$-O); 1.49 (2H, m, $CH_3CH_2CH_2CH_2$-); 1.82 (2H, t, $CH_3CH_2CH_2CH_2$-) ; 2.70 (3H, s, CH_3Ar); 3.85 (3H, s, OCH_3); 4.05 (2H, t, J=6.48 Hz, $CH_3CH_2CH_2CH_2$-O); 6.98 (2H, d, J=6.88 Hz, Ar); 7.98 (2H, d, J=6.75 Hz, Ar).

NMR: ^{13}C NMR (270MHz): δ($CDCl_3$): 12.6 (CH_3Ar); 13.9 ($CH_3(CH_2)_2CH_2$-O); 19.3 ($CH_3CH_2CH_2CH_2$-O); 31.2 ($CH_3CH_2CH_2CH_2$-O); 52.5 (OCH_3); 68.3 ($CH_3CH_2CH_2CH_2$-O); 115.0 (C_7, C_{11}); 122.7 (C_6); 130.2 (C_8, C_{10}); 131.9 (C_4); 138.8 (C_5); 156.6 (C_2); 161.2 (C_9); 163.8 ($COOCH_3$); 164.6 (CONH).

CHN Analysis: Found: C, 56.79; H, 5.67; N, 7.50; S, 8.47 (Required for $C_{17}H_{20}N_2O_4S$: C, 58.60; H, 5.79; N, 8.04; S, 9.20).

Methyl 2-(2-(2,4-dichlorophenoxy)acetamido)-5-methylthiazole-4-carboxylate (66)

The title compound was obtained as a light brown powder (3.5g, 55.8%) using general procedure B.



M.P. 153-155 °C.

IR: ATR, υ_{max} (cm^{-1}): 3210, NH stretch, primary amide; 1693, C=O stretch, conjugated ester; 1662, C=O stretch, primary amide.

MS: FAB/NOBA, (M+2): 375.0, $C_{14}H_{12}N_2O_4SCl_2$; (m/z 100%): 79.0.

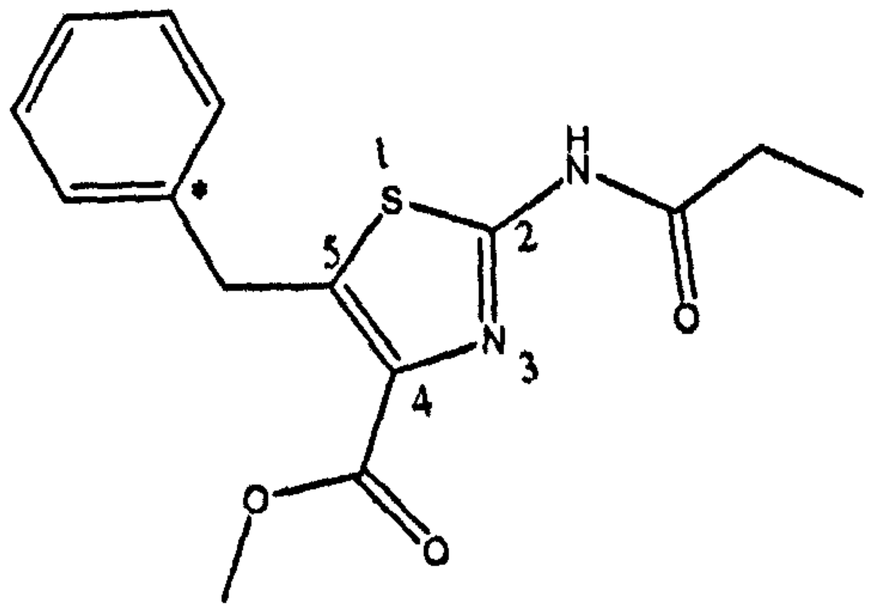
NMR: ^{1}H NMR (270MHz): δ($CDCl_3$): 2.61 (3H, s, CH_3Ar); 3.79 (3H, s, OCH_3); 4.98 (2H, s, -OCH_2CO); 7.1 (1H, d, J=8.94 Hz, Ar); 7.34 (1H, d, J=8.89 Hz, Ar); 7.60 (1H, s, Ar).

NMR: ^{13}C NMR (270MHz): δ($CDCl_3$): 11.7 (CH_3Ar); 51.2 (OCH_3); 66.3 (-OCH_2CO); 114.8-129.0 (5C, Ar); 134.8 (C_4); 137.5 (C_5); 152.0 (C^*); 152.5 (C_2); 162.0 ($COOCH_3$); 166.1 (CONH).

CHN Analysis: Found: C, 44.20; H, 3.33; N, 7.31; S, 8.83; Cl, 18.83 (Required for $C_{14}H_{12}N_2O_4SCl_2$: C, 44.81; H, 3.22; N, 7.47; S, 8.55; Cl, 18.90).

Methyl 5-benzyl-2-propionamidothiazole-4-carboxylate (67)

The title compound was obtained as a white powder (1.1g, 30.0%) using general procedure **B**.



M.P. 210-212 °C.

IR: ATR, υ_{max} (cm^{-1}): 3263, NH stretch, primary amide; 1713, C=O stretch, conjugated ester; 1686, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 305.2, $C_{15}H_{16}N_2O_3S$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

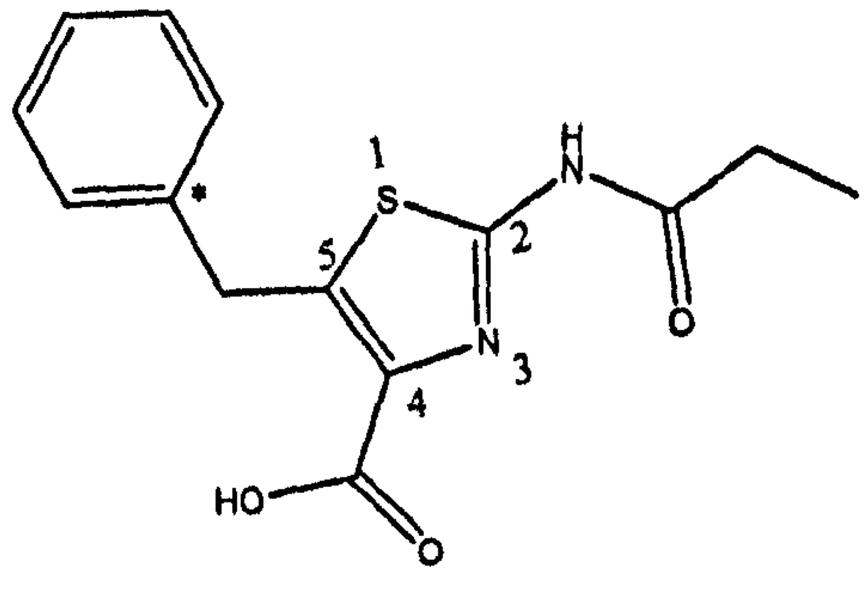
NMR: ^{1}H NMR (270MHz): δ($CDCl_3$): 1.19 (3H, t, J=6.48 Hz, CH_3CH_2-); 2.42 (2H, q, J=7.56 Hz, CH_3CH_2-); 3.90 (3H, s, OCH_3); 4.50 (2H, s, CH_2-Ar); 7.24-7.31 (5H, m, Ar); 9.8 (1H, s, NH).

NMR: ^{13}C NMR (270MHz): δ($CDCl_3$): 9.0 (CH_3CH_2CO); 28.0 (CH_3CH_2CO); 32.0 (Ar-CH_2-); 51.0 (OCH_3); 128.0 – 130.0 (5C, Ar); 136.4 (C_4); 140.5 (C_5); 141.5 (C^*); 156.0 (C_2); 162.0 ($COOCH_3$); 171.0 (CONH).

CHN Analysis: Found: C, 59.04; H, 5.16; N, 9.13; S, 10.85 (Required for $C_{15}H_{16}N_2O_3S$: C, 59.19; H, 5.30; N, 9.20; S, 10.54).

5-Benzyl-2-propionamidothiazole-4-carboxylic acid (68)

The title compound was obtained as a white powder (0.7g, 72.6%) using general procedure **C**.



M.P. 272-274 °C.

IR: ATR, υ_{max} (cm^{-1}): 3185, NH stretch, primary amide; 1702, C=O stretch, carboxylic acid; 1661, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 291.2, $C_{14}H_{14}N_2O_3S$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

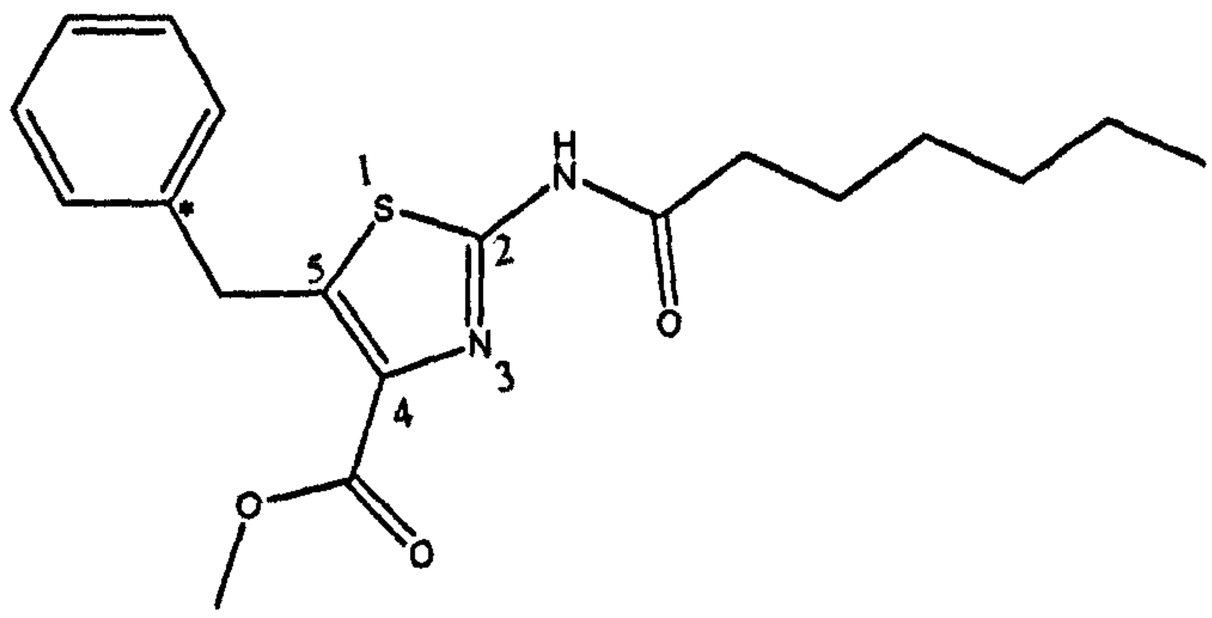
NMR: ^{1}**H NMR** (400MHz): δ(DMSO-d_6): 1.06 (3H, t, J=7.04 Hz, CH_3CH_2-); 2.36 (2H, q, J=7.48 Hz, CH_3CH_2-); 4.47 (2H, s, CH_2-Ar); 7.28-7.31 (5H, m, Ar).

NMR: ^{13}**C NMR** (400MHz): δ(DMSO-d_6): 9.6 (CH_3CH_2CO); 28.7 (CH_3CH_2CO); 32.5 (Ar-CH_2-); 127.1–129.1 (5C, Ar); 136.0 (C_4); 140.4 (C_5); 141.5 (C^*); 154.8 (C_2); 164.2 (COOH); 173.0 (CONH).

CHN Analysis: Found: C, 58.08; H, 4.90; N, 9.90; S, 12.01 (Required for $C_{14}H_{14}N_2O_3S$: C, 57.92; H, 4.86; N, 9.65; S, 11.04).

Methyl 5-benzyl-2-heptanamidothiazole-4-carboxylate (69)

The title compound was obtained as a pale yellow powder (1.2g, 26.6%) using general procedure **B**.



M.P. 130-132 °C.

IR: ATR, υ_{max} (cm^{-1}): 3263, NH stretch, primary amide; 1715, C=O stretch, conjugated ester; 1689, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 361.2, $C_{19}H_{24}N_2O_3S$; (m/z 100%): 383.2, $C_{14}H_{22}N_2O_3S$ + Na.

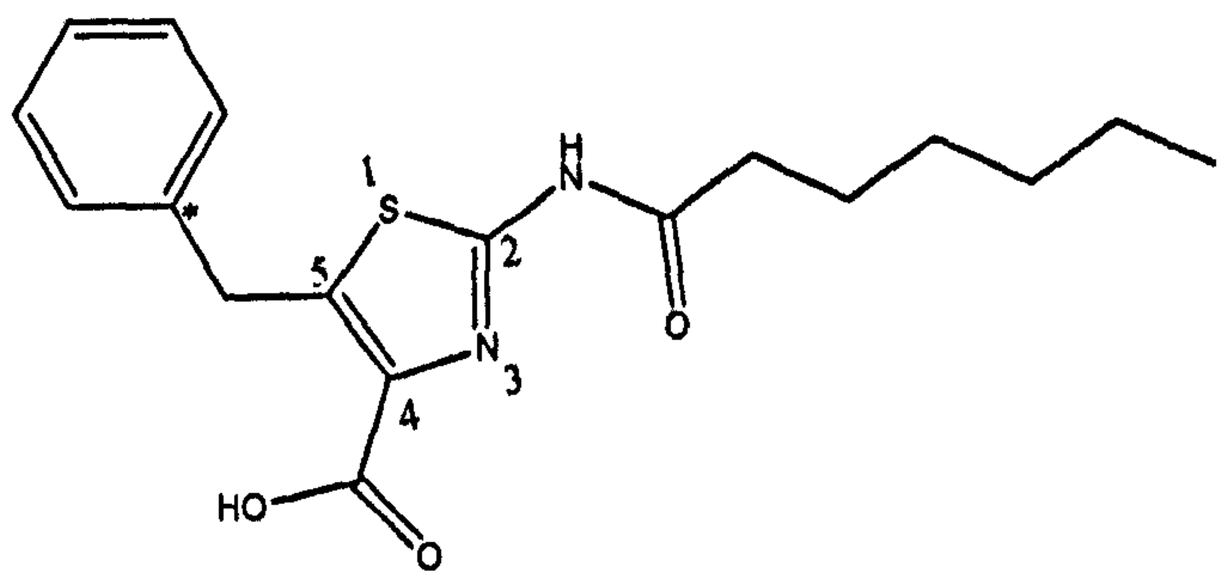
NMR: ^{1}**H NMR** (270MHz): δ($CDCl_3$): 0.86 (3H, t, $CH_3(CH_2)_4CH_2$-); 1.25 (6H, m, $CH_3(CH_2)_3CH_2CH_2$-); 1.66(2H,m,$CH_3(CH_2)_3CH_2CH_2$-); 2.39 (2H, t, J=7.29 Hz, $CH_3(CH_2)_3CH_2CH_2$-) ; 3.90 (3H, s, OCH_3); 4.55 (2H, s, CH_2-Ar); 7.23-7.38 (5H, m, Ar); 9.7 (1H, s, NH).

NMR: ^{13}**C NMR** (270MHz): δ($CDCl_3$): 14.6 ($CH_3(CH_2)_4CH_2$-); 23.0 ($CH_3CH_2(CH_2)_3CH_2$-); 25.5 ($CH_3CH_2CH_2(CH_2)_2CH_2$-); 29.3 ($CH_3(CH_2)_2CH_2CH_2$-CH_2-); 32.0 ($CH_3(CH_2)_2CH_2CH_2CH_2$-); 33.6 (Ar-CH_2-); 36.7 ($CH_3(CH_2)_2CH_2CH_2$-CH_2-); 51.0 (OCH_3); 127.0–129.0 (5C, Ar); 136.4 (C_4); 141.5 (C_5) 144.6 (C^*); 153.0 (C_2); 163.0 ($COOCH_3$); 171.9 (CONH).

CHN Analysis: Found: C, 63.39; H, 6.56; N, 7.65; S, 9.20 (Required for $C_{19}H_{24}N_2O_3S$: C, 63.31; H, 6.71; N, 7.77; S, 8.90).

5-Benzyl-2-heptanamidothiazole-4-carboxylic acid (70)

The title compound was obtained as a white powder (0.3g, 39.6%) using general procedure **C**.



M.P. 216-219 °C.

IR: ATR, υ_{max} (cm^{-1}): 3185, NH stretch, primary amide; 1693, C=O stretch, carboxylic acid; 1661, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 347.0, $C_{18}H_{22}N_2O_3S$; (m/z 100%): 217.0.

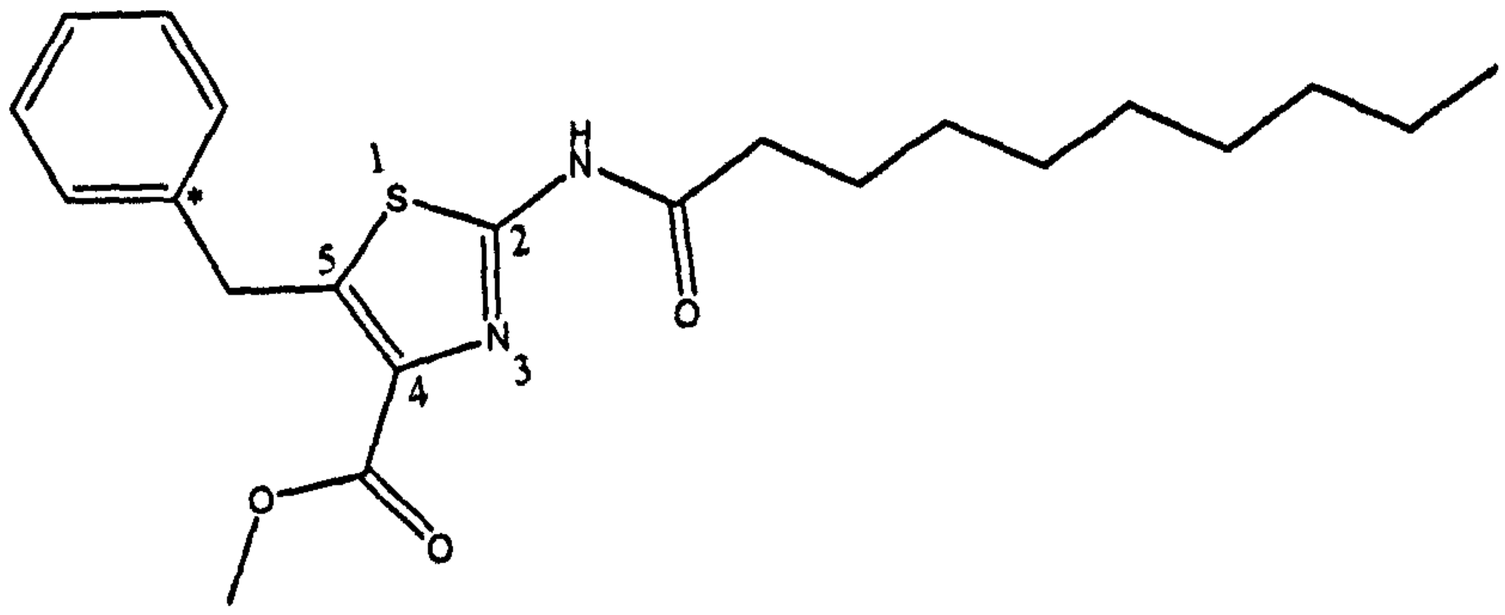
NMR: ^{1}**H NMR** (400MHz): δ(DMSO-d_6): 0.83 (3H, t, J=6.80 Hz, $CH_3(CH_2)_4CH_2$-); 1.22-1.27 (6H, m, $CH_3(CH_2)_3CH_2CH_2$-); 1.55(2H,m,$CH_3(CH_2)_3CH_2CH_2$-); 2.34 (2H, t, J=7.20 Hz, $CH_3(CH_2)_3CH_2CH_2$-) ; 4.47 (2H, s, CH_2-Ar); 7.28-7.31 (5H, m, Ar); 12.3 (1H, s, NH); 12.9 (1H, s, OH) .

NMR: ^{13}**C NMR** (400MHz): δ(DMSO-d_6): 14.4 (**C**$H_3(CH_2)_4CH_2$-); 22.4 (CH_3**C**$H_2(CH_2)_3CH_2$-); 25.1 (CH_3CH_2**C**$H_2(CH_2)_2CH_2$-); 28.6 ($CH_3(CH_2)_2$**C**H_2**C**H_2. CH_2-); 31.4 ($CH_3(CH_2)_2CH_2CH_2CH_2$-); 32.5 (Ar-CH_2-); 35.3 ($CH_3(CH_2)_2CH_2CH_2$. CH_2-); 127.1–129.3 (5C, Ar); 136.3 (C_4); 140.5 (C_5); 141.5 (C^*); 154.7 (C_2); 164.2 (**C**OOH); 172.3 (**C**ONH).

CHN Analysis: Found: C, 62.37; H, 6.20; N, 8.02; S, 9.37 (Required for $C_{18}H_{22}N_2O_3S$: C, 62.40; H, 6.40; N, 8.09; S, 9.26).

Methyl 5-benzyl-2-decanamidothiazole-4-carboxylate (71)

The title compound was obtained as a pale yellow powder (1.9g, 40.0%) using general procedure **B**.



M.P. 182-184 °C.

IR: ATR, υ_{max} (cm^{-1}): 3267, NH stretch, primary amide; 1715, C=O stretch, conjugated ester; 1685, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 403.3, $C_{22}H_{30}N_2O_3S$; (m/z 100%): 425.3, $C_{22}H_{30}N_2O_3S$ + 23.

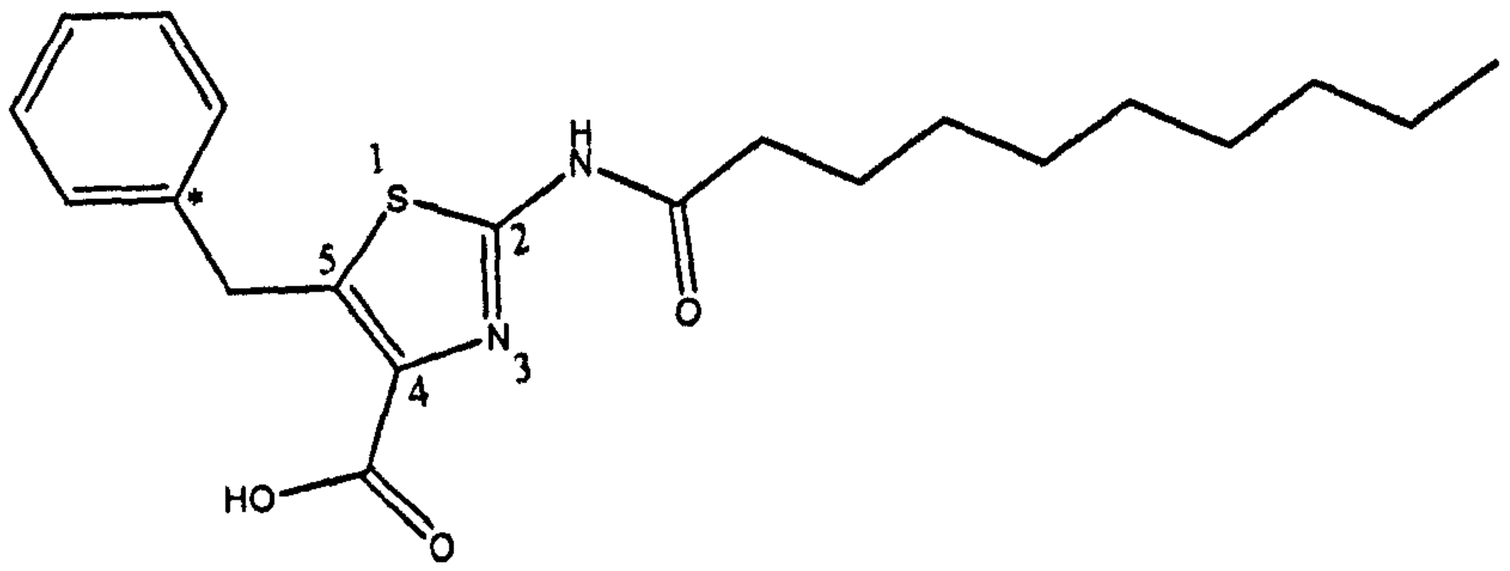
NMR: ^{1}**H NMR** (270MHz): δ($CDCl_3$): 0.87 (3H, t, J=6.39 Hz, C**H**$_3(CH_2)_7CH_2$-); 1.24 (12H, m, $CH_3(CH_2)_6$C**H**$_2$C**H**$_2$-);1.64 (2H,m,$CH_3(CH_2)_6$C**H**$_2$C**H**$_2$-); 2.37 (2H, t, J=7.29 Hz, $CH_3(CH_2)_6CH_2$C**H**$_2$-) ; 3.90 (3H, s, OC**H**$_3$) ; 4.52 (2H, s, C**H**$_2$-Ar); 7.23-7.31 (5H, m, Ar); 9.86 (1H, s, NH).

NMR: ^{13}**C NMR** (270MHz): δ($CDCl_3$): 14.7 ($CH_3(CH_2)_7CH_2$-); 23.3 ($CH_3CH_2(CH_2)_6CH_2$-); 25.5 ($CH_3CH_2CH_2(CH_2)_5CH_2$-); 29.7- 29.9 ($CH_3CH_2CH_2(CH_2)_4$ CH_2CH_2-); 32.4 (Ar-CH_2-); 33.6 ($CH_3(CH_2)_5CH_2CH_2CH_2$-); 36.7 ($CH_3(CH_2)_5CH_2CH_2CH_2$-); 52.7 (OCH_3); 127.6–129.0 (5C, Ar); 133.6 (C_4); 138.5 (C_5);143.9 (C^*); 155.8 (C_2); 163.3 ($COOCH_3$); 172.0 (CONH).

CHN Analysis: Found: C, 65.97; H, 7.12; N, 6.93; S, 7.84 (Required for $C_{22}H_{30}N_2O_3S$: C, 65.64; H, 7.51; N, 6.96; S, 7.97).

5-Benzyl-2-decanamidothiazole-4-carboxylic acid (72)

The title compound was obtained as a white powder (0.6g, 65.2%) using general procedure **C**.



M.P. 182-184 °C.

IR: ATR, υ_{max} (cm^{-1}): 3183, NH stretch, primary amide; 1689, C=O stretch, carboxylic acid; 1662, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 389.2, $C_{21}H_{28}N_2O_3S$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

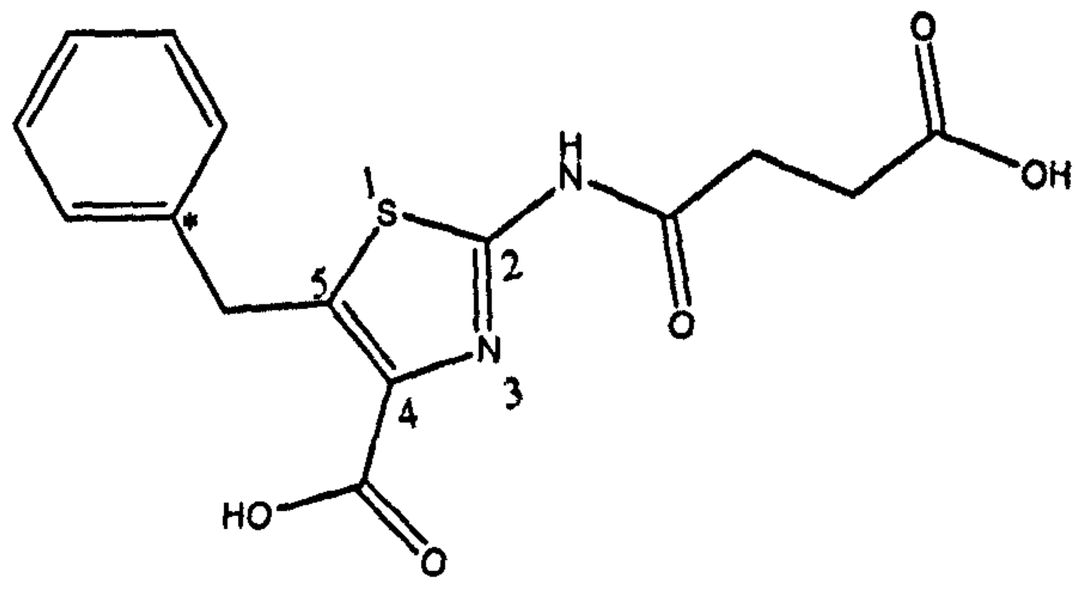
NMR: ^{1}**H NMR** (400MHz): δ($CDCl_3$): 0.84 (3H, t, J=6.6 Hz, $CH_3(CH_2)_7CH_2$-); 1.24 (12H, m, $CH_3(CH_2)_6CH_2CH_2$-);1.72 (2H,m,$CH_3(CH_2)_6CH_2CH_2$-); 2.49 (2H, t, J=7.48 Hz, $CH_3(CH_2)_6CH_2CH_2$-) ; 4.56 (2H, s, CH_2-Ar); 7.25-7.29 (5H, m, Ar); 12.5 (1H, s, NH); 15.3 (1H, s, OH).

NMR: ^{13}**C NMR** (400MHz): δ($CDCl_3$): 14.2 ($\mathbf{C}H_3(CH_2)_7CH_2$-); 22.7 ($CH_3\mathbf{C}H_2(CH_2)_6CH_2$-); 25.1 ($CH_3CH_2\mathbf{C}H_2(CH_2)_5CH_2$-); 29.3- 29.5 ($CH_3CH_2CH_2(\mathbf{C}H_2)_4$ CH_2CH_2-); 31.9 (Ar-CH_2-); 32.7 ($CH_3(CH_2)_5CH_2\mathbf{C}H_2CH_2$-); 36.2 ($CH_3(CH_2)_5CH_2CH_2\mathbf{C}H_2$-); 127.2–129.6 (5C, Ar); 133.9 (C_4); 138.9 (C_5); 143.0 (C^*); 157.2 (C_2); 166.3 (**C**OOH); 172.2 (**C**ONH).

CHN Analysis: Found: C, 64.72; H, 7.00; N, 7.12; S, 8.30 (Required for $C_{21}H_{28}N_2O_3S$: C, 64.92; H, 7.26; N, 7.21; S, 8.25).

5-Benzyl-2-(3-carboxypropanamido)thiazole-4-carboxylic acid (74)

The title compound was obtained as a white powder (0.2g, 25.0%) using general procedure **C**.



M.P. 258-260 °C.

IR: ATR, υ_{max} (cm^{-1}): 3185, NH stretch, primary amide; 1724, C=O stretch, carboxylic acid; 1671, carboxylic acid; 1654, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 335.0, $C_{15}H_{14}N_2O_5S$; (m/z 100%): 79.0.

NMR: ^{1}**H NMR** (400MHz): δ(DMSO-d_6): 2.5 (2H, m, OCC**H**$_2$$CH_2$COOH); 2.60 (2H, m, OC$CH_2$C**H**$_2$COOH); 4.47 (2H, s, C**H**$_2$-Ar); 7.26-7.31 (5H, m, Ar); 12.40 (1H, s, N**H**); 12.60 (1H, s, O**H**).

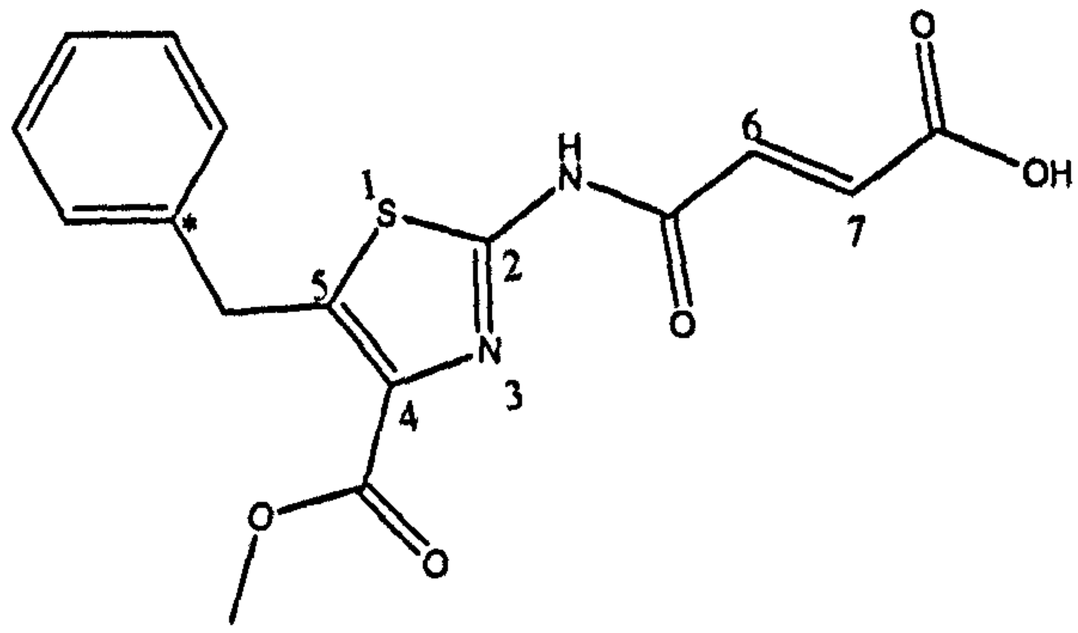
NMR: ^{13}**C NMR** (400MHz): δ(DMSO-d_6): 28.8 (Ar-CH_2-); 30.3 (NHCO$CH_2$$CH_2$COOH); 32.5 (NHCO$CH_2$**C**$H_2$COOH); 127.1–136.4 (5C, Ar); 136.4

(C_4); 140.5 (C_5); 141.5 (C^*); 154.7 (C_2); 164.0 (Ar-COOH); 171.3 (COOH), 174.0 (CONH).

CHN Analysis: Found: C, 54.46; H, 4.15; N, 8.49; S, 9.52 (Required for $C_{15}H_{14}N_2O_5S$: C, 53.88; H, 4.22; N, 8.38; S, 9.59).

4-(5-Benzyl-4-(methoxycarbonyl)thiazol-2-ylamino)-4-oxobut-2-enoic acid (75)

The title compound was obtained as a white powder (0.8g, 19.8%) using general procedure **E**.



M.P. 96-98 °C.

IR: ATR, υ_{max} (cm^{-1}): 3200, NH stretch, primary amide, OH carboxylic acid; 1723, C=O stretch, conjugated ester; 1701, carboxylic acid; 1690, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 347.2, $C_{16}H_{14}N_2O_5S$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

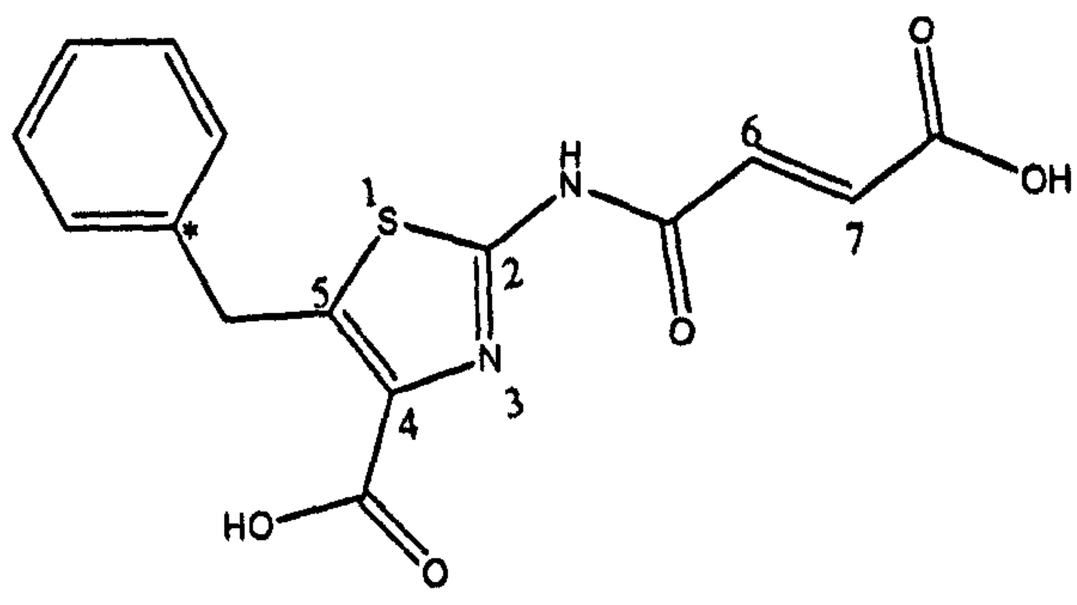
NMR: ^{1}H NMR (270MHz): δ(DMSO-d_6): 3.81 (3H, s, OCH_3); 4.48 (2H, s, CH_2-Ar); 6.37-6.48 (2H, dd, J=11.88 Hz, OCCHCHCOOH); 7.21-7.35 (5H, m, Ar); 12.75 (1H, s, NH).

NMR: ^{13}C NMR (400MHz): δ(DMSO-d_6): 32.5 (Ar-CH_2-); 52.3 (OCH_3); 127.3–129.3 (5C, Ar); 128.4 (C_6); 133.3 (C_7); 135.4 (C_4); 140.2 (C_5); 143.1 (C^*); 154.7 (C_2); 162.9 ($COOCH_3$); 163.6 (COOH), 167.4 (CONH).

CHN Analysis: Found: C, 55.46; H, 4.13; N, 7.88; S, 9.31 (Required for $C_{16}H_{14}N_2O_5S$: C, 55.48; H, 4.07; N, 8.09; S, 9.26).

5-Benzyl-2-(3-carboxyacrylamido)thiazole-4-carboxylic acid (76)

The title compound was obtained as a white powder (0.9g, 95.8%) using general procedure **C**.



M.P. 220-222 °C.

IR: ATR, υ_{max} (cm^{-1}): 3225, NH stretch, primary amide, OH carboxylic acid; 1700, C=O stretch, carboxylic acid; 1700, carboxylic acid; 1629, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 333.0, $C_{15}H_{12}N_2O_5S$; (m/z 100%): 79.0.

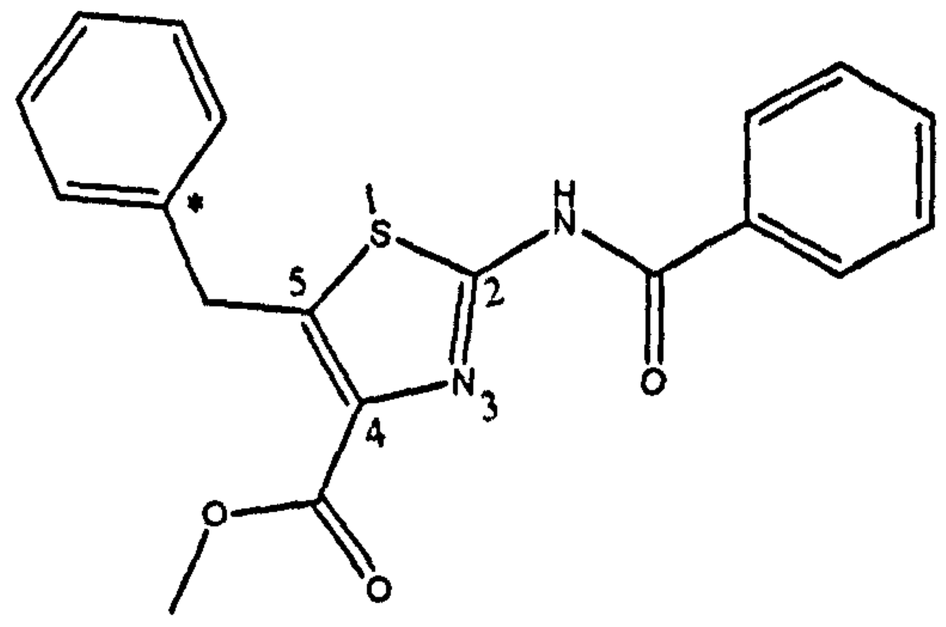
NMR: ^{1}H NMR (400MHz): δ(DMSO-d_6): 4.60 (2H, s, **CH$_2$**-Ar); 6.30-6.47 (2H, dd, J=11.88 Hz,OCC**H**C**H**COOH); 7.23-7.34 (5H, m, Ar).

NMR: ^{13}C NMR (400MHz): δ(DMSO-d_6): 32.6 (Ar-CH_2-); 127.2–129.2 (5C, Ar); 128.5 (C_6) 133.1 (C_7); 136.6 (C_4); 140.4 (C_5); 142.3 (C^*); 154.3 (C_2); 163.6 (Ar-**C**OOH); 164.1 (**C**OOH), 167.5 (**C**ONH).

CHN Analysis: Found: C, 54.29; H, 3.50; N, 8.45; S, 10.70 (Required for $C_{15}H_{12}N_2O_5S$: C, 54.21; H, 3.64; N, 8.43; S, 9.65).

Methyl 2-benzamido-5-benzylthiazole-4-carboxylate (77)

The title compound was obtained as a white powder (2.9g, 67.8%) using general procedure **B**.



M.P. 112-114 °C.

IR: ATR, υ_{max} (cm^{-1}): 3431, NH stretch, primary amide; 1725, C=O stretch, conjugated ester; 1660, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 353.2, $C_{19}H_{16}N_2O_3S$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

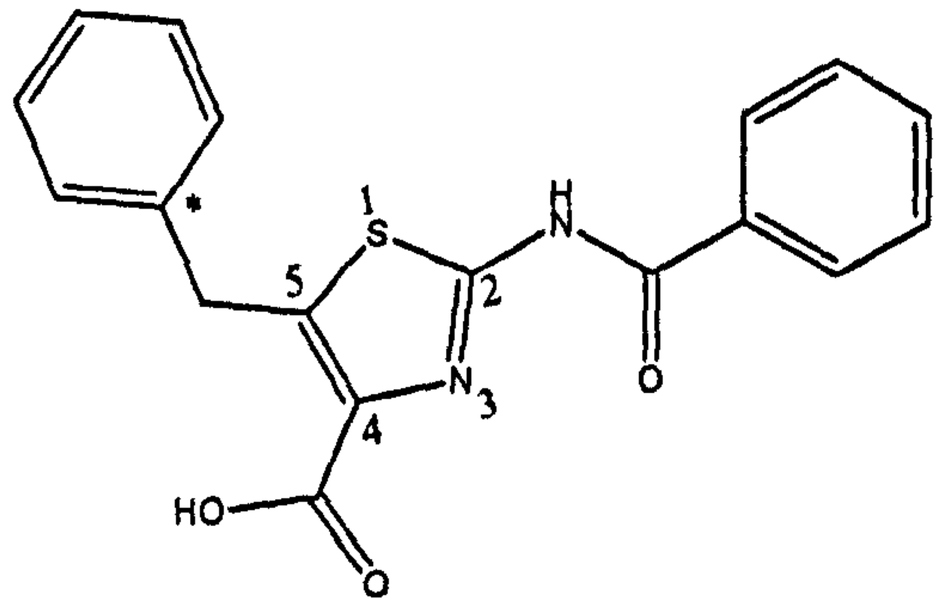
NMR: ^{1}H NMR (270MHz): δ($CDCl_3$): 3.78 (3H, s, OCH_3); 4.56 (2H, s, CH_2-Ar); 7.27-7.35 (5H, m, Ar); 7.35-7.38 (2H, t, Ar); 7.38-7.64 (1H, t, Ar); 7.97 (2H, d, Ar).

NMR: ^{13}C NMR (270MHz): δ($CDCl_3$): 33.5 (Ar-CH_2-); 53.0 (OCH_3); 128.0–132.3 (11C, Ar); 133.3 (C_4); 138.8 (C_5); 144.4 (C^*); 158.0 (C_2); 161.5 ($COOCH_3$); 165.8 (CONH).

CHN Analysis: Found: C, 60.26; H, 4.84; N, 7.27; S, 8.45 (Required for $C_{19}H_{16}N_2O_3S$: C, 64.76; H, 4.58; N, 7.95; S, 9.10).

2-Benzamido-5-benzylthiazole-4-carboxylic acid (78)

The title compound was obtained as a white powder (0.8g, 84.4%) using general procedure **C**.



M.P. 306-308 °C.

IR: ATR, υ_{max} (cm^{-1}): 3186, NH stretch, primary amide; 1670, C=O stretch, carboxylic acid; 1670, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 339.3, $C_{18}H_{14}N_2O_3S$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

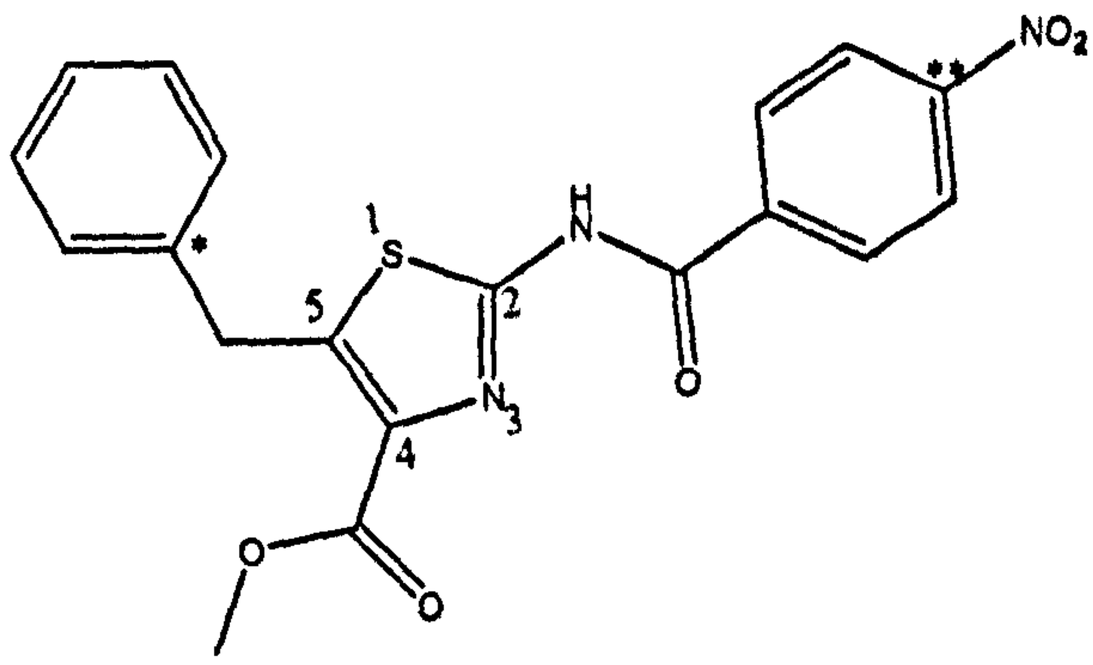
NMR: ^{1}H NMR (400MHz): δ(DMSO-d_6): 4.50 (2H, s, **CH$_2$**-Ar); 7.32-7.33 (5H, m, Ar); 7.50-7.53 (2H, t, Ar); 7.60-7.61 (1H, t, Ar); 8.06 (2H, d, Ar); 12.90 (1H, s, **OH**).

NMR: ^{13}C NMR (270MHz): δ($CDCl_3$): 32.5 (Ar-CH_2-); 127.2–132.0 (11C, Ar); 133.3 (C_4); 140.5 (C_5); 142.1 (C^*); 155.5 (C_2); 164.2 (**C**OOH); 165.9 (**C**ONH).

CHN Analysis: Found: C, 64.05; H, 4.17; N, 8.44; S, 9.56 (Required for $C_{18}H_{14}N_2O_3S$: C, 63.89; H, 4.17; N, 8.28; S, 9.48).

Methyl 5-benzyl-2-(4-nitrobenzamido)thiazole-4-carboxylate (79)

The title compound was obtained as a yellow powder (1.6g, 34.4%) using general procedure **B**.



M.P. 210-214 °C.

IR: ATR, υ_{max} (cm^{-1}): 3376, NH stretch, primary amide; 1699, C=O stretch, conjugated ester; 1668, C=O stretch, primary amide.

MS: +EI, (m/z): 397.1, $C_{19}H_{15}N_3O_5S$; (m/z 100%): 286.1.

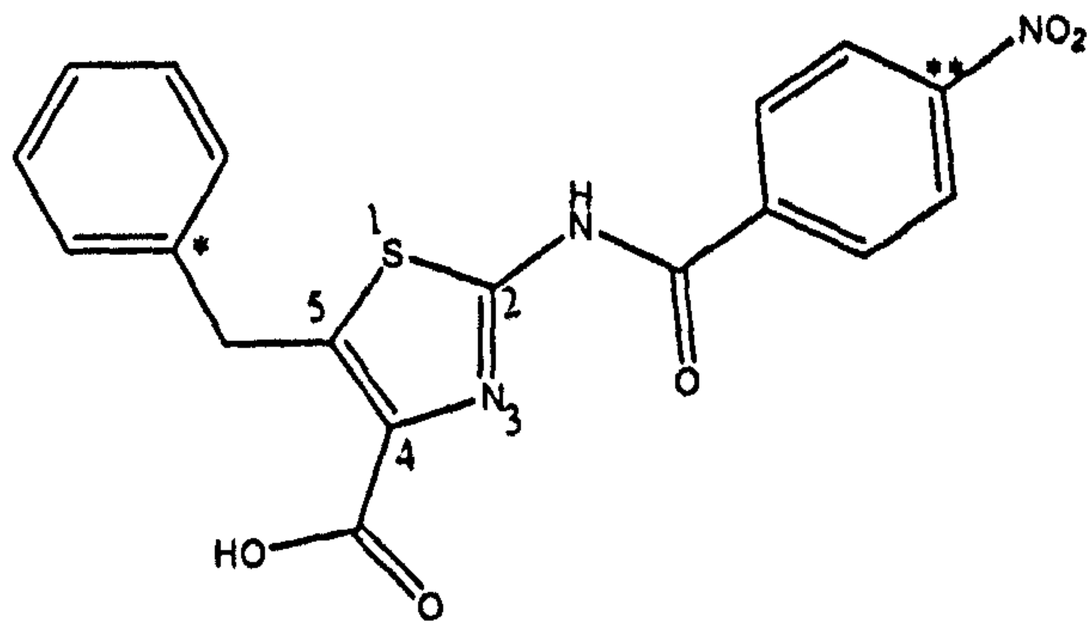
NMR: ^{1}H NMR (270MHz): δ($CDCl_3$): 3.65 (3H, s, OCH_3); 4.55 (2H, s, CH_2-Ar); 7.27-7.39 (5H, m, Ar); 8.03 (2H, d, J=8.80 Hz, Ar); 8.29 (2H, d, J=9.04 Hz, Ar); 11.35 (1H, s, NH).

NMR: ^{13}C NMR (270MHz): δ($CDCl_3$): 33.0 (Ar-CH_2-); 51.9 (OCH_3); 123.9–130.2 (10C, Ar); 137.9 (C_5); 140.3 (C_5); 142.5 (C^*); 152.0 (C^{**}); 157.0 (C_2); 163.0 ($COOCH_3$); 165.0 (CONH).

CHN Analysis: Found: C, 57.11; H, 3.71; N, 10.46; S, 8.11 (Required for $C_{19}H_{15}N_3O_5S$: C, 57.42; H, 3.80; N, 10.57; S, 8.07).

5-Benzyl-2-(4-nitrobenzamido)thiazole-4-carboxylic acid (80)

The title compound was obtained as a yellow powder (0.5g, 59.4%) using general procedure **C**.



M.P. 316-320 °C.

IR: ATR, υ_{max} (cm^{-1}): 3196, NH stretch, primary amide; 1669, C=O stretch, carboxylic acid; 1669, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 384.1, $C_{18}H_{13}N_3O_5S$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

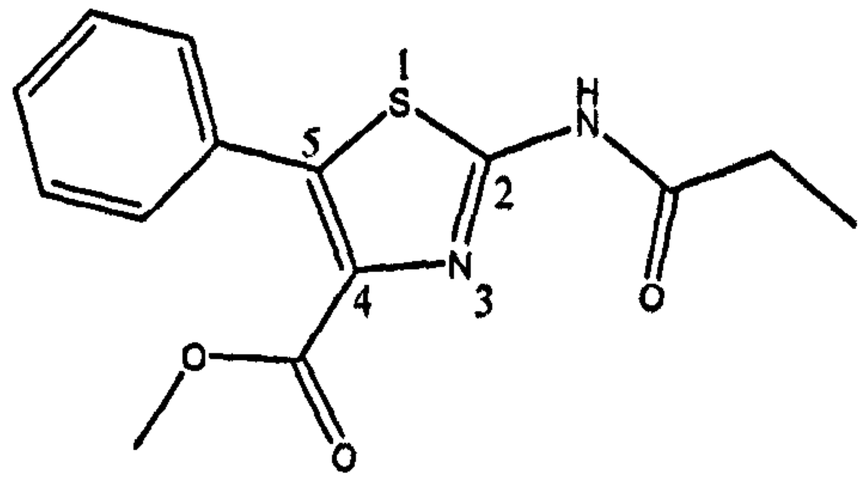
NMR: ^{1}H NMR (400MHz): δ(DMSO-d_6): 4.51 (2H, s, C**H**$_2$-Ar); 7.23-7.32 (5H, m, Ar); 8.24 (2H, d, J=8.80 Hz, Ar); 8.33 (2H, d, J=9.24 Hz, Ar).

NMR: ^{13}C NMR (270MHz): δ($CDCl_3$): 32.5 (Ar-CH_2-); 124.1–130.2 (10C, Ar); 137.3 (C_4); 140.5 (C_5); 142.0 (C^*); 151.2 (C^{**}); 157.0 (C_2); 164.0 (**C**OOH); 165.0 (**C**ONH).

CHN Analysis: Found: C, 56.30; H, 3.06; N, 10.99; S, 8.44 (Required for $C_{18}H_{13}N_3O_5S$: C, 56.39; H, 3.42; N, 10.96; S, 8.36).

Methyl 5-phenyl-2-propionamidothiazole-4-carboxylate (81)

The title compound was obtained as a white powder (1.0g, 25.6%) using general procedure **B**.



M.P. 238-242 °C.

IR: ATR, υ_{max} (cm^{-1}): 3245, NH stretch, primary amide; 1714, C=O stretch, conjugated ester; 1690, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 291.0, $C_{14}H_{14}N_2O_3S$; (m/z 100%): 291.0, $C_{14}H_{14}N_2O_3S$.

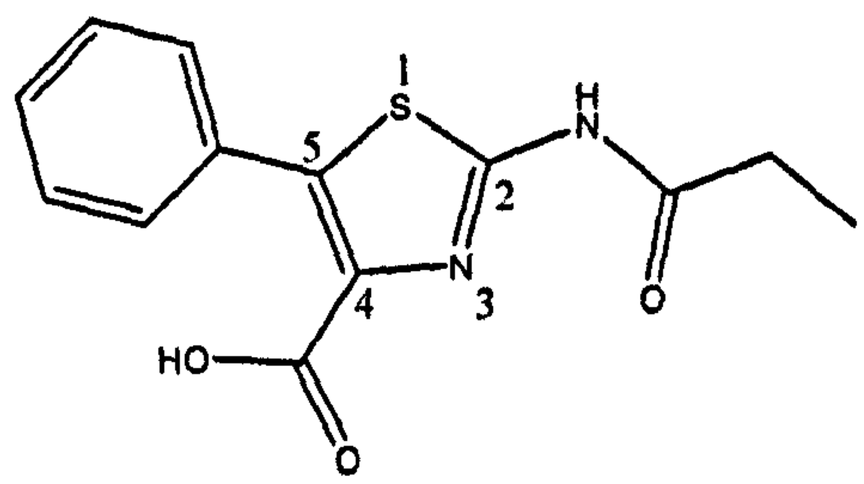
NMR: ^{1}H NMR (400MHz): δ(DMSO-d_6): 1.09 (3H, t, J=7.48 Hz, CH_3C**H**$_2$-); 2.46 (2H, q, J=7.48 Hz, CH_3C**H**$_2$-); 3.70 (3H, s, OC**H**$_3$); 7.42-7.48 (5H, m, Ar); 12.7 (1H, s, N**H**).

NMR: ^{13}**C NMR** (400MHz): δ(DMSO-d_6): 9.6 (CH_3CH_2CO); 28.7 (CH_3CH_2CO); 52.2 (OCH_3); 128.8 – 130.3 (6C, Ar); 134.0 (C_4); 139.0 (C_5); 156.0 (C_2); 163.0 ($COOCH_3$); 173.0 (CONH).

CHN Analysis: Found: C, 57.16; H, 4.33; N, 9.47; S, 11.19 (Required for $C_{14}H_{14}N_2O_3S$: C, 57.92; H, 4.86; N, 9.65; S, 11.04).

5-Phenyl-2-propionamidothiazole-4-carboxylic acid (82)

The title compound was obtained as a white powder (0.8g, 85.1%) using general procedure **C**.



M.P. 262-264 °C.

IR: ATR, υ_{max} (cm^{-1}): 3180, NH stretch, primary amide; 1681, C=O stretch, carboxylic acid; 1681, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 277.0, $C_{13}H_{12}N_2O_3S$; (m/z 100%): 45.0.

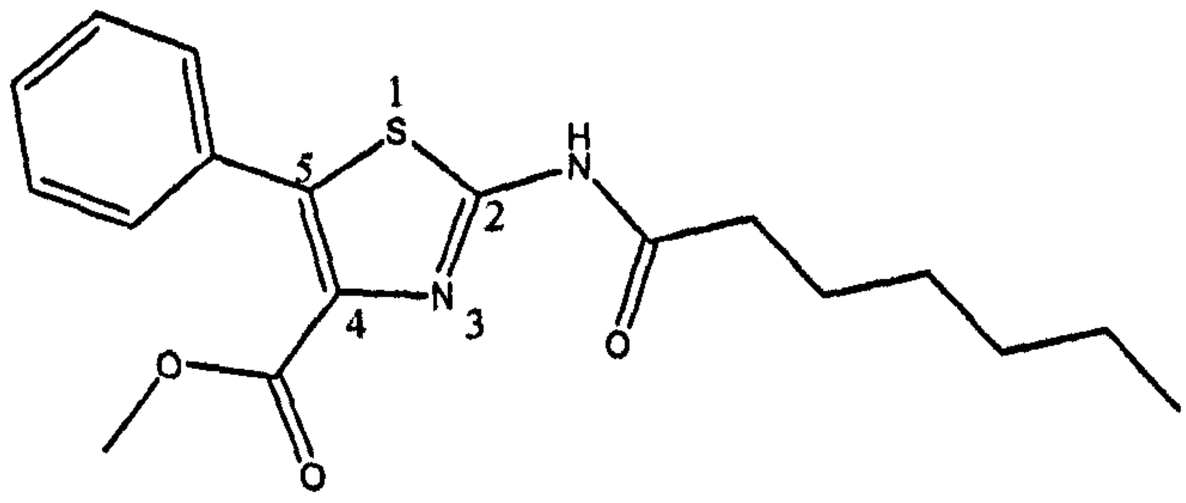
NMR: ^{1}**H NMR** (400MHz): δ(DMSO-d_6): 1.09 (3H, t, J=7.48 Hz, CH_3CH_2-); 2.46 (2H, q, J=7.48 Hz, CH_3CH_2-); 7.42-7.48 (5H, m, Ar); 12.5 (1H, s, NH); 12.8 (1H, s, OH).

NMR: ^{13}**C NMR** (400MHz): δ(DMSO-d_6): 9.6 (CH_3CH_2CO); 28.7 (CH_3CH_2CO); 128.8 – 131.2 (6C, Ar); 136.2 (C_4); 137.6 (C_5); 155.7 (C_2); 163.9 (COOH); 173.3 (CONH).

CHN Analysis: Found: C, 56.49; H, 3.37; N, 10.17; S, 11.85 (Required for $C_{13}H_{12}N_2O_3S$: C, 56.51; H, 4.38; N, 10.14; S, 11.60).

Methyl 2-heptanamido-5-phenylthiazole-4-carboxylate (83)

The title compound was obtained as a white powder (1.1g, 23.6%) using general procedure **B**.



M.P. 198-200 °C.

IR: ATR, υ_{max} (cm^{-1}): 3266, NH stretch, primary amide; 1716, C=O stretch, conjugated ester; 1684, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 347.0, $C_{18}H_{22}N_2O_3S$; (m/z 100%): 369.0, $C_{18}H_{22}N_2O_3S$ + Na.

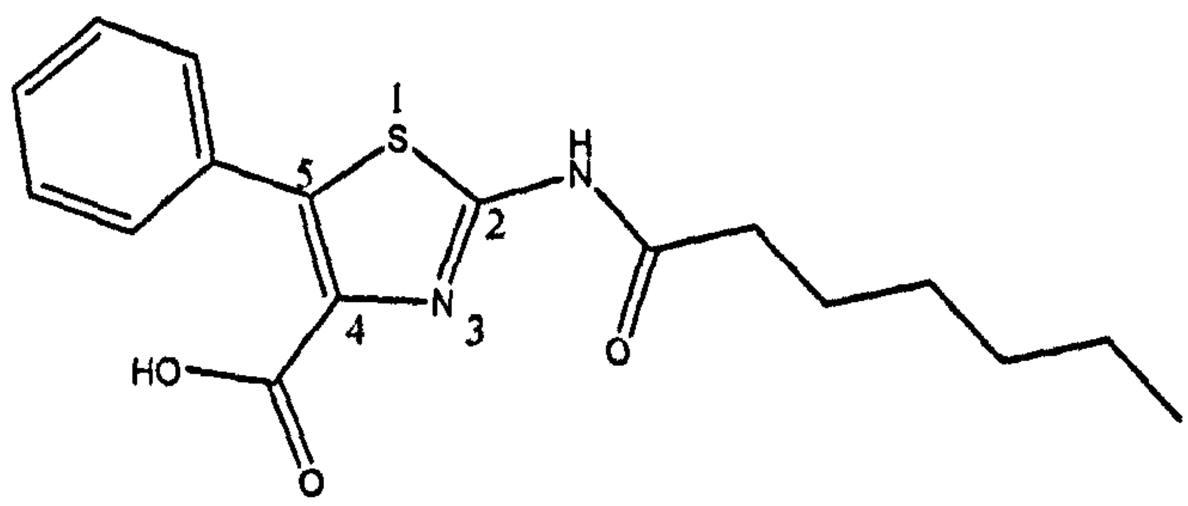
NMR: ^{1}**H NMR** (400MHz): δ($CDCl_3$): 0.86 (3H, t, J=6.6 Hz, $CH_3(CH_2)_4CH_2$-); 1.28 (6H, m, $CH_3(CH_2)_3CH_2CH_2$-); 1.69 (2H,m,$CH_3(CH_2)_3CH_2CH_2$-); 2.47 (2H, t, J=7.9 Hz, $CH_3(CH_2)_3CH_2CH_2$-) ; 3.76 (3H, s, OCH_3); 7.39-7.49 (5H, m, Ar); 9.9 (1H, s, NH) .

NMR: ^{13}**C NMR** (400MHz): δ($CDCl_3$): 14.0 ($CH_3(CH_2)_4CH_2$-); 22.5 ($CH_3CH_2(CH_2)_3CH_2$-); 25.0 ($CH_3CH_2CH_2(CH_2)_2CH_2$-); 28.8 ($CH_3(CH_2)_2CH_2CH_2$-CH_2-); 31.4 ($CH_3(CH_2)_2CH_2CH_2CH_2$-); 36.2 ($CH_3(CH_2)_2CH_2CH_2CH_2$-); 52.1 (OCH_3); 128.2–130.0 (6C, Ar); 134.0 (C_4); 141.0 (C_5); 156.0 (C_2); 163.0 ($COOCH_3$); 171.9 (CONH).

CHN Analysis: Found: C, 62.01; H, 6.66; N, 7.74; S, 9.18 (Required for $C_{18}H_{22}N_2O_3S$: C, 62.40; H, 6.40; N, 8.09; S, 9.26).

2-Heptanamido-5-phenylthiazole-4-carboxylic acid (84)

The title compound was obtained as a white powder (0.8g, 83.2%) using general procedure **C**.



M.P. 292-294 °C.

IR: ATR, υ_{max} (cm^{-1}): 3170, NH stretch, primary amide; 1678, C=O stretch, carboxylic acid; 1678, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 333.0, $C_{17}H_{20}N_2O_3S$; (m/z 100%): 78.

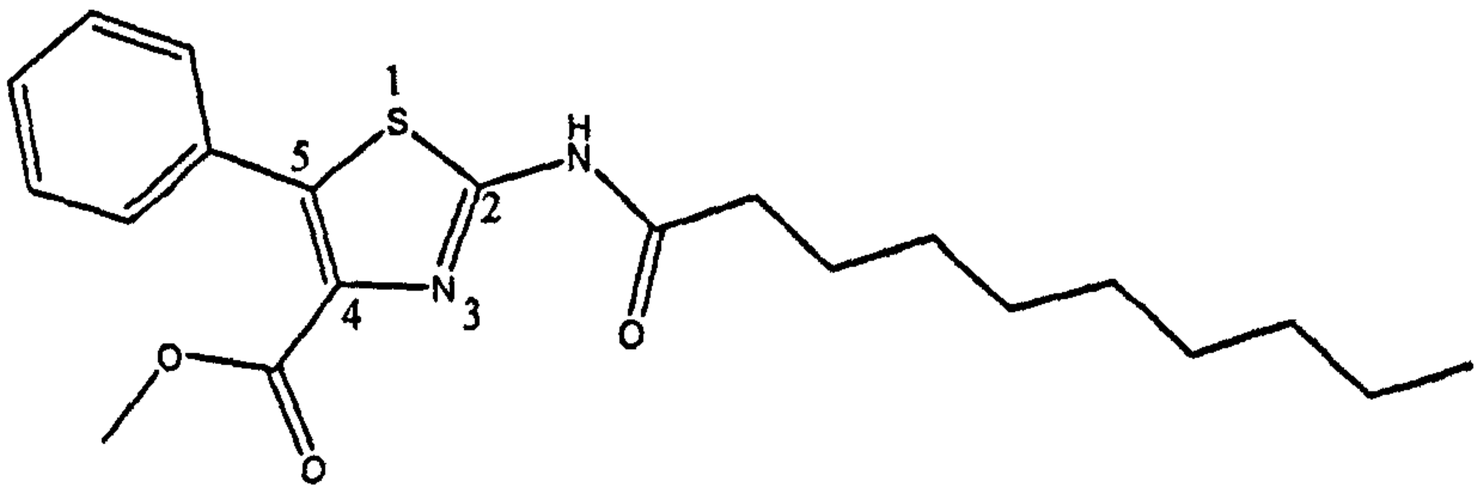
NMR: ^{1}H NMR (400MHz): δ($CDCl_3$): 0.89 (3H, t, J=7.0 Hz, $CH_3(CH_2)_4CH_2$-); 1.24 (6H, m, $CH_3(CH_2)_3CH_2CH_2$-); 1.62 (2H,m,$CH_3(CH_2)_3CH_2CH_2$-); 2.29 (2H, t, J=7.9 Hz, $CH_3(CH_2)_3CH_2CH_2$-) ; 7.40-7.60 (5H, m, Ar); 12.5 (1H, s, NH), 15.1 (1H, s, OH).

NMR: ^{13}C NMR (400MHz): δ($CDCl_3$): 14.5 ($CH_3(CH_2)_4CH_2$-); 22.6 ($CH_3CH_2(CH_2)_3CH_2$-); 24.8 ($CH_3CH_2CH_2(CH_2)_2CH_2$-); 28.9 ($CH_3(CH_2)_2CH_2CH_2$. CH_2-); 31.4 ($CH_3(CH_2)_2CH_2CH_2CH_2$-); 35.8 ($CH_3(CH_2)_2CH_2CH_2CH_2$-); 127.9–130.5 (6C, Ar); 132.9 (C_5); 141.0 (C_4); 157.7 (C_2); 165.5 (COOH); 172.3 (CONH).

CHN Analysis: Found: C, 61.35; H, 5.52; N, 8.49; S, 9.92 (Required for $C_{17}H_{20}N_2O_3S$: C, 61.42; H, 6.06; N, 8.43; S, 9.65).

Methyl 2-decanamido-5-phenylthiazole-4-carboxylate (85)

The title compound was obtained as a white powder (1.5g, 30.6%) using general procedure **B**.



M.P. 98-100 °C.

IR: ATR, υ_{max} (cm^{-1}): 3251, NH stretch, primary amide; 1700, C=O stretch, conjugated ester; 1683, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 389.0, $C_{21}H_{28}N_2O_3S$; (m/z 100%): 411.0, $C_{21}H_{28}N_2O_3S$ + Na.

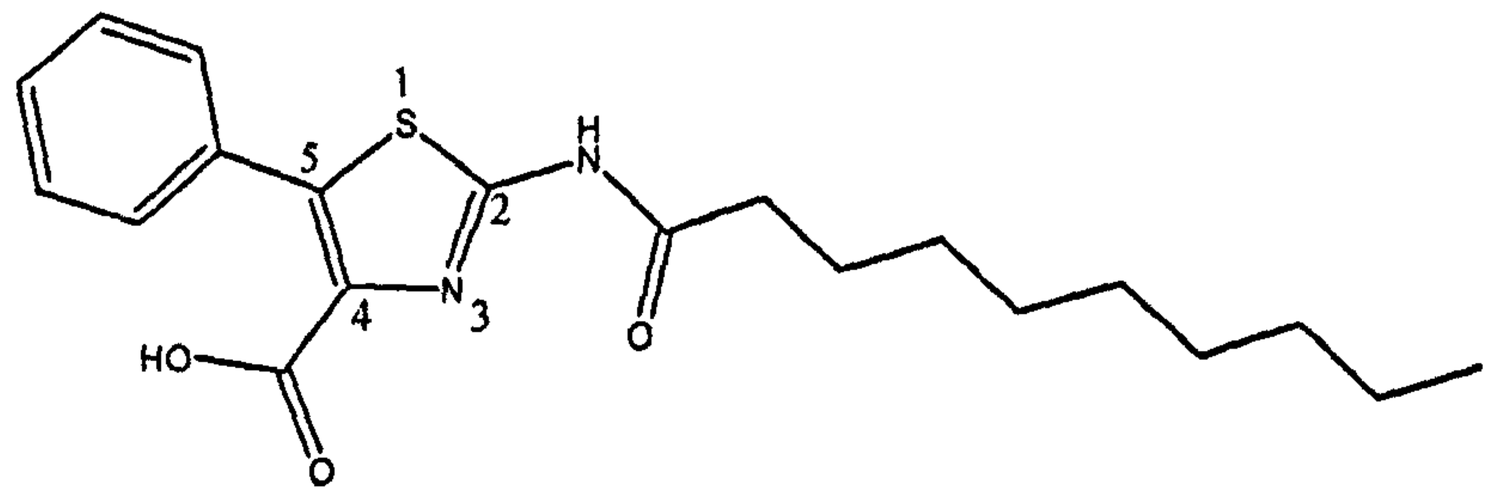
NMR: ^{1}H NMR (400MHz): δ($CDCl_3$): 0.86 (3H, t, J=6.6 Hz, $CH_3(CH_2)_7CH_2$-); 1.26 (12H, m, $CH_3(CH_2)_6CH_2CH_2$-);1.68 (2H,m,$CH_3(CH_2)_6CH_2CH_2$-); 2.42 (2H, t, J=7.8 Hz, $CH_3(CH_2)_6CH_2CH_2$-) ; 3.76 (3H, s, OCH_3) ; 7.40-7.48 (5H, m, Ar); 10.1 (1H, s, NH).

NMR: ^{13}C NMR (400MHz): δ($CDCl_3$): 14.1 ($CH_3(CH_2)_7CH_2$-); 22.7 ($CH_3CH_2(CH_2)_6CH_2$-); 25.0 ($CH_3CH_2CH_2(CH_2)_5CH_2$-); 29.2- 29.45 ($CH_3CH_2CH_2(CH_2)_4$ CH_2CH_2-); 31.9 ($CH_3(CH_2)_5CH_2CH_2CH_2$-); 36.2 ($CH_3(CH_2$.$)_5CH_2CH_2CH_2$-); 52.1 (OCH_3); 128.2–130.3 (6C, Ar); 133.9 (C_4); 141.3 (C_5); 156.3 (C_2); 162.4 ($COOCH_3$); 171.8 (CONH).

CHN Analysis: Found: C, 65.42; H, 7.01; N, 6.97; S, 9.21 (Required for $C_{21}H_{28}N_2O_3S$: C, 64.92; H, 7.26; N, 7.21; S, 8.25).

2-Decanamido-5-phenylthiazole-4-carboxylic acid (86)

The title compound was obtained as a white powder (0.8g, 82.1%) using general procedure **C**.



M.P. 220-222 °C.

IR: ATR, υ_{max} (cm^{-1}): 3170, NH stretch, primary amide; 1676, C=O stretch, carboxylic acid; 1676, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 375.0, $C_{20}H_{26}N_2O_3S$; (m/z 100%): 78.

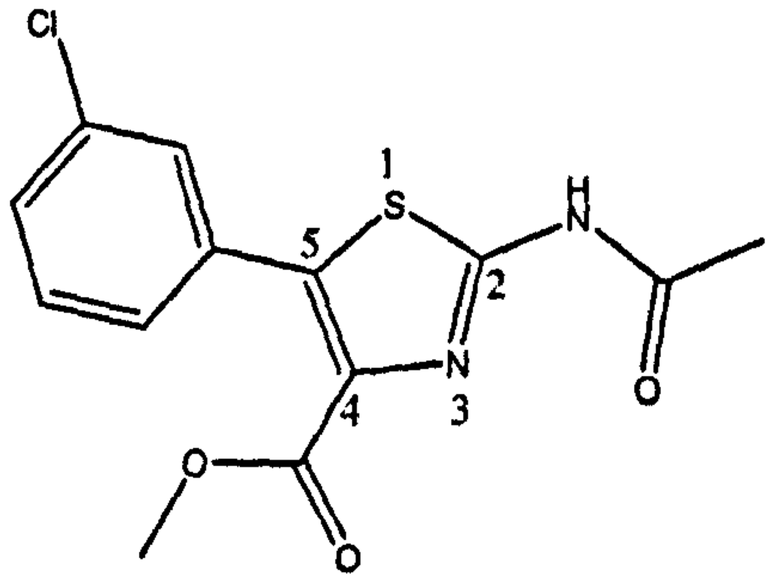
NMR: ^{1}H NMR (400MHz): δ($CDCl_3$): 0.87 (3H, t, J=7.04 Hz, $CH_3(CH_2)_7CH_2$-); 1.27 (12H, m, $CH_3(CH_2)_6CH_2CH_2$-);1.62 (2H,m,$CH_3(CH_2)_6CH_2CH_2$-); 2.25 (2H, t, J=7.48 Hz, $CH_3(CH_2)_6CH_2CH_2$-) ; 7.48-7.58 (5H, m, Ar); 12.5 (1H, s, NH); 15.1 (1H, s, OH).

NMR: ^{13}C NMR (400MHz): δ($CDCl_3$): 14.2 ($CH_3(CH_2)_7CH_2$-); 22.8 ($CH_3CH_2(CH_2)_6CH_2$-); 24.8 ($CH_3CH_2CH_2(CH_2)_5CH_2$-); 29.2- 29.7 ($CH_3CH_2CH_2(CH_2)_4$ CH_2CH_2-); 32.0 ($CH_3(CH_2)_5CH_2CH_2CH_2$-); 35.8 ($CH_3(CH_2$-$)_5CH_2CH_2CH_2$-); 127.9–130.5 (6C, Ar); 132.9 (C_4); 141.5 (C_5); 157.7 (C_2); 165.5 (COOH); 172.3 (CONH);

CHN Analysis: Found: C, 63.73; H, 6.68; N, 7.37; S, 8.46 (Required for $C_{20}H_{26}N_2O_3S$: C, 64.14; H, 7.00; N, 7.48; S, 8.56).

Methyl 2-acetamido-5-(3-chlorophenyl)thiazole-4-carboxylate (87)

The title compound was obtained as a white powder (2.9g, 85.0%) using general procedure **B**.



M.P. 245-248 °C.

IR: ATR, υ_{max} (cm^{-1}): 3224, NH stretch, primary amide; 1723, C=O stretch, conjugated ester; 1723, C=O stretch, primary amide.

MS: CI/ISO, (M+H): 311.3, $C_{13}H_{11}N_2O_3SCl$; (M+H 100%): 311.3, $C_{13}H_{11}N_2O_3SCl$.

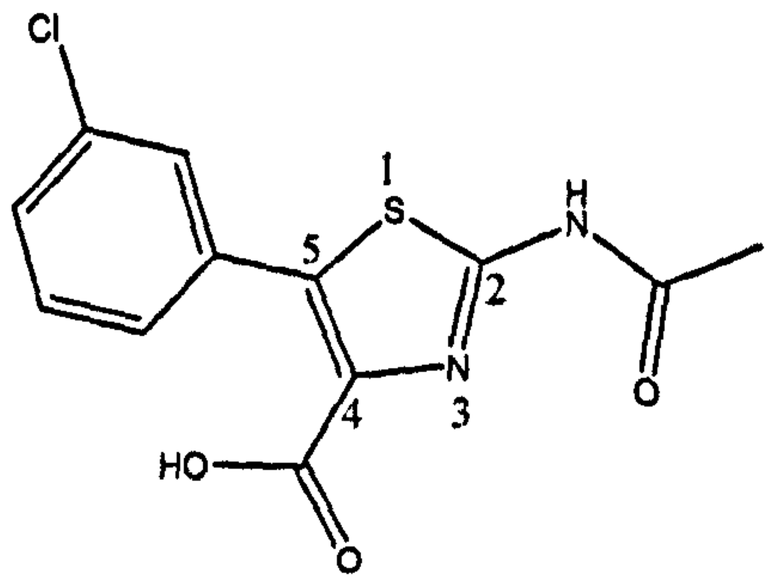
NMR: ^{1}H NMR (400MHz): δ(DMSO-d_6): 2.16 (3H, s, **CH$_3$**CO-); 3.72 (3H, s, -O**CH$_3$**); 7.43-7.47 (3H, m, Ar); 7.59 (1H, s, Ar).

NMR: ^{13}C NMR (400MHz): δ(DMSO-d_6): 22.8 (**CH$_3$**CO); 52.3 (O**CH$_3$**); 129.1 – 133.3 (6C, Ar); 135.3 (C_4); 137.1 (C_5); 156.3 (C_2); 162.5 (**C**OOCH$_3$); 169.7 (**C**ONH).

CHN Analysis: Found: C, 50.22; H, 3.17; N, 8.91; S, 10.64; Cl, 15.05 (Required for $C_{13}H_{11}N_2O_3SCl$: C, 50.24; H, 3.57; N, 9.01; S, 10.32; Cl, 11.41).

2-Acetamido-5-(3-chlorophenyl)thiazole-4-carboxylic acid (88)

The title compound was obtained as a white powder (0.6g, 71.5%) using general procedure **C**.



M.P. 264-266 °C.

IR: ATR, υ_{max} (cm^{-1}): 3176, NH stretch, primary amide; 1699, C=O stretch, carboxylic acid; 1669, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 297.1, $C_{12}H_9N_2O_3SCl$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

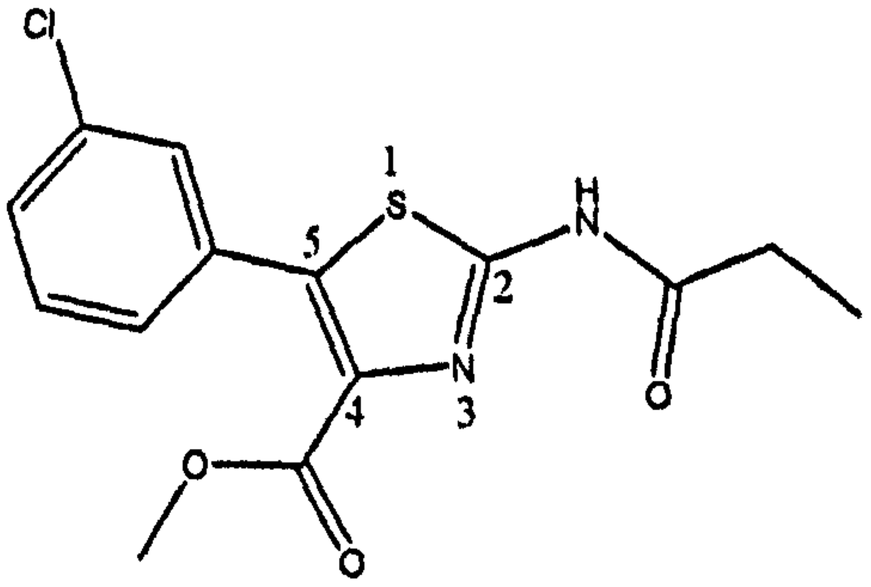
NMR: ^{1}H NMR (400MHz): δ(DMSO-d_6): 2.20 (3H, s, CH_3CO-); 7.44-7.47 (3H, m, Ar); 7.58 (1H, s, Ar).

NMR: ^{13}C NMR (400MHz): δ(DMSO-d_6): 22.9 (CH_3CO); 128.9 – 133.4 (6C, Ar); 135.9 (C_4); 136.9 (C_5); 156.1 (C_2); 163.6 (COOH); 169.7 (CONH).

CHN Analysis: Found: C, 47.29; H, 2.41; N, 9.28; S, 10.79; Cl, 13.24 (Required for $C_{12}H_9N_2O_3SCl$: C, 48.57; H, 3.06; N, 9.44; S, 10.81; Cl, 11.95).

Methyl 5-(3-chlorophenyl)-2-propionamidothiazole-4-carboxylate (89)

The title compound was obtained as a white powder (2.6g, 73.0%) using general procedure **B**.



M.P. 220-222 °C.

IR: ATR, υ_{max} (cm^{-1}): 3258, NH stretch, primary amide; 1718, C=O stretch, conjugated ester; 1685, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 325.0, $C_{14}H_{13}N_2O_3SCl$; (m/z 100%): 63.0.

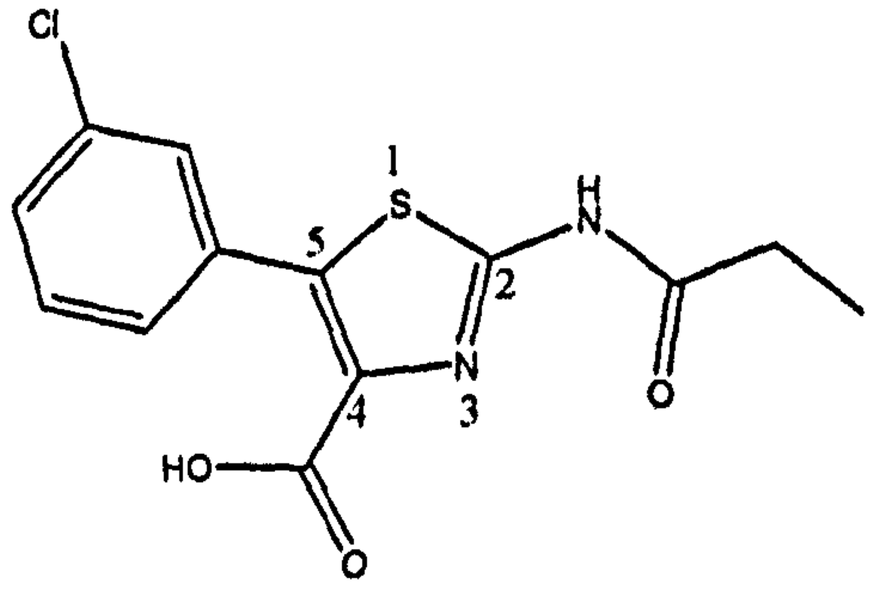
NMR: ^{1}H NMR (400MHz): δ(DMSO-d_6): 1.09 (3H, t, J=7.80 Hz, CH_3CH_2-); 2.46 (2H, q, J=7.88 Hz CH_3CH_2-); 3.72 (3H, s, -OCH_3); 7.44-7.47 (3H, m, Ar); 7.60 (1H, s, Ar).

NMR: ^{13}C NMR (400MHz): δ(DMSO-d_6): 9.5 (CH_3CH_2CO); 28.7 (CH_3CH_2CO); 52.3 (OCH_3); 129.1 – 133.3 (6C, Ar); 135.3 (C_4); 137.0 (C_5); 156.4 (C_2); 162.5 ($COOCH_3$); 173.4 (CONH).

CHN Analysis: Found: C, 51.65; H, 3.99; N, 8.64; S, 10.37; Cl, 11.15 (Required for $C_{14}H_{13}N_2O_3SCl$: C, 51.77; H, 4.03; N, 8.63; S, 9.87; Cl, 10.92).

5-(3-Chlorophenyl)-2-propionamidothiazole-4-carboxylic acid (90)

The title compound was obtained as a white powder (0.4g, 48.1%) using general procedure **C**.



M.P. 250-252 °C.

IR: KBr disk, υ_{max} (cm^{-1}): 3180, NH stretch, primary amide; 1696, C=O strctch, carboxylic acid; 1676, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 311.1, $C_{13}H_{11}N_2O_3SCl$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

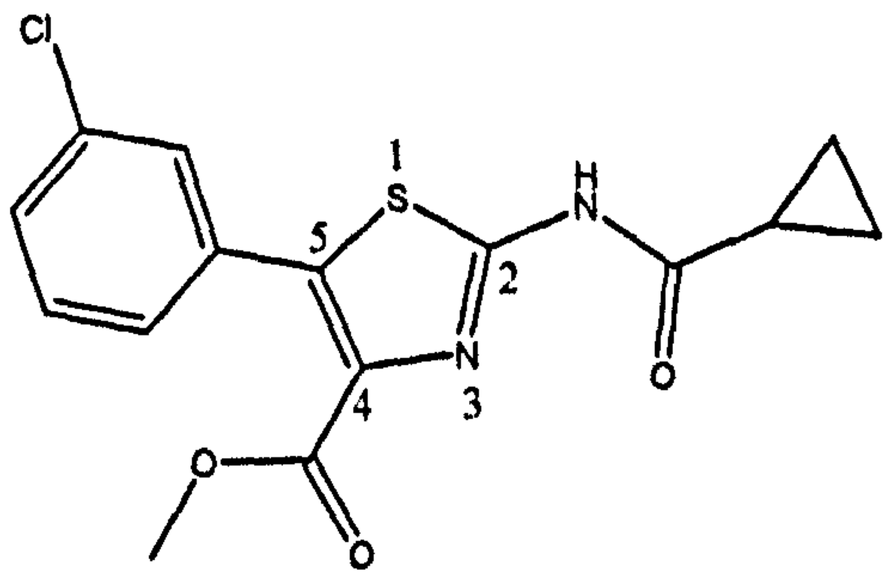
NMR: ^{1}**H NMR** (400MHz): δ(DMSO-d_6): 1.09 (3H, t, J=8.80 Hz, CH_3CH_2-); 2.50 (2H, q, J=7.60 Hz, CH_3CH_2-); 7.44-7.47 (3H, m, Ar); 7.58 (1H, s, Ar).

NMR: ^{13}**C NMR** (400MHz): δ(DMSO-d_6): 9.6 (CH_3CH_2CO); 28.7 (CH_3CH_2CO); 128.9 – 133.4 (6C, Ar); 135.8 (C_4); 136.9 (C_5); 156.1 (C_2); 163.6 ($COOCH_3$); 173.3 (CONH).

CHN Analysis: Found: C, 50.56; H, 3.26; N, 8.99; S, 10.24; Cl, 11.50 (Required for $C_{13}H_{11}N_2O_3SCl$: C, 50.24; H, 3.57; N, 9.01; S, 10.32; Cl, 11.41).

Methyl 5-(3-chlorophenyl)-2-(cyclopropanecarboxamido)thiazole-4-carboxylate (91)

The title compound was obtained as a white powder (2.3g, 62.0%) using general procedure **B**.



M.P. 198-200 °C.

IR: ATR, υ_{max} (cm^{-1}): 3219, NH stretch, primary amide; 1721, C=O stretch, conjugated ester; 1654, C=O stretch, primary amide.

MS: CI/ISO, (M+H): 337.2, $C_{15}H_{13}N_2O_3SCl$; (M+H 100%): 71.1, $C_3H_6N_2$.

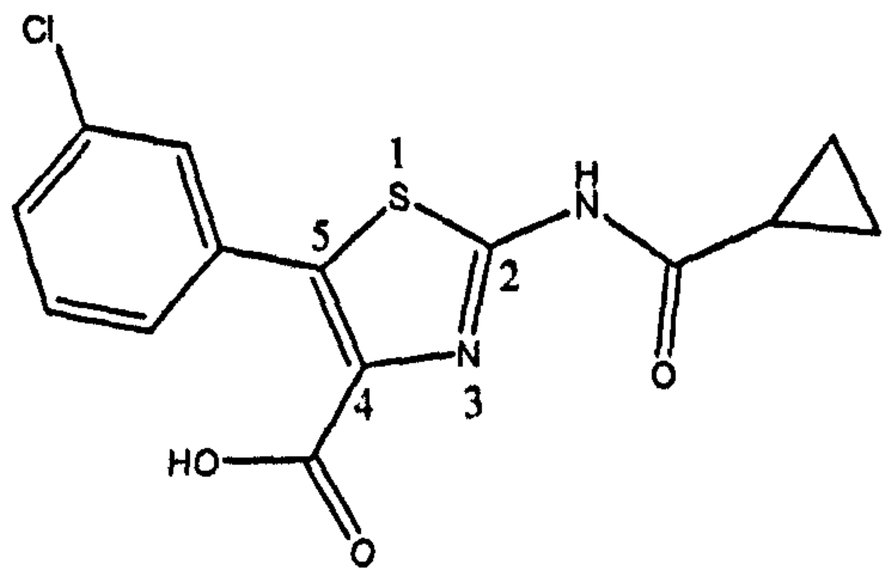
NMR: ^{1}**H NMR** (400MHz): δ(DMSO-d_6): 0.92-0.98 (4H, m, cyclopropyl); 1.93 (1H, m, cyclopropyl); 3.70 (3H, s, -OCH_3); 7.44-7.46 (3H, m, Ar); 7.57 (1H, s, Ar).

NMR: ^{13}**C NMR** (400MHz): δ(DMSO-d_6): 9.0 (2C, cyclopropyl); 14.2 (1C, cycloproyl); 52.2 (OCH_3); 129.0 – 133.4 (6C, Ar); 135.6 (C_4); 136.9 (C_5); 156.4 (C_2); 162.5 (**C**$OOCH_3$); 173.2 (**C**ONH).

CHN Analysis: Found: C, 53.31; H, 3.39; N, 8.25; S, 9.90; Cl, 10.74 (Required for $C_{15}H_{13}N_2O_3SCl$: C, 53.49; H, 3.89; N, 8.32; S, 9.52; Cl, 10.53).

5-(3-Chlorophenyl)-2-(cyclopropanecarboxamido)thiazole-4-carboxylic acid (92)

The title compound was obtained as a white powder (0.2g, 21.9%) using general procedure **C**.



M.P. 270-272 °C.

IR: ATR, υ_{max} (cm^{-1}): 3185, NH stretch, primary amide; 1696, C=O stretch, carboxylic acid; 1662, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 323.2, $C_{14}H_{11}N_2O_3SCl$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

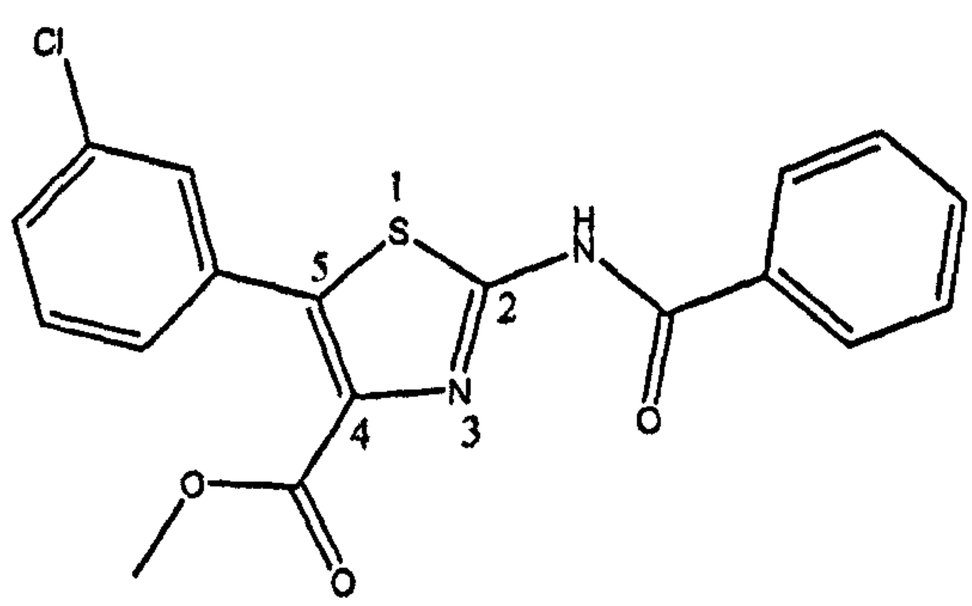
NMR: ^{1}**H NMR** (400MHz): δ(DMSO-d_6): 0.92-0.98 (4H, m, Cyclopropyl); 1.90 (1H, m, Cyclopropyl); 7.44-7.47 (3H, m, Ar); 7.57 (1H, s, Ar); 12.90 (1H, s, OH).

NMR: ^{13}**C NMR** (400MHz): δ(DMSO-d_6): 9.0 (2C, Cyclopropyl); 14.0 (1C, Cyclopropyl); 129.0 – 133.0 (6C, Ar); 135.5 (C_4); 137.0 (C_5); 156.5 (C_2); 164.5 (**C**OOH); 173.5 (**C**ONH).

CHN Analysis: Found: C, 52.03; H, 3.19; N, 8.62; S, 10.10; Cl, 10.96 (Required for $C_{14}H_{11}N_2O_3SCl$: C, 52.10; H, 3.44; N, 8.68; S, 9.93; Cl, 10.98).

Methyl 2-benzamido-5-(3-chlorophenyl)thiazole-4-carboxylate (93)

The title compound was obtained as a white powder (0.8g, 20.0%) using general procedure **B**.



M.P. 150-152 °C.

IR: ATR, υ_{max} (cm^{-1}): 3247, NH stretch, primary amide; 1695, C=O stretch, conjugated ester; 1684, C=O stretch, primary amide.

MS: CI/ISO, (M+H): 373.2, $C_{18}H_{13}N_2O_3SCl$; (M+H 100%): 311.2, $C_{16}H_{14}N_2O_3S$.

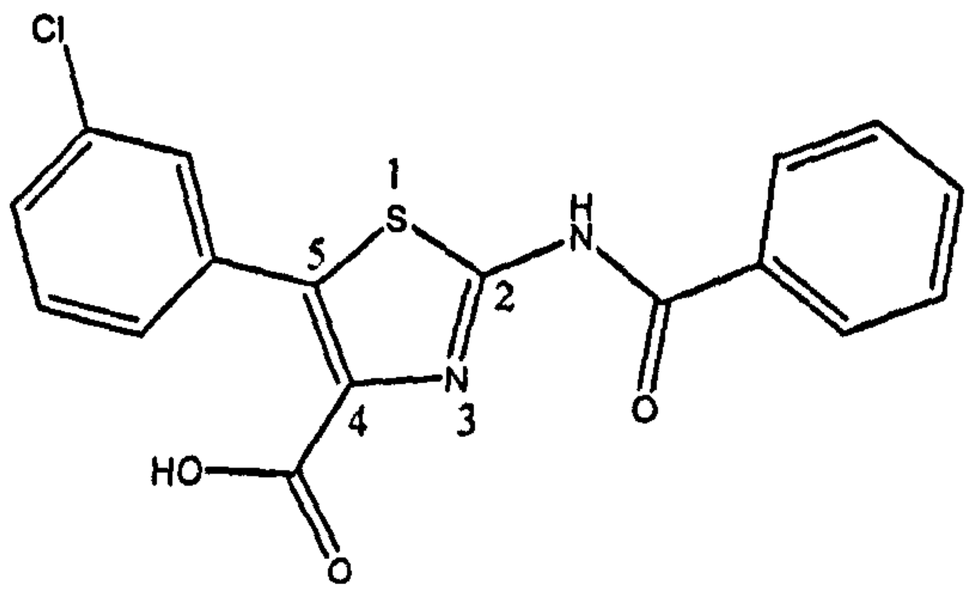
NMR: ^{1}H NMR (400MHz): δ(DMSO-d_6): 3.71 (3H, s, OCH_3); 7.48-7.55 (5H, m, Ar); 7.63-4.64 (2H, m, Ar); 8.12 (2H, d, Ar).

NMR: ^{13}C NMR (400MHz): δ(DMSO-d_6): 52.2 (OCH_3); 128.8 – 133.4 (12C, Ar); 135.0 (C_4); 137.5 (C_5); 157.1 (C_2); 162.5 ($COOCH_3$); 166.2 (CONH).

CHN Analysis: Found: C, 52.82; H, 3.22; N, 6.80; S, 7.93; Cl, 17.38 (Required for $C_{18}H_{13}N_2O_3SCl$: C, 57.99; H, 3.51; N, 7.51; S, 8.60; Cl, 9.51). There are two water molecules in the structure. The required values are correct (52.83; H, 3.17; N, 6.84; S, 7.82).

2-Benzamido-5-(3-chlorophenyl)thiazole-4-carboxylic acid (94)

The title compound was obtained as a white powder (0.2g, 26.8%) using general procedure **C**.



M.P. 280-282 °C.

IR: ATR, υ_{max} (cm^{-1}): 3200, NH stretch, primary amide; 1662, C=O stretch, carboxylic acid; 1662, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 359.0, $C_{17}H_{11}N_2O_3SCl$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

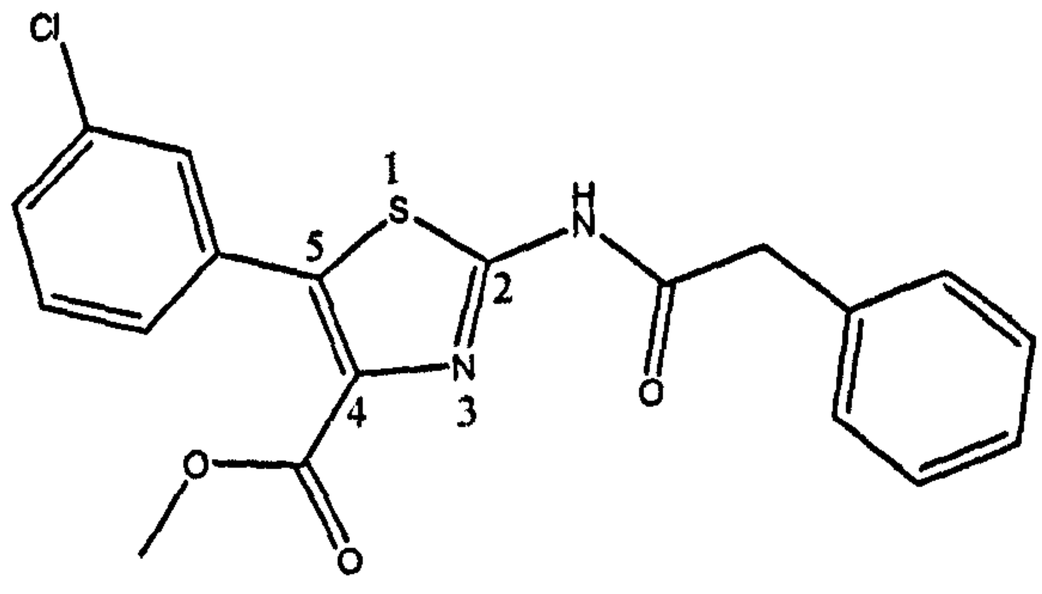
NMR: ^{1}H NMR (400MHz): δ(DMSO-d_6): 7.48-7.55 (7H, m, Ar); 8.13 (2H, d, Ar); 13.00 (1H, s, OH).

NMR: ^{13}C NMR (400MHz): δ(DMSO-d_6): 129.0 – 133.0 (12C, Ar); 136.0 (C_4); 138.0 (C_5); 157.0 (C_2); 163.5 (COOH); 167.0 (CONH).

CHN Analysis: Found: C, 57.14; H, 2.54; N, 7.69; S, 9.17; Cl, 9.90 (Required for $C_{17}H_{11}N_2O_3SCl$: C, 56.91; H, 3.09; N, 7.81; S, 8.94; Cl, 9.88).

Methyl 5-(3-chlorophenyl)-2-(2-phenylacetamido)thiazole-4-carboxylate (95)

The title compound was obtained as a white powder (4.1g, 97.0%) using general procedure **B**.



M.P. 168-170 °C.

IR: ATR, υ_{max} (cm^{-1}): 3247, NH stretch, primary amide; 1684, C=O stretch, conjugated ester; 1684, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 387.2, $C_{19}H_{15}N_2O_3SCl$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

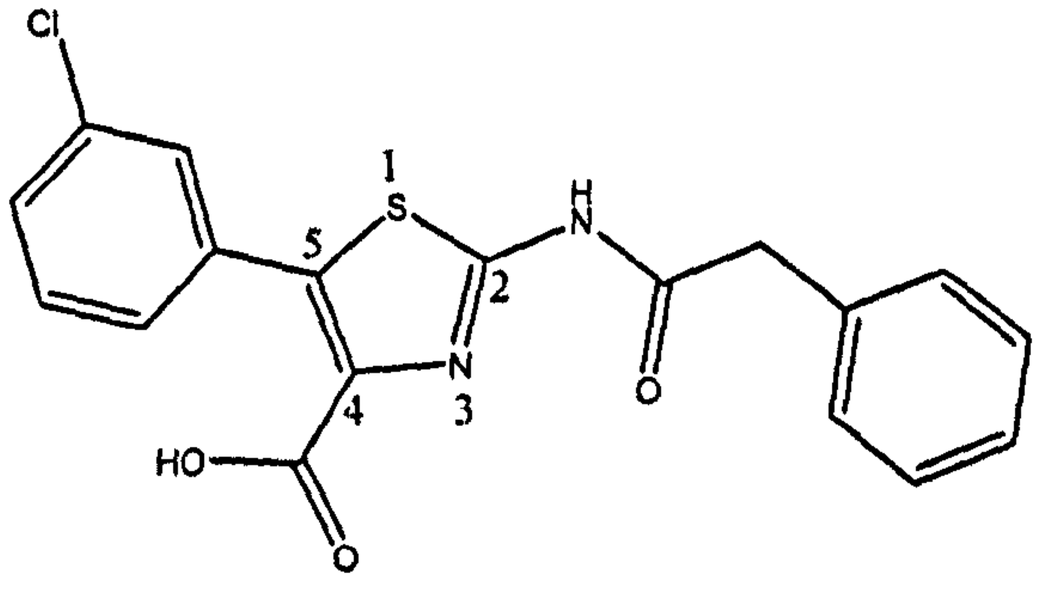
NMR: ^{1}H NMR (400MHz): δ(DMSO-d_6): 3.70 (3H, s, OCH_3); 3.84 (2H, s, CH_2-Ar); 7.22-7.34 (5H, m, Ar); 7.43-7.50 (3H, m, Ar); 7.59 (1H, s, Ar); 12.60 (1H, s, NH).

NMR: ^{13}C NMR (400MHz): δ(DMSO-d_6): 42.5 (CH_2-Ar); 52.2 (OCH_3); 127.4 – 132.9 (12C, Ar); 133.4 (C_4); 135.1 (C_5); 156.4 (C_2); 162.5 ($COOCH_3$); 170.5 (CONH).

CHN Analysis: Found: C, 58.74; H, 3.93; N, 7.16; S, 8.11; Cl, 9.06 (Required for $C_{19}H_{15}N_2O_3SCl$: C, 58.99; H, 3.91; N, 7.24; S, 8.29; Cl, 9.16).

5-(3-Chlorophenyl)-2-(2-phenylacetamido)thiazole-4-carboxylic acid (96)

The title compound was obtained as a white powder (0.4g, 43.3%) using general procedure **C**.



M.P. 230-232 °C.

IR: KBr disk, υ_{max} (cm^{-1}): 3169, NH stretch, primary amide; 1678, C=O stretch, carboxylic acid; 1678, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 373.8, $C_{18}H_{13}N_2O_3SCl$; (m/z 100%): 79.0.

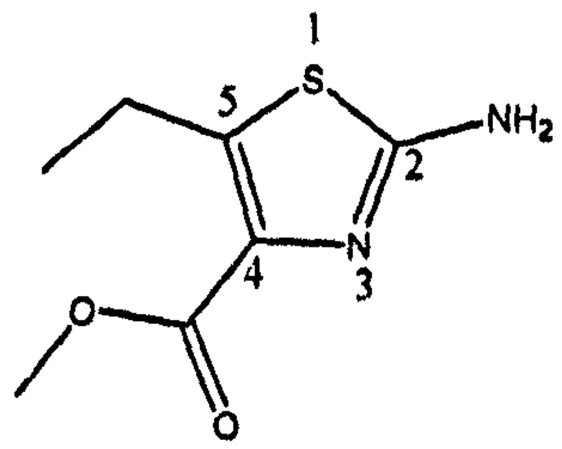
NMR: ^{1}H NMR (400MHz): δ(DMSO-d_6): 3.78 (2H, s, **CH_2**-Ar); 7.25-7.34 (5H, m, Ar); 7.42-7.45 (3H, m, Ar); 7.50 (1H, s, Ar).

NMR: ^{13}C NMR (400MHz): δ(DMSO-d_6): 42.5 (**CH_2**-Ar); 127.4 – 133.3 (12C, Ar); 135.2 (C_4); 137.0 (C_5); 156.0 (C_2); 163.6 (COOH); 170.5 (CONH).

CHN Analysis: Found: C, 58.03; H, 3.50; N, 7.37; S, 8.63; Cl, 9.57 (Required for $C_{18}H_{13}N_2O_3SCl$: C, 57.99; H, 3.51; N, 7.51; S, 8.60; Cl, 9.51).

Methyl 2-amino-5-ethylthiazole-4-carboxylate (97)

The title compound was obtained as a yellow powder (1.9g, 28.4%) using general procedure A.



M.P. 128-130 °C. [[Lit: 131-133 °C].[111]

IR: ATR, υ_{max} (cm^{-1}): 3425, NH stretch, amine; 1689, C=O stretch, conjugated ester.

MS: CI/ISO, (M+H): 187.15, $C_7H_{10}N_2O_2S$; (M+H 100%): 187.15, $C_7H_{10}N_2O_2S$.

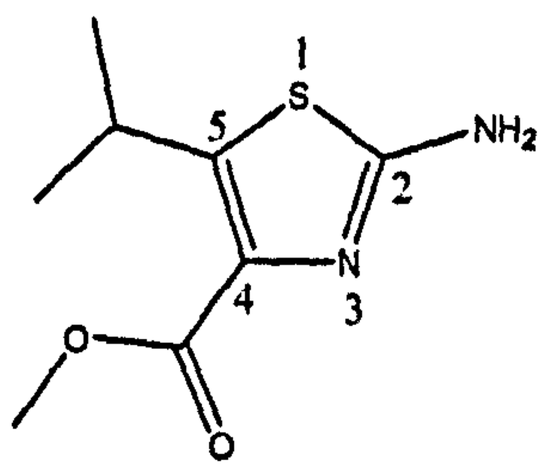
NMR: ^{1}H NMR (270MHz): δ($CDCl_3$): 1.2 (3H, t, J=8.1 Hz, CH_3CH_2-); 3.07 (2H, q, J=8.1 Hz, CH_3CH_2-); 3.80 (3H, s, OCH_3); 5.38 (2H, s, NH_2).

NMR: ^{13}C NMR (270MHz): δ($CDCl_3$): 16.0 (CH_3CH_2); 20.9 (CH_3CH_2); 51.9 (OCH_3); 135.6 (C_4); 143.8 (C_5); 163.0 (CO); 164.5 (C_2).

CHN Analysis: Found: C, 45.31; H, 5.48; N, 14.88; S, 17.31 (Required for $C_7H_{10}N_2O_2S$: C, 45.15; H, 5.41; N, 15.04; S, 17.22).

Methyl 2-amino-5-isopropylthiazole-4-carboxylate (98)

The title compound was obtained as a yellow powder (3.9g, 54.8%) using general procedure A.



M.P. 146-148 °C. [Lit: 150-151 °C][110]

IR: ATR, υ_{max} (cm^{-1}): 3423, NH stretch, amine; 1689, C=O stretch, conjugated ester.

MS: CI/ISO, (M+H): 201.2, $C_8H_{12}N_2O_2S$; (M+H 100%): 201.2, $C_8H_{12}N_2O_2S$.

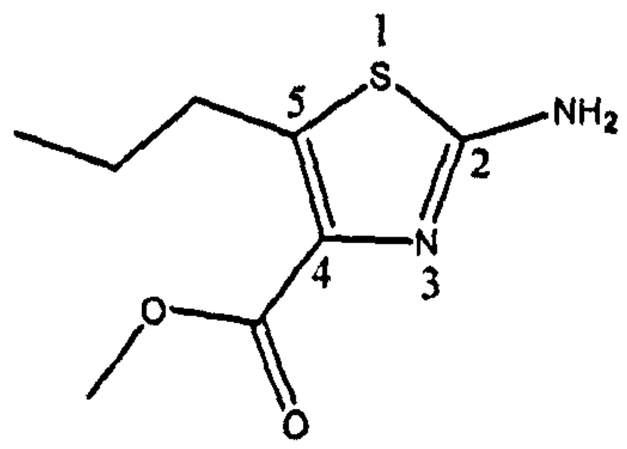
NMR: ^{1}H NMR (270MHz): δ($CDCl_3$): 1.23-1.26 (6H, d, J=8.1 Hz, $(CH_3)_2CH$-); 3.85 (3H, s, OCH_3); 3.90 (1H, m, J=8.1 Hz, $(CH_3)_2CH$-); 5.40 (2H, s, NH_2).

NMR: ^{13}C NMR (270MHz): δ($CDCl_3$): 25.1 (2C, $(CH_3)_2CH$-); 28.5 ($(CH_3)_2CH$-); 52.1 (OCH_3); 134.0 (C_4); 147.0 (C_5); 163.0 (CO); 164.5 (C_2).

CHN Analysis: Found: C, 47.63; H, 6.04; N, 13.82; S, 16.10 (Required for $C_8H_{12}N_2O_2S$: C, 47.98; H, 6.04; N, 13.99; S, 16.01).

Methyl 2-amino-5-propylthiazole-4-carboxylate (99)

The title compound was obtained as a yellow powder (2.5g, 34.1%) using general procedure A.



M.P. 90-92 °C.

IR: ATR, υ_{max} (cm^{-1}): 3401, NH stretch, amine; 16948, C=O stretch, conjugated ester.

MS: FAB/NOBA, (M+H): 201.6, $C_8H_{12}N_2O_2S$; (m/z 100%): 201.6, $C_8H_{12}N_2O_2S$.

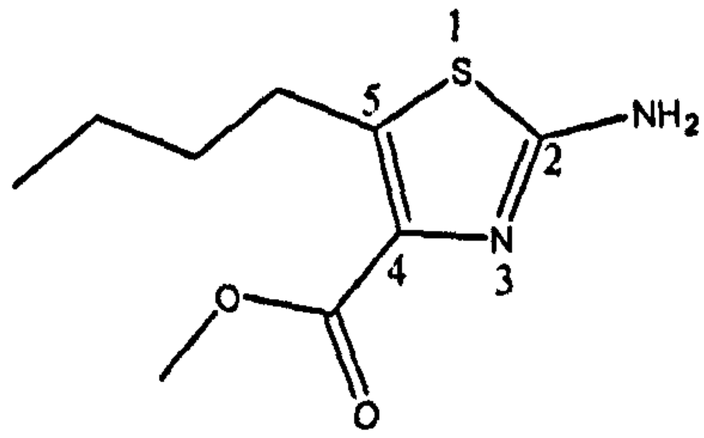
NMR: ^{1}H NMR (270MHz): δ(DMSO-d_6): 0.89 (3H, t, J=7.29 Hz, $CH_3(CH_2)_2$-); 1.55 (2H, m, J=7.56 Hz,$CH_3CH_2CH_2$-); 2.93 (2H, t, J=7.29 Hz,$CH_3CH_2CH_2$-); 3.70 (3H, s, OCH_3) ; 6.99 (2H, s, NH_2).

NMR: ^{13}C NMR (270MHz): δ(DMSO-d_6): 13.1 ($CH_3CH_2CH_2$-); 24.0 ($CH_3CH_2CH_2$-); 27.8 ($CH_3CH_2CH_2$-); 50.8 (OCH_3); 135.3 (C_4); 137.6 (C_5); 162.1 (CO); 166.6 (C_2).

CHN Analysis: Found: C, 47.95; H, 5.74; N, 13.56; S, 15.94 (Required for $C_8H_{12}N_2O_2S$: C, 47.98; H, 6.04; N, 13.99; S, 16.01).

Methyl 2-amino-5-butylthiazole-4-carboxylate (100)

The title compound was obtained as a yellow powder (0.8g, 10.6%) using general procedure A.



M.P. 49-51 °C.

IR: ATR, υ_{max} (cm^{-1}): 3412, NH stretch, amine; 1699, C=O stretch, conjugated ester.

MS: FAB/NOBA, (M+H): 215.5, $C_9H_{14}N_2O_2S$; (m/z 100%): 215.5, $C_9H_{14}N_2O_2S$.

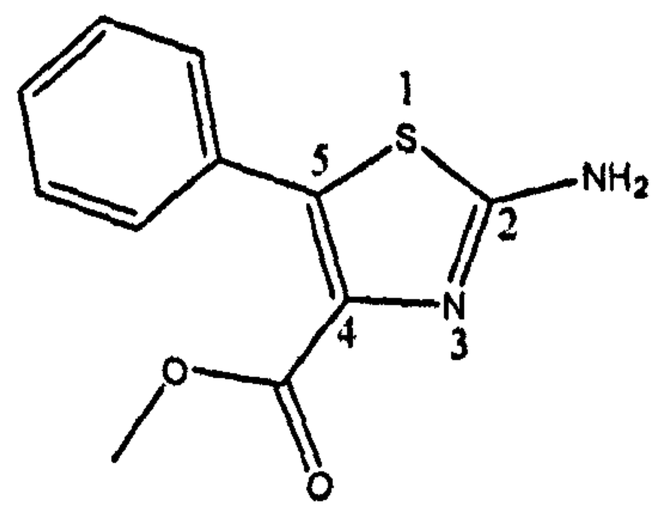
NMR: ^{1}H NMR (270MHz): δ(DMSO-d_6): 1.20 (3H, t, J=7.02 Hz, $CH_3(CH_2)_3$-); 1.62 (2H, m, J=7.56 Hz, $CH_3CH_2CH_2CH_2$-); 1.82 (2H, m, $CH_3CH_2CH_2CH_2$-); 3.28 (2H, t, J=7.29 Hz,$CH_3CH_2CH_2CH_2$-); 3.67 (3H, s, OCH_3) ; 7.10 (2H, s, NH_2).

NMR: ^{13}C NMR (270MHz): δ(DMSO-d_6): 13.2 ($CH_3CH_2CH_2CH_2$-); 21.2 ($CH_3CH_2CH_2CH_2$-); 25.5 ($CH_3CH_2CH_2CH_2$-); 32.8 ($CH_3CH_2CH_2CH_2$-); 50.8 (OCH_3); 135.2 (C_4); 137.9 (C_5); 163.1 (CO); 166.5 (C_2).

CHN Analysis: Found: C, 50.04; H, 6.90; N, 12.61; S, 13.99 (Required for $C_9H_{14}N_2O_2S$: C, 50.45; H, 6.59; N, 13.07; S, 14.96).

Methyl 2-amino-5-phenylthiazole-4-carboxylate (101)

The title compound was obtained as a pale yellow powder (1.7g, 20.4%) using general procedure A.



M.P. 218-221 °C. [Lit: 223 °C][112]

IR: ATR, υ_{max} (cm^{-1}): 3411, NH stretch, amine; 1696, C=O stretch, conjugated ester.

MS: FAB/NOBA, (M+H): 235.3, $C_{11}H_{10}N_2O_2S$; (m/z 100%): 81.1, $C_2H_6N_2$ + Na.

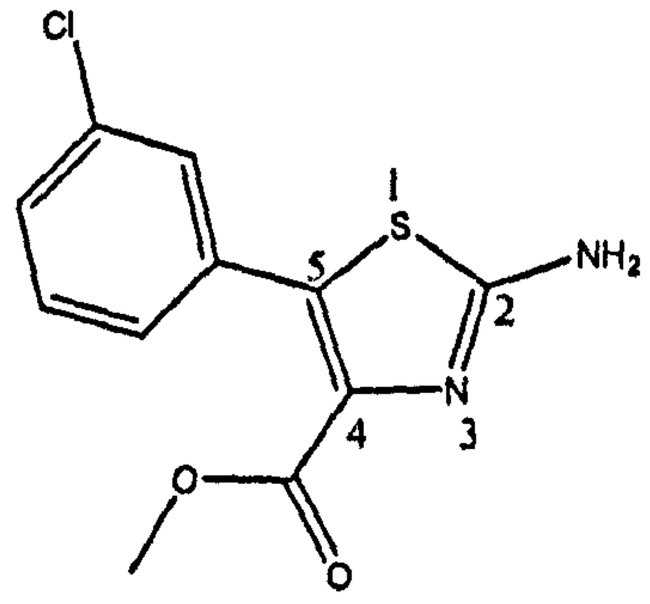
NMR: ^{1}H NMR (270MHz): δ(DMSO-d_6): 3.65 (3H, s, OCH_3) ; 7.25 (2H, s, NH_2); 7.29-7.39 (5H, m, Ar).

NMR: ^{13}C NMR (270MHz): δ(DMSO-d_6): 51.0 (OCH_3); 127.6 – 130.7 (6C, Ar); 132.0 (C_4); 135.1 (C_5); 162.2 (CO); 165.4 (C_2).

CHN Analysis: Found: C, 56.27; H, 4.10; N, 11.68; S, 13.58 (Required for $C_{11}H_{10}N_2O_2S$: C, 56.39; H, 4.30; N, 11.96; S, 13.69).

Methyl 2-amino-5-(3-chlorophenyl)thiazole-4-carboxylate (102)

The title compound was obtained as a pale yellow powder (2.3g, 50.7%) using general procedure A.



M.P. 229-232 °C.

IR: ATR, υ_{max} (cm^{-1}): 3417, NH stretch, amine; 1699, C=O stretch, conjugated ester.

MS: FAB/NOBA, (M+H): 269.1, $C_{11}H_9N_2O_2SCl$; (m/z 100%): 81.1, $C_2H_6N_2$ + Na.

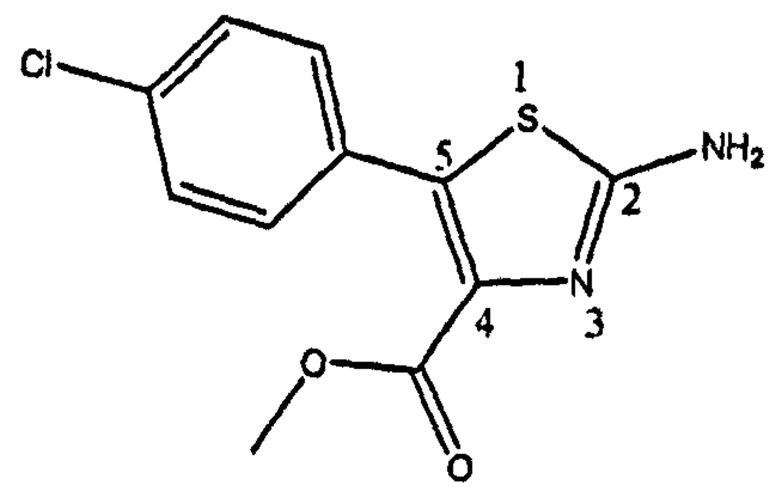
NMR: ^{1}H NMR (270MHz): δ(DMSO-d_6): 3.70 (3H, s, OCH_3); 7.67-7.76 (3H, m, Ar); 7.81 (1H, s, Ar).

NMR: ^{13}C NMR (400MHz): δ(DMSO-d_6): 52.0 (OCH_3); 128.4 – 133.2 (6C, Ar); 133.7 (C_4); 136.7 (C_5); 162.8 (CO); 166.7 (C_2).

CHN Analysis: Found: C, 49.44; H, 3.51; N, 10.15; S, 12.19; Cl, 13.43 (Required for $C_{11}H_9N_2O_2SCl$: C, 49.17; H, 3.38; N, 10.42; S, 11.93; Cl, 13.19).

Methyl 2-amino-5-(4-chlorophenyl)thiazole-4-carboxylate (103)

The title compound was obtained as a white powder (2.1g, 45.5%) using general procedure A.



M.P. 238-240 °C.

IR: ATR, υ_{max} (cm^{-1}): 3411, NH stretch, amine; 1696, C=O stretch, conjugated ester.

MS: FAB/NOBA, (M+H): 269.1, $C_{11}H_9N_2O_2SCl$; (m/z 100%): 81.1, $C_2H_6N_2$ + Na.

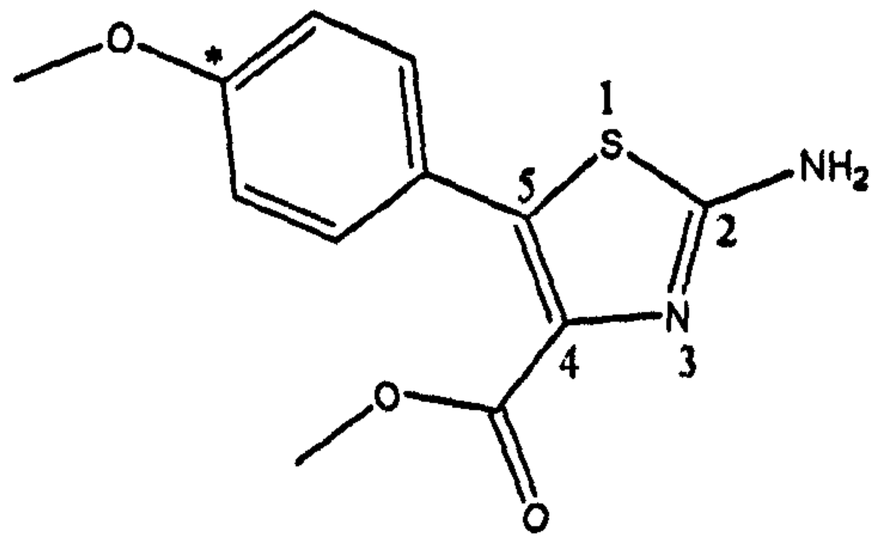
NMR: ^{1}H NMR (270MHz): δ(DMSO-d_6): 3.35 (3H, s, OCH_3) ; 7.35 (2H, s, NH_2); 7.42 (4H, s, Ar).

NMR: ^{13}C NMR (270MHz): δ(DMSO-d_6): 52.1 (OCH_3); 128.8-130.0 (6C, Ar); 133.0 (C_4); 135.0 (C_5); 163.0 (CO); 166.5 (C_2).

CHN Analysis: Found: C, 48.93; H, 3.56; N, 10.46; S, 11.96; Cl, 12.94 (Required for $C_{11}H_9N_2O_2SCl$: C, 49.17; H, 3.38; N, 10.42; S, 11.93; Cl, 13.09).

Methyl 2-amino-5-(4-methoxyphenyl)thiazole-4-carboxylate (104)

The title compound was obtained as a pale yellow powder (1.1g, 26.4%) using general procedure A.



M.P. 188-190 °C.

IR: ATR, υ_{max} (cm^{-1}): 3405, NH stretch, amine; 1696, C=O stretch, conjugated ester.

MS: FAB/NOBA, (M+H): 265.2, $C_{12}H_{12}N_2O_3S$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

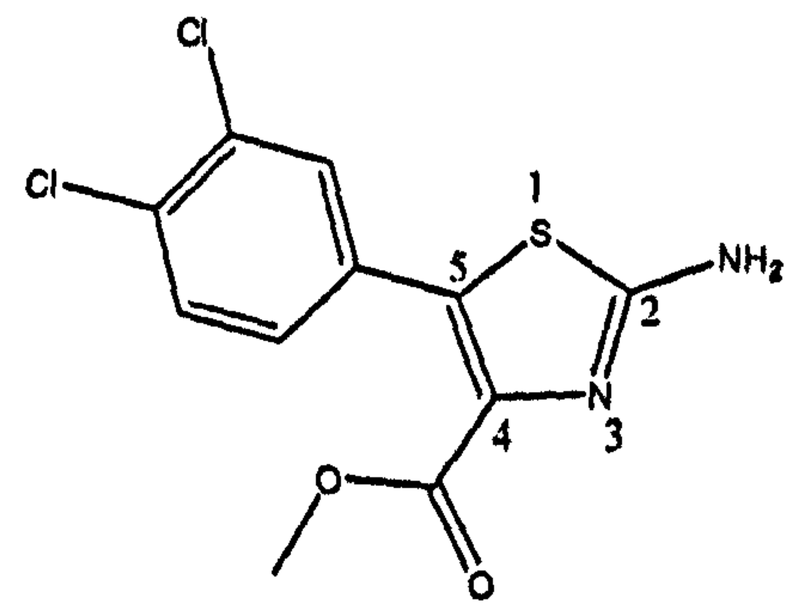
NMR: ^{1}H NMR (270MHz): δ(DMSO-d_6): 3.61 (3H, s, OCH_3) ; 3.76 (3H, s, Ar-OCH_3); 6.90 (2H, d, Ar);7.24 (2H, s, NH_2); 7.33 (2H, d, Ar).

NMR: ^{13}C NMR (270MHz): δ(DMSO-d_6): 51.3 ($COOCH_3$); 55.1 (Ar-OCH_3); 113.5-130.7 (5C, Ar); 133.0 (C_4); 135.0 (C_5); 159.1 (Ar-C^*); 162.5 (CO); 166.1 (C_2).

CHN Analysis: Found: C, 54.99; H, 4.32; N, 10.34; S, 11.93 (Required for $C_{12}H_{12}N_2O_3S$: C, 54.53; H, 4.58; N, 10.60; S, 12.13).

Methyl 2-amino-5-(3,4-dichlorophenyl)thiazole-4-carboxylate (105)

The title compound was obtained as a yellow powder (0.7g, 14.5%) using general procedure A.



M.P. 239-241 °C.

IR: ATR, υ_{max} (cm^{-1}): 3414, NH stretch, amine; 1697, C=O stretch, conjugated ester.

MS: FAB/NOBA, (M+H): 304.1, $C_{11}H_8N_2O_2SCl_2$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

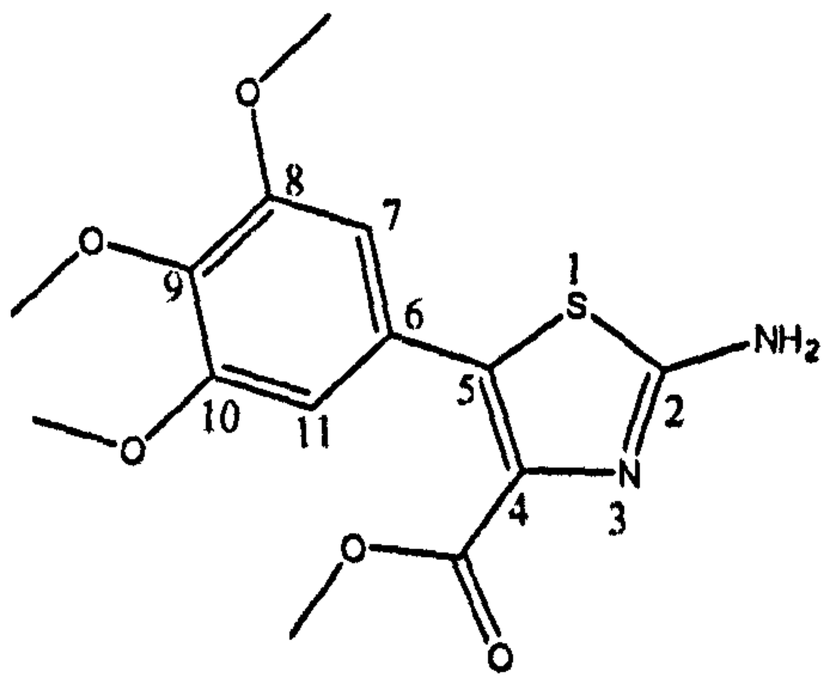
NMR: ^{1}H NMR (270MHz): δ(DMSO-d_6): 3.97 (3H, s, OCH_3) ; 7.70 (1H, d, Ar); 7.96 (1H, d, Ar); 8.03 (1H, s, Ar).

NMR: ^{13}C NMR (270MHz): δ(DMSO-d_6): 51.5 (OCH_3); 128.8 – 131.8 (6C, Ar); 131.8 (C_4); 136.4 (C_5); 162.1 (CO); 166.2 (C_2).

CHN Analysis: Found: C, 43.61; H, 2.54; N, 8.97; S, 10.53; Cl, 23.21 (Required for $C_{11}H_8N_2O_2SCl_2$: C, 43.58; H, 2.66; N, 9.24; S, 10.58; Cl, 23.39).

Methyl 2-amino-5-(3,4,5-trimethoxyphenyl)thiazole-4-carboxylate (106)

The title compound was obtained as a yellow powder (1.2g, 22.6%) using general procedure A.



M.P. 174-176 °C.

IR: ATR, υ_{max} (cm^{-1}): 3446, NH stretch, amine; 1718, C=O stretch, conjugated ester.

MS: FAB/NOBA, (M+H): 325.2, $C_{14}H_{16}N_2O_5S$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

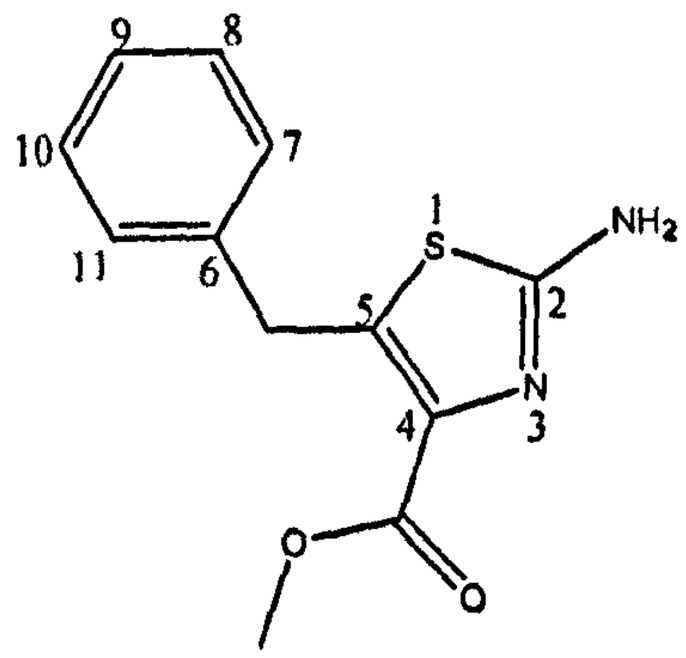
NMR: ^{1}H NMR (270MHz): δ(DMSO-d_6): 3.60 (3H, s, OCH_3) ; 3.68 (3H, s, Ar-OCH_3); 3.76 (3H, s, Ar-OCH_3); 3.87 (3H, s, Ar-OCH_3); 6.87 (1H, s, Ar);7.25 (1H, s, Ar).

NMR: ^{13}**C NMR** (270MHz): δ(DMSO-d_6): 51.2 (OCH_3CO), 55.9, 56.0, 60.0 (3C, OCH_3Ar), 106.7, 107.0 (C_7, C_{11}); 126.3 (C_6); 132.3 (C_4); 135.4 (C_5); 137.4 (C_9); 152.4 (C_8, C_{10}); 162.5 ($COOCH_3$); 165.4 (C_2).

CHN Analysis: Found: C, 51.97; H, 4.82; N, 8.57; S, 9.62 (Required for $C_{14}H_{16}N_2O_5S$: C, 51.84; H, 4.97; N, 8.64; S, 9.89).

Methyl 2-amino-5-benzylthiazole-4-carboxylate (107)

The title compound was obtained as a yellow powder (4.0g, 44.9%) using general procedure A.



M.P. 92-94 °C.

IR: ATR, υ_{max} (cm^{-1}): 3465, NH stretch, amine; 1711, C=O stretch, conjugated ester.

MS: FAB/NOBA, (M+H): 249.3, $C_{12}H_{12}N_2O_2S$; (m/z 100%): 249.3, $C_{12}H_{12}N_2O_2S$.

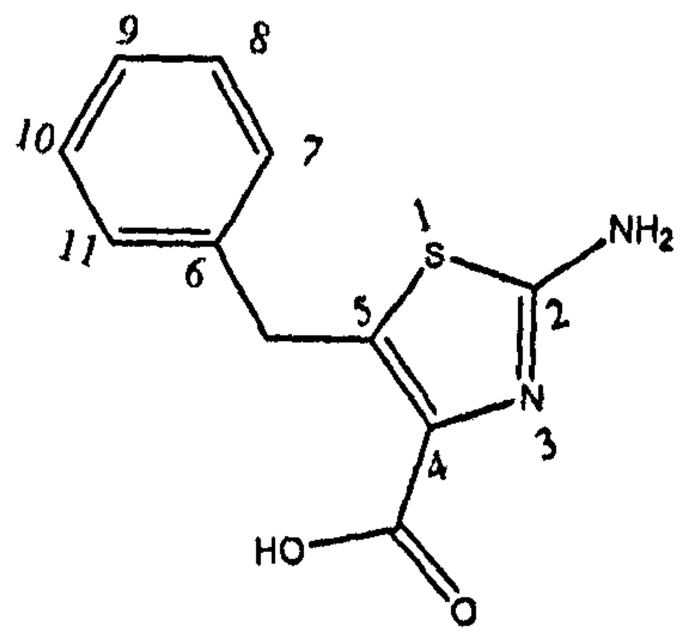
NMR: ^{1}**H NMR** (270MHz): δ(DMSO-d_6): 3.74 (3H, s, OCH_3); 4.32 (2H, s, CH_2-Ar); 7.02 (2H, s, NH_2); 7.20-7.32 (5H, m, Ar).

NMR: ^{13}**C NMR** (270MHz): δ(DMSO-d_6): 31.7 (Ar-CH_2-); 51.0 (OCH_3); 126.1 (Ar C_9); 127.9, 128.8 (C_7,C_8,C_{10},C_{11});135.5 (C_4); 136.4 (C_5); 139.7 (C_6); 162.3 (CO); 164.3 (C_2).

CHN Analysis: Found: C, 58.55; H, 4.73; N, 10.92; S, 13.23 (Required for $C_{12}H_{12}N_2O_2S$: C, 58.05; H, 4.87; N, 11.28; S, 12.91).

Methyl 2-amino-5-benzylthiazole-4-carboxylate (108)

The title compound was obtained as a yellow powder (0.6g, 68.0%) using general procedure C.



M.P. 300-302 °C.

IR: ATR, υ_{max} (cm^{-1}): 3465, NH stretch, amine; 1711, C=O stretch, conjugated ester; C=O stretch, carboxylic acid.

MS: FAB/NOBA, (M+H): 235.0, $C_{11}H_{10}N_2O_2S$; (m/z 100%): 232.0.

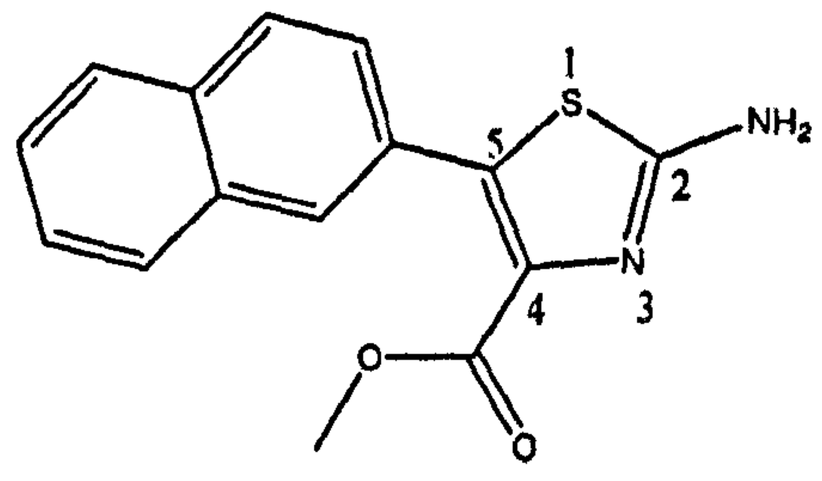
NMR: ^{1}H NMR (400MHz): δ(DMSO-d_6): 4.32 (2H, s, CH_2-Ar); 7.02 (2H, s, NH_2); 7.20-7.32 (5H, m, Ar).

NMR: ^{13}C NMR (270MHz): δ(DMSO-d_6): 32.6 (Ar-CH_2-); 127.0 (Ar C_9); 128.8, 129.1 (C_7,C_8,C_{10},C_{11});136.4 (C_4); 137.6 (C_5); 140.9 (C_6); 164.2 (CO); 165.1 (C_2).

CHN Analysis: Found: C, 56.72; H, 4.13; N, 12.04; S, 13.28 (Required for $C_{11}H_{10}N_2O_2S$: C, 56.39; H, 4.30; N, 11.96; S, 13.69).

Methyl 2-amino-5-(naphthalen-2-yl)thiazole-4-carboxylate (109)

The title compound was obtained as a yellow powder (2.2g, 46.6%) using general procedure A.



M.P. 240-242 °C.

IR: ATR, υ_{max} (cm^{-1}): 3410, NH stretch, amine; 1695, C=O stretch, conjugated ester.

MS: FAB/NOBA, (M+H): 285.3, $C_{15}H_{12}N_2O_2S$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

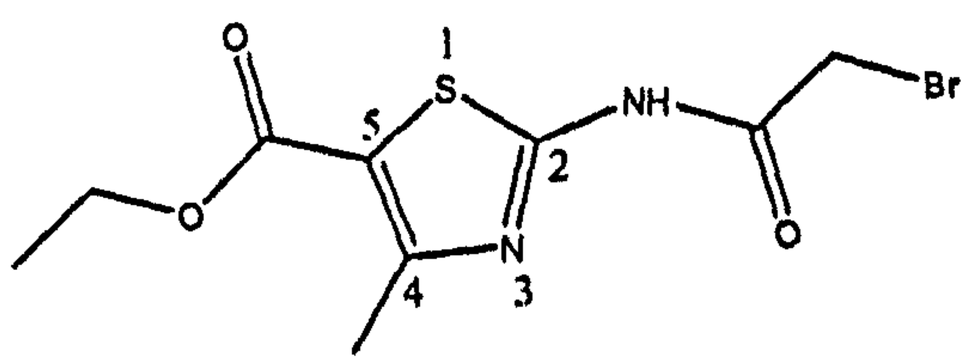
NMR: ^{1}H NMR (400MHz): δ(DMSO-d_6): 3.63 (3H, s, OCH_3) ; 7.36 (2H, s, NH_2); 7.52-7.54 (3H, m, Ar); 7.88-7.95 (4H, m, Ar).

NMR: ^{13}C NMR (400MHz): δ(DMSO-d_6): 52.0 ($COOCH_3$); 127.1-132.8 (10C, Ar); 133.1 (C_4); 136.3 (C_5); 163.1 ($COOCH_3$) ; 166.4 (C_2).

CHN Analysis: Found: C, 62.94; H, 4.16; N, 9.93; S, 11.17 (Required for $C_{15}H_{12}N_2O_2S$: C, 63.36; H, 4.25; N, 9.85; S, 11.28).

Ethyl 2-(2-bromoacetamido)-4-methylthiazole-5-carboxylate (116)

The title compound was obtained as a pale yellow powder (3.5g, 43.0%) using general procedure B.



M.P. 178-180 °C.

IR: ATR, υ_{max} (cm^{-1}): 3243, NH stretch, primary amide; 1706, C=O stretch, conjugated ester; 1668, C=O stretch, primary amide.

MS: FAB/NOBA, (M+2): 309.1, $C_9H_{11}BrN_2O_3S$; (m/z 100%): 309.1, $C_9H_{11}BrN_2O_3S$.

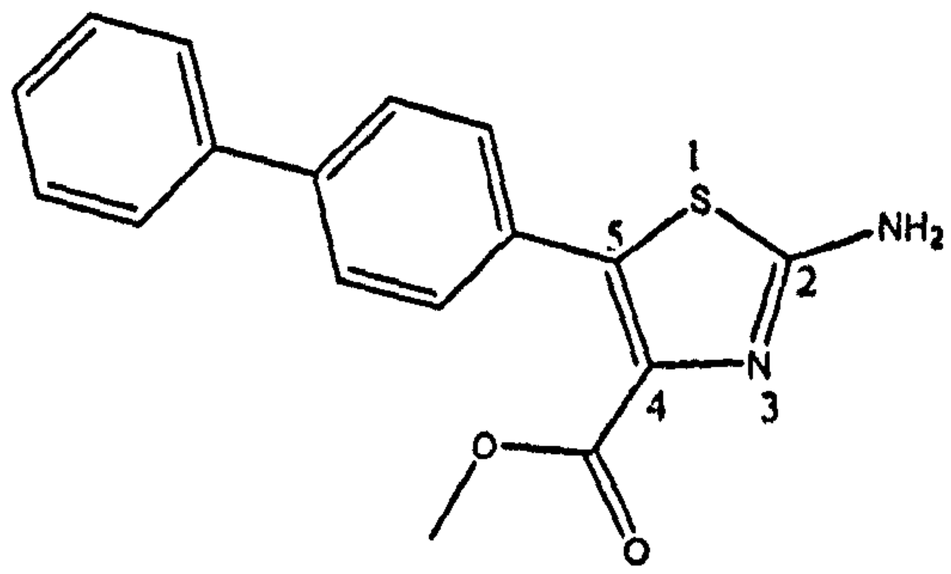
NMR: ^{1}H NMR (400MHz): δ($CDCl_3$): 1.32 (3H, t, J=7.48 Hz, OCH_2CH_3) ;2.63 (3H, s, CH_3-Ar) 4.11 (2H, s, CH_2-Br); 4.32 (2H, q, J=7.60 Hz, OCH_2CH_3).

NMR: ^{13}C NMR (400MHz): δ($CDCl_3$): 14.4 (CH_3CH_2CO); 17.2 (CH_3Ar); 27.5 ($COCH_2Br$); 61.1 (CH_3CH_2CO); 117.0 (C_5); 156.4 (C_4); 158.5 (C_2); 162.5 ($COOCH_2CH_3$); 164.0 (CONH).

CHN Analysis: Found: C, 35.37; H, 3.40; N, 9.08; S, 10.35; Br, 26.18 (Required for $C_9H_{11}BrN_2O_3S$: C, 35.19; H, 3.61; N, 9.12; S, 10.44; Br, 26.01).

Methyl 2-amino-5-(biphenyl)thiazole-4-carboxylate (110)

The title compound was obtained as a yellow powder (0.9g, 17.0%) using general procedure A.



M.P. 198-200 °C.

IR: ATR, υ_{max} (cm^{-1}): 3461, 3461, asymmetrical NH stretch, amine; 1716, C=O stretch, conjugated ester.

MS: FAB/NOBA, (M+H): 311.2, $C_{17}H_{14}N_2O_2S$; (m/z 100%): $C_{16}H_{11}N_2OS$.

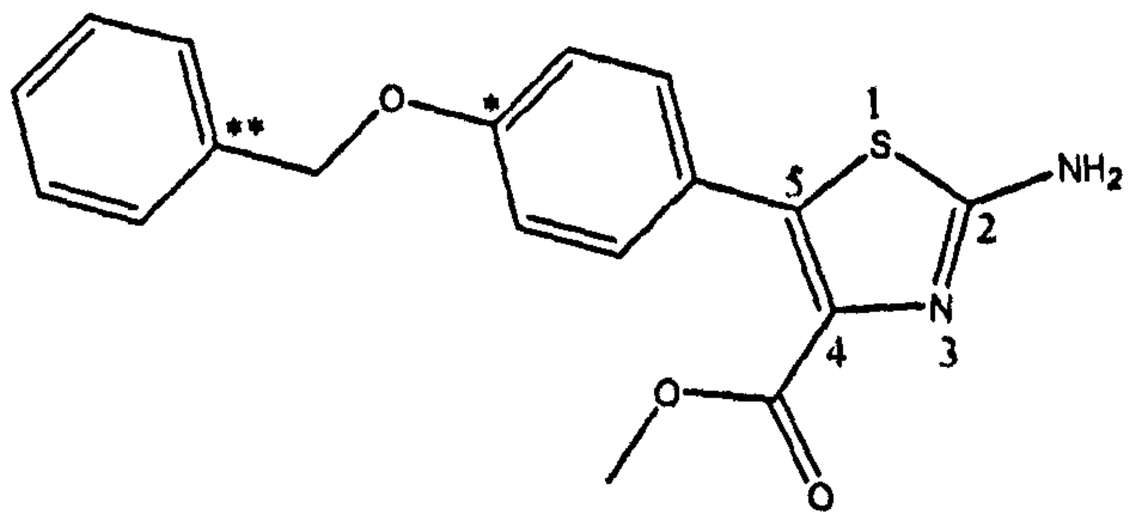
NMR: ^{1}H NMR (270MHz): δ(DMSO-d_6): 3.65 (3H, s, OCH_3); 7.32 (2H, s, NH_2); 7.37-7.50 (5H, m, Ar); 7.65-7.71 (4H, m, Ar).

NMR: ^{13}**C NMR** (270MHz): δ(DMSO-d_6): 51.4 (OCH_3); 126.3 – 130.1 (12C, Ar); 132.0 (C_4); 135.6 (C_5); 162.6 (CO); 165.7 (C_2).

CHN Analysis: Found: C, 65.94; H, 4.17; N, 8.83; S, 10.33 (Required for $C_{17}H_{14}N_2O_2S$: C, 65.79; H, 4.55; N, 9.03; S, 10.33).

Methyl 2-amino-5-(4-(benzyloxy)phenyl)thiazole-4-carboxylate (111)

The title compound was obtained as a yellow powder (1.0g, 17.4%) using general procedure A.



M.P. 198-200 °C.

IR: ATR, υ_{max} (cm^{-1}): 3405, NH stretch, amine; 1696, C=O stretch, conjugated ester.

MS: FAB/NOBA, (M+H): 341.2, $C_{18}H_{16}N_2O_3S$; (m/z 100%): 81.0, $C_2H_6N_2$ + Na.

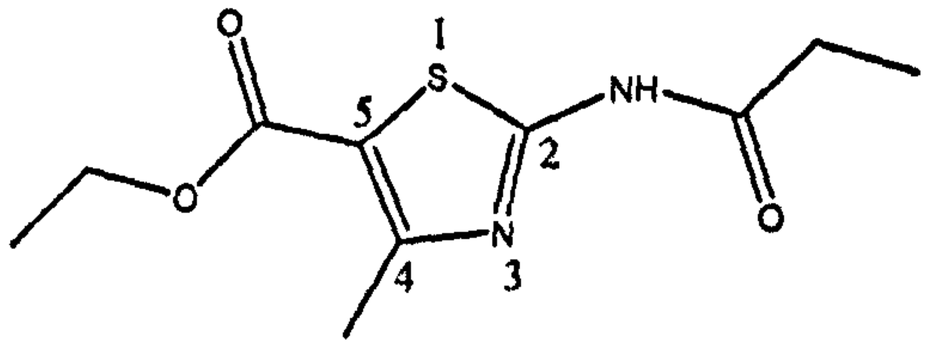
NMR: ^{1}**H NMR** (270MHz): δ(DMSO-d_6): 3.94 (3H, s, OCH_3) ; 5.45 (2H, s, Ar-CH_2OAr); 7.34 (2H, d, Ar); 7.53 (2H, d, Ar); 7.64-7.77 (5H, m, Ar); 8.64 (1H, s, NH).

NMR: ^{13}**C NMR** (400MHz): δ(DMSO-d_6): 51.8 (OCH_3); 69.8 (CH_2O-Ar); 114.9 – 132.3 (10C, Ar); 133.6 (C_4); 135.3 (C_5); 137.4 (C^{**}); 158.7 (C^{*}); 163.1 (CO); 165.7 (C_2).

CHN Analysis: Found: C, 62.88; H, 4.73; N, 8.08; S, 9.47 (Required for $C_{18}H_{16}N_2O_3S$: C, 62.51; H, 4.74; N, 8.23; S, 9.42).

Ethyl 4-methyl-2-propionamidothiazole-5-carboxylate (112)

The title compound was obtained as a brown powder (2.8g, 43.7%) using general procedure B.



M.P. 165-167 °C.

IR: ATR, υ_{max} (cm^{-1}): 3266, NH stretch, primary amide; 1679, C=O stretch, conjugated ester; 1679, C=O stretch, primary amide.

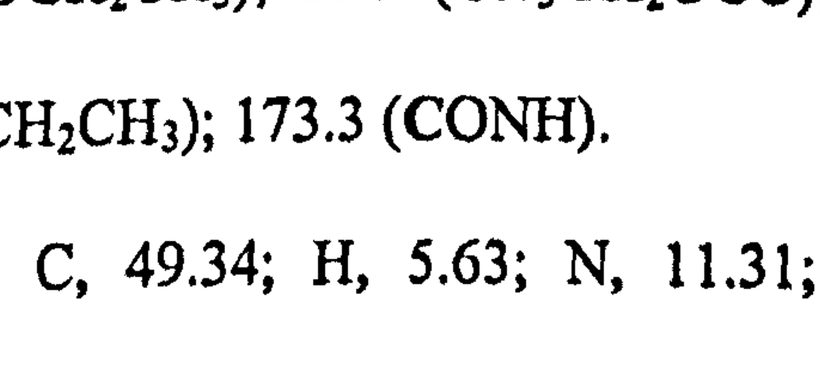
NMR: ^{1}H NMR (400MHz): δ(DMSO-d_6): 1.10 (3H, t, J=7.20 Hz, O=CCH$_2$C**H**$_3$); 1.28 (3H, t, J=7.20 Hz, OCH$_2$C**H**$_3$) ; 2.47 (2H, q, J=7.20 Hz, O=CC**H**$_2$CH$_3$); 2.50 (3H, s, C**H**$_3$-Ar); 4.24 (2H, q, J=7.20 Hz, OC**H**$_2$CH$_3$).

NMR: ^{13}C NMR (400MHz): δ(DMSO-d_6): 9.4 (COCH$_2$**C**H$_3$); 14.7 (**C**H$_3$CH$_2$OCO); 17.5 (**C**H$_3$Ar); 28.8 (COCH$_2$CH$_3$); 60.9 (CH$_3$**C**H$_2$OCO); 114.5 (C$_5$); 156.7 (C$_4$); 160.2 (C$_2$); 162.7 (**C**OOCH$_2$CH$_3$); 173.3 (**C**ONH).

CHN Analysis: Found: C, 49.34; H, 5.63; N, 11.31; S, 13.26 (Required for $C_{10}H_{14}N_2O_3S$: C, 49.57; H, 5.82; N, 11.56; S, 13.23).

4-Methyl-2-propionamidothiazole-5-carboxylic acid (113)

The title compound was obtained as a yellow powder (0.2g, 21.5%) using general procedure C.



M.P. > 350 °C.

IR: ATR, υ_{max} (cm^{-1}): 3170, NH stretch, primary amide; 1695, C=O stretch, carboxylic acid; 1695, C=O stretch, primary amide.

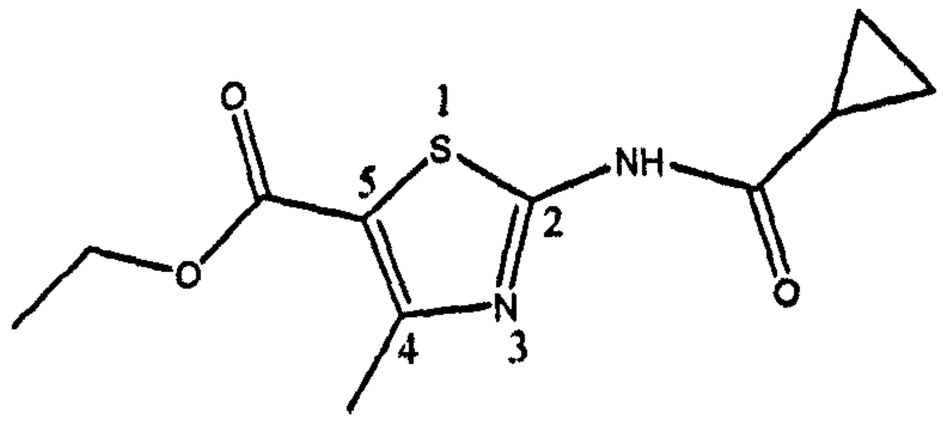
NMR: ^{1}**H NMR** (400MHz): δ(DMSO-d_6): 1.10 (3H, t, J=10.12 Hz, O=CCH$_2$C**H**$_3$); 2.40 (2H, q, J=7.20 Hz, O=CC**H**$_2$CH$_3$); 2.50 (3H, s, C**H**$_3$-Ar).

NMR: ^{13}**C NMR** (400MHz): δ(DMSO-d_6): 9.4 (COCH$_2$CH$_3$); 17.4 (**C**H$_3$Ar); 28.8 (COCH$_2$CH$_3$); 115.6 (C_5); 155.9 (C_4); 159.8 (C_2); 164.2 (**C**OOH); 173.2 (**C**ONH).

CHN Analysis: Found: C, 43.25; H, 4.58; N, 13.03; S, 15.42 (Required for $C_8H_{10}N_2O_3S$: C, 44.85; H, 4.70; N, 13.08; S, 14.97).

Ethyl 2-(cyclopropanecarboxamido)-4-methylthiazole-5-carboxylate (114)

The title compound was obtained as a pale yellow powder (2.9g, 42.5%) using general procedure **B**.



M.P. 185-187 °C.

IR: ATR, υ_{max} (cm^{-1}): 3265, NH stretch, primary amide; 1667, C=O stretch, conjugated ester; 1667, C=O stretch, primary amide.

MS: FAB/NOBA, (M+H): 255.0, $C_{11}H_{14}N_2O_3S$; (m/z 100%): 45.0.

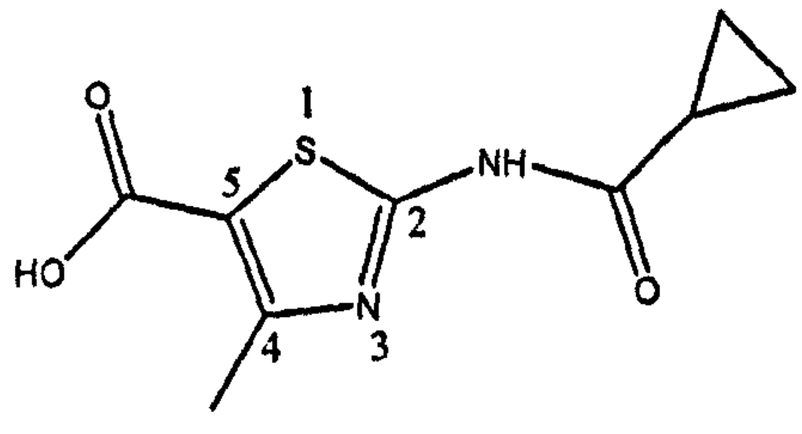
NMR: ^{1}**H NMR** (400MHz): δ(DMSO-d_6): 0.90-0.92 (4H, m, cyclopropyl); 1.27 (3H, t, J=6.80 Hz, OCH$_2$C**H**$_3$); 1.93 (1H, m, cyclopropyl); 2.50 (3H, s, C**H**$_3$-Ar); 4.20 (2H, q, J=7.20 Hz, OC**H**$_2$CH$_3$).

NMR: ^{13}**C NMR** (400MHz): δ(DMSO-d_6): 9.3 (2C, cyclopropyl); 14.2 (1C, cyclopropyl); 14.7 (**C**H_3CH_2OCO); 17.5 (CH_3Ar); 60.9 (CH_3CH_2OCO); 114.3 (C_5); 156.6 (C_4); 160.1 (C_2); 162.6 (**C**$OOCH_2CH_3$); 173.2 (**C**ONH).

CHN Analysis: Found: C, 52.12; H, 5.82; N, 11.23; S, 12.58 (Required for $C_{11}H_{14}N_2O_3S$: C, 51.95; H, 5.55; N, 11.02; S, 12.61).

2-(Cyclopropanecarboxamido)-4-methylthiazole-5-carboxylic acid (115)

The title compound was obtained as a pale yellow powder (0.3g, 30.6%) using general procedure **C**.



M.P. 324-326 °C.

IR: ATR, υ_{max} (cm^{-1}): 3170, NH stretch, primary amide; 1692, C=O stretch, carboxylic acid; 1692, C=O stretch, primary amide.

NMR: ^{1}**H NMR** (400MHz): δ(DMSO-d_6): 0.90-0.94 (4H, m, cyclopropyl); 1.93 (1H, m, cyclopropyl); 2.50 (3H, s, CH_3-Ar).

NMR: ^{13}**C NMR** (400MHz): δ(DMSO-d_6): 9.3 (2C, cyclopropyl); 14.7 (1C, cycloproyl); 17.5 (CH_3Ar); 116.0 (C_5); 156.6 (C_4); 160.1 (C_2); 162.6 (**C**OOH); 173.2 (**C**ONH).

CHN Analysis: Found: C, 46.67; H, 4.80; N, 12.27; S, 14.22 (Required for $C_9H_{10}N_2O_3S$: C, 47.78; H, 4.45; N, 12.38; S, 14.17).

3.3 Biological evaluation

3.3.1 Reagents and solvents

DMSO (Sigma Aldrich, UK), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Lancaster, UK), Cation Adjusted Mueller Hinton Broth with TES (CAMHB) (Sensititre®, Trek-Diagnostics Systems, East Grinstead, UK), Columbia agar (Fluka, UK), horse blood (Oxoid, Basingstoke, UK), Ethambutol (Abbott Laboratories, Queenborough, UK), and flat-bottomed 96-well plates (Nunclon®, Nunc Brand Products).

3.3.2 Bacteria preparation

The bacteria for each experiment are prepared freshly from stock. *M. aurum* cultured at 37 °C on Columbia agar slants supplemented with 5% horse blood. For assays, organisms were subcultured once and incubated for 3 days at 37°C. Inocula were prepared by transferring colonies to saline (0.9% w/v NaCl) in a glass vial containing glass beads. Bacterial suspensions were mixed vigorously to disrupt visible clumps and left to settle. Supernatants, containing dispersed bacterial cells, were diluted to match a McFarland 0.5 standard.

3.3.3 Sample preparation

To get a concentration of 256 μg/ml, 2.048 mg of the compounds added to 120 μL of DMSO to ensure that the final concentration of DMSO in the well not to exceed 5%.

3.3.4 MIC determination

Microdilution susceptibility testing was performed in flat-bottomed 96-well plates containing 100 μL of cation-adjusted Mueller–Hinton broth in each well. The

bacterial inoculum (100 μL) was added to give about 2.5×10^5 CFU/ml. Ethambutol was used as standard. Wells containing the organic solvents employed in sample preparation were also included in order to monitor sample sterility and to determine any antibacterial effects of solvents. No inhibitory effects were observed in the presence of any of the organic solvents at the highest concentrations used (1.5% v/v). The plates were sealed with a plastic cover and incubated at 37°C for 5 days. Inhibition of growth was detected following addition (20 μL) of a methanol solution (5 mg/ml) of the tetrazolium redox dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide followed by incubation at 37 °C for 20–50 min. Viable bacteria reduced the yellow dye to a purple color. MIC was defined as the lowest sample concentration that prevented this change and exhibited complete inhibition of macroscopic growth. Each sample was tested in duplicate and on two separate days. Reading are taken and converted to μM by dividing the value over the molecular weight of the compound and multiplying by 1000.[113]

3.4 Molecular modelling: docking studies using GOLD

3.4.1 Definition of active site

The active site determines the search space taken into consideration during the docking process and must be large enough to accommodate all the possible docked poses. The active site was defined as all protein atoms within 20 Å of the sulphur atom of Cys112.

3.4.2 Ligand and protein flexibility

All rotatable bonds of the ligand were randomized at the beginning of the GOLD docking and treated as completely flexible during the docking runs. Bond lengths, angles and torsions associated with non-rotatable bonds in the ligand were held. The protein in GOLD is normally treated as rigid, with partial flexibility applied to hydroxyl protons and NH_3^+ groups, to allow rotation for hydrogen bond optimization.

3.4.3 GOLD docking

For each independent genetic algorithm (GA) run, a maximum number of 100,000 operations were performed on a population of 5 islands of 100 individuals. Operator weights for crossover, mutation and migration were set to 95, 95 and 10 respectively. Van der Waals contacts and hydrogen bonds were assigned as 4.0 Å and 2.5 Å respectively, whilst the fitness function was chosen to be GOLD score. All the poses (50) were saved and visualized inside the active site to understand the binding pattern. The protein was loaded as a pdb file and the ligands were loaded as sdf files.

Figure 3.1 shows the GOLD interface including the parameters that have been used for docking our compounds. The interface is divided into four sections; the top one, by which the operator can start, end and save the chosen parameters. The second is the Input Parameters and Files, it enables the operator to choose the protein, the ligands and provide options for determining the reference point or points in the protein to perform docking around, with the facility to choose the size of the cavity around the reference point. Fitness Function and Search Setting provides easy choice of the scoring algorithm in addition to the optimal distances for both Van der Waals

and H-bonding with the possibility of adding some types of constrains over the ligand throughout the docking process. The final section only used for the parameters that are related to the genetic algorithm applied in the docking, which is recommended to be left as determined by the GOLD designer company.

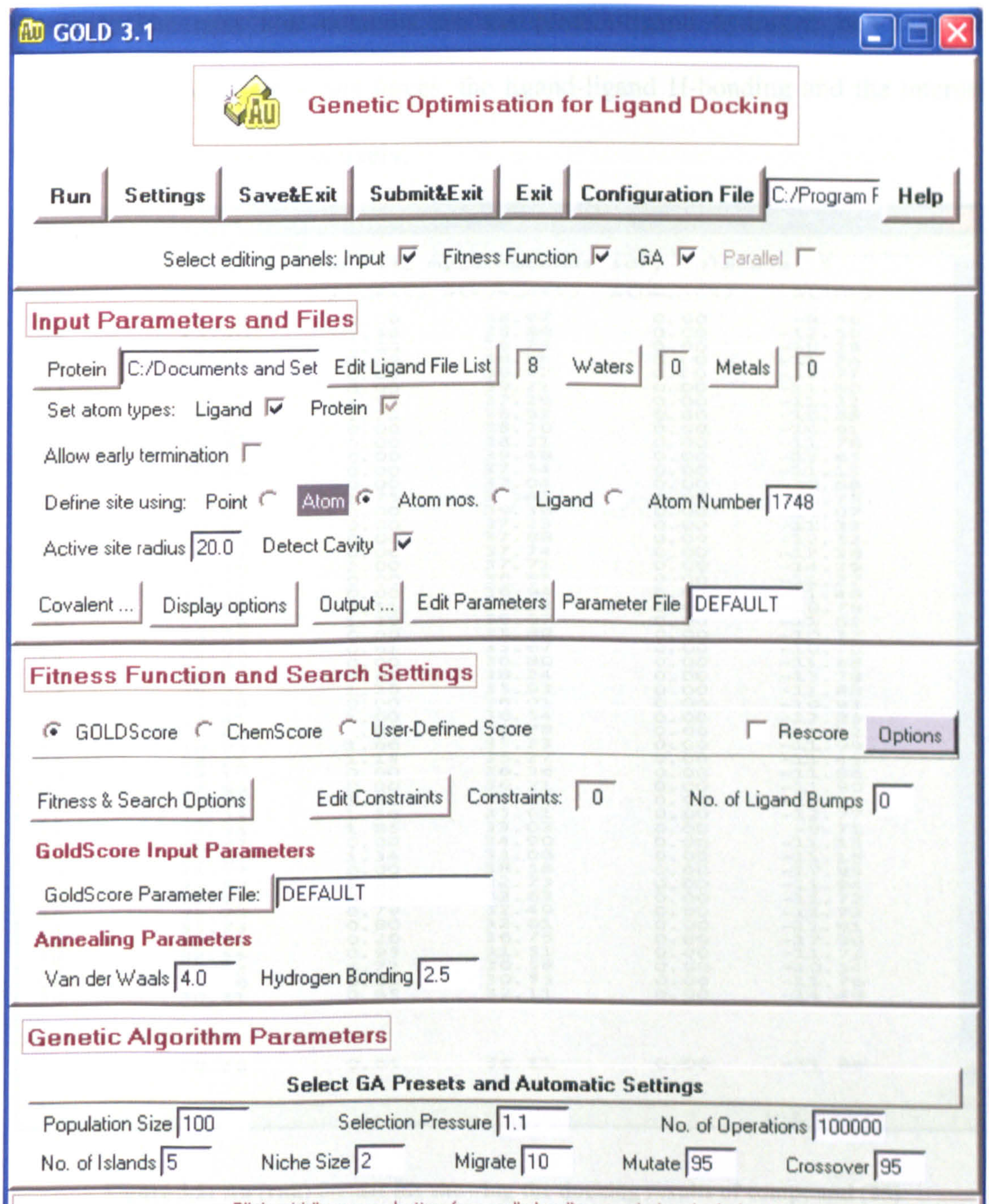


Figure 3.1: Friendly user GOLD interface for Docking ligands in the protein active site.

After the docking being finished, the results are stored in an (rnk) format files, which can be opened by Notepad or WordPad. As it can be seen in figure 3.2, there are six columns; the molecule number (Mol. No.) gives the number of the conformers generated by the genetic algorithm, the next column is the total score of the next four columns. The next four columns are the protein-ligand hydrogen bonding, the protein-ligand Van der Waals forces, the ligand-ligand H-bonding and the internal Van der Waals forces respectively.

my_compounds_m25.rnk - Notepad

File Edit Format View Help

Fitness list for ligand file my_compounds.sdf, molecule 25

Mol No	Fitness	S(hb_ext)	S(vdw_ext)	S(hb_int)	S(int)
14	44.02	0.00	38.97	0.00	-9.56
7	42.30	1.38	38.86	0.00	-12.50
19	42.28	0.00	38.23	0.00	-10.28
13	42.13	0.00	38.83	0.00	-11.26
30	42.12	0.00	39.92	0.00	-12.77
32	42.11	0.00	39.21	0.00	-11.80
21	41.73	1.71	37.26	0.00	-11.22
1	41.68	1.78	36.93	0.00	-10.88
50	41.61	0.00	38.20	0.00	-10.91
4	40.90	0.00	38.31	0.00	-11.78
39	40.63	0.00	39.48	0.00	-13.65
11	40.49	0.00	37.54	0.00	-11.13
6	40.25	0.00	37.04	0.00	-10.68
5	40.07	1.75	36.41	0.00	-11.75
47	40.05	0.00	39.36	0.00	-14.07
41	39.87	0.00	37.23	0.00	-11.33
43	39.56	0.00	37.88	0.00	-12.52
42	39.53	0.00	37.74	0.00	-12.36
36	39.46	2.00	37.71	0.00	-14.39
44	39.39	0.00	36.85	0.00	-11.27
17	39.31	0.00	36.94	0.00	-11.48
27	39.18	1.51	35.23	0.00	-10.76
23	39.06	1.29	36.07	0.00	-11.82
28	39.02	0.00	35.89	0.00	-10.32
22	38.96	0.61	35.67	0.00	-10.70
35	38.93	0.00	36.80	0.00	-11.68
37	38.80	0.00	38.24	0.00	-13.78
8	38.71	1.20	35.93	0.00	-11.88
33	38.59	0.00	36.01	0.00	-10.93
2	38.41	1.00	36.54	0.00	-12.83
3	38.40	1.80	34.78	0.00	-11.22
49	38.25	1.66	35.33	0.00	-11.99
15	38.21	1.15	35.27	0.00	-11.43
18	38.13	0.00	36.36	0.00	-11.86
31	37.89	0.00	36.21	0.00	-11.90
20	37.80	1.85	35.09	0.00	-12.30
25	37.75	1.56	35.61	0.00	-12.77
45	37.56	1.85	33.03	0.00	-9.71
38	36.87	0.00	34.90	0.00	-11.13
34	35.67	1.34	33.08	0.00	-11.15
29	35.22	0.00	38.58	0.00	-17.83
9	33.97	0.90	32.73	0.00	-11.94
10	33.92	0.79	32.63	0.00	-11.72
40	33.75	0.79	32.49	0.00	-11.72
24	33.73	0.80	32.61	0.00	-11.91
46	32.83	0.20	36.75	0.00	-17.90
48	32.56	0.95	31.51	0.00	-11.73
12	32.23	0.72	30.58	0.00	-10.54
16	31.06	0.81	30.55	0.00	-11.76
26	30.31	0.65	32.11	0.00	-14.49
Average Values:					
	38.30	0.64	36.11	0.00	-11.99

Ln 2, Col 1

Figure 3.2: An (rnk) format file showing the docking results of compound (25)

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