CHARLES UNIVERSITY IN PRAGUE

FACULTY OF PHARMACY IN HRADEC KRÁLOVÉ

PREPARATION AND EVALUATION OF NEW POTENTIAL ANTIMICROBIAL DRUGS AND PRODRUGS

PhD THESIS

2007 Ing. Imramovský Aleš

Charles University in Prague
Faculty of Pharmacy in Hradec Králové
Department of Inorganic and Organic Chemistry

Ing. Imramovský Aleš

PREPARATION AND EVALUATION OF NEW POTENTIAL ANTIMICROBIAL DRUGS AND PRODRUGS

PhD THESIS

Hradec Králové

March 2007

ACKNOWLEDGEMENTS

Firstly, I would like to thank my supervisor Associate Professor Jarmila Vinšová, PhD for her valuable advice and constant support throughout my study.

Secondly, I would like to thank my family for their support and all my friends and colleagues at the Department of Inorganic and Organic Chemistry who have made these years memorable.

My special thanks to Professor Dr. Slovenko Polanc from Faculty of Chemistry and Chemical Technology in Ljubljana for a stimulating and fruitful collaboration and co-supervisory during my two stays in Slovenia.

I would especially like to thank Juana Monreal Férriz for being great companion in the lab.

My thanks are also given to Mr. Petr Jančárek, who recorded the NMR spectra, Assoc. Assoc. Prof. Jiří Kuneš, PhD and Prof. Milan Pour, PhD for measurement and help with interpretation of NMR spectras and Mrs. Iva Vencovská, who measured the IR spectras.

I am very thankful to Katherin Monks for thoroughly checking English grammar of the body of this thesis.

Last but not least I thank the Ministry of Education for the financial support making me possible to attend PhD courses.

Imramovský Aleš

March 2007

List of publications

This thesis is based on the following publications, which are referred to in the text by the Roman numerals I – VI and experiments that need to be accomplish by some other biological evaluation.

I. Intramolekulární cyklizace využívané k uvolňování účinných látek z proléčiv. Vinšová, J.; Imramovský, A.
Chem. Listy, **2005**, *99*, 21-29.

II. Salicylanilidy – stále aktuální skupina s potenciální antibakteriální aktivitou. Vinšová, J.; Imramovský, A.
Cesk. Slov. Farm. **2004**, *53*, 294-299.

III. Salicylanilide acetates: Synthesis and Antibacterial Evaluation.
Vinšová, J.; Imramovský, A.; Buchta, V.; Čečková, M; Doležal, M; Štaud, F.; Jampílek, J.; Kaustová, J.
Molecules, **2007**, *12*, 1-12.

IV. Salicylanilide esterification: unexpected formation of novel 7-membered rings.
Imramovský, A.; Vinšová, J.; Monreal Férriz, J.; Kuneš, J.; Pour, M.; Doležal, M. *Tetrahedron Lett.*, **2006**, *47*, 5007-5011.

V. Recent Advances on Isoniazide Derivatives
Vinšová, J.; Imramovský, A.; Jampílek, J.; Monreal, J. F.; Doležal M.
Curr. Med. Chem. under revision **2007.**

VI. A new modification of anti-tubercular active molecules.
Imramovský, A.; Polanc, S.; Vinšová, J.; Kočevar, M.; Jampílek, J.; Rečková, Z.; Kaustová, J.
Bioorg. Med. Chem., **2007**, *15*, 2551-2559.

CONTENT

LIST OF ABBREVIATIONS

Abs.	Absorbance
AcOH	Acetic acid
Anal.	Analysis
Calcd	Calculated
clog*P*	Calculated octanol/water partition
CNCTC	Czech National Collection of Type Cultures
CO	Carbonyl group
CPF	Ciprofloxacine
d	Days
DCI	Dicyclohexylcarbodiimide
DEE	Diethyl ether
DEMA	Diethoxymethyl acetate
DMF	*N*,*N*-dimethylformamide
DMFDMA	*N*,*N*-dimethylformamide dimethyl acetate
DMSO-*d6*	Hexadeuterodimethyl sulfoxide
DNA	Deoxyribonucleic acid
e.g.	*exempli grata*
EMB	Ethambutol
EU	European Union
Fig.	Figure
HIV	Human Immunodeficiency Virus
HPLC	High Pressure Liquid Chromatography
i.e.	*id est*
INH	Isoniazid
IR	Infra red
Log*P*	Octanol/water partition
M^+	Molecular ion
M.	*Mycobacterium*
M. tbc.	*Mycobacterium tuberculosis*
MDR	Multi Drug Resistant
MIC	Minimal inhibitory concentration
mp	Melting point

MS	Mass spectroscopy
NIAID	National Institute of Allergy and Infectious Disease
NMR	Nuclear magnetic resonance
No.	Number
p.a.	*pro analysi*
PAS	*para*-Aminosalicylic acid
POA	Pyrazine-2-carboxylic acid
ppm	*parts per million*
PZA	Pyrazinamide
RIF	Rifampin
TAACF	Tuberculosis Antimicrobial Acquisition Coordinating Facility
TCS	Two-component regulatory system
TB	Tuberculosis
UV	Ultra violet
ver.	Version
WHO	Word Health Organisation
Z-	Benzyloxycarbonyl group

1. INTRODUCTION

The common pathogens, that are to blame for various types of infections, nowadays have become more and more resistant to the current drugs. Resistance to antimicrobial agents is an unavoidable side effect of their use and goes hand in hand with an inexorable drive of bacterial evolution. WHO, the EU and the Ministry of Health Czech Republic are engaged in solving this imminent danger. The European Union proclaimed in the 7th Frame programme („Higly innovative approaches for research into host-pathogen interaction in tuberculosis“)[1], in our republic, for example, was proclaimed the priority of program NI – Research and development in the field of infectious diseases, microbiology, epidemiology and immunology predominantly concerned with virus hepatitis, tuberculosis, borreliosis and AIDS.[2] The emerging combat against drug-resistant bacteria leads to the search for a new type of active molecule with a different or novel mechanism of action. The research is focused mainly on structure modification or finding a completely new type of active agent. The Department of Inorganic and Organic Chemistry of the Faculty of Pharmacy is intensively engaged in the field of searching for a novel potential antimicrobial active compound with high activity against MDR bacteria, predominantly against *Mycobacterium tuberculosis* and/or *M. avium*, *M. kansasii*, and *M. intracellulare*. In the recent years there also has been a dramatic increase in the incidence of fungal infections (mainly systemic candidiasis, aspergillosis). Nosocomial infections are a serious growing problem in medicine as well. Therefore, this very relevant issue is discussed in this dissertation.

1.1. Tuberculosis

Tuberculosis (TB for Tubercle Bacillus) is a chronic infectious disease caused by the bacterium *Mycobacterium tuberculosis*, which most commonly affects the lungs (Pulmonary TB), but can also affect the central nervous system, lymphatic system, circulatory system, genitourinary system, bones and joints.

1.2. World TB incidence

Over one third of the world's population is infected with the TB bacteria and every second a new case of the infection occurs.[3] Not everyone who is infected develops the active form; an asymptomatic latent form of TB infection is insidious. However, one in ten latent infections will progress to the active TB disease which, if left untreated, will kill more than half of its victims. An increasing drug resistance problem together with the HIV-induced

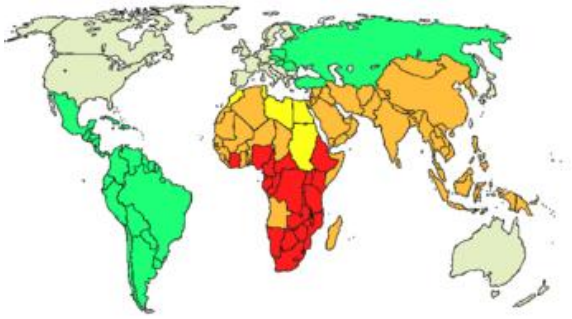


Fig. 1: Cases per 100,000; Red = >300, orange = 200-300; yellow = 100-200; green 50-100 and grey <50. Data from WHO, 2006. [4]

acquired immunodeficiency syndrome is leading to further epidemic spread among the population. In 2004, 14.6 million people suffered from active TB and there were 8.9 million new cases and 1.7 million deaths,[3] mostly in developing countries (**Fig. 1**).[4] The emergence of multiple drug resistant (MDR) TB; the TB/HIV combination and sleeping latent forms of *M. tuberculosis* are alarming reasons to find new, more acceptable drugs with a new mechanism of action that would shorten the treatment regime. The global incidence of TB has been on the increase for the last 6 years (**Fig. 2**). Thus, research efforts are required to overcome all concomitant symptoms.

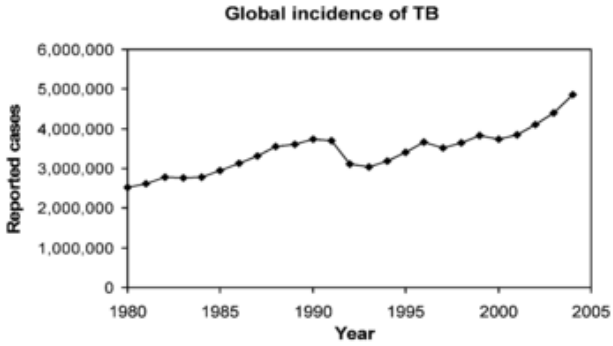


Fig. 2: Annual number of new reported TB cases
Data from WHO.[4]

1.3. TB treatment

Chemotherapy of TB started in the 1940s. A number of agents have been discovered since then, including *para*-aminosalicylic acid (PAS), isoniazid (INH), pyrazinamide (PZA), cycloserine, ethionamide, rifampin (RIF), and ethambutol (EMB). The majority of these drugs were discovered through broad random screening. Current treatment is made up of a cocktail of the first-line drugs (INH, RIF, PZA and EMB), given for six months.[8] If the treatment fails as a result of bacterial drug resistance, or intolerance to one or more drugs, second-line drugs are used, such as PAS, kanamycin, fluoroquinolones, capreomycin, ethionamide and cycloserine. These drugs are generally either less effective or more toxic, with serious side effects.[5] Treatment is made quite

difficult by the presence of metabolically silent, persistent or dormant bacteria within host lesions which are not susceptible to the antimycobacterial drugs that usually kill growing bacteria.[6] During the interaction between mycobacteria and host cells, a cyclic reinfection of host macrophages by tubercle bacillus can occur, allowing for the prolonged survival and persistence of the bacilli.[7] Thus, it is almost impossible to achieve complete sterilization of lesions. This unique ability of the bacilli to withstand chemotherapeutic and host cell immune attack allows surviving bacilli to persist for decades before reactivation making tuberculosis so difficult to treat and eradicate. Due to the heterogeneous bacterial populations in the tuberculosis lesions and perhaps also to insufficient host immunity, treatment with a combination of drugs must be given for an extended period of time to prevent reactivation of the disease by persisting bacilli.

The key to improving therapy is to develop new agents with potent sterilizing activity that will lead to a shortening of the duration of the chemotherapy.[8] The drugs currently used as antituberculosis treatments and than the most advanced compounds of last ten years undergoing clinical trials were described in comprehensive review.[9]

1.4. Antifungal issue

Fungi are one of the most neglected pathogens apparent from the fact that the Amphotericin B, a polyene antibiotic, discovered way back in 1956 is still used as a "gold standard" for antifungal therapy. The past two decades have witnessed a dramatic rise in the incidences of life threatening systemic fungal infections. This can be ascribed to the increase in the number of immuno-compromised patients due to a rising in HIV infected population, cancer chemotherapy and indiscriminate use of antibiotics. The majority of clinically used antifungals suffer from various drawbacks in terms of toxicity, efficacy and cost, and their frequent use has led to the emergence of resistant strains. Hence, there is a great demand for new antifungal agents belonging to wide range of structural classes, selectively acting on novel targets with fewer side effects[10]. Therefore discovering novel antifungal drug targets and investigational molecules acting on them is also very important.

1.5. Progress in drug discovery (Paper I)

Current research involves the testing of new or reformulated drugs (such as rifampin, fluoroquinolones, macrolides[11] and isoniazid[12]; combinations of drugs with a different mechanism of action, supplementation and enhancement of existing drugs, including the identification of drug targets (such as persistent gene) using micro array

analysis and molecular biology tools. Other approaches include structure based design and *in vitro* and *in vivo* screening to identify new drugs; evaluation of novel drug combinations; alternatively the order of drugs given in treatment. Besides chemotherapy, immunotherapeutic approaches such as DNA vaccines[13,14] and cytokines used in combination with chemotherapy also offer a promising prospect for improved treatment of TB.[14]

The development of novel slow-release drug delivery systems that could reduce the frequency and the amount of drug necessity during treatment[15] and also research into molecular targets is up to date.

The design of prodrugs is a widely used strategy to improve the delivery of drugs to the site of their therapeutic action, to increase drug bioavailability and selectivity, and to overcome many problems associated with the oral bioavailability and cellular permeability. In our comprehensive review[16] (see the whole text in Czech language as **paper I**) we have discussed some modern approaches to develop of active molecules by cyclization, which is non enzymatic (chemical) liberation of the drug from its appropriate prodrug form. Possible modes of development are discussed and include cyclic elimination caused basic eventually amidic group or carboxylate function. Investigation of this area is not dependent on individual variability of metabolism and brings usefull information for preparing of new prodrugs form. Targeting drugs to the specific site of action provides several advantages over non-targeted drugs. The main advantages are the prevention of drug side effects on healthy tissues and the enhancement of drug uptake by target cells. Prodrugs release the parent drugs either by chemical or enzymatic activation. Although enzymatic activation is preferred when the objective is the site-specific drug delivery (e.g. by designing a prodrug that is selectively activated by a specific enzyme), chemical prodrug activation has advantage of not being affected by biological variability.[17] Esters are one of the most frequently used modifications of an active molecule[18,19] and one of the main focus points of the thesis.

Generally, the targets for anti-TB drugs include the biosynthetic pathways which are involved in the production of macromolecules (the proteins, the nucleic acids, or cell wall polymers). Many well-known anti-TB drugs target the biosynthesis of these macromolecules. Recent developments in genetic engineering of *M. tuberculosis* have determined the genome sequence of *M. tuberculosis*.[20] The genome comprises 4,411,529 base pairs, contains around 4,000 genes, and has a very high guanine and cytosine content that is reflected in the biased amino-acid content of these proteins. *M. tuberculosis* differs

radically from other bacteria in that a very large portion of its coding capacity is devoted to the production of enzymes involved in lipogenesis and lipolysis, and to two new families of glycine-rich proteins with a repetitive structure that may represent a source of antigenic variation (Fig. 3.).

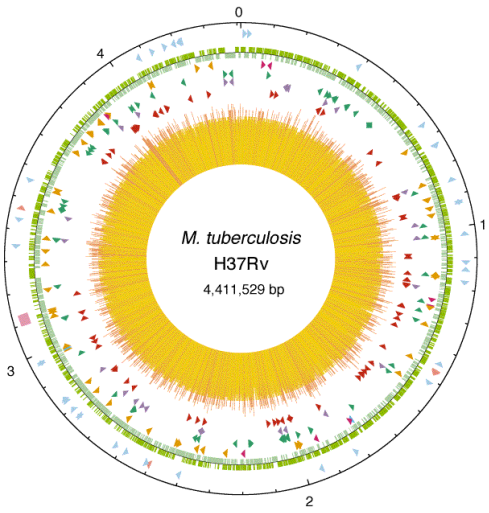


Fig. 3: Circular map of chromosome of *M. tuberculosis* $H_{37}Rv$. The picture was taken from http://genolist.pasteur.fr/TubercuList/.

To pass through the highly lipophilic bacterial cell wall the potential drug must have the appropriate physicochemical parameters. In general, there exists the ”Lipinski rule of 5”[21] which says that the potential active molecule should have: no more than 5 H-bond donors, no more than 10 H-bond acceptors, no higher molecule weight than 500 and clog*P* (calculated octanol/water partition) no higher than 5. No more than one of mentioned rules should be broken in order to obtain a good perspective of investigated compounds as potential antituberculotic drugs.

2. AIM OF THE THESIS

The overall aim of this thesis was to investigate new prodrug forms of antimicrobial agents mainly from the group of anti-tuberculosis and antifungal active compounds and preparation of new potentially antibacterial active compounds. Prodrug form allows better bioavailability of drug, helps to overcome many barriers, make easily transport to the site of action and decreases associated toxicity. Prodrug can be defined as pharmacologically inert chemical derivative that can be converted *in vivo* to the active drug molecule, enzymatically or nonenzymatically/chemically, to exert a therapeutic effect. Ideally, the prodrug should be converted to the origin drug as soon as the goal is achieved, followed by the subsequent rapid elimination of the released derivatizing group. The low membrane permeability and polar solubility is very often overcome by amino acid or short peptide esterification. Peptide transporters are very attractive targets. They have several advantages, broad substrate specifity and high capacity, physiologic advantage going to structural modification possibility. All our investigations were based on a literary study of both groups – salicylanilides and isoniazid derivatives that led to survey articles.

The specific objectives of this study were:

- Releasing of active molecule by intramolecular cyclization.
- Literary review of biological active salicylanilides, the nowadays trend of their development.
- Searching and synthesis of applicable biodegradable prodrugs of anti-tuberculosis active salicylanilides.
- Development methods for synthesis of amino acid esters of salicylanilides.
- Study of rearrangement that leads to benzoxazepine-2,5-diones.
- Survey of derivatives having isoniazid moiety in their molecule, structure activity relationships and the most efficient substitution.
- Development and synthetic methods for synthesis methylenehydrazono INH derivatives and pyrazinamide derivatives.

3. SALICYLANILIDES MODIFICATION (paper II, III, IV)

Salicylanilides have shown manifold biological activities. On the basis of a literary search our comprehensive review was published[22] (see the whole text in **paper II**). Many of these compounds were prepared in our department with a good antifungal activity and a broad spectrum of antimycobacterial activity.[23,24,25,26] Salicylanilides have other important biological activities, they are employed in practise as herbicides (mainly the nitro derivates) in rice cultivation, halogenated derivates are employed as antiparasite agents in veterinary practice against *Fasciaola hepatica*. The most successful 5,2´-dichloro-4´-nitrosalicylanilide (baluscide) is a commonly used molluscicide.[27,28,29,30] In 1998, a new mechanism of their action based on the inhibition of a two-component regulatory system (TCS) in bacteria was discovered.[31,32] Compounds that inhibit TCS could block important bacterial signaling pathways that may lead to the death of the bacterial cell. For the biological effect a salicylanilide pharmacophore, an electron withdrawing substituent on the salicylic moiety and hydrophobic group on the anilide moiety, is essential. The most active salicylanilides have shown significant *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis* and non tuberculosis strains *M. avium* and *M. kansasii* (MIC 1-4 μg/ml)[33] which are resistant to the first-line drug, isoniazid. Hence, salicylanilides are a perspective group of potential drugs for the treatment of tuberculosis caused by these strains. Several research groups intensively investigated new derivates of salicylanilides as well as their heterocyclic isosters,[34] acryloylamino – salicylanilides[35] or benzylsalicylamide derivates.[36]

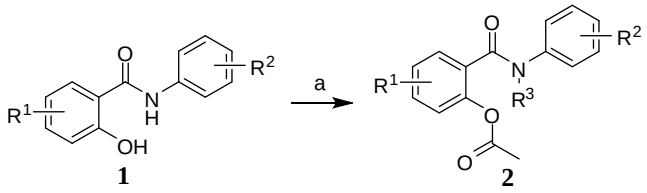
Our strategy in the design of potential antimycobacterial active agents has been mainly oriented towards the synthesis of the prodrug form of the most anti-tuberculosis active salicylanilides, by attaching an amino acid and later on a short peptide to the active molecule.

3.1. Acetylation

Physicochemical properties of salicylanilides such as low solubility mean that this group of promising potential drugs is still discriminated in clinical practice. The common approach in drug modifications is to prepare esters of this potential active group as a prodrug form with improved physicochemical properties and better biodegradability of active molecules.

For the phenol group protection an analogy of salicylic/acetylsalicylic acid was chosen. The acetyl group was introduced to the phenol moiety in the $C_{(2)}$ position of the salicylic nucleus of the starting antimicrobial active salicylanilides to obtain a new series of ten compounds with more convenient physicochemical properties as well as to get a better form for biotransformation of antimycobacterial and antifungal active salicylanilides.

Esterification by acetic acid was performed either by activation of the carboxyl group of acetic acid with *N,N´*-dicyclohexylcarbodiimide (DCI), or by reaction of phenolate and acetanhydride (**Scheme 1**). Esterification with acetyl chloride failed, the isolated product was *N,O*-diacetyl salicylanilide.



Scheme 1: Synthesis of acetyl salicylanilides.

a = DMF, DCI, AcOH -15 °C; or $NaOH/H_2O$, $(CH_3CO)_2O$, 0 °C; or chlorobenzene, CH_3COCl, reflux 6 hours, $R^3 = COCH_3$

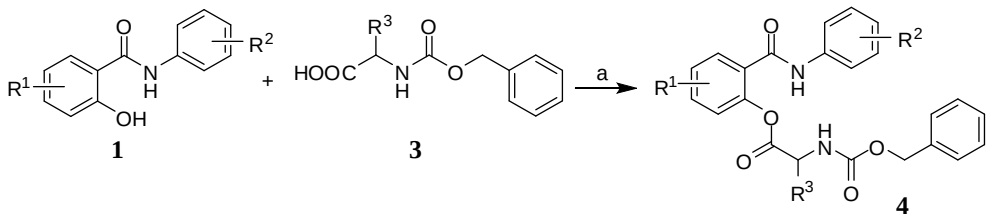
All prepared acetyl salicylanilides **2** were characterized by ^1H NMR, ^{13}C NMR and IR spectroscopy, purity was checked by elemental analysis. *In vitro* biological evaluation was carried out against *Mycobacterium tuberculosis* My 331/88, *M. avium* My 330/88 and two strains of *M. kansasii* My 235/80 and My 6 509/96. All strains were obtained from the Czech National Collection of type cultures (CNCTC) except My 6 509/96 which was clinically isolated. Antifungal properties were evaluated against the following strains: CA-*Candida albicans* ATCC 44859, CT-*Candida tropicalis* 156, CK-*Candida krusei* E28, CG-*Candida glabrata* 20/I, TB-*Trichosporon beigelii* 1188, AF-*Aspergillus fumigatus* 231, AC-*Absidia corymbifera* 272 and TM-*Trichophyton mentagrophytes* 445. The HPLC separation module Waters Alliance 2695 XE and Waters Photodiode Array Detector 2996 (Waters Corp., Milford, MA, U.S.A.) were used for Log *K* determination. (For details see **paper III**).

3.2. Amino acid esterification

The phenolic hydroxyl group of the most antitubercular active salicylanilides **1** was esterified by several *N*-benzyloxycarbonyl α-amino acids **3**. We have chosen more

lipophilic amino acids such as Glycine, (*R*, *S*)-Alanine, (*R*, *S*)-Valine and (*R*, *S*)-Phenylalanine. Salicylanilide esters of amino acids **4** can be considered as prodrug forms with better bioavailabilty done by hydroxyl group protection. The type of amino acid influences physico-chemical properties and lipophilicity that also play very important role in the distribution of drugs through the lipid mycobacterial cell membrane.

General methods for salicylanilide esterification such as reaction of amino acid chloride with salicylanilide phenolate failed. The most efficient was found to be DCI-mediated condensation of salicylanilides with *N*-protected amino acids. In most cases, the reaction produced the required esters (**Scheme 2**). In two cases (**3f** and **3m**) the yields of this reaction were very low, even though experiments were repeated. The whole amount of both prepared compounds was used for biological evaluation and further steps, as deprotection combined with the following rearrangement were not done.

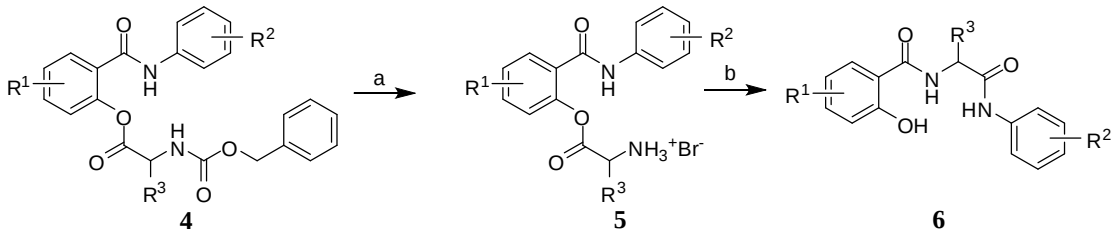


1 **3** **4**

Scheme 2: Synthesis of Z-amino acid esters and substituted salicylanilides derivates

a = DMF, DCI, -15 °C

While *N*-deprotection of esters **4** by hydrogenolysis on Pd/C was unsuccessful, an acidolysis (33% HBr in anhydrous acetic acid) gave appropriate hydrobromide amino salts **5**. Subsequent amino group liberation by triethylamine under anhydrous conditions yielded unexpected product **6** possessing neither ester nor free amino groups, but the presence of a phenolic hydroxyl was clearly apparent. The structure of this product was unequivocally corroborated by 2D NMR. Unexpected rearrangement products after amino group liberation were identified as substituted hydroxy-*N*-(phenylamino)-oxo-alkyl)benzamides **6** (**Scheme 3**).

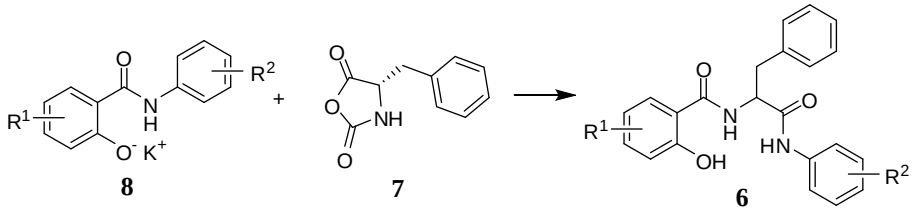


4 **5** **6**

Scheme 3: Unexpected products of *N*-deprotection

a = AcOH/HBr, b = Et_3N

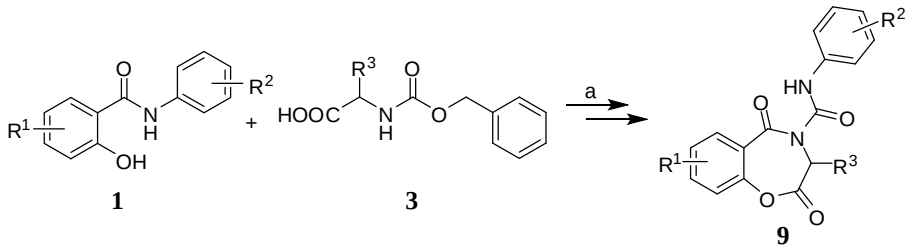
Another possible way for preparation of esters of α-amino acids and salicylanilides is the reaction of *N*-carboxyanhydrides of α-amino acids (Leuchs anhydrides) with appropriate salt of salicylanilides. In these compounds activation and protection are combined in a single –CO-O-grouping. Leuch anhydride is a compound generated from α-amino acid where amino group is protected and at the same time the carboxylic group is activated. These important cyclic compounds can serve for one step preparation of esters[37] or for high molecular weight polypeptide preparation. Leuchs anhydrides are prepared by fairly simple procedure such as treatment of the suspension of an amino acid in non-polar solvent with the phosgene or thermal elimination of benzyl chloride from benzyloxycarbonylamino acid chlorides. The safest one, which we have used, is preparation using bis(trichloromethyl)carbonate (triphosgene) under anhydrous conditions.[38] Attack by nucleophile (phenolate) on the carbonyl of amino acid and acylation is immediately followed by decarboxylation of thus formed carbamoic acid derivative. The regenerated amino group is ready for acylation by a second *N*-carboxanhydride. The sole by-product, carbon dioxide, escapes from the reaction mixture. Therefore we have applied this elegant simple reaction to prepare directly an appropriate ester. Thus, Leuch anhydride of *S*-phenylalanine **7** was combined with salicylanilide potassium salt **8** (**Scheme 4**). The reaction led to the amide **6**, the same product that was obtained from the reaction of *N*-benzyloxycarbonyl-*S*-phenylalanine, DCI and salicylanilide, after deprotection of amino group. The structure was confirmed by all accessible spectral method inclusive of 2D NMR spectra.



8 **7** **6**

Scheme 4: Reaction of Leuch anhydride of *S*-phenylalanine (**7**) with salicylanilide salt (**8**)

When Z-Glycine and (*S*)-Z-Alanine (**3**) were esterified by the following salicylanilides **1** (5-Cl, 4´-Cl; 5-Cl, 4´-Br; 5-Cl, 4´-CF_3, 5-Cl, 3´-Cl); 7-*exo*-trig cyclization proceeded benzoxazepine-diones **9 (Scheme 5**). The hypothesis of a possible mechanism of their formation has been presented in **paper IV**.



1 **3** **9**

Scheme 5: Salicylanilide esterification – formation of benzoxazepine-2,5-diones
a = DMF, DCI, -15 °C

All prepared compounds (esters of Z-amino acids and substituted salicylanilides **4,** their hydrobromide salts **5**, substituted hydroxy-*N*-(phenylamino)-oxo-alkyl)benzamides **6** and benzoxazepine-2,5-diones **9**) were characterized by ^{1}H NMR, ^{13}C NMR and IR spectroscopy. The purity was checked by elemental analysis except in the case of the hydrobromide salts that have been too hygroscopic and their measurement did not give precise values. Mass spectra were recorded for several representative examples from every group (benzoxazepines, hydrobromide salts of salicylanilide amino acid esters and hydroxy-*N*-(phenylamino)-oxo-alkyl)benzamides). General procedures and possible cyclization modes of representative compounds mentioned above were carefully described in **paper IV**.

Biological testing was provided for *N*-protected esters of amino acids and salicylanilides and unexpected products after amino group liberation. *In vitro* biological evaluation for antimycobacterial properties of all *N*-protected esters of amino acids and salicylanilides **4** and unexpected products after amino group liberation **6** was carried out against *Mycobacterium tuberculosis* My 331/88, *M. avium* My 330/88 and two strains of *M. kansasii* My 235/80 and My 6 509/96. All compounds were sent for *in vitro* testing of their antitubercular activity at Hansen´s Disease Center (Colorado State University) as part of the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) screening program for the discovery of novel drugs for the treatment of tuberculosis. Currently only particular results of it are available. Investigation of antifungal properties

against the following strains: CA-*Candida albicans* ATCC 44859, CT-*Candida tropicalis* 156, CK-*Candida krusei* E28, CG- *Candida glabrata* 20/I, TB-*Trichosporon beigelii* 1188, AF-*Aspergillus fumigatus* 231, AC-*Absidia corymbifera* 272 and TM-*Trichophyton mentagrophytes* 445, is already in process. The HPLC separation module Waters Alliance 2695 XE and Waters Photodiode Array Detector 2996 (Waters Corp., Milford, MA, U.S.A.) were used for Log *K* determination.

General schemes, chemical characteristics and currently known results of biological evaluation for benzoxazepine-diones, *N*-protected esters of amino acids and salicylanilides, hydrobromide salts of esters of amino acids and salicylanilides (only chemical characteristics) and substituted hydroxy-*N*-(phenylamino)-oxo-alkyl)benzamides as unexpected products after amino group liberation are in the following part.

3.3. Experimental part

3.3.1. General

The chemicals were purchased from commercial sources (Aldrich, Merck, Fluka). Substituted salicylanilides were synthesized in microwave reactor MicroSYNTH MLS ETHOS 16OO URM. Melting points (uncorrected) were determined on a Kofler micro-hot-stage. Infrared spectra were recorded on a Nicolet Impact 400 apparatus in KBr pellets. NMR spectra were measured in $CDCl_3$ or DMSO-d_6 solutions (if not specified otherwise) on a Varian Merkury – Vxbb 300 (300 MHz for ^1H and 75.5 MHz for ^{13}C; Varian Comp. Palo Alto, CA, USA). The chemical shifts δ are given in ppm, related to tetramethylsilane (TMS) as an internal standard. The coupling constants (*J*) are reported in Hz. Elemental analyses (C, H, N) were performed on an automatic microanalyser CHNS-O CE instrument (FISONS EA 1110, Milano, Italy). Optical activities were measured on polarimeter ADP 220 BS Bellingham Stanley Ltd. The reactions were monitored and the purity of the products was checked by TLC (Fluka silica gel/TLC cards 60 PF_{254}). The plates were visualized using UV light. Mass spectra were recorded on ABI/MSD SCIEX API 3000^{TM}LC/MS/MS System (MSD SCIEX, Concord, ON, Canada). Names of the mentioned compounds were generated and structures were drawn with ChemDraw Ultra 10.0 and are formatted as ACS Document 1996. General structures of prepared compounds and their substituents are summarised in **Tables 1, 2, 5, 9, 10.**

3.3.2. Biological evaluation

Antimycobacterial evaluation made in TAACF (U.S.A)

The compounds were screened for their *in vitro* antituberculotic activity under the direction of the US National Institute of Health, NIAD Division, under protection of the program Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF). Primary screening was conducted at a single concentration 6.25 μg/mL against *M. tuberculosis* $H_{37}Rv$ (ATCC2729) in BACTEC 12B medium using a broth microdilution assay, the Microplate Almar Blue Assay (MABA). Compounds demonstrating at least 90% inhibition in the primary screening (MIC<6.25 μg/mL) were tested at a lower concentration against *M. tuberculosis* $H_{37}Rv$ to determine the MIC by MABA. The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 99% relative to the controls.[39] Results are summarized in the **Table 3**.

Antimycobacterial evaluation made in National Reference Laboratory (Czech Republic)

The *in vitro* antimycobacterial activity of all prepared compounds was evaluated against *M. tuberculosis* CNCTC My 331/88 (dilution of the strain was 10^{-3} μmol/L), *Mycobacterium kansasii* CNCTC My 235/80 (dilution of the strain was 10^{-4} μmol/L), *M. kansasii* 6509/96 (dilution of the strain was 10^{-4} μmol/L) and *Mycobacterium avium* CNCTC My 330/88 (dilution of the strain was 10^{-5} μmol/L) in The National Reference Laboratory for *Mycobacterium kansasii*, Regional Institute of Hygiene, Ostrava, Czech Republic. All strains were obtained from the Czech National Collection of Type Cultures (CNCTC), except *M. kansasii* 6509/96, which was clinically isolated. The antimycobacterial activities were determined in a Šula semisynthetic medium (SEVAC, Prague, Czech Republic). The compounds were added to the medium as dimethyl sulphoxide solutions. The following concentrations were used: 250, 125, 62, 32, 16, 8, 4, 2 and 1 μmol/L. The MIC values were determined after incubation at 37 °C for 7, 14 and 21 days. The MIC (μmol/L) was the lowest concentration of a substance at which the inhibition of the growth of mycobacteria occurred. Results are shown in the **Tables 6, 11.**

Antifungal evaluation made in Faculty of Pharmacy

For antifungal *in vitro* evaluation the broth microdilution test M27-A [40] was used. Antifungal activity of the synthesized compounds was assessed against *Candida albicans* ATCC 44859 (CA), *Candida tropicalis* 156 (CT), *Candida krusei* E28 (CK), *Candida*

glabrata 20/I (CG), *Trichosporon beigelii* 1188 (TB), *Aspergillus fumigatus* 231 (AF), *Absidia corymbifera* 272 (AC), and *Trichophyton mentagrophytes* 445 (TM). Fluconazole was used as a reference drug. The procedure was performed with twofold dilution of the compounds in RPMI 1640 medium (Sevapharma, Prague, Czech Republic) buffered to pH 7.0 with 0.165 mol of 3-morpholinopropane-1-sulphonic acid. Drug-free controls were included. The minimal inhibitory concentrations (MICs) were determined after 24 h and 48 h of static incubation at 35 °C. With *T. mentagrophytes*, the final MICs were determined after 72 h and 120 h of incubation. The results of all *in vitro* tested compounds are summarized in **Table 7**.

3.3.3. Liphophilicity determination

The logarithm of the partition coefficient for *n*-octanol/water (Log *P),* was calculated using the programs CS ChemOffice, ChemDraw Ultra version 10.0 (CambridgeSoft, Cambridge, MA, U.S.A.) and ACD/Log *P* ver. 1.0 (Advanced Chemistry Development Inc., Toronto, Canada). The clog *P* values (the logarithm of *n*-octanol/water partition coefficient based on established chemical interactions) were generated by means of CS ChemOffice Ultra version 7.0 (CambridgeSoft, Cambridge, MA, U.S.A.) software.

For HPLC determination the separation module Waters Alliance 2695 XE and Waters Photodiode Array Detector 2996 were used (Waters Corp., Milford, MA, U.S.A.). The chromatographic column Symmetry® C_{18} 5 μm, 4.6×250 mm, Part No. WAT054275, (Waters Corp., Milford, MA, U.S.A.) was used. The HPLC separation process was monitored by Millennium32® Chromatography Manager Software, Waters 2004 (Waters Corp., Milford, MA, U.S.A.). As a mobile phase the mixture of MeOH p.a. (30.0%) and H_2O-HPLC – Mili-Q grade (70.0%) was used for all the compounds. The total flow of the column was 0.7 ml/min, injection 30 μl, column temperature 22 °C, the sample temperature was 10 °C. The detection wavelength was 265 nm. The KI methanolic solution was used for the dead-time (T_D) determination. Retention times (T_R) were measured in minutes.[41]

The capacity factors *K* were calculated using the Millennium32® Chromatography Manager Software according to the formula $K = (T_R-T_D)/T_D$, where T_R is the retention time of the solute, whereas T_D denotes the dead time obtained via an unretained analyte. Log *K*, calculated from the capacity factor *K*, is used as the lipophilicity index converted to the log *P* scale.

Calculated and determinates values of liphophilicity factors are shown in **Tables 1, 4, 8, 12.**

3.3.4. Purity determination

Purity of starting salicylanilides and final products hydroxy-*N*-(phenylamino)-oxo-alkyl benzamides **6** were set by HPLC at the same time as liphophilicity determination on the base of UV absorption. The conditions and equipment were the same as in 3.3.3.

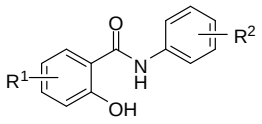
3.3.5. Experimental results

3.3.5.1. **Starting salicylanilides 1**[24]

General procedure:

The starting salicylanilides **1** were routinely prepared by the reaction of substituted salicylic acids (0.13 mol) with the appropriate anilines (0.13 mol) in chlorobenzene (450 mL) in presence of PCl_3 (0.65 mol) using the microwave reactor MicroSYNTH MLS ETHOS 16OO URM (530W for 20 min). After cooling of the reaction mixture, the crude product was filtered off and recrystalized from ethanol 96%.

Table 1: Salicylanilides **1**, their purity and liphophilicity.



Compound	R^1	R^2	Purity[a] (%)	log *K*	log *P*/Clog*P* ChemOffice	log *P* ACD/Log *P*
1a	5-Cl	3-Cl	99.79	0.4927	5.44 ± 0.42	3.57 / 5.08085
1b	5-Cl	4-Cl	99.89	0.4021	5.40 ± 0.42	3.57 / 5.08085
1c	5-Cl	4-Br	99.85	0.4698	5.58 ± 0.49	3.84 / 5.23085
1d	5-Cl	3,4-diCl	99.90	0.6558	6.31 ± 0.44	4.12 / 5.75025
1e	4-Cl	4-Cl	99.97	0.2806	5.35 ± 0.42	3.57 / 5.08085

[a] Purity was determinated by RP-HPLC.

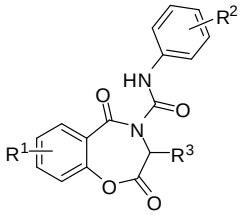
3.3.5.2 **Benzoxazepine-2,5-diones 9**

General procedure

N-Benzyloxycarbonyl protected α-amino acid (10 mmol) and substituted salicylanilide (10 mmol) were dissolved in dry *N*,*N*-dimethylformamide (DMF, 45 ml). The solution was cooled to -10 °C and *N*,*N*-dicyclohexylcarbodiimide (DCI, 11 mmol) was added in three portions over 1 h. The mixture was stirred for 3 h at the same temperature

and stored at +4 °C for 20 h. The precipitate of *N,N´*-dicyclohexylurea was removed by filtration and the solvent was evaporated *in vacuo*. The crude product was purified by crystallization from ethyl acetate – hexane.

Table 2: Benzoxazepine-2,5-diones **9**



Compound	R^1	R^2	R^3
9a	7-Cl	4-Cl	H
9b	7-Cl	4-Br	H
9c	8-Cl	4-Cl	H
9d	8-Cl	3-Cl	H
9e	7-Cl	4-CF_3	H
9f	7-Cl	4-Cl	CH_3
9g	7-Cl	4-Br	CH_3

Data of prepared benzoxazepine-2,5-diones 9:

7-Chloro-*N*-(4-chlorophenyl)-2,5-dioxo-2,3-dihydrobenzo[*f*][1,4]oxazepine-4(5*H*)-carboxamide **9a**.
White solid; yield 35%; mp 246-249 °C. IR (KBr pellet): 3412, 3345, 2931, 2853, 1760 (CO ester), 1705 (CO amide), 1611, 1549, 1493, 1446, 1402, 1362, 1305, 1234, 1157, 1093, 824, 746, 714, 678, 590, 533 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ 10.44 (1H, bs, NH), 8.01 (1H, d, *J*=8.4 Hz, H9), 7.79 (1H, m, H6), 7.59-7.54 (3H, m, H2´, H6´, H8), 7.38-7.35 (2H, m, H3´, H5´), 4.67 (2H, s, CH_2). ^{13}C NMR (75 MHz, DMSO) δ 164.9, 159.5, 151.2, 147.6, 137.6, 136.7, 130.0, 129.0, 127.5, 126.6, 121.1, 119.2, 115.5, 45.0. ^{15}N NMR (DMSO, 500 MHz) δ -252.4 (1N, $J(^{15}N, ^1H)$=90.3 Hz, NH), -231.8 (N). MS (EI): *m/z* (%) 364.2 (M^+, 25), 210.1 (100), 127.1 (92). Anal. Calcd for $C_{16}H_{10}Cl_2N_2O_4$ (365.17): C, 52.63; H, 2.76; N, 7.67; Cl, 19.42; O, 17.53. Found: C, 52.40; H, 3.27; N, 7.90.

N-(4-Bromophenyl)-7-chloro-2,5-dioxo-2,3-dihydrobenzo[*f*][1,4]oxazepine-4(5*H*)-carboxamide **9b**.
White solid; yield 18%; mp 240-242 °C. IR (KBr pellet):3329, 2928, 2851, 1761 (CO ester), 1705 (CO amide), 1575, 1447, 1312, 1245, 1088, 892, 641 cm^{-1}. UV (EtOH 96%) $Abs._{max}$ 256.50, 221.00 nm.^1H NMR (300 MHz, DMSO) δ 10.43 (1H, bs, NH), 7.98 (1H, d, *J*=2.5 Hz, H6), 7.93 (1H, dd, *J*=8.8 Hz, *J*=2.5 Hz, H8),7.59 (1H, d, *J*=8.8 Hz, H9), 7.56-7.46 (4H, m, H2´, H3´, H5´, H6´), 4.69 (2H, s, CH_2). ^{13}C NMR (75 MHz, DMSO) δ 164.9 159.5, 151.2, 147.6, 138.0, 136.7, 131.9, 131.8, 130.0, 126.6, 121.5, 119.2, 115.5, 45.1. Anal. Calcd for $C_{16}H_{10}BrClN_2O_4$ (409.62): C, 46.91; H, 2.46; N, 6.84. Found: C, 46.895; H, 2.815; N, 6.66.

8-Chloro-*N*-(4-chlorophenyl)-2,5-dioxo-2,3-dihydrobenzo[*f*][1,4]oxazepine-4(5*H*)-carboxamide **9c**.
White solid; yield 15%; mp 257-260 °C. IR (KBr pellet):3336, 2930, 2852, 1774 (CO ester) 1701 (CO amide), 1611, 1541, 1431, 1378, 1304, 1235, 10920, 952, 830, 749, 504 cm^{-1}. UV (EtOH 96%) $Abs._{max}$ 286.50, 251.50 nm. ^{1}H NMR (300 MHz, DMSO) δ 10.44 (1H, bs, NH), 8.01 (1H, d, *J*=8.4 Hz, H6), 7.79 (1H, m, H9), 7.59-7.54 (3H, m AA´, BB´, H2´, H6´, H7), 7.38-7.35 (2H, m AA´, BB´, H3´, H5´), 4.67 (2H, s, CH_2). ^{13}C NMR (75 MHz, DMSO) δ 164.9, 159.7, 156.8, 152.9, 147.6, 141.0, 137.6, 129.0, 127.5, 126.4, 121.1, 117.1, 113.1, 44.9. Anal. Calcd for $C_{16}H_{10}Cl_2N_2O_4$ (365.17): C, 52.63; H, 2.76; N, 7.67. Found: C, 52.25; H, 4.27; N, 7.63.

8-chloro-*N*-(3-chlorophenyl)-2,5-dioxo-2,3-dihydrobenzo[*f*][1,4]oxazepine-4(5*H*)-carboxamide **9d**.
White solid; yield 43%; mp 217-219 °C. IR (KBr pellet):3257, 1781 (CO amide), 1705 (CO amide), 1675, 1611, 1593, 1546, 1431, 1376, 1337, 1270, 1235, 1204, 1109, 1076, 983, 953, 873, 782, 763, 746, 682, 464 cm^{-1}. UV (EtOH 96%) $Abs._{max}$ 211.00, 249.50 nm. ^{1}H NMR (300 MHz, DMSO) δ 10.49 (1H, bs, NH), 8.01 (1H, d, *J*=8.4 Hz, H6), 7.79 (1H, d, *J*=1.9 Hz, H9), 7.76-7.71 (1H, m, H2´), 7.55 (1H, dd, *J*=8.4 Hz, *J*=1.9 Hz, H7), 7.45-7.39 (1H, m, H6´), 7.35 (1H, t, *J*=8.0 Hz, H5´), 7.17-7.11 (1H, m, H4´), 4.70 (2H, s, CH_2). ^{13}C NMR (75 MHz, DMSO) δ 165.2, 159.7, 152.9, 147.6, 141.1, 140.0, 133.4, 130.8, 129.3, 126.4, 123.7, 119.0, 117.9, 117.1, 113.0, 45.0. Anal. Calcd for $C_{16}H_{10}Cl_2N_2O_4$ (365.17): C, 52.63; H, 2.76; N, 7.67. Found: C, 52.955; H, 3.565; N, 7.91.

7-Chloro-2,5-dioxo-*N*-(4-(trifluoromethyl)phenyl)-2,3-dihydrobenzo[*f*][1,4]oxazepine-4(5*H*)-carboxamide **9e**.
White solid; yield 8%; mp 240-242 °C. IR (KBr pellet): 3324, 2930, 2852, 1774 (CO ester), 1698 (CO amide), 1612, 1534, 1433, 1375, 1326, 1257, 1235, 1164, 1122, 1069, 1018, 952, 844, 746, 692, 675, 640, 595, 446 cm^{-1}. UV (EtOH 96%) $Abs._{max}$ 252.00, 206.50 nm. ^{1}H NMR (300 MHz, DMSO) δ 10.68 (1H, bs, NH), 8.01 (1H, d, *J*=8.5 Hz, H9), 7.79 (1H, d, *J*=1.8 Hz, H6), 7.78-7.72 (2H, m, AA´, BB´, H2´, H6´), 7.72-7.66 (2H, m, AA´, BB´, H3´, H5´), 7.55 (1H, dd, *J*=8.5 Hz, , *J*=1.8 Hz, H8), 4.74 (2H, s, CH_2). ^{13}C NMR (75 MHz, DMSO) δ 165.4, 159.4, 152.9, 147.6, 142.2, 141.1, 129.3, 126.4, 126.3, 124.2, 123.7, 119.4, 117.1, 113.0, 45.0. Anal. Calcd for $C_{17}H_{10}ClF_3N_2O_4$ (398.72): C, 51.21; H, 2.53; N, 7.03; Cl, 8.89; F, 14.29; O, 16.05. Found: C, 51.00; H, 2.895; N, 7.085.

(*S*)-7-chloro-N-(4-chlorophenyl)-3-methyl-2,5-dioxo-2,3-dihydrobenzo[f][1,4]oxazepine-4(5H)-carboxamide **9f** (published as a sample compound in **paper IV**).
White solid; yield 8.2%; mp 188-191 °C; $[\alpha]_D^{24}$ -67.8 (*c* 2.2; DMSO). IR (KBr pellet): 3455, 1763 (ester), 1708 (amide), 1655, 1533, 1493, 1437, 1343, 1271, 1092, 827. ^{1}H NMR (300 MHz, $(CD_3)_2CO$) δ 9.30 (1H, bs, NH), 7.97 (1H, d, *J*=2.6 Hz, H6), 7.84 (1H, dd, *J*=8.8 Hz, *J*=2.6 Hz, H8), 7.58-7.52 (2H, m, AA´, BB´, H2´, H6´), 7.44 (1H, d, *J*=8.8 Hz, H9), 7.30-7.24 (2H, m, AA´, BB´, H3´, H5´), 5.57 (1H, q, *J*=6.9 Hz, CH), 1.66 (3H, d, *J*=6.9 Hz, CH_3). ^{13}C NMR (75 MHz, $(CD_3)_2CO$) δ 167.8, 160.4, 152.3, 138.6, 136.9, 131.0, 129.3, 128.8, 127.6, 122.4, 122.3, 119.3, 117.1, 53.4, 13.8. Anal. Calcd for $C_{17}H_{12}Cl_2N_2O_4$ (379.17): C, 53.85; H, 3.19; N, 7.39. Found: C, 53.98; H, 3.48; N, 7.50.

(*S*)-N-(4-bromophenyl)-7-chloro-3-methyl-2,5-dioxo-2,3-dihydrobenzo[f][1,4]oxazepine-4(5H)-carboxamide **9g**.
White solid; yield 18%; mp 218-220 °C; $[\alpha]_D^{25}$ -57.4 (*c* 2.0; DMSO). IR (KBr pellet): 3331,

2942, 1760 (CO ester), 1711 (CO amide), 1657, 1612, 1600, 1530, 1489, 1435, 1396, 1340, 1271, 1241, 1130, 1072, 1008, 825, 775, 750, 674, 536, 525 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 9.79 (1H, bs, NH), 7.96 (1H, d, *J*=2.7 Hz, H9), 7.90 (1H, dd, *J*=8.7 Hz, , *J*=2.7 Hz, H7), 7.56 (1H, d, *J*=8.7 Hz, H6), 7.461 (4H, m, H2′, H3′, H5′, H6′), 5.57 (1H, q, *J*=6.9 Hz, CH), 1.49 (3H, d, *J*=6.9 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO) δ 164.9, 159.3, 151.3, 147.1, 138.3, 136.2, 131.6, 129.6, 129.3, 126.5, 122.2, 118.9, 115.4, 52.4, 13.7. Anal. Calcd for $C_{17}H_{12}BrClN_2O_4$ (423.65): C, 48.20; H, 2.86; N, 6.61. Found: C, 48.50; H, 2.895; N, 6.905.

Biological evaluation of benzoxazepine-2,5-diones 9

Antimycobacterial evaluation

Table 3: Primary assay of benzoxazepine-2,5-diones made in TAACF

	TAACF (*M. tbc H$_{37}$Rv*)		
Compound N°	Inh. [%]	MIC [μg/mL]	Activity
9a	0	>6.25	-
9b	0	>6.25	-
9c	0	>6.25	-
9d	0	>6.25	-
9e	0	>6.25	-
9f	0	>6.25	-
9g	0	>6.25	-

Calculated and determinate values of liphophilicity factor

Table 4: Experimental and calculated values of liphophilicity factor of **9**.

Compound	Purity[a] (%)	log *K*	log *P* ACD/Log *P*	log *P*/Clog *P* ChemOffice
9a	98.73	0.3286	2.35 ± 0.64	2.97 / 3.89584
9b	98.21	0.3874	2.53 ± 0.66	3.24 / 4.04584
9c	99.03	0.3130	2.63 ± 0.64	2.97 / 3.89584
9d	99.48	0.3076	2.67 ± 0.64	2.97 / 3.89584
9e	98.97	0.4497	2.37 ± 0.65	3.33 / 4.06584
9f	99.16	0.3562	2.84 ± 0.64	3.46 / 4.41484
9g	99.09	0.4132	3.02 ± 0.66	3.73 / 4.56484

[a] Purity was determinate by RP-HPLC

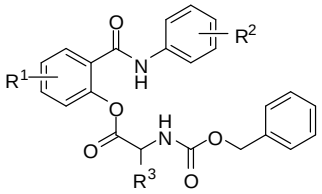
3.3.5.3 **Esters of Z-α-amino acids and substituted salicylanilides 4**

General procedure

N-Benzyloxycarbonyl (Z) protected α-amino acid **3** (10 mmol) and substituted salicylanilide **1** (10 mmol) were dissolved in dry *N*,*N*-dimethylformamide (DMF, 45 ml). The solution was cooled to -10 °C and *N*,*N*′-dicyclohexylcarbodiimide (DCI, 11 mmol)

was added in three portions during 1 h. The mixture was then stirred for 3 h at the same temperature and stored at +4 °C for 20 h. The precipitate of *N,N´*-dicyclohexylurea was removed by filtration and the solvent was evaporated *in vacuo*. The crude product **4** was purified by crystallization from ethyl acetate – hexane.

Table 5: Z-amino acid esters **4**.



Compound	R^1	R^2	R^3
4a	4-Cl	4-Cl	(*R*)-CH_3
4b	4-Cl	4-Cl	(*S*)-CH-$(CH_3)_2$
4c	4-Cl	4-Cl	(*R*)-CH-$(CH_3)_2$
4d	4-Cl	4-Cl	(*S*)-CH_2-phenyl
4e	4-Cl	4-Cl	(*R*)-CH_2-phenyl
4f	4-Cl	4-Br	(*R*)-CH_3
4g	4-Cl	4-Br	(*S*)-CH-$(CH_3)_2$
4h	4-Cl	4-Br	(*R*)-CH-$(CH_3)_2$
4i	4-Cl	4-Br	(*S*)-CH_2-phenyl
4j	4-Cl	4-Br	(*R*)-CH_2-phenyl
4k	4-Cl	4,3-diCl	H
4l	4-Cl	4,3-diCl	(*S*)-CH_3
4m	4-Cl	4,3-diCl	(*R*)-CH_3
4n	4-Cl	4,3-diCl	(*S*)-CH-$(CH_3)_2$
4o	4-Cl	4,3-diCl	(*R*)-CH-$(CH_3)_2$
4p	4-Cl	4,3-diCl	(*S*)-CH_2-phenyl
4q	4-Cl	4,3-diCl	(*R*)-CH_2-phenyl
4r	5-Cl	4-Cl	(*S*)-CH_3
4s	5-Cl	4-Cl	(*R*)-CH_3
4t	5-Cl	4-Cl	(*S*)-CH-$(CH_3)_2$
4u	5-Cl	4-Cl	(*R*)-CH-$(CH_3)_2$
4v	5-Cl	4-Cl	(*S*)-CH_2-phenyl
4w	5-Cl	4-Cl	(*R*)-CH_2-phenyl
4x	4-Cl	3-Cl	H
4y	4-Cl	3-Cl	(*S*)-CH_3
4z	4-Cl	3-Cl	(*R*)-CH_3
4aa	4-Cl	3-Cl	(*S*)-CH-$(CH_3)_2$
4bb	4-Cl	3-Cl	(*R*)-CH-$(CH_3)_2$
4cc	4-Cl	3-Cl	(*S*)-CH_2-phenyl
4dd	4-Cl	3-Cl	(*R*)-CH_2-phenyl

Data of prepared compounds 4

(*R*)-4-Chloro-2-(4-chlorophenylcarbamoyl)phenyl 2-(benzyloxycarbonylamino)-propanoate **4a**.

White solid; yield 12%; mp 137-139 °C; $[\alpha]_D^{25}$ 37.0 (*c* 2.3; $CHCl_3$). IR (KBr pellet): 3307, 1768 (CO ester), 1698, 1657, 1538, 1533, 1493, 1455, 1404, 1315, 1262, 1196, 1101, 1065, 825, 736, 697, 508 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8.13 (1H, bs, NH), 7.77 (1H, m, H3), 7.56-7.28 (10H, m, H2′, H6′, H4, H3′, H5′, H2′′, H3′′, H4′′, H5′′, H6′′), 7.11 (1H, d, *J*=8.7 Hz, H6), 5.30 (1H, d, *J*=6.9 Hz, NH), 5.11 (1H, d, *J*=12.0 Hz, OCH_2), 5.04 (1H, d, *J*=12.0 Hz, OCH_2), 4.54 (1H, m, NCH), 1.48 (3H, d, *J*=7.2 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 162.2, 155.8, 145.8, 136.0, 135.9, 132.3, 132.1, 130.0, 129.9, 129.7, 129.0, 128.6, 128.3, 128.1, 124.5, 121.7, 67.3, 50.1, 17.5. Anal. Calcd for $C_{24}H_{20}Cl_2N_2O_5$ (487.33): C, 59.19; H, 4.14; N, 5.75. Found: C, 59.02; H, 4.43; N, 5.85.

(*S*)-4-Chloro-2-(4-chlorophenylcarbamoyl)phenyl 2-(benzyloxycarbonylamino)-3-methylbutanoate **4b**.

White solid; yield 56%; mp 133-136 °C; $[\alpha]_D^{30}$ -32.4 (*c* 4.8; $CHCl_3$). IR (KBr pellet): 3383 (NH), 3267, 2967, 1751 (CO ester), 1690 (CO amide), 1597, 1530, 1492, 1405, 1312, 1283, 1251, 1193, 1100, 1047, 1011, 978, 827, 752, 694, 507 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8.22 (1H, bs, NH), 7.78 (1H, d, *J*=2.5 Hz, H3), 7.61-7.50 (2H, m, H2′, H6′), 7.44 (1H, dd, *J*=8.5 Hz, *J*=2.5 Hz, H5), 7.38-7.22 (7H, m, H3′, H5′, H2′′, H3′′, H4′′, H5′′, H6′′), 7.11 (1H, d, *J*=8.5 Hz, H6), 5.30 (1H, d, *J*=8.1 Hz, NH), 5.10 (1H, d, *J*=12.2 Hz, OCH_2), 5.00 (1H, d, *J*=12.2 Hz, OCH_2), 4.40 (1H, dd, *J*=8.1 Hz, *J*=5.5 Hz, NCH), 2.35-2.17 (1H, m, CH), 1.03 (3H, d, *J*=6.9 Hz, CH_3), 0.92 (3H, d, *J*=6.9 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 162.2, 156.4, 145.6, 136.1, 135.8, 132.2, 131.9, 130.0, 129.9, 129.8, 129.0, 128.6, 128.3, 128.1, 124.3, 121.8, 67.4, 59.7, 30.5, 19.2, 17.6. Anal. Calcd for $C_{26}H_{24}Cl_2N_2O_5$ (515.4): C, 60.59; H, 4.69; N, 5.44. Found: C, 60.865; H, 4.965; N, 5.595.

(*R*)-4-Chloro-2-(4-chlorophenylcarbamoyl)phenyl 2-(benzyloxycarbonylamino)-3-methylbutanoate **4c.**

White solid; yield 75%; mp 138-141 °C; $[\alpha]_D^{23}$ 22.3 (*c* 5.6; $CHCl_3$). IR (KBr pellet): 3401, 3324 (NH), 2966, 1764 (CO ester), 1707 (CONH), 1664, 1597, 1533, 1493, 1401, 1314, 1242, 1199, 1100, 828, 697, 509 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8.19 (1H, bs, NH), 7.80 (1H, d, *J*=2.3 Hz, H3), 7.60-7.51 (2H, m, H2′, H6′), 7.44 (1H, dd, *J*=8.8 Hz, *J*=2.3 Hz, H5), 7.38-7.24 (7H, m, H3′, H5′, H2′′, H3′′, H4′′, H5′′, H6′′), 7.12 (1H, d, *J*=8.8 Hz, H6), 5.28 (1H, d, *J*=8.2 Hz, NH), 5.10 (1H, d, *J*=12.1 Hz, OCH_2), 5.01 (1H, d, *J*=12.1 Hz, OCH_2), 4.39 (1H, dd, *J*=8.2 Hz, *J*=5.5 Hz, NCH), 2.35-2.18 (1H, m, CH), 1.03 (3H, d, *J*=6.7 Hz, CH_3), 0.93 (3H, d, *J*=6.7 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 162.2, 156.4, 145.6, 136.1, 135.8, 132.2, 132.0, 130.0, 129.9, 129.8, 129.0, 128.6, 128.3, 128.1, 124.3, 121.8, 67.4, 60.0, 30.5, 19.2, 17.6. Anal. Calcd for $C_{26}H_{24}Cl_2N_2O_5$ (515.40): C, 60.59; H, 4.69; N, 5.44. Found: C, 60.38; H, 4.97; N, 5.52.

(*S*)-4-Chloro-2-(4-chlorophenylcarbamoyl)phenyl 2-(benzyloxycarbonylamino)-3-phenylpropanoate **4d** (published as a sample compound in **paper IV**).

White solid; yield 62%; mp 151-153 °C; $[\alpha]_D^{23}$ -13.2 (*c* 1.0; $CHCl_3$). IR (KBr pellet): 3420, 1767 (CO ester), 1706 (CO amide), 1659, 1597, 1531, 1493, 1455, 1402, 1315, 1259, 1199, 1102, 1053, 828, 750, 699, 508 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8.22 (1H, bs, NH), 7.78 (1H, d, *J*=2.5 Hz, H3), 7.59-7.46 (2H, m, H2′, H6′), 7.40 (1H, dd, *J*=8.8 Hz,

J=2.5 Hz, H5), 7.36-7.21 (10H, m, H3´, H5´, +Ar- phenyl), 7.19-7.11 (2H, m, Ar-phenyl), 6.92 (1H, d, *J*=8.5 Hz, H6), 5.29 (1H, d, *J*=7.1 Hz, NH), 5.04 (1H, d, *J*=12.1 Hz, OCH_2), 4.96 (1H, d, *J*=12.1 Hz, OCH_2), 4.75 (1H, q, *J*=7.4 Hz, NCH), 3.23 (1H, dd, *J*=13.9 Hz, *J*=6.3 Hz, CH_2), 3.10 (1H, dd, *J*=13.9 Hz, *J*=7.4 Hz, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1, 162.1, 155.9, 145.7, 136.0, 135.7, 134.9, 132.2, 132.0, 129.9, 129.5, 129.1 129.0, 128.9, 128.5, 128.4, 128.3 128.0, 127.5, 124.3, 121.9, 67.3, 55.3, 37.3. Anal. Calcd for $C_{30}H_{24}Cl_2N_2O_5$ (563.43): C, 63.95; H, 4.29; N. Found: C, 63.83; H, 4.69; N, 5.115.

(*R*)-4-Chloro-2-(4-chlorophenylcarbamoyl)phenyl 2-(benzyloxycarbonylamino)-3-phenylpropanoate **4e.**

White solid; yield 46%; mp 163-166 °C; $[\alpha]_D^{25}$ 18.4 (*c* 3.5; $CHCl_3$). IR (KBr pellet): 3226, 2922, 2851, 1762 (CO ester), 1706, 1663, 1597, 1534, 1493, 1403, 1313, 1260, 1198, 1147, 1101, 826, 751, 699 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 (1H, bs, NH), 7.81 (1H, d, *J*=2.5 Hz, H3), 7.55-7.53 (2H, m, H2´, H6´), 7.40 (1H, dd, *J*=8.5 Hz, *J*=2.5 Hz, H5), 7.36-7.21 (10H, m, H3´, H5´, +Ar- phenyl), 7.19-7.11 (2H, m, Ar-phenyl), 6.93 (1H, d, *J*=8.7 Hz, H6), 5.25 (1H, d, *J*=7.2 Hz, NH), 5.04 (1H, d, *J*=12.0 Hz, OCH_2), 4.97 (1H, d, *J*=12.3 Hz, OCH_2), 4.75 (1H, q, *J*=7.2 Hz, NCH), 3.22 (1H, dd, *J*=13.5 Hz, *J*=6.4 Hz, CH_2), 3.10 (1H, dd, *J*=13.5 Hz, *J*=7.4 Hz, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1, 162.1, 155.9, 145.7, 135.9, 13,5.8, 134.9, 132.3, 132.1, 130.0, 129.5, 129.2, 129.0, 128.9, 128.6, 128.4, 128.3, 128.1, 127.6, 124.3, 121.9, 67.4, 55.4, 37.4. Anal. Calcd for $C_{30}H_{24}Cl_2N_2O_5$ (563.43): C, 63.95; H, 4.29; N, 4.97. Found: C, 64.285; H, 4.57; N, 5.13.

(*R*)-2-(4-Bromophenylcarbamoyl)-4-chlorophenyl 2-(benzyloxycarbonylamino)propanoate **4f**.

White solid; yield 3%; mp 190-192 °C; $[\alpha]_D^{25}$ 38.1 (*c* 1.8; $CHCl_3$). IR (KBr pellet): 3313, 2935, 1767 (CO ester), 1697, 1657, 1533, 1489, 1455, 1403, 1314, 1262, 1196, 1166, 1102, 1071, 1010, 882, 822, 736, 696, 506 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (1H, bs, NH), 7.76 (1H, m, H3), 7.52-7.33 (10H, m, H5, H2´, H6´, H3´, H5´, H2´´, H3´´, H4´´, H5´´, H6´´), 7.11 (1H, d, *J*=8.7 Hz, H6), 5.31 (1H, d, *J*=7.2 Hz, NH), 5.11 (1H, d, *J*=12.3 Hz, OCH_2), 5.03 (1H, d, *J*=12.3 Hz, OCH_2), 4.53 (1H, m, NCH), 1.48 (3H, d, *J*=7.2 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 162.2, 155.8, 145.8, 136.5, 135.6, 132.3, 132.1, 132.0, 129.7, 128.6, 128.3, 128.1, 124.5, 122.0, 117.6, 67.3, 50.1, 33.8, 17.5. Anal. Calcd for $C_{24}H_{20}BrClN_2O_5$ (531.78): C, 54.21; H, 3.79; N, 5.27. Found: C, 54.28; H, 3.92; N, 5.42.

(*S*)-2-(4-Bromophenylcarbamoyl)-4-chlorophenyl 2-(benzyloxycarbonylamino)-3-methylbutanoate **4g**.

White solid; yield 48%; mp 144-146 °C; $[\alpha]_D^{26}$ -24.3 (*c* 2.2; $CHCl_3$). IR (KBr pellet): 3308, 2960, 1748 (CO ester), 1707, 1668, 1531, 1489, 1394, 1315, 1252, 1189, 1105, 1069, 1008, 815, 744, 695 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 8.17 (1H, bs, NH), 7.82-7.77 (1H, m, H3), 7.54-7.28 (10H, m, H5, H2´, H3´, H5´, H6´, H2´´, H3´´, H4´´, H5´´, H6´´), 7.12 (1H, d, *J*=8.8 Hz, H6), 5.27 (1H, d, *J*=8.2 Hz, NH), 5.10 (1H, d, *J*=12.4 Hz, OCH_2), 5.01 (1H, d, *J*=12.4 Hz, OCH_2), 4.39 (1H, dd, *J*=8.2 Hz, *J*=5.5 Hz, CH), 2.35-2.18 (1H, m, CH), 1.03 (3H, d, *J*=6.9 Hz, CH_3), 0.93 (3H, d, *J*=6.9 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 162.2, 156.4, 145.6, 136.5, 135.8, 132.2, 132.0, 131.9, 130.0, 129.8, 128.6, 128.4, 128.1, 124.3, 122.1, 117.6, 67.4, 59.7, 30.5, 19.2, 17.6. Anal. Calcd for $C_{26}H_{24}BrClN_2O_5$ (559.83): C, 55.78; H, 4.32; N, 5.00. Found: C, 56.065, H, 4.565; N, 4.965.

(*R*)-2-(4-Bromophenylcarbamoyl)-4-chlorophenyl 2-(benzyloxycarbonylamino)-3-methylbutanoate **4h.**

White solid; yield 32%; mp 147-150 °C; $[\alpha]_D^{26}$ 16.4 (*c* 2.1; $CHCl_3$). IR (KBr pellet): 3331, 2964, 1763 (CO ester), 1705, 1669, 1601, 1533, 1489, 1394, 1315, 1253, 1199, 1105, 1070, 1008, 827, 696, 507 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8.18 (1H, bs, NH), 7.79 (1H, m, H3), 7.52-7.32 (10H, m, H5, H2´, H6´, H3´, H5´, H2´´, H3´´, H4´´, H5´´, H6´´), 7.12 (1H, d, *J*=8.7 Hz, H6), 5.28 (1H, d, *J*=8.4 Hz, NH), 5.10 (1H, d, *J*=12.3 Hz, OCH_2), 5.00 (1H, d, *J*=12.0 Hz, OCH_2), 4.39 (1H, dd, *J*=4.8 Hz, *J*=1.4 Hz, NCH), 2.30-2.23 (1H, m, CH), 1.03 (3H, d, *J*=6.9 Hz, CH_3), 0.93 (3H, d, *J*=6.9 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 162.2, 156.4, 145.6, 136.5, 135.8, 132.2, 132.0, 131.9, 130.0, 129.8, 128.6, 128.4, 128.1, 124.3, 122.1, 117.6, 67.4, 59.7, 30.5, 19.2, 17.6. Anal. Calcd for $C_{26}H_{24}BrClN_2O_5$ (559.84): C, 55.78; H, 4.32; N, 5.00. Found: C, 55.79; H, 4.61; N, 5.11.

(*S*)-2-(4-Bromophenylcarbamoyl)-4-chlorophenyl 2-(benzyloxycarbonylamino)-3-phenylpropanoate **4i.**

White solid; yield 32%; mp 168-170 °C; $[\alpha]_D^{26}$ -15.2 (*c* 1.65; $CHCl_3$). IR (KBr pellet): 3312, 1761 (CO ester), 1704, 1659, 1593, 1533, 1489, 1456, 1402, 1313, 1261, 1199, 11014, 1050, 1010, 820, 750, 698, 504 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8.15 (1H, bs, NH), 7.80 (1H, d, *J*=2.4 Hz, H3), 7.49 (2H, m, H2´, H6´), 7.50-7.31 (11H, m, H5, H3´, H5´, +Ar- phenyl), 7.16 (2H, m, Ar-phenyl), 6.92 (1H, d, *J*=8.7 Hz, H6), 5.25 (1H, d, *J*=6.9 Hz, NH), 5.04 (1H, d, *J*=12.3 Hz, OCH_2), 4.96 (1H, d, *J*=12.3 Hz, OCH_2), 4.75 (1H, q, *J*=6.9 Hz, NCH), 3.23 (1H, dd, *J*=14.0 Hz, *J*=6.2 Hz, CH_2), 3.10 (1H, dd, *J*=14.0 Hz, *J*=7.5 Hz, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1, 162.1, 155.9, 145.7, 136.5, 135.7, 134.9, 132.3, 132.1, 131.9, 130.0, 129.4, 129.2, 128.9, 128.6, 128.3, 128.1, 127.6, 124.3, 122.3, 117.6, 67.4, 55.3, 37.4. Anal. Calcd for $C_{30}H_{24}BrClN_2O_5$ (607.88): C, 59.28; H, 3.98; N, 4.61. Found: C, 59.23; H, 4.00; N, 4.595.

(*R*)-2-(4-Bromophenylcarbamoyl)-4-chlorophenyl 2-(benzyloxycarbonylamino)-3-phenylpropanoate **4j**.

White solid; yield 37%; mp 169-171 °C; $[\alpha]_D^{26}$ 17.8 (*c* 0.9; $CHCl_3$). IR (KBr pellet): 3309, 1762 (CO ester), 1704, 1659, 1533, 1489, 1455, 1401, 1313, 1261, 1198, 1140, 1104, 1029, 1010, 822, 750, 698 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8.19 (1H, bs, NH), 7.79 (1H, d, *J*=2.4 Hz, H3), 7.48 (2H, m, H2´, H6´), 7.50-7.28 (11H, m, H5, H3´, H5´, +Ar-phenyl), 7.16 (2H, m, Ar-phenyl), 6.92 (1H, d, *J*=8.7 Hz, H6), 5.27 (1H, d, *J*=7.2 Hz, NH), 5.04 (1H, d, *J*=12.3 Hz, OCH_2), 4.96 (1H, d, *J*=12.3 Hz, OCH_2), 4.75 (1H, q, *J*=7.2 Hz, NCH), 3.23 (1H, dd, *J*=14.0 Hz, *J*=6.2 Hz, CH_2), 3.10 (1H, dd, *J*=14.0 Hz, *J*=7.2 Hz, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1, 162.1, 155.9, 145.7, 136.5, 135.7, 134.9, 132.2, 132.1, 131.9, 130.0, 129.5, 129.2, 128.9, 128.6, 128.3, 128.1, 127.5, 124.3, 122.3, 117.6, 67.4, 55.3, 37.4. Anal. Calcd for $C_{30}H_{24}BrClN_2O_5$ (607.88): C, 59.28; H, 3.98; N, 4.61. Found: C, 59.08; H, 4.275; N, 4.70.

4-Chloro-2-(3,4-dichlorophenylcarbamoyl)phenyl 2-(benzyloxycarbonylamino)acetate **4k**.

White solid; yield 39%; mp 138-139 °C. IR (KBr pellet): 3342, 3283, 1768 (CO ester), 1704 (CO amide), 1589, 1477, 1379, 1304, 1260, 1103, 1052, 895, 822, 751, 697, 613, 577 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8.24 (1H, bs, NH), 7.86-7.81 (1H, m, H2´), 7.71 (1H, d, *J*=1.7 Hz, H3), 7.48-7.24 (8H, m, H5, H5´, H6´, H2´´, H3´´, H4´´, H5´´, H6´´), 7.10 (1H, d, *J*=8.8 Hz, H6), 5.41 (1H, t, *J*=5.6 Hz, NH), 5.10 (2H, s, OCH_2), 4.18 (2H, d, *J*=5.6 Hz, NCH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.5, 162.4, 156.5, 146.0, 136.8, 135.8, 132.8, 132.4, 132.3, 130.5, 129.5, 129.0, 128.6, 128.3, 128.3, 128.1, 124.6, 122.3, 119.8, 67.4,

43.1. Anal. Calcd for $C_{23}H_{17}Cl_3N_2O_5$ (507.75): C, 54.41; H, 3.37; N, 5.52. Found: C, 54.785; H, 3.92; N, 5.715.

(*S*)-4-Chloro-2-(3,4-dichlorophenylcarbamoyl)phenyl 2-(benzyloxycarbonylamino)-propanoate **4l.**

White solid; yield 13%; mp 136-138 °C; $[\alpha]_D^{26}$ -36.1 (*c* 1.7; $CHCl_3$). IR (KBr pellet): 3305, 1768 (CO ester), 1700, 1659, 1589, 1525, 1477, 1454, 1381, 1307, 1257, 1199, 1103, 1065, 1028, 881, 818, 736, 697 cm^{-1} ^1H NMR (300 MHz, $CDCl_3$) δ 8.32 (1H, bs, NH), 7. 86-7.75 (2H, m, H3, H2′), 7.48-7.44 (2H, m, H5, H6′), 7.35-7.33 (6H, m, H5′, H2′′, H3′′, H4′′, H5′′, H6′′), 7.10 (1H, d, *J*=8.7 Hz, H6), 5.32 (1H, d, *J*=6.9 Hz, NH), 5.12 (1H, d, *J*=12.0 Hz, OCH_2), 5.02 (1H, d, *J*=12.0 Hz, OCH_2), 4.51 (1H, m, NCH), 1.49 (3H, d, *J*=7.2 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 162.3, 155.9, 145.8, 136.9, 135.8, 132.7, 132.2, 131.0, 130.4, 129.9, 129.2, 128.6, 128.3, 128.1, 127.0, 124.4, 122.4, 119.9, 67.4, 50.1, 17.3. Anal. Calcd for $C_{24}H_{19}Cl_3N_2O_5$ (521.78): C, 55.25; H, 3.67; N, 5.37. Found: C, 55.15; H, 3.75; N, 5.44.

(*R*)-4-Chloro-2-(3,4-dichlorophenylcarbamoyl)phenyl 2-(benzyloxycarbonylamino)-propanoate **4m**.

White solid; yield 24%; mp 134-136 °C; $[\alpha]_D^{25}$ 34.5 (*c* 2.0; $CHCl_3$). IR (KBr pellet): 3305, 1768, 1699, 1659, 1589, 1526, 1477, 1455, 1381, 1300, 1261, 1200, 1103, 1067, 1029, 881, 819, 736, 697 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8.30 (1H, bs, NH), 7.87-7.77 (2H, m, H3, H2′), 7.48-7.32 (8H, m, H5, H5′, H6′, H2′′, H3′′, H4′′, H5′′, H6′′), 7.10 (1H, d, *J*=8.7 Hz, H6), 5.30 (1H, d, *J*=6.6 Hz, NH), 5.11 (1H, d, *J*=12.0 Hz, OCH_2), 5.02 (1H, d, *J*=12.3 Hz, OCH_2), 4.52 (1H, m, NCH), 1.50 (3H, d, *J*=7.2 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 162.3, 156.0, 145.8, 137.0, 135.8, 132.3, 130.4, 130.0, 129.7, 129.2, 128.6, 128.4, 128.1, 127.0, 126,7, 124.4, 122.4, 119.9, 67.4, 50.1, 17.3. Anal. Calcd for $C_{24}H_{19}Cl_3N_2O_5$ (521.78): C, 55.25; H, 3.67; N, 5.37. Found: C, 55.575; H, 3.815; N, 5.52.

(*S*)-4-Chloro-2-(3-chlorophenylcarbamoyl)phenyl 2-(benzyloxycarbonylamino)-3-methylbutanoate **4n**.

White solid; yield 33%; mp 144-147 °C; $[\alpha]_D^{26}$ -20.9 (*c* 1.5; $CHCl_3$). IR (KBr pellet): 3324, 2966, 1763 (CO ester), 1706, 1669, 1587, 1523, 1477, 1382, 1308, 1200, 1104, 1027, 820, 745, 698, 578 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ 8.32 (1H, bs, NH), 7.88 (1H, d, *J*=2.0 Hz, H3), 7.81 (1H, d, *J*=2.2 Hz, H2′), 7.52-7.41 (2H, m, H5, H5′), 7.38-7.29 (6H, m, H6′, H2′′, H3′′, H4′′, H5′′, H6), 7.13 (1H, d, *J*=8.5 Hz, H6), 5.27 (1H, d, *J*=7.7 Hz, NH), 5.12 (1H, d, *J*=12.2 Hz, OCH_2), 5.02 (1H, d, *J*=12.2 Hz, OCH_2), 4.35 (1H, dd, *J*=7.7 Hz, *J*=5.5 Hz, CH), 2.36-2.19 (1H, m, CH), 1.06 (3H, d, *J*=6.9 Hz, CH_3), 0.97 (3H, d, *J*=6.9 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 162.3, 156.5, 145.6, 137.0, 135.7, 134.4, 132.6, 132.3, 132.2, 130.4, 130.2, 129.3, 128.6, 128.4, 128.1, 124.3, 122.5, 120.0, 67.5, 59.9, 30.4, 19.2, 17.8. Anal. Calcd for $C_{26}H_{23}Cl_3N_2O_5$ (549.83): C, 56.80; H, 4.22; N, 5.09. Found: C, 56.74; H, 4.56; N, 5.14.

(*R*)-4-Chloro-2-(3,4-dichlorophenylcarbamoyl)phenyl 2-(benzyloxycarbonylamino)-3-methylbutanoate **4o**.

White solid; yield 15%; mp 150-152 °C; $[\alpha]_D^{26}$ 19.5 (*c* 2.7; $CHCl_3$). IR (KBr pellet): 3324, 2967, 1767 (CO ester), 1704, 1670, 1588, 1525, 1477, 1382, 1308, 1202, 1104, 1028, 821, 698 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ 8.32 (1H, bs, NH), 7.88 (1H, d, *J*=2.0 Hz, H3), 7.81 (1H, d, *J*=2.2 Hz, H2′), 7.52-7.41 (2H, m, H5, H5′), 7.38-7.29 (6H, m, H6′, H2′′,

H3´´, H4´´, H5´´, H6´´), 7.13 (1H, d, *J*=8.5 Hz, H6), 5.27 (1H, d, *J*=7.7 Hz, NH), 5.12 (1H, d, *J*=12.2 Hz, OCH_2), 5.02 (1H, d, *J*=12.2 Hz, OCH_2), 4.35 (1H, dd, *J*=7.7 Hz, *J*=5.5 Hz, CH), 2.36-2.19 (1H, m, CH), 1.06 (3H, d, *J*=6.9 Hz, CH_3), 0.97 (3H, d, *J*=6.9 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 162.3, 156.5, 145.6, 137.0, 135.7, 134.4, 132.6, 132.3, 132.2, 130.4, 130.2, 129.3, 128.6, 128.4, 128.1, 124.3, 122.5, 120.0, 67.5, 59.9, 30.4, 19.2, 17.8. Anal. Calcd for $C_{26}H_{23}Cl_3N_2O_5$ (549.83): C, 56.80; H, 4.22; N, 5.09. Found: C, 57.20; H, 4.22; N, 5.12.

(*S*)-4-Chloro-2-(3,4-dichlorophenylcarbamoyl)phenyl 2-(benzyloxycarbonylamino)-3-phenylpropanoate **4p**.

White solid; yield 27%; mp 169-172 °C; $[\alpha]_D^{26}$ -10.1 (*c* 2.1; $CHCl_3$). IR (KBr pellet): 3308, 1761 (CO ester), 1703, 1658, 1592, 1477, 1456, 1401, 1378, 1306, 1262, 1200, 1139, 1104, 1053, 1029, 1053, 1029, 883, 813, 698 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.33 (1H, bs, NH), 7.84 (1H, d, *J*=1.9 Hz, H3), 7.80 (1H, d, *J*=2.1 Hz, H2´), 7.46 (1H, dd, *J*=8.7 Hz, *J*=2.0 Hz, H5), 7.42 (1H, dd, *J*=8.7 Hz, *J*=2.4 Hz, H6´), 7.34-7.27 (9H, m, H5´, Ar-phenyl), 7.18 (2H, m, Ar-phenyl), 6.90 (1H, d, *J*=8.7 Hz, H6), 5.27 (1H, d, *J*=6.9 Hz, NH), 5.06 (1H, d, *J*=12.3 Hz, OCH_2), 4.99 (1H, d, *J*=12.3 Hz, OCH_2), 4.73 (1H, q, *J*=7.2 Hz, NCH), 3.23 (1H, dd, *J*=13.8 Hz, *J*=6.3 Hz, CH_2), 3.10 (1H, dd, *J*=13.8 Hz, *J*=7.5 Hz, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 162.1, 156.0, 145.7, 136.9, 135.6, 134.7, 132.6, 132.3, 132.2, 130.4, 130.2, 129.8, 129.6, 129.1, 129.0, 128.6, 128.4, 128.1, 127.6, 124.4, 122.6, 120.2, 67.5, 55.4, 37.3. Anal. Calcd for $C_{30}H_{23}Cl_3N_2O_5$ (597.87): C, 60.27; H, 3.88; N, 4.69. Found: C, 60.59; H, 4.03; N, 4.82.

(*R*)-4-Chloro-2-(3,4-dichlorophenylcarbamoyl)phenyl 2-(benzyloxycarbonylamino)-3-phenylpropanoate **4q**.

White solid; yield 10%; mp 167-170 °C; $[\alpha]_D^{26}$ 35.9 (*c* 1.6; $CHCl_3$). IR (KBr pellet): 3320, 2929, 2851, 1761 (CO ester), 1703, 1658, 1628, 1588, 1525, 1477, 1378, 1307, 1262, 1200, 1104, 1052, 1029, 814, 749, 698 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.32 (1H, bs, NH), 7.85 (1H, d, *J*=2.1 Hz, H3), 7.80 (1H, d, *J*=2.5 Hz, H2´), 7.47 (1H, dd, *J*=8.7 Hz, *J*=2.1 Hz, H5), 7.42 (1H, dd, *J*=8.7 Hz, *J*=2.4 Hz, H6´), 7.34-7.27 (9H, m, H5´, Ar-phenyl), 7.18 (2H, m, Ar-phenyl), 6.91 (1H, d, *J*=8.7 Hz, H6), 5.26 (1H, d, *J*=6.6 Hz, NH), 5.06 (1H, d, *J*=12.3 Hz, OCH_2), 5.00 (1H, d, *J*=12.3 Hz, OCH_2), 4.73 (1H, q, *J*=7.2 Hz, NCH), 3.23 (1H, dd, *J*=13.8 Hz, *J*=6.3 Hz, CH_2), 3.10 (1H, dd, *J*=13.8 Hz, *J*=7.7 Hz, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 162.1, 156.0, 145.7, 137.0, 135.7, 134.8, 132.6, 132.3, 132.2, 130.4, 130.3, 129.9, 129.6, 129.2, 129.0, 128.6, 128.4, 128.1, 127.6, 124.3, 122.6, 120.2, 67.4, 55.4, 37.3. Anal. Calcd for $C_{30}H_{23}Cl_3N_2O_5$ (597.87): C, 60.27; H, 3.88; N, 4.69. Found: C, 60.65; H, 4.35; N, 5.015.

(*S*)-5-Chloro-2-(4-chlorophenylcarbamoyl)phenyl 2-(benzyloxycarbonylamino)propanoate **4r**.

White solid; yield 57%; mp 157-160 °C; $[\alpha]_D^{26}$ -39.0 (*c* 1.7; $CHCl_3$). IR (KBr pellet): 3325, 1769 (CO ester), 1691, 1663, 1595, 1537, 1493, 1454, 1399, 1313, 1253, 1165, 1095, 1070, 1015, 910, 824, 738, 696, 508 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19 (1H, bs, NH), 7.74 (1H, d, *J*=8.4 Hz, H3), 7.65 (2H, m, H2´, H6´), 7.32-7.29 (8H, m, H4, H3´, H5´, H2´´, H3´´, H4´´, H5´´, H6´´), 7.20 (1H, m, H6), 5.32 (1H, d, *J*=6.9 Hz, NH), 5.12 (1H, d, *J*=12.3 Hz, OCH_2), 5.04 (1H, d, *J*=12.0 Hz, OCH_2), 4.53 (1H, m, NCH), 1.48 (3H, d, *J*=7.2 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.1, 162.7, 155.8, 147.8, 137.7, 136.1, 135.8, 131.0, 129.9, 129.0, 128.6, 128.3, 128.1, 127.0, 126.7, 123.4, 121.8, 37.3, 50.1, 17.4. Anal.

Calcd for $C_{24}H_{20}Cl_2N_2O_5$ (487.33): C, 59.15; H, 4.14; N, 5.75. Found: C, 59.23; H, 4.235; N, 5.78.

(*R*)-5-Chloro-2-(4-chlorophenylcarbamoyl)phenyl 2-(benzyloxycarbonylamino)propanoate **4s**.

White solid; yield 21%; mp 155-157 °C; $[\alpha]_D^{26}$ 42.1 (*c* 1.65; $CHCl_3$), $[\alpha]_D^{23}$ 52.3 (*c* 1.1; ethyl acetate). IR (KBr pellet): 3325, 1769 (CO ester), 1691, 1663, 1595, 1538, 1494, 1454, 1399, 1313, 1267, 1166, 1095, 1071, 824, 738, 696, 507 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ 8.23 (1H, bs, NH), 7.73 (1H, d, *J*=8.2 Hz, H3), 7.61-7.49 (2H, m, H2′, H6′), 7.38-7.17 (9H, m, H4, H6, H3′, H5′, H2′′, H3′′, H4′′, H5′′, H6′′), 5.36 (1H, d, *J*=6.7 Hz, NH), 5.12 (1H, d, *J*=12.1 Hz, OCH_2), 5.03 (1H, d, *J*=12.1 Hz, OCH_2), 4.61-4.44 (1H, m, CH), 1.48 (3H, d, *J*=7.2 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.1, 162.7, 155.9, 147.8, 137.7, 136.1, 135.8, 130.9, 129.8, 129.0, 128.6, 128.3, 128.1, 127.0, 126.7, 123.4, 121.8, 67.3, 50.1, 17.4. Anal. Calcd for $C_{24}H_{20}Cl_2N_2O_5$ (487.33): C, 59.15; H, 4.14; N, 5.75. Found: C, 59.42; H, 4.465; N, 5.895.

(*S*)-5-Chloro-2-(4-chlorophenylcarbamoyl)phenyl 2-(benzyloxycarbonylamino)-3-methylbutanoate **4t**.

White solid; yield 16%; mp 149-151 °C; $[\alpha]_D^{26}$ -25.5 (*c* 2.7; $CHCl_3$), $[\alpha]_D^{23}$ -37.5 (*c* 1.5; ethyl acetate). IR (KBr pellet): 3303, 2968, 1758 (CO ester), 1684, 1684, 1664, 1603, 1534, 1493, 1400, 1350, 1313, 1250, 1196, 1128, 1092, 1073, 1045, 820, 698, 508 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8.23 (1H, bs, NH), 7.78 (1H, d, *J*=8.4 Hz, H3), 7.58-7.55 (2H, m, H2′, H6′), 7.33-7.25 (8H, m, H4, H3′, H5′, H2′′, H3′′, H4′′, H5′′, H6′′), 7.21 (1H, m H6), 5.29 (1H, d, *J*=8.1 Hz, NH), 5.10 (1H, d, *J*=12.3 Hz, OCH_2), 5.01 (1H, d, *J*=12.0 Hz, OCH_2), 4.38 (1H, dd, *J*=5.4 Hz, *J*=2.7 Hz, NCH), 2.26 (1H, m, CH), 1.03 (3H, d, *J*=6.9 Hz, CH_3), 0.93 (3H, d, *J*=6.9 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3, 162.6, 156.4, 147.6, 137.6, 136.1, 135.8, 131.3, 129.8, 128.9, 128.6, 128.4, 128.1, 127.0, 126.8, 123.3, 121.9, 67.4, 59.7, 30.5, 19.2, 17.6. Anal. Calcd for $C_{26}H_{24}Cl_2N_2O_5$ (515.40): C, 60.59; H, 4.69; N, 5.44. Found: C, 60.375; H, 4.97; N, 5.47.

(*R*)-5-Chloro-2-(4-chlorophenylcarbamoyl)phenyl 2-(benzyloxycarbonylamino)-3-methylbutanoate **4u**.

White solid; yield 42%; mp 148-150 °C; $[\alpha]_D^{26}$ 33.0 (*c* 1.3; $CHCl_3$). IR (KBr pellet): 3303, 2968, 1758 (CO ester), 1684, 1664, 1603, 1523, 1534, 1493, 1399, 1350, 1314, 1250, 1196, 1175, 1127, 1092, 1073, 1045, 1014, 827, 698, 659, 508 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8.22 (1H, bs, NH), 7.78 (1H, d, *J*=8.1 Hz, H3), 7.33-7.25 (10H, m, H4,H2′, H6′, H3′, H5′, H2′′, H3′′, H4′′, H5′′, H6′′), 7.21 (1H, m, H6), 5.29 (1H, d, *J*=8.1 Hz, NH), 5.10 (1H, d, *J*=12.0 Hz, OCH_2), 5.01 (1H, d, *J*=12.0 Hz, OCH_2), 4.38 (1H, dd, *J*=5.4 Hz, *J*=3.0 Hz, NCH), 2.29-2.23 (1H, m, CH), 1.04 (3H, d, *J*=6.9 Hz, CH_3), 0.94 (3H, d, *J*=6.9 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3, 162.6, 156.4, 147.6, 137.6, 136.1, 135.8, 131.3, 129.8, 128.9, 128.6, 128.4, 128.1, 127.0, 126.8, 123.3, 121.9, 67.4, 59.7, 30.5, 19.2, 17.6. Anal. Calcd for $C_{26}H_{24}Cl_2N_2O_5$ (515.40): C, 60.59; H, 4.69; N, 5.44. Found: C, 60.665; H, 5.00; N, 5.425.

(*S*)-5-Chloro-2-(4-chlorophenylcarbamoyl)phenyl 2-(benzyloxycarbonylamino)-3-phenylpropanoate **4v**.

White solid; yield 42%; mp 185-187 °C; $[\alpha]_D^{25}$ -14.8 (*c* 1.8; $CHCl_3$). IR (KBr pellet): 3327, 1764 (CO ester), 1705, 1658, 1533, 1494, 1455, 1401, 1316, 1258, 1143, 1093, 1080,

1056, 826, 743, 699, 508 cm^{-1}.^{1}H NMR (300 MHz, $CDCl_3$) δ 10.35 (1H, bs, NH), 8.02 (1H, d, *J*=7.8 Hz, H3), 7.61 (4H, m, H6, H4, H2′, H6′), 7.53 (2H, m, H3′, H5′), 7.32-7.21 (10H, m, Ar-phenyl), 5.17 (1H, d, *J*=11.4 Hz, NH), 4.92 (1H, d, *J*=12.3 Hz, OCH_2), 4.86 (1H, d, *J*=12.3 Hz, OCH_2), 4.47 (1H, q, *J*=5.1 Hz, NCH), 3.23 (1H, dd, *J*=13.9 Hz, *J*=4.5 Hz, CH_2), 2.89 (1H, dd, *J*=13.9 Hz, *J*=10.5 Hz, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 163.3, 156.0, 148.2, 138.1, 137.3, 137.0, 135.4, 129.2, 129.0, 128.8, 128.6, 128.4, 128.2, 128.0, 127.9, 127.7, 126.8, 126,6, 123.4, 122.3, 65.7, 55.8, 36.1. Anal. Calcd for $C_{30}H_{24}Cl_2N_2O_5$ (563.43): C, 63.95; H, 4.29; N, 4.97. Found: C, 64.30; H, 4.65; N, 5.35.

(*R*)-5-Chloro-2-(4-chlorophenylcarbamoyl)phenyl 2-(benzyloxycarbonylamino)-3-phenylpropanoate **4w**.

White solid; yield 44%; mp 181-184 °C; $[\alpha]_D^{26}$ 18.2 (*c* 2.7; $CHCl_3$). IR (KBr pellet): 3328, 1763 (CO ester), 1705, 1657, 1597, 1534, 1493, 1455, 1401, 1316, 1258, 1193, 1143, 1093, 1079, 1056, 908, 826, 743, 699, 508 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-*d*6) δ 8.31 (1H, bs, NH), 10.59 (1H, d, *J*=7.8 Hz, H3), 7.73 (4H, m, H6, H4, H2′, H6′), 7.53 (2H, m, H3′, H5′), 7.39-7.22 (10H, m, Ar-phenyl), 4.95 (1H, d, *J*=10.5 Hz, NH), 4.94 (2H, m, OCH_2), 4.50-4.43 (1H, m, NCH), 3.20 (1H, dd, *J*=13.8 Hz, *J*=4.5 Hz, CH_2), 2.89 (1H, dd, *J*=13.8 Hz, *J*=10.8 Hz, CH_2). ^{13}C NMR (75 MHz, DMSO-*d*6) δ 170.2, 163.3, 156.2, 148.4, 138.1, 137.4, 137.0, 135.5, 129.3, 129.0, 128.8, 128.7, 128.5, 128.2, 128.0, 127.8, 127.6, 126.8, 126,6, 123.3, 122.3, 65.7, 55.8, 36.1. Anal. Calcd for $C_{30}H_{24}Cl_2N_2O_5$ (563.43): C, 63.95; H, 4.29; N, 4.97. Found: C, 64.15; H, 4.40; N, 5.00.

4-Chloro-2-(3-chlorophenylcarbamoyl)phenyl 2-(benzyloxycarbonylamino)acetate **4x**.

White solid; yield 12%; mp 118-122 °C. IR (KBr pellet): 3313, 1775 (CO ester), 1695 (CO amide)1654, 1593, 1534, 1483, 1425, 1383, 1310, 1263, 1205, 1171, 1105, 1056, 990, 978, 896, 781, 699, 681, 612, 535 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (1H, bs, NH), 7.75-7.67 (2H, m, H3, H2′), 7.48-7.38 (2H, m, H5, H4′), 7.35-7.-30 (5H, m, H2′′, H3′′, H4′′, H5′′, H6′′), 7.24 (1H, t, J=8.0 Hz, H5′), 7.16-7.07 (2H, m, H6, H6′), 5.38 (1H, t, *J*=5.9 Hz, NH), 5.10 (2H, s, OCH_2), 4.18 (2H, d, *J*=5.9 Hz, NCH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.5, 162.4, 156.4, 146.0, 138.5, 135.9 ,134.7, 132.3, 132.0, 130.5, 130.1, 129.4, 128.5, 128.3, 128.1, 125.1, 124.7, 120.5, 118.4, 67.4, 43.0. Anal. Calcd for $C_{23}H_{18}Cl_2N_2O_5$ (473.31): C, 58.37; H, 3.83; N, 5.92. Found: C, 58.26; H, 4.16; N, 6.02.

(*S*)-4-Chloro-2-(3-chlorophenylcarbamoyl)phenyl 2-(benzyloxycarbonylamino)propanoate **4y.**

White solid; yield 10%; mp 133-135 °C; $[\alpha]_D^{27}$ -39.1 (*c* 1.6; $CHCl_3$). IR (KBr pellet): 3316, 1766 (CO ester), 1682 (CO amide), 1651, 1594, 1534, 1482, 1452, 1425, 1341, 1256, 1198, 1106, 1052, 883, 777, 697, 681 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (1H, bs, NH), 7.79-7.70 (2H, m, H3, H2′), 7.50-7.40 (2H, m, H5, H4′), 7.35-7.29 (5H, m, H2′′, H3′′, H4′′, H5′′, H6′′), 7.24 (1H, t, J=8.0 Hz, H5′), 7.16-7.08 (2H, m, H6, H6′), 5.30 (1H, d, *J*=6.6 Hz, NH), 5.13 (1H, d, J=12.2 Hz, OCH_2), 5.04 (1H, d, J=12.2 Hz, OCH_2), 4.61-4.46 (1H, m, NCH), 1.48 (3H, d, *J*=7.4 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 162.3, 155.9, 145.8, 138.6, 135.9, 134.6, 132.3, 132.1, 130.7, 130.0, 129.7, 128.6, 128.3, 128.1, 125.0, 124.5, 120.6, 118.5, 67.3, 50.1, 17.4. Anal. Calcd for $C_{24}H_{20}Cl_2N_2O_5$ (487.33): C, 59.15; H, 4.14; N, 5.75. Found: C, 58.955; H, 4.34; N, 5.745.

(*R*)-4-Chloro-2-(3-chlorophenylcarbamoyl)phenyl 2-(benzyloxycarbonylamino)propanoate **4z**.

White solid; yield 31%; mp 133-135 °C; $[\alpha]_D^{24}$ 38.3 (*c* 4.2; $CHCl_3$). IR (KBr pellet): 3316, 1766 (CO ester), 1682 (CO amide), 1651, 1593, 1533, 1481, 1425, 1341, 1304, 1256, 1194, 1106, 1052, 883, 777, 697, 678 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8.19 (1H, bs, NH), 7.78-7.72 (2H, m, H3, H2´), 7.50-7.41 (2H, m, H5, H4´), 7.36-7.29 (5H, m, H2´´, H3´´, H4´´, H5´´, H6´´), 7.24 (1H, t, J=8.1 Hz, H5´), 7.15-7.08 (2H, m, H6, H6´), 5.31 (1H, d, *J*=6.9 Hz, NH), 5.12 (1H, d, J=12.2 Hz, OCH_2), 5.04 (1H, d, J=12.2 Hz, OCH_2), 4.60-4.47 (1H, m, NCH), 1.48 (3H, d, *J*=7.4 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 162.3, 155.9, 145.8, 138.6, 135.9, 134.6, 132.2, 132.1, 130.7, 130.0, 129.7, 128.5, 128.3, 128.1, 125.0, 124.5, 120.6, 118.5, 67.3, 50.1, 17.4. Anal. Calcd for $C_{24}H_{20}Cl_2N_2O_5$ (487.33): C, 59.15; H, 4.14; N, 5.75. Found: C, 59.445; H, 4.4; N, 5.88.

(*S*)-4-Chloro-2-(3-chlorophenylcarbamoyl)phenyl 2-(benzyloxycarbonylamino)-3-methylbutanoate **4aa**.

White solid; yield 47%; mp 149-151 °C; $[\alpha]_D^{27}$ -30.7 (*c* 2.1; $CHCl_3$), $[\alpha]_D^{23}$ -86.0 (*c* 1.4; ethyl acetate). IR (KBr pellet): 3330, 2965, 1764 (CO ester), 1712, 1667, 1592, 1532, 1481, 1424, 1310, 1199, 1104, 896, 781, 752, 700, 530 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ 8.21 (1H, bs, NH), 7.82-7.72 (2H, m, H3, H2´), 7.53-7.40 (2H, m, H5, H6´), 7.36-7.28 (4H, m, H5´, H3´´, H4´´, H5´´), 7.28-7.20 (2H, m, H2´´, H6´´), 7.16-7.09 (2H, m, H4´, H6), 5.28 (1H, d, *J*=8.0 Hz, NH), 5.11 (1H, d, *J*=12.1 Hz, OCH_2), 5.02 (1H, d, *J*=12.1 Hz, OCH_2), 4.39 (1H, dd, *J*=8.0 Hz, *J*=5.2 Hz, CH), 2.37-2.17 (1H, m, CH), 1.03 (3H, d, *J*=6.9 Hz, CH_3), 0.93 (3H, d, *J*=6.9 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 162.8, 156.4, 145.6, 138.6, 135.8, 134.6, 132.2, 132.0, 130.0, 129.9, 129.8, 128.6, 128.3, 128.2, 124.9, 124.3, 120.7, 118.5, 67.4, 59.7, 30.4, 19.2, 17.6. Anal. Calcd for $C_{26}H_{24}Cl_2N_2O_5$ (515.40): C, 60.59; H, 4.69; N, 5.44. Found: C, 60.355; H, 4.99; N, 5.575.

(*R*)-4-Chloro-2-(3-chlorophenylcarbamoyl)phenyl 2-(benzyloxycarbonylamino)-3-methylbutanoate **4bb**.

White solid; yield 34%; mp 159-162 °C; $[\alpha]_D^{26}$ 18.7 (*c* 2.4; $CHCl_3$). IR (KBr pellet): 3332, 2964, 1764 (CO ester), 1734, 1712, 1667, 1592, 1533, 1480, 1423, 1310, 1202, 1104, 1038, 896, 782, 753, 586, 531 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ 8.21 (1H, bs, NH), 7.82-7.72 (2H, m, H3, H2´), 7.53-7.40 (2H, m, H5, H6´), 7.36-7.28 (4H, m, H5´, H3´´, H4´´, H5´´), 7.28-7.20 (2H, m, H2´´, H6´´), 7.16-7.09 (2H, m, H6, H4´), 5.28 (1H, d, *J*=8.0 Hz, NH), 5.11 (1H, d, *J*=12.0 Hz, OCH_2), 5.02 (1H, d, *J*=12.1 Hz, OCH_2), 4.39 (1H, dd, *J*=8.0 Hz, *J*=5.2 Hz, CH), 2.37-2.17 (1H, m, CH), 1.03 (3H, d, *J*=6.9 Hz, CH_3), 0.93 (3H, d, *J*=7.0 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 162.3, 156.4, 145.6, 138.6, 135.8, 134.6, 132.2, 132.0, 130.0, 129.9, 129.8, 128.6, 128.3, 128.2, 124.9, 124.3, 120.7, 118.5, 67.4, 59.7, 30.4, 19.2, 17.6. Anal. Calcd for $C_{26}H_{24}Cl_2N_2O_5$ (515.40): C, 60.59; H, 4.69; N, 5.44. Found: C, 60.59; H, 5.09; N, 5.785.

(*S*)-4-Chloro-2-(3-chlorophenylcarbamoyl)phenyl 2-(benzyloxycarbonylamino)-3-phenylpropanoate **4cc**.

White solid; yield 33%; mp 157-159 °C; $[\alpha]_D^{26}$ -12.2 (*c* 1.8; $CHCl_3$). IR (KBr pellet): 3326, 2929, 2851, 1761 (CO ester), 1705, 1658, 1627, 1594, 1538, 1483, 1424, 1311, 1262, 1200, 1161, 1106, 1082, 1054, 1029, 779, 698, 682 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8.25 (1H, bs, NH), 7.80 (1H, d, *J*=2.1 Hz, H3), 7.73 (1H, m, H2´), 7.49-7.46 (1H, m, H4´), 7.41 (1H, dd, *J*=8.5 Hz, *J*=2.1 Hz, H5), 7.32-7.27 (10H, m, H6´, H5´, Ar- phenyl),

7.22-7.16 (2H, m, Ar- phenyl), 6.93 (1H, d, *J*=8.5 Hz, H6), 5.27 (1H, d, *J*=7.2 Hz, NH), 5.06 (1H, d, *J*=12.3 Hz, OCH_2), 4.99 (1H, d, *J*=12.3 Hz, OCH_2), 4.75 (1H, q, *J*=6.9 Hz, NCH), 3.24 (1H, dd, *J*=13.9 Hz, *J*=6.3 Hz, CH_2), 3.10 (1H, dd, *J*=13.9 Hz, *J*=7.5 Hz, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 162.2, 155.9, 145.7, 138.6, 135.7, 134.9, 134.5, 132.2, 132.1, 130.0, 129.9, 129.4, 129.2, 128.9, 128.5, 128.3, 128.1, 127.5, 124.9, 124.3, 120.8, 118.7, 67.4, 55.4, 37.3. Anal. Calcd for $C_{30}H_{24}Cl_2N_2O_5$ (563.43): C, 63.95; H, 4.29; N, 4.97. Found: C, 64.35; H, 4.50; N, 5.325.

(*R*)-4-Chloro-2-(3-chlorophenylcarbamoyl)phenyl 2-(benzyloxycarbonylamino)-3-phenyl propanoate **4dd**.

White solid; yield 48%; mp 161-163 °C; $[\alpha]_D^{26}$ 19.1 (*c* 2.9; $CHCl_3$). IR (KBr pellet): 3299, 3064, 3033, 1761 (CO ester), 1705, 1658, 1594, 1538, 1483, 1424, 1306, 1262, 1200, 1161, 1148, 1106, 1081, 1054, 883, 779, 698, 682 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.20 (1H, bs, NH), 7.76 (1H, d, *J*=2.4 Hz, H3), 7.73 (1H, m, H2´), 7.48-7.45 (1H, m, H4´), 7.41 (1H, dd, *J*=8.7 Hz, *J*=2.4 Hz, H5), 7.33-7.28 (10H, m, H6´, H5´, Ar- phenyl), 7.23-7.10 (2H, m, Ar- phenyl), 6.93 (1H, d, *J*=8.7 Hz, H6), 5.25 (1H, d, *J*=7.2 Hz, NH), 5.06 (1H, d, *J*=12.0 Hz, OCH_2), 4.99 (1H, d, *J*=12.3 Hz, OCH_2), 4.75 (1H, q, *J*=6.6 Hz, NCH), 3.24 (1H, dd, *J*=14.0 Hz, *J*=6.2 Hz, CH_2), 3.10 (1H, dd, *J*=14.0 Hz, *J*=7.7 Hz, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 162.2, 155.9, 145.7, 138.6, 135.7, 134.9, 134.5, 132.3, 132.1, 130.0, 129.9, 129.4, 129.1, 128.9, 128.5, 128.3, 128.1, 127.5, 125.0, 124.3, 120.8, 118.7, 67.4, 55.4, 37.3. Anal. Calcd for $C_{30}H_{24}Cl_2N_2O_5$ (563.43): C, 63.95; H, 4.29; N, 4.97. Found: C, 64.15; H, 4.655; N, 5.085.

Biological evaluation of esters of Z-amino acids and substituted salicylanilides 4

Antimycobacterial evaluation

Table 6: Antimycobacterial activities of benzyloxycarbonyl esters of amino acids and substituted salicylanilides **4**.

National Reference Laboratory – MIC [μmol/L]										
Comp.	*M. tbc* 331/88		*M. avium* 330/88		*M. kansasii* 235/80			*M. kansasii* 6509/96		
	14d	21d	14d	21d	7d	14d	21d	7d	14d	21d
4a	4	4	16	16	8	16	16	8	8	16
4b	4	4	16	16	8	16	16	8	8	16
4c	4	4	16	16	8	8	16	8	8	16
4d	4	4	16	16	8	16	16	8	16	16
4e	4	4	16	16	8	8	8	8	8	16
4f	2	4	16	16	4	8	8	8	16	16
4g	4	4	8	16	4	8	8	8	8	8
4h	2	4	16	16	4	8	8	8	8	8
4i	4	8	16	16	4	8	8	16	16	16
4j	2	4	8	16	2	4	4	8	8	8
4k	1	2	16	32	8	8	16	8	8	8
4l	2	4	16	32	4	4	8	8	16	16
4m	2	4	16	32	8	8	16	4	8	8
4n	2	4	16	32	4	4	8	8	8	8

To be continued on page 34

Table 6: Continued from page 33

4o	2	4	16	16	4	4	8	8	8	16
4p	2	2	16	32	4	4	8	8	8	8
4q	2	2	16	32	4	4	8	8	8	8
4r	4	8	16	16	4	8	8	8	8	16
4s	4	8	16	16	8	8	8	4	8	16
4t	4	8	16	16	4	8	8	4	8	16
4u	4	4	8	16	4	8	8	4	8	8
4v	4	4	8	8	4	8	8	4	8	8
4w	4	4	8	8	4	8	8	4	8	8
4x	4	8	16	32	16	16	16	8	16	16
4y	4	8	32	62.5	16	16	16	8	16	16
4z	8	8	32	32	16	16	16	8	16	16
4aa	4	4	16	16	8	16	16	8	16	16
4bb	8	8	32	32	8	16	16	8	16	16
4cc	16	16	32	62.5	32	32	32	32	32	32
4dd	4	4	16	32	8	16	16	8	16	16
INH	0.5	0.5	>250	>250	>250	>250	>250	4	8	8

Antifungal evaluation

Table 7: *In vitro* antifungal activity of some benzyloxycarbonyl esters of amino acids and substituted salicylanilides **4** in comparison with standard fluconazole (FLU).

	MIC [μg/mL]							
Comp	CA	CT	CK	CG	TB	AF	AC	TM
	24h	*24h*	*24h*	*24h*	*24h*	*24h*	*24h*	*72h*
	48h	*48h*	*48h*	*48h*	*48h*	*48h*	*48h*	*120h*
4y	31.25	>62.5	31.25	31.25	>62.5	62.5	15.63	7.81
	>62.5	>62.5	31.25	>62.5	>62.5	>62.5	15.63	7.81
4z	62.5	62.5	31.25	62.5	>62.5	>62.5	31.25	7.81
	>62.5	>62.5	31.25	62.5	>62.5	>62.5	31.25	7.81
4c	>62.5	>62.5	31.25	>62.5	>62.5	>62.5	>62.5	15.63
	>62.5	>62.5	31.25	>62.5	>62.5	>62.5	>62.5	15.63
4d	62.5	31.25	15.63	31.25	31.25	>62.5	15.63	7.81
	62.5	31.25	15.63	31.25	62.5	>62.5	15.63	7.81
4b	>62.5	>62.5	31.25	>62.5	>62.5	>62.5	>62.5	15.63
	>62.5	>62.5	15.63	>62.5	31.25	>62.5	>62.5	15.63
4k	>62.5	>62.5	>62.5	>62.5	>62.5	>62.5	62.5	31.25
	>62.5	>62.5	>62.5	>62.5	>62.5	>62.5	>62.5	31.25
4x	62.5	>62.5	62.5	62.5	>62.5	>62.5	62.5	31.25
	>62.5	>62.5	62.5	>62.5	>62.5	>62.5	62.5	31.25
FLU	0.06	0.12	3.91	0.98	0.24	>125	>125	1.95
	0.12	>125	15.62	3.91	0.48	>125	>125	3.91

Calculated and determinated values of liphophilicity factor

Table7: Experimental and calculated values of liphophilicity factor of Z-amino acids esters **4**

Compound	log *K*	log *P* ACD/Log *P*	log *P*/Clog *P* ChemOffice
4a	0.7294	5.35 ± 0.48	5.20 /5.45525
4b	0.9417	6.23 ± 0.48	6.09 / 6.38325
4c	0.9421	6.23 ± 0.48	6.09 / 6.38325
4d	1.0971	7.03 ± 0.58	6.88 / 6.87325
4e	1.0678	7.28 ± 0.40	6.88 /7.19557
4f	0.7527	5.53 ± 0.54	5.47 / 5.60525
4g	0.9803	6.41 ± 0.54	6.36 / 6.53325
4h	0.9805	6.41 ± 0.54	6.36 / 6.53325
4i	1.1258	7.46 ± 0.55	7.15 / 7.02325
4j	1.1267	7.46 ± 0.55	7.15 / 7.02325
4k	0.8639	5.91 ± 0.50	5.27 / 5.7738
4l	0.9408	6.26 ± 0.50	5.76 / 6.0828
4m	0.9380	6.26 ± 0.50	5.76 / 6.0828
4n	1.1567	7.14 ± 0.50	6.65 / 7.0108
4o	1.1559	7.14 ± 0.50	6.65 / 7.0108
4p	1.3056	8.19 ± 0.51	7.44 / 7.5008
4q	1.3030	8.19 ± 0.51	7.44 / 7.5008
4r	0.6337	5.63 ± 0.48	5.20 / 5.45525
4s	0.6334	5.63 ± 0.48	5.20 / 5.45525
4t	0.8804	5.61 ± 0.48	6.09 / 6.38325
4u	0.8816	5.61 ± 0.48	6.09 / 6.38325
4v	1.0304	7.56 ± 0.49	6.88 / 6.87325
4w	1.0315	7.56 ± 0.49	6.88 / 6.87325
4x	0.6119	5.04 ± 0.48	4.71 / 5.14625
4y	0.6995	5.39 ± 0.48	5.20 / 5.45525
4z	0.6990	5.39 ± 0.48	5.20 / 5.45525
4aa	0.8972	6.27 ± 0.48	6.09 / 6.38325
4bb	0.8954	6.27 ± 0.48	6.09 / 6.38325
4cc	1.0465	7.32 ± 0.49	6.88 / 6.87325
4dd	1.0476	7.32 ± 0.49	6.88 / 6.87325

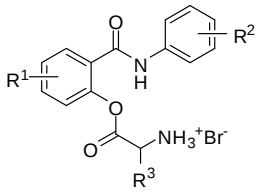
3.3.5.4 **Hydrobromide salts of α-amino acid esters and salicylanilides 5**

General procedure

A solution of hydrogen bromide in acetic acid (33%) (6 mL) was slowly added to *N*-benzyloxycarbonyl-protected esters **4** (2 mmol) with stirring. The suspension was stirred at room temperature for 30 min. during this time, the suspension turned into a clear brown solution, and evolution of carbon dioxide was observed. When the gas evolution ceased,

dry diethyl ether (DEE) was added. The precipitate was collected by filtration, washed with DEE (3 x 15 ml) and dried. The isolated crystals were suspended in dry chloroform at room temperature, filtered and dried *in vacuo* at room temperature. The yield of hydrobromide salt **5** was about 90 %.

Table 9: Hydrobromide salt of esters of α-amino acids and salicylanilides **5**



Compound N°	R^1	R^2	R^3
5a	4-Cl	4-Cl	(*R*)-CH_3
5b	4-Cl	4-Cl	(*S*)-CH-$(CH_3)_2$
5c	4-Cl	4-Cl	(*R*)-CH-$(CH_3)_2$
5d	4-Cl	4-Cl	(*S*)-CH_2-phenyl
5e	4-Cl	4-Cl	(*R*)-CH_2-phenyl
5g	4-Cl	4-Br	(*S*)-CH-$(CH_3)_2$
5h	4-Cl	4-Br	(*R*)-CH-$(CH_3)_2$
5i	4-Cl	4-Br	(*S*)-CH_2-phenyl
5j	4-Cl	4-Br	(*R*)-CH_2-phenyl
5k	4-Cl	4,3-diCl	H
5l	4-Cl	4,3-diCl	(*S*)-CH_3
5n	4-Cl	4,3-diCl	(*S*)-CH-$(CH_3)_2$
5o	4-Cl	4,3-diCl	(*R*)-CH-$(CH_3)_2$
5p	4-Cl	4,3-diCl	(*S*)-CH_2-phenyl
5q	4-Cl	4,3-diCl	(*R*)-CH_2-phenyl
5r	5-Cl	4-Cl	(*S*)-CH_3
5s	5-Cl	4-Cl	(*R*)-CH_3
5t	5-Cl	4-Cl	(*S*)-CH-$(CH_3)_2$
5u	5-Cl	4-Cl	(*R*)-CH-$(CH_3)_2$
5v	5-Cl	4-Cl	(*S*)-CH_2-phenyl
5w	5-Cl	4-Cl	(*R*)-CH_2-phenyl
5x	4-Cl	3-Cl	H
5y	4-Cl	3-Cl	(*S*)-CH_3
5z	4-Cl	3-Cl	(*R*)-CH_3
5aa	4-Cl	3-Cl	(*S*)-CH-$(CH_3)_2$
5bb	4-Cl	3-Cl	(*R*)-CH-$(CH_3)_2$
5cc	4-Cl	3-Cl	(*S*)-CH_2-phenyl
5dd	4-Cl	3-Cl	(*R*)-CH_2-phenyl

Data of prepared hydrobromide salts 5

(*R*)-1-(4-Chloro-2-(4-chlorophenylcarbamoyl)phenoxy)-1-oxopropan-2-aminium bromide **5a**.
White solid; yield 89%; mp 191-194 °C. IR (KBr pellet): 3421, 2939, 1772 (CO ester), 1659, 1595, 1519, 1493, 1399, 1314, 1202, 1105, 1014, 820, 508 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 10.68 (1H, bs, NH), 8.50 (3H, bs, NH_2.HBr), 7.86 (1H, d, *J*=2.7 Hz, H3), 7.75-7.70 (2H, m, AA´, BB´, H2´, H6´), 7.73 (1H, dd, *J*=8.7 Hz, *J*=2.7 Hz, H5), 7.43-7.36 (3H, m, H6, H3´, H5´), 4.36 (1H, m, CH), 1.47 (3H, d, *J*=7.2 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO) δ 168.7, 162.5, 146.1, 137.9, 132.0, 131.0, 130.9, 129.6, 129.3, 129.2, 128.9, 127.9, 125.3, 121.7, 48.3, 15.8.

(*S*)-1-(4-Chloro-2-(4-chlorophenylcarbamoyl)phenoxy)-3-methyl-1-oxobutan-2-aminium bromide **5b**.
White solid; yield 97%; mp 202-204 °C. IR (KBr pellet): 3424, 2969, 2880, 1756 (CO ester), 1663, 1596, 1518, 1493, 1400, 1313, 1198, 1014, 828, 508 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 10.72 (1H, bs, NH), 8.51 (3H, bs, NH_2.HBr), 7.83 (1H, d, *J*=2.4 Hz, H3), 7.74-7.70 (2H, m, AA´, BB´, H2´, H6´), 7.58 (1H, dd, *J*=8.7 Hz, *J*=2.4 Hz, H5), 7.43-7.39 (3H, m, H6, H3´, H5´), 4.20 (1H, m CH), 2.33-2.23 (1H, m, CH), 0.98 (3H, d, *J*=6.6 Hz, CH_3), 0.96 (3H, d, *J*=6.6 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO) δ 167.7, 162.7, 155.2, 145.7, 137.9, 131.7, 131.5, 131.0, 129.1, 129.0, 128.9, 127.8, 125.2, 121.5, 57.6, 29.3, 18.0, 17.9.

(*R*)-1-(4-Chloro-2-(4-chlorophenylcarbamoyl)phenoxy)-3-methyl-1-oxobutan-2-aminium bromide **5c**.
White solid; yield 92%; mp 209-211 °C. IR (KBr pellet): 3415, 2969, 1757 (CO ester), 1660, 1595, 1493, 1399, 1314, 1291, 1198, 1103, 1014, 821, 508 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 10.73 (1H, bs, NH), 8.52 (3H, bs, NH_2.HBr), 7.83 (1H, d, *J*=2.7 Hz, H3), 7.74-7.71 (2H, m, AA´, BB´, H2´, H6´), 7.58 (1H, dd, *J*=8.1 Hz, *J*=2.7 Hz, H5), 7.44-7.38 (3H, m, H6, H3´, H5´), 4.19 (1H, m CH), 2.31-2.23 (1H, m, CH), 0.98 (3H, d, *J*=6.6 Hz, CH_3), 0.96 (3H, d, *J*=6.6 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO) δ 167.7, 162.7, 155.2, 145.8, 137.9, 131.7, 131.5, 131. 0, 129.1, 129.0, 128.9, 127.8, 125.2, 121.5, 57.6, 29.3, 18.0, 17.9.

(*S*)-1-(4-Chloro-2-(4-chlorophenylcarbamoyl)phenoxy)-1-oxo-3-phenylpropan-2-aminium bromide **5d** (published as a sample compound in **paper IV**).
White solid; yield 92%; mp 214-216 °C. IR (KBr pellet): 3421, 1763 (CO ester), 1658, 1595, 1493, 1400, 1204, 1102, 825, 701, 507 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 10.72 (1H, bs, NH), 8.61 (3H, bs, NH_2.HBr) 7.87 (1H, d, *J*=2.7 Hz, H3), 7.78-7.73 (2H, m, AA´, BB´, overlapped, H2´, H6´), 7.73 (1H, dd, overlapped, *J*=8.5 Hz, *J*=2.7 Hz, H4), 7.45-7.39 (2H, m, AA´, BB´, H3´, H5´), 7.33-7.21 (6H, m, H6, H2´´, H3´´, H4´´, H5´´, H6´´), 4.54 (1H, m, CH), 3.25 (1H, dd, *J*=14.4 Hz, *J*=6.7 Hz, CH_2), 3.13 (1H, dd, *J*=14.4 Hz, *J*=6.7 Hz, CH_2). ^{13}C NMR (75 MHz, DMSO) δ 167.5, 162.6, 146.1, 137.9, 134.8, 132.0, 131.0, 130.8, 129.7, 129.3, 128.9, 128.8, 127.9, 127.5, 125.2, 121.7, 53.5, 35.6. MS (ESI): m/z (%) 511.2 ($M+H^+$), 25), 210.1 (100), 127.1 (92).

(*R*)-1-(4-Chloro-2-(4-chlorophenylcarbamoyl)phenoxy)-1-oxo-3-phenylpropan-2-aminium bromide **5e**.
White solid; yield 95%; mp 205-209 °C. IR (KBr pellet): 3409, 1763 (CO ester), 1658, 1595, 1493, 1400, 1313, 1205, 1102, 1014, 824, 702, 508 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 10.72 (1H, bs, NH), 8.61 (3H, bs, NH_2.HBr) 7.87 (1H, d, *J*=2.7 Hz, H3), 7.77-7.72 (2H, m, AA´, BB´overlapped, H2´, H6´), 7.72 (1H, dd overlapped, *J*=8.4 Hz, *J*=2.7 Hz, H4), 7.43-7.40 (2H, m, AA´, BB´, H3´, H5´), 7.31-7.24 (6H, m, H6, H2´´, H3´´, H4´´, H5´´, H6´´), 4.54 (1H, m, CH), 3.29 (1H, dd, *J*=14.4 Hz, *J*=6.6 Hz, CH_2), 3.13 (1H, dd, *J*=14.4 Hz, *J*=6.6 Hz, CH_2). ^{13}C NMR (75 MHz, DMSO) δ 167.5, 162.6, 146.1, 137.9, 134.8, 132.0, 131.0, 130.8, 129.7, 129.4, 128.9, 128.8, 127.9, 127.5, 125.1, 121.7, 53.5, 35.6.

(*S*)-1-(2-(4-Bromophenylcarbamoyl)-4-chlorophenoxy)-3-methyl-1-oxobutan-2-aminium bromide **5g**.
White solid; yield 91%; mp 198-203 °C. IR (KBr pellet): 3420, 2969, 1757 (CO ester), 1663, 1592, 1515, 1490, 1394, 1198, 1105, 1072, 1011, 918, 818, 505 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 10.72 (1H, bs, NH), 8.50 (3H, bs, NH_2.HBr), 7.83 (1H, d, *J*=2.7 Hz, H3), 7.68-7.64 (2H, m, AA´, BB´, H2´, H6´), 7.58 (1H, dd, *J*=8.5 Hz, *J*=2.7 Hz, H5), 7.55-7.52 (3H, m, H6, H3´, H5´), 4.20 (1H, m CH), 2.31-2.25 (1H, m, CH), 0.98 (3H, d, *J*=6.5 Hz, CH_3), 0.96 (3H, d, *J*=6.5 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO) δ 167.7, 162.7, 155.1, 145.7, 138.3, 132.1, 131.8, 131.5, 131.0, 129.3, 129.0, 125.1, 121.8, 115.9, 57.6, 29.3, 18.0, 17.8.

(*R*)-1-(2-(4-Bromophenylcarbamoyl)-4-chlorophenoxy)-3-methyl-1-oxobutan-2-aminium bromide **5h**.
White solid; yield 93%; mp 180-185 °C. IR (KBr pellet): 3421, 2969, 1756 (CO ester), 1667, 1592, 1518, 1490, 1394, 1313, 1291, 1198, 1105, 1072, 1011, 818, 655, 505 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 10.71 (1H, bs, NH), 8.50 (3H, bs, NH_2.HBr), 7.83 (1H, d, *J*=2.7 Hz, H3), 7.74-7.65 (2H, m, AA´, BB´, H2´, H6´), 7.58 (1H, dd, *J*=8.8 Hz, *J*=2.7 Hz, H5), 7.40-7.30 (3H, m, H6, H3´, H5´), 4.20 (1H, m CH), 2.31-2.25 (1H, m, CH), 0.98 (3H, d, *J*=6.5 Hz, CH_3), 0.96 (3H, d, *J*=6.5 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO) δ 167.7, 162.7, 155.1, 145.7, 138.3, 132.2, 131.8, 131.5, 131.0, 129.3, 129.0, 124.1, 121.8, 115.9, 56.6, 29.3, 18.0, 17.9.

(*S*)-1-(2-(4-Bromophenylcarbamoyl)-4-chlorophenoxy)-1-oxo-3-phenylpropan-2-aminium bromide **5i.**
White solid; yield 97%; mp 195-198 °C. IR (KBr pellet): 3309, 1762 (CO ester), 1662, 1591, 1515, 1490, 1395, 1312, 1206, 1104, 1072, 1011, 918, 820, 703, 505 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 10.71 (1H, bs, NH), 8.61 (3H, bs, NH_2.HBr) 7.87 (1H, d, *J*=2.7 Hz, H3), 7.73-7.68 (3H, m, H4, H2´, H6´), 7.38-7.23 (8H, m, H6, H3´, H5´, H2´´, H3´´, H4´´, H5´´, H6´´), 4.54 (1H, m, CH), 3.29 (1H, dd, *J*=14.4 Hz, *J*=6.6 Hz, CH_2), 3.12 (1H, dd, *J*=14.4 Hz, *J*=6.6 Hz, CH_2). ^{13}C NMR (75 MHz, DMSO) δ 167.5, 162.6, 155.1, 138.1, 134.8, 133.6, 132.0, 131.9, 131.8, 131.0, 130.8, 129.5, 128.9, 128.8, 123.3, 122.1, 53.5, 28.3.

(*R*)-1-(2-(4-Bromophenylcarbamoyl)-4-chlorophenoxy)-1-oxo-3-phenylpropan-2-aminium bromide **5j**.
White solid; yield 93%; mp 200-202 °C. IR (KBr pellet): 2857, 1762 (CO ester), 1663, 1591, 1517, 1490, 1395, 1312, 1205, 1105, 1072, 1010, 820, 703 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 10.72 (1H, bs, NH), 8.64 (3H, bs, NH_2.HBr) 7.87 (1H, d, *J*=2.4 Hz, H3),

7.75-7.69 (3H, m, H4, H2′, H6′), 7.33-7.22 (8H, m, H6, H3′, H5′, H2′′, H3′′, H4′′, H5′′, H6′′), 4.54 (1H, m, CH), 3.29 (1H, dd, *J*=14.4 Hz, *J*=6.9 Hz, CH_2), 3.14 (1H, dd, *J*=14.4 Hz, *J*=6.6 Hz, CH_2). ^{13}C NMR (75 MHz, DMSO) δ 167.3, 163.2, 155.1, 142.2, 138.3, 133.6, 132.5, 131.8, 129.7, 129.5, 128.6, 126.9, 123.3, 122.5, 122.1, 118.0, 53.6, 28.3.

2-(4-Chloro-2-(3,4-dichlorophenylcarbamoyl)phenoxy)-2-oxoethanaminium bromide **5k**.
White solid; yield 69%; mp 158-162 °C. IR (KBr pellet): 3420, 1774 (CO ester), 1659, 1589, 1522, 1477, 1389, 1207, 1105, 1029, 814 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 10.81 (1H, bs, NH), 8.42 (3H, bs, NH_2.HBr), 8.09 (1H, d, *J*=1.5 Hz, H2′), 7.91 (1H, d, d, *J*=2.4 Hz, H3), 7.75 (1H, dd, *J*=8.7 Hz, *J*=2.7 Hz, H5), 7.58-7.57 (2H, m, H6′, H5′), 7.36 (1H, d, *J*=8.7 Hz, H6), 4.08 (2H, m, CH_2).). ^{13}C NMR (75 MHz, DMSO) δ 166.7, 162.8, 146.4, 138.9, 132.4, 131.1, 131.0, 130.9, 130.1, 129.5, 125.8, 125.5, 121.6, 120.4, 49.5.

(*S*)-1-(4-Chloro-2-(3,4-dichlorophenylcarbamoyl)phenoxy)-1-oxopropan-2-aminium bromide **5l**.
White solid; yield 67%; mp 177-181 °C. IR (KBr pellet): 3425, 1774 (CO ester), 1659, 1591, 1477, 1380, 1306, 1202, 1105, 1030, 817, 570 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 10.81 (1H, bs, NH), 8.48 (3H, bs, NH_2.HBr), 8.07 (1H, d, *J*=1.8 Hz, H2′), 7.89 (1H, d, *J*=2.4 Hz, H3), 7.75 (1H, dd, *J*=8.7 Hz, *J*=2.7 Hz, H5), 7.62 (2H, m, H6′, H5′), 7.37 (1H, d, *J*=9.0 Hz, H6), 4.39 (1H, m, CH), 1.48 (3H, d, *J*=7.2 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO) δ 167.5, 162.5, 155.2, 146.1, 140.3, 133.1, 132.0, 131.0, 130.7, 129.2, 125.1, 124.0, 119.5, 118.5, 48.2, 15.7.

(*S*)-1-(4-Chloro-2-(3,4-dichlorophenylcarbamoyl)phenoxy)-3-methyl-1-oxobutan-2-aminium bromide **5n**.
White solid; yield 87%; mp 202-204 °C. IR (KBr pellet): 3293, 2969, 1756 (CO ester), 1668, 1592, 1510, 1477, 1387, 1302, 1248, 1194, 1130, 1106, 1029, 815, 732, 576 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 10.88 (1H, bs, NH), 8.52 (3H, bs, NH_2.HBr), 8.08 (1H, d, *J*=1.8 Hz, H2′), 7.86 (1H, d, *J*=2.4 Hz, H3), 7.74 (1H, dd, *J*=8.7 Hz, *J*=2.7 Hz, H5), 7.62 (2H, m, H6′, H5′), 7.40 (1H, d, *J*=8.7 Hz, H6), 4.21 (1H, m, CH), 2.35-2.24 (1H, m, CH), 0.99 (3H, d, *J*=5.1 Hz, CH_3), 0.97 (3H, d, *J*=5.1 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO) δ 167.7, 163.0, 155.4, 145.8, 139.0, 132.0, 131.2, 131.1, 131.0, 129.0, 125.7, 125.3, 121.2, 120.1, 57.6, 29.3, 18.0, 17.9.

(*R*)-1-(4-Chloro-2-(3,4-dichlorophenylcarbamoyl)phenoxy)-3-methyl-1-oxobutan-2-aminium bromide **5o**.
White solid; yield 89%; mp 198-200 °C. IR (KBr pellet): 3494, 2969, 1756 (CO ester), 1668, 1592, 1510, 1477, 1382, 1303, 1194, 1105, 1029, 815 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 10.88 (1H, bs, NH), 8.52 (3H, bs, NH_2.HBr), 8.08 (1H, d, *J*=1.5 Hz, H2′), 7.85 (1H, d, *J*=2.7 Hz, H3), 7.74 (1H, dd, *J*=8.7 Hz, *J*=2.7 Hz, H5), 7.61 (2H, m, H6′, H5′), 7.40 (1H, d, *J*=8.7 Hz, H6), 4.22 (1H, m, CH), 2.35-2.26 (1H, m, CH), 0.99 (3H, d, *J*=5.1 Hz, CH_3), 0.95 (3H, d, *J*=5.1 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO) δ 167.7, 163.0, 155.4, 145.8, 139.0, 132.0, 131.2, 131.1, 131.0, 129.0, 125.7, 125.3, 121.2, 120.1, 57.6, 29.3, 18.0, 17.9.

(*S*)-1-(4-Chloro-2-(3,4-dichlorophenylcarbamoyl)phenoxy)-1-oxo-3-phenylpropan-2-aminium bromide **5p**.
White solid; yield 90%; mp 191-193 °C. IR (KBr pellet): 3417, 1761 (CO ester), 1663, 1585, 1476, 1378, 1305, 1206, 1105, 1031, 813, 702 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 10.86 (1H, bs, NH), 8.61 (3H, bs, NH_2.HBr), 8.11 (1H, d, *J*=1.2 Hz, H2′), 7.83 (1H, d,

J=2.6 Hz, H3), 7.85 (1H, d, *J*=6.2 Hz, H5′), 7.78 (1H, d, *J*=8.7 Hz, H6), 7.74 (1H, dd, *J*=6.4 Hz, *J*=1.3 Hz, H6′), 7.42 (1H, dd, *J*=8.7 Hz, *J*=2.5 Hz, H5), 7.37-7.36 (5H, m, H2′′, H3′′, H4′′, H5′′, H6′′), 4.52 (1H, m, CH), 3.29 (1H, dd, *J*=14.0 Hz, *J*=6.9 Hz, CH_2), 3.14 (1H, dd, *J*=14.0 Hz, *J*=6.9 Hz, CH_2). ^{13}C NMR (75 MHz, DMSO) δ 167.5, 162.9, 154.9, 142.3, 139.0, 133.9, 133.0, 132.4, 131.6, 131.4, 131.2, 131.1, 130.4, 129.7, 129.6, 129.4, 128.9, 127.0, 122.6, 118.1, 53.8, 28.3.

(*R*)-1-(4-Chloro-2-(3,4-dichlorophenylcarbamoyl)phenoxy)-1-oxo-3-phenylpropan-2-aminium bromide **5q**.
White solid; yield 90%; mp 200-203 °C. IR (KBr pellet): 3420, 1762 (CO ester), 1663, 1585, 1476, 1377, 1305, 1206, 1105, 1031, 703 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 10.85 (1H, bs, NH), 8.61 (3H, bs, NH_2.HBr), 8.10 (1H, d, *J*=1.3 Hz, H2′), 7.85 (1H, d, *J*=2.7 Hz, H3), 7.87 (1H, d, *J*=6.4 Hz, H5′), 7.80 (1H, d, *J*=8.7 Hz, H6), 7.75 (1H, dd, *J*=6.5 Hz, *J*=1.1 Hz, H6′), 7.42 (1H, dd, *J*=8.7 Hz, *J*=2.7 Hz, H5), 7.37-7.36 (5H, m, H2′′, H3′′, H4′′, H5′′, H6′′), 4.52 (1H, m, CH), 3.29 (1H, dd, *J*=14.0 Hz, *J*=7.0 Hz, CH_2), 3.14 (1H, dd, *J*=14.0 Hz, *J*=7.0 Hz, CH_2). ^{13}C NMR (75 MHz, DMSO) δ 167.5, 162.9, 154.9, 142.3, 139.0, 136.0, 133.9, 132.9, 131.8, 131.6, 131.3, 131.1, 130.8, 129.9, 129.6, 129.5, 128.9, 127.5, 122.8, 118.3, 53.5, 28.4.

(*S*)-1-(5-Chloro-2-(4-chlorophenylcarbamoyl)phenoxy)-1-oxopropan-2-aminium bromide **5r**.
White solid; yield 85%; mp 185-187 °C. IR (KBr pellet): 3416, 2884, 1773 (CO ester), 1654, 1596, 1522, 1493, 1400, 1314, 1200, 1176, 1105, 1096, 1013, 906, 879, 827, 508 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 10.64 (1H, bs, NH), 8.50 (3H, bs, NH_2.HBr), 7.81 (1H, d, *J*=8.1 Hz, H3), 7.75-7.71 (2H, m, AA′, BB′, H2′, H6′), 7.61-7.56 (2H, m, AA′, BB′, H3′, H5′), 7.42-7.39 (2H, m, H4, H6), 4.36 (1H, m, CH), 1.48 (3H, d, *J*=7.2 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO) δ 168.5, 163.0, 157.1, 148.0, 141.7, 137.9, 134.7, 129.6, 129.1, 129.0, 128.8, 127.1, 124.3, 121.8, 48.3, 15.7.

(*R*)-1-(5-Chloro-2-(4-chlorophenylcarbamoyl)phenoxy)-1-oxopropan-2-aminium bromide **5s**.
White solid; yield 83%; mp 178-181 °C. IR (KBr pellet): 3420, 2887, 1781 (CO ester), 1653, 1596, 1526, 1493, 1400, 1315, 1201, 1177, 1105, 1096, 1014, 906, 827, 508 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 10.64 (1H, bs, NH), 8.51 (3H, bs, NH_2.HBr), 7.81 (1H, d, *J*=8.4 Hz, H3), 7.74-7.71 (2H, m, AA′, BB′, H2′, H6′), 7.61-7.56 (2H, m, AA′, BB′, H3′, H5′), 7.42-7.39 (2H, m, H4, H6), 4.36 (1H, m, CH), 1.48 (3H, d, *J*=7.2 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO) δ 168.6, 163.0, 157.3, 148.0, 142.0, 137.9, 135.8, 131.1, 128.9, 128.3, 127.8, 123.5, 121.7, 119.9, 48.3, 15.7.

(*S*)-1-(5-Chloro-2-(4-chlorophenylcarbamoyl)phenoxy)-3-methyl-1-oxobutan-2-aminium bromide **5t**.
White solid; yield 92%; mp 201-204 °C. IR (KBr pellet): 3422, 3294, 2969, 1759 (CO ester), 1662, 1595, 1515, 1493, 1399, 1312, 1196, 1095, 1014, 910, 827, 508 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 10.68 (1H, bs, NH), 8.52 (3H, bs, NH_2.HBr), 7.75 (1H, d, *J*=8.1 Hz, H3), 7.75-7.71 (2H, m, AA′, BB′, H2′, H6′), 7.60-7.55 (2H, m, AA′, BB′, H3′, H5′), 7.40-7.33 (2H, m, H4, H6), 4.15 (1H, m, CH), 2.32-2.25 (1H, m, CH), 0.98 (3H, d, *J*=5.2 Hz, CH_3), 0.96 (3H, d, *J*=5.2 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO) δ 167.6, 163.2, 155.7, 147.7, 138.0, 135.5, 130.8, 129.2, 128.9, 128.9, 127.8, 127.1, 123.5, 121.5, 57.7, 29.3, 18.0, 17.9.

(*R*)-1-(5-Chloro-2-(4-chlorophenylcarbamoyl)phenoxy)-3-methyl-1-oxobutan-2-aminium bromide **5u**.
White solid; yield 97%; mp 192-198 °C. IR (KBr pellet): 3412, 3294, 2969, 1759 (CO ester), 1662, 1605, 1595, 1515, 1493, 1399, 1312, 1196, 1158, 1095, 1014, 910, 887, 827, 581, 508 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 10.70 (1H, bs, NH), 8.53 (3H, bs, NH_2.HBr), 7.77 (1H, d, *J*=8.1 Hz, H3), 7.74-7.71 (2H, m, AA´, BB´, H2´, H6´), 7.60-7.54 (2H, m, AA´, BB´, H3´, H5´), 7.41-7.36 (2H, m, H4, H6), 4.16 (1H, m, CH), 2.34-2.23 (1H, m, CH), 0.98 (3H, d, *J*=5.1 Hz, CH_3), 0.96 (3H, d, *J*=5.1 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO) δ 167.6, 163.2, 157.1, 147.7, 138.0, 135.5, 130.8, 129.6, 129.2, 128.9, 128.8, 127.1, 123.4, 121.4, 57.7, 21.7, 18.0, 17.9.

(*S*)-1-(5-Chloro-2-(4-chlorophenylcarbamoyl)phenoxy)-1-oxo-3-phenylpropan-2-aminium bromide **5v**.
White solid; yield 96%; mp 184-186 °C. IR (KBr pellet): 3411, 2857, 1771 (CO ester), 1652, 1601, 1526, 1493, 1399, 1315, 1199, 1094, 1013, 906, 827, 752, 701, 508 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 10.68 (1H, bs, NH), 8.61 (3H, bs, NH_2.HBr), 7.82 (1H, d, *J*=8.4 Hz, H3), 7.76-7.74 (2H, m, AA´, BB´, H2´, H6´), 7.37 (1H, dd, *J*=8.4 Hz, *J*=2.1 Hz, H4), 7.43-7.39 (2H, m, AA´, BB´, H3´, H5´), 7.38 (1H, d, *J*=2.1 Hz, H6), 7.33-7.24 (5H, m, H2´´, H3´´, H4´´, H5´´, H6´´), 4.53 (1H, m, CH), 3.30 (1H, dd, *J*=14.1 Hz, *J*=6.6 Hz, CH_2), 3.14 (1H, dd, *J*=14.1 Hz, *J*=6.6 Hz, CH_2). ^{13}C NMR (75 MHz, DMSO) δ 167.3, 163.1, 148.0, 138.0, 135.8, 134.7, 131.2, 129.7, 129.6, 129.3, 128.9, 128.8, 127.6, 126.9, 122.5, 119.6, 53.6, 28.3.

(*R*)-1-(5-Chloro-2-(4-chlorophenylcarbamoyl)phenoxy)-1-oxo-3-phenylpropan-2-aminium bromide **5w**.
White solid; yield 91%; mp 196-198 °C. IR (KBr pellet): 3420, 1771 (CO ester), 1654, 1594, 1523, 1493, 1400, 1315, 1199, 1094, 828, 701, 508 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 10.65 (1H, bs, NH), 8.60 (3H, bs, NH_2.HBr), 7.81 (1H, d, *J*=8.7 Hz, H3), 7.75-7.73 (2H, m, AA´, BB´, H2´, H6´), 7.37 (1H, dd, *J*=8.7 Hz, *J*=2.2 Hz, H4), 7.44-7.39 (2H, m, AA´, BB´, H3´, H5´), 7.38 (1H, d, *J*=2.2 Hz, H6), 7.35-7.25 (5H, m, H2´´, H3´´, H4´´, H5´´, H6´´), 4.52 (1H, m, CH), 3.30 (1H, dd, *J*=14.1 Hz, *J*=6.6 Hz, CH_2), 3.14 (1H, dd, *J*=14.1 Hz, *J*=6.6 Hz, CH_2). ^{13}C NMR (75 MHz, DMSO) δ 167.2, 163.1, 148.0, 138.0, 135.8, 134.9, 131.2, 129.7, 129.6, 128.9, 128.8, 127.8, 127.5, 127.1, 123.4, 121.7, 53.6, 30.6.

2-(4-Chloro-2-(3-chlorophenylcarbamoyl)phenoxy)-2-oxoethanaminium bromide **5x**.
White solid; yield 45%; mp 162-165 °C. IR (KBr pellet): 3420, 1771 (CO ester), 1654, 1594, 1533, 1481, 1424, 1391, 1312, 1211, 1106, 899, 780, 531 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 10.70 (1H, bs, NH), 8.42 (3H, bs, NH_2.HBr), 7.90 (2H, m, H3, H2´), 7.75 (1H, dd, *J*=8.7 Hz, *J*=2.7 Hz, H5), 7.58-7.57 (2H, m, H6´, H4´), 7.50 (1H, d, *J*=8.7 Hz, H6), 7.36 (1H, dd, *J*=7.8 Hz, *J*=2.7 Hz, H5´), 4.08 (2H, m, CH_2). ^{13}C NMR (75 MHz, DMSO) δ 167.9, 162.8, 157.9, 144.8, 140.3, 144.8, 140.3, 133.5, 133.2, 131.2, 130.6, 130.3, 125.7, 125.5, 49.4.

(*S*)-1-(4-Chloro-2-(3-chlorophenylcarbamoyl)phenoxy)-1-oxopropan-2-aminium bromide **5y**.
White solid; yield 90%; mp 182-185 °C. IR (KBr pellet): 3286, 2935, 1775 (CO ester), 1663, 1593, 1525, 1481, 1424, 1391, 1311, 1203, 1107, 999, 928, 876, 781, 728, 680, 532 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 10.72 (1H, bs, NH), 8.50 (3H, bs, NH_2.HBr), 7.89 (1H, t, *J*=1.8 Hz, H2´), 7.86 (1H, d, *J*=2.7 Hz, H3), 7.74 (1H, dd, *J*=8.9 Hz, *J*=2.7 Hz, H5),

7.50 (1H, d, *J*=9.0 Hz, H6), 7.41-7.30 (2H, m, H6´, H4´), 7.18 (1H, dd, *J*=7.8 Hz, *J*=1.2 Hz, H5´), 4.40 (1H, m, CH), 1.48 (3H, d, *J*=7.2 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO) δ 168.7, 162.7, 155.2, 146.1, 140.3, 133.8, 134.7, 131.8, 131.2, 131.1, 131.0, 129.6, 124.0, 118.0, 48.2, 15.8.

(*R*)-1-(4-Chloro-2-(3-chlorophenylcarbamoyl)phenoxy)-1-oxopropan-2-aminium bromide **5z.**
White solid; yield 90%; mp 177-183 °C. IR (KBr pellet): 3286, 2935, 1775 (CO ester), 1663, 1594, 1521, 1481, 1424, 1311, 1203, 1106, 876, 776, 678, 532 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 10.72 (1H, bs, NH), 8.50 (3H, bs, NH_2.HBr), 7.88 (1H, t, *J*=1.9 Hz, H2´), 7.86 (1H, d, *J*=2.5 Hz, H3), 7.74 (1H, dd, *J*=8.8 Hz, *J*=2.5 Hz, H5), 7.50 (1H, d, *J*=8.9 Hz, H6), 7.41-7.30 (2H, m, H6´, H4´), 7.18 (1H, dd, *J*=7.8 Hz, *J*=0.9 Hz, H5´), 4.40 (1H, m, CH), 1.48 (3H, d, *J*=7.2 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO) δ 168.7, 162.7, 155.2, 146.1, 140.3, 133.2, 132.1, 131.0, 130.7, 129.3, 125.2, 124.0, 119.6, 118.5, 48.2, 15.7.

(*S*)-1-(4-Chloro-2-(3-chlorophenylcarbamoyl)phenoxy)-3-methyl-1-oxobutan-2-aminium bromide **5aa**.
White solid; yield 88%; mp 199-202 °C. IR (KBr pellet): 3420, 2969, 1765 (CO ester), 1668, 1593, 1515, 1483, 1422, 1298, 1199, 1107, 777, 677, 539 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 10.77 (1H, bs, NH), 8.54 (3H, bs, NH_2.HBr), 7.90 (1H, t, *J*=1.8 Hz, H2´), 7.85 (1H, d, *J*=2.7 Hz, H3), 7.73 (1H, dd, *J*=8.7 Hz, *J*=2.5 Hz, H5), 7.57 (1H, d, *J*=8.7 Hz, H6), 7.42-7.35 (2H, m, H6´, H4´), 7.17 (1H, dd, *J*=8.0 Hz, *J*=0.9 Hz, H5´), 4.21 (1H, m, CH), 2.35-2.24 (1H, m, CH), 0.99 (3H, d, *J*=6.5 Hz, CH_3), 0.97 (3H, d, *J*=6.5 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO) δ 167.7, 162.9, 155.3, 145.8, 140.4, 133.2, 131.8, 131.0, 130.7, 129.0, 125.3, 124.0, 119.4, 118.4, 57.6, 29.3, 18.0, 17.9.

(*R*)-1-(4-Chloro-2-(3-chlorophenylcarbamoyl)phenoxy)-3-methyl-1-oxobutan-2-aminium bromide **5bb**.
White solid; yield 77%; mp 187-189 °C. IR (KBr pellet): 3317, 2969, 1765 (CO ester), 1668, 1593, 1515, 1483, 1422, 1299, 1199, 1107, 777, 677, 540 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 10.77 (1H, bs, NH), 8.50 (3H, bs, NH_2.HBr), 7.89 (1H, t, *J*=2.1 Hz, H2´), 7.85 (1H, d, *J*=2.7 Hz, H3), 7.73 (1H, dd, *J*=9.0 Hz, *J*=2.5 Hz, H5), 7.55 (1H, d, *J*=8.7 Hz, H6), 7.40-7.37 (2H, m, H6´, H4´), 7.17 (1H, dd, *J*=8.1 Hz, *J*=1.2 Hz, H5´), 4.22 (1H, m, CH), 2.35-2.24 (1H, m, CH), 0.99 (3H, d, *J*=6.6 Hz, CH_3), 0.97 (3H, d, *J*=6.6 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO) δ 167.7, 163.0, 155.3, 145.8, 140.4, 133.2, 131.4, 131.1, 130.7, 130.0, 125.2, 124.0, 119.4, 118.4, 57.6, 29.3, 18.0, 17.9.

(*S*)-1-(4-Chloro-2-(3-chlorophenylcarbamoyl)phenoxy)-1-oxo-3-phenylpropan-2-aminium bromide **5cc**.
White solid; yield 96%; mp 195-197 °C. IR (KBr pellet): 2858, 1762 (CO ester), 1661, 1593, 1529, 1482, 1424, 1309, 1206, 1106, 879, 678 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 10.77 (1H, bs, NH), 8.66 (3H, bs, NH_2.HBr), 7.94 (1H, t, *J*=1.8 Hz, H2´), 7.88 (1H, d, *J*=2.7 Hz, H3), 7.74 (1H, dd, *J*=8.7 Hz, *J*=2.4 Hz, H5), 7.61 (1H, d, *J*=8.6 Hz, H6), 7.39 (1H, t, *J*=8.1 Hz, H5´), 7.33-7.30 (3H, m, H6´, H4´, H4´´), 7.25-7.16 (4H, m, H2´´, H3´´, H5´´, H6´´), 4.57 (1H, m, CH), 3.30 (1H, dd, *J*=14.4 Hz, *J*=6.9 Hz, CH_2), 3.16 (1H, dd, *J*=14.4 Hz, *J*=6.9 Hz, CH_2). ^{13}C NMR (75 MHz, DMSO) δ 170.1, 166.4, 157.6, 140.3, 137.4, 133.5, 133.3, 130.7, 129.4, 128.6, 128.5, 126.8, 123.5, 122.9, 119.4, 119.1, 118.0, 117.7, 55.6, 37.5.

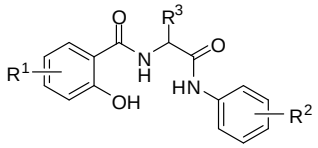
(*R*)-1-(4-Chloro-2-(3-chlorophenylcarbamoyl)phenoxy)-1-oxo-3-phenylpropan-2-aminium bromide **5dd.**
White solid; yield 89%; mp 188-192 °C. IR (KBr pellet): 3424, 2962, 1762 (CO ester), 1661, 1594, 1527, 1482, 1424, 1310, 1205, 1107, 1080, 880, 783, 701 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 10.76 (1H, bs, NH), 8.62 (3H, bs, NH_2.HBr), 7.93 (1H, t, *J*=2.1 Hz, H2´), 7.88 (1H, d, *J*=2.4 Hz, H3), 7.74 (1H, dd, *J*=8.5 Hz, *J*=2.4 Hz, H5), 7.60 (1H, d, *J*=8.4 Hz, H6), 7.37 (1H, t, *J*=8.1 Hz, H5´), 7.33-7.29 (3H, m, H6´, H4´, H4´´), 7.27-7.16 (4H, m, H2´´, H3´´, H5´´, H6´´), 4.57 (1H, m, CH), 3.30 (1H, dd, *J*=14.1 Hz, *J*=6.6 Hz, CH_2), 3.16 (1H, dd, *J*=14.1 Hz, *J*=6.6 Hz, CH_2). ^{13}C NMR (75 MHz, DMSO) δ 167.4, 162.8, 149.9, 146.1, 140.4, 134.8, 133.2, 132.1, 131.0, 130.7, 129.7, 129.4, 128.8, 127.5, 125.2, 124.0, 119.7, 118.6, 53.5, 35.7.

3.3.5.5 **Hydroxy-*N*-(phenylamino)-oxo-alkyl benzamides 6**

General procedure.

Triethylamine (0.95 mmol) was added to a stirred suspension of hydrobromide salt **5** (1 mmol) in dry chloroform (10 ml) at room temperature. After 30 min of stirring, an insoluble material was filtered off and the filtrate was purified by using a Chromatotron® Harrison Research Model 7924T (toluene/ethyl acetate 4:1) or flash chromatography (toluene/ethyl acetate 9:1). Hydroxy-*N*-(phenylamino)-oxo-alkyl benzamides **6** were isolated as unexpected products of amino group liberation.

Table 10: Hydroxy-*N*-(phenylamino)-oxo-alkyl benzamides **6**



Compound	R^1	R^2	R^3
6a	5-Cl	4-Cl	(*R*)-CH_3
6b	5-Cl	4-Cl	(*S*)-CH-$(CH_3)_2$
6c	5-Cl	4-Cl	(*R*)-CH-$(CH_3)_2$
6d	5-Cl	4-Cl	(*S*)-CH_2-phenyl
6e	5-Cl	4-Cl	(*R*)-CH_2-phenyl
6g	5-Cl	4-Br	(*S*)-CH-$(CH_3)_2$
6h	5-Cl	4-Br	(*R*)-CH-$(CH_3)_2$
6i	5-Cl	4-Br	(*S*)-CH_2-phenyl
6j	5-Cl	4-Br	(*R*)-CH_2-phenyl
6k	5-Cl	4,3-diCl	H
6l	5-Cl	4,3-diCl	(*S*)-CH_3
6n	5-Cl	4,3-diCl	(*S*)-CH-$(CH_3)_2$
6o	5-Cl	4,3-diCl	(*R*)-CH-$(CH_3)_2$
6p	5-Cl	4,3-diCl	(*S*)-CH_2-phenyl

To be continued on page 44

Table 10: Continued from page 43

Compound	R^1	R^2	R^3
6q	5-Cl	4,3-diCl	(*R*)-CH_2-phenyl
6r	4-Cl	4-Cl	(*S*)-CH_3
6s	4-Cl	4-Cl	(*R*)-CH_3
6t	4-Cl	4-Cl	(*S*)-CH-$(CH_3)_2$
6u	4-Cl	4-Cl	(*R*)-CH-$(CH_3)_2$
6v	4-Cl	4-Cl	(*S*)-CH_2-phenyl
6w	4-Cl	4-Cl	(*R*)-CH_2-phenyl
6x	5-Cl	3-Cl	H
6y	5-Cl	3-Cl	(*S*)-CH_3
6z	5-Cl	3-Cl	(*R*)-CH_3
6aa	5-Cl	3-Cl	(*S*)-CH-$(CH_3)_2$
6bb	5-Cl	3-Cl	(*R*)-CH-$(CH_3)_2$
6cc	5-Cl	3-Cl	(*S*)-CH_2-phenyl
6dd	5-Cl	3-Cl	(*R*)-CH_2-phenyl

Data of prepared hydroxy-*N*-(phenylamino)-oxo-alkyl benzamides 6

(*R*)-5-Chloro-*N*-(1-(4-chlorophenylamino)-1-oxopropan-2-yl)-2-hydroxybenzamide **6a**.

White solid; yield 49%; mp 241-243 °C, $[\alpha]_D^{24}$ -58.6 (*c* 1.45; ethyl acetate). IR (KBr pellet): 3368, 1683, 1628, 1601, 1534, 1493, 1401, 1301, 1288, 1094, 1015, 825, 650, 508 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 12.20 (1H, s, NH), 10.30 (1H, bs, OH), 9.09 (1H, d, *J*=6.6 Hz, NH), 8.04 (1H, d, *J*=2.2 Hz, H6), 7.65-7.62 (2H, m, AA´, BB´, H2´, H6´), 7.43 (1H, dd, *J*=8.7 Hz, *J*=2.2 Hz, H4), 7.37-7.34 (2H, m, AA´, BB´, H3´, H5´), 6.96 (1H, d, *J*=8.7 Hz, H3), 4.63 (1H, m CH), 1.44 (3H, d, *J*=6.9 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.1, 166.5, 157.9, 138.0, 133.5, 128.9, 128.5, 127.2, 122.8, 121.1, 119.4, 117.5, 49.9, 18.3. Anal. Calcd for $C_{16}H_{14}Cl_2N_2O_3$ (353.20): C, 54.41; H, 4.00; N, 7.93. Found: C, 54.695; H, 3.875; N, 7.94.

(*S*)-5-Chloro-*N*-(1-(4-chlorophenylamino)-3-methyl-1-oxobutan-2-yl)-2-hydroxybenzamide **6b**.

White solid; yield 39%; mp 202-205 °C, $[\alpha]_D^{24}$ 81.9 (*c* 1.8; ethyl acetate). IR (KBr pellet): 3297, 2966, 1672, 1645, 1630, 1598, 1492, 1401, 1235, 1114, 1090, 1014, 827, 651 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 12.03 (1H, s, NH), 10.38 (1H, bs, OH), 8.95 (1H, d, *J*=8.1 Hz, NH), 8.00 (1H, d, *J*=2.7 Hz, H6), 7.65-7.62 (2H, m, AA´, BB´, H2´, H6´), 7.43 (1H, dd, *J*=9.0 Hz, *J*=2.7 Hz, H4), 7.37-7.34 (2H, m, AA´, BB´, H3´, H5´), 6.97 (1H, d, *J*=9.0 Hz, H3), 4.53 (1H, t, *J*=7.8 Hz, CH), 2.22-2.09 (1H, m, CH), 0.95 (6H, d, *J*=6.6 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1, 165.7, 156.9, 137.8, 133.2, 129.2, 128.9, 127.3, 123.0, 121.1, 119.3, 118.6, 59.3, 31.0, 19.4, 18.6. MS (EI+): m/z (%) 380.8 (M^+, 12), 254.1 (100), 225.9 (65), 127.0 (88). Anal. Calcd for $C_{18}H_{18}Cl_2N_2O_3$ (381.2): C, 56.71; H, 4.76; N, 7.35. Found: C, 56.925; H, 4.91; N, 7.40.

(*R*)-5-Chloro-*N*-(1-(4-chlorophenylamino)-3-methyl-1-oxobutan-2-yl)-2-hydroxybenzamide **6c**.

White solid; yield 57%; mp 214-216 °C, $[\alpha]_D^{24}$ -71.7 (*c* 1.5; ethyl acetate). IR (KBr pellet): 3297, 2966, 1668, 1634, 1599, 1534, 1493, 1403, 1288, 1246, 1093, 1014, 825, 650 cm^{-1}.

^{1}H NMR (300 MHz, DMSO) δ 12.04 (1H, s, NH), 10.38 (1H, bs, OH), 8.95 (1H, d, *J*=8.1 Hz, NH), 8.00 (1H, dd, *J*=2.4 Hz, *J*=0.9 Hz, H6), 7.65-7.62 (2H, m, AA´, BB´, H2´, H6´), 7.43 (1H, ddd, *J*=8.8 Hz, *J*=2.7 Hz, *J*=1.2 Hz, H4), 7.37-7.34 (2H, m, AA´, BB´, H3´, H5´), 6.97 (1H, dd, *J*=8.8 Hz, *J*=1.2 Hz, H3), 4.53 (1H, t, *J*=7.8 Hz, CH), 2.20-2.13 (1H, m, CH), 0.96 (6H, d, *J*=6.6 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.3, 167.7, 157.5, 135.7, 133.8, 129.0, 128.9, 125.3, 124.5, 121.1, 119.0, 116.5, 60.8, 31.0, 19.4, 19.0. Anal. Calcd for $C_{18}H_{18}Cl_2N_2O_3$ (381.25): C, 56.71; H, 4.76; N, 7.35. Found: C, 56.77; H, 4.745; N, 7.365.

(*S*)-5-Chloro-*N*-(1-(4-chlorophenylamino)-1-oxo-3-phenylpropan-2-yl)-2-hydroxybenzamide **6d**.

White solid; yield 44%; mp 236-238 °C, $[\alpha]_D^{24}$ 48.4 (*c* 1.2; DMSO). IR (KBr pellet): 3370, 3262, 1671, 1649, 1627, 1536, 1492, 1455, 1401, 1282, 1230, 1091, 1014, 914, 821, 746, 698, 670, 650, 534, 507 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 12.02 (1H, bs, NH), 10.41 (1H, s, OH), 9.11 (1H, d, *J*=7.5 Hz, NH), 7.97 (1H, d, *J*=2.5 Hz, H6), 7.65-7.60 (2H, m, AA´, BB´, H2´, H6´), 7.43 (1H, dd, *J*=8.7 Hz, *J*=2.4 Hz, H4), 7.39-7.34 (2H, m, AA´, BB´, H3´, H5´), 7.32-7.18 (5H, m, H2´´, H3´´, H4´´, H5´´, H6´´), 6.93 (1H, d, *J*=8.7 Hz, H3), 4.90-4.85 (1H, m, CH), 3.20-3.04 (2H, m, CH_2). ^{13}C NMR (75 MHz, DMSO) δ 169.9, 166.3, 157.6, 137.8, 137.5, 133.5, 129.4, 128.9, 128.6, 128.5, 127.4, 126.8, 122.9, 121.2, 119.4, 117.7, 55.5, 37.6. Anal. Calcd for $C_{22}H_{18}Cl_2N_2O_3$ (429.30): C, 61.55; H, 4.23; N, 6.53. Found: C, 61.205; H, 4.62; N, 6.515.

(*R*)-5-Chloro-*N*-(1-(4-chlorophenylamino)-1-oxo-3-phenylpropan-2-yl)-2-hydroxybenzamide **6e**.

White solid; yield 61%; mp 236-238 °C, $[\alpha]_D^{25}$ -42.1 (*c* 1.0; DMSO). IR (KBr pellet): 3372, 3260, 1669, 1647, 1626, 1535, 1492, 1450, 1400, 1283, 1090, 1015, 746, 700, 668, 648, 530, 505 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 11.99 (1H, bs, NH), 10.40 (1H, s, OH), 9.10 (1H, d, *J*=7.5 Hz, NH), 7.97 (1H, d, *J*=2.7 Hz, H6), 7.63-7.59 (2H, m, AA´, BB´, H2´, H6´), 7.42 (1H, dd, *J*=8.7 Hz, *J*=2.7 Hz, H4), 7.38-7.35 (2H, m, AA´, BB´, H3´, H5´), 7.32-7.18 (5H, m, H2´´, H3´´, H4´´, H5´´, H6´´), 6.93 (1H, d, *J*=8.7 Hz, H3), 4.93-4.86 (1H, m, CH), 3.22-3.04 (2H, m, CH_2). ^{13}C NMR (75 MHz, DMSO) δ 169.9, 166.3, 157.6, 137.8, 137.4, 133.5, 129.4, 128.9, 128.5, 128.4, 127.4, 126.8, 122.8, 121.2, 119.4, 117.7, 55.5, 37.6. Anal. Calcd for $C_{22}H_{18}Cl_2N_2O_3$ (429.30): C, 61.55; H, 4.23; N, 6.53. Found: C, 61.17; H, 4.525; N, 6.58.

(*S*)-*N*-(1-(4-Bromophenylamino)-3-methyl-1-oxobutan-2-yl)-5-chloro-2-hydroxybenzamide **6g**.

White solid; yield 56%; mp 226-228 °C, $[\alpha]_D^{26}$ 68.8 (*c* 2.1; ethyl acetate). IR (KBr pellet): 3297, 2966, 1668, 1634, 1604, 1538, 1489, 1398, 1288, 1245, 1114, 1074, 1010, 823, 651 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) δ 12.02 (1H, s, NH), 10.38 (1H, bs, OH), 8.94 (1H, d, *J*=8.1 Hz, NH), 8.00 (1H, dd, *J*=2.4 Hz, *J*=0.9 Hz, H6), 7.60-7.57 (2H, m, AA´, BB´, H2´, H6´), 7.50-7.47 (2H, m, AA´, BB´, H3´, H5´), 7.43 (1H, ddd, *J*=9.0 Hz, *J*=3.0 Hz, *J*=0.9 Hz, H4), 6.97 (1H, dd, *J*=9.0 Hz, *J*=1.2 Hz, H3), 4.53 (1H, t, *J*=7.5 Hz, CH), 2.22-2.11 (1H, m, CH), 0.95 (6H, d, *J*=6.9 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1, 165.7, 156.9, 138.2, 133.2, 131.8, 129.1, 123.0, 121.54, 119.2, 118.6, 115.3, 59.3, 30.9, 19.4, 18.6. Anal. Calcd for $C_{18}H_{18}BrClN_2O_3$ (425.70): C, 50.78; H, 4.26; N, 6.58. Found: C, 50.825; H, 4.435; N, 6.48.

(*R*)-*N*-(1-(4-Bromophenylamino)-3-methyl-1-oxobutan-2-yl)-5-chloro-2-hydroxybenzamide **6h**.

White solid; yield 50%; mp 214-216 °C, $[\alpha]_D^{24}$ -50.1 (*c* 1.4; ethyl acetate). IR (KBr pellet): 3298, 2968, 1668, 1633, 1595, 1537, 1489, 1398, 1286, 1249, 1073, 1011, 817, 648, 505 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ 12.02 (1H, s, NH), 10.38 (1H, bs, OH), 8.94 (1H, d, *J*=8.1 Hz, NH), 8.00 (1H, d, *J*=2.7 Hz, H6), 7.60-7.57 (2H, m, AA´, BB´, H2´, H6´), 7.50-7.47 (2H, m, AA´, BB´, H3´, H5´), 7.43 (1H, ddd, *J*=9.0 Hz, *J*=2.7 Hz, *J*=0.6 Hz, H4), 6.97 (1H, d, *J*=9.0 Hz, H3), 4.53 (1H, t, *J*=7.8 Hz, CH), 2.22-2.11 (1H, m, CH), 0.95 (6H, d, *J*=6.6 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1, 165.7, 156.9, 138.2, 133.2, 131.8, 129.1, 123.1, 121.5, 119.2, 118.6, 115.4, 59.3, 31.0, 19.4, 18.6. Anal. Calcd for $C_{18}H_{18}BrClN_2O_3$ (425.70): C, 50.78; H, 4.26; N, 6.58. Found: C, 51.05; H, 4.40; N, 6.425.

(*S*)-*N*-(1-(4-Bromophenylamino)-1-oxo-3-phenylpropan-2-yl)-5-chloro-2-hydroxybenzamide **6i**.

White solid; yield 39%; mp 238-240 °C, $[\alpha]_D^{26}$ 68.0 (*c* 1.5; DMSO). IR (KBr pellet): 3371, 3266, 1684, 1647, 1627, 1603, 1544, 1530, 1489, 1397, 1284, 1230, 1115, 1071, 1011, 819, 748, 697, 652, 585, 532, 503 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ 12.04 (1H, bs, NH), 10.39 (1H, s, OH), 9.09 (1H, d, *J*=7.4 Hz, NH), 7.99-7.96 (1H, m, H6), 7.60-7.53 (2H, m, AA´, BB´, H2´, H6´), 7.52-7.46 (2H, m, AA´, BB´, H3´, H5´), 7.42 (1H, ddd, *J*=8.8 Hz, *J*=2.5 Hz, *J*=0.7 Hz, H4), 7.34-7.22 (1H, m, H4´´), 7.22-7.13 (4H, m, H2´´, H3´´, H5´´, H6´´), 6.93 (1H, dd, *J*=8.8 Hz, *J*=0.6 Hz, H3), 4.95-4.84 (1H, m, CH), 3.24-3.02 (2H, m, CH_2). ^{13}C NMR (75 MHz, DMSO) δ 169.9, 166.3, 157.6, 138.2, 137.4, 133.5, 131.8, 129.4, 128.5, 128.4, 126.8, 122.8, 121.6, 119.4, 117.7, 115.4, 55.5, 37.6. Anal. Calcd for $C_{22}H_{18}BrClN_2O_3$ (473.75): C, 55.78; H, 3.83; N, 5.91. Found: C, 55.835; H, 3.90; N, 5.885.

(*R*)-*N*-(1-(4-Bromophenylamino)-1-oxo-3-phenylpropan-2-yl)-5-chloro-2-hydroxybenzamide **6j**.

White solid; yield 32%; mp 239-242 °C, $[\alpha]_D^{25}$ -40.1 (*c* 1.6; DMSO). IR (KBr pellet): 3370, 3269, 1675, 1628, 1601, 1591, 1530, 1488, 1397, 1281, 1246, 1230, 1114, 1072, 1010, 825, 746, 700, 651, 536 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ 12.06 (1H, bs, NH), 10.40 (1H, s, OH), 9.11 (1H, d, *J*=7.5 Hz, NH), 7.98 (1H, m, H6), 7.58-7.53 (2H, m, AA´, BB´, H2´, H6´), 7.51-7.48 (2H, m, AA´, BB´, H3´, H5´), 7.42 (1H, ddd, *J*=8.7 Hz, *J*=2.4 Hz, *J*=0.6 Hz, H4), 7.31-7.16 (5H, m, H2´´, H3´´, H4´´, H5´´, H6´´), 6.93 (1H, dd, *J*=8.7 Hz, *J*=0.6 Hz, H3), 4.93-4.86 (1H, m, CH), 3.22-3.05 (2H, m, CH_2). ^{13}C NMR (75 MHz, DMSO) δ 169.9, 166.3, 157.7, 138.2, 137.4, 133.5, 131.8, 129.4, 128.5, 128.4, 126.8, 122.8, 121.6, 119.4, 117.7, 115.4, 55.5, 37.6. Anal. Calcd for $C_{22}H_{18}BrClN_2O_3$ (473.75): C, 55.78; H, 3.83; N, 5.91. Found: C, 55.52; H, 3.92; N, 5.86.

5-Chloro-*N*-(2-(3,4-dichlorophenylamino)-2-oxoethyl)-2-hydroxybenzamide **6k**.

White solid; yield 27%; mp 232-233 °C. IR (KBr pellet): 3346, 1693, 1636, 1593, 1532, 1475, 1395, 1286, 1230, 1130, 1031, 871, 824, 650, 535 cm^{-1}.^1H NMR (300 MHz, DMSO) δ 12.16 (1H, bs, NH), 10.42 (1H, bs, OH), 9.22 (1H, t, *J*=5.4 Hz, NH), 7.97 (1H, d, *J*=2.1 Hz, Ar-phenyl), 7.95 (1H, d, *J*=2.7 Hz, Ar-phenyl), 7.58-7.43 (3H, m, H4, H5´, H6´), 6.98 (1H, d, *J*=8.7 Hz, H3), 4.13 (2H, d, *J*=5.4 Hz, CH_2). ^{13}C NMR (75 MHz, DMSO) δ 167.9, 167.2, 157.9, 139.0, 133.5, 131.2, 131.0, 128.3, 125.0, 122.8, 120.6, 119.5, 119.4, 117.6, 45.9. Anal. Calcd for $C_{15}H_{11}Cl_3N_2O_3$ (373.62): C, 48.22; H, 2.97; N, 7.50; Cl, 28.47; O, 12.85. Found: C, 48.17; H, 2.77; N, 7.395.

(*S*)-5-Chloro-*N*-(1-(3,4-dichlorophenylamino)-1-oxopropan-2-yl)-2-hydroxybenzamide **6l**.

White solid; yield 23%; mp 240-242 °C, $[\alpha]_D^{24}$ 34.0 (*c* 1.8; ethyl acetate). IR (KBr pellet): 3347, 3277, 1664, 1635, 1589, 1541, 1525, 1473, 1394, 1342, 1285, 1219, 1136, 1029, 897, 860, 828, 811, 694, 573 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ 12.20 (1H, s, NH), 10.44 (1H, bs, OH), 8.95 (1H, d, *J*=6.6 Hz, NH), 8.04 (1H, d, *J*=2.7 Hz, Ar), 7.99 (1H, d, *J*=2.1 Hz, Ar), 7.70 (1H, d, *J*=8.8 Hz, Ar), 7.51 (1H, dd, *J*=8.8 Hz, *J*=2.2 Hz, Ar), 7.44 (1H, dd, *J*=9.0 Hz, *J*=2.7 Hz, Ar), 6.96 (1H, d, *J*=8.7 Hz, Ar), 4.65-4.56 (1H, m, CH), 1.44 (3H, d, *J*=7.3 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.5, 166.7, 158.0, 139.1, 133.5, 131.2, 131.0, 128.5, 125.1, 122.8, 120,7, 119.6, 119.4, 117.4, 50.0, 18.1. Anal. Calcd for $C_{16}H_{13}Cl_3N_2O_3$ (387.65): C, 49.57; H, 3.38; N, 7.23. Found: C, 49.20; H, 3.675; N, 7.11.

(*S*)-5-Chloro-*N*-(1-(3,4-dichlorophenylamino)-3-methyl-1-oxobutan-2-yl)-2-hydroxybenzamide **6n**.

White solid; yield 53%; mp 201-202 °C, $[\alpha]_D^{26}$ 72.5 (*c* 1.6; ethyl acetate). IR (KBr pellet): 3305, 2966, 1672, 1634, 1592, 1532, 1476, 1393, 1289, 1232, 1134, 1114, 1029, 872, 822, 650, 533 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ 12.02 (1H, s, NH), 10.55 (1H, bs, OH), 8.95 (1H, d, *J*=8.0 Hz, NH), 8.01 (1H, d, *J*=2.2 Hz, Ar), 7.99 (1H, d, *J*=2.8 Hz, Ar), 7.57 (1H, d, *J*=8.8 Hz, Ar), 7.50 (1H, dd, *J*=8.8 Hz, *J*=2.2 Hz, Ar), 7.43 (1H, dd, *J*=8.8 Hz, *J*=2.8 Hz, Ar), 6.97 (1H, d, *J*=8.8 Hz, Ar), 4.51 (1H, t, *J*=7.6 Hz, CH), 2.25-2.09 (1H, m, CH), 0.95 (6H, d, *J*=6.6 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 165.7, 156.9, 138.9, 133.3, 131.3, 131.0, 129.2, 125.2, 123.0, 120.7, 119.6, 119.3, 118.6, 59.4, 30.9, 19.4, 18.6. Anal. Calcd for $C_{18}H_{17}Cl_3N_2O_3$ (415.70): C, 52.01; H, 4.12; N, 6.74. Found: C, 52.015; H, 4.925; N, 7.215.

(*R*)-5-Chloro-*N*-(1-(3,4-dichlorophenylamino)-3-methyl-1-oxobutan-2-yl)-2-hydroxybenzamide **6o**.

White solid; yield 40%; mp 201-203 °C, $[\alpha]_D^{25}$ -57.1 (*c* 1.55; ethyl acetate). IR (KBr pellet): 3304, 2966, 1672, 1634, 1592, 1532, 1476, 1393, 1289, 1232, 1134, 1114, 1030, 871, 821, 650, 533 cm^{-1}.^1H NMR (300 MHz, DMSO) δ 12.03 (1H, s, NH), 10.55 (1H, bs, OH), 8.95 (1H, d, *J*=8.0 Hz, NH), 8.01 (1H, d, *J*=2.2 Hz, Ar), 7.99 (1H, d, *J*=2.8 Hz, Ar), 7.57 (1H, d, *J*=9.0 Hz, Ar), 7.52 (1H, dd, *J*=9.0 Hz, *J*=2.4 Hz, Ar), 7.43 (1H, dd, *J*=8.7 Hz, *J*=2.7 Hz, Ar), 6.98 (1H, d, *J*=9.0 Hz, Ar), 4.51 (1H, t, *J*=7.5 Hz, CH), 2.23-2.12 (1H, m, CH), 0.96 (6H, d, *J*=6.9 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 165.7, 156.9, 138.8, 133.5, 131.3, 131.0, 129.2, 125.2, 123.0, 120.7, 119.6, 119.3, 118.6, 59.4, 30.9, 19.4, 18.6. Anal. Calcd for $C_{18}H_{17}Cl_3N_2O_3$ (415.70): C, 52.01; H, 4.12; N, 6.74. Found: C, 51.65; H, 4.275; N, 6.59.

(*S*)-5-Chloro-*N*-(1-(3,4-dichlorophenylamino)-1-oxo-3-phenylpropan-2-yl)-2-hydroxybenzamide **6p**.

White solid; yield 23%; mp 240-242 °C, $[\alpha]_D^{24}$ 39.1 (*c* 1.4; DMSO). IR (KBr pellet): 3398, 1674, 1633, 1593, 1532, 1476, 1455, 1417, 1375, 1289, 1232, 1134, 1118, 1030, 871, 824, 699, 650, 534 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ 12.01 (1H, bs, NH), 10.56 (1H, bs, OH), 9.09 (1H, d, *J*=7.5 Hz, NH), 7.97 (2H, m, H6, H2′), 7.58 (1H, d, *J*=8.7 Hz, H5′), 7.49 (1H, dd, *J*=8.7 Hz, *J*=2.1 Hz, H6′), 7.42 (1H, ddd, *J*=9.0 Hz, *J*=2.6 Hz, *J*=0.9 Hz, H4), 7.31-7.24 (4H, m, H2′′, H3′′, H4′′, H5′′, H6′′), 7.21-7.16 (1H, m, H4′′), 6.94 (1H, dd, *J*=9.0 Hz, *J*=0.9 Hz, H3), 4.91-4.84 (1H, m, CH), 3.13-3.05 (2H, m, CH_2). ^{13}C NMR (75 MHz, DMSO) δ 170.3, 166.4, 157.6, 138.9, 137.3, 133.5, 131.2, 131.0, 129.4, 128.6,

128.5, 126.8, 125.2, 122.9, 120.8, 119.7, 119.4, 117.7, 55.6, 37.5. Anal. Calcd for $C_{22}H_{17}Cl_3N_2O_3$ (463.74): C, 56.98; H, 3.69; N, 6.04. Found: C, 57.10; H, 3.955; N, 6.23.

(*R*)-5-Chloro-*N*-(1-(3,4-dichlorophenylamino)-1-oxo-3-phenylpropan-2-yl)-2-hydroxybenzamide **6q**.

White solid; yield 34%; mp 238-240 °C, $[\alpha]_D^{24}$ -48.1 (*c* 1.4; DMSO). IR (KBr pellet): 3305, 1684, 1635, 1593, 1539, 1476, 1385, 1289, 1233, 1133, 1030, 824, 699, 650, 534 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ 12.00 (1H, bs, NH), 10.59 (1H, bs, OH), 9.10 (1H, d, *J*=7.5 Hz, NH), 7.98 (2H, m, H6, H2′), 7.58 (1H, d, *J*=8.8 Hz, H5′), 7.50 (1H, dd, *J*=8.8 Hz, *J*=2.4 Hz, H6′), 7.41 (1H, ddd, *J*=8.9 Hz, *J*=2.5 Hz, *J*=1.0 Hz, H4), 7.30-7.23 (4H, m, H2′′, H3′′, H4′′, H5′′, H6′′), 7.21-7.12 (1H, m, H4′′), 6.95 (1H, dd, *J*=8.8 Hz, *J*=1.0 Hz, H3), 4.93-4.84 (1H, m, CH), 3.15-3.07 (2H, m, CH_2). ^{13}C NMR (75 MHz, DMSO) δ 170.3, 166.4, 157.7, 138.9, 137.4, 133.5, 131.2, 131.0, 129.4, 128.7, 128.5, 126.8, 125.1, 122.9, 120.8, 119.7, 119.4, 117.7, 55.6, 37.5. Anal. Calcd for $C_{22}H_{17}Cl_3N_2O_3$ (463.74): C, 56.98; H, 3.69; N, 6.04. Found: C, 56.65; H, 3.255; N, 6.355.

(*S*)-4-Chloro-*N*-(1-(4-chlorophenylamino)-1-oxopropan-2-yl)-2-hydroxybenzamide **6r**.

White solid; yield 64%; mp 223-225 °C, $[\alpha]_D^{26}$ 28.0 (*c* 1.7; ethyl acetate). IR (KBr pellet): 3351, 3262, 1663, 1636, 1588, 1539, 1491, 1401, 1340, 1297, 1219, 1094, 1013, 919, 852, 821, 773, 690, 573, 509 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ 12.44 (1H, s, NH), 10.30 (1H, bs, OH), 9.03 (1H, d, *J*=6.9 Hz, NH), 8.00 (1H, dd, *J*=9.1 Hz, *J*=0.9 Hz, Ar), 7.65-7.62 (2H, m, AA′, BB′, H2′, H6′), 7.41-7.32 (2H, m, AA′, BB′, H3′, H5′), 7.01-6.97 (2H, m, Ar), 4.68-4.60 (1H, m, CH), 1.44 (3H, d, *J*=6.9 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.2, 166.9, 159.9, 138.0, 137.8, 131.0, 128.9, 127.2, 121.0, 119.3, 117.0, 115.4, 49.8, 18.3. Anal. Calcd for $C_{16}H_{14}Cl_2N_2O_3$ (353.20): C, 54.41; H, 4.00; N, 7.93. Found: C, 54.81; H, 3.885; N, 7.955.

(*R*)-4-Chloro-*N*-(1-(4-chlorophenylamino)-1-oxopropan-2-yl)-2-hydroxybenzamide **6s**.

White solid; yield 73%; mp 226-227 °C, $[\alpha]_D^{26}$ -16.4 (*c* 1.6; ethyl acetate). IR (KBr pellet): 3351, 3262, 1663, 1634, 1588, 1538, 1491, 1400 1339, 1297, 1218, 1164, 1094, 1013, 919, 852, 821, 785, 773, 690, 573, 509 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ 12.44 (1H, s, NH), 10.30 (1H, bs, OH), 9.03 (1H, d, *J*=6.9 Hz, NH), 8.00 (1H, dd, *J*=10.2 Hz, *J*=1.2 Hz, Ar), 7.65-7.62 (2H, m, AA′, BB′, H2′, H6′), 7.37-7.35 (2H, m, AA′, BB′, H3′, H5′), 7.01-6.98 (2H, m, Ar), 4.68-4.59 (1H, m, CH), 1.44 (3H, d, *J*=7.2 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.2, 166.9, 159.9, 138.0, 137.8, 131.0, 128.9, 127.2, 121.0, 119.3, 117.0, 115.3, 49.8, 18.3. Anal. Calcd for $C_{16}H_{14}Cl_2N_2O_3$ (353.20): C, 54.41; H, 4.00; N, 7.93. Found: C, 54.245; H, 4.145; N, 7.955.

(*S*)-4-Chloro-*N*-(1-(4-chlorophenylamino)-3-methyl-1-oxobutan-2-yl)-2-hydroxybenzamide **6t**.

White solid; yield 69%; mp 203-205 °C, $[\alpha]_D^{26}$ 38 (*c* 1.5; ethyl acetate). IR (KBr pellet): 3304, 2965, 1667, 1635, 1598, 1531, 1492, 1403, 1300, 1238, 1092, 1014, 916, 824, 860, 824, 767, 678, 506 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ 12.24 (1H, s, NH), 10.38 (1H, bs, OH), 8. 85 (1H, d, *J*=8.3 Hz, NH), 7.99 (1H, d, *J*=9.0 Hz, H6), 7.66-7.60 (2H, m, AA′, BB′, H2′, H6′), 7.38-7.32 (2H, m, AA′, BB′, H3′, H5′), 7.00-6.96 (2H, m, H3, H5), 4.53 (1H, t, *J*=7.6 Hz, CH), 2.22-2.11 (1H, m, CH), 0.96 (6H, d, *J*=6.6 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 166.1, 158.8, 137.8, 137.4, 131.7, 128.9, 127.3, 121.1, 119.5, 116.9,

116.4, 59.2, 30.9, 19.4, 18.6. Anal. Calcd for $C_{18}H_{18}Cl_2N_2O_3$ (381.25): C, 56.71; H, 4.76; N, 7.35. Found: C, 57.065; H, 5.07; N, 7.32.

(*R*)-4-Chloro-*N*-(1-(4-chlorophenylamino)-3-methyl-1-oxobutan-2-yl)-2-hydroxybenzamide **6u**.

White solid; yield 48%; mp 204-205 °C, $[\alpha]_D^{24}$ -74.1 (*c* 1.8; ethyl acetate). IR (KBr pellet): 3304, 2966, 1669, 1635, 1598, 1541, 1492, 1403, 1349, 1299, 1241, 1216, 1092, 1013, 916, 827, 768, 508 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ 12.23 (1H, s, NH), 10.38 (1H, bs, OH), 8. 85 (1H, d, *J*=8.1 Hz, NH), 7.99 (1H, d, *J*=9.0 Hz, H6), 7.65-7.62 (2H, m, AA′, BB′, H2′, H6′), 7.37-7.34 (2H, m, AA′, BB′, H3′, H5′), 7.00-6.97 (2H, m, H3, H5), 4.53 (1H, t, *J*=7.5 Hz, CH), 2.22-2.11 (1H, m, CH), 0.95 (6H, d, *J*=6.6 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 166.1, 158.8, 137.8, 137.4, 131.6, 128.8, 127.3, 121.1, 119.6, 116.7, 116.3, 59.2, 30.9, 19.4, 18.6. Anal. Calcd for $C_{18}H_{18}Cl_2N_2O_3$ (381.25): C, 56.71; H, 4.76; N, 7.35. Found: C, 56.99; H, 4.99; N, 7.30.

(*S*)-4-Chloro-*N*-(1-(4-chlorophenylamino)-1-oxo-3-phenylpropan-2-yl)-2-hydroxybenzamide **6v**.

White solid; yield 40%; mp 232-234 °C, $[\alpha]_D^{24}$ 56.4 (*c* 1.35; DMSO). IR (KBr pellet): 3376, 3270, 1676, 1637, 1597, 1534, 1492, 1402, 1299, 1232, 1092, 1014, 918, 862, 825, 744, 700, 572 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ 12.29 (1H, bs, NH), 10.40 (1H, s, OH), 9.03 (1H, d, *J*=7.5 Hz, NH), 7.94 (1H, d, *J*=8.4 Hz, H6), 7.63-7.59 (2H, m, AA′, BB′, H2′, H6′), 7.39-7.35 (2H, m, AA′, BB′, H3′, H5′), 7.38-7.15 (5H, m, H5, H3, H2′′, H4′′, H6′′), 7.00-6.96 (2H, m, H3′′, H5′′), 4.92-4.84 (1H, m, CH), 3.21-3.05 (2H, m, CH_2). ^{13}C NMR (75 MHz, DMSO) δ 170.0, 166.8, 159.7, 137.8, 137.5, 131.0, 129.4, 128.9, 128.4, 127.5, 127.4, 126.8, 121.2, 119.4, 117.0, 115.4, 55.5, 37.6. Anal. Calcd for $C_{22}H_{18}Cl_2N_2O_3$ (429.30): C, 61.55; H, 4.23; N, 6.53. Found: C, 61.30; H, 4.435; N, 6.475.

(*R*)-4-Chloro-*N*-(1-(4-chlorophenylamino)-1-oxo-3-phenylpropan-2-yl)-2-hydroxybenzamide **6w**.

White solid; yield 40%; mp 232-234 °C, $[\alpha]_D^{24}$ 43.7 (*c* 1.6; DMSO). IR (KBr pellet): 3377, 3266, 1677, 1599, 1532, 1492, 1402, 1299, 1233, 1091, 1014, 918, 825, 744, 700, 571, 496 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ 12.33 (1H, bs, NH), 10.40 (1H, s, OH), 9.00 (1H, d, *J*=7.3 Hz, NH), 7.94 (1H, d, *J*=8.7 Hz, H6), 7.63-7.59 (2H, m, AA′, BB′, H2′, H6′), 7.39-7.35 (2H, m, AA′, BB′, H3′, H5′), 7.37-7.17 (5H, m, H5, H3, H2′′, H4′′, H6′′), 7.05-6.99 (2H, m, H3′′, H5′′), 4.90-4.81 (1H, m, CH), 3.20-3.06 (2H, m, CH_2). ^{13}C NMR (75 MHz, DMSO) δ 170.0, 166.9, 159.7, 137.8, 137.7, 137.5, 129.4, 128.9, 128.4, 128.5, 127.3, 126.8, 121.2, 119.4, 117.0, 115.4, 55.5, 37.6. Anal. Calcd for $C_{22}H_{18}Cl_2N_2O_3$ (429.30): C, 61.55; H, 4.23; N, 6.53. Found: C, 60.57; H, 4.43; N, 6.46.

5-Chloro-*N*-(2-(3-chlorophenylamino)-2-oxoethyl)-2-hydroxybenzamide **6x**.

White solid; yield 32%; mp 244-245 °C. IR (KBr pellet): 3343, 3308, 1690, 1633, 1596, 1538, 1483, 1431, 1285, 1270, 1221, 1198, 1120, 999, 974, 901, 870, 823, 776, 678, 535 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ 12.19 (1H, s, NH), 10.32 (1H, bs, OH), 9.22 (1H, d, *J*=9.2 Hz, NH), 7.95 (1H, d, *J*=2.7 Hz, H6), 7.80 (1H, t, *J*=2.5 Hz, H2′), 7.45 (2H, dd, *J*=8.0 Hz, *J*=2.5 Hz, H4′, H6′), 7.34 (1H, t, *J*=8.0 Hz, H5′), 7.11 (1H, dd, *J*=8.7 Hz, *J*=2.7 Hz, H4), 6.98 (1H, d, *J*=8.7 Hz, H3), 4.13 (2H, d, *J*=5.4 Hz, CH_2). ^{13}C NMR (75 MHz, DMSO) δ 167.7, 167.1, 158.0, 140.4, 133.5, 33.3, 130.8, 128.4, 123.3, 122.8, 119.5, 118.8, 117.7, 117.6, 43.4. Anal. Calcd for $C_{15}H_{12}Cl_2N_2O_3$ (339.17): C, 53.12; H, 3.57; N, 8.26. Found: C, 53.19; H, 3.775; N, 8.17.

(*S*)-5-Chloro-*N*-(1-(3-chlorophenylamino)-1-oxopropan-2-yl)-2-hydroxybenzamide **6y**.

White solid; yield 43%; mp 222-224 °C, $[\alpha]_D^{26}$ 37.0 (*c* 1.6; ethyl acetate). IR (KBr pellet): 3367, 3285, 3060, 1679, 1627, 1578, 1598, 1483, 1413, 1363, 1280, 1235, 1212, 1115, 1077, 1047, 890, 826, 787, 779, 676, 651, 598, 535 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ 12.19 (1H, s, NH), 10.34 (1H, bs, OH), 9.10 (1H, d, *J*=6.9 Hz, NH), 8.04 (1H, d, *J*=2.5 Hz, H6), 7.81 (1H, t, *J*=2.0 Hz, H2´), 7.50-7.47 (1H, m, H6´), 7.44 (1H, dd, *J*=8.8 Hz, *J*=2.5 Hz, H4), 7.34 (1H, t, *J*=8.0 Hz, H5´), 7.15-7.08 (1H, m, H4´), 6.96 (1H, d, *J*=8.8 Hz, H3), 4.69-4.55 (1H, m, CH), 1.44 (3H, d, *J*=7.2 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 166.6, 158.0, 140.5, 133.5, 133.3, 130.7, 128.5, 123.4, 122.8, 119.4, 119.0, 117.9, 117.5, 50.0, 18.2. Anal. Calcd for $C_{16}H_{14}Cl_2N_2O_3$ (353.20): C, 54.41; H, 4.00; N, 7.93. Found: C, 54.00; H, 4.16; N, 7.965.

(*R*)-5-Chloro-*N*-(1-(3-chlorophenylamino)-1-oxopropan-2-yl)-2-hydroxybenzamide **6z**.

White solid; yield 3%; mp 225-226 °C, $[\alpha]_D^{24}$ -43.1 (*c* 1.4; ethyl acetate). IR (KBr pellet): 3368, 3285, 1679, 1627, 1598, 1579, 1541, 1483, 1413, 1295, 1213, 1115, 890, 826, 787, 676, 535 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ 12.21 (1H, s, NH), 10.35 (1H, bs, OH), 9.10 (1H, d, *J*=6.9 Hz, NH), 8.00 (1H, d, *J*=2.5 Hz, H6), 7.81 (1H, t, *J*=2.0 Hz, H2´), 7.52-7.46 (1H, m, H6´), 7.43 (1H, dd, *J*=8.8 Hz, *J*=2.5 Hz, H4), 7.34 (1H, t, *J*=8.0 Hz, H5´), 7.16-7.08 (1H, m, H4´), 6.98 (1H, d, *J*=8.8 Hz, H3), 4.71-4.55 (1H, m, CH), 1.45 (3H, d, *J*=7.2 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 166.6, 158.0, 140.5, 133.5, 133.3, 130.7, 128.4, 123.3, 122.9, 119.4, 119.1, 117.8, 117.5, 50.0, 18.2. Anal. Calcd for $C_{16}H_{14}Cl_2N_2O_3$ (353.20): C, 54.41; H, 4.00; N, 7.93. Found: C, 54.64; H, 4.10; N, 7.895.

(*S*)-5-Chloro-*N*-(1-(3-chlorophenylamino)-3-methyl-1-oxobutan-2-yl)-2-hydroxybenzamide **6aa.**

White solid; yield 46%; mp 244-245 °C, $[\alpha]_D^{24}$ 70.2 (*c* 1.5; ethyl acetate), $[\alpha]_D^{24}$ 46.9 (*c* 2.2; DMSO). IR (KBr pellet): 3282, 1660, 1632, 1595m, 1535, 1481, 1418, 1375, 1355, 1279, 1251, 1200, 1170, 867, 822, 790, 771, 721, 649 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ 12.01 (1H, s, NH), 10.44 (1H, bs, OH), 8.94 (1H, d, *J*=8.1 Hz, NH), 8.00 (1H, d, *J*=2.4 Hz, H6), 7.83 (1H, t, *J*=2.0 Hz, H2´), 7.48-7.41 (2H, m, H6´, H4´), 7.33 (1H, t, *J*=8.1 Hz, H5´), 7.11 (1H, ddd, *J*=8.7 Hz, *J*=2.2 Hz, *J*=1.0 Hz, H4), 6.97 (1H, d, *J*=8.7 Hz, H3), 4.53 (1H, t, *J*=7.5 Hz, CH), 2.23-2.12 (1H, m, CH), 0.96 (6H, d, *J*=6.6 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 165.7, 156.9, 140.2, 133.3, 133.2, 130.7, 129.2, 123.5, 123.1, 119.2, 119.0, 118.6, 117.9, 59.3, 30.9, 19.4, 18.6. Anal. Calcd for $C_{18}H_{18}Cl_2N_2O_3$ (381.25): C, 56.71; H, 4.76; N, 7.35. Found: C, 57.105; H, 4.965; N, 7.245.

(*R*)-5-Chloro-*N*-(1-(3-chlorophenylamino)-3-methyl-1-oxobutan-2-yl)-2-hydroxybenzamide **6bb**.

White solid; yield 39%; mp 211-212 °C, $[\alpha]_D^{26}$ -44.1 (*c* 1.7; ethyl acetate). IR (KBr pellet): 3310, 2966, 1674, 1637, 1595, 1542, 1482, 1426, 1292, 1212, 1099, 822, 779, 680, 651 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ 12.02 (1H, s, NH), 10.44 (1H, bs, OH), 8.94 (1H, d, *J*=8.1 Hz, NH), 8.00 (1H, d, *J*=2.2 Hz, H6), 7.83 (1H, t, *J*=2.0 Hz, H2´), 7.48-7.41 (2H, m, H6´, H4´), 7.34 (1H, t, *J*=7.6 Hz H5´), 7.12 (1H, ddd, *J*=8.7 Hz, *J*=2.2 Hz, *J*=1.2 Hz, H4), 6.98 (1H, d, *J*=9.0 Hz, H3), 4.53 (1H, t, *J*=7.5 Hz, CH), 2.23-2.11 (1H, m, CH), 0.96 (6H, d, *J*=6.6 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 165.7, 156.9, 140.2, 133.3, 133.2, 130.7, 129.2, 123.5, 123.1, 119.2, 119.0, 118.6, 117.9, 59.3, 30.9, 19.4, 18.6. Anal. Calcd

for $C_{18}H_{18}Cl_2N_2O_3$ (381.25): C, 56.71; H, 4.76; N, 7.35. Found: C, 56.35; H, 4.925; N, 7.215.

(*S*)-5-Chloro-*N*-(1-(3-chlorophenylamino)-1-oxo-3-phenylpropan-2-yl)-2-hydroxybenzamide **6cc** (published as a sample compound in **paper IV**).

White solid; yield 60%; mp 181-183 °C, $[\alpha]_D^{24}$ 60.1 (*c* 1.5; DMSO). IR (KBr pellet): 3297, 1672, 1633, 1594, 1536, 1483, 1416, 1288, 1236, 1180, 865, 823, 746, 693, 675, 535 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ 12.03 (1H, bs, NH), 10.45 (1H, bs, OH), 9.09 (1H, d, *J*=7.4 Hz, NH), 7.97 (1H, d, *J*=2.5 Hz, H6), 7.79 (1H, t, *J*=1.9 Hz, H2′), 7.49-7.09 (9H, m, H4, H4′, H5′, H6′, H2′′, H3′′, H4′′, H5′′, H6′′), 6.94 (1H, d, *J*=8.8 Hz, H3), 4.95-4.81 (1H, m, CH), 3.24-3.03 (2H, m, CH_2). ^{13}C NMR (75 MHz, DMSO) δ 170.1, 166.4, 157.6, 140.3, 137.4, 133.5, 133.3, 130.7, 129.4, 128.6, 128.5, 126.8, 123.5, 122.9, 119.4, 119.1, 118.0, 117.7, 55.6, 37.5. Anal. Calcd for $C_{22}H_{18}Cl_2N_2O_3$ (429.30): C, 61.55; H, 4.23; N, 6.53. Found: C, 61.735; H, 4.33; N, 6.595.

(*R*)-5-Chloro-*N*-(1-(3-chlorophenylamino)-1-oxo-3-phenylpropan-2-yl)-2-hydroxybenzamide **6dd**.

White solid; yield 7.5%; mp 182-184 °C, $[\alpha]_D^{24}$ -71.4 (*c* 1.9; DMSO). IR (KBr pellet): 3305, 1672, 1636, 1595, 1537, 1483, 1426, 1418, 1289, 1230, 1101, 824, 778, 699, 680, 650, 535 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ 12.03 (1H, bs, NH), 10.50 (1H, bs, OH), 9.09 (1H, d, *J*=7.4 Hz, NH), 7.97 (1H, d, *J*=2.7 Hz, H6), 7.79 (1H, t, *J*=2.1 Hz, H2′), 7.47-7.11 (9H, m, H4, H4′, H5′, H6′, H2′′, H3′′, H4′′, H5′′, H6′′), 6.93 (1H, d, *J*=8.7 Hz, H3), 4.93-4.85 (1H, m, CH), 3.22-3.05 (2H, m, CH_2). ^{13}C NMR (75 MHz, DMSO) δ ^{13}C NMR (75 MHz, DMSO) δ 170.1, 166.4, 157.6, 140.3, 137.4, 133.5, 133.3, 130.7, 129.4, 128.6, 128.5, 126.8, 123.5, 122.9, 119.4, 119.1, 118.0, 117.7, 55.6, 37.5. Anal. Calcd for $C_{22}H_{18}Cl_2N_2O_3$ (429.30): C, 61.55; H, 4.23; N, 6.53. Found: C, 61.31; H, 4.32; N, 6.475.

Antimycobacterial evaluation

Table 11: Antimycobacterial evaluation of Hydroxy-*N*-(phenylamino)-oxo-alkyl benzamides **6** made in National Reference Laboratory.

National Reference Laboratory – MIC [μmol/L]										
Comp.	*M. tbc* 331/88		*M. avium* 330/88		*M. kansasii* 235/80			*M. kansasii* 6509/96		
	14d	21d	14d	21d	7d	14d	21d	7d	14d	21d
6a	8	16	62.5	125	32	125	250	62.5	125	250
6b	32	32	62.5	62.5	32	62.5	62.5	32	62.5	62.5
6c	32	32	62.5	62.5	32	62.5	62.5	32	62.5	62.5
6d	16	16	32	32	32	32	62.5	16	32	62.5
6e	16	16	32	32	16	32	32	32	32	62.5
6g	62.5	62.5	62.5	62.5	62.5	62.5	125	62.5	62.5	62.5
6h	62.5	62.5	125	125	62.5	62.5	125	32	62.5	62.5
6i	32	32	250	250	32	62.5	500	62.5	125	250
6j	32	32	250	250	32	32	32	32	32	62.5
6k	125	500	62.5	500	62.5	125	500	62.5	250	500
6l	32	32	62.5	32	32	62.5	>500	62.5	62.5	32
6n	32	32	62.5	125	16	32	62.5	16	32	62.5
6o	16	32	62.5	125	16	32	62.5	16	32	32
6p	125	>1000	125	>1000	125	250	>1000	62.5	250	500
6q	125	>1000	125	>1000	32	62.5	500	32	62.5	500
6r	62.5	62.5	125	125	62.5	125	125	125	62.5	62.5
6s	32	62.5	125	125	62.5	125	125	62.5	62.5	62.5
6t	62.5	62.5	125	125	32	62.5	62.5	32	62.5	62.5
6u	62.5	62.5	62.5	62.5	16	32	62.5	32	62.5	62.5
6v	32	32	32	62.5	32	32	32	32	32	32
6w	32	32	62.5	62.5	32	32	62.5	32	62.5	62.5
6x	125	125	62.5	62.5	125	250	250	62.5	250	250
6y	250	500	500	500	125	500	500	250	500	500
6z	62.5	125	125	250	62.5	62.5	62.5	32	62.5	62.5
6aa	32	62.5	125	125	62.5	62.5	125	32	62.5	62.5
6bb	32	62.5	62.5	125	62.5	62.5	125	32	62.5	62.5
6cc	62.5	125	125	250	32	62.5	125	16	32	32
6dd	62.5	62.5	32	32	62.5	62.5	62.5	32	62.5	62.5
INH	0.5	0.5	>250	>250	>250	>250	>250	2	4	4

Calculated and determinated values of liphophilicity factor

Table12: Experimental and calculated values of liphophilicity factor of **6**

Compound	Purity[a] (%)	log *K*	log *P* ACD/Log *P*	log *P*/Clog *P* ChemOffice
6a	99.95	0.4308	4.64 ± 0.47	2.91 / 4.64056
6b	99.97	0.6371	5.52 ± 0.48	3.80 / 5.56856
6c	98.92	0.6125	5.52 ± 0.48	3.80 / 5.56856
6d	96.68	0.7394	6.57 ± 0.49	4.58 / 6.05856
6e	99.67	0.7329	6.57 ± 0.49	4.58 / 6.05856
6g	99.83	0.6646	5.76 ± 0.54	4.07 / 5.71856
6h	98.87	0.6709	5.76 ± 0.54	4.07 / 5.71856
6i	99.66	0.7779	6.81 ± 0.55	4.86 / 6.20856
6j	99.90	0.7673	6.81 ± 0.55	4.86 / 6.20856
6k	99.63	0.4971	5.20 ± 0.49	2.98 / 5.01472
6l	99.78	0.6698	5.55 ± 0.50	3.47 / 5.32372
6n	99.73	0.8614	6.43 ± 0.50	4.35 / 6.25172
6o	99.73	0.8623	6.43 ± 0.50	4.35 / 6.25172
6p	99.81	0.9485	7.48 ± 0.51	5.14 / 6.74172
6q	98.72	0.9408	7.48 ± 0.51	5.14 / 6.74172
6r	99.89	0.4921	4.54 ± 0.47	2.91 / 4.64056
6s	99.96	0.4934	4.54 ± 0.47	2.91 / 4.64056
6t	98.59	0.6832	5.41 ± 0.48	3.80 / 5.56856
6u	99.55	0.6839	5.41 ± 0.48	3.80 / 5.56856
6v	99.90	0.7540	6.47 ± 0.49	4.58 / 6.05856
6w	99.63	0.7479	6.47 ± 0.49	4.58 / 6.05856
6x			4.36 ± 0.47	2.42 / 4.33156
6y	99.95	0.4166	4.71 ± 0.48	2.91 / 4.64056
6z	99.42	0.4153	4.71 ± 0.48	2.91 / 4.64056
6aa	99.23	0.6114	5.59 ± 0.48	3.80 / 5.56856
6bb	99.29	0.6114	5.59 ± 0.48	3.80 / 5.56856
6cc	96.82	0.7066	6.64 ± 0.49	4.58 / 6.05856
6dd	98.39	0.7098	6.64 ± 0.49	4.58 / 6.05856

[a] Purity was determinated by RP-HPLC.

Over the course of esterification of Z-amino acids with chosen salicylanilides benzoxazepine-2,5-diones **9** were obtained as unexpected product of reaction of Z-Glycine and (*S*)-Z-Alanine and some salicylanilides in the presence of DCI. These products were tested in TAACF (USA). These compounds are without antimycobacterial activity.

In cases where "higher" amino acids were esterified or different substitution of salicylanilides was used, esters of Z-amino acid and substituted salicylanilides **4** were acquired. The compounds show better antimycobacterial activity (MIC = 4 – 32 μmol/L) than standard isoniazid against *M. avium* 330/88, *M. kansasii* 235/80 (Tested in National

Reference Laboratory for *M. kansasii* in Ostrava). Antifungal screening of some derivates **4** show better activity than standard Fluconazole against AF – *Aspergillus fumigatus* and AC – *Absidia corymbifera* MIC = 15.63 – 62.5 μg/mL (Tested at The Department of Biological and Medical Science, Faculty of Pharmacy Charles University in Prague).

Acidolytic liberation of amino group of compounds **4** correspond to hydroxy-*N*-(phenylamino)-oxo-alkyl benzamides **6**. Across this series, compound, with substitution R^2 = 4-Cl and R^3 = (*R, S*) – CH_2-phenyl show higher activity (MIC = 16 – 32 μmol/L) against *M. avium* 330/88 and *M. kansasii* 235/80 (Tested in National Reference Laboratory for *M. kansasii* in Ostrava) than standard isoniazid. Its activity against the mentioned strains is higher than 250 μmol/L. Antifungal screening of the compounds **6** is still in progress.

Optical activity measurement of prepared compounds **4** and **6** has brought interesting results. Derivates **4** showed activities in the following order: when (*R*)-Z-amino acids were used as starting material for esterification of salicylanilides specific optical rotation has positive values (measured in chloroform as solvent, several sample compounds also in DMSO). In cases of (*S*)-Z-amino acids values of $[\alpha]_D$ were negative. On the other hand appropriate compounds **6** showed reverse sign as compound **4** (DMSO or ethyl acetate as solvent). These results led us to hypothesis about mechanism of rearrangement process. If absolute configuration changed, the most probably mechanism of this rearrangement was bimolecular substitution. Confirmation of the proposal mechanism can bring X-ray analysis, which is in process for sample compounds. Spatial orientation of substituents (Table 10) and chemical names of compounds **6** remained for clarity in concordance with the starting amino acids.

4. NEW MODIFICATIONS OF ANTI-TUBERCULAR ACTIVE MOLECULES (PAPER V, VI)

Isonicotinic acid hydrazide (Isoniazid, INH) and pyrazin-2-carboxamide (Pyrazinamide, PZA) are widely applied as first-line drugs for the treatment of tuberculosis and usually are employed in combination with other drugs (Rifampicine, Ethambutol). Isoniazid is also used for the prevention of TB and in this case may be given alone.

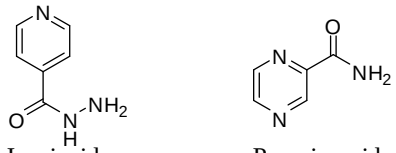


Fig. 4: Structure of isoniazid and pyrazinamide.

4.1. Isoniazid – mechanism of action and its resistance

INH is a synthetic prodrug that requires activation by mycobacterial catalase-peroxidase (*KatG*) for its action.[42] The main target of INH is a mycolic acids synthesis where a 2-*trans*-enoyl-acyl carrier protein reductase is inhibited, called *InhA*, which belongs to the FAS-II (fatty acid synthethase II) system – an essential step in fatty acid synthesis.[43] Moreover, compounds with *InhA* inhibition block the biosynthesis of mycolic acids, which are major constitutional lipids of the mycobacterial envelope.

The number of cases, where the treatment with INH failed, has been growing in recent years. Cause of it is bacterial chromosomal mutations which induce antibiotic resistance. Most INH resistance in clinical isolates results from blocking prodrug activation through mutation in the gene *KatG* that alter or eliminate mycobacterial catalase – peroxidase activity.[44] Mutation in 2-*trans*-enoyl-acyl carrier protein (*InhA*) also caused INH resistance.[45] Such strains are called INH resistant. To increase activity mainly against resistant strains, the INH molecule has been broadly modified by various substitutions and the INH moiety has been incorporated into complex structures. Many analogues featuring the isoniazid structure have been synthesized and are still the subject of works.[46,47,48,49,50] A critical review of such approaches has been published recently.[12] Most published modifications that have led to active structures are summarized in our review **paper V.**

4.2. Pyrazinamide – mechanism of action and its resistance

Pyrazinamide, an analog of nicotinamide, is a cornerstone in drug combination and plays a key role in shortening TB therapy from 9–12 months to the current 6 months. The ability of pyrazinamide to shorten TB therapy is related to its activity against a population of non-growing, persistent *tubercle bacilli* residing in an acidic pH environment that are not killed by other TB drugs. Pyrazinamide is also a prodrug that requires activation or conversion to its active form, pyrazinoic acid (POA), by the PZase/nicotinamidase enzyme encoded by *pncA* gene of susceptible *M. tuberculosis*. The target of PZA/POA appears to be the membrane.[51] Mutation in *pncA* is a major mechanism of PZA resistence in *M. tuberculosis*.[52]

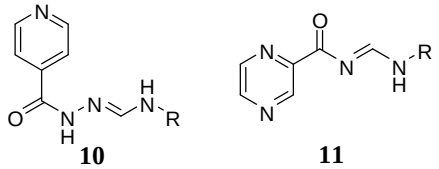
Despite its high *in vivo* sterilizing activity, paradoxically, pyrazinamide is not active *in vitro* under normal culture conditions near neutral pH, it is only active under acidic conditions (e.g. pH 5.5), with an MIC of 50–100 mg/L at pH 5.5–6.0.[53]

4.3. Isoniazid modification (paper V)

The **paper V** offers a compilation of comprehensive literature on advances of the isoniazid moiety and research efforts towards the discovery of new chemical compounds as potential anti-TB agents. A great amount of work has been done in order to acquire useful knowledge about the mechanism of action and of resistance of isoniazid. We have found more than 500 derivatives with the INH moiety, prepared during the past several years as new anti-TB agents. We have chosen the most active derivatives and tried to discuss some structure activity relationship in accordance to their lipophilicity.

4.4. Our approach to INH and PZA modification (paper VI)

Recent results concerning the synergetic effect of the isonicotinoyl hydrazide/hydrazone combination[54] as well as the report dealing with active isonicotinoyl hydrazones[55] led us to the preparation of new methylenehydrazono INH derivatives **10** or PZA derivates **11**, where R reports *N*-nucleophile



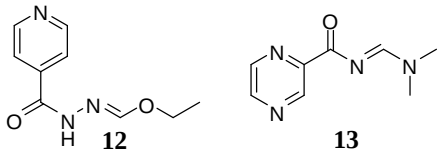
10

11

Fig. 5: General structures of INH and PZA derivates.

This type of "double active" molecule can play the role of prodrug with prolonged liberation, possible synergism could be generated[56,8] or a new active molecule formed.

The best starting derivative to introduce the group possessing nitrogen functionality has been found to be isonicotinoylethoxymethylenehydrazone **12.** An ethoxy group was substituted with an appropriate amino group belonging to the molecule of already known tuberculostatic agent (pyrazinamide, *p*-amino salicylic acid, ciprofloxacine) or some amines like morpholine, benzylamine, picoline amine. The product contains two active fragments connected by the CH functionality. Such compounds hydrolyze under aqueous conditions slowly liberating the two active drugs. They play the role of prodrugs and depot form. Compounds containing other *N*-nucleophile can be classified as new drugs, where activity of these compounds is liable to synergetic effect of INH/INH hydrazone combination.[54] The same approach was also used for PZA modification where *N*-((dimethylamino)methylene)-pyrazine-2-carboxamide **13** (**Figure 6**) was found as an optimal starting derivate for these transformations. From this intermediate were prepared derivates with the general structure **11** where molecule of PZA was connected with other anti-TB drug by a simple CH bridge (for general structure see **Figure 5**).



12

13

Fig. 6: Starting points for INH and PZA modifications

In the **paper VI** we are presenting several examples of compounds where some of them involve two current anti-TB drugs. Liphophilicity and basic screening of stability was checked by RP-HPLC. Promising results of biological evaluation (Tuberculosis Antimicrobial Acquisition and Coordinating Facility and National Reference Laboratory for *Mycobacterium kansasii*), make this research an attractive starting point for other combinations.

5. SUMMARY

This thesis deals with the synthesis of antimicrobial active compounds from the group of salicylanilide derivates that were found to be highly antituberculosis active compounds against atypical and resistant strains of *Mycobacterium tuberculosis*. These derivates served as the starting substances for the synthesis of prodrugs of acetic acid and amino acid esters, with the aim to reduce toxicity and to optimize physico-chemical properties and transport to the site of action. The second group under investigation was new modifications of some contemporary antituberculosis drug (isoniazide and pyrazinamide), the combination of either of these two compounds with second active molecule through an easily splitting methane bridge, which together could serve as depot forms, after release of both parts they may act as synergists.

The outcomes of a literary search, that forewent synthesis, were published as survey articles in scientific papers. They deal with: a) cyclization forms of prodrugs (**paper I**), b) salicylanilides and their research trends (**paper II**) and c) isoniazide, its mechanism of activity and structure modifications published till now (**paper V**).
The main scope of the thesis is experimental parts whose results were parcially published and belong to enclosed articles (**paper III**, **IV** and **VI**). Unpublished experimental results from chapter 3, that is supplemented with the results of biological evaluation and experimentally determinate values of the liphophilicity parameter. After receiving biological evaluation results from TAACF, this part will be completed and sent to scientific journal.

Within the frame of this dissertation 126 compounds were prepared, 112 are original till this time in literature blank organic adducts. The structures of all prepared compounds were electronically checked by Beilstein Commander and SciFinder Scholar electronic version of Chemical Abstracts.

Esterification of α-amino acids with salicylanilides was not easy. Firstly, esters of acetic acid and salicylanilides were prepared by various modifications of the reaction. These procedures were applied on several chosen amino acid. The method from peptide chemistry where carboxylic group of *N*-benzyloxycarbonyl-α-amino acid was activated

with *N*,*N*´-dicyclohexylcarbodiimide was found to be optimal one. In some cases, unexpected benzoxazepine-2,5-diones were formed. Unfortunately, these compounds did not report any antitubercular activity. In those cases, where esterification of *N*-benzyloxycarbonyl protected α-amino acid was successful, the following amino group liberation led to an unexpected hydroxy-*N*-(phenylamino)-oxo-alkyl benzamides formed by quick nucleophilic attack of the free amino group on amidic carbonyl and rearrangement. Identical compounds were also the products of reaction where both protection of amino group and activation of carboxylic group of amino acid were done in one step by Leuchs anhydride preparation, which reacted with appropriate salicylanilides. This way confirms the predicted cyclic mechanism of rearrangement. *N*-Protected esters and hydroxy-*N*-(phenylamino)-oxo-alkyl benzamides have shown very high activity against atypical strains of *Mycobacterium*. This group is still under intensive research, where the aim is the preparation of di- respectively tri peptide esters.

The synthesis of isoniazid and pyrazinamide *N*-hydrazone derivates has started in the University of Ljubljana as an international *Socrates/Erasmus* program. The combination of INH and pyrazinamide with other antitubercular agents or other molecules which are *N*-nucleophiles has led to the preparation of depot forms with possible synergetic action known from the literature. Results of biological evaluation show this group of compounds as perspective for finding other possible combinations, which will be highly active against a wide range of Mycobacterial strains.

SOUHRN

Práce se zabývá syntézou antimikrobiálně aktivních sloučenin ze skupiny derivátů salicylanilidů, u nichž byla nalezena vysoká antituberkulotická aktivita rovněž vůči atypickým a rezistentním kmenům *Mycobacterium tuberculosis*. Tyto deriváty sloužily jako výchozí sloučeniny pro syntézu proléčiv typu esterů kyseliny octové a aminokyselin, s cílem snížit toxicitu, optimalizovat fyzikálně chemické vlastnosti a transport na místo účinku. Druhou studovanou skupinou jsou nové modifikace některých současných antituberkulotik (isoniazidu a pyrazinamidu), jejich kombinace přes methinový můstek s druhou aktivní molekulou, které mohou sloužit jako depotní formy, po uvolnění obou částí mohou působit synergicky.

Výsledky literární rešerše, která předcházela syntézu, byly publikovány jako přehledné články v odborných časopisech. Jsou zaměřeny na: a) cyklizační formy proléčiv (**publikace I**), b) salicylanilidy a směry jejich výzkumu (**publikace II**) a c) isoniazid, jeho mechanismus účinku a dosud publikované modifikace struktury (**publikace V**).
Hlavní náplní práce je experimentální část jejíž parciální výsledky jsou součástí přiložených prací (**publikace III**, **IV** a **VI**). Nepublikované experimentální výsledky tvoří kapitolu 3, která je doplněna doposud získanými výsledky biologického hodnocení a experimentálně zjištěnými hodnotami parametru lipofility. Po získání všech výsledků z TAACF, bude i tato část odeslána do tisku.

V rámci této disertační práce bylo připraveno celkem 126 sloučenin, z toho je 112 originálních, dosud v literatuře nepopsaných organických sloučenin. Struktury všech připravených látek byly konfrontovány s elektronickými databázemi Beilstein Commander a SciFinder Scholar elektronickou verzí Chemical Abstracts.

Příprava esterů aminokyselin s vybranými salicylanilidy nebyla snadná. Nejprve byly provedeny modifikace esterifikace kyseliny octové a salicylanilidů a ty pak aplikovány na vybrané aminokyseliny. Pro aminokyseliny byla shledána jako nejúspěšnější metoda z peptidové chemie, aktivace karboxylu *N*,*N*´-dicyklohexylkarbodiimidem. U některých aminokyselin došlo ke vzniku neočekávaných benzoxazepindionových cyklů. Tyto sloučeniny jsou bohužel antimikrobiálně neúčinné. U těch derivátů, u nichž esterifikace *N*-

chráněné aminokyseliny proběhla, aminoskupina po uvolnění atakovala amidový karbonylový uhlík za nečekaného přesmyku na hydroxy-*N*-(fenylamino)-oxo-alkyl benzamidy. Stejné produkty byly získány při současném chránění aminoskupiny a aktivaci karboxylové kyseliny u typu Leuchových anhydridů, což potvrdilo navržený cyklizační mechanismus reakce. *N*-Chráněné estery a získané diamidy vykazují velmi dobrou aktivitu zejména vůči atypickým kmenům *Mycobacterium*. V této skupině modifikací se dále pokračuje přípravou esterů di- a tripeptidů.

Příprava *N*-hydrazonových derivátů isoniazidu a pyrazinamidu byla zahájena na odborné stáži ve Slovinsku. Kombinace INH a pyrazinamidu s nukleofily typu dalšího antituberkulotika či jiné molekuly vedou k přípravě depotních forem s možností zvýšení účinku synergickým způsobem. Výsledky biologického hodnocení ukázaly tuto skupinu jako perspektivní pro hledání dalších možných kombinací, působících vůči širší škále kmenů*Mycobacteria*.

6. REFERENCES:

[1] http://rp7.ffg.at/upload/medialibrary/a_ct_200701_en.pdf (January 2007)
[2] http://www.vyzkum.cz/FrontClanek.aspx?idsekce=1458#kapIV1.1 (January 2007)
[3] World Health Organization (WHO). Tuberculosis Fact Sheet N°104 – Global and Regional incidence. March 2006.
[4] World Health Organization (WHO). Global tuberculosis control – surveillance, planning, financing WHO Report 2006.
[5] Blumberg, H. M.; Burman, W. J.; Chaisson, R. E.; Daley, C. L.; Etkind, S. C.; Friedman, L. N.; Fujiwara, P.; Grzemska, M.; Hopewell, P. C.; Iseman, M. D.; Jasmer, R. M.; Koppaka, V. R.; Menzies, R. I.; O´brien, R. J.; Reves, R. R.; Reichman, L. B.; Simone, P. M.; Starke, J. R.; Vernon, A. A. American Thoracic Society/Centres for disease Control and prevention/infections disease Society of America: treatment of tuberculosis. *Am. J. Respir. Crit. Care Med.* **2003**, *167*, 603-662.
[6] Zhang, Y. Persistent and dormant tubercle bacilli and latent tuberculosis. *Front Biosci.* **2004**, *9*, 1136-1156.
[7] Global Alliance for Tuberculosis Drug Development; Executive summary of the scientific Blueprint for TB Drug Development. *Tuberculosis (Edinb.)* **2001**, *81*(Suppl.), 1-52.
[8] Duncan, K; and C. E. Barry III Prospects for new antitubercular drugs. *Curr. Opin. Microbiol.* **2004**, *7*, 460-465.
[9] Janin,Y. L. Antituberculosis drugs: Ten years of research. *Bioorg. Med. Chem.* (**2007**), doi:10.1016/j.bmc.2007.01.030.
[10] Sundriyal, S.; Sharma, R. K.; Jain, R. Current advances in antifungal targets and drug development. *Curr. Med. Chem.* **2006**, *13*, 1321-1335.
[11] Cohen, J. New TB Drug Promises Shorter, Simpler Treatment. *Science* **2004**, *306*, 1872.
[12] Scior, T.; Garcés-Eisele, S. J. Isoniazid is not a Lead Compound for its Pyridyl Ring Derivates, Isonicotinoyl Amide, Hydrazides, Hydrazones: A Critical Review. *Curr. Med. Chem.* **2006**, *13*, 2205-2219.
[13] Ha, S-J.; Jeon, B. Y.; Joun, J. I.; Kim, S. C.; Cho, S. N.; Sung, S.C. Protective effect of DNA vaccine during chemotherapy on reactivation and reinfection of *Mycobacterium tuberculosis*. *Gene Ther*. **2005**, *12*, 634-638.
[14] Flynn, J.L. Immunology of tuberculosis and implication in vaccine development. *Tuberculosis (Edinb.)* **2004**, *84*, 93-101.
[15] Barrow, W. W. Microsphere technology for chemotherapy of mycobacterial infections. *Curr. Pharm. Des.* **2004**, *10*, 3275-3284.
[16] Vinšová, J.; Imramovský, A. Intramolekulární cyklizace využívané k uvolňování účinných látek z proléčiv. *Chem. Listy*, **2005**, *99*(1), 21-29
[17] Li, X.; Taylor, J.S. General strategy for the preparation of membrane permeable fluorogenic peptide ester conjugates for in vivo studies of ester prodrug stability. *Bioorg. Med. Chem*. **2004**, *12*, 545-552.
[18] Sriram, D.; Yogeeswari, P.; Srichakravarthy, N.; Bal, T.R. Synthesis of Stavudine Amino acid Ester Prodrugs with Broad-Spectrum Chemotherapeutic Properties for the Effective Treatment of HIV/AIDS. *Bioorg. Med. Chem. Lett*. **2004**, *14*, 1085-1087.
[19] Gomes, P.; Gomes, J. R. B.; Rodrigues, M.; Moreira, R. Amino acids as selective sulfonamide acylating agents. *Tetrahedron* **2003**, *59*, 7473-7480.
[20] Cole, S. T.; Brosch, R.; Parkhill, J.; Garnier, T.; Churcher, C.; Hartus. D.; Gordon, S. V.; Eiglmeier, N. K.; Gas, C.; Barry, E.; Tekaia F.; Badcock, K.; Baham, D.; Brown D.; Chillingworth, T.; Conner, R.; Davies, R.; Delvin, K.; Feltwell, T.; Gentles, S.; Hamlin,

N.; Holroyd, S.; Hornsby, T.; Jagels, K.; Krogh, A.; McLean, J.; Moule, S.; Murphy, L.; Olivek, K.; Osborne, J.; Quail, M. A.; Rajandream, M. A.; Rogers, J.; Rutter, S.; Server, K.; Skeleton, J.; Squares, R.; Squares, S.; Sulston, J. E.; Tailor, K.;Whitehead, S.; Barrell, B. G. Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence. *Nature* **1998**, *393*, 537-544.

[21] Lipinski, C. A.; Lombardo, F.; Dominy, B.W.; Feeney, P.J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.*, **1997**, *23*, 3-26.

[22] Vinšová, J.; Imramovský, A. Salicylanilidy – stále aktuální skupina s potenciální antibakteriální aktivitou. *Ces. Slov. Farm.* **2004**, *53*, 294-299.

[23] Kubicová, L.; Waisser, K. Biologická aktivita salicylanilidů. *Ces. Slov. Farm*. **1992**, *41*, 208.

[24] Waisser, K.; Hladůvková, J.; Kuneš, J.; Kubicová, L.; Klimešová, V.; Synthesis and Antimycobacterial Activity of Salicylanilides Substituted in Position 5. *Chem. Pap.* **2001**, *55*, 121-129.

[25] Waisser, K.; Bureš, O.; Holý, P.; Kuneš, J.; Oswald, R.; Jirásková, L.; Pour, M.; Klimešová, V.; Kubicová, L.; Kaustová, J. Relationship between the Structure and Antimycobacterial Activity of Substituted Salicylanilides. *Arch. Pharm. Pharm. Med. Chem.* **2003**, *1*, 53.

[26] Waisser, K.; Matyk, J.; Divišová, H.; Husáková, P.; Kuneš, J.; Klimešová, V.; Kaustová, J.; Möllmann, U.; Dahse, H.-M.; Miko, M. The Oriented Development of Antituberculotics: Salicylanilides. *Arch. Pharm. Chem. Life Sci.* **2006**, *339*, 616-620.

[27] Lupea, A. X.; Popescu, L.; Tarabasanu, C. Synthesis of new 2-[2-(4-chlorophenylcarbamoyl)]-phenoxyalkanoic acid derivates. *Rev. Roum. Chim.* **2006**, *51*, 517-521.

[28] De La Fuente, R. D.; Sonawane, N.; Arumainayagam, D. Small molecules with antimicrobial activity against *E-coli* and *P-aeruginosa* identified by high throughput screening. *Br. J. Pharmacol.* **2006**, *149*, 551-559.

[29] Nawwar, G.; Chabaka, L. M.; Shafik, N.A. Oximinosalicylanilide like analogues as molluscicidal agents. *Afinidad* **2006**, *63*, 153-158.

[30] Liechti, C.; Séquin, U.; Bold, G.; Furet, P.; Meyer, T.; Traxler, P. Salicylanilides as inhibitors of the protein tyrosine kinase epidermal growth factor receptor. *Eur. J. Med. Chem*. **2004**, *39*, 11-26.

[31] Macielag, M. J.; Demers, J. P.; Fraga-Spano, S.A.; Hlasta, D. J.; Johnson, S. G.; Kanojia, R. M.; Russell, R. K.; Sui, Z.; Weidner-Wells, M.A.; Werblood, H.; Foleno, B. D.; Goldschmidt, R. M.; Loeloff, M. J.; Webb, G. C.; Barrett, J. F. Substituted Salicylanilides as Inhibitors of Two-Component Regulatory Systems in Bacteria. *J. Med. Chem.* **1998**, *41*, 2939-2945.

[32] Hlasta, D. J.; Demers, J. P.; Foleno, B. D.; Fraga-Spano, S. A.; Guan, J.; Hilliard, J. J.; Macielag, M. J.; Ohemeng, K. A.; Sheppard, C. M.; Sui, Z.; Webb, G. C.; Weidner-Wells, M. A.; Barret, J. F. Novel inhibitors of bacterial two-component systems with gram positive antibacterial activity: Pharmacophore identification based on the screening hit closantel. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 1923-1928.

[33] Vinšová, J.; Imramovský, A.; Buchta, V.; Doležal, M.; Jampílek, J. Biodegradable esters of salicylanilides with significant antimicrobial activity. 14th European Symposium on Organic Chemistry, July 4-8, **2005**, Helsinki, Finland, ISBN 952-10-2553-0, p. 258.

[34] Matyk, J.; Waisser, K.; Dražková, K.; Kuneš, J.; Klimešová, V.; Palát, K. Jr.; Kaustová, J. Heterocyclic isosters of antimycobacterial salicylanilides. *Il Farmaco* **2005**, *60*, 399-408.

[35] Deng, W.; Guo, Z.; Feng, Z.; Juany, Y.; Chu, F. Acryloylamino-salicylanilides as EGFR PTK inhibitors. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 469-472.
[36] Waisser, K.; Peřina, M.; Klimešová, V.; Kaustová, J. On the relationship between the structure and antimycobacterial activity of substituted *N*-benzylsalicylamides. *Collect. Czech. Chem. Commun.* **2003**, *68*, 1275-1293.
[37] Rivero, I. A.; Heredia, S.; Ochoa, A. Esterification of amino acids and monoacids using triphosgene. *Synth. Commun.* **2001**, *31*, 2169-2175.
[38] Daly, W. H.; Poché, D. The preparation of *N*-caboxyanhydrides of α-amino acids using bis(trichloromethyl)carbonate. *Tetrahedron Lett.* **1988**, *29*, 5859-5862.
[39] Collins, L. A., Franzblau, S. G. Microplate alamar blue assay versus BACTEC 460 system for high- throughput screening of compounds against Mycobacterium tuberculosis and Mycobacterium avium. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004–1009.
[40] National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of yeast. Approved Standard, NCCLS document, M27-A; NCCLS: Villanova, PA, U.S.A., **1997**.
[41] Doležal, M.; Pálek, L.; Vinšová, J.; Buchta, V.; Jampílek, J.; Kral'ová K. Substituted Pyrazinecarboxamides: synthesis and Biological Evaluation. *Molecules* **2006**, *11*, 242–256.
[42] Zhang, Y; Heym, B.; Allen, B.; Zouny, D.; Cole, S. The catalase-peroxidase gene and isoniazid resistance of Mycobacterium tuberculosis. *Nature* **1992**, *358*, 501-593.
[43] Marrakchi, H.; Laneelle, G.; Quemard, A. InhA, a target of the antituberculous drug isoniazid, is involved in a mycobacterial fatty acid elongation system, FAS-II. *Microbiology* **2000**, *146*, 289–296.
[44] Slayden, R. A.; Lee, R. E.; Barry C. E. The genetics and biochemistry of isoniazid resistence in *Mycobacterium tuberculosis*. *Microbes Infect.* **2000**, *2*, 659-669.
[45] Banerjee, A.; Dubnau, E.; Quemard, A.; Balasubramanian. V.; UM, K. S.; Wilson, T.; Collins, D.; de Lisle, G.; Jacobs, W. R. Jr. Since overexpression of inhA confers INH resistance in *Mycobacterium smegmatis*. *Science* **1994**, *263*, 227-230.
[46] Hearn, M. J. Patent WO 0243668 *Chem. Abstr.* **2002** 137: 20296b.
[47] Sriram, D.; Yogeeswari, P.; Madhu, K. Synthesis and *in vitro* and *in vivo* antimycobacterial activity of isonicotinoyl hydrazones. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4502-4505.
[48] Shaharyar, M.; Siddiqui, A. A.; Ali, M. A.; Sriram, D.; Yogeeswari, P. Synthesis and *in vitro* antimycobacterial activity of N^1-nicotinoyl-3-(4´-hydroxy-3´-methyl phenyl)-5-[(sub)phenyl]-2-pyrazolines. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3947-3949.
[49] Velikorodov, A. V.; Urlyapva, N. G.; Daudova, A. D. Synthesis and antituberculous activity *in vitro* of amidine and hydrazidine analogs of pyrazinamide and isoniazid. *Pharm. Chem. J.* **2005**, *39*, 126-128.
[50] Kamal, A.; Srinivasa Reddy, K.; Kaleem Ahmed, S.; Khan, M. N.; Sinha, R. K.; Yadav, J. S.; Arora, S. K. Anti-tubercular Agents. Part 3. Benzothiadiazine as a novel scaffold for anti-*Mycobacterium* activity. *Bioorg. Med. Chem.* **2006**, *14*, 650-658.
[51] Zhang, Y.; Mazel, L. M. Tuberculosis Drug Targets. *Curr. Drug Targets* **2002**, *3*, 131-154.
[52] Scorpio, A.; Zhang, Y. Mutations in pncA, a gene encoding pyrazinamidase/nicotinamidase, cause resistance to the antituberculous drug pyrazinamide in tubercle bacillus. *Nat. Med.* **1996**, *2*, 662-667.
[53] Wade, M. M.; Zhang, Y. Effects of weak acids, UV and proton motive force inhibitors on pyrazinamide activity against *Mycobacterium tuberculosis in vitro*. *J. Antimicrob. Chemother*. **2006** *58*: 936-941.

[54] De Logu, A.; Onnis, V.; Saddi, B.; Congiu, C.; Schivo, M. L.; Cocco, M. T. Activity of a new class of isonicotinoylhydrazones used alone and in combination with isoniazid, rifampicin, ethambutol, *para*-aminosalicylic acid and clofazimine against *Mycobacterium tuberculosis*. *J. Antimicrob. Chemother*. **2002**, *49*, 275-282.

[55] Sinha, N.; Jain, S.; Tilekar, A.; Upadhayaya, R. S.; Kishore, N.; Jana, G. H.; Arora, S. K. Synthesis of isonicotinic acid *N*′-arylidene-*N*-[2-oxo-2-(4-aryl-piperazin-1-yl)-ethyl]-hydrazides as antituberculosis agents. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1573-1576.

[56] Zhang, Y. J.; Wade, M. M.; Scorpio, A.; Zhang, H.; Sun, Z. Mode of action of pyrazinamide: disruption of *Mycobacterium tuberculosis* membrane transport and energetics by pyrazinoic acid. *J. Antimicrob. Chemother*. **2003**, *52*, 790-795.