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DEVELOPMENT OF NEW POTENTIAL ANTIMYCOBACTERIAL ACTIVE AGENTS BASED ON THE GROUP OF SALICYLANILIDES

Doctoral Thesis

Hradec Králové

November 2009

Acknowledgements

Firstly I would like to thank my head supervisor professor Jarmila Vinšová for her valuable advice and constant support throughout my study.

Secondly, I would like to thank my family for their support and my boyfriend and colleague Jakub Novotný for his support and advice during these years.

I would especially like to thank Aleš Imramovský and Eva Vavříková for being great companions in the lab. Also thanks to Kateřina Vávrová for a fruitful collaboration.

Thanks to all my friends and colleagues at the department of organic and inorganic chemistry who have made these years memorable.

Finally, my thanks are also given to Mrs. Iva Vencovská for measuring of IR spectra and Doc. PharmDr. Jiří Kuneš, CSc. for NMR spectra measurement.

Abstract

Salicylanilides are an important class of aromatic compounds with a wide range of pharmacological activities, such as antibacterial, antifungal and anti-inflammatory, among others. Furthermore; several studies reported their potent antimycobacterial effect. Their activity results from multiple mechanisms. They are therefore interesting compounds for medicinal chemists. As phenolic-containing drugs, we hypothesised that a prodrug approach will make possible the improvement of the pharmaceutical, pharmacokinetic and/or pharmacodynamic properties of salicylanilides.

This thesis describes the development of new potential antimycobacterial active agents based on this group that have shown interesting antimycobacterial activity against *Mycobacterium tuberculosis*, and some atypical strains.

As the starting point for our research, the different strategies used in order to overcome the limited bioavailability of phenolic drugs were reviewed. Then new potentially antibacterial active prodrugs of salicylanilides, particularly *N*-benzyloxycarbonyl-ester and alkyl-carbamate derivatives of salicylanilide, active against *M. tbc.*, MDR-TB strains or non-TB strains such as *M. avium* and *M. kansasii*, were prepared. Finally the physicochemical and pharmacokinetic properties of the most active synthesised compounds were explored. For the esterification, *N*-protected, mainly *N*-benzyloxycarbonyl amino acids were used. The synthesised esters result to have comparable activity to the starting salicylanilides. In the course of our research, we have also investigated and elucidated the mechanism for the unexpected formation of novel seven-membered ring benzoxazepines and 2-hydroxy-*N*-(1-(oxo-(phenylamino)-alkan-2-yl)benzamides. All the studied alkyl-carbamate derivatives of salicylanilide exhibited higher activity against *M. tbc., M. kansasii* and *M. avium* compared to the starting salicylanilide and promising activity against five MDR-TB strains, with MIC values between 0.5-2 μmol/L. Furthermore, as prodrug forms, they seem to fulfil better conditions than the corresponding synthesised esters, due to the increment of the half time of hydrolysis to several hours and to the stability in acidic environment.

All these results have been submitted, accepted or already published in several impact-scientific journals and presented in different national and international symposia.

Abstrakt

Salicylanilidy jsou důležitou skupinou aromatických sloučenin, které vykazují široké spektrum různých farmakologických aktivit, především antibakteriální, antifungální a protizánětlivý efekt. Několik studií presentuje rovněž jejich významný antimykobakteriální účinek a mechanismus působení, lišící se od ostatních antimykobakteriálně aktivních sloučenin. Vzhledem k fenolickému charakteru těchto sloučenin lze předpokládat, že tvorba vhodné formy proléčiva zlepší jejich farmakochemické, farmakokinetické a farmakodynamické vlastnosti.

Tato práce se zabývá vývojem nových forem proléčiv antimykobakteriálně aktivních sloučenin na bázi salicylanilidů, které vykazují srovnatelné aktivity s běžnými antituberkulotiky vůči *Mycobacterium tuberculosis*, ale také vůči některým netuberkulózním kmenům jako je *M. avium* a *M. kansasii,* popř. kmenům, rezistentním na běžná antituberkulotika, kde jsou dokonce účinnější.

Na základě literární rešerše, která byla podkladem pro sepsání přehledného článku o literárně dostupných modifikacích léčiv, majících fenolickou hydroxyskupinu, byla cílem této práce příprava vhodné formy proléčiv antimykobakteriálně aktivních salicylanilidů, které by překonaly limitovanou biodostupnost a zlepšily fyzikálně chemické a farmakokinecké vlastnosti těchto fenolických sloučenin formou esterů a karbamátů. Pro esterifikaci byly použity N-chráněné, nejčastěji N-benzyloxykarbonylové aminokyseliny. Jejich estery se ukázaly být srovnatelně aktivní s výchozími salicylanilidy. Během těchto reakcí došlo ke tvorbě sedmičlenných benzoxazepindionů a 2-hydroxy-*N*-(1-(oxo-(fenylamino)-alkan-2-yl)benzamidů, vzniklých nečekaným přesmykem. Na základě experimentálních studií a díky izolaci a charakterizaci pětičlenného imidazolinonového meziproduktu byl potvrzen jeden z navržených mechanismů přesmyku. Salicylanilidové karbamáty vykázaly dokonce vyšší antimykobakteriální aktivitu oproti výchozím salicylanilidům. Působí rovněž na 5 rezistentních kmenů v MIC 0,5-2 μmol/L a jako formy proléčiv se zdají být daleko vhodnější ve srovnání s estery, díky poločasu hydrolýzy, který se pohybuje v rozmezí několika hodin.

Všechny presentované výsledky jsou předmětem několika publikovaných sděleních v mezinárodních impaktovaných časopisech, popř. byly zaslány či jsou přijaty k publikaci a vyjdou v nejbližší době.

List of publications

This thesis is based on the following publications, which are referred to in the text by the Roman numerals I – VI.

I. Prodrug design of phenolic drugs.
Juana Monreal Férriz, Jarmila Vinšova.
Curr. Pharm. Design, **2009**. Under revision.

II. Salicylanilide esters of *N*-protected amino acids as novel antimicrobial agents.
Aleš Imramovský, Jarmila Vinšová, Juana Monreal Férriz, Vladimír Buchta, Josef Jampílek.
Bioorg. Med. Chem. Lett., **2009**, *19*, 348-351.

III. New antituberculotics originated from salicylanilides with promising *in vitro* activity against atypical mycobacterial strains.
Aleš Imramovský, Jarmila Vinšová, Juana Monreal Férriz, Rafael Doležal, Josef Jampílek, Jarmila Kaustová, Filip Kunc.
Bioorg. Med. Chem., **2009**, *17*, 3572-3579.

IV. Salicylanilide esterification: unexpected formation of novel seven-membered rings.
Aleš Imramovský, Jarmila Vinšová, Juana Monreal Férriz, Jiří Kuneš, Milan Pour, Martin Doležal.
Tetrahedron Lett., **2006**, *47*, 5007-5011.

V. Salicylanilide carbamates: Promising antitubercular agents active against multidrug resistant *Mycobacterium tuberculosis* strains.
Juana Monreal Férriz, Kateřina Vávrová, Filip Kunc, Aleš Imramovský, Jiřina Stolaříková, Eva Vavříková, Jarmila Vinšová.
Bioorg. Med. Chem., **2009**. Under revision.

VI. Unprecedented rearrangement of salicylanilide derivatives: imidazolinone intermediate formation.
Jarmila Vinšová, Aleš Imramovský, Martin Krátký, Juana Monreal Férriz, Karel Palát, Antonín Lyčka, Aleš Růžička
Tetrahedron Lett., **2009**. doi:10.1016/j.tetlet.2009.10.084.

Table of Contents

List of Abbreviations

AA	Amino acid
AcOH	Acetic acid
ACN	Acetonitrile
ACOM	Alkylcarbonyloxymethyl
ADEPT	Antibody-directed enzyme prodrug therapy
AK	Amikacin
AOC	Alkyloxycarbonyl
APAP	Acetaminophen
BBB	Blood-brain barrier
Boc	*tert*-Butyloxycarbonyl
Cbz	Benzyloxycarbonyl
CNS	Central nervous system
CFZ	Clofazimine
DCC	*N*,*N*'-Dicyclohexylcarbodiimide
DEE	Diethylether
DKP	Piperazine-2,5-diones
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
EC_{50}	Concentration inhibiting 50% of the cell growth
EMB	Ethambutol
GTM	Gentamicin
Gly	Glycine
HBr	Hydrogen bromide
HD_{50}	Hypnotic dose

HIV	Human immunodeficiency virus
INH	Isoniazid
IC_{50}	Half maximal inhibitory concentration
IPM	Isopropyl mirystate
i.v.	Intra venous
LD_{50}	Lethal dose
MIC	Minimum inhibition concentration
MDR-TB	Multidrug-resistant tuberculosis
M.	*Mycobacterium*
M. tbc.	*Mycobacterium tuberculosis*
NANAOCAM	*N*-alkyloxycarbonylaminomethyl
N-Cbz-AA	*N*-Benzyloxycarbonyl amino acids
OFX	Ofloxacin
PAS	*para*-Amino-salicylic acid
PD	Parkinson's disease
Phe	Phenylalanine
PTM	Prodrug mono-therapy
PZA	Pyrazinamide
k_{obs}	Rate constant
RMP	Rifampin
SAL	Salicylanilide
SI	Selectivity index
STM	Streptomycin
$t_{1/2}$	Half-time
TEA	Triethylamine
TB	Tuberculosis

UV/vis	Ultra violet/visible		
Val	Valine		
XDR-TB	Extensively	drug-resistant	tuberculosis

1. Introduction.

1.1. Tuberculosis.

Tuberculosis (TB) is an infectious bacterial disease caused by *Mycobacterium tuberculosis (M. tbc.)*, which most commonly affects the lungs but can also affect the central nervous system, the lymphatic system, the circulatory system, the genitourinary system, the gastrointestinal system, bones, joints, and even the skin.

1.1.1. World TB incidence.

Nearly 2 billion people are presently infected with *M. tbc*. Not everyone who is infected develops the active form; an asymptomatic latent form of TB is insidious. However, one in ten latent infectious will progress to the active TB disease which, if left untreated, will kill more than half of its victims. Every year around 9 million people develop the active and contagious pulmonary TB and 20% of these victims die of their infection, mostly in developing countries [1,2]. WHO estimates than 9.27 million new cases of TB occurred in 2007 (139 per 100,000 population), more than 4% of all the world-wide TB patients are resistant to at least one of the current first line drugs [3]. The recent worldwide emergence of drug-resistant TB, caused by poorly managed TB treatment is a growing problem of serious matter in many countries around the world, especially the increase of multidrug-resistant TB (MDR-TB) [4,5] and, most recently, extensively drug-resistant TB (XDR-TB) [6]. Every year almost 500,000 people are infected with MDR-TB and there are estimated 40,000 new cases of XDR-TB annually [7,8]. TB in co-occurrence with the spread of human immunodeficiency virus (HIV) infection [9] belongs amongst the most serious worldwide health threats. Among the 9.27 million incident cases of TB in 2007, an estimated 1.37 million (14.8%) were HIV-positive. Therefore, effective new drugs [10] and strategies [11] to treat the TB bacilli as well as its resistance pattern are an urgent demanding task.

1.1.2. TB treatment.

Modern TB chemotherapy started with the discovery of streptomycin (STM) in 1944. Two years later, *para*-amino-salicylic acid (PAS) was discovered. In 1952, two important front-line TB drugs, isoniazid (INH) and pyrazinamide (PZA) were introduced in the clinical treatment of TB. Ethambutol (EMB), a synthetic anti-TB, was discovered in 1961, followed by the introduction of rifampin (RIF) in 1967; both are effective TB drugs. An important observation in the early days of TB chemotherapy was that the use of a combination of two or more drugs greatly reduced emergence of drug resistance. Current treatment is made up of a cocktail of the first-line drugs (INH, RIF, PZA and EMB) for a period of six months [12] .

The principles of treatment for MDR-TB and for XDR-TB are the same. The main difference is that XDR-TB is associated with a much higher mortality rate than MDR-TB, because of a reduced number of effective treatment options [13]. Treatment courses are a minimum of 18 months and may last years, 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 [14].

The drugs currently used as anti-TB agents and new drug candidates and therapeutic targets for TB chemotherapy have been extensively described. See for example, Zhang et al. [15,16], Protopopova et al. [17].

2. Aim of the thesis.

Clearly there is a need for antibacterial agents with novel mechanism of action and thus salicylanilides (SAL) are promising candidates. The overall aim of this thesis was to investigate the most suitable prodrug forms based on this group that have shown interesting antimycobacterial activity against *M. tbc*. and some atypical strains, where INH is non-active. As a phenolic-containing drug, SAL are subjected to extensive first pass metabolism and, non-lipophilic character results in poor bio-membrane passage. Alternatively, the presence of a phenolic group should bring the possibility of a prodrug approach making possible the improvement of the pharmaceutical, pharmacokinetic and/or pharmacodynamic properties of the SAL. As the starting point for our research, the different strategies used in order to overcome the limited bioavailability of phenolic drugs were reviewed. Then, new potentially antibacterial active prodrugs of SAL, particularly active against *M. tbc*., MDR-TB strains or non-TB strains such as *M. avium* and *M. kansasii* were prepared to finally explore their physicochemical and pharmacokinetic properties.

The specific objectives of this study were:

- Literary review of prodrug design of phenolic-containing drugs (Paper I).
- Development methods for the synthesis of amino acid esters of salicylanilides and biological evaluation of the synthesized compounds (Paper II, III).
- Development methods for the synthesis of alkyl carbamate esters of salicylanilides and biological evaluation of the synthesized compounds (Paper V).
- Study of the unexpected formation of novel benzoxazepine seven-membered rings (Paper IV).
- Study of the rearrangement that leads to 2-Hydroxy-*N*-(1-(oxo-(phenylamino)-alkan-2-yl)benzamides (Paper VI).

3. Prodrug design of phenolic drugs (Paper I).

The phenolic functional group is one of the most common functional groups found in drug molecules such as narcotic agonists and antagonists, estrogens, neurotransmitters, anticancer agents or antibacterial agents, among others. However, phenolic drugs usually present limited bioavailability and thus limited effectivity.

Due to their poor delivery characteristics caused by their metabolic instability, they are often subjected to extensive first pass metabolism and in general, non-lipophilic character results in poor bio-membrane passage. Solubility limitations have been also frequently encountered in the development of commercial phenolic drugs. Alternatively, the presence of phenolic groups brings the possibility of prodrug approach which can result in effective, safe and efficient delivery of the parent drug to the systemic circulation and to the desire site of action. Several strategies have been used in order to overcome the limited bioavailability of phenolic drugs. Examples of ester, sulphate, carbamate, phosphate and ether prodrugs as well as the limitations of these prodrug strategies are reviewed in this chapter.

3.1. Introduction.

The principal mechanism used by the human body to eliminate low molecular weight environmental pollutants is the use of non-specific enzymes, which transform the extraneous compounds into polar molecules, excreted by natural bodily processes. Despite the fact that this mechanism can be highly preferable to rid the body of xenobiotics, it can represent a problem when the foreign agent is a drug that should enter and be retained in the body enough time to be effective. The enzymatic biotransformation of drugs is known as drug metabolism. When a drug is taken orally, it is usually absorbed through the membrane of the small intestine or from the stomach. Then it is carried by the bloodstream to the liver where it is often first metabolized. The complete deactivation of the drug may result if metabolism by liver enzymes occurs before the drug reaches the systemic circulation (presystemic or first-pass effect). Two undesirable effects of drug metabolism are, on one hand the larger or multiple doses of the drug that will be required to accomplish the desired effect and on the other hand the possible toxic metabolites originated in these processes, even though the parent drug will be not toxic. The first-pass effect can be avoided either changing the route of drug administration or via structural modifications approaches in drug design. Once the drug has reached its site of action and achieved the desired objective, should be metabolized and eliminated at a reasonable rate, otherwise, it can stay in the body and produce a longer effect

than desired or accumulate and became toxic for the cells [18]. Drug metabolism reactions are divided into two general categories, named phase I and phase II reactions. Phase I transformations involve reactions that introduce or unmask a functional group, such as oxygenation or hydrolysis. Phase II transformations mostly generate highly polar derivatives, such as glucuronides and sulphate esters, for excretion in the urine.

Phenolic drugs are often subjected to phase II metabolism [18,19]. This phase II modification is responsible for deactivation of drugs, can change their physicochemical properties and produce water-soluble metabolites that are excreted in the urine or bile. Therefore, phenolic drugs show low bioavailability after oral administration and thus limited effectivity. The inactivation of these drugs in the gut and/or liver is due to glucuronidation, sulphatation or methylation. Glucuronic acid conjugation is the most common mammalian conjugation pathway. Four general classes of glucuronides have been established, the *O*-, *N*-, *S*- and *C*-glucuronides. Sulphate conjugation occurs less frequently than glucuronidation. The main substrates involved in sulphatation are phenols but alcohols, amines and to a much lesser extent, thiols are also sulphated. It tends to predominate at low doses, when there is less to diffuse into membranes and with smaller, less lipid-soluble molecules. Methyl conjugation is relatively minor conjugation pathway in drug metabolism; in general, xenobiotics that undergo methylation do share marked structural similarities to the endogenous substrates that are methylated.

Alternatively, the presence of phenolic groups brings the possibility of prodrug approach [20,21] making possible the improvement of the pharmaceutical, pharmacokinetic and/or pharmacodynamic properties of the parent drug.

One of the most important pharmacokinetic characteristic of a new drug candidate is its oral bioavailability [22,23]. As it was mentioned above, phenolic drugs have often limited systemic bioavailability due to extensive presystemic metabolism. In this context, one strategy would be to administrate the drug orally as a prodrug able to pass intact through the potential sites of metabolism, converting to the parent drug in an organ other than the intestine or the liver.

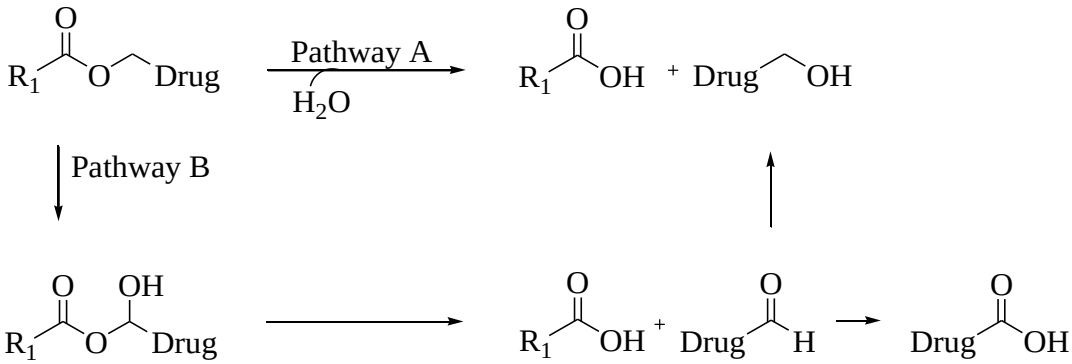
Another prodrug strategy would be to temporally mask polar groups resulting in an increased lipophilic molecule, which would promote membrane permeability. Esterification of the hydroxyl group(s) has been one of the preferred prodrug strategies for this purpose. As a case in point, consider lipophilic prodrugs designed for intestinal lymphatic transport [24], prodrugs for improved the central nervous system (CNS) delivery [25] or, among other promising nonoral, nonparentenal routes, prodrugs for nasal delivery [26].

Cancer chemotherapy is closely associated with serious side effects due to the high toxicity of the used drugs. In order to minimize systemic toxicity of the parent drug, a number of prodrugs of phenolic group-containing have been targeted toward molecular receptors on tumour cells for in situ activation. The different prodrugs strategies in cancer therapy have been examined extensively in the literature [27,28,29,30,31].

Herein, we offer a summary of the various prodrug strategies design to modify the phenolic group, focusing on the physicochemical properties and pharmacokinetics of the representative compounds, showing the biochemical pathways involved in prodrug activation and illustrating these with relevant examples.

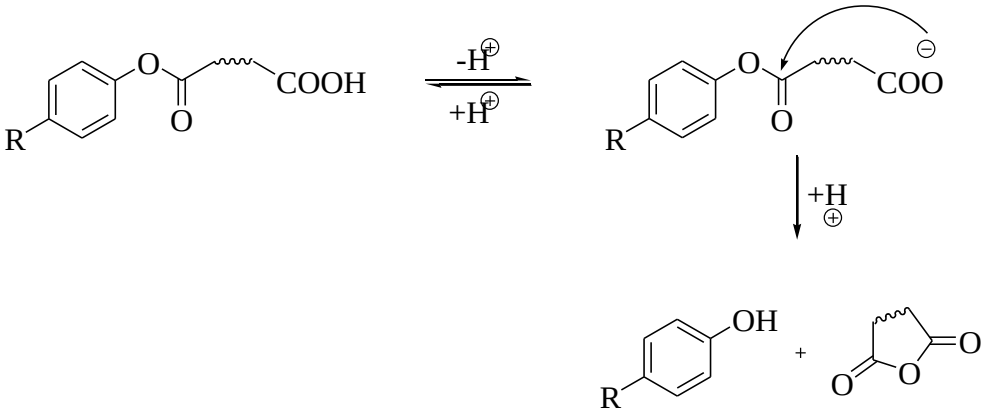
3.2. Ester Prodrugs of Phenols.

The aim of the ester prodrug approach is to mask polar ionisable groups (phenolic, hydroxyl or carboxylic group) within a molecule, enhancing the aqueous solubility and stability of the compound [32] to provide a sustained-release derivative of the parent drug or increasing its lipophilicity [33] to promote permeability and oral absorption. When the ester bond of the prodrug is cleaved, the active drug is released. This cleavage usually occurs through hydrolysis or oxidation [34]. The hydrolysis of the prodrug is usually catalyzed by different esterases, including carboxylesterase, acetylcholinesterase, butyrylcholinesterase, paraoxonase and arylesterase. The oxidation of ester-based prodrugs is catalyzed by cytochrome P450s (**Scheme 1**).



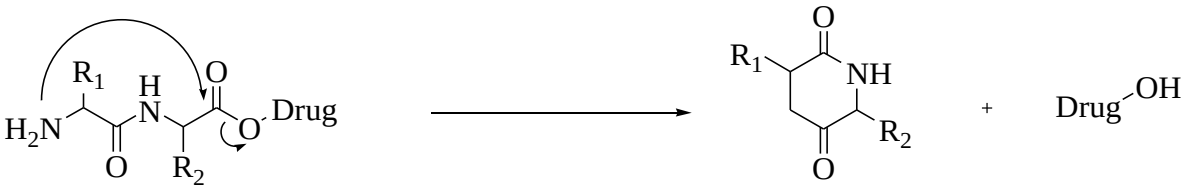
Scheme 1. Pathways for ester bond cleavage. Pathway A: Esterases or proteins (HAS, carboxypeptidase A, aldehyde dehydrogenase, carbonic anhydrase B and C, lipases) having esterase activity. Pathway B: Cytochrome P450s [34].

As an alternative approach, the parent drug can be regenerated through non-enzymatic pathways, particularly via cyclization-elimination strategies [35,36]. Begtrup and co-workers [37] developed hemiesters of aliphatic dicarboxylic acids as cyclization-activated prodrugs of phenols that were released after the attack of the carboxylate on the ester bond (**Scheme 2**).



Scheme 2. Carboxylate nucleophiles in the intramolecular activation of phenol prodrugs [36].

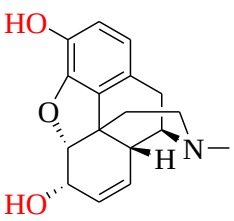
Another example of non-enzymatic pathway was presented by Gomes and co-workers [38], they have considered that dipeptides might play a crucial role as carriers for hydroxyl-containing drugs. Dipeptide esters and amides can deliver the parent drug through an intramolecular cyclization to form the corresponding piperazine-2,5-diones (DKP) (**Scheme 3**) [35,36].



Scheme 3. Prodrug intramolecular activation via DKP formation [36].

The bioconversion to the active drug determines its utility as a prodrug. The rate of biotransformation is particularly important due to its role for the onset, duration and intensity of the drug action. Many factors may contribute to the prodrug conversion rate as genetic differences, environmental effects and disease states. Although some of these factors, such as structural aspects of prodrugs, can be optimized in drug discovery, others are intrinsically related to individuals, adding complexity of the attempt to predict drug stability.

After oral administration, morphine (**1**) (**Figure 1**) is almost completely absorbed from the gastro-intestinal tract, however, due to extensive first pass metabolism the systemic bioavailability is low and variable (19-47%) [39]. Following parenteral administration side-effects may occur as a consequence of high pick plasma levels. Bioavailability of morphine after buccal and sublingual administration is as well quite limited, apparently cause of its poor lipophilicity and polar properties at physiological pH. A prodrug approach to enhance penetration of morphine through the buccal mucosa was achieved by esterification of its 3-phenolic group [40]. All the morphine esters studied underwent enzymatic hydrolysis, the acetoyl prodrug (**2**) (**Figure 1**) was found to be the most stable derivative in both plasma and saliva. The ester prodrugs tested were able to permeate porcine buccal mucosa, whereas the parent drug **1** was not at any measurable extent. Even if permeation of **1** can be markedly improved by using ester prodrugs with higher lipophilicity, the enzymatic stability of the prodrugs in saliva do also play an important role for the overall improvement in absorption properties. Similar approach has been used to improve transdermal penetration where various alkyl esters formed at the 3- and/or 6-hydroxy group in **1** were prepared, showing higher penetrating capacity due to higher water and lipid solubility than the parent drug **1** [41]. The hexanoyl prodrugs (**3** and **4**) (**Figure 1**) delivered only **1** while propionyl prodrug (**5**) delivered 50:50 intact prodrug:morphine. This correlates with other reports [42] where it is demonstrated that the medium alkyl chain length homologues are more completely hydrolyzed to the parent drug than the shorter or longer alkyl chain ones.



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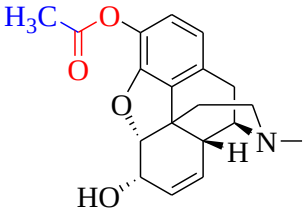

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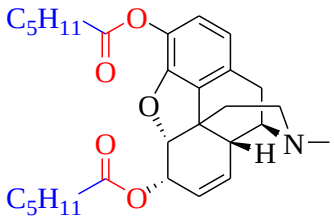

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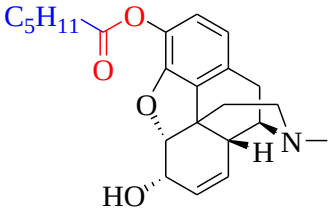

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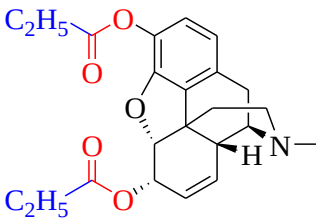

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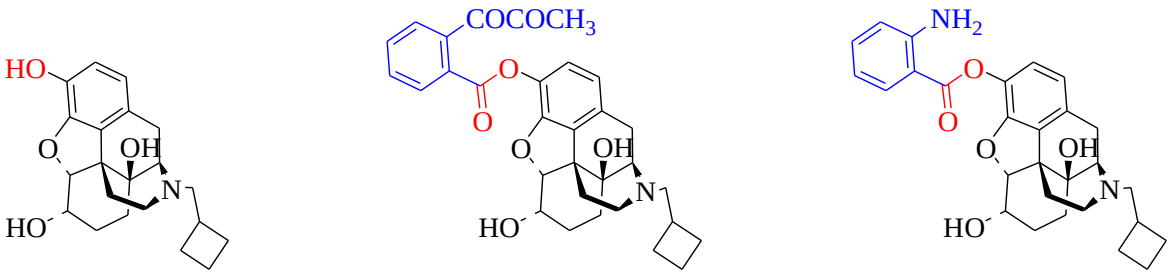
Figure 1. Morphine (**1**) and its ester prodrugs (**2**, **3**, **4**, **5**).

Nalbuphine (**6**) (**Figure 2**) is a synthetic opioid used commercially as an analgesic that present incomplete oral bioavailability in animals and humans attributable to extensive presystemic metabolism via conjugation on the phenolic hydroxyl group. Two prodrugs were presented [43] in preclinical studies as candidates with improved oral bioavailability, the acetylsalicylate (**7**) and anthranilate (**8**) esters (**Figure 2**). The increment of bioavailability after oral administration in dogs was demonstrated, from 5-7% for **6** to approximately17-24% for **7** or 50% for **8**. Furthermore, the plasma concentrations of conjugated **6** were reduced after dosing with the prodrugs, elucidating that first-pass metabolism was reduced.

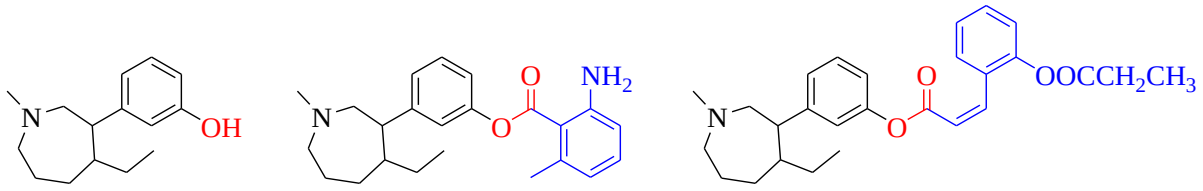


6 **7** **8**

Figure 2. Nalbuphine (**6**) and its ester prodrugs (**7**, **8**).

Contrary to other typical opiates such as **1** (**Figure 1**), meptazinol (**9**) (**Figure 3**) accompanies less respiratory depression and addictive potential. Nevertheless, **9** displayed poor oral bioavailability (8.96%) [44] because of serious first-pass effect in liver similar to the opiate described above. Qiu and co-workers [45] reported the synthesis of three benzoyl esters of **9** with the aim to protect this drug from enzyme metabolism and enhance hydrophobicity as well as to improve its pharmacokinetic properties. 2-Amino-6-methylbenzoyl prodrug (**10**) (**Figure 3**) showed significant increase in looped duodenum, jenunum and colon absorption than **9** which correlated well with its enhanced bioavailability. Notice here that the carbonyl group is surrounded by both the 2´-amino and the 6´-methyl group which could thusly protect the ester bond from hydrolysis. As part of a continuing effort to develop novel **9** prodrugs, a coumarin-based esterase-sensitive prodrug approach was reported [46]. Its application for the preparation of prodrugs of amines and cyclic prodrugs of peptides has been discussed extensively in the literature [47,48,49,50,51].The major advantage of the prodrug system is that hydrolysate coumarin seems to be relatively non-toxic. The propionyloxy group-

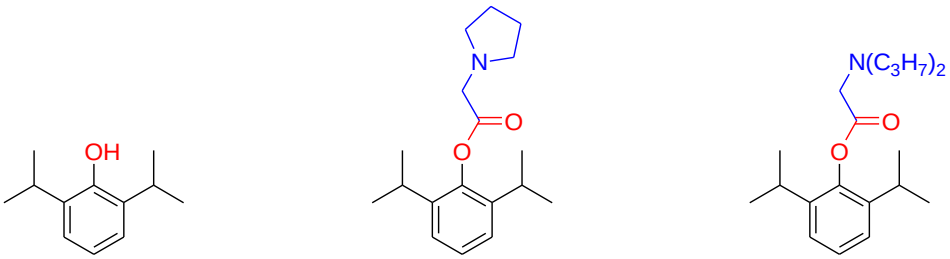
substituted phenyl propenoic acid was chosen as carrier molecule. 3-(2-(prop-1-en-2-ylperoxy)phenyl)acryloyl prodrug (**11**) (**Figure 3**) showed a superior oral bioavailability to the earlier reported benzoyl esters [45]. These results encourage the use of coumaring-based esterase-sensitive system as a prodrug template for other drug molecules besides **9**.



9 **10** **11**

Figure 3. Meptazinol (**9**) and its ester prodrugs (**10**, **11**).

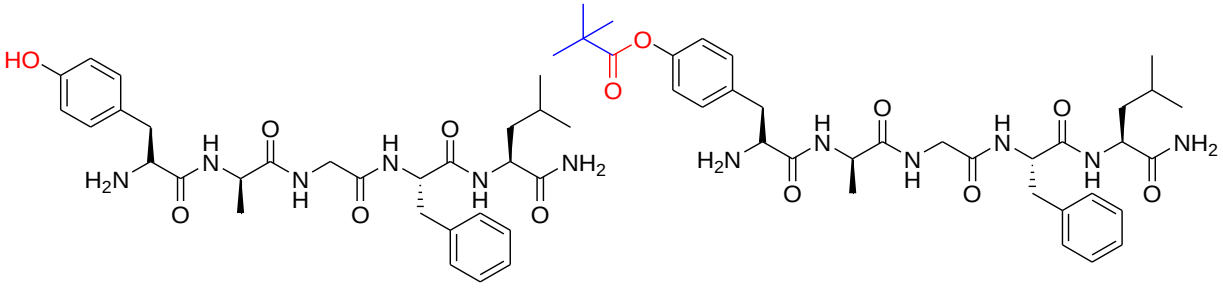
Propofol (**12**) (**Figure 4**) is an intravenous (*i.v.*) agent with very low aqueous solubility. At present, the anaesthetic agent is formulated as 1% w/v of oil/water emulsion of soya bean oil, glycerol and purified egg phosphatide (Diprivan®). Unfortunately, these emulsions present several limitations including poor physical stability, potential for embolism and pain on injection [52]. In order to increase the aqueous solubility and to reduce side effects as well as to prolong the drug action, several prodrug strategies have been described. *N*,*N*-disubstituted-α-amino acid esters were synthesized [53] and their physicochemical properties were studied. Among them, the pyrrolidinoacetyl ester (**13**) (**Figure 4**) showed half maximal inhibitory concentration (IC_{50}) value comparable to **12** as well as the highest solubility in aqueous solution, however, after the study of enzymatic stability, it was found that these candidates can not be considered as prodrugs since they are not readily converted to the parent drug in physiological conditions. In a later study, cyclic amino acid esters of **12** were synthesized [54]. L-proline ester of propofol (**14**) (**Figure 4**) was found to protect animals against pentylentetrazol-convulsions and to induce in a shorter time an anaesthetic action with comparable duration as marketed emulsion Diprivan®. Its high solubility and stability in water at physiological pH would aloud freeze-dried formulations to be used for parenteral administration. Its susceptibility to enzymatic cleavage by esters hydrolases in plasma and liver should afford a rapid conversion to the parent drug. Finally, the comparable IC_{50} values with **12** showed that **14** is a propofol prodrug candidate for developing formulations useful for *i.v.* administration.



12 **13** **14**

Figure 4. Propofol (**12**) and its ester prodrugs (**13**, **14**).

Application of peptides as clinically useful drugs represent an important task due to their poor delivery characteristics caused by their metabolic instability and in general a non-lipophilic character resulting in poor membrane absorption. This leads to bioavailabilities less than 1-2% [55]. Clinical development of drug candidates that are peptide based has therefore been restricted. In the recent past, the prodrug approach has been used in order to improve bioavailability of peptides [56,57,58]. With this purpose, study of ester prodrugs of the tyrosine phenolic group of a Leu-enkephalin analogue (**15**) (**Figure 5**) was reported [59]. This analogue already shows favourable properties in comparison with Leu-enkephalin. The substitution of Gly^2 with $D\text{-}Ala^2$ significantly decreases the enzymatic hydrolysis of the Tyr^1-Gly^2. Amidation of the C-terminal offers protection against degradation by carboxypeptidases [60,61]. The most favourable prodrug candidate in this study was the pivaloyl ester of Leu-enkephalin analogue (**16**) (**Figure 5**), showing 18 times higher transport compare to the analogue itself, what suggests that more sterically hindered prodrugs improve stability and thereby penetration through the intestine and blood-brain barrier (BBB).



15 **16**

Figure 5. Leu-enkephalin analogue (**15**) and its ester prodrug (**16**).

The extensive first-pass metabolism of paracetamol (**17**) "Fig. (**6**)" can lead to serious hepatic and renal toxic effects [62]. Esterification of **17** with some non-steroidal anti-inflammatory

carboxylic acids was reported as mutual prodrug in order to prevent the severe hepatotoxicity of the drug at high doses [63]. According to this study, 2-(4-isobutylphenyl)propanoyl ester of paracetamol (**18**) (**Figure 6**) was found to be more bioavailable orally than the parent drugs in physical mixture due to improved lipophilicity; furthermore, ulcerogenic liability was reduced providing the corresponding improvement in the therapeutic index (TI) of the parent drugs.

In a recent study in prodrug design, dipeptide esters of **17** were reported [38]. In contrast to the previous study, where mutual prodrugs were found to be susceptible to enzymatic hydrolysis; the delivery of the parent drug is, in this case, through enzyme-independent processes such as an intramolecular cyclization to form the corresponding DKP [64]. Compounds **19** (Phe-Gly-trifluoroacetate), **20** (Gly-Val-trifluoraoacetate), **21** (Gly-Phe-trifluoroacetate) (**Figure 6**) were proved to efficiently reduce hepatotoxicity of **17**. However, the selection of the dipeptide carrier must consider the potential side effects of the corresponding DKP formed during the cyclization.

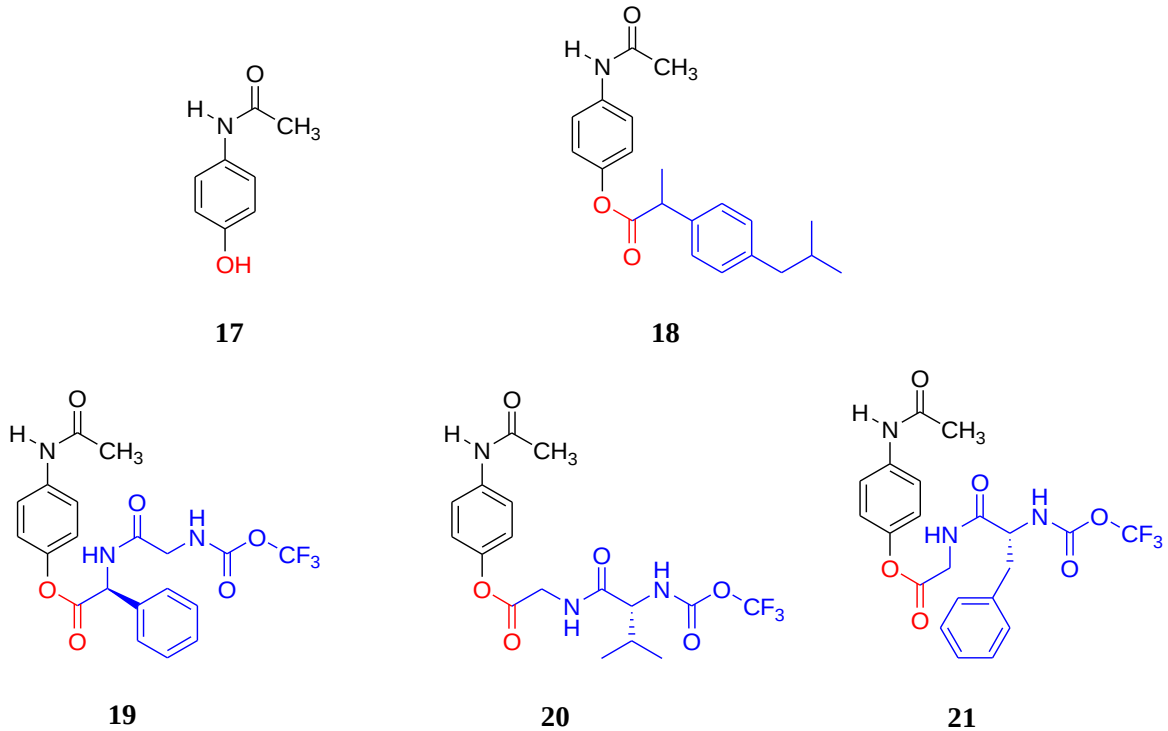
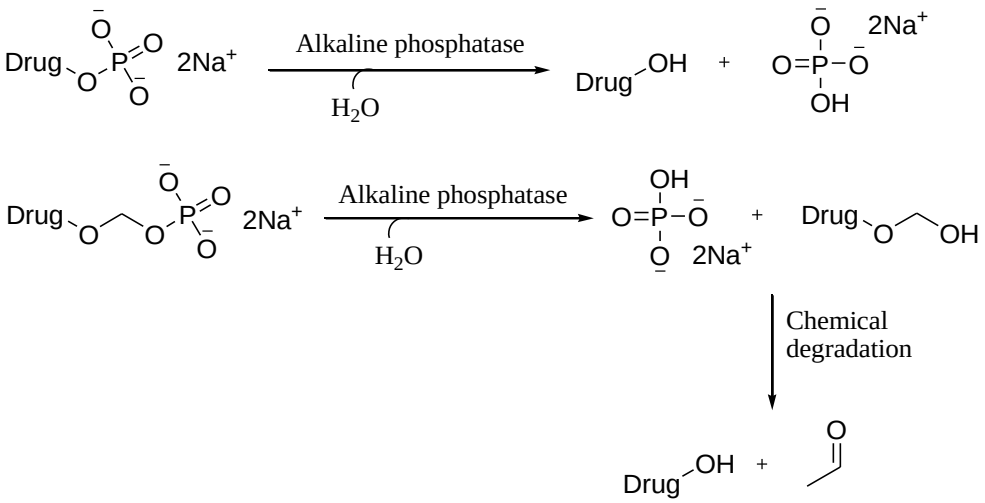


Figure 6. Paracetamol (**17**) and its ester prodrugs (**18**, **19**, **20**, **21**).

3.3. Phosphate Ester Prodrugs of Phenols

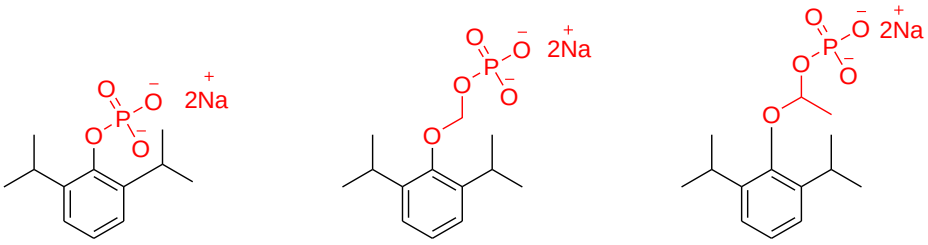
Phosphate ester prodrug approach has overcome numerous drug delivery problems of sparingly water soluble potential drugs. The phosphate moiety can be directly attached to the parent drug to form a phosphomonoester which undergoes an alkaline phosphatase-catalyzed bioconversion *in vivo* to release the parent phenol drug and inorganic phosphate, or via a chemical linker such a oxymethyl spacers, usually used to increase the space around the enzymatically cleavage bond [65]. These prodrugs undergo rapid spontaneous chemical hydrolysis most notably by endothelial cell surface alkaline phosphatases liberating the parent drug, phosphate and formaldehyde (**Scheme 4**). This enzyme is responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids and cleaves the phosphomonoester at the phosphorous-oxygen bond with high catalytic efficiency [66].



Scheme 4. Pathway for phosphate ester bond cleavage.

We have previously shown how the anaesthetic drug **12** (**Figure 4**) has lead to the development of more water-soluble prodrugs for *i.v.* administration [53,54]. For this purpose, at least three phosphate prodrugs of **12** have been described in the literature. Firstly, one with the phosphate group attached directly to the phenolic functionality of **12**, named PP (**22**) (**Figure 7**) [67], well defined water-soluble prodrug of **12** that is enzymatically converted to the parent drug and non-toxic inorganic phosphate. In contrast with **12**, **22** is formulated on water as a sodium salt (3.1% wt/vol; pH 7.4) and sterilized by filtration (0.2 μm) and then is ready for *i.v.* administration. Although the **22** hypnotic dose (HD_{50}) was found to be around

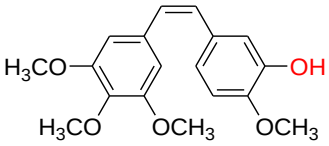
10 times larger than the HD_{50} values reported for **12**, the PP lethal dose (LD_{50}) was as well bigger in mice, rats and pigs; this implies lower hypnotic activity and lower toxicity so it would be possible to applied larger doses of **22** to achieve the same level of sedation as with **12**. The second prodrug of **12** presented was one with an oxymethyl spacer between the parent drug and the phosphate promoiety, named GPI 15715 (Aquavan®) (**23**) (**Figure 7**) [68]. The enzymatic hydrolysis of **23** is faster than that of **22**, however **22** does not liberate the toxic formaldehyde, which in addition to its toxicity may alter homeostasis within cells. Finally, it was presented one with an ethyl dioxy-spacer between **12** and the phosphate promoiety that liberates the much less toxic compound, acetaldehyde, from the prodrug structure [69]. Ethyl dioxy phosphate of propofol (**24**) (**Figure 7**) greatly enhanced the aqueous solubility of **12**, being sufficient for *i.v.* administration. Furthermore, **24** was bio-converted to **12** as efficiently as **23** and much faster and more effective than **22**. Nevertheless, larger doses would be needed to achieve complete sedation. This effect is similar to that reported earlier in previous cases.



22 **23** **24**

Figure 7. Propofol phosphate ester prodrugs (**22**, **23**, **24**).

Cobretastatins are natural compounds isolated from the Combretum caffrum willow tree in South Africa. One of them CA-4 (**25**) (**Figure 8**), named as tubulin-binding agent, can induce vascular damage and subsequent hemorrhagic necrosis in tumours. This agent causes the destabilization of the tubulin polymers of the cytoskeleton [70]. It was shown that a more soluble prodrug of **25**, CA-4-P (**26**) (**Figure 8**) [71,72], which can now be delivered systematically, can cause vascular shut-down in the murine adenocarcinoma NT and the human breast carcinoma xenografts MDA-MB-231. This compound is cleaved to the active **25** by endogenous non specific phosphatases and its effects can be achieved using relatively non-toxic doses. Further investigations showed that the vascular-damaging effect of **26** were larger in tumour tissue than in any of the normal tissues investigated [73]. **26** is currently in Phase I/II clinical trials.



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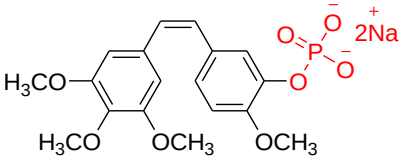

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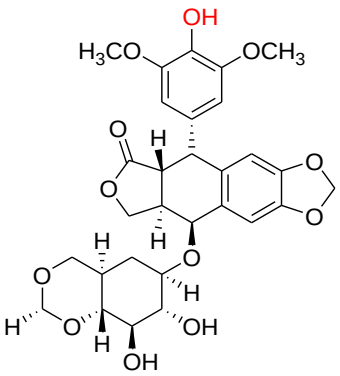
Figure 8. Cobretastatin CA-4 (**25**) and its phosphate ester prodrug (**26**).

Etoposide (**27**) (**Figure 9**) is a semi-synthetic derivative of podophyllotoxin that is given as a treatment for some kinds of cancers. Possessing potent antineoplastic properties, binds to and inhibits topoisomerase II and its function in ligating cleaved DNA molecules, resulting in the accumulation of single- or double-strand DNA breaks, the inhibition of DNA replication and transcription and apoptotic cell death. The equilibrium aqueous solubility of **27** is low, 0.15-0.7 mg/mL, making necessary the use of organic solvents and big volumes of saline for *i.v.* administration, therefore, a phosphate prodrug of **27** was developed [74]. Etoposide phosphate (Etopophos®) (**28**) (**Figure 9**) is water soluble (20mg/mL) and is available for *i.v.* infusion as a sterile lyophile in single-dose vials containing **28** equivalent to 100 mg of **27**, 32.7mg sodium citrate USP, and 300 mg dextran 40. **28** quantitatively converts to the parent drug after *i.v* administration in humans and has equivalent TI and toxicity than **27** [75,76]. **28** is actually used for the treatment of small cell lung cancer and testicular carcinoma.



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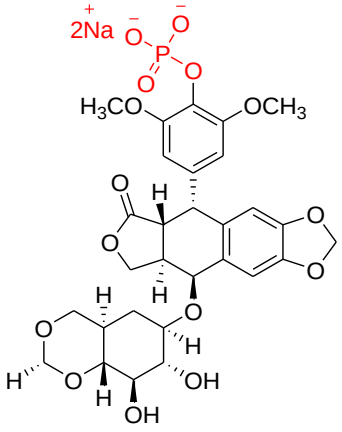

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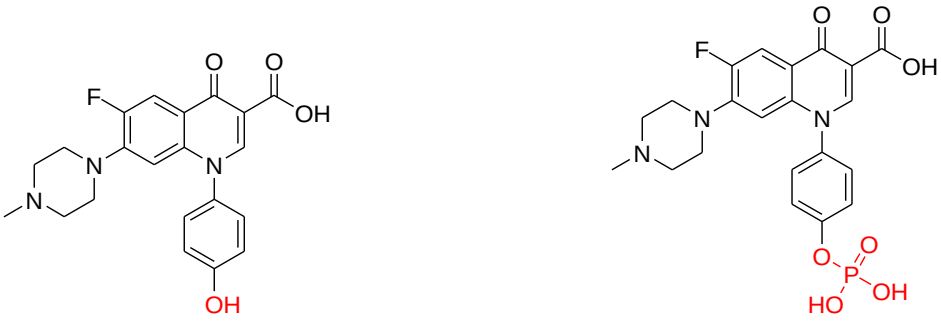
Figure 9. Etoposide (**27**) and its phosphate ester prodrug (**28**).

The fluoroquinolone PA2789 (**29**) (**Figure 10**) is an effective antibacterial agent for lung infections; however, it was later determined to have poor oral bioavailability. The low oral

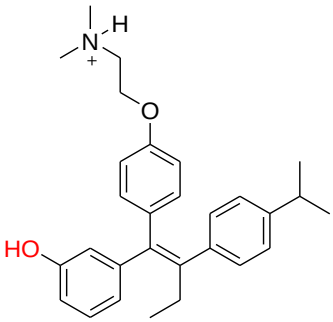
bioavailability of **29** precludes high systemic levels of **29** during aerosol delivery, confining the drug to pulmonary tissue. Although this property appears to be desirable, as the goal is to deliver active compound to the lungs and minimize systemic exposure from any compound entering the stomach during inhalation, poor water solubility of this drug restrains its use as an aerosol. With the aim of improving the water solubility of **29**, the prodrug PA2808 (**30**) (**Figure 10**) which contains a phosphate group attached to the phenolic group at the *N*-1 position, was published [77]. **30** presented increased solubility achieve at pHs that are physiologically acceptable (pH range 4.5-8.7, out of this range inhaled aerosols can cause coughing or bronchoconstriction) [78], displayed excellent activity against the organism tested and was converted into **29** after phosphate cleavage by alkaline phosphatase.



29 **30**

Figure 10. Fluoroquinolone PA2789 (**29**) and its phosphate ester prodrug (**30**).

DP-TAT-59 (**31**) (**Figure 11**) is a practically insoluble analogue of tamoxifen, an antagonist of the estrogen receptor in breast tissue, therefore used in the treatment of breast cancer. In order to increase the solubility of **31**, a phosphate prodrug strategy was developed. Miproxifene phosphate (TAT-59) (**32**) (**Figure 11**) was synthesized [79,80] and actually is in phase II-III trials. Surprisingly **32** showed unusual low solubility comparing to most phosphate esters freely soluble in water [81,82]. Despite the relatively low solubility of **32**, its solubility and dissolution rate were much higher than those of the parent drug, making succeed the prodrug approach. The dephosphorylation *in vivo* of **32** was rather quick as it was shown in pharmacokinetics studies. The bioavailability of **31** after oral **32** dosing was 28.8% in rats and 23.8% in dogs. Furthermore, no intact prodrug was detected in plasma of either species. However, no bioavailability data have been published for humans.



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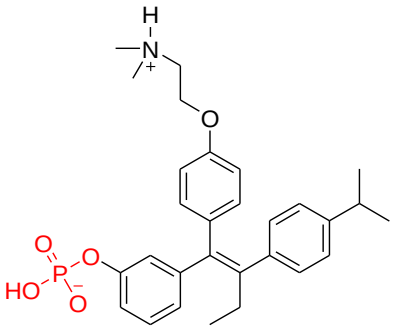

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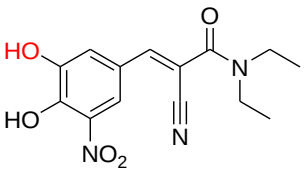
Figure 11. Tamoxifen analogue DP-TAT-59 (**31**) and its phosphate prodrug (**32**).

Entacapone (Comtan®) (**33**) (**Figure 12**) is used in the treatment of Parkinson's disease (PD) and works as a catechol-O-methyl transferase inhibitor. It is used as a support in the levodopa/carbidopa (Sinemet®) therapy [83]. Common treatment of PD involves oral administration of a combination of levodopa, a precursor of dopamine that can cross the BBB and carbidopa, an inhibitor of dopa decarboxylase, which inhibits decarboxylation of levodopa in the periphery. **33** presents a low oral bioavailability (~25-35%). Although the factors contributing to its poor bioavailability are not resolved, it was suggested that might be caused by its low aqueous solubility and slow dissolution at the pH range of the stomach and low intestine [84]. With the aim of increasing the solubility and dissolution rate, a phosphate ester of entacapone was presented [85]. Phosphate ester of entacapone (**34**) (**Figure 12**) notably increased the aqueous solubility of the parent drug (over 30 mg/mL at both pH 1.2 and 7.4 in contrast with that of **33**, 0.017 mg/mL at pH 1.2 and 1.75 mg/mL at pH 7.4 and 37 °C) and showed high stability in both phosphate buffer and human serum, realising quantitatively the parent drug in liver homogenate. However, the prodrug strategy failed as the prodrug did not yield higher **33** plasma levels in rats [82], which may be result of **33**'s low intestinal permeability.



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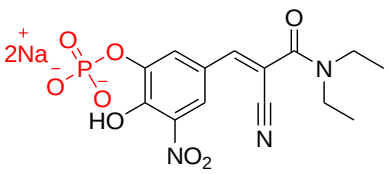

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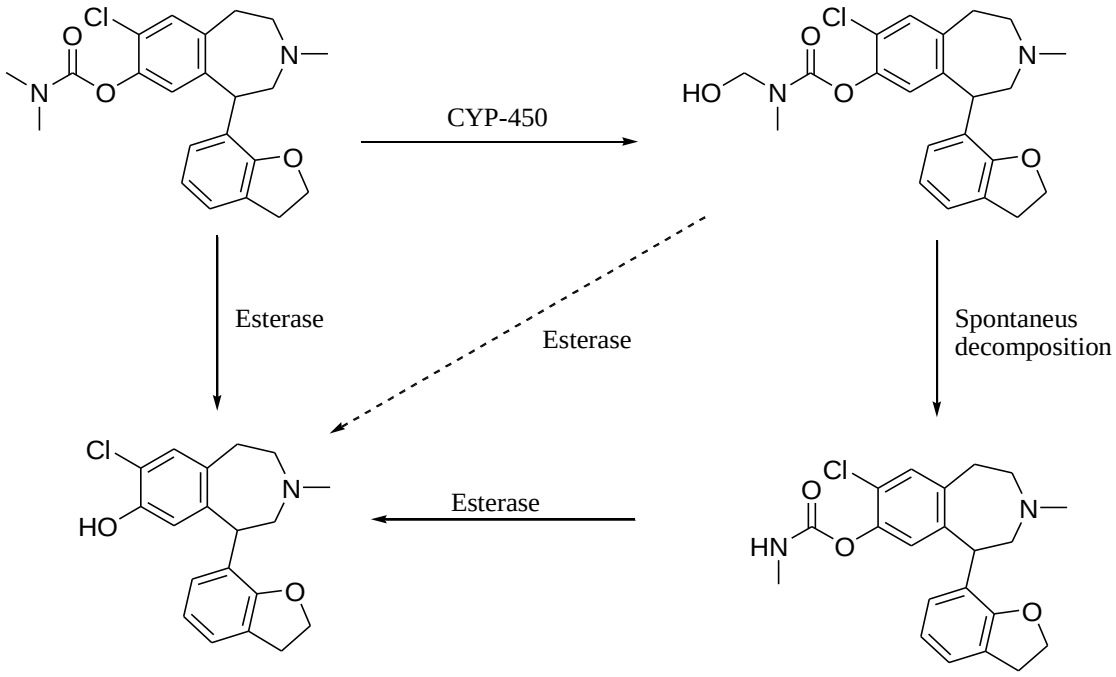
Figure 12. Entacapone (Comtan®) (**33**) and its phosphate ester prodrug (**34**).

These results suggest the influence of the intestinal permeability of the parent drug in the success of a phosphate prodrug strategy.

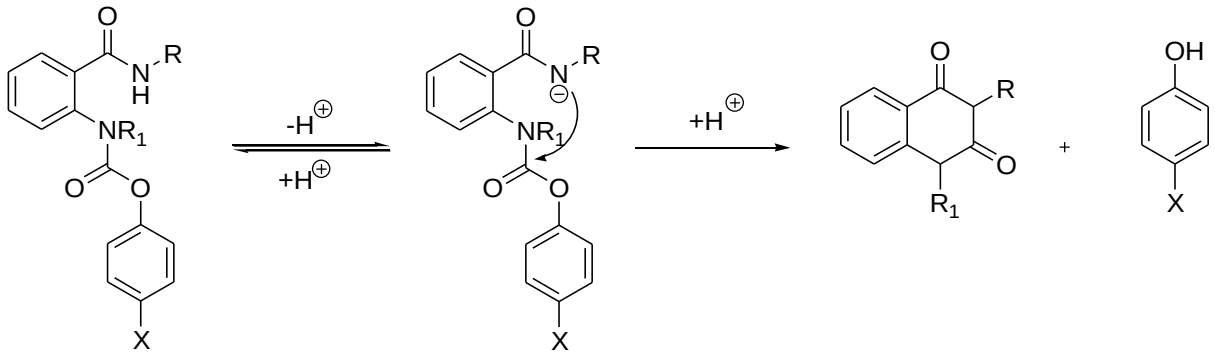
3.4. Carbamate Ester Prodrugs of Phenols.

Carbamate ester prodrugs are usually designed with the aim of either protecting the parent phenols against first-pass metabolism following oral administration or as substrates of specific enzymes. After hydrolysis, these carbamate esters release the parent drug and carbamic acid which is chemically unstable, undergoing to decomposition to amine and CO_2. Hansen and co-workers [86] suggested the bioconversion of the prodrugs of two dopaminergic 7-hydroxy[3]benzazepines via direct carboxylesterase-mediated hydrolysis and cytochrome P450-catalyzed hydroxylation to give an *N*-hydroxymethyl derivative that spontaneously decomposes to the *N*-monomethylcarbamate (**Scheme 5**).



Scheme 5. Pathways for carbamate ester bond cleavage [87].

Carbamate prodrugs may as well undergo conversion to their parent drug via a non-enzymatic/chemical process. Thomsen and Bundgaard [88] were pioneers in this approach, through the preparation and study of cyclization-activated phenyl carbamate prodrugs of phenols for protecting them against first-pass metabolism (**Scheme 6**).



Scheme 6. Intramolecular activation of phenyl carbamate prodrugs of phenols [36].

However, the selection of non-enzymatically activated strategies must consider the competing enzymatic reactions interfering with the intramolecular cyclization process as well as the adverse effects in absorption of the prodrug through biological membranes due to the increase in molecular weigh.

The limited effectivity after oral administration of a class of dopaminergic drugs with potential antipsychotic effect, 5-substituted 8-chloro-7-hydroxy-3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepines, due to glucuronidation of the 7-phenolic group, makes them good candidates for prodrug approach. Hansen and co-workers [86] presented various carbamic acid esters of this class of drugs. Particularly, carbamate ester prodrug approach was applied to 8-chloro-5-(2,3-dihydrobenzofuran-7-yl)-3-methyl-2,3,4,5-tetrahydro-1H-benzo[d]azepin-7-ol (**35**) (**Figure 13**) which shows a bioavailability of only ~5%. In this study it was found that *N*,*N*-dimethylcarbamate prodrug (**36**) (**Figure 13**) may be a potentially useful prodrug for protection against first-pass metabolism. The enzymatic bioconversion of **36** (**Scheme 5**) results in almost quantitative recovery of the parent drug and later studies in dogs [89] confirmed that the oral administration of prodrug **36** significantly improves the bioavailability to approximately 20%.

35 **36**

Figure 13. 8-chloro-5-(2,3-dihydrobenzofuran-7-yl)-3-methyl-2,3,4,5-tetrahydro-1H-benzo-[d]azepin-7-ol (**35**) and its carbamate ester prodrug (**36**).

As it was shown before, **33** (**Figure 12**) presents a low bioavailability after oral administration (25-35%). Although the reason of its poor bioavailability is unknown, it was suggested that it may be the acidic character of **33** (pKa 4.5) which makes it ionise at the pH of small intestine [84]. As is well known, molecules with an ionisable group are better absorbed through the intestinal wall when they are present in un-ionised form. On the other hand, low aqueous solubility at low pH may obstruct its absorption from the stomach. With the aim to enhance its poor bioavailability, Savolainen and co-workers [90] presented various alkyl carbamate esters as potential prodrugs of **33**. Carbamates of **33** were prepared to increase the lipophilicity of **33** at neutral pH and to increase the aqueous solubility at an acidic pH. The *N*-*terc*-butyl carbamate ester (**37**) (**Figure 14**) presented higher aqueous solubility at pH 5 as well as higher apparent partition coefficient at pH 5 and 7.4 compared to **33**. However, the low oral bioavailability of **33** was not improved via this strategy. This result agrees with those obtained previously [82], where it was concluded that **33** absorption might be controlled by permeability.

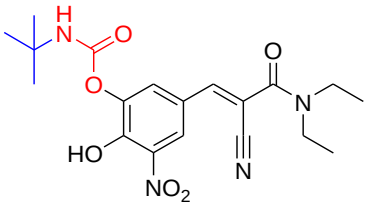


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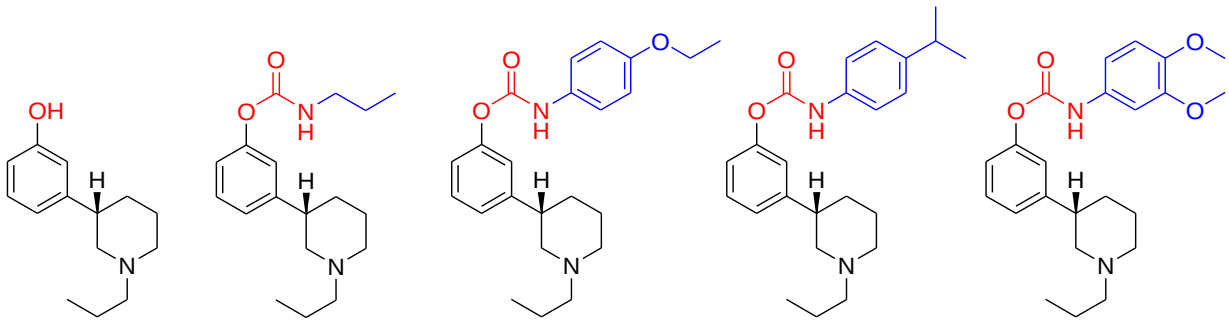
Figure 14. Encacapone carbamate ester prodrug (**37**).

The presynaptic dopamine autoreceptor agonist (-)-3-(hydroxyphenyl)-*N*-propylpiperidine[(-)-3-PPP] (**38**) (**Figure 15**) was developed as an alternative antipsychotic agent in humans [91]. Following oral administration, **38** was found to have a low bioavailability due to first-pass metabolism in the intestinal mucosa and /or in the liver. In order to escape the first-pass metabolism in the hepatoportal system and generate high plasma and tissue levels of the parent drug, a series of alkyl and arylcarbamate ester derivatives was reported [92]. Although the alkylcarbamate ester **39** was found to be one of the best prodrug candidates with regard to high plasma levels of the parent drug, it was as well found that causes convulsions in rats. Prodrugs based on aryl carbamate derivates of **38** were proven to achieve the goal of this study. The nature of the substituents in the phenyl ring of the aromatic carbamate derivatives seemed to play an important role on the levels of **38** in plasma. As a case in point, more

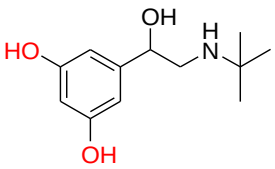
electron donating substituents as in compounds **40** (4-ethoxyphenylcarbamoyl), **41** (4-isopropylphenylcarbamoyl) or **42** (3,4-dimethoxyphenylcarbamoyl) (**Figure 15**), strongly enhanced the levels of **38** in plasma.



38 **39** **40** **41** **42**

Figure 15. 3-(Hydroxyphenyl)-*N*-propylpiperidine [(-)-3-PPP] (**38**) and its carbamate ester prodrugs (**39**, **40**, **41**, **42**).

Terbutaline (**43**) (**Figure 16**) is a β_2-adrenergic receptor agonist, used as a fast-acting bronchodilator (often used as a short-term asthma treatment) and as a tocolytic to delay premature labour. Due to its high hydrophilicity, oral absorption varies between 25% and 80% of the dose, furthermore, undergoes extensive first-pass sulphate conjugation (69%), causing low bioavailability (7% to 26%) [93]. Attempts to increase first-pass hydrolytic stability, it would be worthwhile to use a carbamate function to protect the phenolic resorcinol hydroxyl groups in **43**, since the carbamate group is well known moiety which has been used to achieve cholinesterase-inhibiting properties of various compounds. Bambuterol (Bambec®) (**44**) (**Figure 16**) was prepared to examine whether the desired properties could be obtained [94,95]. **44** was found to be a selective cholinesterase inhibitor, resulting in a controlled rate of hydrolysis. Furthermore, the dimethylcarbamate moieties resulted to be targets for the mixed-function oxidases in the liver, where new carbamate prodrugs of **43** are formed by oxidative metabolism. In this respect, **44** may work as a prodrug-prodrug. Finally, **44** and its prodrug metabolites seem to facilitate delivery of **43** to the target tissue, the lung.



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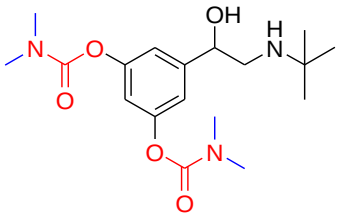

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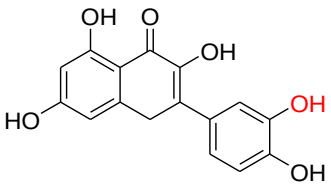
Figure 16. Terbutaline (**43**) and its carbamate ester prodrug (**44**).

Quercetin (**45**) (**Figure 17**) is a plant-derived flavonoid with many biological activities including inhibition of a number of tyrosine kinases. Due to its extreme water insolubility, formulation in dimethylsulfoxide (DMSO) is required. As is well known the use of high doses of DMSO causes dose-dependent haemolysis [96,97]. To overcome these limitations Qc12 (**46**) (**Figure 17**), a water soluble glycine ester prodrug of **45** was presented [98]. After oral administration, neither **46** nor **45** was found in serum. However, after *i.v.* administration, both compounds were detected in the serum. The relative availability of **45** obtained following *i.v.* dosing of **46** was found to be in the range of 20-25%. The biggest advantage obtained by the utilization of **46** is its high aqueous solubility, eliminating the necessity of DMSO formulation.



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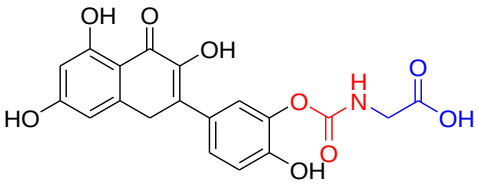
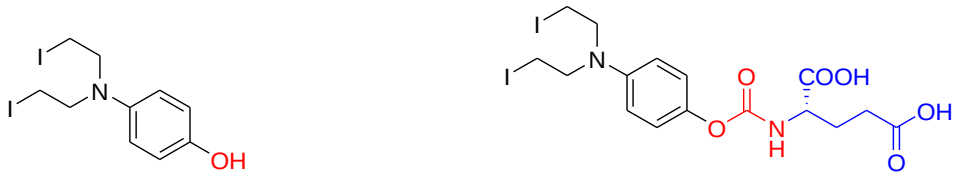


46

Figure 17. Quercetin (**45**) and its carbamate ester prodrug (**46**).

Nitrogen mustard drugs are in common clinical use in cancer chemotherapy as non-specific DNA alkylating agents [99]. However, their clinical efficacy has been limited due to their toxicity to normal tissues. Therefore, selective tumour generation of potent cytotoxic nitrogen mustard from a relatively inactive prodrug is desirable. The antibody-directed enzyme prodrug therapy (ADEPT) [29] approach was proposed as a solution to the selective liberation of the drugs at the surface of the tumour cells. The ADEPT strategy is a two component system. In the first step, a tumour selective antibody-enzyme conjugate is administrated. Time is allowed to optimize conjugate localization at the tumour. In the second step, a relatively non-toxic prodrug is administrated. This prodrug liberates the active drug by enzymatic cleavage at the tumour site. In order to optimize the differential cytotoxicity, enzyme kinetics

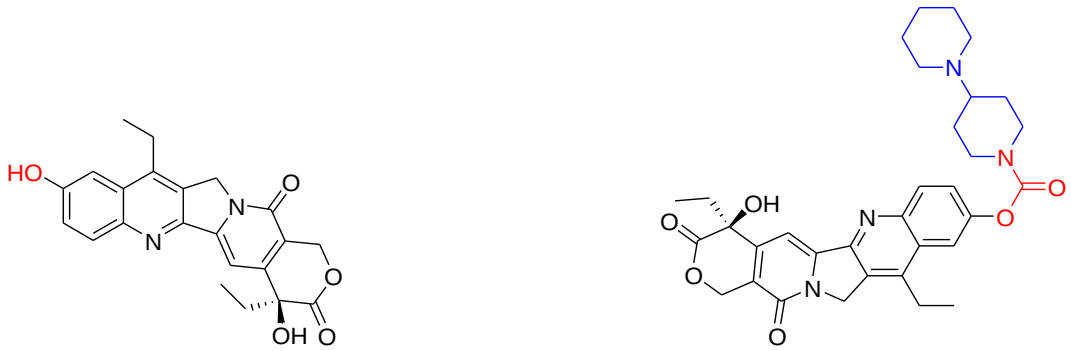
and chemical stability, Springer and co-workers [100] presented a new clinical candidate prodrug ZD2767 (**48**) (**Figure 18**) for ADEPT. The diiodoethylaminophenol mustard glutamate prodrug **48** is hydrolyzed at the carbamate bond by the enzyme carboxypeptidase G2, releasing the potent anticancer drug 4-bis(2-iodoethyl)aminophenol mustard (**47**) (**Figure 18**). The novel compound **48** was found to be 100-fold less cytotoxic than the parent drug **47** when measured in LoVo cells. Furthermore, extremely short life of the candidate (~2min), circumvent the potential drawback to leak back into the general circulation after formation at the tumour.



47 **48**

Figure 18. 4-Bis(2-iodoethyl)aminophenol mustard (**47**) and its carbamate ester prodrug (**48**).

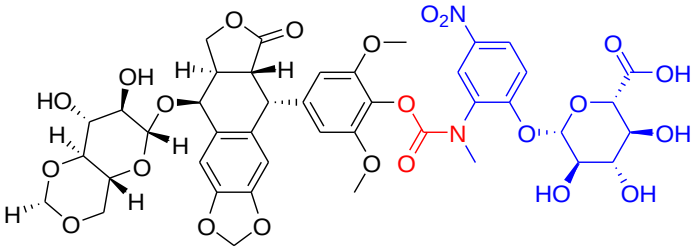
Irinotecan hydrochloride trihydrate (Camptosar®) (**50**) (**Figure 18**) is a water-soluble carbamate prodrug of the lipophilic antineoplastic agent SN-38 (**49**) (**Figure 18**) [101]. Its main use is in colon cancer as an inhibitor of type-I DNA topoisomerase, particularly in combination with other chemotherapy agents, this includes the regimen FOLFIRI which consists of infusional 5-fluorouracil, leucovorin, and **50** and its clinically administrated as a short *i.v.* infusion. Human liver microsomal carboxylesterases, CES 1A1 and CES2, cleave the ester bond of **50** realising the ionisable piperidinopiperidine promoiety and the parent drug **49** which is 1000-fold more potent inhibitor *in vitro* of type I DNA topoisomerase than **50** [102].



49 **50**

Figure 19. SN-38 (**49**) and its carbamate ester prodrug (**50**).

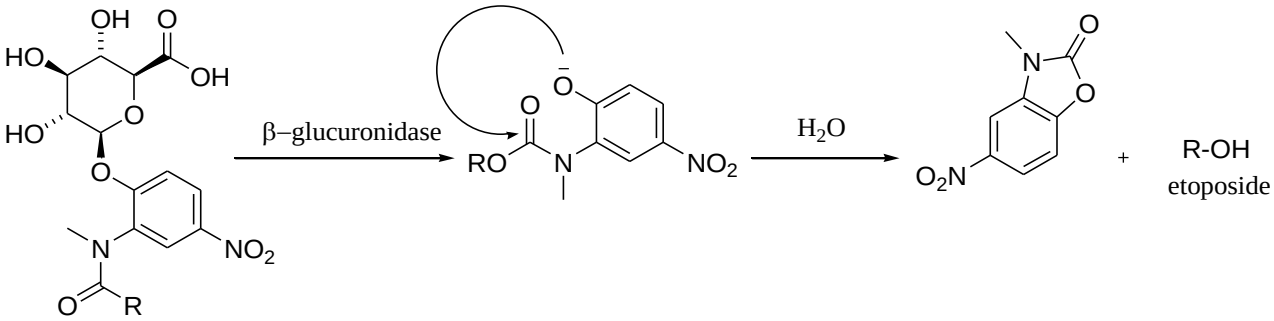
Previously in this paper it was shown how due to the poor water solubility of **27** (**Fig. 9**), a phosphate prodrug was developed, **28** (**Figure 9**) [74,75,76]. **28** is rapidly converted to the parent drug *in vivo* and therefore, has been introduced in clinics with the same profile as **27** itself. A later study [103], presented a prodrug of **27** connected to the β-glucuronic moiety by a self-immolative spacer (**51**) (**Figure 20**), remember that these spacers are used to facilitate the enzymatic cleavage of the prodrug.



51

Figure 20. Etoposide carbamate ester prodrug (**51**).

For this purpose, the therapeutic procedure called prodrug mono therapy (PTM) [104] was applied. The prerequisite in this therapy, was to identify an enzyme specifically localize around the tumour cells. It was found that necrotic areas in human cancers are the site in which lysosomal β-glucuronidase is liberated extracellularly in high local concentrations. This has led some authors to consider this strategy as available to increase the delivery of oncostatic drugs in human tumours [105]. The prodrug **51** presented all the requirements *in vitro* for use in a PTM strategy in cancer chemotherapy. It is less toxic than the parent drug and quantitatively releases **27** by the action of β-glucuronidase (**Scheme 7**).



Scheme 7. Release of etoposide (**27**) from prodrug **51** [103].

3.5. Sulphamate Ester Prodrugs of Phenols.

Natural and synthetic estrogens are used as contraceptives and in tumour therapy. Orally administrated estrogens undergo extensive-pass metabolism in the liver resulting in low bioavailability and high hepatotoxicity [106]. The incorporation of a sulphamate group into the steroid nucleus of estradiol (**52**), estrone (**54**) and ethinylestradiol (**56**) (**Figure 21**) was proposed [107] in order to enhance systemic estrogenicity and reduce their hepatotropic estrogenic effects at oral administration. 3-sulphamates of estradiol, estrone and ethinylestradiol (**53**, **55**, **57**) (**Fig**ure **21**) accumulate in the erythrocytes and pass the liver in this compartment without extraction, metabolism and hepatic hormone action, releasing later from the erythrocyte compartment to convert into the active compound by the systemic hydrolysis of the sulphamate ester (**Scheme 8**).

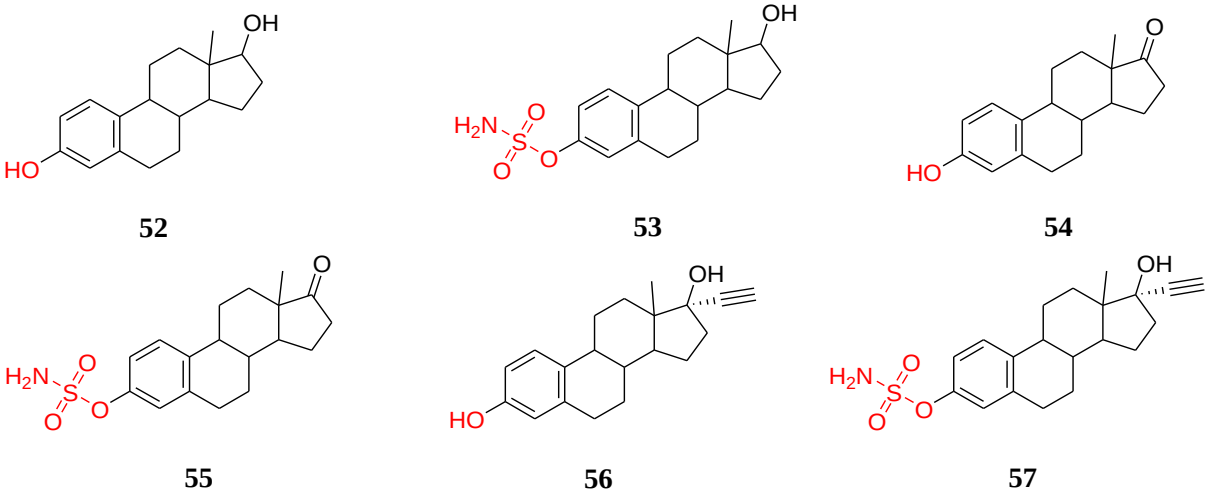
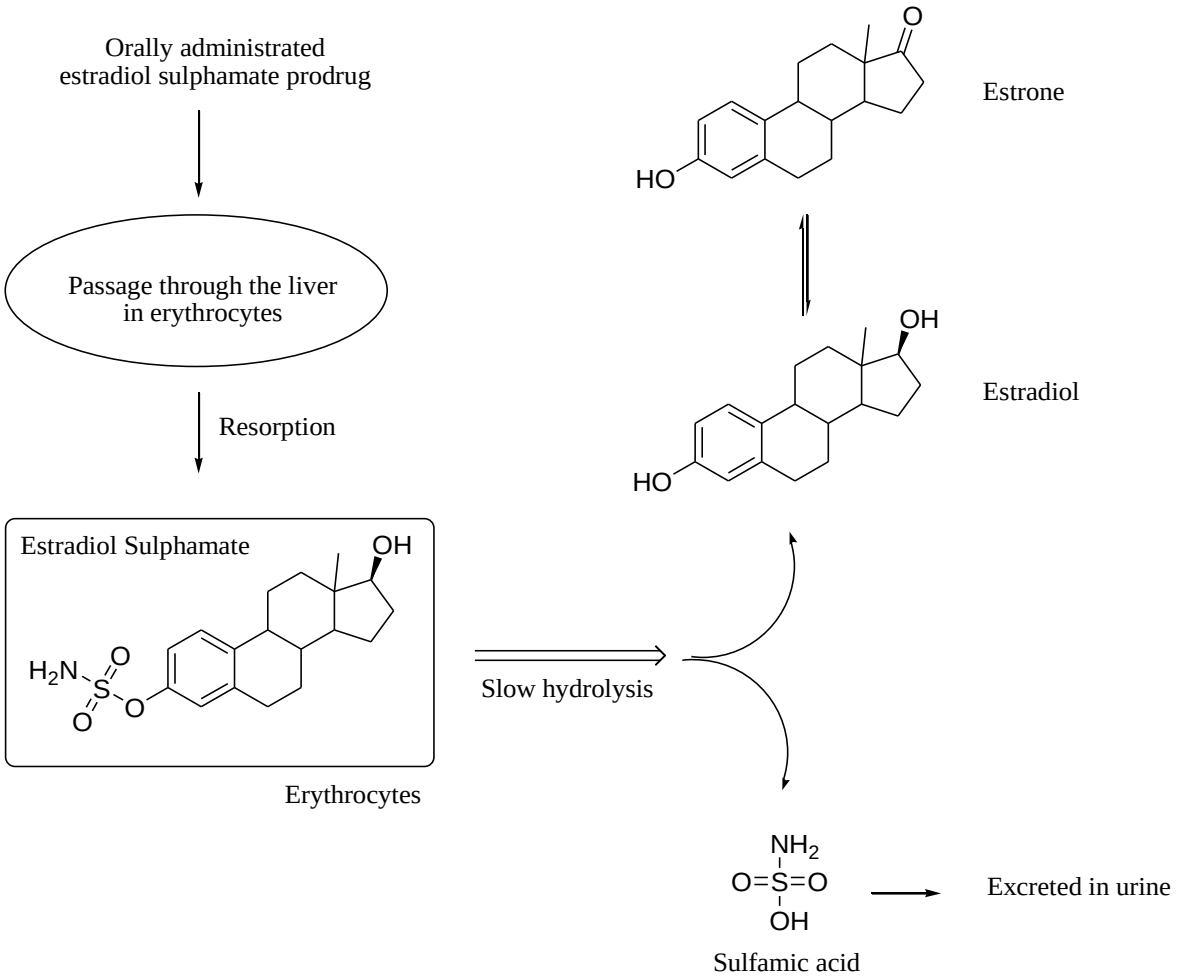


Figure 21. Estradiol (**52**) and its sulphamate ester prodrug (**53**); estrone (**54**) and its sulphamate ester prodrug (**55**); ethinylestradiol (**56**) and its sulphamate ester prodrug (**57**).

Erythrocyte transport is clearly responsible for the significantly improved balance of systemic estrogenic activity, which is increased and hepatic estrogenicity, which is much reduced. Furthermore, later studies [108] suggested that this type of liver bypass is not associated with any measurable adverse effect on erythrocyte oxygen binding, circulatory and blood gas functions. In conclusion, steroidal estrogen prodrug approach offers an interesting option to overcome limitations of the natural and synthetic steroidal estrogens used in current contraceptive regimens.



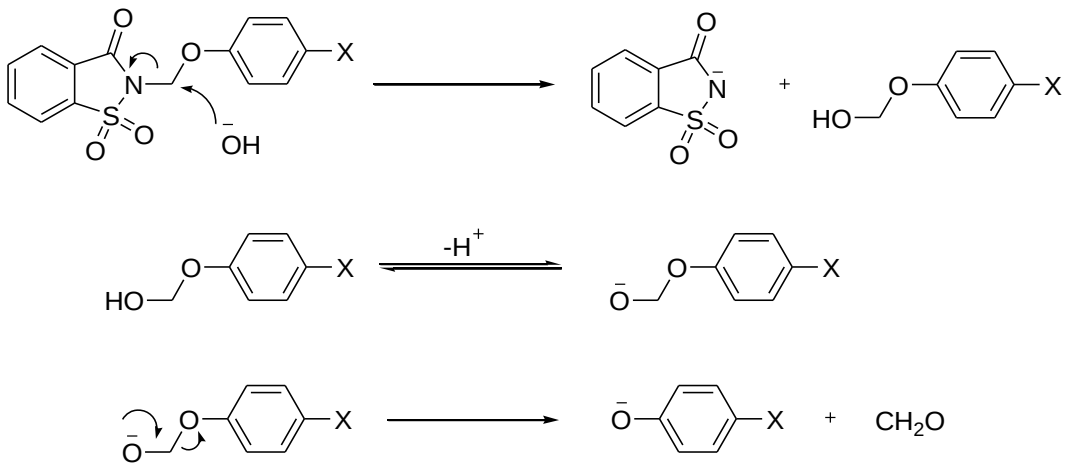
Scheme 8. Pathway for orally administrated estradiol sulphamate prodrug [109].

3.6. Ether Prodrugs of Phenols.

Ether prodrug approach has been used to enhance lipophilicity, favouring the permeation into cellular membranes and the BBB, and/or to improve the pharmacokinetic properties of the parent drug.

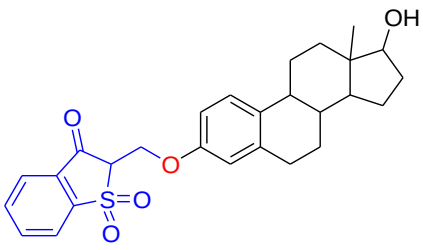
Many phenolic group-containing drugs cannot be administrated orally due to their rapid metabolism by the enzymes in the intestinal tract and liver [19] and when, ester prodrug approach is applied, these derivatives undergo presystemic esterase cleavage in the gut wall to liberate the parent drug prematurely. Therefore, a number of types of prodrugs which could undergo chemical hydrolysis and not be dependent on esterase activity for their reversion to their parent phenols can offer an advantage. One of these types of derivatives is the *O*-(imidomethyl) type of prodrug, which undergoes rapid hydrolysis by an S_N2 mechanism [110]. In this study, the fastest rate of chemical hydrolysis of the *O*-(imidomethyl) type

derivatives was observed for the *O*-(saccharinylmethyl) derivative of model phenols (**Scheme 9**).



Scheme 9. A S_N2 Reaction of cleavage for saccharinylmethyl ether prodrugs of phenols [110].

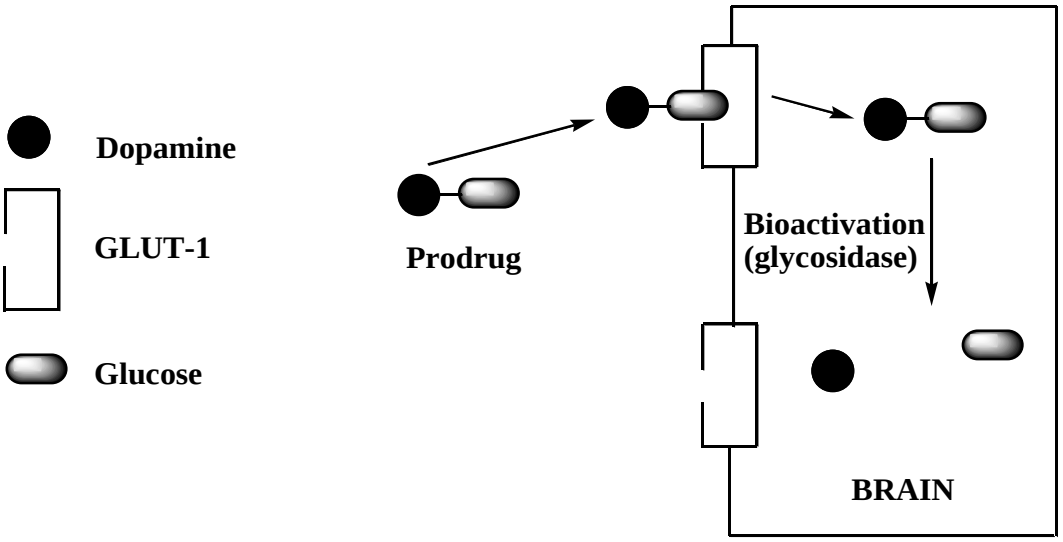
In this context, Prankerd and co-workers [111] reported an *O*-(saccharinylmethyl) prodrug (**58**) (**Figure 22**) of the model compound **52** (**Figure 21**). As it was said in the last section, **52** undergoes extensive first-pass metabolism, resulting in an oral bioavailability of only 11% [112]. **58** was found to be totally dependent in chemical and not enzymatic hydrolysis for the reversion to its parent phenolic drug. On the other hand, **58** had shown a fast rate of hydrolysis in biological media, insuring the release of **52** faster than it is conjugated and excreted. Subsequent studies [113] of the oral potency of **58** have suggested that *O*-(saccharinyl-methyl) promoiety is a promising candidate for protecting **52** from extensive first-pass metabolism and increasing their oral potency. Furthermore, this approach can be extended to other phenolic drugs and, another imidomethyl promoieties can be used to form chemically labile prodrugs that are insensitive to enzymatic hydrolysis.



58

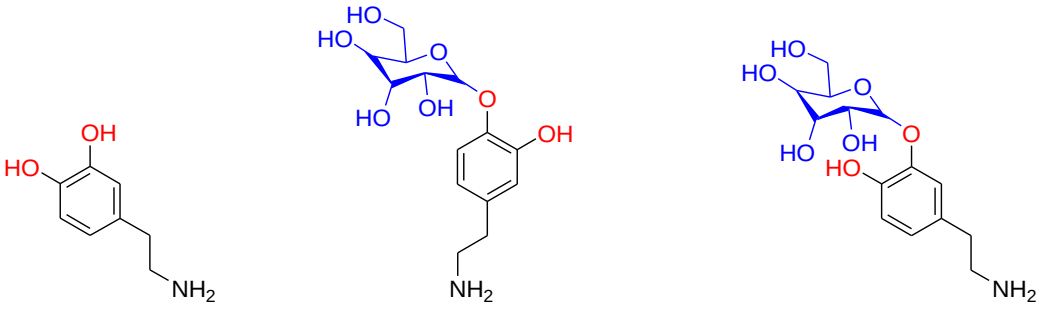
Figure 22. 17β-estradiol ether prodrug (**58**).

PD is a progressive disorder of the central CNS characterized by a reduced concentration of the neurotransmitter, dopamine (**59**) (**Figure 23**) to the brain. A possible therapy based on the used of **59** itself is limited by inability of this substance to cross the BBB. This limitation has stimulated the search for prodrug strategies that rely on dopamine-derivatives able to penetrate the BBB, by making use of specific transport systems. Ferdández-Mayoralas and co-workers [114] initiated studies on a new approach to deliver **59** by linking the neurotransmitter to a sugar molecule, resulting in a glycoconjugate that may cross the BBB using the glucose carrier GLUT-1. As is well known, glucose is the brain's source of energy and other hexoses are transported across the BBB by GLUT-1 [115]. Once transported into the CNS the prodrug should be enzymatically cleaved to release the active compound (**Scheme 10**).



Scheme 10. Schematic representation of the new strategy to deliver dopamine into the CNS [114].

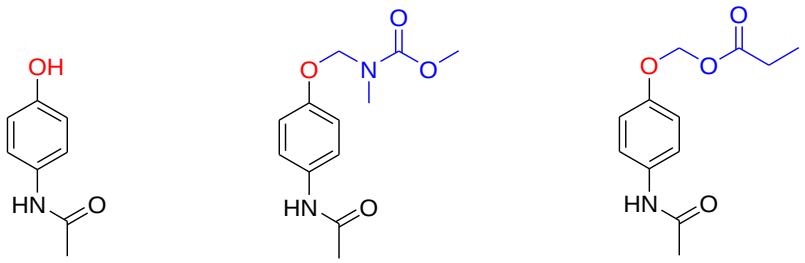
Two prodrugs of dopamine (**60, 61**) (**Figure 23**) where the sugar was attached to the phenolic groups of **59** were presented. Glycosides **60** and **61** fulfilled the prodrug criteria since they showed high stability in plasma and a sustained release of dopamine in brain extract through an activation mechanism catalysed by glycosidase. Unfortunately they did not exhibit *in vivo* antiparkinsonian properties. Later studies [116] demonstrated that the factor responsible for the absence of *in vivo* activity is the lack of affinity of the glycosyl derivatives **60** and **61** for the glucose carrier GLUT-1.



59 **60** **61**

Figure 23. Dopamine (**59**) and its ether prodrugs (**60**, **61**).

Approaches to increasing topical delivery by transiently masking a phenolic functionality have been recently investigated. The soft alkyl analogues of alkyoxycarbonyl (AOC) prodrugs obtained by inserting an –NR´CH_2– between the carbonyl carbon and the phenol oxygen to form *N*-alkyloxycarbonylaminomethyl (NANAOCAM) using the model drug acetaminophenol (APAP) (**62**) (**Figure 24**) was presented [117]. It was found that the insertion of –NR´CH_2– into the AOC promoiety increased the biphasic solubility and the flux of **62** compared with AOC-APAP [118]. The most effective derivative (*N*-methyl-*N*-methyloxycarbonylamino-methyl prodrug) (**63**) (**Figure 24**) at enhancing the delivery of total **62** species through mousses skin from isopropyl mirystate (IPM) was found to be the most water soluble member of this more lipid soluble series. However NANAOCAM derivatives of **62** were poorly converted to **62** in diffusion cell experiments. The poor bioconversion of these derivatives limits their application in topical delivery. Recently, Sloan and co-workers [119] have presented a series of alkylcarbonyloxymethyl (ACOM) derivatives of **62**, where a –OCH_2– spacer separates the carbonyl moiety from the parent drug. In this study, the flux of the most permeable derivative (ethylcarbonyloxymethyl prodrug) (**64**) (**Figure 24**)" was 3.6 times greater than that of **62** and just 9% of the intact prodrug was found in the receptor phase. High stability in IPM solution of theses derivatives showed that the absence of intact prodrug in the receptor phase was due to extensive enzymatic hydrolysis in the skin and was not the result of substantial chemical hydrolysis in the receptor or donor phases. The results presented in this study [119] demonstrate for the first time that ACOM derivatives are capable of improving the topical delivery of a phenol.



62 **63** **64**

Figure 24. Acetaminophen (**62**) and its ether prodrugs (**63**, **64**).

3.7. Conclusion.

Prodrugs continue to be an exciting area of research due to the significant delivery challenges that present more and more drug candidates. The examples provided in this review demonstrate the diverse array of prodrugs options in improving poor delivery characteristics and solubility limitations of phenolic drugs.

While this chapter does not represent an exhaustive review of all the prodrug approaches for improved the characteristics of phenolic group-containing drugs, it does provide a suitably general view of the different prodrug strategies to give the readers a comprehension for the choice and limitations of this approach and the tasks scientists are dealing with in improving phenolic group-containing therapeutics with the use of prodrugs.

4. Salicylanilide Modification.

4.1. Introduction.

SAL are an important class of aromatic compounds with a wide range of pharmacological activities, such as antibacterial[120,121,122,123], antifungal [124] and anti-inflammatory [125], among others. Furthermore; several studies reported their potent antimycobacterial [123,126] effect. Their activity results from multiple mechanisms. SAL were identified as inhibitors of the two-component regulatory systems [127] of bacteria [128,129] by a mechanism related to the effects on uncoupling oxidative phosphorylation. Later on, they were found to be inhibitors of the protein kinase epidermal growth factor receptor [130,131], by the competition with ATP for binding at the catalytic domain of tyrosine kinase and to stop the tumour growth. The pseudo six-member ring facilitated by a strong intramolecular phenolic hydroxyl-carbonyl hydrogen bond seems to be significant for their activity [132]. In recent studies, they were also found to be selective inhibitors of interleukin-12p40 production that play an specific role in the initiation, expansion and control of the cellular response to TB as well [133,134].

In general, an electron-withdrawing group on the salicyloyl ring and hydrophobic groups on the anilide moiety, as well as the 2-hydroxy group, are essential for the optimal antimicrobial effect. The salicylanilides substituted with halogens in both parts meet the requirements and form the most active derivatives with anti-TB activity against *M. tbc*. and some atypical strains of mycobacteria[120].

Although the presence of phenolic hydroxyls seems to be necessary for the activity of these compounds also limits their bioavailability and thus their effectivity.

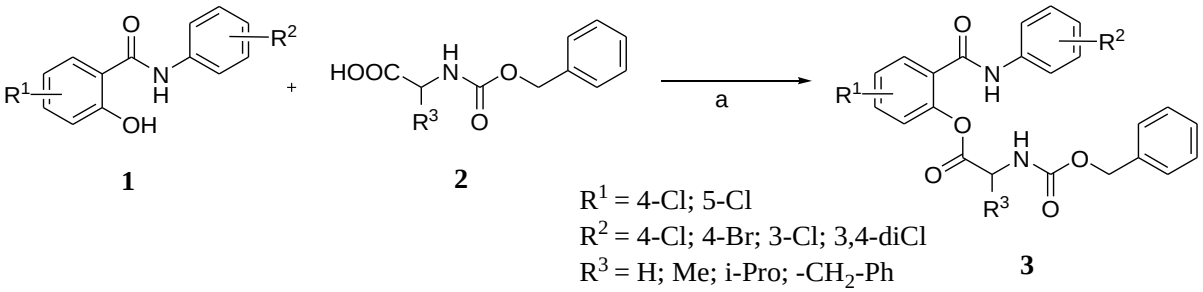
Our strategy in the design of new potential antimycobacterial active agents has been mainly orientated towards the synthesis of prodrug forms of the most anti-TB active SAL in order to improve their physico-chemical and pharmacokinetic properties, enlarge their activity profile and protect the phenolic hydroxyl against first-pass metabolism.

4.2. Amino acid esterification (Paper II, Paper III).

Esterification of the hydroxyl(s) group has been one of the preferred prodrug strategies in order to temporally mask polar groups resulting in an increased lipophilic molecule, which would promote membrane permeability.

For the development of a general synthetic method for SAL esterification, protected lipophilic simple amino acids (AA) were chosen for being natural molecules which will increase lipophilicity of the parent corresponding SAL.

The series of the above-mentioned salicylanilide derivatives was synthesized in two steps. The starting SAL **1** were selected according to previous results showing high *in vitro* activity against *M. tbc*. [135]. They were routinely prepared by the reaction of 4 or 5-chlorosalicylic acid with the appropriate aniline in chlorobenzene with PCl_3. By using microwave irradiation, the reaction time was shortened from several hours to minutes. In a second step, *N*-benzyloxycarbonyl AA (*N*-Cbz-AA) **2** were esterified with the corresponding SAL **1** by using *N*,*N*'-dicyclohexylcarbodiimide (DCC) activation as an optimal method [136]. Lipophilic *N*-Cbz-AA such as glycine (Gly), *R/S*-alanine (Ala), *R/S*-valine (Val) and *R/S*-phenylalanine (Phe) were used for esterification. As expected, in most of the cases the reaction gave the desired esters **3** in high yields. The general synthetic pathway is shown in **Scheme 11**.



Scheme 11. Synthesis of investigated salicylanilide derivatives. Reagents and conditions: (a) DCC, DMF, -10°C.

All the prepared *N*-Cbz-AA esters **3** were tested *in vitro* for their antimycobacterial activity in the National Reference Laboratory for *M. kansasii*, against *M. tbc*. (331/88) and against some non-TB strains such as *M. avium* (330/88) and *M. kansasii* (235/80 and 6509/96), where the first line drug INH shows no activity. All the 30 SAL esters showed high antimycobacterial activity against all tested mycobaterial strains with minimum inhibition concentration (MIC) values in the range of 1 – 32 μmol/L. The anti-TB screening results of the most active compounds are summarized in **Table 1**.

Hydrophobicities (log *P*/Clog *P* values) of the studied compounds **3** were calculated using two commercially available programs (CHEMDRAW Ultra 10.0 and ACD/logP) and measured by means of RP-HPLC determination of capacity factors *k* with subsequent calculation of log *k*

[137]. In general, the levels of anti-TB activity of the synthesized compounds **3** suggest that lipophilicity is a secondary parameter for anti-TB activity and there is no correlation between log *k* and the anti-TB activity of these compounds (See **Table 1**).

Table 1. Antimycobacterial activity and lipophilicity of the most active SAL Cbz-amino acid esters **3.**

	R_1	R_2	R_3	MIC (μM)										Lipophilicity factor		
				M. tbc 331/88		*M. avium* 330/88		*M. kansasii* 235/80			*M. kansasii* 6509/96			log *k*	log *P* ACD/log *P*	log *P*/Clog *P* ChemOffice
				14d	21d	14d	21d	7d	14d	21d	7d	14d	21d			
3a	4-Cl	4-Br	(*S*)-CH-$(CH_3)_2$	4	4	8	16	4	8	8	8	8	8	0.980	6.41±0.54	6.36/6.5332
3b	4-Cl	4-Br	(*R*)-CH-$(CH_3)_2$	2	4	16	16	4	8	8	8	8	8	0.980	6.41±0.54	6.36/6.5332
3c	4-Cl	4-Br	(*R*)-CH_2-Ph	2	4	8	16	2	4	4	8	8	8	1.126	7.46±0.55	7.15/7.0232
3d	5-Cl	4-Cl	(*R*)-CH-$(CH_3)_2$	4	4	8	16	4	8	8	4	8	8	0.881	5.61±0.48	6.09/6.3832
3e	5-Cl	4-Cl	(*S*)-CH_2-Ph	4	4	8	8	4	8	8	4	8	8	1.030	7.56±0.49	6.88/6.8732
3f	5-Cl	4-Cl	(*R*)-CH_2-Ph	4	4	8	8	4	8	8	4	8	8	1.030	7.56±0.49	6.88/6.8732
INH				0.5	0.5	>250	>250	>250	>250	>250	4	8	8			

The most active compounds (**3a**-**3f**) were chosen for further investigation. Cytotoxicity assessment made on human intestinal cell line HCT-8 (ECACC, UK) showed medium cytotoxicity for all the measured compounds in comparison to the standard INH. Furthermore, the selectivity index (SI), defined as the ratio of the concentration inhibiting 50% of the cell growth (EC_{50}) to MIC, was higher than 10 (see **Table 2**), what makes them good candidates for additional screening [138].

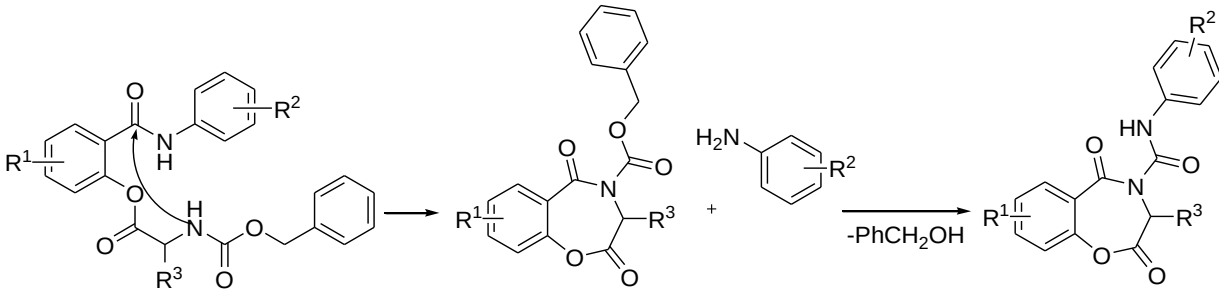
The hydrolytic stability of the most active compounds (**3a**-**3f**) was as well investigated. To simulate blood or serum and afflicted tissue, the stability in two types of phosphate buffer at pH 7.4 (7×10^{-2} M, 37°C) and pH 5.5 (7×10^{-2} M, 37°C) was measured using UV/Vis spectroscopy [139]. From experimental data measured at 224 nm, the half-times ($t_{1/2}$) and rate constants (k_{obs}) were calculated and the results are summarized in **Table 2**. The experimental results for both types of hydrolysis are comparable, being acidic hydrolysis slightly faster.

Table 2. Rate constants and half-times of the most active compounds in phosphate buffer solution pH 7.4 and 5.5.

	Rate constant k_{obs} (s^{-1}) pH 7.4	$t_{1/2}$ (s) for pH 7.4	Rate constant k_{obs} (s^{-1}) pH 5.5	$t_{1/2}$ (s) for pH 5.5	EC_{50} (μM)	SI for *M. tbc*.
3a	7.79×10^{-4}	889.94	8.06×10^{-4}	860.10	121.8	16.74
3b	7.70×10^{-4}	897.47	7.81×10^{-4}	887.25	121.3	33.35
3c	3.37×10^{-4}	2057.70	3.56×10^{-4}	1948.50	35.5	10.61
3d	5.65×10^{-4}	1226.10	5.69×10^{-4}	1218.10	106.7	13.75
3e	4.56×10^{-4}	1520.80	5.29×10^{-4}	1311.50	82.9	20.73
3f	5.24×10^{-4}	1323.30	4.35×10^{-4}	1074.00	104.2	26.05

4.2.1. Unexpected formation of novel seven-membered ring benzoxazepines (Paper IV).

In the course of our research, we found that *N*-Cbz-Gly **2** (R^3=H) or *N*-Cbz-(*S*)-Ala (R^3=CH_3), when esterified with salicylanilides **2** (R^1=5-Cl, R^2=4-Cl, 4-Br, 4-CF_3, 3-Cl) underwent to seven-membered rings of benzoxazepine-2,5-diones **5.** The structure of the final product was unequivocally determined by mass spectroscopy and 2D NMR experiment (gHMBC). We proposed a possible mechanism for this rearrangement where the attack of the amide carbonyl by the carbamic NH group would give rise to a seven-membered cyclic intermediate **4**, which would further react with the substituted aniline released as a leaving group in the previous step, to result in the final product **5** (See **Scheme 12**).



3 **4** **5**

R^1=4-Cl; 5-Cl
R^2=4-Cl; 4-Br; 4-CF_3; 3-Cl
R^3=H; CH_3

Scheme 12. Possible mode of cyclization.

Biological testing was provided for the series of 9 unexpected bezoxazepine-2,5-diones **5** against *M. tbc*. (331/88), *M. avium* (330/88) and *M. kansasii* (235/80 and 6509/96). Unluckily, these cycles doesn't present anti-TB activity.

4.3. Alkyl carbamate esterification (Paper V).

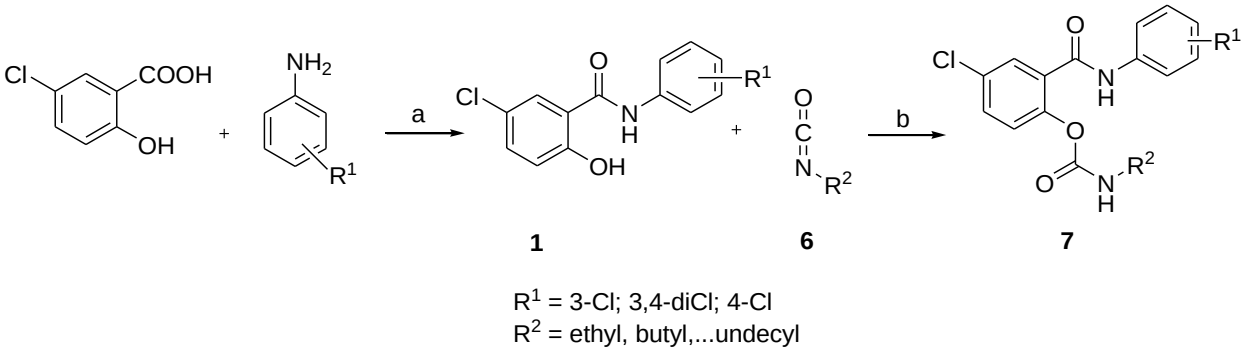
In the recent past, a number of organic carbamates [140] have been found as potential antibacterial and antiviral agents. The carbamate residue present in these new molecules either contributes as a core component [141] or incorporated into a known molecule, contributes to the improvement of its pharmacodynamic and pharmacokinetic properties [142,143]. In particular, carbamate was successfully used to protect phenolic drugs [144,145].

Thus, we hypothesised that masking the phenolic hydroxyl in SAL by carbamate formation may protect the molecule against extensive first-pass metabolism following oral administration, broaden its activity profile and improve its physicochemical and

pharmacokinetic properties. In this context, the aim of this article was to describe the synthesis, antimycobacterial activity and cytotoxicity of a series of SAL carbamates as well as to evaluate their stability against chemical hydrolysis at various pH values.

The preparation of the carbamates is outlined in **Scheme 13**. The synthesis of the starting SAL **1** has been described in the previous chapter.

For the synthesis of the corresponding carbamates **7**, a suspension of SAL **1** in acetonitrile (ACN) was treated with one equivalent of triethylamine (TEA), adding then the corresponding isocyanate **6**. This reaction was performed at room temperature due to thermal instability of the products. The prepared carbamates **7** belong into three series: those having chlorine at position 3, at position 4, and at positions 3 and 4, respectively, of the anilide ring.



Scheme 13. Synthesis of 4-chloro-2-(R^1-chlorophenylcarbamoyl)phenyl alkylcarbamates. Reagents and conditions: (a) PCl_3, chlorobenzene, microwave irradiation (530 W); (b) TEA, ACN, rt.

The prepared carbamates **7** were tested *in vitro* for their antimycobacterial activity in against *M. tbc*. (331/88) and against some non-TB strains such as *M. avium* (330/88) and *M. kansasii* (235/80 and 6509/96). The anti-TB screening results of the most active compounds of this series are summarized in **Table 3**.

Table 3. Antimycobacterial activity and calculated lipophilicity of the most active SAL alkylcarbamates **7**.

	R^1	R^2	MIC [μmol/L]								log *P*/Clog*P*
			M. tbc 331/88		*M. avium* 330/88		*M. kansasii* 235/80		*M. kansasii* 6509/96		
			14 d	21 d	14 d	21 d	14 d	21 d	14 d	21 d	
7a	3-Cl	hexyl	0.5	1	8	8	4	4	4	4	5.53/6.31
7b	3,4-diCl	ethyl	0.5	1	16	32	2	4	2	4	4.35/4.82
7c	3,4-diCl	butyl	0.5	1	8	16	2	2	2	4	5.25/5.88
7d	3,4-diCl	pentyl	0.5	0.5	8	16	2	2	2	4	5.67/6.41
7e	3,4-diCl	hexyl	0.5	0.5	4	8	2	2	1	2	6.08/6.93
7f	3,4-diCl	heptyl	0.5	1	4	8	2	2	2	4	6.50/6.46
7g	3,4-diCl	octyl	0.5	1	4	8	2	4	2	4	6.92/7.99
7h	4-Cl	pentyl	0.5	0.5	8	8	2	4	4	4	5.11/5.78
INH	-	-	0.5	0.5	>250	>250	>250	>250	4	4	-

In this study, the most active derivatives resulted to be those with log*P* values of approximately 6. Although the higher lipophilicity may be the reason for the increase in the activities of the carbamates **7** against *M. tbc., kansasii* and *avium* compared to the starting SAL [135], it cannot explain their higher potency compared to the corresponding *N*-protected AA ester derivatives **3**, whose lipophilicities are comparable [146]. For example, compound **7e** was 16 times more potent than the parent SAL and 4 times more active than the most active corresponding *N*-protected AA ester derivative against *M. tbc.* (331/88).

The most active derivatives of this series were tested as well against five clinically isolated MDR-TB strains, including *M. tbc.* 7357/98, resistant to INH, RMP, EMB, STM, ofloxacin (OFX) and ansamycin, *M. tbc.* 9449/06, resistant to IHN, STM, RMP and ansamycin, *M. tbc.* 2092/05, resistant to INH, RMP, EMB, STM, OFX and ansamycin, *M. tbc.* Praha 1, resistant to INH, RMP, EMB, STM, clofazimine (CFZ) and ansamycin, and *M. tbc.* Praha 128 resistant to IHN, RMP, EMB, STM, gentamicin (GTM), CFZ, ansamycin and amikacin (AK). All the studied compounds exhibited high activity against the MDR-TB strains, with MIC values between 0.5-2 μmol/L (**Table 4**). These activities are comparable with that presented by compounds undergoing PhaseII clinical trials such a nitroimidazopyran PA-824 [147,148], with MIC values between 0.1-0.7 μmol/L against mono and MDR-TB strains or the diamine analogue of EMB, SQ109 [149,150], with MIC values between 0.5-1.8 μmol/L against strains resistant to EMB, IHN and RMP.

Table 4. Activity of selected carbamates against MDR-TB strains.

	MIC [μmol/L]												EC_{50} [μmol /L]	SI	SI
	M. tbc 331/88		*M. tbc* 7357/98[a]		*M. tbc* 9449/06[b]		*M. tbc* 2092/05[c]		*M. tbc* Praha 1[d]		*M. tbc* Praha 128[e]			For MTB	For MDR-TB
	14 d	21 d	14 d	21 d	14 d	21 d	14 d	21 d	14 d	21 d	14 d	21 d			
7a	0.5	1	1	1	1	1	1	2	1	2	1	2	14.9	14.9	7.4-14.9
7b	1	1	0.5	1	0.5	0.5	0.5	0.5	0.5	1	0.5	0.5	31.0	31.0	31-62
7c	0.5	1	0.5	1	1	0.5	0.5	1	0.5	1	0.5	1	38.0	38.0	38-76
7d	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1	0.5	0.5	40.0	80.0	40-80
7e	0.5	0.5	0.5	1	0.5	0.5	0.5	0.5	0.5	1	0.5	0.5	27.9	55.8	28-56
7f	0.5	1	0.5	0.5	0.5	0.5	0.5	1	0.5	1	0.5	1	43.4	43.4	43.4-86.8
7g	1	1	1	1	1	0.5	0.5	1	1	2	0.5	1	48.5	48.5	24-97
7h	0.5	0.5	1	2	1	1	1	1	1	2	1	1	17.4	34.8	8.7-17.4
INH	0.5	0.5	16	16	16	16	16	16	16	16	16	16	>100	>200	>6.25

[a]Resistant to INH, RMP, EMB, STM, OFX and ansamycin
[b]Resistant to INH, STM, RMP and ansamycin
[c]Resistant to INH, RMP, EMB, STM, OFX and ansamycin
[d]Resistant to INH, RMP, EMB, STM, CFZ and ansamycin
[e]Resistant to INH, RMP, EMB, STM, GTM, CFZ, ansamycin and AK

The *in vitro* mammalian cell toxicity of the most active compounds **7** was assessed on human intestinal cell line HCT-8 (ECACC, UK) using the XTT assay [151]. The cytotoxicity results presented in **Table 4** are expressed as the EC_{50}. The studied compounds show moderate cytotoxicity in comparison to the standard INH with the EC_{50} values in the range of 14.9 – 48.5 μmol/L. Nevertheless, all the studied compounds displayed the SI value higher than 10 for both TB and MDR-TB strains (**Table 4**), what means that they should be seriously considered for further screening [138].

The chemical hydrolysis of compound **7e** was studied in aqueous buffer solutions at pH from 3 to 8 at 37 °C. The decomposition of **7e** and the release of the parent SAL were monitored by HPLC for 48 h. The carbamate bond was stable at acidic pH values but decomposed in alkaline environment following a pseudo-first order kinetics (**Table 5**).

At physiological pH of 7.4, the carbamate **7e** showed a $t_{1/2}$ of almost 2 days. Although these data describe chemical hydrolysis only, we hypothesize that these compounds may also be stable in plasma for sufficient time to reach its site of action, *i. e*. the mycobacteria. This assumption is supported by the relative stability of the carbamate bond towards esterases [152]. Moreover, in a previous study, a carbamate of a similar structure displayed a $t_{1/2}$ of 15.9 and 2.7 h in a buffer and plasma, respectively [90]. In addition, due to the stability in acidic environment, these compounds may be good candidates for oral administration. Furthermore, protection of the phenolic hydroxyl may render the compound less susceptible towards first-pass metabolism. However, the stability of the synthesized SAL carbamates in biological environments and their bioavailability warrants further investigation.

Table 5. Half-lives ($t_{1/2}$) of the carbamate **7e** at various pH values.

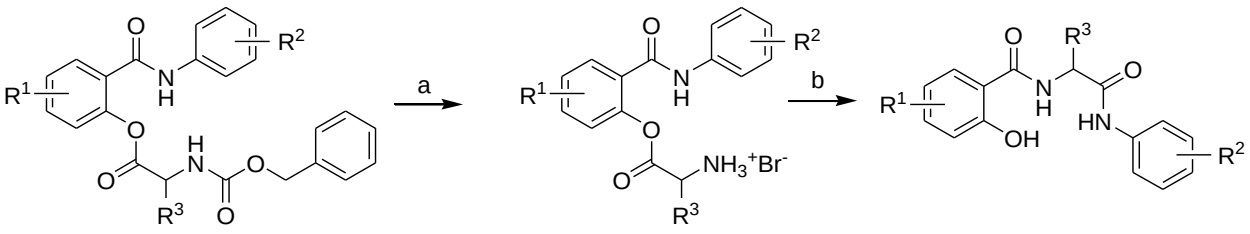
pH	$t_{1/2}$ (h)
3	nd
4	nd
5	nd
6	185 ± 68
7	64 ± 8
7.4	43 ± 6
8	5 ± 2

Mean ± SEM, n = 3
nd = no significant decomposition observed ($p < 0.05$)

5. Rearrangement of salicylanilide derivatives

5.1. Unexpected formation of 2-Hydroxy-N-(1-(oxo-(phenylamino)-alkan-2-yl)benzamides.

As a part of our ongoing search for new antituberculosis active molecules of SAL esters, we proceeded to the deprotection of the synthesized *N*-Cbz-AA esters **3**. The *N*-deprotection of the amino group failed under generous conditions of hydrogenolysis (H_2/Pd) – the ester linkage was found unstable even if different reaction conditions and solvents were used (temperature modification, toluene, ethylacetate, methanol solvents). Amino group liberation was finally realized using 33 % solution of hydrogen bromide (HBr) in glacial acetic acid (AcOH) in absence of solvent. Diethyl ether (DEE) addition precipitated the appropriate HBr salts of SAL-AA esters as white hygroscopic solids **8** in almost quantitative yields. Subsequent amino group liberation by TEA under anhydrous conditions yielded the unexpected product **9** in which there was no signal either of ester or of free amino group, only the presence of a phenolic hydroxyl was clearly detected. The structure of **9** was unequivocally corroborated by HMBC 2D NMR experiments. Unexpected rearrangement products after amino group liberation were identified as substituted hydroxy-*N*-(phenylamino)-oxo-alkyl)benzamides, "diamides" **9** (**Scheme 14**).



3 **8** **9**

R^1 = 4-Cl; 5-Cl
R^2 = 4-Cl; 4-Br; 3-Cl; 3,4-diCl
R^3 = H; Me; i-Pro; -CH_2-Ph

Scheme 14. Synthesis of substituted hydroxy-*N*-(phenylamino)-oxo-alkyl)benzamides **9**. Reagents and conditions: (a) AcOH/HBr, rt; (b) TEA, rt.

These series of hydroxy-*N*-(phenylamino)-oxo-alkyl)benzamides **9** were tested *in vitro* for their antimycobacterial activity in the National Reference Laboratory for *M. kansasii*, against *M. tbc*. (331/88) and against some non-TB strains such as *M. avium* (330/88) and *M. kansasii* (235/80 and 6509/96). The results are presented in **Table 6**.

Table 6. Antimycobacterial evaluation of Hydroxy-*N*-(phenylamino)-oxo-alkyl benzamides **9.**

	R_1	R_2	R_3	MIC (μM)									
				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
9a	4-Cl	4-Br	(*S*)-CH-$(CH_3)_2$	62.5	62.5	62.5	62.5	62.5	62.5	125	62.5	62.5	62.5
9b	4-Cl	4-Br	(*R*)-CH-$(CH_3)_2$	62.5	62.5	125	125	62.5	62.5	125	32	62.5	62.5
9c	4-Cl	4-Br	(*R*)-CH_2-Ph	32	32	250	250	32	62.5	500	62.5	125	250
9d	5-Cl	4-Cl	(*R*)-CH-$(CH_3)_2$	62.5	62.5	62.5	62.5	16	32	62.5	32	62.5	62.5
9e	5-Cl	4-Cl	(*S*)-CH_2-Ph	32	32	32	62.5	32	32	32	32	32	32
9f	5-Cl	4-Cl	(*R*)-CH_2-Ph	32	32	62.5	62.5	32	32	62.5	32	62.5	62.5
9g	5-Cl	4-Cl	(*R*)-CH_3	8	16	62.5	125	32	125	250	62.5	125	250
9h	5-Cl	4-Cl	(*S*)-CH-$(CH_3)_2$	32	32	62.5	62.5	32	62.5	62.5	32	62.5	62.5
9i	5-Cl	4-Cl	(*R*)-CH-$(CH_3)_2$	32	32	62.5	62.5	32	62.5	62.5	32	62.5	62.5
9j	5-Cl	4-Cl	(*S*)-CH_2-Ph	16	16	32	32	32	32	62.5	16	32	62.5
9k	5-Cl	4-Cl	(*R*)-CH_2-Ph	16	16	32	32	16	32	32	32	32	62.5
9l	5-Cl	4-Br	(*S*)-CH_2-Ph	32	32	250	250	32	62.5	500	62.5	125	250
9m	5-Cl	3,4-diCl	H	125	500	62.5	500	62.5	125	500	62.5	250	500
9n	5-Cl	3,4-diCl	(*S*)-CH_3	32	32	62.5	32	32	62.5	>500	62.5	62.5	32
9o	5-Cl	3,4-diCl	(*S*)-CH-$(CH_3)_2$	32	32	62.5	125	16	32	62.5	16	32	62.5
9p	5-Cl	3,4-diCl	(*R*)-CH-$(CH_3)_2$	16	32	62.5	125	16	32	62.5	16	32	32
9q	5-Cl	3,4-diCl	(*S*)-CH_2-Ph	125	>1000	125	>1000	125	250	>1000	62.5	250	500
9r	5-Cl	3,4-diCl	(*R*)-CH_2-Ph	125	>1000	125	>1000	32	62.5	500	32	62.5	500
9s	4-Cl	4-Cl	(*S*)-CH_3	62.5	62.5	125	125	62.5	125	125	125	62.5	62.5
9t	4-Cl	4-Cl	(*R*)-CH_3	32	62.5	125	125	62.5	125	125	62.5	62.5	62.5
9u	4-Cl	4-Cl	(*S*)-CH-$(CH_3)_2$	62.5	62.5	125	125	32	62.5	62.5	32	62.5	62.5
9v	5-Cl	3-Cl	H	125	125	62.5	62.5	125	250	250	62.5	250	250
9w	5-Cl	3-Cl	(*S*)-CH_3	250	500	500	500	125	500	500	250	500	500
9x	5-Cl	3-Cl	(*R*)-CH_3	62.5	125	125	250	62.5	62.5	62.5	32	62.5	62.5
9y	5-Cl	3-Cl	(*S*)-CH-$(CH_3)_2$	32	62.5	125	125	62.5	62.5	125	32	62.5	62.5
9z	5-Cl	3-Cl	(*R*)-CH-$(CH_3)_2$	32	62.5	62.5	125	62.5	62.5	125	32	62.5	62.5
9aa	5-Cl	3-Cl	(*S*)-CH_2-Ph	62.5	125	125	250	32	62.5	125	16	32	32
9bb	5-Cl	3-Cl	(*R*)-CH_2-Ph	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	4	8	8

Although synthesized compounds **9** show some activity, we observed an important decrease of the activity compared with the starting SAL [135] or the corresponding *N*-Cbz-AA ester derivatives **3 [**146]. Take as example the compound **9c**, which presented 2 times less potency than the parent SAL and 8 times less potency than the corresponding *N*-Cbz-AA ester.

However, we have developed an alternative synthetic route for this kind of "diamides". In the recent past, for example, Shibasaki and co-workers [153] have presented the diamide (*S*)-2-hydroxy-*N*-(1-(2-hydroxyphenylamino)-3-methyl-1-oxobutan-2-yl)benzamide as a promising ligand for the amination of succinimide in the catalytic asymmetric synthesis of AS-3201. AS-3201 was identified as a structurally novel aldose-reductase inhibitor used in the treatment of diabetic disorders.

5.1.1. Hydrobromide salts of α-amino acid esters and salicylanilides **8**.

General procedure

A solution of hydrogen bromide in acetic acid (33%) (6 mL) was slowly added to *N*-benzyloxycarbonyl-protected esters **3** (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 **8** was about 90 %.

Data of prepared hydrobromide salts **8**

(*S*)-1-(2-(4-Bromophenylcarbamoyl)-4-chlorophenoxy)-3-methyl-1-oxobutan-2-aminium bromide **8a**.

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 **8b**.

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.

(*R*)-1-(2-(4-Bromophenylcarbamoyl)-4-chlorophenoxy)-1-oxo-3-phenylpropan-2-aminium bromide **8c**.

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.

(*R*)-1-(5-Chloro-2-(4-chlorophenylcarbamoyl)phenoxy)-3-methyl-1-oxobutan-2-aminium bromide **8d**.

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 **8e**.

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 **8f**.

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.

(*R*)-1-(4-Chloro-2-(4-chlorophenylcarbamoyl)phenoxy)-1-oxopropan-2-aminium bromide **8g**.

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 **8h**.

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 **8i**.

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 **8j**.

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 **8k**.

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)-1-oxo-3-phenylpropan-2-aminium bromide **8l.**

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.

2-(4-Chloro-2-(3,4-dichlorophenylcarbamoyl)phenoxy)-2-oxoethanaminium bromide **8m**.

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 **8n**.

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 **8o**.

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 **8p**.

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 **8q**.

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 **8r**.

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 **8s**.

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}. ^1H 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 **8t**.

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}. ^1H 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 **8u**.

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}. ^1H 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.

2-(4-Chloro-2-(3-chlorophenylcarbamoyl)phenoxy)-2-oxoethanaminium bromide **8v**.

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}. ^1H 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 **8w**.

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}. ^1H 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 **8x.**

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}. ^1H 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 **8y**.

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 **8z**.

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 **8aa**.

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 **8bb.**

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.

5.1.2. Hydroxy-*N*-(phenylamino)-oxo-alkyl benzamides **9**.

General procedure

Triethylamine (0.95 mmol) was added to a stirred suspension of hydrobromide salt **8** (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 **9** were isolated as unexpected products of amino group liberation.

Data of prepared hydroxy-*N*-(phenylamino)-oxo-alkyl benzamides **9**

(*S*)-*N*-(1-(4-Bromophenylamino)-3-methyl-1-oxobutan-2-yl)-5-chloro-2-hydroxybenzamide **9a**.

White solid; yield 56%; mp 226-228 °C. 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 **9b**.

White solid; yield 50%; mp 214-216 °C. IR (KBr pellet): 3298, 2968, 1668, 1633, 1595, 1537, 1489, 1398, 1286, 1249, 1073, 1011, 817, 648, 505 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, 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.

(*R*)-*N*-(1-(4-Bromophenylamino)-1-oxo-3-phenylpropan-2-yl)-5-chloro-2-hydroxybenzamide **9c**.

White solid; yield 32%; mp 239-242 °C. 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}. ^{1}H 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.

(*R*)-4-Chloro-*N*-(1-(4-chlorophenylamino)-3-methyl-1-oxobutan-2-yl)-2-hydroxybenzamide **9d**.

White solid; yield 48%; mp 204-205 °C. 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 **9e**.

White solid; yield 40%; mp 232-234 °C. 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 **9f**.

White solid; yield 40%; mp 232-234 °C. 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.

(*R*)-5-Chloro-*N*-(1-(4-chlorophenylamino)-1-oxopropan-2-yl)-2-hydroxybenzamide **9g**.

White solid; yield 49%; mp 241-243 °C. IR (KBr pellet): 3368, 1683, 1628, 1601, 1534, 1493, 1401, 1301, 1288, 1094, 1015, 825, 650, 508 cm^{-1}. ^1H 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 **9h**.

White solid; yield 39%; mp 202-205 °C. 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 **9i**.

White solid; yield 57%; mp 214-216 °C. 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 **9j**.

White solid; yield 44%; mp 236-238 °C. 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 **9k**.

White solid; yield 61%; mp 236-238 °C. 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)-1-oxo-3-phenylpropan-2-yl)-5-chloro-2-hydroxybenzamide **9l**.

White solid; yield 39%; mp 238-240 °C. 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}. ^{1}H 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.

5-Chloro-*N*-(2-(3,4-dichlorophenylamino)-2-oxoethyl)-2-hydroxybenzamide **9m**.

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}.^{1}H 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 **9n**.

White solid; yield 23%; mp 240-242 °C. 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}. ^{1}H 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 **9o**.

White solid; yield 53%; mp 201-202 °C. IR (KBr pellet): 3305, 2966, 1672, 1634, 1592, 1532, 1476, 1393, 1289, 1232, 1134, 1114, 1029, 872, 822, 650, 533 cm^{-1}. ^{1}H 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 **9p**.

White solid; yield 40%; mp 201-203 °C. IR (KBr pellet): 3304, 2966, 1672, 1634, 1592, 1532, 1476, 1393, 1289, 1232, 1134, 1114, 1030, 871, 821, 650, 533 cm^{-1}.^{1}H 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 **9q**.

White solid; yield 23%; mp 240-242 °C. 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 **9r**.

White solid; yield 34%; mp 238-240 °C. 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 **9s**.

White solid; yield 64%; mp 223-225 °C. 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 **9t**.

White solid; yield 73%; mp 226-227 °C. 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 **9u**.

White solid; yield 69%; mp 203-205 °C. 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.

5-Chloro-*N*-(2-(3-chlorophenylamino)-2-oxoethyl)-2-hydroxybenzamide **9v**.

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}. ^{1}H 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 **9w**.

White solid; yield 43%; mp 222-224 °C. 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}. ^{1}H 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 **9x**.

White solid; yield 3%; mp 225-226 °C. IR (KBr pellet): 3368, 3285, 1679, 1627, 1598, 1579, 1541, 1483, 1413, 1295, 1213, 1115, 890, 826, 787, 676, 535 cm^{-1}. ^{1}H 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 **9y**.

White solid; yield 46%; mp 244-245 °C. IR (KBr pellet): 3282, 1660, 1632, 1595m, 1535, 1481, 1418, 1375, 1355, 1279, 1251, 1200, 1170, 867, 822, 790, 771, 721, 649 cm^{-1}.

^{1}H 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 **9z**.

White solid; yield 39%; mp 211-212 °C. 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 **9aa**.

White solid; yield 60%; mp 181-183 °C. 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 **9bb**.

White solid; yield 7.5%; mp 182-184 °C. 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.

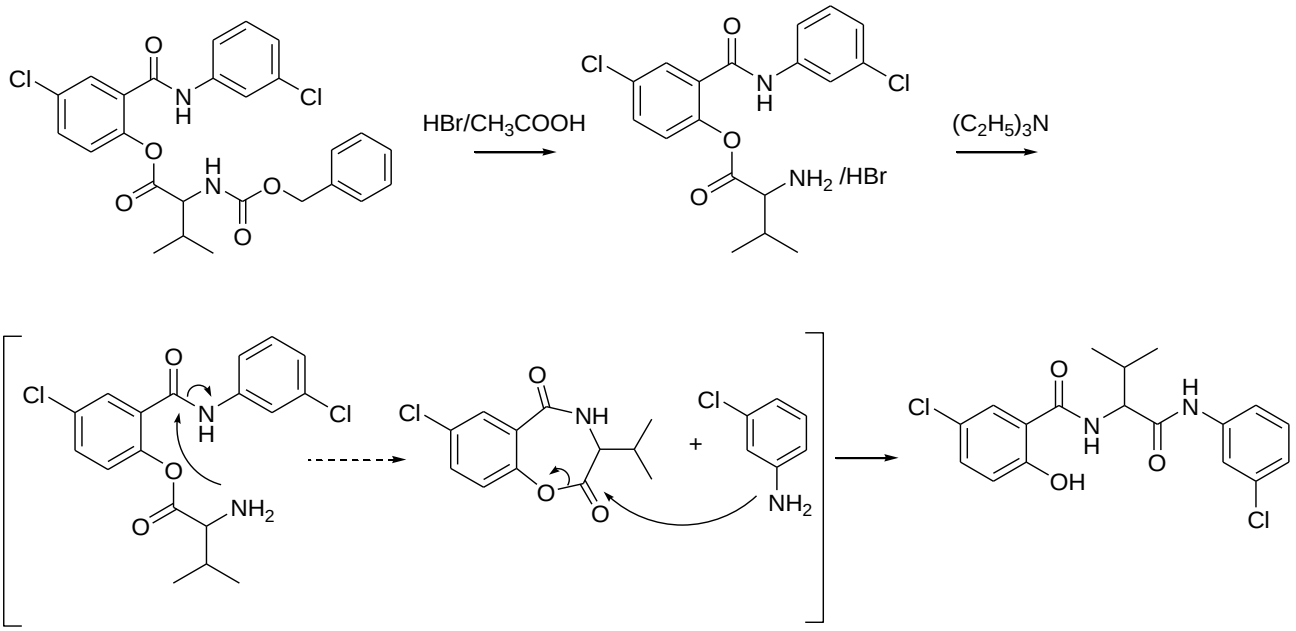
5.2. Study of the mechanism of the rearrangement: imidazolinone intermediate formation (Paper VI).

With the aim of elucidating the mechanism of the rearrangement and study its extension, several experiments were performed.

It was proved that the rearrangement occurs as well with salicylanilides without any substituent on both rings – salicylic and aniline or with electron activating methyl and methoxy group on aniline moiety. Furthermore, it is not limited only for salicylanilide esters

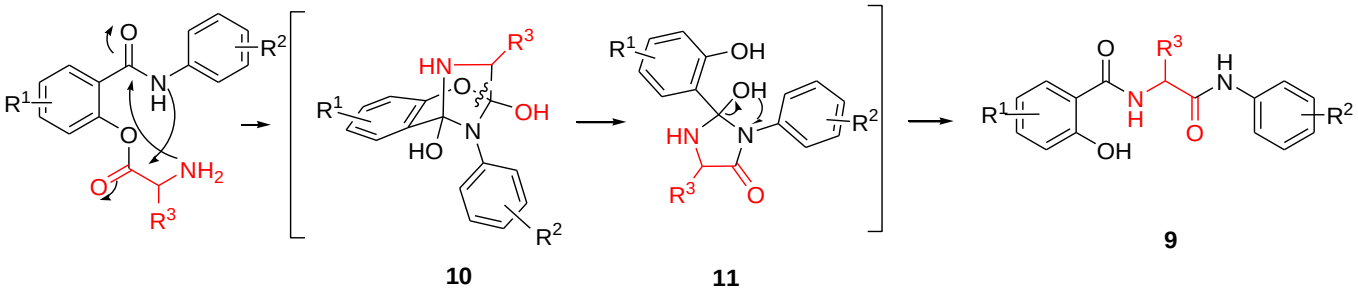
of Cbz protected AA. We have used *tert*-butyloxycarbonyl (Boc) protected Val and Leuch anhydride of Phe [154] for esterification and the appropriate SAL esters gave after *N*-deprotection the rearranged diamides which were isolated and characterized.

In a previous publication [154] it was proposed a possible mechanism for this rearrangement where the deprotected amino group immediately attacks the carbonyl of amide and forms a cyclic seven-membered ester which is opened by the action of the released aniline to produce diamide (**Scheme 15**) . In order to confirm the above-proposed mechanism, a large number of experiments were performed where activated anilines bearing a stronger nucleophilic substituent in the position 4, were added to the reaction mixture. None of the recombinant diamides was isolated, only the product with the original aniline moiety **9**. Therefore, an alternative mechanism involving the reorganization of the molecule without liberation of aniline moiety was investigated.



Scheme 15. First proposed mechanism for the rearrangement.

We hypothesised that the free amino group attacks the amidic carbonyl and amidic nitrogen attacks the ester carbonyl to generate the bicyclic intermediate 7 which is spontaneously transformed into a five-membered hydroxyimidazolinone **11** which opens under formation of diamide **9** (**Scheme 16**).



Scheme 16. Second proposed mechanism for the rearrangement.

Support for a five-membered imidazoline ring as an intermediate in this rearrangement came from our experimental results. During the purification by column chromatography of the corresponding diamide **9a**, we isolated a side-product that resulted to be a dehydrated form of this five-membered ring intermediate as a racemic mixture, 2-(5-chloro-2-hydroxyphenyl)-1-(3-chlorophenyl)-4-isopropyl-1*H*-imidazol-5(4*H*)-one **11a** (**Figure 25**). The structure of **11a** was confirmed by MS, IR, 2D NMR and the X-ray study. The same type of substituted 1*H*-imidazol-5(4*H*)-one was isolated during the purification of **9b**, 4-benzyl-2-(5-chloro-2-hydroxyphenyl)-1-(3-chlorophenyl)-1*H*-imidazol-5(4*H*)-one **11b** (**Figure 25**) and determined by IR, ^{1}H NMR and ^{13}C NMR.

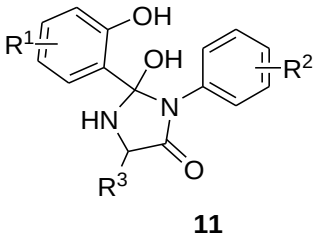


Figure 25. Isolated dehydrated reaction intermediates.

Additional structure determination of 2-(5-chloro-2-hydroxyphenyl)-1-(3-chlorophenyl)-4-isopropyl-1*H*-imidazol-5(4*H*)-one **9a** was made by ^{1}H NMR, ^{13}C NMR ^{15}N NMR in 2D experiments (gradient-selected (gs)-COSY, gs-HMQC, gs-HMBC) as well as by the construction of the computer model using a DFT method (B3LYP/6-31G(d)). Based on this model was calculated IR and ^{1}H and ^{13}C NMR spectra. Finally, the compound **11a** was also studied by X-ray diffraction techniques (**Figure 26**).

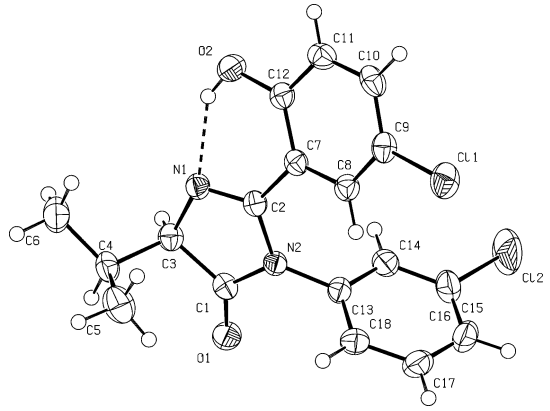


Figure 26. The ORTEP view of the compound **11a**.

6. Conclusions.

The outcomes of the literary research that summarizes all main kinds of prodrug modifications of phenolic-containing drugs is summarised in a review-article (paper I) which was sent for publication after its abstract acceptance to Pharmaceutical Drug Design journal.

The main scope of the thesis is summarised in the enclosed articles II, III and V. The results obtained in this study show that although *N*-Cbz-AA esters of SAL showed high antimycobacterial activity against all tested mycobacterial strains, their limited stability compared with the later synthesised carbamate ester of SAL make them not so interesting candidates.

The derivatization of the phenolic group of SAL in the form of carbamate appears to be the most useful approach, increasing the antimycobacterial activity and pharmacokinetic properties of SAL. Whether this is a simple effect of an increased hydrophobicity and therefore better permeability through the lipophilic mycobacterial cell wall or a direct effect of the carbamate moiety remains to be elucidated.

However, the high potencies of these newly prepared compounds, particularly those against the MDR-TB strains, together with their favourable selectivity and the stability in acidic environment which converts these compounds in good candidates for oral administration makes these SAL-derived carbamates promising drug candidates which will be further investigated.

In the course of our research, unexpected benzoxazepine-diones were formed. Unluckily, these compounds did not report any antitubercular activity. The unexpected formation of novel seven membered rings is reported in the enclosed paper IV.

The deprotection of the synthesized *N*-Cbz-AA esters yielded an unexpected product later identified as hydroxy-*N*-(phenylamino)-oxo-alkyl)benzamides. Although these compounds show some activity, an important decrease of the activity was observed compared with the starting SAL or the corresponding *N*-Cbz-AA ester derivatives. The report of this study is now being prepared for publication. The mechanism of this rearrangement was elucidated and its extension proved by the performance of several experiments. All this results were recently reported and enclosed as paper VI.

7. References.

[1] http://www.who.int/tb/en/

[2] Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. Global burden of tuberculosis - Estimated incidence, prevalence, and mortality by country. Jama 1999; 282: 677-86.

[3] Janin YL. Antituberculosis drugs: Ten years of research. Bioorg Med Chem 2007; 15: 2479-513.

[4] Petrini B, Hoffner S. Drug-resistant and multidrug-resistant tubercle bacilli. Int J Antimicrob Agents 1999; 13: 93-7.

[5] Espinal MA. The global situation of MDR-TB. Tuberculosis (Edinb) 2003; 83: 44-51.

[6] Shenoi S, Friedland G. Extensively Drug-Resistant Tuberculosis: A New Face to an Old Pathogen. Annu Rev Med 2009; 60: 307-20.

[7] Dye C, Floyd K, Gunneberg C, Hosseini M, Lonnroth K, Nathanson E, Pantoky A, Piatek M, Uplekar M, Watt C, Williams B, Wright A, Zignol M. Global tuberculosis control: surveillance, planning, financing. 1st ed Geneva: World Health Organization 2007.

[8] http://www.who.int/tb/publications/2008/factsheet_april08.pdf.

[9] Breen RA, Swaden L, Ballinger J, Lipman MC. Tuberculosis and HIV co-infection: a practical therapeutic approach. Drugs 2006; 66: 2299-308.

[10] Zhang Y, Post-Martens K, Denkin S. New drug candidates and therapeutic targets for tuberculosis therapy. Drug Discov Today 2006; 11: 21-7.

[11] Brown ED, Wright GD. New targets and screening approaches in antimicrobial drug discovery. Chem Rev 2005; 105: 759-74.

[12] Duncan K, Barry CE. Prospects for new antitubercular drugs. Curr Opin Microbiol 2004; 7: 460-5.

[13] http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5511a2.htm.

[14] Blumberg HM, Burman WJ, Chaisson RE, Daley CL, Etkind SC, Friedman LN, Fujiwara P, Grzemska M, Hopewell PC, Iseman MD, Jasmer RM, Koppaka VR, Vernon AA. American thoracic society, centers for disease control and prevention, infections diseases society of America: treatment of tuberculosis. Am. J Resir Crit Care Med 2003; 167: 603-62.

[15] Zhan Y, Amzel M. Tuberculosis drug targets. Curr Drug Targ 2002; 3: 131-54.

[16] Zhan Y, Post-Martens K, Denkin S. New drug candidates and therapeutic targets for tuberculosis therapy. Drug Discov Today 2006; 11: 21-7.

[17] Protopopova M, Bogatcheva E, Nikonenko B, Hundert S, Einck L, Nacy CA. In Search of New Cures for Tuberculosis. Med Chem 2007; 3: 301-16.

[18] Silverman RB. The Organic Chemistry of Drug Design and Drug Action. Drug Metabolism. New York, Elsevier Academic Press. 2004; 405-98

[19] Georgie GF. Drug Metabolism by the Gastrointestinal Mucosa. Clin Pharmacokinet 1981; 6: 259-74.

[20] Testa B. Prodrug research: futile or fertile. Biochem Pharmacol 2004; 68: 2097-106.

[21] Stella VJ, Borchardt RT, Hageman MJ, Oliyai R, Maag H, Tilley JW. Prodrugs: Challenges and Rewards Part 1. New York, Springer Science-Business Media. 2007.

[22] Fleisher D, Bong R, Stewart BH. Improved oral drug delivery: solubility limitations overcome by the use of prodrugs. Adv Drug Deliver Rev 1996; 19: 115-30.

[23] Stella VJ, Nti-Addae KW. Prodrug strategies to overcome poor water solubility. Adv Drug Deliver Rev 2007; 59: 677-94.

[24]. Charman WN, Porter CJH. Lipophilic prodrugs design for intestinal lymphatic transport. Adv Drug Deliver Rev 1996; 19: 149-69.

[25] Anderson BD. Prodrugs for improved CNS delivery. Adv Drug Deliver Rev 1996; 19: 171-202.

[26] Krishnamoorthy R, Mitra AK. Prodrugs for nasal drug delivery. Adv Drug Deliver Rev 1998; 29: 135-46.

[27] Takakura Y, Hashida M. Macromolecular drug carrier system in cancer chemotherapy: macromolecular prodrugs. Crit Rev Oncol Hemat 1995; 18: 207-31.

[28] Sinhababu AK, Thakker DR. Prodrugs of anticancer agents. Adv Drug Deliver Rev 1996; 19: 241-73.

[29] Niculescu-Duvaz I, Springer CJ. Antibody-directed enzyme prodrug therapy (ADEPT): a review. Adv Drug Deliver Rev 1997; 26:151-72.

[30] Denny WA. Prodrug strategies in cancer therapy. Eur J Med Chem 2001; 36: 577-95.

[31] Greenwald RB. PEG drugs: an overview. J Control Release 2001; 74: 159-71.

[32] Johansen M, Bundgaard H. Prodrugs as drug delivery systems. XVI. Novel water-soluble prodrug types of chlozoxazone by esterification of the N-hydroxymethyl derivatives. Arch Pharm Chem Sci Edn. 1981; 8: 43-54.

[33] Beaumont K, Webster R, Gardner I, Dack K. Design of ester prodrugs to enhance oral absorption of poorly permeable compounds: Challenges to the discovery scientist. Curr Drug Metab 2003; 4: 461-85.

[34] Liederer BM, Borchardt RT. Enzymes involved in the bioconversion of ester-based prodrugs. J Pharm Sci 2006; 95: 1177-95.

[35] Vinšová J, Imramovský A. Intramolekulární cyklizace využívané k uvolňovaní účinných látek z proléčiv. Chem Listy 2005; 99: 21-9.

[36] Gomes P, Vale N, Moreira R. Cyclization-activated prodrugs. Molecules 2007; 12: 2484-506.

[37] Fredholt K, Mork N, Begtrup M. Hemiesters of aliphatic dicarboxylic acids as cyclization-activated prodrug forms for protecting phenols against first-pass metabolism. Int J Pharm 1995; 123: 209-16.

[38] Santos C, Mateus ML, dos Santos AP, Moreira R, Oliveira E, Gomes P. Cyclization-activated prodrugs. Synthesis, reactivity and toxicity of dipeptide esters of paracetamol. Bioorg Med Chem Lett 2005; 15: 1595-8.

[39] Osborne R, Joel S, Trew D, Slevin M. Morphine and metabolite behaviour after different routes of morphine administration: Demonstration of the importance of the active metabolite morphine-6-glucuronide. Clin Pharmacol Ther 1990; 47: 12-9.

[40] Christrup LL, Christensen CB, Trios GJ, Jorgensen A. Improvement of buccal delivery of morphine using the prodrug approach. Int J Pharm 1997; 154: 157-65.

[41] Drustrup J, Fullerton A, Christup L, Bundgaard H. Utilization of prodrugs to enhance the transdermal absorption of morphine. Int J Pharm 1991; 71: 105-16.

[42] Kawaguchi T, Fukushima S, Hayashi Y, Nakano M. Nonenzymatic and Enzymatic hydrolysis of 5-Fluoro-2´-deoxyuridine (FudR) esters. Pharm Res 1988; 5: 741-4.

[43] Aungst BJ, Myers MJ, Shefter E, Shami EG. Prodrugs for improved oral nalbuphine bioavailability: Inter-species differences in the disposition of nalbuphine and its acetylsalicylate and anthranilate esters. Int J Pharm 1987; 38: 199-209.

[44] Norbury HM, Franklin RA, Graham DF. Pharmacokinetics of the new analgesic, meptazinol, after oral and intravenous administration to volunteers. Eur J Clin Pharmacol 1983; 25: 77-80.

[45] Lu M, Zhang C, Hao J, Qiu Z. Synthesis and relative bioavailability of meptazinol benzoyl esters as prodrugs. Bioorg Med Chem Lett 2005; 15: 2607-9.

[46] Xie Q, Wang X, Wang X, Jiang Z, Qiu Z. Design, synthesis and bioavailability evaluation of coumarin-based prodrug of meptazinol. Bioorg Med Chem Lett 2005; 15: 4953-6.

[47] Camenisch GP, Wang W, Wang B, Borchardt RT. A comparison of the bioconversion rates and the Caco-2 cell permeation characteristics of coumarin-based cyclic prodrugs and methylester-based linear prodrugs of RGD peptidomimetics. Pharm Res 1998; 15: 1174-81.

[48] Gudmundsson O, Pauletti GM, Wang W, Shan D, Zhan H, Wang B, Borchardt RT. Coumarinic acid-based cyclic prodrugs of opioid peptides that exhibit metabolic stability to peptidases and excellent cellular permeability. Pharm Res 1999; 16: 7-15.

[49] Wang W, Camenisch G, Sane DC, Zhang H, Hugger E, Wheeler GL, Borchardt RT, Wang B. A coumarin-based prodrug strategy to improve the oral absorption of RGD peptidomimetics. J Control Release 2000; 65: 245-51.

[50] Tang FX, Borchardt RT. Characterization of the efflux transporter(s) responsible for restricting intestinal mucosa permeation of the coumarinic acid-based cyclic prodrug of the opioid peptide DADLE. Pharm Res 2002; 19: 787-93.

[51] Zych LA, Yang WQ, Liao Y, Griffin KR, Wang B. The effect of substitution patterns on the release rates of opioid peptides DADLE and [Leu(5)]-enkephalin from coumarin prodrug moieties. Bioorg Chem 2004; 32: 109-23.

[52] Prankerd RD, Stella VJ. Use of oil-in-water emulsions as a vehicle for parenteral drug administration. J Parent Sci Technol 1990; 44: 139-49.

[53] Trapani G, Latrofa A, Franco M, Lopedota A, Maciocco E, Liso G. Water-soluble salts of aminoacids esters of the anaesthetic agent Propofol. Int J Pharm 1998; 175: 195-204.

[54] Altomare C, Trapani G, Latrofa A, Serra M, Sanna E, Biggio G, Liso G. Highly water-soluble derivatives of the anesthetic agent propofol: in vitro and in vivo evaluation of cyclic amino acid esters. Eur J Pharm Sci 2003; 20: 17-26.

[55] Humphrey MJ, Ringrose PS. Peptides and related drugs: a review of their absorption, metabolism and excretion. Drug Met Rev 1986; 17: 283-310.

[56] Bundgaard H. Means to enhance penetration. Prodrugs as a means to improve the delivery of peptide drugs. Adv Drug Del Rev 1992; 8: 1-38.

[57] Gangwar S, Pauletti GM, Wang B, Siahaan TJ, Stella VJ, Borchardt RT. Prodrugs strategies to enhance the intestinal absorption of peptides. Drug Discov Today 1997; 2: 148-55.

[58] Oliyai R. Prodrugs of peptides and peptidomimetics for improved formulation and delivery. Adv Drug Del Rev 1996; 19: 275-86.

[59] Fredholt K, Adrian C, Just L, Larsen DH, Weng S, Moss B, Trios GJ. Chemical and enzymatic stability as well as transport properties of a Leu-enkephalin analogue and ester prodrugs thereof. J Control Release 2000; 63: 261-73.

[60] Friedman DI, Amidon GL. Oral absorption of peptides: Influence of pH and inhibitors on the intestinal hydrolysis of Leu-enkephalin analogues. Pharm Res 1991; 8: 93-6.

[61] Hartsuck JA, Lipscomb WN. Carboxypeptidase A, In: Boyer PD Ed, The enzymes Vol. 3. New York, Academic Press. 1971; 1-56.

[62] Boyer TD, Rouff SL. Acethaminophen-induced hepatic necrosis adrenal failure. J Am Med Assoc 1971; 218: 440-1.

[63] Fadl TA, Omar FA. Paracetamol (Acetaminophen) esters of some non-esteroidal anti-inflammatory carboxylic acids as mutual prodrugs with improved therapeutic index. Inflammopharm 1998; 6: 143-57.

[64] Goolcharran C, Borchardt RT. Kinetics of diketopiperazine formation using model peptides. J Pharm Sci 1998; 87: 283-8.

[65] Safari M, Oliyai R, Stella VJ. Phosphoryloxymethyl carbamates and carbonates-novel water-soluble prodrugs for amines and hindered alcohols. Pharm Res 1993; 10: 1350-5.

[66] Gani D, Wilkie J. Stereochemical, mechanistic and structural features of enzyme-catalyzed phosphate monoester hydrolyses. Chem Soc Rev 1995; 24: 55-63.

[67] Banaszczyk MG, Carlo AT, Milan V, Lindsey A, Moss R, Carlo DJ, Hendler SS. Propofol phosphate, a water-soluble propofol prodrug: In vivo evaluation. Anesth Analg 2002; 95: 1285-92.

[68] Schywalsky M, Ihmsen H, Tzabazis A, Fechner J, Burak E, Vornov J, Schwilden H. Pharmacokinetics and pharmacodynamics of the new propofol gpi 15715 in rats. Eur J Anaesthesiol 2003; 20: 182-90.

[69] Kumpulainen H, Järvinen T, Mannila A, Leppänen J, Nevalainen T, Mäntylä A, Vepsäläinen J, Rautio J. Synthesis, in vitro and in vivo characterization of novel ethyl dioxy phosphate prodrug of propofol. Eur J Pharm Sci 2008; 34: 110-7.

[70] Pettit GR, Singh SB, Hamel E, Lin CM, Alberts DS, Garia-Kendall D. Isolation and structure of the strong cell growth and tubulin inhibitor combretastatin A4. Experientia 1989; 45: 205-11.

[71] Dorr RT, Dvorakova K, Snead K, Alberts DS, Salmon SE, Pettit GR. Antitumor activity of combretastatin-A4 phosphate, a natural product tubulin tumor inhibitor. Invest New Drug 1996; 14: 131-7.

[72] Petit GR, Temple C, Narayanan VL, Varma R, Simpson MJ, Boyd MR, Rener GA, Bansal N. Antineoplastic agents 322. Synthesis of cobretastatin A-4 prodrugs. Anti-Cancer Drug Des 1995; 10: 299-309.

[73] Tozer MT, Prise VE, Wilson J, Locke RJ, Vojnovic B, Stratford MRL, Dennis MF, Chaplin DJ. Cobretastatin A-4 phosphate as a tumor vascular-targeting agent: Early effects in tumors and normal tissues. Cancer Res 1999; 59: 1626-34.

[74] Schacter L. Etoposide Phosphate: What, why, where and how? Semin Oncol 1996; 23: 1-7.

[75] Fields SZ, Igwemezie LN, Kaul S, Schacter LP, Schiller PP, Himpler BS, McAleer C, Wright J, Barbhaiya RH. Phase I Study of etoposide phosphate (Etopophos) as a 30-minute infusion on days 1, 3 and 5. Clin Cancer Res 1995; 1: 105-11.

[76] Fields SZ, Budman DR, Young RR, Kreis W, Ingram R, Schulman P, Cherny RC, Wright J, Behr J, Snow C, Schecter LP. Phase I Study of high-dose etoposide phosphate in man. Bone Marrow Transpl 1996; 18: 851-6.

[77] Baker WR, Cai S, Dimitroff M, Fang L, Huh KK, Ryckman DR, Shang X, Shawar RM, Therrien JH. A prodrug approach toward the development of water soluble fluoroquinolones and structure-activity relationships of quinoline-3-carboxylic acids. J Med Chem 2004; 47: 4693-709.

[78] Godden DJ, Borland C, Lowry R, Higenbottan TW. Chemical specificity of coughing in man. Clin Sci 1986; 70: 301-6.

[79] Nomura Y, Abe O, Enomoto K, Fujiwara K, Tominaga T, Hayashi K, Uchino J, Takahashi M, Hayasaka A, Asaishi K, Okazaki M, Abe R, Kimishima I, Kajiwara T, Haga S, Shimizu T, Miyazaki I, Noguchi M, Yoshida M, Miura S, Taguchi T, Oota J, Sakai K, Kinoshita H, Tashiro H. Phase I Study of TAT-59 (a new antiestrogen) in breast cancer. Gan To Kagaku Ryoho 1998; 25: 553-61.

[80] Nomura Y, Nakajima M, Tominaga T, Abe O. Late phase II Study of TAT-59 (miproxifene phosphate) in advanced or recurrent breast cancer patients (a double-blind comparative study with tamoxifen citrate). Gan To Kagaku Ryoho 1998; 25: 1045-63.

[81] Heimbach T, Oh D-M, Li LY, Rodriguez-Hornedo N, Garcia G, Fleisher D. Enzyme-Mediated precipitation of parent drugs from their phosphate prodrugs. Int J Pharm 2003; 261: 81-92.

[82] Heimbach T, Oh D-M, Li L. Y, Forsberg M, Leppänen J, Matsunaga Y, Flynn G, Fleisher D. Absorption rate limit considerations for oral phosphate prodrugs. Pharm Res 2003; 20: 848-56.

[83] Henchcliffe C, Waters C. Entacapone in the management of Parkinson's disease. Expert Opin Pharmacother 2002; 3: 957-63.

[84] Savolain J, Forsberg M, Taipale H, Mannisto PT, Jarvinen K, Gynther J, Jarho P, Jarvinen T. Effects of aqueous solubility and dissolution characteristics on oral bioavailability of entacapone. Drug Dev Res 2000; 49: 238-44.

[85] Leppänen JM, Huuskonen J, Savolainen J, Nevalainen T, Taipale H, Vepsalainen J, Gynther J, Jarvinen T. Synthesis of a water-soluble prodrug of entacapone. Bioorg Med Chem Lett 2000; 10: 1967-9.

[86] Hansen J, Mork N, Bundgaard H. Phenyl carbamates of amino acids as prodrug forms for protecting phenols against first-pass metabolism. Int J Pharm 1992; 81: 253-61.

[87] Stella VJ, Borchardt RT, Hageman MJ, Oliyai R, Maag H, Tilley JW. Prodrugs: Challenges and rewards Part 2. New York, Springer Science-Business Media. 2007; 54.

[88] Thomsen KF, Bundgaard H. Cyclization-activated phenyl carbamate prodrug forms for protecting phenols against first-pass metabolism. Int J Pharm 1993; 91: 39-49.

[89] Hansen KT, Jansen JA, Mengel H, Christensen JV, Bundgaard H. Enhanced bioavailability of a new class of dopamine-D-1 antagonists following oral administration of their carbamic acid ester prodrugs to dogs. Int J Pharm 1992; 79: 205-12.

[90] Savolainen J, Leppänen J, Forsberg M, Taipale H, Nevalainen T, Huuskonen J, Gynther J, Mannisto PT, Jarvinen T. Synthesis and in vitro/in vivo evaluation of novel oral N-alkyl and N,N-dialkyl-carbamate esters of entacapone. Life Sci 2000; 67: 205-16.

[91] Nilsson JLG, Carlsson A. Dopamine-receptor agonist with apparent selectivity for autoreceptors: a new principle for antipsychotic action? Trends Pharmacol Sci 1981; 3: 322-5.

[92] Thorberg S-O, Berg S, Lundström J, Petterson B, Wijkström A, Sanchez D, Lindberg P, Nilsson JLG. Carbamates ester derivatives as potencial prodrugs of the presynaptic dopamine autoreceptor agonist (-)-3-(hydroxyphenyl)-N-propylpiperidine. J Med Chem 1987; 30: 2008-12.

[93] Nyberg L. Pharmacokinetic parameters of terbutaline in healthy man. An overview. Eur J Respir Dis 1984; 134: 149-60.

[94] Olsson T, Svensson LÅ. New lipophilic terbutaline ester prodrugs with long effect duration. Pharm Res 1984; 1: 19-23.

[95]. Svensson LÅ, Tunek A. The design and bioactivation of the presystemically stable prodrugs. Pharm Res 1988; 19: 165-94.

[96] Muther RS, Bennet WM. Effects of dimethyl sulfoxide on renal function in man. JAMA 1980; 244: 2081-3.

[97] Santos NC, Figueira-Coelho J, Martins-Silva J, Saldanha C. Multidisciplinary utilization of dimethyl sulfoxide: Pharmacological, cellular and molecular aspects. Biochem Pharmacol 2003; 65: 1035-41.

[98] Mulholland PJ, Ferry DR, Anderson D, Hussain SA, Young AM, Cook JE, Hodgkin E, Seymour LW, Kerr DJ. Pre-Clinical and Clinical Study of Qc12, a water-soluble prodrug of quercetin. Ann Oncol 2001; 12: 245-8.

[99] Frei E. III Curative cancer chemotherapy. Cancer Res 1985; 45: 6532-8.

[100] Springer CJ, Dowell R, Burke PJ, Hadley E, Davies DH, Blakey DC, Melton RG, Niculescu-Duvaz I. Optimization of alkylating agent prodrugs derived from phenol and aniline mustards: A new clinical candidate prodrug (ZD2767) for antibody-directed enzyme prodrug therapy (ADEPT). J Med Chem 1995; 38: 5051-65.

[101] Stella VJ, Borchardt RT, Hageman MJ, Oliyai R, Maag H, Tilley JW. Prodrugs: Challenges and rewards Part 2. New York, Springer Science-Business Media. 2007; 56.

[102] Kunimoto T, Nitta K, Tanaka T, Uehara N, Baba H, Takeuchi M, Yokokura T, Sawada S, Miyasaka T, Mutai M. Antitumor activity of 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin, a novel water-soluble derivative of camptothecin, against murine tumors. Cancer Res 1987; 47: 5944-7.

[103] Schmidt F, Monneret C. Prodrug mono therapy: Synthesis and biological evaluation of an etoposide glucuronide-prodrug. Bioor Med Chem 2003; 11: 2277-83.

[104] Bosslet K, Strub R, Blumrich M, Czech J, Gerken M, Sperker B, Kroemer HK, Gesson J-P, Koch M, Monneret C. Elucidation of the mechanism enabling tumor selective prodrug monotherapy. Cancer Res 1998; 57: 1195-201.

[105] de Groot FMH, Damen EWP, Scheeren HW. Anticancer prodrugs for application in monotherapy: Targeting hypoxia, tumor-associated enzymes, and receptors. Curr Med Chem 2001; 8: 1093-122.

[106] Lobo RA, Cassident DL. Pharmacokinetics of oral 17b-estradiol. J Reprod Med 1992; 37: 77-84.

[107] Elger W, Schwarz S, Hedden A, Reddersen G, Schneider B. Sulfamates of various estrogens are prodrugs with increased systemic and reduced hepatic estrogenicity at oral application. J Steroid Biochem Biol 1995; 55: 395-403.

[108] Bauer K, Elger W, Schneider B, Krahl E, Buer R. Effect of estradiol sulphamate (ES-J995) on affinity of haemoglobin for oxygen, cardiovascular function and acid-base balance in ovariectomized rats. Exp Toxic Pathol 2003; 55: 301-7.

[109] Stella VJ, Borchardt RT, Hageman MJ, Oliyai R, Maag H, Tilley JW. Prodrugs: Challenges and rewards Part 2. New York, Springer Science-Business Media. 2007; 59.

[110] Getz JJ, Prankerd RJ, Sloan KB. Mechanism of hydrolysis of O-imidomethyl derivatives of phenols. J Org Chem 1993; 58: 4913-8.

[111] Patel JU, Prankerd RJ, Sloan KB. A prodrug approach to increasing the oral potency of a phenolic drug. Part 1. Synthesis, caracterization and stability of an O-(imidomethyl) derivative of 17β-estradiol. J Pharm Sci 1994; 83: 1477-81.

[112] Longcope C, Gorbach S, Goldin B, Woods MJ, Dwyer J, Warram J. The metabolism of estradiol- Oral compared to intravenous administration. J Steroid Biochem 1985; 23: 1065-70.

[113] Patel J, Katovich MJ, Sloan KB, Curry SH, Pranker RJ. A prodrug approach to increasing the oral potency of a phenolic drug. Part 2. Pharmacodynamics and preliminary bioavailability of an orally administered O-(iodomethyl) derivative of 17β-estradiol. J Pharm Sci 1994; 84: 174-8.

[114] Fenández C, Nieto O, Rival E, Montenegro G, Fontenla JA, Fernández-Mayoralas A. Synthesis and biological studies of glycosyl dopamine derivatives as potential antiparkinsonian agents. Carbohid Res 2000; 327: 353-65.

[115] Mueckler M. Facilitative glucose transporters. Eur J Biochem 1994; 219: 713-25.

[116] Fernández C, Nieto O, Fontenla JA, Rivas MLC, Fernández-Mayoralas A. Synthesis of glycosyl derivatives as dopamine prodrugs: interaction with glucose carrier GLUT-1. Org Biomol Chem 2003; 1: 767-71.

[117] Majumdar S, Sloan KB. Synthesis and topical delivery of N-alkyloxycarbonylaminomethyl prodrugs of a model phenolic drug: Acetaminophen. Int J Pharm 2007; 337: 48-55.

[118] Wasdo S, Sloan KB. Topical delivery of a model phenolic drug: alkyoxycarbonyl prodrugs of acetaminophen. Pharm Res 2004; 21: 940-6.

[119] Thomas JD, Sloan KB. In vitro evaluation of alkylcarbonyloxymethyl (ACOM) ethers as novel prodrugs of phenols for topical delivery: ACOM prodrugs of acetaminophen. Int J Pharm 2008; 346: 80-8.

[120] Vinsova J, Imramovsky A. Salicylanilides: still a potential antibacterially active group. Cesk Slov Farm 2004; 53: 294-9.

[121] De la Fuente R, Sonawane ND, Arumainayagam D, Verkman AS. Small molecules with antimicrobial activity against E. coli and P. aeruginosa identified by high-throughput screening. Br J Pharmacol 2006; 149: 551-9.

[122] Dahlgren MK, Kauppi AM, Olsson IM, Linusson A, Elofsson M. Design, synthesis, and Multivariate quantitative structure-activity relationship of Salicylanilides-Potent inhibitors of type III secretion in Yersinia. J Med Chem 2007; 50: 6177-88.

[123] Daidone G, Maggio B, Schillaci D. Salicylanilide and its heterocyclic-analogs: a comparative study of their antimicrobial activity. Pharmazie 1990; 45: 441-2.

[124] Vinsova J, Imramovsky A, Buchta V, Ceckova M, Dolezal M, Staud F, Jampilek J, Kaustova J. Salicylanilide acetates: synthesis and antibacterial evaluation. Molecules 2007; 12: 1-12.

[125] Raffa D, Plescia S, Matera M, Caruso A, Leone V, Amico-Roxas M. Synthesis and evaluation of the analgesic and antiinflammatory activities of N-substituted salicylanilide. Farmaco 1989; 44: 465-73.

[126] Waisser K, Matyk J, Divisova H, Husakova P, Kunes J, Klimesova V, Kaustova J, Mollmann U, Dahse HM, Miko M. The oriented development of antituberculotics: salicylanilides. Arch Pharm (Weinheim) 2006; 339: 616-20.

[127] Stephenson K, Yamaguchi Y, Hoch JA. The mechanism of action of inhibitors of bacterial two-component signal transduction systems. J Biol Chem 2000; 275: 38900-4.

[128] Macielag MJ, Demers JP, Fraga-Spano SA, Hlasta DJ, Johnson SG, Kanojia RM, Russell RK, Sui Z, Weidner-Wells MA, Werblood H, Foleno BD, Goldschmidt RM, Loeloff MJ, Webb GC, Barrett JF. Substituted salicylanilides as inhibitors of two-component regulatory systems in bacteria. J Med Chem 1998; 41: 2939-45.

[129] Kamath S, Buolamwini JK. Targeting EGFR and HER-2 receptor tyrosine kinases for cancer drug discovery and development. Med Res Rev 2006; 26: 569-94.

[130] Liechti C, Sequin 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.

[131] Deng W, Guo ZR, Guo YS, Feng ZK, Jiang Y, Chu FM. Acryloylamino-salicylanilides as EGFR PTK inhibitors. Bioorg Med Chem Lett 2006; 16: 469-72.

[132] Guo L, Wang QL, Jiang QQ, Jiang QJ, Jiang YB. Anion-triggered substituent-dependent conformational switching of salicylanilides. New hints for understanding the inhibitory mechanism of salicylanilides. J Org Chem 2007; 72: 9947-53.

[133] Brown ME, Fitzner JN, Stevens T, Chin W, Wright CD, Boyce JP. Salicylanilides: Selective inhibitors of interleukin-12p40 production. Bioorg Med Chem 2008; 16: 8760-4.

[134] Boyce JP, Brown ME, Chin W, Fitzner JN, Paxton RJ, Shen M, Stevens T, Wolfson MF, Wright CD. Identification of 14-3-3 zeta by chemical affinity with salicylanilide inhibitors of interleukin-12p40 production. Bioconjugate Chem 2008; 19: 1775-84.

[135] Waisser K, Bures O, Holy P, Kunes J, Oswald R, Jiraskova L, Pour M, Klimesova V, Kubicova L, Kaustova J. Relationship between the structure and antimycobacterial activity of substituteds salicylanilides. Arch Pharm (Weinheim) 2003; 336: 53-71.

[136] Bodamský M. Principle of peptide synthesis, 2nd ed.; Springer: Berlin, 1993.

[137] Doležal M, Pálek L, Vinšová J, Buchta L, Jampílek J, Králová K. Substituted pyrazinecarboxamides: synthesis and biological evaluation. Molecules 2006; 11: 242-56.

[138] http://www.taacf.org/Process-text.htm

[139] Sedlák M, Pravda M, Štaud F, Kubicová L, Tyčova K, Ventura K. Synthesis of pH-sensitive amphotericin B-poly(ethylene glycol) conjugates and study of their controlled release in vitro. Bioorg Med Chem2007; 15: 4069-76.

[140] Ray S, Pathak SR, Chaturvedi D. Organic carbamates in drug development. Part II: antimicrobial agents recent reports. Drug Future 2005; 30: 161-232.

[141] Agouridas C, Denis A, Auger JM, Benedetti Y, Bonnefoy A, Bretin F, Chantot JF, Dussarat A, Fromentin C, D'Ambrieres SG, Lachaud S, Laurin P, Le Martret O, Loyau V, Tessot N. Synthesis and antibacterial activity of ketolides (6-O-methyl-3-oxoerythromycin derivatives): A new class of antibacterials highly potent against macrolide-resistant and -susceptible respiratory pathogens. J Med Chem 1998; 41: 4080-100.

[142] Albrecht HA, Beskid G, Christenson JG, Georgopapadakou NH, Keith DD, Konzelmann FM, Pruess DL, Rossman PL, Wei CC. Dual-action cephalosporins: cephalosporin 3'-quinolone carbamates. J Med Chem 1991; 34: 2857-64.

[143] Hansen JM, Mørk N, Bundgaard H. Phenyl carbamates of amino acids as prodrug forms for protecting phenols against first-pass metabolism. Int J Pharm 1992; 81: 253-61.

[144] Thorberg S, Berg S, Lundstrom L, Pettersson B, Wijkstrom A, Sanchez D, Lindberg P, Nilsson JG. Carbamate esters derivatives as potential prodrugs of the presynaptic dopamine autoreceptor agonist (-)-3-(3-hydroxyphenyl)-N-propylpiperidine. J Med Chem 1987; 30: 2008-12.

[145] Olsson T, Svensson LÅ. New Lipophilic Terbutaline Ester Prodrugs with Long Effect Duration. Pharm Res 1984; 1: 19-23.

[146] Imramovsky A, Vinsova J, Ferriz JM, Buchta V, Jampilek J. Salicylanilide esters of N-protected amino acids as novel antimicrobial agents. Bioorg Med Chem Lett 2009; 19: 348-51.

[147] Lenaerts AJ, Gruppo V, Marietta KS, Johnson CM, Driscoll DK, Tompkins NM, Rose JD, Reynolds RC, Orme IM. Preclinical testing of the nitroimidazopyran PA-824 for activity against Mycobacterium tuberculosis in a series of in vitro and in vivo models. Antimicrob Agents Chemother 2005; 49: 2294-301.

[148] Stover CK, Warrener P, VanDevanter DR, Sherman DR, Arain TM, Langhorne MH, Anderson SW, Towel JA, Yuan Y, Mc Murray DN, Kreiswirth BN, Barry CE, Baker WR. A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. Nature 2000; 405: 962-6.

[149] Nikonenko BV, Protopopova M, Sarnala R, Einck L, Nacy CA. Drug therapy of experimental tuberculosis (TB): Improved outcome by combining SQ109, a new diamine antibiotic, with existing TB drugs. Antimicrob Agents Chemother 2007; 51: 1563-5.

[150] Jia L, Tomaszewski JE, Hanrahan C, Coward L, Noker P, Gorman G, Nikonenko B, Protopopova M. Pharmacodynamic and pharmacokinetic characteristics of SQ109, a new diamine-based anti-tubercular drug. Br J Pharmacol 2005; 144: 80-7.

[151] Scudiero DA, Shoemaker RH. Evaluation of a soluble tetrazolium formazan assay for cell-growth and drug sensitivity in culture using human and other tumor-cell lines. Cancer Res1988; 48: 4827-33.

[152] D'Souza AJ, Topp EM. Release from polymeric prodrugs: linkages and their degradation. J Pharm Sci 2004; 93: 1962-79.

[153] Mashiko T, Hara K, Tanaka D, Fujiwara Y, Kumagai N, Shibasaki M. En Route to an Efficient Catalytic Asymmetric Synthesis of AS-3201. J Am Chem Soc 2007; 129: 11342-3.

[154] Imramovsky A, Vinsova J, Ferriz JM, Kunes J, Pour M, Dolezal M. Salilcylanilide esterification: unexpected formation of novel seven-membered rings. Tetrahedron Lett 2006; 47: 5007-11.