Salicylanilides and Their Derivates as Perspective Anti-tuberculosis Drugs: Synthetic Routes and Biological Evaluations

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Abstract: Salicylanilides are a well-known family of pharmacological compounds, which are under renewed investigation because of the discovery of novel interesting biological activities and mechanisms of action over the last decade. This comprehensive mini-review describes the biological and pharmacological properties of salicylanilides, their activity against atypical and multi-drug resistant mycobacterial strains, and synthetic routes for their preparation. In particular, this review focuses on the synthesis and biological properties of salicylanilides and *O*-substituted derivates reported between 2000 and 2010, which have displayed the highest antituberculosis or antifungal activity.

Keywords: Anti-MDR tuberculotics, anti-tuberculotic agents, hydroxybenzamides, salicylanilides, salicylanilide derivates.

1. INTRODUCTION

Salicylanilides (*N*-substituted hydroxy benzamides) are well-known organic pharmacological compounds with numerous biological activities, which were initially investigated for their antimicrobial [1] and antifungal activities [2], as well as their usefulness as topical antimycotics and antiplaque agents [3]. Salicylanilides have also found use as molluscicidal [4] or anthelmintic agents [5] in human and veterinary practise. The most successful of these, 5,2´-dichloro-4´-nitrosalicylanilide (Niclosamide), is a member of a group of common molluscicides [6] with anthelmintic properties, and is also effective in the treatment of diphyllobothriasis and hymenolepiasis [7]. Closantel is another broad-spectrum anthelmintic salicylanilide, which has been used as an anti-trematode, anti-nematode and anti-arthropod, in combination with benzimidazole anthelmintics such as Mebendazole [8]. Rafoxanide is highly active against *Fasciola Hepatica* [9] and commonly used in veterinary practise. Salicylanilides have also been investigated for their ability to cause photoallergic contact dermatitis [10]. The structures of common salicylanilide derivates in clinical or veterinary use are presented in Fig. (**1**).

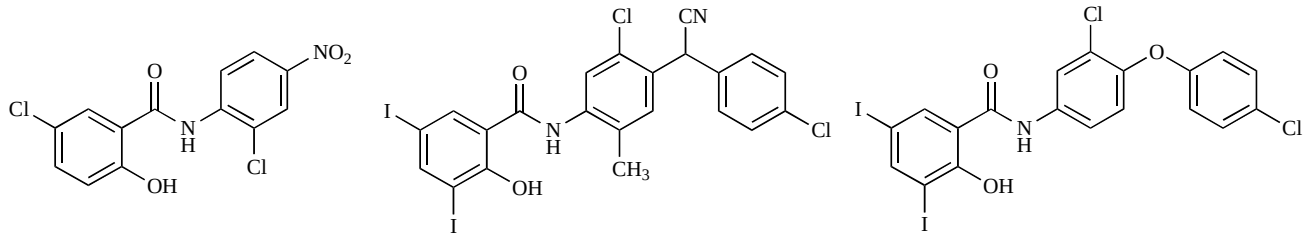

Fig. (1). Common salicylanilide derivates used in clinical or veterinary practise.

2. SALICYLANILIDES: THE DISCOVERY OF NOVEL MECHANISMS OF PHARMACOLOGICAL ACTION

Interestingly, salicylanilides were reported to contain novel anti-bacterial properties in 1998, which appear to function through inhibition of the two-component regulatory system (TCS) in bacteria [11]. Compounds that inhibit TCS block important bacterial signalling pathways, possibly resulting bacterial cell death. In addition, both an electron withdrawing substituent on the salicylic moiety and a hydrophobic group on the anilide moiety have been shown to be essential for this salicylanilide-mediated biological effect.

Research over the last decade has advanced our knowledge of the mechanisms of action of several salicylanilide derivates, while at the same time uncovering new properties of these important compounds.

Protein Tyrosine Kinase (PTK) activity is essential for fundamental signal transduction pathways, and PTK was found to be deregulated in many proliferative diseases (*e.g.* cancer, psoriasis, restenosis etc.) [12]. In fact, dysfunctional growth factor receptor PTKs are associated with a number of tumour types. As such, tyrosine kinases have become an attractive target for pharmacological inhibition, which might stop tumour growth. Compounds such as genistein (**1**) (Fig. **2**) [13], which influence PTK activity through inhibition of epidermal growth factor receptors (EGFR), may be of great therapeutic value.

Hypothetical models of the ATP-binding site of PTK, based on molecular modelling, were postulated by Furet *et al.* [14] and Palmer *et al.* [15], and have been successfully used for the development of several ATP competitive inhibitors of EGFR PTK, such as 4-(phenylamino)pyrrolo[2,3-*d*]pyrimidines (**2**) (Fig. **2**) [16], 4-(phenylamino)pyrazolo[3,4-*d*]pyrimidines (**3**) [17] and 4-(phenylamino)quinazolines (**4**) [18]. The formation of a pseudo six-membered ring in 2-hydroxy-4,5-dimethoxy-*N*-phenylbenzamide

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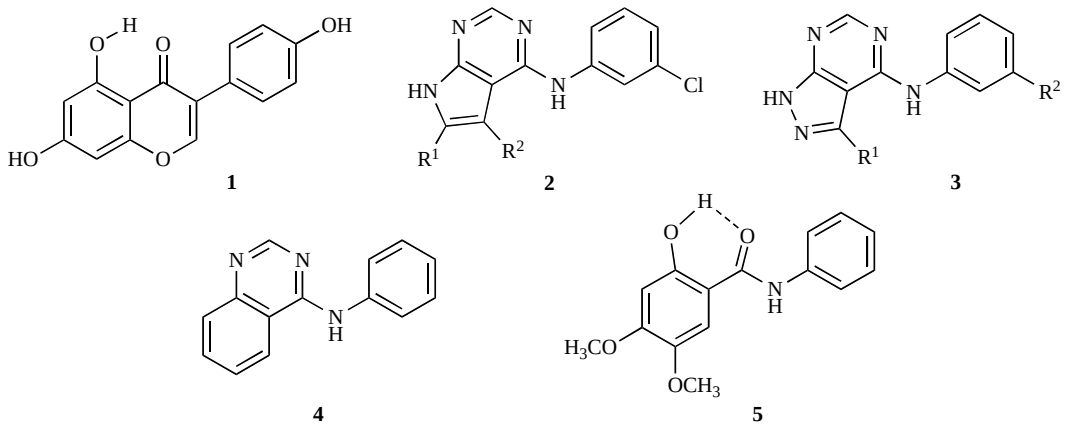


Fig. (2). Comparison of the structures of known EGFR PTK inhibitors with the pseudo six-membered ring in salicylanilide **5**.

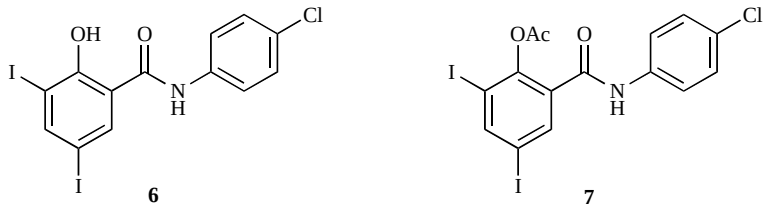


Fig. (3). Single hit from the class *Yersinia* protein secretion type III inhibitors.

(**5**), which is formed by an intramolecular hydrogen bond, has been proposed to function as a quinazoline pyrimidine ring mimic [19]. In support of this, salicylamide (**5)** superimposes very well with quinazoline (**4)**. Moreover, salicylanilides with an alkylated phenolic OH group, which prevents intramolecular hydrogen bond formation, are unable to inhibit PTK EGFR [20]. Based on these studies, a series of appropriately substituted salicylanilide EGFR PTK inhibitors were developed, with IC50 values in the 23-71 nM range [20].

More recently, Elofsson *et al.* (2007) reported identification of salicylanilides with inhibitory properties against type III protein secretion (T3S) in *Yersinia* [21]. Members of the *Yersinia* genus, such as *Y. Pestis* and *Y. pseudotuberculosis*, cause adenitis and septicaemia, while *Y. Enterocolitica* inflicts a broad range of gastrointestinal syndromes. T3S is a virulence system which is shared by many other pathogenic bacteria as well, including *Salmonella* spp., *Pseudomonas aeruginosa*, *Chlamydia Shigella* spp., and enteropathogenic *Escherichia coli* [22]. T3S is essential for bacterial penetration into host cells, allowing pathogenic bacteria to avoid the host immune response, and T3S inhibitors have been shown to significantly reduce the virulence of bacteria which use this secretion system.

Lead molecules from a series of *N*-(4-chlorophenyl)-2-hydroxy-3,5-diiodobenzamide (**6**) compounds, their *O*-acetylated derivates (**7)** (Fig. **3**), and 48 analogues, were tested for biological activity against *Yersinia pseudotuberculosis* serotype III (YPIII) strain pIB29 (*yopE-luxAB*) at four different concentrations. Based on these studies, an electronegative substituent (chlorine or iodine) at position 3 was found to be essential for the highest biological activity in *O*-acetylated derivates, while compounds without *O*-acetylation require substitution on the salicylic ring at positions 3 and 5 (see starting compound **7,** Fig. **3**). Interestingly, acetylated salicylanilides were found to be active even when the iodine at position 5 is replaced by a hydrogen [21].

The salicylanilide compounds included in this study were also shown to inhibit the growth of bacteria from the *Yersinia* family, by blocking ATP synthesis via disruption of oxidative phosphorylation, although the concentrations required were reported to be higher than that required for complete inhibition of T3S.

As mentioned above, non-acetylated salicylanilides, with substitution patterns that are highly similar to the compounds presented in this study, have been shown to inhibit two-component systems (TCSs) in gram-positive bacteria [11]. Although there are a number of TCSs in *Y. pseudotuberculosis*, no connection between TCS and T3S has been reported. However, a link between TCSs and T3S *has* been established in *Pseudomonas syringae* and *Salmonella* [23], and TCSs control the virulence response in a wide variety of pathogenic bacteria. Compounds (including salicylanilides) which target bacterial virulence have a high chance of being effective against resistant strains, and there is a possibility that virulence inhibitors will remain effective for a long time before resistance becomes an issue [21].

Azole-fused salicylamides **(8)** (Fig. **4**) have been prepared, where the azole NH was designed to replace the formamide NH in Antimycin A_1 **(9)**. Antimycin is a bislactone salicylamide, which was isolated from *Streptomyces sp.* [24]. Antimycin is a mitochondrial inhibitor, which targets the energy-coupling site of the respiratory system. Specifically, antimycin works by inhibiting the flow of electrons from cytochrome b to cytochrome c_1, and was first discovered as a potent fungicide produced by a species of *Streptomyces.* Azole-fused salicylanilides have shown excellent activity against the Qi site of complex III (*bc*1 complex) in the mitochondrial respiratory chain. In fact, the *in vitro* activity of compound **8** was tested against the mitochondrial electron transport and cellular growth of *Septoria nodorum*, and the benzotriazole analogue **(8)** is nearly equipotent with the natural product. These functional mimics might find application elsewhere, such as in Bcl2 binding for cancer chemotherapy [25].

3. SYNTHESIS OF SALICYLANILIDES

Numerous classical synthetic approaches have been reported for the synthesis of salicylanilides, which may be differ in dependance on the form of starting compound (salicylic acid), and number of synthetic steps. Of course, "one-pot synthesis" are preferred. As

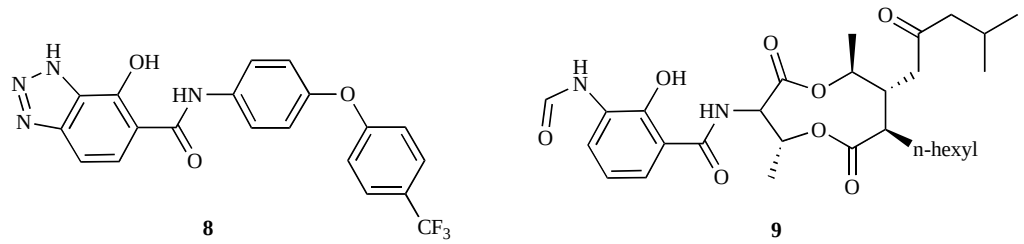
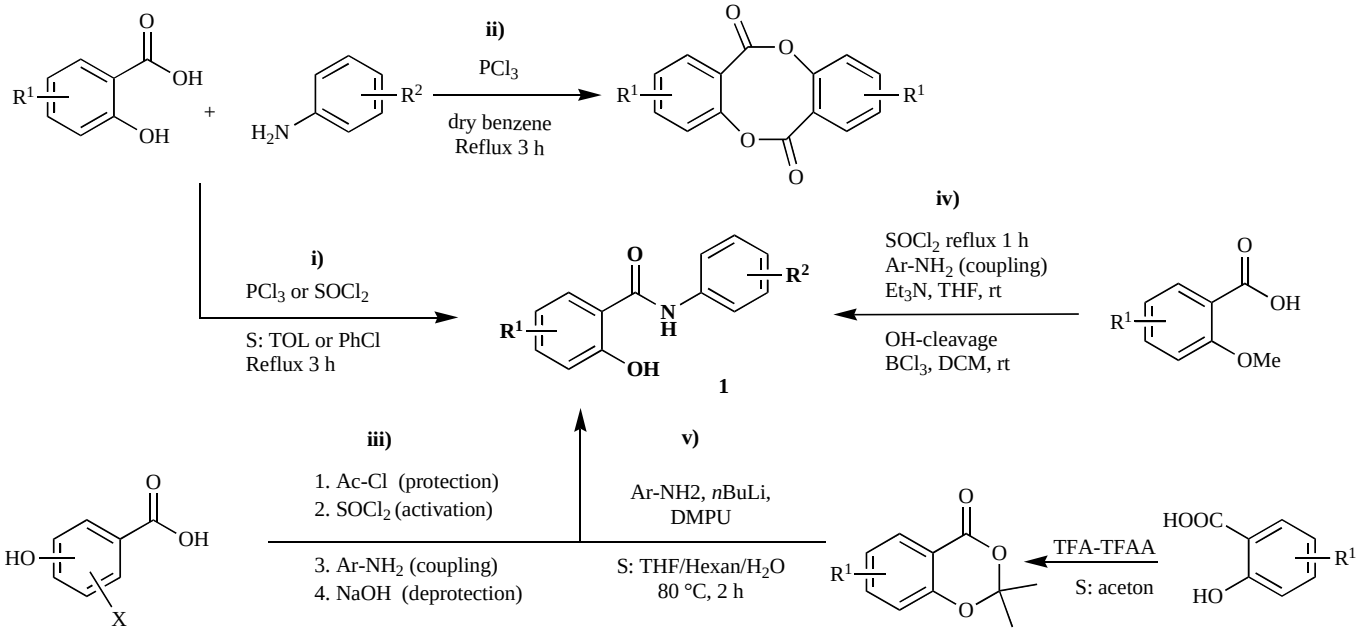
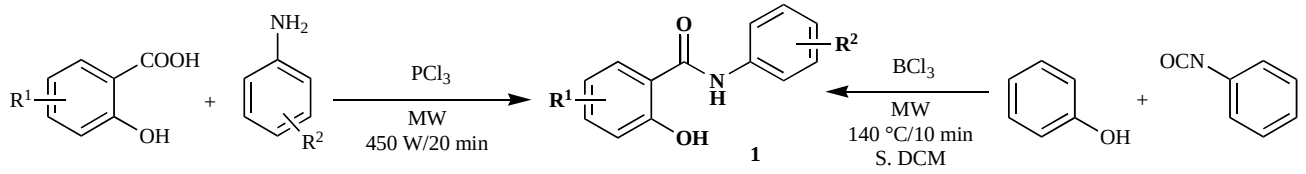


Fig. (4). Comparison of the most active azole-fused salicylamides derivate with antimycin A_1.



Scheme 1. Classical synthetic pathways for salicylanilides **(1)**.



Scheme 2. Microwave-assisted synthesis of salicylanilides.

shown in Scheme **1**: i) substituted salicylic acid and an appropriate aniline are mixed in the presence of phosphorus trichloride [26, 27] or thionyl chloride [28, 29], and refluxed in chlorobenzene or toluene. ii) Dry benzene is an unsuitable solvent because of health risks and the possible formation of undesired by-products [28]. iii) Preparations can also start from variously substituted acetylsalicylic acids, where the carboxylic group is activated with thionyl chloride, followed by coupling with aniline, and deacetylation with aqueous sodium hydroxide [30]. iv) The hydroxyl group on the starting salicylic acid can be protected as in methyl ethers and salicylic acid is activated using thionyl chloride (reflux 1 h), followed by coupling with aniline at room temperature. These mild reaction conditions are also suitable for thermally labile anilines. "OH" group cleavage is quite simple, and can be conducted at room temperature in the presence of BCl_3, using dichloromethane as a solvent [29]. v) Salicylic acid reacts in acetone with a mixture of trifluoroacetic acid (TFA) and trifluoroacetic acid anhydride (TFAA) resulting in formation of 2,2-dimethyl-4*H*-benzo[*d*][1,3]dioxin-4-one [31]. This compound is a suitable starting material for the quick reaction with aniline in the presence of *n*-BuLi and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (DMPU) in THF, yielding the required salicylanilide [32].

A more modern method for salicylanilide preparation is Microwave-assisted (MW) synthesis. This procedure is very similar to the classical method described above, but requires significantly less time. Using MW synthesis, total reaction time is reduced from several hours to minutes, while resulting in higher yields of the desired compounds [33]. The influence of solvent on MW synthesis of salicylanilides was also investigated, and a wide range of solvents have been found to be effective, including chlorobenzene, toluene, tetrahydrofurane, dimethylformamide and acetonitrile [34]. Direct MW preparation of salicylanilides is also possible via BCl_3 mediated coupling, where microwave irradiation significantly reduces the reaction time [35]. General reaction schemes for microwave-assisted preparation of salicylanilides **1** are shown in Scheme **2**.

4. BIOLOGICAL PROPERTIES OF SALICYLANILIDES

Salicylanilides have numerous biological activities, and new knowledge of their mechanisms of action have resulted in a "renaissance" of these compounds, as several research groups have been intensively investigating new derivates as potential "weapons" in the war against microbial infections. In addition, their atypical mechanisms of action and high activity against atypical mycobacte-

Table 1. Anti-TB Activities of Selected Salicylanilides

Compounds		MIC (mmol/L) Incubation time 14/21 d				
R^1	R^2	*M. tuberculosis* My 331/88	*M. kansasii* My 235/88	*M. avium* My 330/88	*M. kansasii* My 6509/96	Ref.
4-Cl	4-Cl	4/4	4/8	8/8	-	[27]
4-Cl	3-Cl	4/4	4/8	16/16	-	[27]
4-Cl	4-Br	4/4	4/4	16/16	-	[27]
5-Cl	4-CF_3	2/2	1/1	8/8	-	[27]
5-F	3,4-diCl	4/4	4/4	8/8	-	[27]
5-Br	4-Cl	32/32	32/32	16/16	32/32	[38]
5-Cl	4-Cl	32/32	32/32	16/16	32/32	[38]
5-Br	3,4-diCl	16/32	32/32	16/32	32/32	[38]
H	3-Cl	16/16	8/8	31/31	-	[2]
H	3,4-diCl	28/8	4/8	16/31	-	[2]
5-Cl	4-CH_3	16/32	4/4/	16/16	-	[36]
5-Cl	4-Br	8/16	4/4	8/8	-	[36]
4-Cl	4,3-diCl	4/4	4/4	16/16	-	[36]
5-Cl	4-NO_2	4/8	4/8	8/8	-	[36]
5-F	3,4-diCl	4/4	4/4	4/8	-	[36]
5-Cl	4-*n*Bu	4/4	8/8	8/8	4/8	[37]
5-Cl	4-*n*heptyl	4/4	8/8	4/8	8/8	[37]
5-Br	4-*n*octyl	2/4	4/4	8/8	4/4	[37]
INH		0.5/1	250/250	250/250	4/4	[27]

rial strains add to their attractiveness. For example, Waisser *et al.* reported antituberculotic (anti-TB) activity for more than 215 salicylanilide derivates over the past 10 years. The antituberculotic activities of several representative salicylanilides are shown in Table **1** [36, 37].

5. SALICYLANILIDE DERIVATES: THE INFLUENCE OF PHYSICO-CHEMICAL PROPERTIES

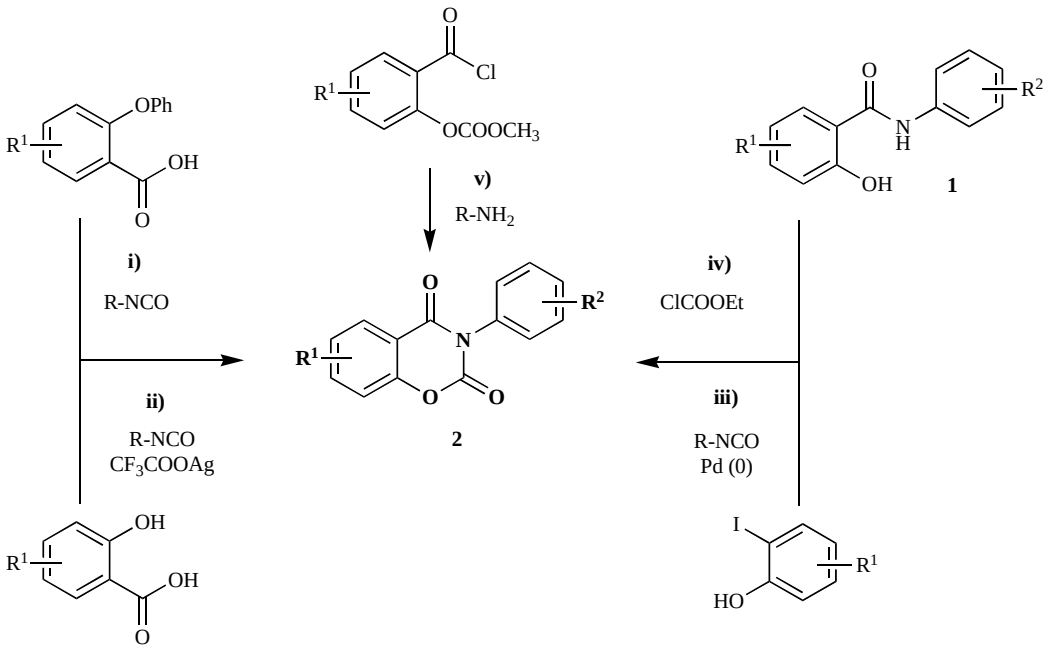
In spite of their promise as potential drugs, the physico-chemical properties of salicylanilides, such as low solubility, have prevented their widespread use in clinical practice. Thus, improving the physico-chemical properties of salicylanilides is an interesting and vital area of research. In fact, several research groups are intensively investigating new salicylanilide derivates, as well as heterocyclic salicylanilide isosters [38], acryloylamino – salicylanilides [39] and benzylsalicylamide derivates [40]. Specifically, modifications to the phenolic hydroxyl group have been shown to improve the physico-chemical properties of salicylanilide molecules. In addition, salicylanilides can be synthesised as active molecules or as inactive molecules that are then chemically or enzymatically activatable. The following text describes specific modifications of the phenolic hydroxyl group in salicylanilide synthesis.

5.1 Benzoxazepine: the Simplest Salicylanilide Derivates

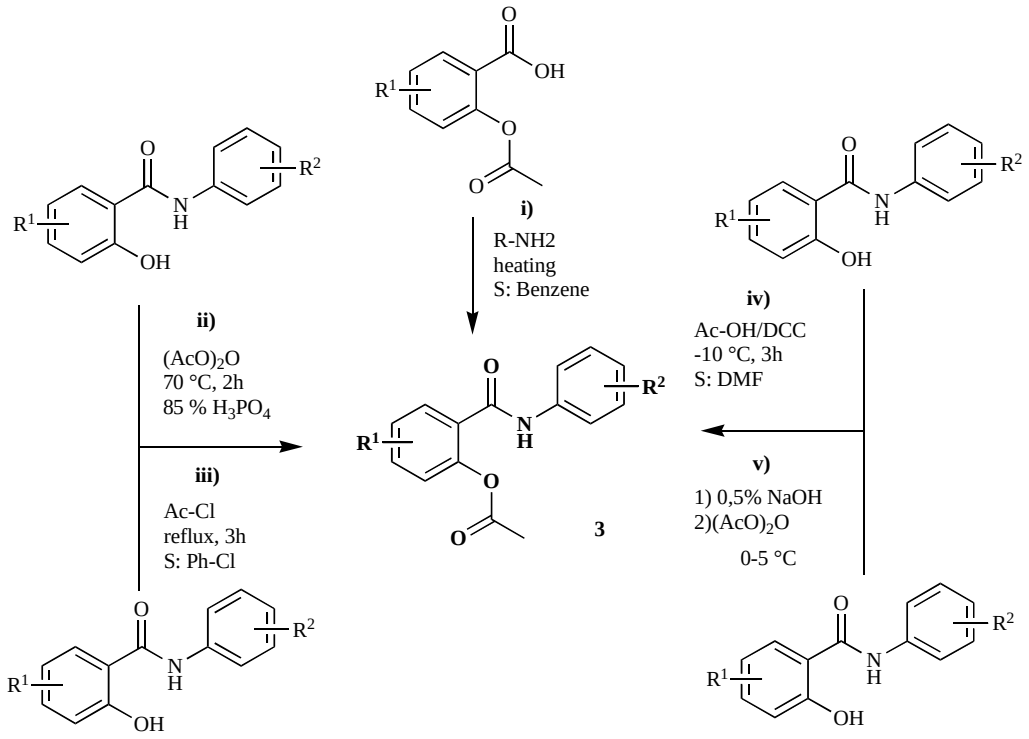
The simplest salicylanilide derivates are benzoxazines, where a carbonyl group is implemented to the salicylanilide molecule. Methods (Scheme **3**) for the synthesis of substituted benzoxazine-2,4-diones (**2**) reported in the literature include: i) reaction of phenyl salicylates with isocyanates [41]; ii) silver trifluoroacetate mediated reaction of salicylic acid with isocyanates [42]; iii) palladium catalysed cyclocarbonylation of *o*-iodophenols [43]; reaction of salicylanilides with ClCOOEt [44-47]; and v) from 2-(methoxycarbonyloxy) benzoyl chloride [48]. Waisser *et al.* (1999) reported the anti-TB properties (including QSAR between the chemical structure and antimycobacterial activity against atypical strains) of a series of these compounds [44]. In addition, a more recent study described benzoxazines as potential antifungal agents [46]. Furthermore, release of salicylanilides from the appropriate benzoxazines under basic conditions is quick and simple [49]. The biological properties of benzoxazine derivates are presented and discussed in Table **2**.

5.2 Salicylanilide Acetates: a Promising Group of anti-TB Compounds

In other salicylanilide derivates, the phenolic hydroxyl group is protected with simple groups, yielding the corresponding ester or, especially, acetate. Various methods for the synthesis of salicylanilide acetates **(3)** are summarised in Scheme **4**. Generally, i) acetylsalicylic chloride and an appropriate aniline can be used as starting material for the synthesis [50]. Other synthetic routes start from salicylanilides. ii) Heating in phosphoric acid, in the presence of acetic anhydride, gives the desired acetate at 70-85 % yield [21]. iii) We have developed other synthesis methods starting from salicylanilides, based on refluxing starting material in chlorobenzene in the presence of excess of acetylchloride for several hours. A possible side product of this reaction is an *O*,*N*-diacetylated product [51]. Therefore, another method derived from peptide chemistry was applied. iv) This reaction was carried out in dry DMF in the presence of DCC, resulting in moderate to good yields (25-75%) [51]. It is also possible to perform the reaction in water under the rules of Green Chemistry. In this case salicylanilides are first transformed to their sodium salt using sodium hydroxide. Subsequent addition of acetic anhydride is followed by precipitation of the desired acety-



Scheme 3. Reported methods for the synthesis of substituted benzoxazine-2,4-diones **(2)**.



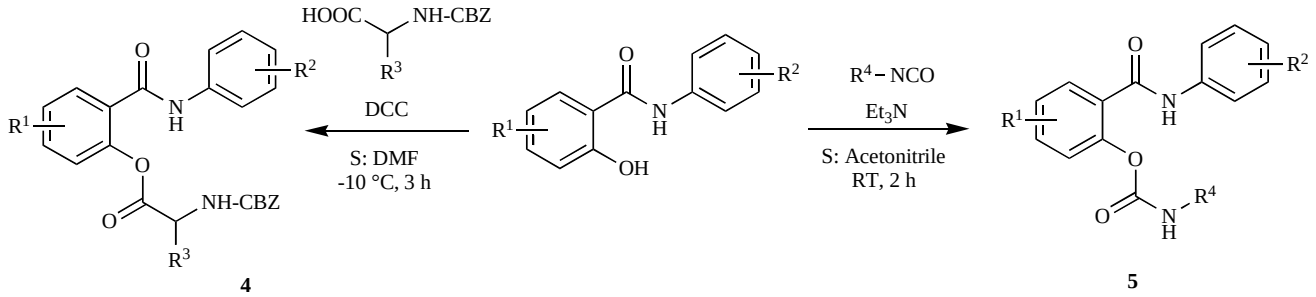
Scheme 4. Reported synthetic pathways yielding salicylanilide acetates.

lated products. Both the reaction and isolation of the desired product is carried out in cold solution (T < 5°C) and yields are approximately 50% [51]. Possible synthetic pathways for the preparation of the above mentioned acetates are described in Scheme **4**. Specific acetates were tested [51] for their antifungal and anti-TB activity, and promising results from this evaluation were used to guide the synthesis of more advanced esters, such as carbamates of salicylanilides. The biological properties of both salicylanilide esters of amino acids and carbamates are discussed below.

5.3. Amino Acid Salicylanilide Esters

Based on promising results from biological testing, our group has started to prepare a series of amino acids esters and appropriate salicylanilide carbamates.

The phenolic hydroxyl group of the salicylanilide **(1)** with the most antituberculosis activity was esterified using several *N*-benzyloxycarbonyl α-amino acids. For this purpose we have chosen more lipophilic amino acids such as Glycine, (*R* and *S*)-Alanine, (*R* and *S*)-Valine and (*R* and *S*)-Phenylalanine. Salicylanilide esters of



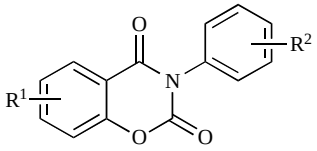
Scheme 5. Salicylanilide derivates, *N*-protected amino acid esters **(4)** and carbamates **(5)**.

amino acids can be considered to be prodrug forms, with better bioavailability due to hydroxyl group protection. The type of amino acid used for esterification influences the final physico-chemical properties and lipophilicity, which in turn affects the distribution of these drugs through the lipid mycobacterial cell membrane. Common methods for salicylanilide esterification, such as reaction of amino acid chlorides with salicylanilide phenolates failed. The most efficient reaction scheme involved DCC-mediated condensation of salicylanilides with *N*-protected amino acids. (Scheme **5**) *N*-CBZ-amino acid esters synthesised from salicylanilides displaying the most antituberculosis activity, were evaluated as antimicrobial agents [52] and anti-TB compounds [53]. Activity screening results were very interesting and are presented in Table 2. Deprotection of esters in HBr 33% in glacial acetic acid yields the appropriate hydrobromide amino salts. However, subsequent amino group liberation by triethylamine under anhydrous conditions yielded an unexpected product. The unexpected rearrangement products were identified as substituted hydroxy-*N*-(phenylamino)-oxoalkyl) benzamides [33]. Recently, we described the synthesis of these compounds [54], and proposed a mechanism for the observed rearrangement [55].

5.4. Carbamates of Salicylanilides

A similar approach for masking phenolic hydroxyl groups in salicylanilide molecules involves formation of carbamates. More practically, carbamates also satisfy requirements for easy decomposition and liberation of active molecules. Furthermore, lipophilicity of targeted molecules can also be influenced by the alkyl chain of the isocyanate molecule. The synthesis of these salicylanilides **(1)** derivates is quick and easy, as shown in Scheme **5** [56].

Table 2. Recently Reported Activities of Salicylanilide Derivates



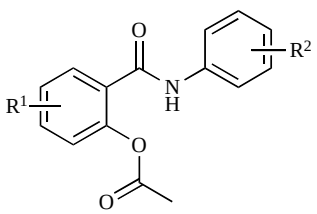
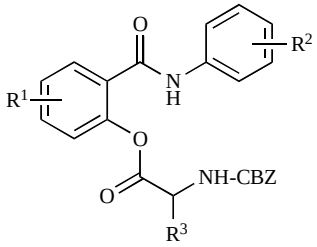
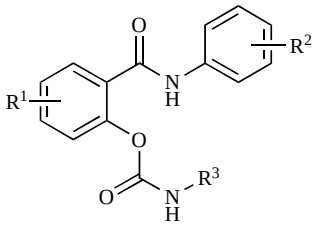
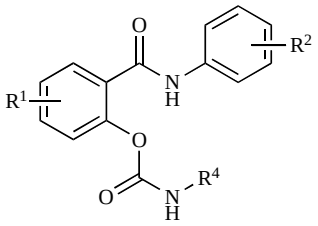
Compounds		MIC (μmol/L) Incubation time 14/21 d						
R^1	R^2	R^3	*M. tbc* My 331/88	*M. kansasii* My 235/88	*M. avium* My 330/88	*M. kansasii* My 6509/96	IC_{50} μg/mL	Lit.
6-Cl	4-octyl	-	8/8	4/8	8/8	4/4	ND	[47]
7-Cl	4-pent.	-	8/8	8/8	4/8	8/16	ND	[47]
7-Cl	4-hexyl	-	4/8	8/8	4/8	8/8	ND	[47]
6-Br	4-butyl	-	4/4	8/8	8/8	8/8	ND	[47]
6-Br	4-hept	-	4/8	8/16	4/4	4/8	ND	[47]
INH			1/2	250/250	250/250	8/8	ND	[47]
								
4-Cl	4-CF_3		4/4	2/2	4/4	2/4	12.60	[51]
4-Cl	3,4-diCl		1/1	4/4	8/8	4/4	0.82	[51]
5-Cl	4-CF_3		4/4	2/4	8/8	4/4	0.27	[51]
5-Cl	3,4-diCl		2/2	4/4	8/8	4/4	1.18	[51]
4-Cl	4-Cl		2/2	4/8	8/8	4/4	5.19	[51]
INH			0.5/1	>250/>250	>250/>250	4/4	>100	[51]

Table 2. contd…

Compounds		MIC (μmol/L) Incubation time 14/21 d						
R^1	**R^2**	**R^3**	***M. tbc* My 331/88**	***M. kansasii* My 235/88**	***M. avium* My 330/88**	***M. kansasii* My 6509/96**	**IC_{50} μg/mL**	**Lit.**
							**EC_{50} μ mol/L**	
4-Cl	4,3-diCl	(*S*)-Me	2/4	4/4	16/32	8/16	ND	[53]
4-Cl	4,3-diCl	(*S*)-*i*Pro	2/4	4/4	16/16	8/8	ND	[53]
4-Cl	4,3-diCl	(*S*)-CH_2-Ph	2/2	4/4	16/32	8/8	ND	[53]
4-Cl	4-Br	(*S*)-*i*Pro	4/4	4/8	8/16	8/8	121.8	[53]
5-Cl	4-Br	(*S*)-CH_2-Ph	4/8	4/8	16/16	16/16	35.5	[53]
5-Cl	4-Cl	(*R*)-*i*Pro	4/4	4/8	8/16	4/8	106.7	[53]
INH			0.5/0.5	>250/>250	>250/>250	4/8	ND	[53]
								
4-Cl	3-Cl	Hexyl	0.5/1	4/4	8/8	4/4	14.9	[56]
4-Cl	3,4-diCl	Ethyl	0.5/1	2/4	16/32	2/4	31.0	[56]
4-Cl	3,4-diCl	Pentyl	0.5/0.5	2/2	8/16	2/4	40.0	[56]
4-Cl	3,4-diCl	Hexyl	0.5/0.5	2/2	4/8	1/2	27.9	[56]
4-Cl	3,4-diCl	Octyl	0.5/1	2/4	4/8	2/4	48.5	[56]
4-Cl	4-Cl	Pentyl	0.5/0.5	2/4	8/8	4/4	17.4	[56]
INH			0.5/0.5	>250/>250	>250/>250	4/4	>100	[56]

ND – cytotoxicity was not determined

Table 3. Activities of Salicylanilide Carbamates Against MDR-TB Strains [56]



Compounds			MIC (μmol/L) Incubation time 14/21 d				
R^1	**R^2**	**R^3**	***M. tbc.* 7357/98**	***M. tbc.* 9449/06**	***M. tbc.* 2092/05**	***M. tbc.* Praha 1**	***M. tbc.* Praha 128**
4-Cl	3-Cl	Hexyl	1/1	1/1	1/2	1/2	1/2
4-Cl	3,4-diCl	Ethyl	0.5/1	0.5/0.5	0.5/0.5	0.5/1	0.5/0.5
4-Cl	3,4-diCl	Butyl	0.5/1	1/0.5	0.5/1	0.5/1	0.5/1
4-Cl	3,4-diCl	Pentyl	0.5/0.5	0.5/0.5	0.5/0.5	0.5/0.5	0.5/0.5
4-Cl	3,4-diCl	Hexyl	0.5/1	0.5/0.5	0.5/0.5	0.5/0.5	0.5/0.5

Table 3. contd…

Compounds			MIC (μmol/L) Incubation time 14/21 d				
R^1	R^2	R^3	*M. tbc.* 7357/98	*M. tbc.* 9449/06	*M. tbc.* 2092/05	*M. tbc.* Praha 1	*M. tbc.* Praha 128
4-Cl	3,4-diCl	Heptyl	0.5/0.5	0.5/0.5	0.5/1	0.5/1	0.5/1
4-Cl	3,4-diCl	Octyl	1/1	1/0.5	0.5/1	1/2	0.5/1
4-Cl	4-Cl	Pentyl	1/2	1/1	1/1	1/2	1/1
INH			16/16	16/16	16/16	16/16	16/16

5.5. Biological Evaluation of Salicylanilides Derivates

As mentioned above, halogenated salicylanilides have been modified in order to improve their physico-chemical properties while maintaining high pharmacological activity. A number of derivates were prepared and their antituberculosis activity was investigated. The activity of these derivates was found to be comparable to or higher than the starting salicylanilide compounds [46, 47, 51, 52, 53, 56]. In addition, these derivates clearly exceed isoniazid (INH) as a first-line antituberculotic drug against atypical mycobacterial strains such as *M. avium* or *M. kansasii*. Cytotoxicity assays indicate that salicylanilide carbamates, such as salicylanilide carbamates **5,** are moderately toxic compounds (EC_{50} was measured to be in the range of 15-50 μmol/L) in comparison with INH (EC_{50}>100 μmol/L). In contrast, the cytotoxicity of active salicylanilide *N*-protected amino acid esters (**4)** is comparable with INH (EC_{50} was in the range 82-120 μmol/L).

Acetylated salicylanilides were also tested for their anti-TB properties. Biological evaluation demonstrated very high activity against both *M tbc*. and atypical mycobacterial strains [51]. Cytotoxicity assays determined that IC_{50} values (range 60 – 0.82 μg/mL) and compounds are less toxic than first line drug INH .

The most active salicylanilide carbamates were also tested against clinically isolated multidrug resistant mycobacterial strains, including: *M. tuberculosis* 7357/98, which is resistant to INH, rifampicine (RMP), ethambutol (ETA), streptomycin (STM), ofloxacin (OFX) and ansamycin; *M. tuberculosis* 9449/06, which is resistant to INH, STM, RMP and ansamycin; *M. tuberculosis* 2092/05, which is resistant to INH, RMP, ETA, STM, OFX and ansamycin; *M. tuberculosis Praha 1*, which is resistant to INH, RMP, ETA, STM, clofazimine (CFZ) and ansamycin; and *M. tuberculosis Praha 128*, which is resistant to IHN, RMP, ETA, STM, gentamicin (GTM), CFZ, ansamycin and amikacin (AK). All of the compounds investigated exhibited high activity against the MDR-TB strains, with MIC values between 0.5–2 mol/L [56]. These activities are comparable with compounds undergoing Phase II clinical trials, such as nitroimidazopyran PA-824 [57]. Recently published results [56] from MDR-TB screening are shown in Table **3**.

Several salicylanilides and selected derivates were also tested as antifungal agents, and showed medium activity against tested fungal strains [46, 52, 56].

6. CONCLUSION

This comprehensive review summarises synthetic routes for the preparations of biologically active 2-hydroxy benzamides, generally known as salicylanilides, which have been reported in the literature between 2000 and 2010. Over the last decade, these antibacterial compounds have been "rediscovered", sparked by reports of novel, atypical mechanisms of action against pathogenic bacterial strains. Another reason for the increasingly high interest in this group of compounds is their antituberculosis activity against atypical mycobacterial strains. For example, the anti-TB activity measured against *Mycobacterium kansasii* for almost all published derivates exceeds the first-line antituberculotic drug isoniazid. Several series of salicylanilide derivates were prepared from a common scaffold. In particular, the hydroxy group of this active molecule was variously modified, to obtain benzoxazines, acetic acid or *N*-protected amino acids esters, and eventually various carbamates. Easily preparable salicylanilide carbamates show higher anti-TB activity with a low cytotoxicity profile. Moreover, their activities against clinically isolated MDR-TB strains were highly promising. *In vivo* tests are required to compare the pharmacological profiles of these promising salicylanilide derivates.

The salicylanilides and their derivates presented in this review represent a perspective group of compounds with high activity against atypical and MDR mycobacterial strains. However, there is an urgent need for further investigation into the pharmacological activity and toxicity of these important compounds.

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REFERENCES

[1] Singh, H.; Singh, A.K.; Sharma, S.; Iyer, R.N. Synthesis of 5-chloro-3´-nitro-4-substituted salicylanilides, a new series of anthelmintic and antimicrobial agents. *J. Org. Chem.*, **1977**, *20*(6), 826-829.

[2] Waisser, K.; Perina, M.; Holy, P.; Pour, M.; Bures, O.; Kunes, J.; Klimesova, V.; Buchta, V.; Kubanova, P.; Kaustova, J. Anti mycobacterial and antifungal isosters of salicylamides. *Arch. Pharm. (Weinheim)*, **2003**, *336*(6-7), 322-335.

[3] Clark, M.T.; Coburn, R.A.; Evans, R.T.; Genco, R.J. 5-(Alkylsulfonyl)salicylanilides as potential dental antiplaque agents. *J. Med. Chem.*, **1986**, *29*(1), 25-29.

[4] Nawwar, G. Salicylanilides containg amino-acid or pyran moieties with molluscicidal activity. *Arch. Pharm. (Weinheim)*, **1994**, *327*(4), 201-205.

[5] Sjogren, E.B.; Rider, M.A.; Nelson, P.H.; Bingham, S.; Poulton, A.L.; Emanuel, M.A.; Komuniecki, R. Synthesis and biological aktivity of a series of diaryl susbtituted alpha-cyano-beta hydroxypenamides, a new class of anthelmintik agents. *J. Med. Chem.*, **1991**, *34*(11), 3295-3301.

[6] (a) Lupea, A.X.; Popescu, L.; Tarabasanu, C. Synthesis of new 2-[2-(4-chlorophenylcarbamoyl)]-phenoxyalkanoic acid derivates. *Rev. Roum. Chim.* **2006**, *51*(6), 517-521. b) De La Fuente, R.D.; Sonawane, N.; Arumainayagam, D. Small molecules with antimicrobial activity against *E-coli* and *P-aeruginosa* identified by high throughput screening. *Br. J. Pharmacol.* **2006**, *149*(5), 551-559. c) Nawwar, G.; Chabaka, L.M.; Shafik, N.A. Oximinosalicylanilide like analogues as molluscicidal agents. *Afinidad* **2006**, *63*(522), 153-158.

[7] Ruttenber, A.J.; Weniger, B.G.; Sorvillo, F.; Murray, R.A.; Ford, S.L. Diphyllobothriasis associated with salmon consumption in pacific coast states. *Am. J. Trop. Med. Hyg.* **1984**, *33*(3), 455-459.

[8] Hall, C.A.; McDonnel, P.A.; Graham, J.M. Anthelmintic activity of closanthel against benzimidazole resistant strains of haemonchus-contortus and trichostrongylus-colubriformis in sheep. *Aust Vet. J.*, **1980**, *56*(9), 461-462.

[9] Campbell, N.J.; Brotowidjoyo, M.D. Efficiency of clioxanide and rafoxanide against fasciola-hepatica in sheep by different routes of administration. *Aust. Vet. J.* **1975**, *51*(11), 500-503.

[10] Harber, L.C.; Harris, H.; Baer, R.L. Photoallergic contact dermatitis - due to halogenated salicylanilides and related compounds. *Arch. Dermatol.* **1966**, *94*(3), 295-262.

[11] (a) Macielag, M.J.; Demers, J.P.; Fraga-Spano, S.A.; Hlasta, D.J.; Johnson, S.G.; Kanojia, R.M.; Russell, R.K.; Sui, Z.; Weidner-Wells, M.A.; Wer-

blood, H.; Foleno, B.D.; Goldschmidt, R.M.; Loeloff, M.J.; Webb, G.C.; Barrett, J.F. Substituted salicylanilides as inhibitors of two-component regulatory systems in bacteria. *J. Med. Chem.* **1998**, *41*(16), 2939-2945. b) Hlasta, D.J.; Demers, J.P.; Foleno, B.D.; Fraga-Spano, S.A.; Guan, J.; Hilliard, J.J.; Macielag, M.J.; Ohemeng, K.A.; Sheppard, C.M.; Sui, Z.; Webb, G.C.; Weidner-Wells, M.A.; Barret, J.F. Novel inhibitors of bacterial two-component systems with gram positive antibacterial activity: Pharmacophore identification based on the screening hit closantel. *Bioorg. Med. Chem. Lett.* **1998**, *8*(14), 1923-1928. c) Hillard, J.J.; Goldschmidt, R.M.; Licata, L.; Baum, E.Z.; Bush, K. Multiple mechanism of action for inhibitors of histidine protein kinases from bacterial two-component systems. *Antimicrob. Agents Chemother.* **1999** , *43*(7), 1693-1699.

[12] Aaronson, S.A. Growth-factors and cancer. *Science* **1991**, *254*(5035), 1146-1153.

[13] Masatoshi, H.; Shigeo, I.; Toshio, T.; Kazuo, N.; Masaaki, I.; Hiroyoshi, H. Differential effects of flavonoids as inhibitors of tyrosine protein kinases and serine/threonine protein kinases. *Biochem. Pharmacol.* **1988**, *37*(15), 2987-2992.

[14] Furet, P.; Caravatti, G.; Lydon, N.; Priestle, J.P.; Sowadski, J.M.; Trinks, U.; Traxler, P. Modelling study of protein kinase inhibitors: Binding mode of staurosporine and origin of the selectivity of CGP 52411. *J. Comput.-Aided Mol. Des.* **1995**, *9*(6), 465-472.

[15] Palmer, B.D.; Trumpp-Kallmeyer, S.; Fry, D.W.; Nelson, J.M.; Showalter, H.D.H.; Denny, W.A. Tyrosine kinase inhibitors .11. Soluble analogues of pyrrolo- and pyrazoloquinazolines as epidermal growth factor receptor inhibitors: Synthesis, biological evaluation, and modeling of the mode of binding. *J. Med. Chem.* **1997**, *40*(10), 1519-1529.

[16] Traxler, P.M.; Furet, P.; Mett, H.; Buchdunger, E.; Meyer, T.; Lydon, N. 4-(Phenylamino)pyrrolopyrimidines: Potent and selective, ATP site directed inhibitors of the EGF-receptor protein tyrosine kinase. *J. Med. Chem.* **1996**, *39*(12), 2285-2292.

[17] Traxler, P.; Bold, G.; Frei, J.; Lang, M.; lydon, N.; Hett, H.; Buchdunger, E.; Meyer, T.; Mueller, M.; Furet, P. Use of a pharmacophore model for the design of EGF-R tyrosine kinase inhibitors: 4-(Phenylamino)pyrazolo[3,4-d]pyrimidines. *J. Med. Chem.* **1997**, *40*(22), 3601-3616.

[18] Hennequin, L.F.; Stokes, E.S.E.; Thomas, A.P.; Johnstone, C.; Ple, C.A.; Ogilvie, D.J.; Dukes, M.; Wedge, S.R.; Kendrew, J.; Curwen, J. O. Novel 4-anilinoquinazolines with C-7 basic side chains: Design and structure activity relationship of a series of potent, orally active, VEGF receptor tyrosine kinase inhibitors. *J. Med. Chem.* **2002**, *45*(6), 1300-1312.

[19] Hodge, C.N.; Pierce, J. A diazine heterocycle replaces a six-membered hydrogen-bonded array in the active site of scytalone dehydratase. *Bioorg. Med. Chem. Lett.* **1993**, *3*(8), 1605-1608.

[20] 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*(1), 11-26.

[21] Dahlgren, M.K.; Kauppi, A.M.; Olsson, I.M.; 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*(24), 6177-6188.

[22] Hueck, C.J. Type III protein secretion systems in bacterial pathogens of animals and plants. *Microbiol. Mol. Biol. Rev.* **1998**, *62*(2), 379-433.

[23] Kim, C.C.; Falkow, S. Delineation of upstream signaling events in the Salmonella pathogenicity island 2 transcriptional activation pathway. *J. Bacteriol.* **2004**, *186*(14), 4694-4704.

[24] Slater, E.C. Mechanism of action of the respiratory inhibitor, antimycin. *Biochim. Biophys. Acta Rev. Bioenerg.* **1973**, *301*(2), 129-154.

[25] (a) Liu, W.; Bulgaru, A.; Haigentz, M.; Stein, C.A.; Perez-Soler, R.; Mani, S. The BCL2 -family of protein ligands as cancer drugs: The next generation of therapeutics. *Curr. Med. Chem.: Anti-Cancer Agents* **2003**, *3*(3), 217-223. b) Daniel, P.T.; Koert, U.; Schuppan, J., Apoptolidin: induction of apoptosis by a natural product. *Angew. Chem., Int. Ed.* **2006**, *45*(6), 872-893.

[26] Gong, D.; Li, J.; Yuan, C.; Yuan, J. Synthetic study of substituted arylsulfonylphenylbenzamides. *Synth. Commun.* **2005**, *35*(1), 55-66.

[27] Waisser, K.; Perina, M.; Holy, P.; Pour, M.; Bures, O.; Kunes, J.; Klimesova, V.; Buchta, V.; Kubanova, P.; kaustova, J. Anti mycobacterial and antifungal isosters of salicylamides. *Arch. Pharm. Med. Chem.* **2003**, *336*(6-7), 322-335.

[28] Hassan, G.S.; Hegazy, G.H.; Safwat, H.M. Synthesis of furo-salicylanilides and their heterocyclic derivatives with anticipated molluscicidal activity. *Arch. Pharm. Med. Chem.* **2006**, *339*(8), 448-455.

[29] Deng, W.; Guo, Z.R.; Guo, Y.S.; Feng, Z.Q.; Jiang, Y.; Chu, F.M. Acryloylamino-salicylanilides as EGFR PTK inhibitors. *Bioorg. Med.Chem. Lett.* **2006**, *16*(2), 469-472.

[30] Gschneidner, D.; Corvino, J.; Freeman, J.; O'Toole, D.; Shields, L.; Wang, E. Use of oligosalicylates in the preparation of phenolic amido acids. *Synth. Commun.* **2005**, *35*(12), 1567-1575.

[31] Russell, G.D.; Danishefsky, S.J. Total syntheses of KS-501, KS-502, and Their Enantiomers. *J. Am. Chem. Soc.* **1992**, *114*, 655-659.

[32] Souto, J.A.; Conte, M.; Alvarez, R.; Nebbioso, A.; Carafa, V.; Altucci, L.; de Lera, A. Synthesis of benzamides related to anacardic acid and their histone Acetyltransferase (HAT) inhibitory activities. *ChemMedChem* **2008**, *3*(9), 1435-1442.

[33] Imramovsky, A.; Vinsova, J.; Ferriz, J.M.; Kunes, J.; Pour, M.; Dolezal, M. Salicylanilide esterification: unexpected formation of novel seven-membered rings. *Tetrahedron Lett.* **2006**, *47*(29), 5007-5011.

[34] Colombo, M.; Bossolo, S.; Aramini, A. Phosphorus trichloride-mediated and microwave-assisted synthesis of a small collection of amides bearing strong electron-withdrawing group substituted anilines. *J. Comb. Chem.* **2009**, *11*(3), 335-337.

[35] Zhang, L.; Zhang, J.Y. Microwave-assisted synthesis of salicylamide via BCl3 mediated coupling. *J. Comb. Chem.* **2005**, *7*(4), 622-626.

[36] Waisser, K.; Hladuvkova, J.; Kunes, J.; Kubicova, L.; Klimesova, V.; Karajannis, P.; Kaustova, J. Synthesis and antimycobacterial activity of salicylanilides substituted in position 5. *Chem. Pap.* **2001**, *55*(2), 121-129.

[37] Waisser, K.; Matyk, J.; Divisova, H.; Husakova, P.; Kunes, J.; klimesova, V.; Mollmann, U.; Dahse, H. M.; Miko, M. The oriented development of antituberculotics: Salicylanilides. *Arch. Pharm. (Weinheim, Ger.)* **2006**, *339*(11), 616-620.

[38] Matyk, J.; Waisser, K.; Drazkova, K.; Kunea, J.; Klimesova, V.; Palat, K. Jr.; Kaustova, J. Heterocyclic isosters of antimycobacterial salicylanilides. *Il Farmaco* **2005**, *60*(5), 399-408.

[39] Deng, W.; Guo, Z.; Feng, Z.; Juany, Y.; Chu, F. Acryloylamino-salicylanilides as EGFR PTK inhibitors. *Bioorg. Med. Chem. Lett.* **2006**, *16*(2), 469-472.

[40] Waisser, K.; Peřina, M.; Klimešová, V.; Kaustová, J. On the relationship between the structure and antimycobacterial activity of substituted *N*-benzylsalicylamides. *Collect. Czech. Chem. Commun.* **2003**, *68*(7), 1275-1293.

[41] Boontheung, P.; Perlmutter, P. A highly-Efficient synthesis of benzoxazine-2,4-diones. *Tetrahedron Lett.* **1998**, *39*(17), 2629-2630.

[42] Shibuya, I.; Goto, M.; Shimizu, M.; Yanagisawa, M.; Gama, Y. Silver(I) ion-mediated desulfurization-cyclization of isothiocyanates with several hydroxy acids and *N*-substituted amino acids. *Heterocycles* **1999**, *51*(11), 2667-2673.

[43] Larksarp, C.; Alper, H. Palladium-catalyzed cyclocarbonylation of o-iodophenols and 2-hydroxy-3-iodopyridine with heterocumulenes: Regioselective synthesis of benzo[e]-1,3-oxazin-4-one and pyrido[3,2-e]-1,3-oxazin-4-one derivatives. *J. Org. Chem.* **1999**, *64*(25), 9194-9200.

[44] Waisser, K.; Machacek, M.; Dostal, H.; Gregor, J.; Kubicova, L.; Klimesova, V.; Kunes, J.; Palat, K. Jr.; Hladuvkova, J.; Kaustova, J.; Mollmann, U. : Relationships between the chemical structure of substances and their antimycobacterial activity against atypical strains. Part 18. 3-phenyl-2H-1,3-benzoxazine-2,4(3H)-diones and isosteric 3-phenylquinazoline-2,4 (1H, 3H)-diones. *Collect. Czech. Chem. Commun.* **1999**, *64*(11), 1902-1924.

[45] Zhu, X.; Yu, Q.S.; Greig, N.H.; Flippen-Anderson, J.L.; Brossi, A.A simple one-pot synthesis of benzoxazine-2,4-diones and benzothiazine-2,4-diones. *Heterocycles* **2003**, *59*(1), 115-128.

[46] Skala, P.; Machacek, M.; Vejsova, M.; Kubicova, L.; Kunes, J.; Waisser, K. Synthesis and antifungal evaluation of hydroxy-3-phenyl-2H-1,3-benzoxazine-2,4(3H)-diones and their thioanalogs. *J. Heterocycl. Chem.* **2009**, *46*(5), 873-880.

[47] Waisser, K.; Matyk, J.; Divisova, H.; Husakova, P.; Kunes, J.; Klimesova, V.; Palat, K.; Kaustova, J. The oriented development of antituberculotics (Part II): Halogenated 3-(4-alkylphenyl)-1,3-benzoxazine-2,4-(3H)-diones. *Arch. Pharm. (Weinheim, Ger.)* **2007**, *340*(5), 264-267.

[48] Heinisch, L.; Wittman, S.; Stoiber, T.; Berg, A.; Ankel-Fuchs, D.; Mollmann, U. Highly antibacterial active aminoacyl penicillin conjugates with acylated bis-catecholate siderophores based on secondary diamino acids and related compounds. *J. Med. Chem.* **2002**, *45*(14), 3032-3040.

[49] Kowalski, P.; Jakowska, J.; Bojarski, A.J.; Duszynska, B. The synthesis of cyclic and acyclic long-chain arylpiperazine derivatives of salicylamide as serotonin receptor ligands. *J. Heterocycl. Chem.* **2008**, *45*(1), 209-214.

[50] Biaggi, G.; Giorgi, I.; Livi, O.; Scartoni, V.; Barili, P.L.; Calderone, V.; Martinotti, E. New 5-substituted-1-(2-hydroxybenzoyl)-benzotriazoles, potassium channel activators. IV. *Farmaco* **2001**, *56*(11), 827-834.

[51] 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), 1-12.

[52] Imramovsky, A.; Vinsova, J.; Ferriz, J.M.; Buchta, V.; Jampilek, J. Salicylanilide esters of *N*-protected amino acids as novel antimicrobial agents *Bioorg. Med. Chem. Lett.* **2009**, *19*(2), 348-351.

[53] Imramovsky, A.; Vinsova, J.; Ferriz, J.M.; Dolezal, R.; Jampilek, J.; Kaustova, J.; Kunc, F. New antituberculotics originated from salicylanilides with promising *in vitro* activity against atypical mycobacterial strains. *Bioorg. Med. Chem.* **2009**, *17*(10), 3572-3579.

[54] Imramovsky, A.; Ferriz, J.M.; Pauk, K.; Kratky, M.; Vinsova, J. Synthetic Route for the Preparation of 2-Hydroxy-*N*-[1-(2-hydroxyphenylamino)- 1-oxoalkan-2-yl]benzamides. *J. Comb. Chem.* **2010**, *12*(4), 414-416.

[55] Vinsova, J.; Imramovsky, A.; Kratky, M.; Ferriz, J.M.; Palat, K. Jr.; Lycka, A.; Ruzicka, A. An unprecedented rearrangement of salicylanilide derivatives: imidazolinone intermediate formation. *Tetrahedron Lett.* **2010**, *51*(1), 23-26.

[56] Ferriz, J.M.; Vavrova, K.; Kunc, F.; Imramovsky, A.; Stolarikova, J.; Vavrikova, E.; Vinsova, J. Salicylanilide carbamates: Antitubercular agents active against multidrug-resistant Mycobacterium tuberculosis strains. *Bioorg. Med. Chem.* **2010**, *18*(3), 1054-1061.

[57] Lenaerts, A.J.; Gruppo, V.; Marietta, K.S.; Johnson, C.M.; Driscoll, D.K.; Tompkins, N.M.; Rose, J.D.; Reynolds, R.C.; Orme, I.M. 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*(6), 2294-2301.

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