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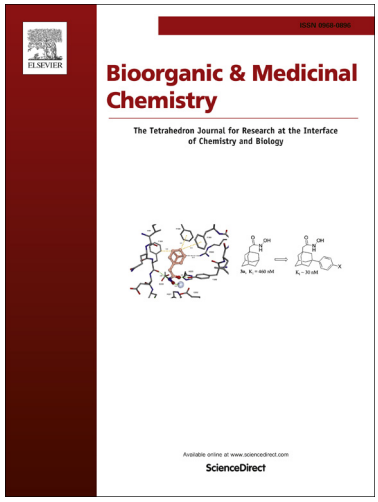

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Synthesis and Biological Activity of New Salicylanilide *N*,*N*-Disubstituted Carbamates and Thiocarbamates

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Abstract

The development of novel antimicrobial drugs represents a cutting edge research topic. In this study, 20 salicylanilide *N*,*N*-disubstituted carbamates and thiocarbamates were designed, synthesised and characterised by IR, ^{1}H NMR and ^{13}C NMR. The compounds were evaluated *in vitro* as potential antimicrobial agents against *Mycobacterium tuberculosis* and nontuberculous mycobacteria (*M. avium* and *M. kansasii*) as well as against eight bacterial and fungal strains. Additionally, we investigated the inhibitory effect of these compounds on mycobacterial isocitrate lyase and cellular toxicity. The minimum inhibitory concentrations (MICs) against mycobacteria were from 4 μM for thiocarbamates and from 16 μM for carbamates. Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus*, were inhibited with MICs from 0.49 μM by thiocarbamates, whilst Gram-negative bacteria and most of the fungi did not display any significant susceptibility. All (thio)carbamates mildly inhibited isocitrate lyase (up to 22%) at a concentration of 10 μM. The (thio)carbamoylation of the parent salicylanilides led to considerably decreased cytotoxicity and thus improved the selectivity indices (up to 175). These values indicate that some derivatives are attractive candidates for future research.

Keywords

antimicrobial activity; antimycobacterial activity; cytotoxicity; isocitrate lyase inhibition; salicylanilide carbamate; salicylanilide thiocarbamate

1. Introduction

In the 1970s, tuberculosis (TB) was thought to have been nearly eradicated; however, it is currently one of the most deadly infectious diseases in the world. The main reasons for this unpleasant reality are co-infection with HIV and the development of drug resistance. It is estimated that 5% of the more than 9 million people who develop TB annually are infected with multidrug-resistant tuberculosis (MDR-TB), i.e., a strain that is resistant to at least isoniazid (isonicotinohydrazide, INH; Fig. 1) and rifampicin (RIF). Other drug-resistant forms of tuberculosis have been described and defined. Many reasons and risk factors promoting the development of drug resistance have been proposed and explored. The treatment of resistant TB is more complicated. In 2006, the WHO declared an ambitious global plan to eradicate TB by the year 2050. This plan should involve increased research efforts including the development and testing of novel potential antimycobacterial drugs and/or vaccines.[1]

Nontuberculous (atypical) mycobacteria are increasingly recognised as causative agents of various opportunistic human infections. In general, their drug treatment is long and complicated due to additional factors including the high levels of natural and acquired antibiotic resistance conferred by a range of mechanisms. The current treatment regimens for diseases caused by nontuberculous mycobacteria share limited efficacy.[2]

Similar to mycobacterial species, problems with drug-resistance have been reported for many other bacterial strains, although it is possible to observe particular amelioration in some cases. Methicillin-resistant *Staphylococcus aureus* (MRSA), *Streptococcus pneumoniae*, enterococci, *Pseudomonas aeruginosa* or the family of *Enterobacteriaceae* that produce extended-spectrum β-lactamases (ESBL) represent common pathogens with problematic drug susceptibility or nosocomial strains.[3] Mycoses account for the most frequent infections in humans, and they have become an important public health issue because of the increasing number of immunocompromised patients. The development of drug resistance also complicates therapy.[4]

The reported facts justify an intensive future search for antimicrobial chemotherapeutics, especially those with unique mechanisms of action without any cross-resistance to approved and clinically established drugs. The development of those antibiotic agents should also overcome organisational, economic and marketing obstacles.

Some *O*-aromatic carbamates and thiocarbamates have been reported as potential antimicrobial agents, in some cases with excellent activity. For example, tolnaftate and tolciclate (Fig. 1), two thiocarbamate inhibitors of squalene epoxidase, represent clinically used antifungal agents against dermatophytes.[5,6] The analogues of the antituberculosis drug, PA-824, in which the OCH_2 linkage is replaced with carbamate functionality, exhibited more favourable properties[7]; the introduction of the *N*,*N*-dimethylthiocarbamoyl moiety into the aromatic molecule of aureol substantially increased its antimycobacterial potency.[8] In addition, carbamates and thiocarbamates represent scaffolds that are often incorporated in the prodrug design including those oriented on antimicrobial drugs.[9-13] *O*-substituted salicylanilides have exhibited significant antimicrobial activity against mycobacteria, Gram-positive bacteria and moulds; however, they share pronounced cytotoxicity.[14]

The main impetus for this study was the work of Férriz et al.,[15] which reported promising *in vitro* activity of chlorinated salicylanilide *N*-alkyl carbamates with minimum inhibitory concentrations (MICs) against both drug-sensitive and MDR *M. tuberculosis* within the range of 0.5 to 4 μM; nontuberculous mycobacteria were inhibited within the range of 2 to 32 μM. Moreover, they displayed acceptable cytotoxicity and stability profiles. It was suggested that the lipophilicity of the carbamates plays an important role in the biological activity of these compounds.

Various benzanilides, salicylanilides and their esters have been described as being mostly mild inhibitors of mycobacterial isocitrate lyase (ICL); some of these compounds were comparable or superior to 3-nitropropionic acid (3-NP), a known ICL inhibitor.[16-18] ICL represents one of the glyoxylate-shunt-pathway enzymes, which is considered to be an attractive drug target especially for persistent mycobacteria with an assumed potential to shorten and simplify the TB treatment. In particular, compounds with dual activity against actively growing and non-replicating mycobacterial subpopulations should be more beneficial.

Based on presented findings, we designed and evaluated a series of salicylanilide *N*,*N*-dialkyl/aryl carbamates and *N*,*N*-dimethylthiocarbamates to determine the influence of *N*,*N*-disubstitution by various alkyls/aryls and thionation on antimicrobial activity, cytotoxicity and isocitrate lyase inhibition.

2. Results and discussion

2.1 Chemistry

*Parent salicylanilides (**SAL-1-SAL-5**) were synthesised from salicylic and 5-chlorosalicylic acids and appropriate anilines using microwave irradiation and phosphorus trichloride via a previously described method.*[19] Based on previously reported structure-activity relationships,[14,15,17] we selected salicylanilides derived from 5-chlorosalicylic acid and 4-substituted anilines; unsubstituted salicylanilide **SAL-5** was used for the comparison.

Salicylanilide carbamates and thiocarbamates (**1**-**5**) were synthesised by direct carbamoylation of *in situ* generated salicylanilide triethylammonium salts by (thio)carbamoyl chlorides in dichloromethane (DCM) at rt: 48 h for carbamates and 72 h for thiocarbamates. Yields ranged from 55 to 99%, and thiocarbamates produced generally less yields than corresponding carbamates. The synthetic plan is depicted in Scheme 1.

When dry acetonitrile (MeCN) was used as the solvent instead of DCM at rt, it was possible to shorten the reaction time to 18 h for carbamates and 24 h for thiocarbamates. All these reaction conditions produced desired compounds in similar yields.

We synthesised five parent salicylanilides (**SAL-1**-**SAL-5**), fifteen salicylanilide carbamates and five thiocarbamates. The presented compounds are summarised in Table 1.

2.2 Antimycobacterial activity

Salicylanilides, their carbamates and thiocarbamates were evaluated for their *in vitro* antimycobacterial activity against *M. tuberculosis* 331/88 ($H_{37}Rv$), *Mycobacterium avium* 330/88 and two strains of *M. kansasii*: 235/80 and a clinical isolate, 6509/96. The first-line antituberculosis drug, isoniazid (INH), and *p*-aminosalicylic acid (PAS; Fig. 1) as a structurally similar second-line drug were chosen as the reference compounds.

Salicylanilide *N*,*N*-disubstituted carbamates and thiocarbamates (**1**-**5**) showed mostly a significant antimycobacterial activity (MIC $\geq$ 4 μM; Table 1). One molecule (**5b**) did not show any activity at a concentration of 1000 μM and for four derivatives (**3d**, **4a**, **4c**, **4d**), it was impossible to determine the exact MIC values because of the precipitation in the testing medium. When parent salicylanilides were compared, 4-trifluoromethyl group bearing salicylanilide **SAL-4** expressed the lowest MIC, followed by chlorinated **SAL-1** and brominated **SAL-2** salicylanilides, which is consistent with previous results. The halogenation of the salicylanilide scaffold enhanced the antimycobacterial activity (**SAL-5** vs. other salicylanilides).

Mycobacterium tuberculosis showed the highest susceptibility (MICs $\geq$ 4 μM with **4b** and **1b** superiority), whereas *M. avium* with MICs $\geq$ 16 μM (reached by three thiocarbamates **1b**, **2b** and **3b**) was shown to be the least susceptible; these findings reflect the activity of parent salicylanilides (**SAL-1**-**SAL-5**). The growth of both *M. kansasii* strains was suppressed most effectively by thiocarbamate **1b** with the lowest MIC values of 8-16 μM. No *O*-salicylanilide derivative exceeded the MIC of isoniazid against *M. tuberculosis* $H_{37}Rv$ and clinically isolated strain of *M. kansasii* 6509/96, whereas four carbamates (**1a**, **2a**, **3a**, **5d**) and thiocarbamates (**1b**, **2b**, **3b**, **4b**) exhibited clearly lower MICs for *M. avium* than INH. Thirteen newly synthesised compounds (**1a-d**, **2a-d**, **3a-c**, **4b** and **5d**) inhibited *M. kansasii* 235/80 at lower concentrations than this substantial first-line anti-TB drug. All halogenated (thio)carbamates with presented MIC values surpassed *p*-aminosalicylic acid, a second-line oral drug with a similar structural fragment, towards *M. tuberculosis* and *M. kansasii* 235/80, but only thiocarbamates produced the same results as well for *M. avium*.

Surprisingly, the conversion of salicylanilides to their *N*,*N*-disubstituted (thio)carbamates did not produce noticeable improvement in antimycobacterial activity, as it was reported previously for *N*-

alkyl carbamates with excellent *in vitro* activity.[15] With one exception (**5d**), no (thio)carbamate showed significantly lower MICs than the original molecule; only in some cases, the activity of thiocarbamates was comparable (i.e., equal or with a difference of one dilution) to parent salicylanilides (**SAL-1**-**SAL-5**) for all or some strains (**1b**, **2b**, **3b**). Modification of the most potent salicylanilide **4** produced carbamates with sharply reduced activity (**4a**, **4c**, **4d**). Thiocarbamates of halogenated salicylanilides exhibited decreased MIC values than the corresponding carbamate isosters (**1a** vs. **1b**, **2a** vs. **2b**, **3a** vs. **3b**, **4a** vs. **4b**) indicating that the presence of sulphur is favourable for the antimycobacterial action. The reason may be related to the enhanced lipophilicity (but it is not a general rule because more lipophilic *N*,*N*-diphenylcarbamates do not produce the best growth inhibition), modified steric parameters or different hydrolytic stability. For *M. tuberculosis* and *M. avium*, *N*-substitution by two methyl groups represents the most convenient pattern; substitution of one methyl by phenyl led to the mild reduction of the activity, whereas the presence of two phenyls on carbamate nitrogen abolished the antimycobacterial properties completely at low concentrations (**1d**, **2d**, **3d**, **4d**). It seems that bulkier *N*-substitution negatively modulates the described biological activity. For *M. kansasii*, the activity is not substantially influenced by the *N*-substitution and *N*,*N*-dimethyl, *N*-methyl-*N*-phenyl and *N*,*N*-diphenyl carbamates share similar MICs.

Interestingly, derivatives of unsubstituted salicylanilide **5** expressed a mostly inverse structure-activity relationship: *N*,*N*-dimethylthiocarbamate **5b** is less active than analogous *N*,*N*-dimethylcarbamate **5a**; *N*,*N*-diphenylcarbamate **5d** possessed lower MIC values than other carbamates **5a** and **5c**.

Based on the comparison of highly antimycobacterial active *N*-alkylcarbamates (MIC for *M. tuberculosis* within the range of 0.5 to 4 μM)[15] and less antimycobacterial active salicylanilide *N*,*N*-disubstituted carbamates in this series, we propose three possible hypotheses to explain this phenomenon. First, the presence of one hydrogen on the carbamate nitrogen is necessary for effective binding to the cellular target(s), which are currently still not exactly known, most likely *via* hydrogen bonding. Second, the longer lipophilic aliphatic tail is more convenient for binding to the target site, which is the hydrophobic pocket or cavity. The third hypothesis may involve somewhat different hydrolytic behaviour of various carbamates. Previously, it was demonstrated that aryl-O-CO-NH-alkyl carbamates show a higher chemical and metabolic lability than carbamates with a general aryl-O-CO-N(alkyl)$_2$ structure.[20-21] Based on this third hypothesis, carbamate prodrugs serve as temporary salicylanilide depot pool.

2.3 Isocitrate lyase inhibition

Salicylanilide carbamates and thiocarbamates (**1**-**5**) were evaluated for their inhibition of mycobacterial isocitrate lyase (Table 1). The isocitrate lyase (ICL-1) inhibition activity was assayed *via* glyoxylate phenyl hydrazone formation at the concentration of investigated compounds being 10 μM. INH was employed as a negative control (inhibition of 0%), when 3-nitropropionic acid (3-NP) served as a positive control.

(Thio)carbamates expressed consistent but mild inhibition within the range of 3 to 22% at the concentration of 10 μM. Three carbamates (**1d**, **3d**, **4d**) demonstrated ≥ 20% ICL inhibition with *N*,*N*-diphenylcarbamate **3d** superiority (22%), thus being slightly less active than 3-NP (25%). Halogenated salicylanilides and their derivatives seem to be stronger inhibitors than those based on unsubstituted salicylanilide **SAL-5**; the trend of the inhibition activity decrease of the parent compound **SAL-5** is observed with *O*-(thio)carbamoylation, which is in contrast to 4-trifluoromethylaniline-substituted salicylanilide **SAL-4** and its derivatives **4a**-**4d**, generally the most potent inhibitor scaffold. In the case of 4-monohalogenated salicylanilides **SAL-1**, **SAL-2** and **SAL-3**, their dimethyl(thio)carbamates share lower inhibition, while phenylcarbamates enhance this action when compared to parent compounds. For halogenated salicylanilides, the following is the inhibitory potency in decreasing order: diphenylcarbamates > (methyl)phenylcarbamates > dimethylcarbamates ≈ dimethylthiocarbamates; this trend indicates that bulkier and/or aromatic nitrogen substituents improve enzymatic inhibition. In contrast to the antimicrobial activity,

thiocarbamates did not produce better results (i.e., higher inhibition rates) than corresponding carbamates.

We tried to determine IC_{50} for the most potent inhibitors (**1d**, **2d**, **3d** and **4d**), but the sharply increased lipophilicity of these molecules (Clog*P values of* 6.61-7.37) caused problems with the enzymatic assessment. Diphenylcarbamates precipitated after a very short period in the testing medium at the concentrations higher than 50 μM; therefore it was not possible to find exact IC_{50} values.

Similar to other salicylanilide esters,[16-18] presented (thio)carbamates act as moderate ICL inhibitors with no superiority to 3-NP. Thus, this inhibition represents probably only a minor benefit in addition to the action against actively growing mycobacteria. As expected and observed previously for other benzanilides, there is not a clear relationship of *in vitro* MICs and ICL inhibition. Here reported MIC values were obtained for actively growing mycobacteria, while ICL inhibition has displayed the potential against persistent or non-growing mycobacterial subpopulations.

2.4 Antibacterial and antifungal activity evaluation

All of the salicylanilide carbamates and thiocarbamates (**1**-**5**) were assayed *in vitro* against eight bacterial strains; four of them were Gram-positive [*Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, *Enterococcus* sp.], and four were Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, ESBL-positive *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). Benzylpenicillin (PNC) and bacitracin (BAC) were used as comparative drugs.

None of the salicylanilide derivatives inhibited the growth of Gram-negative bacteria *at a concentration of 125* μM *or lower, whereas Gram-positive cocci were inhibited by some derivatives at 0.49-125* μM. *Compounds, which are not reported in Table 2, were found sharing all MIC values ≥ 125* μM*; compound **4a** precipitated in the testing medium. O*-{2-[(4-Bromophenyl)carbamoyl]-4-chlorophenyl} dimethylcarbamothioate **2b** showed best MICs (0.49 μM) against *Staphyloccus* sp. including MRSA*, superior to benzylpenicillin). Three thiocarbamates (**1b**, **2b**, **4b**) expressed mostly lower MIC values than bacitracin, an established antibacterial drug used particularly in the topical treatment of infections caused by Gram-positive bacteria. Despite the presence of drug-resistance, the most susceptible strain was S. aureus.*

*It is obvious that halogenation of the salicylanilide core improves the antibacterial activity when compared to unsubstituted salicylanilide (**5** vs. **1a**, **1b**, **2b**, **3a**, **3b**, **4b**) with bromine as the most superior (**2b**), followed by chlorine (**1b**) as aniline ring substitution patterns in accordance with increasing lipophilicity. The thiocarbamates possess a stronger in vitro antibacterial activity than corresponding carbamates (**1a** vs. **1b**, **2a** vs. **2b**, **3a** vs. **3b**). The possible explanation may consist in significantly increased lipophilicity or changed steric parameters. The substitution of carbamate nitrogen by small alkyls is a necessary requirement for this biological activity, while the replacement of one or both methyls by phenyl(s) results in its complete abolition.*

The antifungal properties of investigated compounds were evaluated *in vitro* against eight species: *Candida albicans*, *C. tropicalis*, *C. krusei*, *C. glabrata*, *Trichosporon asahii*, *Aspergillus fumigatus*, *Absidia corymbifera* and *Trichophyton mentagrophytes*. Fluconazole (FLU) and amphotericin B (AMB) were used as reference drugs.

Among all strains, only *T. mentagrophytes* was inhibited by carbamates and thiocarbamates at the concentration of 125 μM and lower; MIC values range of 1.95 to 500 μM. MICs of derivatives not involved in Table 2 exceeded 125 μM; compound **4a** precipitated in the testing medium. Under our conditions, two thiocarbamates **1b** and **2b** share the identical *in vitro* antifungal activity as an established and highly effective drug amphotericin B and superior to fluconazole.

The structure-activity relationship, although based on limited data, revealed similar results as for antibacterial activity: thiocarbamates expressed more potent MICs than corresponding carbamates (**1a** vs. **1b**, **2a** vs. **2b**, **3a** vs. **3b**), the monohalogen substitution of aniline ring improves the activity (with chlorine **1b** and bromine **2b** superiority) and the presence of any phenyl on carbamic nitrogen prevents *in vitro* antifungal properties at low concentrations.

2.5 Cytotoxicity evaluation

Parent salicylanilides (**SAL**) and their derivatives (**1**-**5**) underwent cytotoxicity determination in HepG2 cell model using a standard colorimetric method measuring a tetrazolium salt reduction. Their cytotoxicity is expressed as IC_{50}, *i.e.*, concentration, which reduces the viability of the cells to 50% of the maximal viability.

IC_{50} of parent salicylanilides (**SAL-1**-**SAL-5**) ranged between 0.36 and 89.8 μM, and the halogenation decreased the values to 0.36 μM for trifluoromethyl derivative **4**. All carbamates showed an IC_{50} that was higher than 188 μM and thiocarbamates ≥ 67.3 μM. Six compounds (**1d**, **2d**, **3d**, **4c**, **4d** and **5b**) started to crystallise from water-containing testing medium just in non-cytotoxic concentrations; therefore, it was not possible to determine the exact IC_{50} values, although the highest non-cytotoxic and fully soluble concentration is reported (Table 3). The less toxic carbamates were dimethyl derivatives **4a** and **5a**.

Importantly, carbamoylation and thiocarbamoylation of parent salicylanilides sharply decreases their substantial cytotoxicity – e.g., regarding **4** and **4a,** the cytotoxicity was decreased more than 18,077-fold. Thiocarbamates of halogenated salicylanilides showed a higher cytotoxicity (≥ 67.3 μM) than corresponding carbamates (≥ 188.7 μM); the replacement of oxygen by sulphur leads to 2.3-97.6 times higher cytotoxicity. For halogenated salicylanilides (**SAL-1**-**SAL-4**), the following is the cytotoxicity reduction in decreasing order: (methyl)phenylcarbamate > dimethylcarbamate > diphenylcarbamate (limited validity due to not exact data) > dimethylthiocarbamate. Interestingly, the order is reversed for unsubstituted salicylanilide **5** derivatives.

While the *O*-modification of parent phenolic salicylanilides did not markedly increase the antimycobacterial activity, it mitigated the cytotoxicity in the HepG2 model, thus improving the selectivity indices (SI). SI is calculated as a ratio of IC_{50}/MIC, and its values higher than 10 indicate rather acceptable toxicity (based on the analogy of the therapeutic index). The SI of the parent salicylanilides, **SAL-1**-**SAL-5**, ranged from 0.05 to 5.4 for mycobacteria and 0.05 to 16.0 for *T. mentagrophytes*. The SI of (thio)carbamates was up to 29.5 for mycobacteria, up to 174.8 for *S. aureus* including MRSA and up to 43.9 for *T. mentagrophytes*. Seven molecules, carbamates **1a**, **2a**, **2c**, **3a** and thiocarbamates **1b**, **2b**, **4b**, showed both SI values higher than 10 for *M. tuberculosis* with **2c** superiority (29.5); two derivatives (**1b**, **2c**) exhibited at least one value exceeding this threshold for nontuberculous mycobacteria. Two thiocarbamates with a significant *in vitro* antibacterial activity share SI values for *Staphylococci* over 10: **1b** and especially **2b** (174.8), which selectivity index is superior to bacitracin. Three thiocarbamates (**1b**, **2b**, **3b**) also exhibited a satisfactory ratio in the case of *T. mentagrophytes*, even in comparison with fluconazole and especially amphotericin B.

In general, derivatives of 4-Cl and 4-bromoanilines demonstrated more convenient activity/toxicity data than other salicylanilide-based compounds.

3. Conclusions

In this study, we synthesised twenty salicylanilide carbamates and thiocarbamates from (thio)carbamoyl chlorides and salicylanilide triethylammonium salts. New compounds were characterised, and all of them underwent a set of biological tests as potential antimicrobial agents against mycobacteria, other bacterial and fungal strains, regarding their cytotoxicity and inhibition of mycobacterial isocitrate lyase.

The presented derivatives inhibited the growth of both tuberculous and nontuberculous mycobacteria in the micromolar concentration range; however, they did not exceed antimycobacterial activity of parent salicylanilides. On the other hand, the modification of the salicylic phenol group by (thio)carbamoylation markedly alleviated the substantial toxicity of salicylanilides against HepG2 cells, thus improving the selectivity indices and the toxicity profile. Several derivatives exhibited low micromolar MICs for *Staphylococci* including MRSA and *T. mentagrophytes*. Some structure-activity relationships have been identified; for example, *N*,*N*-dimethylthiocarbamates share more enhanced antimicrobial and cytotoxic properties than

corresponding *N*,*N*-dimethylcarbamates. Together, some carbamates and thiocarbamates may represent attractive molecules for future investigation, especially with respect to *M. tuberculosis*, Gram-positive cocci and *Trichophyton mentagrophytes* fungus.

4. Experimental part

4.1 Chemistry

4.1.1 General methods

All of the reagents and solvents were purchased from Sigma-Aldrich (Darmstadt, Germany) or Penta Chemicals (Prague, Czech Republic), and they were used as received. Reactions and the purity of the products were monitored by thin layer chromatography with a toluene/ethyl acetate 4:1 or toluene/methanol 9:1 mixture as eluent; plates were coated with 0.2 mm Merck 60 F254 silica gel and were visualised by UV irradiation (254 nm). Melting points were determined on a Büchi Melting Point machine B-540 apparatus using open capillaries, and the reported values are uncorrected.

Elemental analysis (C, H, N) was performed on an automatic microanalyser CHNS-O CE instrument (FISONS EA 1110, Milano, Italy). Infrared spectra (ATR) were recorded on FT-IR spectrometer Nicolet 6700 FT-IR in the range of 400 to 4,000 cm^{-1}. The NMR spectra were measured in $CDCl_3$ at ambient temperature on a Varian VNMR S500 instrument (500 MHz for ^1H and 125 MHz for ^{13}C; Varian Comp. Palo Alto, CA, USA). The chemical shifts, δ, are given in ppm, with respect to tetramethylsilane as an internal standard. The coupling constants (*J*) are reported in Hz.

The calculated log*P* values (Clog*P*), that are the logarithms of the partition coefficients for octan-1-ol/water, were determined using the program CS ChemOffice Ultra version 12.0 (CambridgeSoft, Cambridge, MA, USA).

4.1.2 Synthesis

Parent salicylanilides (**SAL-1**-**SAL-5**) were synthesised *via* a previously described method.[19] This microwave-assisted synthesis was carried out in a microwave reactor (530 W, 600 rpm; MicroSYNTH Milestone) for 22 min to refluxing.

An equivalent of appropriate salicylanilide (1 mmol) was suspended under vigorous stirring in dry dichloromethane (DCM; 10 mL), and then triethylamine (1.5 of equivalents) was added in one portion. The mixture was stirred for 5 minutes to allow complete dissolution of the salicylanilide due to formation of its triethylammonium salt. Then, appropriate (thio)carbamoyl chloride (1.5 of equivalents) was added in one portion, and the mixture was stirred at room temperature for 48 h (carbamates) or 72 h (thiocarbamates). The reaction was monitored using TLC. Then, the solution was evaporated till dryness, ethyl acetate was added and the suspension was stored at 4 °C for approximately 30 min. Then, the precipitate was removed by filtration, the filtrate was collected, partly evaporated and then *n*-hexane was added to initiate crystallisation. After 24 hours at 4 °C, the precipitate was filtered to give resulting (thio)carbamates **1-5**. Products were recrystallised from ethyl acetate, if necessary.

When dry acetonitrile (MeCN) was used as the solvent at rt, the reaction time was shortened to 18 h for carbamates and 24 h for thiocarbamates. Under refluxing in acetonitrile, the reaction time of 2.5 h was sufficient for carbamates and 5 h for thiocarbamates. However, these methods produced similar yields and purity of the product as the described general method performed in dichloromethane at rt.

4-Chloro-2-[(4-chlorophenyl)carbamoyl]phenyl dimethylcarbamate (**1a**). White solid; yield 94%; mp 171.5-173.5 °C. IR (ATR): 3301 (NH), 1710 (O-CO-N), 1676 (CO-NH), 1598, 1534, 1492, 1400, 1388, 1318, 1216, 1174, 1143, 1103, 1016, 862, 824, 809, 801, 737, 665 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 8.68 (1H, bs, NH), 7.70 (1H, d, *J* = 2.6 Hz, H3), 7.56-7.50 (2H, m, H2´, H6´), 7.40 (1H, dd, *J* = 2.6 Hz, *J* = 8.7 Hz, H5), 7.32-7.28 (2H, m, H3′, H5′), 7.06 (1H, d, *J* = 8.7 Hz, H6), 3.09 (3H, s, CH_3), 3.01 (3H, s, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): 162.9, 154.9, 146.6, 136.4, 131.8,

131.7, 131.5, 129.8, 129.5, 129.0, 124.7, 120.9, 37.0, 36.7. Anal. Calcd. for $C_{16}H_{14}Cl_2N_2O_3$ (353.20): C, 54.41; H, 4.00; N, 7.93. Found: C, 54.23; H, 3.86; N, 8.12.

O-{4-Chloro-2-[(4-chlorophenyl)carbamoyl]phenyl} dimethylcarbamothioate (**1b**). Yellowish solid; yield 55%; mp 144-145 °C. IR (ATR): 3272 (NH), 1645 (CO-NH), 1599, 1541 (O-CS-N), 1534, 1492, 1477, 1403, 1389, 1313, 1287, 1205, 1123, 1097, 1012, 822, 814, 739 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 9.13 (1H, bs, NH), 7.68 (1H, d, *J* = 2.6 Hz, H3), 7.16-7.55 (2H, m, H2´, H6´), 7.45 (1H, dd, *J* = 2.7 Hz, *J* = 8.7 Hz, H5), 7.32-7.26 (2H, m, H3′, H5′), 6.97 (1H, d, *J* = 8.7 Hz, H6), 3.44 (3H, s, CH_3), 3.29 (3H, s, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): 186.9, 162.8, 148.8, 136.6, 132.4, 132.0, 131.7, 129.8, 129.4, 129.0, 125.3, 120.6, 43.5, 39.2. Anal. Calcd. for $C_{16}H_{14}Cl_2N_2O_2S$ (369.27): C, 52.04; H, 3.82; N, 7.59. Found: C, 52.23; H, 3.76; N, 7.40.

4-Chloro-2-[(4-chlorophenyl)carbamoyl]phenyl methyl(phenyl)carbamate (**1c**). White solid; yield 93%; mp 126.5-127.5 °C. IR (ATR): 3316 (NH), 1698 (O-CO-N), 1676 (CO-NH), 1597, 1531, 1493, 1400, 1373, 1312, 1223, 1140, 1103, 1014, 971, 917, 839, 818, 797, 768, 730, 699 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 8.20 (1H, bs, NH), 7.76 (1H, d, *J* = 2.6 Hz, H3), 7.42-7.23 (10H, m, H5, H2´, H6´, H3´, H5´, H2´´, H6´´, H3´´, H5´´, H4´´), 7.12 (1H, d, *J* = 8.7 Hz, H6), 3.37 (3H, s, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): 162.3, 153.2, 146.6, 141.9, 136.1, 131.8, 131.6, 130.1, 129.6, 129.5, 128.9, 127.5, 126.1, 124.5, 121.4, 38.8. Anal. Calcd. for $C_{21}H_{16}Cl_2N_2O_3$ (415.27): C, 60.74; H, 3.88; N, 6.75. Found: C, 60.97; H, 3.89; N, 6.92.

4-Chloro-2-[(4-chlorophenyl)carbamoyl]phenyl diphenylcarbamate (**1d**). White solid; yield 91%; mp 141-143 °C. IR (ATR): 3342 (NH), 1699 (O-CO-N), 1666 (CO-NH), 1596, 1528, 1492, 1400, 1355, 1313, 1239, 1210, 1104, 1015, 825, 762, 729, 698 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 8.32 (1H, bs, NH), 7.66 (1H, d, *J* = 2.6 Hz, H3), 7.39-7.23 (13H, m, H5, H2´, H6´, H2´´, H6´´, H3´´, H5´´, H4´´, H2´´´, H6´´´, H3´´´, H5´´´, H4´´´), 7.16-7.12 (3H, m, H6, H3′, H5′). ^{13}C NMR (125 MHz, $CDCl_3$): 162.7, 152.5, 146.5, 141.6, 136.1, 131.7, 131.4, 130.0, 129.6, 129.5, 129.3, 128.7, 127.2, 127.0, 124.2, 121.4. Anal. Calcd. for $C_{26}H_{18}Cl_2N_2O_3$ (477.34): C, 65.42; H, 3.80; N, 5.87. Found: C, 65.19; H, 3.98; N, 5.55.

2-[(4-Bromophenyl)carbamoyl]-4-chlorophenyl dimethylcarbamate (**2a**). White solid; yield 89%; mp 176-177 °C. IR (ATR): 3305 (NH), 1709 (O-CO-N), 1676 (CO-NH), 1593, 1533, 1488, 1475, 1387, 1316, 1252, 1215, 1173, 1142, 1103, 1070, 1012, 862, 822, 808, 800, 736, 663 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 8.70 (1H, bs, NH), 7.69 (1H, d, *J* = 2.6 Hz, H3), 7.48-7.42 (4H, m, H2´, H6´, H3′, H5′), 7.39 (1H, dd, *J* = 2.6 Hz, *J* = 8.7 Hz, H5), 7.05 (1H, d, *J* = 8.7 Hz, H6), 3.09 (3H, s, CH_3), 3.00 (3H, s, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): 162.9, 154.9, 146.6, 136.9, 132.0, 131.8, 131.4, 129.7, 124.7, 121.3, 121.2, 117.1, 37.0, 36.7. Anal. Calcd. for $C_{16}H_{14}BrClN_2O_3$ (397.65): C, 48.33; H, 3.55; N, 7.04. Found: C, 48.54; H, 3.80; N, 7.01.

O-{2-[(4-Bromophenyl)carbamoyl]-4-chlorophenyl} dimethylcarbamothioate (**2b**). Yellowish solid; yield 81%; mp 146-148.5 °C. IR (ATR): 3292 (NH), 1679 (CO-NH), 1590, 1538 (O-CS-N), 1514, 1489, 1479, 1396, 1388, 1310, 1285, 1220, 1151, 1135, 1102, 1070, 1013, 890, 816, 746 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 9.12 (1H, bs, NH), 7.68 (1H, d, *J* = 2.7 Hz, H3), 7.55-7.51 (2H, m, H2´, H6´), 7.46-7.43 (3H, m, H5, H3′, H5′), 6.97 (1H, d, *J* = 8.7 Hz, H6), 3.44 (3H, s, CH_3), 3.29 (3H, s, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): 186.9, 162.8, 148.8, 137.1, 132.4, 132.0, 131.9, 131.7, 129.8, 125.3, 121.0, 117.0, 43.6, 39.2. Anal. Calcd. for $C_{16}H_{14}BrClN_2O_2S$ (413.72): C, 46.45; H, 3.41; N, 6.77. Found: C, 46.26; H, 3.50; N, 6.51.

2-[(4-Bromophenyl)carbamoyl]-4-chlorophenyl methyl(phenyl)carbamate (**2c**). White solid; yield 89%; mp 154.5-155.5 °C. IR (ATR): 3316 (NH), 1698 (O-CO-N), 1680 (CO-NH), 1597, 1531, 1489, 1395, 1372, 1313, 1208, 1136, 1104, 1071, 1010, 816, 765, 746, 729, 698, 668 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 8.20 (1H, bs, NH), 7.76 (1H, d, *J* = 2.7 Hz, H3), 7.39 (1H, dd, *J* = 2.7 Hz, *J* =

8.8 Hz, H5), 7.37-7.22 (9H, m, H2´, H6´, H3´, H5´, H2´´, H6´´, H3´´, H5´´, H4´´), 7.12 (1H, d, *J* = 8.7 Hz, H6), 3.37 (3H, s, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): 162.2, 152.9, 146.6, 141.8, 136.6, 131.8, 131.6, 130.1, 129.7, 129.5, 127.5, 126.1, 124.5, 121.7, 117.2, 38.8. Anal. Calcd. for $C_{21}H_{16}BrClN_2O_3$ (459.72): C, 54.86; H, 3.51; N, 6.09. Found: C, 55.07; H, 3.68; N, 6.21.

2-[(4-Bromophenyl)carbamoyl]-4-chlorophenyl diphenylcarbamate (**2d**). White solid; yield 98%; mp 175.5-177.5 °C. IR (ATR): 3341 (NH), 1699 (O-CO-N), 1669 (CO-NH), 1593, 1529, 1490, 1393, 1355, 1312, 1238, 1211, 1104, 1072, 1039, 1015, 822, 762, 729, 698 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.26 (1H, bs, NH), 7.68 (1H, d, *J* = 2.6 Hz, H3), 7.38 (1H, dd, *J* = 2.7 Hz, *J* = 8.8 Hz, H5), 7.36-7.24 (12H, m, H2´, H6´, H2´´, H6´´, H3´´, H5´´, H4´´, H2´´´, H6´´´, H3´´´, H5´´´, H4´´´), 7.22-7.18 (2H, m, H3′, H5′), 7.15 (1H, d, *J* = 8.7 Hz, H6). ^{13}C NMR (125 MHz, $CDCl_3$): 162.6, 152.5, 146.4, 141.6, 136.6, 131.8, 131.7, 131.5, 130.1, 129.7, 129.3, 127.2, 124.3, 121.7, 121.6, 117.3. Anal. Calcd. for $C_{26}H_{18}BrClN_2O_3$ (521.79): C, 59.85; H, 3.48; N, 5.37. Found: C, 59.70; H, 3.63; N, 5.59.

4-Chloro-2-[(4-fluorophenyl)carbamoyl]phenyl dimethylcarbamate (**3a**). White solid; yield 87%; mp 148.5-149.5 °C. IR (ATR): 3296 (NH), 1702 (O-CO-N), 1665 (CO-NH), 1613, 1536, 1510, 1408, 1390, 1317, 1215, 1177, 1103, 1017, 893, 859, 828, 810, 747, 734 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.70 (1H, bs, NH), 7.67 (1H, d, *J* = 2.6 Hz, H3), 7.52-7.48 (2H, m, H2´, H6´), 7.34 (1H, dd, *J* = 2.6 Hz, *J* = 8.7 Hz, H5), 7.04–6.99 (3H, m, H6, H3′, H5′), 3.09 (3H, s, CH_3), 3.00 (3H, s, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): 162.9, 159.4 (d, *J* = 243.9 Hz), 154.8, 146.7, 133.8 (d, *J* = 2.8 Hz), 131.6, 131.5, 131.2, 129.6, 124.6, 121.6 (d, *J* = 7.9 Hz), 115.6 (d, *J* = 22.5 Hz), 36.9, 36.7. Anal. Calcd. for $C_{16}H_{14}ClFN_2O_3$ (336.75): C, 57.07; H, 4.19; N, 8.32. Found: C, 56.88; H, 3.99; N, 8.39.

O-{4-Chloro-2-[(4-fluorophenyl)carbamoyl]phenyl} dimethylcarbamothioate (**3b**). Yellowish solid; yield 56%; mp 164-165.5 °C. IR (ATR): 3291 (NH), 1643 (CO-NH), 1541 (O-CS-N), 1509, 1473, 1412, 1390, 1288, 1236, 1204, 1159, 1143, 1124, 1097, 1055, 826, 814, 791, 773, 714 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.05 (1H, bs, NH), 7.69 (1H, d, *J* = 2.6 Hz, H3), 7.61-7.57 (2H, m, H2´, H6´), 7.45 (1H, dd, *J* = 2.7 Hz, *J* = 8.8 Hz, H5), 7.03 (2H, dt, *J* = 2.1 Hz, *J* = 8.7 Hz, H3′, H5′), 6.97 (1H, d, *J* = 8.6 Hz, H6), 3.45 (3H, s, CH_3), 3.30 (3H, s, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): 187.0, 162.7, 159.4 (d, *J* = 243.7 Hz), 148.8, 134.1 (d, *J* = 2.9 Hz), 132.4, 132.1, 131.6, 129.9, 125.3, 121.1 (d, *J* = 7.8 Hz), 115.7 (d, *J* = 22.6 Hz), 43.5, 39.2. Anal. Calcd. for $C_{16}H_{14}ClFN_2O_2S$ (352.81): C, 54.47; H, 4.00; N, 7.94. Found: C, 54.69; H, 3.80; N, 7.79.

4-Chloro-2-[(4-fluorophenyl)carbamoyl]phenyl methyl(phenyl)carbamate (**3c**). White solid; yield 99%; mp 150.5-151 °C. IR (ATR): 3321 (NH), 1687 (O-CO-N), 1672 (CO-NH), 1599, 1532, 1509, 1501, 1408, 1375, 1282, 1215, 1138, 1102, 822, 767, 730, 701 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.15 (1H, bs, NH), 7.78 (1H, d, *J* = 2.6 Hz, H3), 7.39 (1H, dd, *J* = 2.6 Hz, *J* = 8.6 Hz, H5), 7.37-7.24 (7H, m, H2´, H6´, H2´´, H6´´, H3´´, H5´´, H4´´), 7.13 (1H, d, *J* = 8.6 Hz, H6), 6.99 (2H, t, *J* = 8.5 Hz, H3′, H5′), 3.37 (3H, s, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): 162.2, 159.4 (d, *J* = 244.3 Hz), 153.0, 146.4, 141.9, 133.5 (d, *J* = 2.8 Hz), 131.7, 131.6, 130.1, 129.5, 127.5, 126.1, 124.5, 122.1 (d, *J* = 7.8 Hz), 115.6 (d, *J* = 22.5 Hz), 38.8. Anal. Calcd. for $C_{21}H_{16}ClFN_2O_3$ (398.81): C, 63.24; H, 4.04; N, 7.02. Found: C, 63.50; H, 3.90; N, 6.85.

4-Chloro-2-[(4-fluorophenyl)carbamoyl]phenyl diphenylcarbamate (**3d**). White solid; yield 93%; mp 171.5-172.5 °C. IR (ATR): 3330 (NH), 1703 (O-CO-N), 1669 (CO-NH), 1530, 1509, 1493, 1406, 1358, 1319, 1260, 1213, 1105, 1013, 845, 833, 758, 706, 693, 666 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.20 (1H, bs, NH), 7.69 (1H, d, *J* = 2.6 Hz, H3), 7.39-7.24 (13H, m, H5, H2´, H6´, H2´´, H6´´, H3´´, H5´´, H4´´, H2´´´, H6´´´, H3´´´, H5´´´, H4´´´), 7.15 (1H, d, *J* = 8.5 Hz, H6), 6.99 (2H, t, *J* = 8.2 Hz, H3′, H5′). ^{13}C NMR (125 MHz, $CDCl_3$): 162.6, 159.5 (d, *J* = 243.9 Hz), 152.5, 146.5, 141.6, 133.5 (d, *J* = 2.6 Hz), 131.7, 131.5, 130.1, 129.7, 129.3, 127.1, 124.3, 122.1 (d, *J* = 8.1 Hz),

115.4 (d, *J* = 22.4 Hz). Anal. Calcd. for $C_{26}H_{18}ClFN_2O_3$ (460.88): C, 67.76; H, 3.94; N, 6.08. Found: C, 67.54; H, 4.12; N, 6.25.

4-Chloro-2-{[4-(trifluoromethyl)phenyl]carbamoyl}phenyl dimethylcarbamate (**4a**). White solid; yield 94%; mp 183.5-185 °C. IR (ATR): 3286 (NH), 1694 (O-CO-N), 1676 (CO-NH), 1604, 1532, 1405, 1390, 1317, 1257, 1221, 1178, 1167, 1110, 1064, 1015, 885, 847, 836, 728 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 8.98 (1H, bs, NH), 7.68-7.65 (3H, m, H3, H2´, H6´), 7.55 (2H, d, *J* = 8.6 Hz, H3′, H5′), 7.36 (1H, dd, *J* = 2.6 Hz, *J* = 8.7 Hz, H5), 7.04 (1H, d, *J* = 8.6 Hz, H6), 3.11 (3H, s, CH_3), 3.02 (3H, s, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): 163.2, 154.9, 146.7, 140.9, 131.9, 131.6, 131.1, 129.6, 126.2 (q, *J* = 32.8 Hz), 126.1 (q, *J* = 3.8 Hz), 124.6, 124.0 (q, *J* = 271.5 Hz), 119.4, 36.9, 36.7. Anal. Calcd. for $C_{17}H_{14}ClF_3N_2O_3$ (386.75): C, 52.79; H, 3.65; N, 7.24. Found: C, 52.98; H, 3.42; N, 7.37.

O-(4-Chloro-2-{[4-(trifluoromethyl)phenyl]carbamoyl}phenyl) dimethylcarbamothioate (**4b**). Yellowish solid; yield 91%; mp 138.5-139.5 °C. IR (ATR): 3391 (NH), 1666 (CO-NH), 1599, 1536 (O-CS-N), 1473, 1411, 1392, 1332, 1283, 1255, 1196, 1166, 1090, 1064, 1015, 850, 821, 721 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 9.36 (1H, bs, NH), 7.75 (2H, d, *J* = 8.5 Hz, H2´, H6´), 7.69 (1H, d, *J* = 2.6 Hz, H3), 7.59 (2H, d, *J* = 8.5 Hz, H3′, H5′), 7.46 (1H, dd, *J* = 2.6 Hz, *J* = 8.6 Hz, H5), 6.98 (1H, d, *J* = 8.5 Hz, H6), 3.45 (3H, s, CH_3), 3.30 (3H, s, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): 186.9, 163.1, 148.8, 141.0, 132.5, 131.9, 131.8, 129.9, 126.3 (q, *J* = 4.0 Hz), 126.1 (q, *J* = 31.8 Hz), 125.3, 124.0 (q, *J* = 271.5 Hz), 119.1, 43.6, 39.2. Anal. Calcd. for $C_{17}H_{14}ClF_3N_2O_2S$ (402.81): C, 50.69; H, 3.50; N, 6.95. Found: C, 50.90; H, 3.72; N, 6.78.

4-Chloro-2-{[4-(trifluoromethyl)phenyl]carbamoyl}phenyl methyl(phenyl)carbamate (**4c**). White solid; yield 84%; mp 129-130 °C. IR (ATR): 3334 (NH), 1704 (O-CO-N), 1676 (CO-NH), 1606, 1540, 1409, 1373, 1336, 1323, 1265, 1214, 1165, 1148, 1138, 1105, 1067, 1016, 841, 767, 731, 695, 675 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 8.46 (1H, bs, NH), 7.77 (1H, d, *J* = 2.6 Hz, H3), 7.58-7.25 (10H, m, H5, H2´, H6´, H3′, H5′, H2´´, H6´´, H3´´, H5´´, H4´´), 7.13 (1H, d, *J* = 8.7 Hz, H6), 3.44 (3H, s, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): 162.5, 153.0, 146.7, 141.9, 140.6, 132.0, 131.6, 130.1, 129.5, 127.5, 126.3-125.8 (m), 126.0, 124.5, 124.0 (q, *J* = 271.5 Hz), 119.8, 38.8. Anal. Calcd. for $C_{22}H_{16}ClF_3N_2O_3$ (448.81): C, 58.78; H, 3.59; N, 6.24. Found: C, 59.01; H, 3.41; N, 6.52.

4-Chloro-2-{[4-(trifluoromethyl)phenyl]carbamoyl}phenyl diphenylcarbamate (**4d**). White solid; yield 95%; mp 155.5-157 °C. IR (ATR): 3397 (NH), 1707 (O-CO-N), 1675 (CO-NH), 1605, 1536, 1493, 1411, 1345, 1322, 1257, 1214, 1189, 1172, 1152, 1112, 1067, 1016, 996, 838, 758, 701, 694 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 8.62 (1H, bs, NH), 7.53 (1H, d, *J* = 2.6 Hz, H3), 7.38-7.22 (15H, m, H5, H2´, H6´, H3´, H5´, H2´´, H6´´, H3´´, H5´´, H4´´, H2´´´, H6´´´, H3´´´, H5´´´, H4´´´), 7.06 (1H, d, *J* = 8.6 Hz, H6). ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.1, 152.6, 146.7, 141.6, 140.6, 131.8, 131.0, 129.6, 129.3, 129.2, 127.2, 126.3 (q, *J* = 32.7 Hz), 125.7 (q, *J* = 3.9 Hz), 125.6, 123.9 (q, *J* = 271.0 Hz), 123.4, 119.6. Anal. Calcd. for $C_{27}H_{18}ClF_3N_2O_3$ (510.88): C, 63.48; H, 3.55; N, 5.48. Found: C, 63.19; H, 3.26; N, 5.44.

2-(Phenylcarbamoyl)phenyl dimethylcarbamate (**5a**)[22]. White solid; yield 93%; mp 115.5-117 °C. IR (ATR): 3280 (NH), 1721 (O-CO-N), 1652 (CO-NH).

O-[2-(Phenylcarbamoyl)phenyl] dimethylcarbamothioate (**5b**). Yellowish solid; yield 77%; mp 136.5-137.5 °C. IR (ATR): 3291 (NH), 1676 (CO-NH), 1594, 1545 (O-CS-N), 1509, 1493, 1439, 1400, 1318, 1286, 1253, 1212, 1173, 1146, 778, 768, 759, 697 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 9.06 (1H, bs, NH), 7.74 (1H, dd, *J* = 1.7 Hz, *J* = 7.6 Hz, H3), 7.65 (2H, d, *J* = 7.8 Hz, H2´, H6´), 7.53-7.47 (1H, m, H4), 7.41-7.30 (3H, m, H5, H3´, H5´), 7.15-7.09 (1H, m, H4´), 7.04 (1H, d, *J* = 8.1 Hz, H6), 3.45 (3H, s, CH_3), 3.29 (3H, s, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 187.3, 164.2,

150.3, 138.3, 131.5, 130.9, 130.0, 129.0, 126.7, 124.2, 123.8, 119.4, 43.4, 39.1. Anal. Calcd. for $C_{16}H_{16}N_2O_2S$ (300.38): C, 63.98; H, 5.37; N, 9.33. Found: C, 64.13; H, 5.55; N, 9.20.

2-(Phenylcarbamoyl)phenyl methyl(phenyl)carbamate (**5c**). White solid; yield 93%; mp 141.5-142.5 °C. IR (ATR): 3318 (NH), 1714 (O-CO-N), 1682 (CO-NH), 1598, 1536, 1496, 1453, 1442, 1378, 1318, 1311, 1201, 1139, 969, 775, 744, 703, 692, 669, 658 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 8.16 (1H, bs, NH), 7.82 (1H, d, *J* = 7.9 Hz, H3), 7.50-7.39 (3H, m, H4, H2´, H6´), 7.36-7.23 (8H, m, H5, H3´, H5´, H2´´, H6´´, H3´´, H5´´, H4´´), 7.19 (1H, d, *J* = 7.9 Hz, H6), 7.15 (1H, t, *J* = 7.3 Hz, H4´), 3.38 (3H, s, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.7, 153.5, 148.0, 142.1, 137.8, 131.8, 130.3, 129.3, 129.0, 128.9, 127.3, 126.2, 124.4, 123.1, 120.1, 38.6. Anal. Calcd. for $C_{21}H_{18}N_2O_3$ (346.38): C, 72.82; H, 5.24; N, 8.09. Found: C, 72.57; H, 5.03; N, 8.24.

2-(Phenylcarbamoyl)phenyl diphenylcarbamate (**5d**). White solid; yield 91%; mp 170-172 °C. IR (ATR): 3373 (NH), 1702 (O-CO-N), 1676 (CO-NH), 1601, 1542, 1491, 1442, 1349, 1328, 1202, 1191, 1012, 772, 775, 745, 702, 692, 680, 653 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 8.10 (1H, bs, NH), 7.82 (1H, dd, *J* = 1.9 Hz, *J* = 7.9 Hz, H3), 7.53-7.43 (3H, m, H4, H2´, H6´), 7.37-7.21 (14H, m, H5, H6, H3´, H5´, H2´´, H6´´, H3´´, H5´´, H4´´, H2´´´, H6´´´, H3´´´, H5´´´, H4´´´), 7.15 (1H, t, *J* = 7.4 Hz, H4´). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.8, 152.9, 147.8, 141.7, 137.7, 131.8, 130.1, 129.3, 129.2, 128.9, 126.9, 126.3, 124.5, 123.1, 120.2. Anal. Calcd. for $C_{26}H_{20}ClF_3N_2O_3$ (408.45): C, 76.45; H, 4.94; N, 6.86. Found: C, 76.19; H, 4.75; N, 6.84.

4.2 Biological activity

4.2.1 *In vitro* antimycobacterial susceptibility determination

Salicylanilide carbamates and thiocarbamates were evaluated for their *in vitro* antimycobacterial activity against *M. tuberculosis* 331/88 ($H_{37}Rv$; dilution of this strain was 10^{-3}), *Mycobacterium avium* 330/88 (resistant to INH, RIF, ofloxacin and ethambutol; dilution 10^{-5}) and two strains of *M. kansasii*: 235/80 (dilution 10^{-4}) and the clinically isolated strain 6509/96 (dilution 10^{-5}). The used method is described in ref.[17]. The following concentrations were used: 1000, 500, 250, 125, 62.5, 32, 16, 8, 4, 2, 1, 0.5, 0.25, and 0.125 μM. MIC (reported in μM) was the lowest concentration at which the complete inhibition of mycobacterial growth occurred. Isoniazid (INH) and *p*-aminosalicylic acid (4-amino-2-hydroxybenzoic acid, PAS) as a structurally similar second-line drug were chosen as the reference compounds. For each compound, MICs were determined in quadruplicate and repeated twice.

4.2.2 *In vitro* antibacterial activity determination

The *in vitro* antibacterial activity was assayed against eight Gram-positive and Gram-negative strains: *Staphylococcus aureus* CCM 4516/08, methicillin-resistant *Staphylococcus aureus* H 5996/08 (MRSA), *Staphylococcus epidermidis* H 6966/08, *Enterococcus* sp. J 14365/08; *Escherichia coli* CCM 4517, *Klebsiella pneumoniae* D 11750/08, ESBL-positive *Klebsiella pneumoniae* J 14368/08, and *Pseudomonas aeruginosa* CCM 1961.

The microdilution broth method in Mueller-Hinton broth was used. The tested compounds were dissolved in DMSO to the final concentrations ranging from 500 to 0.49 μM. Benzylpenicillin (penicillin G; PNC) and bacitracin (BAC) were used as the comparative drugs. The minimum inhibitory concentrations were assayed as 95% (IC_{95}) or higher reduction of growth compared to the control. For each compound, MIC determination was performed twice. The used method is described in ref.[17].

4.2.3 *In vitro* antifungal activity determination

The antifungal properties of all synthesised compounds were evaluated *in vitro* against four *Candida* strains (*Candida albicans* ATCC 44859, *Candida tropicalis* 156, *Candida krusei* E28, and *Candida glabrata* 20/I), *Trichosporon asahii* 1188 and three filamentous fungi (*Aspergillus fumigatus* 231, *Absidia corymbifera* 272, and *Trichophyton mentagrophytes* 445). The

microdilution broth method was used in RPMI 1640 with glutamine. Fluconazole (FLU) and amphotericin B (AMB) were used as the reference drugs. The MICs were assayed as an 80% (IC_{80}) or higher reduction of growth in comparison to the control; for filamentous fungi, MICs are expressed as IC_{50} values. For each compound, MIC determination was performed twice. The used method is described in ref.[19].

4.2.4 Isocitrate lyase inhibition assay (ICL1)

The isocitrate lyase (ICL) activity was assayed according to the protocol reported by Dixon and Kornberg (glyoxylate phenyl hydrazone formation)[23] at the investigated compounds concentration of 10 μM. IC_{50} data represent an average of triplicate experiments ± SD. Isoniazid was employed as a negative control (inhibition of 0%), and 3-nitropropionic acid (3-NP) served as a positive control.[17]

4.2.5 Cytotoxicity evaluation (HepG2 cells)

All compounds were tested for their cytotoxicity in the human hepatocellular liver carcinoma cell line HepG2 (passage 3–4; ECACC, Salisbury, UK) using a standard colorimetric method that involves measuring a tetrazolium salt reduction (CellTiter(R) 96 AQueous One Solution Assay, Promega G3580, Madison, WI, USA).

The cells were routinely cultured in Eagle's minimum essential media supplemented with 10% foetal bovine serum, 1% L-glutamine solution, and a non-essential amino acid solution. The investigated compounds were dissolved in a very small amount of DMSO, and a small volume was added to the cell culture. The tested compounds were prepared in triplicate at eight incubation concentrations. The following types of controls were included: determination of 100% viability and 0% viability (the cells treated by 10% DMSO), no cell control, control for the determination of possible interaction of tested compounds with reagents, control of the setting of incubation medium and the control of the toxicity of DMSO.

The results are expressed as the inhibitory concentration that reduces cell viability to 50% of the maximal (control) viability (IC_{50}). IC_{50} was calculated in each of the tested substances using GraphPad Prism software (version 5.02; GraphPad Software Inc., San Diego, CA, USA) and Microsoft Excel 2010.

This method is described in depth in ref.[17].

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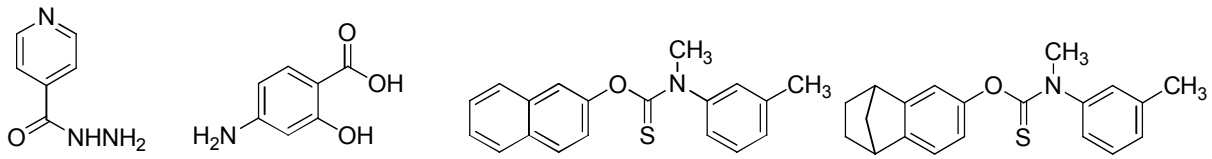
Conflict of Interest

The authors declare no conflict of interest.

References

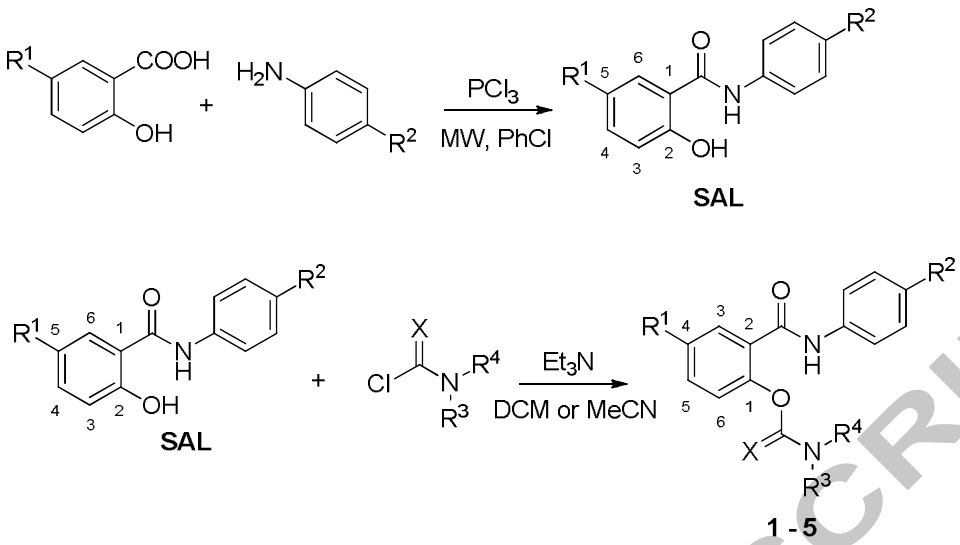
1. Loddenkemper, R.; Hauer, B. *Dtsch. Arztebl. Int.* **2010**, *107*, 10.
2. van Ingen, J.; Boeree, M. J.; van Soolingen, D.; Mouton, J. W. *Drug Resist. Updat.* **2012**, *15*, 149.
3. Livermore, D. M. *Int. J. Antimicrob. Agents* **2012**, *39*, 283.

4. Peres, N. T. D.; Maranhão, F. C. A.; Rossi, A.; Martinez-Rossi, N. M. *An. Bras. Dermatol.* **2010**, *85*, 657.
5. Ryder, N. S.; Frank, I.; Dupont, M. C. *Antimicrob. Agents Chemother.* **1986**, *29*, 858.
6. Mukherjee, P. K.; Leidich, S. D.; Isham, N.; Leitner, I.; Ryder, N. S.; Ghannoum, M. A. *Antimicrob. Agents Chemother.* **2003**, *47*, 82.
7. Blaser, A.; Palmer, B. D.; Sutherland, H. S.; Kmentova, I.; Franzblau, S. G.; Wan, B.; Wang, Y.; Ma, Z.; Thompson, A. M.; Denny, W. A. *J. Med. Chem.* **2012**, *55*, 312.
8. Hu, J.-F.; Schetz, J. A.; Kelly, M.; Peng, J.-N.; Ang, K. K. H.; Flotow, H.; Leong, C. Y.; Ng, S. B.; Buss, A. D.; Wilkins, S. P.; Hamann, M. T. *J. Nat. Prod.* **2002**, *65*, 476.
9. Meng, Q. Y.; Luo, H. B.; Liu, Y. B.; Li, W.; Zhang, W.; Yao, Q.Z. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2808.
10. Férriz, J. M.; Vinšová, J. *Curr. Pharm. Des.* **2010**, *16*, 2033.
11. Solyev, P. N.; Shipitsin, A. V.; Karpenko, I. L.; Nosik, D. N.; Kalnina, L. B.; Kochetkov, S. N.; Kukhanova, M. K.; Jasko, M. V. *Chem. Biol. Drug. Des.* **2012**, *80*, 947.
12. Yang, Y. H.; Voak, A.; Wilkinson, S. R.; Hu, L. Q. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 6583.
13. Gomez, C.; Ponien, P.; Serradji, N.; Lamouri, A.; Pantel, A.; Capton, E.; Jarlier, V.; Anquetin, G.; Aubry, A. *Bioorg. Med. Chem.* **2013**, *21*, 948.
14. Krátký, M.; Vinšová, J. *Curr. Pharm. Des.* **2011**, *17*, 3494.
15. Férriz, J.M.; Vávrová, K.; Kunc, F.; Imramovský, A.; Stolaříková, J.; Vavříková, E.; Vinšová, J. *Bioorg. Med. Chem.* **2010**, *18*, 1054.
16. Krátký, M.; Vinšová, J.; Novotná, E.; Mandíková, J.; Wsól, V.; Trejtnar, F.; Ulmann, V.; Stolaříková, J.; Fernandes, S.; Bhat, S.; Liu, J.O. *Tuberculosis (Edinb).* **2012**, *92*, 434.
17. Krátký, M.; Vinšová, J.; Novotná, E.; Mandíková, J.; Trejtnar, F.; Stolaříková, J. *Molecules* **2013**, *18*, 3674.
18. Krátký, M.; Vinšová, J. *Curr. Med. Chem.* **2012**, *19*, 6126.
19. Krátký, M.; Vinšová, J. *Molecules* **2012**, *17*, 9426.
20. Vacondio, F.; Silva, C.; Mor, M.; Testa, B. *Drug Metab. Rev.* **2010**, *42*, 551.
21. Hansen, K. T.; Faarup, P.; Bundgaard, H. *J. Pharm. Sci.* **1991**, *80*, 793.
22. Kumar, G. S.; Maheswari, C. U.; Kumar, R. A.; Kantam, M. L.; Reddy, K. R. *Angew. Chem. Int. Ed. Engl.* **2011**, *50*, 11748.
23. Dixon, G. H.; Kornberg, H. L. *Biochem. J.* **1959**, *72*, P3.



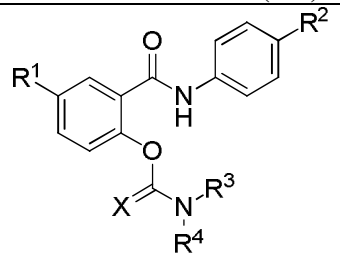
isoniazid *p*-aminosalicylic acid tolnaftate tolciclate

Figure 1. Structures of some antimycobacterial and thiocarbamate-based antimycotic drugs



Scheme 1. Synthesis of salicylanilides (**SAL-1**-**SAL-5**) and their carbamates and thiocarbamates (**1-5**). [R^1 = H, Cl; R^2 = H, Cl, Br, F, CF_3; R^3 and R^4 = CH_3, phenyl; X = O (carbamates), S (thiocarbamates); MW: microwave irradiation (530 W, 600 rpm, 22 min); PhCl: chlorobenzene; DCM: dichloromethane; MeCN: acetonitrile; Et_3N: triethylamine].

Table 1. Antimycobacterial activity of salicylanilide carbamates and thiocarbamates (**1**-**5**)



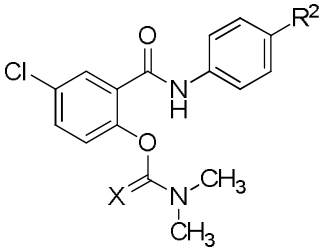
Code	R^1	R^2	R^3	R^4	X	MIC [μM]										% ICL inhibition at 10 μM (± SD)	Clog*P*
						M. tuberculosis 331/88		*M. avium* 330/88		*M. kansasii* 235/80			*M. kansasii* 6509/96				
						14 d	21 d	14 d	21 d	7 d	14 d	21 d	7 d	14 d	21 d		
SAL-1	Cl	Cl	parent salicylanilide			4	4	8	16	4	8	8	4	8	8	13 ± 2.90	3.57
1a	Cl	Cl	CH_3	CH_3	O	16	16	62.5	62.5	62.5	62.5	62.5	62.5	125	125	6 ± 1.36	3.69
1b	Cl	Cl	CH_3	CH_3	S	**4**	**8**	**16**	**16**	**8**	**16**	**16**	**16**	**16**	**16**	6 ± 1.92	4.67
1c	Cl	Cl	CH_3	Ph	O	32	32	250*	250*	62.5	125*	125*	32	62.5	125	17 ± 1.63	5.35
1d	Cl	Cl	Ph	Ph	O	250*	250*	250*	250*	32	125*	125*	32	62.5*	62.5*	**20** ± 1.08	7.01
SAL-2	Cl	Br	parent salicylanilide			4	4	8	16	4	8	8	4	8	8	13 ± 2.04	3.84
2a	Cl	Br	CH_3	CH_3	O	16	16	62.5	62.5	62.5	125*	125*	62.5	62.5	62.5	9 ± 1.43	3.96
2b	Cl	Br	CH_3	CH_3	S	**8**	**8**	**16**	**16**	**16**	**16**	**16**	**16**	32	32	11 ± 0.50	4.94
2c	Cl	Br	CH_3	Ph	O	32	32	250*	250*	32	125*	125*	32	62.5	125	14 ± 2.70	5.62
2d	Cl	Br	Ph	Ph	O	250*	250*	250*	250*	32	125*	125*	32	62.5*	62.5*	19 ± 0.32	7.28
SAL-3	Cl	F	parent salicylanilide			8	8	16	32	8	16	16	8	16	16	14 ± 3.01	3.17
3a	Cl	F	CH_3	CH_3	O	32	32	125	125	62.5	125	125	62.5	125	125	8 ± 1.64	3.29
3b	Cl	F	CH_3	CH_3	S	**8**	16	32	32	**16**	32	32	**16**	32	32	7 ± 0.33	4.27
3c	Cl	F	CH_3	Ph	O	32	62.5	250*	250*	62.5	125	125*	62.5	125	125*	14 ± 1.09	4.95
3d	Cl	F	Ph	Ph	O	250*	250*	250*	250*	62.5*	62.5*	62.5*	125*	125*	125*	**22** ± 2.02	6.61
SAL-4	Cl	CF_3	parent salicylanilide			1	2	4	8	2	4	4	2	4	4	6 ± 1.9	3.93
4a	Cl	CF_3	CH_3	CH_3	O	>1000	>1000	250*	250*	250*	250*	250*	250*	250*	250*	11 ± 0.50	4.05
4b	Cl	CF_3	CH_3	CH_3	S	**4**	**4**	**16**	**16**	**16**	32	32	**16**	32	32	12 ± 1.97	5.03
4c	Cl	CF_3	CH_3	Ph	O	125*	125*	250*	250*	125*	125*	125*	125*	125*	125*	16 ± 2.17	5.71
4d	Cl	CF_3	Ph	Ph	O	125*	125*	250*	250*	125*	125*	125*	125*	125*	125*	**21** ± 1.12	7.37
SAL-5	H	H	parent salicylanilide			32	125	125	250	125	250	250	125	250	250	9 ± 3.8	3.29
5a	H	H	CH_3	CH_3	O	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	10 ± 1.12	2.57
5b	H	H	CH_3	CH_3	S	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	6 ± 1.53	3.55
5c	H	H	CH_3	Ph	O	250	250	>1000	>1000	250	500	500	250	500	1000	3 ± 0.58	4.23
5d	H	H	Ph	Ph	O	125	125	62.5	62.5	62.5	125	125	125	125	125	8 ± 0.74	5.9
INH						0.5	1	>250	>250	>250	>250	>250	2	4	4 or 8	0	-0.6

PAS	62.5	62.5	32	125	125	1000	>1000	32	125	500	10 ± 1.60	0.88
3-NP	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	25 ± 4.1	-0.4

Ph: phenyl; INH: isoniazid; PAS: *p*-aminosalicylic acid; 3-NP = 3-nitropropionic acid. NT – not tested. * at the specified concentration, the growth of the tested strain was observed; at duplex concentration, precipitate and/or turbidity was present, therefore it was not possible to determine the exact MIC value.

The best values for each strain are provided in bold.

Table 2. Antibacterial and antifungal activity of salicylanilide carbamates and thiocarbamates (**1**-**5**)

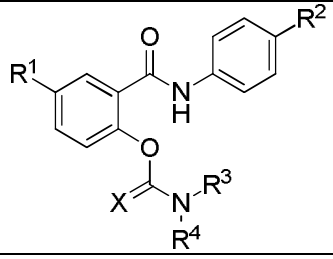


Code	R^2	X	MIC/IC_{95}/IC_{50}[μM]									
			SA		MRSA		SE		EF		TM	
			24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	72 h	120 h
1a	Cl	O	62.5	125	62.5	125	125	125	**125**	>125	31.25	31.25
1b	Cl	S	**3.9**	7.81	**3.9**	7.81	**7.81**	31.25	**125**	>125	**1.95**	**1.95**
2b	Br	S	**0.49**	**0.49**	**0.49**	**0.49**	**0.49**	**0.49**	>500	>500	**1.95**	**1.95**
3a	F	O	125	>125	>125	>125	>125	>125	**125**	>125	125	125
3b	F	S	15.62	31.25	31.25	31.25	31.25	31.25	>500	>500	7.81	7.81
4b	CF_3	S	7.81	7.81	7.81	7.81	31.25	31.25	>500	>500	500	500
	PNC		0.98	0.98	62.5	125	250	250	7.81	15.62	-	-
	BAC		7.81	15.62	15.62	15.62	15.62	31.25	15.62	62.5	-	-
	FLU		-	-	-	-	-	-	-	-	7.81	125
	AMB		-	-	-	-	-	-	-	-	1.95	1.95

SA: *Staphylococcus aureus* CCM 4516/08; MRSA: methicillin-resistant *Staphylococcus aureus* H 5996/08; SE: *Staphylococcus epidermidis* H 6966/08; EF: *Enterococcus* sp. J 14365/08. TM: *Trichophyton mentagrophytes* 445. PNC: benzylpenicillin; BAC: bacitracin, FLU: fluconazole, AMB: amphotericin B.

The best values for each strain are provided in bold.

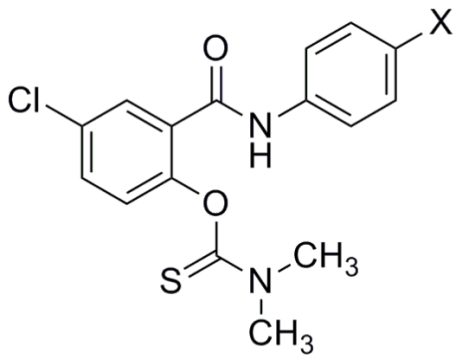
Table 3. Cytotoxicity and selectivity indices of parent salicylanilides (**SAL-1**-**SAL-5**), carbamates and thiocarbamates (**1**-**5**)



Code	R^1	R^2	R^3	R^4	X	IC_{50} [μM]	SI for *M. tuberculosis*	SI for atypical mycobacteria	SI for *S. aureus*	SI for TM
SAL-1	Cl	Cl	parent salicylanilide			21.71	5.4	1.4-5.4	-	**11.1**
1a	Cl	Cl	CH_3	CH_3	O	188.7	**11.8**	1.5-3.0	1.5-3.0	6.0
1b	Cl	Cl	CH_3	CH_3	S	83.51	**10.4-20.9**	5.2-**10.4**	**10.7**-**21.4**	**42.8**
1c	Cl	Cl	CH_3	phenyl	O	289.0	9.0	≤9.0	-	-
1d	Cl	Cl	phenyl	phenyl	O	>100	-	-	-	-
SAL-2	Cl	Br	parent salicylanilide			15.72	3.9	1.0-3.9	-	**16.0**
2a	Cl	Br	CH_3	CH_3	O	342.5	**21.4**	≤5.5	-	-
2b	Cl	Br	CH_3	CH_3	S	85.64	**10.7**	2.7-5.4	**174.8**	**43.9**
2c	Cl	Br	CH_3	phenyl	O	945.1	**29.5**	≤**29.5**	-	-
2d	Cl	Br	phenyl	phenyl	O	>100	-	-	-	-
SAL-3	Cl	F	parent salicylanilide			26.06	3.3	0.8-3.3	-	3.3
3a	Cl	F	CH_3	CH_3	O	444.5	**14.0**	3.6-7.1	≤3.6	3.6
3b	Cl	F	CH_3	CH_3	S	116.8	7.3-**14.6**	3.7-7.3	3.7-7.5	**15.0**
3c	Cl	F	CH_3	phenyl	O	512.0	8.2-**16**	≤8.2	-	-
3d	Cl	F	phenyl	phenyl	O	>100	-	-	-	-
SAL-4	Cl	CF_3	parent salicylanilide			0.36	0.18-0.36	0.05-0.18	-	0.05-0.09
4a	Cl	CF_3	CH_3	CH_3	O	6508.0	-	-	-	-
4b	Cl	CF_3	CH_3	CH_3	S	67.29	**16.8**	2.1-4.2	8.6	<1
4c	Cl	CF_3	CH_3	phenyl	O	>100	-	-	-	-
4d	Cl	CF_3	phenyl	phenyl	O	>100	-	-	-	-
SAL-5	H	H	parent salicylanilide			89.80	0.7-2.08	0.36-0.72	-	-
5a	H	H	CH_3	CH_3	O	5985.9	6.0	6.0	-	-
5b	H	H	CH_3	CH_3	S	>500	-	-	-	-
5c	H	H	CH_3	phenyl	O	279.9	1.1	≤1.1	-	-
5d	H	H	phenyl	phenyl	O	211.1	1.7	1.7-3.4	-	-
INH						>250	>250	≥31.3 (only *Mk*)	-	-
PAS						2,240	35.8	<2.2-70	-	-
3-NP						692.5	<0.7	<0.7	-	-
PNC						>10,000	-	-	>40->10,204	-
BAC						254.6	-	-	8.1-32.6	-
FLU						>300	-	-	-	>2.4->38.4
AMB						2.5	-	-	-	1.3

SI = IC50/MIC. SI values higher than 10 are given in bold. *Mk* = *M. kansasii* 6509/96. MICs of salicylanilides **1**, **2**, **3** and **4** against *T. mentagrophytes* were reported previously.19

Graphical abstract



M. tuberculosis:
MICs 4-16 µmol/L, SI up to 29.5
Staphylococcus aureus (including MRSA):
MICs from 0.49 µmol/L, SI up to 174.8
Trichophyton mentagrophytes:
MICs from 1.95 µmol/L, SI up to 43.9

X = Cl, Br, F, CF_3