Bioorganic & Medicinal Chemistry 23 (2015) 7292–7301

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Novel derivatives of nitro-substituted salicylic acids: Synthesis, antimicrobial activity and cytotoxicity

Georgios Paraskevopoulos [a], Martin Krátký [a], Jana Mandíková [b], František Trejtnar [b], Jiřina Stolaříková [c], Petr Pávek [b], Gurdyal Besra [d], Jarmila Vinšová [a,*]

[a] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy, Charles University in Prague, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic
[b] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Charles University in Prague, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic
[c] Laboratory for Mycobacterial Diagnostics and Tuberculosis, Regional Institute of Public Health in Ostrava, Partyzánské náměstí 7, 702 00 Ostrava, Czech Republic
[d] School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, United Kingdom

ARTICLE INFO

Article history:
Received 21 August 2015
Revised 13 October 2015
Accepted 22 October 2015
Available online 24 October 2015

Keywords:
Salicylanilides
Nitro group
In silico ADMET prediction
In vitro antimycobacterial activity
In vitro antimicrobial activity
Cytotoxicity

ABSTRACT

Inspired by the high antituberculous activity of novel nitro-substituted derivatives and based on promising predicted ADMET properties we have synthesized a series of 33 salicylanilides containing nitro-group in their salicylic part and evaluated them for their in vitro antimycobacterial, antimicrobial and antifungal activities. The presence of nitro-group in position 4 of the salicylic acid was found to be beneficial and the resulting molecules exhibited minimum inhibitory concentrations (MICs) ranging from 2 to 32 μM against *Mycobacterium tuberculosis*. The best activity was found for 2-hydroxy-4-nitro-*N*-[4-(trifluoromethyl)phenyl]benzamide (MIC = 2 μM). 4-Nitrosalicylanilides were also found to be active against all *Staphylococcus* species tested while for MRSA strain 2-hydroxy-4-nitro-*N*-[4-(trifluoromethyl)phenyl]benzamide's MIC was 0.98 μM. None of the nitrosalicylanilides was active against *Enterococcus* sp. J 14365/08 and no considerable activity was found against Gram-negative bacteria or fungi. The hepatotoxicity of all nitrosalicylanilides was found to be in the range of their MICs for HepG2 cells.

1. Introduction

According to the World Health Organization, tuberculosis (TB) is second only to Human Immunodeficiency Virus (HIV) as the greatest killer worldwide due to a single infectious agent.[1] The numbers reported at the Global Tuberculosis Report for 2014 are highlighting the severity of the disease. During 2013, 9 million people fell ill with TB and 1.5 million people died. Whilst TB is treatable and curable, standard anti-TB drugs, like isoniazid (INH) and rifampicin (RMP), have been used for decades, and resistance to the medicines is widespread. Clinical strains that are resistant to a single anti-TB drug have been documented in every country surveyed giving rise to multidrug-resistant tuberculosis (MDR-TB), extensively drug-resistant tuberculosis (XDR-TB) and the recently reported totally drug-resistant tuberculosis (TDR-TB).[2–4] In addition, the standard treatment for adult respiratory TB is a regimen of drugs developed many years ago and is connected with several adverse effects like hepatotoxicity, skin reactions, gastrointestinal and neurological disorders.[5] Among them hepatotoxicity is the most serious one,[6] directing the current research towards the need to enrich the pipeline of available drugs against TB, preferably with candidates offering lower cytotoxicity and with new mode of action in comparison to existing drugs.

On the contrary, various opportunistic human infections are caused by nontuberculous mycobacteria (NTM) and, according to the predictions, they will continue to increase their incidence at least up to year 2050 mainly due to an increasing elderly population.[7] The treatment of NTM caused infections is complicated and involves multiple medications due to the high levels of natural and acquired antibiotic resistance. Furthermore, their current treatment share limited efficacy,[8] and therefore there is a need for new therapeutic strategies to be developed. Moreover, severe hospital-acquired infections can be caused due to notable drug-resistance complications that have been reported for Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), *Streptococcus pneumoniae* and *Enterococci*.[9]

Salicylanilides (2-hydroxy-*N*-phenylbenzamides) have been the subject of several medicinal chemistry studies due to their significant in vitro antimicrobial and antifungal activities.[10–12] A number of salicylanilide derivatives have been developed so far with proven activity against *Mycobacterium tuberculosis* (Mtb) and INH-resistant strains.[13–15] However, the precise molecular mode of action of salicylanilides is not clear. It has been previously reported

* Corresponding author. Tel.: +420 495067343, fax: +420 495067166.
E-mail address: jarmila.vinsova@faf.cuni.cz (J. Vinšová).

http://dx.doi.org/10.1016/j.bmc.2015.10.029
0968-0896/

that a free phenolic hydroxyl on the salicylic acid moiety is required for activity and suggested that they might function as proton shuttles that kill bacterial cells by destroying the cellular proton gradient.[16] On the other hand, salicylanilides and their ester derivatives have been reported to act as moderate inhibitors of mycobacterial and human methionine aminopeptidases and may target the function of mycobacterial isocitrate lyase which could enhance its efficacy due to its particular relevance to mycobacterial latency.[17] These findings have suggested that salicylanilides affect multiple targets to exert their antimicrobial properties. Furthermore, salicylanilide esters, carbamates and additional derivatives resulting from their conjugation with existing antimicrobial agents, have been suggested to work as pro-drugs that are hydrolyzed in order to express their activity.[18]

Although salicylanilides and their derivatives (esters, carbamates etc.) are very potent against Mtb, they often share a non-preferable cytotoxic profile.[16,19] This drawback has limited their utility as potential therapeutics so far. Recent work has focused on the discovery of salicylanilide derivatives with decreased cytotoxicity and, under this concept, novel 2-(phenylcarbamoyl)phenyl 4-substituted benzoates were synthesized and found to be active against several mycobacteria (0.125–8 μM) while they exhibited no cytotoxicity at concentrations of up to 50 μM against a hepatocyte cell line (HepG2).[20] The esterification products of 5-halogenated salicylanilides with 4-substituted benzoic acids were found to be, not only more potent inhibitors of Mtb growth but also to share a preferable cytotoxic profile in contrast with the parent salicylanilides. The increased lipophilicity of the afforded derivatives limited their solubility in aqueous media and hence their drugability.

Our ongoing efforts explore the influence of different substitutions on the parent structure of salicylanilides, in terms of their activity and cytotoxicity. The introduction of several substituents is under investigation. In general, it has been previously reported that an electron withdrawing group is favored in the aniline part while the influence of the substituents from the acyl moiety is more complex.[21] The best substituents of the parent salicylanilide structure reported so far is –Br or –Cl atoms in position 5 of the salicylic acid part while a $-CF_3$ group is favoured in position 4 of the aniline part. More specifically, 5-bromo-2-hydroxy-*N*-[4-(trifluoromethyl)phenyl]benzamide and 5-chloro-2-hydroxy-*N*-[4-(trifluoromethyl)phenyl]benzamide are inhibiting the growth of Mtb at the concentrations of 1 and 2 μM, respectively.

Current research in the field of novel antituberculotics on their way to the clinic has revealed that many nitro-substituted compounds owe their antimycobacterial activity to the presence of a nitro group in their scaffold.[22,23] For example, the nitro-reduction of nitroimidazole OPC-67683 (also known as delamanid)[24] and PA-824[25] by F_{420}-deazaflavin-dependent nitroreductase (Ddn) releases nitric oxide, which is thought to inhibit cytochrome oxidase and other targets. In addition, benzothiazinones (BTZ), e.g., BTZ-043[26] and BTZ-derived inhibitors, e.g., CT139[27] may interact through their nitro-group with a cysteine residue of decaprenylphosphoryl-β-D-ribose 2′-epimerase (DprE1), either covalently or not, thus blocking the first step in the epimerization reaction of decaprenyl-phosphoryl-D-ribose (DPR) to decaprenyl-phosphoryl-D-arabinose (DPA) the arabinan donor for arabinogalactan. In a similar manner dinitrobenzamides, e.g., DNB1[28] are forming a covalent bond with Cys387 within the active site of DprE1. More recently, novel dinitrobenzyl-bearing benzazole and tetrazole derivatives exhibited high and selective antimycobacterial activity.[29,30]

Herein we describe the influence of the introduction of a nitro-group at the salicylic part of salicylanilides to their antimycobacterial, antimicrobial and antifungal activities. To the best of our knowledge, a structure activity relationship (SAR) concerning the preferable nitro-substitution of the salicylic part of salicylanilides has not been reported in the literature to date, even if some nitro-substituted salicylanilides have been previously presented.[16,21,31–34]

2. Results and discussion

2.1. ADMET properties prediction

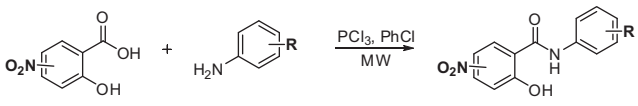
The novel salicylanilides were screened in silico, prior to their synthesis, for their ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties. The software of choice was ADMET Predictor (Simulation Plus, Lancaster, CA), one of the leading computer software for advanced predictive modeling of ADMET properties.[35] The program uses molecular description values as inputs to independent mathematical models (generally, nonlinear machine learning technics) in order to generate estimates for each of the ADMET properties.[36] ADMET Predictor not only rapidly estimates a number of vital ADMET properties from molecular structures, but it is able to validate a series of 'ADMET Risk' or 'Tox Risk' parameters. They are parameterized to include thresholds for a wide range of calculated and predicted properties that represent potential obstacles to a compound being successfully developed as a drug.

For the majority of the compounds, the analyses indicated no potential risk of either ADME/absorption problems related with physico-chemical properties or any stability risk related to fast hepatic metabolism. Furthermore, no potential interaction related to inhibition of drug biotransformation enzymes was indicated. Most importantly, the tested compounds were not found to be potentially related with toxicity or carcinogenicity as indicated by parameters of acute toxicity and carcinogenicity in rats expressed as TD_{50} and LD_{50} values, respectively, mutagenic chromosomal aberrations, or maximum recommended therapeutic dose (MRTD).

More specifically, 17 out of 33 compounds showed a preferable ADMET Risk (score 0–2). 5 out of 33 compounds presented an acceptable ADMET Risk (score 3), limited by potential high lipophilicity and absorption related risk. 11 out of 33 compounds showed a non-preferable ADMET Risk profile (score 4–5). On the other hand, 28 out of 33 compounds showed no potential risk of toxicity (score 0–2) and only 5 out of 33 compounds showed an acceptable risk of toxicity (score 3). There was no direct correlation between the position of the nitro group and the potential risk of toxicity. Furthermore, the substituent on the aniline part is more likely affecting the potential ADMET properties, as halogens in position 3 is preferred while trifluoromethyl group and nitro group are not preferable. The ADMET Risk, as well as the Tox Risk scores of the screened compounds are summarized in Table S1. With the encouraging prediction results the synthesis of the molecules was decided.

2.2. Chemistry

The synthesis of nitro-containing salicylanilides (**1–33**) is described in Scheme 1. The final compounds were products of the conjugation of nitro-salicylic acids with selected anilines in the presence of PCl_3 under microwave irradiation. All compounds were isolated with good to moderate yields.



Scheme 1. Synthesis of nitro-substituted salicylanilides.