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Salicylanilide diethyl phosphates: Synthesis, antimicrobial activity and cytotoxicity

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ABSTRACT

A series of 27 salicylanilide diethyl phosphates was prepared as a part of our on-going search for new antimicrobial active drugs. All compounds exhibited in vitro activity against *Mycobacterium tuberculosis*, *Mycobacterium kansasii* and *Mycobacterium avium* strains, with minimum inhibitory concentration (MIC) values of 0.5–62.5 μmol/L. Selected salicylanilide diethyl phosphates also inhibit multidrug-resistant tuberculous strains at the concentration of 1 μmol/L. Salicylanilide diethyl phosphates also exhibited mostly the activity against Gram-positive bacteria (MICs $\geqslant$1.95 μmol/L), whereas their antifungal activity is significantly lower. The IC_{50} values for Hep G2 cells were within the range of 1.56–33.82 μmol/L, but there is no direct correlation with MICs for mycobacteria.

1. Introduction

The worldwide increased number of multidrug-resistant tuberculosis (MDR-TB) cases, emergence of extensively drug-resistant tuberculosis (XDR-TB) and recently reported totally drug-resistant (TDR-TB) strains become a very serious health and social problem. Therefore the development of new drugs which should be able to shorten the treatment of TB and stop emerging resistance is required.

Nontuberculous (atypical) mycobacteria represent causative agents of various opportunistic human infections; their treatment is complicated, particularly due to the high level of antibiotic resistance. The problems with drug-resistance have been reported also for many other bacterial and fungal strains, including methicillin-resistant *Staphylococcus aureus* (MRSA), enterococci, *Pseudomonas aeruginosa* or the family of *Enterobacteriaceae*.

Salicylanilide esters with carboxylic acids[1–4] as well as benzenesulfonic acid[5] have been described sharing a significant antimycobacterial activity including against nontuberculous mycobacteria, MDR- and XDR-TB strains in micromolar or even in submicromolar range. Moreover, they have exhibited a mild inhibition of mycobacterial isocitrate lyase, an enzyme essential for the maintenance of latent TB infection.[1,4] Some of the salicylanilide esters were also reported as agents with a significant antimicrobial activity especially against Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* and filamentous fungi.[2–4]

The modification of parent molecules leading to the prodrugs or more active derivatives may help to overcome undesired properties. Phosphate triesters (phosphotriesters) approach have been applied successfully e.g. in the design of nucleoside prodrugs.[6,7] Phosphate-based prodrugs have been developed, inter alia, for the modification of solubility, which represents one critical parameter for the drug administration. Many phosphate esters often show both good chemical stability and rapid and easy enzymatic hydrolysis.[8]

Substituted diethyl phenyl phosphates have been reported to exhibit various biological effects and applications including insecticides inhibiting acetylcholinesterase,[9–11] herbicides,[12] fungicidal,[13] antiviral and cytotoxic[14] agents. A well-known acetylcholinesterase inhibitor paraoxone, diethyl (4-nitrophenyl) phosphate, the member of diethyl phenyl phosphate group, has revealed also some interesting metabolic and physiological impacts.[15–17] However, as an organophosphorus compound, it displays a high toxicity for humans with a potential threat of misuse

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as military nerve poison. The main mechanism of action has been generally attributed to the central and peripheral inhibition of human acetylcholinesterase, but other additional mechanisms have been identified.[18]

Despite the organophosphate toxicity, we selected salicylanilide diethyl phosphates (diethyl [2-(phenylcarbamoyl)phenyl] phosphates) as a new group of salicylanilide phosphoric acid-derived molecules as potential inhibitors of mycobacterial and other microbes growth and enzymatic reactions. Despite of various reports dealing with particular molecular and cellular effects of salicylanilide derivatives (e.g.,[1,2,19]), the exact mechanism(s) of salicylanilide action as antimicrobial agents is not still completely elucidated, probably combining specific and non-specific aspects.

2. Results and discussion

2.1. Chemistry

Series of substituted diethyl [(2-phenylcarbamoyl)phenyl] phosphates **1** was prepared by two synthetic steps. Condensation of 4/5-chloro and 5-bromosalicylic acids with substituted anilines was done in a microwave reactor according to the lit.[20] Salicylanilides were esterified by diethyl chlorophosphate in the presence of triethylamine (TEA) in dichloromethane (Scheme 1). This procedure and subsequent isolation and purification gave yields within the range of 11–78%.

Based on the literature data,[6–9,21,22] we can propose two possible pathways of diethyl salicylanilide phosphates **1**, as possible salicylanilide prodrugs, decomposition in organisms (Fig. 1). Firstly, it could be cleft by a two-step process. After cleavage of two molecules of ethanol from phosphotriesters, resulting 2-(phenylcarbamoyl)phenyl phosphate as anion with two negative charges is much more soluble in water and it can be a convenient soluble transport form of parent salicylanilides. Then, the phosphate group may be removed hydrolytically by enzymes like phosphatase and released salicylanilide may cross through biological barriers.

In the case, when diethyl [2-(phenylcarbamoyl)phenyl] phosphate reaches the proximity of targeted cells unhydrolysed, it may penetrate their biomembranes and it should be cleaved into charged phosphate within the cell. This polar form should be concentrated in the cytoplasm due to potentially abolished salicylanilide membrane shuttling.

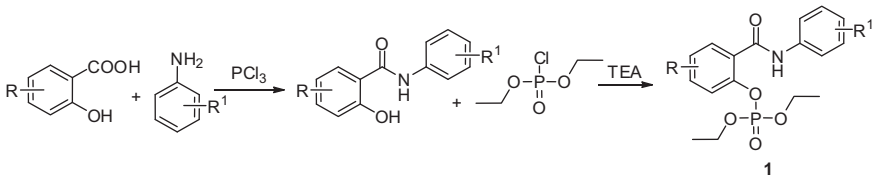
In the second way, the phosphotriester prodrug as a depot form could release salicylanilide directly by the hydrolysis of P–O(–Ar) bond. This step is catalysed by phosphotriesterase enzyme paraoxonase (PON1), which also inactivates highly toxic organophosphates like paraoxon, soman, tabun or sarin as well as other different substrates including aromatic esters. Paraoxonase is located in plasma, liver, brain, kidney and, importantly, in lungs.[22,23] However, it was described that replacement of the nitro group from paraoxon as well as electronic changes converted these analogues into inhibitors of PON1. Hydrophobicity was detrimental to hydrolysis by PON1.[24]

2.2. Antimycobacterial activities

All compounds were tested in vitro for their antimycobacterial activity against *Mycobacterium tuberculosis* (*Mtb.*) strain $H_{37}Rv$, *Mycobaterium avium* and two *Mycobacterium kansasii* strains; *M. kansasii* 6509/96 was isolated from a patient. The screened minimum inhibitory concentration (MIC) values are summarized in Table 1.

Results revealed that all tested compounds displayed an activity comparable with INH against *Mtb.* (five compounds—**1d**, **1f**, **1o**, **1r** and **1zz**—exhibited identical MIC as INH of 1 μmol/L), very high efficiency against *M. avium* (4–62.5 μmol/L, all esters superior to INH) and both strains of *M. kansasii* (0.5–32 μmol/L; all phosphates **1** showed a better inhibition of the strain 235/80 than INH and most of them were comparable or superior to isoniazid for the clinical isolate) and also much better activity against all evaluated mycobacteria than *para*-aminosalicylic acid (PAS), a second-line oral drug sharing a structural similarity.

The most active 5-chloro-2-{[4-(trifluoromethyl)phenyl]carbamoyl}phenyl diethyl phosphate (**1zz**) has expressed the best activity against all tested strains (from 0.5 to 4 μmol/L), closely followed by its 4-chloro isomer **1r** and then 4-bromo derivative **1f**. In general, it can be postulated that activity is increased by 4-trifluoromethyl (**1f**, **1r**, **1zz**) and 3,4-dichloro (**1d**, **1o** and less **1y**) substitution on aniline part, followed by 3-CF_3 (especially **1e** and **1q**) and 4-Br. On the other site, fluorination of aniline led to the derivatives with the highest MICs (**1a**, **1i**, **1l**, **1m**, **1u**, **1v**) when compared to other monohalogen substitution patterns. When focused on position isomerism, derivatives of 4-chloroaniline showed a somewhat lower MICs than those of 3-chloro one (**1b** vs **1c**, **1j** vs **1k** and **1s** vs



Scheme 1. Synthesis of diethyl (2-phenylcarbamoyl)phosphates **1** (R for esters **1** = 4-Cl, 4-Br, 5-Cl, R^1 = 3-Cl, 4-Cl, 3,4-diCl, 3-Br, 4-Br, 3-F, 4-F, 3-CF_3, 4-CF_3).

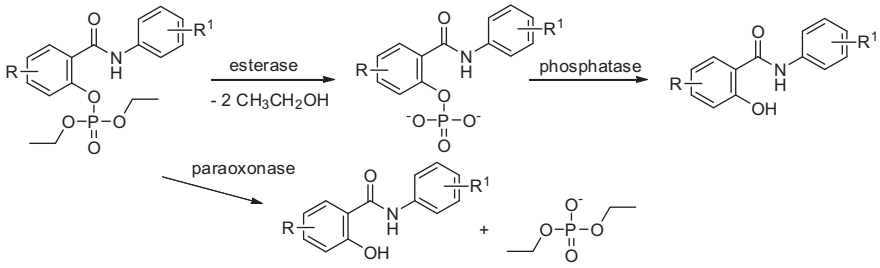


Figure 1. Two expected metabolic pathways of diethyl salicylanilide phosphates **1**.