

***In vitro* Evaluation of 2-/3-Alkoxyphenylcarbamic Acid Esters Containing *N*-Arylpiperazine Moiety Against Some Nontuberculous Mycobacteria**

Ivan Malik¹, Iveta Zadrazilova² and Josef Jampilek²

¹Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Comenius University, Odbojárov 10, 832 32 Bratislava, Slovak Republic

²Department of Chemical Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences Palackého 1/3, 612 42 Brno, Czech Republic

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Nontuberculous mycobacteria (NTM) are a diverse group of ubiquitous environmental organisms and include more than 125 identified species broadly classifiable into “slow” and “rapid” growers. NTM are present in the environment, mainly in water, and are occasionally responsible for opportunistic infections in humans. Despite the fact that NTM are characterized by a moderate pathogenicity, the diseases caused by NTM at various body sites are increasing worldwide.^{1,2} Pulmonary involvement is the most common disease manifestation of NTM.^{3,4} Furthermore, patients with cystic fibrosis, the most frequent lethal genetic disorder of White persons, face numerous infectious pathogens over the course of their lifespan, increasing attention has recently been paid to NTM.^{5,6}

Treatment of infections caused by NTM is either by drug therapy, surgery, or both. Drug therapy of NTM diseases is long, costly, and often associated with drug-related toxicities. The treatment regimens differ by species with the most important distinction being that between slow and rapid growing NTM.⁷

Searching for novel structures that NTM have very rarely been presented with before, the aim of current research is to *in vitro* investigate the efficiency of 2-/3-alkoxyphenylcarbamic acid esters containing 4-(pyrimidin-2-yl) piperazin-1-yl fragment within their structure (Table 1) against some members of NTM strains, namely *Mycobacterium marinum* CAMP 5644, *M. kansasii* DSM 44162 and *M. smegmatis* ATCC 700084 and try to identify some structural features which could be beneficial in terms of their potency. The molecules **1-4** have been previously investigated against Gram-positive bacteria *Staphylococcus aureus* ATCC 6538 (*Micrococcaceae*), Gram-negative bacteria *Escherichia coli* CNCTC 377/79 (*Enterobacteriaceae*) and yeast *Candida albicans* CCM 8186 as well.⁸

The derivatives under the study **1-4** (Table 1), chemically 1-[3-(2-/3-alkoxyphenylcarbamoxyloxy)-2-hydroxypropyl]-4-(pyrimidin-2-yl)piperazinium chlorides (where alkoxy=methoxy or ethoxy), were obtained from Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Comenius University in Bratislava, Slovak Republic.

The evaluation of *in vitro* antimycobacterial activity of the compounds was performed against *M. marinum* CAMP 5644, *M. kansasii* DSM 44162 and *M. smegmatis* ATCC 700084. The broth dilution

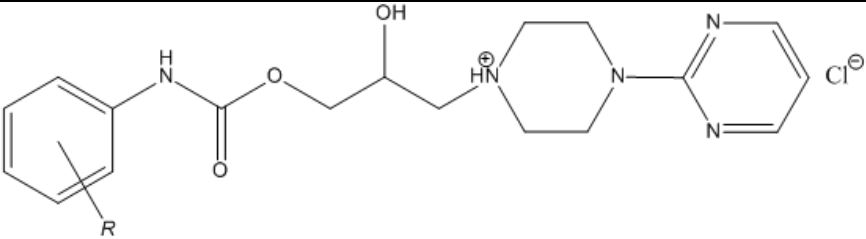
Correspondence to: Ivan Malik
Tel.: +421-2-50 117 226; Fax: +421-2-50 117 100;
E-mail: malikivan001@gmail.com

micromethod in Middlebrook 7H9 medium (Difco, USA) supplemented with BD BBL Middlebrook ADC Enrichment (Becton, USA) was used to determine the minimum inhibitory concentration (MIC) as previously described.⁹ The compounds were dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich, Germany), and the final concentration of DMSO did not exceed 2.5% of the total solution composition. The final concentrations of the evaluated compounds ranging from 256 to 0.125 µg/mL were obtained by two-fold serial dilution of the stock solution in microtiter plate with sterile medium. Isoniazid (*INH*; Sigma-Aldrich, Germany) and rifampicin (*RIF*; Sigma-Aldrich, Germany) were used as the standards as they are clinically used antimycobacterial drugs. Bacterial inocula were prepared by transferring colonies from culture to sterile water. The cell density was adjusted to 0.5 McFarland units using a densitometer (Densi-

La-Meter, LIAP, Latvia). The final inoculum was made by 1:1000 dilution of the suspension with sterile water. Drug-free controls, sterility controls and controls consisted of medium and DMSO alone were included. The determination of results was performed visually after 3 days of static incubation in the darkness at 37 °C in an aerobic atmosphere for *M. smegmatis*, after 7 days of static incubation in the darkness at 37 °C in an aerobic atmosphere for *M. kansasii* and after 21 days of static incubation in the darkness at 28 °C in an aerobic atmosphere for *M. marinum*.¹⁰

The MICs were defined as the lowest concentration of the compound at which no visible bacterial growth was observed. The MIC readout is routinely and widely used in bacterial assays and is a standard detection limit according to the Clinical and Laboratory Standards Institute (CLSI, www.clsi.org). The results are summarized in table 1.

Table 1. Activity of the *in vitro* investigated compounds 1-4 against some nontuberculous mycobacterial strains expressed by MIC values (µmol/l).



Entry	R	MIC (µmol/l)		
		<i>M. marinum</i>	<i>M. kansasii</i>	<i>M. smegmatis</i>
1	2-OCH ₃	>256	>256	>256
2	2-OC ₂ H ₃	>256	128	>256
3	3-OCH ₃	>256	>256	>256
4	3-OC ₂ H ₃	>256	128	>256
<i>INH</i>	–	467	29.2	117
<i>RIF</i>	–	2.43	0.15	19.4

To be the best of authors' knowledge, current paper presents the first report dealing with the activity of 2-/3-alkoxyphenylcarbamic acid esters containing 2-hydroxypropane-1,3-diyl connecting chain and *N*-arylpiperazine group against *M. marinum* CAMP 5644, *M. kansasii* DSM 44162 and *M. smegmatis* ATCC 700084 as well.

Following chemical structure of currently *in vitro* investigated compounds 1-4 (Table 1), their potency against given NTM might be influenced, among others, by: (i) 2-/3-alkoxy side chain isomerism, (ii) their lipohydrophilic profile, (iii) nature of salt forming moiety. Possible impacts of

aforementioned factors will be discussed in the parts below.

As current results clearly indicated (Table 1), positional isomerism of alkoxy group did not affect the potency of the derivatives under the study against all the inspected mycobacteria. In terms of 2-alkoxy substituted derivatives, proximity of attached 2-alkoxy chain to carbamate bond led to the twist of the phenyl ring plain towards carbamate group. Described process resulted in the planarity violation of such molecule(s) to provide subsequent conjugation of π -bonds of aromatic ring over NH fragment up to carbonyl group. The outcome of this conjugation was different electronic density (charge) at carbonyl moiety which could be one of possible binding sites to reactive membrane locations. In the series of 3-alkoxy substituted compounds, given secondary steric effect did not manifest. Current results confirmed that described notable intramolecular interactions did not influenced the activity. Following given preliminary data, it was not clear if such lipophilic moiety (alkoxyphenyl group) could be regarded as essential part of pharmacophore. Taking into account effect of the positional isomerism, further experimental determinations will be carried out for more comprehensive conclusions.

All the investigated derivatives were considered more efficient against *M. marinum* than *INH*. Furthermore, elongation of attached alkoxy side chain in both series of 2- and 3-substituted molecules, i.e. the increase in lipophilicity, led to more promising results for *M. kansasii* strain only. On the other hand, both compounds **2** and **4** have shown higher MIC outputs than used standards *INH* and *RIF* not only for *M. kansasii* but also for *M. smegmatis*. The standard *RIF* were regarded as the most active compound of entire tested set against all *in vitro* inspected NTM (Table).

In general, it seemed that lipophilicity could be important factor affecting the potency of alkoxyphenylcarbamic acid esters containing 2-hydroxypropane-1,3-diyl and *N*-arylpiperazine moiety not only against given *Mycobacterium* but

also against previously *in vitro* tested *M. tuberculosis* H₃₇R_v and *C. albicans*.^{11,12}

Furthermore, it could be suggested that the differences in assumed electronic and hydrophobic interactions with effector sites of tested NTM involved by various substituents attached to piperazin-1,4-diyl would be decisive for the activity of thus alkoxyphenylcarbamic acid-based substances. Following conclusions from previous research, the presence of pyrimidin-2-yl in the chemical structure of *N*-arylpiperazine compounds would be favorable in terms of their toxicological profile due to formation of 1-(pyrimidin-2-yl) piperazine, an active metabolite, which is converted to 5-hydroxy-1-(pyrimidin-2-yl) piperazine in human liver microsomes.^{13,14} On the other hand, both atoms of nitrogen have shown the ability to polarize the π -electron system of pyrimidin-2-yl, resulting in slightly decreased electron density on the ring carbons. It would be probable that incorporation of (suitably substituted) phenyl instead of heterocyclic pyrimidin-2-yl would lead to more promising results.

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