The *in vitro* Antimycobacterial Investigation of Some Basic *meta*-/*para*-Alkoxyphenylcarbamic Esters Bearing 4-(4-Fluoro/2-methoxyphenyl)piperazin-1-yl Fragment

Ivan Malik[1], Eva Sedlarova[1], Jozef Csollei[1,2], Lukas Stanzel[1], Lubica Sichrovska[1] and Jirina Stolarikova[3]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Comenius University Odbojárov 10, 832 32 Bratislava, Slovak Republic
[2]Department of Chemical Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences,Palackého 1-3, 612 42 Brno, Czech Republic
[3]Laboratory for Mycobacterial Diagnosis and Tuberculosis, Institute of Public Health in Ostrava Partyzánské nám. 7, 702 00 Ostrava, Czech Republic

ABSTRACT: Following our previous systematic research with *ortho*-/*meta*-/*para*-alkoxyphenylcarbamic acid esters as antimycobacterially active compounds, the set of the derivatives containing *meta*-/*para*-alkoxyphenylcarbamoyloxy and 4-(4-fluorophenyl/2-methoxyphenyl)piperazin-1-yl moieties were tested for their *in vitro* activity against *Mycobacterium tuberculosis* CNCTC My 331/88 as well as against potential pathogenic strains of *M. kansasii* CNCTC My 235/80, *M. avium* CNCTC My 330/88 and *M. kansasii* 6 509/96 by applying the micromethod for the determination of the minimum inhibitory concentration (MIC). In terms of estimated MIC readouts, the advantage of the molecules under study has been the activity against *Mycobacterium tuberculosis*.

Key words: Antimycobacterial activity, *meta*-/*para*-alkoxyphenylcarbamic acid esters, arylpiperazines.

INTRODUCTION

The idea to investigate basic esters of *ortho*-/*meta*-/*para*-alkoxyphenylcarbamic acids as potential antimycobacterial agents has been probably primarily connected with the publishing of research papers of Čižmárik *et al.*[1] and Waisser *et al.*[2] in which the authors have evaluated the *in vitro* efficiency of piperidino and pyrrolidino ethyl esters against *Mycobacterium* (*M.*) *tuberculosis* CNCTC My 331/88, *M. kansasii* CNCTC My 235/80, *M. avium* CNCTC My 330/88 and *M. kansasii* 6 509/96 strains. Within another study, the authors mentioned above have analyzed antimycobacterial activity – structure relationships within the series of 124 basic esters which basic compartment has been formed by aliphatic dimethylamino group or by the cyclic one, *i.e.* piperidin-1-yl, pyrrolidin-1-yl, 4-morpholin-1-yl or azepan-1-yl moiety, respectively.[3] Generally, such activity has been potentiated by the elongation of alkoxy side chain attached to lipophilic aromatic ring. It has been also observed that *meta*- and *para*-alkoxy substituted derivatives (substituent attached to position 3 or 4 at the aromate within lipophilic part) have been more effective than the *ortho*-alkoxy substituted ones (substituent attached to position 2). Additionally, the most lipophilic azepan-1-yl fragment has shown the most positive influence while the compounds containing 4-morpholin-1-yl have been regarded as less active. However, the linear correlation of the contribution of mentioned basic substituents with the lipophilicity has been considered very questionable.[3] The researchers have also previously proved that the elongation of ethane-1,2-diyl connecting chain to the propane-1,3-diyl one

Correspondence to: Ivan Malik
Tel.: +421-2-50 117 226; Fax: +421-2-50 117 100

has led to the maintenance of antimycobacterial potency *in vitro* (or even in the increase in the activity) against *M. tuberculosis* of such prepared derivatives as well as to the decrease in the efficacy against potentially pathogenic strains.[4] Similarly, the embranchment of connecting chain within the chemical structure of inspected compounds has also meant a maintenance or even an enhancing their *in vitro* effectiveness.[5] A few years ago, by the applying linear, parabolic and sigmoidal calculation model as well, Waisser *et al*.[6] have documented that the increase in the activity in keeping with the lipophilicity enhancement has been limited due to a cut-off effect observation.[6]

As the continuation of outlined research considering *ortho*-/*meta*-/*para*-alkoxyphenylcarbamic acid esters as promising antituberculotics, the aim of current study has been to investigate antimycobacterial activity profile of some derivatives which basic part has been formed by substituted *N*-phenylpiperazine moiety. It should be mentioned that antimycobacterial profile of some structurally similar molecules has been already published,[7,8] so the current investigation could also be regarded as the contribution to more complex knowledge in terms of structure – activity relationships study within given class of the compounds.

MATERIALS AND METHODS

Chemicals and reagents. Currently evaluated compounds labelled as **1-5** (Table 1), chemically 1-[3-(/3-/ 4 - alkoxyphenylcarbamoyloxy) - 2- hydroxypropyl]-4-(4-fluorophenyl/2-methoxyphenyl) piperazinium chlorides (alkoxy=methoxy or ethoxy), were previously synthesized at the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Comenius University, Bratislava, Slovak Republic as well as at the Department of Chemical Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic. The substances under the study were prepared and *in vitro* tested as the racemates, not as pure enantiomers. The compound used as the control, first-line antituberculosis drug isoniazide (INH), was purchased from Sigma-Aldrich (Germany).

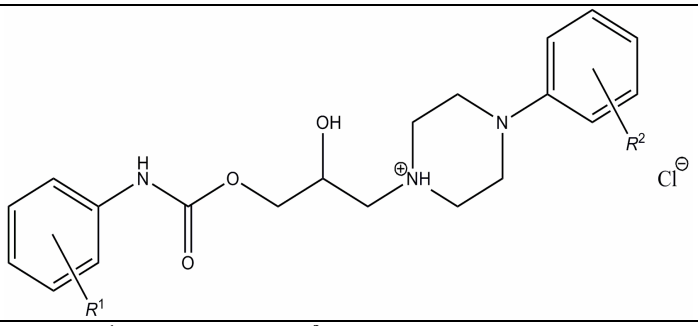
***In vitro* antimycobacterial assay.** The *in vitro* antimycobacterial activity of the inspected compounds 1-5 was determined against *Mycobacterium tuberculosis* My 331/88 (10^{-3} dilution of the strain), *M. avium* My 330/88 (10^{-5} dilution of the strain), *M. kansasii* My 235/80 (10^{-4} dilution of the strain) and *M. kansasii* 6509/96 (10^{-4} dilution of the strain) following the procedure reported earlier.[9] All of the strains were obtained from the Czech National Collection of Type Cultures (CNCTC) except of *M. kansasii* 6509/96, which was clinically isolated. The antimycobacterial activity of the compounds was determined in a Sula´s semisynthetic medium (SEVAC, Prague, Czech Republic) *via* the micromethod for the determination of the minimum inhibitory concentration (*MIC*) at 37ºC after 14 (14 d) and 21 days (21 d) of the incubation for the *M. tuberculosis* and *M. avium* strains, and after 7 (7 d), 14 (14 d) and 21 (21 d; these abbreviations have been used in Table 2) days of the incubation for both *M. kansasii* strains, respectively. The tested compounds were added to the medium as dimethylsulfoxide (DMSO) solutions and INH was used as a standard in sterile water solution. The following concentrations of inspected molecules were used: 1000, 500, 250, 125, 62.5, 32, 16, 8, 4, 2 and 1 μmol/l, INH was applied in the concentration range of 250-0.5 μmol/l.

RESULTS AND DISCUSSION

The efficiency against *M. tuberculosis* (My 331/88). Following currently estimated values of the *MIC* (Table 2), the elongation of alkoxy side chain has led to the increase in the activity within both evaluated series, *i.e.* for *meta*-alkoxy (compounds **1** and **2**) as well as *para*-alkoxy substituted derivatives (compounds **3** and **4**). Current results have also indicated no differences in the *MIC* readouts of investigated derivatives **1-4** due to positional *meta*-/*para*-alkoxy side chain isomerism, as can be seen from the data in Table 2. On the other hand, previously estimated *MIC* output for *meta*-butoxy substituted homologue (*MIC*=500 μmol/l for both 14-

day and 21-day incubation) could confirmed the proposal that the increase in the activity with the elongation of side string has been limited due to possible cut-off effect.[7] On the other hand, taking into account previously estimated *MIC*s for other *para*-alkoxy substituted homologues, *i.e.* the *para*-propoxy (*MIC*=16 and 32 μmol/l, respectively) and the *para*-butoxy (*MIC*=8 μmol/l for both 14-day and 21-day incubation) ones, the enhancement in the lipophilicity has meant lower values of the *MIC*.[7] Additionally, the replacement of 4-(4-fluorophenyl)piperazin-1-yl by 4-(2-methoxyphenyl)piperazin-1-yl has not led to lower values of the *MIC* (compound **5**, Table 2). On the contrary, all the determined *MIC* readouts within entire set of tested molecules **1-5** have been substantially higher than the ones which has been simultaneously estimated for reference standard, INH (Table 2).

Table 1. The chemical structure of antimycobacterially investigated compounds 1-5.



Entry	R^1	R^2	Formula	M_r
1	3-OCH_3	4´-F	$C_{21}H_{27}FClN_3O_4$	439.91
2	3-OC_2H_5	4´-F	$C_{22}H_{29}FClN_3O_4$	453.94
3	4-OCH_3	4´-F	$C_{21}H_{27}FClN_3O_4$	439.91
4	4-OC_2H_5	4´-F	$C_{22}H_{29}FClN_3O_4$	453.94
5	3-OC_2H_5	2´-OCH_3	$C_{23}H_{32}ClN_3O_5$	465.98

Table 2. Antimycobacterial activity of the compounds under the study against selected tuberculous and non-tuberculous mycobacterial strains.

Entry	*M. tuberculosis* My 331/88		*M. avium* My 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
1	125[a]	250[a]	250[b]	500[b]	62.5	62.5[b]	62.5[b]	250	250[b]	500
2	62.5	125	500	1000	32	62.5	62.5	*n*[b]	*n*[b]	*n*[b]
3	125[a]	250[a]	500[b]	500	62.5	125[b]	250[b]	125	250	500[b]
4	62.5[a]	125[a]	125	250	125	250	500	62.5	125	250
5	62.5	125	500	1000	250	500	500	125	250	500
INH	0.5	1	>250	>250	>250	>250	>250	2	4	8

[a]The data have been previously published in the paper[7]
[b]The data have been previously published in the paper[8]
n - impossible to determine due to low solubility of the compound or low growth of the mycobacteria

Efficiency against *M. avium* (My 330/88). As can be deduced from the results summarized in Table 2, all the inspected compounds **1-5** have been considered practically inactive (the determined *MIC*s have been noticed in the interval of 125 - 1000 μmol/l) against given bacterial strain regardless the type of the substitution within basic *N*-phenylpiperazin-1-yl fragment. For *para*-alkoxy substituted derivatives, the replacement of methyl by ethyl has caused the decrease in the *MIC*s. For the complexity of information, the non-tuberculous *M. avium* My 330/88 strain has been even resistant against INH, as corresponding *MIC* outputs have clearly revealed (Table 2).

Efficiency against *M. kansasii* (My 235/80). As current observation has indicated, positional *meta*-/*para*-alkoxy side string isomerism could be considered important factor in terms of the activity against given non-tuberculous mycobacterial strain. *meta*-alkoxy substituted derivatives **1** and **2** have been slightly more effective, the *MIC*s have been observed in the range of 32 - 62.5 μmol/l, than the corresponding *para*-alkoxy substituted ones, *i.e.* compounds **3** and **4** with their *MIC*s in the area of 62.5 - 500 μmol/l (Table 2). It could be stated that both tested *meta*-alkoxy substituted derivatives have shown comparable effectiveness against concerned mycobacterial strain. Within the series of *para*-alkoxy substituted substances, the increase in the lipophilicity has not seemed to be a crucial factor which has influenced such potency (Table 2). The modification within basic part, i.e. the replacement of the fluorine atom attached to *para*-position (compound **2**) by methoxy group placed at *ortho*-position (compound **5**) has caused the increase in the *MIC* output of such derivative (Table 2). Following current results it could be suggested that (i) the presence of the substituent which has shown primarily electron-donating effect toward aromatic ring has led to the decrease in the potency. It has been well known that positive mesomeric effect (i.e. electron-donating + *M*-effect) has prevailed over negative inductive influence (*i.e.* electron-withdrawing-*I*-effect) for methoxy group directly attached to phenyl ring.[10,11] Additionally, it could be also proposed that (ii) the presence of less sterically bulky substituent (atom of fluorine) has been regarded as more convenient than the substitution by the sterically bulkier one (methoxy group);[12] (iii) the distribution and the size of the charge have seemed to be more important for the activity against given non-tuberculous strain compared to the chosen tuberculous one. The most effective compound within evaluated set, *meta*-ethoxy derivative **2**, has been also more potent than INH (Table 2).

Efficiency against *M. kansasii* (6509/96). As the results summarized in Table 2 have outlined, *para*-position of alkoxy side chain would be more favourable in terms of the activity against concerned clinically isolated mycobacterial strain than the *meta*-one. The increase in the lipophilicity has led to more effective substance, as the outputs for the compounds **3** and **4** have indicated. On the contrary, it could be seemed that the presence of electron-donating group within basic *N*-phenylpiperazin-1-yl moiety has not meant notable improvement in the activity profile of such substituted molecule. The most active *para*-ethoxy derivative has been regarded as less active than reference standard, INH (Table 2).

In conclusion, this study has brought to light potential antimycobacterially active class of the compounds contaning *meta*-/*para*-alkoxyphenyl-carbamoyloxy as well as (substituted) *N*-phenylpiperazin-1-yl fragments which have possessed relatively promising activity especially against *M. tuberculosis* My 331/88 and *M. kansasii* My 235/80 strains. Despite limited set of evaluated compounds, such effectiveness has appeared to be dependent at least on these factors: (i) on the position and the length of present alkoxy side string directly attached to lipophilic aromatic ring (*M. tuberculosis* My 331/88) and (ii) on the distribution as well as the size of the charge within basic fragment (*M. kansasii* My 235/80).

ACKNOWLEDGEMENT

The authors are very grateful to Slovak Grant Agency for Science for supporting by the VEGA Grant Project No. 1/0039/12.

REFERENCES

1. Čižmárik, J., Švejnochová, M. and Schwartz, E. 1986. Tuberkulostatische Wirkung der Piperidinoethylesters der Alkoxyphenylcarbamidsäuren. *Pharmazie* **41**, 744-745.
2. Waisser, K., Čižmárik, J., Dražková, K. and Kaustová, J. 2002. Antimycobacterial effects of pyrrolidine esters of alkoxysubstituted phenylcarbamic acids. *Čes. slov. Farm.* **51**, 140-144.
3. Waisser, K., Dražková, K., Čižmárik, J. and Kaustová, J. 2003. Antimycobacterial activity of basic ethyl esters of alkoxy-substituted phenylcarbamic acids. *Folia Microbiol.* **48**, 45-50.
4. Waisser, K., Dražková, K., Čižmárik, J. and Kaustová, J. 2003. Antimycobacterial activity of piperidinylpropyl esters

of alkoxy-substituted phenylcarbamic acids. *Folia Microbiol.* **48**, 585-587.

5. Waisser, K., Dražková, K., Čižmárik, J. and Kaustová, J. 2004. A new group of potential antituberculotics: Hydrochlorides of piperidinylalkyl esters of alkoxy-substituted phenylcarbamic acids. *Folia Microbiol.* **49**, 265-268.
6. Waisser, K., Doležal, R. and Čižmárik, J. 2009. Graphic demonstration of the structure – antimycobacterial activity relationships in the series of ester phenylcarbamid acids with piperidine or pyrrolidine moiety. *Folia Pharm. Univ. Carol.* **37**, 65-76.
7. Waisser, K., Doležal, R., Čižmárik, J., Malík, I. and Kaustová, J. 2007. The potential antituberculotics of the series of 2-hydroxy-3-(4-phenylpiperazin-1-yl)-propyl-phenylcarbamates. *Folia Pharm. Univ. Carol.* **35-36**, 45-48.
8. Waisser, K., Doležal, R., Čižmárik, J., Malík, I. and Kaustová, J. 2007. The antimycobacterial derivatives against potential pathogenic strains: 2-Hydroxy-3-(4-phenylpiperazin-1-yl)-propylphenylcarbamates. *Sci. Pharm.* **75**, 55-61.
9. Kozic, J., Novotná, E., Volková, M., Stolaříková, J., Trejtnar, F. and Vinšová J. 2012. Synthesis and *in vitro* antimycobacterial activity of 2-methoxybenzanilides and their thioxo analogues. *Eur. J. Med. Chem.* **56**, 387-395.
10. Todeschini, R. and Consoni, V. 2000. Handbook of molecular descriptors. In: *Methods and Principles in Medicinal Chemistry. Vol. 11* (Eds. Mannhold, R., Kubinyi, H. and Timmerman, H.), Wiley-VCh Verlag, Weinheim, 667 pp.
11. Kubinyi, H. 1993. *QSAR: Hansch Analysis and Related Approaches*, Wiley-VCh Verlag, Weinheim, 240 pp.
12. Skagerberg, B., Bonelli, D., Clementi, S., Cruciani, G. and Ebert, C. 1989. Principal properties for aromatic substituents. A multivariate approach for design in QSAR. *Quant. Struct.-Act. Relat.* **8**, 32-38.