PŮVODNÍ PRÁCE

Antimycobacterial activity of novel derivatives of arylcarbonyloxyaminopropanols

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SUMMARY

Antimycobacterial activity of novel derivatives of arylcarbonyloxyaminopropanols

The study examined the antimicrobial activity of 2-hydroxy-3-[4-(2-fluoro, 4-fluoro, and 2-metoxyphenyl)-piperazin-1-yl]-propyl-4-[(alkoxycarbonyl)amino] benzoates, their salts with hydrochloric acid, with one to four carbon atoms in the alkoxy group of the carbamoyl group studied and described. The series of these substances were evaluated *in vitro* against *Mycobacterium tuberculosis, Mycobacterium avium, Mycobacterium kansasii* and against clinically isolated strains of *Mycobacterium kansasii* 6 509/96. The correlation of this activity with lipophilicity (log k') was used to describe the structure-antimycobacterial activity relationships (QSARs). The tests have shown that practically all the substances under study possess antituberculotic activity. *In vitro* antimycobacterial activity increased with increasing lipophilicity. According to the obtained results, the substances can be considered as potential antituberculotics.

Key words: antimycobacterial activity – antituberculotic activity – potential antituberculotics – arylcarbonyloxyaminopropanols – lipophilicity – phenylcarbamates

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SÚHRN

Antimykobakteriálna aktivita nových derivátov arylkarbonyloxyaminopropanolov

V práci je opísaná antimikrobiálna aktivita 2-hydroxy-3-[4-(2-fluór, 4-fluór, a 2-metoxyfenyl)--piperazín-1-yl]-propyl-4-[(alkoxykarbonyl)amino] benzoátov, ich solí s kyselinou chlorovodíkovou, s jedným až štyrmi atómami uhlíka v alkoxy skupine karbamoylovej skupiny. Série týchto látok boli hodnotené *in vitro* vzhľadom na *Mycobacterium tuberculosis, Mycobacterium avium, Mycobacterium kansasii* a klinicky izolovaný kmeň *Mycobacterium kansasii* 6 509/96. Korelácia aktivity s lipofilitou (log k') bola použitá pre popis vzťahov štruktúra-antimykobakteriálna aktivita (QSAR).Testy ukázali, že prakticky všetky študované látky majú antituberkulotickú aktivitu. *In vitro* sa antimikrobiálna aktivita zvyšovala so zvyšujúcou sa lipofilitou. Na základe dosiahnutých výsledkov možno študované látky považovať za potenciálne antituberkulotiká.

Kľúčové slová: antimykobakteriálna aktivita – antituberkulotická aktivita – potenciálne antituberkulotiká – arylkarbonyloxyaminopropanoly – lipofilita – fenylkarbamáty

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Introduction

In consideration of minutely increasing counts of the multiresistant strains of *Mycobacterium tuberculosis*, the design, preparation and study of new potential antituberculotics with structures different from those used in clinical praxis nowadays, belongs to the topical tasks of the drug research and development.

The task is really topical because there is also another source of the threat for European inhabitants – the occasionally pathogenic mycobacterial strains, such as *Mycobacterium avium*, *Mycobacterium kansasii* and *Mycobacterium intercellulare* [1].

The paper [1] described the antimycobacterial activity of 2-(4-alkylpiperazin-yl)-ethyl esters of 2-heptyl-oxyphenylcarbamic acid. The series of these substances was evaluated *in vitro* against *Mycobacterium tuberculosis*, *Mycobacterium kansasii*, *Mycobacterium avium* and against clinically isolated strains of *Mycobacterium kansasii* 6 509/96. According to the obtained results, all the studied substances possessed antituberculotic activity. The substances under study have in their chemical structure a fragment of substituted ester of phenylcarbamic acid and 4-substituted piperazine. These two groups are present in the studied structures and participate in the formation of some common metabolites.

The aim of this paper was to verify whether the studied structures exerted an antimycobacterial effect against hereinbefore mentioned strains.

EXPERIMENTAL PART

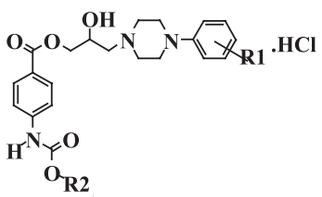
Synthesis

All of the compounds under study were prepared according to [2] and published in the papers [3, 4]. The structure of the substances studied is given in Table 1.

Microbiology

For the evaluation of the antimycobacterial activity *in vitro*, the following strains were used: *Mycobacterium tuberculosis* CNCTC My 331/88, *Mycobacterium avium* CNCTC My 330/88,

Table 1. Chemical structure of the compounds under study



Compd.	R1	R2	Compd.	R1	R2	Compd.	R1	R2
5a1	4-F	$-CH_3$	**5a2**	2-F	$-CH_3$	**5a3**	$2-OCH_3$	$-CH_3$
5b1	4-F	$-C_2H_5$	**5b2**	2-F	$-C_2H_5$	**5b3**	$2-OCH_3$	$-C_2H_5$
5c1	4-F	$-C_3H_7$	**5c2**	2-F	$-C_3H_7$	**5c3**	$2-OCH_3$	$-C_3H_7$
5d1	4-F	$-C_4H_9$	**5d2**	2-F	$-C_4H_9$	**5d3**	$2-OCH_3$	$-C_4H_9$

Table 2. The minimum inhibitory concentration (MIC) and the logarithm of the retention factor k (log k) of the substances under study

Compd.	log k	MIC (μmol/l) Time of inhibition 14 d/21 d			
		M. tuberculosis My 331/88	*M. avium* My 330/88	*M. kansasii* My 235/80	*M. kansasii* My 6 509/96
5a1	-0.42	62.5/125	125/250	250/250	125/250
5b1	-0.38	62.5/125	62.5/250	125/250	125/250
5c1	-0.32	32/62.5	125/250	125/125	62.5/125
5d1	-0.27	32/32	32/62.5	62.5/62.5	32/62.5
5a2	-0.35	125/250	250/500	250/500	62.5/125
5b2	-0.31	125/250	125/250	125/125	125/125
5c2	-0.26	32/62.5	62.5/125	125/125	62.5/125
5d2	-0.20	32/32	62.5/125	62.5/125	32/62.5
5a3	-0.36	125/250	250/250	500/>500	125/250
5b3	-0.32	32/62.5	250/250	500/>500	62.5/125
5c3	-0.27	32/62.5	125/250	125/125	32/62.5
5d3	-0.22	16/32	125/125	62.5/125	32/32
Ison.	–	0.5/0.5	>250/>250	>250/>250	2/4

Mycobacterium kansasii CNCTC My 235/80 obtained from the Czech National Collection of Type Cultures (CNCTC), National Institute of Public Health, Prague, and the clinically isolated strain of *Mycobacterium kansasii* 6 509/96. Antimycobacterial activity was determined in Šula semisynthetic medium (SEVAC, Prague). The substances were added to the medium in a Me_2SO solution. The final concentrations of the compounds were 1–500 μmol/l. The minimum inhibitory concentration (MIC, i.e. the lowest concentration of a substance at which the inhibition of growth occurred) [5] was determined after incubation at 37 °C for 14 and 21 days. The results are summarized in Table 2. The antituberculotic activity of isoniazide was used as the reference standard.

Determination of lipophilic properties

Since many years, lipophilicity is recognized as a meaningful parameter in many structure-activity and structure-ADME relationships. It is also the single most informative and successful physicochemical property in medicinal chemistry [6].

The chromatographic system for HPLC analysis for the retention factor k determination consisted of a pump Delta Chrom SDS (Watrex, Slovakia) with an injection valve and a UV detector Delta Chrom UVD 200 (Watrex, Slovakia). The analytical chromatography column was a Sepharon SGX C_{18} (254 × 4 mm, particle size 7 μm). The mobile phase was a mixture of 94 % methanol in an amount of 500 ml and 3.4 g of natrium acetate, the pH value was adjusted with acetic acid to pH = 6.0. The flow rate of the mobile phase was 0.6 $ml.min^{-1}$, the injection volume was 1.10^{-2} ml, the chromatograms were scanned at the wavelength of 274 nm. The solution of $NaNO_2$ ($c = 0.1\ mol.l^{-1}$) was used to determine the dead time t_0, and the solution of the analyzed compound in methanol medium ($c = 0.4 . 10^{-3}\ mol.l^{-1}$) was used for the determination of t_R.

Table 3. Linear regressions (5a1–5d1 derivatives)

Mycobacterium	Time of inhibition	a	b	r^2	s	n
***tuberculosis* My 331/88**	14 d	-2.329	0.800	0.843	0.167	4
	21 d	3.903	2.724	0.569	0.342	4
***avium* My 330/88**	14 d	-2.752	0.912	0.413	0.283	4
	21 d	-3.557	1.004	0.613	0.300	4
***kansasii* My 235/80**	14 d	-3.442	0.894	0.861	0.245	4
	21 d	-4.188	0.709	0.927	0.287	4
***kansasii* My 6509/96**	14 d	-4.128	0.433	0.928	0.283	4
	21 d	-4.188	0.709	0.926	0.287	4

Table 4. Parabolic regressions (5a1–5d1 derivatives)

Mycobacterium	Time of inhibition	a	b	c	r^2	s	n
***tuberculosis* My 331/88**	14 d	4.482	0.762	1.354	0.849	0.167	4
	21 d	-40.999	-24.370	2.016	0.676	0.342	4
***avium* My 330/88**	14 d	-33.967	-26.174	-3.016	0.519	0.283	4
	21 d	-62.442	-46.615	-6.215	0.934	0.300	4
***kansasii* My 235/80**	14 d	0.850	-2.856	0.993	0.861	0.245	4
	21 d	-26.584	-22.519	-2.364	0.989	0.287	4
***kansasii* My 6509/96**	14 d	-25.544	-21.742	-2.519	0.989	0.283	4
	21 d	-26.584	-22.519	-2.364	0.989	0.287	4

Table 5. Linear regressions (5a2–5d2 derivatives)

Mycobacterium	Time of inhibition	a	b	r^2	s	n
***tuberculosis* My 331/88**	14 d	-4.683	0.484	0.794	0.341	4
	21 d	-6.603	0.169	0.920	0.446	4
***avium* My 330/88**	14 d	-4.048	0.882	0.834	0.287	4
	21 d	-4.048	1.182	0.834	0.287	4
***kansasii* My 235/80**	14 d	-3.572	1.090	0.893	0.245	4
	21 d	-3.334	1.307	0.519	0.300	4
***kansasii* My 6509/96**	14 d	-2.556	1.077	0.473	0.241	4
	21 d	-1.905	1.482	0.677	0.150	4

Table 6. Parabolic regressions (5a2–5d2 derivatives)

Mycobacterium	Time of inhibition	a	b	c	r^2	s	n
***tuberculosis* My 331/88**	14 d	16.652	4.445	1.682	0.813	0.341	4
	21 d	-10.302	-12.249	-0.573	0.925	0.446	4
***avium* My 330/88**	14 d	40.146	17.956	3.769	0.994	0.287	4
	21 d	40.146	17.956	4.069	0.994	0.287	4
***kansasii* My 235/80**	14 d	3.504	-1.651	1.342	0.895	0.245	4
	21 d	63.358	31.393	5.863	0.882	0.300	4
***kansasii* My 6509/96**	14 d	-50.448	-30.206	-2.551	0.831	0.241	4
	21 d	-28.175	-17.347	-0.545	0.965	0.150	4

Table 7. Linear regressions (5a3–5d3 derivatives)

Mycobacterium	Time of inhibition	a	b	r^2	s	n
***tuberculosis* My 331/88**	14 d	-5.559	-0.054	0.821	0.373	4
	21 d	-5.556	0.243	0.814	0.374	4
***avium* My 330/88**	14 d	-2.574	1.488	0.815	0.173	4
	21 d	-1.964	1.741	0.633	0.150	4
***kansasii* My 235/80**	14 d	-7.111	0.235	0.922	0.450	4
	21 d	-5.147	0.885	0.815	0.347	4
***kansasii* My 6509/96**	14 d	-4.316	0.458	0.865	0.282	4
	21 d	-6.300	0.099	0.997	0.384	4

Table 8. Parabolic regressions (5a3–5d3 derivatives)

Mycobacterium	Time of inhibition	a	b	c	r^2	s	n
***tuberculosis* My 331/88**	14 d	36.572	15.623	2.912	0.879	0.373	4
	21 d	38.907	16.980	3.398	0.879	0.374	4
***avium* My 330/88**	14 d	5.364	0.534	1.923	0.821	0.173	4
	21 d	-34.120	-21.726	-1.027	0.947	0.150	4
***kansasii* My 235/80**	14 d	-23.390	-20.660	-1.662	0.938	0.450	4
	21 d	10.728	1.067	1.755	0.821	0.347	4
***kansasii* My 6509/96**	14 d	41.130	19.507	3.793	0.994	0.282	4
	21 d	8.327	-1.478	0.775	0.999	0.384	4

The quantitative structure-activity relationships (QSARs)

The quantitative structure-activity relationships were determined according to two computing models of linear regression (log MIC = a log k' + b) and parabolic regression (log MIC = a $(\log k')^2$ + b $(\log k')^2$ + c). Both types of the calculation were carried out using the Microsoft Excel program. The results are summarized in Tables 3–8.

RESULTS AND DISCUSSION

The paper focused on the antimicrobial activity of the new compounds.

The logarithm of the retention factor k, like the lipophilicity parameter, was obtained by the HPLC method. The log k values of the substances under evaluation increased with the increasing number of carbon atoms in the alkoxy chain of the carbamoyl group. The results of the analysis confirm that the activity of the compounds increases with increasing lipophilicity.

The conclusions indicate that practically all of the derivatives show expected antimycobacterial activity in consideration of all of the strains studied, and some of them are more efficient than the standard isoniazide.

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REFERENCES

1. **Čižmárik, J., Waisser, K., Doležal, R., Kaustová, J.:** Farm. Obzor, 2007; 76, 144–146.
2. **Mokrý, P., Zemanová, M., Csöllei, J., Račanská, E., Tumová, I.:** Pharmazie, 2003; 58, 18–21.
3. **Kečkéšová, S., Sedlárová, E., Csöllei, J., Mokrý, P.:** Čes. a Slov. Farm., 2008; 57, 160–164.
4. **Kečkéšová, S., Sedlárová, E., Csöllei, J., Mokrý, P., Vančo, J., Vanko, M.:** Acta Facult. Pharm. Univ. Com., 2008; 55, 122–128.
5. **Waisser, K., Dražková, K., Čižmárik, J., Kaustová, J.:** Folia Microbiol., 2003; 48, 45–50.
6. **Malík, I., Sedlárová, E., Csöllei, J., Andriamainty, F., Čižmárik, J., Kečkéšová, S.:** Acta Facult. Pharm. Univ. Com., 2007; 54, 136–145.