Laboratory of Structure and Interactions of Biologically Active Molecules[1], Department of Inorganic and Organic Chemistry, Department of Inorganic and Organic Chemistry[2], Faculty of Pharmacy, Charles University, Hradec Králové, Institute of Public Health[3], Prague, Czech Republik

Quinoxaline derivatives as potential antituberculotic agents

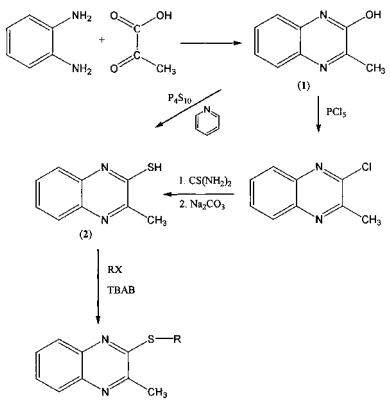
J. Kuneš[1], M. Špulák[2], K. Waisser[2], M. Šlosárek[3] and J. Janota[3]

Following the results of research and development of potential antituberculotics, Waisser et al. concluded in 1995 that the alkylsulfanyl group linked to an electron-deficient carbon is a pharmacophore of antituberculotic activity [1]. This hypothesis was corroborated on a broad variety of compounds, the structures of which fit this reqiurement, such as 2-alkylsulfanylbenzothiazoles [1], tetrazole derivates (1-aryl-5alkylsulfanyl-1,2,3,4-tetrazoles) [2, 3], and substituted pyridine derivatives [4].

The aim of this work was to find out whether this hypothesis is also valid for quinoxaline derivatives, namely 3-methyl-2-alkylsulfanylquinoxalines. Thus, our goal was to prepare a series of these derivatives, and to evaluate their antimycobacterial activity against various strains of mycobacteria.

The compounds were made as depicted in the Scheme.

Scheme



3-Methyl-2-quinoxalinol (**1**) was prepared by the condensation of *o*-fenylendiamine with pyruvic acid. The hydroxy group was then converted to the sulfanyl moity using two different procedures. Firstly, the starting compound **1** was treated with phosphorus(V)sulfide in an pyridine to yield 3-methyl-2-quinoxalinethiol **3**. Secondly, compound **1** was melted with phosphorus(V)chloride, and the resultant chloroderivative was subjected to reaction with thiourea which afforded the corresponding isothiuronic salt. Finally, the base-catalyzed cleavage of the salt gave rise to 3-methyl-2-quinoxalinethiol.

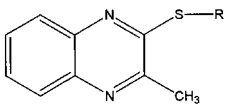
Two alkylation methods were employed to prepare 2-alkylthio-3-methylquinoxaline **2**: alkylation under the conditions of classical nucleophilic substitution, and under the conditions of phase-transfer catalysis using tetrabutylammonium bromide as a catalyst.

Overall, we prepared 11 target compounds, out of which 6 had not been described in the literature as yet. Their structures were confirmed by ^{1}H NMR, ^{13}C NMR, and IR spectra. IR spectra of the substances showed characteristic C−H$_{arom}$ vibrations in the range of 3055–3065 cm^{-1}, C−H$_{alif}$ in the range of 2850–2890 cm^{-1}, and the bands of C−C and C−N bonds of quinoxaline in the range of 1050–1550 cm^{-1}. In the ^{1}H NMR spectra, the signals of aromatic hydrogens appeared as multiplets in the range 7.95–7.85 ppm and 7.65–7.52 ppm.

The substances were evaluated for antituberculotic activity. The evaluation was carried out *in vitro* against the following strains of mycobacteria: *Mycobacterium tuberculosis, Mycobacterium kansasii, Mycobacterium fortuitum, Mycobacterium avium* and *Mycobacterium intracellulare*. The results of this evaluation (Table) allow a few conclusions.

For the derivatives with an unbranched alkyl in the molecule, the activity depends on the length of the chain, and reaches its maximum in the case of a four-carbon residue. The activity rapidly declines with prolonging of the chain: the pentyl and hexyl derivatives as well as the cetyl derivative possesses virtually no activity. The introduction of the benzyl group also results in obtaining an inactive compound, but the prolonging of the alkyl link between sulphur and the benzene ring increases the antituberculotic activity. The branching of the alkyl chain gives rise to a increase in the activity as compared to a linear alkyl. Allyl, on the other hand, slightly increases the activity. In

Table: Antimycobacterial activity (MIC, μmol/l) of 2-alkylthio-3-methylquinoxalines (INH = isoniazid)



R	*M. tuberculosis* TBC 1/47	*M. kansasii* My 235/80	*M. fortuitum* My 187/73	*M. avium* My 66/72	*M. avium* D5/93	*M. avium* My 80/72	*M. intracellulare* D 39/93	*M. intracellulare* D 38/92
C_2H_5	250	250	63	250	125	63	250	63
C_3H_7	250	250	125	250	500	250	500	63
C_4H_9	125	63	63	250	63	125	250	63
C_5H_{11}	500	250	250	1000	1000	1000	500	250
C_6H_{13}	500	500	>1000	250	500	1000	1000	63
$C_{16}H_{33}$	500	500	>1000	>1000	1000	1000	500	500
$CH_2C_6H_5$	1000	1000	>1000	>1000	>1000	>1000	>1000	63
$CH_2CH_2C_6H_5$	125	125	>1000	250	63	250	250	125
CH_2CHCH_2	250	125	250	63	250	500	500	125
$CH(CH_2)_2$	500	250	1000	125	500	500	500	500
$CH_2CH(CH_2)_2$	1000	500	500	125	1000	1000	1000	250
INH	4	500	63	250	63	125	250	63

summary, the most active compounds were 3-methyl-2-ethylsulfanylquinoxaline and 3-methyl-2-butylsulfanylquinoxaline. These substances showed higher activity against some atypical strains of mycobacteria as compared to the commercially used antituberculotic drug isoniazide.
This study thus represents a further confirmation of the hypothesis of the alkylsulfanyl group bond to an electron-deficient carbon atom being a pharmacophore of antituberculotic activity, as the results fall well in line with it.

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Dr. Jiří Kuneš
Department of Inorganic and Organic Chemistry
Faculty of Pharmacy, Charles University
Heyrovského 1203
50005 Hradec Králové
Czech Republic
kunes@faf.cuni.cz

Department of Analytical Chemistry, Faculty of Chemical Technology, Bratislava, Slovakia

Determination of flurbiprofen in serum by capillary isotachophoresis

J. Sádecká, A. Hercegová and J. Polonský

Flurbiprofen is non-steroidal anti-inflammatory drug which has a therapeutic range of 2–12 mg/l (0.008–0.049 mmol/l) in serum [1]. HPLC [2–6], high-performance thin-layer chromatography [7] and capillary zone electrophoresis [1] have been used to determine flurbiprofen in plasma, serum and urine. Each of these methods requires a sample preparation based on simple acetonitrile deproteinization [6], liquid-liquid extraction [2, 5, 7] or on-line dialysis [1]. The aim of this work was the development of a isotachophoretic method for the determination of flurbiprofen in serum samples. The method involved deproteinization of the biological sample with ethanol.
A series of standard curves ($n = 5$) of flurbiprofen were prepared both in ethanol and in serum over a concentration range of 0.006–0.060 mmol $\cdot$ l^{-1}. The mean values of correlation coefficient (r^2), line slope (zone length $\cdot$ l/mmol) and intercept (zone length) were 0.999 ± 0.002 (standard deviation, SD), 170.1 ± 1.63 (SD), and 0.02 ± 0.01 (SD) for standard curve in ethanol, while those in serum showed mean values of 0.998 ± 0.003 (SD), 160.1 ± 4.15 (SD), and 0.24 ± 0.01 (SD), respectively. The limit of detection for flurbiprofen in serum was found to be 0.003 mmol $\cdot$ l^{-1}. The limit of quantitation was 0.006 mmol $\cdot$ l^{-1}. The accuracy and precision (Table) of the method were evaluated by analyzing five replicates of spiked serum at each concentration against calibration curve. Accuracy was given by the % bias (mean of measured – mean of added/mean of added) $\times$ 100. The mean bias was −7.4%. The precision expressed as relative standard deviation (RSD) was 2.7%. The recovery of flurbiprofen was determined by comparing the zone length from drug-free serum spiked with known amounts of flurbiprofen with the zone length of the same concentration prepared in ethanol. The use of a protein denaturation with ethanol resulted in mean recovery of 93.7%. The utility of the method was assessed by determining the serum concentration of flurbiprofen following single oral administration of flurbiprofen in a set of patient's samples. A representative isotachopherogram of a serum sample from a patient who had received a single oral dose of 50 mg of flurbiprofen is demonstrated in the Fig. No interfering metabolite zones were observed in serum. Drugs which did not interfere with the assay are: amiloride, diclofenac, fenoprofen, ibuprofen, ketoprofen, labetalol, metoprolol, and naproxen.

Table: Accuracy, precision and recovery of the analytical procedure for flurbiprofen

Added	Found	Accuracy	Precision	Recovery	
(mmol $\cdot$ l^{-1})	(mmol $\cdot$ l^{-1})	Bias (%)	RSD (%)	Mean (%)	RSD (%)
0.0060	0.0054	−10	3.7	90.1	3.7
0.0100	0.0095	−5.0	4.2	94.9	3.8
0.0015	0.0142	−5.3	1.8	94.1	2.1
0.0200	0.0190	−5.0	2.2	95.0	2.1
0.0300	0.0288	−12.0	4.0	96.0	4.0
0.0500	0.0455	−9.0	1.8	91.1	1.7
0.0600	0.0568	−5.3	1.3	94.7	1.4
	Mean	−7.4	2.7	93.7	2.4