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Thiosemicarbazones of Acetylpyrazines: Preparation and Their Hydrophobic Properties

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Abstract: Some substituted acetylpyrazine derivatives were prepared as the starting materials for the subsequent synthesis of thiosemicarbazones. General synthetic approach of all newly synthesized compounds is presented. All the thiosemicarbazone derivatives of acetylpyrazines were analyzed using the reversed phase high performance liquid chromatography (RP-HPLC) method for the lipophilicity measurement. The procedure was performed under isocratic conditions with methanol as an organic modifier in the mobile phase using end-capped non-polar C_{18} stationary RP column. In the present study the correlation between RP-HPLC retention parameter Log *K* (the logarithm of capacity factor *K*) and various calculated Log *P* values is shown. The relationships between the lipophilicity and the chemical structure of the studied compounds are discussed as well.

Keywords: Acetylpyrazines; Thiosemicarbazones; Lipophilicity measurement; Structure-lipophilicity relationships

Introduction

Acetylpyrazines are used as food flavourings and intermediates for synthesis of various pyrazine derivatives [1-5]. They are formed from sugars and amino acids via Maillard reactions [6-8]. Synthetic methods for the preparation of acetylpyrazines have been reviewed [9].

Thiosemicarbazones present a wide range of bioactivities, and their chemistry, pharmacological applications, and physicochemical properties have been extensively investigated. Thiosemicarbazones and their complexes with transition metals have been studied as potential antiviral, antibacterial, antimycobacterial, antiprotozoal, antifungal, and antineoplastic agents [10, 11]. Their anticonvulsant and neurotropic effects were reported as well [11]. The thiosemicarbazones of acetylpyrazines reported in this paper showed antifungal, antimycobacterial, and antiproliferative activity [12].

One of the major prerequisites for pharmacological screening and drug development is the prediction of absorption, e. g. the transport of a molecule through cellular membranes. The drugs mostly (most frequently) cross the biological barriers by the passive transport, which strongly depends on the lipophilicity. Therefore hydrophobicity is one of the most important physical properties of biologically active compounds. This thermodynamic parameter describes the partitioning of a compound between an aqueous and an organic phase and is characterized by the partition (Log *P*) or distribution (Log *D*) coefficient [13, 14]. Classical methods for the determination of these constants are time consuming and not always sufficiently reliable. Therefore, reversed phase high performance liquid chromatography (RP-HPLC) methods have become popular and widely used for lipophilicity measurement in spite of being more expensive. A general procedure is the measurement of the directly accessible retention time under isocratic conditions with varying amounts of an organic modifier in the mobile phase using end-capped non-polar C_{18} stationary RP columns and calculating the capacity factor K ($K = (T_R - T_D) / T_D$, where T_R is the retention time of the solute, whereas T_D denotes the dead time obtained via an unretained analyte). Log K, calculated from the capacity factor K, is used as the lipophilicity index converted to Log *P* scale [15].

This study is a follow-up work to the previous papers and deals with the synthesis and physicochemical properties of the newly prepared *N*-heterocyclic compounds as potential drugs [16-28].

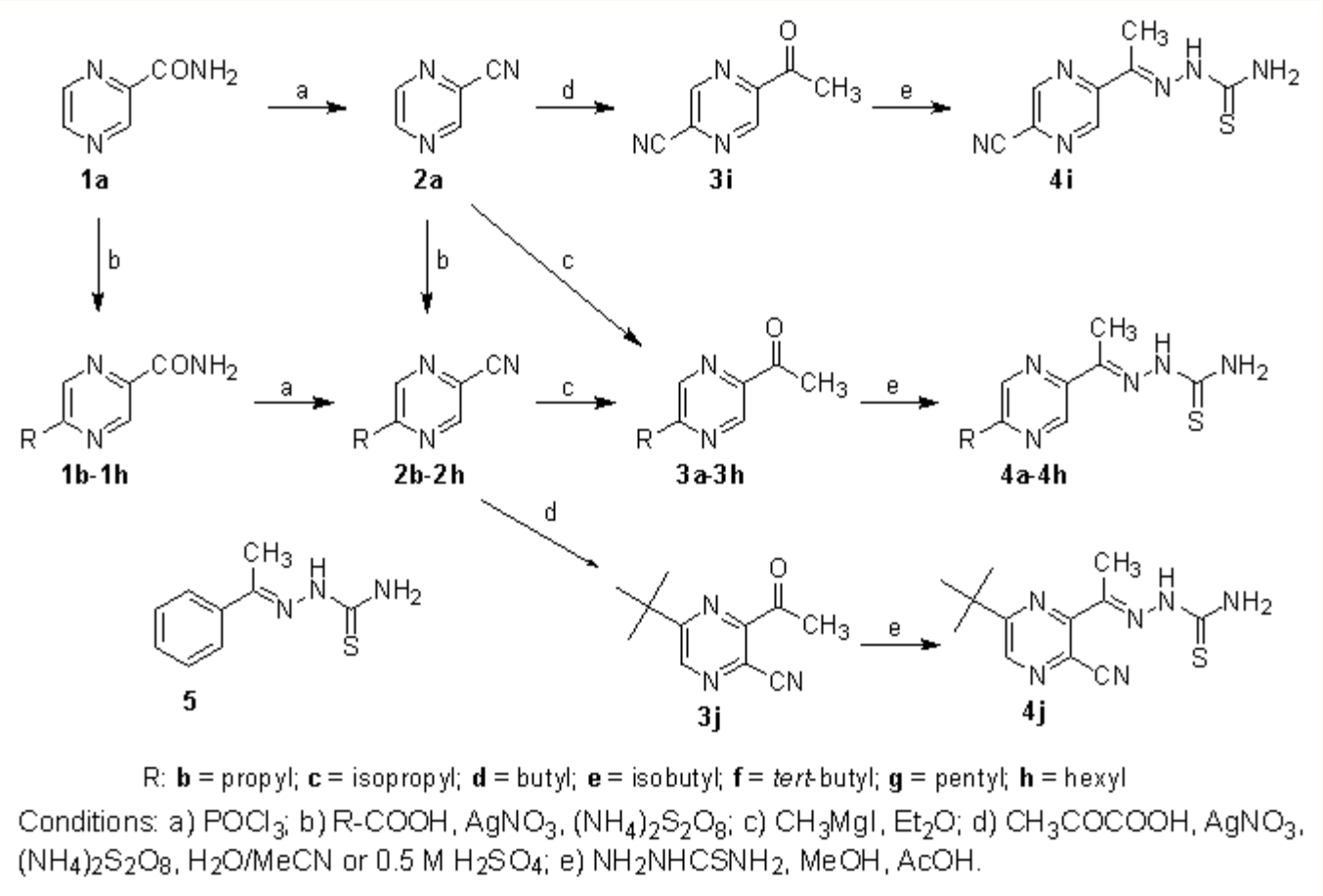
Results and Discussion

The carboxamide moieties in the starting material **1a** and substituted pyrazine-2-carboxamides **1b**-**1h** were dehydrated to the corresponding nitrile groups (compounds **2a** and **2b**-**2h**) by means of phosphoryl chloride. Alkylation of the amide **1a** or the nitrile **2a** was performed using the mixture of a carboxylic acid as a source of the alkyl radical, ammonium peroxydisulfate and silver nitrate. Pyrazine-2-carbonitrile (**2a**) and its 5-alkylated analogues **2b**-**2h** were then converted to the corresponding acetyl derivatives **3a**-**3h** via the Grignard reaction [3, 5].

Compound **3i** was obtained by homolytic acetylation of pyrazine-2-carbonitrile (**2a**) using pyruvic acid as a source of acetyl radical [1]. The same reaction with 5-*tert*-butylpyrazine-2-carbonitrile (**2f**) resulted in the disubstituted acetylpyrazine **3j** [12]. Acetylpyrazines **3a**-**3j** with thiosemicarbazide yielded the final thiosemicarbazones **4a**-**4j** [10, 12]. Acetophenone thiosemicarbazone **5** was prepared for comparison in the same synthetic pathway. The general synthetic approach of all newly synthesized compounds is shown in Scheme 1.

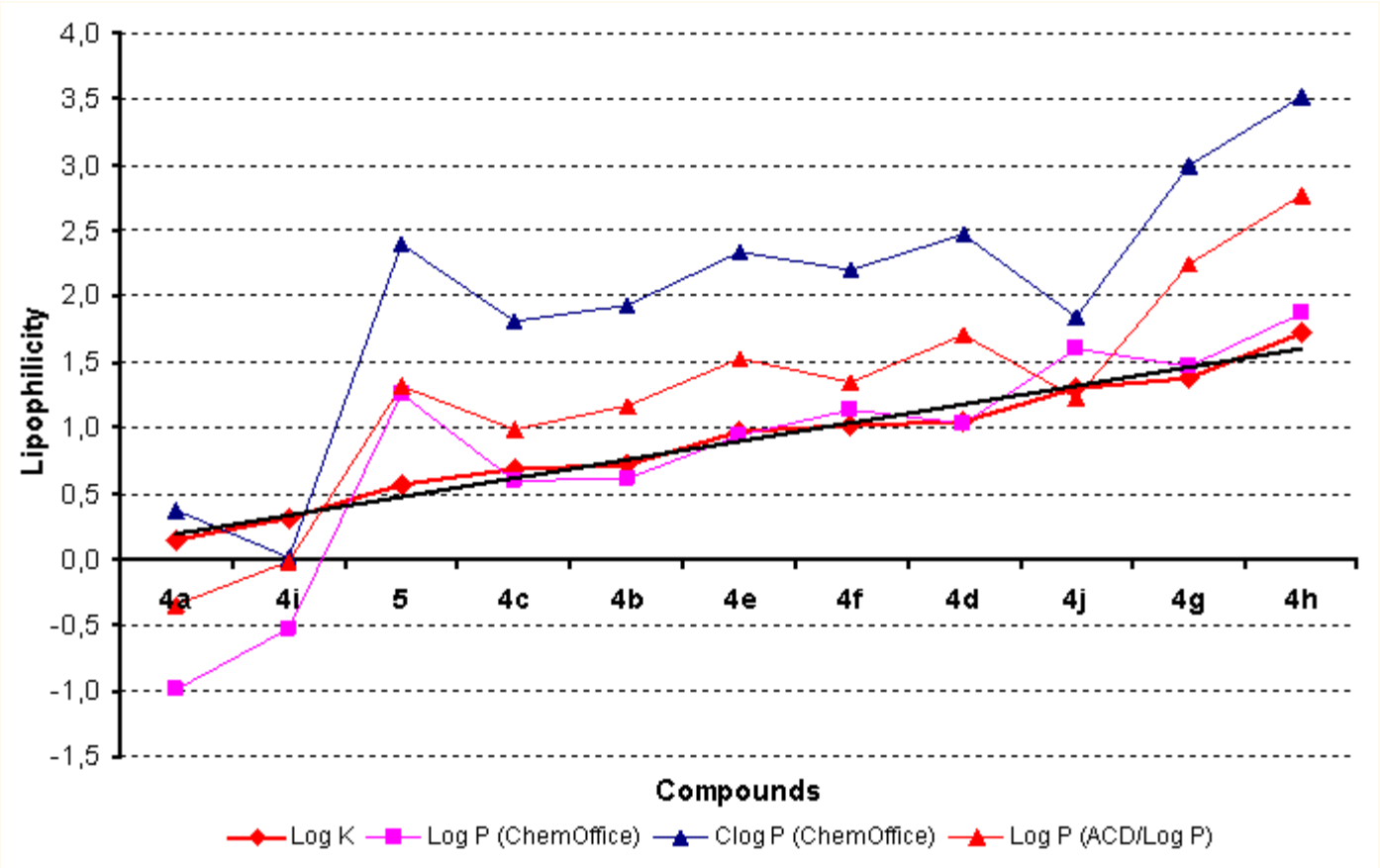
According to ^{1}H NMR experiments all the final products are *E*-isomers. The thioxo moiety of all thiosemicarbazones was confirmed by means of IR spectroscopy [12].

Scheme 1: Synthesis and structures of thiosemicarbazones **4a**-**4j** and **5**.



Hydrophobicities (Log *P* / CLog *P* values) of the studied compounds were calculated using two commercially available programs and measured by means of RP-HPLC determination of capacity factors *K* with a subsequent calculation of Log *K*. The results are shown in Table 1 and illustrated in Figure 1.

Figure 1: Comparison of the computed Log *P* / CLog *P* values using the two programs and the experimentally found Log *K* values.



The results show that the experimentally determined Log *K* values correlate well with Log *P* values calculated either by ChemOffice Ultra software or ACD/Log *P* program, whilst according to the calculated CLog *P* data the hydrophobicity of most compounds (with exception of **4a** and **4i** should be substantially higher than that actually found in RP-HPLC measurements. As expected, the dependence between Log *K* and the length of the non-branched alkyl substituents in compounds **4a**, **4b**, **4d**, **4g**, **4h** (H, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}) is approximately linear. 1-(5-Isopropylpyrazin-2-yl)ethan-1-one thiosemicarbazone **4c** is slightly less lipophilic than the corresponding propyl derivative **4b**. Similarly, 1-(5-isobutylpyrazin-2-yl)ethan-1-one thiosemicarbazone **4e** is slightly less lipophilic than its congeners **4d** (R^1 = butyl) and **4f** (R^1 = *tert*-butyl). This is in a good agreement with the results of our previous study [24] in which the lipophilicity of a series of 3-phenyl-1-pyrazin-2-ylpropen-1-ones was evaluated by means of RP-TLC.

Great differences between the experimental and calculated lipophilicity parameters could be observed for the compound **5** (acetophenone thiosemicarbazone), see Fig. 1. The non-heterocyclic derivative **5** is situated between **4i** (R^1 = CN) and **4c** (R^1 = isopropyl) according to Log *K* and shows relatively low lipophilicity, but according to all calculated data it seems to be much more hydrophobic. In contrast, for compound **4i** Log *K* is higher than the calculated lipohilicity parameters. The differences cannot be explained on the basis of the results presented here.

Experimetal

Lipophilicity HPLC determination (capacity factor K / calculated Log K)

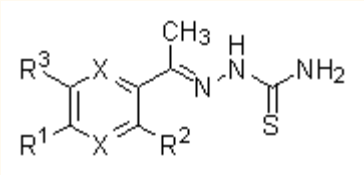
The HPLC separation module Waters Alliance 2695 XE and Waters Photodiode Array Detector 2996 (Waters Corp., Milford, MA, U.S.A.) were used. The chromatographic column Symmetry® C_{18} 5 µm, 4.6×250 mm, Part No. WAT054275, (Waters Corp., Milford, MA, U.S.A.) was used. The HPLC separation process was monitored by Millennium32® Chromatography Manager Software, Waters 2004 (Waters Corp., Milford, MA, U.S.A.). The mixture of MeOH p.a. (50.0%) and H_2O-HPLC -- Mili-Q Grade (50.0%) was used as a mobile phase. The total flow of the column was 0.9 ml/min, injection 30 µl, column temperature 22 °C and sample temperature 10 °C. The detection wavelength 210 nm was chosen. The KI methanolic solution was used for the dead time (T_D) determination.

The capacity factors *K* were calculated using the Millennium32® Chromatography Manager Software. The Log *K* values of the individual compounds are shown in Table 1.

Lipophilicity calculations

Log *P*, *i. e.* the logarithm of the partition coefficient for *n*-octanol / water, was calculated using the programs CS ChemOffice Ultra ver. 7.0 (CambridgeSoft, Cambridge, MA, U.S.A.) and ACD/Log *P* ver. 1.0 (Advanced Chemistry Development Inc., Toronto, Canada). CLog *P* values (the logarithm of *n*-octanol / water partition coefficient based on established chemical interactions) were generated by means of CS ChemOffice Ultra ver. 7.0 (CambridgeSoft, Cambridge, MA, U.S.A.) software. The results are shown in Table 1.

Table 1: Comparison of calculated lipophilicities (Log *P* / CLog *P*) and determined Log *K* of compounds **4a**-**4j** and **5**.



Compound	X	R^1	R^2	R^3	Log *P* / CLog *P* ChemOffice	Log *P* ACD/Log P	Log *K*
4a	N	H	H	H	-0.99 / 0.37975	-0.35 ± 0.61	0.1437
4b	N	C_3H_7	H	H	0.62 / 1.93675	1.17 ± 0.61	0.7232
4c	N	$(CH_3)_2CH$	H	H	0.60 / 1.80675	0.99 ± 0.61	0.6953
4d	N	C_4H_9	H	H	1.04 / 2.46575	1.70 ± 0.61	1.0480
4e	N	$(CH_3)_2CHCH_2$	H	H	0.95 / 2.33575	1.52 ± 0.61	0.9754
4f	N	$(CH_3)_3C$	H	H	1.14 / 2.20575	1.34 ± 0.61	1.0224
4g	N	C_5H_{11}	H	H	1.46 / 2.99475	2.24 ± 0.61	1.3707
4h	N	C_6H_{13}	H	H	1.87 / 3.52375	2.77 ± 0.61	1.7246
4i	N	CN	H	H	-0.53 / 0.0188925	-0.01 ± 0.63	0.3063
4j	N	H	CN	$(CH_3)_3C$	1.60 / 1.84489	1.23 ± 0.64	1.2999
5	C	H	H	H	1.26 / 2.401	1.32 ± 0.58	0.5657

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