Chromatographic and Computational study of Hydrophobic Properties of Ring Substituted Pyrazinecarbonitriles and Acetylpyrazines

Veronika Opletalova[1]*, Josef Jampilek[2], Marta Chlupacova[1], Jan Dolezel[1], Tatjana Grafnetterova[2], Jiri Dohnal[2]

[1] Department of Pharmaceutical Chemistry and Drug Control, Charles University in Prague, Faculty of Pharmacy in Hradec Kralove, 500 05 Hradec Kralove, Czech Republic; e-mail: Veronika.Opletalova@faf.cuni.cz, tel.: 420-495067372, fax: 420-495512423

[2] Zentiva a. s., U kabelovny 130, 102 37 Prague 10, Czech Republic

* Author to whom correspondence should be addressed

Abstract: Pyrazinecarbonitriles and acetylpyrazines have widely been used as intermediates in the synthesis of various heterocyclic derivatives. Acetylpyrazines also serve as food and tobacco flavourants. In our laboratory, the title compounds have been used as synthetic intermediates for the preparation of potential antifungal and antimycobacterial drugs. Homolytic alkylation of commercially available pyrazine-2-carbonitrile yielded a series of 5-alkylpyrazine-2-carbonitriles that were then converted to the corresponding 1-(5-alkylpyrazin-2-yl)-1-ethan-1-ones (5-alkylpyrazine-2-acetylpyrazines) *via*

the Grignard reaction. Homolytic acetylation of pyrazine-2-carbonitrile yielded 5-acetylpyrazine-2-carbonitrile. By means of the same procedure 3-acetyl-5-*tert*-butylpyrazine-2-carbonitrile was obtained using 5-*tert*-butylpyrazine-2-carbonitrile as a starting material. The hydrophobicity of all the pyrazinecarbonitriles and acetylpyrazines was determined using the reversed phase high performance liquid chromatography (RP-HPLC) method (isocratic elution with methanol as an organic modifier in the mobile phase, end-capped non-polar C_{18} stationary RP column). Experimentally derived Log *K* values (the logarithm of capacity factor *K*) were that compared with Log *P* values calculated by commercially available programmes (CS ChemOffice Ultra ver. 7.0 and ACD/Log *P* ver. 1.0).

Keywords: Pyrazinecarbonitriles; Acetylpyrazines; RP-HPLC Lipophilicity Determination; Lipophilicity Calculations

INTRODUCTION

Pyrazinecarbonitriles and acetylpyrazines have widely been used as intermediates in the synthesis of various heterocyclic compounds [1]. Acetylpyrazines also serve as food and tobacco flavourants [1, 2]. As a part of our studies focused on the synthesis and biological evaluation of pyrazine derivatives various ring substituted pyrazinecarbonitriles and acetylpyrazines were prepared [2-13]. Since electron-deficient nitrogen heteroaromatics, including pyrazine, are inert to the electrophilic substitution [14], homolytic alkylation and acylation were applied to get the title compounds. In this communication we wish to report on the evaluation of their hydrophobicity by means of RP-HPLC and computational techniques [15, 16].

EXPERIMENTAL

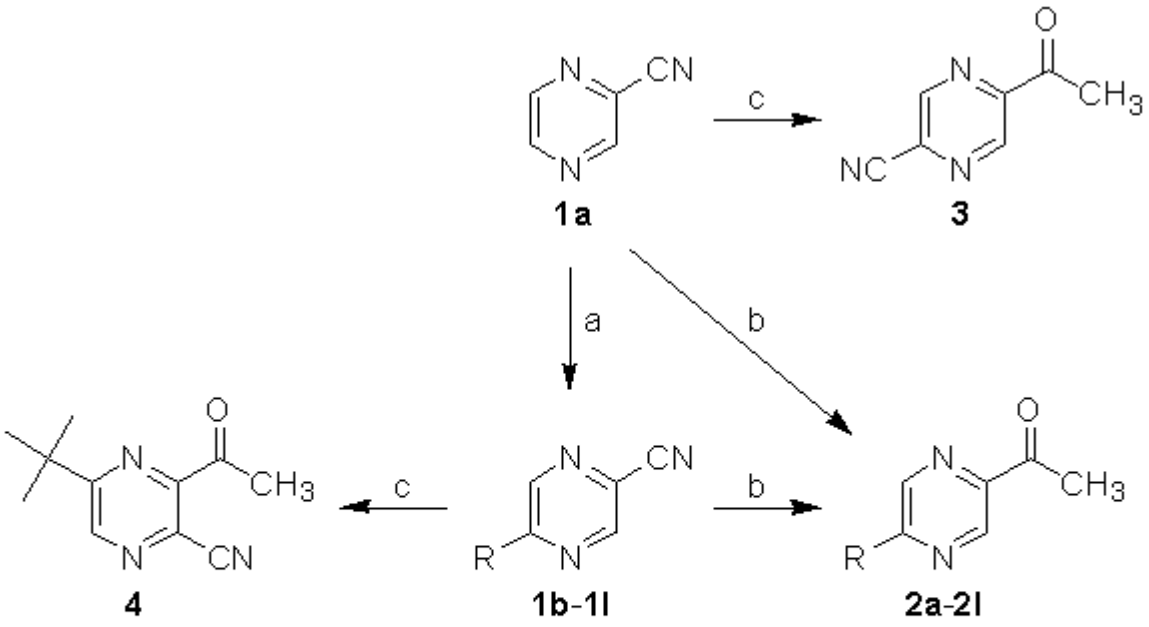
Synthesis of the title compounds

Homolytic alkylation of pyrazine-2-carbonitrile (**1a**) gave 5-alkylpyrazine-2-carbonitriles **1b**-**1l**. The Grignard reaction of **1a** yielded 1-pyrazin-2-yl-1-ethan-1-one (**2a**). Application of the same procedure to the alkylated nitriles **1b**-**1l** resulted in 1-(5-alkylpyrazin-2-yl)-1-ethan-1-ones **2b**-**2l**. Detailed information

concerning reaction conditions is available in ref. [6, 13].

Acetylpyrazine **3** was obtained by homolytic acetylation of pyrazine-2-carbonitrile (**1a**) using pyruvic acid as a source of acetyl radical [9]. The same reaction with 5-*tert*-butylpyrazine-2-carbonitrile (**1f**) resulted in the disubstituted acetylpyrazine **4** [10, 17]. The general synthetic approach of all prepared compounds is shown in Scheme 1.

Scheme 1: Synthesis and structures of the studied compounds **1a**-**2l**, **3** and **4**.



R: **b** = propyl; **c** = isopropyl; **d** = butyl; **e** = isobutyl; **f** = *tert*-butyl; **g** = pentyl; **h** = hexyl; **i** = heptyl; **j** = octyl; **k** = nonyl; **l** = benzyl

Conditions: a) R-COOH, $AgNO_3$, $(NH_4)_2S_2O_8$; b) CH_3MgI, Et_2O; c) $CH_3COCOOH$, $AgNO_3$, $(NH_4)_2S_2O_8$, H_2O/MeCN or 0.5 M H_2SO_4.

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

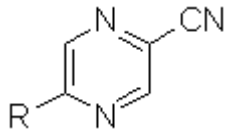
dead time (T_D) determination.

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 and Table 2.

Lipophilicity calculations

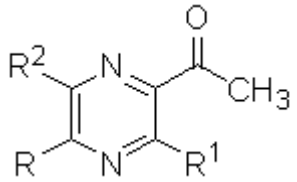
Log *P*, *i. e.* the logarithm of the partition coefficient for *n*-octanol/water, was calculated using the programs CS ChemOffice Ultra ver. 7.0 (CambridgeSoft, Cambridge, MA, U.S.A.) and ACD/Log *P* ver. 1.0 (Advanced Chemistry Development Inc., Toronto, Canada). CLog *P* values (the logarithm of *n*-octanol/water 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 and Table 2.

Table 1: Comparison of calculated lipophilicities (Log *P* / CLog *P*) and determined Log *K* of the ring substituted pyrazine-2-carbonitriles **1a**-**1l**.



Comp.	**R**	**Log *K***	**Log *P* / CLog *P*** ChemOffice	**Log *P*** ACD/Log *P*
1a	H	0.1061	-0.18 / -0.590275	-0.15 ± 0.35
1b	C_3H_7	0.5978	1.42 / 0.966725	1.37 ± 0.35
1c	$(CH_3)_2CH$	0.6840	1.41 / 0.836725	1.19 ± 0.35
1d	C_4H_9	0.9037	1.84 / 1.49573	1.90 ± 0.35
1e	$(CH_3)_2CHCH_2$	0.8378	1.75 / 1.36573	1.72 ± 0.35
1f	$(CH_3)_3C$	0.9151	1.94 / 1.23573	1.54 ± 0.36
1g	C_5H_{11}	1.3266	2.26 / 2.02472	2.43 ± 0.35
1h	C_6H_{13}	1.6876	2.68 / 2.55373	2.96 ± 0.35
1i	C_7H_{15}	2.0556	3.09 / 3.08273	3.49 ± 0.35
1j	C_8H_{17}	2.4231	3.51 / 3.61172	4.03 ± 0.35
1k	C_9H_{19}	2.7878	3.93 / 4.14072	4.56 ± 0.35
1l	benzyl	0.9959	2.20 / 1.47673	1.84 ± 0.35

Table 2: Comparison of calculated lipophilicities (Log *P* / CLog *P*) and determined Log *K* of the ring substituted acetylpyrazines **2a**-**2l**, **3** and **4**.

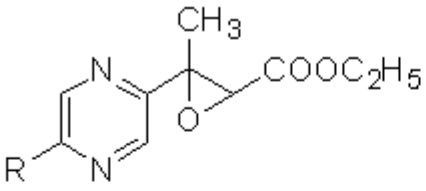


Comp.	R	R^1	R^2	Log *K*	Log *P* / CLog *P* ChemOffice	Log *P* ACD/Log *P*
2a	H	H	H	0.1697	-0.90 / -0.075335	0.16 ± 0.34
2b	C_3H_7	H	H	0.7348	0.70 / 1.48167	1.68 ± 0.34
2c	$(CH_3)_2CH$	H	H	0.7882	0.68 / 1.35167	1.50 ± 0.35
2d	C_4H_9	H	H	1.0626	1.12 / 2.01066	2.21 ± 0.34
2e	$(CH_3)_2CHCH_2$	H	H	0.9901	1.03 / 1.88066	2.03 ± 0.35
2f	$(CH_3)_3C$	H	H	1.0818	1.22 / 1.75066	1.85 ± 0.35
2g	C_5H_{11}	H	H	1.4694	1.54 / 2.53967	2.75 ± 0.34
2h	C_6H_{13}	H	H	1.8301	1.96 / 3.06867	3.28 ± 0.34
2i	C_7H_{15}	H	H	2.1987	2.37 / 3.59767	3.81 ± 0.34
2j	C_8H_{17}	H	H	2.5618	2.79 / 4.12666	4.34 ± 0.34
2k	C_9H_{19}	H	H	2.9264	3.21 / 4.65566	4.87 ± 0.34
2l	benzyl	H	H	1.1093	1.47 / 1.99166	2.15 ± 0.35
3	CN	H	H	0.2742	-0.45 / -0.596925	0.41 ± 0.38
4	H	CN	$(CH_3)_3C$	1.02066	1.68 / 1.42908	1.74 ± 0.39

RESULTS AND DISCUSSION

Synthetic reactions were repeated many times to get sufficient amounts of the intermediates for subsequent reactions. So far, acetylpyrazines reported here

were used for the synthesis of ethyl 3-(5-alkylpyrazin-2-yl)-2,3-epoxybutanoates **5** [18], 3-phenyl-1-pyrazin-2-ylprop-2-en-1-ones **6** [19-22] and their derivatives **7** [23], thiosemicarbazones **8** [17], and 5-(1-pyrazin-2-yl) ethyliden-2-thioxo-1,3-thiazolidin-4-ones **9** [24]. The yields of homolytic alkylation were in the range 80% – 10%. Due to poor solubility of the higher fatty acids in water, the yield decreased with the increasing number of carbon atoms in the alkyl substituent. Conversion of 5-alkylpyrazin-2-carbonitriles to the corresponding 1-(5-alkylpyrazin-2-yl)-1-ethan-1-ones proceeded with 60% to 40% yields. Homolytic acetylation gave rather poor yields (approximately 30%) even if it was performed under nitrogen. Structure of all products was confirmed by means of spectral data (IR, ^{1}H NMR, ^{13}C NMR and MS) and the purity of the compounds was checked by HPLC.



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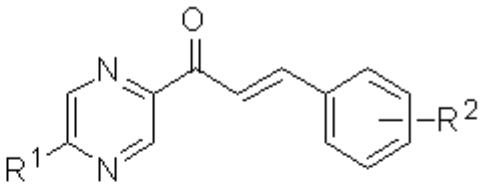

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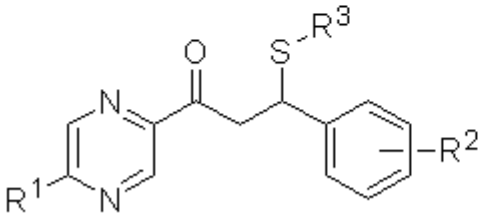

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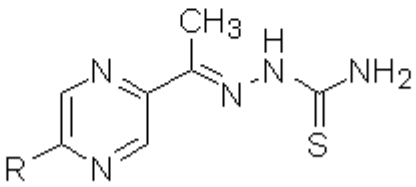

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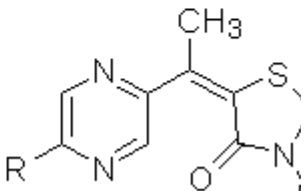

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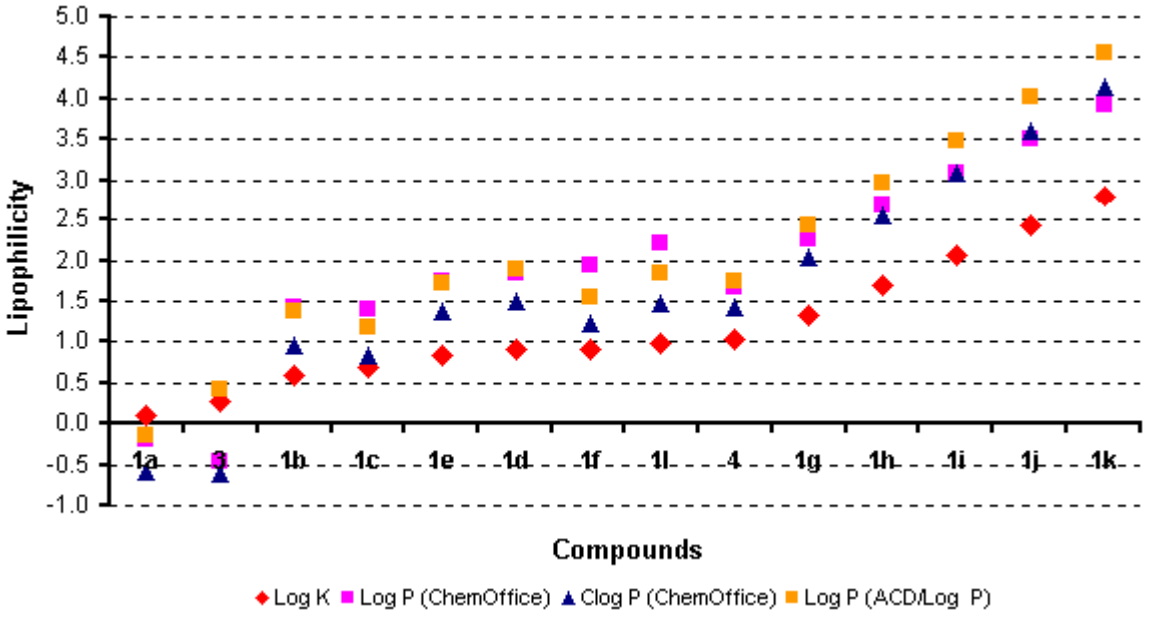
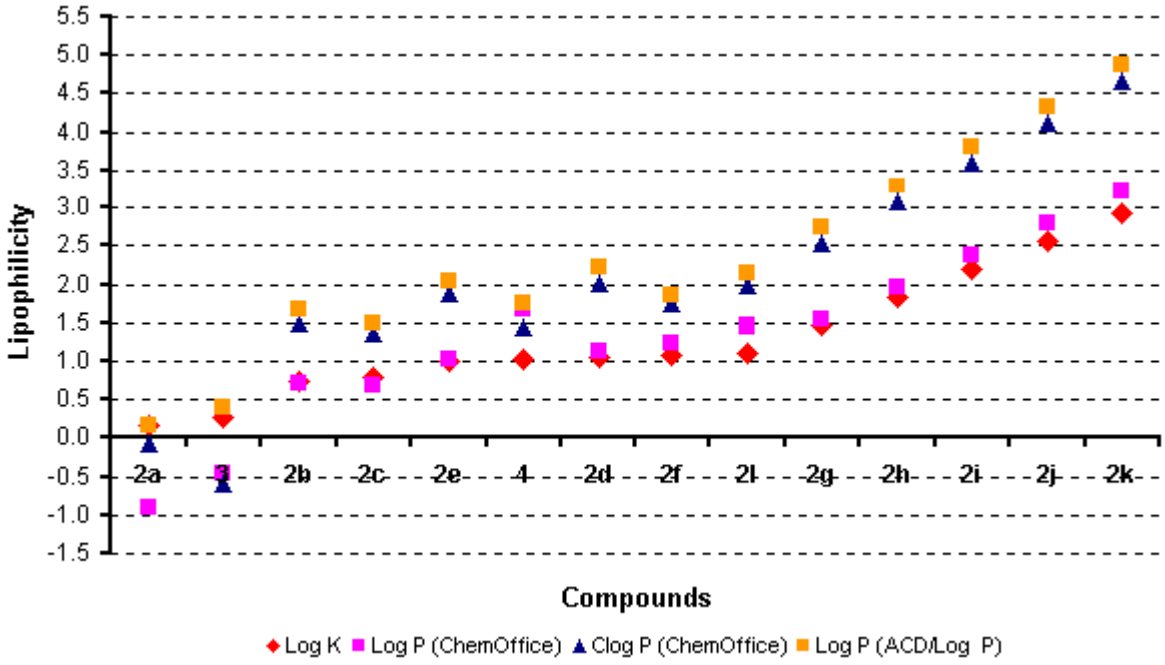
Hydrophobicities (Log *P* / CLog *P* values) of the studied compounds **1a**-**4** were calculated using two commercially available programs and measured by means of RP-HPLC determination of capacity factors *K* with a subsequent calculation of Log *K*. The results are shown in Tables 1, 2 and illustrated in Figures 1, 2.

Figure 1: Comparison of the computed Log *P* / CLog *P* values using the two programs and the experimentally found Log *K* values of the ring substituted pyrazinecarbonitriles **1a**-**1l**, **3** and **4**.



The results obtained with nitriles show that the experimentally determined lipophilicities (Log *K* values) are in most cases lower than those indicated by the calculated Log *P* or CLog *P*, but the correlation is good with the exception of **1a**, **1c** and **3**. As expected, the dependence between Log *K* and the length of the non-branched alkyl substituents in compounds **1a**, **1b**, **1d**, **1g**-**1k** (H, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, C_9H_{19}) is approximately linear. 5-Isopropylpyrazin-2-carbonitrile (**1c**) should be slightly less lipophilic than the corresponding propyl derivative **1b** according to the calculated values, but experimental Log *K* for **1c** is actually higher that for **1b**. The lipophilicity sequence of **1e** (R = isobutyl), **1d** (R = butyl), and **1f** (R = *tert*-butyl) is the same as for determined Log *K* as for calculated Log *P* (ChemOffice). For compound **3**, very good agreement was observed between experimental Log *K* and ACD/Log *P*, whilst for **1a** experimentally determined hydrophobicity was higher than predicted by the calculated data.

Figure 2: Comparison of the computed Log *P* / CLog *P* values using the two programs and the experimentally found Log *K* values of the ring substituted acetylpyrazines **2a**-**2l**, **3** and **4**.



For acetylpyrazines, the results show that the experimentally determined Log *K* values correlate best with Log *P* values calculated by ChemOffice Ultra software. Nonetheless, for **2a** and **3** the correlation is better with ACD/Log *P* parameters, and for **4** experimental Log *K* is different from all calculated values. According CLog *P* (ChemOffice Ultra) and Log *P* (ACD/Log *P*) the hydrophobicity of most compounds should be substantially higher than that actually found in RP-HPLC measurements, calculated values being twice as high than Log *K* values in some cases (e.g. **2b**, **2c**, **2d**, **2e**). As expected, the dependence between Log *K* and the length of the non-branched alkyl substituents in compounds **2a**, **2b**, **2d**, **2g**-**2k** (H, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, C_9H_{19}) is approximately linear. Similar to nitriles, 1-(5-isopropylpyrazin-2-yl)ethan-1-one **2c** is slightly more lipophilic than the corresponding propyl derivative **2b** according to Log *K*, but less lipophilic according to the calculated parameters. In contrast, 1-(5-isobutylpyrazin-2-yl)ethan-1-one (**2e**) is slightly less lipophilic than its congener **2d** (R = butyl) according to both experimental and calculated parameters.

In general, acetylated derivatives **2a**-**2l**, **3** and **4** are slightly more lipophilic than the corresponding nitriles **1a**-**1l**.

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REFERENCES

1. Cheeseman, G.W.H.; Werstiuk, E.S.G. In *Advances in Heterocyclic Chemistry.* Vol 14. (Katritzky, A.R., Boulton, A.J., Eds.). Academic Press, New York-London 1972, pp. 99-209.
2. Opletalova, V.; Domonhedo, C.: *Chem. Listy* **1999**, *93*, 15.
3. Dlabal, K.; Palat, K.; Lycka, A.; Odlerova, Z.: *Collect. Czech. Chem. Commun.* **1990**, *55*, 2493.
4. Dolezal, M.; Hartl, J.; Lycka, A.; Buchta, V.; Odlerova, Z.: *Collect. Czech. Chem. Commun*. **1995**, *60*, 1236.
5. Opletalova, V.; Hartl, J.; Patel, A.; Boulton, M.: *Collect. Czech. Chem. Commun.* **1995**, *60*, 1551.
6. Opletalova, V.; Patel, A.; Boulton, M.; Dundrova, A.; Lacinova, E.; Prevorova, M.; Appeltauerova, M.; Coufalova, M.: *Collect. Czech. Chem. Commun.* **1996**, *61*, 1093.
7. Dolezal, M.; Hartl, J.; Lycka, A.; Buchta, V.; Odlerova, Z: *Collect. Czech. Chem. Commun.* **1996**, *61*, 1102.
8. Hartl, J.; Dolezal, M.; Krinkova, J.; Lycka, A.; Odlerova, Z.: *Collect. Czech. Chem. Commun.* **1996**, *61*, 1109.
9. Opletalova, V.; Hartl, J.; Domonhedo, C., Patel, A.: *Folia Pharm. Univ. Carol.* **1999**, *24*, 29.
10. Svandova, M.: *Diploma thesis*. Faculty of Pharmacy, Hradec Kralove 2001.
11. Poslednikova, M.: *Diploma thesis*. Faculty of Pharmacy, Hradec Kralove 2002.
12. Zobalova, D.: *Diploma thesis*. Faculty of Pharmacy, Hradec Kralove 2003.
13. Opletalova, V.; Chlupacova, M.; Poslednikova, M.; Zobalova, D.; Kunes, J.; Vorisek, V.: *Book of Abstracts of the 56th Meeting of Chemical Societies*, Ostrava (Czech Republic), September 6–9, 2004. In: *Chem. Listy* **2004**, *98*, 669.
14. Sato, N.; Kadota, H.: *J. Heterocycl. Chem.* **1992**, *29*, 1685.
15. Valko, K.: *J. Chromatogr. A* **2004**, *1037*, 299.

16. Hartmann, T.; Schmitt, J.: *Drug Disc. Today Technol.* **2004**, *1*, 431.

17. Opletalova, V.; Pour, M.; Kunes, J.; Silva, L.; Buchta, V.; Jampilek, J.; Richardson, D.R.; Kalinowski, D.; Svandova, M.; Zobalova, D.: *Book of Abstracts of the 11th Blue Danube Symposium on Heterocyclic Chemistry*, Brno (Czech Republic), August 28 – September 1, 2005, PO-70.

18. Opletalova, V.; Hartl, J.; Lycka, A.: *Symposium on Heterocyclic Compounds*, Hradec Kralove (Czech Republic), June 28 – July 1 1995. In: *Folia Pharm. Univ. Carol*. **1995**, *18* (Suppl.), 171.

19. Opletalova, V.; Hartl, J.; Patel, A.; Palat, K.; Buchta, V.: *Farmaco* **2002**, *57*, 135.

20. Opletalova, V.; Chlupacova, M.; Poslednikova, M.; Kunes, J.; Silva, L.; Buchta, V.; Jun, D.: *Book of Abstracts of the Joint Meeting of the Czech Pharmaceutical Society, German Pharmaceutical Society and Austrian Pharmaceutical Society*, Regensburg (Germany), October 6–9, 2004, p. 121.

21. Chlupacova, M.; Opletalova, V.; Dosedel, M.; Svedova, L.; Silva, L.; Buchta, V.: *Book of Abstracts of the 34th Conference Synthesis and Analysis of Drugs*, Brno (Czech Republic), September12–14, 2005, p. 66.

22. Opletalova, V.; Pour, M.; Buchta, V.; Silva, L.; Kralova, K.; Chlupacova, M.; Meltrova, D.; Peterka, M.; Poslednikova, M.: *Collect. Czech. Chem. Commun.*, in press.

23. Chlupacova, M.; Opletalova, V.; Silva, L.; Buchta, V.; Kunes, J.: *Book of Abstracts of the Joint Meeting of the Czech Pharmaceutical Society, German Pharmaceutical Society and Austrian Pharmaceutical Society*, Regensburg (Germany), October 6–9, 2004, p. 100.

24. Dolezel, J.; Opletalova, V.; Silva, L.; Buchta, V.: *Book of Abstracts of the 34th Conference Synthesis and Analysis of Drugs*, Brno (Czech Republic), September 12–14, 2005, p. 54.