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N,N-Dimethylthiosemicarbazones 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 *N*,*N*-dimethylthiosemicarbazones. General synthetic approach of all newly synthesized compounds is presented. All the *N*,*N*-dimethylthiosemicarbazone 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 log *P* values calculated in various ways is discussed as well as the relationships between the lipophilicity and the chemical structure of the studied compounds.

Keywords: *N*,*N*-dimethylthiosemicarbazones; Lipophilicity measurement; Structure-lipophilicity relationships.

Introduction

Thiosemicarbazones are mostly prepared by the condensation of aldehydes or ketones with thiosemicarbazide. Acetone thiosemicarbazone, which decomposes easily, can be used instead of the free thiosemicarbazide in some cases [1, 2]. Thiosemicarbazones substituted on the terminal nitrogen are usually obtained by the reaction of methyl hydrazinecarbodithioates with amines [3-5]. Klayman and Lin [6, 7] described the preparation of a variety of *N*-mono and *N*,*N*-disubstituted thiosemicarbazones by the displacement of the dimethylamino function of the corresponding *N*,*N*-dimethylthiosemicarbazones by a primary or secondary amine. The reaction was further improved by Scovill [8] who used *N*-methyl-*N*-phenylthiosemicarbazones as the starting compounds for transamination. Preparation methods, synthetic applications and biological importance of thiosemicarbazones have been reviewed recently [9, 10]. Examining biologically active derivatives of pyrazine, we have found that acetylpyrazine thiosemicarbazones exhibited promising biological effects [11, 12]. Since the potency of thiosemicarbazones often increases by the replacement of the terminal NH_2 by a substituted amino group, analogous *N*,*N*-dimethylthiosemicarbazones were prepared and tested for tested for antifungal, antimycobacterial and antiproliferative activity [13].

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 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*) coefficient. 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. 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*. Log *K*, calculated from the capacity factor *K*, is used as the lipophilicity index converted to log *P* scale [14].

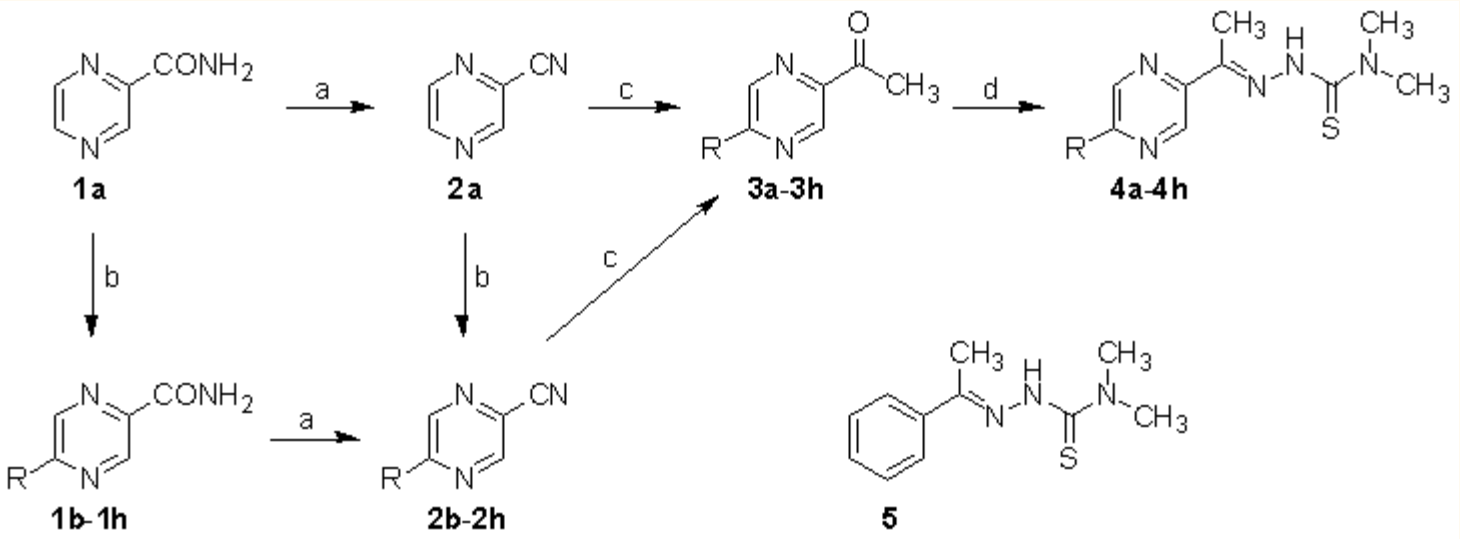
This contribution is a follow-up work to the previous papers [15-28] aimed at the synthesis, physicochemical properties and biological testing of newly prepared potential drugs based on nitrogen containing heterocycles.

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, ammonium peroxydisulfate and silver nitrate as a source of the alkyl radical. 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 [29, 30]. Acetylpyrazines **3a**–**3h** with *N*,*N*-dimethylthiosemicarbazide yielded the final thiosemicarbazones **4a**–**4h** [3, 13]. Analogous acetophenone *N*,*N*-dimethylthiosemicarbazone **5** was prepared for comparison in the same synthetic pathway. The general synthetic approach to 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 [13].

Scheme 1. Synthesis and structures of the target substituted *N*,*N*-dimethylthiosemicarbazones **4a**-**4h** and **5**.



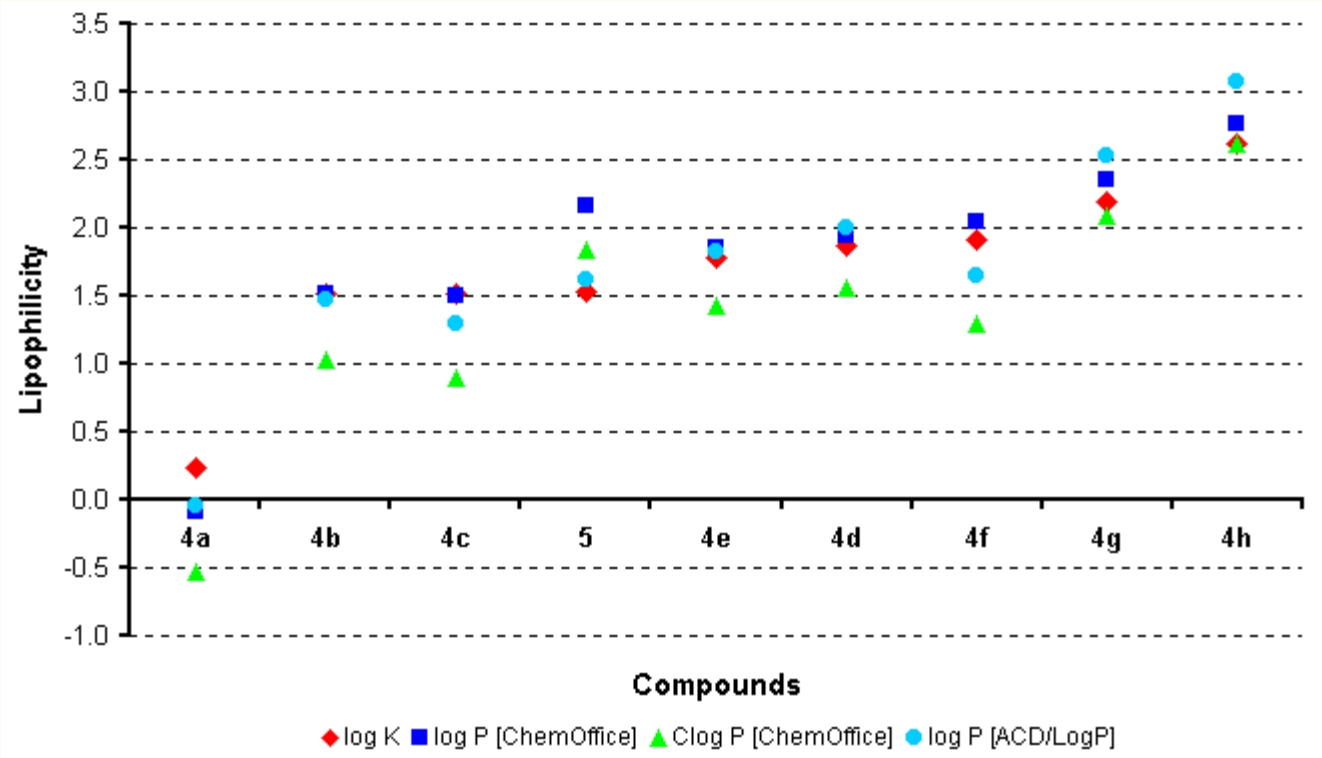
R: **a** = H; **b** = propyl; **c** = isopropyl; **d** = butyl; **e** = isobutyl; **f** = *tert*-butyl; **g** = pentyl; **h** = hexyl

Conditions: a) $POCl_3$; b) R-COOH, $AgNO_3$, $(NH_4)_2S_2O_8$; c) CH_3MgI, Et_2O; d) $NH_2NHCSN(CH_3)_2$, MeOH, AcOH.

Hydrophobicities (log *P* / Clog *P* data) 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 Figure 1. In the figure they are arranged in the ascending manner according to the experimental log *K* values.

N,*N*-Dimethylthiosemicarbazones reported here are much more hydrophobic (log K = 0.2335 – 2.6160) than the thiosemicarbazones described previously (log K = 0.1437 – 1.7246) [11, 12]. However, the higher lipophilicity did not automatically result in higher biological activity. Antifungal activity was only improved with the compound **4a**. In all other cases it remained the same or was lowered. The same applies for antiproliferative activity where compound **4a** was the most potent. Nonetheless, in the antiproliferative assay the potency of alkylated derivatives **4b**–**4h** gradually increased with the number of atoms in the alkyl chain and the lipophilicity, and the high activity of **4a** was rather surprising. Antimycobacterial activity of *N*,*N*-dimethylthiosemicarbazones was mostly lower compared to thiosemicarbazones, except for **4g** and **4h**. Compound **5** was inactive in all performed bioassays [13]. These results clearly indicate that the lipophilicity is only one of the factors affecting biological activity of thiosemicarbazones.

Figure 1. Comparison of the log *P* / Clog *P* data calculated using the two programs with the experimentally found log *K* values. The discussed compounds are ordered according to the log *K* values increase.



The results show that the experimentally determined log *K* values correlate well with log *P* values calculated either by ChemOffice Ultra software or ACD/LogP program, whilst according to the calculated Clog *P* data the hydrophobicity of most compounds (with exception of **5**) should be substantially lower 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 **4b**, **4d**, **4g** and **4h** (C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}) is approximately linear. Unsubstituted compound **4a** shows significantly lower hydrophobicity than linear, see Figure 1. 1-(5-Isopropylpyrazin-2-yl)ethan-1-one *N*,*N*-dimethylthiosemicarbazone **4c** is slightly more lipophilic than the corresponding propyl derivative **4b**, contrary to all the computed data. 1-(5-Isobutylpyrazin-2-yl)ethan-1-one *N*,*N*-dimethylthiosemicarbazone **4e** is slightly less lipophilic than its congeners **4d** (R = butyl) and **4f** (R = *tert*-butyl). This is in a good agreement with the results of our previous studies [12, 22], as well as with the computed log *P* data.

Great differences between the experimental and calculated log *P* (ChemOffice) could be observed for the compound **5** (acetophenone *N*,*N*-dimethylthiosemicarbazone), see Figure 1. The non-heterocyclic derivative **5** is situated between **4c** (R = isopropyl) and **4e** (R = isobutyl) according to log *K* and shows relatively low lipophilicity. According to the calculated log *P* (ChemOffice), its hydrophobicity should rather correspond to that of **4f** (R = isobutyl) or **4g** (R = pentyl).

Experimetal

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

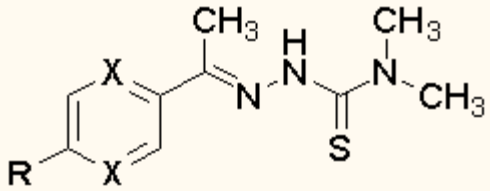
The HPLC separation module Waters Alliance 2695 XE and Waters Photodiode Array Detector 2996 (Waters Corp., Milford, MA, U.S.A.) were used. The chromatographic column Symmetry® C_{18} 5 µm, 4.6 × 250 mm, Part No. WAT054275, (Waters Corp., Milford, MA, U.S.A.) was used. The HPLC separation process was 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 25 °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. Retention times (T_R) were measured in minutes.

The capacity factors *K* were calculated using the Millennium32® Chromatography Manager Software according to the formula $K = (T_R - T_D) / T_D$, where T_R is the retention time of the solute, whereas T_D denotes the dead time obtained via an unretained analyte. The log *K* values of the individual compounds, calculated from the capacity factor *K*, 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. 9.0 (CambridgeSoft, Cambridge, MA, U.S.A.) and ACD/LogP 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. 9.0 (CambridgeSoft, Cambridge, MA, U.S.A.) software. The results are shown in Table 1.

Table 1. Calculated lipophilicities (log *P* / Clog *P*) and determined log *K* of the studied compounds **4a**-**4h** and **5**.



Compound	X	R	log *K*	log *P* / Clog *P* ChemOffice	log *P* ACD/LogP
4a	N	H	0.2335	-0.09 / -0.5291	-0.05 ± 0.62
4b	N	C_3H_7	1.5108	1.52 / 1.0279	1.47 ± 0.62
4c	N	$(CH_3)_2CH$	1.5161	1.50 / 0.8979	1.29 ± 0.62
4d	N	C_4H_9	1.8745	1.94 / 1.5569	2.00 ± 0.62
4e	N	$(CH_3)_2CHCH_2$	1.7859	1.85 / 1.4269	1.82 ± 0.62
4f	N	$(CH_3)_3C$	1.9130	2.04 / 1.2969	1.64 ± 0.62
4g	N	C_5H_{11}	2.1984	2.35 / 2.0859	2.53 ± 0.62

4h	N	C_6H_{13}	2.6160	2.77 / 2.6149	3.07 ± 0.62
5	C	H	1.5360	2.16 / 1.834	1.62 ± 0.59

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References

1. *Methoden der Organischen Chemie* (Houben-Weyl), Band 7/1. Sauerstoff-verbindungen II. Teil 1. Aldehyde. Mueller, E. (Edit.). 4. Auflage. Georg Thieme Verlag, Stuttgart 1954.
2. *Methoden der Organischen Chemie* (Houben-Weyl), Band 9. Schwefel-, Selen-, Tellur-verbindungen. Mueller, E. (Edit.). 4. Auflage. Georg Thieme Verlag, Stuttgart 1955.
3. Easmon, J., Heinisch, G., Holzer, W., Rosenwirth, B.: Novel thiosemicarbazones derived from formyl- and acyldiazines: synthesis, effects on cell proliferation, and synergism with antiviral agents. *J. Med. Chem.* **1992**, *35*, 3288-3296.
4. Milczarska, B., Foks, H., Trapkowski, Z., Milzynska-Kolaczek, A., Janowiec, M., Zwolska, Z., Andrzejczyk, Z.: Studies on pyrazine derivatives. XXXII. Synthesis and tuberculostatic activity of acetylpyrazine thiosemicarbazone derivatives. *Acta Pol. Pharm. - Drug Res.* **1998**, *55*, 289-295.
5. Milczarska, B., Foks, H., Sokolowska, J., Janowiec, M., Zwolska, Z., Andrzejczyk, Z.: Studies on pyrazine derivatives. XXXIII. Synthesis and tuberculostatic activity of 1-[1-(2-pyrazinyl)ethyl]-4-*N*-substituted thiosemicarbazide derivatives. *Acta Pol. Pharm. - Drug Res.* **1999**, *56*, 121-126.
6. Klayman, D. L., Lin, A. J.: Facile synthesis of N^4-mono and N^4,N^4-disubstituted 2-acetylpyridine thiosemicarbazones via transamination. *Heterocycles* **1983**, *20*, 392-393.
7. Klayman, D. L., Lin, A. J.: Thiocarbonyl-activated transamination. A facile synthesis of N^4-mono and N^4,N^4-disubstituted thiosemicarbazones. *Org. Prep. Proced. Int.* **1984**, *16*, 79-83.
8. Scovill, J. P.: A facile synthesis of thiosemicarbazides and thiosemicarbazones by the transamination of 4-methyl-4-phenyl-3-thiosemicarbazide. *Phosphorus, Sulfur Silicon Relat. Elem.* **1991**, *60*, 15-19.
9. Tenorio, R. P., Goes, A. J. S., De Lima, J. G., De Faria, A. R., Alves, A. J., De Aquino, T. M.: Thiosemicarbazones: preparation methods, synthetic applications and biological importance. *Quim. Nova* **2005**, *28*, 1030-1037.
10. Beraldo, H., Gambino, D.: The wide pharmacological versatility of semicarbazones, thiosemicarbazones and their metal complexes. *Mini Rev. Med. Chem.* **2004**, *4*, 31-39.
11. Opletalova, V., Pour, M., Kunes, J., Silva, L., Buchta, V., Jampilek, J., Richardson, D. R., Kalinowski, D. S., Svandova, M., Zobalova, D.: Thiosemicarbazones of acetylpyrazines as potential drugs. Book of Abstracts of the *11th Blue Danube Symposium on Heterocyclic Chemistry*, Brno (Czech Republic), August 28 - September 1, 2005, PO-70.
12. Jampilek, J., Opletalova, V., Grafnetterova, T., Dohnal, J.: Thiosemicarbazones of acetylpyrazines: preparation and their hydrophobic properties. *9th International Electronic Conference on Synthetic Organic Chemistry*

(*ECSOC-9*). November 1-30, 2005, A001; http://www.usc.es/congresos/ecsoc/9/GOS/a001/index.htm.

13. Jampilek, J., Opletalova, V., Pour, M., Kunes, J., Silva, L., Buchta, V., Richardson, D. R., Kalinowski, D. S., Al-Hallak, L., Honcova, D.: *N*,*N*-Dimethylthiosemicarbazones of acetylpyrazines as potential drugs. Book of Abstract of the *Joint Meeting 2006*, Marburg (Germany), October 4-7, 2006, C-76.
14. Pliska, V. In: *Lipophilicity in drug action and toxicology* (Methods and Principles in Medicinal Chemistry, Vol. 4). Pliska, V., Testa, B., van der Waterbeemd, H. (Edits.). 1st Ed. Wiley-VCH, Weinheim 1996, pp. 1-6.
15. Chlupacova, M., Opletalova, V., Kunes, J., Silva, L., Buchta, V., Dusková, L., Kralova, K.: Synthesis and biological evaluation of some ring-substituted (*E*)-3-aryl-1-pyrazin-2-ylprop-2-en-1-ones. *Folia Pharm. Univ. Carol.* **2005**, *33*, 31-43.
16. Opletalova, V., Pour, M., Kunes, J., Buchta, V., Silva, L., Kralova, K., Chlupacova, M., Meltrova, D., Peterka, M., Poslednikova, M.: Synthesis and biological evaluation of (*E*)-3-(nitrophenyl)-1-(pyrazin-2-yl)prop-2-en-1-ones. *Collect. Czech. Chem. Commun.* **2006**, *71*, 44-58.
17. Dolezal, M., Jampilek, J., Osicka, Z., Kunes, J., Buchta, V., Vichova, P.: Substituted 5-aroylpyrazine-2-carboxylic acid derivatives: Synthesis and biological activity. *Farmaco* **2003**, *58*, 1105-1111.
18. Jampilek, J., Dolezal, M., Osicka, Z., Kunes, J., Kralova, K.: Substituted pyrazine-2-carboxamides: preparation and their photosynthesis-inhibiting activity. *7th International Electronic Conference on Synthetic Organic Chemistry* (*ECSOC-7*), November 1-30, 2003, C001; http://www.mdpi.net/ec/ec_article.php?Id=81&file=papers/ecsoc-7/C001/C001.htm.
19. Jampilek, J., Dolezal, M., Kunes, J., Buchta, V.: Quinaldine Derivatives Preparation and Their Antifungal Activity. *8th International Electronic Conference on Synthetic Organic Chemistry* (*ECSOC-8*), November 1-30, 2004, C005; http://www.lugo.usc.es/~qoseijas/ECSOC-8/BOCNP/005/index.htm.
20. Jampilek, J., Dolezal, M., Kunes, J., Satinsky, D., Raich, I.: Novel regioselective preparation of 5-chloropyrazine-2-carbonitrile from pyrazine-2-carboxamide and coupling study of substituted phenylsulfanylpyrazine-2-carboxylic acid derivatives. *Curr. Org. Chem.* **2005**, *9*, 49-60.
21. Jampilek, J., Dolezal, M., Kunes, J., Buchta, V., Silva, L., Kralova, K.: Quinaldine derivatives: preparation and biological activity. *Med. Chem.* **2005**, *1*, 591-599.
22. Opletalova, V., Jampilek, J., Chlupacova, M., Dolezel, J., Dohnal, J.: Chromatographic and computational study of hydrophobic properties of ring substituted pyrazinecarbonitriles and acetylpyrazines. *9th International Electronic Conference on Synthetic Organic Chemistry* (*ECSOC-9*). November 1-30, 2005, C004; http://www.usc.es/congresos/ecsoc/9/BOCNP/c004/index.htm.
23. Jampilek, J., Vinsova, J., Grafnetterova, T., Dohnal, J.: Synthesis and Hydrophobic Properties of Benzoxazoles. *9th International Electronic Conference on Synthetic Organic Chemistry* (*ECSOC-9*). November 1-30, 2005, A008; http://www.usc.es/congresos/ecsoc/9/GOS/a008/index.htm.
24. Musiol, R., Jampilek, J., Kralova, K., Podeszwa, B., Finster, J., Niedbala, H., Palka, A., Polanski, J.: New Quinoline Derivatives Possessing Herbicidal Activity. *9th International Electronic Conference on Synthetic Organic Chemistry* (*ECSOC-9*). November 1-30, 2005, C005; http://www.usc.es/congresos/ecsoc/9/BOCNP/c005/index.htm.
25. Dolezal, M., Cmedlova, P., Palek, L., Kunes, J., Buchta, V., Jampilek, J., Kralova, K.: Synthesis and biological evaluation of pyrazinecarboxamides. *9th International Electronic Conference on Synthetic Organic Chemistry* (*ECSOC-9*). November 1-30, 2005, C020; http://www.usc.es/congresos/ecsoc/9/BOCNP/c020/index.htm.

26. Dolezal, M., Palek, L., Vinsova, J., Buchta, V., Jampilek, J., Kralova, K.: Substituted pyrazinecarboxamides. Synthesis and biological evaluation. *Molecules* **2006**, *11*, 242-256; http://www.mdpi.org/molecules/papers/11040242.pdf.
27. Musiol, R., Jampilek, J., Buchta, V., Silva, L., Niedbala, H., Podeszwa, B., Palka, A., Majerz-Maniecka, K., Oleksyn, B., Polanski, J.: Antifungal properties of new series of quinoline derivatives. *Bioorg. Med. Chem.* **2006**, *14*, 3592-3598.
28. Vinsova, J., Cermakova, K., Tomeckova, A., Ceckova, M., Jampilek, J., Cermak, P., Kunes, J., Dolezal, M., Staud, F.: Synthesis and antimicrobial evaluation of new 2-substituted 5,7-di-*tert*-butylbenzoxazoles. *Bioorg. Med. Chem.* **2006**, *14*, 5850-5865.
29. Opletalova, V., Patel, A., Boulton, M., Dundrova, A., Lacinova, E., Prevorova, M., Appeltauerova, M., Coufalova, M.: 5-Alkyl-2-pyrazinecarboxamides, 5-alkyl-2-pyrazinecarbonitriles and 5-alkyl-2-acetylpyrazines as synthetic intermediates for antiinflammatory agents. *Collect. Czech. Chem. Commun.* **1996**, *61*, 1093-1101.
30. Opletalova, V., Chlupacova, M., Poslednikova, M., Zobalova, D., Kunes, J., Vorisek, V.: 5-Alkylpyrazin-2-carbonitriles as intermediates for the synthesis of potential drugs. Book of Abstracts of the *56th Meeting of Chemical Societies*, Ostrava (Czech Republic), September 6-9, 2004. In: *Chem. Listy* **2004**, *98*, 669.