Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. *77* (1) 9–16 (2012)
JSCS–4244
UDC 547.78+547.233+547.282.1:615.28–188
Original scientific paper

New oxadiazole derivatives of isonicotinohydrazide in the search for antimicrobial agents: Synthesis and *in vitro* evaluation

MANAV MALHOTRA[1], MOHIT SANDUJA[2], ABDUL SAMAD[3] and AAKASH DEEP[4]*

[1]*Department of Pharmaceutical Chemistry, Meerut Institute of Engineering and Technology, Bypass Road-Baghpat Crossing, Meerut-250005, Uttar Pradesh, India,* [2]*Department of Pharmaceutical Chemistry, ISF College of Pharmacy, Ferozepur Road, Moga-142001, India,* [3]*Department of Pharmaceutical Chemistry, College of Pharmacy in Al-Kharj, King Saud University, Riyadh, Saudi Arabia and* [4]*Department of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak-124001, India*

(Received 23 January, revised 24 June 2011)

Abstract: Structural modifications of the front line antitubercular drug isoniazid provide lipophilic adaptations of the drug in which the hydrazide moiety of isoniazid is replaced by 1,3,4-oxadiazole heterocycles to eliminate *in vivo* acetylation by arylamine *N*-acetyltransferase, which results in the formation of inactive acetylated drug. In the present study, a series of sixteen oxadiazole derivatives were synthesized and characterized by IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral studies. All the synthesized compounds were evaluated for their antimicrobial activity by broth dilution method against two Gram-positive bacterial strains (*Bacillus subtilis* and *Staphylococcus aureus*), two Gram-negative bacterial strains (*Pseudomonas aeruginosa* and *Escherichia coli*) and two fungal strains (*Candida albicans* and *Aspergillus niger*). The minimum inhibitory concentrations of the compounds were in the range of 1.56–50 μg ml^{-1} against the bacterial and fungal strains. The results revealed that all the synthesized compounds have a significant biological activity against the tested microorganisms. Among the synthesized derivatives **4g**, **4h**, **4m** and **4p** were found to be the most effective antimicrobial compounds.

Keywords: 1,3,4-oxadiazoles; antimicrobial activity; isoniazid; Mannich bases; lipophilicity.

INTRODUCTION

One of the key objectives of organic and medicinal chemists is to design and synthesize molecules having potent therapeutic values.[1] The rapid development of resistance to existing antimicrobial drugs generates a serious challenge to the scientific community. Consequently, there is a vital need for the development of new antimicrobial agents having potent activity against the resistant microorga-

* Corresponding author. E-mail: aakashdeep82@gmail.com
doi: 10.2298/JSC110123155M

nism.[2–5] 1,3,4-Oxadiazoles have played an important role in medicinal chemistry, pesticide chemistry, polymer science and they are the building blocks in the construction of new molecular systems for biologically active molecules.[6] Many 1,3,4-oxadiazoles display a remarkable biological activity, such as antimicrobial,[7,8] anti-HIV,[9] antitubercular,[10] antimalarial,[11] analgesic,[12] anti-inflammatory.[13] The oxadiazole pharmacophore has a key property that influences the ability of a drug to reach the target by transmembrane diffusion and show potent antimicrobial activity.[14] Inspired by the above facts and in continuation of an ongoing research program in the field of the synthesis and determination of the antimicrobial activity of medicinally important compounds,[15,16] the synthesis and antimicrobial evaluation of new oxadiazole derivatives are reported herein.

EXPERIMENTAL

Material and methods

Melting points of the synthesized compounds were determined in open-glass capillaries on Stuart SMP10 melting point apparatus and are uncorrected. The purity of the compounds was checked by thin layer chromatography (TLC). Silica gel, 0.25 mm, 60 GF_{254}, precoated sheets obtained from Merck, (Germany) were used for the TLC and the spots were visualized by iodine vapor/ultraviolet light. The IR spectra were obtained on a Perkin-Elmer 1600 FTIR spectrometer in KBr pellets. The ^{1}H-NMR spectra were recorded in DMSO-d_6 solutions on a Varian-Mercury 300 MHz spectrometer using tetramethylsilane as the internal reference. The ^{13}C-NMR spectra were recorded in DMSO-d_6 solutions on a Bruker Avance II 400 spectrometer at using tetramethylsilane as the internal reference. The mass spectra were recorded on a Shimadzu GCMS-QP 1000 EX. The elemental analyses were performed on an ECS 4010 elemental combustion system. The necessary chemicals were purchased from Loba Chemie, Fluka and Aldrich.

(E)-N'-*(2-Methoxybenzylidene)isonicotinohydrazide (**1a**)*

A mixture of 2-methoxybenzaldehyde (1.36 g, 0.01 mol) and isoniazid (1.37 g, 0.0100 mol) in 15 ml of absolute ethanol was refluxed for 7 h. The completion of reaction was confirmed by TLC. The reaction mixture was then poured into ice-cold water and the obtained precipitate was filtered and dried in an oven at a low temperature. The product was recrystallized from absolute ethanol.[17] Yield 68 %; m.p. 204–207 °C.

*(*E*)-*N*'-3-((Dimethylamino)methyl)-2-methoxybenzylidene)isonicotinohydrazide (**2a**)*

(*E*)-*N*'-(2-Methoxybenzylidene)isonicotinohydrazide (612 mg, 0.00240 mol) along with (0.10 ml, 0.0036 mol) of formaldehyde and (0.0024 mol) of dimethylamine was placed in 100 ml round bottom flask to which 50 ml of absolute ethanol was added. The pH was adjusted to 4 with hydrochloric acid and the mixture refluxed for 35 h. Completion of the reaction was confirmed by TLC. The reaction mixture was then poured into a beaker and concentrated on a water bath. The reaction mixture was allowed to cool to room temperature and then diethyl ether was added. The reaction mixture was kept for 3–5 h in a refrigerator, filtered and washed with *n*-hexane. The product was recrystallized from absolute ethanol. Yield 78 %; m.p. 222––225 °C.

*1-(2-(3-((Dimethylamino)methyl)-2-methoxyphenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol--3(*2H*)-yl)ethanone (**3a**)*

A mixture of (*E*)-*N*'-3-((dimethyamino)methyl)-2-methoxybenzylidene)isonicotinohydrazide (3.57 g, 0.0100 mol) with an excess of acetic anhydride was refluxed for 7 h until the completion of the reaction, which was confirmed by TLC. The excess acetic anhydride was distilled off and the residue was poured onto crushed ice. The solid thus obtained was filtered; washed with water and then recrystallized with aqueous methanol. Yield 75 %; m.p. 168–170 °C.

*General procedure for synthesis of the substituted oxadiazoles **4a**–**p***

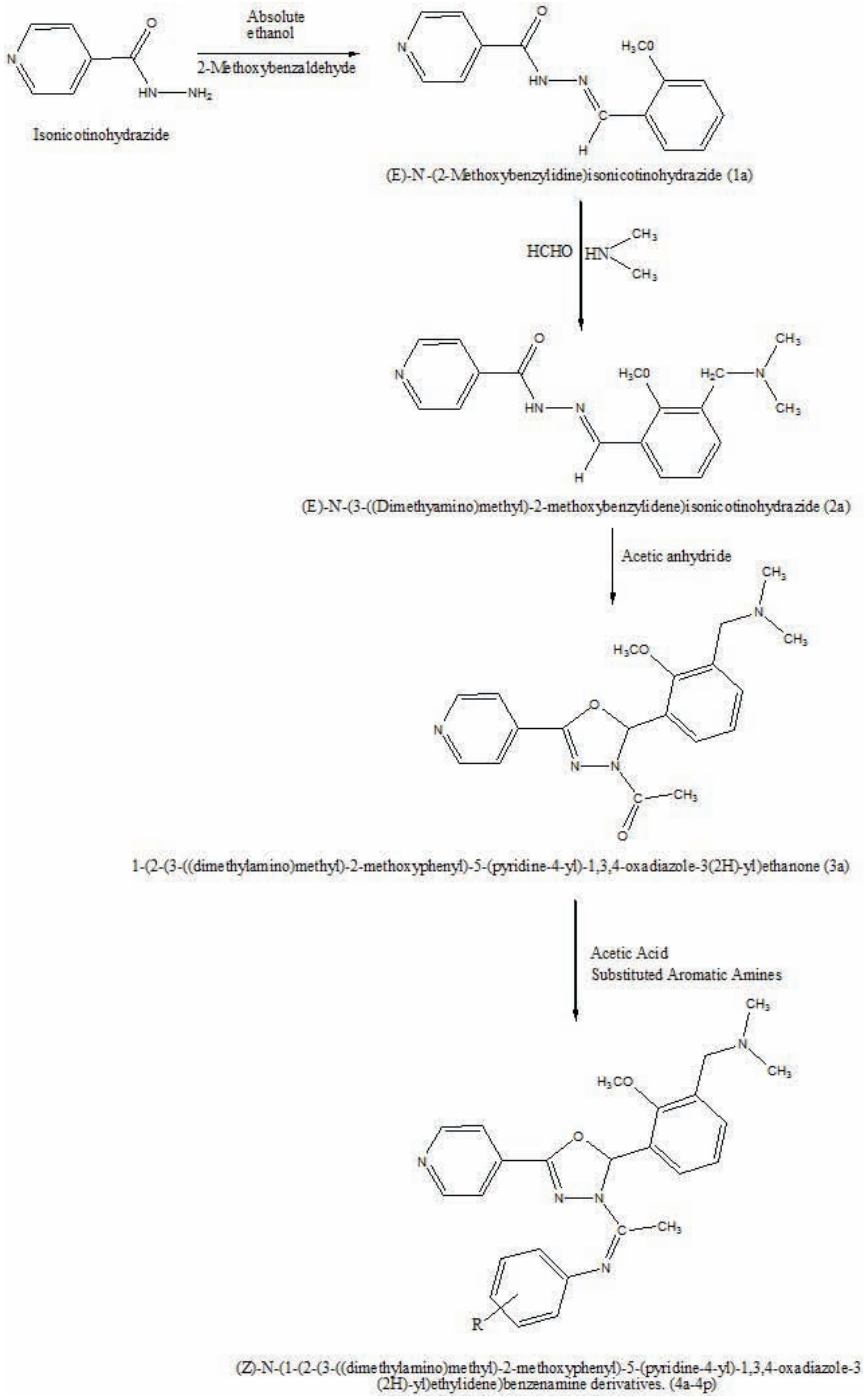
A mixture of **3a** (0.010 mol) and an equimolar amount of an appropriate aromatic amine (0.010 mol) was added to 25 ml absolute ethanol with a drop of glacial acetic acid and heated under reflux for 7–9 h. The obtained precipitate was filtered off; washed with ethanol and recrystallized from absolute ethanol to obtain compounds **4a**–**p**.

Antimicrobial activity

The synthesized compounds were evaluated for their *in vitro* antimicrobial activity against the Gram-positive bacteria *Staphylococcus aureus* (MTCC 96) and *Bacillus subtilis* (MTCC 121), the Gram-negative bacteria *Escherichia coli* (MTCC 40) and *Pseudomonas aeruginosa* (MTCC 2453) and the fungal strains *Candida albicans* (MTCC 227) and *Aspergillus niger* (MTCC 8189). Antimicrobial activity was assessed by the serial two-fold dilution technique. Amoxicillin was used as the standard drug for the antibacterial activity and nystatin was used as the standard drug for the antifungal activity. All the compounds were dissolved in dimethyl sulfoxide to give a concentration of 10 μg ml^{-1}. The two-fold dilutions of the test and standard compounds were prepared in double strength nutrient broth I.P. (bacteria) or Sabouraud dextrose broth I.P. (fungi).[18] The stock solution was serially diluted to give concentrations of 50–0.78 μg ml^{-1} in the nutrient broth. The inoculum size was approximately 10^6 colony forming units (CFU ml^{-1}). The tubes were incubated at 37±1°C for 24 h (bacteria), 25 °C for *C. albicans* and 35°C for *A. niger* for 7 days. Subsequently, the inoculated culture tubes were macroscopically examined for turbidity. The culture tube showing turbidity (lower concentration) and the culture tube showing no turbidity (higher concentration) gave the minimum inhibitory concentration (*MIC*) for the compound.

RESULTS AND DISCUSSION

The syntheses of the target compounds were performed according to the outline given in Scheme 1. Compounds **4a**–**p** were readily prepared in good yield and purity. Firstly, an equimolar mixture of 2-methoxybenzaldehyde and isonicotinohydrazide was refluxed to form (*E*)-*N*'-(2-methoxybenzylidene)isonicotinohydrazide (**1a**), then its reaction with formaldehyde and dimethylamine formed (*E*)-*N*'-(3-((dimethyamino)methyl)-2-methoxybenzylidene)isonicotinohydrazide (**2a**), which on treatment with acetic anhydride yielded 1-(2-(3-((dimethylamino)methyl)-2-methoxyphenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)ethanone (**3a**) and in the last reaction with substituted aromatic amines, it afforded a series of (*Z*)-*N*-(1-(2-(3-((dimethylamino)methyl)-2-methoxyphenyl)-5-(pyridin-4-yl)--1,3,4-oxadiazol-3(2*H*)-yl)ethylidene)benzenamine derivatives (**4a**–**p**). The structure, melting points and yields the synthesized compounds **4a**–**p** are given in Table I. The purity of the compounds was checked by TLC and elemental analyses.



Scheme 1. Synthetic route for the formation of the title compounds.

TABLE I. Physical data of the synthesized compounds **4a**–**4p**

Compd.	R	Molecular formula	Molecular weight	Melting point, °C	Yield, %
4a	H	$C_{25}H_{27}N_5O_2$	429.5	205–207	72
4b	2-F	$C_{25}H_{26}FN_5O_2$	447.5	195–197	69
4c	3-F	$C_{25}H_{26}FN_5O_2$	447.5	187–189	72
4d	4-F	$C_{25}H_{26}FN_5O_2$	447.5	212–214	64
4e	2-Cl	$C_{25}H_{26}ClN_5O_2$	463.9	178–180	65
4f	3-Cl	$C_{25}H_{26}ClN_5O_2$	463.9	185–187	59
4g	4-Cl	$C_{25}H_{26}ClN_5O_2$	463.9	218–220	69
4h	2-Br	$C_{25}H_{26}BrN_5O_2$	508.4	228–230	77
4i	3-Br	$C_{25}H_{26}BrN_5O_2$	508.4	235–237	64
4j	4-Br	$C_{25}H_{26}BrN_5O_2$	508.4	215–217	65
4k	2-NO_2	$C_{25}H_{26}N_6O_4$	474.5	223–225	79
4l	3-NO_2	$C_{25}H_{26}N_6O_4$	474.5	210–212	65
4m	4-NO_2	$C_{25}H_{26}N_6O_4$	474.5	217–219	73
4n	2-OCH_3	$C_{26}H_{29}N_5O_3$	459.2	213–215	56
4o	3-OCH_3	$C_{26}H_{29}N_5O_3$	459.2	207–209	73
4p	4-OCH_3	$C_{26}H_{29}N_5O_3$	459.2	210–212	65

Nevertheless, the structures of all new compounds synthesized were confirmed by IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy. The analytic and spectral data for all the compounds synthesized in this study are given in the Supplementary material to this paper. The IR spectra of all the compounds **4a**–**p** showed absorption bands at around 2993–2955, 2868–2839, 1679–1664, 1589–1557, 1189––1174, 1097–1018 cm^{-1} regions, confirming the presence of CH, CH_2, C=N, C=C, C–N, C–O groups, respectively. The, the chemical shifts, multiplicities, and coupling constants of the signals in the ^{1}H-NMR spectra of the respective derivatives **4a**–**p** verified their structures. The spectra of most compounds showed the characteristic 4H protons of pyridine at around δ 8.98–8.15 ppm, the characteristic protons of phenyl at δ 7.95–6.45 ppm, the 1H proton of oxadiazole at around δ 5.73–5.37 ppm, the 3H protons of O–CH_3 at around δ 3.88–3.64 ppm, the 2H protons of Ar–CH_2–N at around δ 3.69–3.52 ppm, the 6H protons of N–2CH_3 at around δ 2.49–2.26 ppm and the 3H protons of CH_3 at δ 1.21–1.05 ppm. The ^{13}C-NMR spectra of compounds **4a–p** exhibited characteristic signals of –N=**C**–CH_3 at around δ 164.77–164.18 ppm, phenyl at δ 155.87–119.15 ppm, pyridine at δ 149.78–124.11 ppm, oxadiazole at δ 155.17–154.13, O–CH_3 at δ 57.63–54.29, Ar–CH_2–N at δ 54.73–53.92 ppm, N-2CH_3 at δ 47.38–45.49 ppm and N–C–**C**H_3 at δ 22.77–15.13 ppm.

Antimicrobial activity

The compounds were evaluated for their antimicrobial properties in comparison to the control antibacterial drug amoxicillin and antifungal drug nystatin. The determined *MIC* values of compounds **4a**–**p** are listed in Table II. The investigation of antibacterial screening data revealed that all the tested compounds

showed moderate to good bacterial inhibition. The compounds **4g**, **4h**, **4m** and **4p** displayed excellent activity against the Gram-positive bacteria *B. subtilis* and *S. aureus* and good activity against the Gram-negative bacteria *P. aeruginosa* and *E. coli*. Compounds **4c**, **4d** and **4f** showed moderate antibacterial activity, while compounds **4a**, **4b**, **4e** and **4o** were less active against the tested bacterial strains. Of all the synthesized derivatives, compound **4a** was found to be the least active compound against most of the bacterial strain. From, these results, it could be generalized that the *p*-chloro-, *o*-bromo-, *p*-nitro- and *p*-methoxy-substituted oxadiazole derivatives showed higher antibacterial activity compared to the other analogues.

TABLE II. Antimicrobial screening results of the tested compounds (minimum inhibitory concentration, μg ml^{-1})

Compound	Gram-positive bacteria		Gram-negative bacteria		Fungal strain	
	B. subtilis (MTCC121)	*S. aureus* (MTCC96)	*P. aeruginosa* (MTCC2453)	*E. coli* (MTCC40)	*C. albicans* (MTCC227)	*A. nigar* (MTCC8189)
4a	6.25	12.5	50	3.12	12.5	25
4b	12.5	50	25	12.5	3.12	12.5
4c	6.25	6.25	12.5	3.12	6.25	3.12
4d	6.25	6.25	6.25	12.5	3.12	12.5
4e	12.5	6.25	3.12	12.5	6.25	12.5
4f	12.5	6.25	6.25	3.12	6.25	3.12
4g	3.12	1.56	1.56	3.12	6.25	3.12
4h	12.5	6.25	3.12	6.25	3.12	6.25
4i	3.12	12.5	12.5	25	50	12.5
4j	3.12	12.5	6.25	25	25	12.5
4k	6.25	25	12.5	6.25	12.5	25
4l	3.12	12.5	12.5	25	6.25	12.5
4m	1.56	3.12	3.12	1.56	6.25	12.5
4n	6.25	12.5	25	12.5	6.25	12.5
4o	25	12.5	12.5	6.25	12.5	6.25
4p	3.12	6.25	1.56	3.12	6.25	1.56
Amoxicillin	0.12	0.25	0.15	0.20	–	–
Nystatin	–	–	–	–	0.30	0.78

Concerning the antifungal activity of the tested compounds, only two fungal strains were selected *C. albicans* and *A. niger* and the result of antifungal screening data revealed that all the synthesized compounds showed variable degrees of inhibition against the tested fungi. The investigation of antibacterial screening data revealed that all the tested compounds showed moderate to good fungal inhibition as compare to standard drug nystatin. Of the screened compounds, **4g**, **4h**, **4m** and **4p** exhibited the highest antifungal activity against both fungal strains, while compounds **4e**, **4l** and **4n** showed moderate antifungal activity. Among all the synthesized derivatives, compound **4i** was found to be the least active com-

pound against the fungal strains. From these result, it could be generalized that the *p*-fluoro-, *o*-bromo-, *p*-nitro- and *m*-methoxy-substituted oxadiazole derivatives **4g**, **4h**, **4m** and **4p** showed higher antifungal activity than the other analogues. It was also observed that the derivatives having a chloro-, bromo-, nitro- and methoxy- substituent at the *ortho* and *meta* positions were not as potent as the derivatives having same substituent at the *para* position. Thus, from the obtained results it was found that the nature and position of the substituent had marked effects on antibacterial and antifungal activity.

SUPPLEMENTARY MATERIAL

Analytical and spectral data of synthesized compounds are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

И З В О Д

НОВИ ОКСАДИАЗОЛНИ ДЕРИВАТИ ИЗОНИКОТИНОХИДРАЗИДА У ПОТРАЗИ ЗА АНТИМИКРОБНИМ АГЕНСИМА: СИНТЕЗА И *IN VITRO* ЕВАЛУАЦИЈА

MANAV MALHOTRA[1], MOHIT SANDUJA[2], ABDUL SAMAD[3] и AAKASH DEEP[4]

[1]Department of Pharmaceutical Chemistry, Meerut Institute of Engineering and Technology, Bypass Road--Baghpat Crossing, Meerut-250005, Uttar Pradesh, India, [2]Department of Pharmaceutical Chemistry, ISF College of Pharmacy, Ferozepur Road, Moga-142001, India, [3]Department of Pharmaceutical Chemistry, College of Pharmacy in Al-Kharj, King Saud University, Riyadh, Saudi Arabia и [4]Department of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak-124001, India

Структурним модификацијама најважнијег лека против туберкулозе, изонијазида, добијени су деривати који имају израженије липофилне особине услед замене хидразидне функције 1,3,4-оксадиазолским хетероцикличним делом структуре. На тај начин се спречава *in vivo* ацетиловање ензимом ариламин-*N*-ацетилтрансфераза чиме су добијани неактивни ацетиловани деривати. У овом раду приказано је шеснаест нових оксадиазолских деривата који су окарактерисани спектралним анализама (IR, ^{1}H-NMR, ^{13}C-NMR и MS). Испитана је антимикробна активност синтетисаних деривата према Грам-позитивним (*Bacillus subtilis* и *Staphylococcus aureus*), Грам-негативним сојевима (*Pseudomonas aeruginosa* и *Escherichia coli*) и сојевима гљива (*Candida albicans* и *Aspergillus niger*). Минималне инхибиторне концентрација једињења налазе се у опсегу 1,56–50 μg ml^{-1} према сојевима бактерија и гљива. Резултати показују да једињења показују изражену активност, од којих су најактивнији деривати **4g, 4h, 4m** и **4p**.

(Примљено 23. јануара , ревидирано 24. јуна 2011)

REFERENCES

1. A. Verma, S. K. Saraf, *Eur. J. Med. Chem.* **43** (2008) 897
2. M. Koca, S. Servi, C. Kirilimis, M. Ahmedzade, C. Kazaz, B. Ozbek, G. Otuk, *Eur. J. Med. Chem.* **40** (2005) 1351
3. C. Bonde, N. J. Gaikwad, *Bioorg. Med. Chem.* **12** (2004) 2151
4. O. Prakash, M. Kumar, R. Kumar, C. Sharma, K. R. Aneja, *Eur. J. Med. Chem.* **45** (2010) 4252
5. N. N. Farshori, M. R. Banday, A. Ahmad, A. U. Khan, A. Rauf, *Bioorg. Med. Chem. Lett.* **20** (2010) 1933

6. G. C. Ramaprasad, B. Kalluraya, B. S. Kumar, R. K. Hunnur, *Eur. J. Med. Chem.* **45** (2010) 4587
7. K. Manjunthatha, B. Poojary, P. L. Lobo, J. Fernandes, N. S. Kumari, *Eur. J. Med. Chem.* **45** (2010) 5225
8. K. K. Jha, A. Samad, Y. Kumar, M. Shaharyar, K. L. Khosa, J. Jain, V. Kumar, P. Singh, *Eur. J. Med. Chem.* **45** (2010) 4963
9. A. A. El-Emam, O. A. Al-Deeb, M. Al-Omar, J. Lehmann, *Bioorg. Med. Chem.* **12** (2004) 5107
10. S. G. Kucukguzel, E. E. Oruc, S. Rollas, F. Sahin, A. Ozbek, *Eur. J. Med. Chem.* **37** (2002) 197
11. B. Chandrakantha, P. Shetty, V. Nambiyar, N. Isloor, A. M. Isloor, *Eur. J. Med. Chem.* **45** (2010) 1206
12. M. Akhter, A. Husain, B. Azad, M. Ajmal, *Eur. J. Med. Chem.* **44** (2009) 2372
13. V. Padmavathi, S. N. Reddy, G. D. Reddy, A. Padmaja, *Eur. J. Med. Chem.* **45** (2010) 4246
14. B. Testa, P. Crivori, M. Reist, P. A. Carrupt, *Perspect. Drug Discovery Des.* **19** (2000) 179
15. A. Madhukar, N. Kannappan, A. Deep, P. Kumar, M. Kumar, P. Verma, *Int. J. Chem. Tech. Res.* **1** (2009) 1376
16. A. Deep, S. Jain, P. C. Sharma, P. Verma, M. Kumar, C. P. Dora, *Acta Pol. Pharm.* **67** (2010) 255
17. M. Malhotra, R. Sharma, V. Monga, A. Deep, K. Sahu, A. Samad, *Lett. Drug Des. Discovery* **8** (2011) 579
18. *Pharmacopoeia of India*, Vol. II, Ministry of Health Department of India, New Delhi, 1996, p. A-88.