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European Journal of Medicinal Chemistry 43 (2008) 2831–2834
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Original article

Synthesis and antimicrobial activities of a new series of 4-*S*-[4^1-amino-5^1-oxo-6^1-substituted benzyl-4^1,5^1-dihydro-1^1,2^1,4^1-triazin-3-yl]mercaptoacetyl-3-arylsydnones

Jyothi C Hegde, K.S Girisha, Adithya Adhikari, Balakrishna Kalluraya*

Department of Studies in Chemistry, Mangalore University, Mangalagangothri 574 199, Karnataka, India

Received 29 August 2007; received in revised form 4 February 2008; accepted 7 February 2008
Available online 4 March 2008

Abstract

The synthesis of some 4-*S*-(4^1-amino-5^1-oxo-6^1-substituted benzyl-4^1,5^1-dihydro-1^1,2^1,4^1-triazin-3-yl)mercaptoacetyl-3-arylsydnones by the reaction of 3-aryl-4-bromoacetylsydnones with 6-substituted-4-amino-3-mercapto-1,2,4-triazin-5-ones is described. The IR, ^{1}H NMR, mass spectra and elemental analysis characterized the newly synthesized compounds. The synthesized compounds were screened for their antimicrobial activity. All the compounds showed higher activity than that of standard drug during antimicrobial studies and the activity was comparable with the standard drug for antifungal activity.

Keywords: Synthesis; Sydnones; Triazines; Antimicrobial activity

1. Introduction

Rapid development of resistance to the existing antimicrobial drugs poses a major threat to the public health. Recent reports suggest that bacteria and fungi are developing resistance to existing drugs [1,2]. Consequently, there is a pressing need to develop new antimicrobial agents, which have a broad spectrum of activity against the resistant micro-organisms.

Since their discovery, sydnones have shown diverse biological activities and it is thought that the meso-ionic nature of sydnone ring promote significant interactions with biological systems. Some of its derivatives are reported to possess activities such as anticancer, antimicrobial and anti-inflammatory [3–6]. Similarly, 1,2,4-triazinone derivatives are known to be endowed with wide spectrum of biological activities such as antitumor and antimicrobial properties [7–9]. Keeping in view of these observations and in continuation of our search for biologically active sydnone derivatives [10,11], we synthesized a series of the title compounds and studied their biological activity.

2. Chemistry

The synthetic routes followed for the preparation of triazinones **3** and the title compounds **5** are outlined in Schemes 1 and 2. Arylidine oxazolones **1** and acetyl sydnones were prepared following the literature methods [12,13]. Photochemical bromination of 3-aryl-4-acetylsydnone afforded 3-aryl-4-bromoacetylsydnones **4** [10]. 6-Substituted-4-amino-3-mercapto-1,2,4-triazine-5-ones **3** were obtained by direct one-pot condensation of 4-arylidine-2-methyl-1,3-oxazol-5-ones **1** with thiocarbohydrazide **2** (Scheme 1). The condensation of 6-substituted-4-amino-3-mecapto-1,2,4-triazine-5-ones **3** with 3-aryl-4-bromoacetylsydnones **4** yielded S-substituted triazinone derivatives **5a–l** (Scheme 2). We expected the formation of cyclized triazinothiadiazines, but unexpectedly S-substituted products were obtained instead of cyclized product. Attempts were made to cyclize the newly synthesized products, employing a variety of catalysts. However, our all efforts did not succeed due to the sensitivity of the sydnone ring towards acid, base and heat. The newly synthesized compounds were well characterized by spectral and analytical data.

* Corresponding author. Tel.: +91 824 228 7262; fax: +91 824 228 7367.
E-mail address: bkalluraya_2001@yahoo.com (B. Kalluraya).

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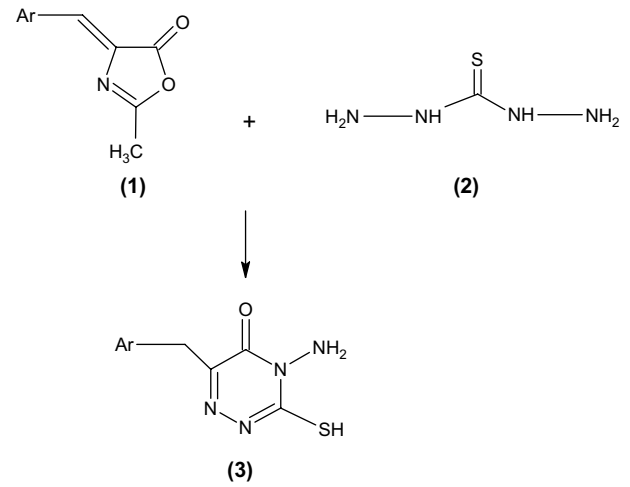
doi:10.1016/j.ejmech.2008.02.003



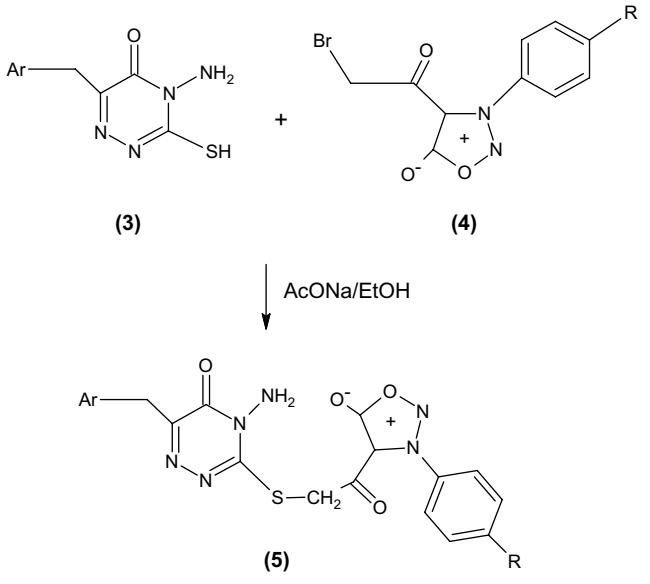
Scheme 1.

3. Results and discussion

3.1. Chemistry

Reaction of 3-aryl-4-bromoacetylsydnones **4** with 6-substituted-4-amino-3-mercapto-1,2,4-triazine-5-ones **3** gave S-substituted triazinone derivatives **5a–l**. The IR spectra of compounds **5** showed absorption bands at 1780–1790, 1680–1690 and 1624–1637 cm^{-1} for the C=O stretching frequency of sydnone, triazinone and $-CH_2-CO-$, respectively. The peaks between 3200 and 3320 cm^{-1} showed the presence of $-NH_2$ group thereby indicating that cyclization has not taken place.

In a typical example, ^1H NMR of **5j** showed a singlet at δ, 4.4 integrating for 2 protons of $-SCH_2$ group. The signal due to NH_2 protons appeared at δ, 4.7 while CH_2 protons came into resonance at δ, 4.0 as a singlet. The $-OCH_3$ protons appeared as a singlet at δ, 3.78 integrating for 3 protons. Two doublets centered at δ, 6.86 and δ, 7.36 integrating for 2 protons each corresponding to *ortho* and *meta* protons of *p*-anisyl moiety. The aromatic protons of phenyl ring resonated as multiplets at δ, 7.16–7.33 integrating for 5 protons. Further evidence for the assigned structure has been drawn from the mass spectral data.



Scheme 2.

3.2. Antimicrobial activity

The new compounds **5a–l** were subjected for *in vitro* antibacterial and antifungal studies by serial dilution method [14,15] against both Gram +ve and Gram −ve bacteria. Antibacterial activity was determined against *Staphylococus aureus* (ATCC-25923), *Bacillus subtilis* (Recultured), *P. aeruginosa* (ATCC-27853) and *Eschercia coli* (ATCC-25922), and antifungal activity was determined against *Candida albicans* (NCIM No. 3100). Nitrofurazone and Fluconazole were employed as standard drugs. Peptone–water and DMF were used as medium and solvent control. The results of biological study are summarized in Table 1. From the data, it is clear that all the compounds showed good activity against both fungus and bacteria, particularly showed a significant antimicrobial activity much higher than that of the standard drug.

4. Conclusion

In conclusion the reaction of 6-substituted-4-amino-3-mercapto-1,2,4-triazine-5-ones **3** with 3-aryl-4-bromoacetylsydnones **4** gave 4-*S*-(4^1-amino-5^1-oxo-6^1-substituted benzyl-4^1,5^1-dihydro-1^1,2^1,4^1-triazin-3-yl)mercaptoacetyl-3-arylsydnones **5** rather than the cyclized products. The microbial evaluation of these compounds indicated that they are more potent antimicrobial agents than the standard drug.

5. Experimental section

5.1. General

Melting points were determined in open capillary tubes and are uncorrected. The purity of the compounds is confirmed by thin layer chromatography using silica gel plates. ^1H NMR spectra were recorded on a 300 MHz NMR spectrometer and the ^{13}C NMR spectra were recorded on a 400 MHz NMR spectrometer. TMS was employed as internal standard. Chemical shift values are expressed in δ scale down field from TMS. The IR spectra [KBr disc] were recorded on JASCO FTIR-430 spectrophotometer and the mass spectra were recorded on a Jeol-JMS D-300 mass spectrometer operating at 70 eV. CHN analysis was carried on a Vairo-El-Elementa model analyzer.

5.1.1. Synthesis of 6-substituted-4-amino-3-mecapto-1,2,4-triazin-5-ones 3

A solution of thiocarbohydrazide **2** (0.1 mol) dissolved in minimum amount of hot water was added dropwise to a solution of oxazole derivative **1** (0.1 mol) in ethanol (50 ml). The

Table 1
Antibacterial and Antifungal activity data in MIC (μg/ml)

Compound no.	Antibacterial activity data				Antifungal activity data
	S. aureus	*P. aeruginosa*	*E. coli*	*B. Subtilis*	*C. albicans*
5a	0.25	0.25	0.25	0.25	0.25
5b	0.25	0.25	0.25	0.25	0.25
5c	0.25	0.25	0.25	0.25	0.25
5d	0.25	0.25	0.25	0.25	0.25
5e	0.5	0.25	0.25	0.25	0.25
5f	0.25	0.25	0.25	0.25	0.25
5g	0.25	0.25	0.25	0.25	0.25
5h	0.25	0.25	0.25	0.25	0.25
5i	0.25	0.25	0.25	0.25	0.25
5j	0.25	0.25	0.25	0.25	0.25
5k	0.25	0.25	0.25	0.25	0.25
5l	0.25	0.25	0.25	0.25	0.25
Nitrofurazone (Std.)	0.5	0.5	0.25	0.5	–
Fluconazole (Std.)	–	–	–	–	0.25
Solvent control (DMF)	–	–	–	–	–

Index for antibacterial and antifungal activity – Method: minimum inhibitory concentration by serial dilution method. Medium used: peptone–water. Solvent control: DMF. Std. for antibacterial: nitrofurazone. Std. for antifungal: fluconazole.

reaction mixture was refluxed for 2 h. Solid separated upon cooling was collected by filtration. Further, the products were purified by crystallization in ethanol–dioxane mixture and characterized by their melting points and reference to literature [16].

Compound **3a**: 6-benzyl-4-amino-3-mercapto-1,2,4-triazine-5-ones. Yield 78%, m.p. 204–205 °C (lit. m.p. 205 °C).

Compound **3b**: 6-(*p*-bromobenzyl)-4-amino-3-mercapto-1,2,4-triazine-5-ones. Yield 82%, m.p. 220–221 °C (lit. m.p. 220–223 °C).

Compound **3c**: 6-(*p*-chlorobenzyl)-4-amino-3-mercapto-1,2,4-triazine-5-ones. Yield 80%, m.p. 217 °C (lit. m.p. 218 °C).

Compound **3d**: 6-(3,4-methylenedioxybenzyl)-4-amino-3-mercapto-1,2,4-triazine-5-ones. Yield 69%, m.p. 221–223 °C (lit. m.p. 223 °C).

Compound **3e**: 6-(*p*-nitrobenzyl)-4-amino-3-mercapto-1,2,4-triazine-5-ones. Yield 75%, m.p. 204–206 °C (lit. m.p. 205 °C).

5.1.2. Synthesis of 4-S-[4^1-amino-5^1-oxo-6^1-substituted benzyl-4^1,5^1-dihydro-1^1,2^1,4^1-triazin-3-yl] mercaptoacetyl-3-arylsydnone ***5a–l***

To a solution of triazinenone **3** (0.01 mol) and bromoacetylsydnones **4** (0.01 mol) in ethanol (20 ml), anhydrous sodium acetate (0.41 g, 0.005 mol) was added. The solution was stirred at room temperature for 2–3 h. The solid product separated was collected by filtration, washed with water, dried and recrystallised from ethanol. Compounds prepared as per this procedure are as follows.

Compound **5a**: Ar = phenyl, R = H; m.p. = 188–189 °C; yield 68%; CHN analysis – Found: C: 55.01, H: 3.56, N: 19.19; Calculated: C: 55.04, H: 3.66, N: 19.26; ^{1}H NMR 300 MHz, $CDCl_3$: δ: 4.09 (s, 2H, CH_2), δ: 4.46 (s, 2H, S–CH_2), δ: 4.79 (s, 2H, NH_2); δ: 7.30–7.60 (m, 10H, ArH). ^{13}C NMR 400 MHz, DMSO: δ: 39.76 (S–CH_2–), δ: 40.18 (Ar–CH_2–), δ: 107 (triazine-3C), δ: 125–136 (10C-aromatic), δ: 152 (triazine-6C), δ: 156 (Sydnone-4C), δ: 161 (Sydnone-5C), δ: 166 (–CO–, triazine-5C), δ: 179 (–CO–). IR (KBr): ν_{CO} (sydnone) 1784 cm^{-1}, ν_{CO} (triazinone) 1685 cm^{-1}, ν_{C-O} (–CH_2–CO–) 1631 cm^{-1} Mass: *m/z*, 436 (MF: $C_{20}H_{16}N_6O_4S$).

Compound **5b**: Ar = *p*-bromophenyl, R = H; m.p. = 178–180 °C; yield 65%; CHN analysis – Found: C: 46.61, H: 2.81, N: 16.30; Calculated: C: 46.69, H: 2.91, N: 16.34; ^{1}H NMR 300 MHz, $CDCl_3$: δ: 4.05 (s, 2H, CH_2), δ: 4.49 (s, 2H, S–CH_2), δ: 4.82 (s, 2H, NH_2), δ: 7.24 (d, 2H, *ortho* protons of *p*-bromophenyl), δ: 7.43 (d, 2H, *meta* protons of *p*-bromophenyl), δ: 7.49–7.66 (m, 5H, ArH); Mass: *m/z*, 514/516 (MF: $C_{20}H_{15}BrN_6O_4S$).

Compound **5c**: Ar = *p*-chlorophenyl, R = H; m.p. = 183–184 °C; yield 67%; CHN analysis – Found: C: 51.12, H: 3.14, N: 17.78; Calculated: C: 51.06, H: 3.18, N: 17.85; ^{1}H NMR 300 MHz, $CDCl_3$: δ: 4.12 (s, 2H, CH_2), δ: 4.38 (s, 2H, S–CH_2), δ: 4.91 (s, 2H, NH_2), δ: 7.04 (d, 2H, *ortho* protons of *p*-chlorophenyl), δ: 7.51 (d, 2H, *meta* protons of *p*-chlorophenyl), δ: 7.54–7.68 (m, 5H, Ar–H); Mass: *m/z*, 470/472 (MF: $C_{20}H_{15}ClN_6O_4S$).

Compound **5d**: Ar = 3,4-methylenedioxyphenyl, R = H; m.p. = 162–163 °C; yield 72%; CHN analysis – Found: C: 52.23, H: 3.28, N: 17.47 Calculated: C: 52.50, H: 3.33, N: 17.50; ^{1}H NMR 300 MHz, $CDCl_3$: δ: 4.01 (s, 2H, CH_2), δ: 4.49 (s, 2H, S–CH_2), δ: 4.82 (s, 2H, NH_2), δ: 5.93 (s, 2H, O–CH_2–O), δ: 6.76–7.53 (m, 8H, ArH); Mass: *m/z*, 480 (MF: $C_{21}H_{16}N_6O_6S$).

Compound **5e**: Ar = *p*-nitrophenyl, R = H; m.p. = 211–212 °C; yield 69%; CHN analysis – Found: C: 49.78, H: 3.06, N: 20.42; Calculated: C: 49.89, H: 3.11, N: 17.37; ^{1}H NMR 300 MHz, $CDCl_3$: δ: 3.98 (s, 2H, CH_2), δ: 4.73 (s, 2H, S–CH_2), δ: 5.01 (s, 2H, NH_2), δ: 6.81–7.07 (m, 9H, Ar–H); IR (KBr): ν_{CO} (sydnone) 1780 cm^{-1}, ν_{CO} (triazinone) 1689 cm^{-1}, ν_{C-O} (–CH_2–CO–) 1637 cm^{-1}; Mass: *m/z*, 481 (MF: $C_{20}H_{15}N_7O_6S$).

Compound **5f**: Ar = phenyl, R = CH_3; m.p. = 188–189 °C; yield 65%; CHN analysis – Found: C: 56.11, H: 4.07, N: 18.58; Calculated: C: 56.00, H: 4.00, N: 18.66; IR (KBr): ν_{CO} (sydnone) 1787 cm^{-1}, ν_{CO} (triazinone) 1689 cm^{-1}, ν_{C-O} (–CH_2–CO–) 1624 cm^{-1}; ^{1}H NMR 300 MHz, $CDCl_3$: δ: 2.42 (s, 3H, CH_3), δ: 4.09 (s, 2H, CH_2), δ: 4.51 (s, 2H, S–CH_2), δ: 4.79 (s, 2H, NH_2), δ: 7.22–7.39 (m, 9H, ArH); Mass: *m/z*, 450 (MF: $C_{21}H_{18}N_6O_4S$).

Compound **5g**: Ar = *p*-bromophenyl, R = CH_3; m.p. = 194–195 °C; yield 67%; CHN analysis – Found: C: 47.64, H: 3.29, N: 15.83; Calculated: C: 47.72, H: 3.21, N: 19.90; IR (KBr): ν_{CO} (sydnone) 1786 cm^{-1}, ν_{CO} (triazinone) 1683 cm^{-1}, ν_{C-O} (–CH_2–CO–) 1626 cm^{-1}; ^{1}H NMR 300 MHz, $CDCl_3$: δ: 2.51 (s, 3H, CH_3), δ: 4.24 (s, 2H, CH_2), δ: 4.63 (s, 2H, S–CH_2), δ: 4.83 (s, 2H, NH_2), δ: 6.98 (d, 2H, *ortho* protons of *p*-bromophenyl), δ: 7.68 (d, 2H,

meta protons of *p*-bromophenyl), δ: 7.11 (d, 2H, *ortho* protons of *p*-tolyl), δ: 7.34 (d, 2H, *meta* protons of *p*-tolyl); Mass: *m/z*, 528/530 (MF: $C_{21}H_{17}BrN_6O_4S$).

Compound **5h**: Ar = *p*-chlorophenyl, R = CH_3; m.p. = 188–189 °C; yield 71%; CHN analysis – Found: C: 52.14, H: 3.54, N: 17.41; Calculated: C: 52.01, H: 3.50, N: 17.33; IR (KBr) ν_{CO} (sydnone) 1790 cm^{-1}, ν_{CO} (triazinone) 1680 cm^{-1}, ν_{C-O} (–CH_2–CO–) 1631 cm^{-1}; ^1H NMR 300 MHz, $CDCl_3$: δ: 2.31 (s, 3H, CH_3), δ: 4.23 (s, 2H, CH_2), δ: 4.63 (s, 2H, S–CH_2), δ: 5.03 (s, 2H, NH_2), δ: 7.86–7.58 (m, 8H, Ar–H); Mass: *m/z*, 484/486 (MF: $C_{21}H_{17}ClN_6O_4S$).

Compound **5i**: Ar = 3,4-methylenedioxyphenyl, R = CH_3; m.p. = 184–185 °C; yield 69%; CHN analysis – Found: C: 53.35, H: 3.57, N: 17.21; Calculated: C: 53.44, H: 3.64, N: 17.28; IR (KBr) (sydnone) 1789 cm^{-1}, ν_{CO} (triazinone) 1686 cm^{-1}, ν_{C-O} (–CH_2–CO–) 1636 cm^{-1}; Mass: *m/z*, 494 (MF: $C_{22}H_{18}N_6O_6S$).

Compound **5j**: Ar = phenyl, R = OCH_3; m.p. = 175–176 °C; yield 69%; CHN analysis – Found: C: 54.15, H: 3.92, N: 18.13; Calculated: C: 54.07, H: 3.86, N: 18.02; IR (KBr): ν_{CO} (sydnone) 1793 cm^{-1}, ν_{CO} (triazinone) 1683 cm^{-1}, ν_{C-O} (–CH_2–CO–) 1631 cm^{-1}; Mass: *m/z*, 466 (MF: $C_{21}H_{18}N_6O_5S$).

Compound **5k**: Ar = *p*-bromophenyl, R = OCH_3; m.p. = 194–195 °C; yield 71%; CHN analysis – Found: C: 56.26, H: 3.03, N: 15.49; Calculated: C: 56.32, H: 3.12, N: 15.44; IR (KBr): ν_{CO} (sydnone) 1779 cm^{-1}, ν_{CO} (triazinone) 1680 cm^{-1}, ν_{C-O} (–CH_2–CO–) 1625 cm^{-1}; ^1H NMR 300 MHz, $CDCl_3$: δ: 3.81 (s, 3H, O–CH_3), δ: 4.3 (s, 2H, CH_2), δ: 4.58 (s, 2H, S–CH_2), δ: 4.89 (s, 2H, NH_2), δ: 6.93–7.88 (m, 8H, Ar–H); Mass: *m/z*, 544/546 (MF: $C_{21}H_{17}BrN_6O_5S$).

Compound **5l**: Ar = *p*-chlorophenyl, R = OCH_3; m.p. = 196–197 °C; yield 66%; CHN analysis – Found: C: 50.38, H: 3.30, N: 16.71; Calculated: C: 50.34, H: 3.39, N: 16.78; IR (KBr): ν_{CO} (sydnone) 1780 cm^{-1}, ν_{CO} (triazinone) 1685 cm^{-1}, ν_{C-O} (–CH_2–CO–) 1627 cm^{-1}; ^1H NMR 300 MHz, $CDCl_3$: δ: 3.93 (s, 3H, O–CH_3), δ: 4.21 (s, 2H, CH_2), δ: 4.49 (s, 2H, S–CH_2), δ: 5.01 (s, 2H, NH_2), δ: 6.74–7.03 (m, 8H, Ar–H); Mass: *m/z*, 500/502 (MF: $C_{21}H_{17}ClN_6O_5S$).

References

[1] D. Niccolai, L. Tarsi, R.J. Thomas, Chem. Commun. (1997) 2333–2342.
[2] D.T.W. Chu, J.J. Plattner, L. Kats, J. Med. Chem. 39 (1996) 3853–3874.
[3] S.G. Mallur, B.V. Badami, Il Farmaco 55 (1) (2000) 65–67.
[4] M.A. Moustafa, M.N. Nasr, M.M. Gineinah, W.A.H. Bayoumi, Arch. Pharm. Pharm. Med. Chem. 337 (2004) 164–170.
[5] M.A. Moustafa, M.N. Nasr, M.M. Gineinah, W.A.H. Bayoumi, Arch. Pharm. Pharm. Med. Chem. 337 (2004) 427–433.
[6] C.S. Dunkley, C.J. Thoman, Bioorg. Med. Chem. Lett. 13 (2003) 2899–2901.
[7] A.M. Abdel-Halim, Z. El-Gendy, R.M. Abdel-Rahman, Pharmazie 50 (1995) 726–729.
[8] M. Kidwai, R. Kumar, A. Srivastava, H.P. Gupta, Bioorg. Chem. 26 (1998) 289–294.
[9] B.S. Holla, M.K. Shivananda, B. Veerendra, B.K. Subramanya, Indian J. Chem. 42B (2003) 2649–2651.
[10] B. Kalluraya, A.M. Rahiman, Pol. J. Chem. 71 (1997) 1049.
[11] B. Kalluraya, A.M. Rahiman, D. Banji, Arch. Pharm. Pharm. Med. Chem. 334 (2001) 263–268.
[12] B.S. Furniss, A.J. Hannaford, P.W.G. Smith, Vogel's Text Book of Organic Chemistry, fifth ed. Longman Group Limited, London, 1989, p. 1155 (Chapter 8).
[13] C.Y. Gerco, J. Tobias, L.B. Kier, J. Heterocycl. Chem. 4 (1967) 160.
[14] Z.K. Khan, In vitro and vivo screening techniques for bioactivity screening and evaluation, Proceedings of the International Workshop, UNIDO – CDRI, 1997, p. 210.
[15] R.S. Verma (Ed.), Antifungal Agents: Past, Present and Future Prospectus, National Academy of Chemistry and Biology, Lucknow, India, 1998.
[16] B.S. Holla, R. Gonsalves, B.K. Sarojini, Indian J. Chem. 36B (1997) 943–946.