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Original article

Synthesis and *in vitro* study of methylene-bis-tetrahydro[1,3]thiazolo[4,5-*c*] isoxazoles as potential nematicidal agents

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ABSTRACT

A series of methylene-bis-tetrahydro[1,3]thiazolo[4,5-*c*]isoxazoles **6** were synthesized by the reaction of chalcone derivative of methylene-bis-thiazolidinone **5** with hydroxylamine hydrochloride. The chemical structures of newly synthesized compounds were elucidated by IR, ^{1}H, ^{13}C NMR, MS and elemental analyses. The compounds **6a**–**g** were evaluated for their nematicidal activity against *Ditylenchus myceliophagus* and *Caenorhabditis elegans*, compound **6e** and **6f** showed appreciable nematicidal activity. Further the compounds **6a**–**g** were screened for their antifungal activity against *Candida albicans* (ATCC 10231), *Aspergillus fumigatus* (HIC 6094), *Trichophyton rubrum* (IFO 9185) and *Trichophyton mentagrophytes* (IFO 40996). The compounds **6b** and **6f** displayed notable antifungal activity against all the microorganisms employed. The activity of these two compounds is almost equal to the standard. It is also interesting to note that the compounds **6b**, **6f** and **6g** showed activity towards *C. albicans* at the concentration of 3.75 μM, which is less than the concentration of the standard Amphotericin B.

1. Introduction

Thiazoles are familiar group of heterocyclic compounds possessing a wide variety of biological activities and their utility as medicine is very much established [1]. Thiazole nucleus is also integral part of all the available penicillins which have revolutionized the therapy of bacterial diseases [2]. Further the chemistry of thiazolidinone ring system is of considerable interest as it is a core structure in various synthetic pharmaceuticals displaying a broad spectrum of biological activities [3]. The thiazolidinone nucleus also appears frequently in the structure of various natural products, notably thiamine, compounds possessing cardiac and glycemic benefits such as troglitazone [4] and many metabolic products of fungi and primitive marine animals, including 2-(aminoalkyl)-thiazole-4-carboxylic acids [5]. Numerous thiazolidinone derivatives have shown significant bioactivities such as antidiarrhoeal [6], anticonvulsant [7], antimicrobial [8], antidiabetic [9], antihistaminic [10], anticancer [11], anti HIV [12], Ca^{2+} channel blocker [13], PAF antagonist [14], cardioprotective [15], antiischemic [16], COX inhibitory [17], anti-platelet activating factor [18], non-peptide thrombin receptor antagonist [19], tumor necrosis factor-α antagonist [20] and nematicidal activities. Moreover, isoxazole derivatives are an important class of bioactive molecules, which exhibit significant activities such as antifungal [21], Aβ precursor protein [22], protein tyrosine phosphatase 1B inhibitors [23], antiviral [24], antihelmintics [25], anti-inflammatory [26], anticonvulsant [27], insecticidal [28], antitubercular [29], immunomodulatory [30] and hypolipemics [31].

Nematodes are tiny worms, some of them are plant parasites, and can play an important role in the predisposition of the host plant to the invasion by secondary pathogens [32]. Plants attacked by nematodes show retarded growth and development, as well as loss in the quality and quantity of the harvest. The nematicide use is slated for reduction due to environmental problems, and human and animal health concerns. For example, effective nematicides such as DBCP (dibromochloropropane) and EDB (ethylenedibromide) have been withdrawn from the market due to their deleterious effects on humans and the environment. Methyl bromide, the most effective and widely used fumigant for soil borne pests, including nematodes, has already been banned. The use of nonfumigant nematicides, based on organophosphates and carbamates, is expected to increase the withdrawal of methyl bromide, which will bring about new environmental concerns. In fact, the highly toxic aldicarb used to control insects and nematodes has been detected in ground water [33]. Therefore, alternative nematode control methods or less toxic nematicides need to be developed [34]. One way of searching for such nematicidal compounds is to screen naturally occurring compounds in plants. Several such compounds, e.g. alkaloids, phenols, sesquiterpenes, diterpenes, polyacetylenes and thienyl derivatives have nematicidal activity

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[35]. For example, α-terthienyl is a highly effective nematicidal compound [36]. Other compounds with nematicidal activity have been isolated from plants, mainly from the family Asteraceae [35]. However, compounds of plant origin and their analogs have not been developed into commercial nematicides, hence there is a need to develop commercial synthesis.

Following the successful introduction of antimicrobial and nematicidal agents, inspired by the biological profile of thiozolidinones, isoxazoles and their increasing importance in pharmaceutical and biological fields, and in continuation of our research on biologically active heterocycles [37], and in order to enhance the biological activity of both isoxazole and thiazolidinone, it was thought of interest to accommodate thiazolidinone and isoxazole moieties in a single molecular frame work. In this article, we wish to report the synthesis of a new class of methylene-bis-tetrahydro [1,3]thiazolo[4,5-*c*]isoxazoles **6** in good yields from methylene-bis-thiazolidinones **4** through their chalcone derivatives **5** (Schemes 1 and 2) and their evaluated *in vitro* antifungal and nematicidal activity.

2. Chemistry

The key intermediate, **4** required for the synthesis of title compounds was prepared according to the procedure outlined in the Scheme 1. Condensation of salicylaldehyde **1** with trioxane in the presence of a mixture of conc. H_2SO_4 and AcOH gave methylene-bis-salicylaldehyde **2** in good yield [38]. Compound **2** was then reacted with methyl iodide in the presence of K_2CO_3 in DMF at room temperature to give 5-(3-formyl-4-methoxybenzyl)-2-methoxybenzaldehyde **3**. The one-pot synthesis of methylene-bis-thiazolidinone derivatives **4** was carried out by the condensation–cyclization reaction between compound **3**, primary aromatic amine and mercaptoacetic acid in the presence of $ZnCl_2$ under microwave irradiation/conventional heating conditions (Scheme 1). In the "classical" method, the reactions were performed in dry toluene at reflux for a long time (2–4 h), often leading to degradation processes and consequent low yields of isolated products, whereas with the application of microwave assisted technology, the reaction completed in 5–7 min and the compounds, isolated by conventional work-up, obtained in satisfactory yields, often higher than those achieved by the traditional methods [37k]. Compound **4** was then reacted with *p*-fluorobenzaldehyde in the presence of anhydrous NaOAc in glacial AcOH at reflux temperature to give chalcone derivatives of methylene-bis-thiazolidinones **5**. Compound **5** on cyclo condensation with hydroxylamine hydrochloride in the presence of anhydrous NaOAc in glacial AcOH at reflux temperature gave methylene-bis-tetrahydro[1,3]thiazole[4,5-*c*]isoxazole derivative **6** in good to excellent yields (Scheme 2). The structures of the synthesized compounds were confirmed by their IR, ^1H, ^{13}C, MS and elemental analyses.

3. Results and discussion

In the present work, a series of new compounds were synthesized, Schemes 1 and 2 illustrates the path used for the preparation of target compounds. As starting materials, salicylaldehyde and trioxane were used to prepare thiazolo-isoxazole derivatives. The structure of the compounds was elucidated by IR, ^1H, ^{13}C NMR, MS and elemental analyses. In the IR spectra of compounds **6a**–**g**, disappearance of amide carbonyl (C=O) absorption band at ~1700 cm^{-1}, which was present in compound **5**, confirmed the cyclization or involvement of α,β-unsaturated carbonyl system. The C=N, N–O bands of the isoxazole moiety were observed at about 1600 and 1470 cm^{-1} respectively.

In the ^1H NMR spectra of compounds **6a**–**g**, recorded in DMSO-d_6, the signal due to methylene bridge protons appeared at 3.98–4.06 ppm as a singlet, the N–CH–S proton of thiazole ring at 7.37–7.40 ppm as a doublet, 5-CH fused proton at 4.70–4.80 ppm as a doublet and CH–O proton of isoxazole ring at 5.70–5.80 ppm as a doublet. These signals demonstrate that the cyclization step had occurred. All the other aromatic and aliphatic protons of **6a**–**g** were observed at the expected regions.

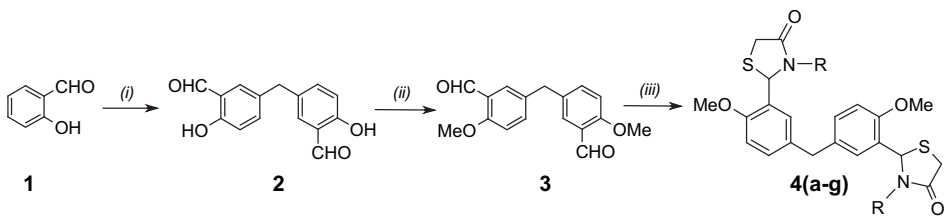
In the ^{13}C NMR spectrum of compounds **6a**–**g**, recovered in DMSO-d_6, the prominent signals corresponding to the carbons of thiazolo-isoxazole ring in all compounds observed nearly at 59.2, 64.7, 80.2 and 145.7 ppm, are proof of further evidence of their structures. Mass spectra of all the synthesized compounds showed M^+/M^{+1} peaks, in agreement with their molecular formulae.

4. Pharmacology

4.1. Antifungal studies

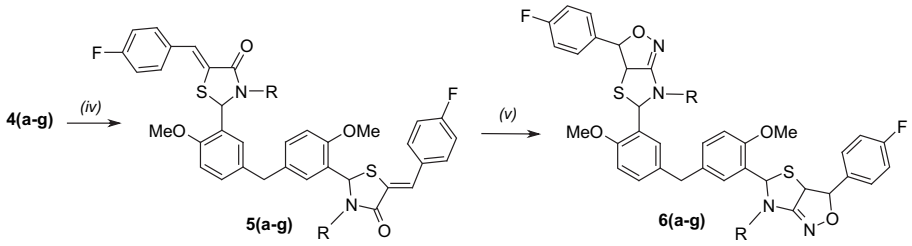
The newly prepared compounds **6a**–**g** were screened for their antifungal activity against four fungal organisms *viz. Candida albicans* (ATCC 10231), *Aspergillus fumigatus* (HIC 6094), *Trichophyton rubrum* (IFO 9185) and *Trichophyton mentagrophytes* (IFO 40996) by the broth dilution method, recommended by National Committee for Clinical Laboratory Standards (NCCLS) [39]. The antifungal activity of each compound was compared with the standard drug Amphotericin B. Minimum inhibitory concentration (MIC, μM) was measured and compared with controls; the MIC values of the compounds screened are presented in Table 1.

The antifungal screening data showed moderate activity of the test compounds, among the screened **6b**, **6f** in which thiazolo-isoxazole moiety bearing *p*-chlorophenyl and *p*-fluorophenyl nucleus on nitrogen respectively showed high activity against all the microorganisms employed. The activities of these two compounds are almost equal to the standard. It is also interesting to note that the compounds **6b**, **6f** and **6g** showed activity towards *C. albicans* at the concentration of 3.75 μM, which is less than the concentration of the standard Amphotericin B. The remaining compounds showed moderate to good antifungal activity. Further,



4: R = **a**) C_6H_5; **b**) 4-Cl-C_6H_4; **c**) 4-NO_2-C_6H_4; **d**) 4-Me-C_6H_4; **e**) 4-HO-C_6H_4; **f**) 4-F-C_6H_4; **g**) 2-Me-C_6H_4

Scheme 1. Reagents and conditions: (i) trioxane, H_2SO_4/AcOH, reflux, 81%; (ii) MeI, K_2CO_3, DMF, rt, 83%; (iii) R–NH_2, HS–CH_2–COOH, $ZnCl_2$/PhMe, reflux/MWI, 81–90%.



5/6: R = **a**) C_6H_5; **b**) 4-Cl-C_6H_4; **c**) 4-NO_2-C_6H_4; **d**) 4-Me-C_6H_4; **e**) 4-HO-C_6H_4; **f**) 4-F-C_6H_4; **g**) 2-Me-C_6H_4

Scheme 2. Reagents and conditions: (iv) 4-F–C_6H_4–CHO, AcOH/NaOAc, reflux, 82–88%; (v) $NH_2OH.HCl$, NaOAc/AcOH, reflux, 78–84%.

the antifungal activities of dimeric compounds **6a**–**g** were compared to their monomeric compounds. The results reveal that almost all the dimeric compounds showed enhanced activity than their monomeric compounds (Table 1).

4.2. Nematicidal studies

The compounds synthesized **6a**–**g** in this study were also screened for their nematicidal activity against *Ditylenchus myceliophagus* and *Caenorhabditis elegans* by aqueous *in vitro* screening technique [40] at various concentrations. The nematicidal activity of each test compound was compared with the standard drug Levamisole. The results have been expressed in terms of LD_{50} *i.e.* median lethal dose at which 50% nematodes became immobile (dead). The screened data reveal that, compounds **6e** and **6f** are the most effective against *D. myceliophagus* and *C. elegans* with LD_{50} 190 and 220 $\times 10^{-6}$, respectively, the other test compounds showed moderate activity. The LD_{50} values of the compounds screened are presented in Table 1.

5. Conclusion

In conclusion, a series of a new class of methylene-bis-tetrahydro[1,3]thiazolo[4,5-*c*]isoxazole **6a**–**g** has been synthesized. The antifungal activity of these compounds was evaluated against various fungi. Among the synthesized compounds **6b** and **6f** showed good activity against test fungi and emerged as potential molecules for further development. The compounds were also evaluated for their nematicidal activity, **6e** and **6f** showed an appreciable nematicidal activity.

6. Experimental section

Research chemicals were either purchased from Aldrich Co. or Fluka and used without further purification in the reactions or were prepared according to procedures described in the literature. Reactions were monitored by thin layer chromatography (TLC) on silica gel plated (60 F_{254}; Merck) visualizing with ultraviolet light or iodine. Column chromatography was performed on silica gel 60 (0.043–0.060 mm), Merck. Melting points were determined with a Fisher–Johns apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer FTIR 5000 spectrometer, using KBr pellet. ^1H, ^{13}C NMR spectra were recorded on a Varian Gemini spectrometer, operating at 300, 75 MHz respectively. Chemical shifts (δ) are reported in parts per million downfield from tetramethyl silane. Mass spectra were obtained on a VG micro mass 7070H spectrometer. Elemental analyses were performed on a Perkin–Elmer 240 CHN elemental analyzer.

6.1. 5-[(Z)-1-(4-Fluorophenyl)methylidene]-2-[5-(3-5-[(Z)-1-(4-fluorophenyl)methylidene]-4-oxo-3-phenyl-1,3-thiazolan-2-yl-4-methoxybenzyl)-2-methoxyphenyl]-3-phenyl-1,3-thiazolan-4-one (*5a*)

A mixture of compound **4a** (0.01 mol), *p*-fluorobenzaldehyde (0.02 mol) and sodium acetate (0.01 mol) in anhydrous glacial acetic acid (20 mL), was refluxed for 3 h. The reaction mixture was concentrated and then poured into ice cold water, the solid thus separated, was filtered, washed with water and crystallized from glacial acetic acid to afford pure **5a** (83% yield) as yellow solid: m.p. 145–47 °C; IR (KBr): 3026, 2985, 1721, 1535, 1482, 686 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.81 (s, 2H, CH_2), 4.11 (s, 6H, OCH_3), 6.61 (d, J = 9.1 Hz, 2H, ArH), 6.69 (s, 2H, ArH), 6.87–6.92 (m, 6H, ArH + CH–S), 7.09 (d, J = 9.1 Hz, 2H, ArH), 7.21–7.30 (m, 6H, ArH), 7.40–7.45 (m, 8H, ArH), 7.80 (s, 2H, CH=C); ^{13}C NMR (DMSO-d_6): δ 42.0, 56.0, 64.1, 112.7, 117.0, 122.7, 124.9, 126.3, 128.4, 128.9, 129.7, 130.4, 131.1, 134.5, 135.6, 137.9, 140.8, 154.0, 161.8, 165.7; MS: *m*/*z* 795 (M^+ +1). *Anal. Calcd*. for $C_{45}H_{36}F_2N_2O_4S_2$: C, 71.01; H, 4.56; N,

Table 1
Antifungal and Nematicidal activity of compounds **6a**–**g**.

Compd.	Antifungal activity				Nematicidal activity	
	Minimum inhibitory concentration (MIC, μM)				LD_{50} values ($\times 10^{-6}$)	
	Candida albicans	*Aspergillus fumigatus*	*Trichophton rubrum*	*Trichophyton mentagrophytes*	*D. myceliophagus*	*C.elegans*
6a	30.0[a] (120.0)[b]	60.0[a] (—)[b]	30.0[a] (—)[b]	60.0[a] (—)[b]	790	760
6b	3.75 (30.0)	3.75 (30.0)	3.75 (30.0)	3.75 (30.0)	850	770
6c	15.0 (30.0)	30.0 (60.0)	30.0 (120.0)	7.50 (30.0)	560	360
6d	7.50 (60.0)	30.0 (120.0)	— (–)	15.0 (120.0)	600	550
6e	15.0 (60.0)	15.0 (60.0)	7.50 (–)	7.50 (60.0)	190	240
6f	3.75 (120.0)	3.75 (60.0)	3.75 (60.0)	3.75 (60.0)	270	220
6g	3.75 (60.0)	3.75 (30.0)	3.75 (15.0)	3.75 (30.0)	950	870
Amphotericin B	7.50	3.75	3.75	3.75	—	—
Levamisole	—	—	—	—	160	180

—Indicates fungi are resistant to the compound >120 μM conc.
[a] Activity of dimeric compounds.
[b] Activity of their monomeric compounds.

3.52. Found: C, 69.91; H, 4.51; N, 3.55. The other compounds **5b**–**g** were also prepared by the similar procedure.

*6.2. 3-(4-Chlorophenyl)-2-[5-(3-3-(4-chlorophenyl)-5-[(Z)-1-(4-fluorophenyl)methylidene]-4-oxo-1,3-thiazolan-2-yl-4-methoxybenzyl)-2-methoxyphenyl]-5-[(Z)-1-(4-fluorophenyl) methylidene]-1,3-thiazolan-4-one (**5b**)*

This compound was obtained as dark yellow solid: m.p. 153–55 °C; IR (KBr): 3035, 2989, 1719, 1535, 1182, 747, 686 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.81 (s, 2H, CH_2), 4.12 (s, 6H, OCH_3), 6.61 (d, J = 9.1 Hz, 2H, ArH), 6.69 (s, 2H, ArH), 6.92 (s, 2H, CH–S), 7.12 (d, J = 9.1 Hz, 2H, ArH), 7.23–7.29 (m, 8H, ArH), 7.51 (d, J = 8.3 Hz, 4H, ArH), 7.63 (d, J = 8.6 Hz, 4H, ArH), 7.71 (s, 2H, CH=C); ^{13}C NMR (DMSO-d_6): δ 42.0, 56.1, 64.0, 112.7, 117.0, 122.7, 124.9, 126.6, 127.1, 128.9, 130.6, 131.7, 133.4, 134.5, 135.3, 137.9, 139.1, 154.0, 161.8, 165.7; MS: m/z 864 (M^+). *Anal. Calcd*. for $C_{47}H_{34}Cl_2F_2N_2O_4S_2$: C, 65.35; H, 3.97; N, 3.24. Found: C, 65.37; H, 3.91; N, 3.19.

*6.3. 5-[(Z)-1-(4-Fluorophenyl)methylidene]-2-(5-3-[5-[(Z)-1-(4-fluorophenyl)methylidene]-3-(4-nitrophenyl)-4-oxo-1,3-thiazolan-2-yl]-4-methoxybenzyl-2-methoxyphenyl)-3-(4-nitrophenyl)-1,3-thiazolan-4-one (**5c**)*

This compound was obtained as brown solid: m.p. 189–91 °C; IR (KBr): 3036, 2995, 1720, 1542, 1535, 1340, 1187, 684 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.80 (s, 2H, CH_2), 4.19 (s, 6H, OCH_3), 6.57–6.60 (4H, m, ArH), 6.92 (s, 2H, CH–S), 7.14 (d, J = 9.1 Hz, 2H, ArH), 7. 32 (d, J = 8.4 Hz, 4H, ArH), 7.47 (d, J = 8.4 Hz, 4H, ArH), 7.70–7.80 (m, 6H, ArH + C=CH), 8.18 (d, J = 8.7 Hz, 4H, ArH); ^{13}C NMR (DMSO-d_6): δ 42.0, 56.1, 64.0, 112.5, 117.1, 123.1, 125.1, 126.7, 128.0, 128.7, 129.3, 130.7, 134.5, 135.2, 137.8, 145.6, 146.3, 154.0, 161.7, 165.6; MS: m/z 884 (M^+). *Anal. Calcd*. for $C_{47}H_{34}F_2N_4O_8S_2$: C, 63.79; H, 3.87; N, 6.33. Found: C, 63.73; H, 3.89; N, 6.27.

*6.4. 5-[(Z)-1-(4-Fluorophenyl)methylidene]-2-(5-3-[5-[(Z)-1-(4-fluorophenyl)methylidene]-3-(4-methylphenyl)-4-oxo-1-3-thiazolan-2-yl]-4-methoxybenzyl-2-methoxyphenyl)-3-(4-methylphenyl)-1,3-thiazolan-4-one (**5d**)*

This compound was obtained as brown solid: m.p. 167–69 °C; IR (KBr): 3062, 2990, 1720, 1530, 1270, 685 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.10 (s, 6H, CH_3), 3.81 (s, 2H, CH_2), 4.10 (s, 6H, OCH_3), 6.61 (d, J = 9.1 Hz, 2H, ArH), 6.70 (s, 2H, ArH), 6.90–7.15 (m, 8H, ArH + CH–S), 7.50–7.55 (m, 8H, ArH), 7.27 (d, J = 8.3 Hz, 4H, ArH), 7.81 (s, 2H, CH=C); ^{13}C NMR (DMSO-d_6): δ 20.3, 42.0, 56.1, 64.0, 112.5, 117.1, 123.2, 125.0, 126.9, 128.6, 130.5, 131.9, 134.5, 135.7, 136.7, 137.8, 142.7, 154.0, 161.3, 165.4; MS: m/z 822 (M^+). *Anal. Calcd*. for $C_{49}H_{40}F_2N_2O_4S_2$: C, 71.51; H, 4.90; N, 3.40. Found: C, 71.46; H, 4.92; N, 3.34.

*6.5. 5-[(Z)-1-(4-Fluorophenyl)methylidene]-2-(5-3-[5-[(Z)-1-(4-fluorophenyl)methylidene]-3-(4-hydroxyphenyl)-4-oxo-1,3-thiazolan-2-yl]-4-methoxybenzyl-2-methoxyphenyl)-3-(4-hydroxyphenyl)-1,3-thiazolan-4-one (**5e**)*

This compound was obtained as brown solid: m.p. 171–73 °C; IR (KBr): 3410, 3035, 2962, 1722, 1530, 1271, 682 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.82 (s, 2H, CH_2), 4.12 (s, 6H, OCH_3), 5.07 (s, 2H, OH), 6.62 (d, J = 9.1 Hz, 2H, ArH), 6.70 (s, 2H, ArH), 6.80–6.92 (m, 6H, ArH + CH–S), 7.09 (d, J = 9.1 Hz, 2H, ArH), 7.30–7.40 (m, 8H, ArH), 7.45 (d, J = 8.3 Hz, 4H, ArH), 7.80 (s, 2H, CH=C); ^{13}C NMR (DMSO-d_6): δ 42.0, 56.1, 64.0, 112.6, 117.2, 119.1, 121.7, 122.6, 124.8, 126.5, 128.9, 130.0, 134.5, 135.2, 136.1, 137.9, 154.0, 157.1, 161.4, 165.5; MS: m/z 826 (M^+). *Anal. Calcd*. for $C_{47}H_{36}F_2N_2O_6S_2$: C, 68.27; H, 4.39; N, 3.39. Found: C, 68.24; H, 4.35; N, 3.44.

*6.6. 3-(4-Fluorophenyl)-2-[5-(3-3-(4-fluorophenyl)-5-[(Z)-1-(4-fluorophenyl)methylidene]-4-oxo-1,3-thiazolan-2-yl-4-methoxybenzyl)-2-methoxyphenyl]-5-[(Z)-1-(4-fluorophenyl) methylidene]-1,3-thiazolan-4-one (**5f**)*

This compound was obtained as gray solid: m.p. 181–83 °C; IR (KBr): 3065, 2992, 1720, 1530, 1270, 686 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.81 (s, 2H, CH_2), 4.11 (s, 6H, OCH_3), 6.60 (d, J = 9.0 Hz, 2H, ArH), 6.69 (s, 2H, ArH), 6.89 (s, 2H, CH–S), 7.10–7.15 (m, 6H, ArH), 7.22 (d, J = 8.7 Hz, 4H, ArH), 7.29 (d, J = 8.4 Hz, 4H, ArH), 7.57 (d, J = 8.4 Hz, 4H, ArH), 7.79 (s, 2H, CH=C); ^{13}C NMR (DMSO-d_6): δ 42.0, 56.1, 64.0, 112.0, 117.2, 118.1, 122.7, 124.6, 126.3, 129.0, 130.5, 130.7, 134.5, 135.6, 137.9, 138.1, 154.0, 159.3, 161.4, 165.7; MS: m/z 830 (M^+). *Anal. Calcd*. for $C_{47}H_{34}F_4N_2O_4S_2$: C, 67.90; H, 4.12; N, 3.37. Found: C, 67.84; H, 4.10; N, 3.40.

*6.7. 5-[(Z)-1-(4-Fluorophenyl)methylidene]-2-(5-3-[5-[(Z)-1-(4-fluorophenyl)methylidene]-3-(2-methylphenyl)-4-oxo-1, 3-thiazolan-2-yl]-4-methoxybenzyl-2-methoxyphenyl)-3-(2-methylphenyl)-1,3-thiazolan-4-one (**5g**)*

This compound was obtained as dark yellow solid: m.p. 157–59 °C; IR (KBr): 3062, 2967, 1722, 1532, 1269, 685 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.21 (s, 6H, CH_3), 3.81 (s, 2H, CH_2), 4.12 (s, 6H, OCH_3), 6.60 (d, J = 9.0 Hz, 2H, ArH), 6.69 (s, 2H, ArH), 6.90–7.15 (m, 8H, ArH + CH–S), 7.29 (d, J = 8.4 Hz, 4H, ArH), 7.47–7.52 (m, 6H, ArH), 7.79 (s, 2H, CH=C); ^{13}C NMR (DMSO-d_6): δ 18.7, 42.0, 56.1, 64.0, 113.0, 117.1, 122.9, 124.8, 126.3, 126.8, 127.0, 128.6, 129.8, 130.3, 131.9, 133.5, 134.5, 135.8, 137.8142.7, 154.0, 161.4, 165.3; MS: m/z 822 (M^+). *Anal. Calcd*. for $C_{49}H_{40}F_2N_2O_4S_2$: C, 71.51; H, 4.90; N, 3.40. Found: C, 7.46; H, 4.85; N, 3.39.

*6.8. 3-(4-Fluorophenyl)-5-(5-3-[3-(4-fluorophenyl)-6-phenyl-3,3a,5,6-tetrahydro[1,3]thiazolo[4,5-c]isoxazol-5-yl]-4-methoxybenzyl-2-methoxyphenyl)-6-phenyl-3,3a,5,6-tetrahydro [1,3]thiazolo[4,5-c]isoxazole (**6a**)*

A mixture of compound **5a** (0.01 mol), hydroxylamine hydrochloride (0.02 mol) and sodium acetate (0.01 mol) in anhydrous glacial acetic acid (20 mL), was refluxed for 8 h. The reaction mixture was concentrated and then poured into ice cold water, the solid thus separated, was filtered, washed with water and crystallized from ethanol to afford pure **6a** (87% yield) as brown solid: m.p. 179–81 °C; IR (KBr): 3065, 2970, 1600, 1562, 1470, 1270, 1065, 820 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.40 (s, 2H, CH_2), 4.17 (s, 6H, OCH_3), 4.69 (d, J = 2.2 Hz, 2H, S–CH), 5.70 (d, J = 2.2 Hz, 2H, CH–O), 6.64 (s, 2H, ArH), 6.75 (d, J = 9.1 Hz, 2H, ArH), 7.00 (d, J = 8.6 Hz, 4H, ArH), 7.10–7.19 (m, 12H, ArH), 7.32 (d, J = 8.6 Hz, 4H, ArH), 7.45 (s, 2H, CH–N); ^{13}C NMR (DMSO-d_6): δ 42.0, 56.0, 60.2, 67.1, 82.1, 111.3, 116.2, 123.1, 125.3, 126.5, 128.1, 128.7, 129.7, 130.1, 135.1, 136.7, 142.3, 146.7, 153.9, 165.0; MS: m/z 825 ($M^+ + 1$). *Anal. Calcd*. for $C_{47}H_{38}F_2N_4O_4S_2$: C, 68.43; H, 4.64; N, 6.79. Found: C, 68.40; H, 4.58; N, 6.70.

*6.9. 6-(4-Chlorophenyl)-5-(5-3-[6-(4-chlorophenyl)-3-(4-fluorophenyl)-3,3a,5,6-tetrahydro[1,3]thiazolo[4,5-c]isoxazol-5-yl]-4-methoxybenzyl-2-methoxyphenyl)-3-(4-fluorophenyl)-3,3a,5,6-tetrahydro[1,3]thiazolo[4,5-c]isoxazole (**6b**)*

This compound was obtained as dark yellow solid: m.p. 167–69 °C; IR (KBr): 3070, 2962, 1600, 1472, 1570, 1271, 1065, 820, 686 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.40 (s, 2H, CH_2), 4.17 (s, 6H, OCH_3), 4.69 (d, J = 2.2 Hz, 2H, S–CH), 5.70 (d, J = 2.2 Hz, 2H, CH–O), 6.64 (s,

2H, ArH), 6.75 (d, $J = 9.1$ Hz, 2H, ArH), 7.00 (d, $J = 8.6$ Hz, 4H, ArH), 7.10–7.19 (m, 6H, ArH), 7.34–7.40 (m, 8H, ArH), 7.45 (s, 2H, CH–N); ^{13}C NMR (DMSO-d_6): δ42.0, 56.0, 60.2, 67.2, 82.3, 111.1, 116.2, 123.2, 125.3, 128.1, 128.7, 129.6, 132.0, 132.4, 135.2, 136.7, 142.3, 146.7, 153.8, 165.0; MS: m/z 894 (M^+). *Anal. Calcd*. for $C_{47}H_{36}Cl_2F_2N_4O_4S_2$: C, 63.16; H, 4.06; N, 6.27. Found: C, 63.10; H, 4.10; N, 6.21.

*6.10. 3-(4-Fluorophenyl)-5-(5-3-[3-(4-fluorophenyl)-6-(4-nitrophenyl)-3,3a,5,6-tetrahydro[1,3]thiazolo[4,5-c]isoxazol-5-yl]-4-methoxybenzyl-2-methoxyphenyl)-6-(4-nitrophenyl)-3,3a,5,6-tetrahydro[1,3]thiazolo[4,5-c]isoxazole (**6c**)*

This compound was obtained as red solid: m.p. 210–11 °C; IR (KBr): 3065, 2990, 1570, 1600, 1472, 1340, 1271, 1046, 820 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 3.40 (s, 2H, CH_2), 4.17 (s, 6H, OCH_3), 4.69 (d, $J = 2.2$ Hz, 2H, S–CH), 5.70 (d, $J = 2.2$ Hz, 2H, CH–O), 6.64 (s, 2H, ArH), 6.75 (d, $J = 9.1$ Hz, 2H, ArH), 7.00 (d, $J = 8.6$ Hz, 4H, ArH), 7.21 (d, $J = 9.1$ Hz, 2H, ArH), 7.34–7.40 (m, 8H, ArH), 7.34 (d, $J = 8.6$ Hz, 4H, ArH), 7.45 (s, 2H, CH–N), 8.20 (d, $J = 9.1$ Hz, 4H, ArH); ^{13}C NMR (DMSO-d_6): δ42.0, 56.0, 60.2, 67.0, 82.0, 111.4, 116.4, 123.1, 125.3, 126.6, 127.3, 128.0, 128.7, 135.3, 136.8, 140.7, 146.0, 146.7, 154.0, 165.0; MS: m/z 915 ($M^+ + 1$). *Anal. Calcd*. for $C_{47}H_{36}F_2N_6O_8S_2$: C, 61.70; H, 3.97; N, 9.19. Found: C, 61.74; H, 3.92; N, 9.12.

*6.11. 3-(4-Fluorophenyl)-5-(5-3-[3-(4-fluorophenyl)-6-(4-methylphenyl)-3,3a,5,6-tetrahydro[1,3]thiazolo[4,5-c]isoxazol-5-yl]-4-methoxybenzyl-2-methoxyphenyl)-6-(4-methylphenyl)-3,3a,5,6-tetrahydro[1,3]thiazolo[4,5-c]isoxazole (**6d**)*

This compound was obtained as yellow solid: m.p. 152–54 °C; IR (KBr): 3062, 2991, 1600, 1472, 1570, 1271, 1045, 821 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.30 (s, 6H, CH_3), 3.40 (s, 2H, CH_2), 4.17 (s, 6H, OCH_3), 4.69 (d, $J = 2.2$ Hz, 2H, S–CH), 5.70 (d, $J = 2.2$ Hz, 2H, CH–O), 6.64 (s, 2H, ArH), 6.75 (d, $J = 9.1$ Hz, 2H, ArH), 7.00 (d, $J = 8.6$ Hz, 4H, ArH), 7.10–7.20 (m, 10H, ArH), 7.34 (d, $J = 8.6$ Hz, 4H, ArH), 7.45 (s, 2H, CH–N); ^{13}C NMR (DMSO-d_6): δ 22.0, 42.1, 56.0, 60.2, 67.1, 82.1, 111.4, 116.3, 123.2, 125.3, 128.3, 128.6, 129.7, 129.0, 135.2, 136.6, 137.4, 146.7, 143.8, 153.3, 165.1; MS: m/z 854 (M^+). *Anal. Calcd*. for $C_{49}H_{42}F_2N_4O_4S_2$: C, 69.00; H, 4.96; N, 6.57. Found: C, 68.94; H, 4.98; N, 6.51.

*6.12. 4-[3-(4-Fluorophenyl)-5-(5-3-[3-(4-fluorophenyl)-6-(4-hydroxyphenyl)-3,3a,5,6-tetrahydro[1,3]thiazolo[4,5-c] isoxazol-5-yl]-4-methoxybenzyl-2-methoxyphenyl)-3,3a,5,6-tetrahydro[1,3] thiazolo[4,5-c]isoxazol-6-yl]phenol (**6e**)*

This compound was obtained as brown solid: m.p. 164–66 °C; IR (KBr): 3410, 3062, 2970, 1600, 1472, 1570, 1269, 1042, 822 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 3.40 (s, 2H, CH_2), 4.17 (s, 6H, OCH_3), 4.69 (d, $J = 2.2$ Hz, 2H, S–CH), 5.10 (s, 2H, OH), 5.70 (d, $J = 2.2$ Hz, 2H, CH–O), 6.64 (s, 2H, ArH), 6.75 (d, $J = 9.1$ Hz, 2H, ArH), 6.80–7.00 (m, 12H, ArH), 7.19 (d, $J = 9.1$ Hz, 2H, ArH), 7.34 (d, $J = 8.6$ Hz, 4H, ArH), 7.45 (s, 2H, CH–N); ^{13}C NMR (DMSO-d_6): δ42.0, 56.0, 60.2, 67.2, 82.3, 111.3, 116.2, 117.3, 123.1, 125.3, 128.1, 128.4, 128.9, 135.2, 136.7, 137.8, 146.7, 154.0, 154.8, 165.1; MS: m/z 856 (M^+). *Anal. Calcd*. for $C_{47}H_{38}F_2N_4O_6S_2$: C, 65.87; H, 4.47; N, 6.54. Found: C, 65.90; H, 4.42; N, 6.50.

*6.13. 5-(5-3-[3,6-di(4-Fluorophenyl)-3,3a,5,6-tetrahydro [1,3] thiazolo[4,5-c]isoxazol-5-yl]-4-methoxybenzenzyl-2-methoxyphenyl)-3,6-di(4-fluorophenyl)-3,3a,5,6-tetrahydro-[1,3] thiazolo[4,5-c]isoxazole (**6f**)*

This compound was obtained as gray solid: m.p. 176–78 °C; IR (KBr): 3064, 2970, 1600, 1570, 1470, 1269, 1046, 821 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 3.40 (s, 2H, CH_2), 4.17 (s, 6H, OCH_3), 4.69 (d, $J = 2.2$ Hz, 2H, S–CH), 5.70 (d, $J = 2.2$ Hz, 2H, CH–O), 6.64 (s, 2H, ArH), 6.75 (d, $J = 9.1$ Hz, 2H, ArH), 6.90–7.00 (m, 4H, ArH), 7.19–7.22 (m, 6H, ArH), 7.34 (d, $J = 8.6$ Hz, 4H, ArH), 7.45 (s, 2H, CH–N); ^{13}C NMR (DMSO-d_6): δ 42.0, 56.0, 60.2, 67.0, 82.4, 111.2, 115.0, 116.1, 123.2, 125.3, 128.2, 128.9, 129.3, 135.1, 136.6, 141.4, 146.7, 153.2, 164.2, 165.2; MS: m/z 860 (M^+). *Anal. Calcd*. for $C_{47}H_{36}F_4N_4O_4S_2$: C, 65.57; H, 4.21; N, 6.51. Found: C, 65.60; H, 4.17; N, 6.45.

*6.14. 3-(4-Fluorophenyl)-5-(5-3-[3-(4-fluorophenyl)-6-(2-methylphenyl)-3,3a,5,6-tetrahydro[1,3]thiazolo[4,5-c]isoxazol-5-yl]-4-methoxybenzyl-2-methoxyphenyl)-6-(2-methylphenyl)-3,3a,5,6-tetrahydro[1,3]thiazolo[4,5-c]isoxazole (**6g**)*

This compound was obtained as brown solid: m.p. 156–58 °C; IR (KBr): 3062, 2990, 1600, 1570, 1470, 1271, 1040, 822 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.15 (s, 6H, CH_3), 3.40 (s, 2H, CH_2), 4.17 (s, 6H, OCH_3), 4.69 (d, $J = 2.2$ Hz, 2H, S–CH), 5.70 (d, $J = 2.2$ Hz, 2H, CH–O), 6.64 (s, 2H, ArH), 6.75 (d, $J = 9.1$ Hz, 2H, ArH), 6.90–7.00 (m, 4H, ArH), 7.10–7.20 (m, 8H, ArH), 7.34 (d, $J = 8.6$ Hz, 4H, ArH), 7.45 (s, 2H, CH–N); ^{13}C NMR (DMSO-d_6): δ 17.1, 42.0, 56.0, 60.2, 67.1, 82.1, 111.1, 116.3, 120.1, 123.1, 124.1, 125.4, 128.3, 128.7, 129.0, 129.7, 131.0, 135.2, 136.7, 139.6, 146.7, 153.1, 165.4; MS: m/z 854 (M^+). *Anal. Calcd*. for $C_{49}H_{42}F_2N_4O_4S_2$: C, 69.00; H, 4.96; N, 6.57. Found: C, 68.95; H, 4.91; N, 6.60.

6.15. Antifungal assay

The newly prepared compounds **6a**–**g** were screened for their antifungal activity against four fungal organisms viz. *C. albicans* (ATCC 10231), *A. fumigatus* (HIC 6094), *Trichophyton rubrum* (IFO 9185) and *T. mentagrophytes* (IFO 40996) by the broth dilution method. The *C. albicans* was grown for 48 h at 28 °C in YPD broth (1% yeast extract, 2% peptone, and 2% dextrose), harvested by centrifugation and then washed twice with sterile distilled water. *A. fumigatus*, *T. rubrum* and *T. mentagrophytes* were plated in potato dextrose agar (PDA) (Difco) and incubated at 28 °C for two weeks. Spores were washed three times with sterile distilled water and resuspended in distilled water to obtain an initial inoculum size of 10^5 spores/mL. Each test compound was dissolved in DMSO and diluted with potato dextrose broth (Difco) to prepare serial two-fold dilutions in the range 120–0.9 μM. Ten micro liters of the broth containing about 10^3 (for yeast) and 10^4 (for filamentous fungi) cells/mL of test fungi was added to each well of a 96-well microtiter plate. Culture plates were incubated for ~48–72 h at 28 °C. Minimum inhibitory concentration (MIC, μM) was measured and compared with controls; the MIC values of the compounds screened are presented in Table 1.

6.16. Nematicidal assay

The compounds synthesized **6a**–**g** was screened for their nematicidal activity against *D. myceliophagus* and *Caenorhabitis elegans* by aqueous *in vitro* screening technique. The *D. myceliophagus* was extracted form the cultivated mushrooms (Agaricus bisporus) infected with the nematode, and *C. elegans*, was grown on 10 cm 8P plates on an Na22 bacteria diet, which grow in a very thick layer and constitute an abundant food source for large quantities on nematode. The nematode water suspension was collected in Petri dishes. Suspension of adult worms from five day old culture was diluted with approximately 100–250 nematodes/mL of water. 100 μL of the nematode suspension was introduced into a solution of each test compound at various concentrations in a well of 24-well plates and incubated at 25 °C. The percentage of immobile

nematodes was recorded after two days. The LD_{50} values of the compounds screened are presented in Table 1.

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References

[1] S. Andrew, M. Susan, F. Michael, W. Matthew, L. Penny, L. Paul, G. Anne, B. Chris, M. John, T. Donald, S. Dennis, B. Tesfaye, Bioorg. Med. Chem. Lett. 18 (2008) 5263.
[2] S. Onca, M. Punar, H. Erakosy, Chemotherapy 50 (2004) 98.
[3] C.V. Dave, M.C. Shukla, Indian J. Chem. 39B (2000) 210.
[4] M.N. Ghazzi, J.E. Perez, T.K. Antonucci, J.H. Driscoll, S.M. Huang, B.W. Faja, Diabetes 46 (1997) 433.
[5] U. Scmidt, R. Utz, A. Lieberknecht, H. Griesser, B. Potzolli, J. Bahr, K. Wagner, P. Fischer, Synthesis (1987) 233.
[6] M.V. Diurno, O. Mazzoni, A.A. Izzo, A. Bolognese, Il Farmaco 52 (1997) 237.
[7] N. Ergene, G. Capan, Il Farmaco 49 (1994) 449.
[8] J.S. Viswajanani, S. Ajay, S. Smita, K. Seema, P. Manisha, B. Pragya, M. Tarun, R. Ashok, K. Jag Mohan, M. Anitha, ARKIVOC (2005) 46.
[9] H. Ueno, T. Oe, I. Snehiro, S. Nakamura, US Patent, 1997, 5594116.
[10] T. Previtera, M.G. Vigorita, M. Bisila, F. Orsini, F. Benetolla, G. Bombieri, Eur. J. Med. Chem. 29 (1994) 317.
[11] M.Y. Ebeid, O.A. Fathallah, M.I. El-Zaher, M.M. Kamel, W.A. Abdon, M.M. Anwar, Bull. Fac. Pharm. 34 (1996) 125.
[12] R.K. Rawal, Y.S. Prabhakar, S.B. Katti, E. De Clercq, Bioorg. Med. Chem. 13 (2005) 6771.
[13] T. Kato, T. Ozaki, K. Tamura, J. Med. Chem. 42 (1999) 3134.
[14] Y. Tanabe, G. Suzukamo, Y. Komuro, N. Imanishi, S. Morooka, M. Enomoto, A. Kojima, Y. Sanemitsu, M. Mizutani, Tetrahedron Lett. 32 (1991) 379.
[15] T. Kato, T. Ozaki, N. Ohi, Tetrahedron Asymmetry 10 (1999) 3963.
[16] Y. Adachi, Y. Suzuki, N. Homma, M. Fukazawa, K. Tamura, I. Nishie, O. Kuromaru, Eur. J. Pharmacol. 367 (1999) 267.
[17] R. Ottana, E. Mazzon, L. Dugo, F. Monforte, R. Maccari, L. Sautebin, G. De Luca, M.G. Vigorita, S. Alcaro, F. Ortuso, Eur. J. Pharmacol. 448 (2002) 71.
[18] Y. Tanabe, H. Yamamoto, M. Murakami, K. Yanagi, Y. Kubota, H. Okumura, Y. Sanemitsu, G. Suzukamo, J. Chem. Soc., Perkin Trans. 1 7 (1995) 935.
[19] Y. Kato, Y. Kita, M. Nishio, Y. Hirasawa, K. Ito, T. Yamanaka, Y. Motoyama, J. Seki, Eur. J. Pharmacol 384 (1999) 197.
[20] M.E. Voss, P.H. Carter, A.J. Tebben, P.A. Scherle, G.D. Brown, L.A. Thampson, M. Xu, Y.C. Lo, R.R.Q. Yang-Liu, Bioorg. Med. Chem. Lett. 13 (2003) 533.
[21] J.T. Desai, C.K. Desai, K.R. Desai, J. Iran Chem. Soc. 5 (2008) 67.
[22] N. Rajeshwar, P. Marcus, L. Stefanie, B. Karlheinz, K. Sabine, D. Thomas, W. Sascha, M. Eckhard, S. Boris, Chem. Med. Chem. (2007) 1.
[23] Y.C. Sung, H.A. Jin, D.H. Jae, K.K. Seung, Y.B. Ji, S.H. Sang, Y.S. Eun, S.K. Sung, R. K. Kwang, G.C. Hyae, K.C. Joong, Bull. Kor. Chem. Soc. 24 (2003) 1455.
[24] Y.S. Lee, S.M. Park, B.H. Kim, Bioorg. Med. Chem. Lett. 19 (2009) 1126.
[25] J.F. Hansen, S.A. Stronge, J. Heterocycl. Chem. 14 (1977) 1289.
[26] T.K. Adhikari, A. Vasudeva, M. Girisha, Indian J. Chem. 48B (2009) 430.
[27] S. Balalaie, A. Sharifi, B. Ahangarian, Indian J. Heterocycl. Chem. 10 (2000) 149.
[28] H. Kai, T. Ichiba, M. Tomida, H. Nakai, K. Morita, J. Pest. Sci. 25 (2000) 267.
[29] V.V. Kachhadia, M.R. Patel, H.S. Joshi, J. Sci. I. R. Iran 15 (2004) 47.
[30] M. Marcin, Z. Michal, D.S. Ewa, R. Stanislaw, Cell. Mol. Biol. Lett. 10 (2005) 613.
[31] N.R. Nagar, V.H. Shan, Indian J. Heterocycl. Chem. 13 (2003) 173.
[32] U.L.B. Jayasinghe, B.M.M. Kumarihamy, A.G.D. Bandara, E.A. Vasquez, W. Kraus, Nat. Prod. Res. 17 (2003) 259.
[33] M.H. Xaki, D. Moran, D. Harris, Am. J. Public Health 72 (1982) 1391.
[34] J.W. Noling, J.O. Becker, J. Nematol. 26 (1994) 573.
[35] O. Yuji, N. Sengual, P. Eli, R. Uzi, Y. Zohara, S. Yitzhak, Phytopathalogy 90 (2000) 710.
[36] J. Kagan, P.A. Kagan, H.E. Buhse Jr., J. Chem. Ecol. 10 (1984) 1115.
[37] (a) Ch. Sanjeeva Reddy, G. Smitha, Synth. Commun. 21 (2004) 3997;
(b) Ch. Sanjeeva Reddy, G. Smitha, Synth. Commun. 36 (2006) 1795;
(c) Ch. Sanjeeva Reddy, A. Nagaraj, Heterocycl. Commun. 13 (2007) 67;
(d) Ch. Sanjeeva Reddy, A. Nagaraj, Chin. Chem. Lett. 18 (2007) 1431;
(e) Ch. Sanjeeva Reddy, A. Nagaraj, P. Jalapathi, Chin. Chem. Lett. 18 (2007) 1213;
(f) Ch. Sanjeeva Reddy, A. Nagaraj, J. Heterocycl. Chem. 44 (2007) 1357;
(g) Ch. Sanjeeva Reddy, A. Nagaraj, J. Heterocycl. Chem. 44 (2007) 1181;
(h) Ch. Sanjeeva Reddy, A. Nagaraj, Indian J. Chem. 47B (2008) 319;
(i) Ch. Sanjeeva Reddy, A. Nagaraj, J. Iran. Chem. Soc. 5 (2008) 262;
(j) Ch. Sanjeeva Reddy, A. Srinivas, A. Nagaraj, Indian J. Chem. 47B (2008) 787;
(k) Ch. Sanjeeva Reddy, A. Srinivas, A. Nagaraj, J. Heterocycl. Chem. 45 (2008) 999;
(l) Ch. Sanjeeva Reddy, A. Srinivas, A. Nagaraj, J. Heterocycl. Chem. 45 (2008) 1121;
(m) Ch. Sanjeeva Reddy, M. Raghu, A. Nagaraj, J. Heterocycl. Chem. 45 (2008) 1115;
(n) Ch. Sanjeeva Reddy, A. Nagaraj, Indian J. Chem. 47B (2008) 1154.
[38] C.S. Marvel, N. Tarkoy, J. Am. Chem. Soc. 79 (1957) 6000.
[39] National Committee for Clinical Laboratory Standards (NCCLS), Standard Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria, Which Grows Aerobically. Nat. Comm. Lab. Stands, Villanova, 1982, 242.
[40] C.W. McBeth, G.B. Bergeson, Phytopathology 43 (1953) 264.