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Short Communication

Synthesis and antimicrobial study of novel heterocyclic compounds from hydroxybenzophenones

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Abstract

The triazolothiadiazine analogues **6a–e** were obtained via a multistep synthesis sequences beginning with the hydroxybenzophenones **1a–e**. Hydroxybenzophenones on reaction with ethyl chloroacetate affords ethyl (2-aroylaryloxy)acetates **2a–e** which on treatment with hydrazine hydrate yields 2-(2-aroylaryloxy)acetohydrazides **3a–e.** Intramolecular cyclization of **3a–e** with carbon disulfide affords 5-(2-aroylaryloxy)methyl-1,3,4-oxadiazole-2-(3H)thiones **4a–e**, which on treatment with hydrazine hydrate yields 4-amino-5-(2-aroyl aryloxy)methyl-1,2,4-triazole-3-(2H)thiones **5a–e.** Condensation of **5a–e** with α-halocarbonyl compound results in 3-(2-aroylaryloxy)methyl-6-phenyl-1,2,4-triazolo[3,4-b][1,3,4] thiadiazine **6a–e** analogues. The compounds **4a–e**, **5a–e** and **6a–e** were tested against variety of fungal and bacterial strains in comparison to fluconazole and chloramphenicol, respectively.

Keywords: 1,3,4-Oxadiazole-2-(3H)thiones; 1,2,4-Triazole-3-(2H)thiones; 1,2,4-Triazolo thiadiazines; Synthesis; Antimicrobial activity

1. Introduction

Over the past several years the emergence of organisms resistant to nearly all the class of antimicrobial agents has become a serious public health concern [1,2]. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. [3].

Since past two decades there has been significant increase in the frequency of systematic fungal infection in man. The first orally active antifungal agent that was effective against a broad array of systematic and superficial fungal infections was ketoconazole [4]. Further a number of azole antifungal agents viz., itraconazole [5], fluconazole [6], voriconazole [7], ravuconazole [8] etc., and glucan synthesis inhibitor caspofungin [9] have been introduced to the clinic. Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses not only because many of them produce toxic reactions but also due to emergence of drug resistant bacteria. It is essential to investigate newer drugs with lesser resistance [10].

During the past years extensive evidences have been accumulated to establish the efficiency of benzophenone analogues as antimicrobial agent [11–14]. Benzophenone analogue (garcinol) has been isolated from the stem bark of *Garcinia huillensis* grown in Zaire and used in central-African traditional medicine and this has been shown to exhibit chemotherapeutical activity against gram-positive and gram-negative *cocci*, mycobacteria and fungi [15]. Recently Selvi et al have shown antifungal activity of benzophenone analogues, at its lower concentration [16]. Besides chloro substituted benzophenones have exhibited more antifungal activity [17]. Moreover, a large number of oxadiazoles [18,19], triazoles [20] and triazolothiadiazines [21] have been shown to exhibit significant antimicrobial activity against *S. aureus, C. albicans, C. krusei, C. parapsilosis, T. paradoxa, E. Coli, B. subtilis* and *P. aeruginosa*. These initial reports, thereafter stimulated us to integrate 1,3,4-oxadiazole-2-(3H)thione and

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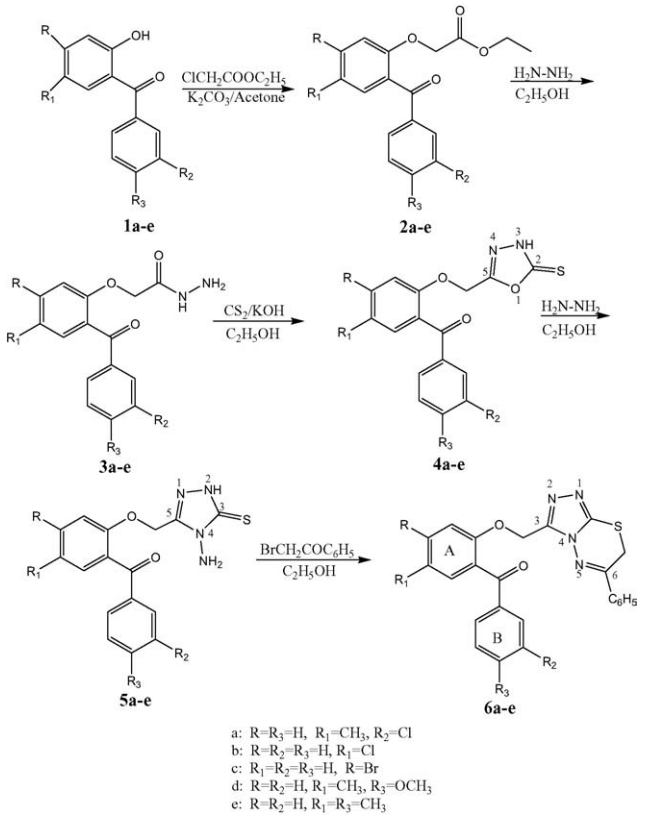

Scheme 1.

triazolothiadiazine moieties in benzophenone framework, since these systems possess well documented antimicrobial activity.

2. Chemistry

The synthesis of the hitherto unreported title compounds is as outlined in Scheme 1 in 70% yield. Hydroxybenzophenones **1a–e** on reaction with ethyl chloroacetate affords ethyl (2-aroylaryloxy)acetates **2a–e** in excellent yield [22–25], which on treatment with hydrazine hydrate yields corresponding 2-(2-aroylaryloxy)acetohydrazides **3a–e** [24,25]. Intramolecular cyclization of **3a–e** with carbon disulfide resulted 5-(2-aroylaryloxy)methyl-1,3,4-oxadiazole-2-(3H)thiones **4a–e** [19,26]. Compounds **4a–e** were further treated with hydrazine hydrate to obtain compounds 4-amino-5-(2-aroylaryloxy)methyl-1,2,4-triazole-3-(2H)thiones **5a–e** [23,25]. The preparations of novel 3-(2-aroylaryloxy)methyl-6-phenyl-1,2,4-triazolo[3,4-b][1,3,4] thiadiazine **6a–e** were achieved from **5a–e** with phenacyl bromide [21].

3. Results and discussion

The structures of the compounds were elucidated by IR, NMR and microanalyses. The IR spectra of 2-(2-aroylaryloxy)acetohydrazides **3a–e** have amide C=O and NH_2 stretching bands at 1655–1670 and 3110–3223 cm^{-1}, respectively. The disappearance of amide C=O and NH_2 stretching bands of **3a–e** and detection of strong C–O–C, C=S, and C=N stretching bands at about 1130–1137, 1235–1245 and 1610–1647 cm^{-1}, respectively, are evidences for ring closure of 1,3,4-oxadiazoles-2(3H) thiones **4a–e.** The disappearance of C–O–C stretching bands of **4a–e** and detection of strong NH_2 bands at 3400–3412 cm^{-1} are evidences for conversion of **4a–e** to 4-amino-5-(2-aroylaryloxy)methyl-1,2,4-triazole-3-(2H)thiones **5a–e**. Similarly disappearance of NH_2 stretching bands of **5a–e** and detection of strong C–S–C bands at 750–760 cm^{-1} are evidences for conversion of **5a–e** to triazolothiadiazine analogues **6a–e.** In ^1H NMR spectra, all protons were seen according to the expected chemical shift and integral values. Aromatic methyl, phenoxymethyl and aromatic ring protons were seen at 2.0–2.32, 4.42–4.85 and 6.65–7.8 ppm. The NH protons of **4a–e** and **5a–e** were seen at about 9.0–9.15 ppm, respectively, and the NH_2 protons of **5a–e** were seen at 3.7–3.73 ppm. The S-CH_2 protons of **6a–e** were seen at 3.2–3.23 ppm. The results of microanalyses were within ± 0.4% error.

The antimicrobial activities of compounds **4a–e**, **5a–e** and **6a–e** were evaluated in vitro against some fungi such as *C. albicans, C. krusei, C. parapsilosis, A. flavus, A. ochraceous, F. moniliforme* and *C. gloeosporioides*. Bacteria such as *E. coli, P. solanacearum, P. fluorescens* and *B. subtilis* were evaluated by serial tube dilution technique and the results are summarized in Tables 1 and 2. The antifungal screening results have shown that the halo substituted compounds **4a**, **4b**, **5a**, **5b**, **5c**, **6a**, **6b** and **6c** exhibit in general growth inhibitory activity more relevant than that of the reference fluconazole against *F. moniliforme* and *C. gloeosporioides*. It is worth noting that when chloro group is at meta position in ring B as in compounds **4a** and **6a** have shown more activity than fluconazole against *A. ochraceous*. Besides, when chloro group is at para position in ring A as in compounds **4b**, **5b** and **6b** and at meta position in ring B as in compounds **4a**, **5a** and **6a**, also when bromo group is at meta position in ring A as in compounds **5c** and **6c**, have shown more activity compared to fluconazole against *F. moniliforme*. Nevertheless, with *A. flavus* only bromo and chloro substituted compounds **5c** and **6b**, respectively, have exhibited more activity than fluconazole. This is an example, which shows how the biological properties are influenced by even minor structural modifications. With *C. albicans* chloro substituted compound **5b** and with *C. krusei* chloro compounds **5a**, **5b** and **6b** have shown more activity compared to fluconazole. Correspondingly, with *C. gloeosporioides* chloro compounds **4a**, **4b**, **5a**, **5b**, **6a**, and **6b**, bromo compounds **5c** and **6c** and methyl substituted compounds **5e** and **6e** have shown more activity compared to standard drug. On the contrary, with *C. parapsilosis* all compounds have shown less antifungal activity compared to fluconazole.

In case of antibacterial activity with few exceptional cases all the compounds have shown higher activity compared to chloramphenicol against *E. coli, P. solanacearum, P. fluorescens* and *B. subtilis*. It is worth noting that compound **5e**

Table 1
The minimum inhibitory concentration (MIC)[a] of **4a–e**, **5a–e** and **6a–e** for antifungal activity

Compounds	Tested fungi (MIC in mM)						
	C. albicans	*C. krusei*	*C. parapsilosis*	*A. flavus*	*A. ochraceous*	*F. moniliforme*	*C. gloeosporioides*
4a	7.0	6.0	11.5	12.0	1.0	4.5	2.5
4b	6.0	5.5	9.5	6.5	10.0	1.5	1.0
4c	3.0	7.0	9.5	8.0	6.5	8.5	9.5
4d	8.0	8.0	11.0	9.0	4.0	11.5	10.0
4e	12.0	12.0	11.5	7.5	3.5	8.5	6.5
5a	3.0	4.0	6.0	7.5	10.5	2.0	1.5
5b	2.5	3.0	5.0	11.0	11.0	4.0	2.0
5c	4.0	5.5	8.0	1.0	12.0	5.0	3.0
5d	7.0	5.0	9.5	2.0	6.5	8.0	6.5
5e	10.5	10.5	5.5	6.0	5.0	6.0	6.0
6a	3.0	5.0	11.5	9.0	1.0	4.5	2.5
6b	3.0	4.5	9.0	1.0	7.0	1.0	1.5
6c	5.5	5.5	8.5	10.0	7.5	5.5	3.5
6d	8.0	6.5	8.0	7.0	12.0	11.0	12.0
6e	11.5	5.5	11.5	6.5	9.5	6.5	3.0
Fluconazole	3.0	5.0	2.5	2.0	2.0	6.0	6.5

[a] Average of at least three determinations.

Table 2
The MIC[a] of **4a–e**, **5a–e** and **6a–e** for antibacterial activity

Compounds	Tested bacteria (MIC in mM)			
	E. coli	*P. solanacearum*	*P. fluorescens*	*B. subtilis*
4a	2.0	12.0	8.0	2.5
4b	4.5	2.5	2.0	1.5
4c	3.0	5.5	5.5	4.5
4d	4.0	3.5	6.0	6.5
4e	5.0	4.0	6.5	5.5
5a	6.0	2.5	2.5	0.5
5b	3.0	7.5	1.5	2.5
5c	0.5	6.5	11.5	11.0
5d	4.5	3.0	3.5	0.5
5e	11.5	12.0	1.0	8.0
6a	0.5	4.0	1.0	3.0
6b	2.5	1.0	8.0	3.0
6c	5.5	11.0	3.5	4.0
6d	4.0	3.5	3.5	1.0
6e	2.0	1.0	1.0	3.5
Chloramphenicol	10.5	7.0	10.0	5.0

[a] Average of at least three determinations.

with two methyl groups at para position in ring A and B has shown lesser activity compared to other substituents against *Escherichia coli.* Whereas chloro substituted compounds **4a** and **5b**, bromo substituted compound **6c** and methyl substituted compound **5e**, exhibit lesser activity compared to other compounds against *P. solanacearum.* Nevertheless with *P. fluorescens* strain bromo compound **5c** has shown lesser activity. Besides with *B. subtilis* compounds with two methyl groups **4e** and **5e**, with methyl and methoxy groups **4d** and with bromo group **5c** have shown lesser activity compared to the other compounds. In general these compounds are found to possess more antibacterial activity than antifungal activity.

4. Conclusion

In conclusion our study shows a strong evidence for the antimicrobial activity of halo substituted 1,3,4-oxadiazole-2-(3H)thiones, 1,2,4-triazole-3-thiones and triazolothiadiazines linked benzophenones. It is interesting and significant to note from the antifungal data in Table 1 that one or the other chloro substituted compounds exhibit in general growth inhibitory activity more relevant than that of the reference compound against the strains. Besides, compounds with methyl and methoxy groups in ring A and B, respectively, in general exhibited lower growth inhibitory activity when compared to reference compound against all the strains. It is interesting to note from the antibacterial data in Table 2 that all the compounds have shown higher activity compared to reference compound against all the strains with few exceptional cases. In general, compound **5e** with two methyl groups in ring A and B has exhibit lower growth inhibitory activity compared to reference compound against all the strains except with *P. fluorescens*. One can conclude that these compounds posses more antibacterial activity than antifungal activity. In retrospect, there exists a bright prospect for the discovery of many a new drug for the treatment of antibacterial and antifungal activity.

5. Experimental

5.1. Chemistry

TLC was performed on aluminum-backed silica plated with visualization by UV-light. IR spectra were determined with a FT IR Shimadzu 8300 spectrophotometer using a potassium bromide wafer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 300 and 100 MHz, respectively. Chemical shifts are in ppm relative to internal TMS. Melting points were determined on a Thomas Hoover capillary melting point apparatus with a digital thermometer.

*5.1.1. Synthesis of ethyl [2-(3-chlorobenzoyl)-4-methylphenoxy]acetate (**2a**)*

A mixture of **1a** (5 g, 0.02 mol), ethyl chloroacetate (2.4 g, 0.02 mol) in dry acetone (60 ml) and anhydrous potassium carbonate (2.8 g, 0.02 mol) was refluxed for 8 h then cooled and the solvent removed under reduced pressure. The residual mass was triturated with ice water to remove potassium carbonate and extracted with ether (3 × 50 ml) and the ether layer was washed with 10% sodium hydroxide solution (3 × 30 ml) followed by water (3 × 30 ml) and then dried over anhydrous sodium sulfate and evaporated to dryness to get crude solid, which on recrystallization with ethanol gave **2a** (5.39 g, 80%) as white flakes.

2a: M.p. 60–62 °C; IR (KBr): 1670 (C=O), 1735 cm^{-1} (ester, C=O); ^1H NMR ($CDCl_3$): δ 1.2 (t, J = 7 Hz, 3H, CH_3 of ester), 2.3 (s, 3H, CH_3), 4.2 (q, J = 6 Hz, 2H, CH_2 of ester), 4.45 (s, 2H, CH_2), 7.2–7.6 (m, 7H, Ar-H); ^{13}C NMR ($CDCl_3$): δ 13.6 (q), 20.9 (q), 59.5 (t), 75.6 (t), 113.7 (d), 123.3 (s), 128.2 (d), 129.61 (d), 129.7 (s), 130.5 (d), 131.8 (d), 132.6 (d), 133.5 (s), 139.2 (s). 133.9 (d), 160.6 (s), 171.0 (s), 187.0 (s). Anal. Calcd. for $C_{18}H_{17}O_4Cl$ (332.5): C, 64.96; H, 5.11; Cl, 10.67. Found: C, 64.94; H, 5.07; Cl, 10.64%.

2b: Oily product[23]; IR (KBr): 1672 (C=O), 1738 cm^{-1} (ester, C=O); ^1H NMR ($CDCl_3$): δ 1.21 (t, J = 7 Hz, 3H, CH_3 of ester), 4.23 (q, J = 6 Hz, 2H, CH_2 of ester), 4.5 (s, 2H, CH_2), 7.2–7.75 (m, 8H, Ar-H); ^{13}C NMR ($CDCl_3$): δ 13.62 (q), 59.52 (t), 75.61 (t), 115.2 (d), 124.8 (s), 125.8 (s), 128.22 (d), 130.1 (d), 131.5 (d), 132.21 (d), 133.6 (d), 137.8 (s), 161.7 (s), 171.8 (s), 187.03 (s). Anal. Calcd. for $C_{17}H_{15}ClO_4$ (318.5): C, 64.05; H, 4.70; Cl, 11.14. Found: C, 64.02; H, 4.67; Cl, 11.11%.

2c: M.p. 69–71 °C; IR (KBr): 1672 (C=O), 1736 cm^{-1} (ester, C=O); ^1H NMR ($CDCl_3$): δ 1.2 (t, J = 7 Hz, 3H, CH_3 of ester), 4.22 (q, J = 6 Hz, 2H, CH_2 of ester), 4.45 (s, 2H, CH_2), 7.22–7.8 (m, 8H, Ar-H); ^{13}C NMR ($CDCl_3$): δ 13.61 (q), 59.51 (t), 75.61 (t), 117.1 (d), 122.4(s), 123.8 (d), 127.8 (s), 128.21 (d), 130.1 (d), 132.2 (d), 133.3 (d), 137.8 (s), 165.8 (s), 171.0 (s), 187.03 (s). Anal. Calcd. for $C_{17}H_{15}BrO_4$ (363): C, 56.19; H, 4.13; Br, 22.03. Found: C, 56.17; H, 4.10; Br, 22.0%.

2d: M.p. 58–60 °C; IR (KBr): 1660 (C=O), 1730 cm^{-1} (ester, C=O); ^1H NMR ($CDCl_3$): δ 1.2 (t, J = 7 Hz, 3H, CH_3 of ester), 2.25 (s, 3H, CH_3), 3.8 (s, 3H, OCH_3), 4.2 (q, J = 6 Hz, 2H, CH_2 of ester), 4.42 (s, 2H, CH_2), 7.0–7.6 (m, 7H, Ar-H); ^{13}C NMR ($CDCl_3$): δ 13.63 (q), 20.92 (q), 56.0 (q), 59.53 (t), 75.6 (t), 113.71 (d), 113.8 (d), 123.32 (s), 129.7 (s), 130.1 (s), 131.1 (d), 131.81 (d), 133.91 (d), 160.62 (s), 165.7 (s), 171.02 (s), 187.04 (s). Anal. Calcd. for $C_{19}H_{20}O_5$ (328): C, 69.51; H, 6.09. Found: C, 69.49; H, 6.05%.

2e: M.p. 57–59 °C IR (KBr): 1740 (ester, C=O), 1665 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 1.2 (t, J = 7 Hz, 3H, CH_3 of ester), 2.3–2.35 (d, J = 7 Hz, 6H, 2Ar-CH_3), 4.25 (q, J = 6 Hz, 2H, CH_2 of ester), 4.45 (s, 2H, OCH_2), 7.2–7.8 (bm, 7H, Ar-H); ^{13}C NMR ($CDCl_3$): δ 13.61 (q), 20.92 (q), 59.51 (t), 75.61 (t), 113.71 (d), 123.31 (s), 128.9 (d), 129.7 (s), 130.0 (d), 131.8 (d), 133.9 (d), 134.8 (s), 141.4 (s), 160.61 (s), 171.01 (s), 187.02 (s). Anal. Calcd. for: $C_{19}H_{20}O_4$: C, 73.07; H, 6.41. Found: C, 73.04; H, 6.38%.

*5.1.2. Synthesis of 2-[2-(3-chlorobenzoyl)-4-methylphenoxy]acetohydrazide (**3a**)*

To **2a** (2 g, 6 mmol) in methanol (10 ml), 80% hydrazine hydrate (0.3 g, 6 mmol) was added in drops and stirred for 1 h at room temperature. A white solid separated, which on recrystallization with ethanol gave **3a** (1.43 g, 75%) as white needles.

3a: M.p. 177–180 °C; IR (KBr): 1620 (C=O), 1655 (amide, C=O), 3110–3215 cm^{-1} (NH–NH_2); ^1H NMR ($CDCl_3$): δ 2.3 (s, 3H, CH_3), 3.7 (bs, 2H, NH_2), 4.5 (s, 2H, CH_2), 7.1–7.6 (m, 7H, Ar-H), 9.25 (bs, 1H, CONH); ^{13}C NMR ($CDCl_3$): δ 20.91 (q), 78.0 (t), 113.7 (d), 123.3 (s), 128.21 (d), 129.62 (d), 129.7 (s), 130.5 (d), 131.8 (d), 132.6 (d), 133.51 (s), 133.9 (d), 139.2 (d), 160.6 (s), 170.3 (s), 187.0 (s). Anal. Calcd. for $C_{16}H_{15}ClN_2O_3$ (318.5): C, 60.28; H, 4.70; Cl, 11.14; N, 8.79. Found: C, 60.24; H, 4.67; Cl, 11.10; N, 8.75%.

3b: M.p. 181–183 °C; IR (KBr): 1630 (C=O), 1668 (amide, C=O), 3120–3223 cm^{-1} (NH–NH_2); ^1H NMR ($CDCl_3$): δ 3.72 (bs, 2H, NH_2), 4.55 (s, 2H, CH_2), 7.15–7.75 (m, 8H, Ar-H), 9.35 (bs, 1H, CONH); ^{13}C NMR ($CDCl_3$): δ 78.01 (t), 115.2 (d), 124.8 (s), 125.8 (s), 128.21 (d), 130.1 (d), 131.51 (d), 132.62 (d), 133.61 (d), 137.81 (s), 161.7 (s), 170.31 (s), 187.03 (s). Anal. Cal. for $C_{15}H_{13}ClN_2O_3$ (304.5): C, 59.11; H, 4.26; Cl, 11.65; N, 9.19. Found: C, 59.12; H, 4.24; Cl, 11.67; N, 9.17%.

3c: M.p. 182–185 °C; IR (KBr): 1625 (C=O), 1670 (amide, C=O), 3115–3220 cm^{-1} (NH–NH_2); ^1H NMR ($CDCl_3$): δ 3.72 (bs, 2H, NH_2), 4.53 (s, 2H, CH_2), 7.15–7.76 (m, 8H, Ar-H), 9.35 (bs, 1H, CONH); ^{13}C NMR ($CDCl_3$): δ 78.01 (t), 117.1 (d), 122.4 (s), 123.8 (d), 127.8 (s), 128.2 (d), 130.1 (d), 132.2 (d), 133.3 (d), 137.8 (s), 165.8 (s), 170.3 (s), 187.03 (s). Anal. Cal. for $C_{15}H_{13}BrN_2O_3$(349): C, 51.57; H, 3.72; Br, 23.92; N, 8.02. Found: C, 51.54; H, 3.70; Br, 23.89; N, 8.0%.

3d: M.p. 175–177 °C; IR (KBr): 1610 (C=O), 1645 (amide, C=O), 3100–3205 cm^{-1} (NH–NH_2); ^1H NMR ($CDCl_3$): δ 2.2 (s, 3H, CH_3), 3.5 (bs, 2H, NH_2), 3.9 (s, 3H, OCH_3), 4.55 (s, 2H, CH_2), 7.2–7.9 (m, 7H, Ar-H), 9.4 (bs, 1H, CONH); ^{13}C NMR ($CDCl_3$): δ 20.92 (q), 56.0 (q), 78.02 (t), 113.72 (d), 113.82 (d), 129.6 (d), 129.72 (s), 130.1 (s), 131.1 (d), 131.8 (d), 133.9 (d), 160.62 (s), 165.7 (s), 170.3 (s), 187.04 (s). Anal. Cal. for $C_{17}H_{18}N_2O_4$(314): C, 64.96; H, 5.73; N, 8.91. Found: C, 64.94; H, 5.70; N, 8.89%.

3e: M.p. 182–85 °C; IR (KBr): 1630 (C=O), 1670 (amide, C=O), 3120–3220 cm^{-1} (NH–NH_2); ^1H NMR ($CDCl_3$): δ 2.2–2.3 (d, J = 7 Hz, 6H, 2CH_3), 3.55 (bs, 2H, NH_2), 4.6 (s, 2H, CH_2), 7.2–7.8 (m, 7H, Ar-H), 9.35 (bs, 1H, CONH); ^{13}C NMR ($CDCl_3$): δ 20.91 (q), 78.01 (t), 113.71 (d), 123.3 (s), 128.9 (d), 129.7 (s), 130.0 (d), 131.8 (d), 133.9 (d), 134.8 (s), 141.4 (s), 160.61 (s), 170.31 (s), 187.01 (s). Anal. Cal. for $C_{17}H_{18}N_2O_3$: C, 68.41; H, 6.0; N, 9.35. Found: C, 68.45; H, 6.04; N, 9.39%.

*5.1.3. Synthesis of 5-[2-(3-chlorobenzoyl)-4-methyl-phenoxymethyl] 1,3,4-oxadiazole-2- (3H)thione (**4a**)*

To a 0 °C solution of **3a** (1 g, 3.13 mmol) and carbon disulfide (0.47 g, 6.27 mmol) in absolute ethanol (15 ml), potassium hydroxide (0.20 g, 3.13 mmol) was added in one portion. The resulting mixture was stirred and refluxed for 8 h. The solvent was removed in vacuo and the residue was acidified with 2 M hydrochloric acid and extracted with ethyl acetate (2 × 20 ml). Organic layers were washed with water and dried with anhydrous sodium sulfate. Filtration and concentration in vacuo gave a solid, which was recrystallized from ethanol to give **4a** (0.75 g, 72%) as yellow solid.

4a: M.p. 112–114 °C; IR (KBr): 1130 (C–O–C linkage), 1235 (C=S), 1610 (C=N), 1640 (C=O), 3310 cm^{-1} (N–H); 1H NMR ($CDCl_3$): δ 2.31 (s, 3H, CH_3), 4.51 (s, 2H, CH_2), 6.8–7.5 (m, 7H, Ar-H), 9.0 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 20.91 (q), 78.02 (t), 113.7 (d), 123.3 (s), 128.21 (d), 129.62 (d), 129.7 (s), 130.5 (d), 131.8 (d), 132.6 (d), 133.51 (s), 133.9 (d), 139.2 (s), 155.01 (s), 157.0 (s), 160.6 (s), 187.0 (s). Anal. Calcd. for $C_{17}H_{13}ClN_2O_3S$ (360.5): C, 56.59; H, 3.63; Cl, 9.83; N, 7.76; S, 8.89. Found: C, 56.57; H, 3.65; Cl, 9.85; N, 7.78; S, 8.88%.

4b: M.p. 117–119 °C; IR (KBr): 1133 (C–O–C linkage), 1238 (C=S), 1612 (C=N), 1643 (C=O), 3315 cm^{-1} (N–H); ^1H NMR ($CDCl_3$): δ 4.53 (s, 2H, CH_2), 6.95–7.7 (m, 8H, Ar-H), 9.1 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 78.03 (t), 115.2 (d), 124.8 (s), 125.8 (s), 128.2 (d), 130.1 (d), 131.5 (d), 132.2 (d), 133.6 (d), 137.8 (s), 155 .02 (s), 157.02 (s), 161.7 (s), 187.01 (s). Anal. Calcd. for $C_{16}H_{11}ClN_2O_3S$ (346.8): C, 55.41; H, 3.20; Cl, 10.22; N, 8.08; S, 9.25. Found: C, 55.40; H, 3.22; Cl, 10.25; N, 8.10; S, 9.22%.

4c: M.p. 122–124 °C; IR (KBr): 1132 (C–O–C linkage), 1237 (C=S), 1610 (C=N), 1641 (C=O), 3310 cm^{-1} (N–H); ^1H NMR ($CDCl_3$): δ 4.51 (s, 2H, CH_2), 6.85–7.76 (m, 8H, Ar-H), 9.05 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 78.02 (t), 117.1 (d), 122.4 (s), 123.8 (d), 127.8 (s), 128.2 (d), 130.1 (d), 132.21 (d), 133.3 (d), 137.82 (s), 155 .02 (s), 157.02 (s), 165.8 (s), 187.02 (s). Anal. Calcd. for $C_{16}H_{11}BrN_2O_3S$ (391.25): C, 49.12; H, 2.83; Br, 20.42; N, 7.16; S, 8.20. Found: C, 49.15; H, 2.81; Br, 20.44; N, 7.18; S, 8.22%.

4d: M.p. 114–116 °C; IR (KBr): 1130 (C–O–C linkage), 1236 (C=S), 1610 (C=N), 1640 (C=O), 3312 cm^{-1} (N–H); 1H NMR ($CDCl_3$): δ 2.3 (s, 3H, CH_3), 3.8 (s, 3H, OCH_3), 4.5 (s, 2H, CH_2), 6.9–7.6 (m, 7H, Ar-H), 9.1 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 20.92 (q), 56.0 (q), 78.0 (t), 113.7 (d), 113.8 (d), 123.31 (s), 129.7 (s), 130.11 (s), 131.1 (d), 131.8 (d), 133.9 (d), 155.02 (s), 157.02 (s), 160.61 (s), 165.7 (s), 187.03 (s). Anal. Calcd. for $C_{18}H_{16}N_2O_4S$ (356.4): C, 60.66; H, 4.53; N, 7.86; S, 9.0. Found: C, 60.68; H, 4.55; N, 7.88; S, 9.03%.

4e: M.p. 125–127 °C; IR (KBr): 1139 (C–O–C linkage), 1245 (C=S), 1620 (C=N), 1645 (C=O), 3323 cm^{-1} (N–H); 1H NMR ($CDCl_3$): δ 2.3–2.35 (d, J = 7 Hz, 6H, CH_3), 4.51 (s, 2H, CH_2), 7.0–7.8 (m, 7H, Ar-H), 9.13 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 20.91 (q), 78.01 (t), 113.71 (d), 123.32 (s), 128.9 (d), 129.7 (s) 130.01 (d), 131.8 (d), 133.91 (d), 134.8 (s), 141.4 (s), 155.01 (s), 157.02 (s), 160.62 (s), 187.02 (s). Anal. Calcd. for $C_{18}H_{16}N_2O_3S$ (340.4): C, 63.51; H, 4.74; N, 8.23; S, 9.42. Found: C, 63.53; H, 4.75; N, 8.21; S, 9.40%.

*5.1.4. Synthesis of 4-amino-5-[2-(3-chlorobenzoyl)-4-methylphenoxy]methyl-1,2,4-triazole- 3-(2H)thiones (**5a**)*

To a mixture of **4a** (0.8 g, 2.51 mmol) in ethanol (10 ml), 0.23 ml of 24% hydrazine hydrate was added drop wise and the mixture was refluxed for 5 h. After cooling water was added and the mixture was acidified by excess of 3N HCl, the separated solid was filtered off, washed with water and crystallized from ethanol to give **5a** (0.706 g, 75%) as white solid.

5a: M.p. 205–207 °C; IR (KBr): 1230 (C=S), 1625 (C=N), 1642 (C=O), 3330 (N–H), 3400 cm^{-1} (NH_2); ^1H NMR ($CDCl_3$): δ 2.31 (s, 3H, CH_3), 3.7 (bs, 2H, NH_2), 4.51 (s, 2H, CH_2), 6.9–7.55 (m, 7H, Ar-H), 9.1 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 20.92 (q), 74.1 (t), 113.71 (d), 123.31 (s), 128.2 (d), 129.61 (d), 129.71(s), 130.51 (d), 131.81 (d), 132.61 (d), 133.51 (s), 133.91 (d), 139.21 (s), 155.01 (s), 160.61 (s), 186.0 (s), 187.01 (s). Anal. Calcd. for $C_{17}H_{15}ClN_4O_2S$ (374.8): C, 54.47; H, 4.03; Cl, 9.46; N, 14.95; S, 8.55. Found: C, 54.46; H, 4.05; Cl, 9.45; N, 14.92; S, 8.53%.

5b: M.p. 210–212 °C; IR (KBr): 1233 (C=S), 1630 (C=N), 1647 (C=O), 3335 (N–H), 3408 cm^{-1} (NH_2); ^1H NMR ($CDCl_3$): δ 3.71 (bs, 2H, NH_2), 4.52 (s, 2H, CH_2), 6.95–7.6 (m, 8H, Ar-H), 9.13 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 74.12 (t), 115.2 (d), 124.8 (s), 125.8 (s), 128.2 (d), 130.1 (d), 131.5 (d), 132.2 (d), 133.6 (d), 137.8 (s), 155.02 (s), 161.7 (s), 186.01 (s), 187.03 (s). Anal. Calcd. for $C_{16}H_{13}ClN_4O_2S$ (360.8): C, 53.26; H, 3.63; Cl, 9.83; N, 15.53; S, 8.89. Found: C, 53.25; H, 3.65; Cl, 9.81; N, 15.50; S, 8.90%.

5c: M.p. 220–222 °C; IR (KBr): 1232 (C=S), 1622 (C=N), 1645 (C=O), 3332 (N–H), 3404 cm^{-1} (NH_2); ^1H NMR ($CDCl_3$): δ 3.71 (bs, 2H, NH_2), 4.51 (s, 2H, CH_2), 6.9–7.7 (m, 8H, Ar-H), 9.12 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 74.11 (t), 117.1 (d), 122.4 (s), 123.8 (d), 127.8 (s), 128.2 (d), 130.1 (d), 132.21 (d), 133.3 (d), 137.81 (s), 155.01 (s), 165.8 (s), 186.02 (s), 187.04 (s). Anal. Calcd. for $C_{16}H_{13}BrN_4O_2S$ (405.2): C, 47.42; H, 3.23; Br, 19.72; N, 13.82; S, 7.91. Found: C, 47.44; H, 3.21; Br, 19.75; N, 13.32; S, 7.93%.

5d: M.p. 225–227 °C; IR (KBr): 1230 (C=S), 1621 (C=O), 1640 (C=N), 3331 (N–H), 3409 cm^{-1} (NH_2); ^1H NMR ($CDCl_3$): δ 2.3 (s, 3H, CH_3), 3.7 (bs, 2H, NH_2), 3.85 (s, 3H, OCH_3), 4.51 (s, 2H, CH_2), 6.8–7.65 (m, 7H, Ar-H), 9.12 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 21.2 (q), 56.01 (q), 74.12 (t), 113.8 (d), 114.5 (d), 120.4 (s), 121.2 (d), 130.1 (s), 131.01 (d), 131.1 (d), 142.4 (s), 155.01 (s), 163.5 (s), 165.7 (s), 186.03 (s), 187.04 (s). Anal. Calcd. for $C_{18}H_{18}N_4O_3S$ (370.4): C, 58.36; H, 4.90; N, 15.12; S, 8.66. Found: C, 58.34; H, 4.92; N, 15.15; S, 8.68%.

5e: M.p. 208–210 °C; IR (KBr): 1240 (C=S), 1638 (C=N), 1652 (C=O), 3340 (N–H), 3412 cm^{-1} (NH_2); ^1H NMR ($CDCl_3$): δ 2.25–2.31 (d, J = 7 Hz, 6H, CH_3), 3.73 (bs, 2H, NH_2), 4.52 (s, 2H, CH_2), 7.0–7.78 (m, 7H, Ar-H), 9.15 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 20.91 (q), 21.21 (q), 74.11 (t), 114.5 (d), 120.41 (s), 121.2 (d), 128.9 (d), 130.01 (d), 131.01 (d), 134.8 (s), 141.4 (s), 142.41 (s), 155.02 (s), 163.51

(s), 186.02 (s), 187.03 (s). Anal. Calcd. for $C_{18}H_{18}N_4O_2S$ (354.4): C, 61.0; H, 5.12; N, 15.81; S, 9.05. Found: C, 61.03; H, 5.15; N, 15.80; S, 9.03%.

*5.1.5. Synthesis of 3-[2-(3-chlorobenzoyl)-4-methylphenoxy]methyl-1,2,4-triazolo[3,4-b] [1,3,4]thiadiazine (**6a**)*

A mixture of **5a** (0.5 g, 1.33 mmol) and phenacyl bromide (0.19 g, 1.33 mmol) in anhydrous ethanol (10 ml) was refluxed for 5 h. The solvent was removed under reduced pressure, diethyl ether (15 ml) was added and the reaction mixture was left at 0 °C overnight. The precipitated solid was filtered off, dried ad recrystallized with ethanol to give **6a** (0.44 g, 70%) as white needles.

6a: M.p. 142–144 °C; IR (KBr): 750 (C–S–C), 1630 (C=N), 1645 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 2.3 (s, 3H, CH_3), 3.2 (s, 2H, CH_2), 4.8 (s, 2H, OCH_2), 6.75–7.6 (m, 12H, Ar-H); ^{13}C NMR ($CDCl_3$): δ 20.91 (q), 36.3 (t), 63.0 (t), 113.7 (d), 123.31 (s), 128.2 (d), 128.61 (d), 129.0 (d), 129.6 (d), 129.7 (s), 130.5 (d), 130.8 (d), 131.2 (s), 131.8 (d), 132.6 (d), 133.5 (s), 133.9 (d), 139.2 (s), 147.0 (s), 160.61 (s), 162.01 (s), 164.6 (s), 187.01 (s). Anal. Calcd. for $C_{25}H_{19}ClN_4O_2S$ (474.9): C, 63.22; H, 4.03; Cl, 7.46; N, 11.80; S, 6.75. Found: C, 63.24; H, 4.04; Cl, 7.44; N, 11.82; S, 6.73%.

6b: M.p. 151–153 °C; IR (KBr): 752 (C–S–C), 1633 (C=N), 1648 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 3.22 (s, 2H, CH_2), 4.83 (s, 2H, OCH_2), 6.8–7.65 (m, 13H, Ar-H); ^{13}C NMR ($CDCl_3$): δ 36.32 (t), 63.01 (t), 115.2 (d), 124.8 (s), 125.8 (s), 128.2 (d), 128.62 (d), 129.02 (d), 129.72 (s), 130.1 (d), 130.82 (d), 131.21 (s), 131.5 (d), 132.2 (d), 133.6 (d), 137.8 (s), 147.01 (s), 161.7 (s), 162.01 (s), 164.62 (s), 187.01 (s). Anal. Calcd. for $C_{24}H_{17}ClN_4O_2S$ (460.9): C, 62.54; H, 4.72; Cl, 7.69; N, 12.16; S, 6.96. Found: C, 62.55; H, 4.74; Cl, 7.66; N, 12.14; S, 6.94%.

6c: M.p. 157–159 °C; IR (KBr): 748 (C–S–C), 1632 (C=N), 1648 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 3.21 (s, 2H, CH_2), 4.84 (s, 2H, OCH_2), 6.85–7.7 (m, 13H, Ar-H); ^{13}C NMR ($CDCl_3$): δ 36.31 (t), 63.01 (t), 117.1 (d), 122.4 (s), 123.8 (d), 127.8 (s), 128.2 (d), 128.6 (d), 129.0 (d), 130.1 (d), 130.81 (d), 131.22 (s), 132.21 (d), 133.3 (d), 137.83 (s), 147.01 (s), 162.01 (s), 164.61 (s), 165.82 (s), 187.03 (s). Anal. Calcd. for $C_{24}H_{17}BrN_4O_2S$ (505.3): C, 57.04; H, 3.39; Br, 15.81; N, 11.09; S, 6.34. Found: C, 57.06; H, 3.37; Br, 15.83; N, 11.09; S, 6.31%.

6d: M.p. 162–164 °C; IR (KBr): 740 (C–S–C), 1630 (C=N), 1645 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 2.31 (s, 3H, CH_3), 3.2 (s, 2H, CH_2), 3.75 (s, 3H, OCH_3), 4.75 (s, 2H, OCH_2), 6.8–7.65 (m, 12H, Ar-H); ^{13}C NMR ($CDCl_3$): δ 20.92 (q), 36.33 (t), 56.04 (q), 63.02 (t), 113.71 (d), 113.8 (d), 123.33 (s), 128.6 (d), 129.02 (d), 129.72 (s), 130.1 (s), 130.83 (d), 131.1 (d), 131.2 (s), 131.8 (d), 133.9 (d), 147.02 (s), 160.61 (s), 162.03 (s), 164.6 (s), 165.7 (s), 187.03 (s). Anal. Calcd. for $C_{26}H_{22}N_4O_3S$ (470.5): C, 66.37; H, 4.71; N, 11.91; S, 6.81. Found: C, 66.35; H, 4.73; N, 11.93; S, 6.83%.

6e: M.p. 171–173 °C; IR (KBr): 755 (C–S–C), 1645 (C=N), 1652 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 2.25–2.3 (d, J = 7 Hz, 6H, $2CH_3$), 3.23 (s, 2H, CH_2), 4.83 (s, 2H, OCH_2), 6.8–7.72 (m, 12H, Ar-H); ^{13}C NMR ($CDCl_3$): δ 20.91 (q), 36.32 (t), 63.02 (t), 113.71 (d), 123.33 (s), 128.6 (d), 128.9 (d), 129.03 (d), 129.7 (s), 130.0 (d), 130.82 (d), 131.22 (s), 131.83 (d), 133.9 (d), 134.8 (s), 141.42 (s), 147.02 (s), 160.62 (s), 162.03 (s), 164.63 (s), 187.04 (s). Anal. Calcd. for $C_{26}H_{22}N_4O_2S$ (454.5): C, 68.70; H, 4.88; N, 12.33; S, 7.05. Found: C, 68.72; H, 4.86; N, 12.31; S, 7.03%.

5.2. Antimicrobial activity

The antimicrobial activities of compounds **4a**–**e**, **5a**–**e** and **6a**–**e** were evaluated in vitro by serial tube dilution technique [27,28] at different concentrations (0.5, 1.0, 1.5,.....12 mM). Some fungi such as *C. albicans, C. krusei* and *C. parapsilosis, A. flavus, A. ochraceous, F. moniliforme* and *C. gloeosporioides* and bacteria such as *E. coli, P. solanacearum, P. fluorescens* and *B. subtilis* were used. Fluconazole and chloramphenicol were used as reference standard in antifungal and antibacterial activity studies, respectively. The stock solutions of the compounds were prepared in chloroform. To the culture tubes containing 1.9 ml of media, 0.1 ml of test solution was added at sterile conditions. To all the tubes including standard and controls, the fresh inoculums was added using Himedia flexiloop 4 calibrated to 0.001 ml. After incubating all the tubes at 37 °C for 24 h, their absorbance was recorded at 640 nm along with reference. Percentage of inhibition was calculated using the following equation.

$$\% \text{ Inhibition} = 100\ (m - n)/m$$

where m = absorbance without the test sample and n = absorbance with test sample.

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References

[1] H.S. Gold, R.C. Moellering, N. Engl. J. Med. Chem. 335 (1996) 1445–1453.
[2] H. Vanden. Bossche, P. Marichal, F.C. Odds, Trends Microbiol. 10 (1994) 393–400.
[3] M.L. Cohen, Science 257 (1992) 1050–1055.
[4] R.C. Heel, R.N. Brogden, A. Carmine, P.A. Morley, T.M. Speight, G.S. Avery, Drugs 23 (1982) 1–36.
[5] D.J. Sheehan, C.A. Hitchcock, C.M. Sibley, Clin. Microbiol. Rev. 12 (1999) 40–79.
[6] C.A. Lyman, T.J. Walsh, Drugs 44 (1992) 9–35.
[7] C.J. Clancy, M.H. Nguyen, Eur. J. Clin. Microbiol. Infect. Dis. 17 (1998) 573–575.
[8] J.C.E. Fung-Tomc, B. Huczko, D.P. Minassian, Bonner, Antimicrob. Agents Chemother. 42 (1998) 313–318.
[9] A. Espinel-Ingroff, J. Clin. Microbiol. 36 (1998) 2950–2956.

[10] A. Sarkar, K.A. Kumar, N.K. Dutta, P. Chakraborty, S.G. Dastidar, Indian J. Med. Microbiol. 21 (2003) 172–178.
[11] B. Trusheva, M. Popova, H. Naydenski, I. Tsvetkova, G.J. Rodriguez, V. Bankova, Fitoterapia 75 (2004) 683–689.
[12] J. Lokvam, J.F. Braddock, P.B. Reichardt, T.P. Clausen, Phytochemistry 55 (2000) 29–34.
[13] J. Curtze, C.H.G. Rudolph, L. Schroder, G. Albert, A.E.E. Rehnig, E.G. Sieverding, U. Pat, Chem. Abstr. 129 (1998) 108898 (577366).
[14] L. Ke, Z. Wannian, Z. Wu, W. Xiaoyan, Chem. Abstr. 136 (2002) 167122 (Zhongguo Yiyao Gongye Zazhi 32 (2001) 115–117).
[15] P. Bakana, M. Claeys, J. Totte, L.A. Pieters, L. Van Hoof, D.A. Tamba-Vemba, V.d. Berghe, A.J. Vlietinck J. Ethnopharmacol. 21 (1987) 75–84.
[16] A. Tamil Selvi, G.S. Joseph, G.K. Jayaprakasha, Food Microbiol. 20 (2003) 455–460.
[17] T. Grote, A. Gypser, J. Rheinheimer, I. Rose, P. Schaefer, F. Schieweck, N. Goetz, K. Eicken, E. Ammermann, S. Strathmann, G. Lorenz, R. Stierl, Chem. Abstr. 137 (2002) 232443 (WO Patent (2002) 2002072523).
[18] S. Giri, H. Singh, L.D.S. Yadav, Agric. Biol. Chem. 40 (1976) 17–21.
[19] G. Sahin, E. Palaska, M. Ekizoglu, M. Ozalp, Farmaco 57 (2002) 539–542.
[20] B.S. Holla, K.N. Poojary, B. Kalluraya, Farmaco 51 (1996) 793–799.
[21] B.S. Holla, P.M. Akberali, M.K. Shivananda, Farmaco 56 (2001) 919–927.
[22] M.R. Friedman, K.J. Toyne, J.W. Goodby, M. Hird, J. Mater. Chem. 11 (2001) 2759–2772.
[23] G.N. Walker, R.T. Smith, J. Org. Chem. 36 (1971) 305–308.
[24] G. Sahin, E. Palaska, P. Kelicen, R. Demirdamar, G. Altinok, Arzneim. Forsch, Drug Res. 51 (2001) 478–484.
[25] E. Palaska, G. Sahin, P. Kelicen, N.T. Durlu, G. Altinok, Farmaco 57 (2002) 101–107.
[26] M.D. Mullican, M.W. Wilson, D.T. Connor, C.R. Kostlan, D.J. Schrier, R.D. Dyer, J. Med. Chem. 36 (1993) 1090–1099.
[27] C.H. Collins, P.M. Lyne, J.M. Grange, J.O. Falkinham, Collins and Lyne's Microbiological Methods, Butterworth, London, 1970.
[28] B.G. Mullen, R.T. Decory, T.J. Mitchell, D.S. Allen, C.R. Kinsolving Vassil St, B. Georgiev, J. Med. Chem. 31 (1988) 2008–2014.