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Analgesic and antimicrobial studies of some 2,4-dichloro-5-fluorophenyl containing arylidenetriazolothiadiazines

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Abstract 2,4-Dichloro-5-fluorophenyl containing 7-arylidenetriazolothiadiazines were obtained by the reaction of 4-amino-3-(2,4-dichloro-5-fluorophenyl)-5-mercapto-1,2,4-triazole with 2,3-dibromo-1,3-diarylpropan-1-ones, and also by the reaction of 4-amino-3-(2,4-dichloro-5-fluorophenyl)-5-mercapto-1,2,4-triazole with α-bromopropenones in the presence of a base. The structure of the 7-arylidenetriazolothiadiazines was confirmed by an alternative synthesis. A plausible mechanism for the formation of 7-arylidenetriazolothiadiazines is proposed. All newly synthesized compounds were screened for their analgesic and antimicrobial activities. Compounds bearing 4-chlorophenyl or 3,4-methylenedioxyphenyl moieties at position 7 of the arylidenetriazolothiadiazines showed excellent analgesic activity. Arylidenetriazolothiadiazines carrying a phenyl, 4-chlorophenyl, 4-methylphenyl, 3,4-dimethoxyphenyl, and 2,4-dichlorophenyl moieties at position 7 showed excellent antibacterial and antifungal activities.

Keywords 2,4-Dichloro-5-fluorophenyltriazole; Dibromopropan-1-ones; α-Bromopropenones; Analgesic; Antimicrobial activities.

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Introduction

In recent days, active research has been initiated on halogen containing heterocycles, particularly on fluorine containing heterocycles. 2,4-Dichloro-5-fluoroacetophenone is used in the synthesis of drugs like Ciprofloxacin and their analogues [1]. Moreover, incorporation of a fluorine atom can alter the course of reaction as well as biological activities [2–4]. Furthermore, the introduction of a fluorine atom or a CF_3 group into an organic molecule largely enhances the pharmacological properties as compared with the non-fluorinated analogues.

Incorporation of fluorine may also lead to increased lipid solubility thereby enhancing the rates of absorption and transport of drugs *in vivo*. The replacement of hydrogen or a hydroxyl group by fluorine is a strategy widely used in drug development to alter biological function. Despite the fact that fluorine is bigger than hydrogen, several studies have demonstrated that fluorine is a reasonable hydrogen mimic and exerts only a minor steric demand at receptor sites [5].

1,2,4-Triazole derivatives are known to exhibit antibacterial, antifungal [6], antitubercular [7], anticancer [8], anticonvulsant [9], anti-inflammatory, and analgesic properties [10]. Among the pharmacological profiles of 1,2,4-triazoles, their antimicrobial, anticonvulsant, and antidepressant properties seem to be best documented. The arrangement of three basic

nitrogen atoms in the triazole ring induces antiviral activity in the compounds containing a triazole ring [11]. The 1,2,4-triazole nucleus has been incorporated into a wide variety of therapeutically interesting drug candidates including H_1/H_2 histamine receptor blockers, cholinesterase active agents, CNS stimulants, antianxiety, and sedatives agents [12]. Some of the modern day drugs with triazole nucleus are Ribavirin (antiviral agent), Alprazolam (anxiolytic agent), Flucanozole, Itraconazole (antifungal agent) [13], and Rizatriptan (antimigrane agent).

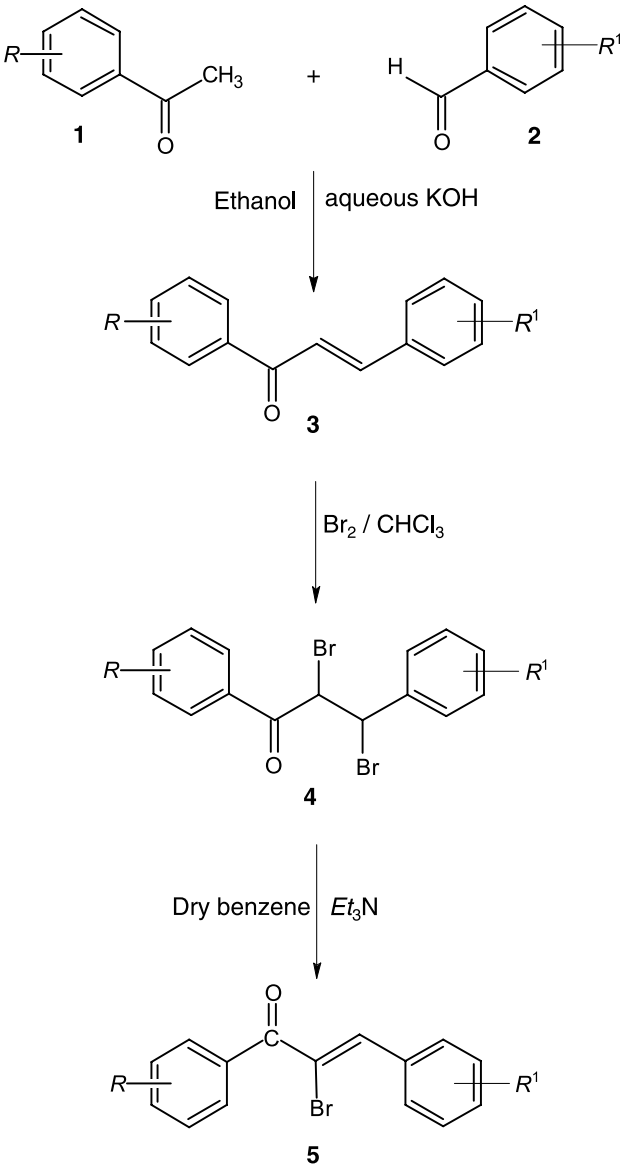
The ambident nucleophilic centers present in 3-substituted-4-amino-5-mercapto-1,2,4-triazoles render them as useful synthons for the synthesis of various N-bridged heterocycles. Moreover, synthesis of triazole fused to other heterocycles has attracted attention widely due to their diverse applications. Recently, a number of N-bridged heterocycles are also derived from substituted chalcones and have been reported to possess some interesting biological properties [14–16].

Prompted by these observations, it was contemplated to synthesize some dichlorofluorophenyl containing arylidenetriazolothiadiazines with a view to explore their potency as better chemotherapeutic agents. All newly synthesized compounds were screened for the analgesic, antibacterial, and antifungal activities.

Results and discussion

Chemistry

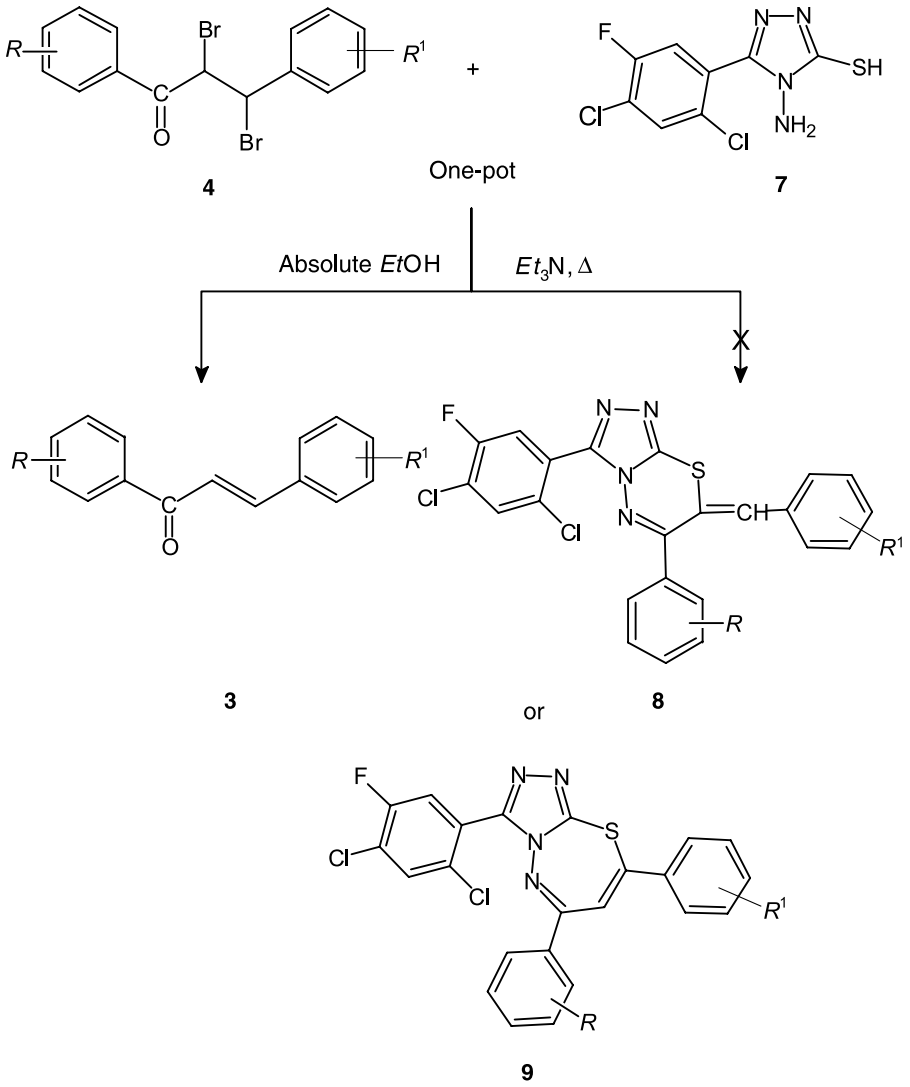
Substituted acetophenones **1** and substituted benzaldehydes **2** were allowed to react in the presence of aqueous potassium hydroxide to yield 1,3-diaryl-2-propen-1-ones **3**. Bromination of these propenones **3** employing bromine in chloroform yielded 2,3-dibromo-1,3-diarylpropan-1-ones **4**. The dibromopropanones **4** on dehydrobromination using triethylamine in dry benzene yielded 1,3-diaryl-2-bromo-2-propen-1-ones **5** [17, 18]. The reaction sequences are outlined in Scheme 1. 4-Amino-3-(2,4-dichloro-5-fluorophenyl)-5-mercapto-1,2,4-triazole **7** was obtained from 2,4-dichloro-5-fluorobenzoic acid according to Refs. [19, 20].



Scheme 1

In a one-pot reaction between 2,3-dibromo-1,3-diarylpropan-1-ones **4** and 4-amino-3-(2,4-dichloro-5-fluorophenyl)-5-mercapto-1,2,4-triazole **7** in presence of triethylamine in absolute ethanol neither arylidene-triazolothiadiazines **8** nor triazolothiadiazepines **9**, but unexpectedly chalcones **3** were obtained as the major products, which was confirmed by spectroscopic and analytical data. The possibility could be that 2,3-dibromo-1,3-diarylpropan-1-ones **4** might undergo debromination under mild basic condition and yielded chalcones **3** as the major products (Scheme 2).

The same reaction was carried out in two steps. Initially triethylamine was added to 2,3-dibromo-1,3-diarylpropan-1-ones **4** in absolute ethanol and refluxed for 1 h. To this reaction mixture 4-amino-3-(2,4-dichloro-5-fluorophenyl)-5-mercapto-1,2,4-triazole **7** was added and further refluxed for 6 h,



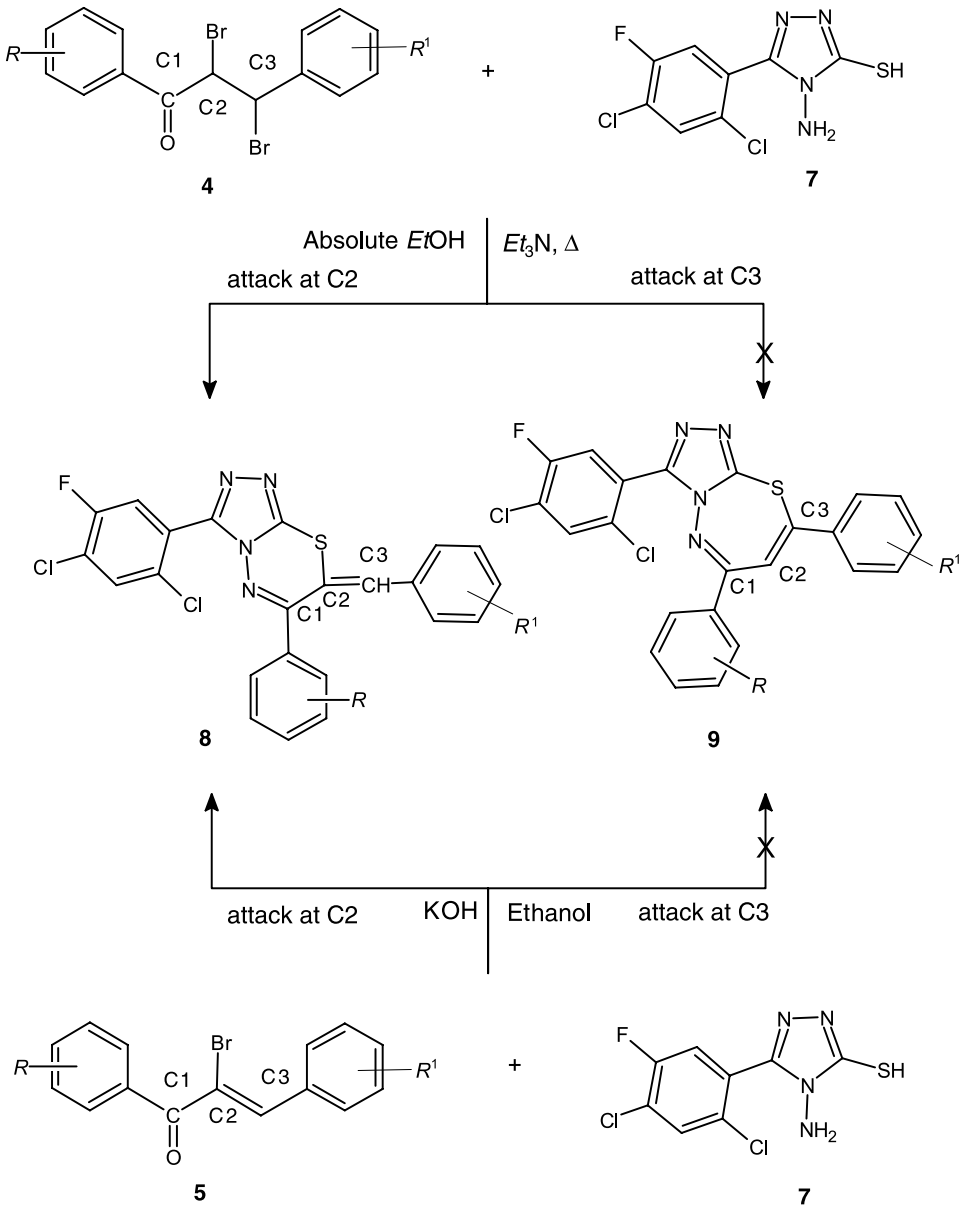
Scheme 2

which yielded arylidenetriazolothiadiazines **8** rather than triazolothiadiazepines **9** (Scheme 3).

When α-bromopropenones **5** were treated with 4-amino-3-(2,4-dichloro-5-fluorophenyl)-5-mercapto-1,2,4-triazole **7** in presence of potassium hydroxide in ethanol, arylidenetriazolothiadiazines **8** rather than triazolothiadiazepines **9** were formed (Scheme 3).

Arylidenetriazolothiadiazines **8** were also synthesized by an alternate route to confirm their formation. 6-Substituted-3-(2,4-dichloro-5-fluorophenyl)-7*H*-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines **11** were obtained by the initial reaction of 4-amino-3-(2,4-dichloro-5-fluorophenyl)-5-mercapto-1,2,4-triazole (**7**) with phenacyl bromides [21] **10** in presence of sodium acetate in absolute ethanol. Condensation of 6-substituted 3-(2,4-dichloro-5-fluorophenyl)-7*H*-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines **11** with substituted benzaldehyde in presence of piperidine yielded arylidenetriazolothiadiazines **8** (Scheme 4).

Compounds **8** obtained by the above method were found to be identical with the compounds prepared by the direct condensation of triazole **7** with 2,3-dibromo-1,3-diaryl propan-1-ones **4** and triazole **7** with α-bromopropenones **5**. By the mixed melting point and superimposable IR spectra the compounds formed were found to be arylidenetriazolothiadiazines **8** and not triazolothiadiazepines **9**. Although stereochemistry of **8** was not investigated, analytical data suggest that only one diastereomer was formed which is due to previous work [22] very likely to be the (*Z*)-isomer.

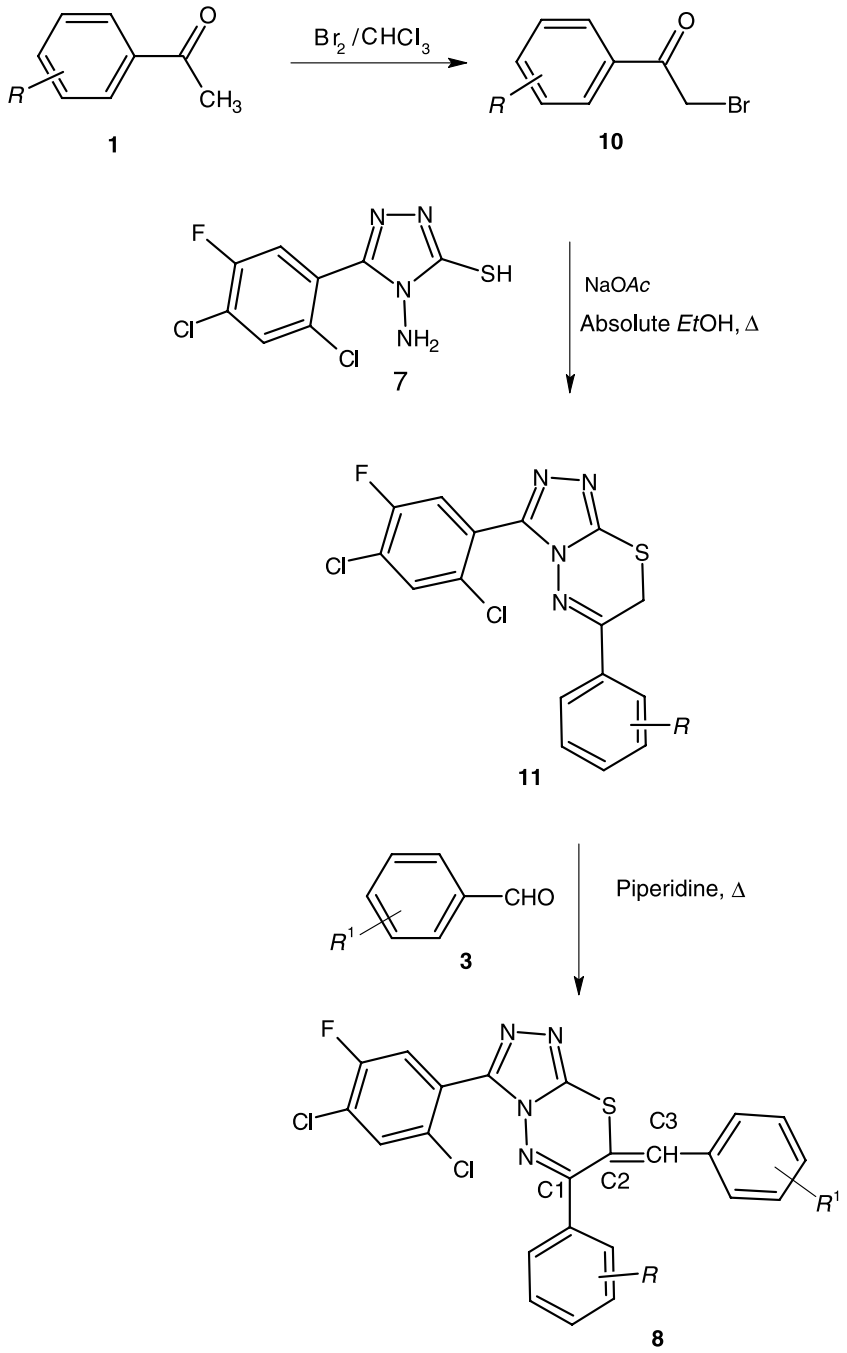


Compd. No.	*R*	R^1	Compd. No.	*R*	R^1
8a	2,4-Cl_2-5-F	H	**8h**	4-Cl	4-Cl
8b	2,4-Cl_2-5-F	4-OCH_3	**8i**	4-Cl	4-$N(CH_3)_2$
8c	2,4-Cl_2-5-F	4-Cl	**8j**	4-Cl	2,4-Cl_2
8d	2,4-Cl_2-5-F	2,4-Cl_2	**8k**	4-CH_3	4-Cl
8e	2,4-Cl_2-5-F	3,4-OCH_2O	**8l**	4-CH_3	2,4-Cl_2
8f	2,4-Cl_2-5-F	3,4-$(OCH_3)_2$			

Scheme 3

Mechanism

A plausible mechanism for the formation of arylidenetriazolothiadiazines **8** was proposed on the basis of earlier observations. α-Bromopropenones were generated *in situ* by refluxing 2,3-dibromo-1,3-diarylpropan-1-ones **4** with triethylamine in absolute ethanol. When triazole **7** was added to the reaction mixture *Schiff* base was formed, further nucleophilic attack at



Scheme 4

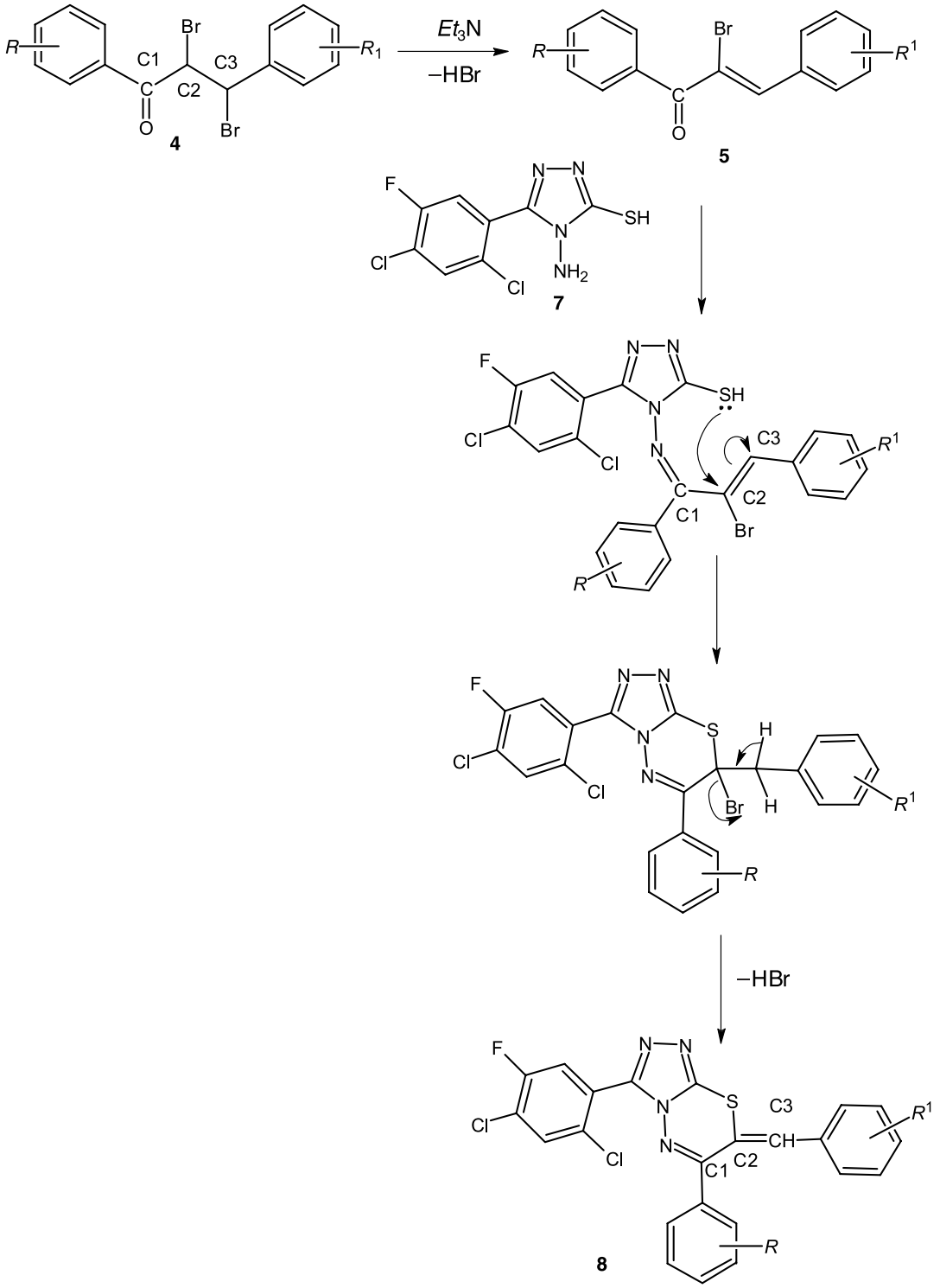
C2 resulted in cyclization and subsequent dehydrobromination yielding arylidenetriazolothiadiazines **8** rather than triazolothiadiazepines **9** (Scheme 5).

Formation of arylidenetriazolothiadiazines **8** was confirmed on the basis of IR, ^{1}H NMR, mass spectral data, and elemental analysis. All the newly synthesized compounds were screened for their biological activities.

Pharmacological studies

Analgesic studies

Selected compounds were screened for analgesic activity by the hot plate test according to *Eddy* and *Leimbach* [23]. The analgesic screening results are given in Table 1. The analgesic screening results revealed that compounds **8c**, **8e**, and



Scheme 5

8h showed excellent analgesic activity whereas compounds **8b**, **8d**, **8f**, **8g**, and **8k** showed moderate to good analgesic activity compared with Pethidine.

Antibacterial studies

The newly prepared compounds were screened for their antibacterial activity against *Staphylococcus aureus* (ATCC-25923), *Escherichia coli* (ATCC-

Table 1 Analgesic activity data of arylidenetriazolothiadiazines **8a–8l**

Compd. no.	Dose/ mg kg^{-1}	Time of reaction to pain stimulus at time/h [s] ± SEM		
		0	1	3
8a	50	8.6	8.86	9.14
8b	50	7.5	11.71	10.76
8c	50	8.5	14.41	13.3
8d	50	9.2	10.21	10.7
8e	50	9.4	14.9	13.5
8f	50	8.5	11.1	10.7
8g	50	8.9	10.4	11.5
8h	50	8.2	14.1	13.31
8i	50	8.8	10.21	11.04
8k	50	9.1	11.4	12.4
control	10	8.25	8.2	8.8
standard*	5	8.5	16.4	14.3

* Pethidine is used as the standard

25922), *Pseudomonas aeruginosa* (ATCC-27853), and *Klebsiella pneumoniae* (recultured) bacterial strains by the disc diffusion method [24, 25]. The diameter of the zone of inhibition and minimum inhibitory concentration *MIC i.e.*, the lowest concentration to completely inhibit bacterial growth values are given in Table 2. The antibacterial screening data revealed that the compounds **8a**, **8c**, **8f**, **8g**, **8j**, and **8l** exhibited good antibacterial activity against all tested bacterial strains almost equivalent to that of the standard drug Norfloxacin.

Antifungal studies

The newly prepared compounds were screened for their antifungal activity against *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans*, and *Trichophyton mentagrophytes* (recultured) in *DMSO* by the agar diffusion method [26, 27]. The diameter of zone of inhibition and minimum inhibitory concentration values are given in Table 3. The antifungal screening data showed that compounds **8e** showed good activity against *Trichophyton mentagrophytes* at 6.25 μg cm^{-3} concentration Compounds **8a**, **8c**, **8f**, **8g**, and **8j** exhibited good antifungal activity against all tested fungal strains almost equivalent to that of the standard drug Griseofulvin.

Conclusion

The analgesic activity study revealed that compounds with 4-chlorophenyl, and 3,4-methylenedioxyphenyl moieties at position 7 of the arylidenetriazolothiadiazines showed excellent analgesic activity. The investigation of antibacterial screening data revealed that among the twelve compounds screened six compounds showed good bacterial inhibition almost equivalent to that of the standard. Only five of the compounds displayed good antifungal activity. Arylidenetriazolothiadiazines carrying phenyl, 4-chlorophenyl, 4-methylphenyl, 3,4-dimethoxyphenyl, and 2,4-dichlorophenyl moieties at position 7 of the arylidenetriazolothiadia-

Table 2 Antibacterial activity data of arylidenetriazolothiadiazines **8a–8l**

Compd. no.	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*
8a	21 (6.25)	25 (12.5)	20 (6.25)	25 (6.25)
8b	14 (25)	17 (6.25)	12 (25)	15 (12.5)
8c	18 (6.25)	27 (6.25)	23 (6.25)	21 (6.25)
8d	12 (25)	17 (6.25)	11 (25)	19 (6.25)
8e	14 (12.5)	19 (6.25)	15 (12.5)	12 (25)
8f	22 (6.25)	28 (6.25)	20 (6.25)	23 (12.5)
8g	20 (6.25)	24 (6.25)	18 (6.25)	25 (6.25)
8h	12 (12.5)	18 (6.25)	13 (25)	9 (25)
8i	17 (6.25)	12 (25)	16 (6.25)	19 (6.25)
8j	22 (6.25)	26 (6.25)	24 (6.25)	22 (6.25)
8k	11 (25)	19 (6.25)	10 (25)	17 (6.25)
8l	20 (6.25)	27 (6.25)	20 (6.25)	23 (6.25)
standard*	22 (6.25)	28 (6.25)	24 (6.25)	25 (6.25)

* Norfloxacin is used as the standard. Diameter/mm of zone of inhibition. *MIC*/μg cm^{-3} values are given in brackets.

Table 3 Antifungal activity data of arylidenetriazolothiadiazines **8a–8l**

Compd. no.	*Aspergillus niger*	*Aspergillus flavus*	*Candida albicans*	*Trichophyton mentagrophytes*
8a	28 (6.25)	25 (6.25)	33 (6.25)	25 (6.25)
8b	10 (25)	19 (6.25)	30 (6.25)	12 (12.5)
8c	29 (6.25)	27 (6.25)	28 (6.25)	21 (6.25)
8d	23 (6.25)	12 (12.5)	20 (12.5)	17 (6.25)
8e	15 (12.5)	10 (25)	24 (6.25)	21 (6.25)
8f	28 (6.25)	25 (12.5)	34 (6.25)	24 (6.25)
8g	30 (6.25)	28 (12.5)	29 (6.25)	20 (6.25)
8h	16 (12.5)	15 (12.5)	19 (12.5)	18 (6.25)
8i	23 (6.25)	22 (6.25)	24 (6.25)	15 (12.5)
8j	27 (6.25)	26 (6.25)	32 (6.25)	23 (6.25)
8k	20 (12.5)	20 (6.25)	18 (12.5)	12 (25)
8l	15 (12.5)	19 (6.25)	21 (12.5)	10 (25)
standard*	30 (6.25)	28 (6.25)	33 (6.25)	25 (6.25)

* Griseofulvin is used as the standard. Diameter/mm of zone of inhibition. $MIC/\mu g\,cm^{-3}$ values are given in brackets.

zines emerged as active compounds in both antibacterial and antifungal screening.

Experimental

Melting points were determined by open capillary method. The IR spectra (in KBr pellets) were recorded on a Shimadzu FT-IR 157 spectrophotometer. ^{1}H NMR spectra were recorded (in $CDCl_3$/*DMSO*-d_6) on a Bruker 400/300 MHz NMR spectrometer using *TMS* as an internal standard. The mass spectra were recorded on a MASPEC/FAB mass spectrometer operating at 70 eV. The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel plates using a mixture of petroleum ether and ethyl acetate. Iodine was used as visualizing agent. Compounds **3**, **4**, and **5** were prepared according to Refs. [17, 18] and 4-amino-3-(2,4-dichloro-5-fluorophenyl)-5-mercapto-1,2,4-triazole (**7**) was prepared according to the reported method [19, 20].

*Procedure for the preparation of 6-(substituted phenyl)-7-(arylidene)-3-(2,4-dichloro-5-fluorophenyl)-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazines **8***

A mixture of 0.01 mol of 1,3-diaryl-2,3-dibromo-propan-1-ones **4** and 0.02 mol triethylamine in 35 cm^3 absolute ethanol was refluxed for 1 h. To the resulting reaction mixture 0.01 mol triazole **7** was added and refluxed for 6 h. The resulting solid was filtered off, washed with water, and recrystallized from a mixture of ethanol and dimethylformamide.

*General procedure of alternative route for preparation of 6-(substituted phenyl)-7-(arylidene)-3-(2,4-dichloro-5-fluorophenyl)-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazines **8***

A mixture of 0.01 mol 4-amino-3-(2,4-dichloro-5-fluorophenyl)-5-mercapto-1,2,4-triazole (**7**), 0.01 mol 1,3-diaryl-2-bromo-2-propen-1-ones **5**, and 0.015 mol alcoholic KOH was refluxed in ethanol for 6 h. The precipitated solid was filtered off, washed with water, and recrystallized from a mixture of ethanol and dimethylformamide to yield the title compound **8**.

Substituted phenacyl bromides **10** were prepared according to the reported procedure [21].

*General procedure for preparation of 6-(substituted phenyl)-3-(2,4-dichloro-5-fluorophenyl)-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines **11***

A mixture of 0.01 mol triazole **7**, 0.01 mol substituted phenacyl bromides **10**, and 0.01 mol anhydrous sodium acetate in 30 cm^3 absolute ethanol was refluxed on a water bath for 5 h. The reaction mixture was cooled, the precipitated solid was filtered off, and recrystallized from ethanol.

*General procedure for preparation of 6-(substituted phenyl)-7-(arylidene)-3-(2,4-dichloro-5-fluorophenyl)-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazines **8** from 6-(substituted phenyl)-3-(2,4-dichloro-5-fluorophenyl)-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines **11***

A mixture of 0.01 mol 6-(substituted phenyl)-3-(2,4-dichloro-5-fluorophenyl)-7*H*-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazine **11**, 0.01 mol substituted benzaldehyde **2**, and 0.01 mol piperidine in 25 cm^3 absolute ethanol was refluxed for 5 h. The reaction mixture was cooled. The precipitated solid was filtered off, washed with water, and recrystallized from a mixture of absolute ethanol and dimethylformamide to yield the title compound **8**. This product was identical with the sample prepared by the direct condensation of triazole **7** with 1,3-diaryl-2,3-dibromopropan-1-ones **4**.

7-Benzylidene-3,6-bis(2,4-dichloro-5-fluorophenyl)-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine (**8a**, $C_{23}H_{10}Cl_4F_2N_4S$) Yield 87%; mp 200–02°C (*Et*OH:*DMF* = 2:1); IR (KBr): $\bar{\nu} = 3090$ (*Ar*–H), 2935 (C–H), 1567 (C=N), 1096 (C–F), 734 (C–Cl) cm^{-1}; ^{1}H NMR (400 MHz, *DMSO*-d_6): $\delta = 7.11$ (s, 1H- exocyclic vinylic), 7.53 (m, 5H-phenyl), 7.8, 7.89 (d, 2H-dichlorofluorophenyl, $J_{H-F_{ortho}} = 9.3$ Hz), 8.04, 8.08 (d,

2H-dichlorofluorophenyl, $J_{H-F_{meta}} = 6.6$ Hz) ppm; FABMS: $m/z = 552$ (M^+).

3,6-Bis(2,4-dichloro-5-fluorophenyl)-7-(4-methoxybenzylidene)-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine (**8b**, $C_{24}H_{12}Cl_4F_2N_4OS$)
Yield 68%; mp 183–85°C (*Et*OH:*DMF* = 2:1); IR (KBr): $\bar{\nu} = 3085$ (*Ar*–H), 1583 (C=N), 1087 (C–F), 825, 727 (C–Cl) cm^{-1}; ^1H NMR (400 MHz, *DMSO*-d_6): $\delta = 3.82$ (s, OCH_3), 7.00 (s, 1H- exocyclic vinylic proton), 7.09 (m, 2H-*p*-anisyl), 7.59 (m, 2H-*p*-anisyl), 7.78, 7.88 (d, 1H, dichlorofluorophenyl, $J_{H-F_{ortho}} = 9.2$ Hz), 8.06 (m, 2H-dichlorofluorophenyl) ppm; FABMS: $m/z = 582$ (M^+, 11), 583 ($M^+ + 1$, 42), 460 (18).

7-(4-Chlorobenzylidene)-3,6-bis(2,4-dichloro-5-fluorophenyl)-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine (**8c**, $C_{23}H_9Cl_5F_2N_4S$)
Yield 75%; mp 199–201°C (*Et*OH:*DMF* = 2:1); IR (KBr): $\bar{\nu} = 3098$ (*Ar*–H), 1566 (C=N), 1106 (C–F), 735 (C–Cl) cm^{-1}; ^1H NMR (400 MHz, *DMSO*-d_6): $\delta = 7.11$ (s, 1H-exocyclic vinylic), 7.61 (m, 4H-*p*-chlorophenyl), 7.78 and 7.88 (d, 1H-dichlorofluorophenyl, $J_{H-F_{ortho}} = 9$ Hz), 8.05 and 8.08 (d, 1H, dichlorofluorophenyl proton, $J_{H-F_{meta}} = 6.6$ Hz) ppm; FABMS: $m/z = 586$ (M^+, 10), 587 ($M^+ + 1$, 38), 589 ($M^+ + 2$, 60), 594 ($M^+ + 4$, 41).

7-(2,4-Dichlorobenzylidene)-3,6-bis(2,4-dichloro-5-fluorophenyl)-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine (**8d**, $C_{23}H_8Cl_6F_2N_4S$)
Yield 81%; mp 248–50°C (*Et*OH:*DMF* = 2:1); IR (KBr): $\bar{\nu} = 3090$ (*Ar*–H), 1589 (C=N), 1105 (C-F), 736 (C-Cl) cm^{-1}; ^1H NMR (400 MHz, *DMSO*-d_6): $\delta = 6.99$ (s, 1H-exocyclic vinylic), 7.63 (m, 2H-dichlorophenyl), 7.83 (m, 2H, dichlorophenyl and dichlorofluorophenyl), 7.89 (d, 1H- dichlorofluorophenyl, $J_{H-F_{ortho}} = 9.3$ Hz), 8.08 (m, 2H-dichlorofluorophenyl) ppm; FABMS: $m/z = 620$ (M^+, 10), 621 ($M^+ + 1$, 55), 589 ($M^+ + 2$, 30), 594 ($M^+ + 4$, 25).

3,6-Bis(2,4-dichloro-5-fluorophenyl)-7-(3,4-dimethoybenzylidene)-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine (**8f**, $C_{25}H_{14}Cl_4F_2N_4O_2S$)
Yield 70%; mp 196–98°C (*Et*OH:*DMF* = 2:1); IR (KBr): $\bar{\nu} = 3091$ (*Ar*–H), 1577 (C=N), 1097 (C–F) 812, 732 (C–Cl) cm^{-1}; ^1H NMR (300 MHz, *DMSO*-d_6): $\delta = 3.78$ (s, OCH_3), 3.83 (s, OCH_3), 7.01 (s, 1H-exocyclic vinylic), 7.14 (m, 2H-3,4-dimethoxyphenyl), 7.26 (d, 1H-3,4-dimethoxyphenyl, $J = 8.4$ Hz), 7.80, 7.90 (d, 1H-dichlorofluorophenyl, $J_{H-F_{ortho}} = 9$ Hz), 8.06 (m, 2H-dichlorofluorophenyl) ppm; FABMS: $m/z = 612$ (M^+, 15), 613 ($M^+ + 1$, 45).

6-(4-Chlorophenyl)-3-(2,4-dichloro-5-fluorophenyl)-7-(4-methylbenzylidene)-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine (**8g**, $C_{24}H_{14}Cl_3FN_4S$)
Yield 78%; mp 232–34°C (*Et*OH:*DMF* = 2:1); IR (KBr): $\bar{\nu} = 3088$ (*Ar*–H), 1585 (C=N), 1097 (C–F), 743 (C–Cl) cm^{-1}; ^1H NMR (300 MHz, *DMSO*-d_6): $\delta = 2.42$ (s, CH_3), 7.18 (s, 1H-exocyclic vinylic), 7.29 (d, 2H-*p*-tolyl, $J =$ 8 Hz), 7.40 (d, 2H-*p*-tolyl, $J = 8$ Hz), 7.48 (m, 3H-*p*-chlorophenyl, dichlorofluorophenyl), 7.65 (m, 3H-*p*-chlorophenyl, dichlorofluorophenyl) ppm.

6-(4-Chlorophenyl)-3-(2,4-dichloro-5-fluorophenyl)-7-(4-N,N-dimethylamino benzylidene)-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine (**8i**, $C_{25}H_{17}Cl_3FN_5S$)
Yield 75%; mp 242–44°C (*Et*OH:*DMF* = 2:1); IR (KBr): $\bar{\nu} = 3087$ (Ar–H), 1567 (C=N), 1093 (C–F) 816, 736 (C–Cl) cm^{-1}; ^1H NMR (300 MHz, *DMSO*-d_6): $\delta = 3.03$ (s, $N(CH_3)$), 6.82, 7.52 (d, 2H-N,N-dimethylamino, $J = 8.7$ Hz), 7.16 (s, 1H-exocyclic vinylic), 7.64 and 7.91 (d, 2H-*p*-chlorophenyl), 7.69 (d, 1H-dichlorofluorophenyl, $J_{H-F_{ortho}} = 9.3$ Hz), 7.93 (d, 2H-dichlorofluorophenyl, $J_{H-F_{meta}} = 6.8$ Hz) ppm; FABMS: $m/z = 544$ (M^+).

Analgesic assay
Male Albino mice of either sex with weight between 20 and 25 g were used for analgesic study. The animals were divided into 12 experimental groups each consisted of 6 animals. Gum acacia (2%) was administered to group 1. Group 2 received Pethidine at a dose 5 mg/kg by intraperitoneal injection. Other groups were given the test compounds (**8a**, **8b**, **8c**, **8d**, **8e**, **8f**, **8g**, **8h**, **8i**, and **8k**) at a dose of 50 mg/kg orally. The animals were housed and fed in laboratory kept at constant temperature of 22°C under standard conditions (12:12 h light-dark cycle, standard pellet diet, tap water). In this test, the reaction of mice to painful stimulus was measured. Mice were placed on the metal plate heated to 55 ± 0.4°C and covered with a glass cylinder (25 cm high, 15 cm in diameter). The time(s) elapsing to the first pain response (licking or jumping) was determined and then recorded as response latency, *prior to* 60, 180 min following the *po* administration of the investigated compounds. Institutional ethics committee approved all the experiments.

Antibacterial assay
The newly prepared compounds were screened for their antibacterial activity against five bacterial strains by disc diffusion method. A standard inoculum ($1–2 \times 10^7$ c.f.u./cm^3 0.5 *McFarland* standards) was introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculum. The discs measuring 6.25 mm in diameter were prepared from *Whatman* no. 1 filter paper and sterilized by dry heat at 140°C for 1 h. The sterile disc previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were kept. The plates were inverted and incubated for 24 h at 37°C. The inhibition zones were measured and compared with the controls. Minimum inhibitory concentration (*MIC*) was determined by broth dilution technique. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls were inoculated with approximately 5×10^5 c.f.u. of actively dividing bacteria cells. The cultures were incubated for 24 h at 37°C and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest

the growth of bacteria was regarded as minimum inhibitory concentrations (*MIC*). Norfloxacin was used as the standard drug.

Antifungal assay

The newly prepared compounds were screened for their antifungal activity against five fungal strains by the agar diffusion method. *Sabouraud*'s agar media was prepared by dissolving 1 g peptone, 4 g D-glucose, and 2 g agar in 100 cm^3 distilled water, and adjusting *pH* to 5.7 using buffer. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 cm^3 saline to get a suspension of corresponding species. 20 cm^3 of agar media was poured into each *Petri* dish. Excess of suspension was decanted and the plates were dried by placing them in an incubator at 37°C for 1 h. Using an agar punch, wells were made and each well was labeled. A control was also prepared in triplicate and maintained at 37°C for 3–4 d. The inhibition zones in diameter were measured and compared with the controls. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls were inoculated with approximately 1.6×10^4–6×10^4 c.f.u. cm^{-3}. The cultures were incubated for 48 h at 35°C and the growth was monitored. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as minimum inhibitory concentrations (*MIC*). Griseofulvin was used as the standard drug.

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