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Synthesis and biological evaluation of dihydroindeno and indeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazines as antimicrobial agents

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ABSTRACT

Two series of compounds namely, dihydroindeno and indeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadizines (**9a**–**l** & **11a-l**) were synthesized by cyclocondensation between α-bromoindanones (**7a**–**b**) or/and α,α-dibromoindanones (**8a**–**b**) and various 3-alkyl/aryl-4-amino-5-mercapto-1,2,4-s-triazoles (**3a**–**f**) in methanol with an aim to explore their effect on *in vitro* growth of microorganism causing microbial infection. *In vitro* antibacterial activity was performed against four strains namely, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and antifungal activity against three fungal strains namely, *Aspergillus niger*, *Aspergillus flavus*, *Penicillium species*. Of all the compounds screened for activity some of the compounds were associated with considerably higher antibacterial and antifungal activity than commercial antibiotics.

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1. Introduction

Synthesis of nitrogen containing heterocyclic systems has been attracting increasing interest over the past decade because of their utility in various applications. In recent years, a special attention has been given to nitrogen bridged heterocyclic systems derived from 1,2,4-triazoles owing to their promising biological activities as antihypertensive, antimicrobial, anticonvulsant, antidepressant, analgesic and antitumour [1–13] etc. The fused ring of triazoles and thiadiazines called triazolothiadiazines represent an important class of nitrogen bridgehead heterocyclic compounds with wide range of activities such as antifungal, antibacterial, antiviral, anthelmintic, antitumour, antitubercular, diuretics, analgesic, anti-inflammatory, antiviral, CNS-stimulant, PDE4 inhibitors and hypoglycemic agents [14–29], etc.

These results inspired us to synthesize compounds containing a system which involves combination of these pharmacophores in one molecular framework to give the title structure for screening their antimicrobial activities. Synthesis of 1,3,4-thiadiazines is mainly based on cyclocondensation of heterocyclic amino thiols with bifunctional reagents such as α-halo/tosyloxy carbonyl compounds, dihalides, α-halo nitriles [30–38]. α-Halo ketones are among the most versatile intermediates in organic synthesis [39–44] and their high reactivity makes them prone to react with large number of nucleophiles to provide a variety of biologically active heterocyclic compounds. In an important development, it has been observed that α,α-dihalo ketones behave as synthetic equivalents of their corresponding α-halo ketones [45–51]. Being non-lachrymatory, generally solid at room temperature and soluble in commonly used reaction solvents, these compounds are easy to work with and can be handled easily.

In view of the above facts, it was considered worthwhile to extend the application of α-halo and α,α-dihalo ketones based methodology to the synthesis of two series of polycyclic fused thiadiazines containing 1,2,4-triazoles namely, dihydroindeno and indeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazines of the type **9** and **11** to evaluate their antimicrobial activity. The structure of the compounds **9** and **11** were modified from 6-phenyl-7*H*-[1,2,4]

Abbreviations: MIC, Minimum inhibitory concentration; *B. subtilis*, *Bacillus subtilis*; *E. coli*, *Escherichia coli*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *S. aureus*, *Staphylococcus aureus*; *A. niger*, *Aspergillus niger*; *A. flavus*, *Aspergillus flavus*; *Penicillium sp.*, *Penicillium species*; s, singlet; d, doublet; t, triplet; dd, doublet of doublet; m, multiplet; dec, decomposition.

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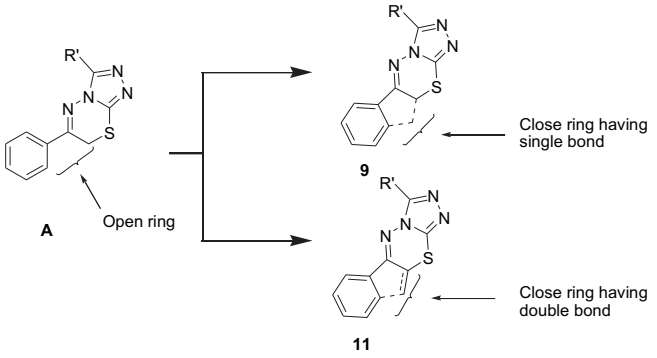
triazolo [3,4-b] [1,3,4]thiadiazines (**A**), which were known to possess good antimicrobial activity (Scheme 1).



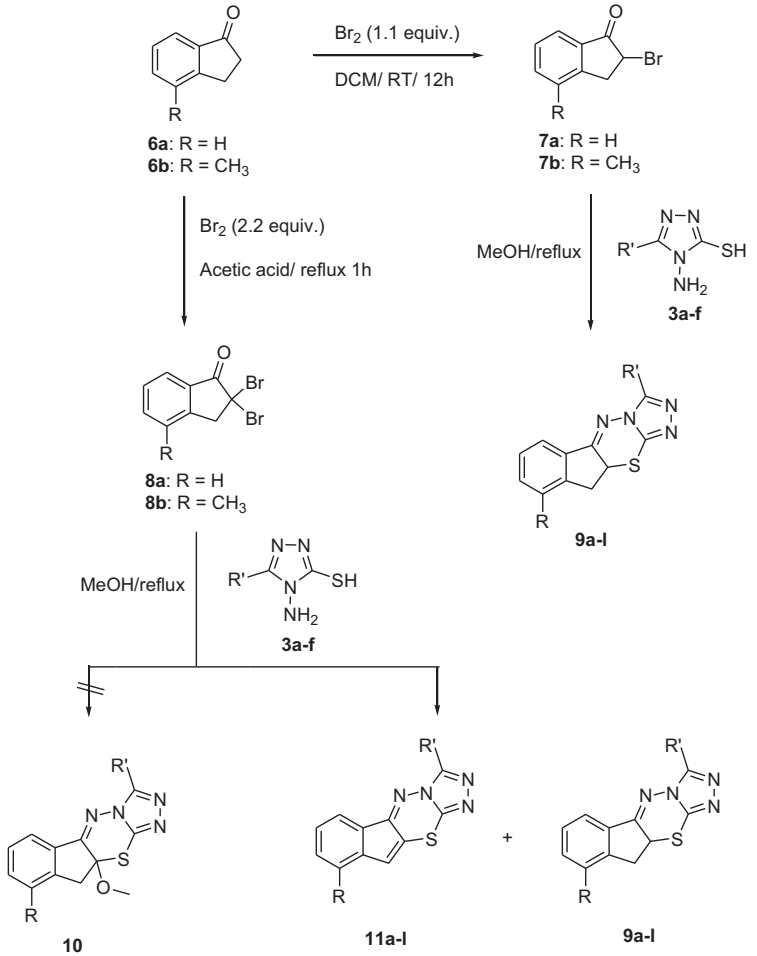
Scheme 1. Modification in the series.

2. Results and discussion

2.1. Chemistry

First, we synthesized α-bromo and α,α-dibromoindanone by using bromine (1.1 eq) in dichloromethane (DCM) and by refluxing indanone with bromine (2.2 eq) in acetic acid. Then, we performed the reaction of α-bromoindanones (**7a**–**b**) with 3-alkyl/aryl-4-amino-5-mercapto-1,2,4-s-triazoles (**3a**–**f**) in methanol. IR spectra of the products showed disappearance of bands corresponding to CO and NH_2 stretch and characterization by the spectral (^{1}H NMR, ^{13}C NMR, IR) and elemental analytical data of the products confirmed the formation of 3-alkyl/aryl-9-methyl-10,10a-dihydroindeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazines (**9a**–**l**) (Scheme 2) [59]. For example, ^{1}H NMR of the compound **9h** showed dd of three protons at δ value ranges between 2.850 and 4.236 ppm apart from other aliphatic and aromatic signals (see Supplementary materials).

Then, we carried out reaction of α,α-dibromoindanones (**8a**–**b**) with 3-alkyl/aryl-4-amino-5-mercapto-1,2,4-s-triazoles (**3a**–**f**). During the progress of the reaction, it was observed that colour of the reaction mixture changed from colourless to red. The reactions were monitored by tlc and the formation of two products were observed in each case. Column chromatography using silica gel (100–200 mesh) as stationary phase and pet ether (boiling range 60–80 °C)/ethyl acetate as mobile phase was run for separation of mixture of products. From tlc comparison, data analysis and melting points determination it was found that the one of the product **9** in all cases was identical to that obtained from cyclocondensation of α-bromoindanones (**7a**–**b**) and substituted 3-alkyl/aryl-4-amino-5-mercapto-1,2,4-s-triazoles (**3a**–**f**). The complete spectral (^{1}H NMR, ^{13}C NMR, IR) and elemental analytical data of the second product confirmed the formation of new 3-alkyl/aryl-9-methylindeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazines (**11a**–**l**) rather than expected alkoxy derivatives (**10**) (Scheme 2) as



Scheme 2. Synthesis of the title compounds **9a**–**l** and **11a**–**l**.

reported earlier in the case of α,α-dibromoacetophenones (**2**) [38] (Scheme 3). A careful comparison of ^{1}H NMR of **11a**–**l** and **9a**–**l** showed that doublet of doublet (dd) of three protons which appeared at δ value in the range of 3.1–3.3, 3.6–3.8 and 4.1–4.4 of **9a**–**l** disappeared in **11a**–**l**. Instead, a singlet integrating to one proton appeared in the range of 6.9–7.1. For example, ^{1}H NMR of the compound **11h** showed a singlet at δ value 7.082 ppm apart from other characteristic signals (see Supplementary materials).

It is worthwhile here to mention that the reaction of α-bromoacetophenone (**1**) with 4-amino-5-mercapto-s-triazoles (**3**) in ethanol under reflux gave triazolothiadiazines (**4**) which is reported in the literature [37]. In our previous work dealing with the reactivity of α,α-dibromoacetophenones (**2**) with 4-amino-5-mercapto-s-triazoles (**3**), we arrived at the result that fate of reaction is decided by the alcohol employed in the reaction (Scheme 3) [38]. In case of ethanol and *i*-propanol only alkoxy derivatives **5** were formed. However in case of methanol and propanol, in addition to alkoxy derivatives, reduced products were also formed (Scheme 3). In view of this, we continued our studies and performed the reaction of α-bromoindanones and α,α-dibromoindanones (**8a**–**b**) with various substituted 3-alkyl/aryl-4-amino-5-mercapto-1,2,4-s-triazoles (**3a**–**f**). Based on our earlier observations, it was anticipated that this reaction might afford triazolothiadiazines **9** and their alkoxy derivatives **10**. However, the reaction did not follow similar trends and in this case in addition to expected dihydroindeno triazolothiadiazines **9**, we obtained new indeno triazolothiadiazines **11** (Scheme 2).

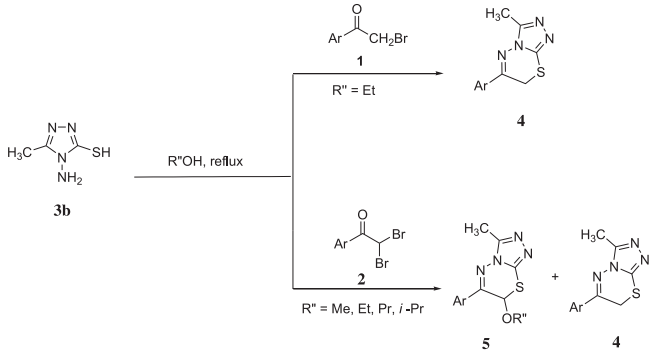
Possible mechanistic pathways for the conversions of **8** to **9** and **11** are analogous to the literature report on the reactions of α,α-dibromoacetophenones (**2**) (For detailed mechanism see Supplementary materials) [45,52,53].

The physical data of all the synthesized compounds are summarized in Table 1.

2.2. Biological results and discussion

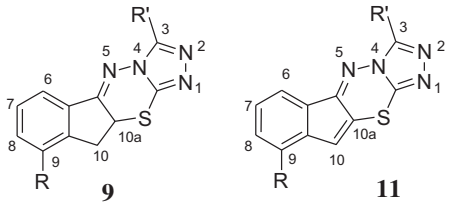
2.2.1. In vitro antibacterial activity

All the twenty four compounds **9a**–**l** and **11a**–**l** were screened for their *in vitro* antibacterial activity against two Gram-positive bacteria namely *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 121) and two Gram-negative bacteria namely *Escherichia coli* (MTCC 1652), *Pseudomonas aeruginosa* (MTCC 741) by agar well diffusion method (Table 2). Standard antibiotics namely ciprofloxacin was used for comparison of antibacterial activity shown by compounds **9a**–**l** and **11a**–**l**. The results were recorded for each tested compound as average diameter of zone of inhibition of bacterial growth adjoining the well in millimetres (Table 2, Fig. 1). Minimum inhibitory concentration (MIC) measurements were



Scheme 3. Reaction of **3b** with α-bromoacetophenones **1** and α,α-dibromoacetophenones **2**.

Table 1
Physical data of the synthesized compounds **9a**–**l** and **11a**–**l**.



Entry	R	R′	Product	Time (h)	Yield (%)	Mp (°C)
1	H	H	**9a**	5.0	92[a]/50[b]	215–216
2	H	Me	**9b**	5.0	90[a]/60[b]	154–155
3	H	Et	**9c**	4.5	93[a]/62[b]	115–116
4	H	Pr	**9d**	4.0	84[a]/55[b]	129–130
5	H	*i*-Pr	**9e**	4.5	86[a]/40[b]	114–115
6	H	Ph	**9f**	5.0	89[a]/58[b]	214–215
7	Me	H	**9g**	5.0	90[a]/52[b]	207–208
8	Me	Me	**9h**	5.0	92[a]/62[b]	219–220
9	Me	Et	**9i**	4.5	91[a]/63[b]	223–225
10	Me	Pr	**9j**	4.0	84[a]/58[b]	80–81
11	Me	*i*-Pr	**9k**	4.5	83[a]/32[b]	187–188
12	Me	Ph	**9l**	5.0	88[a]/60[b]	~255 (dec)
13	H	H	**11a**	7.0	25[b]	154–156
14	H	Me	**11b**	6.0	30[b]	163–165
15	H	Et	**11c**	6.5	32[b]	130–132
16	H	Pr	**11d**	6.0	31[b]	133–135
17	H	*i*-Pr	**11e**	6.5	25[b]	~160 (dec)
18	H	Ph	**11f**	6.5	30[b]	169–171
19	Me	H	**11g**	6.0	27[b]	202–204
20	Me	Me	**11h**	6.5	28[b]	239–241
21	Me	Et	**11i**	7.0	30[b]	167–169
22	Me	Pr	**11j**	6.5	32[b]	151–152
23	Me	*i*-Pr	**11k**	6.5	22[b]	~165 (dec)
24	Me	Ph	**11l**	6.5	28[b]	210–212

[a] Isolated yield obtained from the reaction between **3** and **7**.
[b] Isolated yield obtained from the reaction between **3** and **8**.

Table 2
In vitro antibacterial activity of the compounds **9a**–**l** and **11a**–**l**.

Entry	Compounds	Diameter of growth of inhibition zone (mm)[a]			
		Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*
1	**9a**	15.3	17.6	–	–
2	**9b**	14.6	16.3	–	–
3	**9c**	25.3	21.6	18.3	16.0
4	**9d**	27.3	25.0	19.3	18.6
5	**9e**	28.6	24.6	20.6	19.3
6	**9f**	20.6	16.3	18.3	14.6
7	**9g**	26.3	30.6	22.3	20.3
8	**9h**	27.6	31.3	20.6	18.6
9	**9i**	26.6	28.0	21.0	19.3
10	**9j**	25.3	21.6	19.3	17.6
11	**9k**	28.6	27.0	21.6	20.3
12	**9l**	22.3	21.6	17.3	15.6
13	**11a**	14.3	16.0	–	–
14	**11b**	14.6	15.3	–	–
15	**11c**	16.3	17.6	–	–
16	**11d**	25.6	24.3	18.6	16.3
17	**11e**	15.6	17.3	–	–
18	**11f**	14.6	15.0	–	–
19	**11g**	25.3	28.6	20.6	18.3
20	**11h**	20.3	18.6	18.6	15.6
21	**11i**	21.6	23.0	19.3	17.3
22	**11j**	16.3	18.6	15.6	14.3
23	**11k**	17.6	19.3	15.0	14.6
24	**11l**	18.6	22.6	15.6	15.3
25	Ciprofloxacin	26.6	24.0	25.0	22.0

–: No activity.
[a] Values including diameter of the well (8 mm) are means of three replicates.

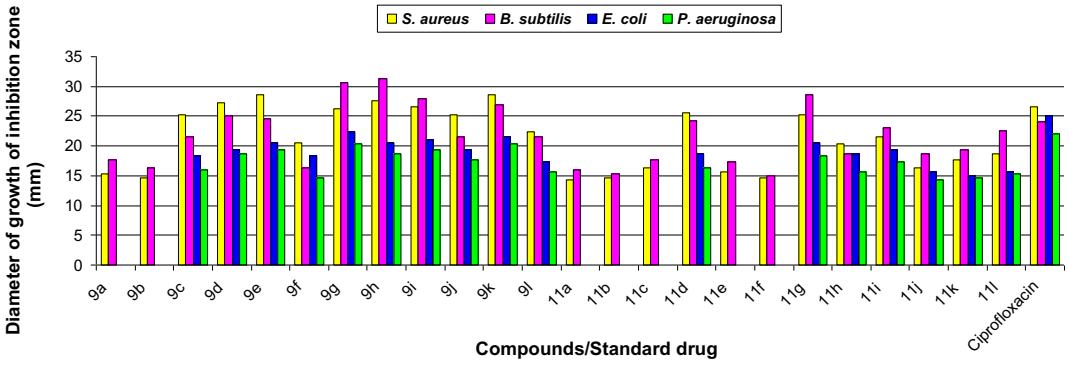


Fig. 1. Comparisons of diameter of growth of inhibition zone (mm) of compounds **9a**–**l** and **11a**–**l** with standard drug ciprofloxacin.

performed using a modified agar well diffusion method (Table 3, Fig. 2). MIC of those compounds was determined which were showing activity in primary screening.

Results revealed that in general, all the tested compounds possessed moderate to excellent antibacterial activity against both Gram-positive (*S. aureus*, *B. subtilis*) and Gram-negative bacteria (*E. coli*, *P. aeruginosa*). However, out of the twenty four compounds tested only seven compounds **9a**–**b**, **11a**–**c** and **11e**–**f** did not show activity against Gram-negative bacteria (*E. coli*, *P. aeruginosa*). On the basis of zone of inhibition against test bacterium, eleven compounds **9c**–**e**, **9g**–**l**, **11d** and **11h** showed excellent activity against *S. aureus* showing the maximum zone of inhibition >25.0 mm as compared with standard drug ciprofloxacin which showed the zone of inhibition of 26.6 mm against *S. aureus*. It should be noted that out of these eleven compounds, four compounds **9d**–**e**, **9i** and **9k** were found to be most potent member showing zone of inhibition against *S. aureus* even greater than the standard drug (Table 2, Fig. 1).

Similarly, on the basis of zone of inhibition thirteen compounds **9c**–**e**, **9g**–**l**, **11d**, **11g**, **11i**, and **11l** were found to be most effective against *B. subtilis* showing maximum zone of inhibition >20.0 mm as compared to standard drug ciprofloxacin which showed the zone of inhibition of 24.0 mm against *B. subtilis*. In this case also, out of these thirteen compounds eight compounds **9d**–**e**, **9g**–**i**, **9k, 11d** and **11g** showed greater activity than the standard drug. However, in case of the Gram-negative bacteria all the compounds showed moderate to good antibacterial activity against the test bacteria *E. coli* and *P. aeruginosa* as shown in Table 2. In case of *E. coli*, six compounds **9e**, **9g**–**i**, **9k** and **11g** showed maximum zone of inhibition >20 mm in comparison to ciprofloxacin which showed 25 mm. Furthermore, in case of *P. aeruginosa*, seven compounds **9d**–**e**, **9g**–**i**, **9k** and **11g** sowed the maximum zone of inhibition >18 mm in comparison to standard drug, ciprofloxacin, which showed 22 mm (Table 2, Fig. 1).

In the whole series, MIC of the synthesized compounds ranged between 2 and 256 μg/mL against the Gram-positive bacteria (Table 3, Fig. 2). In case of *S. aureus*, compounds **9d**–**e**, **9g**–**i**, **9k** are most potent member having MIC ranged between 4 and 8 μg/mL in comparison to standard drug having MIC of 5 μg/mL. Other compounds showing good activity are **9c**, **9j** and **11d** having MIC of 16 μg/mL and the compounds showing moderate activity are **9c**, **9j**, **9l**, **11i** and **11l** having MIC of 32 μg/mL. In case of *B. subtilis*, most potent members having MIC ranged between 2 and 8 μg/mL are **9g**–**i**, **9k** and **11g**. Other compounds showing good to moderate activity having MIC ranged between 16 and 32 μg/mL are **9c**–**e**, **9j**, **9l**, **11d**, **11i**, and **11l**. All other compounds showed reasonable activity against Gram-positive bacteria. In case of Gram-negative bacteria, MIC ranged between 32 and 256 μg/mL and the most potent members among all the tested compounds are **9g**, **9i** and **9k** having MIC of 32 μg/mL (Table 3, Fig. 2).

A comparison of the activity data of the compounds **9a**–**l** and **11a**–**l** indicate that the compounds **9a**–**l** are more potent than the compounds **11a**–**l**. Further, it is also noteworthy that the substituted indanones **9g**–**l** are more biologically active than parent indanones (**9a**–**f**).

Table 3
MIC of the compounds **9a**–**l** and **11a**–**l**.

Entry	Compounds	Minimum inhibitory concentration (MIC) in μg/mL			
		Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*
1	**9a**	128	128	–	–
2	**9b**	256	128	–	–
3	**9c**	16	32	64	128
4	**9d**	8	16	64	64
5	**9e**	4	16	64	32
6	**9f**	64	128	64	256
7	**9g**	8	2	32	32
8	**9h**	8	2	64	64
9	**9i**	8	4	32	32
10	**9j**	16	32	64	64
11	**9k**	4	8	32	32
12	**9l**	32	32	128	128
13	**11a**	256	128	–	–
14	**11b**	256	256	–	–
15	**11c**	128	128	–	–
16	**11d**	16	16	64	128
17	**11e**	128	128	–	–
18	**11f**	256	256	–	–
19	**11g**	16	4	64	128
20	**11h**	64	64	64	128
21	**11i**	32	32	64	64
22	**11j**	128	64	128	256
23	**11k**	128	64	128	256
24	**11l**	64	32	128	128
25	Ciprofloxacin	5	5	5	5

–: Not tested.

2.2.2. Antifungal activity

All the twenty four compounds **9a-l** and **11a-l** were also tested for their *in vitro* antifungal activity against three fungal strains, namely, *A. niger*, *A. flavus* and *Penicillium species* through poisoned food method. Standard drug fluconazole was used for comparison with antifungal activity shown by the compounds **9a**–**l** and **11a**–**l** and results were recorded as percentage (%) of mycelial growth inhibition. It has been revealed from the Table 4 that all the compounds **9a**–**l** and **11a**–**l** showed variable antifungal activity against three pathogens. From the careful comparison of the results, it has been revealed that mainly the three compounds **9h**, **11g** and **11j** showed excellent antifungal activity with >65% inhibition of mycelial growth against all the

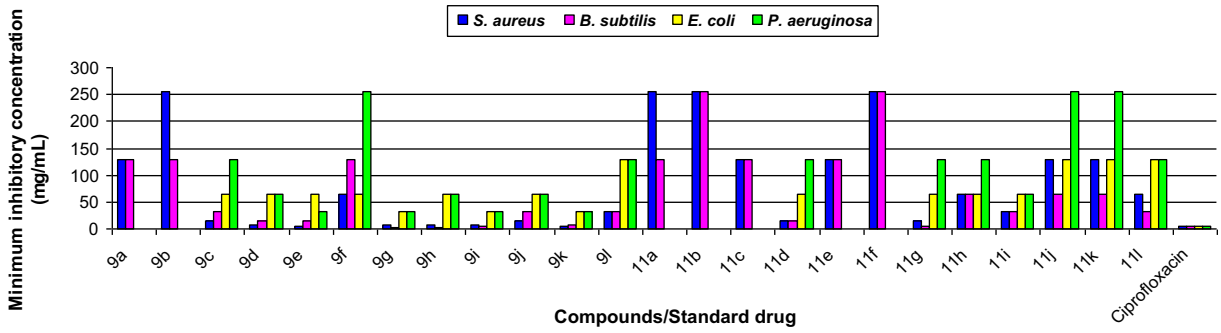


Fig. 2. Comparison of MIC of the compounds **9a**–**l** and **11a**–**l**.

Table 4
In vitro antifungal activity of the compounds **9a**–**l** and **11a**–**l**.

Entry	Compounds	Mycelial growth inhibition (%)		
		Aspergillus niger	*Aspergillus flavus*	*Penicillium species*
1	**9a**	51.1	52.5	51.1
2	**9b**	53.3	51.1	55.5
3	**9c**	58.8	55.5	57.7
4	**9d**	55.5	52.5	53.3
5	**9e**	53.3	51.1	51.1
6	**9f**	51.1	48.8	50.0
7	**9g**	63.3	65.5	62.5
8	**9h**	67.7	68.8	67.7
9	**9i**	64.4	63.3	65.5
10	**9j**	58.8	55.5	61.1
11	**9k**	63.3	61.1	65.5
12	**9l**	55.5	58.8	60
13	**11a**	53.3	50	51.1
14	**11b**	50.0	48.8	52.5
15	**11c**	48.8	45.5	50.0
16	**11d**	51.1	48.8	47.7
17	**11e**	48.8	50	44.4
18	**11f**	45.5	48.8	51.1
19	**11g**	71.1	68.8	66.6
20	**11h**	53.3	50	51.1
21	**11i**	65.5	62.5	63.3
22	**11j**	68.8	65.5	65.5
23	**11k**	65.5	61.1	63.3
24	**11l**	55.5	51.1	48.8
25	Fluconazole	81.1	77.7	83.3

three fungi in comparison with the standard drug (*Aspergillus niger* 81.1%, *Aspergillus flavus* 77.7% and *Penicillium* sp. 83.3%). In addition to these, three more compounds which showed >65% inhibition are **9g**, **9i** and **11i** against *A. flavus*, *Penicillium* sp. and *A. niger* respectively. There are many other compounds which showed good and fair antifungal activities are summarized in Table 4. Comparisons of antifungal activity of all the synthesized compounds with reference drug in terms of % mycelial growth inhibition are also shown in Fig. 3.

3. Conclusion

In conclusion, the present study offers an application of α-bromo and α,α-dibromoindanones in an efficient and convenient synthesis of dihydroindeno and indeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazines (**9** and **11**). Biological evaluation of these compounds proves them potent antimicrobial agents. Some of the compounds tested are superior to the reference drug. Thus, the present work will have a good impact on chemist and bio-chemist and can be further used in pharmaceutical industry for mankind, as an antimicrobial agent, after testing its toxicity to human beings.

4. Experimental

4.1. General

Melting points were taken on slides in an electrical apparatus Labindia visual melting range apparatus and are uncorrected. Benzoic acid was taken as reference for calibration of instrument. The infrared spectra (IR) were recorded on a Perkin–Elmer 1800 FT-IR spectrophotometer using KBr Pellets technique. The ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on Bruker Nuclear Magnetic Resonance (NMR) spectrometer at 300 and 75 MHz respectively, using tetramethylsilane (TMS) as an internal standard. Chemical shifts are expressed in δ ppm. Purification of the compounds was done by the column chromatography using silica gel (100–200 mesh) as stationary phase and petroleum ether/ethyl acetate mixture as eluent. The purity of the compounds were checked by elemental analysis and thin layer chromatography (TLC) using silica gel aluminium plates of Merck using a mixture of petroleum ether and ethyl acetate as mobile phase. Iodine and

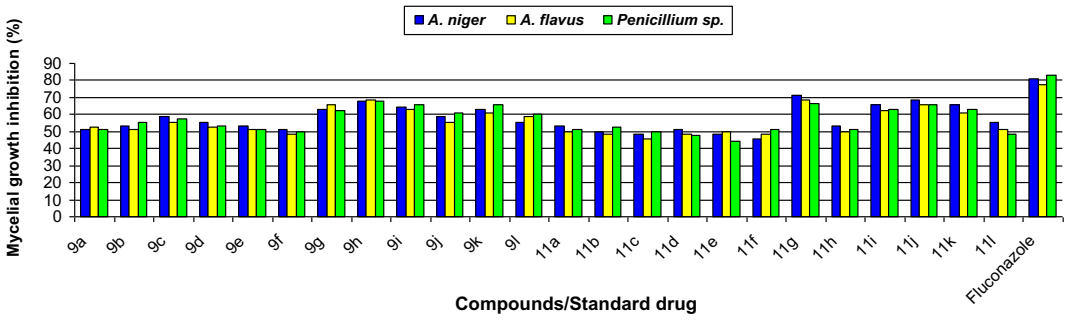


Fig. 3. Comparisons of antifungal activity of compounds **9a**–**l** and **11a**–**l** with standard drug fluconazole.

Ultraviolet (UV) lamp was used for visualisation of the compounds. All other chemicals used as such as procured from supplier. The physical data of all the synthesized compounds are summarized in Table 1.

4.2. Synthesis of α-bromoindanones (**7a**–**b**)

4.2.1. 2-Bromo-2,3-dihydroinden-1-one (**7a**)

To a solution of 1-indanone **(6a**, 5 g, 37.8 mmol) in 50 mL DCM, bromine (5.5 g, 34.3 mmol) was added and the reaction mixture was stirred at room temperature for 12 h. Reaction was monitored by thin layer chromatography. After completion of reaction the reaction mixture was quenched with water and separated the organic layer. The organic layer was washed with saturated aqueous sodium metabisulphite solution (30 mL × 3), brine and finally with water. After evaporation of solvent a solid was obtained in good yield (**7a**, 7.29 g, 83%), Mp. 40–42 °C(Lit. Mp.39–40 °C) [39–41].

The other derivative **7b** was prepared in a similar manner.

4.2.2. 2-Bromo-2,3-dihydro-4-methylinden-1-one (**7b**)

Mp. 69–71 °C; yield 80%; IR (ν_{max}, cm^{-1}): 1720 (C=O); ^1H NMR (δ, $CDCl_3$, 300 MHz,): 7.707–7.682 (d, 1H, ArH, J = 7.5 Hz), 7.506–7.481 (m, 1H, ArH), 7.393–7.368 (m, 1H, ArH), 4.698–4.663 (dd, 1H, J_1 = 3.0 Hz, J_2 = 7.5 Hz), 3.797–3.712 (dd, 1H, J_2 = 7.5 Hz, J_3 = 18 Hz), 3.349–3.289 (m, 1H), 2.360 (s, 3H, CH_3).

4.3. Synthesis of α,α-dibromoindanones (**8a**–**b**)

4.3.1. 2,2-Dibromo-2,3-dihydroinden-1-one (**8a**)

To a solution of 1-indanone **(6a**, 5 g, 37.8 mmol) in acetic acid (50 mL), bromine (13.3 g, 83.3 mmol) was added and the solution was refluxed for 1 h. After cooling to room temperature, the solution was poured on the crushed ice (100 g). A solid thus obtained was filtered and washed with cold water and dried to give the desired product **8a** (9.66 g, 88%). Mp. 133 °C (Lit. Mp. 131–134 °C) [42–44].

The other derivative **8b** was prepared in a similar manner.

4.3.2. 2,2-Dibromo-2,3-dihydro-4-methylinden-1-one (**8b**)

Mp. 66–68 °C; yield 85%; IR (ν_{max}, cm^{-1}): 1728 (C=O str.);^1H NMR (δ, $CDCl_3$, 300 MHz): 7.807–7.782 (d, 1H, ArH, J = 7.5 Hz), 7.566–7.542 (d, 1H, ArH, J = 7.2 Hz), 7.45–7.4 (m, 1H, ArH), 4.213 (s, 2H, CH_2), 2.337 (s, 3H, CH_3).

4.4. Synthesis of 3-alkyl/aryl-9-methyl-10,10a-dihydroindeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazines (**9a**–**l**)

4.4.1. Typical procedure

To a solution of **7a** (1g, 3.31 mmol) in 30 mL ethanol was added 4-amino-5-mercapto-1,2,4-triazole (**3a,** 0.384 g, 3.31 mmol) and the reaction mixture was refluxed for 4–5 h. The reaction mixture was poured onto crushed ice (100 g), followed by basification with ammonia solution. The solid product thus obtained was filtered, washed with water and recrystalized from ethanol to give pure 10,10a-dihydroindeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazine (**9a,** 0.67 g, 90%).

All other derivatives were synthesized by adopting the similar procedure.

*4.4.1.1. 10,10a-Dihydroindeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4] thiadiazine (**9a**).* IR (ν_{max}, cm^{-1}): transparent in the region of C=O str., 1628, 1605, 1481, 1443, 1366, 1304, 1281, 1287, 1188, 1157, 1095; ^1H NMR (δ, $CDCl_3$, 300 MHz): 8.758 (s, 1H, triazole H), 7.954 (m, 1H, ArH), 7.601–7.494 (m, 3H, ArH), 4.414–4.398 (m, 1H), 3.823–3.731 (m, 1H), 3.193–3.026 (m, 1H); ^{13}C NMR: 163.15, 158.45, 147.56, 133.90, 132.92, 128.72, 125.85, 123.67, 37.01, 35.06; Anal. Calcd. for $C_{11}H_8N_4S$: C 57.88, H 3.53, N 24.54; Found: C 57.80, H 3.50, N 24.44.

*4.4.1.2. 3-Methyl-10,10a-dihydroindeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazine (**9b**).* IR (ν_{max}, cm^{-1}): transparent in the region of C=O str., 1612, 1535, 1466, 1435, 1373, 1304, 1203, 1149, 1095; ^1H NMR (δ, $CDCl_3$, 300 MHz): 7.981 (bs, 1H, ArH), 7.599–7.489 (m, 3H, ArH), 4.163 (bs, 1H), 3.759–3.705 (m, 1H), 3.177–3.127 (m, 1H), 2.633 (s, 3H, CH_3); ^{13}C NMR: 163.46, 1587.23, 147.42, 133.57, 133.33, 128.53, 125.80, 123.57, 36.28, 34.95, 18.40; Anal. Calcd. for $C_{12}H_{10}N_4S$: C 59.48, H 4.16, N 23.12; Found: C 57.44, H 4.10, N 23.10.

*4.4.1.3. 3-Ethyl-10,10a-dihydroindeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazine (**9c**).* IR (ν_{max}, cm^{-1}): transparent in the region of C=O str., 1605, 1528, 1458, 1381, 1311, 1273, 1188, 1157, 1095; ^1H NMR (δ, $CDCl_3$, 300 MHz): 8.005–7.979 (d, 1H, ArH, J = 7.8 Hz), 7.627–7.580 (m, 1H, ArH), 7.496 (m, 2H, ArH), 4.243–4.153 (m, 1H), 3.763–3.677 (dd, 1H, J_1 = 8.4 Hz, J_2 = 17.4 Hz), 3.193–2.946 (m, 3H), 1.481–1.431 (t, 3H, CH_3, J = 7.5 Hz), ^{13}C NMR: 162.36, 158.32, 147.42, 133.57, 133.33, 128.53, 125.80, 123.57, 36.28, 34.95, 18.40, 11.30; Anal. Calcd. for $C_{13}H_{12}N_4S$: C 60.91, H 4.72, N 21.86; Found: C 60.88, H 4.67, N 21.78.

*4.4.1.4. 3-Propyl-10,10a-dihydroindeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazine (**9d**).* IR (ν_{max}, cm^{-1}): transparent in the region of C=O str., 1605,1528, 1458, 1389, 1311, 1265, 1196, 1149, 1095, 1057, 1026; ^1H NMR (δ, $CDCl_3$, 300 MHz): 7.980 (m, 1H, ArH), 7.578–7.475 (m, 3H, ArH), 4.166 (m, 1H), 3.696 (m, 1H), 3.160–3.127 (m, 1H), 2.975 (m, 2H, CH_2), 1.886 (m, 2H, CH_2), 1.069 (m, 3H, CH_3); ^{13}C NMR: 162.61, 154.26, 147.43, 139.32, 133.58, 133.52, 128.52, 125.80, 123.56, 36.29, 34.94, 26.57, 20.48, 13.84; Anal. Calcd. for $C_{14}H_{14}N_4S$: C 62.20, H 5.22, N 20.72; Found: C 62.17, H 5.18, N 20.69.

*4.4.1.5. 3-Isopropyl-10,10a-dihydroindeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazine (**9e**).* IR (ν_{max}, cm^{-1}): transparent in the region of C=O str., 1605, 1520, 1458, 1389, 1304, 1196, 1157, 1095; ^1H NMR (δ, $CDCl_3$, 300 MHz): 7.976–7.951 (d, 1H, ArH, J = 7.5 Hz), 7.585–7.560 (d, 1H, ArH, J = 7.5 Hz), 7.489–7.415 (m, 2H, ArH), 4.195–4.149 (dd, 1H, J_1 = 5.4 Hz, J_3 = 8.4 Hz), 3.746–3.662 (m, 1H), 3.448–3.378 (m, 1H), 3.161–3.086 (dd, 1H, J_1 = 5.4 Hz, J_2 = 17.1 Hz), 1.502–1.491 (d, 3H, CH_3, J = 7.5 Hz), 1.426–1.402 (d, 3H, CH_3, J = 7.5 Hz); ^{13}C NMR: 162.11, 158.40, 147.47, 139.63, 133.60, 133.31, 128.52, 125.83, 123.52, 36.21, 34.95, 25.50, 20.81, 19.79; Anal. Calcd. for $C_{14}H_{14}N_4S$: C 62.20, H 5.22, N 20.72; Found: C 62.18, H 5.18 N 20.66.

*4.4.1.6. 3-Phenyl-10,10a-dihydroindeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazine (**9f**).* IR (ν_{max}, cm^{-1}): transparent in the region of C=O str., 1597, 1466, 1366, 1296, 1196, 1165, 1103, 1072, 1026; ^1H NMR (δ, $CDCl_3$, 300 MHz): 8.209 (m, 2H, ArH), 8.015–7.992 (m, 1H, ArH), 7.612–7.489 (m, 6H, ArH), 4.232 (bs, 1H), 3.815–3.731 (dd, 1H, J_1 = 8.1 Hz, J_2 = 17.1 Hz), 3.220–3.163 (m, 1H); ^{13}C NMR: 163.05, 152.18, 147.63, 140.97, 133.81, 133.21, 130.23, 128.61, 128.57, 128.25, 126.21, 125.87, 123.78, 35.86, 34.98; Anal. Calcd. for $C_{17}H_{12}N_4S$: C 67.08, H 3.97, N 18.41; Found: C 67.02, H 3.93, N 18.38.

*4.4.1.7. 9-Methyl-10,10a-dihydroindeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazine (**9g**).* IR (ν_{max}, cm^{-1}): transparent in the region of C=O str., 1597, 1481, 1443, 1373, 1288, 1149, 1065, 1034; ^1H NMR (δ, $CDCl_3$, 300 MHz): 8.637 (s, 1H, triazole H), 7.777–7.620 (bs, 1H, ArH), 7.415–7.312 (m, 2H, ArH), 4.250–4.207 (m, 1H), 3.696–3.612 (dd, 1H, J = 8.1 Hz, J = 16.5 Hz), 3.137–3.005 (m, 1H), 2.377 (s, 3H, CH_3); ^{13}C NMR: 163.80, 146.72, 142.25, 139.96, 135.44, 134.48, 132.60, 128.97, 121.01, 36.91, 33.92, 18.35; Anal. Calcd. for $C_{12}H_{10}N_4S$: C 59.48, H 4.16, N 23.12; Found: C 59.44, H, 4.10, N 23.08.

*4.4.1.8. 3-Methyl-9-methyl-10,10a-dihydroindeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazine (**9h**).* IR (ν_{max}, cm^{-1}): transparent in the region of C=O str., 1597, 1535, 1458, 1373, 1311, 1203, 1057; ^1H NMR (δ, $CDCl_3$, 300 MHz): 7.851–7.802 (m, 1H, ArH); 7.412–7.398 (m, 2H, ArH), 4.184–4.138 (dd, 1H, J_1 = 5.4 Hz, J_3 = 8.4 Hz), 3.690–3.605 (dd, 1H, J_2 = 16.8 Hz, J_3 = 8.4 Hz), 3.053–2.979 (dd, 1H, J_1 = 5.4 Hz, J_2 = 16.8 Hz), 2.636 (s, 3H, CH_3), 2.380 (s, 3H, CH_3); ^{13}C NMR: 162.97, 150.10, 146.56, 138.81, 134.96, 133.68, 132.34, 128.24, 120.25, 35.64, 33.18, 17.85, 9.80; Anal. Calcd. for $C_{13}H_{12}N_4S$: C 60.91, H 4.72, N 21.86. Found: C 60.85, H 4.67, N 21.77.

*4.4.1.9. 3-Ethyl-9-methyl-10,10a-dihydroindeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazine (**9i**).* IR (ν_{max}, cm^{-1}): transparent in the region of C=O str., 1636, 1597, 1528, 1458, 1381, 1350, 1311 1281, 1203, 1165, 1065, 1034; ^1H NMR (δ, $CDCl_3$, 300 MHz): 7.825–7.799 (m, 1H, ArH), 7.403–7.390 (m, 2H, ArH), 4.190–4.145 (m, 1H), 3.683–3.598 (dd, 1H, J_1 = 8.4 Hz, J_2 = 17.1 Hz), 3.131–2.910 (m, 3H), 2.376 (s, 3H, CH_3), 1.470–1.421 (t, 3H, CH_3, J = 7.5 Hz), ^{13}C NMR: 162.70, 155.30, 146.51, 139.50, 135.35, 134.13, 133.06, 128.82, 120.92, 36.20, 33.81, 18.41, 18.35, 11.30; Anal. Calcd. for $C_{14}H_{14}N_4S$: C 62.20, H 5.22, N 20.72; Found: C 62.16, H 5.18, N 20.68.

*4.4.1.10. 3-Propyl-9-methyl-10,10a-dihydroindeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazine (**9j**).* IR (ν_{max}, cm^{-1}): transparent in the region of C=O str., 1597, 1520, 1458, 1389, 1342, 1304, 1273, 1203, 1034; ^1H NMR (δ, $CDCl_3$, 300 MHz): 7.800–7.293 (m, 3H, ArH), 4.230–4.201 (m, 1H), 3.720–3.510 (m, 1H), 3.101–2.910 (m, 3H), 2.245 (s, 3H, CH_3), 1.928–1.801 (m, 2H, CH_2), 1.102–0.912 (m, 3H, CH_3); ^{13}C NMR: 163.42, 146.70, 139.77, 135.42, 134.35, 132.88, 128.85, 121.01, 36.19, 33.85, 26.45, 20.43, 18.36, 13.86; Anal. Calcd. for $C_{15}H_{16}N_4S$: C 63.35, H 5.67, N 19.70. Found: C 63.30, H 5.64, N 19.65.

*4.4.1.11. 3-Isopropyl-9-methyl-10,10a-dihydroindeno [1,2-e] [1,2,4] triazolo [3,4-b] [1,3,4]thiadiazine (**9k**).* IR (ν_{max}, cm^{-1}): transparent in the region of C=O str., 1597, 1520, 1458, 1389, 1350, 1311, 1281, 1203, 1157, 1095, 1065, 1034; ^1H NMR (δ, $CDCl_3$, 300 MHz): 7.800–7.293 (m, 3H, ArH), 4.217 (bs, 1H), 3.682–3.450 (m, 2H), 3.025–2.969 (m, 1H), 1.602–1.501 (m, 6H, $(CH_3)_2$), 2.865 (s, 3H); ^{13}C NMR: 162.76, 158.32, 146.60, 139.76, 135.38, 134.19, 133.01, 128.81, 120.89, 36.13, 33.83, 25.49, 20.80, 19.77, 18.35; Anal. Calcd. for $C_{15}H_{16}N_4S$: C 63.35, H 6.67, N 19.70; Found: C 63.28, H 6.62, N 19.65.

*4.4.1.12. 3-Phenyl-9-methyl-10,10a-dihydroindeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazine (**9l**).* IR (ν_{max}, cm^{-1}): transparent in the region of C=O str., 1597, 1528, 1458, 1373, 1304, 1203, 1119, 1034; ^1H NMR (δ, $CDCl_3$, 300 MHz): 8.612–7.441 (m, 8H, ArH), 4.274 (bs, 1H), 3.687 (bs, 1H), 3.070–3.019 (m, 1H), 2.384 (s, 3H, CH_3); ^{13}C NMR: 161.45, 157.22, 146.43, 134.35, 132.96, 130.22, 128.90, 128.57, 128.23, 121.15, 35.79, 33.91, 18.39; Anal. Calcd. for $C_{18}H_{14}N_4S$: C 67.90, H 4.43, N 17.60; Found: C 67.84, H 4.40, N 17.56.

*4.5. Synthesis of 3-alkyl/aryl-9-methylindeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazines (**11a–l**)*

4.5.1. Typical procedure

To a solution of **8a** (1 g, 3.45 mmol) in 50 mL methanol was added 4-amino-5-mercapto-1,2,4-triazole (**3a,** 0.79 g, 6.90 mmol) and the reaction mixture was refluxed for 5 h. The colour of the reaction mixture changed from colourless to red as the reaction proceeded. The reaction mixture was poured onto crushed ice (100 g), followed by basification with ammonia solution to give the crude product as red solid, filtered, washed with water and dried (mixture of the two products by tlc). The crude product was chromatographed over silica gel (100–200 mesh) using petroleum ether-ethyl acetate (4:1) as eluent to give **9a** (0.413 g, 50%) and **11a** (0.206 g, 25%).

All other derivatives were synthesized by adopting the similar procedure and purified by column chromatography.

*4.5.1.1. Indeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazine (**11a**).* IR (ν_{max}, cm^{-1}, neat): transparent in the region of C=O str., 1605, 1535, 1450, 1388, 1319; ^1H NMR (δ, $CDCl_3$, 300 MHz): 8.791 (s, 1H, triazole H), 7.951–7.432 (m, 4H, ArH), 6.985 (s, 1H,=CH), ^{13}C NMR: 151.26, 142.75, 134.91, 132.45, 130.28, 128.05, 126.07, 122.44, 121.13, 114.88; Anal. Calculated for $C_{11}H_6N_4S$: C 58.39, H 2.67, N 24.76; Found: C 58.26, H 2.60, N 24.69.

*4.5.1.2. 3-Methylindeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazine (**11b**).* IR (ν_{max}, cm^{-1}, neat): transparent in the region of C=O str., 1605, 1532, 1450, 1389, 1319; ^1H NMR (δ, $CDCl_3$, 300 MHz): 7.948–7.923 (d, 1H, ArH, J = 7.5 Hz), 7.502–7.452 (m, 1H, ArH), 7.330–7.306 (m, 2H, ArH), 6.982 (s, 1H, =CH), 2.734 (s, 3H, CH_3); ^{13}C NMR: 152.85, 151.25, 142.75, 139.27, 132.45, 130.28, 128.05, 126.06, 122.43, 121.13, 114.87, 10.13; Anal. Calculated for $C_{12}H_8N_4S$: C 59.98, H 3.36, N 23.32; Found: C 59.92, H 3.28, N 23.29.

*4.5.1.3. 3-Ethylindeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazine (**11c**).* IR (ν_{max}, cm^{-1}, neat): transparent in the region of C=O str., 1605, 1535, 1450, 1388, 1319; ^1H NMR (δ, $CDCl_3$, 300 MHz): 7.934–7.909 (d, 1H, ArH, J = 7.5 Hz), 7.492–7.442 (m, 1H, ArH), 7.321–7.272 (m, 2H, ArH), 6.971 (s, 1H, =CH), 3.166–3.090 (q, 2H, CH_2, J = 7.5 Hz), 1.520–1.470 (t, 3H, CH_3, J = 7.5 Hz); ^{13}C NMR: 155.44, 152.67, 142.76, 134.97, 132.42, 130.32, 128.0, 126.03, 122.38, 121.11, 114.95, 18.19, 11.34; Anal. Calculated for $C_{13}H_{10}N_4S$: C 61.40, H 3.96, N 22.03; Found: C 61.35, H 3.89, N 21.95.

*4.5.1.4. 3-Propylindeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazine (**11d**).* IR (ν_{max}, cm^{-1}, neat): transparent in the region of C=O str., 1605, 1535, 1450, 1389, 1319; ^1H NMR (δ, $CDCl_3$, 300 MHz): 7.946–7.922 (d, 1H, ArH, J = 7.2 Hz), 7.498–7.447 (m, 1H, ArH), 7.328–7.304 (m, 2H, ArH), 6.979 (s, 1H, =CH), 3.115–3.064 (t, 2H, CH_2, J = 7.5 Hz), 2.015–1.717 (m, 2H, CH_2), 1.129–1.080 (t, 3H, CH_3, J = 7.5 Hz); ^{13}C NMR: 155.41, 152.66, 142.77, 134.85, 132.41, 130.35, 127.97, 126.02, 122.39, 121.11, 114.99, 26.29, 20.53, 13.84; Anal. Calculated for $C_{14}H_{12}N_4S$: C 62.66, H 4.51, N 20.88; Found: C 62.60, H 4.44, N 20.83.

*4.5.1.5. 3-Isopropylindeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazine (**11e**).* IR (ν_{max}, cm^{-1}, neat): transparent in the region of C=O str., 1605, 1534, 1450, 1389, 1319; ^1H NMR (δ, $CDCl_3$, 300 MHz): 7.939–7.915 (d, 1H, ArH, J = 7.2 Hz), 7.668–7.303 (m, 3H, ArH), 6.978 (s, 1H, =CH), 3.582–3.542 (m, 1H, CH), 1.574–1.270 (6H, m, $(CH_3)_2$); ^{13}C NMR: 155.87, 154.25, 132.41, 127.97, 126.02, 122.34, 121.12, 114.70, 25.33, 22.68, 20.37; Anal. Calculated for $C_{14}H_{12}N_4S$: C 62.66, H 4.51, N 20.88; Found: C 62.62, H 4.45, N 20.81.

4.5.1.6. 3-Phenylindeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazine (11f). IR (ν_{max}, cm^{-1}, neat): transparent in the region of C=O str., 1605, 1520, 1450, 1373, 1319; ^1H NMR (δ, $CDCl_3$, 300 MHz): 8.268–8.235 (m, 2H, ArH), 7.930–7.904 (d, 1H, ArH, J = 7.8 Hz), 7.606–7.559 (m, 3H, ArH), 7.501–7.447 (m, 1H, ArH), 7.321–7.271 (m, 2H, ArH), 7.014 (s, 1H, =CH); ^{13}C NMR: 153.09, 152.25, 142.78, 135.84, 132.57, 130.58, 130.32, 128.79, 128.66, 128.29, 126.12, 125.70, 122.58, 121.19, 114.35; Anal. Calculated for $C_{17}H_{10}N_4S$: C 67.53, H 3.33, N 18.53; Found: C 67.47, H 3.29, N 18.51.

*4.5.1.7. 9-Methylindeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazine (**11g**).* IR (ν_{max}, cm^{-1}, neat): transparent in the region of C=O

str., 1620, 1474, 1443, 1381, 1327; ^{1}H NMR (δ, $CDCl_3$, 300 MHz): 8.783 (s, 1H, triazole H), 7.762–7.738 (d, 1H, ArH, J = 7.2 Hz), 7.310–7.194 (m, 2H, ArH), 7.105 (s, 1H, =CH), 2.423 (s, 3H, CH_3); ^{13}C NMR: 154.14, 142.71, 141.28, 135.46, 134.20, 130.72, 129.86, 127.26, 126.37, 120.30, 114.46, 14.11; Anal. Calculated for $C_{12}H_8N_4S$: C 59.98, H 3.36, N 23.32; Found: C 59.95, H 3.32, N 23.28.

*4.5.1.8. 3,9-Dimethylindeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazine (**11h**).* IR (ν_{max}, cm^{-1}, neat): transparent in the region of C=O str., 1605, 1543, 1458, 1381, 1327; ^{1}H NMR (δ, $CDCl_3$, 300 MHz): 7.796–7.772 (d, 1H, ArH, J = 7.2 Hz), 7.3–7.28 (m, 1H, ArH), 7.238–7.188 (m, 1H, ArH), 7.082 (s, 1H, =CH), 2.735 (s, 3H, CH_3), 2.424 (s, 3H, CH_3); ^{13}C NMR: 154.24, 153.16, 142.61, 141.27, 133.91, 130.53, 126.54, 126.12, 120.10, 14.11, 10.15; Anal. Calculated for $C_{13}H_{10}N_4S$: C 61.40, H 3.96, N 22.03; Found: C 61.37, H 3.91, N 21.98.

*4.5.1.9. 3-Ethyl-9-methylindeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4] thiadiazine (**11i**).* IR (ν_{max}, cm^{-1}, neat): transparent in the region of C=O str., 1620, 1458, 1389, 1311; ^{1}H NMR (δ, $CDCl_3$, 300 MHz): 7.766–7.742 (d, 1H, ArH, J = 7.2 Hz), 7.255–7.167 (m, 2H, ArH), 7.053 (s, 1H, =CH), 3.154–3.04 (q, 2H, CH_2, J = 7.5 Hz), 2.406 (s, 3H, CH_3), 1.517–1.467 (t, 3H, CH_3, J = 7.5 Hz); ^{13}C NMR: 154.22, 153.11 142.81, 141.38, 126.23, 120.06, 17.90, 11.34; Anal. Calculated for $C_{14}H_{12}N_4S$: C 62.66, H 4.51, N 20.88; Found: C 62.62, H 4.46, N 20.84.

*4.5.1.10. 9-Methyl-3-propylindeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazine (**11j**).* IR (ν_{max}, cm^{-1}, neat): transparent in the region of C=O str., 1620, 1458, 1389, 1319; ^{1}H NMR (δ, $CDCl_3$, 300 MHz): 7.788–7.764 (d, 2H, ArH, J = 7.2 Hz), 7.293–7.181 (m, 2H, ArH), 7.074 (s, 1H, =CH), 3.113–3.062 (t, 2H, CH_2, J = 7.5 Hz), 2.419 (s, 3H, CH_3,), 2.014–1.890 (m, 2H, CH_2), 1.129–1.080 (t, 3H, CH_3, J = 7.5 Hz); ^{13}C NMR: 154.38, 153.05, 141.36, 134.96, 133.88, 130.51, 126.50, 120.06, 114.13, 26.29, 20.52, 17.78, 13.84; Anal. Calculated for $C_{15}H_{14}N_4S$: C 63.80, H 5.0, N 19.84; Found: C 63.77, H 4.95, N 19.81.

*4.5.1.11. 3-Isopropyl-9-methylindeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazine (**11k**).* IR (ν_{max}, cm^{-1}, neat): transparent in the region of C=O str., 1620, 1458, 1384, 1311; ^{1}H NMR (δ, $CDCl_3$, 300 MHz): 7.923–7.322 (m, 3H, ArH), 6.892 (s, 1H, =CH), 3.572–3.531 (m, 1H, CH), 2.5 (s, 3H, CH_3), 1.583–1.258 (m, 6H, $(CH_3)_2$); ^{13}C NMR: 155.87, 154.25, 132.41, 127.97, 126.02, 122.34, 121.12, 114.70, 25.33, 22.68, 20.37, 14.24; Anal. Calculated for $C_{15}H_{14}N_4S$: C 63.80, H 5.0, N 19.84; Found: C 63.76, H 4.95, N 19.80.

*4.5.1.12. 9-Methyl-3-phenylindeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazine (**11l**).* IR (ν_{max}, cm^{-1}, neat): transparent in the region of C=O str., 1605, 1543, 1458, 1381, 1327; ^{1}H NMR (δ, $CDCl_3$, 300 MHz): 8.081–7.842 (m, 4H, ArH), 7.422–7.396 (m, 4H, ArH), 7.087 (s, 1H, =CH) 2.5 (s, 3H, CH_3); ^{13}C NMR: 163.54, 152.12, 146.71, 141.03, 135.43, 134.34, 139.95, 130.20, 128.89, 128.53, 128.22, 126.23, 121.12, 18.35; Anal. Calculated for $C_{18}H_{12}N_4S$: C 68.33, H 3.82, N 17.71; Found: C 68.28, H 3.78, N 17.67.

4.6. Antimicrobial assay

4.6.1. Test microorganisms

Total seven microbial strains were selected on the basis of their clinical importance in causing diseases in humans. Two Gram-positive bacteria (*S. aureus* MTCC 96 and *B. subtilis* MTCC 121); two Gram-negative bacteria (*E. coli* MTCC 1652 and *P. aeruginosa* MTCC 741) and three fungi, (*A. niger*, *A. flavus* and *Penicillium* sp.) the ear pathogens isolated from the patients of Kurukshetra [54] were used in the present study for evaluation of antimicrobial activity of the compounds. All the bacterial cultures were procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh. The bacteria were subcultured on Nutrient agar whereas fungi on Sabouraud dextrose agar.

4.6.2. Antibacterial activity

The antibacterial activity of twenty four compounds was evaluated by the agar well diffusion method. All the microbial cultures were adjusted to 0.5 McFarland standard, which is visually comparable to a microbial suspension of approximately 1.5×10^8 cfu/mL 20 mL of Mueller Hinton agar medium was poured into each petri plate and plates were swabbed with 100 μL inocula of the test microorganisms and kept for 15 min for adsorption. Using sterile cork borer of 8 mm diameter, wells were bored into the seeded agar plates and these were loaded with a 100 μL volume with concentration of 2.0 mg/mL of each compound reconstituted in the dimethylsulphoxide (DMSO). All the plates were incubated at 37 °C for 24 h. Antibacterial activity of each synthetic compound was evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (Hi Antibiotic zone scale). DMSO was used as a negative control whereas ciprofloxacin was used as positive control. This procedure was performed in three replicate plates for each organism [55,56].

4.6.3. Determination of minimum inhibitory concentration (MIC)

MIC is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation. MIC of the various compounds against bacterial strains was tested through a modified agar well diffusion method [57]. In this method, a two fold serial dilution of each compound was prepared by first reconstituting the compound in DMSO followed by dilution in sterile distilled water to achieve a decreasing concentration range of 256 to 0.5 μg/mL. A 100 μL volume of each dilution was introduced into wells (in triplicate) in the agar plates already seeded with 100 μL of standardized inoculum (10^6 cfu/mL) of the test microbial strain. All test plates were incubated aerobically at 37 °C for 24 h and observed for the inhibition zones. MIC, taken as the lowest concentration of the chemical compound that completely inhibited the growth of the microbe, showed by a clear zone of inhibition, was recorded for each test organism. Ciprofloxacin was used as positive control while DMSO as negative control.

4.6.4. Antifungal activity

The antifungal activity of chemical compounds was evaluated by poisoned food technique. The moulds were grown on Sabouraud dextrose agar (SDA) at 25 °C for 7 days and used as inocula. The 15 mL of molten SDA (45 °C) was poisoned by the addition of 100 μL volume of each compound having concentration of 4.0 mg/mL reconstituted in the DMSO, poured into a sterile Petri plate and allowed it to solidify at room temperature. The solidified poisoned agar plates were inoculated at the centre with fungal plugs (8 mm diameter) obtained from the colony margins and incubated at 25 °C for 7 days. DMSO was used as the negative control whereas fluconazole was used as the positive control. The experiments were performed in triplicates. Diameter of fungal colonies was measured and expressed as percent mycelial inhibition by applying the formula [58].

$$\text{Percent inhibition of myelial growth} = (dc - dt)/dc \times 100$$

where dc = average diameter of fungal colony in negative control plates; dt = average diameter fungal colony in experimental plates.

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Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2011.08.019.

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