ORIGINAL ARTICLE

Synthesis and antimicrobial activity of some novel fused heterocyclic 1,2,4-triazolo [3,4-*b*][1,3,4] thiadiazine derivatives

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ABSTRACT

In the present investigation, the synthesis and antimicrobial evaluation of 1,2,4-triazolo [3,4-*b*][1,3,4] thiadiazine including different pharmacophores are aimed at. In this study, a series of 6-aryl-3- (3,4 -dialkoxyphenyl)-7*H* -[1,2,4]triazolo [3,4-*b*][1,3,4] thiadiazine (7a-7k) was synthesized by condensing 4-amino-5-(3,4-dialkoxyphenyl)-4*H*-[1,2,4]-triazole-3-thiol (6) with various aromatic carboxylic acids in the presence of phenacyl bromides through one-pot reaction. Eleven fused heterocyclic derivatives were successfully synthesized. The structures of these newly synthesized compounds were characterized by IR, ^{1}H NMR and mass spectroscopic studies. All the synthesized compounds were screened for their antimicrobial evaluation. Some of the compounds exhibited promising antimicrobial activity. From the present study it may be concluded that synthesized compounds are fruitful in terms of their structural novelty and marked biological activities. These compounds could be further modified to develop potential and safer antifungal agents.

Key words: Antimicrobial screening, fused heterocycles, triazolothiadiazines

INTRODUCTION

In recent years, 1,2,4-triazole moiety and their fused heterocyclic derivatives have received much attention due to their synthetic and effective biological importance. For example, a large number of 1,2,4-triazole-containing ring system have been incorporated into a wide variety of therapeutically interesting drug candidates including anti-inflammatory, CNS stimulants, sedatives, antianxiety, antimicrobial agents[1,2] and antimycotic activity such as fluconazole, intraconazole, voriconazole.[3] Also, there are known drugs containing the 1,2,4- triazole group e.g. triazolam, alprazolam, etizolam and furacylin.[4] Moreover, a variety of biological activities have been reported for a large number of their derivatives, such as antimicrobial,[5-10] antitubercular,[11] anticancer,[12,13] anticonvulsant,[14] hypoglycemic,[15] anti-inflammatory and analgesic activities.[16]

In the design of new drugs, the combination of two or more biologically active heterocyclic rings, either in condensed form or coupled form, results in augmentation of biological activity of such compounds by many folds. In the present study, prompted by these observations, the synthesis and antimicrobial evaluation of 1,2,4-triazolo [3,4-*b*][1,3,4] thiadiazine including different pharmacophores are aimed at.

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MATERIALS AND METHODS

Chemistry

Melting points were determined in open glass capillary tube and are uncorrected. Purity of the compounds was checked by thin layer chromatography (TLC) on silica gel G-coated plates using ethyl acetate and n hexane (1:1, v/v); iodine chamber and observed in UV light. IR spectra were recorded on a BRUKER 375-FTIR Spectrometer. ^{1}H NMR spectra

were recorded on a Bruker BioSpin Avance III 700MHz FT-NMR spectrometer using TMS as an internal standard. Chemical shifts are reported in parts per million (d) and signals are described as singlet (s), doublet (d), triplet (t), and multiplet (m). The mass spectra were recorded on a Q-TRAP 1400 spectrophotometer on Turbo spray mode. Solvents and reagents were purchased from the commercial vendors in the appropriate grade and were used without purification. Reaction sequence employed for the synthesis of title compounds is shown in Figure 1. Physico-chemical data of all new compounds are summarized in Table 1.

Synthesis of the compounds

General method for the synthesis of 3,4-dialkoxy benzoic acid (2)

To a solution of substituted benzaldehyde (0.176 mol) in 30 ml water, a solution of potassium permanganate (0.191 mol) in 60 ml water was added at 70-80°C over a period of 2 hours. The reaction mass was stirred at the same temperature for 2 h and filtered. The filtrate was acidified using conc. hydrochloric acid at 0-5°C. The product obtained was filtered, washed with water and dried. The crude product was recrystallized from methanol.[17]

General method for the synthesis of 3,4-dialkoxy benzoate (3)

To a solution of substituted benzoic acid in methanol (absolute), conc. sulphuric acid was added slowly at 0-5°C over a period of 30 min and refluxed for 2 hours. After quenching into cold water (in some reactions bicarbonates also added), precipitated solid was filtered, washed with water and dried.[17]

General method for the synthesis of 3,4-dialkoxyphenyl carbohydrazide (4)

The methyl esters of substituted aromatic acids (0.1 M), in 30 ml of methanol were dissolved and hydrazine hydrate (0.1 M) was added drop wise to the mixture with stirring. The resulting mixture was allowed to reflux for 6 h and the contents were allowed to cool. The crystalline residue formed was filtered, washed thoroughly with water and dried. The completion of the reaction was monitored on TLC by using silica gel-G coated plates by using ethyl acetate and n hexane (1:1) as the eluent and observed in UV light.[17]

General method for the synthesis of 4-amino-5-(3,4-dialkoxy phenyl)-4H-[1,2,4]- triazole-3-thiol (6)

Substituted phenylcarbohydrazide was treated with a solution of potassium hydroxide (7 g) dissolved in methanol (50 ml) at 0-5°C under 10 hour stirring. Carbon disulfide (10 g) was then added slowly and the reaction mixture was stirred overnight at room temperature. The solid product of potassium dithiocarbazinate was filtered, washed with anhydrous ether and dried.

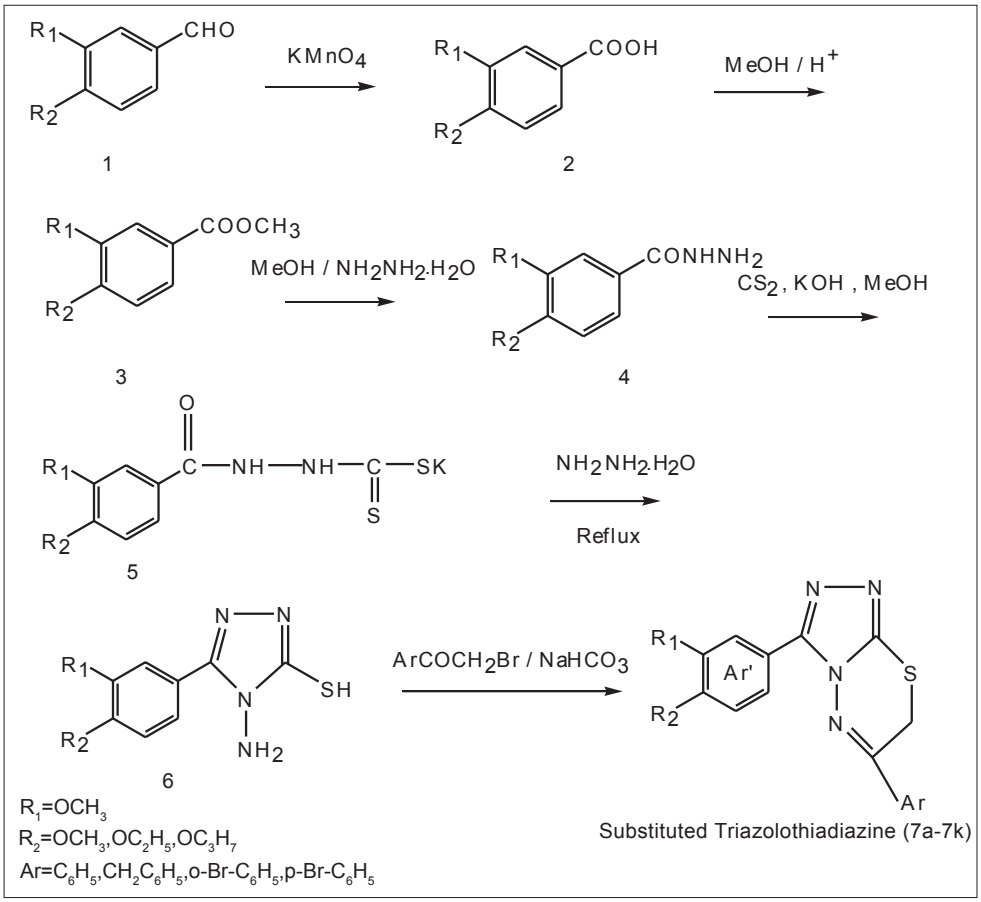

Figure 1: Synthesis of substituted triazolothiadiazines

Table 1: Physico-chemical data of substituted -[1,2,4]triazolo [3,4-*b*][1,3,4] thiadiazine (7a-7k)

Comp code	R_1	R_2	Ar	Molecular formula	Molecular weight	Melting point (°C)	Yield (%)
7a	OCH_3	OCH_3	C_6H_5	$C_{18}H_{16}N_4O_2S$	352	232	61
7b	OCH_3	OC_2H_5	C_6H_5	$C_{19}H_{18}N_4O_2S$	366	189	69
7c	OCH_3	OC_3H_7	C_6H_5	$C_{20}H_{20}N_4O_2S$	380	176	67
7d	OCH_3	OC_2H_5	$CH_2C_6H_5$	$C_{20}H_{20}N_4O_2S$	380	276	78
7e	OCH_3	OC_3H_7	$CH_2C_6H_5$	$C_{21}H_{22}N_4O_2S$	394	280	71
7f	OCH_3	OCH_3	o-Br-C_6H_5	$C_{18}H_{15}BrN_4O_2S$	431	287	81
7g	OCH_3	OC_2H_5	o-Br-C_6H_5	$C_{19}H_{17}BrN_4O_2S$	445	242	60
7h	OCH_3	OC_3H_7	o-Br-C_6H_5	$C_{20}H_{19}BrN_4O_2S$	459	256	80
7i	OCH_3	OCH_3	p-Br-C_6H_5	$C_{18}H_{15}BrN_4O_2S$	431	289	57
7j	OCH_3	OC_2H_5	p-Br-C_6H_5	$C_{19}H_{17}BrN_4O_2S$	445	248	73
7k	OCH_3	OC_3H_7	p-Br-C_6H_5	$C_{20}H_{19}BrN_4O_2S$	459	226	70

A suspension of potassium dithiocarbazinate of respective aromatic esters (3) (0.1 M) in water (5 ml) and hydrazine hydrate (15 ml, 0.3 M) was refluxed for 6-7 h with occasional shaking. The color of the reaction mixture changed to green with the evolution of hydrogen sulfide gas. A homogenous reaction mixture was obtained during the reaction process. The reaction mixture was cooled to room temperature and diluted with water (100 ml). On acidification with concentrated hydrochloric acid, the corresponding triazole was precipitated which was recrystallized with methanol.[17]

General method for the synthesis of 6-aryl-3-(3,4-dialkoxyphenyl)-7H -[1,2,4]triazolo [3,4-b][1,3,4] thiadiazine (7a-7k)

A mixture of 4-amino-5-(3,4-dialkoxyphenyl)-4*H*-[1,2,4]-triazole-3-thiol (1 mmol) and substituted phenacyl bromides (1.2 mmol) in 15 ml of absolute ethanol was refluxed for 8 hours. The reaction mass was poured into crushed ice and neutralized with sodium bicarbonate and potassium hydroxide to pH 8. The residue obtained was filtered, washed with water, dried and recrystallized from absolute ethanol (7a-7k).

3- (3,4 -dimethoxyphenyl)- 6-phenyl-7H -[1,2,4]triazolo [3,4-b] [1,3,4] thiadiazine (7a)

IR (KBr, cm^{-1}): 3097(C-H, aromatic),2961(C-H aliphatic), 1394(C = N str),796(aromatic C-H bend out of plane), 721(C-S-C);^{1}H-NMR ($CDCl_3$) ∂ ppm: 7.36-7.64 (m, 5H, ArH), 6.69-6.93 (m, 3H, Ar'H), 3.73 (s, 6H, OCH_3), 3.02 (s, 2H, SCH_2) ; m/z, %: 353.4 (M^+).

3- (4-ethoxy-3 -methoxyphenyl)-6-phenyl-7H -[1,2,4]triazolo [3,4-b] [1,3,4] thiadiazine (7b)

IR (KBr, cm^{-1}): 3061(C-H, aromatic),2926 and 2856(C-H aliphatic), 1398(C = N str),760(aromatic C-H bend out of plane), 683(C-S-C); ^{1}H-NMR ($CDCl_3$) ∂ ppm: 7.36-7.64 (m, 5H, ArH), 7.07-7.17 (m, 3H, Ar'H), 3.51 (s, 3H, OCH_3), 3.06 (s, 2H, SCH_2), 1.27 (s, 3H, CH_3); m/z, %: 367.4 (M^+).

3- (3-methoxy -4 -propoxyphenyl)-6-phenyl-7H -[1,2,4]triazolo [3,4-b][1,3,4] thiadiazine (7c)

IR (KBr, cm^{-1}): 3060(C-H, aromatic),2920 and 2854(C-H aliphatic), 1394(C = N str),758(aromatic C-H bend out of plane), 689(C-S-C); ^{1}H-NMR ($CDCl_3$) ∂ ppm: 7.33-7.64 (m, 5H, ArH), 6.72-6.93 (m, 3H, Ar'H), 3.73 (s, 3H, OCH_3), 3.00 (s, 2H, SCH_2), 1.75 (s, 2H, CH_2); m/z, %: 381.4 (M^+).

6-benzyl-3- (4-ethoxy -3 -methoxyphenyl)-7H -[1,2,4]triazolo [3,4-b] [1,3,4] thiadiazine (7d)

IR (KBr, cm^{-1}): 3092(C-H, aromatic),2919(C-H aliphatic), 1396(C = N str), 757 (aromatic C-H bend out of plane), 691(C-S-C); ^{1}H-NMR ($CDCl_3$) ∂ ppm: 7.13-7.14 (m, 5H, ArH), 6.72-7.07 (m, 3H, Ar'H), 3.94 (s, 2H, CH_2), 3.73 (s, 3H, OCH_3), 3.00 (s, 2H, SCH_2), 2.63 (s, 2H, $CH_2C_6H_5$), 1.33 (s, 2H, CH_2); m/z, %: 381.4 (M^+).

6-benzyl-3- (3-methoxy-4 -propoxyphenyl)-7H- -[1,2,4]triazolo [3,4-b][1,3,4] thiadiazine (7e)

IR (KBr, cm^{-1}): 2936(C-H aliphatic), 1275(C = N str), 687(C-S-C); ^{1}H-NMR ($CDCl_3$) ∂ ppm: 7.22-7.57 (m, 5H, ArH), 6.72-6.93 (m, 3H, Ar'H), 3.93 (s, 2H, CH_2), 3.73 (s, 3H, OCH_3), 3.03 (s, 2H, SCH_2), 2.63 (s, 2H, $CH_2C_6H_5$),1.75 (s, 2H, CH_2) ; m/z, %: 395.5 (M^+).

6-(2-bromophenyl) -3- (3,4-dimethoxyphenyl)-7H -[1,2,4]triazolo [3,4-b][1,3,4] thiadiazine (7f)

IR (KBr, cm^{-1}): 3060(C-H, aromatic),2917(C-H aliphatic), 1407(C = N str), 759(aromatic C-H bend out of plane), 691(C-S-C), 529 (C-Br str); ^{1}H-NMR ($CDCl_3$) ∂ ppm: 7.22-7.56 (m, 4H, ArH), 6.72-6.93 (m, 3H, Ar'H), 3.73 (s, 6H, OCH_3), 3.02 (s, 2H, SCH_2); m/z, %: 417.2 (M^+).

6-(2-bromophenyl) -3- (4-ethoxy-3 -methoxyphenyl)-7H -[1,2,4] triazolo [3,4-b][1,3,4] thiadiazine (7g)

IR (KBr, cm^{-1}): 2941(C-H aliphatic), 1385(C = N str), 745(aromatic C-H bend out of plane), 671(C-S-C), 564 (C-Br str); ^{1}H-NMR ($CDCl_3$) ∂ ppm: 7.32-7.56 (m, 4H, ArH), 6.72-6.93 (m, 3H, Ar'H), 3.73 (s, 3H, OCH_3), 3.02 (s, 2H, SCH_2), 1.33 (s, 3H, CH_3); m/z, %: 445.3 (M^+).

6-(2-bromophenyl) -3- (3-methoxy-4 -propoxyphenyl)-7H -[1,2,4] triazolo [3,4-b][1,3,4] thiadiazine (7h)

IR (KBr, cm^{-1}): 3064(C-H, aromatic),2929(C-H aliphatic),

1375(C = N str), 746(aromatic C-H bend out of plane); ^{1}H-NMR ($CDCl_3$) ∂ ppm: 7.22-7.57 (m, 4H, ArH), 6.72-6.93 (m, 3H, Ar'H), 3.93 (s, 2H, CH_2), 3.73 (s, 3H, OCH_3), 3.03 (s, 2H, SCH_2), 1.75 (s, 2H, CH_2); m/z, %: 459.8 (M^+).

6-(4-bromophenyl) -3- (3,4-dimethoxyphenyl)-7H -[1,2,4]triazolo [3,4-b][1,3,4] thiadiazine (7i)

IR (KBr, cm^{-1}): 3097(C-H, aromatic),2925(C-H aliphatic), 1391(C = N str), 760(aromatic C-H bend out of plane), 691(C-S-C), 570 (C-Br str); ^{1}H-NMR ($CDCl_3$) ∂ ppm: 7.51-7.59 (m, 4H, ArH), 6.72-6.92 (m, 3H, Ar'H), 3.73 (s, 6H, OCH_3), 3.02 (s, 2H, SCH_2); m/z, %: 431.3 (M^+).

6-(4-bromophenyl) -3- (4-ethoxy-3 -methoxyphenyl)-7H -[1,2,4] triazolo [3,4-b][1,3,4] thiadiazine (7j)

IR (KBr, cm^{-1}): 3060(C-H, aromatic),2984 and 2887(C-H aliphatic), 1394(C = N str), 796(aromatic C-H bend out of plane), 717(C-S-C), 668 (C-Br str); ^{1}H-NMR ($CDCl_3$) ∂ ppm: 7.28-7.73 (m, 4H, ArH), 7.07 (m, 3H, Ar'H), 3.80 (s, 3H, OCH_3), 3.32 (s, 2H, CH_2), 2.74 (s, 2H, SCH_2), 1.28 (s, 3H, CH_3); m/z, %: 445.3 (M^+).

6-(4-bromophenyl) -3- (3-methoxy-4 -propoxyphenyl)-7H -[1,2,4] triazolo [3,4-b][1,3,4] thiadiazine (7k)

IR (KBr, cm^{-1}): 3062(C-H, aromatic),2925 and 2854(C-H aliphatic),1714(C-O str), 1394(C = N str), 796(aromatic C-H bend out of plane), 638 (C-Br str); ^{1}H-NMR ($CDCl_3$) ∂ ppm: 7.28-7.49 (m, 4H, ArH), 7.07 (m, 3H, Ar'H), 3.97 (s, 2H, CH_2), 3.94 (s, 3H, OCH_3), 3.06 (s, 2H, SCH_2), 1.28 (s, 2H, CH_2), 0.91 (s, 3H, CH_3); m/z, %: 459.8 (M^+).

Biological Evaluation

Antibacterial activity

The newly synthesized compounds were evaluated for their antibacterial activity against *Staphylococcus aureus* (MTCC No 3160), *Bacillus cereus* (MTCC No 9786), *Escherichia coli* (MTCC No 118) and *Pseudomonas aeruginosa* (MTCC No 4673) bacterial strains by the serial broth dilution method.[4,18] Activity of each compound was compared with Gentamycin as standard. Required bacterial strains were procured from Institute of Microbial Technology, Chandigarh.

Serial dilutions of the drug in Nutrient broth were taken and their pH was adjusted to 7.2-7.4. Standardized suspension of the test bacterium was inoculated and incubated for 24 h at 37°C. The minimum inhibitory concentration (MIC) was noted by observing the lowest concentration of the drug at which there was no visible growth. Test compounds and standard drug Gentamycin were dissolved in 10% dimethyl sulfoxide (DMSO) to give a concentration of 2000 µg/ml. Nutrient Agar was poured into petri dish, excess of suspension was decanted and placing in incubator at 37°C for 24 hours. After specified time of incubation of solid slant culture, a loop full of grown culture was transferred into 5-6 ml of fresh nutrient broth. This was incubated at appropriate temperature of 35-37°C for 24 h (10^7-10^8CFU/ml). From this 0.1 ml was withdrawn and was diluted to 10 ml with sterile water and this served as working inoculum for screening the newly synthesized compounds for their antibacterial activity.

Sterilized test tubes were numbered 1 through 9. All of the following steps were carried out using aseptic technique. A solution of 0.2 ml of 2000 µg/ml test stock solution in DMSO was transferred to a first sterile test tube containing 3.8 ml of nutrient broth to arrive 100 µg/ml as starting dose and the remaining test tubes 2-9 were filled with 2 ml of nutrient broth. DMSO as a control has no effect at 10% concentration against bacteria. These test tubes were serially diluted to give a concentration of 100, 50, 25, 12.5, 6.25, 3.125 and 1.56 µg/ml. One test tube with no test compound but with equal volume of solvent DMSO (10%) served as the vehicle control. One test tube with no test compound and no vehicle but only with nutrient media served as the positive control to ensure the growth property of media. To all the test tubes 0.1 ml of suspension of bacteria (working inocula) was added and the test tubes were incubated at 35-37°C for 24 hours. The highest dilution of the test compound that completely inhibited the growth of test organism was considered as the MIC value of the test compound and was expressed in µg/ml and the results are summarized in Table 2.

Antifungal activity

Newly prepared compounds were screened for their antifungal activity against *Candida albicans* (MTCC No 1637) and *Aspergillus niger* (MTCC No 282), in DMSO by serial broth dilution method.[4,19] Activity of each compound was compared with Miconazole as standard. Required bacterial strains were procured from Institute of Microbial Technology, Chandigarh.

Serial dilutions of the drug in Sabouraud dextrose broth were taken and their pH was adjusted to 5-6. Standardized

Table 2: Antibacterial activity data of synthesized compounds (7a-7k)

Comp	Minimum Inhibitory Concentration (MIC)* in µg/ml			
	S. aureus	*B. cereus*	*E. coli*	*P. aeruginosa*
7a	6.25	6.25	6.25	6.25
7b	6.25	6.25	6.25	6.25
7c	50	50	25	25
7d	25	25	25	25
7e	25	6.25	25	25
7f	25	25	25	25
7g	12.5	25	25	25
7h	100	100	25	25
7i	6.25	6.25	6.25	6.25
7j	6.25	25	6.25	25
7k	6.25	25	6.25	25
Gentamycin	1.56	6.25	1.56	6.25

*MIC values were evaluated at concentration range, 1.56-100 µg/ml

suspension of the test fungi was inoculated and incubated for 48 h at 25-27°C. The minimum inhibitory concentration (MIC) was noted by observing the lowest concentration of the drug at which there was no visible growth. Test compounds and standard drug Miconazole were dissolved in 10% DMSO to give a concentration of 2000 µg/ml. Sabouraud dextrose agar was poured into petri dish, excess of suspension was decanted and placing in incubator at 25-27°C for 48 hours. After specified time of incubation of solid slant culture, a loop full of grown culture was transferred into 5-6 ml of fresh Sabouraud dextrose broth. This was incubated at appropriate temperature of 25-27°C for 48 h (10^6-10^5 CFU/ml). From this 0.1 ml was withdrawn and was diluted to 10 ml with sterile water and this served as working inoculum for screening the newly synthesized compounds for their antifungal activity.

Sterilized test tubes were numbered 1 through 9. All of the following steps were carried out using aseptic technique. A solution of 0.2 ml of 2000 µg/ml test stock solution in DMSO was transferred to a first sterile test tube containing 3.8 ml of Sabouraud dextrose broth to arrive 100 µg/ml as starting dose and the remaining test tubes 2-9 were filled with 2 ml of Sabouraud dextrose broth. DMSO as a control has no effect at 10% concentration against bacteria. These test tubes were serially diluted to give a concentration of 100, 50, 25, 12.5, 6.25, 3.125 and 1.56 µg/ml. One test tube with no test compound but with equal volume of solvent DMSO (10%) served as the vehicle control. One test tube with no test compound and no vehicle but only with Sabouraud dextrose media served as the positive control to ensure the growth property of media. To all the test tubes 0.1 ml of suspension of fungi (working inocula) was added and the test tubes were incubated at 25-27°C for 48 hours. The highest dilution of the test compound that completely inhibited the growth of test organism was considered as the MIC value of the test compound and was expressed in µg/ml and the results are summarized in Table 3.

RESULTS AND DISCUSSION

Synthesis

The synthesis of 6-aryl-3- (3,4 -dialkoxyphenyl)-7*H* -[1,2,4] triazolo [3,4-*b*][1,3,4] thiadiazines (7a-7k) is shown in Figure 1. All synthesized compounds were obtained in good yields as shown in Table 1 and the structure of all newly synthesized compounds 7a-7k were confirmed by IR, ^{1}H NMR and mass spectral data.

Biological Evaluation

Antibacterial activity

The antibacterial activity of the newly synthesized compounds 7a-7k were reported as minimum inhibitory concentration (MIC) at the concentration range, 1.56-100 µg/ml against *S. aureus*, *B. cereus* (gram-positive bacteria) and *E. coli*, *P. aeruginosa* (gram-negative bacteria) using Gentamycin as standard and the results are summarized in Table 2. The compounds 7a, 7b and 7i exhibited highest activity against all tested bacteria. Compounds 7j and 7k showed comparatively good activity against S. aureus, Compound 7e showed moderate to good activity against B. cereus, Compounds 7j and 7k exhibited moderate to good activity against E. coli and Compounds 7a, 7b and 7i exhibited significant activity against *P. aeruginosa*.

Table 3: Antifungal activity data of synthesized compounds (7a-7k)

Comp	Minimum Inhibitory Concentration (MIC)* in µg/ml	
	C. albicans	*A. niger*
7a	6.25	6.25
7b	6.25	6.25
7c	50	25
7d	25	25
7e	25	25
7f	25	25
7g	12.5	25
7h	100	25
7i	6.25	6.25
7j	6.25	25
7k	12.5	25
Miconazole	1.56	1.56

*MIC values were evaluated at concentration range, 1.56-100 µg/ml

Antifungal activity

The antifungal data of the synthesized compounds 7a-7k were reported as minimum inhibitory concentration (MIC) at the concentration range, 1.56-100 µg/ml against *Candida albicans* and *Aspergillus niger* using Miconazole as standard and the results are summarized in Table 3. The compounds 7a, 7b and 7i exhibited highest activity against all tested fungi. Compounds 7g and 7j showed good activity against *C. albicans* while Compound 7j exhibited moderate to good activity against *A. niger*.

It was observed that the triazolo thiadiazine derivatives having phenyl group (7a and 7b) and p-bromo phenyl group (7i) at position C-6 possess high activity. Replacement of these groups by o-bromo phenyl resulted in a decrease in antibacterial and antifungal activity.

CONCLUSION

Eleven fused heterocyclic derivatives [Figure 1 and Table 1] were successfully synthesized. Among the newer analogs, three compounds, 3- (3,4 -dimethoxyphenyl)-6-phenyl-7*H* -[1,2,4]triazolo [3,4-*b*][1,3,4] thiadiazine **7a,** 3- (4-ethoxy-3 -methoxyphenyl)-6-phenyl-7*H* -[1,2,4]triazolo [3,4-*b*][1,3,4] thiadiazine **7b** and 6-(4-bromophenyl) -3-(3,4-dimethoxyphenyl)-7*H* -[1,2,4]triazolo [3,4-*b*][1,3,4]

thiadiazine **7i** exhibited promising antimicrobial activity. These compounds could be further modified to develop potential and safer antifungal agents.

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REFERENCES

1. Heindel ND, Reid JR. 4-Amino-3-mercapto-4H-1,2,4-triazoles and propargyl aldehydes: A new route to 3-R-8-Aryl-1,2,4-triazole [3,4-b]-1,3,4-thiadiazepines. J Heterocycl Chem 1980;17:1087-8.
2. Holla BS, Kalluraya B, Sridhar KR, Drake E, Thomas LM, Bhandary KK, *et al*. Synthesis, structural characterization, crystallographic analysis and antibacterial properties of somenitrofuryltriazolo [3,4-b]-1,3,4- thiadiazines. Eur J Med Chem 1994;29:301-8.
3. Haber J. Present status and perspectives on antimycoties with systematic effects. Cas Lek Cesk 2001;140:596.
4. Karegoudar P, Prasad DJ, Ashok M, Mahalinga M, Poojary B, Holla BS. Synthesis, antimicrobial and antiinflammatory activities of some -[1,2,4]triazolo [3,4-b][1,3,4] thiadiazoles and [1,2,4] triazolo [3,4-b][1,3,4] thiadiazines bearing trichlorophenyl moiety. Eur J Med Chem 2008;43:808-15.
5. Basavaraja HS, Nagamani JE, Vijay Kumar M, Padmashali B. Antimicrobial evaluation of novel substituted pyrimidinopyrazoles and pyrimidinotriazoles. J Adv Pharm Technol Res 2010;1:236-44.
6. Holla BS, Gonsalves R, Shenoy S. Studies on some N-bridged heterocycles derived from bis-[4-amino-5-mercapto-1,2,4-triazol-3-yl] alkanes. Farmaco 1998;53:574-8.
7. Holla BS, Veerendra B, Shivananda MK, Poojary B. Synthesis characterization and anticancer activity studies on some Mannich bases derived from 1,2,4-triazoles. Eur J Med Chem 2003;38:759-67.
8. Ashok M, Holla BS. Convenient synthesis of some triazolothiadiazoles and triazolothiadiazines carrying 4-methylthiobenzyl moiety as possible antimicrobial agents. J Pharmacol Toxicol 2007;2:256-63.
9. Zitouni GT, Kaplancikli ZA, Yildiz MT, Chevallet P, Kaya D. Synthesis and antimicrobial activity of 4-phenyl/cyclohexyl-5-(1-phenoxyethyl)-3-[N-(2-thiazolyl) acetamido] thio-4H-1,2,4-triazole derivative. Eur J Med Chem 2005;40:607-13.
10. Prasad DJ, Ashok M, Karegoudar P, Poojary B, Holla BS, Kumari NS. Synthesis and antimicrobial activities of some new triazolothiadiazoles bearing 4-methylthiobenzl moiety. Eur J Med Chem 2009;44:551-7.
11. Walczak K, Gondela A, Suwinski J. Synthesis and anti-tuberculosis activity of N-aryl-C-nitroazoles. Eur J Med Chem 2004;39:849-53.
12. Holla BS, Poojary KN, Rao BS, Shivananda MK. New bis-aminomercaptotriazoles and bis-triazolothiadiazoles as possible anticancer agents. Eur J Med Chem 2002;37:511-7.
13. Holla BS, Veerendra B, Shivananda MK, Kumari NS. Synthesis and biological activity of some bistriozole derivatives. Ind J Chem 2003;42B: 2010-4.
14. Amir M, Shikha K. Synthesis and anti-inflammatory, analgesic, ulcerogenic and lipid peroxidation activities of some new 2-[(2,6-dichloroanilino) phenyl] acetic acid derivatives. Eur J Med Chem 2004;39:535-45.
15. Mhasalkar MY, Shah MH, Nikam ST, Deliwda CV. 4-Alkyl-5-aryl-4H-1,2,4-triazole-3-thiols as hypoglycemic agents. J Med Chem 1970;13:672-4.
16. Almasirad A, Tabatabai SA, Faizi M, Kebriaeezadeh A, Mehrabi N, Dalvandi A, *et al*. Synthesis and anticonvulsant activity of new 2-substituted-5- [2-(2-fluorophenoxy) phenyl]-1,3,4-oxadiazoles and 1,2,4- triazoles. Bioorg Med Chem Lett 2004;14:6057-9.
17. Mathew V, Keshavayya J, Vaidya VP. Heterocyclic system containing bridgehead nitrogen atom: Synthesis and pharmacological activities of some substituted 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazoles. Eur J Med Chem 2006;41:1048-58.
18. Barry A. Procedures and theoretical considerations for testing antimicrobial agents in agar media, p. 1-16. In: V. Lorian (Editor), Antibiotics in Laboratory Medicine, 5th ed. Baltimore: Williams and Wilkins; (MD, 1); 1991.
19. Verma RS, Khan IK, Singh AP, (Editors). Antifungal agents: Past, present and future Prospects. National Academy of Chemistry and Biology, Lucknow, India 1998, p. 55-128.

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