Journal of Applied Pharmaceutical Science Vol. 4 (02), pp. 081-086, February, 2014
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2014.40214
ISSN 2231-3354

Synthesis of Some Novel Heterocyclic 1,2,4-triazolo [3,4-b][1,3,4] Thiadiazole Derivatives as Possible Antimicrobial Agents

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ARTICLE INFO

Article history:
Received on: 23/10/2013
Revised on: 29/11/2013
Accepted on: 12/12/2013
Available online: 27/02/2014

Key words:
Fused heterocycles;
Triazolothiadiazoles;
Antibacterial activity,
Antifungal activity.

ABSTRACT

A series of 6-aryl-3- (3,4–dialkoxyphenyl) -[1,2,4]triazole [3,4-b][1,3,4] thiadiazole (7a-7o) were synthesized by condensing 4-amino-5-(3,4-dialkoxyphenyl)-4H-[1,2,4]- triazole-3-thiol (6) with various aromatic carboxylic acids in the presence of phosphorous oxychloride through one-pot reaction. The structures of these newly synthesized compounds were confirmed on the basis of IR, ^{1}H NMR and mass spectral studies. All the synthesized compounds were screened for their antimicrobial activity against a variety of microorganisms.

INTRODUCTION

In recent years, a large number of fused heterocycles derived from 1,2,4-triazole moiety has received much attention due to their synthetic and effective biological importance. 1,2,4-triazole-containing ring system has been incorporated into a wide variety of therapeutically interesting drug candidates including anti-inflammatory, CNS stimulants, sedatives, antianxiety, cardiotonic, antimicrobial agents (Heindal *et al.,* 1980; Vio *et al.,* 1989; Holla *et al.,* 1994), and antimycotic activity such as fluconazole, itraconazole, voriconazole (Haber *et al.,* 2001). Also, there are known drugs containing the 1,2,4- triazole group e.g. triazolam, alprazolam, etizolam and furacylin (Karegoudar *et al.,* 2008). Moreover, a variety of biological activities have been reported for a large number of their derivatives, such as antimicrobial (Ram et al.,1990; Holla *et al.,* 1998; Holla *et al.,* 2003; Zitouni *et al.,* 2005; Prasad *et al.,* 2007), antitubercular (Walczak *et al.,* 2004), anticancer (Holla *et al.,* 2002; Holla *et al.,* 2003), anticonvulsant (Amir *et al.,* 2004), antiplatlet and antithrombotic properties (Rehse *et al.;* 1998), hypoglycemic (Mhasalkar *et al.,* 1970), anti-inflammatory and analgesic activities (Almasirad *et al.,* 2004). 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] thiadiazoles including different pharmacophores are aimed at.

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 BRUKER 375-FTIR Spectrometer. ^{1}H NMR spectra 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

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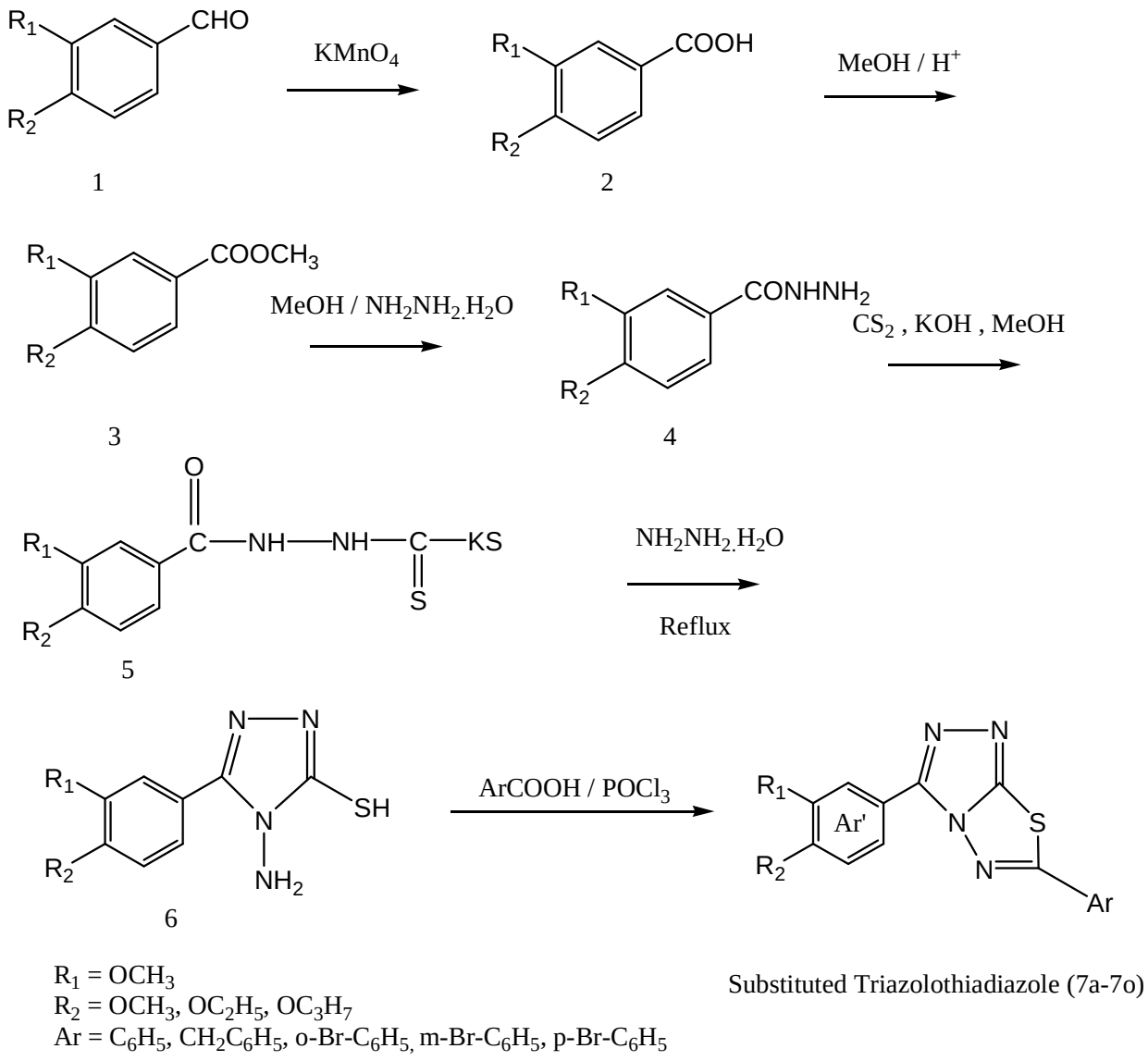

1 2 3 4 5 6

$R_1 = OCH_3$
$R_2 = OCH_3, OC_2H_5, OC_3H_7$
$Ar = C_6H_5, CH_2C_6H_5$, o-Br-C_6H_5, m-Br-C_6H_5, p-Br-C_6H_5

Substituted Triazolothiadiazole (7a-7o)

Scheme 1. Synthesis of substituted triazolothiadiazoles

appropriate grade and were used without purification. Reaction sequence employed for the synthesis of title compounds is shown in scheme 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 h. The reaction mass was stirred at 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 (Mathew *et al.*, 2006).

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 h. After quenching into cold water (in some reactions bicarbonates also added), precipitated solid was filtered, washed with water and dried. The completion of reaction was monitored by using TLC (Mathew *et al.*, 2006).

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 crystals 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 (Mathew *et al.*, 2006).

General method for the synthesis of 4-amino-5-(3,4-dialkoxyphenyl)-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.

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 (lead acetate paper and odor). 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 (Mathew *et al.*, 2006).

General method for the synthesis of 6-aryl-3- (3,4–dialkoxyphenyl) -[1,2,4]triazolo [3,4-b][1,3,4] thiadiazole (7a-7o)

A mixture of 4-amino-5-(3,4-dialkoxyphenyl)-4H-[1,2,4]- triazole-3-thiol (1 mmol) and substituted benzoic acid (1.1 mmol) in $POCl_3$ (5 ml) was refluxed for 8 h. The reaction mixture was slowly poured into crushed ice with stirring and neutralized with solid sodium bicarbonate and sodium hydroxide.

Solid residue was precipitated with evolution of gas. It was collected after 12 hours standing, was filtered, washed with cold water, dried and recrystallized from ethanol (7a-7o).

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

IR (KBr, cm^{-1}): 3097(C-H, aromatic), 2961(C-H, aliphatic), 1655(C=N str), 721(C-S-C) ; ^{1}H-NMR ($CDCl_3$) ∂ ppm : 7.32-7.48 (m, 5H, ArH), 6.72-6.93 (m, 3H, Ar'H), 3.73 (s, 6H, OCH_3); m/z, %: 339.3 (M^+).

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

IR (KBr, cm^{-1}) : 3023(C-H, aromatic),2904 & 2810(C-H aliphatic), 1669(C=N str), 698(C-S-C); ^{1}H-NMR ($CDCl_3$) ∂ ppm : 7.14-7.80 (m, 5H, ArH), 6.71-6.97 (m, 3H, Ar'H), 3.87 (s, 2H, CH_2), 3.66 (s, 3H, OCH_3), 2.00 (s, 3H, CH_3); m/z, %: 353.4 (M^+).

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

IR (KBr, cm^{-1}) : 3079(C-H, aromatic),2928 & 2873(C-H aliphatic), 1698(C=N str), 749(C-S-C); ^{1}H-NMR ($CDCl_3$) ∂ ppm : 7.22-7.48 (m, 5H, ArH), 6.72-6.93 (m, 3H, Ar'H), 3.94 (s, 2H, CH_2), 3.72 (s, 3H, OCH_3), 1.75 (s, 2H, CH_2), 0.89 (s, 3H, CH_3); m/z, %: 367.4 (M^+).

6-benzyl-3- (3,4 –dimethoxyphenyl) -[1,2,4]triazolo [3,4-b][1,3,4] thiadiazole (7d)

IR (KBr, cm^{-1}) : 3053(C-H, aromatic),2928(C-H aliphatic), 1655(C=N str),1456(CH_2 bend), 721(C-S-C); ^{1}H-NMR ($CDCl_3$) ∂ ppm : 7.07-7.49 (m, 5H, ArH), 6.58-7.01 (m, 3H, Ar'H), 3.88 (s, 2H, $CH_2C_6H_5$), 3.73 (s, 6H, OCH_3) ; m/z, %: 353.4 (M^+).

6-benzyl-3- (4-ethoxy-3-methoxyphenyl) -[1,2,4]triazolo [3,4-b][1,3,4] thiadiazole (7e)

IR (KBr, cm^{-1}) : 3079(C-H, aromatic),2974 & 2924(C-H aliphatic), 1645(C=N str), 750(C-S-C); ^{1}H-NMR ($CDCl_3$) ∂ ppm : 7.06-7.28 (m, 5H, ArH), 6.72-7.07 (m, 3H, Ar'H), 4.14 (s, 2H, CH_2), 3.78 (s, 2H, $CH_2C_6H_5$), 3.76 (s, 3H, OCH_3), 1.30 (s, 2H, CH_2); m/z, %: 367.4 (M^+).

6-benzyl-3- (3-methoxy-4-propoxyphenyl) -[1,2,4]triazolo [3,4-b][1,3,4] thiadiazole (7f)

IR (KBr, cm^{-1}) : 3031(C-H, aromatic),2924 & 2856(C-H aliphatic), 1652(C=N str), 721(C-S-C); ^{1}H-NMR ($CDCl_3$) ∂ ppm : 7.07-7.74 (m, 5H, ArH), 6.27-6.89 (m, 3H, Ar'H), 4.62 (s, 2H, CH_2), 3.76 (s, 2H, $CH_2C_6H_5$), 3.63 (s, 3H, OCH_3),1.92 (s, 2H, CH_2), 0.91 (s, 3H, CH_3); m/z, %: 381.4 (M^+).

6-(o-bromophenyl) -3- (3,4-dimethoxyphenyl) -[1,2,4]triazolo [3,4-b][1,3,4] thiadiazole (7g)

IR (KBr, cm^{-1}) : 2933(C-H aliphatic), 1654(C=N str), 751(C-S-C) ; ^{1}H-NMR ($CDCl_3$) ∂ ppm : 7.03-7.26 (m, 4H, ArH), 6.72-6.93 (m, 3H, Ar'H), 3.73 (s, 6H, OCH_3) ; m/z, %: 417.2 (M^+).

6-(o-bromophenyl) -3- (4-ethoxy-3-methoxyphenyl) -[1,2,4]triazolo [3,4-b][1,3,4] thiadiazole (7h)

IR (KBr, cm^{-1}) : 3061(C-H, aromatic),2918(C-H aliphatic), 1659(C=N str), 759(C-S-C), 562 (C-Br str); ^{1}H-NMR ($CDCl_3$) ∂ ppm : 7.11-7.49 (m, 4H, ArH), 6.72-6.93 (m, 3H, Ar'H), 3.88 (s, 2H, CH_2), 3.73 (s, 3H, OCH_3), , 1.33 (s, 3H, CH_3); m/z, %: 431.3 (M^+).

6-(o-bromophenyl) -3- (3-methoxy-4-propoxyphenyl) -[1,2,4]triazolo [3,4-b][1,3,4] thiadiazole (7i)

IR (KBr, cm^{-1}) : 3061(C-H, aromatic),2915(C-H aliphatic), 1614(C=N str), 751(C-S-C), 537 (C-Br str); ^{1}H-NMR

Table. 1: Physico-chemical data of substituted-[1,2,4]triazolo [3,4-b][1,3,4] thiadiazole (7a-7o) .

Comp code	R_1	R_2	Ar	Molecular Formula	Molecular Weight	Melting Point (°C)	Yield (%)
7a	OCH_3	OCH_3	C_6H_5	$C_{17}H_{14}N_4O_2S$	338	156	65
7b	OCH_3	OC_2H_5	C_6H_5	$C_{18}H_{16}N_4O_2S$	352	192	76
7c	OCH_3	OC_3H_7	C_6H_5	$C_{19}H_{18}N_4O_2S$	366	169	54
7d	OCH_3	OCH_3	$CH_2C_6H_5$	$C_{18}H_{16}N_4O_2S$	352	223	70
7e	OCH_3	OC_2H_5	$CH_2C_6H_5$	$C_{19}H_{18}N_4O_2S$	366	245	78
7f	OCH_3	OC_3H_7	$CH_2C_6H_5$	$C_{20}H_{20}N_4O_2S$	380	145	58
7g	OCH_3	OCH_3	o-Br-C_6H_5	$C_{17}H_{13}BrN_4O_2S$	417	221	69
7h	OCH_3	OC_2H_5	o-Br-C_6H_5	$C_{18}H_{15}BrN_4O_2S$	431	211	67
7i	OCH_3	OC_3H_7	o-Br-C_6H_5	$C_{19}H_{17}BrN_4O_2S$	445	167	73
7j	OCH_3	OCH_3	m-Br-C_6H_5	$C_{17}H_{13}BrN_4O_2S$	417	162	52
7k	OCH_3	OC_2H_5	m-Br-C_6H_5	$C_{18}H_{15}BrN_4O_2S$	431	160	56
7l	OCH_3	OC_3H_7	m-Br-C_6H_5	$C_{19}H_{17}BrN_4O_2S$	445	156	58
7m	OCH_3	OCH_3	p-Br-C_6H_5	$C_{17}H_{13}BrN_4O_2S$	417	174	61
7n	OCH_3	OC_2H_5	p-Br-C_6H_5	$C_{18}H_{15}BrN_4O_2S$	431	198	69
7o	OCH_3	OC_3H_7	p-Br-C_6H_5	$C_{19}H_{17}BrN_4O_2S$	445	223	70

($CDCl_3$) ∂ ppm : 7.11-7.49 (m, 4H, ArH), 6.72-6.93 (m, 3H, Ar'H), 3.98 (s, 2H, CH_2), 3.73 (s, 3H, OCH_3), 1.31 (s, 3H, CH_3); m/z, %: 431.3 (M^+).

6-(m-bromophenyl) -3- (3,4-dimethoxyphenyl) -[1,2,4]triazolo [3,4-b][1,3,4] thiadiazole (7j)

IR (KBr, cm^{-1}) : 2934(C-H aliphatic), 1644(C=N str), 583 (C-Br str); ^{1}H-NMR ($CDCl_3$) ∂ ppm : 7.21-7.65 (m, 4H, ArH), 6.72-6.93 (m, 3H, Ar'H), 3.73 (s, 6H, OCH_3) ; m/z, %:417.2 (M^+).

6-(m-bromophenyl) -3- (4-ethoxy-3-methoxyphenyl) - [1,2,4]triazolo [3,4-b][1,3,4] thiadiazole (7k)

IR (KBr, cm^{-1}) : 3060(C-H, aromatic),2944 & 2865(C-H aliphatic), 625 (C-Br str);
^{1}H-NMR ($CDCl_3$) ∂ ppm : 7.39-7.65 (m, 4H, ArH), 6.72-6.95 (m, 3H, Ar'H), 3.94 (s, 2H, CH_2), 3.72 (s, 3H, OCH_3), 1.75 (s, 3H, CH_3); m/z, %: 431.3 (M^+).

6-(m-bromophenyl) -3- (3-methoxy-4-propoxyphenyl) - [1,2,4]triazolo [3,4-b][1,3,4] thiadiazole (7l)

IR (KBr, cm^{-1}) : 3168(C-H, aromatic),2984 & 2846(C-H aliphatic), 1661(C=N str), 736(C-S-C), 632 (C-Br str); ^{1}H-NMR ($CDCl_3$) ∂ ppm : 7.21-7.42 (m, 4H, ArH), 6.72-6.96 (m, 3H, Ar'H), 3.97 (s, 2H, CH_2), 3.73 (s, 3H, OCH_3), 1.75 (s, 2H, CH_2), 0.93 (s, 3H, CH_3); m/z, %: 445.3 (M^+).

6-(p-bromophenyl) -3- (3,4-dimethoxyphenyl) -[1,2,4]triazolo [3,4-b][1,3,4] thiadiazole (7m)

IR (KBr, cm^{-1}) : 3010(C-H, aromatic),2947 & 2870(C-H aliphatic), 1698(C=N str), 737(C-S-C), 669 (C-Br str); ^{1}H-NMR ($CDCl_3$) ∂ ppm : 7.07-7.49 (m, 4H, ArH), 6.72-6.93 (m, 3H, Ar'H), 3.73 (s, 6H, OCH_3) ; m/z, %: 417.2 (M^+).

6-(p-bromophenyl) -3- (4-ethoxy-3-methoxyphenyl) - [1,2,4]triazolo [3,4-b][1,3,4] thiadiazole (7n)

IR (KBr, cm^{-1}): 3140(C-H, aromatic),2918(C-H aliphatic), 1673(C=N str), 757(C-S-C), 691 (C-Br str); ^{1}H-NMR ($CDCl_3$) ∂ ppm : 7.11-7.49 (m, 4H, ArH), 6.72-6.93 (m, 3H, Ar'H), 3.98 (s, 2H, CH_2), 3.73 (s, 3H, OCH_3), 1.33 (s, 3H, CH_3); m/z, %: 431.3 (M^+).

6-(p-bromophenyl) -3- (3-methoxy-4-propoxyphenyl) - [1,2,4]triazolo [3,4-b][1,3,4] thiadiazole (7o)

IR (KBr, cm^{-1}) : 3166(C-H, aromatic),2931(C-H aliphatic), 1652(C=N str), 731(C-S-C), 686 (C-Br str); ^{1}H-NMR ($CDCl_3$) ∂ ppm : 7.28-7.84 (m, 4H, ArH), 6.39-6.41 (m, 3H, Ar'H), 4.21 (s, 2H, CH_2), 3.65 (s, 3H, OCH_3), 1.73 (s, 2H, CH_2), 1.08 (s, 3H, CH_3); m/z, %: 445.3 (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 1698) and *Pseudomonas aeruginosa* (MTCC No 4673) bacterial strains by serial plate dilution method (Barry, 1991; Karegoudar *et al.*, 2008). Serial dilutions of the drug in Muller-Hinton broth were taken in tubes and their pH was adjusted to 5.0 using phosphate buffer. Standardized suspension of the test bacterium was inoculated and incubated for 16-18 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. A number of antimicrobial discs are placed on the agar for the sole purpose of producing zones of inhibition in the bacterial lawn. Agar media were poured into each petri dish. Excess of suspension was decanted and placing in incubator at 37 °C for 1 h dried the plates. Using an agar punch, wells were made on these seeded agar plates and minimum inhibitory concentrations of the test compounds in dimethylsulfoxide (DMSO) were added into each labelled well. A control was also prepared for the plates in the same way using solvent DMSO. The petri dishes were prepared in triplicate and maintained at 37 °C for 3-4 days. Antibacterial activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with Gentamycin as standard. Zone of inhibition was determined for (7a-7o) and the results are summarized in table no 2.

Antifungal activity

Newly prepared compounds were screened for their antifungal activity against *Candida albicans* (MTCC No 1637) and

Aspergillus niger (MTCC No 10180), in DMSO by serial plate dilution method (Verma, 1998; Karegoudar *et al.,* 2008). Agar media were prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 ml) and adjusting the pH to 5.7. Normal saline was used to make a suspension of spore of fungal strains for lawning. A loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of corresponding species. Agar media of 20 ml were poured into each petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37°C for 1 h. Using an agar punch each labelled well were made on these seeded agar plates and minimum inhibitory concentrations of the test compounds in dimethylsulfoxide (DMSO) were added into each labelled well. A control was also prepared for the plates in the same way using solvent DMSO. The petri dish were prepared in triplicate and maintained at 37 °C for 3-4 days. Antifungal activity was determined by measuring the diameter of the inhibition zone. Activity of each compound was compared with Miconazole as standard. Zones of inhibition were determined for (7a-7o) and the results are summarized in table no 3.

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

Comp	MIC* in µg/ml and zone of inhibition (mm)			
	S. aureus	***B. cereus***	***E. coli***	***P. aeruginosa***
7a	6.25 (08)	25 (< 5)	25 (< 5)	25 (< 5)
7b	25 (< 5)	25 (< 5)	25 (< 5)	25 (< 5)
7c	25 (< 5)	25 (< 5)	25 (< 5)	25 (< 5)
7d	12.5 (06)	12.5 (06)	12.5 (< 5)	25 (05)
7e	12.5 (05)	25 (< 5)	25 (< 5)	25 (< 5)
7f	12.5 (06)	25 (< 5)	25 (< 5)	25 (< 5)
7g	25 (05)	12.5 (06)	25 (< 5)	25 (< 5)
7h	25 (< 5)	25 (< 5)	25 (< 5)	25 (< 5)
7i	25 (< 5)	25 (< 5)	25 (< 5)	25 (< 5)
7j	25 (< 5)	25 (< 5)	25 (< 5)	25 (< 5)
7k	12.5 (06)	25 (< 5)	12.5 (05)	25 (< 5)
7l	25 (< 5)	25 (< 5)	25 (< 5)	25 (< 5)
7m	6.25 (08)	6.25 (08)	12.5 (06)	25 (< 5)
7n	12.5 (06)	25 (< 5)	25 (< 5)	25 (< 5)
7o	12.5 (06)	25 (< 5)	25 (< 5)	25 (< 5)
Gentamycin	1.56 (16)	6.25 (10)	1.56 (16)	6.25 (10)

* the MIC values were evaluated at concentration range, 1.56 – 25 µg/ml

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

Comp	MIC* in µg/ml and zone of inhibition (mm)	
	C. albicans	***A. niger***
7a	12.5 (11)	6.25 (12)
7b	25 (< 5)	25 (< 5)
7c	25 (< 5)	25 (< 5)
7d	12.5 (07)	25 (< 5)
7e	25 (< 5)	25 (< 5)
7f	25 (< 5)	25 (< 5)
7g	25 (< 5)	25 (05)
7h	25 (< 5)	25 (05)
7i	25 (< 5)	25 (< 5)
7j	25 (< 5)	25 (< 5)
7k	25 (< 5)	25 (< 5)
7l	25 (< 5)	25 (< 5)
7m	25 (< 5)	12.5 (06)
7n	25 (< 5)	12.5 (08)
7o	25 (< 5)	12.5 (06)
Miconazole	1.56 (14)	1.56 (12)

***** the MIC values were evaluated at concentration range, 1.56 – 25 µg/ml

RESULTS AND DISCUSSION

Synthesis

The synthesis of 6-aryl-3- (3,4–dialkoxyphenyl) -[1,2,4]triazole [3,4-b][1,3,4] thiadiazoles (7a-7o) is shown in scheme 1. All synthesized compounds were obtained in good yields as shown in table no 1 and the structure of all newly synthesized compounds 7a-7o were confirmed by IR, ^{1}H NMR and Mass spectral data.

Biological Evaluation

Antibacterial activity

The antibacterial activity of the newly synthesized compounds 7a-7o were reported as minimum inhibitory concentration (MIC) at the concentration range, 1.56 – 25 µg/ml against *Staphylococcus aureus* , *Bacillus cereus* (Gram-positive bacteria) and *Escherichia coli*, *Pseudomonas aeruginosa* (Gram-negative bacteria) using gentamycin as standard and the results are summarized in table no 2. Compounds **7a, 7d** and **7m** showed comparatively good activity against *S. aureus* and *B. cereus*, Compound **7g** showed moderate to good activity against *B. cereus* and Compound **7m** exhibited moderate to good activity against *E. coli*.

Antifungal activity

The antifungal data of the synthesized compounds 7a-7o were reported as minimum inhibitory concentration (MIC) at the concentration range, 1.56 – 25 µg/ml against *Candida albicans* and *Aspergillus niger* using miconazole as standard and the results are summarized in table no 3. The compound **7a** exihibited highest activity against all tested fungi. Compound **7d** showed good activity against *C. albicans* while Compounds **7m, 7n** and **7o** exhibited moderate to good activity against *A. niger*.

CONCLUSION

Fifteen fused heterocyclic derivatives (Scheme 1 and Table No 1) were successfully synthesized. Among the newer analogues, three compounds, 3- (3,4-dimethoxyphenyl) -6-phenyl-[1,2,4]triazolo [3,4-b][1,3,4] thiadiazole **7a,** 6-benzyl-3-(2,3 –dimethoxyphenyl) -[1,2,4]triazolo [3,4-b][1,3,4] thiadiazole **7d** and 6-(p-bromophenyl) -3- (3,4-dimethoxyphenyl) -[1,2,4]triazolo [3,4-b][1,3,4] thiadiazole **7m** exhibited promising antimicrobial activity. These compounds could be further modified to develop potential and safer antifungal agents.

ACKNOWLEDGEMENT

The authors are thankful to IISER, Bhopal and School of Pharmaceutical Sciences, RGPV, Bhopal for providing IR, ^{1}H NMR and Mass spectra of compounds. The authors are also thankful to Institute of Microbial Technology, Chandigarh for providing bacterial and fungal strains for the compounds reported herein.

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How to cite this article:

Jagdish K. Sahu, Swastika Ganguly, Atul Kaushik. Synthesis of Some Novel Heterocyclic 1,2,4-triazolo [3,4-b][1,3,4] Thiadiazole Derivatives as Possible Antimicrobial Agents. J App Pharm Sci, 2014; 4 (02): 081-086.