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Research Article

Synthesis, Characterization and Biological Evaluation of Some Novel N-Mannich Bases of Heterocyclic 1,3,4-thiadiazole.

Singh Charanjeet [1*]

[1]*Biyani Institute of Pharmaceutical Sciences, Jaipur, Rajasthan (INDIA)*

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ABSTRACT

A series of some novel N-Mannich bases of heterocyclic 1,3,4-thiadiazole were synthesized through the condensation reaction of 1,3,4-thiadiazole containing a aromatic secondary amine, aromatic aldehydes and cyclic compounds employing Mannich reaction and using conventional synthesis. All the synthesized compounds were obtained in the range of 57.41-83.3 % yield. The structures of synthesized compounds were confirmed by UV, IR, ^{1}H NMR spectroscopy. the essential structural features responsible for interaction with receptor site are established within a suggested pharmacophore. The *in vitro* antibacterial activity of the synthesized compounds was determined, against two Gram-positive bacteria, viz. *S. aureus* & *B. subtilis* and Gram-negative, viz. *E. coli* and *K. pneumoniae*, by cup-plate method using the standard drug ciprofloxacin. Minimum inhibitory concentrations (MIC) changed in the range of 1.56_ _ 200 mg mL_1. Compound 3b exhibited excellent activity against both bacteria. The *in vitro* antifungal activity of the synthesized compound was also evaluated by cup-plate method against the fungi A. *niger* and *C. albicans* compared with the standard drug Fluconazole. Compound 4a, 8a exhibited excellent activity against both fungi. The result has show that the compounds are quite active against pathogens under study and were nontoxic. The anti-inflammatory activity of the compound was evaluated, on albino rats, by carageenan induced rat paw oedema method using the standard drug diclofenac sodium. Compound 7b and 8c exhibited excellent anti-inflammatory and analgesic pharmacological activities. Structurally the compound 7b has a greater number of unsaturated hydrocarbons in schiff base, which shows good lipophilic properties within electron rich morpholine ring in Mannich base. Statistical significance of differences between group was determined by one-way analysis of variance (ANOVA).Among the synthesized compounds 3a, 4b, 5c, 7b, 8a and 8c were found be the most active. All the synthesized compounds were found to be low lethal as ascertained by LD_{50} test.

Keywords: N- Mannich bases of heterocyclic 1,3,4-thiadiazole derivatives; Mannich reaction; antimicrobial agents; anti-inflammatory activity

INTRODUCTION

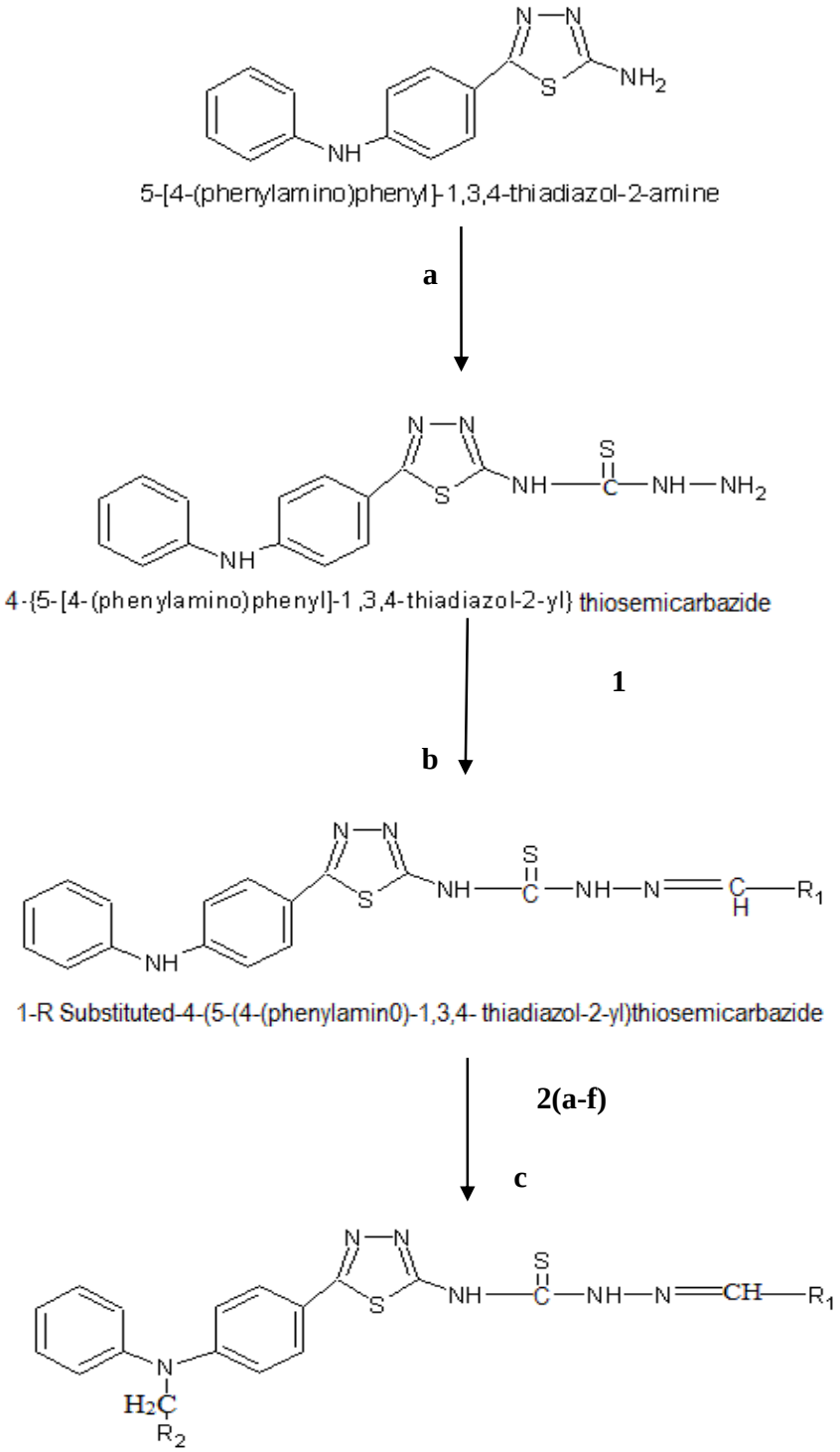
A lot of research has been done on N-Mannich bases derivatives and more research is going on in present time. From the literature it was concluded that the derivatives of N-Mannich bases of 1,3,4-thiadiazole shown diver type of activities viz antimicrobial, anti-inflammatory activities. Furthermore N-Mannich bases of 1,3,4-thiadiazole which contain an azomethine group attract much interest due to their synthetic along with antitumor (Dhapalapur MG et al.,1968) antimycobacterial (Patole J et al., 2006) antihypertensive (Turner S et al., 1988, Turner S et al., 1988), properties. The thiadiazole nucleus with N-C-S linkage exhibits a large number of biological activities (Kurtzer F et al.,1965). The treatment of microbial infections, especially bacterial infection, has become an important problem to solve due to the emergence of mulidrug resistance. As a contribution to the new antibacterial drug development we have previously synthesized N-Mannich bases of 1,3,4-thiadiazole derivatives. The aim of this study was to synthesize some N-Mannich bases having inhibition against *microbial infections*. An additionally anti-inflammatory activity was evaluated.

The interesting properties of many of these heteroccycles have increase the need for rapid synthesis of new, potentially useful sulfur-nitrogen containing heterocycles.

Chemistry

A lone pair of electrone is present on nitrogen atom. 1,3,4-thiadiazole is a weak base. Electrone density on the nitrogen atom and facilitative reaction undergoes with eletrophilic reagents. It has a common structure which may consist of an aromatic secondary amine of 1,3,4-thiadiazole ring, a thionyl carbon (none of the valencies of which is satisfied by hydrogen) asa thiosemicarbazide and Schiff base between the flat carbon (-N=CH-). From the considerations of the above facts , it was planned to synthesize Mannich bases of 1-substituted-4-(4-(phenylamino)phenyl)-1,3,4-thiadiazole-2-yl)thiosemicarbazide by carrying out reaction between 1-substituted-4-(5-(4-(phenylamino)phenyl)-1,3,4-thiodiazole-2-yl)thiosemicarbazide,formaldehyde and dimethylamine, morpholin, piperidine. Basic moiety 4-(5-(4-(phenylamino)phenyl)-1,3,4-thiodiazol-2-yl)thiosemicarbazide (**1**) was synthesized from 5-(-4-(phenylamino)phenyl)-1,3,4-thiodiazole-2-amine, into their corresponding carbon disulphide in presence of

**Author for Correspondence: charanjeet2004@gmail.com*

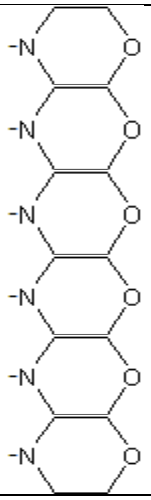
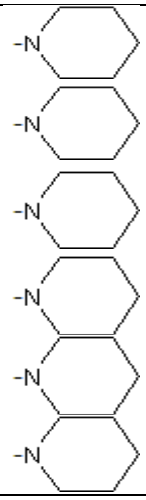


4-(5-(4-(N-phenyl-N-(R2-substituted)methylamino)phenyl)-1,3,4-thiadiazol-2-yl)-1-R1-substituted-thiosemicarbazide, 3(a-c), 4(a-c), 5(a-c), 6(a-c), 7(a-c), 8(a-c).

Fig. 1 Graphical abstract of synthesized title compounds.

alcoholic N_aOH TO obtain the sodium salts, followed by the addition of the hydrazine hydrate gives the thiosemicarbazide compound, which involved the reported reaction mechanism of Nucleophilic Addition Reaction (Ananthanaryan R et al., 2005). Further derivatives of methylene-4-(5-(-(phenylamino) phenyl-1,3,4-thiadiazol-2-yl)thiosemicarbazide 2(a-f), were synthesized by 4-(-5-(4-(-phenylamino)phenyl-1,3,4-thiadiazol-2-yl)thiosemicarbazide and different aromatic and heterocycle aldehydes. Reaction mechanism of this step undergoes Nucleophilic Addition Reaction (Morrison R T rt al.,2005). Title compounds **3(a-c), 4(a-c), 5(a-c), 6(a-c),**

Table 1: Compounds synthesized

Compound	R_1	R_2		
		a	**b**	**C**
3	C_6H_5	$N(CH_3)_2$		
4	o-C_6H_4-OH	$N(CH_3)_2$		
5	p-C_6H_4-Cl	$N(CH_3)_2$		
6	p-C_6H_4-$N(CH_3)_2$	$N(CH_3)_2$		
7	CH=CH-C_6H_5	$N(CH_3)_2$		
8	C_4H_3O*	$N(CH_3)_2$		

*Furan

(a) NaOH, DMF, CS2, $NH_2NH_2.H_2O$, 60^0C. **(b)** Aromatic aldehydes, Few drops CH_3COOH, C_2H_5OH. **(c)** Tetrahydrofuran, Formaldehyde, concentrated hydrochloric acid, Dimethylamine, Morpholine and Piperidine (a-c), Room temperature for 1hr.

7(a-c), 8(a-c) derivatives of N-Mannich bases of heterocyclic 1,3,4-thiadiazol were prepared from 1-R_1Substituted-4-(5-(4-(phenylamino)phenyl)-1,3,4)thiosemicarbazide and formaldehyde, dimethylamine, morpholine, piperidine. Reaction mechanism of this step undergoes N-Mannich Base Reaction (Agarwal O P, 2006). Mannich reaction is an approach to bring about condensation between a compound containing atleast one active hydrogen ayom , formaldehyde and ammonia or a primary amine or a econdary amine. An example of Mnnich reaction is reaction between acetophenone or another aryl amino [(diphenyl amine) compound containing active hydrogen], formaldehyde and secondary amine (Adam R., 1996).

MATERIALS AND METHODS

Preparation of 4-(5-(4-(phenylamino)phenyl)-1,3,4-thiadiazol-2-yl)thiosemicarbazide (1)

3.76g (0.014mol) 5-(4-(phenylamino) phenyl)-1,3,4-thiadiazol-2-amine was taken in 15ml of

Table 2 Characterization of synthesized compounds.

Compound name	Specroscopic data
3(a-c)	IR ATR (cm^{-1})= 3340(N-H), 3004(Ar.C-H), 2910(Ali-c-h), 1576(C=N), 1667(C-N), 1497(N-N), 104(C==S); ^{1}H-NMR (DMSO-d6, ppm): 2.5(s, 2H, NH), 6.6-6.8(m, 9H, CH, Ar-H, DEPHENYLAMINE), 7.0-7.8(m, 5H, CH, Ar-H, C_6H_5); Electron absorption spectra (UV) max 296.76(methanol)
4(a-c)	IR ATR(CM^{-1})=3390(N-H),3105(Ar.C-H),2878(Ali-C-H),1560(C=N),1650(C-N), 1524(N-N),1214(C-S);^{1}H-NMR(DMSO-d6,δ ppm):2.1(s,2H,NH),2.6(s,1H,Ar-OH),6.8-6.99(m,9H,CH,Ar-H,diphenylamine),7.0-7.8(m,4H,Ar-H,C_6H_4),8.0-8.7(s,1H,CH,α- to C_6H_4-OH);Electron absorption spectra (UV)λ_{max} 328 (methanol).
5(a-c)	IR ATR(CM^{-1})=3420(N-H),3020(Ar-C-H),2915(Ali-C-H),1720(C=N),1570(C-N),1645(Ar-C=C),1440(N-N),1210(C-S),1095(Ar .C-Cl);^{1}H-NMR (DMSO-d6,δ ppm):2.5(s,2H,NH),2.5-2.8(t,3H,$N(CH_3)_2$),6.6-6.8(m,9H,CH,Ar-H,diphenylamine);Electron absorption spectra (UV) λ_{max} 334.6 (methanol).
6(a-c)	IR ATR(CM^{-1})=3420(N-H),3000(Ar-CH),2950(Ali-C-H),1540(C=N),1540(C-N),1550(N-N),1210(C-S),^{1}H-NMR (DMSO-d6,δ ppm):2.1(t,3H,CH_3,Aliph-H,dimethylamine),2.4(s,1H,NH),6.5-6.9(m,9H,CH,Ar-H,diphenylamine),7.2-7.4(m,Ar-CH,C_6H_4);Electron absorption spectra (UV)λ_{max} 315.8 (methanol).
7(a-c)	IR ATR(CM^{-1})=3130(N-H),3010(Ar-C-H),2096(Ali-CH)1660(C=N),1580(C-N),1510(Ar-C-C),1515(N-N);^{1}H-NMR (DMSO-d6,δ ppm):2.1(s,1H,NH),6.6(s,1H,CH,Aliph.-H,α- to C_6H_5)7.0-7.5(m,9H,CH,Ar-H,diphenylamine);Electron absorption spectra (UV) λ_{max} 246.46 (methanol).
8(a-c)	IR ATR(CM^{-1})=3240(N-H),3060(Ar-C-H),2910(CH_2),1620(C=N),1610(C-N),1540(Ar-C-C),1540(N-N),1486(C-O),1210(C-S);^{1}H-NMR (DMSO-d6,δ ppm):3.3(s,1H,NH),5.9(m,3H,CH,heter.-h,furan) 6.5-6.9(m,9H,CH,Ar-H,diphenylamine);Electron absorption spectra (UV)λ_{max} 326.6 (methanol).

Table 3 physiochemical properties of synthesized compounds.

Comp.	M.formula	M.wt	Rast's M.wt.	Depression of m.p.ΔT(^{0}C)	B.P.[K]	Log P	MR[CM^3/mol]	HENRY'S LAW	Gibbs Energy[kJ/mol]
3a	$C_{25}H_{25}N_7S_2$	488	484.9	81.87	1342.73	7.39	147.48	6.31	298.15
3b	$C_{27}H_{27}N_7OS_2$	530	532.5	74.55	1435.39	6.99	156.53	6.31	298.15
3c	$C_{28}H_{29}N_7S_2$	528	529.3	75.00	1431.32	8.12	159.4	6.31	298.15
4a	$C_{25}H_{25}N_7OS_2$	504	506.4	78.39	1423.35	7	149.29	6.31	298.15
4b	$C_{27}H_{27}N_7O_2S_2$	546	544.3	72.94	1516.01	6.6	158.34	6.31	298.15
4c	$C_{28}H_{29}N_7OS_2$	544	546.8	72.63	1511.94	7.73	161.21	6.31	298.15
5a	$C_{25}H_{24}ClN_7S_2$	521.5	524.5	75.54	1385.14	7.95	152.08	6.31	298.15
5b	$C_{27}H_{26}ClN_7OS_2$	563.5	565.3	70.10	1477.8	7.55	161.13	6.31	298.15
5c	$CH_{28}H_{28}ClN_7S_2$	561.5	558.2	71.50	1473.73	8.68	164.01	6.31	298.15
6a	$C_{27}H_{30}N_8S_2$	531	533.5	74.42	1405.91	7.67	162.66	6.31	298.15
6b	$C_{29}H_{32}N_8OS_2$	573	570.1	69.63	1498.57	7.27	171.71	6.31	298.15
6c	$C_{30}H_{34}N_8S_2$	571	573.4	69.24	1494.5	8.41	174.58	6.31	298.15
7a	$C_{27}H_{27}N_7S_2$	514	513.5	77.31	1392.65	7.27	157.53	6.31	298.15
7b	$C_{29}H_{29}N_7OS_2$	556	558.6	71.07	1485.31	6.87	166.58	6.31	298.15
7c	$C_{30}H_{31}N_7S_2$	554	556.2	71.38	1481.24	8.01	169.58	6.31	298.15
8a	$C_{23}H_{23}N_7OS_2$	478	475.8	83.43	1320.49	6	140	6.31	298.15
8b	$C_{25}H_{25}N_7O_2S_2$	520	518.1	76.62	1413.15	5.6	149.05	6.31	298.15
8c	$C_{26}H_{27}N_7OS_2$	518	521.1	76.19	1409.08	6.74	151.92	6.31	298.15

dimethylformamide (DMF) in a flat bottom flask. To this sodium hydroxide (0.014mol), carbon disulphide(0.014mol) were added. The mixture was stirred at 15-20^{0}C for 1h, to the stirred mixture was added hydrazine hydrate (0.014mol) and stirred continue for 1 h more at 60^{0}C. On adding water, a pale-yellow solid separated out which was recrystallized from DMF-Ethanol. T he pale yellow coloured product obtained, yield 78%, melting point 251^{0}C and calculated R^f 0.72 (Pandeya S N, et al.,1999).

Derivatives of methylene-4-(5-(4-(phenylamino)phenyl)-1,3,4-thiadiazol-2-yl)thiosemicarbazide 2(a-f)

In a dry flat bottom flask 3.42g 4-(5-(4-(phenylamino)phenyl)-1,3,4-thiadiazole-2-yl)thiosemicarbazide was taken in 35ml of ethanol. This solution was added an equimolar quantity of the appropriate different aromatic and heterocyclic aldehydes in a small quqntity of alcohol. Then added a few drops of glacial acetic acid, stirring was done for 5 min. Immediate precipitation occurred and solid was filtered, dried and

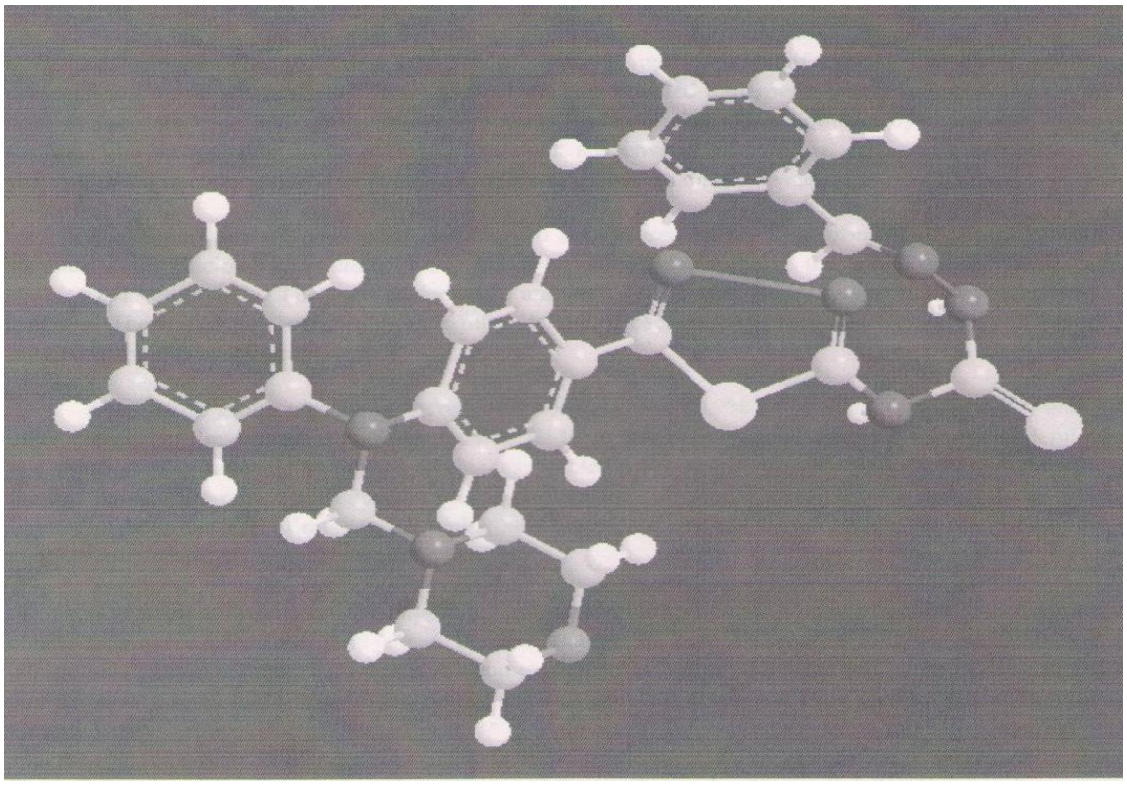


Fig. 2 Three dimension structure of title compound with energy minima.

Table 3 Results of in vitroantimicrobial activity of synthesized compunds 3-8(a-c)

Comp.	Gram +ve bacteria				Gram –ve bacteria			
	S. aureus		B. subtilis		E. coli		K. Pneumoniae	
	ZOI (mm)	% Inhibition	ZOI (mm)	% Inhibition	ZOI (mm)	% Inhibition	ZOI (mm)	% Inhibition
5a	12	52.17	18	85.71	10	43.47	16	69.56
5b	19	82.60	20	95.23	08	34.78	15	65.21
5c	06	26.08	08	38.09	14	60.86	13	56.52
6a	19	82.60	12	57.14	12	52.17	13	56.52
6b	15	65.21	06	28.57	08	34.78	06	26.08
6c	04	17.39	14	66.66	06	26.08	14	60.86
7a	04	17.39	12	57.14	06	26.08	14	60.86
7b	16	69.56	10	47.61	06	26.08	10	43.47
7c	06	26.08	06	28.57	08	34.78	12	52.17
8a	09	39.13	08	38.09	08	34.78	10	43.47
8b	12	52.17	08	38.09	12	52.17	06	26.08
8c	05	21.73	12	57.14	08	34.78	09	39.13
9a	05	21.73	06	28.57	12	52.17	15	65.21
9b	03	13.04	08	38.09	08	34.78	15	65.21
9c	12	52.17	06	28.57	14	60.86	15	65.21
10a	06	26.08	06	28.57	08	34.78	11	47.82
10b	08	34.78	12	57.14	10	43.47	13	56.52
10c	06	26.08	06	28.57	16	69.56	09	39.13
Standard (ciprofloxacin)	23	100.00	21	100.00	23	100.00	23	100.00

recrystellized from hot ethanol. The white powder product obtained, yield 73%, melting point 243^{0}C andclculated R^f 0.68 (Yogeeswari Pet al.,2004).

Synthesis of N-Maanich bases of heterocyclic 1,3,4-thiadiazol derivatives, 3(a-c), 4(a-c), 5(a-c), 6(a-c), 7(a-c), 8(a-c) (Pandeya S N et al.,1999).

Preparation of 4-(5-(4-(N-(substituted)methyl)-N-phenylamino)phenyl)-1,3,4-thiadiazol-2-yl)-1-benzylidenethiosemicarbazide(3a-c)

2.44g (0.005mol) 1-benezylidene-4-(5-(4-(phenylamino)phenyl)-1,3,4-thiadiazol-2-yl)thiosemicarbazide was placed in a 250ml round bottom flask in 8ml tetrahydronfuran. To this solution was added 2ml formaldehyde and 2-3 drops of concentrated hydrochloric acid. To this mixture (0.005mol) dimethylamine, morpholine and piperidine(a-c) were added with cooling and shaking respectively. The reaction mixture was allowed to stand at room temperature for 1hr with occasional shaking, after which it was warmed on a steam bath for 15 min. At the end of the period, the contents were cooled and the product obtained was recrystallized with choloroform : petroleum ether (50:50). Synthesized compounds (3a-c)were obtained with yield 64.2%, 72.7% and 68.4% respectively. The colored product observed, melting point 241^{0}C and calculated R^f 0.74.

Graph 1 Comparison of in vitro antibacterial activity exhibited by the test compounds 3-8(a-c) and standard drug ciprofloxacin

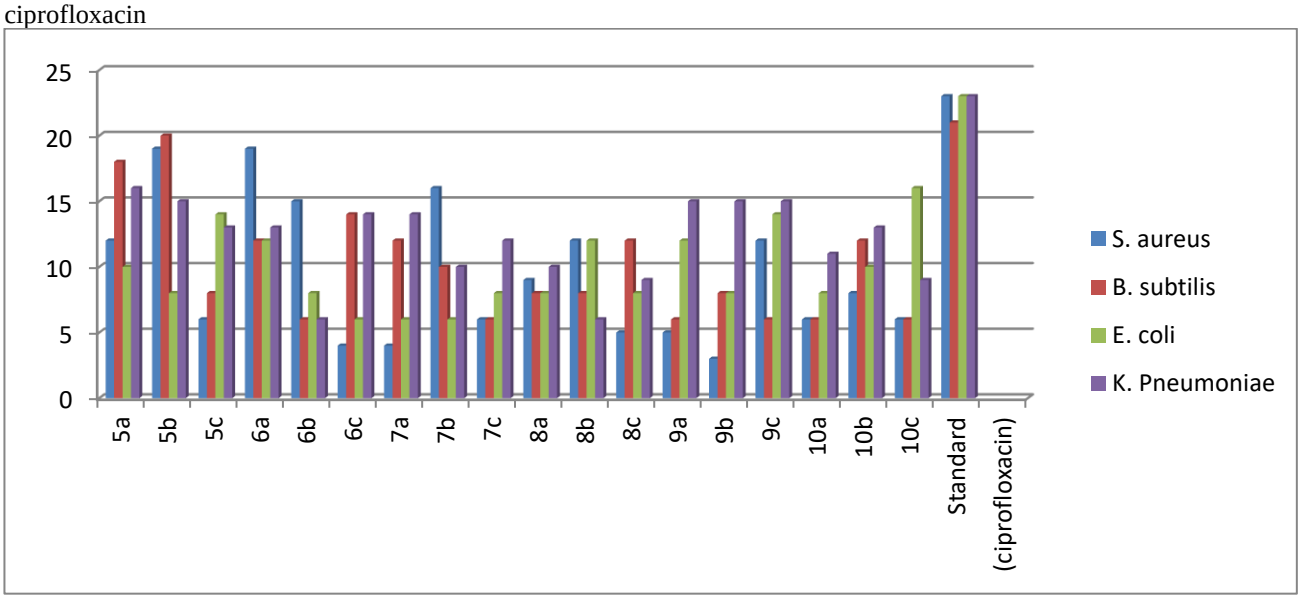


Table 4 Results of in vitro antifungal activity of synthesized compunds

Comp.	A.niger		C.albicans	
	Zone of inhibition (mm)	% inhibition	Zone of inhibition (mm)	% inhibition
3a	13	68.42	05	31.25
3b	10	52.63	06	37.50
3c	11	57.89	08	50.00
4a	18	94.73	15	93.75
4b	10	52.63	17	106.25
4c	11	57.89	04	25.00
5a	14	73.68	09	56.25
5b	11	57.89	09	56.25
5c	12	63.15	12	75.00
6a	09	47.36	07	43.75
6b	12	63.15	13	81.25
6c	10	52.63	08	50.00
7a	10	52.63	04	25.00
7b	12	63.15	06	37.50
7c	07	36.84	04	37.50
8a	13	68.42	17	106.25
8b	06	31.57	10	62.50
8c	09	47.36	16	100.00
Std(flucon azole)	19	100.00	16	100.00

Preparation of 1-(o-hydroxybenzylidene)-4-(5-(4-(N-(substituted)-N-phenylamino)phenyl)-1,3,4-thiadiazol-2-yl)thiosemecarbazide(4a-c)

2.23g (0.005mol) 1-(o-htdroxybenzylidene)-4(5-(4-(phenylamino)phenyl)-1,3,4-thiadiazol-2-yl) thiosemecarbazide was placed in a 250ml round bottom flask in 8 ml tetrahydrofuran. To this solution was added 2ml formaldehyde and 2-3 drops of concentrated hydrochloric acid. To this mixture (0.005mol)dimethylamine, morpholine and piperidine (a-c) were added with cooling and shaking respectively. The reaction mixture was allowed to stand at room temperature for 1hr with occasional shaking, after which it was warmed on a steam bath for 15 min. At the end of the period, the contents were cooled and the product obtained was recrystallized with chloroform : petroleum (50:50). Synthesized compounds(4a-c)were obtained with yield 57.41%, 66.7% and63.6%respectively. The colourless product observed, melting point 230^0C and calculated R^f 0.54.

Preparation f 1-(4-chlorobenzylidene)-4-(5-(4-(N-(substituted)methyl)-N-phenylamino)phenyl)-1,3,4-thiadiazol-2-yl)thiosemecarbazide(5a-c)
thiadiazol-2-yl)thiosemecarbazide(5a-c)

2.32g (0.005mol) 1-(4-chlorobenzylidene)-4-(5-(4-(phenylamino)phenyl)-1,3,4-thiadiazol-2-yl)thiosemecarbazide was placed in 250ml round bottom flask in 8ml tetrahydrofuran. To this solution was added 2ml formaldehyde and 2-3 drops of concentrated hydrochloric acid. To this (0.005mol) dimethylamine, morpholine and piperidine (a-c)were added with cooling and shaking respsctively. The reaction mixture was allowed to stand at room tempersture for 1hr with occasional shaking, after which it was warmed on the steam bath for 15min. At the end of the period, the contents were cooled and the product obtained was recrystallized with chloroform : petroleum (50:50). Synthesised compounds 95A-C0were obtained with yield 58%, 65.4% and 61.6% respectively. The colourless product observed, melting point 240^0c and calculated R^f 0.46.

Preparation of 1-(4-(dimethylamino)benzylidene)-4-(5-(4-(N-substituted)methyl)-N-phenylamino)phenyl)1,3,4-thiadiazol-2-yl)thiosemicarbazide(6a-c)

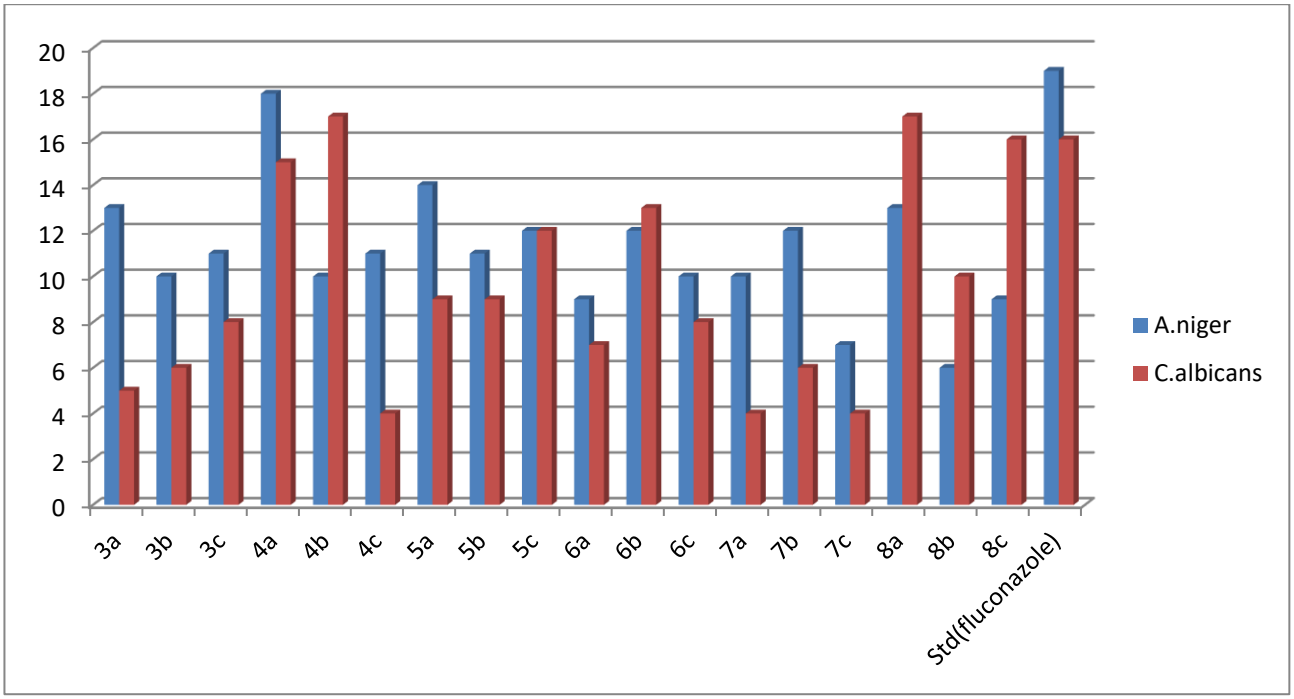
2.37g 0.005mol 1-(4-(dimethylamino)benzylidene)-4-(5-(4-(phenylamino)phenyl)-1,3,4-thiadiazol-2-yl)thiosemicarbazide was placed in 250ml round bottm flask in 8ml tetrahyrofurane. To this solution was added 2ml formaldehyde and 2-3 drops of concentrated hydrochloric acid. To this (0.005mol) dimethylamine , morpholine and piperidine (a-c) were added with cooling and shaking respectively. The reaction mixture was allowed to stand at room temperature for 1hr with occasional shaking, after whicw it was warmed on a steam bath for 15min. At the end of the period, the contents were cooled and the product obtained was recrystallized with chloroform : petroleum (50:50). Synthesised compounds (6a-c)were found with yield 69.8%, 73.7% and 70% respectivele. T he white crystalline product observed, melting point 243^0C and calculated R^f 0.63.

Preparation of 4-(-5-(4-(N-(substituted)methyl)-N-phenylamino)phenyl)-1,3,4-thiadiazol-2-yl)-1-(3-phenylallylidene)thiosemicarbazide (7a-c)

2.28g (0.005mol) 1-(3-phenylallylidene)-4-(5-(4-(phenylamino)phenyl)-1,3,4-thiadiazol-2-yl)thiosemicarbazide was placed in a round bottom flask in 8ml tetrahydrofuran. To this solution was added 2ml formaldehyde and 2-3 drops of concentrared hydrochloric acid. Tot this (0.005mol) dimethylamine, morpholie and piperiden (a-c) were added with cooling and shaking respectively. The reaction mixture was allowed to stand at room temperature for 1hr with occasional shaking, after which it was warmed on steam bath for 15min. At the end of the period, the contents were cooled and the product obtained was recrystallized with chloroform : petroleum (50:50). Synthesised compounds (7a-c) were obtained with yield 75%, 76.8% and 74% respectively. The pale yellow product observed, melting point 227^0C and calculated R^f 0.71.

Preparation of 4-(5-(4-(N-(substituted)methyl-N-phenylamino)phenyl)-1,3,4-thiadiazol-2-yl)-1-(furan-2-yl)methylene)thiosemicarbazide (8a-c)

2.10g (0.005mol) 1-(furan-2-yl)methylene)-4-(5-(4-(phenylamino)phenyl)-1,3,4-thiadiazol-2-yl)thiosemicarbazide was placed in round bottom flask in 8ml tetrahydrofuran. TOthis solution was added 2ml formaldehyde and 2-3 drops of concentrated hydrochloric acid. To this (0.005mol) dimethylamine, morpholine and piperidine (a-c)were added with cooling and shaking respectively. The reaction mixture was allowed to stand at room temperature for 1hr with occasional shaking, after which it was warmed on a steam bath for 15min. At the



Graph 2 Comparison of the in vitro antifungal activity exhibited by the test compounds and standard drugs fluconazole

end of the period, the contents were cooled and the product obtained was recrystallized with chloroform : petroleum (50:50). Synthesised compounds (8a-c) were obtained with yield 78.8%, 83.3% and 79.6% respectively. The colouless product observed, melting point 249^{0}C and calculated R^f 0.59.

Characterization of the synthesized compounds

Synthesised compounds N-Mannich bases of heterocyclic 1,3,4-thiadiazol derivatives **3(a-c), 4(a-c), 5(a-c), 6(a-c), 7(a-c), 8(a-c)** were synthesized by the reaction between 1-R1Substituted-4-(5-(4-(phenylamino)phenyl)-1,3,4-thiadiazol-2-yl)thiosemicarbazide and formaldehyde, dimethylamine, morpholine, piperidine using reported method. All melting points (m.p) were determined in open capillary method using Jindal point apparatus and were uncorrected.The purity of the compounds was routinely checked by thin layer chromatography (TLC) using silica gel G (Merck). The instruments used for spectroscopy data are FTIR : Bruker tensor-27 spectrophotometer (ATR) with diffuse reflectance method. H^1NMR :JEOL GSX-400, 60MHz spectrometer in $CDCl_3$, TMS (tetra methyl silane) as an internal standard. H^1NMR and IR spectra were consistent with the assigned structure. The results obtain which are shown in table 1 indicates, derivatives of N-Mannich bases of heterocyclic 1,3,4-thiadiazol **3(a-c), 4(a-c), 5(a-c), 6(a-c), 7(a-c), 8(a-c)** were synthesized under conventional method under reaction suitable conditions. All compounds were conformed to the structures envisaged. Thermodynamics properties, physic-chemical properties and costant are given table 2and 3D structure of syhthesised title compounds are shown fig 2.

Biological Evaluation

1. Antimicrobial Activity (Pelczar J. M. et al., 2003 and Ananthanaryan R et al., 2005).

In an approach to develop and synthesised new antimicrobial and anti-inflammatory agents,derivatives of N-Mannich bases of heterocyclic 1,3,4-thiadiazole 3(a-c),4(a-c),5(a-c),6(a-c),7(a-c),8(a-c). The in vitro antimicrobial activity of the synthesized compounds was screened by cup plate method against two gram positive bacteria viz. Staphylococcus aureus& Bacillus subtilis and two gram negative bacteria viz. Escherichia coli, Klebsiella pneumoniae and two pathogenic fungi Candida albicans, Aspergillus niger. The standard antibacterial agent used in the study was Ciprofloxacin, for the antifungal standard drug fluconazole. Twenty five milliliter of molten nutrient agar [Sabouraud's Dextrose Agar (at pH 6.8)] was poured into pre sterilized Petri-dishes and allowed to solidify at room temperature. Broth cultures of the test microbial were used as inoculums under sterile conditions. Dimethyl formamide (DMF) was used as control and as solvent to prepare the stock solutions of the synthesized compounds. The concentration of the prepared stock solutions was 100 µg/ ml. Then 250µl of the stock solution was poured in to each cup, the Petri dishes were incubated at 25^{0}C$\pm$2^{0}C for 48 hours and were examined for zone of inhibition (in mm) and % inhibition, exhibited by the test and standard compounds, which is given in Tables 3 & 4.

2. Anti-inflammatory Activity

Carrageenin induced rat paw oedema model

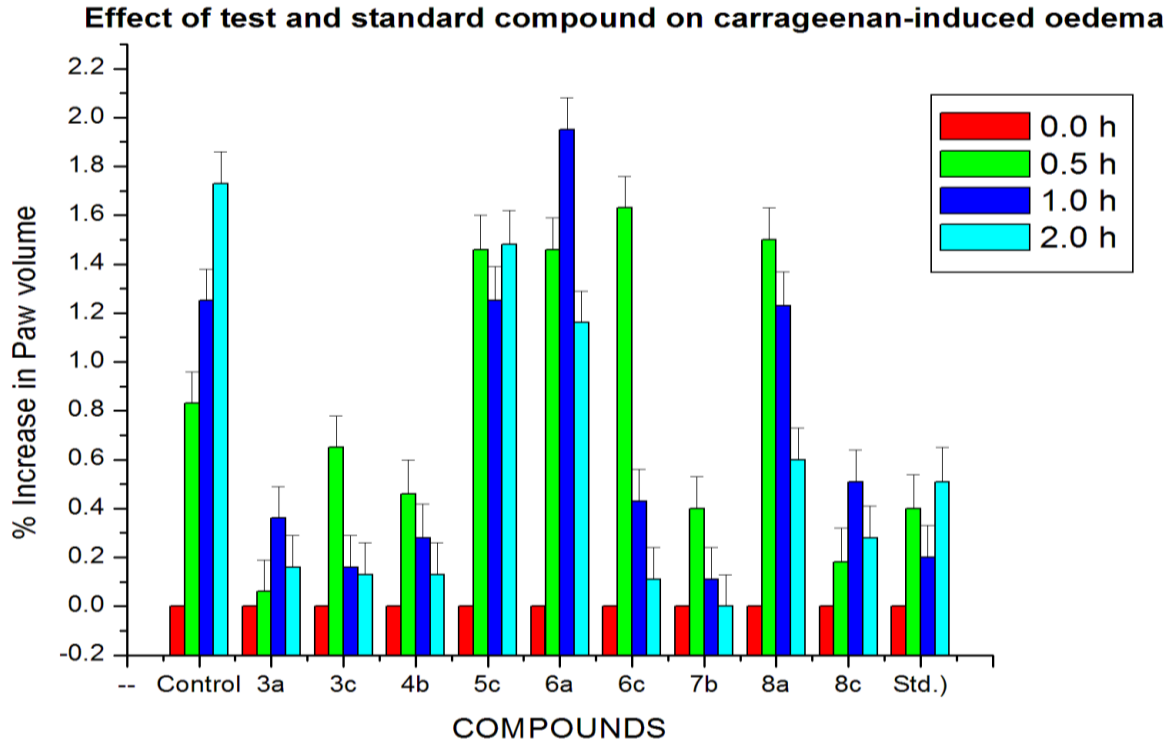
The anti-inflammatory activity of the standard drug diclofenac sodium and synthesized compounds, **3a, 3c,4b, 5c,6a,6c,7b,8a, and 8c,** was determined against carrageenan induced acute paw oedema in albino rats (66 numbers weighing 200-225 g). The 1% w/v solution of carrageenan for injection was prepared in normal saline (0.9%NaCl) and 0.1 ml was injected underneath planter region. The 50 mg/kg dose of standard drug and synthesized compounds was administered in animals, by

Table 5 effect of test and standard compound on carrageenan-induced oedema

Comp.	Dose (mg/kg,p.o)	Increase in paw volume (Mean±SEM)in ml at			
		0.0 h	0.5 h	1.0 h	2.0 h
Control(N/saline)	2.5/kg	0.0	0.83±0.13	1.25±0.13	1.73±0.13
3a	50mg/kg	0.0	0.06±0.13**	0.36±0.13**	0.16±0.13**
3c	50mg/kg	0.0	0.65±0.13*	0.16±0.13**	0.13±0.13**
4b	50mg/kg	0.0	0.46±0.14**	0.28±0.14**	0.13±0.13**
5c	50mg/kg	0.0	1.46±0.14	1.25±0.14	1.48±0.14
6a	50mg/kg	0.0	1.46±0.14	1.95±0.13	1.16±0.13
6c	50mg/kg	0.0	1.63±0.13	0.43±0.13	0.11±0.13
7b	50mg/kg	0.0	0.4±0.13**	0.11±0.13**	0.0±0.13**
8a	50mg/kg	0.0	1.5±0.13	1.23±0.14	0.6±0.13
8c	50mg/kg	0.0	0.18±0.14**	0.51±0.13**	0.28±0.13*
Std.(diclofenac)	50mg/kg	0.0	0.4±14**	0.2±0.13**	0.51±0.14**

The statistical significance of differences assessed with an analysis of variance (ANOVA), followed by Bonferroni t-test test.Results are expressed in mean +_SEM. (N=6) significance levels *P<0.05 and **P<0.01, compared with control respectively.

oral route using oral feeding tube through tuberculim syringe. The stock suspensions of standard and synthesized compounds were prepared in concentration of 10 mg/ml of 2% w/v CMC in distilled water. The albino rats were weighed and marked and housed as six in a group. One group was for the standard drug (diclofenac sodium), one group served as control and the other groups were for the synthesized compounds. The hind paws (right and left) of the rats were marked just beyond tibio-tarsal junction. The initial paw volume (both right and left) of each rat was recorded by mercury displacement method using Plethysmograph. The control group was administered with normal saline (0.9% w/v NaCl) (2.5 ml/kg) orally and other groups with respective drug suspension in CMS as per body weight. After 30 minutes, 0.1 ml of 1% w/v solution of carrageenan in normal saline was injected using No. 26 gauge needle, in the planter region of the left paw of the rats. The right paw served as reference non-inflammed paw for comparison. The paw volume of both the legs of rats treated with control, standard and test compounds were recorded at 30, 60, and 120 minutes after carrageenan challenge. The percent difference in the right and left paw volumes of each animal of control, standard and test compounds were calculated and meen percent change in paw volume in control, standard and test compounds treated animals were compared and expressed



Graph 3 comparison results are expressed in mean ± SEM for statistical significance of differences of above compounds.

as percent ooedema inhibition of drug in Table 5 and percent oedema inhibition shown by the test and standard compounds are compared and expressed in
Graph 3.

RESULT AND DISCUSSION

Antimicrobial activity.

Derivatives of 1-methyl-4(5-(4-(phenyl amino) phenyl)-1, 3,4-thiadiazol-2-e thiosemicar bazide (2a-f)were reacted with corresponding reactant dimity lamina, morph line and piper dine (a-c) in the presence of formaldehyde and concentrated acid. All synthesized compounds were obtained in the range of 57.41-66.7%yield. Purity and homogeneity of the synthesized compound were routinely checked by sharp melting point and TLC. Melting point of the all synthesized compound was observes between at 240.0-251.0-^{0}c.

57.41-66,7 % yield purity and homogeneity of the synthesized compound were routinely checked by sharp melting point and TLC .melting point of the all synthesized compounds was observed 240.0 t 251.0-^{0}c .

The *in vitro* antimicrobial activity of the synthesized compound was evaluated by cup plate method for bacteria viz. S. *aurreus, B subtilis , E. coli, K. pneumonia and fungi c. albecans , A Niger* .some compounds exhibited potent pharmacological activities as compared to the standard drugs.

The compounds 3b and 4a exhibited highest activity i.e. each with 82.60% inhibition against *S. aureous* while the compound 4b , 5b, 6b, 7c showed 65.21, 69.56, 52.17 and 52.17% inhibition respectively against *S. aureus.* Against *B. subtiles* the compound 3a, 3b, and 4c showed 85.71 , 95.23, 66.66% inhibition respectively and were the highest activity compound. While the compound for 4a, 5a, 6c, 8b(each with 57.41% inhibition) and 5b (47.61% inhibition)showed activity.

When tested against *E.coli* the compound 3c, 4a, 6b, 7a and 7c exhibited 60.86, 52.17,52.17,52.17 and 60.86% inhibition respectively and these compound showed Good activity among the tested compound. The highest activity was observed with 8c (with 69.56% inhibition).

Against *K. pneumoniae* the compound 3b (65.21%),4c(60.86% inhibition), 5a (60.86% inhibition), 7a (65,21% inhibition), 7b (65.21% inhibition) and 7c (65.21% inhibition) exhibited good activity. Among the highest ac tested compounds 3a(69.56%)showed activity.

All the synthesized compound were screened in vitro against the pathogenic fungal strains *A.niger* and *c.albicans*. The compound 3a (68.42% inhibition), 5a (73.68% inhibition), 5c (63.15 inhibition), 6b, 7b (each with 63.15% inhibition) and 8a (68.42% inhibition) showed excellent activities.

The anti-inflammatory activity of the selected compound 3a ,3c, 4b, 5c ,6a, 6c, 7b, 8a and 8c was screened by carrageen induced rat paw oedema method against the standard drug diclofenac sodium. The activity was evaluated over a time frame of 2h, after the carrageen challenge, at intervals of 0.5 h., 1.0 h. and 2.o 0.5 h.

After 0.5 h, the compound 3a,7b and 8c showed 92.77,51.80 and 78.31% oedema inhibition as compared to 51.80% oedema inhibition shown by the standard drug diclofenac sodium. the data indicate the short onset of action and comparable activity of these compound.

The standard drug declofenac sodium showed the maximum anti-inflammatory activity (84.00% oedema inhibition). comparable activity at this time interval was exhibited by the compound 3a (71.2% oedema inhibition), 3c (87.2% oedema inhibition), 4b (77.6% oedema inhibition) and 7b (91.2% oedema inhibition).

At 2.0 h time interval 3a,3c,4b,6c,7b, and 8c exhibited 90.75,92.48,92.48,93.64,100 and 83.81% oedema inhibition respectively in comparison to 70.52% oedema inhibition shown by the standard drug. in brief compound 3a and 7b showed excellent anti-inflammatory activity at time interval of 0.5,1.0 and 2.0 h.

Statistical Analysis

Table 5 result are expressed as mean±standard error of mean (SEM) of at least 6 animals per group. Statistical significance of differences between group was determined by one-way analysis of variance (ANOVA) followed by bonfeeroni t-test. For the statistical determination, statistical computerized software SIGMASTAT was used. Statistical analysis was assessed for the test compound 3a, 3c, 4b, 6a, 7b, 8a & 8c compare with control respectively. In pharmacological study anti-inflammatory effect of synthesized compounds were observed and found to be statically significant at the levels *P<0.05 and **P<0.01 compared with control normal saline treated animals' group. Probability value less than 0.01 (P<0.01) was significant.

Test compound 3a, 3c, 4b, 7b and 8c showed significant decrease in oedema on rat's paw compared with the test compound 5c, 6a, 6c and 8a at significance level. The F_{cal} (6.98933)>F_{tab}(4.8317) hence there is a statistically significant difference (P<0.01) in the mean values of the treated groups .The test compound 3a,3c,4b,7band8c found as significant compound .However, the profile of compound was at the expected level.

SUMMARY AND CONCLUSION

In summary ,designed and synthesized a series of N-Mannich bases of heterocyclic 1,3,4-thiadiazole 3-8(a-c) and characterization of the synthesized compound was carried was carried out by determining their melting points, R_f value ,UV,IR, Spectra, 1H-NMR and evaluated for in-vitro anti-microbial and ant-inflammatory activities .The compound 3b and 4a showed highest activities against *S. aureus* while the compound 3a and 3b were highly active against *B.subtilis*. It revealed that compound 3b exhibited excellent activity against both bacteria. The compounds 3c, 7c and 8c showed very comparable activity as compare to the standard drug ciprofloxacin against the pathogen *E.coli*. It can be assumed that pipiridine ring is necessary in mannich base for antibacterial activity against Gram-negative *E.coli.*

The compound 3a was observed as the most active against *K.pneumoniae.*

The *in vitro* antifungal activity of the synthesized compounds was also evaluated by cup-plate method.The

study revealed that the compounds 3a,4a,5a and 8a are the most active against the fungi A.niger .Attribution of structure dimethylamine group is necessary in Mannich base compounds for antifungal activity against A.niger. When compard with the standard drug Fluconazole, the compounds 4a,4b, 5c,6b,8a and 8c showed excellent activity against the fungi *C.albicans*. The anti-inflammatory activity of the randomly selected compounds was evaluated, on albino rats, by carrageen induced rat paw oedema method using the standard drug diclofenac sodium. The study was carried out over a frame of 2h at intervals of 0.5, 1.0and 2.0h. Fluconazole, the compounds 3a,4b,7b and 8c possess more potent anti-inflammatory activity compared with the standard drug.

To summarize the overall performance, the compound 7b exhibited excellent anti-inflammatory pharmacological activity. Structurally the compound 7b has a greater number of unsaturated hydrocarbons in Schiff base which shows good lipophilic properties with electron rich morpholine ring in Mannich base.

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