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Research Article

Synthesis, characterization and biological evaluation of some novel N-Mannich bases of heterocyclic 1,3,4-thiadiazole.

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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, ¹H 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 shown 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 to be the most active. All the synthesized compounds were found to be low lethal as ascertained by LD₅₀ test.

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

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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 multidrug 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 heterocycles have increased the need for rapid synthesis of new, potentially useful sulfur-nitrogen containing heterocycles.

Chemistry

A lone pair of electron is present on nitrogen atom. 1,3,4-thiadiazole is a weak base. Electron density on the nitrogen atom and facilitative reaction undergoes with electrophilic

reagents. It has a common structure, ie

$$\text{Ar}-\text{C}_2\text{N}_2\text{S}-\overset{\text{S}}{\underset{\text{H}}{\text{N}}}=\text{CH}-\text{Ar}$$
 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) as a thiosemicarbazide and Schiff base between the flat carbon ($-\text{N}=\text{CH}-$). 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-thiadiazole-2-yl)thiosemicarbazide, formaldehyde and dimethylamine, morpholine, piperidine.

Basic moiety 4-(5-(4-(phenylamino) phenyl)-1,3,4-thiadiazol-2-yl)thiosemicarbazide (**1**) was synthesized from 5-(4-(phenylamino)phenyl)-1,3,4-thiadiazole-2-amine, into their corresponding carbon disulphide in presence of alcoholic NaOH 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-(4-(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 et al., 2005). Title compounds **3(a-c)**, **4(a-c)**, **5(a-c)**, **6(a-c)**, **7(a-c)**, **8(a-c)** derivatives of N-Mannich bases of heterocyclic 1,3,4-thiadiazol were prepared from 1- R_1 Substituted-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 at least one active hydrogen atom, formaldehyde and ammonia or a primary amine or a secondary amine. An example of Mannich 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 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°C for 1h, to the stirred mixture was added hydrazine hydrate (0.014mol) and stirred continue for 1 h more at 60°C. On adding water, a pale-yellow solid separated out which was recrystallized from DMF-Ethanol. The pale yellow coloured product obtained, yield 78%, melting point 251°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-thiadiazol-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 quantity 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 recrystallized from hot ethanol. The white powder product obtained, yield 73%, melting point 243°C and calculated R_f 0.68 (Yogeeswari Pet al., 2004).

Synthesis of 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)** (Pandeya S N et al., 1999).

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

2.44g (0.005mol) 1-benzylidene-4-(5-(4-(phenylamino) phenyl)-1,3,4-thiadiazol-2-yl)thiosemicarbazide was placed in a 250ml round bottom flask in 8ml 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 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°C and calculated R_f 0.74.

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

2.23g (0.005mol) 1-(o-hydroxybenzylidene)-4-(5-(4-(phenylamino) phenyl)-1,3,4-thiadiazol-2-yl) thiosemicarbazide 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% and 63.6% respectively. The colourless product observed, melting point 230°C and calculated R_f 0.54.

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

2.32g (0.005mol) 1-(4-chlorobenzylidene)-4-(5-(4-(phenylamino) phenyl)-1,3,4-thiadiazol-2-yl)thiosemicarbazide 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 respectively. The reaction mixture was allowed to stand at room temperature 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). Synthesized compounds **5A-C** were obtained with yield 58%, 65.4% and 61.6% respectively. The colourless product observed, melting point 240°C 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 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 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 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% respectively. The white crystalline product observed, melting point 243°C 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)

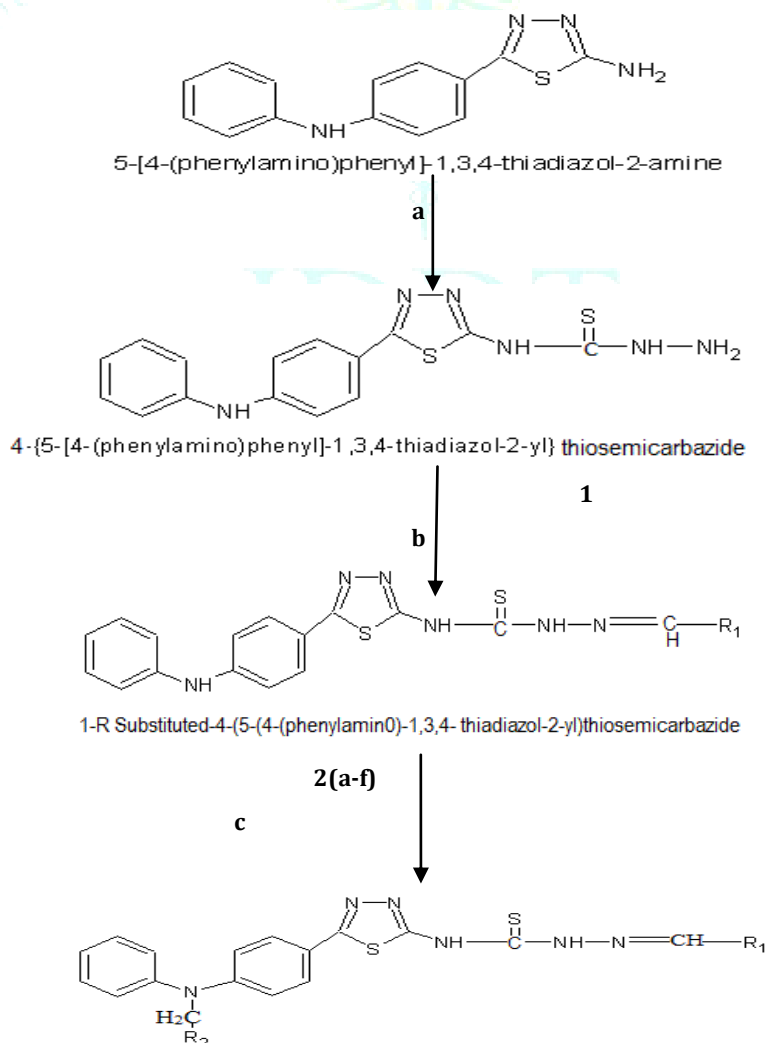
2.28g (0.005mol) 1-(3-phenylallylidene)-4-(5-(4-(phenyl amino)phenyl)-1,3,4-thiadiazol-2-yl)thiosemicarbazide was placed in a round bottom flask in 8ml tetrahydrofuran. To this solution was added 2ml formaldehyde and 2-3 drops of concentrated hydrochloric acid. To this (0.005mol)

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 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°C and calculated R_f 0.71.

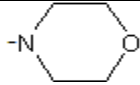

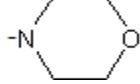

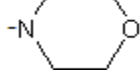
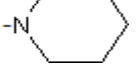
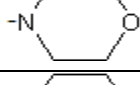

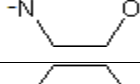

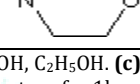
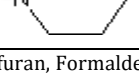
Preparation of 4-(5-(4-(N-(substituted) methyl-N-phenyl amino) phenyl)-1,3,4-thiadiazol-2-yl)-1-(furan-2-yl) methylene) thiosemicarbazide (8a-c)

2.10g (0.005mol) 1-(furan-2-yl)methylene-4-(5-(4-(phenyl amino)phenyl)-1,3,4-thiadiazol-2-yl)thiosemicarbazide was placed in 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 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 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 colourless product observed, melting point 249°C and calculated R_f 0.59.

Fig. 1 Graphical abstract of synthesized title compounds.



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).

COMPOUND	R ₁	R ₂		
		a	b	c
3	C ₆ H ₅	N(CH ₃) ₂		
4	o-C ₆ H ₄ -OH	N(CH ₃) ₂		
5	p-C ₆ H ₄ -Cl	N(CH ₃) ₂		
6	p-C ₆ H ₄ -N(CH ₃) ₂	N(CH ₃) ₂		
7	CH=CH-C ₆ H ₅	N(CH ₃) ₂		
8	C ₄ H ₃ O*	N(CH ₃) ₂		

*Furan (a) NaOH, DMF, CS₂, NH₂NH₂.H₂O, 60°C. (b) Aromatic aldehydes, Few drops CH₃COOH, C₂H₅OH. (c) Tetrahydrofuran, Formaldehyde, concentrated hydrochloric acid, Dimethylamine, Morpholine and Piperidine (a-c), Room temperature for 1hr.

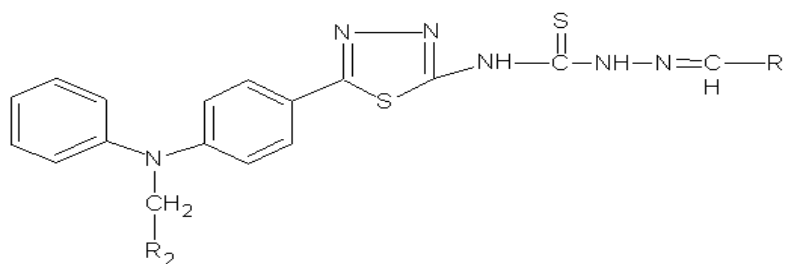
Characterization of the synthesized compounds

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. ¹H-NMR : JEOL GSX-400, 60MHz spectrometer in CDCl₃, TMS (tetra methyl silane) as an internal standard. ¹H-NMR 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 constant are given table 2 and 3D structure of synthesized title compounds are shown fig 2.

Table 1 Characterization of synthesized compounds.

Compound name	Specroscopic data
3(a-c)	IR ATR (cm ⁻¹)= 3340(N-H), 3004(Ar.C-H), 2910(Al-i-c-h), 1576(C=N), 1667(C-N), 1497(N-N), 104(C==S); ¹ H-NMR (DMSO-d ₆ , 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 ₆ H ₅); Electron absorption spectra (UV) λ _{max} 296.76(methanol)
4(a-c)	IR ATR(CM ⁻¹)=3390(N-H),3105(Ar.C-H),2878(Al-i-C-H),1560(C=N),1650(C-N), 1524(N-N),1214(C-S); ¹ H-NMR(DMSO-d ₆ ,8ppm):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 ₆ H ₄),8.0-8.7(s,1H,CH,α- to C ₆ H ₄ -OH);Electron absorption spectra (UV)λ _{max} 328 (methanol).
5(a-c)	IRATR(CM ⁻¹)=3420(N-H),3020(Ar-C-H),2915(Al-i-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-d ₆ ,8ppm):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(Al-i-C-H),1540(C=N),1540(C-N),1550(N-N),1210(C-S); ¹ H-NMR (DMSO-d ₆ ,8 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 ₆ H 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(Al-i-CH)1660(C=N),1580(C-N),1510(Ar-C-C),1515(N-N); ¹ H-NMR (DMSO-d ₆ ,8 ppm):2.1(s,1H,NH),6.6(s,1H,CH,Aliph.-H,α- to C ₆ H 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-d ₆ , 8 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).

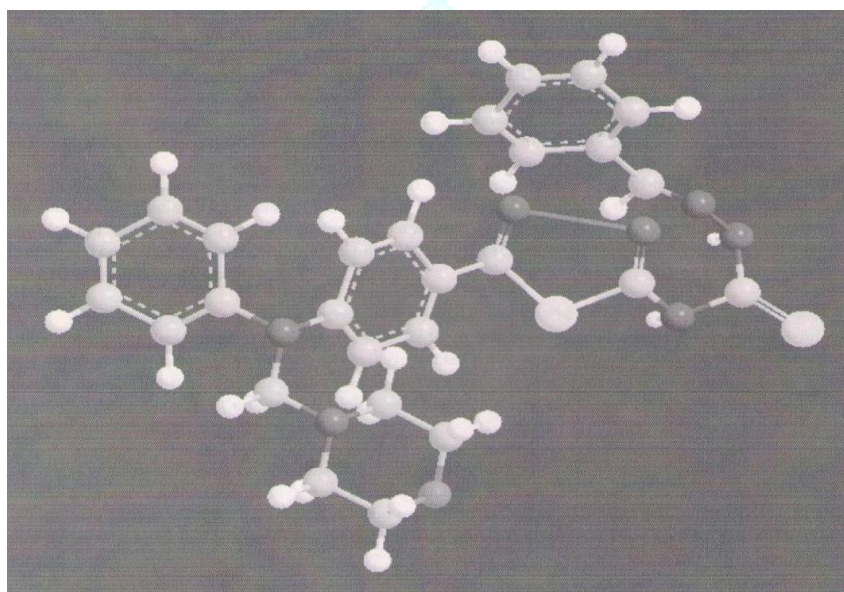


R₂-substituted compounds (N-Mannich bases compounds)

Table 2 physiochemical properties of synthesized compounds.

Comp.	M.formula	M.wt	Rast's M.wt.	Depression of m.p.ΔT(°C)	B.P.[K]	Log P	MR[CM ³ /mol]	HENRY'S LAW	Gibbs Energy[kJ/mol]
3a	C ₂₅ H ₂₅ N ₇ S ₂	488	484.9	81.87	1342.73	7.39	147.48	6.31	298.15
3b	C ₂₇ H ₂₇ N ₇ OS ₂	530	532.5	74.55	1435.39	6.99	156.53	6.31	298.15
3c	C ₂₈ H ₂₉ N ₇ S ₂	528	529.3	75.00	1431.32	8.12	159.4	6.31	298.15
4a	C ₂₅ H ₂₅ N ₇ OS ₂	504	506.4	78.39	1423.35	7	149.29	6.31	298.15
4b	C ₂₇ H ₂₇ N ₇ O ₂ S ₂	546	544.3	72.94	1516.01	6.6	158.34	6.31	298.15
4c	C ₂₈ H ₂₉ N ₇ OS ₂	544	546.8	72.63	1511.94	7.73	161.21	6.31	298.15
5a	C ₂₅ H ₂₄ ClN ₇ S ₂	521.5	524.5	75.54	1385.14	7.95	152.08	6.31	298.15
5b	C ₂₇ H ₂₆ ClN ₇ OS ₂	563.5	565.3	70.10	1477.8	7.55	161.13	6.31	298.15
5c	CH ₂₈ H ₂₈ ClN ₇ S ₂	561.5	558.2	71.50	1473.73	8.68	164.01	6.31	298.15
6a	C ₂₇ H ₃₀ N ₈ S ₂	531	533.5	74.42	1405.91	7.67	162.66	6.31	298.15
6b	C ₂₉ H ₃₂ N ₈ OS ₂	573	570.1	69.63	1498.57	7.27	171.71	6.31	298.15
6c	C ₃₀ H ₃₄ N ₈ S ₂	571	573.4	69.24	1494.5	8.41	174.58	6.31	298.15
7a	C ₂₇ H ₂₇ N ₇ S ₂	514	513.5	77.31	1392.65	7.27	157.53	6.31	298.15
7b	C ₂₉ H ₂₉ N ₇ OS ₂	556	558.6	71.07	1485.31	6.87	166.58	6.31	298.15
7c	C ₃₀ H ₃₁ N ₇ S ₂	554	556.2	71.38	1481.24	8.01	169.58	6.31	298.15
8a	C ₂₃ H ₂₃ N ₇ OS ₂	478	475.8	83.43	1320.49	6	140	6.31	298.15
8b	C ₂₅ H ₂₅ N ₇ O ₂ S ₂	520	518.1	76.62	1413.15	5.6	149.05	6.31	298.15
8c	C ₂₆ H ₂₇ N ₇ OS ₂	518	521.1	76.19	1409.08	6.74	151.92	6.31	298.15

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



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°C±2°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 (Kulkarni S.K,1999 and Winter C.A., et al., 1962)

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 oral route using oral feeding tube through tuberculin 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.

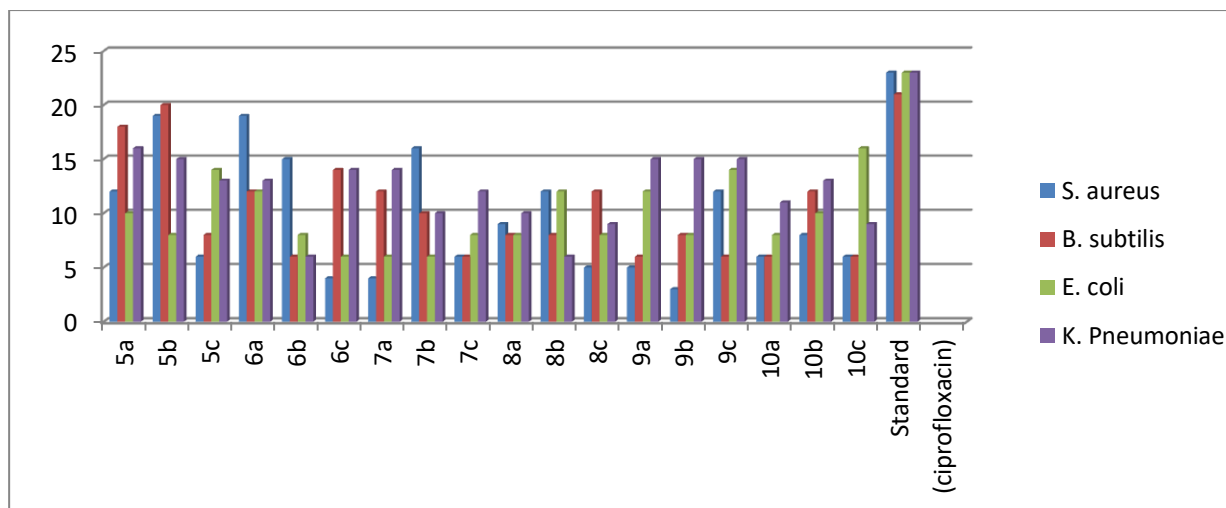
The albino rats were weighed and marked and housed as six in a group. One group was for the standard drug (diclofenac sodium), one group served as control and the other groups were for the synthesized compounds. The hind paws (right and left) of the rats were marked just beyond tibio-tarsal junction. 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-inflamed 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.

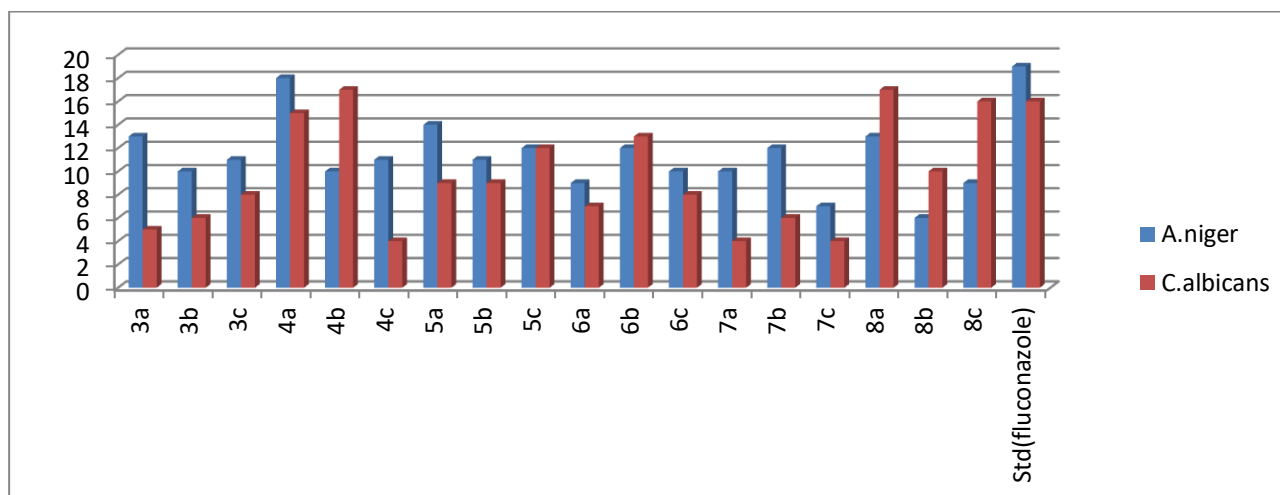
The percent difference in the right and left paw volumes of each animal of control, standard and test compounds were calculated and mean percent change in paw volume in control, standard and test compounds treated animals were compared and expressed as percent oedema inhibition of drug in Table 5 and percent oedema inhibition shown by the test and standard compounds are compared and expressed in Graph 3.

Table 3 Results of in vitro antimicrobial activity of synthesized compounds 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

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 compounds

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(fluconazole)	19	100.00	16	100.00

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

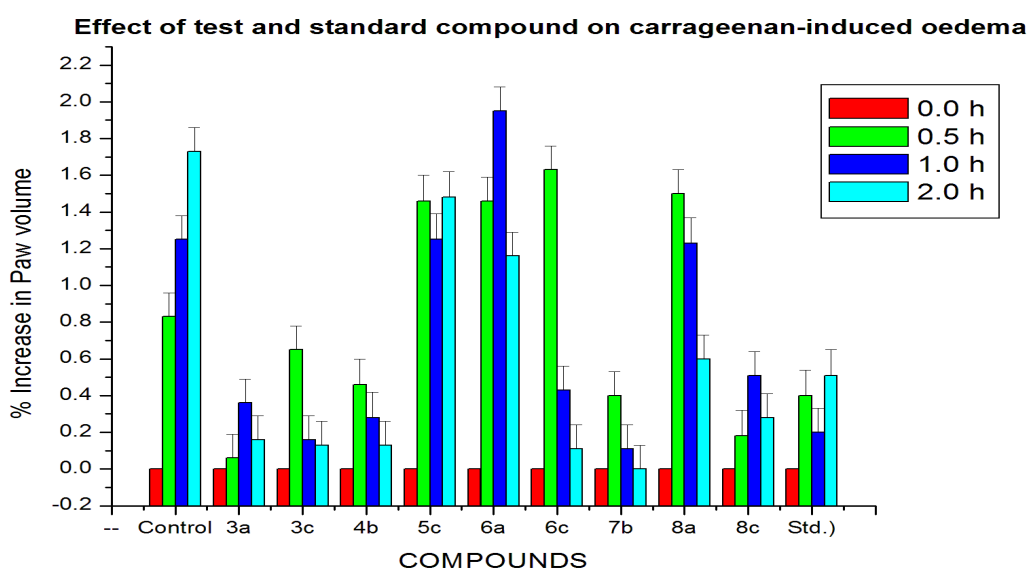
Anti-inflammatory activity

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±0.14**	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. Results are expressed in mean +_SEM. (N=6) significance levels *P<0.05 and **P<0.01, compared with control respectively.

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



RESULT AND DISCUSSION

Antimicrobial activity.

Derivatives of 1-methyl-4(5-(4-(phenyl amino) phenyl)-1,3,4-thiadiazol-2-yl)thiosemicarbazide (2a-f) were reacted with corresponding reactant dimethylamine, morpholine and piperidine (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 observed between at 240.0-251.0-°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 to 251.0-°C.

The *in vitro* antimicrobial activity of the synthesized compound was evaluated by cup plate method for bacteria viz. *S. aureus*, *B. subtilis*, *E. coli*, *K. pneumonia* and fungi *C. albicans*, *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. aureus* while the compound 4b, 5b, 6b, 7c showed 65.21, 69.56, 52.17 and 52.17% inhibition respectively against *S. aureus*. Against *B. subtilis* 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 compounds showed good activity among the tested compounds. 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 tested compounds 3a (69.56%) showed activity.

All the synthesized compounds 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 2.0 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 compounds.

The standard drug diclofenac 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 \pm 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 bonferroni 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, 7b and 8c 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 out by determining their melting points, R_f value, UV, IR, Spectra, 1H -NMR and evaluated for in-vitro antimicrobial and anti-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 compared to the standard drug

ciprofloxacin against the pathogen *E. coli*. It can be assumed that pipridine 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 compared 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, 1.0 and 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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