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Synthesis and antibacterial activity of novel 4-(5-phenylthiophen-2-yl) methyleneamino)-2-(phenylamino)methyl)-5-ethyl-2*H*-1,2,4-triazole-3(4*H*)-thione derivatives

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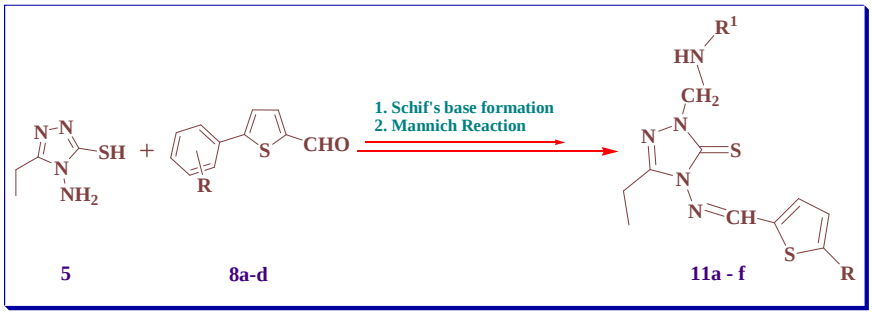
ABSTRACT

A series of 4-(5-phenylthiophen-2-yl)methyleneamino)-2-(phenylamino)methyl)-5-ethyl-2H-1,2,4-triazole-3(4H)-thionederivatives were prepared and screened for their antimicrobial activity. As per the obtained results, the compounds containing fluorine at para position have showed a significant action against B.Subtilis and K.Aerogenes. The structure of the newly synthesized compounds was established by means of spectral data.In IR band, the C=N stretching has observed at 1710 cm^{-1} and NH stretching at 1589 cm^{-1}. Whereas in ^{1}H NMR spectra the chiral carbons have appeared as doublets.

Graphical Abstract



A series of 4-(5-phenylthiophen-2-yl)methyleneamino)-2-(phenylamino)methyl)-5-ethyl-2*H*-1,2,4-triazole-3(4*H*)-thione derivatives (11a-f) were prepared through Mannich reaction and screened for their antimicrobial activity.

Keywords: Triazole, Diazotisation, Schiff's base, Mannich reaction, Antibacterial activity.

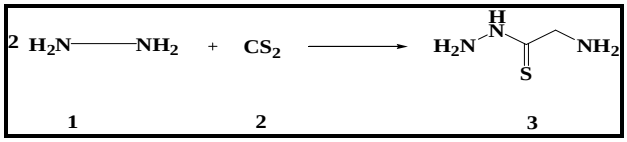
INTRODUCTION

Mannich reaction is a nucleophilic addition reaction, in which an active hydrogen of one compound is combined with a primary or secondary amine and a formaldehyde or any aldehyde gives a beta-amino ketone [**1-5**]. Mannich bases are used in the synthesis of various synthetically and pharmaceutically potent aminoalkyl chains of very high medicinal value, for example, cocaine, fluoxetine, atropine, ethacrynic acid, trihexyphenidyl, procyclidine, ranitidine, and biperiden etc. are the examples of clinically useful Mannich bases [**6-8**]. According to the literature, Mannich bases are highly reactive and pretty handy to convert them into many other useful compounds; for example, physiologically active amino alcohols are formed by just reducing a suitable Mannich base [**9**]. Mannich bases are known to possess potent biological activities like anti-inflammatory [**10, 11**], anticancer [**12, 13**], antifilarial [**10**], antibacterial [**14, 15**], antifungal [**15, 16**], anticonvulsant [**17**], anthelmintic [**18**], antitubercular [**19, 20**], analgesic [**21**], anti-HIV [**19**], antimalarial [**22**], antipsychotic [**23**] and antiviral [**22**] activities etc. On the other hand, the triazoles have paid significant contributions in the field of medicinal chemistry [**24-26**].

MATERIALS AND METHODS

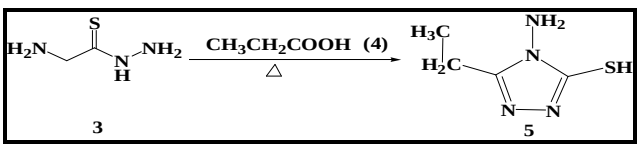
The required chemicals/reagents/solvents of high grade were bought from Avra Synthesis Pvt. Ltd, Merck Pvt. Ltd. India. Melting points of the newly synthesized compounds were determined by capillary method and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on Bruker Avance 300 MHz spectrometer and the chemical shift (δ) values are in parts per million (ppm) with reference to TMS (δ = 0ppm) as an internal standard. Interactions between the adjacent protons i.e., coupling constants (J) are expressed in Hertz (Hz). Types of protons and number of protons were identified through their s = singlet/d = doublet/t = triplet/q = quartet/m = multiplet/dd = double doublets/bs = broad singlets in their respective ^{1}HNMR. Mass spectra were recorded on a Jeol JMS – D-300 mass spectrometer operating at 70ev.

Synthesis of thiocarbohydrazide (3): To a vigorously stirred solution of hydrazine hydrate (**1**) [99%, 250g, 5 mol] in water (150 mL), carbon disulphide (**2**) (76 g, 1 mol) was added in dropwise at below 60°C. After the addition, mixture was refluxed for about 30 min, cooled at room temperature and then cooled on an ice salt mixture. The precipitate of thiocarbohydrazide obtained was filtered, washed with ethanol, ether and then dried. The filtrate of ethanol and water mixture was used as a solvent to repeat the procedure for two more times. The separated thiocarbohydrazide (**3**) of all the three batches was collected, recrystallized from minimum amount of water and acidified with drops of conc.HCl, Scheme 1 [**25**].



Scheme 1. Synthesis of thiocarbohydrazide (3).

Synthesis of 3-methyl -4-amino-5-ethyl-4*H*-1,2,4-triazole-3-thiol (5): A mixture of thiocarbohydrazide (**3**) (0.094 mole, 10 g) and propionic acid (**4**) (60ml) was refluxed for 4 h. The solid mass that separated out was collected by filtration and recrystallized from hot water, Scheme 2 and Table 1.

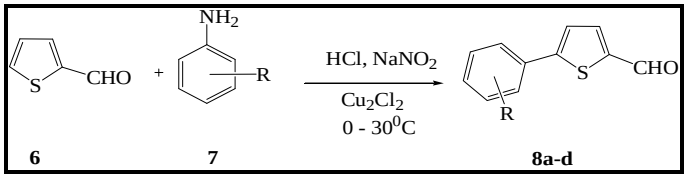


Scheme 2. Synthesis of 3-methyl -4-amino-5-ethyl-4*H*-1,2,4-triazole-3-thiol (5).

Table 1. Melting point and yield of 4-amino-5-ethyl-4*H*-1,2,4-triazole-3-thiol (5).

Compound	Yield (%)	M.P^{0}C	Appearance
5	90%	121^{0}C	White crystals

Synthesis of 5- substituted -2-thiophene derivatives (8a-d): A hot mixture of substituted 4-fluoro aniline (**6**) (0.1mole, 11.2 mL), 15% hydrochloric acid (60 mL) and water (90 mL) was cooled to 0°C and diazotized with 30% sodium nitrite solution (24 mL). To the diazotised solution under 0°C, water (50 mL), thiophenaldehyde (**7**) (11.2 mL, 0.1 mol) were added and then drop wise an aqueous solution of cuprous chloride (0.018 mole,2.55g) was added. Stirred the mixture for 4 h at 20- 30°C and kept overnight. The inorganic solid that formed was separated by filtration. Fractional distillation of the filtratein the presence of sodium carbonate solution gave 5-phenyl-2-thiophene carbaldehyde. Similarly, the other derivatives (**8b-d**) were prepared Scheme 3 and Table 2.

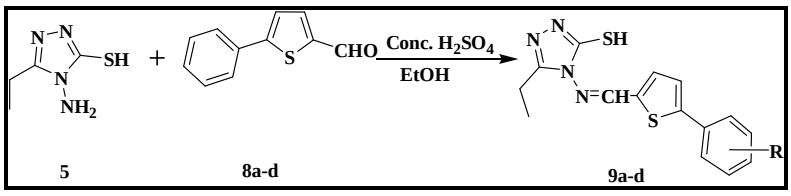


Scheme 3. Synthesis of 5- substituted -2-thiophene derivatives (8a-d).

Table 2. Melting point/Boiling point and yield of 5-phenylthiophene-2-carbaldehyde derivatives (8a-d)

Compound	R	Yield	M.P/B. P°C
8a	4-fluoro	80%	B. P.: 179
8b	2-fluoro	74%	B. P.: 194
8c	4-chloro	85%	M.P.:174
8d	2-H	80%	M. P. :167

Synthesis of 4-((5-phenylthiophen-2-yl)methyleneamino)-5-ethyl-4*H*-1,2,4-triazole-3-thiol derivatives (9a-d): The compound **9a** was prepared by refluxing a mixture mercapto triazole (**5**) (0.1 mole, 13 g) with 5-(4-fluorophenyl)thiophene-2-carbaldehyde (**8a**) (0.01 mole, 2.2 mL) in the presence of a catalytic amount of concentrated sulphuric acid (1 mL) in an ethanol medium (20 mL) for about 4h. The completion of the reaction was monitored by TLC, after completion, the reaction mixture was poured on to crushed ice and cold water. The solid of compound **9a** that separated was filtered and recrystallized from ethanol. Similarly, the other and derivatives (**9b-d**) were prepared.

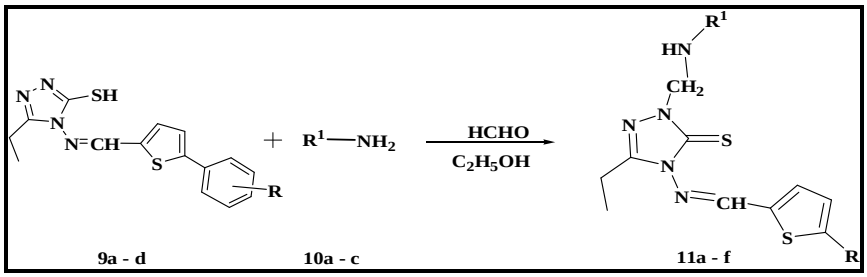


Scheme-4. Synthesis of 4-((5-phenylthiophen-2-yl)methyleneamino)-5-ethyl-4*H*-1,2,4-triazole-3-thiol derivatives (9a-d).

Table 3. Synthesis of 4-((5-phenylthiophen-2-yl)methyleneamino) -5-ethyl-4*H*-1,2,4-triazole-3-thiol derivatives (9a-d)

Comp.	R	Yield in %	Appearance	M.P in ^{0}C
9a	4-Fluro	80	Yellow crystal	133-134
9b	2-Fluro	70	Yellow crystal	136
9c	4-Chloro	85	White amorphous	138
9d	H	82	Grey crystalline	139.5

Synthesis of 4-(5-phenylthiophen-2-yl)methyleneamino)-2-(phenylamino)methyl)-5-ethyl-2*H*-1,2,4-triazole-3(4*H*)-thione derivatives (10a-f): The Mannich base **11a** was prepared by stirring a mixture of a Schiff's base **9a** (0.05 mole, 16.55 g), 40% formaldehyde (0.04 mole, 1.2 mL) and 4-chloroaniline (**10a**) (0.05 mole, 6.35 g) in ethanol 30 mL) for about 5h. Solid that obtained was filtered and recrystallized from ethanol and that gave a pure form of the compound **11a** in 70% yield. Similarly, the other derivatives (**10b-f**) were prepared. Scheme 5, Table 4.



Scheme 5. Yield, M.P and appearance of 4-(5-phenylthiophen-2-yl)methyleneamino)-2-(phenylamino)methyl)-5-ethyl-2*H*-1,2,4-triazole-3(4*H*)-thione derivatives [11a-f]

Table 4. Yield, M.P and appearance of 4-(5-phenylthiophen-2-yl)methyleneamino)-2-(phenylamino)methyl)-5-ethyl-2*H*-1,2,4-triazole-3(4*H*)-thione derivatives [11a-f]

Comp.	R	R^1	Yield (%)	Appearance	M.P in°C
11a	4-F-Ph	4-Cl-Ph	70	Yellow Crystals	128°C
11b	2-F-Ph	4-Cl-Ph	75	Yellow Crystals	139°C
11c	4-Cl-Ph	Ph	83	Yellow Flakes	158°C
11d	Ph	4-F-Ph	72	Yellow Flakes	168°C
11e	4-F-Ph	4-F-Ph	60	Yellow Needles	103°C
11f	2-F-Ph	Ph	65	Orange Crystals	102°C

^{1}HNMR and Mass spectral data of the compounds 10a-f.

Compound 10a: H^1 NMR: 12.02(s,Ar-OH,2H), 7.51(dd, 7.3Hz, ,2H), 7.65(dd, ,2H), 7.57(dd,2H), 7.16 (s,2H), 6.86(dd, 2H), 3.84(t, 4H), 2.50(s ,6H), 2.40–2.33(m, 2H). **Mass spectral values (m/z)(%)**: 471(M^++1). **IR, (KBr, ν(cm^{-1})):** 1726(C=N), 1587(NH), 1552(C=C), 1022(CN–C).

Compound 10b: H^1 NMR: 12.12(s,Ar-OH,2H), 7.49(dd, 7.3Hz,2H), 7.65(dd, 2H), 7.58(dd,2H), 7.26 (s,2H), 6.87(dd, 2H), 3.85(t, 4H), 2.49(s ,6H), 2.19–2.22(m, 2H).**Mass spectral values (m/z)(%)**: 471(M^++1). **IR, (KBr, ν(cm^{-1})):** 1716(C= N), 1584(NH), 1551(C=C), 1021(CN–C).

Compound 10c: H^1 NMR: 12.19(s, Ar-OH,2H), 7.39(dd, 7.3Hz, ,2H), 7.67(dd, 2H), 7.59(dd,2H), 7.28(s,2H), 6.85(dd, 2H), 3.84(t, 4H), 2.48(s,6H), 2.15–2.17(m, 2H).**Mass spectral values (m/z)(%):** 455(M^++1). **IR, (KBr, ν(cm^{-1})):** 1716(C= N), 1584(NH), 1551(C=C), 1021(CN–C).

Compound 10d: H^1NMR: 12.0(s, Ar-OH,2H),7.32(dd,7.3 Hz,2H),7.57(dd, 2H), 7.46(dd,2H), 7.28 (s,2H), 6.85(dd,2H),3.84(t,4H),2.48(s,6H), 2.15-2.16(m,2H).**Mass spectral values (m/z) (%)**: 437(M^++1). **IR, (KBr, ν(cm^{-1})):** 1712 (C=N), 1582 (NH), 1550 (C=C), 1024 (CN–C).

Compound 10e: H^1NMR: 12.11(s, Ar-OH,2H), 7.31(dd,7.3Hz, 2H), 7.57(dd, 2H), 7.51(dd,2H), 7.22(s,2H), 6.75(dd, 2H), 3.56(t, 4H), 2.43(s, 6H), 2.09–2.12(m, 2H).**Mass spectral values (m/z) (%)**:455(M^++1). **IR, (KBr, ν(cm^{-1})):**1702(C=N),1520(NH),1542(C=C),1012(CN–C).

Compound 10f: H^1 NMR: 12.06 (s, Ar-OH,2H), 7.21(dd, 7.3Hz, 2H), 7.36(dd,2H), 7.44(dd,2H), 7.18(s,2H), 6.65(dd, 2H), 3.43(t, 4H), 2.21(s,6H), 2.02–2.08(m, 2H).**Mass spectral values (m/z) (%)**: 437(M^++1). **IR, (KBr, ν(cm^{-1})):** 1699(C=N),1502(NH),1534 (C = C),1011(CN–C).

Biological Activity: Antibacterial bacterial activity of the final compounds was studied against the bacteria *B.Subtilis* and *A.Aerogenes* by using plate scattering technique [23]. The circles of each obsession were placed in three-overlap on supplement agar medium developed with new bacterial social orders independently. The agonizing was finished at 37^0c for 24 hrs. The results are depicted in table 5.

Table 5. Antibacterial bacterial activity of the compounds **10a-f** against the bacteria *B. Subtilis* and *A. Aerogenes*

Comp.	Minimum inhibitory concentration Mg/disk (diameter of Zone of inhibition in mm)	
	B. Subtilis	*A. Aerogenes*
10a	5(10.2)	5(9.7)
10b	<5(7.4)	5(8.2)
10c	5(11.4)	10(10.2)
10d	10(9.2)	<5(7.1)
10e	<5(7.8)	5(9.1)
10f	5(9.1)	5(8.6)

Screening impact showed that, the compounds of fluorine at para position **(10a, 10d, 10e)** have showed more dynamic antibacterial activity than compared to other derivatives.

RESULTS AND DISCUSSION

A series of 4-(5-phenylthiophen-2-yl)methyleneamino)-2-(phenylamino)methyl)-5-ethyl-2*H*-1,2,4-triazole-3(4*H*)-thione derivatives [**10a-f**] were prepared by means of multistep conventional chemical reactions. The reactions of final compounds were clean. The isolation and purification of all the final compounds (**10a-f**) was carried out by simple recrystallization process by using economically affordable solvent like ethanol. All the final compounds were confirmed by means of their ^{1}HNMR, mass and IR spectra.

The required starting material **3** was obtained by the reaction of hydrazine hydrate and carbon disulphide by using ethanol-water mixture as solvent and it was purified by recrystallisation with water. Here remarkably, the ethanol-water mixture was recovered by filtration and recycled for two more batches of preparation of compound **3**. The compound **5** was prepared by the reaction of the compound **3** and **4** under reflux condition. And it (**5**) was purified by recrystallization with hot water. Later on, the compounds **8a-d** were prepared by diazotisation and coupling reaction of aromatic primary amines (**7a-d**) with thiophene-2-carbaldehyde (**6**) in the presence of sodium nitrite as diazotising agent and cuprous iodide as reducing agent at 0°C to 30°C. The obtained compounds (**8a-d**) were purified by means of fractional distillation process. The prefinal derivatives **9a-d** were prepared by the refluxed mixtures of the compound **5** with different 5-phenylthiophene-2-carbaldehyde derivatives (**8a-d**). The compounds **9a-d** was purified by recrystallization from ethanol. Then the final compounds (**11a-f**) were prepared by the Mannich reaction of the compounds **9a-d**, formaldehyde and different aromatic primary amines (**10a-c**) in ethanol. The obtained final compounds (**11a-f**) were prepared purified by recrystallizing with ethanol.

The synthesized final compounds (**10a-f**) were screened for their antibacterial activity by using bacterial organisms *Bacillus subtilis (B. Subtilis* is a Gram-positive bacterium*)* and *Aerobacter Aerogenes (A. Aerogenes* is a Gram-negative bacterium*)*by plate dispersion method. It was found that, the compounds of fluorine at para position **(10a, 10d, 10e)** have showed significant activity than compared to others.

APPLICATION

Manuscript contains the successful execution of many organic reactions like condensation, condensation followed by cyclization, diazotization and coupling reaction, Schiff's base synthesis and synthesis of Mannich bases by Mannich reactions, and all the synthesized compounds were systematically characterized. Perhaps it provides a good scope for the application to synthesis of various organic compounds through conventional organic synthesis. Moreover, all the final compounds were screened for their antibacterial activity.

CONCLUSION

In the present study, synthesis of a series of novel 4-(5-phenylthiophen-2-yl)methyleneamino)-2-(phenylamino)methyl)-5-ethyl-2*H*-1,2,4-triazole-3(4*H*)-thione derivatives having pharmacologically important chlorine/fluorine in different position inthe phenyl ring. Which were evaluated for their antibacterial activity. It was found that compounds containing fluorine at para position showed significant activity than others.

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