Synthesis, Structural Elucidation of Novel Thieno [2,3-d] Pyrimidine Core Unit Containing 1,2,4-Triazoles and Thiophenes as Potent Antimicrobial Activity

Virupakshi Prabhakar1*, Sudhakar Babu Kondra2, Srivivasula Reddy Maddula3, Parandhama G2 and Latha J4

1Faculty of Engineering Chemistry, SVR Engineering College, Jawaharlal Nehru Technological University-Anantapuramu, Nandyal, Kurnool Pin 518502, Andhra Pradesh, India
2Department of Chemistry, Sri Krishnadevaraya University, Anantapuramu, Andhra Pradesh, India
3Praja Generics Private Limited, Hyderabad, Telangana, India
4Department of Environmental Science, College of Engineering and Technology, Sri Krishnadevaraya University, Anantapuramu, Andhra Pradesh, India

*Corresponding author: Virupakshi Prabhakar, Faculty of Engineering Chemistry, SVR Engineering College, Jawaharlal Nehru Technological University - Anantapuramu (JNTU-A), Nandyal, Kurnool Pin 518502, Andhra Pradesh, India, Tel: +918297140295; E-mail: Virupakshi.prabhakar@gmail.com

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Abstract

Several new thieno[2,3-d] Pyrimidine derivatives 3-Substituted phenyl-5-(thiophen-2-yl) thieno[3,2-e] [1,2,4] triazolo[4,3-c] pyrimidine 8(a-j), were synthesized starting from thieno[2,3-d] pyrimidine-2,4-diol (2). The characterization of the newly synthesized compounds was established by IR, 1H NMR, 13C NMR and Mass Spectral analysis. The final compounds were screened for their anti-bacterial activity against Bacillus subtilis and Staphylococcus aureus from Gram positive group of bacteria and Escherichia coli and Klebsiella pneumonia from Gram negative group of bacteria and antifungal activity against Candida albicans and Aspergillus flavus. Anti-bacterial and anti-fungal activities were Evaluated and compared with the standard drugs. From anti-bacterial and anti-fungal activity screening results, it has been observed that compounds 8i, 8j, 8e possess good activity.

Keywords: Thiieno [2, 3-d] pyrimidine; Antibacterial; Antifungal activity; Cyclisation; Suzuki cross coupling

Introduction

Pyrimidine has always been a unique interesting heterocyclic moiety for the medicinal chemists; an exhaustive research has been done on the pyrimidines that led to the discovery and introduction of several drugs into the market. From the standpoint of biological activity, fused hetero aromatic systems are often of much greater interest than the constituent monocyclic compounds. The appearance of qualitatively new properties of an annulated molecule, enlargement of the possibility of varying pharmacophore groups in different positions of the molecule and the ability of the latter to interact with a wider spectrum of receptors adopting various conformations are apparently of crucial importance. In addition, the structure of the molecule can be varied by annealing at different positions of individual Hetero cyclic fragments. Fused pyrimidines have also been attracted a considerable interest in medicinal chemistry research due to their versatility and a broad bioactive potential. Thieno pyrimidine is among those fused pyrimidines found to have a wide variety of pharmacological and biological applications. Since last four decades research has been focused on the design and synthesis of novel thienopyrimidines as medicinal agents, a large number of reports have been documented on thieno pyrimidines as they found to exhibit a variety of biological activities such as antimicrobial, anti-inflammatory, bronchodilatory activity, inhibition of Phosphodiesterases, tyrosine kinase and VEGFR kinase. It is evident that purine as an endogenous scaffold plays an important biochemical role in variety of regular physiological functions such as respiration, inflammation, cell proliferation and so forth. As a bio isoster to purines, thieno[2,3-d] pyrimidines were also found to exhibit numerous biological activities probably due to the interaction with various physiological elements. Thieno pyrimidine is a bi cyclic heterocyclic compound consists of a five membered thiohene ring is fused to a six membered hetero cyclic ring with two nitrogen atoms. The fusion may occur in three different orientations that results in three important types of thieno pyrimidines namely; Thieno[2,3-d] Pyrimidine (a), thieno[3,2-d] Pyrimidine (b) and thieno[3,4-d] pyrimidine (c) (Figure 1). Most of the isomeric thienopyrimidines occur as colored amorphous form, some exists as crystalline form. Synthetic approaches for the construction of a number of thieno pyrimidines are well established. There exist three possible types of fusion of thiophene to pyrimidines ring results in corresponding isomeric thienopyrimidines namely; thieno [3,2-d] pyrimidines, thieno[3,4-d] pyrimidines and thieno[3,2-d] pyrimidines.
Thieno pyrimidine derivatives, which are structure analogues of purines, have been of great interest because of their large range of pharmacological activities [1] as antibacterial, [2,3] antifungal [4], analgesic [5-7], antipyretic [8,9], anti-inflammatory [10], anti-tubercular [11,12], anti-cancer [13-15], radioprotective [16,17]. Many thieno [2,3-d] pyrimidine derivatives were reported as phosphodiesterase inhibitors [18], also exhibited good H1 receptor antagonistic activities [19], 4-amino derivatives showed insecticidal, pesticidal and acaricidal activities [20]. Numerous thieno[2,3-d] pyrimidines have been proved to use in case of cerebral ischemia, malaria, tuberculosis, Alzheimer’s and Parkinson’s diseases [21].

In this context, thienopyrimidine containing 1,2,4-triazole derivatives with similar structural qualities would be projected to result in newer molecular systems with increased efficacy. Definitely, 1,2,4-triazole template has been known to express considerable antimicrobial [22], antitubercular [23] and anticancer activities [24]. In continuation to extend our research [25,26] it was our thought of interest to design and synthesize thienopyrimidine 1,2,4 tri azole derivatives hoping to go a step forward in the field of antioxidant agents. Taking the above points in consideration, we have studied the antimicrobial action of the resultant thieno pyrimidine 1,2,4-triazole and Thiophene derivatives against wide range of different microorganisms.

This work aimed to synthesize some new thieno[2,3-d] pyrimidine derivatives starting with thieno[2,3-d] pyrimidine-2,4-diol and to evaluate their biological activities. Encouraged by the diverse biological activities of Thiено [2,3-d] pyrimidine Heterocyclic compounds, it was decided to prepare a new series of Thiено[2,3-d] pyrimidine Heterocyclic compounds. Literature survey revealed that incorporation of different groups in Thiено[2,3-d] pyrimidine Heterocyclic ring enhanced antibacterial and antifungal activity. In the present communication 2-chloro-4-hydrazinyl thieno[2,3-d] pyrimidine (4) was reacted with different substituted acids (5a-j) in POCl3 at Reflux Temperature to form 1,2,4 tri azole Thiено Pyrimidine derivatives 6 (a-j), which were further reacted with thiophen-2-ylboronic acid (7) under Suzuki reaction conditions to get target compounds (8a-8j). The synthesis of the compounds as per the following Figure 2 given below. The synthetic route was depicted in Figure 2.

The structures of all synthesized compounds were assigned on the basis of IR, Mass, 1H and 13C NMR spectral data analysis. Further these compounds were subjected for antifungal and antibacterial activity.

Materials and Methods

In this Investigation chemicals were purchased from local dealer with S.D fine make was used. Chemicals were 99% pure; purity has been checked by thin layer chromatography and melting point. Conventional method has been used for synthesis of thieno[2,3-d] pyrimidine derivatives. Stirring and reflux method were used for synthesis of thieno[2,3-d] pyrimidine derivatives 8 (a-j) respectively. The synthetic route was depicted in Figure 2. The title compounds 8(a-j) were synthesized in five sequential steps using different reagents and reaction conditions (Figures 2 and 3), the 8(a-j) were obtained in moderate yields. The structure was established by spectral (IR, 1H-NMR, 13C-NMR and mass) and analytical data.

R=-Phenyl, -4 Methyl phenyl, -4 Methoxy phenyl, -4 tri fluoro methoxy phenyl, -4 Tri fluoro phenyl, -4 Chloro phenyl, -4 Bromo phenyl, -4 Nitro phenyl, -2 Indole, iso nicotinic acids

Figure 2: Synthetic path way for compounds 8a-8j. Synthetic path way of preparation of 1,2,4-triazole thienopyrimidine derivatives. Reagents and Reaction conditions: (a) Urea 200°C, 3 hrs (b) POCl3, N-ethyl - N, N di isopropyl amine Reflux, 6 hrs (b) Hydrazine hydrate, Tri Ethyl Amine, Ethanol, 0°C-RT, 3 hrs. (d) POCl3, Reflux, 6 hrs (e) 1,4-Di oxane, Na2CO3, Water, Pd(PPh3)4, RT-110°C, 12 hrs.

Figure 1: Three important types of Thieno pyrimidines.
Experimental Section

All reactions were carried out under argon in oven-dried glassware with magnetic stirring. Unless otherwise noted, all materials were obtained from commercial suppliers and were used without further purification. All solvents were reagent grade. THF was distilled from sodium benzo phenone ketyl and degassed thoroughly with dry argon directly before use. Unless otherwise noted, organic extracts were dried with anhydrous Na$_2$SO$_4$, filtered through a fitted glass funnel, and concentrated with a rotary evaporator (20-30 Torr). Flash chromatography was performed with silica gel (200-300 mesh) by using the mobile phase indicated. The NMR spectra were measured with a 400 MHz Bruker Avance spectrometer at 400.1 and 100.6 MHz, recorded using tetra methyl silane (TMS) in the solvent of CDCl$_3$ referenced to the residual proton resonances of the solvents. Proton and carbon magnetic resonance spectra (H NMR and 13C NMR) were recorded using tetra methyl silane (TMS) in the solvent of CDCl$_3$-d$_3$ or DMSO-d$_6$ as the internal standard (H NMR: TMS; at 0.00 ppm, CDCl$_3$ at 7.26 ppm, DMSO at 2.50 ppm; 13C NMR: CDCl$_3$ at 77.16 ppm, DMSO at 40.00 ppm).

General procedure for synthesis of thieno[2,3-d] pyrimidine-2,4-diol [compound (2)]

Methyl 2-aminothiophene-3-carboxylate (0.01 m.mol) and 0.05 m.mol of urea, were intimately mixed with each other, the mixture was heated for two hours at 200°C. A clear, brown molten mass was obtained from commercial suppliers and were used without further purification. The excess unreacted phosphorus oxy chloride was refluxed for ten hours, whereby a clear solution was formed. The reaction mass was allowed to stir at room temperature for 3 hrs, after completion of starting compound, the excess amount of methanol and Tri Ethyl amine was removed under vacuum. The residue was washed with water, finally petroleum ether then they obtained solid was filtered and Dried under vacuum (Figures 4, 5 and 6).

Yield: 90% (white color solid); IR (KBr, cm$^{-1}$): 3440 (-OH), 1160 (C=O-C Stretching), 3090 (Ar C-H), 1630 (Ar C=C Stretching).

$^1$H NMR (400 MHz; CDCl$_3$): δH 11.44 (s, 1H, -OH), 9.18 (s, 1H, -OH), 6.94 (d, 1H, J$_{HH}$=8.0 Hz, Ar-H$_3$), 7.29 (d, J$_{HH}$=8.0 Hz, 1H, Ar-H).

$^{13}$C NMR (100 MHz; CDCl$_3$): δC 128.92, 124.03, 128.11, 159.62, 161.67, 154.75.

LC-MS (70 eV): m/z=169 (M+H$^+$).

General procedure for synthesis of 2,4-dichlorothieno[2,3-d] pyrimidine [compound (3)]

A mixture consisting of 8.4 gm (0.05 mol) of 2,4-di hydroxythieno[2,3-d] Pyrimidine (2) and 100 ml. of phosphorus oxychloride was refluxed for two hours, whereby a clear solution was formed. Thereafter, the excess unreacted phosphorus oxy chloride was evaporated in vacuo, the residual oil was poured into ice water, and the aqueous mixture was extracted with chloroform [29]. The chloroform phase was isolated, washed with water until neutral, then dried over Sodium sulfate, the chloroform was evaporated in vacuo, and the solid residue was re-crystallized from ethanol. 7.6 gm. (75% of yield) of 2,4-dichlorothieno[2,3-d] pyrimidine, M.P. 161-162°C, were obtained.

IR (KBr, cm$^{-1}$): 740(-C-Cl), 3110(Ar C-H), 1660 (Ar C=C Stretching), 1310 (C=C Stretching), 3090 (Ar C-H), 1630 (Ar C=C Stretching).

$^1$H NMR (400 MHz; CDCl$_3$): δH 6.98 (d, 1H, J$_{HH}$=7.0 Hz, 1H, Ar-H$_3$), 7.39 (d, J$_{HH}$=7.0 Hz, 1H, Ar-H$_3$).

$^{13}$C NMR (100 MHz; CDCl$_3$): δC 126.92, 123.03, 126.11, 153.62, 161.67, 154.75.

LC-MS (70 eV): m/z=205(M+H$^+$), 207(M+2), 209(M+4), 9:6:1 It indicates molecule contain two chlorine atoms.

General procedure for synthesis of 2-chloro-4-hydrazinylthieno [2,3-d] pyrimidine [compound (4)]

A mixture of 2,4-dichlorothieno[2,3-d] pyrimidine [compound (2)] (Compound 2) (0.1 mol) in methanol was taken and cooled to 0°C-5°C. A mixture of 2,4-dichlorothieno[2,3-d] pyrimidine (2) and 100 ml. of phosphorus oxychloride was refluxed for ten hours, whereby a clear solution was formed. The reaction mass was allowed to stir at room temperature for 3 hrs, after completion of starting compound, the excess amount of methanol and Tri Ethyl amine was removed under vacuum. The residue was washed with water, finally petroleum ether then they obtained solid was filtered and Dried under vacuum (Figures 4, 5 and 6).

Yield: 84% (pale brown color solid); m.p. 202-204°C.

IR (KBr, cm$^{-1}$): 760(-C-Cl), 3102(Ar C-H), 1650 (Ar C=C Stretching), 3340 (-N-H Stretching, two bands indicates 1°Amine).

$^1$H NMR (400 MHz; CDCl$_3$): δH 4.68 (s, 2H), 7.60 (s, 2H, Ar-H$_3$), 9.6 (1H, S).
General procedure for synthesis of

Compound (4) (Figure 7) (0.1 m. mol) and substituted benzoic acids (or) Heterocyclic Acids (5a-j) (0.13 m.mol) were taken in POCl₃ (5 ml) and heated to reflux for 6 hrs. The reaction mass was concentrated under reduced pressure and then quenched in ice water. The Solid obtained was filtered off, washed with water, dried and crystallized from methanol/Ethanol solvent.

The following compounds were synthesized using this method.

Yield: 84% (brown color solid); m.p. 212-214°C.

IR (KBr, cm⁻¹): 755(-C-Cl), 3100(Ar C-H), 1570 (Ar C=C Stretching).

¹H NMR (400 MHz; CDCl₃): δH 7.40-7.53 (m, 3H, Ar-H), 8.3(2H,t, Ar-H), 6.96 (1H, d, Ar-H), 7.3(1H,d, Ar-H).

¹³C NMR (100 MHz; CDCl₃): δC 126.92, 123.03, 126.11, 173.62, 158.67, 154.25 MS (70 eV): m/z=201(M+H) +, 203(M+2), 3:1 It indicates molecule contain one chlorine atom (Figure 7).

5-chloro-3-phenylthieno[3,2-e][1,2,4] triazolo[4,3-c]pyrimidine (6a)
Yield: 81% (yellow color solid); m.p. 210-212°C.

IR (KBr, cm\(^{-1}\)): 745 (-C-Cl), 3110 (Ar C-H), 1590 (Ar C=C Stretching), 2960 (SP\(_3\) C-H Stretching).

\(^1\)H NMR (400 MHz; CDCl\(_3\)): \(\delta\)H 6.96 (1H, d, J\(_{HH}\)=7.0 Hz, Ar-H), 7.3(1H,d, J\(_{HH}\)=7.0 Hz, Ar-H), 8.5(2H, d, J\(_{HH}\)=7.4 Hz, Ar-H), 7.3((2H, d, J\(_{HH}\)=7.4 Hz, Ar-H), 2.36(3H,S).

\(^{13}\)C NMR (100 MHz; CDCl\(_3\)): \(\delta\)C 126.92, 123.03, 126.11, 153.62, 160.67, 154.25, 134, 128, 131.

LC-MS (70 eV): m/z=301(M+H)+, 303(M+2), 3:1. It indicates molecule contain one chlorine atom (Figure 8).

5-chloro-3-(4-methoxyphenyl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine (6b):

Yield: 84% (Greenish yellow color solid); m.p. 190-191°C.

IR (KBr, cm\(^{-1}\)): 755 (-C-Cl), 1155(C-O-C Stretching), 3095(Ar C-H), 1580 (Ar C=C Stretching).

\(^1\)H NMR (400 MHz; CDCl\(_3\)): \(\delta\)H 6.96 (1H, d, J\(_{HH}\)=7.0 Hz, Ar-H), 7.3(1H,d, J\(_{HH}\)=7.0 Hz, Ar-H), 7.95(2H, d, J\(_{HH}\)=7.8 Hz, Ar-H), 7.03((2H, d, J\(_{HH}\)=7.8 Hz, Ar-H).

\(^{13}\)C NMR (100 MHz; CDCl\(_3\)): \(\delta\)C 126.92, 123.03, 126.11, 153.62, 160.67, 154.25, 134, 128, 131.

LC-MS (70 eV): m/z=371(M+H)+, 373(M+2), 3:1. It indicates molecule contain one chlorine atom (Figure 10).

5-chloro-3-(4-((trifluoromethoxy)phenyl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine (6d):

Yield: 81% ((brown color solid); m.p. 216-218°C.

IR (KBr, cm\(^{-1}\)): 765(-C-Cl), 1340(C-F), 303(M+2), 3:1. It indicates molecule contain one chlorine atom (Figure 11).

5-chloro-3-(4-((trifluoromethyl)phenyl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine (6e)
**Figure 11:** 5-chloro-3-(4-(trifluoromethyl)phenyl)thieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidine. Yield: 81% (pale brown color solid); m.p. 186-188°C.

IR (KBr, cm\(^{-1}\)): 768(-C-Cl), 3120(Ar C-H), 1590 (Ar C=C Stretching).

\(^1\)H NMR (400 MHz; CDCl\(_3\)): δH 6.96 (1H, d, J\(_{HH}\)=7.0 Hz, Ar-H), 7.3(1H,d, J\(_{HH}\)=7.0 Hz, Ar-H), 8.25(2H, d, J\(_{HH}\)=7.8 Hz, Ar-H), 7.53((2H, d, J\(_{HH}\)=7.8 Hz, Ar-H).

\(^13\)C NMR (100 MHz; CDCl\(_3\)): δC 127.92, 123.03, 126.11, 153.62, 160.67, 154.25, 134, 128, 131, 124.

LC-MS (70 eV): m/z=320(M+H)+, 322(M+2), 324(M+4) 9:6:1 It indicates molecule contain two chlorine atoms (Figure 12).

**Figure 12:** 5-chloro-3-(4-chlorophenyl)thieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidine (6f).

Yield: 80% (pale yellow color solid); m.p. 218-220°C.

IR (KBr, cm\(^{-1}\)): 748(-C-Cl), 540(C-Br), 3120(Ar C-H), 1560 (Ar C=C Stretching).

\(^1\)H NMR (400 MHz; CDCl\(_3\)): δH 6.96 (1H, d, J\(_{HH}\)=7.0 Hz, Ar-H), 7.3(1H,d, J\(_{HH}\)=7.0 Hz, Ar-H), 8.15(2H, d, J\(_{HH}\)=7.0 Hz, Ar-H), 8.53((2H, d, J\(_{HH}\)=7.0 Hz, Ar-H).

\(^13\)C NMR (100 MHz; CDCl\(_3\)): δC 127.92, 123.03, 126.11, 153.62, 160.67, 154.25, 144, 128, 124, 150.

LC-MS (70 eV): m/z=330(M-H)+, 332(M+2) 3:1 It indicates molecule contain one chlorine atom (Figure 13).

**Figure 13:** 3-(4-bromophenyl)-5-chlorothieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine (6g).

Yield: 80% (yellow color solid); m.p. 219-221°C;

IR (KBr, cm\(^{-1}\)): 768(-C-Cl), 1540 and 1350 (N-O Stretching in Nitro group), 3120(Ar C-H), 1590 (Ar C=C Stretching).

\(^1\)H NMR (400 MHz; CDCl\(_3\)): δH 6.76 (1H, d, J\(_{HH}\)=7.0 Hz, Ar-H), 7.2(1H,d, J\(_{HH}\)=7.0 Hz, Ar-H), 8.15(2H, d, J\(_{HH}\)=7.0 Hz, Ar-H), 8.53((2H, d, J\(_{HH}\)=7.0 Hz, Ar-H).

\(^13\)C NMR (100 MHz; CDCl\(_3\)): δC 127.92, 123.03, 126.11, 153.62, 160.67, 154.25, 144, 128, 124, 131, 124.

LC-MS (70 eV): m/z=326(M+H)+, 328(M+2) 3:1 It indicates molecule contain one chlorine atom (Figure 14).

**Figure 14:** 5-chloro-3-(4-nitrophenyl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine (6h).

Yield: 70% (greenish yellow color solid); m.p. 239-241°C.

IR (KBr, cm\(^{-1}\)): 768(-C-Cl), 3320 (N-H Stretching), 3120(Ar C-H), 1586 (Ar C=C Stretching).

\(^1\)H NMR (400 MHz; DMSO-d\(_6\)): δH 6.86 (1H, d, J\(_{HH}\)=7.0 Hz, Ar-H), 7.2(1H,d, J\(_{HH}\)=7.0 Hz, Ar-H), 6.8(1H,S), 6.9(2H, m, Ar-H), 8.15(2H, d, J\(_{HH}\)=7.0 Hz, Ar-H).

\(^13\)C NMR (100 MHz; CDCl\(_3\)): δC 127.92, 123.03, 126.11, 153.62, 160.67, 154.25, 120, 113.

LC-MS (70 eV): m/z=326(M+H)+, 328(M+2) 3:1 It indicates molecule contain one chlorine atom (Figure 15).

**Figure 15:** 5-chloro-3-(1H-indol-2-yl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine(6i).
Yield: 74% (brown color solid); m.p. 240-242°C.

IR (KBr, cm\(^{-1}\)): 758(-C-Cl), 3110(Ar C-H), 1580 (Ar C=C Stretching).

\(^1\)H NMR (400 MHz; DMSO-d\(_6\)): \(\delta H 6.86 (1H, d, J_{HH}=7.0 \text{ Hz}, \text{ Ar-H}), 7.2(1H,d, J_{HH}=7.0 \text{ Hz, Ar-H}), 8.5(1H,d, J_{HH}=7.4 \text{ Hz, Py, Ar-H}), 8.7(1H,d, J_{HH}=7.4 \text{ Hz, Py, Ar-H}), 9.3(1H, S, Py-Ar-H).

\(^13\)C NMR (100 MHz; CDCl\(_3\)): \(\delta C 127.92, 123.03, 126.11, 153.62, 160.67, 154.25, 134, 135, 124, 148, 155.

LC-MS (70 eV): m/z= 333(M-H, 100%)+, 334(M+1, 18%), It indicates molecule contain 17 Carbon atoms (Figure 17).

3-phenyl-5-(thiophen-2-yl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine(8a)

Yield: 51% (Fine brown crystals); m.p. 118-120°C.

IR (KBr, cm\(^{-1}\)): 3110(Ar C-H), 1680 (Ar C=C Stretching), 2980(SP\(_3\)C-H Stretching).

\(^1\)H NMR (400 MHz; DMSO-d\(_6\)): \(\delta H 6.96 (1H, d, J_{HH}=7.0 \text{ Hz, Ar-H}), 7.3(1H,d, J_{HH}=7.0 \text{ Hz, Ar-H}), 8.5(2H, d, J_{HH}=7.4 \text{ Hz, Ar-H}), 7.3((2H, d, J_{HH}=7.4 \text{ Hz, Ar-H}), 7.83(1H,d), 7.23(1H,t), 8.32(1H,d), 2.36(3H,S).

\(^13\)C NMR (100 MHz; CDCl\(_3\)): \(\delta C 126.98, 123.03, 126.11, 156.62, 160.67, 154.25, 134, 135, 124, 131, 23.3.

LC-MS (70 eV): m/z=349(M+H+, 100%), 350(M+1, 19.5%), It indicates molecule contain 18 Carbon atoms (Figure 18).

5-(thiophen-2-yl)-3-p-tolylthieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine(8b)

Yield: 54% (Fine yellow crystals); m.p. 269-270°C.
IR (KBr, cm⁻¹): 1155 (C-O-C Stretching), 3095 (Ar C-H), 1680 (Ar C=C Stretching), 2950 (SP3 C-H Stretching).

¹H NMR (400 MHz; DMSO-d₆): δH 6.96 (1H, d, JHH=7.0 Hz, Ar-H), 7.3 (1H, d, JHH=7.0 Hz, Ar-H), 7.95 (2H, d, JHH=7.8 Hz, Ar-H), 7.03 (2H, d, JHH=7.8 Hz, Ar-H), 3.9 (3H, S).

¹³C NMR (100 MHz; DMSO-d₆): δC 126.92, 123.03, 126.11, 153.62, 160.67, 154.25, 134, 128, 131.

LC-MS (70 eV); m/z=365 (M+H, 100%), 366 (M+1, 19.5%), It indicates molecule contain 18 Carbon atoms (Figure 19).

3-(4-methoxyphenyl)-5-(thiophen-2-yl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine (8c)

![Compound (8c)](image)

Figure 19: 3-(4-methoxyphenyl)-5-(thiophen-2-yl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine (8c)

Yield: 55% (yellow crystals); m.p. 234-235°C.

IR (KBr, cm⁻¹): 1140 (C-O-C Stretching), 1340 (C-F), 3110 (Ar C-H), 1687 (Ar C=C Stretching).

¹H NMR (400 MHz; DMSO-d₆): δH 6.96 (1H, d, JHH=7.0 Hz, Ar-H), 7.3 (1H, d, JHH=7.0 Hz, Ar-H), 8.65 (2H, d, JHH=7.8 Hz, Ar-H), 7.73 (2H, d, JHH=7.8 Hz, Ar-H), 7.8 (1H, d), 8.3 (1H, d).

¹³C NMR (100 MHz; DMSO-d₆): δC 126.92, 123.03, 126.11, 153.62, 160.67, 154.25, 134, 128, 131, 134, 128, 131.

LC-MS (70 eV); m/z=365 (M+H, 100%), 366 (M+1, 19.5%), It indicates molecule contain 18 Carbon atoms (Figure 19).

5-(thiophen-2-yl)-3-(4-(trifluoromethyl)phenyl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine (8d)

![Compound (8d)](image)

Figure 20: 5-(thiophen-2-yl)-3-(4-(trifluoromethyl)phenyl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine (8d)

Yield: 51% (Light brown crystals); m.p. 247-248°C.

IR (KBr, cm⁻¹): 748 (-C-Cl), 3120 (Ar C-H), 1690 (Ar C=C Stretching).

¹H NMR (400 MHz; DMSO-d₆): δH 6.96 (1H, d, JHH=7.0 Hz, Ar-H), 7.3 (1H, d, JHH=7.0 Hz, Ar-H), 8.25 (2H, d, JHH=7.8 Hz, Ar-H), 7.53 (2H, d, JHH=7.8 Hz, Ar-H), 8.1 (1H, d), 8.3 (1H, d).

¹³C NMR (100 MHz; DMSO-d₆): δC 127.92, 123.03, 126.11, 153.62, 160.67, 154.25, 134, 128, 131, 134, 128, 131, 134.

LC-MS (70 eV); m/z=369 (M+H), 371 (M+2), 3:1 It indicates molecule contain one chlorine atom (Figure 22).

5-(thiophen-2-yl)-3-(4-chlorophenyl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine (8e)

![Compound (8e)](image)

Figure 21: 5-(thiophen-2-yl)-3-(4-chlorophenyl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine (8e)

Yield: 51% (Light brown crystals); m.p. 247-248°C.

IR (KBr, cm⁻¹): 1155 (C-O-C Stretching), 3095 (Ar C-H), 1680 (Ar C=C Stretching).

¹H NMR (400 MHz; DMSO-d₆): δH 6.96 (1H, d, JHH=7.0 Hz, Ar-H), 7.3 (1H, d, JHH=7.0 Hz, Ar-H), 8.25 (2H, d, JHH=7.8 Hz, Ar-H), 7.53 (2H, d, JHH=7.8 Hz, Ar-H), 8.1 (1H, d), 8.3 (1H, d).

¹³C NMR (100 MHz; DMSO-d₆): δC 127.92, 123.03, 126.11, 153.62, 160.67, 154.25, 134, 128, 131, 134, 128, 131, 134.

LC-MS (70 eV); m/z=369 (M+H), 371 (M+2), 3:1 It indicates molecule contain one chlorine atom (Figure 22).
Yield: 50% (Yellow powder); m.p. 268-270°C.

IR (KBr, cm⁻¹): 680(-C-S-C Stretching), 540(C-Br), 3120(Ar C-H), 1690 (Ar C=C Stretching).

¹H NMR (400 MHz; DMSO-d₆): δH 6.96 (1H, d, J_H = 7.0 Hz, Ar-H), 7.31(1H,d, J_H = 7.0 Hz, Ar-H), 7.65(2H, d, J_H = 7.8 Hz, Ar-H), 7.53(2H, d, J_H = 7.8 Hz, Ar-H), 7.83(1H,d), 7.26(1H,t), 8.36(1H,d).

¹³C NMR (100 MHz; DMSO-d₆): δC 127.92, 123.03, 126.11, 153.62, 160.67, 154.25, 134, 128, 131, 124.

LC-MS (70 eV): m/z=413(M+H)⁺, 414(M+2, 1:1) It indicates molecule contain one bromine atom (Figure 23).

3-(4-bromophenyl)-5-(thiophen-2-yl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine(8g)

Yield: 48% (Yellow crystals); m.p. 193-195°C.

IR (KBr, cm⁻¹): 685(-C-S-C Stretching), 1540 and 1350 (N-O Stretching in Nitro group), 3160(Ar C-H), 1590 (Ar C=C Stretching).

¹H NMR (400 MHz; DMSO-d₆): δH 6.76 (1H, d, J_H = 7.0 Hz, Ar-H), 7.2(1H, d, J_H = 7.0 Hz, Ar-H), 8.15(2H, d, J_H = 7.0 Hz, Ar-H), 8.53((2H, d, J_H = 7.0 Hz, Ar-H), 7.83(1H,d), 7.23(1H,t), 8.35(1H,d).

¹³C NMR (100 MHz; DMSO-d₆): δC 127.92, 123.03, 126.11, 153.62, 160.67, 143.25, 124, 100, 128, 120, 113.

LC-MS (70 eV): m/z=378(M+H)⁺, 379(M+1) It indicates molecule contain 19 carbon atoms (Figure 24).

3-(1H-indol-2-yl)-5-(thiophen-2-yl) thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine(8i)

Yield: 60% (Light yellow powder); m.p. 205-207°C.

IR (KBr, cm⁻¹): 685(-C-S-C Stretching), 1530 (N-O Stretching in Nitro group), 1350 (N-O Stretching in Nitro group), 1680 (Ar C=C Stretching).

¹H NMR (400 MHz; DMSO-d₆): δH 6.86 (1H, d, J_H = 7.0 Hz, Ar-H), 7.21(1H, d, J_H = 7.0 Hz, Ar-H), 8.15(2H, d, J_H = 7.0 Hz, Ar-H), 8.53((2H, d, J_H = 7.0 Hz, Ar-H), 7.83(1H,d), 7.23(1H,t), 8.35(1H,d).

¹³C NMR (100 MHz; CDCl₃): δC 127.92, 123.03, 126.11, 153.62, 160.67, 154.25, 134, 124, 148, 155.

LC-MS (70 eV): m/z=334(M+H)⁺, 335(M+1) It indicates molecule contain 16 carbon atoms (Figure 26).
Biological Activity

Antibacterial studies

The newly prepared compounds were screened for their antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Escherichia coli* (clinical isolate) bacterial strains by disc diffusion method. A standard inoculum (1-2 × 10^7 cfu/ml 0.5 McFarland standards) were introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculums. The disks measuring 6 mm in diameters were prepared from Whatman No. 1 filter paper and sterilized by dry heat at 140°C for 1 h. The sterile disks previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were kept. Amoxicillin (30 µg) was used as positive control and the disk poured in DMSO was used as negative control and the test compounds were dissolved in DMSO at concentration of 100 and 50 µg/mL. The plates were inverted and incubated for 24 h at 37°C. The susceptibility was assessed on the basis of diameter of zone of inhibition against Gram-positive and Gram-negative strains of bacteria. Inhibition of zone of measured and compared with controls. The bacterial zone of inhibition values is given in Table 1. The order of activity was 8i>8j>8e>8h>8d>8f>8g>>8a>8b>8c.

<table>
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<tr>
<th>Synthesized Compounds</th>
<th>Gram positive</th>
<th></th>
<th></th>
<th>Gram negative</th>
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<td></td>
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<td><em>Klebsiella pneumonia</em></td>
<td><em>Escherichia coli</em></td>
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<td></td>
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<td>100 µg/mL</td>
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<td>8a</td>
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<td>7</td>
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<td></td>
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<tr>
<td>8b</td>
<td>7</td>
<td>4.5</td>
<td>7</td>
<td>4.5</td>
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<tr>
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<td>12.5</td>
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<td>Control (DMSO)</td>
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</table>

Table 1: Anti-bacterial activity of compounds 8(a-j).

Antifungal studies

The newly prepared compounds were screened for their antifungal activity against *Candida albicans* and *Aspergillus flavus* in DMSO by agar diffusion method. Sabourauds agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 ml) and adjusting pH 5.7. Normal saline was used to make suspension of corresponding species. Twenty millilitres of agar media was poured into each Petri dish. Excess of suspension was decanted and the plates were dried by placing in an incubator at 37°C for 1 h using an agar punch, wells were made and each well was labelled. A control was also prepared in triplicate and maintained at 37°C for 3–4
days. The fungal activity of each compound was compared with Ketoconazole as a standard drug. Inhibition zone were measured and compared with the controls. The fungal zone of inhibition values is given in Table 2.

<table>
<thead>
<tr>
<th>Synthetic Compounds</th>
<th>Candida albicans</th>
<th>Candida albicans</th>
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</table>

Table 2: Anti-fungal activity of compounds 8(a-j).

Conclusion
The research study reports the successful synthesis and antimicrobial activity study revealed that all the tested compounds showed good antibacterial and antifungal activities against pathogenic stains. The structure and biological activity relationship of title compounds indicate that the presence of electron donating and nitro attached to the triazole ring and Indole, Iso nicotinic ring and Thiophene rings were responsible for good antimicrobial activity and hence compounds 8i, 8j, 8e Exhibited more potent anti-microbial activity of all tested pathogenic strains.

Acknowledgments
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References