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SYNTHESIS, ANTIMICROBIAL AND ANTI-INFLAMMATORY ACTIVITIES OF SOME 2-(2-CHLOROPHENYL)-6-(SUBSTITUTED PHENYL)-THIAZOLO[3,2-b][1,2,4]TRIAZOLE

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ABSTRACT

The reaction of 2-chlorobenzohydrazide **3** with potassium thiocyanate yield {(2-chlorobenzoyl) aminothiourea **4** followed by cyclization in the presence of base afforded 5-(2-chlorophenyl)-4H-1,2,4-triazole-3-thiol **5**. Condensation of **5** with various 2-bromo-1-(substituted phenyl) ethanone **6** in the presence of base afforded 2-{[5-(2-chlorophenyl)-4H-1,2,4-triazole-3-yl]sulfanyl}-1-(substituted phenyl)ethanone **7** which on further cyclization in the presence of polyphosphoric acid afforded series of fused heterocycles namely 2-(2-chlorophenyl)-6-(substituted phenyl)-thiazolo[3,2-b][1,2,4]triazole (**8a-l).** The structure of all the synthesized compounds were characterized by FT-IR, ^{1}H and ^{13}C NMR, TOF MS ES+ data and elemental (C, H, N, S) analysis. Furthermore, compounds (**8a-l)** were screened for their antibacterial activity against gram negative (*E. coli* and *P. aeruginosa*) and gram positive (*S. aureus* and *B. subtilis*) bacteria, antifungal activity against pathogenic fungal strains (*C. albicans* and *A. niger*) and anti-inflammatory activities. Some of the compounds exhibited promising antibacterial, antifungal and anti-inflammatory activities.

KEYWORDS: Thiazole; Triazole; Thiazolotriazole; Antibacterial activity; Antifungal activity; Anti-inflammatory activity.

INTRODUCTION

We are observing today a dramatic world-wide extension of serious infections by microbes. Infectious diseases are now the second major cause of death and the third leading cause of demise in developed countries.[1] Rapid increase in pathogenic fungi and bacteria that are resisting to several antibiotics has grown major threat in handling of infectious diseases.[2] The incidental interest in the development of new antimicrobial agents can be fractionally impute both to the emergence of bacterial resistance to wonder drug therapy and new emerging pathogens. The different types of antibiotics available for the treatment of bacterial infections and emergence of dose resisting organisms have determined a great question to the scientists. There is real discern emergency for the discovery of fresh compounds endowed with antimicrobial activity, perhaps acting through mechanisms of action, which are different from those of well-understood form of antibacterial agents to which many clinically pertinent pathogens are now resistant.[3]

Non-steroidal anti-inflammatory drugs (NSAIDs) are used in the treatment of pain, inflammation, fever and a number of arthritic diseases such as rheumatoid arthritis and osteoarthritis.[4,5] However, their remedial use are often limited by common side effects, such as gastrointestinal (GI) hemorrhage, perforation and ulceration.[6,7] The incident of clinically significant GI side manifestation due to NSAIDs is high (30%) and causes some patients to cede NSAID therapy[8]. Hence in spite of abundance of NSAIDs in the market, an ideal agent is still a dream and the search continues to develop new drugs that have potent anti-inflammatory activity with minimum side effects. All such problems raise the extremity and make the noteworthy interest of medicinal apothecary in the discovery and evolution of new lead structures.

Thiazoles are an important set of heterocyclic compounds, found in many potent biologically active molecules such as Nizatidine, Sulfatiazole, Ritonavir, Meloxicam, Bleomycine, Fentiazac, and Tiazofurin.[9] Thiazole scaffold is an essential pharmacophore and its shackle with other rings could furnish novel biologically active compounds. Thiazole confine compounds exhibit a broad stroll of biological properties, such as anti-inflammatory[10], antimicrobial[11], antitumor[12], anticonvulsant[13], cardiotonic[14], anti-biofilm[15], analgesic[16] and anticancer[17]. 1,2,4-Triazole represent an overpowering and sharp developing field in recent heterocyclic chemistry. From erudition it is predictable

that, 1,2,4-triazole express important pharmacophores, and play a vital role as medicinal agents. A quality has been imparted to 1,2,4-triazole derivatives due to their wide range of biological activities such as antimicrobial[18], antitubercular[19], anti-inflammatory[20], antioxidant[21] and anticancer[22]. Prompted from therapeutically importance of thiazole and triazole as a part of our ongoing research in the area of antimicrobial and anti-inflammatory agents, it was reflection of interest to combine these two vital rings together into simple brownian framework. We have united an order of 2-(2-chloro-phenyl)-6-(substituted phenyl)-thiazolo[3,2-b][1,2,4]triazole **8a**-l and evaluated for their potential as antibacterial, antifungal and anti-inflammatory agents.

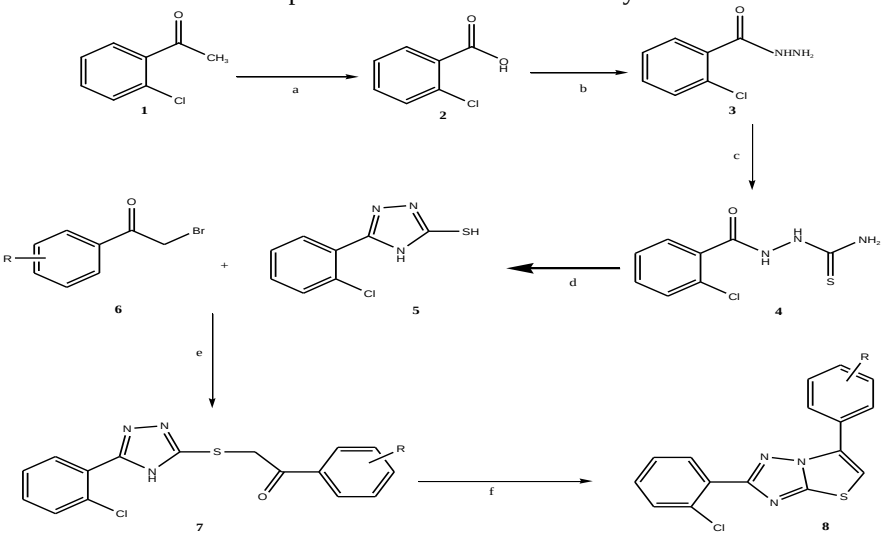
MATERIALS AND METHODS

General

All the reagents and chemicals were purchased from Sigma Aldrich (USA), Merck (Germany), SD Fine Chemicals (India) and used without any purification. Solvents were freshly distilled and used. Melting points were determined on a digital melting point apparatus DBK 10 MPA 03 (India). All the reactions were monitored by TLC performed on 20 × 20 cm aluminium sheets precoated with silica gel 60 F254 (Merck, Germany). The infrared spectra were recorded on a Shimadzu FT-IR-8400S spectrometer (Japan) using KBr optics. ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d6 on a Bruker Avance II-400 MHz spectrometer at frequencies of 400 MHz and 100 MHz respectively. Mass spectra were recorded on Waters, Q-TOF Micromass LC-MS mass spectrometer. Elemental analyses were carried out using Perkin Elmer Series II CHNS/O analyser (PerkinElmer, USA) and found within ± 0.4% of theoretical values.

Chemistry

The reaction sequence involved in the formation of compound **8a**-l is outlined in Scheme 1. The compound, 2-chlorobenzohydrazide **3** was synthesized in an excellent yield from 2-chloro benzoic acid **2**. The reaction of compound **3** with potassium thiocyanate gave {(2-chlorobenzoyl)amino}thiourea **4** which on further cyclization in the presence of base to yield 5-(2-chlorophenyl)-4H-1,2,4-triazole-3-thiol **5.** Then 2-Bromo-1-(substituted phenyl)ethanone **6** were prepared by following reported method[23]. Later, compound **5** was treated with compound **6** in the presence of base in ethanol medium to produce 2-{[5-(2-chlorophenyl)-4H-1,2,4-triazol-3-yl]sulfanyl}-1-(substituted phenyl)ethanone **7** which on further cyclization in the presence of polyphosporic acid to give a series of **8a**-l. All the reaction products were obtained in good yield. The synthesized compounds were purified by flash chromatography using ethyl acetate and hexane. The structures of newly synthesized compounds **(8a-l)** were elucidated by FTIR, ^{1}H and ^{13}C NMR, TOF MS ES+ and elemental analyses.



Compound	R_1	Compound	R_1
8a	H	8g	4-NO_2
8b	2-Cl	8h	4-CH_3
8c	4-Cl	8i	4-OCH_3
8d	4-Br	8j	2-OH
8e	4-NH_2	8k	4-OH
8f	2-NO_2	8l	4-F

Scheme: 1. Synthesis of 2-(2-chlorophenyl)-6-(4-substituted phenyl)-1,3-thiazolo[3,2-b]-1,2,4-triazole **(8a-l).** Reagents and condition: (a) $KMnO_4$, 2 h, 70-80 °C; (b) Conc.H_2SO_4, $NH_2NH_2.H_2O$, reflux, 6 h; (c) KSCN, Conc. HCl, reflux, 2 h; (d) 5% NaOH, reflux, 3 h; (e) EtOH, KOH, reflux, 5 h; (f) EtOH, polyphosphoric acid, reflux, 6 h, 120 °C.

General procedure for the synthesis of 2-chlorobenzohydrazide (3)

To a solution of compound **2** (0.01 mol) in methanol (20 mL), catalytic amount of sulfuric acid (0.5 mL) was added and the reaction mixture was refluxed for 2 h. The resulting ester was refluxed with hydrazine hydrate (0.02 mol) in dry methanol for 6 h and after cooling solid obtained was collected by filtration. The product was washed with cold methanol, dried and recrystallized from methanol.

Yellow solid; Yield 92.74%; mp 113-115 °C; IR (KBr, cm^{-1}): 3286.48 and 3184.26 (NH_2), 3066.41 (Ar-H), 1645.17 (C=O); ^{1}H-NMR (400 MHz, DMSO): δ 9.70 (s, 1H, NH), 7.50 (d, 1H, Ar-H, *J*=1.14 Hz), 7.44 (t, 2H, Ar-H, *J*=6.76 Hz), 7.39 (t, 1H, Ar-H, *J*=7.29 Hz), 4.57 (s, 2H, NH_2); *m/z*: 171.06 (M+1, 100 %); Anal. calcd. for $C_7H_7ClN_2O$: C, 49.32; H, 4.19; N, 16.47, Found: C, 49.28; H, 4.14; N, 16.42.

General procedure for the synthesis of {(2-chlorobenzoyl)amino}thiourea (4)

To a solution of compound **3** (0.01 mol) in water (25 mL), potassium thiocyanate (0.02 mol) and conc. HCl (2 mL) were added. The reaction mixture was refluxed for 2 h and after completion of the reaction; the mass was quenched into ice-water (200 mL). The precipitated product was filtered, dried and recrystallized from ethanol.

Orange brown solid; Yield 85.12%; mp 132-134 °C; IR (KBr, cm^{-1}): 3415.70 (Sec. NH), 3294.19 and 3199.69 (NH_2), 1677.95 (C=O), 1600.81 (Ar C=C); ^{1}H-NMR (400 MHz, DMSO): δ 10.42 (s, 1H, NH), 9.52 (s, 1H, NH of NHC=S), 7.76 (d, 1H, Ar-H, *J*=7.44 Hz), 7.60 (d, 1H, Ar-H, *J*=7.64 Hz), 7.56 -7.37 (m, 1H, Ar-H), 7.34 (s, 1H, Ar-H), 5.29 (s, 2H, NH_2); *m/z*: 230.04 (M+1, 9.69 %); Anal. calcd. for $C_8H_8ClN_3OS$: C, 41.82; H, 3.47; N, 18.03; S, 13.02, Found: C, 41.83; H, 3.51; N, 18.29; S, 13.96.

General procedure for the synthesis of 5-(2-chlorophenyl)-4H-1,2,4-triazole-3-thiol (5)

To a solution of 5% sodium hydroxide (100 mL), compound **4** (0.01 mol) was added and refluxed for 3 h. The reaction mixture was poured onto crushed ice with stirring and neutralized with conc. HCl. The resulting solid was filtered, wash with cold water, dried and recrystallized from ethanol.

White solid; Yield 87.32%; mp 145-147 °C; IR (KBr, cm^{-1}): 3415.70 (Sec. NH), 3056.96 (Ar-H), 2663.51 (S-H), 1604.66 (Ar C=C); ^{1}H-NMR (400 MHz, DMSO): δ 13.61 (s, 1H, SH), 8.21 (s, 1H, NH), 7.70 -7.65 (m, 1H, Ar-H), 7.55 (d, 1H, Ar-H, *J*=7.96 Hz), 7.49 (t, 1H, Ar-H, *J*=7.04 Hz), 7.41 (t, 1H, Ar-H, *J*=7.04 Hz); *m/z*: 212.06 (M+1, 9.97 %); Anal. calcd. for $C_8H_6ClN_3S$: C, 45.45; H, 2.92; N, 19.57; S, 15.48, Found: C, 45.39; H, 2.86; N, 19.85; S, 15.15.

General procedure for the synthesis of 2-bromo-1-(substituted phenyl)ethanone (6)

A solution of acetophenone (0.21 mol) in dry ether (25 mL) was placed in a dry flask fitted with a separating funnel and reflux condenser. The solution was cooled in an ice bath, anhydrous $AlCl_3$ (0.25 g) was introduced and bromine (0.21 mol) was added gradually from a separating funnel with stirring. The solid mass obtained was washed with 1:1 mixture of water and petroleum ether and recrystallized from ethanol. The yields of compound **6a-l** were in between 74 and 95 %.

General procedure for the synthesis of 2-{[5-(2-chlorophenyl)-4H-1,2,4-triazol-3-yl]sulfanyl}-1-(substituted phenyl)ethanone (7)

To a solution of compound **5** (0.01 mol) in ethanol (50 mL), compound **6** (0.01 mol) and potassium hydroxide (0.015 mol) were added and refluxed for 5 h. The reaction mixture was cooled and poured onto crushed ice. The resulting solid was filtered, wash with water and dried. The crude product was purified by flash chromatography using hexane and ethyl acetate (1:1). The purified product was recrystallized from ethanol. The yields of compound **7a-l** were in between 72 and 95 %.

General procedure for the synthesis of 2-(2-chlorophenyl)-6-(4-substituted phenyl)-1,3-thiazolo[3,2-b]-1,2,4-triazole (8a-l)

To a solution of compound **7** (0.01 mol) in ethanol (50 mL), polyphosphoric acid (5 mL) was added and refluxed at 120 °C for 6 h. The reaction mixture was cooled, poured onto crushed ice and neutralized by adding sodium bicarbonate. The resulting solid was filtered, wash with water and dried. The crude product was purified by flash chromatography using hexane and ethyl acetate (1:1) and recrystallized from ethanol.

2-(2-Chlorophenyl)-6-phenyl-thiazolo[3,2-b][1,2,4]triazole (8a)

White solid; Yield 86.71%; mp 196-198 °C; IR (KBr, cm^{-1}): 3068.53 (Ar-H str), 2983.67(C-H str), 1606.59 (C=N str), 1589.23 (Ar C=C str), 846.69 (C-Cl str); ^{1}H-NMR (400 MHz, DMSO): δ 8.28 (s, 1H, thiazole proton), 7.84 (t, 1H, Ar-H, *J*=7.09 Hz), 7.66 (t, 2H, 2-chlorophenyl protos, *J*=8.14 Hz), 7.57-7.43 (m, 2H, 2-chlorophenyl protons), 7.39-7.34 (m, 2H, Ar-H), 7.27 (s, 1H, Ar-H), 7.18 (t, 1H, Ar-H, *J*=7.07 Hz); ^{13}C-NMR (DMSO, 100 MHz): δ 156.50, 150.32, 149.94, 148.80, 134.54, 133.32, 131.54, 131.01, 129.54, 127.32, 126.24, 125.90, 123.70, 115.40, 115.08, 113.38; *m/z*: 312.62 (M+1, 25.99 %); Anal. calcd. for $C_{16}H_{10}ClN_3S$: C, 61.64; H, 3.23; N, 13.48; S, 10.28, Found: C, 61.03; H, 3.29; N, 13.75; S, 10.82.

2,6-Bis(2-chlorophenyl)-thiazolo[3,2-b][1,2,4]triazole (8b)

Dark yellow solid; Yield 84.69%; mp 206-208 °C; IR (KBr, cm^{-1}): 3076.25(Ar-H str), 2991.39 (C-H str), 1602.74 (C=N str), 1554.52 (Ar C=C str), 850.55 (C-Cl

str); ^{1}H-NMR (400 MHz, DMSO): δ 8.25 (s, 1H, thiazole proton), 7.76 (t, 2H, 2-chlorophenyl protons, *J*=7.45 Hz), 7.48 (t, 2H, 2-chlorophenyl protons, *J*=8.11 Hz), 7.39 (s, 2H, Ar-H), 7.32 (d, 1H, Ar-H, *J*=7.86 Hz), 7.18 (d, 1H, Ar-H, *J*=8.52 Hz); ^{13}C-NMR (DMSO, 100 MHz): δ 155.98, 150.18, 149.58, 137.91, 135.95, 135.54, 133.41, 132.54, 131.47, 130.54, 130.07, 127.98, 126.18, 125.58, 124.91, 122.55; *m/z*: 347.57 (M+1, 18.96 %); Anal. calcd. for $C_{16}H_9Cl_2N_3S$: C, 55.50; H, 2.62; N, 12.14; S, 9.26, Found: C, 55.57; H, 2.67; N, 11.92; S, 9.88.

2-(2-Chlorophenyl)-6-(4-chlorophenyl)-thiazolo[3,2-b][1,2,4]triazole (8c)

Dark yellow solid; Yield 81.48%; mp 194-196 °C; IR (KBr, cm^{-1}): 3076.25(Ar-H str), 2991.39 (C-H str), 1602.74 (C=N str), 1554.52 (Ar C=C str), 850.55 (C-Cl str); ^{1}H-NMR (400 MHz, DMSO): δ 8.25 (s, 1H, thiazole proton), 7.84 (t, 1H, 2-chlorophenyl protons, *J*=8.03 Hz), 7.76 (q, 2H, 2-chlorophenyl protons, *J*=8.21 Hz), 7.51 (t, 2H, Ar-H, *J*=9.01 Hz), 7.21 (t, 1H, 2-chlorophenyl protons, *J*=2.14 Hz), 7.18 (d, 2H, Ar-H, *J*=8.63 Hz); ^{13}C-NMR (DMSO, 100 MHz): δ 154.48, 149.19, 148.88, 136.91, 134.45, 133.53, 132.41, 131.14, 128.57, 128.18, 127.77, 126.68, 125.18, 123.58, 122.92, 121.15; *m/z*: 347.55 (M+1, 18.96 %); Anal. calcd. for $C_{16}H_9Cl_2N_3S$: C, 55.50; H, 2.62; N, 12.14; S, 9.26, Found: C, 55.62; H, 2.59; N, 11.96; S, 9.02.

6-(4-Bromophenyl)-2-(2-chlorophenyl)- thiazolo[3,2-b][1,2,4]triazole (8d*)*

Light yellow solid; Yield 87.71%; mp 201-203 °C; IR (KBr, cm^{-1}): 3056.96 (Ar-H str), 2943.17 (C-H str), 1602.74 (C=N str), 1525.59 (Ar C=C str), 850.55 (C-Cl str), 686.61 (C-Br str); ^{1}H-NMR (400 MHz, DMSO): δ 8.26 (s, 1H, thiazole proton), 7.76 (t, 2H, 2-chlorophenyl protons, J=8.89 Hz), 7.48 (q, 2H, 2-chlorophenyl protons, J=9.02 Hz), 7.39 (d, 2H, Ar-H, J=8.12 Hz), 7.22 (d, 1H, Ar-H, J=2.64 Hz), 7.14 (d, 1H, Ar-H, J=7.09 Hz); ^{13}C-NMR (DMSO, 100 MHz): δ 151.44, 146.19, 145.88, 133.96, 131.44, 130.53, 129.42, 125.11, 124.58, 123.18, 122.77, 119.66, 118.15, 117.53, 116.92, 115.25; *m/z*: 391.24 (M+1, 15.80 %); Anal. calcd. for $C_{16}H_9BrClN_3S$: C, 49.19; H, 2.32; N, 10.76; S, 8.21, Found: C, 49.31; H, 2.38; N, 10.02; S, 8.59.

4-[2-(2-Chlorophenyl)-thiazolo[3,2-b][1,2,4]triazol-6-yl]-phenylamine (8e)

White crystal; Yield 89.32%; mp 215-217 °C; IR (KBr, cm^{-1}): 3458.13 and 3367.48 (N-H str), 3060.82 (Ar-H str), 2846.74 (C-H str), 1620.09 (C=N str), 1568.02 (Ar C=C str), 858.26 (C-Cl str); ^{1}H-NMR (400 MHz, DMSO): δ 8.28 (s, 1H, thiazole proton), 7.76 (t, 2H, 2-chlorophenyl protons, *J*=8.09), 7.57-7.39 (m, 2H, 2-chlorophenyl protons), 7.34 (d, 1H, Ar-H, *J*=1.86 Hz), 7.27 (s, 1H, Ar-H), 7.21 (t, 2H, Ar-H, *J*=9.25 Hz), 4.27 (s, 2H, NH_2); ^{13}C-NMR (DMSO, 100 MHz): δ 155.33, 154.87, 153.64, 152.18, 151.77, 147.42, 146.31, 144.41, 143.94, 139.66, 138.37, 137.74, 136.03, 124.81, 123.39, 118.33; *m/z*: 327.11 (M+1, 28.92 %); Anal. calcd. for $C_{16}H_{11}ClN_4S$: C, 58.80; H, 3.39; N, 17.14; S, 9.81, Found: C, 58.89; H, 3.36; N, 17.61; S, 10.06.

2-(2-Chlorophenyl)-6-(2-nitrophenyl)-thiazolo[3,2-b][1,2,4]triazole (8f)

Dark brown crystals; Yield 79.22%; mp 197-199 °C; IR (KBr, cm^{-1}): 3062.75 (Ar-H str), 2995.25 (C-H str), 1604.66 (C=N str), 1533.30 and 1338.51 and (N-O str), 867.91 (C-Cl str); ^{1}H-NMR (400 MHz, DMSO): δ 8.29 (s, 1H, thiazole proton), 7.84 (t, 1H, 2-chlorophenyl protons, *J*=8.61 Hz), 7.71 (t, 1H, 2-chlorophenyl protons, *J*=8.81 Hz), 7.60-7.43 (m, 2H, 2-chlorophenyl protons), 7.39-7.32 (m, 2H, Ar-H), 7.31-7.21 (m, 1H, Ar-H), 7.14 (d, 1H, Ar-H, *J*=8.78 Hz); ^{13}C-NMR (DMSO, 100 MHz): δ 152.04, 151.22, 150.38, 149.84, 148.04, 147.74, 146.97, 145.71, 144.37, 132.55, 131.29, 130.67, 129.74, 128.81, 127.33, 126.97; *m/z*: 357.53 (M+1, 17.79 %); Anal. calcd. for $C_{16}H_9ClN_4O_2S$: C, 53.86; H, 2.54; N, 15.70; S, 8.99, Found: C, 53.94; H, 2.49; N, 15.91; S, 10.21.

2-(2-Chlorophenyl)-6-(4-nitrophenyl)-thiazolo[3,2-b][1,2,4]triazole (8g)

Orange yellow solid; Yield 81.14%; mp 188-190 °C; IR (KBr, cm^{-1}): 3062.75 (Ar-H str), 2995.25 (C-H str), 1604.66 (C=N str), 1533.30 and 1338.51 (N-O str), 867.91 (C-Cl str); ^{1}H-NMR (400 MHz, DMSO): δ 8.26 (s, 1H, thiazole proton), 7.84 (t, 1H, 2-chlorophenyl protons, *J*=7.88 Hz), 7.76 (q, 1H, 2-chlorophenyl protons, *J*=9.08 Hz), 7.57-7.39 (m, 2H, 2-chlorophenyl protons), 7.34 (s, 2H, Ar-H), 7.27 (d, 1H, Ar-H, *J*=8.83 Hz), 7.17 (d, 1H, Ar-H, *J*=8.31 Hz); ^{13}C-NMR (DMSO, 100 MHz): δ 156.36, 155.50, 154.94, 153.04, 152.37, 151.11, 150.68, 149.33, 148.77, 146.37, 143.21, 141.45, 135.80, 132.29, 129.91, 127.34; *m/z*: 357.42 (M+1, 31.49 %); Anal. calcd. for $C_{16}H_9ClN_4O_2S$: C, 53.86; H, 2.54; N, 15.70; S, 8.99, Found: C, 53.95; H, 2.56; N, 15.44; S, 10.15.

2-(2-Chlorophenyl)-6-p-tolyl-thiazolo[3,2-b][1,2,4]triazole (8h)

Yellow solid; Yield 90.61%; mp 214-216 °C; IR (KBr, cm^{-1}): 3043.46 (Ar-H str), 2910.38 (C-H str), 1602.74 (C=N str), 1554.52 (Ar C=C str), 850.55 (C-Cl str); ^{1}H-NMR (400 MHz, DMSO): δ 8.38 (s, 1H, thiazole proton), 7.84 (t, 1H, 2-chlorophenyl protons, *J*=9.12 Hz), 7.66 (t, 2H, 2-chlorophenyl protons, *J*=8.61 Hz), 7.48 (t, 1H, 2-chlorophenyl protons, *J*=7.25 Hz), 7.39 (d, 2H, Ar-H, *J*=8.92 Hz), 7.22 (d, 1H, Ar-H, *J*=7.76 Hz), 7.17 (d, 1H, Ar-H, *J*=8.07 Hz), 2.57 (s, 3H, CH_3); ^{13}C-NMR (DMSO, 100 MHz): δ 154.33, 153.77, 152.37, 151.21, 150.45, 148.80, 147.29, 144.91, 140.34, 137.36, 135.50, 134.94, 133.04, 131.37, 130.11, 129.68, 21.78; *m/z*: 326.241 (M+1, 28.22 %); Anal. calcd. for $C_{17}H_{12}ClN_3S$: C, 62.67; H, 3.71; N, 12.90; S, 9.84, Found: C, 62.74; H, 3.78; N, 12.07; S, 10.02.

2-(2-Chlorophenyl)-6-(4-methoxyphenyl)-thiazolo[3,2-b][1,2,4]triazole (8i)

Yellow-brown solid; Yield 78.85%; mp 204-206 °C; IR (KBr, cm^{-1}): 3029.96 (Ar-H str), 2920.03 (C-H str), 1608.52 (C=N str), 1541.02 (Ar C=C str), 1112.85 (C-O str), 875.62 (C-Cl str); ^{1}H-NMR (400 MHz, DMSO): δ 8.31 (s, 1H, thiazole proton), 7.92 (d, 1H, Ar-H, *J*=8.20 Hz), 7.79-7.60 (m, 2H, 2-chlorophenyl protons), 7.43-7.31 (m, 2H, 2-chlorophenyl protons), 7.27 (s, 2H, Ar-H), 7.18 (d, 1H, Ar-H, *J*=8.21 Hz), 3.67 (s, 3H, OCH_3); ^{13}C-NMR (DMSO, 100 MHz): δ 158.54, 157.53, 155.92, 154.51, 143.50, 142.98, 140.97, 140.44, 138.90, 136.97, 135.75, 135.14, 133.33, 126.31, 113.90, 111.29, 59.48; *m/z*: 342.02 (M+1, 19.20 %); Anal. calcd. for $C_{17}H_{12}ClN_3OS$: C, 59.73; H, 3.54; N, 12.29; S, 9.38, Found: C, 58.98; H, 3.59; N, 12.11; S, 9.52.

2-[2-(2-Chlorophenyl)-thiazolo[3,2-b][1,2,4]triazole-6-yl]-phenol (8j)

Yellow solid; Yield 83.09%; mp 192-194 °C; IR (KBr, cm^{-1}): 3353.98 (O-H str), 3032.81 (Ar-H str), 2924.87 (C-H str), 1597.09 (C=N str), 1522.73 (Ar C=C str), 858.26 (C-Cl str); ^{1}H-NMR (400 MHz, DMSO): δ 9.99 (s, 1H, OH), 8.28 (s, 1H, thiazole proton), 7.89 (t, 1H, Ar-H, *J*=7.21 Hz), 7.79 (t, 1H, Ar-H, *J*=8.39 Hz), 7.62-7.43 (m, 1H, 2-chlorophenyl protons), 7.39-7.27 (m, 3H, 2-chlorophenyl protons), 7.14 (t, 2H, Ar-H, J=8.81 Hz); ^{13}C-NMR (DMSO, 100 MHz): δ 151.58, 150.97, 149.95, 147.54, 137.53, 136.92, 134.90, 133.40, 132.98, 130.96, 120.75, 118.15, 116.53, 116.16, 113.95, 113.51; *m/z*: 328.15 (M+1, 38.05 %); Anal. calcd. for $C_{16}H_{10}ClN_3OS$: C, 58.63; H, 3.07; N, 12.82; S, 9.78, Found: C, 58.02; H, 3.01; N, 11.98; S, 9.23.

4-[2-(2-Chlorophenyl)-thiazolo[3,2-b][1,2,4]triazole-6-yl]-phenol (8k)

Light yellow crystals; Yield 92.21%; mp 222-224 °C; IR (KBr, cm^{-1}): 3353.98 (O-H str), 3033.82 (Ar-H str), 2923.88 (C-H str), 1597.09 (C=N str), 1521.73 (Ar C=C str), 858.26 (C-Cl str); ^{1}H-NMR (400 MHz, DMSO): δ 9.99 (s, 1H, OH), 8.28 (s, 1H, thiazole proton), 7.91 (t, 2H, 2-chlorophenyl protons, *J*=8.34 Hz), 7.79 (t, 1H, Ar-H, *J*=8.56 Hz), 7.45 (t, 2H, 2-chlorophenyl protons, *J*=7.79 Hz), 7.39 (d, 1H, Ar-H, *J*=7.43 Hz), 7.18 (d, 2H, Ar-H, *J*=8.72 Hz); ^{13}C-NMR (DMSO, 100 MHz): δ 158.60, 157.58, 154.05, 153.04, 152.94, 151.92, 150.87, 142.94, 141.99, 140.78, 140.12, 139.69, 136.68, 131.90, 129.95, 129.12; *m/z*: 328.28 (M+1, 55.26 %); Anal. calcd. for $C_{16}H_{10}ClN_3OS$: C, 58.63; H, 3.07; N, 12.82; S, 9.78, Found: C, 58.09; H, 3.03; N, 12.99; S, 9.36.

2-(2-Chlorophenyl)-6-(4-fluorophenyl)-thiazolo[3,2-b][1,2,4]triazole (8l)

Brown solid; Yield 91.28%; mp 225-227 °C; IR (KBr, cm^{-1}): 3043.46 (Ar-H str), 2943.17 (C-H str), 1602.74 (C=N str), 1554.52 (Ar C=C str), 1101.28 (C-F str), 850.55 (C-Cl str); ^{1}H-NMR (400 MHz, DMSO): δ 8.38 (s, 1H, thiazole proton), 7.86 (t, 2H, 2-chlorophenyl protons, *J*=9.08 Hz), 7.71 (t, 1H, 2-chlorophenyl protons, *J*=7.76 Hz), 7.62-7.43 (m, 1H, 2-chlorophenyl protons), 7.39-7.31 (m, 2H, Ar-H), 7.27 (d, 1H, Ar-H, *J*=8.16 Hz), 7.14 (d, 1H, Ar-H, *J*=7.75 Hz); ^{13}C-NMR (DMSO, 100 MHz): δ 155.12, 154.69, 153.68, 151.90, 148.95, 146.12, 143.60, 141.58, 139.05, 138.04, 127.94, 124.92, 120.87, 118.94, 116.62, 115.87; *m/z*: 330.21 (M+1, 24.82 %); Anal. calcd. for $C_{16}H_9ClFN_3S$: C, 58.27; H, 2.75; N, 12.74; S, 9.72, Found: C, 58.91; H, 2.69; N, 12.44; S, 9.49.

Acute toxicity studies

The study was conducted as per the protocol of Organization for Economic Cooperation and Development (OECD, 2008). Female Wistar Albino rats weighing 150-200 g were used for the meditation and randomly cluster abode with three per mew. They were housed in standard polypropylene cages and allowed for acclimation to the experimental conditions for one week. The suspensions of trial derivative were ready in water (methyl cellulose, 0.5%) and managed orally at a dose level 2000 mg/kg. This is the limit trial at one portion clear. The feed was restrained for further 6 h. The treated rats were observed for death once during 30 minute, periodically during the first 24 h, with a distinctive attention given during the first 4 h and daily thereafter for a total of 14 days. None of the test derivative at a limit one potion level for a dose 2000 mg/kg evinces any mortality. No mortality, no body weight shift, no toxic mark were observed during the 14 days era of remark. Therefore, the cut off LD_{50} was > 2000 mg/kg for each trial compound when given orally. The approval of the Institutional Animal Ethics Committee was obtained prior to start of the experiment.

Antibacterial activity

All the new synthesized derivatives **8a-l** were assessed for their *in vitro* antibacterial action. The antibacterial activity was carried out against *E. coli* ATCC 25916, *P. aeruginosa* ATCC 29212 (Gram-negative bacteria) and *S. aureus* ATCC 25912, *B. subtilis* ATCC 6621 (Gram-positive bacteria) by broth dilution method. Bacterial strains were cultured in Mueller-Hinton nutrient broth and Ciprofloxacin was employed as a point of reference measure. The trial derivatives were dethawed in dimethylsulfoxide (DMSO) to receive the desired concentration 1000, 500, 250, 150, 100, 75, 50, 25, 10, 5 and 1 µg/mL. Compounds were dribbled with a pore size of 0.45µm for sterilization and trial compounds at various concentrations were added to culture medium in a sterilised test tube. Test strains were suspended in nutrient agar to give a net density of 5×10^5 cfu/mL. The solvent control (DMSO) was also developed in the same manner and tubes were brooded at 37 °C for 24 h. All studies comprised in triplicate. The turbidity was supervised after 24 h for the development of test organism and MIC values were found from the lowest concentration, at which no growth seen. The MIC values were also checked for the reference measure (ciprofloxacin) to compare the antibacterial activity of trial compounds. The MIC values of trial compounds and reference measure are stated in µg/mL and summarized

in Table 1.

Antifungal activity

The new synthesized derivatives were tested for their antifungal activity against *C. albicans* NCIM 3492 and *A. niger* NCIM 1223 by the sequential dilution method using fluconazole as reference standard. Sabouraud Dextrose Agar (Himedia) media was used for the growth of fungi. Normal saline solution was employed to build a suspension of spore of fungal strain. A loopful of specific fungal strain was shifted to 3 mL saline to acquire a suspension of corresponding species. A solution of agar culture medium (20 mL) was decanted into each petri dish. Excess of suspension was poured out and the plates were dried in an incubator at 37 °C. After drying, wells were created using an agar plug and trial samples, reference standard and control (DMSO) were graded in marked wells in each petri plate. The petri plates were incubated at 37 °C for 48 h. The MIC values were observed and the activeness of each trial compound was equated with fluconazole as standard drug. The results of antifungal action are acceded MIC values as µg/mL and are exemplified in Table 1.

Anti-inflammatory activity

In vivo anti-inflammatory drug action of compound (**8a-l)** was appraised by applying carrageenan induced rat paw edema technique. The percentage of inhibition was compared with standard drug Indomethacin. The results were expressed as % inhibition of edema over the untreated control cluster. The results of anti-inflammatory studies are given in Table 2. For analysis, male albino rats weighing 165-220 g were employed for the experiment. They were put up in light wire netted polypropylene cages in controlled room temperature (25 °C), relative humidity (60-70%) and with 12 h light-dark cycle. The animals were conserved on monetary standard laboratory diet and water ad libitum. The animals were bereft of food 12 h before and during observational hours. The animals were carved up arbitrarily into 26 cluster each cluster held in 6 animals. A mark was produced on the left hind paw just beyond the tibio-tarsal joint; so that the paw was dipped inside the mercury pillar up to mounted mark and constant paw volume was ensured. The initial paw volume of every rat was noted plethysmometrically. First cluster got normal saline and the second cluster got Indomethacin at a dose of 1.5 mg/kg, p.o. The 3rd to 14th cluster was allotted the test compounds (**8a-l)** at a dose of 50 mg/kg, p.o. (suspended in 10 ml/kg of 2% gum acacia). After 30 min from treatment of trial compounds, 0.1 ml of 1 % (w/v) carrageenan was injected inside the subplantar area of the left hind paw. The first paw volume was measured out within 30 s of the injection. The relative gain in paw volume was measured in control, standard and test compounds at 1, 2, 3 and 4 h after the carrageenan injection. The deviation between the two readings was taken as the volume of edema and the percentage inhibition of edema by the drugs was calculated using the formula,

$$\% \text{ Edema inhibition} = 100 - (Vtest/Vcontrol) \times 100$$

RESULT AND DISCUSSION

Chemistry

In the present study a series of compounds **(8a-l)** were synthesized and evaluated for antibacterial, antifungal and anti-inflammatory activity. The compounds (8a-l) were synthesized in good yield as illustrated in Scheme 1 and their structures were characterized by spectral data. The IR spectrum of compound **3** showed a characteristic absorption bands appeared at 3286.48-3184.26 cm^{-1} and 1645.17 cm^{-1} due to the presence of NH and C=O functions, respectively. The ^{1}H-NMR spectrum of compound **3** exhibited a singlet at δ 9.70 which accounted NH. The four aromatic protons resonated at δ 7.50-7.39 and broad singlet at δ 4.57 due to NH_2. The mass spectrum of compound **3** showed molecular ion peak at *m/z* 171.06. The IR spectrum of compound **4** showed a characteristic absorption bands at 3415.70 cm^{-1} due to NH and the other at 3294.19-3199.69 cm^{-1} was attributed to NH_2. The ^{1}H-NMR spectrum of the same displayed a singlet at δ 10.42 accounted for NHC=S, four aromatic protons at δ 7.76-7.34 and singlet at δ 5.29 due to NH_2. The mass spectrum showed molecular ion peak at *m/z* 230.04. In IR spectrum of compound **5,** bands appeared at 3415.70, 3056.96, 2663.51 and 1604.66 cm^{-1} were due to NH, Ar-H, SH and C=C functions, respectively. The 400 MHz ^{1}H-NMR spectrum of compound **5** showed singlet at δ 13.61 due to SH and δ 8.21 attributed to NH. The aromatic protons resonated at δ 7.70-7.41. The mass spectrum showed molecular ion peak at *m/z* 212.06 $(M+1)^+$ which was in consistent with its molecular formula $C_8H_6ClN_3S$. The IR spectra of compound (**8a-l)** showed, in each case, stretching band of Ar-H, C-H, C=N, C=C and C-Cl in the region of 3100-3010 cm^{-1}, 2998-2911 cm^{-1}, 1625-1585 cm^{-1} and 870-810 cm^{-1} respectively. The ^{1}H NMR spectra showed, in each case, the signals as multiplet at δ 7.92-7.14 ppm attributed to Ar-H in addition to the singlet of thiazole ring in the region 8.38-8.20 ppm. The ^{13}C NMR spectra showed, in each case, the carbon of thiazole and triazole ring by signal at δ 160.11-114.85 and δ 155.21-142.32, respectively. The aromatic carbon resonated at δ 158.54-111.29. The mass spectrum of all compound displayed $(M+1)^+$ confirming their molecular weight. The elemental (C, H, N, S) analyses were found within the limit of theoretical values.

Antibacterial studies

The outcome of antibacterial activity is explicit as MIC (µg/mL) and results have been presented in Table 1. Compound 8b and 8f with the electron withdrawing (chloro and nitro) group on 2-position of the phenyl ring showed superior activity against Gram-negative (*E. coli* and *P. aeruginosa*) bacteria, while compound 8h and 8i with the electron donating (methyl and methoxy) group exhibited very good antibacterial activity against Gram-positive (*S. aureus* and *B. subtilis*) bacteria. Investigation of the structure activity relationship study revealed that compound 8l with electron withdrawing (fluoro)

substitution on 4-position of the phenyl ring privileges antibacterial activity against both Gram-negative and Gram-positive bacteria. The good activity may be due to the presence of highly electronegative fluorine in the molecule. Compounds 8a, 8c, 8d and 8g showed moderate antibacterial activity. Compare to standard drugs, compounds 8j and 8k showed insignificant antibacterial activity against both Gram-negative and Gram-positive bacteria. The compound 8e in the series was found to have comparatively poor activity against both bacterial strains.

Table 1: Antibacterial and antifungal activities of compound (8a-l)

Compound	Gram negative bacteria*		Gram positive bacteria*		Fungi*	
	E. coli	***P. aeruginosa***	***S. aureus***	***B. subtilis***	***C. albicans***	***A. niger***
8a	21	25	18	23	17	22
8b	7	6	25	31	5	14
8c	32	28	37	39	46	31
8d	14	19	25	18	21	17
8e	72	84	64	58	28	18
8f	8	11	28	17	25	32
8g	28	27	33	36	44	52
8h	56	68	10	9	>100	94
8i	21	34	12	15	32	39
8j	59	>100	82	94	82	76
8k	>100	>100	76	86	>100	81
8l	03	04	03	02	02	04
Ciprofloxacin	02	02	01	01	-	-
Fluconazole	-	-	-	-	01	02

** Minimum Inhibitory Concentration (MIC) in µg/mL. E. coli: Escherichia coli; P. aeruginisa: Pseudomonas aeruginosa; S. aureus: Staphylococcus aureus; B. subtilis: Bacillus subtilis; C. albicans: Candida albicans; A. niger: Aspergillus niger.*

Antifungal studies: From the antifungal activity data in Table 1, it was observed that compound 8l exhibited maximum activity among all tested compounds against both fungal strains. However, all other compounds in the series were found to have mild to moderate antifungal activity compared to reference standard. Compounds 8a, 8c, 8d, 8e, 8f, 8g and 8i showed moderate antifungal activity, while compound 8b with electron withdrawing (chloro) group on 2-position was found to have very good antifungal activity against *C. albican*. The compounds 8h, 8j and 8k with electron donating (methyl and hydroxyl) group on phenyl ring exhibited very less activity against both the fungal strains.

Anti-inflammatory studies

Anti-inflammatory activity data (Table 2) of compound 8a-l revealed that compound 8l with electron withdrawing fluoro substituent exhibited excellent anti-inflammatory activity whereas compounds 8b, 8c and 8i showed very good anti-inflammatory activity compared to that of standard drug, Indomethacin. Compounds 8a, 8d, 8e, 8f, 8g and 8h showed moderate activity. While, compound 8j and 8k with electron donating hydroxyl group at 2 and 4-position exhibited insignificant anti-inflammatory activity.

Table 2: Anti-inflammatory activity of compound (8a-l)

Compound No.	Dose (mg/kg, p.o.)	% edema inhibition after hour			
		1	**2**	**3**	**4**
8a	50	21.23	26.31	36.76	31.89
8b	50	14.77	30.95	46.43	40.68
8c	50	26.54	33.49	46.23	33.67
8d	50	14.77	16.69	29.69	26.98
8e	50	10.61	33.49	38.02	34.93
8f	50	19.82	25.02	34.67	30.00
8g	50	21.23	33.68	38.05	33.03
8h	50	15.92	33.73	38.02	30.00
8i	50	19.55	30.66	44.59	35.97
8j	50	5.30	6.19	3.34	1.77
8k	50	7.07	4.78	2.22	2.02
8l	50	33.62	55.02	58.77	56.96
Control	10 mL/kg	-	-	-	-
Standard[a]	1.5	46.01	54.06	62.95	58.22

[a] *Indomethacin is used as standard; N=6 in each group.*

CONCLUSION

In this study we have reported an effective and convenient synthesis of a new series of 2-(2-chlorophenyl)-6-(4-substituted phenyl)-1,3-thiazolo[3,2-b]-1,2,4-triazole (**8a-l)**. The structures of these new heterocyclic compounds bearing both thiazole and triazole ring arrangements were supported by spectral (IR, ^{1}H and ^{13}C NMR, Mass) and elemental (C, H, N, S) analysis and were evaluated for their antibacterial, antifungal and anti-inflammatory activities. The results proved that many of the synthesized derivatives exhibited significant antibacterial, antifungal and anti-inflammatory activities. The compounds with the electron withdrawing (fluoro) group on 4-position of the phenyl ring supported the antibacterial, antifungal and anti-inflammatory activities. The substitutions with the electron withdrawing (chloro and nitro) group on 2-position of the phenyl ring favoured antibacterial activity against Gram-negative bacterial strain while the electron donating (methyl and methoxy) group supported for Gram-positive bacterial strain. Compounds with electron withdrawing substituents (fluoro and chloro) on phenyl ring encouraged the antifungal activity. The substitution with electron donating hydroxyl group on 2 and 4-position failed to attract attention. The results of anti-inflammatory studies revealed that substitutions with the electron withdrawing (fluoro) group on the phenyl ring exhibited potential activity. Thus, the significant antibacterial, antifungal and anti-inflammatory profiles of some derivatives offer them as promising lead molecules for further optimization using molecular modelling techniques.

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