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Synthesis of some 7-arylidene-3-(4-(Methylthio)bezyl)-7*H*-thiazolo[2,3-*c*][1,2,4]triazine-4,6-diones and their anticonvulsant and antimicrobial activity

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ABSTRACT

*In the present study two series of novel 4-(4-methylthiobenzyl)-5-oxo-7-(5-aryl-2-furfurylidene/arylidene)-1,2,4-triazino[3,4-b]-thiazole-6-ones were synthesized by the multi component reaction of 6-(4-methylthiobenzyl)-3-mercapto-1,2,4-triazin-5(4H)-one with chloroacetic acid and 5-aryl furfurals/aromatic aldehydes in presence of acetic anhydride and sodium acetate in acetic acid media. The structures of the newly synthesized compounds were characterized by spectral studies. New compounds were screened for their anticonvulsant(Pentylenetetrazole animal model) and antimicrobial activity. Compound **4c** and **4d** exhibited considerable anticonvulsant effect on animals by protecting them for a longer duration.*

Key words: 4-Methylthiobenzyl, anticonvulsant, thiazole, antimicrobial.

INTRODUCTION

Epilepsy is a neurological disorder characterized by unprovoked seizures that affects at least 50 million people worldwide. The search for new anticonvulsant drugs continues to be an active area of investigation in medicinal chemistry since currently available epileptic drugs are effective in only 60-80% of patients. Furthermore, many antiepileptic drugs have serious side effects, increasing their toxic action when lifelong medication is required. As a result intensive research efforts are being devoted to find new antiepileptic compounds with more selective activity and lower toxicity.

1,2,4-Triazines and their derivatives have attracted continuing interest over the years because of their varied biological activities and a significant amount of research activity has been directed towards this class of compounds. Triazinones and their condensation products find important applications in medicinal and agricultural fields [1-6]. Some 1,2,4-triazinone derivatives are reported to possess antidiuretic, neurodepressant and herbicidal properties [7]. Thiazoles are one of the most intensely investigated classes of aromatic five membered heterocycles. Thiazolidin-4-

one and thiazol-4-one derivatives have been widely employed in the investigation of biologically active heterocyclic compounds [8-10]. Therefore,the synthesis of combinatorial libraries of these compounds was elaborated [11-14]. The thiazolidinone derivatives are well known in medicinal and biological chemistry due to their diverse pharmacological displays as anticonvulsant [15], antiviral [16], cardiovascular [17] andantitubercular [18] properties. Furan derivatives have emerged as useful ingredients for the treatment of urinary [19] and digestive tract infections [20]. The presence of 4-methylthiophenyl moiety is found to increase the biological activity of the molecules. Few heterocyclic analogues containing 4-methylthiophenyl moiety have been reported to possess potent antimicrobial activity [21-23].

In view of above mentioned findings and in an effort to identify new candidates that may be useful in designingnew, potent, selectiveand less toxic versatile bioactive agents, it was envisaged that the chemical entities with both 1,2,4-triazine and 1,3- thiazoles would result in compounds of interesting biological activity. It was thought to be interesting to synthesize compounds containing the features namely, 1,2,4-triazine moiety fused with 1,3-thiazole ring and to study their anticonvulsant activities in addition to antimicrobial property.

EXPERIMENTAL SECTION

The melting points were determined by an open capillary method and are uncorrected. The IR spectra (in KBr pellets) were recorded on a Shimadzu FT-IR 157 spectrophotometer. The ^{1}H NMR spectra were recorded on a BRUKER AVANCE II-400(400 MHz) spectrometer using TMS as an internal standard. Mass spectra were determined on a Jeol SX 102/Da-600 mass spectrometer/Data System using Argon/Xenon (6kv, 10 mA) as the FAB gas. The accelerating voltage was 10kV and spectra were recorded at room temperature. The progress of the reaction was checked by thin layer chromatography (TLC) on silica gel plate using n-hexane and ethyl acetate (4:1, v/v). Elemental analyses (CHNS) was performed on the CHNS elementor.

4-methylthiobenzylidene-2-methyloxazol-5-one (2)

A mixture of acetylglycine (0.5 mol), anhydrous sodium acetate (0.34 mol), aromatic aldehyde (0.5 mol) and 95 % acetic anhydride (1.35 mol) was taken in a loosely stoppered Erlenmeyer flask and warmed on a steam bath with occasional stirring until the solution became clear. The solution was refluxed in a steam bath for 2–3 h, cooled and placed overnight in a refrigerator. The solid obtained was filtered, washed with cold water, dried and recrystallized from ethanol.Yield(85%), M. p. 150-152^{0}C.

IR(KBr, γ/cm^{-1}): 2922(C-H),1726 (C=O), 1649(C=N), 1517(C=C),1246(C-O-C), 1087 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.39 (s, 3H, CH_3), 2.52 (s, 3H, SCH_3), 7.08 (s, 1H, =CH), 7.26 (d, 2H,Ar-H, *J*=7.1Hz),7.99 (d, 2H,Ar-H,*J*=7.1Hz);MS (m/z, %):234(M^++1, 80);Anal. Calcd. (%) for $C_{12}H_{11}NO_2S$: C, 61.78; H, 4.75; N, 6.00; Found: C, 61.82; H, 4.78; N, 6.03.

6-(4-Methylthiobenzyl)-3-mercapto-1,2,4-triazin-5(4H)-one (3)

A mixture of 4-methylthiobenzylidene-2-methyloxazol-5-one(**2**, 0.1 mol) and KOH (5g in 150mL of water) was refluxed for 6 h. To the resulting clear solution, thiosemicarbazide(0.1 mol) was added and the mixture was refluxed for another 4 h. Activated charcoal was added to this solution and filtered. The filtrate was acidified to pH 3–4 by the addition of acetic acid to yield the title compound. Yield 90 %. M. p. 160^0 C.

IR(KBr, γ/cm^{-1}):3269(NH), 3076(=C-H), 2916(C-H), 1687 (C=O), 1645(C=N), 1089(C-O); ^{1}H NMR (400 MHz, $CDCl_3$): δ, 2.44 (s, 3H, SCH_3), 3.83 (s, 2H, CH_2), 7.13-7.22(m, 4H,Ar-H), 8.82(s, 1H, NH), 13.17 (s, 1H, SH); MS(m/z, %): 266 (M^++1, 97).Anal. Calcd. (%) for $C_{11}H_{11}N_3OS_2$: C, 49.79; H, 4.18; N, 15.84; Found: C, 49.82; H, 4.20; N, 15.86.

7-Arylidene-3-(4-(methylthio)bezyl)-7H-thiazolo[2,3-c][1,2,4]triazine-4,6-diones (4a-f) and (5a-f)

A mixture of appropriate mercaptotriazinone (0. 01 mol), monochoroacetic acid (0.15 mol), anhydrous sodium acetate (2 g), glacial acetic acid (20 mL), acetic anhydride (15 mL) and 5-aryl furan-2-carboxaldehyde/substituted benzaldehyde (0.01 mol) was heated under reflux for 4–6 h. The reaction mixture was cooled, poured into crushed ice with vigorous stirring. The solid obtained was filtered, washed with water, dried and recrystallized from suitable solvent.

7-(3,4-Dimethoxybenzylidene)-3-[4-(methylsulfanyl)benzyl]-4H-[1,3]thiazolo[2,3-c][1,2,4]-triazine-4,6(7H)-dione (4a)

IR(KBr, γ/cm^{-1}): 3010 (=C-H), 2926 (C-H), 1741(C=O), 1584 (C=N), 1263(C-O-C), 1149 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 2.45 (s, 3H, SCH_3), 4.08 (s, 2H, CH_2), 3.96(s,3H,OCH_3), 3.98 (s,3H,OCH_3), 7.01-7.07 (m,3H, Ar-H), 7.16 (d, 2H, Ar-H, *J*=7.2 Hz), 7.36 (d, 2H, Ar-H, *J*=7.2 Hz), 8.19 (s, 1H, =CH); Ms (m/z, %): 453.5 (M^++1, 60), 452 (M^+, 40), 381 (90), 353.4 (100), 274 (85).Anal.Calcd. (%) for $C_{22}H_{19}N_3O_4S_2$: C, 58.26; H, 4.25; N, 9.29; Found: C, 58.28; H, 4.22; N, 9.27.

7-[4-(Dimethylamino)benzylidene]-3-[4-(methylsulfanyl)benzyl]-4H-[1,3]thiazolo[2,3-c][1,2,4]-triazine-4,6 (7H)-dione (4b)

IR(KBr, γ/cm^{-1}): 3020(=C-H), 2923 (C-H), 1735(C=O), 1602 (C=N), 1167 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 2.35 (s, 3H, SCH_3), 3.23 (s, 6H, CH3),4.08 (s,2H,CH_2), 6.85 (d,2H, Ar-H, *J*=7.4 Hz), 7.54 (d, 2H,Ar-H, *J*=7.8 Hz), 7.23 (d, 2H, Ar-H, *J*=7.8 Hz), 7.62(d, 2H, Ar-H,*J*=7.4 Hz), 8.19 (s, 1H, =CH); Ms (m/z, %): 437 (M^++1, 80), 381 (100), 413 (50), 353 (50), 330.4(39), 274.3 (95).Anal.Calcd. (%) for $C_{22}H_{20}N_4O_2S_2$: C, 60.50; H, 4.64; N, 12.85; Found: C, 60.53; H, 4.62; N, 12.83.

7-(4-Hydroxybenzylidene)-3-[4-(methylsulfanyl)benzyl]-4H-[1,3]thiazolo[2,3-c][1,2,4]- triazine-4,6(7H)-dione (4c)

IR(KBr , γ/cm^{-1}): 3020(=C-H), 2932 (C-H), 1739 (C=O), 3335(-OH), 1676(C=C), 1602 (C=N), 1167 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 2.35 (s, 3H, SCH_3), 2.53 (s, 1H, OH),4.08 (s,2H,CH_2), 7.19 (d,2H, Ar-H, *J*=7.3 Hz), 7.33 (d, 2H, Ar-H, *J*=9.0 Hz), 7.42 (d, 2H, Ar-H, *J*=7.3 Hz), 7.65(d, 2H, Ar-H, *J*=9.0 Hz), 8.19 (s, 1H, =CH); Ms (m/z, %): 409 (M^+, 75), 382 (20), 401 (50), 381.4 (80), 353.4 (100), 331.3 (30), 274.3 (40).Anal.Calcd. (%) for $C_{20}H_{15}N_3O_3S_2$: C, 58.63; H, 3.65; N, 10.24; Found: C, 58.66; H, 3.69; N, 10.26.

7-(4-Bromo-2-hydroxybenzylidene)-3-[4-(methylsulfanyl)benzyl]-4H-[1,3]thiazolo[2,3-c]- [1,2,4]- triazine-4,6 (7H)-dione (4d)

IR(KBr, γ/cm^{-1}): 3012 (-C=C-H), 2926 (C-H), 3335(-OH), 1739 (C=O),1666(C=C),1598 (C=N), 1162 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 2.45 (s, 3H, SCH_3),2.54 (s, 1H, OH), 4.08 (s, 2H, CH_2), 6.90 (s, 1H, Ar-H),7.01(d, 1H, Ar-H,*J*=7.8Hz), 7.15(d, 1H, Ar-H, *J*=7.8Hz), 7.19 (d, 2H, Ar-H, *J*=7.4 Hz), 7.36 (d, 2H, Ar-H, *J*=7.4 Hz), 8.25 (s, 1H, =CH); Ms (m/z, %): 489 (M^++1, 60), 488 (M^+, 80), 381(70),353.4(100), 274(50), 228 (20).Anal.Calcd. (%) for $C_{20}H_{14}BrN_3O_3S_2$: C, 49.15; H, 2.85; N, 8.55; Found: C, 49.19; H, 2.89; N, 8.60.

7-(4-Methoxybenzylidene)-3-[4-(methylsulfanyl)benzyl]-4H-[1,3]thiazolo[2,3-c][1,2,4]-triazine-4,6(7H)-dione (4e)

IR(KBr, γ/cm^{-1}): 3015 (=C-H), 2932 (C-H), 1752 (C=O), 1677(C=C), 1601 (C=N), 1167 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 2.45 (s, 3H, SCH_3), 4.08 (s, 2H, CH_2), 3.96(s,3H,OCH_3), 7.52 (d,2H, Ar-H, *J*=7.9Hz), 7.32(d,2H,Ar-H, *J*=7.9Hz), 7.21 (d, 2H, Ar-H, *J*=7.2 Hz), 7.14 (d, 2H,Ar-H, *J*=7.2 Hz), 8.19 (s, 1H, =CH); Ms (m/z, %): 423 (M^+, 80), 413(40), 381 (82), 353.4 (100), 331 (30), 274.3(45).Anal.Calcd. (%) for $C_{21}H_{17}N_3O_3S_2$: C, 59.51; H, 4.02; N, 9.90; Found: C, 59.56; H, 4.05; N, 9.92.

7-(2,4-Dichlorobenzylidene)-3-[4-(methylsulfanyl)benzyl]-4H-[1,3]thiazolo[2,3-c][1,2,4]-triazine-4,6(7H)-dione (4f)

IR(KBr,γ/cm^{-1}): 3010 (=C-H), 2926 (C-H), 1739 (C=O), 1687(C=C),1598 (C=N), 1162 (C-O), 762(C-Cl); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 2.45 (s, 3H, SCH_3), 4.08 (s, 2H, CH_2), 7.46 (m,3H, Ar-H), 7.23 (d, 2H, Ar-H, *J*=7.3 Hz), 7.15 (d, 2H, Ar-H, *J*=7.3 Hz), 8.25 (s, 1H, =CH); Ms (m/z, %): 462 (M^+, 60), 459 (M^+, 20), 415(50), 381 (100), 353 (45), 274.3(95).Anal.Calcd. (%) for $C_{20}H_{13}Cl_2N_3O_2S_2$: C, 51.92; H, 2.80; N, 9.05; Found: C, 51.95; H, 2.83; N, 9.09.

7-{[5-(4-chlorophenyl)furan-2-yl]methylidene}-3-[4-(methylsulfanyl)benzyl]-4H-[1,3]-thiazolo[2,3-c][1,2,4] triazine-4,6(7H)-dione (5a)

IR(KBr, γ/cm^{-1}): 3126 (=C-H), 2916 (C-H), 1731.4 (C=O),1676(C=C), 1593 (C=N), 1159 (C-O), 810(C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$): δ, 2.47 (s, 3H, SCH_3), 4.06 (s, 2H, CH_2), 7.41(d, 2H, Ar-H, *J*=7.8Hz), 7.76 (d, 2H, Ar-H, *J*=67.8 Hz), 7.33 (d, 2H, Ar-H, *J*=7.3 Hz), 7.12(d, 2H, Ar-H, *J*=7.3 Hz), 7.78 (s, 1H, =CH), 7.40 (d, 1H, *J*=3.7 Hz,Furyl-H), 6.83 (d,1H, *J*=3.7 Hz, Furyl-H); Ms (m/z, %): 494 (M^++1, 60), 495(M^++2, 30) , 493 (M^+, 95), 449 (40), 373 (40), 328 (40), 274 (30), 251 (20), 209 (60), 207(100).Anal.Calcd. (%) for $C_{24}H_{16}ClN_3O_3S_2$: C, 58.33; H, 3.24; N, 8.53; Found: C, 58.35; H, 3.26; N, 8.51.

3-(4-(Methylthio)benzyl)-7-((5-(4-nitrophenyl)furan-2-yl)methylene)-7H-thiazolo[2,3-c][1,2,4]-triazine-4,6-dione (5b)

IR(KBr, γ/cm^{-1}):3113 (=C-H), 2924(C-H), 1730 (C=O), 1681(C=N), 1597 (C=C),1524(-NO_2 asy), 1342(-NO_2sym), 1157 (C-O); ^{1}H NMR (400 Hz, $CDCl_3$): δ, 2.46 (s, 3H, SCH_3), 4.17 (s, 2H, CH_2), 8.33 (d, 2H, Ar-H,*J*=7.8Hz), 7.39 (d, 2H, Ar-H, *J*=7.4 Hz), 7.14 (d, 2H, Ar-H, *J*=7.4Hz),7.97 (d, 2H, Ar-H, *J*=7.8 Hz), 7.77 (s, 1H, =CH-), 7.36 (d, 1H, *J*=3.7 Hz,Furanyl-H), 7.04 (d, 1H, *J*=3.7 Hz, Furanyl-H); Ms (m/z, %): 504 (M^+ ,100), 460 (50), 432 (50), 319 (90), 251 (70), 260 (60), 218 (80).Anal.Calcd. (%) for $C_{24}H_{16}N_4O_5S_2$: C, 57.13; H, 3.22; N, 11.12; S. Found: C, 57.14; H, 3.20; N, 11.10.

7-{[5-(3-Chloro-4-fluorophenyl)furan-2-yl]methylidene}-3-[4-(methylsulfanyl)benzyl]-4H-[1,3]- thiazolo[2,3-c] [1,2,4]triazine-4,6(7H)-dione (5c)

IR(KBr,γ/cm^{-1}): 3114 (=C-H), 2926 (C-H), 1732 (C=O), 1681(C=N), 1598 (C=C), 1162 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 2.45 (s, 3H, SCH_3), 4.08 (s, 2H, CH_2), 7.67 (d, 1H, Ar-H,*J*=7.8Hz), 7.53(d, 1H,Ar-H, *J*=7.8Hz), 7.61(s, 1H, Ar-H), 7.39 (d, 2H, Ar-H, *J*=7.5 Hz), 7.14 (d, 2H, Ar-H, *J*=7.5Hz), 7.36 (d, 1H, *J*=3.7 Hz,Furanyl-H), 7.04 (d, 1H, *J*=3.7 Hz,Furanyl-H), 7.82 (s, 1H, =CH); Ms (m/z, %): 511.9(M^+, 80), 513 (M^++2, 30), 478 (20), 420 (50), 319 (70), 260(60).Anal.Calcd. (%) for $C_{24}H_{15}ClFN_3O_3S_2$: C, 56.28; H, 2.99; N, 8.19;. Found: C, 56.30; H, 2.95; N, 8.91.

3-[4-(Methylsulfanyl)benzyl]-7-{[5-(2,4,5-trichlorophenyl)furan-2-yl]methylidene}-4H-[1,3]-thiazolo[2,3-c] [1,2,4]triazine-4,6(7H)-dione (5d)

IR(KBr, γ/cm^{-1}):3115 (=C-H), 2924 (C-H), 1732 (C=O), 1682 (C=C),1597(C=N), 1159 (C-O); ^{1}H NMR (300 Hz , $CDCl_3$): δ, 2.69 (s, 3H, SCH_3), 4.08 (s, 2H, CH_2), 7.66 (s, 1H, Ar-H), 7.23 (d, 2H, Ar-H, *J*=7.3 Hz),7.05(d, 2H, Ar-H, *J*=7.3Hz), 7.56 (s, 1H, Ar-H), 7.80 (s, 1H, =CH-), 7.34 (d, 1H, *J*=3.6 Hz, Furanyl-H), 7.08 (d, 1H, *J*=3.6 Hz,Furanyl-H); Ms (m/z, %): 563 (M^+,90) , 565(M^++2, 90), 567(M^++4, 40), 569(M^++6, 15), 478(10), 432 (50), 319 (70), 251(68).Anal.Calcd. (%) for $C_{24}H_{14}Cl_3N_3O_3S_2$: C, 51.22; H, 2.54; N, 7.43; Found: C, 51.21; H, 2.51; N, 7.47.

3-(4-(Methylthio)benzyl)-7-((5-(2-methyl-4-nitrophenyl)furan-2-yl)methylidene)-7H-thiazolo-[2,3-c][1,2,4] triazine-4,6-dione (5e)

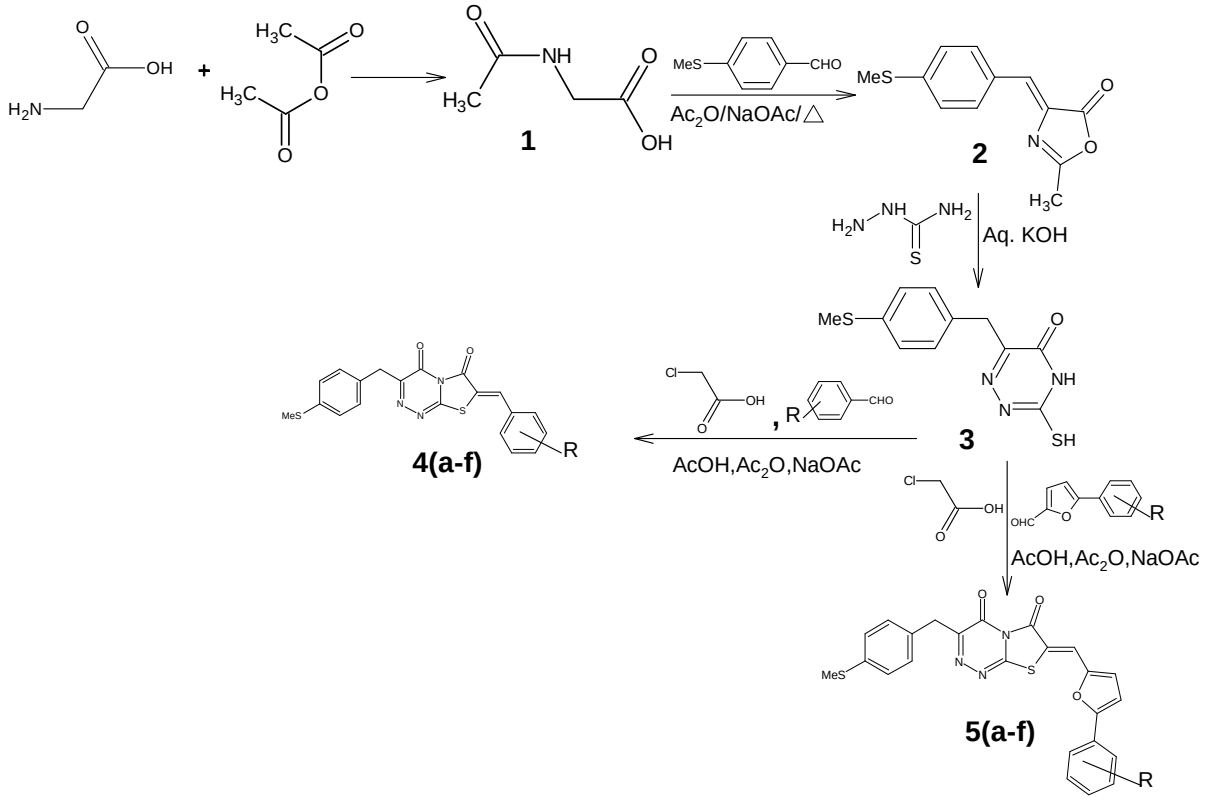
IR(KBr, γ/cm^{-1}):3015 =C-H), 2928 (C-H), 1739 (C=O), 1678(C=C), 1597 (C=N), 1517(-NO_2asym),1349(-NO_2sym), 1162 (C-O); ^{1}H NMR (300 Hz , $CDCl_3$): δ, 1.25 (s,3H, SCH_3), 2.45 (s, 3H, SCH_3), 4.08 (s, 2H, CH_2), 7.86 (s, 1H, Ar-H), 7.20 (d, 2H, Ar-H, *J*=7.3 Hz), 7.56 (d, 2H, Ar-H, *J*=7.3 Hz), 8.01 (d,1H, Ar-H, *J*=7.8Hz), 8.19 (d,1H, Ar-H, *J*=7.8Hz), 7.78 (s, 1H, =CH-), 7.34 (d, 1H, Furan, *J*=3.6 Hz), 7.45 (d, 1H, Furan, *J*=3.6 Hz); Ms (m/z, %): 519 (M^++,80) , 518 (M^+, 70), 494 (30), 451 (40), 377(90), 251(100).Anal.Calcd. (%) for $C_{25}H_{18}N_4O_5S_2$: C, 57.93; H, 3.47; N, 10.83; Found: C, 57.90; H, 3.50; N, 10.80.

3-(4-(Methylthio)benzyl)-7-((5-(4-methoxy-2-nitronitrophenyl)furan-2-yl)methylidene)-7H-thiazolo[2,3-c] [1,2,4]triazine-4,6-dione (5f)

IR(KBr; cm^{-1}):3018 (=C-H), 2936 (C-H), 1739 (C=O), 1597 (C=N),1538(-NO_2asym),1353(-NO_2sym) 1162 (C-O); ^{1}H NMR (300 Hz , $CDCl_3$): δ, 2.45 (s,3H,SCH_3), 3.72 (s, 3H, OCH_3), 4.08 (s, 2H, CH_2), 7.89 (s, 1H, Ar-H), 7.25 (d, 2H, Ar-H, *J*=7.4 Hz), 7.47 (d, 2H, Ar-H, *J*=7.4 Hz), 8.07 (d,1H, Ar-H, *J*=7.8Hz), 8.26 (d,1H, Ar-H, *J*=7.8Hz), 7.79 (s, 1H, =CH-), 7.39 (d, 1H, *J*=3.6 Hz, Furanyl-H), 7.09 (d, 1H, *J*=3.6 Hz,Furanyl-H); Ms (m/z, %): 534.5 (M^+, 80), 511 (M^+, 40), 472 (30), 381 (100), 330 (50), 246(60).Anal.Calcd. (%) for $C_{25}H_{18}N_4O_6S_2$: C, 56.14; H, 3.38; N, 10.46; Found: C, 56.17; H, 3.40; N, 10.48.

CHEMISTRY

The synthetic strategies adopted to obtain the target compounds are depicted in **scheme 1**. The key intermediate 6-(4-methylthiobenzyl)-3-mercapto-[1,2,4]-triazin-5(4*H*)-one **3** was prepared in an excellent yield in two consequence steps by condensing acetyl glycine with acetic anhydride and aromatic aldehyde to afford 4-methylthiobenzylidene-2-methyloxazol-5-one**2** which was added to thiosemicarbazide in KOH. One pot reaction of **3** with chloroacetic acid and substituted benzaldehyde/arylfurfuraldehyde in the presence of acetic anhydride and sodium acetate in acetic acid afforded 4-substituted-5-oxo-7-(5-aryl-2-furylidene/substituted arylidene)-[1,2,4]-triazino[3, 4-*b*]-thiazol-6-ones **4** and **5**.The characterization data of the newly synthesized compounds are presented in **Table 1**.



Scheme 1. Synthesis of 7-Arylidene-3-(4-(methylthio)bezyl)- 7*H*-thiazolo[2,3-c][1,2,4]triazine-4,6-diones (4a-f) and (5a-f)

Table I - Characterization data of compounds 4a-f and 5a-f

Compd.	R	Mol. formula	Mol. Wt.	m.p. (^{0}C)	Yield (%)
4a	4,5-$(OCH_3)_2$-C_6H_3	$C_{22}H_{19}N_3O_4S_2$	453.53	172-73	87
4b	4-$N(CH_3)_2$-C_6H_4	$C_{22}H_{20}N_4O_2S_2$	436.55	160-62	82
4c	4-OH-C_6H_4-	$C_{20}H_{15}N_3O_3S_2$	409.48	156-57	85
4d	4-Br-6-OH- C_6H_3	$C_{20}H_{14}BrN_3O_3S_2$	488.38	126-28	84
4e	4-OCH_3-C_6H_4	$C_{21}H_{17}N_3O_3S_2$	423.51	168-70	75
4f	2,4-Cl_2-C_6H_3	$C_{20}H_{13}Cl_2N_3O_2S_2$	462.37	165-67	60
5a	4-Cl-C_6H_4	$C_{24}H_{16}ClN_3O_3S_2$	493.98	110-12	82
5b	4-NO_2-C_6H_4	$C_{24}H_{16}N_4O_5S_2$	504.54	145-47	80
5c	3-Cl-4-F-C_6H_3	$C_{24}H_{15}ClFN_3O_3S_2$	511.97	96-98	65
5d	2,4,5-Cl_3-C_6H_2	$C_{24}H_{14}Cl_3N_3O_3S_2$	562.87	139-40	75
5e	2-Me-4-NO_2-C_6H_3	$C_{25}H_{18}N_4O_5S_2$	518.56	157-59	80
5f	4-OMe-6-NO_2-C_6H_3	$C_{25}H_{18}N_4O_6S_2$	534.56	192-94	71

BIOLOGICAL ASSAYS

Aniconvulsant activity

Pentylenetetrazole animal model [24]

Swiss albino mice of both sexes weighing around 25-30g were used in the study. They were housed in colony rooms with12/12 h light/dark cycle at 21+2^{o}C and had free access to food and water. Pentylenetetrazole (PTZ, Sigma Chemical, USA) was used as anticonvulsant and diazepam (Ranbaxy Laboratories, India) is used as standard drug. The institutional ethical committee approved the study. Studies were carried out by PTZ animal model. All the results were statistically analyzed and expressed as the mean± S.E.M. Kruskal-Wallis test (Non-parametric ANOVA) followed by Dunn's multiple comparison test was used to analyze the results(the delay of onset of seizures in comparison with the control group). $P<0.05$ is considered as significant.

PTZ was dissolved in normal saline. Mice were divided into 20 groups of 3 each as shown in table 2 and received a dose 4mg/kg.Diazepam and test compounds **4a-f** and **5a-f** were suspended in 2% gum acacia and administered orally

in a volume of 0.1ml/10g body weight by gavage feeding.Normal control received 0.1mL/10g of 2% gum acacia alone orally by gavage feeding. Convulsion was induced 1 hour after the administration of the standard drug or the test compounds by I. P. injection of PTZ(80mg/kg) that was dissolved in saline to a volume of 0.1 mL/10g body weight. The time needed for the development of unequivocal sustained clonic seizure activity involving the limbs was carefully noted.The onset of a general clonus was used as the endpoint. The general clonus was characterized by forelimb clonus followed by full clonus of the body. The duration of clonic convulsions was also noted. Seizure free interval of 1h was considered as protection. The number of animals protected in each group was recorded and percent protection was calculated.

The animals in the control group exhibited seizures at the dose of PTZ used in the study. The onset of seizure (latent period) was found to be 073±13.454 s and mean seizure duration was057±04.256 s. Diazepam protected the animals from developing convulsions at the dose of 4mg/kg body weight in comparison with control group. In compound **4c** and **4d** there was much delay in the onset of seizures i.e. 592±35.539 and853±54.077 respectively.Compounds **4b** and **5c** reduced the duration of seizures in comparison with the control group. Animals were less active after receiving all of the test compounds than the control mice. The results are given in **Table 2**.

Table II - Anticonvulsant activity of the tested compounds(PTZ animal model)

Compound	Latent period(s) Mean ± S. E. M	Duration of seizure(s) Mean ± S. E. M	mortality
4a	227±41.651	065±07.371	100%
4b	094±03.512	026±06.741	100%
4c	592±35.539*	080±08.505	67%
4d	853±54.077*	078±11.465	100%
4e	309±11.724	107±10.786	100%
4f	219±19.342	164±05.239	100%
5a	524±33.232	123±05.457	100%
5b	235±08.686	102±12.129	100%
5c	259±28.614	055±12.129	100%
5d	280±48.855	141±24.552	100%
5e	256±06.360	124±09.821	100%
5f	455±27.339	057±08.876	100%
Control (2% Gum Acacia)	073±13.454	057±04.256	100%
Diazepam	3600	0	0

n=3 in each group
Kruskal-Wallis statistics KW=45.984(corrected for ties)
**p<0.05 when compared to control*

Antibacterial activity

The newly synthesized compounds were screened for their *in-vitro* antibacterial activity against *Escherichia coli (*ATTC-25922*)*, *Staphylococcus aureus* (ATTC-25923), *Pseudomonas aeruginosa* (ATTC-27853*)* and *Klebsiella pneumonia* (recultured) bacterial stains by serial plate dilution method [25, 26]. Serial dilutions of the drug in Muller Hinton broth were taken in tubes and their pH was adjusted to 5.0 using phosphate buffer. A standardized suspension of the test bacterium was inoculated and incubated for 16-18 h at 37^{o}C. The minimum inhibitory concentration (MIC) was noted by seeing the lowest concentration of the drug at which there was no visible growth.

A number of antibacterial discs were placed on the agar for the sole purpose of producing zones of inhibition in the bacterial lawn. Twenty milliliters of agar media was poured into each Petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37 ^{o}C for an hour. Using a punch, wells were made on these seeds agar plates and minimum inhibitory concentrations of the test compounds in dimethyl sulfoxide (DMSO) were added into each labeled well. A control was also prepared for the plates in the same way using DMSO as a solvent. The Petri dishes were prepared in triplicate and maintained a 37 ^{o}C for 3-4 days. Antibacterial activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with ciprofloxacin as standard [27, 28]. Zone of inhibition was determined for **4a-f** and **5a-f**. The results are summarized in **Table 3**.

The MIC values were evaluated at concentration range, 1.56-25 μg/mL. The figures in the table show the MIC values in μg/mL and the corresponding zone of inhibition in mm.

Table III - Antibacterial activity of the newly synthesized compounds 4a-f and 5a-f

Compd	MIC in µg/mL and zone of inhibition in mm			
	S. aureus	***E. coli***	***P. aeruginosa***	***K. pneumoniae***
4a	25(<10)	25(<10)	25(<10)	25(<10)
4b	12.5(11-15)	12.5(11-15)	12.5(11-15)	12.5(11-15)
4c	25(<10)	25(<10)	25(<10)	25(<10)
4d	12.5(11-15)	12.5(11-15)	12.5(11-15)	12.5(11-15)
4e	25(<10)	25(<10)	25(<10)	25(<10)
4f	12.5(11-15)	12.5(11-15)	12.5(11-15)	12.5(11-15)
5a	6.25(16-20)	6.25(16-20)	6.25(16-20)	6.25(16-20)
5b	6.25(16-20)	6.25(16-20)	6.25(16-20)	6.25(16-20)
5c	6.25(16-20)	6.25(16-20)	6.25(16-20)	6.25(16-20)
5d	25(<10)	25(<10)	25(<10)	25(<10)
5e	25(<10)	25(<10)	25(<10)	25(<10)
5f	12.5(11-15)	12.5(11-15)	12.5(11-15)	12.5(11-15)
Standard (Ciprofloxacin)	1.56(22-30)	6.25(30-40)	6.25(25-33)	6.25(23-27)

Antifungal activity

Newly prepared compounds were also screened for their antifungal activity against *Aspergilusflavus (*NCIM No. 524*), Aspergilusfumigatus*(NCIM No. 902*), Penicilliummaneffei (*recultured*)* and *Trichophytonmentagrophytes (*recultured*)* in DMSO by serial plate dilution method [29, 30]. Sabourauds agar media was prepared by dissolving peptone (1g). D-Glucose (4g) and agar (2g) in distilled water (100 mL) and adjusting the pH to 5.7. Normal saline was used to make a suspension of sore of fungal strains for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. Twenty milliliters of agar media was poured into each Petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37 ^{0}C for 1 h. Wells were made on these seeded agar platesusing a punch. Minimum inhibitory concentrations of the test compounds in DMSO were added into each labeled well. A control was also prepared for the plates in the same way using solvent DMSO. The Petri dishes were prepared in triplicate and maintained at 37 ^{0}C for 3-4 days. Antifungal activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with ciclopiroxolamine as standard. Zones of inhibition were determined for **4a-f**and **5a-l**. The results are summarized in **Table 4**.

The MIC values were evaluated at concentration range, 1.56-25 μg/mL. The figures in the table show the MIC values in μg/mL and the corresponding zone of inhibition in mm.

Table IV - Antifungal activity of the newly synthesized compounds 4a-f and 5a-f

Compd	MIC in µg/mL and zone of inhibition in mm			
	P.marneffei	***C. albicans***	***A. flovus***	***A. fumigates***
4a	25(<10)	25(<10)	25(<10)	25(<10)
4b	6.25(16-20)	6.25(16-20)	6.25(16-20)	6.25(16-20)
4c	25(<10)	25(<10)	25(<10)	25(<10)
4d	6.25(16-20)	6.25(16-20)	6.25(16-20)	6.25(16-20)
4e	25(<10)	25(<10)	25(<10)	25(<10)
4f	6.25(16-20)	6.25(16-20)	6.25(16-20)	6.25(16-20)
5a	6.25(16-20)	6.25(16-20)	6.25(16-20)	6.25(16-20)
5b	6.25(16-20)	6.25(16-20)	6.25(16-20)	6.25(16-20)
5c	6.25(16-20)	6.25(16-20)	6.25(16-20)	6.25(16-20)
5d	6.25(16-20)	6.25(16-20)	6.25(16-20)	6.25(16-20)
5e	6.25(16-20)	6.25(16-20)	6.25(16-20)	6.25(16-20)
5f	6.25(16-20)	6.25(16-20)	6.25(16-20)	6.25(16-20)
Standard(Ciclopiroxolamine)	1.56(22-30)	6.25(30-40)	6.25(25-33)	6.25(23-27)

RESULTS AND DISCUSSION

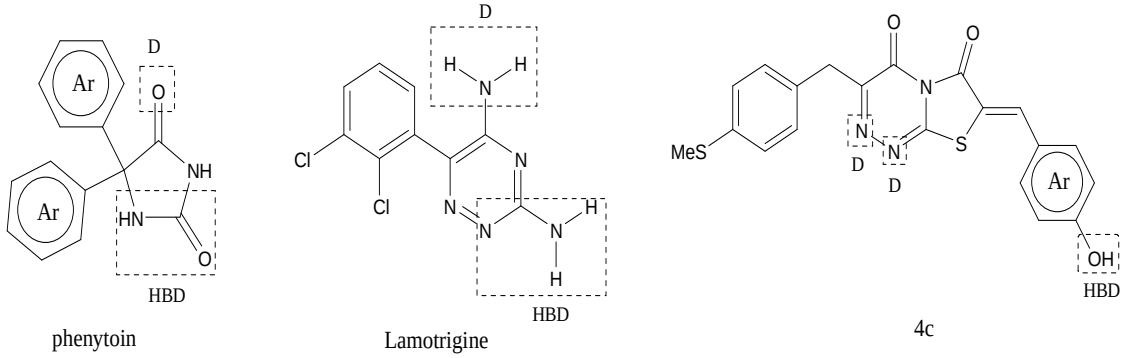
All new compounds reported here were characterized on the basis of complementary spectroscopic (^{1}H NMR, IR and mass) and analytical data. IR spectra of condensed products displayed absence of absorption bands corresponding to N-H at 3320-3400 cm^{-1} thereby indicating the cyclisation process. The absorption bands at 1690 cm^{-1} and 1592 cm^{-1} are characteristic of C=O and C=N functional groups respectively. Absorption bands at 1540 cm^{-1} and 1158 cm^{-1} were due to C=C and C-O groups respectively.

In the ^{1}H NMR spectrum of the same compound **4b**, a singlet appeared at δ 2.45 has been attributed to the three protons of SCH_3 group. The four protons of 4- methylthiophenyl moiety appeared as two doublets centered at δ 6.74 and δ 7.25 with a coupling constant *J*=6.7 Hz. The six protons of $N(CH_3)_2$ moiety appeared as a singlet at δ 3.23. The four protons of 4-$N(CH_3)_2$ phenyl ring appeared as two distinct doublets centered at δ 7.45 and δ 7.55 with coupling constant *J*=7.2 Hz respectively. Singlet observed at δ 4.08 integrating for two protons was due to CH_2 group. The exocyclic methine proton resonated as a sharp singlet at δ 8.19. ^{1}H NMR data agrees very well with its assigned structure. The FAB mass spectrum of the compound **4b** showed molecular ion (M^+) peak at m/z 436.55, which is in accordance with its molecular formula, $C_{22}H_{20}N_4O_2S_2$.The buildup of N-bridged heterocycle **5b** is evidenced by its IR, ^{1}HNMR and mass spectral data. The IR spectrum of **5b** indicates the presence of H-C=C, -C=O, -C=N,-C=C-, -NO_2assym.and-NO_2sym due to the absorption bands at3113, 1681, 1597, 1525, 1509 and 1346 cm^{-1} respectively. Peaks observed at δ 2.45, 4.09 and 9.73 as singlets in its 1HNMR spectrum showed the presence of –SCH_3,-CH_2, =C-H groups respectively. Also, two doublets atδ 7.04 and 7.37 are due to furan ring.Four doublets at δ8.26, 7.82,7.98, and 8.31 are due to aromatic protons. Further, FAB mass spectrum showed the base peak at m/z 504.5 which is in agreement with its molecular formula $C_{24}H_{16}N_4O_5S_2$.

Biological results

In order to reveal anticonvulsant profiles of the synthesized compounds,the scPTZmodel was employed according to the anticonvulsant drug development (ADD) protocols. The results are shown in table 4. None of the tested compounds completely protected the animals from developing seizures. Compounds **4e** and**4f** showed considerable effect on animals by protecting them for a much longer duration when compared to other samples.scPTZ test at a dose 4mg/kg where $p<0.05$ when compared to control. The remaining compounds were found to be devoid of protective activity in the scPTZ test. A structure activity relationship study revealed that compounds bearing OH group (**4e and 4f**) substituents had shown comparatively good activity.

The complete loss of activity in other compounds due to the disappearance of OH function could be explained in terms of the binding site by the pharmacophoric model, which was previously proposed[31](**Scheme 2**).



Scheme 2. Structures of selected anticonvulsants from different classes and synthesized compound 4c showing the general pharmacophore model for anticonvulsant activity. The essential structural requirements are indicated by dotted rectangles (Ar: hydrophobic unit, D: electron donor group, HBD: hydrogen bonding domain).

In this model, it has been reported that the existence of a hydrophobic unit (Ar), an electron donor group and hydrogen bonding domain (HBD) was essential for anticonvulsant activity. As evidenced by the active drugs, such as carbamazepine, lamotrigine, phenytoin fulfilling these demands. As shown in **Scheme 2** replacement of OH groupresponsible for hydrogen bonding resulted in the lack of HBD leading to the abolishment of activity. The result is in accordance with findings that compound **4e** and**4f** had some anticonvulsant properties whereas the remaining compounds were devoid of activity due to inability of hydrogen bonding at the binding site.

From the antimicrobial results obtained, the structure activity relationship can be drawn for the test compounds**4a-f**and **5a-f**. The variation in the antimicrobial activity of the test compounds was explored by varying the substituents. Among the tested compounds, compounds **5a**, **5b** and **5c** exhibited maximum antibacterial activity against all the bacterial pathogens. Compounds **5a**, **5b**,**5c** and **5d** contain Cl, -NO_2or F substituentsat position 4 of the phenyl ring attached to furan ring. On the other hand compounds containing substituents like -OCH_3, -OH, -CH_3 showed moderate activity and in some compounds showed complete reduction in the activity.

The investigation of antifungal screening data revealed that compounds**4b**, **4d**, **4f**, **5a**, **5b**, **5c** and**5d**having 4-Br, 4-Cl, 4-NO_2, 4-F, 4 –$N(CH_3)_2$, 4-NO_2 substituents on benzylidene ring showed maximum antifungal activity.However, the substituents like –CH_3 and –OCH_3 did not show marked activity.

CONCLUSION

A series of 4-substituted-5-oxo-7-(arylidene/5-aryl-2-furylidene)-[1,2,4]-triazino-[3,4-*b*]-thiazol-6-ones were synthesized in good yield by employing a multi component reaction protocol. Anticonvulsant activity results of these compounds indicated that presence of hydrogen bonding domain was very much essential for bioactivity. From the present study two compounds **4e** and **4f** have emerged as the lead compounds with activityin scPTZ test with $p<0.05$ when compared to control. This inference is based on the findings in a pilot study only. Further studies using larger samples have to be done for obtaining conclusive evidence.Further, structural modifications of these molecules might lead to the discovery of more potent anticonvulsant agents.

As revealed from the results, the newly synthesized compounds found to possess significant and potent antifungal activity and moderate antibacterial activity against the tested bacterial and fungal strains. Among the samples screened for their antimicrobial activity, compounds **5a, 5b** and**5c** showed good antimicrobial activity against all the pathogens at 6.25µg/mL concentration equivalent to that of the reference drug, Ciprofloxacin and Ciclopiroxolamine. The good activity may be attributed to the presence of substituents like-Cl, -F or –NO_2at4^{th} position of the phenyl ring attached to furan ring.

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