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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and pharmacological evaluation of newer thiazolo [3,2-a] pyrimidines for anti-inflammatory and antinociceptive activity

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Abstract A new series of thiazolo [3,2-a] pyrimidine derivatives was designed and synthesized using 4-fluoroaniline and ethylacetoacetate as starting material. Anti-inflammatory activity was assessed by the rat paw edema method and antinociceptive activity was evaluated by thermal stimulus technique. The compounds 5-(4-chlorophenyl)-2-(4-fluorobenzylidene)-7-methyl-3-oxo-2,3-dihydro-5*H*-thiazolo [3,2-a]pyrimidine-6-carboxylic acid (4-fluorophenyl)amide (**3l**) and 2-(4-chlorobenzylidene)-5-(4-fluorophenyl)-7-methyl-3-oxo-2,3-dihydro-5*H*-thiazolo[3,2-a] pyrimidine-6-carboxylic acid (4-fluorophenyl)amide (**3q**) were found to possess significant anti-inflammatory and antinociceptive activities. These compounds also showed lower ulcerogenic activity and higher ALD_{50} values. Compounds with an aryl ring substituted with a smaller electron withdrawing group at the fourth position displayed better activity than the other derivatives.

Keywords Thiazolopyrimidine · Anti-inflammatory · Antinociceptive · Acute toxicity

Introduction

Acute and chronic inflammation and different types of arthritis are inflammatory disorders that deal a big blow to humanity, and continual search for newer nonsteroidal anti-inflammatory agents is the only way to protect against this threat. In the past few decades the literature has been enriched with newer agents that are structurally different from the presently known anti-inflammatory drugs, but the

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search for better candidates in terms of higher efficacy and selectivity with lesser side effects continues to be an area of research for scientists worldwide.

A number of heterocyclic moieties are reported to possess anti-inflammatory activity, among which pyrimidine derivatives constitute an important class that displays a number of diverse biological activities including anti-inflammatory and antinociceptive activity (Kowaluk *et al.,* 2000; Orjales *et al.,* 2008; Alagarsamy *et al.,* 2007; Falcão *et al.,* 2006; Jakubkien *et al.,* 2003; Bruno *et al.,* 2002; Jarvis *et al.,* 2002). In addition, thiazole derivatives have acquired conspicuous significance due to their use in inflammation (Bender *et al.,* 1985; Holla *et al.,* 2003).

Since the two heterocyclic moieties constitute two active pharmacophores that are highly active against inflammation and pain, combining the two is expected to have a synergistic effect in dealing with the diseases. This idea has been utilized by some scientists to prepare thiazolopyrimidine derivatives, and their anti-inflammatory activity has been reported (Bekhit *et al.,* 2003; Tozkoparan *et al.,* 1999).

Thiazolopyrimidine derivatives are the biosteric analogues of purines and are potentially bioactive molecules that have shown anti-inflammatory activity comparable to that of some standard drugs in vivo, with no or minimal ulcerogenic effects (Bekhit *et al.,* 2003).

In continuing the search for better anti-inflammatory agents of the thiazolopyrimidine class that can also be used as analgesics and that have minimal side effects, we report here the synthesis of 5-(4-substituted phenyl)-2-(substituted benzylidene)-7-methyl-3-oxo-2,3-dihydro-5*H*-thiazolo[3,2-a]pyrimidine-6-carboxylic acid (4-fluorophenyl)amides (**3a**–**3t**). All the synthesized compounds were tested for anti-inflammatory and antinociceptive activities. Ulcerogenic activity and acute toxicity studies of the most active compounds were also performed.

Materials and methods

Chemistry

Melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded in KBr pellets on a FT-IR (Bio-Rad FTS) spectrophotometer. Elemental analyses (C, H, N) of all compounds were performed on the CHNS Elimentar (Vario EL III; Analysen systime, GmbH, Germany). Proton magnetic resonance spectra (^{1}H-NMR) were recorded on a DRX-300 NMR spectrometer and Bruker 400 Ultra Shield, and chemical shifts (δ) are expressed as parts per million (ppm) using TMS (tetramethylsilane; Me_4Si) as an internal reference. All TLC was carried out using silica gel.

General procedure for synthesis of the title compounds (**3a–3t**)

*N-(4-Fluoro-phenyl)-3-oxo-butyramide (**1**)*

A mixture of ethyl acetatoacetate (0.01 mol) and 4-fluoroaniline (0.01 mol) in 20 ml of ethanol containing 0.3 g of sodium hydroxide was refluxed for 5 h. The

reaction mixture was concentrated and the solid product obtained was filtered off and recrystallized with ethyl acetate. Yield, 76%; m.p., 136°C. IR (KBr, cm^{-1}): 3315, 3070, 1672, 1329. ^1H NMR (DMSO-d_6): δ 2.15 (s, 3H, CH_3), 3.78 (s, 2H, CH_2), 7.12–7.83 (m, 4H, ArH), 8.52 (bs, 1H, NH, D_2O exchangeable).

*6-Methyl-4-(substituted phenyl)-2-thioxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid (4-fluorophenyl)amides (**2a–2e**)*

A mixture of *N*-(4-fluoro-phenyl)-3-oxo-butyramide (**1**), substituted aryl aldehydes (0.01 mol), and thiourea (0.015 mol) in 20 ml of ethanol was refluxed for 7–9 h in the presence of a catalytic amount of concentrated hydrochloric acid. The reaction mixture was kept overnight and the precipitate obtained was filtered and recrystallized with ethanol. Yield, 63%; m.p., 179°C. IR (KBr, cm^{-1}): 3416, 3358, 3340, 3019, 1658, 1304, 1092. ^1H NMR (DMSO-d_6): δ 2.21 (s, 3H, CH_3), 5.11 (s, 1H, CH), 7.09–7.73 (m, 8H, ArH), 9.20 (bs, 1H, NH, D_2O exchangeable), 9.65 (bs, 1H, NH, D_2O exchangeable), 9.86 (bs, 1H, NH, D_2O exchangeable).

*5-(4-Substituted phenyl)-2-(substituted benzylidene)-7-methyl-3-oxo-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylic acid (4-fluorophenyl)amides (**3a–3t**)*

A mixture of compounds **2a**–**2e** (0.01 mol), chloroacetic acid (0.01 mol), substituted aryl aldehydes (0.01 mol), and sodium acetate (1.5 g) in a mixture of acetic acid and acetic anhydride (25 ml; 1:1) was refluxed for 8–10 h. The reaction mixture was concentrated and the solid thus obtained was filtered and recrystallized with ethyl acetate to get the titled compounds.

*5-(4-Bromophenyl)-2-(3,4-dimethoxybenzylidene)-7-methyl-3-oxo-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylic acid (4-fluorophenyl)amide (**3a**)*

Yield, 56%. %CHN found (calc): C, 57.20 (57.24); H, 3.80 (3.81); H, 7.07 (6.91). IR (KBr, cm^{-1}): 3458, 3034, 2930, 1713, 1658, 1304. ^1H NMR (DMSO-d_6): δ 2.46 (s, 3H, CH_3), 3.95 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 5.44 (s, 1H, CH-pyr.), 6.58–6.84 (m, 7H, ArH), 6.92–7.21 (m, 4H, ArH), 7.68 (s, 1H, CH), 9.36 (bs, 1H, NH, D_2O exchangeable).

*5-(4-Bromophenyl)-2-(3,4-dichlorobenzylidene)-7-methyl-3-oxo-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylic acid (4-fluorophenyl)amide (**3b**)*

Yield, 61%. %CHN found (calc): C,52.52 (52.53); H, 2.75 (2.78); N, 6.79 (6.81). IR (KBr, cm^{-1}): 3404, 3015, 2865, 1704, 1646, 1328, 804. ^1H NMR (DMSO-d_6): δ 2.38 (s, 3H, CH_3), 5.49 (s, 1H, CH-pyr.), 6.66–6.98 (m, 7H, ArH), 7.04–7.34 (m, 4H, ArH), 7.71 (s, 1H, CH), 9.41 (bs, 1H, NH, D_2O exchangeable).

*2-Benzylidene-5-(4-bromophenyl)-7-methyl-3-oxo-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylic acid (4-fluorophenyl)amide (**3c**)*

Yield, 59%. %CHN found (calc): C, 59.03 (59.13); H, 3.42 (3.49); N, 7.61 (7.66). IR (KBr, cm^{-1}): 3416, 2958, 2715, 1714, 1679, 1315, 545. ^{1}H NMR (DMSO-d_6): δ 2.46 (s, 3H, CH_3), 5.51 (s, 1H, CH-pyr.), 6.49–7.32 (m, 9H, ArH), 7.48–7.67 (m, 4H, ArH), 7.84 (s, 1H, CH), 9.64 (bs, 1H, NH, D_2O exchangeable).

*5-(4-Bromophenyl)-2-(4-chlorobenzylidene)-7-methyl-3-oxo-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylic acid (4-fluorophenyl)amide (**3d**)*

Yield, 47%. %CHN found (calc): C, 55.61 (55.64); H, 3.15 (3.11); N, 7.27 (7.21). IR (KBr, cm^{-1}): 3455, 2916, 2812, 1694, 1663, 1318, 818, 545. ^{1}H NMR (DMSO-d_6): δ 2.33 (s, 3H, CH_3), 5.45 (s, 1H, CH-pyr.), 6.52–7.16 (m, 8H, ArH), 7.39–7.59 (m, 4H, ArH), 7.78 (s, 1H, CH), 9.59 (bs, 1H, NH, D_2O exchangeable).

*5-(4-Bromophenyl)-7-methyl-2-(4-methylbenzylidene)-3-oxo-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylic acid (4-fluorophenyl)amide (**3e**)*

Yield, 51%. %CHN found. Calc: C, 59.76 (59.79); H, 3.72 (3.76); N, 7.41 (7.47). IR (KBr, cm^{-1}): 3438, 3078, 2768, 1716, 1689, 1352, 598. ^{1}H NMR (DMSO-d_6): δ 2.41 (s, 3H, CH_3), 2.62 (s, 3H, CH_3), 5.48 (s, 1H, CH-pyr.), 6.41–7.28 (m, 8H, ArH), 7.41–7.63 (m, 4H, ArH), 7.69 (s, 1H, CH), 9.46 (bs, 1H, NH, D_2O exchangeable).

*2-(2,4-Dichlorobenzylidene)-7-methyl-5-(4-nitrophenyl)-3-oxo-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylic acid (4-fluorophenyl)amide (**3f**)*

Yield, 59%. %CHN found (calc): C, 55.59 (55.58); H, 2.93 (2.94); N, 9.61 (9.60). IR (KBr, cm^{-1}): 3418, 3006, 2732, 1684, 1646, 1356, 1338, 813. ^{1}H NMR (DMSO-d_6): δ 2.44 (s, 3H, CH_3), 5.46 (s, 1H, CH-pyr.), 6.42–7.31 (m, 7H, ArH), 7.51–7.84 (m, 4H, ArH), 7.89 (s, 1H, CH), 9.64 (bs, 1H, NH, D_2O exchangeable).

*2-(4-Chlorobenzylidene)-7-methyl-5-(4-nitrophenyl)-3-oxo-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylic acid (4-fluorophenyl)amide (**3g**)*

Yield, 61%. %CHN found (calc): C, 59.10 (59.07); H, 3.34 (3.30); N, 10.17 (10.21). IR (KBr, cm^{-1}): 3464, 2956, 2768, 1724, 1664, 1378, 834.; ^{1}H NMR (DMSO-d_6): δ 2.42 (s, 3H, CH_3), 5.51 (s, 1H, CH-pyr.), 6.47–7.04 (m, 8H, ArH), 7.29–7.48 (m, 4H, ArH), 7.67 (s, 1H, CH), 9.67 (bs, 1H, NH, D_2O exchangeable).

*2-(4-Methoxybenzylidene)-7-methyl-5-(4-nitrophenyl)-3-oxo-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylic acid (4-fluorophenyl)amide (**3h**)*

Yield, 58%. %CHN found (calc): C, 61.71 (61.76); H, 3.91 (3.89); N, 10.26 (10.29). IR (KBr, cm^{-1}): 3458, 3016, 2805, 1716, 1654, 1356. ^{1}H NMR (DMSO-d_6): δ 2.47

(s, 3H, CH_3), 3.23 (s, 3H, OCH_3), 5.48 (s, 1H, CH-pyr.), 6.39–7.16 (m, 8H, ArH), 7.31–7.51 (m, 4H, ArH), 7.64 (s, 1H, CH), 9.58 (bs, 1H, NH, D_2O exchangeable).

*2-(2-Chlorobenzylidene)-7-methyl-5-(4-nitrophenyl)-3-oxo-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylic acid (4-fluorophenyl)amide (**3i**)*

Yield, 68%. %CHN found (calc): C, 59.01 (59.07); H, 3.32 (3.30); N, 10.23 (10.21). IR (KBr, cm^{-1}): 3426, 3019, 2889, 1741, 1651, 1245, 854. ^1H NMR (DMSO-d_6): δ 2.51 (s, 3H, CH_3), 5.59 (s, 1H, CH-pyr.), 6.41–7.04 (m, 8H, ArH), 7.42–7.64 (m, 4H, ArH), 7.71 (s, 1H, CH), 9.61 (bs, 1H, NH, D_2O exchangeable).

*2-Benzylidene-7-methyl-5-(4-nitrophenyl)-3-oxo-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylic acid (4-fluorophenyl)amide (**3j**)*

Yield, 56%. %CHN found (calc): C, 62.95 (63.03); H, 3.74 (3.72); N, 10.85 (10.89). IR (KBr, cm^{-1}): 3415, 3056, 2858, 1721, 1668, 1349. ^1H NMR (DMSO-d_6): δ 2.49 (s, 3H, CH_3), 5.63 (s, 1H, CH-pyr.), 6.37–7.46 (m, 9H, ArH), 7.78–8.14 (m, 4H, ArH), 8.23 (s, 1H, CH), 9.78 (bs, 1H, NH, D_2O exchangeable).

*2-(4-Bromobenzylidene)-5-(4-chlorophenyl)-7-methyl-3-oxo-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylic acid (4-fluorophenyl)amide (**3k**)*

Yield, 61%. %CHN found (calc): C, 55.67 (55.64); H, 3.16 (3.11); N, 7.19 (7.21). IR (KBr, cm^{-1}): 3415, 3047, 2804, 1707, 1678, 1317, 854, 563. ^1H NMR (DMSO-d_6): δ 2.41 (s, 3H, CH_3), 5.61 (s, 1H, CH-pyr.), 6.56–7.26 (m, 8H, ArH), 7.41–7.61 (m, 4H, ArH), 7.84 (s, 1H, CH), 9.74 (bs, 1H, NH, D_2O exchangeable).

*5-(4-Chlorophenyl)-2-(4-fluorobenzylidene)-7-methyl-3-oxo-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylic acid (4-fluorophenyl)amide (**3l**)*

Yield, 43%. %CHN found (calc): C, 62.14 (62.13); H, 3.49 (3.48); N, 8.01 (8.05). IR (KBr, cm^{-1}): 3398, 3041, 2848, 1709, 1678, 1304, 1294, 849. ^1H NMR (DMSO-d_6): δ 2.37 (s, 3H, CH_3), 5.54 (s, 1H, CH-pyr.), 6.37–7.21 (m, 8H, ArH), 7.51–7.70 (m, 4H, ArH), 7.84 (s, 1H, CH), 9.78 (bs, 1H, NH, D_2O exchangeable).

*2-(2-Chlorobenzylidene)-5-(4-chlorophenyl)-7-methyl-3-oxo-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylic acid (4-fluorophenyl)amide (**3m**)*

Yield, 59%. %CHN found (calc): C, 60.21 (60.23); H, 3.39 (3.37); N,: 7.79 (7.80). IR (KBr, cm^{-1}): 3314, 3009, 2856, 1745, 1681, 1325, 849, 816.; ^1H NMR (DMSO-d_6): δ 2.41 (s, 3H, CH_3), 5.51 (s, 1H, CH-pyr.), 6.28–7.31 (m, 8H, ArH), 7.47–7.69 (m, 4H, ArH), 7.91 (s, 1H, CH), 9.75 (bs, 1H, NH, D_2O exchangeable).

*5-(4-Chlorophenyl)-2-(2,4-dichlorobenzylidene)-7-methyl-3-oxo-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylic acid (4-fluorophenyl)amide (**3n**)*

Yield, 64%. %CHN found (cal): C, 56.64 (56.61); H, 2.96 (2.99); N, 7.37 (7.34). IR (KBr, cm^{-1}): 3334, 3037, 2816, 1731, 1694, 1341, 849, 831, 828. ^{1}H NMR (DMSO-d_6): δ 2.45 (s, 3H, CH_3), 5.51 (s, 1H, CH-pyr.), 6.34-7.24 (m, 7H, ArH), 7.48-7.64 (m, 4H, ArH), 7.91 (s, 1H, CH), 9.46 (bs, 1H, NH, D_2O exchangeable).

*5-(4-Chlorophenyl)-7-methyl-2-(2-nitrobenzylidene)-3-oxo-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylic acid (4-fluoro-phenyl)-amide (**3o**)*

Yield, 51%. %CHN found (calc): C, 58.99 (59.07); H, 3.32 (3.30); N, 10.23 (10.21). IR (KBr, cm^{-1}): 3318, 3015, 2876, 1745, 1658, 1349, 1315, 849.; ^{1}H NMR (DMSO-d_6): δ 2.46 (s, 3H, CH_3), 5.61 (s, 1H, CH-pyr.), 6.37–7.30 (m, 8H, ArH), 7.44–7.68 (m, 4H, ArH), 7.79 (s, 1H, CH), 9.64 (bs, 1H, NH, D_2O exchangeable).

*5-(4-Fluorophenyl)-7-methyl-2-(4-nitrobenzylidene)-3-oxo-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylic acid (4-fluorophenyl)amide (**3p**)*

Yield, 56%. %CHN found (calc): C, 60.93 (60.90); H, 3.46 (3.41); N, 10.54 (10.52). IR (KBr, cm^{-1}): 3345, 3038, 2887, 1741, 1689, 1338, 1298, 1264. ^{1}H NMR (DMSO-d_6): δ 2.43 (s, 3H, CH_3), 5.59 (s, 1H, CH-pyr.), 6.41–7.09 (m, 8H, ArH), 7.35–7.61 (m, 4H, ArH), 7.78 (s, 1H, CH), 9.56 (bs, 1H, NH, D_2O exchangeable).

*2-(4-Chlorobenzylidene)-5-(4-fluorophenyl)-7-methyl-3-oxo-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylic acid (4-fluorophenyl)amide (**3q**)*

Yield, 67%. %CHN found (calc): C, 62.19 (62.13); H, 3.46 (3.48); N, 8.01 (8.05). IR (KBr, cm^{-1}): 3364, 3015, 2879, 1745, 1667, 1264, 1254, 851. ^{1}H NMR (DMSO-d_6): δ 2.54 (s, 3H, CH_3), 5.51 (s, 1H, CH-pyr.), 6.31–7.04 (m, 8H, ArH), 7.34–7.59 (m, 4H, ArH), 7.97 (s, 1H, CH), 9.64 (bs, 1H, NH, D_2O exchangeable).

*2-(2-Chlorobenzylidene)-7-methyl-5-(2-nitrophenyl)-3-oxo-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylic acid (4-fluorophenyl)amide (**3r**)*

Yield, 46%. %CHN found (calc): C, 59.10 (59.07); H, 3.31 (3.30); N, 10.25 (10.21). IR (KBr, cm^{-1}): 3374, 3019, 2816, 1716, 1645, 1347, 1284, 897. ^{1}H NMR (DMSO-d_6): δ 2.45 (s, 3H, CH_3), 5.61 (s, 1H, CH-pyr.), 6.24–7.11 (m, 8H, ArH), 7.48–7.67 (m, 4H, ArH), 7.67 (s, 1H, CH), 9.69 (bs, 1H, NH, D_2O exchangeable).

*2-(4-Bromobenzylidene)-7-methyl-5-(2-nitrophenyl)-3-oxo-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylic acid (4-fluorophenyl)amide (**3s**)*

Yield, 49%. %CHN found (calc): C, 54.70 (54.65); H, 3.09 (3.06); N, 9.41 (9.44). IR (KBr, cm^{-1}): 3384, 3037, 2816, 1715, 1656, 1345, 1284, 549. ^{1}H NMR

(DMSO-d_6): δ 2.46 (s, 3H, CH_3), 5.51 (s, 1H, CH-pyr.), 6.43–7.16 (m, 8H, ArH), 7.47–7.81 (m, 4H, ArH), 7.91 (s, 1H, CH), 9.64 (bs, 1H, NH, D_2O exchangeable).

*2-(2-Chlorobenzylidene)-5-(4-fluorophenyl)-7-methyl-3-oxo-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylic acid (4-fluorophenyl)amide (**3t**)*

Yield, 51%. %CHN found (calc): C, 62.07 (62.13); H, 3.51 (3.48); N, 8.06 (8.05). IR (KBr, cm^{-1}): 3304, 3009, 2856, 1718, 1659, 1289, 1274, 864. ^{1}H NMR (DMSO-d_6): δ 2.51 (s, 3H, CH_3), 5.51 (s, 1H, CH-pyr.), 6.41–7.11 (m, 8H, ArH), 7.47–7.75 (m, 4H, ArH), 7.98 (s, 1H, CH), 9.61 (bs, 1H, NH, D_2O exchangeable).

Pharmacology

Anti-inflammatory activity

The investigations were conducted on albino rats of either gender (75–125 g). Animals were kept under standard laboratory conditions at an ambient temperature of 25 $\pm$ 2°C and allowed free access to food and water except at the time they were brought out of the cage. All experimental protocols were carried out with permission from the Institutional Animal Ethics Committee (IAEC), form no. 502. Animals were obtained from the Central Animal House Facility, Hamdard University, New Delhi-62. Registration number is 173/CPCSEA; registration date, 28 January 2000.

Preliminary anti-inflammatory activities of the synthesized compounds were evaluated using the Winter *et al.,* (1962) method described previously. It was performed at three graded doses (25, 50, and 100 mg/kg) and compared with the standard drug phenyl butazone. Animals were divided into groups (control, test compounds, and standard) of six animals each. A freshly prepared suspension of carrageenin (1.0% in 0.9% saline), 0.05 ml, was injected under the plantar aponeurosis of the rats' right paw. One group was kept as controls and the animals in other groups were pretreated with the test drugs suspended in a methyl cellulose/water (0.5%) mixture, given orally 1 h before the carrageenin injection. The volume of the foot was measured before and 1 and 3 h after carrageenin treatment by mercury displacement in a plethysmograph. The mean increase in paw volume in each group was measured and the percentage anti-inflammatory activity was calculated according to the formula

$$\text{Percentage anti} - \text{inflammatory activity} = [1 - (V_t/V_c)] \times 100$$

where V_t and V_c are the volumes of edema in the drug-treated and control groups. Phenyl butazone was used as the standard drug for comparison.

Antinociceptive activity

Antinociceptive activity was assessed using the thermal stimulus technique (Kendall *et al.,* 1982). Investigations were performed on albino mice in groups of six each, which were kept under standard laboratory conditions. Test compounds were

suspended in a methyl cellulose/water (0.5%) mixture. Each compound was administered orally at a dose of 20 mg/kg. Antinociceptive activity was assessed 4 h after administration. The tail of each mouse was gently immersed in thermostatically controlled water at 55°C. The parameter measured in test samples was time that elapsed between immersion and the attempt to withdraw the tail from hot water for control as well as treated animals. Diclofenac (20 mg/kg) was used as the standard drug for comparison.

Ulcerogenic activity

Ulcerogenic activity was measured using Djahanguiri's (1969) method. Adult albino rats of either gender were divided into groups of ten animals each. Animals were kept under standard laboratory conditions and fasted 24 h prior to administration of drugs. Water was allowed ad libitum. The most active compounds and the standard drug (phenyl butazone) were given intraperitoneally and the animals sacrificed 8 h after drug treatment. The stomach, duodenum, and jejunum were removed and examined with a hand lens for any evidence of shedding of epithelium, red spots below the skin, bleeding, and erosion or discrete ulceration with or without perforation. The presence of any one of these criteria was considered to be evidence of ulcerogenic activity.

Acute toxicity study

The approximate 50% lethal dose (ALD_{50}) of the promising compounds was determined in albino mice. Animals of either gender weighing between 20 and 25 g were chosen for the study. Drugs were injected intraperitoneally (i.p.) at different dose levels in separate groups of animals. After 24 h of drug administration, percentage mortality in each group was observed. From the data obtained, ALD_{50} was calculated by the method of Smith (1960).

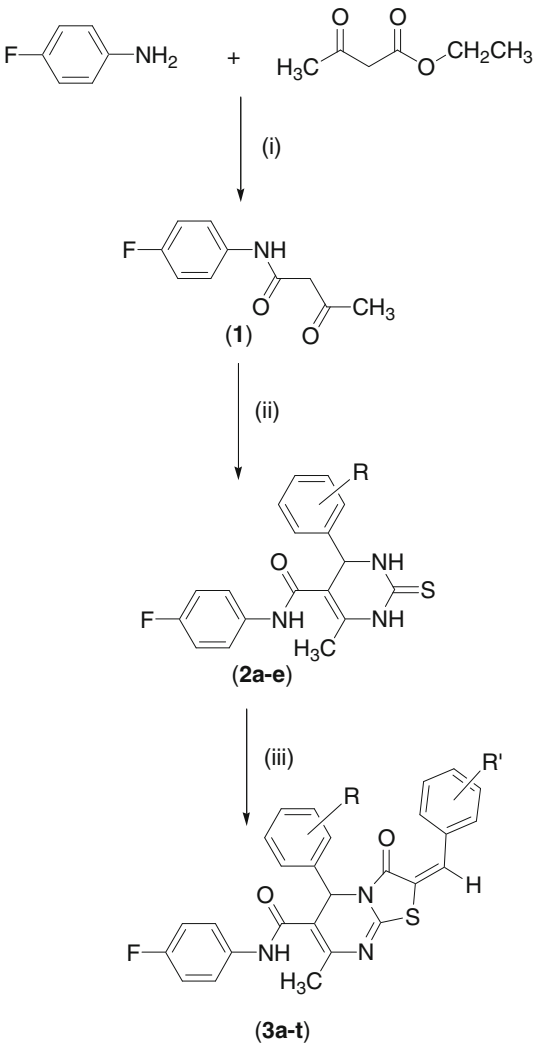
Statistical analyses

All statistical analyses of the data were performed using SigmaStat version 7.0 and ANOVA followed by Dunnett's multiple-comparison test.

Results and discussion

Chemistry

Synthesis of the title compounds (**3a**–**3t**) was carried out according to Scheme 1. Ethylacetoacetate was refluxed with 4-fluoroaniline in ethanol in the presence of sodium hydroxide to yield oxobutyramide (**1**). It was then condensed with thiourea and substituted benzaldehydes in the presence of concentrated hydrochloric acid to get pyrimidine carboxamides (**2a**–**2e**). In the final step, pyrimidine carboxamides were treated with chloroacetic acid and substituted benzaldehydes in a mixture of



Scheme 1 Reagents and conditions: (i) NaOH, ethanol, reflux, 5 h; (ii) thiourea, aromatic aldehydes; (iii) aromatic aldehydes, chloroacetic acid, acetic anhydride, acetic acid

acetic acid and acetic anhydride to afford the title compounds (**3a**–**3t**). All synthesized compounds were characterized by different spectroscopic techniques. Physicochemical properties of compounds are presented in Table 1.

Pharmacology

Anti-inflammatory activity

The anti-inflammatory activity of all the title compounds (**3a**–**3t**) is presented in Table 2. All compounds were tested at a dose of 25 mg/kg p.o and have shown considerable anti-inflammatory activity in the range of 5%–60%. Compounds **3l** and

Table 1 Physicochemical parameters of synthesized compounds (**3a–3t**)

Compound	R	R′	Mol. formula (MW)[a]	R_f value[b]	m.p. (°C)
3a	4-Br	3,4-OCH_3	$C_{29}H_{23}BrFN_3O_4S$ (608.48)	0.69	178–181
3b	4-Br	2,4-Cl	$C_{27}H_{17}BrCl_2FN_3O_2S$ (617.32)	0.78	192–194
3c	4-Br	H	$C_{27}H_{19}BrFN_3O_2S$ (548.43)	0.54	185–187
3d	4-Br	4-Cl	$C_{27}H_{18}BrClFN_3O_2S$ (582.87)	0.61	177–179
3e	4-Br	4-CH_3	$C_{28}H_{21}BrFN_3O_2S$ (562.45)	0.68	191–193
3f	4-NO_2	2,4-Cl	$C_{27}H_{17}C_{l2}FN_4O_4S$ (583.42)	0.74	197–199
3g	4-NO_2	4-Cl	$C_{27}H_{18}ClFN_4O_4S$ (548.97)	0.79	211–213
3h	4-NO_2	4-OCH_3	$C_{28}H_{21}FN_4O_5S$ (544.55)	0.72	226–228
3i	4-NO_2	2-Cl	$C_{27}H_{18}ClFN_4O_4S$ (548.97)	0.64	213–215
3j	4-NO_2	H	$C_{27}H_{19}FN_4O_4S$ (514.53)	0.71	228–230
3k	4-Cl	4-Br	$C_{27}H_{18}BrClFN_3O_2S$ (582.87)	0.50	205–207
3l	4-Cl	4-F	$C_{27}H_{18}ClF_2N_3O_2S$ (521.97)	0.66	185–187
3m	4-Cl	2-Cl	$C_{27}H_{18}Cl_2FN_3O_2S$ (538.42)	0.70	213–215
3n	4-Cl	2,4-Cl	$C_{27}H_{17}Cl_3FN_3O_2S$ (572.87)	0.77	230–232
3o	4-Cl	2-NO_2	$C_{27}H_{18}ClFN_4O_4S$ (548.97)	0.63	176–178
3p	4-F	4-NO_2	$C_{27}H_{18}F_2N_4O_4S$ (532.52)	0.49	163–165
3q	4-F	4-Cl	$C_{27}H_{18}ClF_2N_3O_2S$ (521.97)	0.52	168–170
3r	2-NO_2	2-Cl	$C_{27}H_{18}ClFN_4O_4S$ (548.97)	0.57	233–235
3s	2-NO_2	4-Br	$C_{27}H_{18}BrFN_4O_4S$ (593.42)	0.64	215–217
3t	4-F	2-Cl	$C_{27}H_{18}ClF_2N_3O_2S$ (521.97)	0.53	158–160

[a] Solvent of crystallization: ethanol

[b] Solvent system: toluene/ethyl acetate/formic acid (5:4:1)

3q displayed significant results, comparable to those for the standard drug phenylbutazone (Fig. 1).

Compounds that showed significant anti-inflammatory activity were **3d**, **3g**, **3k**, **3l**, **3o**, and **3q**. These compounds showed >20% protection from inflammation in animals. Among these compounds **3l** and **3q** were found to be the most potent, with 44% and 47% anti-inflammatory activity at 25 mg/kg p.o ($P < 0.001$). These compounds were also tested at 50 and 100 mg/kg p.o. ($P < 0.001$) to determine the potentiality along with the reference drug phenylbutazone. Interestingly, compound **3l** showed comparable activity to phenylbutazone at 25 mg/kg p.o. (44%; $P < 0.001$), better activity at 50 mg/kg p.o. (60%; $P < 0.001$), and even better activity at 100 mg/kg p.o. (80%; $P < 0.001$). Compound **3q** also showed comparable anti-inflammatory activity, at all three graded doses, to the reference drug.

The structure–activity relationship of the compounds was studied and it was found that the halo-substituted compounds were the most active in the series. Substitutions with a halogen atom at both the aryl rings resulted in increased anti-inflammatory activity. Among the halo derivatives, substitutions at the *para* position were more active than the halogen at other positions. The size of the halogen atom also seemed to play an important role in determining the activity. Compounds with smaller halogen atoms (F, Cl) were more potent than the larger bromo derivatives.

Table 2 Pharmacological data on the title compounds (**3a–3t**)

Compound	Anti-inflammatory activity[a]		Antinociceptive activity[b]	
	Mean increase in paw volume ± SE	% Activity	Mean average reaction time ± SE (s)	% Activity
Control	0.78 ± 0.12	–	4.7 ± 0.44	–
3a	0.71 ± 0.11	8	5.8 ± 0.27	23
3b	0.67 ± 0.13	14	6.2 ± 0.31	31
3c	0.74 ± 0.10	5	8.3 ± 0.34	76
3d	0.58 ± 0.08	25	9.6 ± 0.33*	104
3e	0.69 ± 0.11	11	8.1 ± 0.28	72
3f	0.64 ± 0.12	17	10.4 ± 0.16**	121
3g	0.60 ± 0.07*	23	9.6 ± 0.34	104
3h	0.68 ± 0.10	12	6.9 ± 0.25	46
3i	0.63 ± 0.14	19	11.3 ± 0.16**	140
3j	0.72 ± 0.09	7	9.1 ± 0.17	93
3k	0.50 ± 0.13**	35	8.3 ± 0.24	76
3l	0.43 ± 0.15***	44	12.8 ± 0.34***	172
	0.31 ± 0.10***	60[c]		
	0.15 ± 0.18***	80[d]		
3m	0.65 ± 0.11	16	7.7 ± 0.38	63
3n	0.67 ± 0.09	14	6.9 ± 0.39	46
3o	0.65 ± 0.17	16	8.6 ± 0.41	82
3p	0.54 ± 0.06**	30	10.4 ± 0.21*	121
3q	0.41 ± 0.04***	47	13.6 ± 0.15***	189
	0.39 ± 0.08***	50[c]		
	0.17 ± 0.12***	78[d]		
3r	0.68 ± 0.09	12	8.1 ± 0.17	72
3s	0.65 ± 0.07	16	9.4 ± 0.31	100
3t	0.63 ± 0.10	19	10.3 ± 0.35*	119
Phenyl butazone	0.44 ± 0.08***	43	–	–
	0.35 ± 0.13***	55[c]		
	0.23 ± 0.14***	70[d]		
Diclofenac	–	–	17.6 ± 0.25***	274

Note: Number of animals used: six. – Not tested. Solvent used: methyl cellulose/water (0.5%) mixture. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

[a] Tested at a dose of 25 mg/kg

[b] Tested at a dose of 20 mg/kg

[c] Tested at a dose of 50 mg/kg

[d] Tested at a dose of 100 mg/kg

Antinociceptive activity

The antinociceptive activity of all compounds was assessed at 25 mg/kg p.o. and the results are presented in Table 2. Results are expressed as mean average reaction

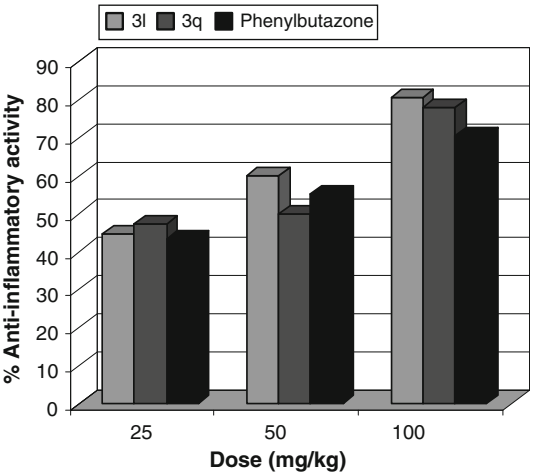


Fig. 1 Anti-inflammatory activity of compounds **3l**, **3q**, and phenylbutazone

time (seconds) ± SE, and the change from control was noted to calculate the percentage analgesia. All compounds showed antinociceptive activity in the range of 23%–189% and were compared to the standard drug diclofenac. The two compounds, **3l** and **3q**, that showed significant anti-inflammatory activity were also found to possess considerable antinociceptive activity (Fig. 2). Compounds that showed significant antinociceptive activity (> 100%) include **3d**, **3f**, **3 g**, **3i**, **3l**, **3p**, **3q**, and **3t**. Among these compounds, **3l** and **3q** were found to be the most potent, with 172% and 189% antinociceptive activity ($P < 0.001$).

All compounds were found to possess lesser activity than the standard drug diclofenac, which was found to show 274% antinociceptive activity. Substitutions with electron-withdrawing functional groups resulted in a significant increase in the activity. Most of the nitro-substituted derivatives were active in the screen. Substitutions at the *para* position increased the activity significantly.

Ulcerogenic activity

The ulcerogenic activity of the most active compounds, **3l** and **3q**, was assessed in albino rats 8 h after compound administration intraperitoneally and the results are

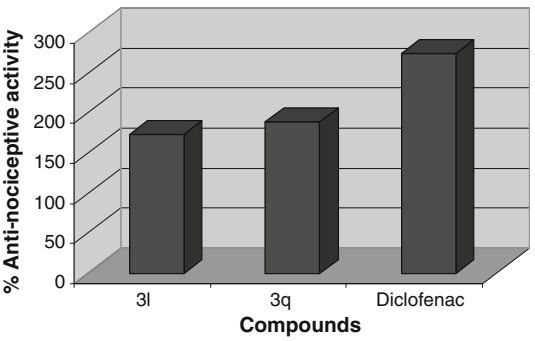


Fig. 2 Antinociceptive activity of compounds **3l**, **3q**, and diclofenac

Table 3 Toxicity studies of the selected compounds

Compound	Ulcerogenic activity (UD_{50}; mg/kg)[a]	ALD_{50} (mg/kg)[b]
3l	240.36	>1000
3q	174.63	>1000
Phenyl butazone	70.25	>1000

Note: Number of animals used: ten. Solvent used: polyethylene glycol (PEG)

[a] Compounds were administered intraperitoneally and animals were assessed 8 h after drug treatment

[b] Approximate lethal dose; percentage mortality was observed 24 h after drug administration

reported in Table 3. Interestingly, both the compounds displayed a better safety profile than the standard drug phenylbutazone. The UD_{50} values of compounds **3l** and **3q** were found to be 240.36 and 174.63 mg/kg, much higher than that of the standard drug ($UD_{50} = 70.25$ mg/kg). This shows that these compounds are less prone to cause ulcers and are relatively safer agents.

Acute toxicity studies

Acute toxicity studies were performed in albino mice and the approximate lethal dose (ALD_{50}) was determined 24 h after drug administration. Results are reported in Table 3. Both compounds showed ALD_{50} values (>1000 mg/kg) comparable to that of the standard drug phenylbutazone. This further supported the wide safety margin of the two compounds.

Conclusion

A new series of thiazolopyrimidines has been identified as anti-inflammatory and anti-nociceptive agents. Two compounds showed significant activities in both screens, comparable to those of the standard drugs phenylbutazone and diclofenac. Interestingly, these compounds displayed higher UD_{50} and ALD_{50} values, indicating the wide safety profile of the compounds. These agents have strong future potential and have proven to be good candidates for further investigations.

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