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Original Research Paper

SYNTHESIS AND ANTIINFLAMMATORY ACTIVITY OF NOVEL PYRIMIDINO BENZOTHIAZOLE AMINE DERIVATIVES

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ABSTRACT

(Benzothiazol-2yl) acetonitrile have been reported to exhibit inhibition of c-Jun N-terminal kinase which is known to play a vital role in cell death (apoptosis) and inflammation. A detail study of SAR of (Benzothiazol-2yl) acetonitrile compounds revealed that Benzothiazol-2-ylidinyl and pyrimidine scaffolds are important for the JNK inhibitory activity. This study intended to hydrolyze the nitrile group in to acid to afford (Benzothiazol-2yl)-2-(2-substituted pyrimidin-4yl) acetic acids (4a-z) and observe for their anti-inflammatory properties. All the compounds have been synthesized by following known literature procedures and characterized by ^{1}H NMR, IR, and Mass and subjected to screening for the anti-inflammatory activity by carrageenan induced *in vivo* rat paw edema model. Compounds 4h and 4q were found to possess significant activity amongst all the compounds.

Keywords: Banzothiazole, Amine derivatives, JNK, Synthesis, Anti-inflammatory, SAR, Rat paw edema model.

INTRODUCTION

Numerous compounds bearing benzothiazole ring are known to possess important of pharmacological activities such as antimicrobial[1,2], anticancer [3,4], antiviral[5], anti HIV[6], antidiabetic.[7] They are also useful as anti-allergic[8], antileishmanial[9] and antitubercular[10, 11] agents. Our group has been working on the design and development of new small molecules as inhibitors of p38 kinase[12-18] which is a validated target in inflammatory diseases and has expanded the kinase inhibitors drug discovery to c-Jun N-terminal kinases (JNKs). The JNKs are members of the mitogen-activated protein kinase (MAPK) family along with p38 mitogen-activated protein kinases. Inhibition of JNK may provide clinical benefit in a wide range of apoptosis-related and inflammatory disorders.[19, 20] The design and development of new JNK inhibitors by our group is under various stages of publication[21, 22] and it is observed that benzothiazol-2-ylacetonitrile pyrimidines possess significant JNK inhibitory activity.[23] The prototype inhibitor AS601245 (figure 1) was found to exhibit significant reduction in joint swelling at 60 mg/kg b.w. Histopathological analysis also revealed that it preserved joint areas by decreasing cartilage erosion and significant reduction in synovial inflammation. We herewith present the synthesis of acid derivatives by hydrolyzing the nitrile

group and incorporating a variety of aryl, heteroaryl and alkyl amines at 2nd position of pyrimidine ring to generate compounds having general structure shown below (figure 2). This manuscript reports the synthesis and anti-inflammatory activity of aforementioned compounds by employing rat paw edema method.

MATERIALS AND METHODS

All the Chemicals and solvents (M/s Sigma Aldrich/ S. D Fine chemical/ Loba) were purchased from local vendors and solvents were purified before being used. Both Pre-coated silica gel F_{254} (Merck) and plates prepared on glass plates were employed to check the TLC for reaction progress and purity. Melting points were recorded in open glass capillaries using Polmon melting point apparatus and are uncorrected. Infrared spectra were recorded on Shimadzu FT IR spectrophotometer in KBr pellet. Mass spectra were obtained on VG-7070H mass spectrometer and ^{1}HNMR spectra were recorded at 300 MHz on a Bruker Avance NMR spectrometer in $CDCl_3$ (δ 7.26) or DMSO- d_6 (δ 2.49).

EXPERIMENTAL

2-(1, 3-Benzothiazol-2-ylidinyl)-2-(2-chloro-pyrimidin4-yl) acetonitrile (2)

To an ice cooled solution of NaH (60% oil, 9.2 g, 0.23 mol) in dry THF (200 mL) was added a solution of 1,3-benzothiazol-2-ylacetonitrile (1**,** 20 g, 0.15 mol) in dry THF (200 mL) and the mixture was stirred for 1 h at room temperature (Scheme). A solution of 2,4-dichloropyrimidine (17.1 g, 0.15 mol) in dry THF (200 mL) was added drop wise and mixture warmed to 70 °C for 1-10 h until complete disappearance of starting material. Upon completion of the reaction, the reaction mixture was quenched by the addition of water and kept aside for about 1 h. Then the pH of the mixture was adjusted to 4 with 1N HCl at 0 °C, and thus formed precipitate was filtered and washed with water, later washed with n-hexane and dried in vacuum to give 2 (97%) as colorless solid.[23]

2-(1,3-Benzothiazol-2-ylidinyl)-2-[(2-substituted aminopyrimidin-4-yl) acetonitriles (3a-z)

An appropriate quantity of 2 in ethanol/dichloromethane was refluxed with different amines (aryl, heteroaryl and alkyl) in presence of triethyl amine as base for 4-6 h monitoring the progress of the reaction using the TLC profile of reaction mixture (for a few compounds, dichloromethane was employed and the reaction temperature was 0 °C-RT). Upon completion of the reaction, ethanol was evaporated and diluted with ethyl acetate and washed with water. The organic solvent was evaporated to get the crude product which was purified over silica column to afford desired compounds 3a-z.

2-(1,3-Benzothiazol-2-ylidinyl)-2-(2-substituted aminopyrimidin-4-yl)acetic acid (4a-z)

A solution of respective acetonitrile intermediate compounds (3a-z, 0.46 mmol) in MeOH (4 mL) was treated with 4N NaOH (4 mL) at 0 °C for 1 h. Later methanol was removed in *vacuo* and adjusted to pH 2-3 with aq. 1N HCl and extracted with EtOAc (2 x 10 ml). The organic layer was dried (Na_2SO_4) and evaporated to give the corresponding acid 4a-z in competitive yields (42-89%) as listed in table 1.

ANTI-INFLAMMATORY ACTIVITY

Anti-inflammatory activity was assessed by the method described by Winter *et. al*[24, 25]. Rats were divided into three groups (control, test compounds and standard drug) of six animals each. The standard Diclofenac sodium (100 mg/kg dose) and synthesized compounds under study (4a-z) were administered orally to all rats. After 30 minuts a freshly prepared suspension of carrageenan (1% in 0.9% saline, 0. 5 mL) was injected under the subplanter tissues of the right hind paw of each rat. The edema volumes of the injected paw were measured at 1st, 2nd, 3rd and 4th hour. The difference between the paw volumes of treated animals were compared with that of the control group and the mean oedema volume was calculated. From the data obtained mean volume of oedema ± SEM (Standard Error Mean**)** and percentage reduction in oedema were calculated. Percentage reduction or inhibition in oedema volume was calculated by using the formula.

Percentage of inhibition of oedema = 1-Vt/Vc **X** 100

Where Vt and Vc are the volumes of oedema in test compound/standard drug treated and control group, respectively. Sigma statistical software was applied for the evaluation of activity data[24, 25].

CHARACTERIZATION

2-(1,3-Benzothiazol-2-ylidinyl)-2--(2-chloropyrimidin-4-yl)acetonitrile 2

Yield,84%; mp 246-48 °C ; IR (KBr): 3110, 2924, 2201, 1630, 1610, 1588, 1533, 1450, 1362, 1298, 1187cm^{-1}; ^{1}H NMR (300 MHz DMSO-d_6): δ 8.26 (d, 1H, aromatic, *J* = 5.7 Hz, C_6 pyrimidine), 7.93 (d, 1H, aromatic, *J* = 8.2 Hz, C_7), 7.62 (d, 1H, *J* = 8.2 Hz, aromatic, C_4), 7.47 – 7.35 (m, 1H, aromatic, C_6), 7.25 (m, 1H, aromatic, *J* = 7.4 Hz, C_5), 7.18-7.09 (d, 1H, aromatic, C_5 Pyrimidine); EI-MS: 287.0 $[M+1]^+$; calculated for $C_{13}H_7ClN_4S$ 287.01, found 287, elemental analysis C 54.4%, H 2.4%, N 19.5%, Cl 12.3%, S 11.1%.

2-(1,3-Benzothiazol-2-ylidinyl)-2-(2-aminopyrimidin-4-yl)acetic acid (4a)

IR (KBr, ν cm^{-1}): ~3300 (OH str), ~3200 (NH, Str.), 3050 (aromatic CH str), 1716 (C=O str), 1637 (C=C), 1580 (NH bnd); ^{1}HNMR (400 MHz, DMSO) δ (ppm): 11.5 (bs, 1H, NH benzothiazole), 9.2 (bs, 1H, OH), 8.26 (d, 1H, aromatic, J = 5.7 Hz, C_6 pyrimidine), 7.93 (d, 1H, aromatic, J = 8.2 Hz, C8), 7.62 (m, 1H, aromatic, C_7), 7.47 – 7.35 (m, 1H, aromatic, C_6), 7.18-7.09 (d, 1H, aromatic, C_5), 6.8 (d, 1H, C_5 pyrimidine), 3.5 (bs, 2H, 1º amine); EI-MS (m/z): 287 $[M]^{+1}$.

2-(1,3-Benzothiazol-2-ylidinyl)-2-(2-cyclo propylamino pyrimidin-4yl)acetic acid (4b)

IR (KBr, ν cm^{-1}): 3396 (OH str), 3260 (NH str), 3010 (aromatic CH str), 2850 (CH_2 str), 1700 (C=O str) 1620 (C=C str), 1420 (C=C str); ^{1}HNMR (500 MHz, DMSO) δ (ppm): 11.3 (bs, 1H, NH benzothiazole), 9.0 (bs, 1H, OH), 8.2 (d, 1H, aromatic, J = 5.7 Hz, C_6 pyrimidine), 8.1 (bs, 1H, NH pyrimidine), 7.95 (d, 1H, aromatic, J = 8.6 Hz, C_7), 7.48 (t, 1H, aromatic, *J* = 7Hz, C_6), 7.32 (t, 1H, *J* = 7 Hz, aromatic, C_5), 7.0 (d, 1H, aromatic C_4), 6.57 (d, 1H, C_5 Pyrimidyl), 0.95 (m, 1H, cyclopropyl), 0.7 (m, 4H, cyclopropyl); EI-MS (m/z): 327 [M+1].

2-(1,3-Benzothiazol-2-ylidinyl)-2-[2-(4-bromo-phenyl amino)pyrimidin-4yl]acetic acid amine (4c)

IR (KBr, ν cm^{-1}): 3365 (OH str), 3255 (NH str), 3050 (aromatic CH str), 1682 (C=O str) 1618 (C=C str); ^{1}HNMR (500 MHz, DMSO) δ (ppm): 11.75 (bs, 1H, NH benzothiazole), 9.5 (bs, 1H, COOH), 8.4 (d, 1H, aromatic, J = 7.4 Hz, C_6 pyrimidine), 8.2 (bs, d, 2H, NH pyrimidine, aromatic, J = 8.5 Hz, C_7), 7.65 (t, 1H, aromatic C_6), 7.26 (d, 2H J = 7.7 Hz, aromatic C'_5, C'_3), 7.0 (d, 2H J = 7.9 Hz, aromatic C'_6, C'_2), 6.8-6.3 (m, 3H, aromatic, C_5, C_4, pyrimidine C_5,); EI-MS (m/z): 441 [M+1].

2-(1,3-Benzothiazol-2-ylidinyl)-2-[2-(3-chloro-4-fluorophenyl amino) pyrimidin-4yl]acetic acid (4d)

IR (KBr, ν cm^{-1}): 3380 (OH str), 3243 (NH str), 3035 (aromatic CH str), 1665 (C=O str) 1610 (C=C str); ^{1}HNMR (500 MHz, DMSO) δ (ppm): 11.40 (bs, 1H, NH benzothiazole), 9.7 (bs, 1H, COOH), 8.3 (d, 1H, aromatic, J = 6.8 Hz, C_6 Pyrimidine), 8.1(bs, d, 2H, NH pyrimidine, aromatic, J = 7.5 Hz, C_7), 7.5 (t, 1H, aromatic C_6), 7.3-7.2 (m, 2H, aromatic C'_6, C'_5), 6.9-6.2 (m, 4H, aromatic, C_5, C_4, pyrimidine C_5, aromatic C'_2); EI-MS (m/z): 415 $[M]^{+1}$.

2-(1,3-Benzothiazol-2-ylidinyl)-2-[2-(2,5-dichloro phenyl amino)pyrimidin-4yl]acetic acid (4e)

IR (KBr, ν cm^{-1}): 3400 (OH str), 3235 (NH str), 3025 (aromatic CH str), 1680 (C=O str) 1614 (C=C str), 1430 (C=C str); ^{1}HNMR (500 MHz, DMSO) δ (ppm): 11.0 (bs, 1H, NH benzothiazol), 8.8 (bs, 1H, OH), 8.1 (d, 1H, aromatic, J = 6.2 Hz, C_6 pyrimidine), 7.99 (d, 1H pyrimidine, aromatic, J = 8.1 Hz, C_7) 7.5-7.1 (m, 4H, aromatic, C_6, C_5, C'_4, C'_3), 6.9-6.6 (m, 3H, aromatic, C_4, pyrimidine C_5, C'_6); EI-MS (m/z): 431 $[M]^{+1}$.

2-(1,3-Benzothiazol-2-ylidinyl)-2-[2-(benzothiazol-2-yl)phenyl amino)pyrimidin-4yl]acetic acid (4f)

IR (KBr, ν cm^{-1}): 3405 (OH str), 3260 (NH str), 3020 (aromatic CH str), 1685 (C=O str) 1615 (C=C str); ^{1}HNMR (500 MHz, DMSO) δ (ppm): 11.65 (bs, 1H, NH benzothiazole), 9.55 (bs, 1H, COOH), 8.5 (d, 1H, aromatic, J = 7.0 Hz, C_6 pyrimidine), 8.25 (bs, m, 3H, NH pyrimidine, aromatic C_7, C''_4), 8.0 (d, 1H, aromatic, *J* = 8.0 Hz, C''_7), 7.65-7.25 (m, 4H, C''_6, C''_5, C'_3, C_5), 7.1-6.3 (m, 6H, C_5, C_4, pyrimidine C_5, C'_6, C'_5, C'_4,), EI-MS (m/z): 495 $[M]^{+1}$.

2-(1,3-Benzothiazol-2-ylidinyl)-2-[2-(2-benzoyl)phenylamino pyrimidin-4yl]acetic acid (4g)

IR (KBr, ν cm^{-1}): 3365 (OH str), 3230 (NH str.), 3050 (aromatic CH str), 1720 (C=O str), 1665 (C=O str) 1610 (C=C str); ^{1}HNMR (500 MHz, DMSO) δ (ppm): 11.4 (bs, 1H, NH benzothiazole), 9.3 (bs, 1H, COOH), 8.7 (bs, 1H, NH pyrimidine), 8.5 (d, 1H, aromatic, J = 7.7 Hz, C_6 pyrimidine), 8.35 (d, 1H, aromatic, J = 7.1 Hz, C'_5), 8.05 (d, 1H, , aromatic, J = 7.6 Hz, C_7), 7.8 (d, 2H, aromatic, *J* = 8.2 Hz, C''_6, C''_2), 7.7-7.2 (m, 6H, C''_5, C''_4, C''_3, C'_3, C'_5, C_6), 7.0-6.5 (m, 4H, C_5, C_4, pyrimidine C_5, C'_4,); EI-MS (m/z):466 $[M]^{+1}$.

2-(1,3-Benzothiazol-2-ylidinyl)-2-[(2-benzylamino) pyrimidin-4yl]acetic acid (4h)

IR (KBr, ν cm^{-1}): 3380 (OH str), 3250 (NH str), 3015 (aromatic CH str), 2860 (CH_2 str), 1695 (C=O str) 1610 (C=C str), 1410 (C=C str); ^{1}HNMR (500 MHz, DMSO) δ (ppm): 11.35 (bs, 1H, NH benzothiazole), 9.6 (bs, 1H, OH), 8.6 (d, 1H, aromatic, J = 6.9 Hz, C_6 pyrimidine), 8.1 (bs, d, 2H, NH pyrimidine, aromatic, J = 7.8 Hz, C_7) 7.5 -7.15 (m, 7H, aromatic C_6, C_5, C'_6, $C'5$, C'_4, C'_3, C'_2), 6.8-6.6 (m, 2H, aromatic C_4, and C_5 pyrimidine), 4.88 (s, 2H, benzylic, -CH_2); EI-MS (m/z): 377 $[M]^{+1}$.

2-(1,3-benzothiazol-2-ylidinyl)-2-[{2-(4-fluorobenzylamino)} pyrimidin-4yl]acetic acid (4i)

IR (KBr, ν cm^{-1}): 3342 (OH str), 3235 (NH str), 3010 (aromatic CH str), 2875 (CH_2 str), 1680 (C=O str); ^{1}HNMR (500 MHz, DMSO) δ (ppm): 11.5 (bs, 1H, NH benzothiazole), 9.4 (bs, 1H, OH), 8.35 (d, 1H, aromatic, J = 6.7 Hz, C_6 pyrimidine), 8.25 (bs, d, 2H, NH pyrimidine, aromatic, J = 7.8 Hz, C_7), 7.4-7.1 (m, 6H, aromatic C_6, C_5, C'_6, $C'5$, C'_3, C'_2), 6.95-6.7 (m, 2H, aromatic C_4, pyrimidine C_5), 4.91 (d, 2H, benzylic, CH_2); EI-MS (m/z): 377 $[M]^{+1}$.

2-(1,3-Benzothiazol-2-ylidinyl)-2-[{2-(4-chlorobenzylamino)} pyrimidin-4yl]acetic acid (4j)

IR (KBr, ν cm^{-1}): 3346 (OH str), 3250 (NH str), 3090 (aromatic CH str), 2860 (CH_2 str), 1692 (C=O str); ^{1}HNMR (500 MHz, DMSO) δ (ppm): 11.3 (bs, 1H, NH benzothiazole), 9.1 (bs, 1H, OH), 8.2 (d, 1H, aromatic, J = 7.0 Hz, C_6 pyrimidine), 8.15 (bs, d, 2H, NH pyrimidine, aromatic, J = 7.3 Hz, C_7), 7.35-7.0 (m, 6H, aromatic C_6, C_5, C'_6, $C'5$, C'_3, C'_2), 6.8-6.7 (m, 2H, aromatic C_4, pyrimidine C_5), 4.4 (d, 2H, benzylic, CH_2); EI-MS (m/z): 412 $[M]^{+1}$.

2-(1,3-Benzothiazol-2-ylidinyl)-2-[{2-(4-methoxy benzylamino)} pyrimidin-4yl]acetic acid (4k)

IR (KBr, ν cm^{-1}): 3370 (OH str), 3235 (NH str), 3060 (aromatic CH str), 2925 (CH_3 str) 2850 (CH_2 str), 1680 (C=O str); ^{1}HNMR (500 MHz, DMSO) δ (ppm): 11.1 (bs, 1H, NH benzothiazole), 9.2 (bs, 1H, OH), 8.3 (d, 1H, aromatic, J = 7.3 Hz, C_6 pyrimidine), 8.0 (bs, d, 2H, NH pyrimidine, aromatic, Hz, C_7), 7. 5-6.4 (m, 9H, aromatic C_6, C_5, C_4, C'_6, pyrimidine C_5, C'_6, C'_5, C'_4, C'_2), 4.86 (s, 2H, benzylic, -CH_2), 3.77 (s, 3H, -OCH_3), EI-MS (m/z): 407 $[M]^{+1}$.

2-(1,3-Benzothiazol-2-ylidinyl)-2-[2-(pyridin-4-ylamino) pyrimidin-4yl]acetic acid (4q)

IR (KBr, ν cm^{-1}): 3390 (OH str), 3255 (NH str), 3080 (aromatic CH str), 3005 (aromatic CH str, pyridyl), 1668 (C=O str); ^{1}HNMR (500 MHz, DMSO) δ (ppm): 11.25 (bs, 1H, NH benzothiazol), 9.0 (bs, 1H, OH), 8.5 (d, 2H, J = 8.5 Hz, C'_6, C'_2 pyridyl), 8.22 (d, 1H, aromatic, J = 7.6 Hz, C_6 pyrimidine), 8.1 (bs, 1H, NH pyrimidine), 7.9 (d, 1H, aromatic J = 8.0 Hz, C_7), 7.4-6.6 (m, 6H, aromatic C_6, C_5, C_4, pyrimidine C_5, pyridine C'_5, C'_3,), EI-MS (m/z): 364 $[M]^{+1}$.

2-(1,3-Benzothiazol-2-ylidinyl)-2-[2-(pyridin-4-yl methylamino) pyrimidin-4yl]acetic acid (4r)

IR (KBr, ν cm^{-1}): 3365 (OH str), 3270 (NH str), 3035 (aromatic CH str), 2850 (CH_2 str), 1670 (C=O str) 1620 (C=C str); ^{1}HNMR (500 MHz, DMSO) δ (ppm): 11.4 (bs, 1H, NH benzothiazol), 9.35 (bs, 1H, COOH), 8.4 (d, 1H, aromatic, J = 7.4 Hz, C_6 pyrimidine), 8.2 (d, 2H, aromatic, J = 8.6 Hz, Pyridyl C_6, C_2), 7.9 (bs, d, 2H, NH pyrimidine, aromatic, J = 6.9 Hz, C_7), 7.5 -7.2 (m, 3H, aromatic C_6, Pyridyl C_5, C_3), 6.9-6.7 (m, 7H, aromatic C_5, C_4, Pyrimidyl C_5), 4.5 (s, 2H, benzylic, CH_2);EI-MS (m/z):378 $[M]^{+1}$.

2-(1,3-Benzothiazol-2-ylidinyl)-2-[2-(2-mercapto benzimidazol-5-ylamino) pyrimidin-4yl]acetic acid (4v)

IR (KBr, ν cm^{-1}): 3375 (OH str), 3270 (NH str), 3040 (aromatic CH str), 1680 (C=O str); ^{1}HNMR (500 MHz, DMSO) δ (ppm): ~13.0 (bs, 1H, SH), 11.5 (bs, 1H, NH benzothiazole), 9.5 (bs, 1H, OH), 8.44 (d, 1H, aromatic, J = 8.0 Hz, C_6 pyrimidine), 8.16 (bs, 1H, NH pyrimidine), 7.94 (d, 1H, aromatic J = 7.8 Hz, C_7), 7.36-6.4 (m, 7H, aromatic C_6, C_5, C_4, pyrimidine C_5, benzimidazole C'_7, C'_5, C'_4); EI-MS (m/z): 435 $[M]^{+1}$.

2-(1,3-Benzothiazol-2-ylidinyl)-2-[2-(2-methyl indol-5-ylamino) pyrimidin-4yl]acetic acid (4w)

IR (KBr, ν cm^{-1}): 3360 (OH str), 3260 (NH str), 3025 (aromatic CH str), 1674 (C=O str); ^{1}HNMR (500 MHz, DMSO) δ (ppm): 12.1 (bs, 1H, NH indole), 11.25 (bs, 1H, NH benzothiazole), 9.2 (bs, 1H, -OH), 8.34 (d, 1H, aromatic, *J* = 7.7 Hz, C_6 pyrimidine), 8.06 (bs, 1H, NH pyrimidine), 7.88 (d, 1H, aromatic J = 7.8 Hz, C_7), 7.36-6.4 (m, 8H, aromatic C_6, C_5, C_4, pyrimidine C_5, indole C'_7, C'_6, C'_4, C'_3), 2.3 (s, 3H, $-CH_3$); EI-MS (m/z): 416 $[M]^{+1}$.

2-(1,3-Benzothiazol-2-ylidinyl)-2-[2-(indazol-5-ylamino) pyrimidin-4yl]acetic acid (4x)

IR (KBr, ν cm^{-1}): 3375 (OH str), 3255 (2°, amine str), 3045 (aromatic CH str), 1670 (C=O str); ^{1}HNMR (500 MHz, DMSO) δ (ppm): ~12.3 (bs, 1H, NH pyrazole), 11.4 (bs, 1H, NH benzothiazole), 9.0 (bs, 1H, COOH), 8.45 (d, 1H, aromatic, *J* = 7.9 Hz, C_6 pyrimidine), 8.17 (s, 1H, pyrazole C'_3), 8.0 (bs, 1H, NH pyrimidine), 7.95 (d, 1H, aromatic J = 8.2 Hz, C_7), 7.78 (d, 1H, aromatic J = 7.3 Hz pyrazole C'_7), 7.3-6.6 (m, 6H, aromatic C_6, C_5, C_4, pyrimidine C_5, pyrazole C'_6, C'_4,); EI-MS (m/z):403 $[M]^{+1}$.

2-(1,3-Benzothiazol-2-ylidinyl)-2-[2-(5-methyl benzothiazol-2ylamino) pyrimidin-4yl]acetic acid (4y)

IR (KBr, ν cm^{-1}): 3350 (OH str), 3240 (NH str), 3035 (aromatic CH str), 2975 (CH_3 str), 1655 (C=O str); ^{1}HNMR (500 MHz, DMSO) δ (ppm): 11.2 (bs, 1H, NH benzothiazole), 9.12 (bs, 1H, COOH), 8.3 (d, 1H, aromatic *J* = 7.7 Hz, C_6 pyrimidine), 8.1 (bs, 1H, NH pyrimidine), 8.0 (d, 1H, aromatic J = 7.8 Hz, C_7), 7.8 (d, 1H, *J* = 7.2 Hz benzothiazolyle C'_7) 7.5-6.5 (m, 6H, aromatic C_6, C_5, C_4, pyrimidine C_5, benzothiazolyle C'_6, C'_4,); EI-MS (m/z):434 $[M]^{+1}$.

2-(1,3-Benzothiazol-2-ylidinyl)-2-[2-(benzimidazol-2-ylamino) pyrimidin-4yl]acetic acid (4z)

IR (KBr, ν cm^{-1}): 3370 (OH str), 3255 (NH str), 3015 (aromatic CH str), 1670 (C=O str); ^{1}HNMR (500 MHz, DMSO) δ (ppm): 11.7 (bs, 1H, NH benzimidazolyle), 11.25 (bs, 1H, NH benzothiazol), 9.3 (bs, 1H, COOH), 8.36 (d, 1H, aromatic *J* = 7.5 Hz, C_6 pyrimidine), 8.2 (bs, 1H, NH pyrimidine), 7.9 (d, 1H, aromatic J = 7.9 Hz, C_7), 7.55 (t, 1H, aromatic C_6), 7.28 (d, 2H, aromatic J = 8.6 Hz, benzimidazole C'_6, C'_5), 7.1-6.7 (m, 5H, aromatic C_5, C_4, pyrimidine C_5, benzimidazole C'_7, C'_4,); EI-MS (m/z): 403 $[M]^{+1}$.

RESULTS AND DISCUSSIONS

In order to prepare the conceptual compounds 4a-z, the keystone compound 2 was synthesized from commercially available 1, 3-benzothiazol-2yl-acetonitrile 1. 2 was synthesized by following a known procedure in which 2,4-dichloro-pyrimidine was made to react with anion of 1 resulted in situ by sodium hydride mediated abstraction of proton in nitrogen atmosphere in THF. The IR spectrum of 2 (KBr pellet) showed a sharp medium intense band at 3385 cm^{-1} due to NH stretching. Aromatic CH stretching band was noticed at 3110 cm^{-1}. C=C stretching peak was found at 1630 cm^{-1} while C=N stretching band was located at 1610 cm^{-1}. NMR spectrum of the

same revealed a broad singlet peak at δ 8.5 is due to NH resonance of benzothiazole ring and a doublet appeared at δ 8.1 is assigned for C6 proton of pyrimidine. Peaks for protons of C4 and C7 of benzothiazole moiety were observed as doublets at 7.93 and 7.62 respectively. Two multiplets integrated for two protons were seen in the range of 7.4-7.2 ppm and assigned for C5 and C6 protons of benzothiazole ring. Comparatively, the proton of C5 of pyrimidine ring was observed downfield at 6.8 ppm as doublet. This molecule was reported already and our observed melting point was coinciding with literature melting point. Compound 2 was further subjected for nucleophilic substitution with numerous primary amines. The displacement of chlorine group was effectively done to get desired compounds 3a-z in good yields in ethanol at reflux temperature in presence of triethylamine which removes the liberated hydrogen chloride. All compounds 3a-z showed similar IR spectral profiles and retained some of the important stretching vibrations. The ^{1}H NMR spectrum of compounds 3a-z showed broad peak in the range of 11.5-13 δ which is due to NH of benzothiazole, a double at ~8.0 ppm is due to C6 of pyrimidine ring. Other distinguished NMR peaks were resultant of respective groups. To highlight some of the important NMR peaks, all the benzyl derivatives 4h-k exhibited the presence of benzylic CH_2 protons in the range of 3.5- 4.0 ppm and compounds 4p, 4v, 4w, 4x and 4z which bear heterocyclic ring with NH group showed additional broad beak at ~ 9.0 ppm. In the final step which resulted in desired compounds 4a-z, alkaline hydrolysis of 3a-z was carried in methanol, subsequently acidified and extracted with ethyl acetate. All the compounds 4a-z displayed a broad band in the range of 3600-3300 cm^{-1} which confirmed the hydrolysis of nitrile group and also noticed the absence of IR band for nitrile group at ~2200 cm^{-1}. Similarly, ^{1}H NMR spectrum of 4a-z reflected the hydrolysis which has been confirmed by the presence of broad peak in the range of δ 8.0-9.0 which is attributed to OH proton.

All the twenty six synthesized compounds have been screened for ascertaining anti-inflammatory activity by adopting carrageenan induced rat paw model and detail profile of activity has been listed in table 2. All the compounds exhibited a trend of increase in activity from 1st hour to second hour. Among them twenty compounds demonstrated peak activity at the second hour and rest of the molecules showed peak activity at 3rd hour of carrageenan challenge. The prototype unsubstituted compound 4a displayed 23.4% and 51.3% at 1st and 2nd hour respectively. Substitution with diverse set of groups at R1 resulted in enhanced anti-inflammatory activity of most of the compounds. However, compounds 4g, 4m, 4s and 4x showed lesser activity at the 2nd hour and 4b, 4j and 4m at the 4th hour when compared 4a. Replacement of amine by cyclopropylamine or 4-bromoaniline has marginally increased the activity at 2nd hour, but compound 4c showed significant antiiflammatory activity at the 3rd hour. Substitution at the 2nd position of phenyl group augmented activity significantly as witnessed with compounds 4e and 4f, nevertheless flexible electron withdrawing group benzophenone as in 4g did not show the comparable activity with respect to aforementioned compounds. In order to know further the effect of flexibility, a methylene group was inserted between amine and aromatic ring as in compounds 4h-4n. With the presence of benzyl group as in 4h, a drastic increase in potency from the 1st hour has been noticed. This compound is found to be most potent among the phenyl and benzyl substituted compounds and to know further the influence of substitution on benzyl ring, a fluoro and chloro group was inserted as in 4i and 4j which resulted in diminished activity however substituting with methoxy group enhanced the activity substantially. A methyl group on α carbon of benzyl group as in 4l was not superior when compared with 4h and even compounds like 4m. Aforementioned results further encouraged to synthesize compounds with diverse set of heterocyclic amines including fused heterocyclic ring systems. Among the five membered heterocyclic amines 4n-p, 4o with isoxazole ring exhibited better activity, this compound demonstrated 81.9% and 78.5%

inhibition at 2nd and 3rd hour respectively. Most of the six membered and fused heterocyclic ring bearing compounds showed greater than 40% activity at the first hour. Compound 4q was found to be most potent among the all synthesized compounds, with 89.4% rat paw protection against the carrageenan induced edema at the 3rd hour and even retained 53% activity at the 4th hour indicating prolonged activity of this compound. Numerous compounds with fused rings retained significant activity; 4u with benzothiadiazole group displayed 80.9% rat paw protection at 2nd hour and further retained 71.3% potency at the 3rd hour. Replacing this group by 2-mercaptobenzimidazole as in 4v retained the significant activity, however it was lesser potent than 4u. Compound 4x with indazole group was inferior among the compounds bearing fused rings, benzothiazole and benzimidazole containing compounds 4y and 4z possessed greater than 70% antiinflammatory activity at the 2nd hour of carrageenan administration.

CONCLUSION

In summary, a new series of compounds containing benzothiazole scaffold has been synthesized, characterized and screened for anti-inflammatory activity in rat paw edema model. Simple and reported chemical reactions were followed to synthesize the desired compounds, the synthesis of intermediates 3a-z afforded higher yields and hydrolysis of 3a-z ceded corresponding acids 4a-z in quantitative yields. Introduction of heterocyclic amine group at 2nd position of pyrimidine influenced to increase the anti-inflammatory activity in numerous compounds and compound 4q was found to be most potent among all synthesized compounds. Further detail investigation about the mechanism of action and pharmacokinetics of 4a-z compounds may further help in improving potency and safety of compounds.

CONFLICT OF INTEREST

The authors express no conflict of interest.

ACKNOWLEDGEMENT

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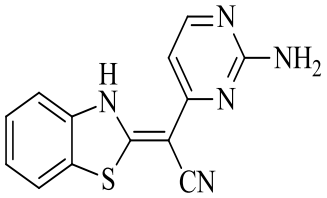


Figure 1: Compound AS601245

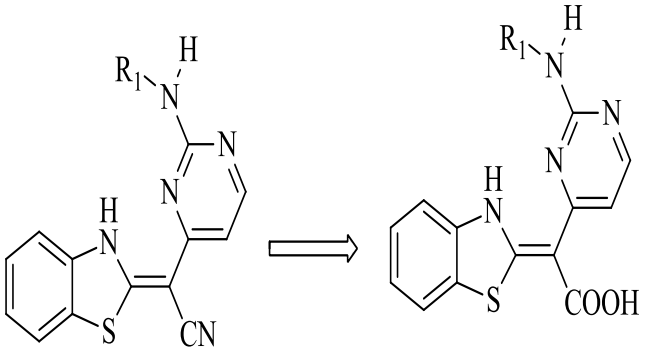
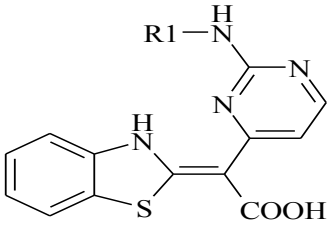


Figure 2: Benzothiazol-2-ylidinyl acetic acid compounds as new anti-inflammatory agents

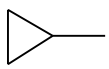
Table 1: Physical properties of benzothiazole derivatives

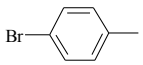
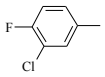
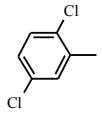
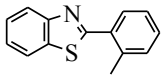
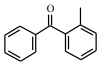
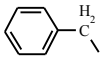
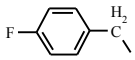
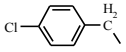
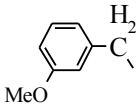
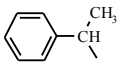
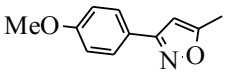
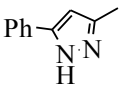
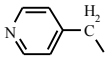
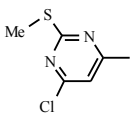
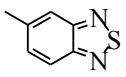
Comp. No	Mol. Form	Mol. Wt	% Yield	m. p. (°C)	R_f	cLogP
4a	$C_{13}H_{10}N_4O_2S$	286.05	66.2	258-260	0.37	1.648
4b	$C_{16}H_{14}N_4O_2S$	326.37	89	184-186	0.46	2.243
4c	$C_{19}H_{13}BrN_4O_2S$	439.99	42.8	194-196	0.27	4.745
4d	$C_{19}H_{12}ClFN_4O_2S$	414.035	66.2	228-230	0.32	4.632
4e	$C_{19}H_{12}Cl_2N_4O_2S$	430.00	57.5	239-241	0.38	5.032
4f	$C_{26}H_{17}N_5O_2S_2$	495. 08	69	218-220	0.28	6.184
4g	$C_{26}H_{18}N_4O_3S$	466.10	78.5	261-263	0.24	5.125
4h	$C_{20}H_{16}N_4O_2S$	376.43	49.1	259-261	0.42	3.68
4i	$C_{20}H_{15}FN_4O_2S$	394.42	73	265-267	0.41	3.838
4j	$C_{20}H_{15}ClN_4O_2S$	410.87	56.8	219-221	0.28	4.238
4k	$C_{21}H_{18}N_4O_3S$	406.46	68.4	197-199	0.29	3.777
4l	$C_{21}H_{18}N_4O_2S$	390.45	42.6	246-248	0.31	3.998
4m	$C_{25}H_{20}N_4O_2S$	440.51	59.9	262-264	0.27	4.996
4n	$C_{17}H_{14}N_4OS$	322	79	216-218	0.39	2.296
4o	$C_{23}H_{17}N_5O_4S$	459.48	67.2	211-213	0.29	4.249
4p	$C_{22}H_{16}N_6O_2S$	428.47	49.7	231-233	0.32	4.463
4q	$C_{18}H_{13}N_5O_2S$	363.39	47.5	199-201	0.39	2.579
4r	$C_{19}H_{15}N_5O_2S$	377.42	67	199-201	0.36	2.343
4s	$C_{17}H_{12}N_6O_2S$	364.38	84	198-200	0.37	1.958
4t	$C_{18}H_{13}ClN_6O_2S_2$	444.92	54.5	201-203	0.28	4.548
4u	$C_{19}H_{12}N_6O_2S_2$	420.47	62.5	216-218	0.36	4.911
4v	$C_{20}H_{14}N_6O_2S_2$	434.49	52.7	242-244	0.31	3.859
4w	$C_{22}H_{17}N_5O_2S$	415	71.6	201-203	0.29	3.793
4x	$C_{20}H_{14}N_6O_2S$	402.08	46.5	197-199	0.38	3.437
4y	$C_{21}H_{15}N_5O_2S_2$	433.51	58.4	216-218	0.23	5.599
4z	$C_{20}H_{14}N_6O_2S$	402.43	56.5	214-216	0.31	3.674

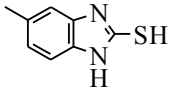
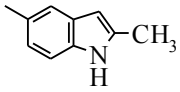
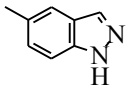
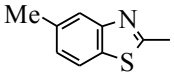
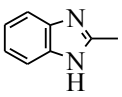
Table 2: Anti-inflammatory activity of benzothizole compounds 4a-z



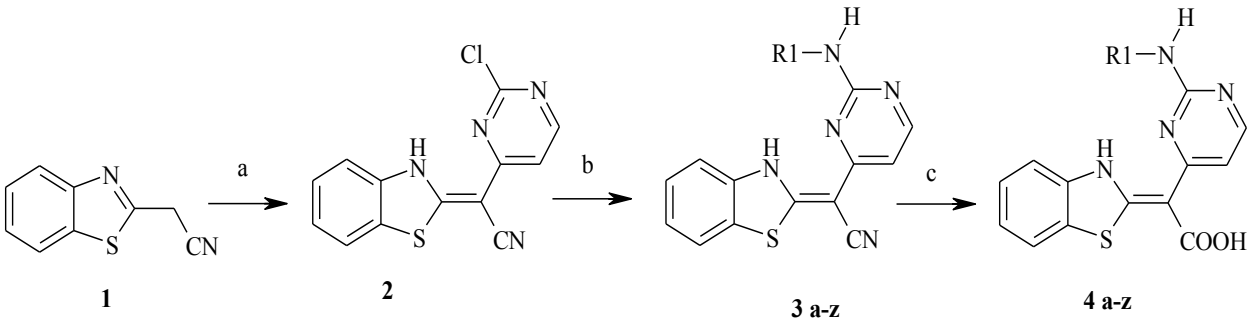
4 a-z

Sr. No.	Substituent	Paw edema, SEM and % inhibition			
		1 hr	2 hr	3 hr	4 hr
4a	$-NH_2$	0.36±0.036 23.4*	0.43 ±0.37 51.3**	0.52±0.027 34.2**	0.38±0.021 28.7*
4b		0.033±0.45 47.7**	0.018± 0.36 57.6**	0.35±0.51 40**	0.24±0.43 27.3*

4c		0.019± 0.37 48.4**	0.044± 0.38 57.3**	0.061± 0.27 68.6***	0.074± 0.35 40.4**
4d		0.036±0.48 46.36**	0.027±0.30 66.3**	0.012±0.34 51.8**	0.024±0.4 36.7*
4e		0.035±0.23 69.7**	0.044±0.27 74.1***	0.027±0.25 70.6***	0.019±0.3 46.3**
4f		0.029± 0.61 66.3**	0.053±0.67 81.9***	0.148±0.63 78.5***	0.17±0.8 52.2**
4g		0.026 ± 0.43 39.4**	0.028± 0.45 47.7**	0.018±0.44 58.8**	0.011±0.48 30.4**
4h		0.042± 0.26 74.3***	0.05 ± 0.67 79.6***	0.14±0.49 80.3***	0.22±0.6 60.2**
4i		0.018 ± 0.41 51.8**	0.017±0.41 53.9**	0.055 ± 0.38 57.3**	0.072 ± 0.5 41.9**
4j		0.055± 0.39 46.2**	0.015± 0.43 55.8**	0.035 ± 0.38 35.8*	0.045± 0.42 20.1*
4k		0.038± 0.28 72.9***	0.03± 0.48 79.6***	0.11±0.29 75.4***	0.2±0.33 54.4**
4l		0.019± 0.27 68.6**	0.025± 0.23 74.1***	0.042 ± 0.26 60.74**	0.055 ± 0.3 40.2**
4m		0.012±0.43 41.7**	0.019±0.42 50.6**	0.024±0.42 43.8**	0.04±0.46 22.4*
4n		0.022± 0.31 63.0**	0.029±0.26 70.8***	0.035±0.33 62.9**	0.045±0.44 37.9**
4o		0.041 ± 0.29 70.3***	0.016±0.35 81.9***	0.017±0.42 78.5***	0.028±0.5 55.1**
4p		0.035± 0.63 60.5**	0.148±0.49 73.46***	0.079±0.41 71.3***	0.13±0.5 56.7**
4q		0.03± 0.49 85.6***	0.03 ± 0.63 87.9***	0.03± 0.7 89.4***	0.09 ± 0.8 53.1**
4r		0.033 ± 0.20 65.5**	0.079± 0.52 73.0***	0.055± 0.42 78.8 ***	0.066± 0.5 54.8**
4s		0.021 ± 0.39 46.2**	0.021±0.42 50.6**	0.032 ± 0.44 48.8**	0.045± 0.41 34.4**
4t		0.025 ± 0.18 72.8***	0.027±0.26 80.9***	0.025±0.17 69.8**	0.032±0.2 43.2**
4u		0.02± 0.41 75.5***	0.01± 0.55 83.4***	0.08 ± 0.5 71.3***	0.11 ± 0.6 53.1**

4v		0.065± 0.29 72.9***	0.115±0.35 79.6***	0.24 ± 0.34 75.4***	0.4± 0.41 51.3**
4w		0.044± 0.28 64.5**	0.02±0.32 71.3***	0.08± 0.3 65.4**	0.15± 0.5 43.2**
4x		0.067 ± 0.41 43.9**	0.054±0.42 50.6**	0.045±0.40 59.2**	0.03±0.5 32.6*
4y		0.024±0.27 59.57**	0.025±0.25 70.6***	0.038±0.27 68.2**	0.05±0.33 41.5**
4z		0.017±0.45 65.5**	0.025 ± 0.56 79.6***	0.079±0.46 71.3***	0.095±0.54 48.9**
Std	Diclofenac sodium	0.029±0.036 (35.59)*	0.0207±0.090 (58.18)**	0.0212±0.069 (62.67)***	0.0216±0.56 (64.47)***

Results expressed in mean ± SEM. (n=6). ANOVA followed by Dunnett's test. ***p<0.001, ** p<0.01, *p<0.05 when compared to control group.



Reagents and Conditions: a) 2, 4-dichloropyrimidines, NaH, THF, 10h;
b) $R1NH_2$, Et_3N, CH_2Cl_2, 3h; c) NaOH, MeOH, 1h

Figure 3: Scheme synthesis of benzothiazolyl acetic acid derivatives

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