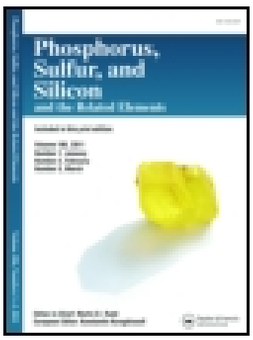

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Synthesis, characterization and evaluation of novel benzothiazole clubbed chromene derivatives for their anti-inflammatory potential

Divyani Gandhi[a], Dinesh Kr. Agarwal[b], Priyanka Kalal[a], Amit Bhargava[b], Dinesh Jangid[c], and Shikha Agarwal[a]

[a]Department of Chemistry, Mohanlal Sukhadia University, Udaipur, India; [b]Department of Pharmacy, Bhupal Noble University, Udaipur, India; [c]Department of Chemistry, University of Rajasthan, Jaipur, India

ABSTRACT

A series of chromene derivatives (5a–f) were prepared by multistep synthesis process using 2-[3-phenyl prop-2-ene nitrile] 1,3-benzothiazole and dimedone using piperidine as catalyst in ethanol. The reaction was found to proceed via Knoevenagel condensation of aldehydes with benzothiazole, followed by the elimination to afford the 2-(benzo[d]thiazol-2-yl)-3-(aryl)acrylonitrile, which then undergoes Michael addition with 5,5-dimethyl-1,3-cyclohexanedione, followed by intramolecular O-cyclization to give the products. The structures of all novel constructed derivatives were corroborated by elemental analysis and spectral data (FT-IR, ^{1}H-NMR, ^{13}C-NMR and Mass). Subsequently, the compounds were tested for their *in-vivo* anti-inflammatory activity. This study revealed that these synthesized derivatives tend to have significantly anti inflammatory activity and shall prove as structural templates in the design and development of new anti inflammatory drugs.

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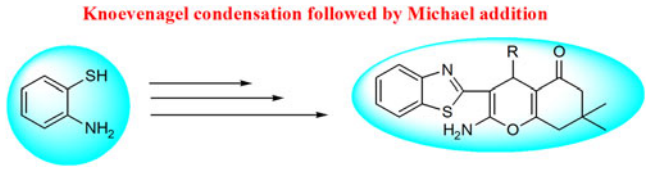
KEYWORDS
2-Aminobenzenethiol; chromene; carrageenan-induced paw edema; anti inflammatory

GRAPHICAL ABSTRACT



Introduction

Inflammation is a characterized response actuated by noxious stimuli and situations such as infection, injury[1] and has been associated with many diseases *viz.* asthma, allergy, diabetes, cancer.[2–5] Numerous anti-inflammatory drugs have been approved for the treatment of inflammation (aspirin, corticosteroids, indomethacin, diclofenac, ibuprofen, pranoprofen, celecoxib, roficoxib).[6] Among these, non-steroidal anti-inflammatory drugs (NSAIDs) are effective anti-inflammatory agents and analgesics that inhibit the biosynthesis or release of prostaglandins from arachidonic acid by suppressing cyclooxygenases.[7,8] Since these drugs inhibit the production of prostaglandins and may cause serious side effectts, such as gastrointestinal irritation, ulceration, hepatotoxicity, acute renal failure, hypertension, and even heart failure.[9–12] Therefore, there is urgent requisite to develop new and safer therapeutic agents and for the purpose, we have synthesized benzothiazole clubbed chromene derivatives as potential anti-inflammatory agents.

Chromene constitute the basic backbone of various types of polyphenols and widely found in natural alkaloids, tocopherols, flavonoids, and anthocyanins.[13] A number of drug molecules bearing chromene unit are biologically active natural products and promising synthetic compounds in the field of medicinal, agrochemical, cosmetics and pigment industries and in the treatment of various ailments such as hypertension, asthma, ischemia and urinary incontinence. They possess important biological properties such as antitumor,[14] antivascular,[15] TNF-α inhibitor,[16] antifungal,[17] anticoagulant,[18] estrogenic,[19] antiviral,[20] anti-helminthic,[21] anticancer,[22] anti-inflammatory,[23] antimalarial[24] and anticonvulsant[25] activities etc.

A literature survey reveals that chromene backbone in assimilation with benzothiazole significantly increases the biological activity and broadens their spectrum of activity Figure 1. The benzothiazole moiety, owing to its diversified molecular design, possesses remarkable biological properties like anticancer,[26] antibacterial,[27] anticonvulsant,[28,29] antidiabetic,[30] anti-HIV,[31] antimalarial,[32] anthelmintic,[33]

CONTACT Shikha Agarwal shikha_urj@yahoo.com
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antifungal,[34] anti-inflammatory,[35] antileishmanial[36] and antitubercular[37] activities. Keeping in light all the above findings, we have reported the series of 2-amino-3-(1,3-benzothiazole-2-yl)-4-phenyl-7,7-dimethyl-4,6,7,8-tetrahydro-5*H*-chromene-5-one derivatives (5a–f).

Result and discussion

Chemistry

The synthesis of compounds 3, 4(a–f), 5(a–f) have been outlined in Schemes 1 and 2. The structures of all the synthesized compounds were confirmed via IR and NMR Spectroscopy. The formation of titled compounds (5a–f) (Figure 2) was achieved in three steps. Initially, synthesis of 2-cynomethylbenzothiazole (3) was undertaken by reacting an equimolar mixture of 2-amino benzenethiol and malononitrile in presence of glacial acetic acid using ethanol as solvent. Different derivatives of 2-[3-phenyl prop-2-ene nitrile] 1,3-benzothiazole (4a–f) were prepared by reacting substituted aldehyde with (3) taking pyridine as catalyst in absolute alcohol. The compounds obtained (4a–f) were further reacted with dimedone in presence of piperidine using ethanol as suitable solvent which resulted in the formation of titled compounds (5a–f) in good yields. The mechanism of reaction proceeds via Knoevenagel condensation of various substituted aldehyde with 2-cynomethylbenzothiazole followed by elimination to obtain 2-[3-phenyl prop-2-ene nitrile] 1,3-benzothiazole (4) which then undergoes Michael addition with 5,5-dimethyl-cyclohexane-1,3-dione to give intermediate followed by an intramolecular ortho cyclization resulting into product (5).[38]

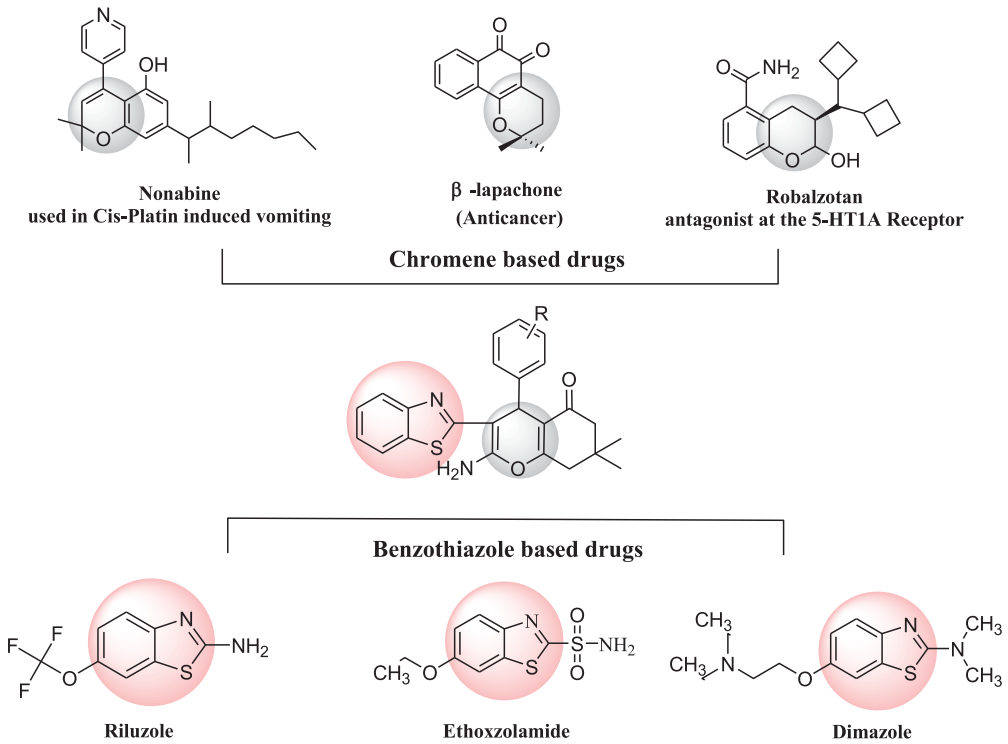
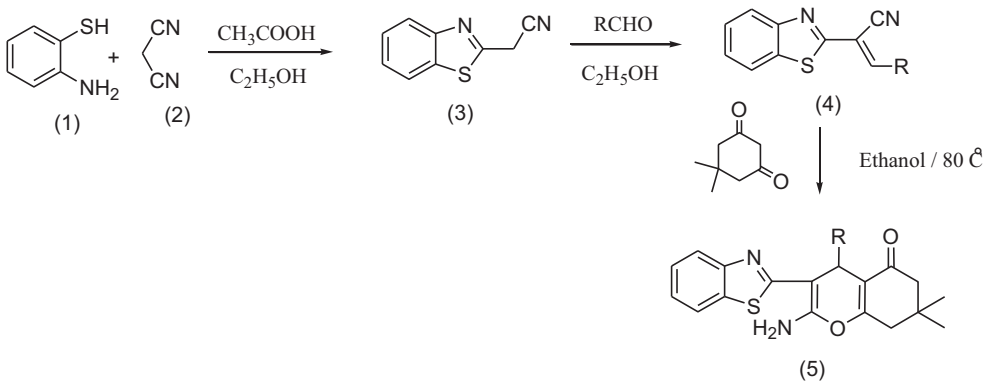
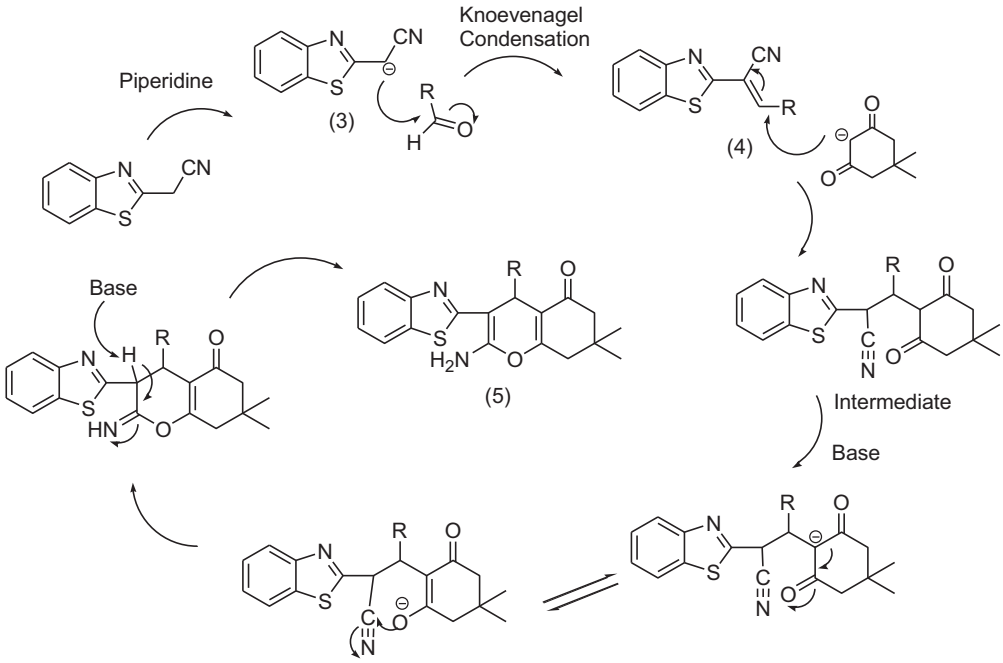


Figure 1. Drug based designing of benzothiazole clubbed chromene.



Scheme 1. Synthesis of 2-amino-3-(1,3-benzothiazole-2-yl)-4-substituted phenyl-7,7-dimethyl-4,6,7,8-tetrahydro-5*H*-chromene-5-ones.



Scheme 2. Plausible mechanism for synthesised compounds.

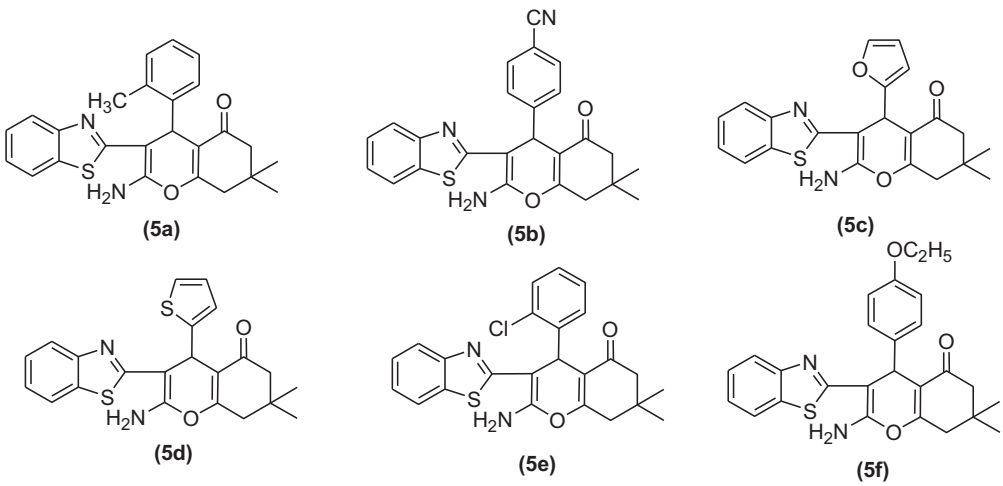


Figure 2. Library of synthesized benzothiazole clubbed chromene derivatives.

FT-IR

FT-IR spectra of (5a–f) showed the appearance of a band at around 3475 cm^{-1} indicating the presence of primary amine group and a weak absorption stretching vibration band observed at 2750 cm^{-1} confirmed the presence of methylene group in the final motif. A sharp intensified band at 1670 cm^{-1} proved the presence of carbonyl group in the structure (5a–f).

NMR

^{1}H NMR spectrum observed for the compounds under investigation exhibited several absorption peaks corresponding to the desired protons. The proton of secondary amine appearing at δ 8.40 ppm confirmed its presence on nitrogen attached to aromatic ring by single bond, whereas the peak appearing particularly at δ 0.9 & 1.1 ppm confirmed the protons of methyl group in the final structure 5(a–f). The protons appearing in the chromene formation confirmed its absorption peak at δ 5.01 ppm indicating the presence of chromene proton attached to the aldehyde. The geometry of each proton attached with C=C double bond of aromatic rings corresponded to the δ values between 7.24 and 7.56 ppm.

13C NMR spectral data helped to confirm the formation of desired structures (5a–f). The carbon atoms in contact to the electronegative element oxygen in the carbonyl group appeared downfield, nearly at δ 195.0 ppm and the carbon in the environment of primary amine and chromene ring was seen to appear at δ 164.0 and 162.5 ppm respectively than the one near to unsaturated carbon.

Anti-inflammatory screening

All the newly synthesized compounds (5a–f) were subjected to in vivo anti-inflammatory screening using the

carrageenan induced paw edema in rats. Diclofenac were used as standard. The results of in vivo anti-inflammatory activity are summarized in (Table S1 and S2, Supplemental Materials). Among the synthesized compounds, compound 5e exhibited 52.77% inhibition of edema after 2 h from time administration and found to be quite superior in activity. Compounds 5f, 5d, 5c and 5a showed 47.05%, 40.0%, 38.88% and 31.25% inhibition of edema after 2 hrs from time of action respectively. Compound 5b showed 18.18% inhibition of edema and was found to be weak in its action. It was also found that activity increased 4 h post carrageenan injection in a similar manner to that of diclofenac with % age of inhibition of paw edema respectively. The experimental data validates with the theoretical data as described in PASS (Table S3). The data also revealed that the activity of compounds follow the order 5d > 5e > 5f > 5c > 5b > 5a.

Pharmocologically, the drug (Diclofenac) is categorized as a NSAID (Nonsteroid anti-inflammatory drug) which acts at the periphery and not at CNS (Central Nervous System). These drugs block the synthesis of ecosanoids by acting at the site of tissue injury which finally blocks the cyclooxygenase (COX) pathway. Thus, probable mechanism of action of carrageenan induced edema is bi-phasic i.e. the first phase is attributed to release of histamine-HT, kinins in the first hour, while the second phase is the release of prostaglandin like substance in 3–4 h. Further the chromene derivatives exhibit structure dependent anti-inflammatory properties which are evidenced by all the six compounds through mechanism like NSAIDS.

Experimental

Chemistry

All the chemicals required for synthesis like 2-aminobenzenethiol, malononitrile, substituted aldehyde, dimedone were commercially procured from Merck Ltd., Sigma-Aldrich and Hi-Media and used without further purification. The melting points were determined in open capillary tubes and are reported uncorrected. The IR spectra were recorded on Shimadzu FT-IR Spectrometer. The ^{1}H NMR spectra (DMSO-d_6) were scanned on a Bruker Avance II (400 MHz) spectrometer using TMS as internal standard and the chemical shifts are expressed in δ, ppm. The mass spectra were recorded on a Waters Xevo G2-S QTof with UPLC, Sophisticated Analytical Instruments Facility, Chandigarh, India. The purity of the synthesized compounds was checked by TLC using silica gel-G plates, n-hexane-ethyl acetate (7:3) as developing solvent system and UV light used as a visualizing agent. The Supplemental Materials contains sample ^{1}H and ^{13}C NMR spectra for products 5 (Figures S1–S7).

Synthesis and physical data

Synthesis of 1,3-benzothiazol-2-ylacetonitrile (3)

A mixture of 2-aminobenzenethiol (0.01 mol), malononitrile (0.01 mol) and glacial acetic acid (0.01 mol) was dissolved in minimum amount of absolute alcohol and the reaction mixture was magnetically stirred at room temperature until no further precipitation was produced and mixture was allowed to stand overnight. Brown colored crystals were obtained, filtered and recrystallized from ethanol.

General procedure for the synthesis of 2-[3-phenyl prop-2-ene nitrile] 1, 3-benzothiazole (4a–4f)

A mixture of 2-cynomethylbenzothiazole (0.01 mol) (3) and thiophene-2-carbaldehyde/furfural/o-chloro benzaldehyde/p-cyano benzaldehyde/p-ethoxy benzaldehyde/o-methyl benzaldehyde (0.01 mol) in a minimum amount of absolute alcohol with a catalytic amount of piperidine (0.5 mL) was magnetically stirred at room temperature until no further precipitation occurred, was filtered, washed with ethanol and recrystallized to yield the products 4.

2-(1,3-benzothiazol-2-yl)-3-(2-methylphenyl)prop-2-enenitrile (4a). Yield: 95%; Yellow; m.p. 146–148 °C; IR (KBr, cm^{-1}): 3083, 3015 (Ar–CH=C, –CH=C Stretching), 2925, 2855 (CH_3, Stretching), 2210 (CN, Stretching); ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 2.13 (s, 3H), 7.20-7.30 (m, 8H, Ar-H), 7.93 (s, 1H, –CH=C). Anal. calcd for $C_{17}H_{12}N_2S$; C, 73.88; H, 4.38; N, 10.14 Found; C, 73.53; H, 4.62; N, 10.55; MS (EI): m/z 276.07 [M+].

2-(1,3-benzothiazol-2-yl)-2-cyanoethenyl]benzonitrile (4b). Yield: 92%; pale yellow; m.p. 182–184 °C; IR (KBr, cm^{-1}): 3093, 3027 (Ar–CH=C, –CH=C Stretching), 2228 (CN, Stretching); ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 7.47-7.63 (m, 8H, Ar-H), 8.32 (s, 1H, –CH=C). Anal. calcd for $C_{17}H_9N_3S$; C, 71.06; H, 3.16; N, 14.62 Found; C, 71.43; H, 3.56; N, 14.78; MS (EI): m/z 287.05 [M+].

2-(1,3-benzothiazol-2-yl)-3-(furan-2-yl)prop-2-enenitrile (4c). Yield: 90%; bright yellow; m.p. 140–142 °C; IR (KBr, cm^{-1}): 3078, 3015 (Ar-CH=C, –CH=C Stretching), 2218 (CN, Stretching), 1275 (C-S, Stretching); ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 6.54 (dd, 1H, J = 3.5, 1.8 Hz), 7.30 (dd, 1H, J = 3.5, 0.9 Hz), 7.33-7.42 (m, 4H, Ar-H), 7.90 (1H, dd, J = 1.8, 0.9 Hz), 7.36 (s, 1H, CH=C). Anal. calcd for $C_{14}H_8N_2OS$; C, 66.65; H, 3.20; N, 11.10 Found; C, 66.32; H, 3.45; N, 11.53; MS (EI): m/z 252.29 [M+].

2-(1,3-benzothiazol-2-yl)-3-(thiophen-2-yl)prop-2-enenitrile (4d). Yield: 96%; dull yellow; m.p. 170–172 °C, IR (KBr, cm^{-1}): 3085, 3020 (Ar–CH=C, –CH=C Stretching), 2222 (CN, Stretching), 1689 (C–O, Stretching). ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 7.30 (1H, dd, J = 7.2, 5.2 Hz), 7.34-7.88 (m, 4H, Ar-H), 7.74 (dd, J = 5.2, 1.3 Hz)), 7.99 (1H, dd, J = 7.2, 1.3 Hz), 7.72 (s, 1H, CH=C). Anal. calcd for $C_{14}H_8N_2S_2$; C, 65.97; H, 4.84; N, 6.41 Found; C, 65.92; H, 4.78; N, 6.55; MS (EI): m/z 268.01 [M+].

2-(1,3-benzothiazol-2-yl)-3-(2-chlorophenyl)prop-2-enenitrile (4e). Yield: 98%; Yellow; m.p. 155–157 °C; IR (KBr, cm^{-1}): 3090, 3020 (Ar–CH=C, –CH=C Stretching), 2220 (CN, Stretching); ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): δ 7.32-

7.53 (m, 8H, Ar-H), 8.08 (s, 1H, –CH=C); Anal. calcd for $C_{16}H_9ClN_2S$; C, 64.75; H, 3.06; N, 9.44 Found; C, 64.29; H, 3.53; N, 9.57; MS (EI): m/z 296.02 [M+], 298.02[M + 2].

2-(1,3-benzothiazol-2-yl)-3-(4-ethoxyphenyl)prop-2-enenitrile (4f). Yield: 88%; Green; m.p. 161–163 °C; IR (KBr, cm^{-1}): 3085, 3022 (Ar–CH=C, –CH=C Stretching), 2215 (CN, Stretching); ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 1.27 (t,3H, J = 7.0 Hz), 4.26 (q, 2H, J = 7.0 Hz), 7.42-7.52 (m, 8H, Ar-H), 8.00 (s, 1H, CH=C). Anal. calcd for $C_{18}H_{14}N_2OS$; C, 70.56; H, 4.61; N, 9.14 Found; C, 70.43; H, 4.32; N, 9.02; MS (EI): m/z 306.08 [M+].

General procedure for the synthesis of 2-amino-3-(1,3-benzothiazole-2-yl)-4-phenyl-7,7-dimethyl-4,6,7,8-tetrahydro-5H-chromene-5-one (5a–5f)

A mixture of 2-[3-phenyl prop-2-ene nitrile] 1, 3-benzothiazole (0.01 mol) (4a–4f) and dimedone (0.01 mol), dissolved in a minimum amount of ethanol was refluxed for 8–10 h using piperidine (0.5 mL) as a catalyst at 80 °C. The progress of the reaction was monitored by thin layer chromatography. After completion of the reaction, the mixture was poured into crushed ice with stirring. The obtained solid was filtered, dried and recrystallized with ethanol.

2-Amino-3-benzothiazol-2-yl-7,7-dimethyl-4-o-tolyl-4,6,7,8-tetrahydro-chromen-5-one (5a). Yield: 87%; Dark brown; m.p. 186–188 °C; IR (KBr, cm^{-1}): 3432 (N–H, Stretching), 3080, 2977, 2882 (Ar–CH=C, Stretching), 1668 (–C=O, Stretching); ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 0.75 (s, 3H, CH_3), 0.82 (s, 3H, CH_3), 2.00 (d, 1H, J = 18.2 Hz), 2.15 (d, 1H, J = 18.2 Hz), 2.23 (s, 3H, CH_3), 2.38 (d, 1H, J = 15.6 Hz), 2.45 (d, 1H, J = 15.6 Hz)), 4.28 (s, 1H, chromene-H), 7.06-7.83 (m, 8H, Ar–H), 8.32 (s, 2H, NH_2); ^{13}C NMR (400 MHz, DMSO-d_6) δ(ppm): 19.60 (CH_3, phenyl group), 28.12 (CH_3 at chromene), 29.52 (CH_3 at chromene), 31.98, 37.85 (CH, chromene), 39.82 (CH_2, chromene), 50.25 ($COCH_2$ chromene), 112.92, 122.85, 122.91, 122.47, 124.96, 126.46, 126.80, 129.23, 129.65, 131.2, 132.32, 134.77, 139.24, 151.54, [162.23, 163.44 (C-O-C)], 164.94 (C=N), 195.56(C=O); Anal. calcd for $C_{25}H_{24}N_2O_2S$; C, 72.09; H, 5.81; N, 6.73 Found; C, 72.06; H, 5.80; N, 6.72; MS (EI): m/z 416.88 [M+].

4-(2-Amino-3-benzothiazol-2-yl-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromen-4-yl)-benzonitrile (5b). Yield: 90%; Coffee brown; m.p. 210–212 °C; IR (KBr, cm^{-1}): 3475 (N–H, Stretching), 3098, 2985, 2892 (Ar–CH=C, Stretching), 1690 (–C=O, Stretching), 2200 (CN, Stretching), ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 1.10 (s, 3H, CH_3), 1.21 (s, 3H, CH_3), 2.20 (d, 1H, J = 18.2 Hz), 2.29 (d, 1H, J = 18.2 Hz), 2.52 (d, 1H, J = 15.6 Hz), 2.56 (d, 1H, J = 15.6 Hz)), 4.89 (s, 1H, chromene-H), 7.30-8.22 (m, 8H, Ar–H), 8.54 (s, 2H, NH_2); ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 28.76 (CH_3 at chromene), 29.65 (CH_3 at chromene), 32.09, 37.99 (CH, chromene), 39.77 (CH_2, chromene), 50.58 ($COCH_2$ chromene), 112.22, 112.78, 118.83 (CN), 122.65, 122.92, 124.75, 126.56, 126.95, 126.45, 129.88, 132.45, 132.98, 134.55, 145.85, 151.44, [162.76, 163.66 (C-O-C)], 164.89 (C=N), 195.93 (C=O); Anal. calcd for $C_{25}H_{21}N_3O_2S$; C, 70.24; H, 4.95; N, 9.83 Found; C, 70.22; H, 4.92; N, 9.80; MS (EI): m/z 427.74 [M+].

2-Amino-3-benzothiazol-2-yl-4-furan-2-yl-7,7-dimethyl-4,6,7,8-tetrahydro-chromen-5-one (5c). Yield: 72%; Black; m.p. 172–174 °C; IR (KBr, cm^{-1}): 3450 (N-H, Stretching), 3075, 2962, 2872 (Ar–CH=C, Stretching), 1675 (–C=O, Stretching), ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 0.99 (s, 3H, CH_3), 1.12 (s, 3H, CH_3), 2.35 (d, 1H, J = 18.2 Hz), 2.40 (d, 1H, J = 18.2 Hz), 2.58 (d, 1H, J = 15.6 Hz), 2.63 (d, 1H, J = 15.6 Hz)), 5.05 (s, 1H, chromene-H), 6.32 (1H, dd, J = 2.9, 1.8 Hz), 6.82 (1H, dd, J = 2.9, 0.8 Hz), 7.12 (1H, dd, J = 1.8, 0.8 Hz), 7.45-8.56 (m, 4H, Ar–H), 8.96 (s, 2H, NH_2); ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 28.65 (CH_3 at chromene), 29.36 (CH_3 at chromene), 31.80, 38.06 (CH, chromene), 39.89 (CH_2, chromene), 50.47 ($COCH_2$ chromene), 104.43, 111.50, 112.65, 122.45, 122.68, 124.74, 126.08, 126.39, 126.87, 126.67, 126.93, 129.34, 129.76, 134.89, 142.13, 145.52, 151.58, 154.16, [162.65, 163.32(C–O–C)], 164.75 (C=N), 195.08 (C=O); Anal. calcd for $C_{22}H_{20}N_2O_3S$; C, 67.33; H, 5.14; N, 7.14 Found; C, 67.30; H, 5.12; N, 7.11; MS (EI): m/z 392.59 [M+].

2-Amino-3-benzothiazol-2-yl-7,7-dimethyl-4-thiophen-2-yl-4,6,7,8-tetrahydro-chromen-5-one (5d). Yield: 84%; Black; m.p. 180–182 °C; IR (KBr, cm^{-1}): 3485 (N-H, Stretching), 3087, 2977, 2878 (Ar–CH=C, Stretching), 1695 (–C=O, Stretching), ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 0.85 (s, 3H, CH_3), 0.93 (s, 3H, CH_3), 2.20 (d, 1H, J = 18.2 Hz), 2.32 (d, 1H, J = 18.2 Hz), 2.38 (d, 1H, J = 15.6 Hz), 2.46 (d, 1H, J = 15.6 Hz)), 4.96 (s, 1H, chromene-H). 7.00 (1H, dd, J = 8.4, 5.0 Hz), 7.21 (1H, dd, J = 8.4, 1.3 Hz), 7.34 (1H, dd, J = 5.0, 1.3 Hz), 7.43-8.55 (m, 4H, Ar-H), 8.72 (s, 2H, NH_2); ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 28.56 (CH_3 at chromene), 29.06 (CH_3 at chromene), 31.83, 40.22 (CH, chromene), 41.02 (CH_2, chromene), 50.42 ($COCH_2$ chromene), 112.98, 122.78, 122.34, 123.98, 124.54, 125.54, 125.95, 126.33, 126.65, 127.76, 127.90, 128.43, 129.53, 133.76, 134.87, 143.79, 145.76, 151.39, [162.02, 163.99 (C–O–C)], 164.55 (C=N), 195.83 (C=O); Anal. calcd for $C_{22}H_{20}N_2O_2S_2$; C, 64.68; H, 4.93; N, 6.86 Found; C, 64.65; H, 4.90; N, 6.88; MS (EI): m/z 408.82 [M+].

2-Amino-3-benzothiazol-2-yl-4-(2-chloro-phenyl)-7,7-dimethyl-4,6,7,8-tetrahydro-chromen-5-one (5e). Yield: 82%; Red-brown; m.p. 203–205 °C; IR (KBr, cm^{-1}): 3456 (N–H, Stretching), 3073, 2970, 2724 (Ar–CH=C, Stretching), 1638 (–C=O, Stretching); ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 0.98 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 2.17 (d, 1H, J = 18.2 Hz), 2.26 (d, 1H, J = 18.2 Hz), 2.53 (d, 1H, J = 15.6 Hz), 2.58 (d, 1H, J = 15.6 Hz)), 4.92 (s, 1H, chromene-H), 7.08-8.13 (m, 8H, Ar–H), 8.40 (s, 2H, NH_2); ^{13}C NMR (400 MHz, DMSO-d_6) δ(ppm): 28.42 (CH_3 at chromene), 29.84 (CH_3 at chromene), 31.61, 37.35 (CH, chromene), 39.55 (CH_2, chromene), 50.17 ($COCH_2$

chromene), 115.73, 120.29, 120.79, 122.85, 123.44, 125.59, 126.16, 127.81, 129.70, 131.61, 132.80, 134.12, 142.52, 154.11, [161.82, 165.16 (C–O–C)], 168.23 (C=N), 195.25 (C=O); Anal. calcd for $C_{24}H_{21}ClN_2O_2S$; C, 65.97; H, 4.84; N, 6.41 Found; C, 65.95; H, 4.81; N, 6.39; MS (EI): m/z 436.98 [M+], 438.98 [M + 2].

2-Amino-3-benzothiazol-2-yl-4-(4-ethoxy-phenyl)-7,7-dimethyl-4,6,7,8-tetrahydro-chromen-5-one (5f).

Yield: 78%; Brick red; m.p. 195–197 °C, IR (KBr, cm^{-1}): 3466 (N-H, Stretching), 3092, 2983, 2888 (Ar–CH=C, Stretching), 1687 (–C=O, Stretching), ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 0.82 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 1.24 (t, 3H, CH_3, $J = 7.0$ Hz), 2.10 (d, 1H, $J = 18.2$ Hz), 2.16 (d, 1H, $J = 18.2$ Hz), 2.46 (d, 1H, $J = 15.6$ Hz), 2.51 (d, 1H, $J = 15.6$ Hz)), 4.02 (q, 2H, CH_2, $J = 7.0$ Hz), 4.66 (s, 1H, chromene-H), 7.12-8.22 (m, 8 H, Ar-H), 8.56 (s, 2H, NH_2); ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 14.73 ($CH_2\underline{C}H_3$), 28.44 (CH_3 at chromene), 29.72 (CH_3 at chromene), 31.67, 38.56 (CH, chromene), 39.09 (CH_2, chromene), 50.65 ($COCH_2$ chromene), 63.54 ($\underline{C}H_2CH_3$), 112.77, 116.27, 116.80, 122.32, 122.76, 124.44, 126.07, 128.12, 128.68, 129.36, 134.40, 145.98, 151.92, 161.56, [162.88, 163.39 (C–O–C)], 164.67 (C=N), 195.88 (C=O); Anal. calcd for $C_{26}H_{26}N_2O_3S$; C, 69.93; H, 5.87; N, 6.27 Found; C, 69.95; H, 5.80; N, 6.17; MS (EI): m/z 446.66 [M+].

Biological evaluation

Theoretical prediction of anti-inflammatory activity

The PASS computer program allows to estimate the probable profile of biological activity of a drug-like organic compound (whose molecular mass ranges from 50 to 1250 Da) based on its structural formula. The categorical description of biological activity as "active" or "inactive" is used in the PASS program. The biological activity types for which the probability to be revealed (Pa) and probability not to be revealed (Pi) are calculated. Pa and Pi values are independent, and their values vary from 0.000 to 1.000. It is reasonable that only those types of activities may be revealed by the compound, where Pa > Pi and so they are put into the biological activity spectrum. If Pa >0.7, the compound is likely to reveal its activity in the experiment, but in this case, the chance of being the analog of the known pharmaceutical agent is high. If Pa <0.5, the compound is unlikely to reveal this activity in the experiment, but if the presence of this activity is confirmed in the experiment, the compound might be a new chemical entity.[39,40]

PASS compares the structure of a new compound with structures of well-known biologically active substance and therefore, it is possible to estimate if a new compound may have a particular effect. It operates with many thousands of substances from the training set and provides a more objective estimation. Since only the structural formula of the chemical compound is necessary to obtain PASS predictions, this approach can be used at the earliest stage of the investigation. Structures of the title compounds were drawn through ACD labs Chem sketch software, submitted to the PASS online program (PASS Online-Way2drug) and the possible mechanisms of action as well as biological activities can be predicted.

Anti-inflammatory activity (carrageenan induced paw edema method)

Anti-inflammatory activity was determined by paw edema method in rats.[41] Experiments were performed on Wistar albino rats-with a body weight between 175 and 225 g at Bhopal Nobles' college of Pharmacy, Udaipur (Rajasthan). All the animal experiments were subjected to Institutional Animal Ethical Committee (870/ac/05/CPCSEA) and were conducted according to the guidelines of Experimental Animal Care issued by the Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The animals were housed in polypropylene cages and maintained on standard chow diet and water *ad libitum* and on 12h/12h light-dark cycle at temperature: 25 ± 2 °C, humidity: 45- 55% and ventilation: 10-12 exchanges/h.

The animals were starved overnight. To insure uniform hydration, the rats received 5 mL of water by stomach tube (controls) or the test drug dissolved or suspended in the same volume. Thirty minutes later, the rats were challenged by a subcutaneous injection of 0.1 mL of 1% solution of carrageenan into the plantar side of the right hind paw. The paw was marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. The paw volume was measured by using plethysmograph immediately after injection, and procedure was repeated at 1, 2, 3, 4 h after carrageenan injection. Rats were divided into nine groups containing six rats each.

In Group I, rats were given only vehicle, in Group II, rats were given carrageenan (0.1 mL of 1% mg/kg, bw) whereas in Group III, animal were given carrageenan (0.1 mL of 1% mg/kg, bw) single dose plus drug diclofenac (12.5 mg/kg bw) and Group IV-Group IX rats were given carrageenan (0.1 mL of 1% mg/kg, bw) plus synthesized compounds 5a–5f (200 mg/kg/day, bw) orally. The mean paw volume at different time intervals was calculated and compared with control and the percentage inhibition was calculated.

The percent reduction value calculated according to the following formula given below:-

$$\text{Anti–inflammatory activity \% reduction of edema} = [1 - Vt/Vc] \times 100$$

Where, Vt and Vc are the mean relative changes in the volume of paw edema in the test and control respectively.

Conclusion

In the present research work we have synthesized benzothiazole clubbed chromene derivatives and most of the compounds were found to possess outstanding anti-inflammatory properties. The practical data however matches with the theoretical (PASS) data and this clearly indicates that the synthesized molecules are potent anti-inflammatory molecules. On the basis of above results, alterations are made to optimize the

lead structure by introducing varied groups on the reactant itself, and this modification in future will help to develop much better anti-inflammatory heterocyclic scaffolds.

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Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Shikha Agarwal http://orcid.org/0000-0003-2946-0409

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