

# Synthesis, Antibacterial, Anti-Inflammatory and Antiplatelet Activities of Some Trisubstituted Thiazoles

**Musatakim Mansuri<sup>1</sup>, Aejaaz Ahmed<sup>2\*</sup>, Arshad Shaikh<sup>2</sup>, Navedul Haque<sup>3</sup>, Avinash K Sheth<sup>4</sup>, Khurshid I Molvi<sup>2</sup>**

**Abstracts:** Sets of trisubstituted thiazoles (*4a-4i*) were synthesized by reaction of substituted N-(piperidinyl/ piperazinyl/N-methylpiperazinyl-4-thioyl) benzamide (*3*) with 3-(bromoacetyl) coumarin, respectively. The (*3*) were synthesized by nucleophilic addition of benzoyl isothiocyanate/ substituted benzoyl isothiocyanate and piperidine/ piperazine/4-methylpiperazine in equimolar quantity at reflux temperature. The synthesized compounds (*4a-4i*) were screened for their *in vitro* antibacterial activity against *S. aureus*, *B. subtilis* and *E. coli* using ciprofloxacin as standard drug. The targeted compounds (*4a-4i*) were evaluated for their *in vivo* anti-inflammatory activity in carrageenin-induced rat hind paw oedema model at three graded doses employed at 10, 20 and 40 mg/kg body weight using ibuprofen standard drug. Compounds (*4a-4i*) were also evaluated for ADP-induced platelet aggregation of platelet-rich plasma aspirin was used as positive control. Among all the targeted compounds *4h* and *4i* showed maximum antibacterial activity against all the three bacteria, while *4b* showed maximum 89.6% antiplatelet activity. Compound *4c* showed maximum anti-inflammatory activity of 33% protection at 10 mg/kg and 57% protection at 20 mg/kg to inflamed paw.

## INTRODUCTION

Bacterial infections are associated with pain and inflammation. In general, chemotherapeutic, analgesic and anti-inflammatory agents are prescribed simultaneously for such condition. However, none of drug possesses these three activities in a single component. Multi-drug treatment of inflammatory conditions associated with microbial infections poses a unique problem especially for patients with impaired liver or kidney functions. Therefore from the pharmacoeconomic and patient compliance points of view, the monotherapy with a drug having both anti-inflammatory and antimicrobial activities is highly desirable [1]. Platelets are essential for normal haemostasis. Activation of the clotting cascade by trauma results in platelet activation, which is followed by aggregation. Platelet aggregation is an important pathogenic marker of inflammation. Hence, one rational approach in the research of anti-inflammatory drugs is to search for compounds causing inhibitions of platelet aggregation [2]. Coumarins with diverse structural features having versatile biological properties such as antibacterial, anti-cancer, anti-inflammatory and anti-HIV activities have been recently reviewed [3]. Coumarin, chromone and their derivatives are well documented as having *in vitro* inhibitory properties on human platelet aggregation in literature [4]. Thiazoles and their derivatives were found to be associated with various biological activities [5-7] such as antibacterial, antifungal, anti-inflammatory activities. Organic compounds bearing thiazoles of different pharmacodynamic nuclei such as 2,4-disubstituted thiazoles [8], imidazolyl thiazoles [9] and

pyrazolyl thiazoles [10] have been recognized as potent anti-inflammatory and analgesic agents. Linkage of various heterocycles at C-4 position in 2-arylamino thiazoles has resulted in novel molecular matrices which were associated with anti-neoplastic activity [11]. Synthesis of many 3-substituted biheterocyclic coumarins with thiazoles and fused thiazoles possessing antimicrobial and anti-inflammatory agents has been reported [12-14]. Thiazole derivatives are also reported as antiplatelet agents [15]. Husain A and coworker has reported anti-inflammatory and analgesic compound with antibacterial activity [16-18].

Earlier we have reported the designing and synthesis of new trisubstituted thiazoles containing coumarin with good antibacterial, anti-inflammatory and antiplatelet activity [19]. In continuation to our previous report, we report here synthesis of trisubstituted thiazoles containing coumarin in which replacement of morpholino moiety by piperidin and piperazine at second position of thiazole to observe the isosteric changes in pharmacological activity by replacing oxygen with carbon and nitrogen, and their *in vitro* antibacterial and antiplatelet activity as well as *in vivo* anti-inflammatory activity of a new series of designed trisubstituted thiazoles molecules (Scheme 1).

## MATERIALS AND METHODS

### Chemistry

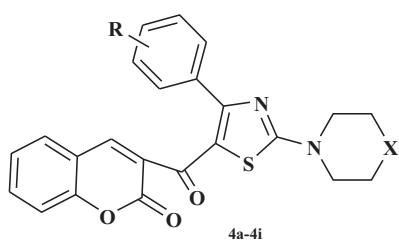
All reagents and solvents were used as obtained from the supplier or recrystallized/ redistilled as necessary. Thin layer chromatography was performed on microscopic slides coated with silica gel G and toluene : acetonitrile as a mobile phase. The spots were visualized by normal TLC and exposure to iodine vapour. Melting points were recorded on open capillary melting point apparatus and were uncorrected. IR spectra were recorded in KBr on SHIMADZU Fourier Transform Infrared 8400 S spectrophotometer. Mass spectra were recorded on Electron impact (EI) on a Jeol JMS-D-300 spectrometer with the ionization potential of 70 eV. Nuclear Magnetic Resonance spectra (<sup>1</sup>H NMR) were recorded in DMSO-d6 on Bruker advance at 400 MHz using Tetramethyl silane (TMS) as internal standard and the chemical shift ( $\delta$ ) were

<sup>1</sup>K. B. Institute of Pharmaceutical Education and Research, Department of Pharmaceutical Chemistry, Gandhinagar, Gujarat, India

<sup>2</sup>Ali-Allana College of Pharmacy, Department of Pharmaceutical Chemistry, Akkalkuwa, Nandurbar-425415 (M.S.) India.  
E-mail: aeajzboraji@rediffmail.com

<sup>3</sup>University Department of Chemistry, B. R. A. Bihar University, Muzaffarpur, Bihar, India.

<sup>4</sup>Department of Pharmacy, Sumandeep Vidyapeeth, Pipariya, Vadodara, Gujarat, India.

**Figure 1:** 3-(4 (substituted) phenyl-2-piperidin/piperine-1-yl-4-thiazole-5-carbonyl)-1-chromon-2-0ne**Table 1: Chemical Structures of Trisubstituted Thiazoles**

Compound No.	R	X
<b>4a</b>	H	-CH <sub>2</sub>
<b>4b</b>	2-Cl	-CH <sub>2</sub>
<b>4c</b>	3-Cl	-CH <sub>2</sub>
<b>4d</b>	4-Cl	-CH <sub>2</sub>
<b>4e</b>	H	-NH
<b>4f</b>	2-Cl	-NH
<b>4g</b>	3-Cl	-NH
<b>4h</b>	4-Cl	-NH
<b>4i</b>	H	-NCH <sub>3</sub>

**Table 2: Antibacterial, Anti-inflammatory and Antiplatelet Activity of Trisubstituted Thiazoles (4a -4i)**

Compound No.	Antimicrobial Activity*			Anti-inflammatory Activity**			Antiplatelet Activity*** %
	MIC in µg/mL (zone of inhibition in mm)	E. coli	S. aureus	B. subtilis	10 mg/kg	20 mg/kg	
<b>4a</b>	13.25 (10)	12.50 (11)	12.50 (10)	19	44	32	2.39
<b>4b</b>	14.25 (10)	12.50 (09)	25.00 (08)	30	55	48	89.6
<b>4c</b>	16.25 (10)	12.50 (10)	25.00 (08)	33	57	52	75.36
<b>4d</b>	12.50 (12)	12.50 (11)	12.50 (10)	32	55	55	49.27
<b>4e</b>	12.50 (12)	12.50 (18)	12.50 (10)	20	24	35	27.17
<b>4f</b>	25.00 (08)	24.50 (08)	22.50 (08)	24	28	45	31.88
<b>4g</b>	12.50 (14)	15.00 (10)	25.00 (08)	23	23	44	3.76
<b>4h</b>	07.50 (16)	08.50 (15)	09.25 (14)	16	18	26	24.13
<b>4i</b>	07.25 (16)	07.25 (16)	08.25 (15)	11	18	20	17.17
Ciprofloxacin	06.25 (18)	06.25 (18)	05.00 (21)	-	-	-	-
Aspirin	-	-	-	-	-	-	90
Ibuprofen (20 mg/kg)	-	-	-	75	-	-	-
Mefanamic acid (100mg/kg)	-	-	-	62	-	-	-

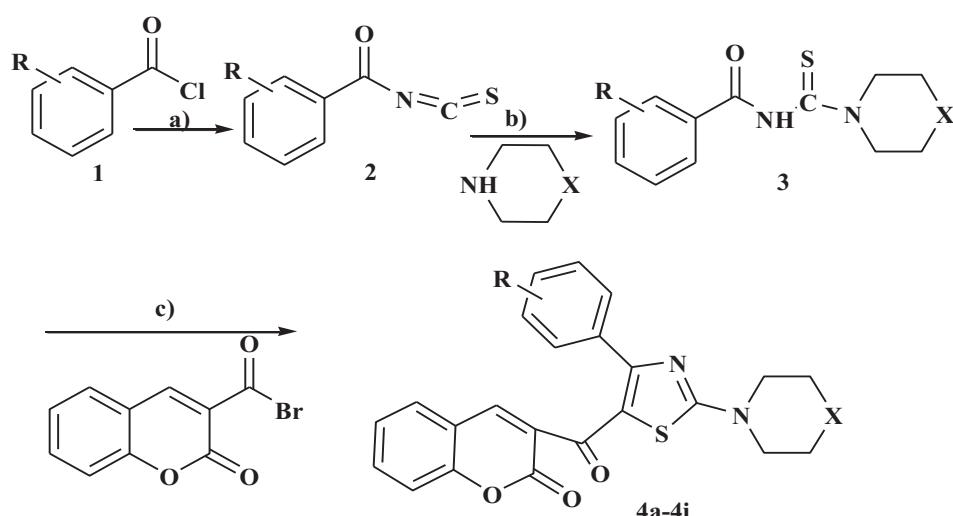
\*MIC values were evaluated at concentration range 1.56 to 75 µg/mL, ciprofloxacin was used as standard drug. \*\*Oral administration for all test compounds, P < 0.05, Student's t-test versus controls, the standard drugs, (dose and % protection) were: ibuprofen (20 mg/kg, 75%) and mefanamic acid (100 mg/kg, 62%). \*\*\*ADP-induced platelet aggregation of platelet-rich plasma (PRP)- Platelet aggregation was expressed as the change in optical density at 5 minutes, compared with PPP as a reference and converted to % aggregation; Aspirin was used as a positive control.

reported in parts per million (ppm). Elemental analysis data were determined using a Carlo-Erba 1108 instrument or Elementar's Vario EL III micro-analyzer.

#### General Method for Synthesis Of Compounds (4a)-(4i)

As shown in Scheme 1, isothiocyanate (2) were obtained by stirring ammonium thiocyanate (0.1379 mol) in 100 mL acetone at room temperature with benzoyl chloride/substituted benzoyl chloride (0.1263 mol) for 15-25 minutes followed by refluxing the reaction mixture for 15 min. Substituted N-(piperidinyl/piperazinyl/N-methylpiperazinyl-4-thioly) benzamide (3) were synthesized by nucleophilic addition of benzoyl

isothiocyanate /substituted benzoyl isothiocyanate and piperidine/piperazine/4-methylpiperazine in equimolar quantity at reflux temperature. The compounds (4a)-(4i) were synthesized as per procedure reported by Reji *et al* [20]. In brief the 3-bromoacetyl coumarin (0.005 mmol) was added to a solution of the adduct (3) (0.005 mmol) in 5 mL dimethyl formamide (DMF). The reaction mixture was heated on a water bath at 80-85°C for 5 min. To this, triethylamine 0.3 mL (0.005 mmol) was added and heating was continued for another 15 min. The reaction mixture was cool and poured into ice-cold water with stirring. A yellow precipitate thus obtained were filtered, wash with water and air-dried, purified by preparative TLC (3:7



**Scheme 1:** Synthesis of 3-(4( substituted) phenyl-2-piperidin/piperine-1-yl-4-thiazole-5-carbonyl)-1-chromen-2-one. Reagent and condition: a) NH<sub>4</sub>SCN, acetone, reflux 15-25 min; b) reflux for 15 min, pour reaction mixture to crushed ice; c) dimethyl amide, stir at 70 °C to 80 °C for 2 hours, pour to crushed ice.

Hexane: Ethyl acetate) corresponding to the (4a-4i) and was characterized as per the analytical data.

**3-(4-Phenyl-2-piperidin-1-yl-thiazole-5-carbonyl)-chromen-2-one (4a).** Yield: 35%; m.p.: 167°C; R<sub>f</sub>: 0.70 (9:1, toluene/methanol); IR (KBr, cm<sup>-1</sup>): 3100-2950 (-CH<sub>2</sub> stretching), 1736 (strong band of -C=O stretching), 1451-1540 (C=C stretching, aromatic), 1376 (C-N), 1250 (C-C[=O]-O symmetric stretching); <sup>1</sup>H NMR : (400 MHz, DMSO) δ (ppm): 1.67 (t, 6H, piperidin CH<sub>2</sub>), 2.51 (t, 4H, piperidin CH<sub>2</sub>), 6.7-7.1 (m, 4H, aromatic proton at 5<sup>th</sup> position of coumarin), 7.2 (d, 1H, aromatic proton at 4<sup>th</sup> position), 7.4 (t, 2H, aromatic proton at 4<sup>th</sup> position), 7.6 (d, 2H, aromatic proton at 4<sup>th</sup> position), 7.9 (s, 1H, aromatic proton at 5<sup>th</sup> position of coumarin); MS: m/z 416 (M<sup>+</sup>); Anal. calcd. for C<sub>24</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub>S: C, 69.05; H, 4.83; N, 6.71; Found: C, 69.01; H, 4.79; N, 6.74%.

**3-(4-(2-Chlorophenyl)-(2-piperidin-1-yl-thiazole-5-carbonyl)-chromen-2-one (4b).** Yield: 40%; m.p.: 215°C; R<sub>f</sub>: 0.65 (9:1, toluene/methanol); IR (KBr, cm<sup>-1</sup>): 3100-3000 (-CH<sub>2</sub> stretching), 1735 (strong band of -C=O stretching), 1456-1539 (C=C stretching), 1375 (C-N), 1249 (C-C[=O]-O symmetric stretching); <sup>1</sup>H NMR: (400 MHz, DMSO) δ (ppm): 1.6 (t, 6H, piperidin CH<sub>2</sub>), 2.53 (t, 4H, piperidin CH<sub>2</sub>), 6.7-7.1 (m, 4H, aromatic proton at 5<sup>th</sup> position of coumarin), 7.2-7.6 (m, 4H, aromatic proton at 4<sup>th</sup> position), 7.9 (s, 1H, aromatic proton at 5<sup>th</sup> position of coumarin); MS: m/z 451 (M<sup>+</sup>); Anal. calcd. for C<sub>24</sub>H<sub>19</sub>ClN<sub>2</sub>O<sub>3</sub>S: C, 63.92; H, 4.25; N, 6.21; Found: C, 63.96; H, 4.21; N, 6.18%.

**3-(4-(3-Chloro-phenyl)-(2-piperidin-1-yl-thiazole-5-carbonyl)-chromen-2-one (4c).** Yield: 48%; m.p.: 166°C; R<sub>f</sub>: 0.62 (3:7, hexane/ethyl acetate); IR (KBr, cm<sup>-1</sup>): 3150-3050 (-CH<sub>2</sub> stretching), 1737 (strong band of -C=O stretching), 1450-1545 (C=C stretching, aromatic), 1375 (C-N), 1249 (C-C[=O]-O symmetric stretching); <sup>1</sup>H NMR: (400 MHz, DMSO) δ (ppm): 1.5 (t, 6H, piperidin CH<sub>2</sub>), 2.5 (t, 4H, piperidin CH<sub>2</sub>), 6.6-7.0 (m, 4H, aromatic proton at 5<sup>th</sup> position of coumarin), 7.2-7.8 (m, 4H, aromatic proton at 4<sup>th</sup> position), 7.95 (s, 1H, aromatic proton at 5<sup>th</sup> position of coumarin); MS: m/z 451 (M<sup>+</sup>), 452 (M+1), 453 (M+2); Anal.

calcd. for C<sub>24</sub>H<sub>19</sub>ClN<sub>2</sub>O<sub>3</sub>S: C, 63.92; H, 4.25; N, 6.21; Found: C, 64.13; H, 4.47; N, 6.27%.

**3-(4-(4-Chlorophenyl)-(2-piperidin-1-yl-thiazole-5-carbonyl)-chromen-2-one (4d).** Yield: 43%; m.p.: 164°C; R<sub>f</sub>: 0.60 (3:7, hexane/ethyl acetate); IR (KBr, cm<sup>-1</sup>): 3180-2969 (-CH<sub>2</sub> stretching), 1730 (strong band of -C=O stretching), 1456-1555 (C=C stretching), 1376 (C-N), 1256 (C-C[=O]-O symmetric stretching); <sup>1</sup>H NMR : (400 MHz, DMSO) δ (ppm): 1.6 (t, 6H, piperidin CH<sub>2</sub>), 2.4 (t, 4H, piperidin CH<sub>2</sub>), 6.8-7.3 (m, 4H, aromatic proton at 5<sup>th</sup> position of coumarin), 7.25 (d, 2H, aromatic proton at 2<sup>nd</sup> and 6<sup>th</sup> position of aromatic phenyl ring), 7.5 (d, 2H, aromatic proton at 3<sup>rd</sup> and 5<sup>th</sup> position of aromatic phenyl ring), 7.9 (s, 1H, aromatic proton at 5<sup>th</sup> position of coumarin); MS: m/z 451 (M<sup>+</sup>), 452 (M+1), 453 (M+2); Anal. calcd. for C<sub>24</sub>H<sub>19</sub>ClN<sub>2</sub>O<sub>3</sub>S: C, 63.92; H, 4.25; N, 6.21; Found: C, 64.05; H, 4.65; N, 6.28%.

**3-(4-Phenyl-2-piperazin-1-yl-thiazole-5-carbonyl)-chromen-2-one (4e).** Yield: 50%; m.p.: 160°C; R<sub>f</sub>: 0.95 (3:7, hexane/ethyl acetate); IR (KBr, cm<sup>-1</sup>): 3500 (-CH<sub>2</sub> stretching), 1680 (strong band of -C=O stretching), 1457-1530 (C=C stretching), 1377 (C-N), 1238 (C-C[=O]-O symmetric stretching); <sup>1</sup>H NMR: (400 MHz, DMSO) δ (ppm): 3.85-4.25 (m, 8H, piperazine), 6.7-7.1 (m, 4H, aromatic proton at 5<sup>th</sup> position of coumarin), 7.2-7.6 (m, 5H, aromatic proton at 4<sup>th</sup> position), 7.9 (s, 1H, aromatic proton at 5<sup>th</sup> position of coumarin), 10.3 (s, 1H, NH piperazine); MS: m/z 418 (M<sup>+</sup>); Anal. calcd. for C<sub>23</sub>H<sub>19</sub>N<sub>3</sub>O<sub>3</sub>S: C, 66.17; H, 4.9; N, 10.07; Found: C, 65.97; H, 4.9; N, 11.04%.

**3-[4-(2-Chloro-phenyl)-2-piperazin-1-yl-thiazole-5-carbonyl]-chromen-2-one (4f).** Yield: 60%; m.p.: 138°C; R<sub>f</sub>: 0.65 (3:7, hexane/ethyl acetate); IR (KBr, cm<sup>-1</sup>): 3032 (-CH<sub>2</sub> stretching-piperazine ring), 1733 (strong band of -C=O stretching), 1457-1533 (C=C stretching, aromatic), 1374 (C-N), 1239 (C-C[=O]-O symmetric stretching); <sup>1</sup>H NMR: (400 MHz, DMSO) δ (ppm): 3.6-4.15 (m, 8H, piperazine), 6.6-7.0 (m, 4H, aromatic proton at 5<sup>th</sup> position of coumarin), 7.0-7.4 (m, 4H, aromatic proton at 4<sup>th</sup> position),

7.9 (s, 1H, aromatic proton at 5<sup>th</sup> position of coumarin), 10.2 (s, 1H, NH piperazine); MS: *m/z* 451 (M+), 452 (M+1), 453 (M+2); Anal. calcd. for C<sub>23</sub>H<sub>18</sub>ClN<sub>3</sub>O<sub>3</sub>S: C, 61.13; H, 4.02; N, 9.30; Found: C, 61.38; H, 4.46; N, 9.44%.

**3-[4-(3-Chloro-phenyl)-2-piperazin-1-yl-thiazole-5-carbonyl]-chromen-2-one (4g).** Yield: 56%; m.p.: 120°C; R<sub>f</sub>: 0.97 (3:7, hexane/ethyl acetate); IR (KBr, cm<sup>-1</sup>): 3032 (-CH<sub>2</sub> stretching-piperazine ring), 1730 (strong band of -C=O stretching), 1456-1533 (C=C stretching, aromatic), 1374 (C-N) 1243, (C-C[=O]-O symmetric stretching); <sup>1</sup>H NMR: (400 MHz, DMSO) δ (ppm): 2.5-3.8 (m, 8H, piperazine), 6.9-7.2 (m, 4H, aromatic proton at 5<sup>th</sup> position of coumarin), 7.1-7.5 (m, 4H, aromatic proton at 4<sup>th</sup> position), 7.8 (s, 1H, aromatic proton at 5<sup>th</sup> position of coumarin), 9.9 (s, 1H, NH piperazine); MS: *m/z* 452 (M+), 453 (M+1), 454 (M+2); Anal. calcd. for C<sub>23</sub>H<sub>18</sub>ClN<sub>3</sub>O<sub>3</sub>S: C, 61.13; H, 4.02; N, 9.30; Found: C, 61.23; H, 4.17; N, 9.56%.

**3-[4-(4-Chloro-phenyl)-2-piperazin-1-yl-thiazole-5-carbonyl]-chromen-2-one (4h).** Yield: 47%; m.p.: 124°C; R<sub>f</sub>: 0.93 (3:7, hexane/ethyl acetate); IR (KBr, cm<sup>-1</sup>): 3040 (-CH<sub>2</sub> stretching-piperazine ring), 1700 (strong band of -C=O stretching), 1450-1530 (C=C stretching, aromatic), 1370 (C-N), 1241 (C-C[=O]-O symmetric stretching); <sup>1</sup>H NMR: (400 MHz, DMSO) δ (ppm): 2.4-3.7 (m, 8H, piperazine), 6.8-7.1 (m, 4H, aromatic proton at 5<sup>th</sup> position of coumarin), 7.25 (d, 2H, aromatic proton at 2<sup>nd</sup> and 6<sup>th</sup> position of aromatic phenyl ring), 7.5 (d, 2H, aromatic proton at 3<sup>rd</sup> and 5<sup>th</sup> position of aromatic phenyl ring), 7.9 (s, 1H, aromatic proton at 5<sup>th</sup> position of coumarin), 10.0 (s, 1H, NH piperazine); MS: *m/z* 452 (M+1), 453 (M+2), 454 (M+3); Anal. calcd. for C<sub>23</sub>H<sub>18</sub>ClN<sub>3</sub>O<sub>3</sub>S: C, 61.13; H, 4.02; N, 9.30; Found: C, 61.22; H, 4.29; N, 9.68%.

**3-[2-(4-Methylpiperazin-1-yl)-4-phenyl-1-yl-thiazole-5-carbonyl]-chromen-2-one (4i).** Yield: 35%; m.p.: 128°C; R<sub>f</sub>: 0.98 (3:7, hexane/ethyl acetate); IR (KBr, cm<sup>-1</sup>): 3100-3000 (-CH<sub>2</sub> stretching-), 1710 (strong band of -C=O stretching), 1457-1533 (C=C stretching, aromatic), 1374 (C-N), 1248 (C-C[=O]-O symmetric stretching); <sup>1</sup>H NMR: (400 MHz, DMSO) δ (ppm): 2.3 (s, 3H, CH<sub>3</sub> of piperazine), 2.50-2.55 (t, 4H, CH<sub>2</sub> of piperazine), 3.05-3.15 (t, 4H, CH<sub>2</sub> of piperazine), 6.7-7.1 (m, 4H, aromatic proton at 5<sup>th</sup> position of coumarin), 7.0 (d, 1H, aromatic proton at 4<sup>th</sup> position), 7.25 (t, 2H, aromatic proton at 4<sup>th</sup> position), 7.4 (d, 2H, aromatic proton at 4<sup>th</sup> position), 7.9 (s, 1H, aromatic proton at 5<sup>th</sup> position of coumarin); MS: *m/z* 432 (M+1), 433 (M+1); Anal. calcd. for C<sub>24</sub>H<sub>21</sub>N<sub>3</sub>O<sub>3</sub>S: C, 66.80; H, 4.91; N, 9.74; Found: C, 65.96; H, 4.9; N, 10.04%.

### Pharmacological Screening

**Animals.** Albino rats (150–250 g) of either sex were provided with pellet diet (Lipton, India) and water *ad libitum* and kept under standard laboratory condition at 25 ± 2°C. The experimental protocol was approved by the institutional ethics committee constituted by the Ministry of Social Justice and Empowerment, (Government of India).

### Antibacterial Activity

The newly synthesized compounds were screened for their antibacterial activity using agar well diffusion method [21].

The antibacterial activity of the test compounds were evaluated against Gram-positive bacteria, *Staphylococcus aureus*, *Bacillus subtilis* and Gram-negative bacteria *Escherichia coli* using ciprofloxacin as standard drug. The solution of the synthesized compounds in dimethylsulfoxide (DMSO) was prepared and used as solvent control. The microorganism was activated by inoculation a loopful of the strain in the nutrient broth (25 mL) and incubating at room temperature in a rotary shaker. The test organism (0.2 mL; 10<sup>8</sup> cells/ as per McFarland standard) was then inoculated into the molten Mueller Hinton agar media. After proper homogenization it was poured into sterile 100 mm petri dishes (Hi-media) and allowed to solidify. A well was made in the seeded plates with the help of a sterile cork borer (8.5 mm). The test solution (0.05 mL) in DMSO was introduced into the well and all the plates were incubated at 37°C for 24 hours. The experiment was performed in triplicate under aseptic conditions. The control was also maintained with 0.05 mL of DMSO under similar conditions and the zone of inhibition of the bacterial growth were measured and recorded. Preliminary screening was conducted for all compounds at 100 µg/mL concentration, against the above mentioned microorganisms. Different series of dilutions of compounds were made (1.56 to 75 µg/mL) to determine the MIC.

### Anti-inflammatory Activity

We have used the method previously described by Winter *et al* [22]. The animals were studied for toxicity of DMSO up to 10% v/v in saline, and 5% DMSO was selected as a vehicle to suspend the standard drugs and the test compounds. Albino rats of either sex weighing between 150 - 250 g were starved for 18 hours prior to the experiment. The animals were weighed, marked for identification and divided into groups of six. The standard drug, ibuprofen (20 mg/kg body weight) and mefanamic acid (100 mg/kg body weight) and the test compounds were given orally (10, 20 and 40 mg/kg body weight) as a suspension using 5% DMSO as a vehicle. One hour later foot paw oedema was induced by injecting 0.1 mL of 1% carrageenin subcutaneously into the planter portion of the right hind paw of each rat. Initial foot paw volume was measured immediately by mercury plethysmometer. Oedema was measured three hours after carrageenin administration. The swelling in test group animals was used to calculate the percent inhibition ± SEM of oedema achieved by the compound at the test dose compared with the vehicle control group. The % protection of oedema was calculated according to the formula, % anti-inflammatory activity = 100 x (1-Vt/Vc) where, Vt and Vc were the volume of oedema in test compounds and control groups, respectively.

### Anti-platelet Activity

ADP-induced platelet aggregation of platelet-rich plasma (PRP) quantitated using optical density filter at 405 nm as a measurement point in kinetic mode. To obtain PRP, a citrated tube of blood was inverted 3 to 5 times for gentle

mixing and centrifuged at room temperature for 10 min at 200 g. After centrifugation, the upper turbid layer of PRP was removed, and the residual blood was centrifuged for 5 min at 2000 g to obtain platelet-poor plasma (PPP). The PPP was used as the baseline optical density for platelet aggregation. A total of 180 µL of PRP containing about  $3 \times 10^8$  platelets/mL was incubated at 37°C in the 96 well plates for 3-5 min, Then 10 µL of test compounds were added in PRP containing wells and incubated for the period of 15 minutes with intermittent shaking. ADP (10 µL) at a final concentration of 10, 20 and 40 µmol/L was added in above wells with intermittent shaking mode. Optical density readings were measured at every 1 minute with intermittent shaking up to 5 minutes. Platelet aggregation was expressed as the change in optical density at 5 minutes, compared with PPP as a reference and converted to % aggregation [23]. Aspirin was used as a positive control.

## RESULT AND DISCUSSION

Some novel trisubstituted thiazole analogues (*4a-4i*) were designed and synthesized. The chemical structures of these compounds are shown in Table 1. The synthesized compounds (*4a-4i*) were evaluated for their *in vitro* antibacterial, *in vivo* anti-inflammatory and *in vitro* anti-platelet activities. The *in vitro* antibacterial activity evaluated against Gram-positive bacteria, *Staphylococcus aureus*, *Bacillus subtilis* and Gram-negative bacteria *Escherichia coli* using ciprofloxacin as standard drug, *in vivo* anti-inflammatory activity was performed in carrageenin-induced rat hind paw oedema model at three graded doses employed at 10, 20 and 40 mg/kg body weight using mefanamic acid and ibuprofen as standard drug and similarly *in vitro* anti-platelet activity was performed in ADP-induced platelet aggregation of PRP, quantitated using optical density filter at 405 nm as a measurement point in kinetic mode using aspirin as a positive control and the results are shown in Table 2. Taking into account the diverse biological activities of coumarin derivatives namely anti-inflammatory activities and *in vitro* inhibitory properties on human platelet aggregation reported in literature and similarly thiazole derivatives which are also extensively reported and clinically used as antimicrobial agents. The compounds *4a-4d* were designed and synthesized by keeping coumarin-3-yl at fifth position and piperidinyl/piperazinyl moiety at second position of thiazole ring. The forth position of thiazole was substituted by introducing electron withdrawing group (-Cl), at different position in phenyl moiety. The compound *4i* was synthesized having methyl group at fourth position of piperazinyl to explore the effect of presence of aliphatic chain on biological activity of profile of the candidate.

Among all the tested compounds *4a-4i*, the *4h* and *4i* displayed a good antibacterial activity against all the Gram-positive and Gram-negative bacteria, and their activities were found to be better than that of *4a-4f* however all the synthesized compounds displayed weaker antibacterial activities compared to those of the standard drug ciprofloxacin. The compound *4i* displayed antibacterial

activity against *E. coli* (7.25 MIC in µg/mL; 16 mm zone of inhibition), *S. Aureus* (7.25 MIC in µg/mL; 16 mm zone of inhibition) and *B. Subtilis* (8.25 MIC in µg/mL; 15 mm zone of inhibition), where as compound *4h* displayed *E. coli* (7.50 MIC in µg/mL; 16 mm zone of inhibition), *S. Aureus* (8.50 MIC in µg/mL; 15 mm zone of inhibition) and *B. Subtilis* (9.25 MIC in µg/mL; 14 mm zone of inhibition). In the series, *4b* showed comparable anti-inflammatory activity at all the three graded doses employed, i.e. 30% at 10 mg/kg, 55% at 20 mg/kg and 48% at 40 mg/kg, for *4c* the % protection was found to be 33% at 10 mg/kg, 57% at 20 mg/kg and 52% at 40 mg/kg, similarly for *4d* the % protection was displayed 32% at 10 mg/kg, 55% at 20 mg/kg and 55% at 40 mg/kg. Compounds *4a* and *4e-4i* showed moderate to less activity as compared to above three compounds. All the targeted trisubstituted thiazole (*4a-4i*) were found to have poor anti-inflammatory activity as compared to the standard drug ibuprofen, which showed 75% protection at 20 mg/kg dose. The targeted compounds (*4a-4i*) were also evaluated for ADP-induced platelet aggregation of platelet-rich plasma (PRP) using aspirin as positive control. The compounds *4b* and *4c* also showed maximum antiplatelet activity of 89.60% and 75.36% respectively; however *4a* and *4d-4i* showed moderate to less antiplatelet activity as compared to above compounds. To summarize these findings, methyl substituent on piperazino moiety and *p*-chlorophenyl at second and forth position of thiazole respectively enhances antibacterial activity against all tested Gram-positive and Gram-negative bacteria. The presence of piperidino moiety and chlorophenyl at second and forth position of thiazole respectively enhances the anti-inflammatory activity and antiplatelet activity. However all targeted trisubstituted thiazole containing coumarin (*4a-4i*) were found to have moderate to poor antibacterial, anti-inflammatory and antiplatelet activities, it is, therefore, suggested that this scheme to be studied further to explore its full structure-activity relationship potential particularly single candidate as monotherapy in the treatment of chronic inflammatory diseases having both anti-inflammatory and antimicrobial activities.

## CONCLUSIONS

The synthesized compounds (*4a-4i*) were evaluated for their *in vitro* antibacterial activity; *in vivo* anti-inflammatory activity and *in vitro* antiplatelet activity. The compounds *4a-4d* were designed and synthesized by keeping coumarin-3-yl at fifth position and piperidinyl/piperazinyl moiety at second position of thiazole ring. The forth position of thiazole was substituted by introducing electron withdrawing group (-Cl), at different position in phenyl moiety. The Compounds *4h* and *4i* displayed a good antibacterial, *4c* showed maximum *in vivo* anti-inflammatory activity and *4b* found to have highest antiplatelet activity. To summarize these findings, the methyl substituent on piperazino moiety and *p*-chlorophenyl at second and forth position of thiazole respectively enhances antibacterial activity against all tested organism. The presence of piperidino moiety and

chlorophenyl at second and forth position of thiazole respectively enhances the anti-inflammatory activity and antiplatelet activity. However all targeted trisubstituted thiazole containing coumarin (*4a-4l*) were found to have moderate to poor antibacterial, anti-inflammatory and antiplatelet activities, it is, therefore, suggested that this scheme to be studied further to explore its full structure-activity relationship potential particularly single candidate as monotherapy in the treatment of chronic inflammatory diseases having both anti-inflammatory and antimicrobial activities.

## REFERENCES AND NOTES

- Bekhit A A, Abdel A T. Design, synthesis and biological evaluation of some pyrazole derivatives as anti-inflammatory antimicrobial agents: Bio. Org. Med. Chem; 12, 1935-1945 2004.
- Sur T K, Biswas T K, Ali L, Mukherjee B. Anti-inflammatory and anti-platelet aggregation activity of human placental extract: Acta. Pharmacol Sin; 24,187-192, 2003.
- Kulkarni M V, Kulkarni G M, Lin C H, Sun C M. Recent advances in coumarins and 1-azacoumarins as versatile biodynamic agents: Curr.Med. Chem; 13 2795-2818, 2006.
- Roma G, Braccio M D, Carrieri A, Grossi G, Leoncini, G, Maria G. Signorello M, Carottib A. Coumarin, chromone, and 4 (3H)-pyrimidinone novel bicyclic and tricyclic derivatives as antiplatelet agents: synthesis, biological evaluation and comparative molecular field analysis: Bio. Org. Med. Chem; 11, 123-138, 2003.
- Franklin, P X. Pillai A D, Rathod P D, Yerande S, Nivsarkar M, Padh H, Vasu, K K, Sudarsanam V. 2-Amino-5-thiazolyl motif: A novel scaffold for designing anti-inflammatory agents of diverse structures: Eur. J. Med. Chem; 43,129-134, 2008.
- Wilson K R, Molloy C J, Bone R, Green D, Randall J. Synthesis of some new 2,4-disubstituted thiazoles as possible antibacterial and anti-inflammatory agents: Bio. Org. Med. Chem. Lett; 11,915-918, 2001.
- Shetty, N S, Khazi I A M, Ahn C. Synthesis anthelmintic and anti-inflammatory activities of some novel imidazothiazole sulfides and sulfones: Bull. Korean. Chem. Soc; 31, 2337-2340, 2010.
- Karegoudar, P, Karthikeyan, M S, Prasad D J, Mahalinga, M, Holla B S, Kumari N S. Synthesis of some novel 2,4-disubstituted thiazoles as possible antimicrobial agents: Eur. J. Med. Chem; 43, 261-267, 2008.
- Sharma, P K, Sawhney S N. Potent antiinflammatory 3-thiazole-4(5)-acetic acids of 1,2-benzisothiazole: Bio. Org. Med. Chem. Lett; 7,2427-2430, 1997.
- Russo F, Guccione S, Romeo G, Ucello B G, Pussi S, Cutuli V. Pyrazolothiazolopyrimidine derivatives as a novel class of anti-inflammatory or antinociceptive agents: synthesis, structural characterization and pharmacological evaluation: Eur. J. Med. Chem; 28,363-376,1993.
- Berlin K D, Herd M D. Novel 2-amino-4-aryl-substituted-and 2-amino-4, 5-disubstituted-thiazoles: Proc. Okla. Acad. Sci; 71, 29-33, 1991.
- Kulkarni, M V. Patil, V D. Nanjappa, S. Biradar, V N. Synthesis and biological properties of some 3-heterocyclic substituted coumarins: Arch. Pharm (Weinheim); 314,435-439,1981.
- Rehse, K. Baselt, T. New 2-amino-thiazole-4-acetamides with antiplatelet activity: Arch. Pharm: (Weinheim); 341,645-54, 2008.
- Kalkhambkar, R G. Kulkarni, G M. Shivkumar, H. Rao, R.N. Synthesis of novel triheterocyclic thiazoles as anti-inflammatory and analgesic agents: Eur. J. Med. Chem; 42,1272-1276, 2007.
- Tanaka, A. Sakai, H. Motoyama, Y. Ishikawa, T. Takasugi, H. Antiplatelet agents based on cyclooxygenase inhibition without ulcerogenesis. Evaluation and synthesis of 4,5-bis (4-methoxyphenyl)-2-substituted-thiazoles: J. Med. Chem; 37,1189-1199, 1994.
- Husain, A. Alam, M M. Zaman, M S. Ismail, M V. Synthesis of 2-arylidene-4-(Substituted aryl) but-3-en-4-olides and evaluation of their antibacterial anti-inflammatory activities: Int. J. Chem. Sci; 6 (3),1535-1541, 2008.
- Husain, A. Khan, M S Y. Hasan, S M. Alam, M M. 2-Arylidene-4-(4-phenoxyphenyl) but-3-en-4-olides: Synthesis, reactions and biological activity: Eur. J. Med. Chem; 40,1394-1404, 2005.
- Husain, A. Hasan, S.M.; Lal, S. Alam, M.M. Synthesis of new 2-Arylidene-4-(4-methylphenyl) but-3-en-4-olides and their pyrrolones derivatives: Ind. J. Het. Chem; 14,163-164, 2004.
- Mansuri, M. Seth, A K.; Molvi, K I. Prajapati, B R. Desai, D G. Synthesis and pharmacological evaluation of some 3-(4-(substituted) 2-morpholino-4-yl-4-phenyl-thiazole-5-carbonyl)-1-benzopyran-2-one: Pharma. Sci. Monitor; 1490-1500, 2011.
- Reji, T F. Devi, S K. Thomas, K K. Shreejalekshmi, K G. Manji, S L. Franscis, M. Philip, S K. Bharathan, A. Rajasekharan, K.N. Synthesis and cytotoxicity studies of thiazole analogs of the anticancer marine alkaloid dendrodoine: Ind. J. Chem; 47B, 1145-1150, 2008.
- Perez, C. Paul, M. Bezerque, P. An antibiotic assay by the agar-well diffusion method: Acta .Biol . Med. Exp; 15,113-115,1990.
- Winter, C A. Risley, E A. Nuss, G W. Carrageenan induced oedema in hind paw of the rat as an assay for anti-inflammatory drugs: Exp. Bio. Med; 111, 544-547, 1962.
- O' Brien, J. R. Study results of new method of platelet aggregation Part II: J. Clin. Pathol; 15,452-455, 1962.

## Acknowledgments

The authors are thankful to president JIIU's G.M.Vastanvi and Dr. V. Sudarsanam for their encouragement and support. We also wish to thanks to Mr. Prashant Patel director, Cambay Organics Pvt. Cambay, Gujarat, India to provide chemicals and Dr. Anita Mehta, Department of Pharmacology, L. M. College of Pharmacy, Ahmedabad, Gujarat, for screening of antiplatelet activity.

**Cite this article as:** Musatakim Mansuri, Aejaq Ahmed, Arshad Shaikh, *et al.* Synthesis, Antibacterial, Anti-Inflammatory and Antiplatelet Activities of Some Trisubstituted Thiazoles. Inventi Rapid: Med Chem, 2012(4): 1-6, 2012.