Research Article

A Facile Microwave Assisted Synthesis and Anti-Inflammatory Activity of Thiophene[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5(6*H*)-one Derivatives

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ABSTRACT

A series of novel substituted triazolo thieno pyrimidines (5-12) was synthesized by employing innovative synthetic methods. Two methods (A and B) were employed for the synthesis of compounds 3-12. Method B (microwave assisted) was found to be facile, economic and less time consuming compared to conventional method (method A) adopted. The microwave irradiation provided an environment-friendly, remarkable rate of acceleration for the reaction with reduced reaction time and in some cases (under MW irradiation) the yields are also substantially higher. Anti-inflammatory activity and mast cell degranulation studies of synthesized compounds were carried out. Indomethacin was taken as a reference standard in carrageenan induced rat paw edema model for anti-inflammatory studies and in mast cell degranulation studies disodium cromoglycate was used for comparison. All compounds showed a good anti-inflammatory response and mast cell stabilization.

Keywords: Microwave, anti-inflammatory, thienopyrimidines, mast cell stabilizers.

INTRODUCTION

Thienopyrimidine, a fused pyrimidine system, represents one of the important class of compounds showing a broad range of biological activity including anti-inflammatory[1,2], antiviral[3-5], analgesic[6,7] and antimicrobial[8,9] prevention of cartilage destruction in articular diseases and properties, antagonism of α_1 adrenoceptors[10], inhibition of cancer cell proliferation[11] and GluR6 antagonism[12]. 1,2,4-triazoles are of considerable research interest because molecules containing these structural features display a variety of biological activities such as antiviral[13], antimicrobial[14], antibacterial[15], antifungal[16], hypnotic[17] and antiasthmatic[18] etc. A report shows that compounds having 1,2,4-triazole moiety can also inhibit the Shiga toxin[19]. Synthesis of triazolothieno pyrimidines by using environment friendly microwave irradiation method is also reported[20].

In view of the above and in continuation of our investigations on the chemistry of thienopyrimidines and triazolothienopyrimidines, we now report a facile microwave-assisted synthesis and anti-inflammatory activity of some new tetracyclic analogues of thienopyrimidines (5-12).

MATERIALS AND METHODS

All the chemicals used were of reagent grade and commercially available. Purification of compounds was carried out by column chromatography using silica gel (100-200 mesh; E. Merck, Mumbai).

All the reactions were monitored by thin layer chromatography (TLC) on precoated silica gel 60 F_{254} (mesh) (E. Merck, Mumbai) and spots were visualized under UV light (254 nm). All the solvents were freshly distilled and dried prior to use according to standard procedures. LABINDIA Melting Point Apparatus (MEPA MP08050204 was used to record the melting points and were uncorrected.

IR spectra were recorded on Perkin Elmer FT-IR Spectrometer (Spectrum RX I) using KBr pellet technique. The UV spectra were recorded on Lamda 25 Spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance II (400 MHz and 100 MHz) spectrometer for solutions in $CDCl_3$ / DMSO-d_6 using TMS as internal standard and reported in parts per million (ppm). Electrospray Ionization Mass spectra (ESI) were recorded on Waters Mass Spectrometer (Micromass Q-TOF Micro) and elemental analyses were performed using Thermo EA 2110 series Elemental Analyzer.

Preparation of Ethyl-3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl-carbamate (3)

Method A

A suspension of 1 (29·7 mmol, 5·28 g) in ethyl chloroformate (148 mmol, 14·1 ml) was heated under reflux for 5 h. The excess ethyl chloroformate was distilled off and toluene (9·6 ml) was added. Slow addition of cyclohexane in cold conditions induced crystallization.

The resulting solid was collected by filtration and rinsed with cyclohexane. Drying under vacuum afforded the product 3 as light yellow crystals; mp 109-110 °C.

Method B

A suspension of 1 (29·7 mmol, 5·28 g) in ethyl chloroformate (148 mmol, 14·1 ml) was heated under

microwave irradiation at 560W for 5 min. A similar work up protocol of the reaction mixture as discussed under Method A gave the product 3.

Preparation of Ethyl-3-cyano-5,6-dihydro-4*H*-cyclopenta[*b*]thiophen-2-yl-carbamate (4)

Method A

A suspension of 2 (29·7 mmol, 4.88 g) in ethyl chloroformate (148 mmol, 14·1 ml) was heated under reflux for 5 h.

The excess ethyl chloroformate was distilled off and toluene (9·6 ml) was added.

Slow addition of cyclohexane in cold conditions induced crystallization.

The resulting solid was collected by filtration and rinsed with cyclo hexane.

Drying under vacuum afforded the compound 4 as light brown crystals; mp 158-160 °C.

Method B

A suspension of 2 (29·7 mmol, 4.88 g) in ethyl chloroformate (148 mmol, 14·1 ml) was heated under microwave irradiation at 560W for 5 min.

A similar work up protocol of the reaction mixture as discussed under Method A gave the product 4.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.63 (br s, 1H, NH), 4.30 (q, *J* = 7.12 *Hz*, 2H, 3'-CH_2), 2.86 (m, 2H, 6-CH_2), 2.77 (m, 2H, 4-CH_2), 2.42 (m, 2H, 5-CH_2), 1.34 (t, *J* = 7.12 *Hz*, 3H, 4'-CH_3).^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 153.01 (C-1'), 152.38 (C-2), 141.12 (C-4a), 133.41 (C-6a), 114.55 (C≡N), 62.89 (C-3), 29.39 (C-6), 28.17 (C-5), 27.98 (C-4), 14.39 (C-4'). IR cm^{-1}: 3222, 3084, 2992, 2916, 2854, 2216, 1724, 1559, 1450, 1230, 1087.UV λ_{max} (methanol): 297.6, 245.6, 216.4 nm.

General procedure for the synthesis of compounds 5-12

Method A

To a solution of 3 (1.5 mmol, 0.375 g) / 4 (1.5 mmol, 0.354 g) in DMF (1.7 ml), was added differently substituted hydrazides [(a (2-chlorobenzohydrazide, 0.256 g) / b (2-hydroxybenzohydrazide, 0.282 g) / c (benzohydrazide, 0.204 g) / d (4-nitrobenzohydrazide, 0.271 g 1.5 mmol) and the resulting mixture was heated at 120°C for 12-24 h under nitrogen (inert) atmosphere.

It was allowed to cool and added slowly to crushed ice to afford the respective products (5-12).

The solids were collected by filtration and washed with water and 2-propanol. The compounds obtained were purified through column on silica gel using n-hexane : ethyl acetate :: 8.5 : 1.5 as eluent to yield pure final products (5-12).

Method B

To a solution of 3 (1.5 mmol, 0.375 g) / 4 (1.5 mmol, 0.354 g) in DMF (1.7 ml), was added differently substituted hydrazides [(a (0.256 g) / b (0.282 g) / c (0.204 g) / d (0.271 g 1.5 mmol) and the resulting mixture was heated under microwave irradiation at 560W for 15-40 min.

A similar work up protocol of the reaction mixture as discussed under Method A gave the final products 5-12.

Synthesis and spectral data of compounds 3 (Ethyl-3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl-carbamate) and 5-8 [2-(2'-chlorophenyl)-8,9,10,11-tetrahydro[*1*]benzothieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5(6*H*)-one (5); 2-(2'-hydroxyphenyl)-8,9,10,11-tetrahydro[*1*]benzothieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5(6*H*)-one (6); 2-phenyl-8,9,10,11-tetrahydro[*1*]benzothieno[3,2-*e*][1,2,4]triazolo[1,5-*c*] pyrimidin-5(6*H*)-one (7) and 2-(4'-nitrophenyl)-8,9,10,11-tetrahydro[*1*]benzothieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5(6*H*)-one (8)] has been published by our research group in J. Chem. Sci.[20]

2-(2'-chlorophenyl)-8,9-dihydro-10H-cyclopenta[b]thiophene[3,2-e][1,2,4]triazolo[1,5-c]pyrimidin-5(6H)-one (9)

Colourless powder. mp 318 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 13.04 (br s, 1H, NH), 8.00 (m, 1H, 6'-ArH), 7.58 (m, 1H, 3'-ArH), 7.47 (m, 2H, 4'-ArH and 5'-ArH), 3.01 (m, 2H, 8-CH_2), 2.75 (m, 2H, 10-CH_2), 1.89 (m, 2H, 9-CH_2).^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 161.77 (C-2), 150.43 (C-5), 143.82 (C-13), 142.73 (C-1'), 132.39 (C-2'), 131.73 (C-6a), 130.82 (C-4'), 130.35 (C-3'), 129.26 (C-12), 128.76 (C-6'), 128.24 (C-7a), 126.71 (C-5'), 109.49 (C-11), 24.64 (C-8), 24.06 (C-9), 22.76 (C-10). IR cm^{-1}: 3061, 2863, 1704, 1650, 1583, 1513, 1454, 1317, 1039, 735. UV, λ_{max} (methanol): 262.0, 202.0 nm.

Anal. Calcd. %: ($C_{16}H_{11}ClN_4OS$) C: 56.06, H: 3.23, N: 16.34; Found %: C: 56.12, H: 3.41, N: 16.52.

2-(2'-hydroxyphenyl)-8,9-dihydro-10H-cyclopenta[b]thiophene[3,2-e][1,2,4]triazolo[1,5-c]pyrimidin-5(6H)-one (10)

Light brown. mp 345 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm : 13.22 (br s, 1H, NH), 10.99 (s, 1H, OH), 8.18 (m, 1H, 6'-ArH), 7.37 (m, 1H, 4'-ArH), 6.98 (m, 2H, 3'-ArH & 5'-ArH), 3.01 (m, 2H, 8-CH_2), 2.76 (m, 2H, 10-CH_2), 1.92 (m, 2H, 9-CH_2). IR cm^{-1}: 3493, 3079, 2939, 2866, 2839, 1683, 1577, 1554, 1377, 1280, 1199, 1158. UV, λ_{max} (methanol): 260.6, 204.4 nm.

Anal. Calcd. %: ($C_{16}H_{12}N_4O_2S$) C: 59.25, H: 3.73, N: 17.27; Found %: C: 59.29, H: 3.52, N: 17.31.

2-phenyl-8,9-dihydro-10H-cyclopenta[b]thiophene[3,2-e][1,2,4]triazolo[1,5-c]pyrimidin-5(6H)-one (11)

Light brown powder. mp 322 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 12.91 (br s, 1H, NH), 8.31 (m, 2H, 2'-ArH & 6'-ArH), 7.49 (m, 3H, 3'-ArH, 4'-ArH & 5'-ArH), 3.11 (m, 2H, 8-CH_2), 2.77 (m, 2H, 10-CH_2), 1.94 (m, 2H, 9-CH_2). IR cm^{-1}: 3085, 2934, 2855, 1694, 1581, 1560, 1203, 1159, 1030, 867,

743. UV, λ_{max} (methanol): 246.6, 204.8 nm. Anal. Calcd. %: ($C_{16}H_{12}N_4OS$) C: 62.32, H: 3.92, N: 18.17; Found %: C: 62.45, H: 3.62, N: 18.31.

2-(4-nitrophenyl)-8,9-dihydro-10H-cyclopenta[b]thiophene[3,2-e][1,2,4]triazolo[1,5-c]pyrimidin-5(6H)-one (12)

Brown crystals. mp 356 °C. Yield ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 13.16 (br s, 1H, NH), 8.48 (d, J = 8.8, 2H, 3'-ArH & 5'-ArH), 8.39 (d, J = 8.8, 2H, 2'-ArH & 6'-ArH), 3.04 (m, 2H, 8-CH_2), 2.77 (m, 2H, 10-CH_2), 1.89 (m, 2H, 9-CH_2). IR cm^{-1}: 3152, 2923, 2857, 1730, 1648, 1609, 1518, 1336, 1197, 1100, 709. UV, λ_{max} (methanol): 284.8, 205.6 nm. Anal. Calcd. %: ($C_{16}H_{11}N_5O_3S$) C: 54.38, H: 3.14, N: 19.82; Found %: C: 54.52, H: 3.01, N: 19.65.

Antiinflammatory Evaluation (*in vivo*)

The anti-inflammatory activities of all the synthesized compounds were carried out in ten groups and the given data is of five animals (female wistar rats) per group. Group-1 referred as control (animals received carrageenan along with the vehicle [0.5% carboxymethylcellulose (CMC)] and Group-2 received standard reference (indomethacin 10 mg/kg) along with the vehicle prior to the administration of carrageenan. Groups 3-10 received the test compounds (5-12, 10 mg/kg suspended in 0.5% of CMC) respectively 1 h prior to the administration of carrageenan (injection of 0.1 ml of freshly prepared carrageenan (1%) in physiological solution (154 mM NaCl) into the sub-planter tissue of hind paw of each rat).

The equivalent volume of carrageenan (1%) in physiological solution was injected into hind paw of the control. The volume was measured three times using water plethysmometer prior to the administration of carrageenan, 2 h and 4 h after the injection. The increase in volume of the paw was adopted as a measure of edema. The antiedematous effects of the compounds were estimated as percentage inhibition of the induced inflammation in comparison with control. Statistical analysis was carried out using a one-way analysis of variance (ANOVA). In all cases, post-hoc comparisons of the means of individual groups were performed using tukey's test. A significance level of $P < 0.001$ denoted the significance in all cases.

The percentage inhibition of edema was calculated using the formula given below:

$$\frac{(V_c - V_t)}{V_c} \times 100$$

Where, V_c is the increase in paw volume of control (in the absence of test compound) and V_t is the increase in paw volume after administration of the test compound.

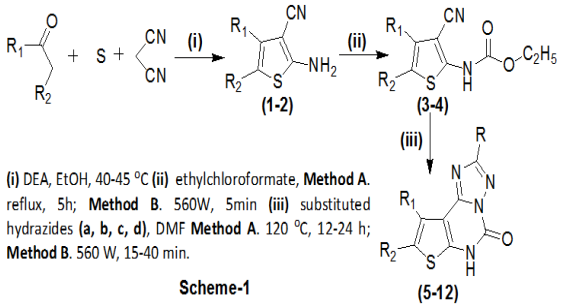
Mast Cell Degranulation Studies (*in vitro*)

Male albino rats were sacrificed by cervical dislocation. The animals were immediately injected with 15 ml of pre-warmed (37 °C) buffered salt solution (NaCl 137 mM; KCl 2.7 mM, $MgCl_2$ 1 mM, $CaCl_2$ 0.5 mM, NaH_2PO_4 0.4 mM, Glucose 5.6 mM, HEPES 10 mM) into the peritoneal cavity and massaged gently in this region for 90 s to facilitate cell recovery. A midline incision was made and the peritonium was exposed. The pale fluid was aspirated using a blunt plastic Pasteur pipette and collected in a plastic centrifuge tube. The fluid was then centrifuged at 1000 rpm for 5 min, and the supernatant discarded to reveal a pale cell pellete. The cell pelletes were re-suspended in fresh buffer and re-centrifuged. Aliquots of the cell suspension were incubated with the test compounds or disodium chromoglycate, before challenge with egg albumin. The aliquotes were carefully spread over glass slides and the mast cells were stained with 1% toluidine blue and counterstained with 0.1% light green. The slides were dried in air and the mast cells were counted from randomly selected high power objective fields (X450). The effect of all the test compounds (5-12) on mast cells was studied by incubating the mast cells for 10 min with all the test compounds in a concentration 20 µg/ml. In another set of experiments the mast cells which were pre-incubated with the test drugs were exposed to the mast cell degranulator, egg albumin (10 µg/ml) and the incubation continued for further 10 min. Then the mast cells were carefully spread over glass slides. The percent degranulation of the mast cells was calculated. The percentage (%) protection of mast cells by all the test compounds was calculated.

RESULTS AND DISCUSSION

Chemistry

The designed molecules fused thieno[3,2-*e*][1,2,4] triazolo[1,5-*c*]pyrimidinone derivatives (5-12) were synthesized (scheme 1) starting from 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-carbonitrile (1) and 2-amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-3-carbonitrile (2), which in turn was prepared following the well-known Gewald process.[21] Although, a solvent-free method to prepare ortho amino ester of thiophene (1 and 2) using microwave irradiation has been reported[22], Gewald's method was found to be more convenient and cost effective as far as the synthesis of compound 1 and 2 is concerned. The formation of thiophene was confirmed by physical and spectral data. The physical data of the compound was corroborating well with the literature.[21,23,24]



(i) DEA, EtOH, 40-45 °C **(ii)** ethylchloroformate, **Method A.** reflux, 5h; **Method B.** 560W, 5min **(iii)** substituted hydrazides **(a, b, c, d)**, DMF **Method A.** 120 °C, 12-24 h; **Method B.** 560 W, 15-40 min.

Scheme-1

Heating of 1 and 2 in excess of ethyl chloroformate gave ethyl-3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophene-2-

ylcarbamate (3) and Ethyl-3-cyano-5,6-dihydro-4*H*-cyclopenta[*b*]thiophen-2-yl-carbamate (4) respectively in good quantity. Compounds 3-4, were synthesized by employing two methods namely Method A (conventional synthetic procedure) and Method B (MW irradiation).[20] This was done to modify and establish the suitability of the synthetic procedure in pursuit of easy work up methods, eco-friendly nature and reduced reaction time. The reaction proceeds *via* nucleophilic substitution as shown in Figure 1.

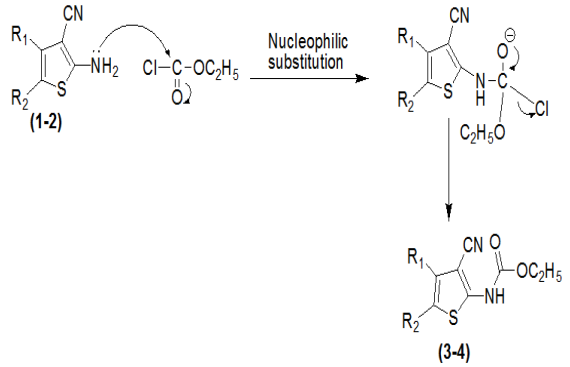


Figure 1: Mechanism involved in the formation of thiophene derivatives **3-4**.

These compounds (3-4) were characterized based on their spectral characteristics. They showed a typical nitrile (C≡N) absorption band at 2214·7 cm^{-1} and 2216 cm^{-1}, a strong signal at 1734·1 cm^{-1} and 1724 cm^{-1} of the carbonyl group and an absorption band at 1560·0 cm^{-1} and 1559 cm^{-1} may be due to the ν (N–H) of ethyl carbamate respectively in IR. Further, prominent triplet signal at d 1·32 ppm and 1.34 ppm and a quartet signal at 4·26–4·32 ppm of ethoxy protons and a broad singlet of NH at d 7·69 ppm and 7.63 ppm in ^{1}H NMR respectively confirmed the presence of ethyl carbamate group as the expected absorption pattern of carbamates.[25]

The compounds 3-4 on a further reaction with differently substituted hydrazides (a-d) (Method A and B) in DMF resulted in the formation of target compounds 5-12 in quantitative yields *via* a mechanism shown in Figure 2.

Under conventional conditions, these reactions have certain disadvantages like long reaction times (11-24 h), high energy consumption and the need for large amount of solvents for work up and purification. The MW assisted reactions were carried out using a Catalyst Microwave Reactor, under constant irradiation power and by varying the temperature (the so-called “power control”).

The best results were obtained when we used 80% of the full power of the magnetron (560W).

The details of the optimized conditions employed, under MW irradiation as well as under conventional method are presented in Table 1.

The target compounds were characterized based on their IR, ^{1}H NMR, ^{13}C NMR and elemental analyses data.

The final products showed no characteristic absorption peak at 2214-2216 cm^{-1} for the nitrile (C≡N) function in their IR spectra indicated the cyclization. All the target compounds showed -C-H stretch in the range 2950-2835 cm^{-1}, carbonyl stretch of cyclic amide between 1683-1718 cm^{-1}, -C=C stretch between 1612-1412 cm^{-1}, NH bend of cyclic amide between 1511-1520 cm^{-1}, aromatic *oop* bending between 975-682 cm^{-1} in their IR spectra. The ^{1}H NMR spectra showed the disappearance of triplet and quartet signals corresponding to ethyl protons of carbamates (3-4). In all the final compounds shifting of broad singlet from 7.63-7.69 ppm (NH of carbamate in compounds 3-4) to 12.74-13.22 ppm for NH of cyclic amide indicating the completion of cyclization and formation of compounds 5-12. Compounds 5-8 showed multiplets in the range 3.02-3.36 ppm (2H), 2.57-2.77 ppm (2H) and 1.86-1.95 ppm (4H) integrated to a total of eight protons were assignable to the methylene protons at 8^{th}, 9^{th}, 10^{th} and 11^{th} position of thienopyrimidine skeleton whereas compounds 9-12 showed multiplets in the range 3.01-3.11 ppm (2H), 2.74-2.77 ppm (2H) and 1.89-1.94 ppm (2H) integrated to a total of six protons were assignable to the methylene protons at 8^{th}, 9^{th} and 10^{th} position of thienopyrimidine skeleton.

A comparative analysis of the data obtained leads to the conclusion that the use of MW resulted in a remarkable acceleration of the reactions, with the reaction times decreasing dramatically, from hours to minutes (15 to 40 min). While optimizing the conditions, it was interesting to note that the reactions could be carried out at considerably lower temperatures (in the most cases by 10 to 30°C). It was also of interest that, in some cases, under MW irradiation the yields were substantially higher (by almost 30%).

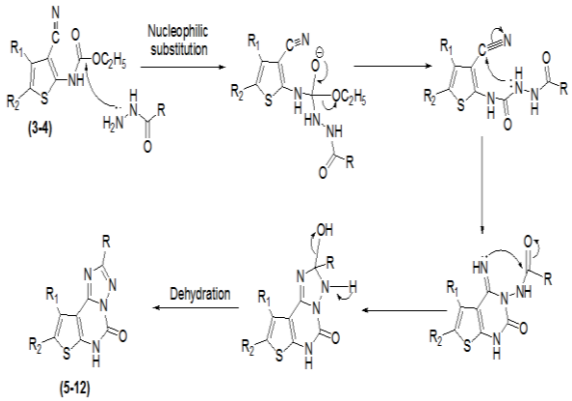


Figure 2: Mechanism involved in the formation of derivatives **5-12**.

Antiinflammatory Activity (*in vivo*)

The *in vivo* antiinflammatory screening for the synthesized compounds was performed by using the functional model of carrageenan-induced rat paw edema[26-28] and was presented as the percentage inhibition of edema at the right hind paw in comparison to the control. Carrageenan-induced edema is a nonspecific inflammation but is highly sensitive to NSAIDs. Indomethacin, a potent NSAID was used as a

reference standard. COX-2-mediated increase in prostaglandin E_2 (PG-E_2) production contributed to the severity of the inflammatory and pain responses in this model. Wistar rats (female) weighing between 190 to 240 g, obtained from Central Animal House, Panjab University, Chandigarh, India, were used in the present study.

Animals were kept in wire-mesh cages and maintained under constant environmental conditions [23 ± 2°C, 12 h, light]. All animals had free access to food and water, *ad libitum* and placed under a constant light-dark cycle. During the course of the experiment, the general behavior of animal was observed and found to be normal.

All the experimental protocols were approved by the Institutional Animal Ethical Committee (IAEC) and experiments were conducted according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India. Unless otherwise stated, the standard conditions were adopted in all experiments. Carrageenan and indomethacin were procured from Sigma Chemical, Co. GraphPad Prism (3.0 version) (GraphPad 2007) has been used for the statistical analysis.

All the test compounds exhibited protection against edema. The protection ranged up to 75.6%, while the reference drug (indomethacin) showed 76.7% at an equivalent dose (Table 2 & Figure 3).

Among the tested analogs, compounds 5 and 9 showed pronounced anti-inflammatory activity comparable with indomethacin in the Carrageenan induced rat paw edema model, suggesting that they might be effective in managing inflammatory conditions. On the other hand, compounds 6, 8 and 10 were nearly effective as indomethacin.

Table 1: Comparative data of conventional (Method-A) and microwave-assisted (Method-B) synthesis of compounds **3-12**.

Compd No.	Groups		R_f value*	Conventional (Method-A)		Microwave-assisted (Method-B)	
	R_1 R_2	R	H:EA :: 9:1	Time (h)	% Yield	Time (min)	% Yield
3	-$(CH_2)_4$-	-	0.62	5	72	5	90
4	-$(CH_2)_3$-	-	0.61	5	76	5	93
5	-$(CH_2)_4$-	-2-Cl-C_6H_4	0.54	12	39.3	15	66.2
6	-$(CH_2)_4$-	-2-OH-C_6H_4	0.52	24	39.6	40	65.6
7	-$(CH_2)_4$-	-C_6H_5	0.41	19	41.3	29	72.8
8	-$(CH_2)_4$-	-4-NO_2-C_6H_4	0.44	16	59.3	20	80.5
9	-$(CH_2)_3$-	-2-Cl-C_6H_4	0.46	11	48.1	15	70.1
10	-$(CH_2)_3$-	-2-OH-C_6H_4	0.39	16	45.2	21	55.3
11	-$(CH_2)_3$-	-C_6H_5	0.45	12	69	15	82.5
12	-$(CH_2)_3$-	-4-NO_2-C_6H_4	0.52	15	65	16	72.3

*Hexane:Ethylacetate::9:1

Table 2: Antiinflammatory data of compounds **5-12** on carrageenan-induced paw edema in rats.

Treatment	Volume of Paw edema (ml)*	
	2 h	4 h
Control (carrageenan treated)	0.86 ± 0.025	0.82 ± 0.028
Indomethacin	0.20 ± 0.011 (76.7)	0.19 ± 0.015 (76.8)
5	0.21 ± 0.027 (75.6)	0.20 ± 0.020 (75.6)
6	0.24 ± 0.012 (72.1)	0.22 ± 0.017 (73.2)
7	0.38 ± 0.017 (55.8)	0.34 ± 0.012 (58.5)
8	0.26 ± 0.019 (69.7)	0.24 ± 0.018 (70.7)
9	0.23 ± 0.021 (73.3)	0.21 ± 0.017 (74.4)
10	0.24 ± 0.025 (72.1)	0.22 ± 0.030 (73.2)
11	0.40 ± 0.028 (53.5)	0.40 ± 0.023 (38.8)
12	0.36 ± 0.014 (58.1)	0.35 ± 0.015 (57.3)

*Values are expressed as mean ± S.E.M (*n*=5) and analyzed by ANOVA.Values in parenthesis (percentage inhibition of edema), 10mg/kg.

Table 3: Effect of synthesized compounds **5-12** on egg albumin treated mast cell degranulation in rat.

Groups	Treatment	Degranulation after Treatment (%)[a]	
		Vehicle	Egg albumin (10 µg/ml)
Control	1% Tween-80	33.4 ± 2.33	85 ± 3.43
Disodium cromoglycate	20 µg/ml	13.2 ± 0.92	25 ± 2.36
5	20 µg/ml	14.5 ± 1.33	26 ± 2.45
6	20 µg/ml	16.4 ± 2.21	28 ± 2.02
7	20 µg/ml	27.9 ± 0.78	55 ± 3.12
8	20 µg/ml	20.6 ± 1.77	32 ± 2.16
9	20 µg/ml	17.4 ± 1.56	30 ± 2.18
10	20 µg/ml	22.1 ± 1.88	33 ± 2.11
11	20 µg/ml	23.6 ± 1.87	50 ± 2.45
12	20 µg/ml	24.2 ± 2.65	40 ± 2.66

[a]Each value represents the mean ± S.E.M. of five observations

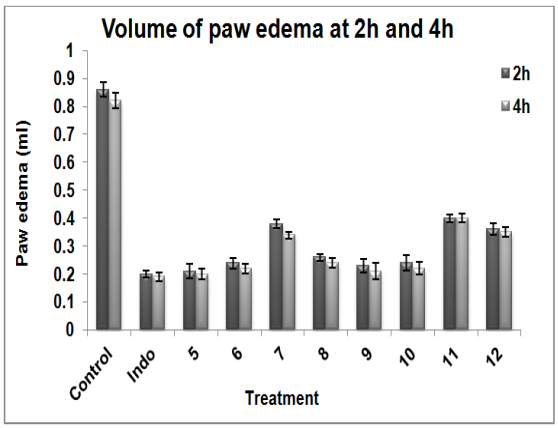


Figure 3: Anti-inflammatory activity: Treated groups (compounds **5-12**) versus paw edema (ml after 2h and after 4h.

Mast Cell Degranulation Studies (*in vitro*)

Mast cell degranulation studies were carried out for newly synthesized compounds to see their anti-inflammatory effect. The experiment was carried out as per the method described by Kaley and Weiner with little modification.[29] Several mast cell mediators are more general amplifiers of the inflammatory response.[30-32] Cromolyn sodium and Nedocromil sodium are the two main drugs that inhibit the release of inflammatory mediators from bronchial mast cells.[33]

So, Disodium cromoglycate (DSCG) (20 µg/ml) was included in one of the study groups for comparison as a reference drug.[33-35] All the synthesized compounds were tested for their abilities to protect the egg albumin-induced degranulation of mast cells. The control group showed (85 ± 3.43) degranulation of mast cell while groups treated with compounds (5-12) and disodium chromoglycate (DSCG) at a concentration of 20 µg/ml significantly (P<0.05) protected degranulation of mast cells (Tables 3 & Figure 4). In particular, the compounds 5 (74%), 6 (72%) and 9 (70%) significantly protect egg albumin-induced degranulation of mast cell comparable to disodium chromoglycate (75%).

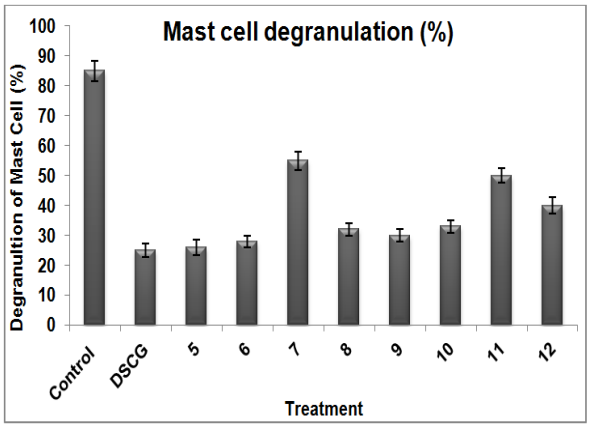


Figure 4: Effect of synthesized compounds **5-12** on egg albumin treated mast cell degranulation (%) in rat.

CONCLUSION

In the present study, a series of new Thiophene[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5(6*H*)-one derivatives have been synthesized by a facile microwave assisted method and evaluated in vivo (rat paw edema) for their antiinflammatory activities and *in vitro* mast cell degranulation studies.

The microwave-assisted synthesis of these compounds was found to be economic, less time consuming with better yield as compared to classical heating conditions.

Among all the tested compounds, compounds 5, 6, 8, 9 and 10 showed remarkable anti-inflammatory activity compared to other derivatives (around 91-97 % of the

standard: indomethacin) and also good protection in mast cell degranulation studies (89-99% of the standard: disodium cromoglycate).

Consequently, this type of compounds would represent a fruitful template for further development of more potent and selective anti-inflammatory agents.

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