



# CHEMISTRY & BIOLOGY INTERFACE

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## Synthesis and anti-inflammatory evaluation of novel 5-methoxy-3-(6'-aryl-methylene-thiazolo-[3,2-b]-1,2,4-triazol-5(6H)-one-2'-yl-methyl)indoles

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**Abstract:** A series of new leads towards potent anti-inflammatory agents, of novel 5-methoxy-3-(6'-aryl-methylene-thiazolo-[3,2-b]-1,2,4-triazol-5(6H)-one-2'-yl-methyl)indoles (**5-14**) have been synthesized from 5-methoxy-3-(5'-mercapto-1,2,4-triazol-3'-yl-methyl) indole (**4**). All synthesized compounds were evaluated for their toxicity profile, anti-inflammatory activity, ulcerogenic liability and the results showed positive anti-inflammatory activity. SAR studies have shown that compound 5-methoxy-3-[6'-(*p*-chlorophenyl)-methylene-thiazolo-[3,2-b]-1,2,4-triazol-5(6H)-one-2'-yl-methyl]indole (**6**) displayed best anti-inflammatory activity.

**Keywords:** Indolylthiazolylthiazoles, Anti-inflammatory activity, Ulcerogenic liability, Acute toxicity, Cyclooxygenase assay

### Introduction

Inflammation is known not only as a symptom of great deal of common ailments but also as an early phase of some serious diseases such as heart vascular diseases [1- 2], cancer [3], cytokines & insulin resistance [4] and various kinds of rheumatoid arthritis [5]. Inflammation was found to be the main problems in ESRD patients [6]. Some reasons like uremic toxins, asymmetric dimethylarginine, homocysteine and modified proteins, retention of cytokines, mechanical stress of the vascular wall, comorbidities such as advanced age and

diabetes and extra-osseous calcification [7,8] for the potentially trigger of the inflammatory response. Some accumulation of the PD catheter itself increases the inflammatory response in the PM and induces fibrosis, angiogenesis and increased peritoneal transport [9]. Inflammatory diseases also comprise different kind of arthritis, respiratory and cutaneous diseases. So, the discovery of novel anti-inflammatory drugs has attracted a lot of interests. The problems in treating these diseases particularly in chronic setting are that these are multifactorial, involve several changes in the system affected by the diseases, have several proteins involved in

the metabolic cascade and are multigenic. Moreover, regularly prescribed drugs viz. NSAIDs and corticosteroids exhibited serious side effects. So, there is not a single drug available, which can be termed as ideal in terms of risk-benefit ratio. Hence, there is still critical need for new anti-inflammatory agents. Thus, as a part of our program of synthesizing new chemical entities possessing anti-inflammatory profile, our interests have been focussed on indole derivatives.

On exhaustive literature survey, indole and its derivatives [10-17] have possessed good anti-inflammatory activity when these were associated with triazole [18-21] and thiazole [22-25] rings. Therefore, in view of above facts and in continuation to search for new lead anti-inflammatory agents, herein, we have reported the synthesis of novel class of indolyltriazolylthiazoles and their biological evaluation such as anti-inflammatory activity and ulcerogenic liability along with toxicity profile.

## Results and Discussion

### Chemistry

The reaction sequence leading to the formation of various indole derivatives is given in the **scheme-1**. The reaction of 5-methoxyindole with ethyl chloroacetate produced 5-methoxy-3-ethylaceto-indole (**2**), which was converted into 5-methoxy-3-(thiosemicarbazido-carbonyl-methyl)indole (**3**) on treatment with thiosemicarbazide. Furthermore, compound **3** was reacted with aq. KOH to afford 5-methoxy-3-(5'-mercapto-1,2,4-triazol-3'-yl-methyl) indole (**4**). Finally, compound **4** was condensed with various aromatic/heteroaromatic aldehydes to yield indolyltriazolylthiazoles (**5-14**). From all, 5-methoxy-3-(6'-aryl-methylene-thiazolo-[3,2-b]-1,2,4-triazol-5-(6*H*)-one-2'-yl-methyl) indoles (**5**) is discussed here, it was synthesized from compound **4** using chloroacetic acid,

benzaldehyde and anhydrous sodium acetate in acetic anhydride and acetic acid under refluxed for 6-8 hours. IR spectra have shown all expected peaks in  $\text{cm}^{-1}$  at 3357(N-H), 3078(C-H aromatic), 2939(C-H aliphatic), 1654(C=O), 1636 (C=C of aromatic ring), 1046(C-O-C) and 659(C-S-C), this was fully supported by  $^1\text{H-NMR}$  have shown expected signals at  $\delta$  11.68 (s, 1H, NH of indole, exchangeable with  $\text{D}_2\text{O}$ ), 8.82 (s, 1H,  $\text{H}_2$  of indole), 8.14-7.59 (m, 3H of indole), 7.57-6.93 (m, 5H, Ar-H), 6.09 (s, 1H, CH-Ar), 5.18 (s, 2H,  $\text{CH}_2$ ), 3.31 (s, 3H,  $-\text{OCH}_3$ ) and finally mass spectrometry was confirmed that compounds have shown molecular ion peak at 388 [ $\text{M}^+$ , 18%].

The structures of all synthesized compounds were confirmed by their spectral studies such as IR,  $^1\text{H}$  NMR and Mass spectrometry and elemental analysis.

### Biological

#### Acute toxicity study

Then, compounds **4-14** were investigated *in vivo* for their pharmacological actions and the results of the study are as follows: compounds **4-14** have been assessed for their toxicity profile. Results of the study revealed that compounds **6** and **7** exhibited  $\text{LD}_{50} > 800 \text{ mg kg}^{-1} \text{ p.o.}$ , whereas compounds **8-11** and **14** showed  $\text{LD}_{50} > 600 \text{ mg kg}^{-1} \text{ p.o.}$  Compounds **4**, **5**, **11** and **13** were found to be more toxic ( $\text{LD}_{50} > 400 \text{ mg kg}^{-1} \text{ p.o.}$ ) as compared to rest compound of the present study (**Table-1**).

#### Anti-inflammatory activity

The compounds **4-14** along with reference drug: indomethacin was assayed for their anti-inflammatory activity at a dose of  $50 \text{ mg kg}^{-1} \text{ p.o.}$  All these tested compounds displayed varying degree of inhibition of oedema and results were found to be statistically significant anti-inflammatory activity (**Table-1**). Out of eleven

compounds tested compounds **6** and **7** were found to possess more potent anti-inflammatory activity (61.22% and 58.00%, respectively) as compared to indomethacin (56.31%) at a dose of 50 mg kg<sup>-1</sup> p.o. Considering their potentiality, compounds **6**, **7** and reference drugs were examined at two more doses of 25 mg kg<sup>-1</sup> and 100 mg kg<sup>-1</sup> for their anti-inflammatory activity. After that experiment, these two compounds showed superior anti-inflammatory activity at all doses tested. However, compound **6** exhibited maximal anti-inflammatory activity.

### Ulcerogenic activity

Two most active compounds **6** and **7** with standard drug, indomethacin were screened for their ulcerogenic liability. Compound **6** was found to be less ulcerogenic as compared to compound **7** and reference drugs.

### Cyclooxygenase assay

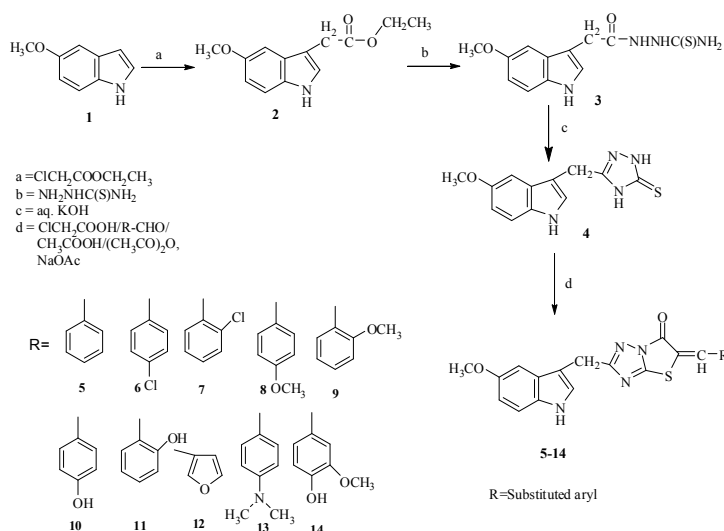
The most promising compounds **6**, **7** and reference drug were underwent a cyclooxygenase assay to explore their most likely mechanism of action. Results given in **Table-1** suggested that these compounds reduce the inflammatory response by inhibiting the prostaglandins' (PG) synthesis by inhibiting the cyclooxygenase

(COX) enzyme.

### Discussion

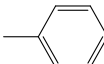
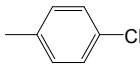
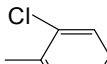
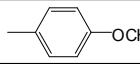
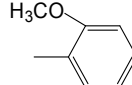
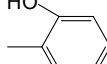
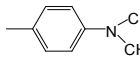
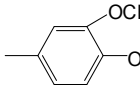
All the compounds exhibited statistically significant anti-inflammatory activity ranging from 22.25%-61.22% (**Table-1**). Structure activity relationship of compounds **4-14** revealed that least inhibition of oedema was found in case of compound **4**, but its conversion into compounds **5-14** having different substituted aryl substituent remarkably increase the inflammation inhibiting property of the compounds.

It is significant to note from the observations that when the compounds **6** and **7** bearing *o*- and *p*-chloro phenyl group, as a substituent, they showed most potent anti-inflammatory activity, where as substitution with *o*- and *p*-methoxyphenyl as seen in compounds **8** and **9** produced appreciable activity. The *o*- and *p*-hydroxyphenyl substituent as seen in compounds **10** and **11** yielded interesting anti-inflammatory profile. Furthermore, compounds **12**, **13** and **14** having furfural, *p*-aminodimethylphenyl and *p*-hydroxy-*m*-methoxyphenyl substitution, respectively, produced lesser degree of anti-inflammatory activity.



Synthetic scheme for indolyltriazolylthiazoles

**Table-1:** Pharmacological results of compounds (4-14):

| Compd. No.            | R                                                                                   | Acute toxicity<br>LD <sub>50</sub> (mg Kg <sup>-1</sup><br>p.o.) | Anti-inflammatory activity               |                                                                   | Ulcerogenic<br>activity<br>UD <sub>50</sub><br>(mg kg <sup>-1</sup> i.p.) | Cyclooxygenas<br>assay (%)<br>inhibition 10 μM) |
|-----------------------|-------------------------------------------------------------------------------------|------------------------------------------------------------------|------------------------------------------|-------------------------------------------------------------------|---------------------------------------------------------------------------|-------------------------------------------------|
|                       |                                                                                     |                                                                  | Dose<br>(mg/kg <sup>-1</sup><br>nump.o.) | % inhibition<br>of oedema                                         |                                                                           |                                                 |
| Control <sup>d)</sup> |                                                                                     | -                                                                | 1 mL                                     | 0.0                                                               | -                                                                         | -                                               |
| 4                     |                                                                                     | >400                                                             | 50                                       | 22.25 <sup>a)</sup>                                               | -                                                                         | -                                               |
| 5                     |    | >800                                                             | 50                                       | 28.53 <sup>a)</sup>                                               | -                                                                         | -                                               |
| 6                     |    | >800                                                             | 25<br>50<br>100                          | 41.53 <sup>b)</sup><br>61.22 <sup>a)</sup><br>84.10 <sup>b)</sup> | 140                                                                       | 85                                              |
| 7                     |    | >800                                                             | 25<br>50<br>100                          | 37.51 <sup>a)</sup><br>58.00 <sup>b)</sup><br>80.21 <sup>b)</sup> | 120                                                                       | 70                                              |
| 8                     |    | >600                                                             | 50                                       | 54.00 <sup>b)</sup>                                               | -                                                                         | -                                               |
| 9                     |   | >600                                                             | 50                                       | 50.10 <sup>b)</sup>                                               | -                                                                         | -                                               |
| 10                    |  | >600                                                             | 50                                       | 42.35 <sup>a)</sup>                                               | -                                                                         | -                                               |
| 11                    |  | >600                                                             | 50                                       | 40.24 <sup>b)</sup>                                               | -                                                                         | -                                               |
| 12                    |  | >400                                                             | 50                                       | 31.50 <sup>a)</sup>                                               | -                                                                         | -                                               |
| 13                    |  | >400                                                             | 50                                       | 36.85 <sup>a)</sup>                                               | -                                                                         | -                                               |
| 14                    |  | >400                                                             | 50                                       | 39.01 <sup>a)</sup>                                               | -                                                                         | -                                               |
| Indomethacin          |                                                                                     | -                                                                | 25<br>50<br>100                          | 35.5 <sup>b)</sup><br>56.31 <sup>c)</sup><br>78.0 <sup>b)</sup>   | 90                                                                        | 86                                              |

<sup>a)</sup> P < 0.05; <sup>b)</sup> P < 0.01; <sup>c)</sup> P < 0.001; n = 6

<sup>d)</sup> Propylene glycol served as control; - denotes that activity was not performed.

## Experimental

### General

All the reagents and solvents were generally received from commercial supplier viz. Spectrochem, Rankem, RFCL, Merck, Qualigens and Sigma-aldrich. All melting points are in centigrade scale, uncorrected and taken on digital melting point apparatus. All organic solvents were dried and distilled before use; petroleum ether used had a boiling range 60-80°C. All ethereal or ethyl acetate extracts were dried over anhydrous sodium sulphate. Solvents were generally removed under reduced pressure.  $R_f$  values were recorded for TLC that was carried out on glass plates coated with 0.2 mm layer of silica gel-G and also on Merck plates. The compounds on the TLC plate were visualized in UV light (254 and 366 nm) or developed in iodine atmosphere and also located by spraying various reagents. Spraying agents used for TLC were 5% aqueous sulphuric acid and 1% ethanolic ferric chloride. Infra-red (IR) spectra were recorded on Shimadzu model 435 spectrophotometer using KBr pellets and only major absorption bands are quoted in  $\text{cm}^{-1}$ . Proton nuclear magnetic resonance ( $^1\text{H}$  NMR) spectra were recorded on JEOL (400MHz) spectrometers with reference to tetramethylsilane as the internal standard. The chemical shifts are expressed in  $\delta$  values and coupling constant ( $J$ ) in Hertz. The abbreviation s, brs, d, t and m indicate the signals as singlet, broad singlet, doublet, triplet and multiplet respectively. The Mass spectra were recorded on a Varian MAT 311A instrument by using electron ionization (EI) at 70eV and TOF MS on LCT micromass and only the major peaks are quoted. Apparatus was dried in electrical oven at 120°C, flushed with nitrogen prior to use. The carbon, hydrogen and nitrogen analysis were performed on Carlo Erba-1108 (Carlo Erba, Milan, Italy) and the results were found within + 0.4% of the theoretical values. All spectral analysis was analyzed in Department

of Chemistry, University of Delhi.

### *General procedure for the synthesis of 5-Methoxy-3-ethylaceto-indole (2)*

A mixture of 5-methoxyindole (**1**), (35g, 238mmol), ethyl chloroacetate (25.47 ml, 238mmol) and anhydrous  $\text{K}_2\text{CO}_3$  (4.0g) in acetone (150ml) was refluxed for 15 hours. Progress and completion of the reaction was monitored by TLC. Then, excess of the solvent was distilled off under reduced pressure and the resulting solid mass poured into ice-water, filtered and the separated solid crystallized from methanol to yield compound **2**.

Yield: 28.07g (80.07%), MP: 51-52°C, IR [ $\text{cm}^{-1}$ , KBr]: 3293(N-H), 3073(C-H aromatic), 2941(C-H aliphatic), 1717(C=O), 1615 (C=C of aromatic ring),  $^1\text{H}$ -NMR [400 MHz,  $\text{CDCl}_3$ -ppm]:  $\delta$ 11.86 (s, 1H, NH of indole, exchangeable with  $\text{D}_2\text{O}$ ), 7.72 (s, 1H,  $\text{H}_2$  of indole), 7.12-7.10 (m, 3H of indole), 4.63 (s, 2H,  $\text{CH}_2$ ), 4.12 (s, 3H of  $-\text{OCH}_3$ ), 2.50-2.48 (q, 2H,  $J=9.6$  Hz,  $-\text{CH}_2$  of ethyl group), 1.15-1.11 (t, 3H,  $J=7.2$  Hz,  $-\text{CH}_3$ ), MS: in  $m/z$  [rel. %]: 233 [ $\text{M}^+$ , 52.3%], Anal. Calcd for  $\text{C}_{13}\text{H}_{15}\text{NO}_3$ : C, 66.95; H, 6.44; N, 6.01. Found: C, 66.86; H, 6.52; N, 6.12.

### *General procedure for the synthesis of 5-Methoxy-3-(thiosemicarbazido-carbonylmethyl)indole (3)*

To a mixture of 5-methoxy-3-ethylaceto-indole (**2**) (27.0g, 115.8mmol) in methanol (80ml), thiosemicarbazide (8.7g, 115.8mmol) added and the resulting mixture was heated under reflux for 10 hours. After completion of the reaction, excess of the solvent was removed under reduced pressure and the formed precipitate were filtered, recrystallized from absolute ethanol to give the compound **3**.

Yield: 21.20g (78.5%), MP: 88-89°C, IR [ $\text{cm}^{-1}$ , KBr]: 3062(N-H), 2924(C-H aromatic), 2852(C-H aliphatic), 1685(C=O), 1649 (C=C of

aromatic ring),  $^1\text{H-NMR}$ [400 MHz,  $\text{DMSO-D}_6$ -ppm]:  $\delta$  12.41(s, 1H, NH of indole, exchangeable with  $\text{D}_2\text{O}$ ), 11.44 (brs, 2H of  $\text{NH}_2$ , exchangeable with  $\text{D}_2\text{O}$ ), 10.87 (s, 1H of CONH), 8.23 (s, 1H of NHCS), 7.49 (s, 1H,  $\text{H}_2$  of indole), 7.08-6.79 (m, aromatic 3H of indole), 5.85 (s, 2H,  $\text{CH}_2$ ), 3.33 (s, 3H,  $-\text{OCH}_3$ ), MS: in m/z [rel.%]: 278 [ $\text{M}^+$ , 34.5%], Anal. Calcd for  $\text{C}_{12}\text{H}_{14}\text{N}_4\text{O}_2\text{S}$ : C, 51.80; H, 5.04; N, 20.14. Found: C, 51.72; H, 5.18; N, 20.25.

*General procedure for the synthesis of 5-Methoxy-3-(5'-mercapto-1,2,4-triazol-3'-yl-methyl)indole (4)*

A solution of compound **3** (20g, 71.9mmol) in 100ml of 10% aq. KOH (4.063g, 71.9mmol), 400ml of water was heated for 3hours, cooled, filtered and the filtrate was acidified with HCl (37%). The precipitated solid was filtered and washed with water. Then the product was crystallized from methanol-ether to furnish compound **4**.

Yield: 15.06g (64.7%), MP: 109-110°C, IR[ $\text{cm}^{-1}$ , KBr]: 3401(N-H), 2924(C-H aromatic), 2852(C-H aliphatic), 1628(C=C of aromatic ring), 1464(C=S),  $^1\text{H-NMR}$  [400 MHz,  $\text{DMSO-D}_6$ -ppm]:  $\delta$  13.04 (s, 1H, NH of indole, exchangeable with  $\text{D}_2\text{O}$ ), 9.88 (s, 1H, NH of triazole, exchangeable with  $\text{D}_2\text{O}$ ), 8.42 (s, 1H, NH of triazole, exchangeable with  $\text{D}_2\text{O}$ ), 7.21-6.85 (m, aromatic 4H of indole), 3.33 (s, 2H,  $\text{CH}_2$ ), 3.33 (s, 3H,  $-\text{OCH}_3$ ), MS: in m/z [rel.%]: 260 [ $\text{M}^+$ , 23%], Anal. Calcd for  $\text{C}_{12}\text{H}_{12}\text{N}_4\text{OS}$ : C, 55.38; H, 4.62; N, 21.54. Found: C, 55.45; H, 4.70; N, 21.46.

*General procedure for the synthesis of 5-methoxy-3-(6'-aryl-methylene-thiazolo-[3,2-b]-1,2,4-triazol-5(6H)-one-2'-yl-methyl)indoles (5-14).*

A solution of compound **4** (1.6g, 6.15mmol), chloroacetic acid (1.16g, 12.3mmol), proper aromatic aldehydes (6.15), anhydrous

sodium acetate (0.70g, 8.53mmol), acetic anhydride (2.5ml, 26.4mmol) and acetic acid (4.5ml, 78.6mmol) was refluxed for 6-8 hours. Then, after refluxing, the mixture was poured into ice water. The precipitated solid was filtered and dissolved in dichloromethane. The organic layer was washed using 6%  $\text{NaHCO}_3$  and brine and then dried over  $\text{Na}_2\text{SO}_4$  and evaporated under reduced pressure. The crude product was recrystallized from appropriate solvents to produce compounds **5-14**. By employing above procedure, compounds **5-14** were synthesized starting from benzaldehyde, 4-chloro benzaldehyde, 2-chlorobenzaldehyde, 4-methoxybenzaldehyde, 2-methoxybenzaldehyde, 4-hydroxybenzaldehyde, 2-hydroxybenzaldehyde, furfuraldehyde, 4-aminodimethyl benzaldehyde and 4-hydroxy-3-methoxybenzaldehyde respectively.

*5-methoxy-3-(6'-(phenyl)-methylene-thiazolo-[3,2-b]-1,2,4-triazol-5(6H)-one-2'-yl-methyl)indole (5)*

Title compound was synthesized from compound **4** (1.6g, 6.15mmol), chloroacetic acid (1.16g, 12.3mmol), benzaldehyde (0.62ml, 6.15mmol), anhydrous sodium acetate (0.70g, 8.53mmol), acetic anhydride (2.5ml, 26.4mmol) and acetic acid (4.5ml, 78.6mmol) at refluxing for 6-8 hours.

Yield: 1.05g (65.4%), MP: 181-182°C, IR[ $\text{cm}^{-1}$ , KBr]: 3357(N-H), 3078(C-H aromatic), 2939(C-H aliphatic), 1654(C=O), 1636 (C=C of aromatic ring), 1046(C-O-C), 659(C-S-C),  $^1\text{H-NMR}$  [400 MHz,  $\text{DMSO-D}_6$ -ppm]:  $\delta$  11.68 (s, 1H, NH of indole, exchangeable with  $\text{D}_2\text{O}$ ), 8.82 (s, 1H,  $\text{H}_2$  of indole), 8.14-7.59 (m, 3H of indole), 7.57-6.93 (m, 5H, Ar-H), 6.09 (s, 1H, CH-Ar), 5.18 (s, 2H,  $\text{CH}_2$ ), 3.31 (s, 3H,  $-\text{OCH}_3$ ), MS: in m/z [rel.%]: 388 [ $\text{M}^+$ , 18%], Anal. Calcd for  $\text{C}_{21}\text{H}_{16}\text{N}_4\text{O}_2\text{S}$ : C, 64.95; H, 4.12; N, 14.43. Found: C, 65.10; H, 4.22; N, 14.50.

*5-Methoxy-3-(6'-(p-chlorophenyl)-methylene-*

*thiazolo-[3,2-b]-1,2,4-triazol-5(6H)-one-2'-yl-methyl) indole (6)*

Title compound was synthesized from compound **4** (1.6g, 6.15mmol), chloroacetic acid (1.16g, 12.3mmol), 4-chlorobenzaldehyde (0.69ml, 6.15mmol), anhydrous sodium acetate (0.70g, 8.53mmol), acetic anhydride (2.5ml, 26.4mmol) and acetic acid (4.5ml, 78.6mmol) at refluxing for 6-8 hours.

Yield: 1.04g (64.8%), MP: 154-155°C, IR[cm<sup>-1</sup>, KBr]: 3433(N-H), 2994(C-H aromatic), 2933(C-H aliphatic), 1648(C=O), 1578 (C=C of aromatic ring), 1038(C-O-C), 1002(N-N), 753(C-Cl), 628(C-S-C), <sup>1</sup>H-NMR[400 MHz, DMSO-D<sub>6</sub>-ppm]: δ 11.74 (s, 1H, NH of indole, exchangeable with D<sub>2</sub>O), 8.03 (s, 1H, H<sub>2</sub> of indole), 7.75-7.62 (m, aromatic 3H of indole), 7.48-6.91 (m, aromatic 4H of benzene), 6.16 (s, 1H, CH-Ar), 5.21 (s, 2H, CH<sub>2</sub>), 3.32 (s, 3H, -OCH<sub>3</sub>), MS: [M<sup>+</sup>] at m/z 422.5, [M+2] at m/z 424.5, Anal. Calcd for C<sub>21</sub>H<sub>15</sub>N<sub>4</sub>O<sub>2</sub>SCl: C, 59.64; H, 3.55; N, 13.25. Found: C, 59.88; H, 3.43; N, 13.35.

*5-methoxy-3-(6'-(o-chlorophenyl)-methylene-thiazolo-[3,2-b]-1,2,4-triazol-5(6H)-one-2'-yl-methyl)indoles (7)*

Title compound was synthesized from compound **4** (1.6g, 6.15mmol), chloroacetic acid (1.16g, 12.3mmol), 2-chlorobenzaldehyde (0.70ml, 6.15mmol), anhydrous sodium acetate (0.70g, 8.53mmol), acetic anhydride (2.5ml, 26.4mmol) and acetic acid (4.5ml, 78.6mmol) at refluxing for 6-8 hours.

Yield: 1.11g (69.2%), MP: 161-162°C, IR[cm<sup>-1</sup>, KBr]: 3432(N-H), 2994(C-H aromatic), 2934(C-H aliphatic), 1685(C=O), 1648 (C=C of aromatic ring), 1069(C-O-C), 1003(N-N), 700(C-Cl), 628(C-S-C), <sup>1</sup>H-NMR[400 MHz, DMSO-D<sub>6</sub>-ppm]: δ 11.83 (s, 1H, NH of indole, exchangeable with D<sub>2</sub>O), 8.04 (s, 1H, H<sub>2</sub> of indole), 7.66-7.61 (m, aromatic 3H of indole),

7.61-7.25 (m, 4H, Ar-H), 7.23 (s, 1H, CH-Ar), 6.04 (s, 2H, CH<sub>2</sub>), 3.37 (s, 3H, -OCH<sub>3</sub>), MS: [M<sup>+</sup>] at m/z 422.5, [M+2] at m/z 424.5, Anal. Calcd for C<sub>21</sub>H<sub>15</sub>N<sub>4</sub>O<sub>2</sub>SCl: C, 59.64; H, 3.55; N, 13.25. Found: C, 59.75; H, 3.69; N, 13.31.

*5-Methoxy-3-(6'-(p-methoxyphenyl)-methylene-thiazolo-[3,2-b]-1,2,4-triazol-5(6H)-one-2'-yl-methyl)indole (8)*

Title compound was synthesized from compound **4** (1.6g, 6.15mmol), chloroacetic acid (1.16g, 12.3mmol), 4-methoxybenzaldehyde (0.75ml, 6.15mmol), anhydrous sodium acetate (0.70g, 8.53mmol), acetic anhydride (2.5ml, 26.4mmol) and acetic acid (4.5ml, 78.6mmol) at refluxing for 6-8 hours.

Yield: 0.99g (62.0%), MP: 188-189°C, IR[cm<sup>-1</sup>, KBr]: 3357(N-H), 3077(C-H aromatic), 2939(C-H aliphatic), 1636(C=O), 1614 (C=C of aromatic ring), 1107(C-O-C), 1013(N-N), 658(C-S-C), <sup>1</sup>H-NMR[400 MHz, DMSO-D<sub>6</sub>-ppm]: δ 11.53 (s, 1H, NH of indole, exchangeable with D<sub>2</sub>O), 7.75 (s, 1H, H<sub>2</sub> of indole), 7.58-7.15 (m, aromatic 3H of indole), 6.93-6.84 (m, 4H, Ar-H), 6.07 (s, 1H, CH-Ar), 5.23 (s, 2H, CH<sub>2</sub>), 3.90 (s, 3H, 2×-OCH<sub>3</sub>), MS: in m/z [rel.%]: 418 [M<sup>+</sup>, 15.5%], Anal. Calcd for C<sub>22</sub>H<sub>18</sub>N<sub>4</sub>O<sub>3</sub>S: C, 63.16; H, 4.31; N, 13.40. Found: C, 63.20; H, 4.42; N, 13.49.

*5-Methoxy-3-(6'-(o-methoxyphenyl)-methylene-thiazolo-[3,2-b]-1,2,4-triazol-5(6H)-one-2'-yl-methyl)indoles (9)*

Title compound was synthesized from compound **4** (1.6g, 6.15mmol), chloroacetic acid (1.16g, 12.3mmol), 2-methoxybenzaldehyde (0.75ml, 6.15mmol), anhydrous sodium acetate (0.70g, 8.53mmol), acetic anhydride (2.5ml, 26.4mmol), acetic acid (4.5ml, 78.6mmol) at refluxing for 6-8 hours.

Yield: 1.06g (66.5%), MP: 195-196°C, IR[cm<sup>-1</sup>, KBr]: 3269(N-H), 3086(C-H aromatic),

2928(C-H aliphatic), 1628(C=O), 1605 (C=C of aromatic ring), 1117(C-O-C), 1053(N-N), 643(C-S-C), <sup>1</sup>H-NMR[400 MHz, DMSO-D<sub>6</sub>-ppm]: δ 11.49 (s, 1H, NH of indole, exchangeable with D<sub>2</sub>O), 7.79 (s, 1H, H<sub>2</sub> of indole), 7.62-6.86 (m, 3H aromatic of indole), 6.78-6.72 (m, 4H, Ar-H), 6.00 (s, 1H, CH-Ar), 5.11 (s, 2H, CH<sub>2</sub>), 3.77 (s, 3H, OCH<sub>3</sub>), 3.71 (s, 3H, OCH<sub>3</sub>), MS: in m/z [rel.%]: 418 [M<sup>+</sup>, 16%], Anal. Calcd for C<sub>22</sub>H<sub>18</sub>N<sub>4</sub>O<sub>3</sub>S: C, 63.16; H, 4.31; N, 13.40. Found: C, 63.22; H, 4.51; N, 13.52.

*5-Methoxy-3-(6'-(p-hydroxyphenyl)-methylene-thiazolo-[3,2-b]-1,2,4-triazol-5(6H)-one-2'-yl-methyl)indole (10)*

Title compound was synthesized from compound **4** (1.6g, 6.15mmol), chloroacetic acid (1.16g, 12.3mmol), 4-hydroxybenzaldehyde (0.81g, 6.15mmol), anhydrous sodium acetate (0.70g, 8.53mmol), acetic anhydride (2.5ml, 26.4mmol) and acetic acid (4.5ml, 78.6mmol) at refluxing for 6-8 hours.

Yield: 1.15g (71.6%), MP: 150-151°C, IR[cm<sup>-1</sup>, KBr]: 3423(OH), 3060(N-H), 2926(C-H aromatic), 2868(C-H aliphatic), 1692 (C=O), 1647(C=C of aromatic ring), 1113(C-O-C), 956(N-N), 640(C-S-C), <sup>1</sup>H-NMR[400 MHz, DMSO-D<sub>6</sub>-ppm]: δ 11.46 (s, 1H, OH, exchangeable with D<sub>2</sub>O), 9.46 (s, 1H, NH of indole, exchangeable with D<sub>2</sub>O), 7.89 (s, 1H, H<sub>2</sub> of indole), 7.68-7.28 (m, aromatic 3H of indole), 6.93-6.79 (m, 4H, Ar-H), 6.19 (s, 1H, CH-Ar), 5.27 (s, 2H, CH<sub>2</sub>), 3.79 (s, 3H, -OCH<sub>3</sub>), MS: in m/z [rel.%]: 404 [M<sup>+</sup>, 26.5%], Anal. Calcd for C<sub>21</sub>H<sub>16</sub>N<sub>4</sub>O<sub>3</sub>S: C, 62.38; H, 3.96; N, 13.86. Found: C, 62.24; H, 3.87; N, 13.80.

*5-Methoxy-3-(6'-(o-hydroxyphenyl)-methylene-thiazolo-[3,2-b]-1,2,4-triazol-5(6H)-one-2'-yl-methyl)indole (11)*

Title compound was synthesized from compound **4** (1.6g, 6.15mmol), chloroacetic acid (1.16g, 12.3mmol), 2-hydroxybenzaldehyde (0.66g,

6.15mmol), anhydrous sodium acetate (0.70g, 8.53mmol), acetic anhydride (2.5ml, 26.4mmol) and acetic acid (4.5ml, 78.6mmol) at refluxing for 6-8 hours.

Yield: 1.21g (75.4%), MP: 143-144°C, IR[cm<sup>-1</sup>, KBr]: 3394 (OH), 3079(N-H), 2989(C-H aromatic), 2907(C-H aliphatic), 1705 (C=O), 1606(C=C of aromatic ring), 1043(C-O-C), 1012(N-N), 673(C-S-C), <sup>1</sup>H-NMR [400 MHz, DMSO-D<sub>6</sub>-ppm]: δ 11.67 (s, 1H, OH, exchangeable with D<sub>2</sub>O), 9.96 (s, 1H, NH of indole, exchangeable with D<sub>2</sub>O), 8.00 (s, 1H, H<sub>2</sub> of indole), 7.81-7.67 (m, 3H, H<sub>4</sub>, H<sub>6</sub> and H<sub>7</sub> of indole), 7.28-6.97 (m, 4H, Ar-H), 6.23 (s, 1H, CH-Ar), 5.29 (s, 2H, CH<sub>2</sub>), 3.33 (s, 3H, -OCH<sub>3</sub>), MS: in m/z [rel.%]: 404 [M<sup>+</sup>, 26%], Anal. Calcd for C<sub>21</sub>H<sub>16</sub>N<sub>4</sub>O<sub>3</sub>S: C, 62.38; H, 3.96; N, 13.86. Found: C, 62.10; H, 4.12; N, 13.95.

*5-Methoxy-3-(6'-(furyl)-methylene-thiazolo-[3,2-b]-1,2,4-triazol-5(6H)-one-2'-yl-methyl)indole (12)*

Title compound was synthesized from compound **4** (1.6g, 6.15mmol), chloroacetic acid (1.16g, 12.3mmol), furfuraldehyde (0.51g, 6.15mmol), anhydrous sodium acetate (0.70g, 8.53mmol), acetic anhydride (2.5ml, 26.4mmol) and acetic acid (4.5ml, 78.6mmol) at refluxing for 6-8 hours.

Yield: 1.08g (67.5%), MP: 175-176°C, IR[cm<sup>-1</sup>, KBr]: 3190(N-H), 3089(C-H aromatic), 2931(C-H aliphatic), 1694(C=O), 1615 (C=C of aromatic ring), 1082(C-O-C), 1023(N-N), 677(C-S-C), <sup>1</sup>H-NMR [400 MHz, DMSO-D<sub>6</sub>-ppm]: δ 11.51 (s, 1H, NH, exchangeable with D<sub>2</sub>O), 8.07 (s, 1H, H<sub>2</sub> of indole), 7.47-7.15 (m, aromatic 3H of indole), 6.94-6.71 (m, 3H, Ar-H), 5.99 (s, 1H, CH-Ar), 5.04 (s, 2H, CH<sub>2</sub>), 3.06 (s, 3H, -OCH<sub>3</sub>), MS: in m/z [rel.%]: 278 [M<sup>+</sup>, 21%], Anal. Calcd for C<sub>19</sub>H<sub>14</sub>N<sub>4</sub>O<sub>3</sub>S: C, 60.32; H, 3.70; N, 14.81. Found: C, 60.12; H, 3.86; N, 14.88.

**5-Methoxy-3-(6'-(p-aminodimethylphenyl)-methylene-thiazolo-[3,2-b]-1,2,4-triazol-5(6H)-one-2'-yl-methyl)indole (13)**

Title compound was synthesized from compound **4** (1.6g, 6.15mmol), chloroacetic acid (1.16g, 12.3mmol), 4-aminodimethylbenzaldehyde (0.92g, 6.15mmol), anhydrous sodium acetate (0.70g, 8.53mmol), acetic anhydride (2.5ml, 26.4mmol) and acetic acid (4.5ml, 78.6mmol) at refluxing for 6-8 hours.

Yield: 0.94g (58.8%), MP: 178-179°C, IR[cm<sup>-1</sup>, KBr]: 3388 (N-H), 3079 (C-H aromatic), 2976(C-H aliphatic), 1705 (C=O), 1606 (C=C of aromatic ring), 1043(C-O-C), 1024(N-N), 673(C-S-C), <sup>1</sup>H-NMR [400 MHz, DMSO-D<sub>6</sub>-ppm]: δ 11.83 (s, 1H, NH, exchangeable with D<sub>2</sub>O), 8.06 (s, 1H, H<sub>2</sub> of indole), 8.04-7.84 (m, 3H, Ar-H), 7.66-7.53 (m, 3H, Ar-H), 7.45-7.23 (m, 2H, Ar-H, CH-Ar), 6.04 (s, 2H, CH<sub>2</sub>), 3.37(s, 6H, 2×-NCH<sub>3</sub>), 2.33 (s, 3H, -OCH<sub>3</sub>), MS: in m/z[rel.%]: 431 [M<sup>+</sup>, 100%], Anal. Calcd for C<sub>23</sub>H<sub>21</sub>N<sub>5</sub>O<sub>2</sub>S: C, 64.04; H, 4.87; N, 16.24. Found: C, 64.12; H, 5.02; N, 16.02.

**5 - M e t h o x y - 3 - ( 6 ' - ( p - h y d r o x y - m - methoxyphenyl)-methylene-thiazolo-[3,2-b]-1,2,4-triazol-5(6H)-one-2'-yl-methyl)indole (14)**

Title compound was synthesized from compound **4** (1.6g, 6.15mmol), chloroacetic acid (1.16g, 12.3mmol), 4-hydroxy-3-methoxybenzaldehyde (0.93g, 6.15mmol), anhydrous sodium acetate (0.70g, 8.53mmol), acetic anhydride (2.5ml, 26.4mmol) and acetic acid (4.5ml, 78.6mmol) at refluxing for 6-8 hours.

Yield: 1.04g (65.2%), MP: 166-167°C, IR[cm<sup>-1</sup>, KBr]: 3305(OH), 3075(N-H), 2915(C-H aromatic), 1702(C=O), 1606(C=C of aromatic ring), 1083(C-O-C), 1018(N-N), 669(C-S-C), <sup>1</sup>H-NMR [400 MHz, DMSO-D<sub>6</sub>-ppm] : δ 11.82 (s, 1H, OH, exchangeable with D<sub>2</sub>O), 11.02 (s,

1H, NH, exchangeable with D<sub>2</sub>O), 8.51 (s, 1H, H<sub>2</sub> of indole), 7.68-7.61 (m, 2H, Ar-H), 7.47-7.45 (d, 1H, J=8.0 Hz, Ar-H), 7.24-7.01 (m, 1H, Ar-H), 6.98-6.82 (m, 3H, Ar-H), 6.16 (s, 1H, -CH<sub>2</sub>), 5.21 (s, 2H, CH<sub>2</sub>), 3.32 (s, 3H, -OCH<sub>3</sub>), MS: in m/z [rel.%] : 434 [M<sup>+</sup>, 11.3%], Anal. Calcd for C<sub>22</sub>H<sub>18</sub>N<sub>4</sub>O<sub>4</sub>S: C, 60.83; H, 4.15; N, 12.90. Found: C, 60.71; H, 4.28; N, 12.97.

**Pharmacology**

All the compounds **4-14** were evaluated for their anti-inflammatory and acute toxicity. The experiments were performed on albino rats of Charles Foster strain of either sex, excluding pregnant females, of 70 to 90 days weighing 68-115g. Acute toxicity of numerous compounds was tested in albino mice (22–28 g). (Rats and mice were purchased from LABO-AIDS, Meerut, U.P., India). Food (chow pallet) and water were given to the animals *ad libitum*. The test compounds were dissolved in propylene glycol. Indomethacin (from commercial source) was used as reference drugs. The biological activities were carried out in Late Dr. Ashok Kumar's (ex supervisor) lab, Medicinal Chemistry Division, Department of Pharmacology, Lala Lajpat Rai Memorial Medical College, Meerut (U.P.), India and the protocol was approved by the ethical committee of Lala Lajpat Rai Memorial Medical College, Meerut (U.P.), India.

**Acute toxicity study**

The test compounds were investigated for acute toxicity profile (LD<sub>50</sub>) in albino mice, according to the method of Smith Q. E. [26]. The test compounds were given orally at different dose levels in separate groups of animals. After 24 hours of drug administration, percent mortality in each group was observed. LD<sub>50</sub> was calculated from the data obtained.

**Anti-inflammatory activity**

This activity was done according to the given procedure by Winter *et al.*, [27]. Indomethacin was used as reference drugs. The rats were divided into three groups (control, drug treated and standard drug) of six animals each. A freshly prepared suspension of carrageenan (1% in 0.9% saline), 0.05 mL was injected under planter aponeurosis of right hind paw of each rat. One group was kept as control and treated with propylene glycol. Test compounds and standard drugs were administered to the animals of drug treated and standard drug group, respectively, 1 h before to carrageenan injection. The paw volume of each rat was measured before 1 and 3 h after carrageenan treatment with the help of Plethysmometer. The percent anti-inflammatory activity was calculated according to the formula given below.

$$\text{Percentage of inhibition of oedema} = (1 - V_t / V_c) \times 100$$

Where,  $V_t$  and  $V_c$  are the mean increase in paw volume of rats of treated group and the control group, respectively. Results obtained were statistically analyzed.

### Ulcerogenic Activity

The ulcerogenic activity was done on albino rats according to the procedure of Verma *et al.*, [28]. The rats were fasted for 24 hours prior to the administration of the test compounds. Water was given to the animals *ad libitum*. The most promising compounds **6**, **7** and reference drugs were given intraperitoneally, and then these animals were sacrificed 8 hours after drug treatment. The stomach, duodenum and jejunum were removed and examined with a hand lens for any evidence of the following: (a) shedding of epithelium, (b) petechial or frank hemorrhage, (c) erosion or discrete ulceration with or without perforation. The presence of any one of these criteria was considered to be an evidence of ulcerogenic liability.

### Cyclooxygenase activity

This test was carried out *in vitro* on the microsomal fraction of mucosal preparations of rabbit distal colon in order to search out the possible mechanism of action of the test compounds by Calderano *et al.*, [29]. Colonic mucosa (=2-3g), stripped as previously described, was minced and homogenized (Potter homogenizer) in 3 volumes of Tris buffer, 0.1 M, pH 8.0. The homogenate was centrifuged for 30 min at 10 000 g. The resulting supernatant was centrifuged for 1 hour at 100 000 g. The precipitate was suspended in Tris buffer, 0.1M, pH 8.0, and re-centrifuged for 1 hour at 100 000 g. The microsomal pellet was used immediately for an enzyme assay; cyclooxygenase activity was assayed by measuring the rate of conversion of arachidonic acid to PGE<sub>2</sub>. Microsomal fractions (50  $\mu$  L) were incubated with test agents for 5 min at 37°C in 30 mL Tris HCl, pH 8.0 containing 2 mM reduced glutathione, 5mM L-tryptophan, and 1 $\mu$ M hematin. The substrate, 20  $\mu$ M arachidonic acid with tracer amount of [1- <sup>14</sup>C] arachidonic acid [=20 000 cpm] was then added and the reaction proceeded for 3 min at 37 °C. The reaction was stopped by addition of 0.2 mL of ethyl ether/methanol /citric acid 0.2 M (30:4:1), which was pre-cooled at -25 °C. PGE<sub>2</sub> was extracted twice into the same mixture. The solvent was evaporated under a N<sub>2</sub> stream and radiolabelled arachidonic acid was separated from radio-labeled PGE<sub>2</sub> by RP-HPLC. HPLC analysis was performed on a Hitachi spectrophotometer (Hitachi High Technologies America, San Jose, CA, USA) equipped with a flow cell. The sample was injected on an ultra sphere column (Beckman Coulter Ltd., High Wycombe, Buckinghamshire, UK) ODS 5 mm, 4.6 mm X 25 cm with 2 mmol unlabelled PGE<sub>2</sub> as an internal standard, PG chromatographic profile was obtained by isocratic elution with 150 mM H<sub>3</sub>PO<sub>4</sub> in water, pH 3.5, containing 30% acetonitrile a flow rate of 1mL min<sup>-1</sup> monitoring the UV absorption at 214 nm. Radioactivity that coeluted with authentic PGE<sub>2</sub> was quantified by

liquid scintillation spectrometry. Test samples were compared to parried control incubations. The percentage of inhibition was calculated as follows:

$$[(\text{cpm control} - \text{cpm test} / (\text{cpm control})) \times 100]$$

## Conclusion

Out of eleven compound examined, compound **6** and **7** were found to be the most promising compounds of the present study. LD<sub>50</sub> of these compounds was very high suggesting good safety margin and also these compounds possessing less ulcerogenic liability as compared to standard drug: indomethacin. However, compound **6** yielded outstanding biological results as compared compound **7**.

At the end, following conclusion can be drawn:

- Presence of *o*- or *p*-chlorophenyl group as a substituent may elicit a remarkable increase in anti-inflammatory activity.
- *o*- or *p*-methoxyphenyl substitution showed significant activity.
- Presence of electronegative atom may play an important role in the modulation of activity.
- Compounds bearing *p*-substitution showed better activity as compared to *o*- substitution.

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## References

1. S. E. Anderson, M. L. Edvinsson and L. Edvinsson, Clin. Sci., 2003, 105, 699-707.
2. Y. W. Kim, X. Z. West and T. V. Byzova, J. Mol. Med., 2013, 91, 323-328.
3. I. Blasko, B. Grubeck-Loebenstein, Drugs Aging, 2003, 20(2), 101-13.
4. V. Wieser, A. R. Moschen and H. Tilg, Arch. Immunol. Ther. Exp., 2013, 61, 119-125.
5. G. Theodoropoulos, I. Papaconstantinou, E. Felekouras, N. Nikiteas, P. Karakitsos, D. Panoussopoulos, C. Lazaris, E. Patsouris, J. Bramis, M. Gazouli, World J. Gastroenterol., 2006, 12, 5037-5043.
6. S. M. A. de Lima, A. Otoni, A. P. Sabino, L. M. S. A. Dusse, K. B. Gomes, S. W. L. Pinto, M. A. S. Marinho, D. R. A. Rios, Clinica Chimica Acta, 2013, 421, 46-50.
7. C. Manzano, L. G. Espinoza, F. M. Campo, P.C. Fortes, R. P. Filho, Perit Dial Int, 2007, 27, 347-352.
8. R. Pecoits, Perit Dial Int, 2005, 25, 432-440.
9. M.F. Flessner, K. Credit, K. Henderson, J Am Soc Nephrol, 2007, 18, 2294-2302.
10. C. Xavier, J. M. Roberta, G. Weilgosza, G. L. Bautta, C. B. Dubigeonb, N. Grimaudb, J. Y. Petitb, Eur. J. Med. Chem., 2001, 36, 639-649.
11. A. Kumar, S. Sharma, B. K. Achana, S. Sharma, H. Panwar, T. Singh, V. K. Srivastava, Bioorg. Med. Chem., 2003, 11, 5293-5299.
12. D. Carcache, E. J. Blancoa, B. Pandita, Z. Hua, J. Shia, A. Lewisa, P. K. Li, Bioorg. Med. Chem. Lett., 2007, 17, 6031-6035.
13. S. Sharma, V. K. Srivastava, A. Kumar, Indian J. Chem., 2002, 41B, 2647-2654.
14. A. Kumar, Archana, S. Sharma, N. Malik, P. Sharma, K. Kaushik, K. K. Saxena, V. K. Srivastava, R. S. Verma, H. Sharma, S. C. Gupta, V. Gupta, R. C. Agarwal, Indian J. Chem., 2004, 43B, 1532-1536.
15. T. Chandra, N. Garg, A. Kumar, International J. Chem. Tech. Res., 2010, 2(2), 762 - 763.
16. K. M. Asif, S. Ahmad, Orient. J. Chem., 2012, 28(2), 927-931.
17. B. Gutmann, M. Gottsponer, P. Elsner, D. Cantillo, D.M. Roberge, C. O. Kappe, Org. Process Res. Dev., 2013, 17, 294-302.
18. M. Shiradhkar, R. Kale, Indian J. Chem., 2006, 45B, 1009-1013.
19. B. Kalluraga, M. A. Rahimann, Indian J. Chem., 2002, 41B, 1712-1717.
20. M. D. Braccio, G. Grossi G. Roma D. Piras F. Mattaioli, M. Gosmar, Eur. J. Med. Chem., 2008, 43(3), 584-594.
21. Z. P. Cao, W. J. Dong, H. S. Dong, Indian J. Chem., 2009, 48B, 873-876.
22. P. X. Franklin, A. D. Pillai, P. D. Rathod, S. Yarande, M. Nivsarkar, H. Padh, K. K. Vasu, V. Suduranam, Eur. J. Med. Chem., 2008, 43, 129-134.

23. E. Bansal, A. Kumar, R. S. Verma, K. K. Saxena, V. K. Srivastava, *Indian J. Heterocycl. Chem.*, 2000, 9, 301-306.
24. Y. Dundar, B. Cakir, E. Kupeli, M. F. Sahin, N. Noyanalpan, *Turk. J. Chem.*, 2007, 31, 301-313.
25. P. X. Franklin, A. D. Pillai, P. D. Rathod, S. Yerande, M. Nivsarkar, H. Padh, K. K. Vasu, V. Sudersanam, *Eur. J. Med. Chem.*, 2008, 43(1), 129-134.
26. Q. E. Smith, *Pharmacol. Screening Tests Progress Medi. Chem.*, Butterworth, London, 1960, 1, 1-33.
27. C. A. Winter, E. A. Risley, G. Nuss, W. Proc. Soc. Exp. Biol., 1962, 111, 544-547.
28. M. Verma, J. N. Sinha, V. R. Gujrati, *Pharma. Res. Commun.* 1981, 13, 967-969.
29. C. Calderaro, C. Parrillo, A. Giovane, R. Greco, M. G. Matera, L. Berrino, R. J. Rossi, *Pharmacol. Exp. Ther.*, 1992, 263, 579-587.