



Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Design, synthesis and biological evaluation of novel diphenylthiazole-thiazolidin-4-one-based derivatives as anti-inflammatory/analgesic agents

Ahmed H. Abdelazeem^{1,2}, Mohammed T. El-Saadi¹, Asmaa G. Safi El-Din¹
and Samir M. El-Moghazy^{3*}

¹Department of Medicinal Chemistry, Faculty of Pharmacy, Beni-Suef University, Beni-Suef 62514, Egypt

²Department of Pharmaceutical Chemistry, College of Pharmacy, Taif University, Taif 21974, Saudi Arabia

³Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Cairo University, Cairo, Egypt

ABSTRACT

A series of novel diphenylthiazole-thiazolidin-4-one-based derivatives was synthesized and characterized by elemental and spectral analysis. The newly synthesized compounds were screened for their analgesic and anti-inflammatory activities using acetic acid-induced writhing test and carrageenan-induced edema assay, respectively. Among the compounds, compound 13 was found to be the most potent analgesic and anti-inflammatory agent among the newly synthesized compounds compared to the standard drug, diclofenac.

Keywords: diphenylthiazole, thiazolidin-4-one, anti-inflammatory, analgesic.

INTRODUCTION

Inflammation is considered unpleasant, at least at times, and an unavoidable phenomenon. It can be classified as either immunological or non-immunological according to the origin of the inflammation, which then sub-classified into acute and chronic [1]. Inflammation is well-known to be mediated by various mediators such as prostaglandins (PGs) and nitric oxide (NO) which are generated by cyclooxygenases (COXs) and nitric oxide synthase (iNOS), respectively [1, 2]. PGE₂ is the important type of prostaglandins contributing in inflammation, which has a key role in the regulation of vascular permeability, platelet aggregation, and thrombus formation through the progression of inflammation. Over the few past decades, it is well documented that there are, at least two designated COX isoforms; COX-1 and COX-2 [3, 4]. The inducible isoform, COX-2, is important in inflammation and pain, while the constitutively expressed isoform, COX-1, not play a significant role in acute inflammatory pain [5]. The original COX-1, is responsible for constitutive PG production under normal conditions, whereas the described isoform, termed COX-2, is up-regulated during inflammation and appears responsible for PG biosynthesis at sites of inflammation [6]. Because of the importance of COX enzymes in the inflammation process, traditional non-steroidal anti-inflammatory drugs (NSAIDs) which inhibit both COX subtypes are still the most widely used medications for inflammation and pain [7]. However, NSAIDs have several serious side effects such as gastric ulceration, renal injury and cardiotoxicity [6, 8]. In several attempts to avoid the GI damage induced by NSAIDs, highly selective COX-2 inhibitors (coxibs) have been developed where they exhibited equivalent anti-inflammatory/analgesic activities to those of non-selective COX inhibitors but with least GI toxicity [9]. Also, there are other main inflammatory mediators incorporating in the process including the inducible transcription factor (NF- κ B) which regulates the expression of several genes such as iNOS, COX-2, and TNF- α [7]. It is well known that diphenylheterocyclic building blocks (Y-shaped) is one of the most common and frequently existing scaffolds in the analgesic/anti-inflammatory drugs, especially COXs inhibitors such as celecoxib [10]. Furthermore, it was noticed that the presence of two methoxy groups on the diphenylheterocycles system as in Mofezolac and FR122047 increase the analgesic activity and COX-1 selectivity with less gastric effect, **Fig. 1** [11].

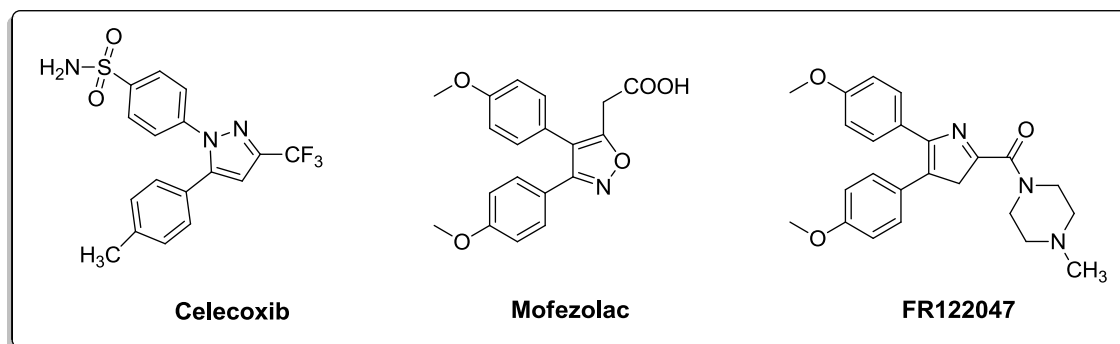


Fig. 1: Some diphenylheterocyclic-based drugs

On the other hand, thiazolidinone can be considered as a magic moiety which is important and frequently used scaffold in drug design and discovery that possess a huge variety of biological activities including antimicrobial activity [12], Anticancer activity [13], analgesic/anti-inflammatory activities [14], Anticonvulsant and antidepressant activity [15], Antidiabetic activity [16], Antiarrhythmic activity [17]. Compounds **1-3**, as thiazolidinone-based derivatives, were reported and found to have good anti-inflammatory and moderate analgesic activities compared to phenylbutazone as a reference drug [12, 18]. Moreover, we have very recently reported a series of thiazolidinone-based diphenylthiazole hybrids with good analgesic/anti-inflammatory activities. These hybrid molecules were found to exert their effect via inhibition of COX enzymes [19].

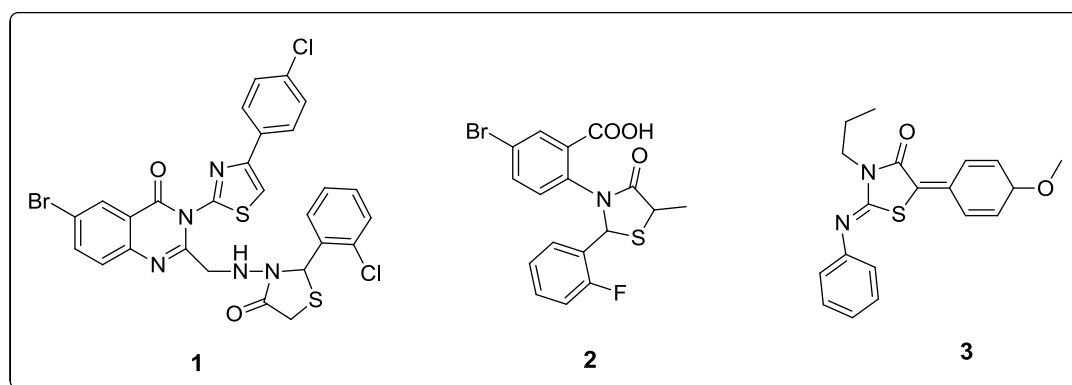


Fig. 2: Some thiazolidinone-based compounds with analgesic/anti-inflammatory activities

Considering the aforementioned data, we herein describe our continuous efforts to design and synthesize novel and safer analgesic/anti-inflammatory conjugates through the combination of thiazolidinone and the p-methoxy substituted diphenylthiazole in one skeleton which subsequently was evaluated *in vivo* for its analgesic/anti-inflammatory activities.

EXPERIMENTAL SECTION

Chemistry:

Melting points were determined on a griffin apparatus and are uncorrected. Infrared (IR) spectra were recorded on a shimaduz 435 Spectrometer, using KBr discs and values were represented in cm^{-1} . ¹H-NMR spectra were measured a Varian Mercury VX-300 NMR spectrometer at 400 MHz and BRUKER APX400 spectrometer at 400 MHz in the specified solvent, chemical shifts were reported on the scale and were related to that of the solvent and J values are given in Hz. ¹³C NMR spectra were obtained on a Bruker APX400 at 100 MHz at the faculty of pharmacy, Beni-Suef University. Mass spectra were run on Hewlett packed 5988 spectrometer. Microanalysis were performed for C, H, N (Micro Analytical center, Al-Azhar university, Egypt) and were within $\pm 0.5\%$ of theoretical values. Progress of the reactions was monitored by TLC using TLC sheets precoated with UV fluorescent silica gel MERCK 60 F 254 that was visualized by UV lamp. 2-hydroxy-1,2-bis(4-methoxyphenyl)ethanone (**4**), 2-chloro-1,2-bis(4-methoxyphenyl)ethanone (**5**), 4,5-bis(4-methoxyphenyl)thiazol-2-amine (**6**) were prepared according to reported procedures [20-23].

N-(4,5-bis(4-methoxyphenyl)thiazole-2-yl)-2-chloroacetamide (**7**). Chloroacetyl chloride (2.9 g, 25.7 mmol) was added dropwise to a stirred solution of compound **8** (6.17 g, 19.8 mmol) and triethylamine (6 g, 59.5 mmol) in

CH₂Cl₂ at 0 °C. The reaction mixture was stirred for 1 h at 0°C then for 5 h at room temperature. The reaction mixture was filtered, then the organic layer was separated and evaporated in vacuum. The residue obtained was crystallized from ethanol to afford off-white solid (80%), m.p. 140 °C. IR (cm⁻¹): 3342(NH); 3040(CH aromatic); 2929(CH aliphatic); 1674(C=O); 1606, 1511(C=C, C=N). ¹H-NMR (400 MHz, DMSO): δ 3.81 (s, 3H), 3.82 (s, 3H), 4.41 (s, 2H), 6.86-7.36 (m, 8H), 12.64 (s, 1H; NH exchangeable with D₂O). ¹³C-NMR (101 MHz, DMSO): δ 42.68, 55.49, 55.59, 114.15, 114.89, 124.21, 124.99, 127.46, 130.03, 131.05, 143.66, 155.06, 159.14, 159.43, 165.54. MS (EI) m/z (%): 388 (M⁺+1). Anal. Calcd. For C₁₉H₁₇ClN₂O₃S: C, 58.68; H, 4.41; N, 7.20. Found: C, 58.81; H, 4.46; N, 7.28.

2-((4,5-bis(4-methoxyphenyl)thiazole-2-yl)imino)thiazolidin-4-one (**8**). Ammonium thiocyanate (7.6 gm, 100 mmol) was added to a stirred solution of compound **7** (19.4 g, 50 mmol) in absolute ethanol, then reflux for 5 hr. the reaction mixture was left overnight, filter, recrystallized from ethanol/acetone to give compound **8** as yellow solid (67%), m.p. 254°C. IR (cm⁻¹): 3432(NH); 3035(CH aromatic); 2927(CH aliphatic); 1707(C=O); 1605, 1511(C=C, C=N). ¹H-NMR (400 MHz, DMSO): δ 3.74 (s, 3H), 3.78 (s, 3H), 4.02 (s, 2H), 6.88-7.44 (m, 8H), 12.12 (s, 1H; NH exchangeable with D₂O). ¹³C-NMR (101 MHz, DMSO) δ 35.45, 55.55, 55.65, 114.19, 114.93, 124.13, 127.35, 129.93, 131.15, 145.39, 159.22, 159.62, 163.38, 166.50, 174.66, 174.69. (EI) m/z (%): 412(M⁺+1). Anal. Calcd. For C₂₀H₁₇N₃O₃S₂: C, 58.38; H, 4.16; N, 10.21. Found: C, 58.49; H, 4.22; N, 10.39.

General Procedure 1. Synthesis of compounds (9-11). A solution of sodium acetate (0.16 g, 1.95 mmol) was added to glacial acetic acid (5 mL), and the solution was stirred for 5 min. A solution of compound **8** (1 mmol) in glacial acetic acid (5-10 mL) was added to the sodium acetate solution. The resulting solution was stirred for 5 min and then respective aldehyde (2 mmol) added. The mixture was refluxed for 6 h. The precipitate was filtered, washed with water and then recrystallized from ethanol to give the final targeted compounds (**9-11**).

5-benzylidene-2-((4,5-bis(4-methoxyphenyl)thiazol-2-yl)imino)thiazolidin-4-one (**9**). General Procedure 1, orange solid, m.p. 220 °C. IR (cm⁻¹): 3426(NH); 3065(CH aromatic); 2925(CH aliphatic); 1719(C=O); 1511, 1485(C=C, C=N). ¹H-NMR (400 MHz, DMSO): δ 3.79 (s, 6H), 6.97-7.72 (m, 13H), 7.76 (s, 1H), 12.78 (s, 1H; NH exchangeable with D₂O). MS (EI) m/z (%): 499(M⁺+1). Anal. Calcd. For C₂₇H₂₁N₃O₃S₂: C, 64.91; H, 4.24; N, 8.41. Found: C, 65.18; H, 4.32; N, 8.49.

2-((4, 5-bis (4-methoxyphenyl) thiazol-2-yl) imino)-5-(4chlorobenzylidene) thiazolidin-4-one (**10**). General procedure 1, yellowish orange solid (65%), m.p. 223 °C. IR (cm⁻¹): 3422 (NH); 3061 (CH aromatic); 2927(CH aliphatic); 1664(C=O); 1589, 1510(C=C). ¹H-NMR (400 MHz, DMSO): δ 3.79 (s, 6H), 6.97 (m, 4H), 6.93-7.68 (m, 12H), 7.71 (s, 1H), 12.67 (s, 1H; NH exchangeable with D₂O). MS (EI) m/z (%): 533 (M⁺+1). Anal. Calcd. For C₂₇H₂₀N₃O₃S₂: C, 60.72; H, 3.77; N, 7.87. Found: C, 60.93; H, 3.74; N, 7.98.

2-((4, 5-bis (4-methoxyphenyl) thiazol-2-yl) imino)-5-(4 methoxybenzylidene) thiazolidin-4-one (**11**). General procedure 1, yellowish orange solid (55%), m.p. 227 °C. IR (cm⁻¹): 3422(NH); 3061(CH aromatic); 2929(CH aliphatic); 1664(C=O); 1589, 1510(C=C). ¹H-NMR (400 MHz, DMSO): δ 3.79 (s, 6H), 3.86 (s, 3H), 6.97-7.65 (m, 12H), 7.71 (s, 1H), 12.67 (s, 1H; NH exchangeable with D₂O). ¹³C-NMR (101 MHz, DMSO) δ 55.64, 55.70, 114.34, 115.00, 115.45, 122.11, 124.00, 126.45, 127.23, 128.02, 129.94, 131.21, 132.52, 132.65, 145.76, 159.40, 159.76, 161.41, 164.81, 165.81, 167.81. MS (EI) m/z (%): 530 (M⁺+1). Anal. Calcd. For C₂₈H₂₃N₃O₄S₂: C, 63.50; H, 4.38, 7.93. Found: C, 63.69; H, 4.36; N, 8.12.

2-((4, 5-bis (4-methoxyphenyl) thiazol-2-yl) imino)-5-(4 methylbenzylidene) thiazolidin-4-one (**12**). General procedure 1, yellowish orange solid (59%), m.p. 232 °C. IR (cm⁻¹): 3422(NH); 3061(CH aromatic); 2927(CH aliphatic); 1664(C=O); 1589, 1510 (C=C). ¹H-NMR (400 MHz, DMSO): δ 2.40 (s, 3H), 3.79 (s, 6H), 6.97-7.65 (m, 12H), 7.71 (s, 1H), 12.67 (s, 1H; NH exchangeable with D₂O). ¹³C-NMR (101 MHz, DMSO) δ 21.57, 55.69, 114.34, 115.00, 115.45, 122.11, 124.00, 126.45, 127.23, 128.02, 129.94, 131.21, 132.52, 132.65, 145.76, 159.40, 159.76, 161.41, 164.81, 165.81, 167.81. MS (EI) m/z (%): 513 (M⁺+1). Anal. Calcd. For C₂₈H₂₃N₃O₃S₂: C, 65.48; H, 4.51; N, 8.18. Found: C, 65.61; H, 4.59; N, 8.32.

2-((4, 5-bis (4-methoxyphenyl) thiazol-2-yl) imino)-5-(furan-2-ylmethylene) thiazolidin-4-one (**13**). General procedure 1, yellowish orange solid (65%), m.p. 235 °C. IR (cm⁻¹): 3431(NH); 3040(CH aromatic); 2923(CH aliphatic); 1709(C=O); 1589, 1510(C=C, C=N). ¹H-NMR (400 MHz, DMSO): δ 3.80 (s, 6H), 6.82 – 6.77 (m, 1H), 6.99 (m, 4H), 7.12 (d, J = 4 Hz, 1H), 7.33-7.51 (m, 4H), 7.57 (s, 1H), 8.16 (s, 1H), 12.62 (s, 1H; NH). ¹³C-NMR (101 MHz, DMSO) δ 55.64, 114.09, 114.41, 114.99, 118.81, 119.02, 122.19, 124.03, 127.22, 127.94, 129.94, 131.21, 145.82, 148.04, 150.23, 157.17, 159.41, 159.74, 165.92, 167.24. MS (EI) m/z (%): 489 (M⁺+1). Anal. Calcd. For C₂₅H₁₉N₃O₄S₂: C, 61.33; H, 3.91; N, 8.58. Found: C, 61.51; H, 3.97; N, 8.72.

Biological screening:

Male albino rats (120-150 g) and male mice (15-25 g) were used. Animals were fasted for 12 h before treatment, but had always free access to water. All compounds were administered orally by a gastric tube, as finely homogenized suspension in 0.5% carboxymethylcellulose (CMC) (1 ml/100 g body weight), at the initial dose of 60 mg/kg. Compounds which exhibited a statistically significant activity at this dose were further tested at doses decreasing by a factor of 2, always in the same volume of 0.5% CMC. Controls received the same volume of CMC dispersion [24].

Carrageenan-induced rat paw edema assay:

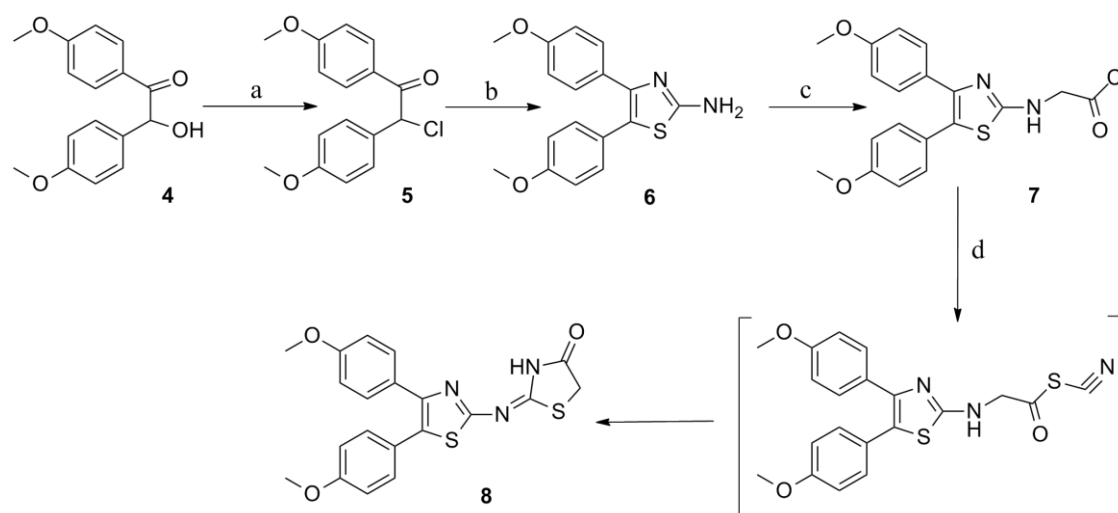
The carrageenan-induced paw edema test was performed in groups of six rats. Sixty minutes after administering the test compound, 0.1 ml of 1% carrageenan suspension in saline was injected into the plantar surface of the right hind paw of each rat. Paw volume, as determined by measuring the volume of water displaced after immersing paw up to the lateral malleolus level, was recorded immediately after the carrageenan injection and again 1 h later. The difference between these two volumes was taken as edema volume. For the most active compounds the edema volume was measured also after 3 h and 7 h. The percent inhibition of the edema of treated rats with respect to control animals was calculated and compared with that produced by diclofenac (60 mg/kg, p.o.), used as reference drug. Statistical significance versus control group was evaluated.

Acetic acid-induced writhing test:

The writhing test was performed in groups of five mice. One hour after the administration of the test compound, 0.01 ml/g of 0.6% acetic acid solution was injected intraperitoneally in each mouse. The writhing movements of each animal were counted for 15 min (between the fifth and 20th min after the injection of the irritant). The antinociceptive effect was expressed as the percent reduction of writhing number compared with the control group. Diclofenac (6 mg/kg, p.o.) was used as reference drug. Statistical significance versus control group was evaluated.

RESULTS AND DISCUSSION**Chemistry**

The synthesis of the new target compounds was straightforward and the general synthetic pathways are outlined in Schemes 1 and 2. Benzoin was reacted with thionyl chloride to form desyl chloride **5** which was subsequently condensed with thiourea in absolute ethanol using Hantzsch-thiazole synthesis method to give the starting material **6**, 4, 5-bis(4-methoxyphenyl)thiazol-2-amine, in high yield [25]. The intermediates **7** was accomplished through the reaction of the starting materials **6** with chloroacetyl chloride in methylene chloride and triethylamine at 0°C. The cyclization of the intermediates **7** was efficiently done using ammonium thiocyanate to furnish 2-((4, 5-bis(4-methoxyphenyl)thiazol-2-yl)imino)thiazolidin-4-one **8**, **Scheme 1**. The mechanism that has been proposed for the formation of thiazolidine-4-one derivatives **8** was previously reported [26, 27].

Scheme 1:

Reaction reagents and conditions: (a) SOCl_2 , pyridine, 1 h; (b) thiourea, ethanol, reflux, 2 h; (c) ClCH_2COCl , CH_2Cl_2 , TEA, 5 h; (d) NH_4SCN , acetone, reflux, 6 h.

Consequently, the final target compounds **9-13** were prepared using Knoevenagel reaction of the intermediates **8** with the appropriate aldehyde in glacial acetic acid in presence of anhydrous sodium acetate, **Scheme 2**. All the new compounds were characterized and confirmed by elemental analysis and several spectral data ($^1\text{H-NMR}$, $^{13}\text{C-NMR}$ and mass).

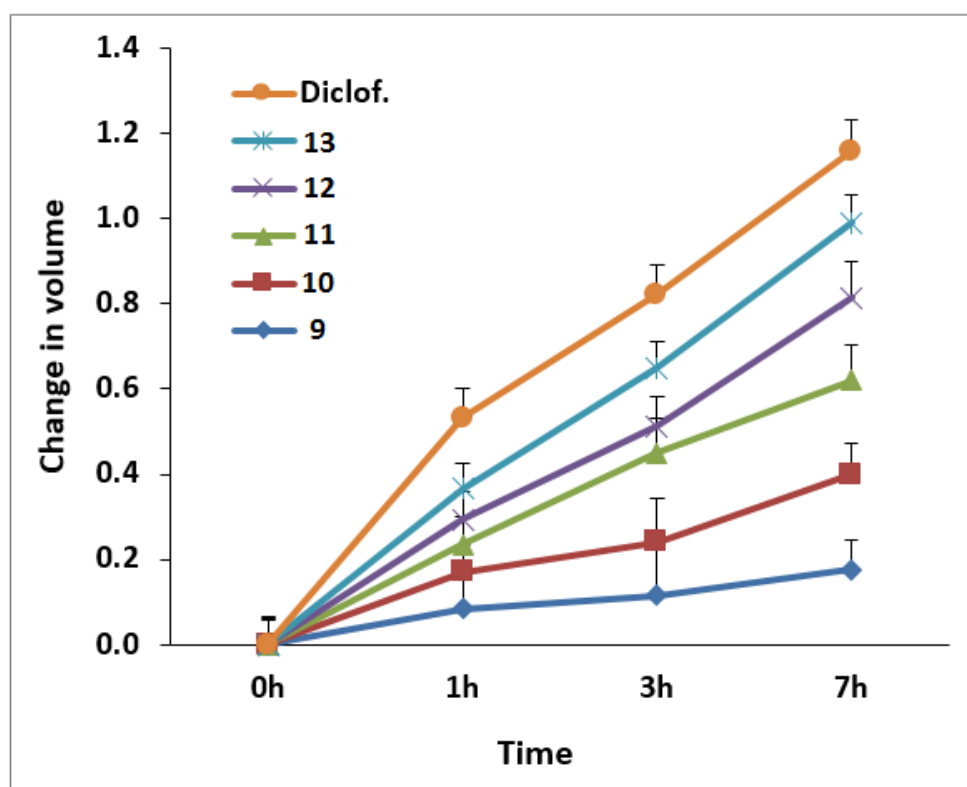
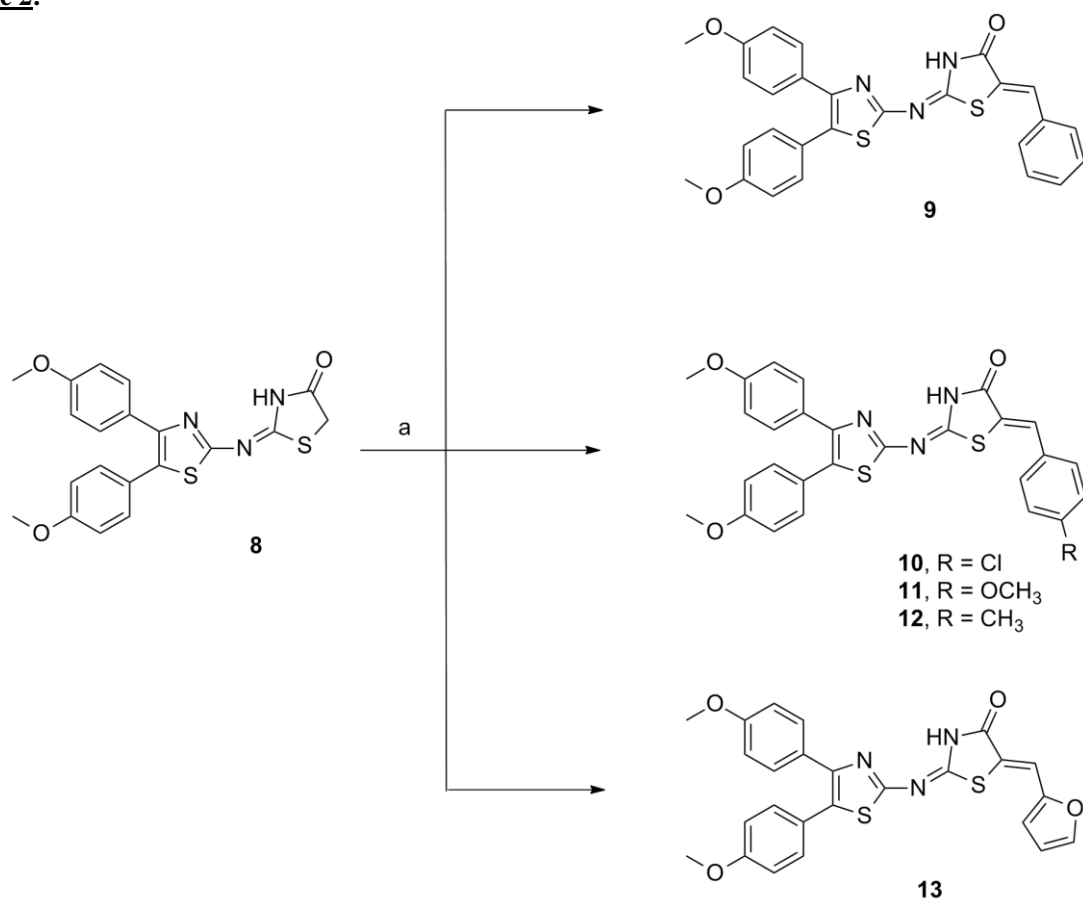
Scheme 2:

Fig. 3: Graphical representation of anti-inflammatory activity of compounds against Diclofenac as standard using paw-edema method

Biological Evaluation:**Carrageenan-induced rat paw edema assay:**

The carrageenan-induced rat paw edema model was used for the assessment of anti-inflammatory activity of the tested compounds using diclofenac as a reference anti-inflammatory drug. Mean changes in paw edema thickness of animals pretreated with the test compounds after 1, 3 and 7 h from induction of inflammation was shown in **Fig. 3**. Almost all the compound showed moderate edema inhibition at the injective dose compared to diclofenac. It was observed that the substitution of 5-benzylidene moiety with electron-donating groups markedly increased the anti-inflammatory activity as in compounds **11** and **12**. However, the substitution of 5-benzylidene moiety with electron-withdrawing groups exhibited slightly affect the anti-inflammatory activity as in compound **10**. Compound **9** with non-substituted 5-benzylidene showed the least activity. Finally, the replacement of the 5-benzylidene moiety with the less bulky furyl moiety in **13** resulted in a sharp increase in the anti-inflammatory activity, **Fig. 3**.

Acetic acid-induced writhing test:

The test compounds were further examined for their analgesic activity using the acetic acid-induced writhing test in mice. Diclofenac was used as a reference standard analgesic drug. It was noteworthy that the electronic effect of the various substitutions on the 5-benzylidene moiety was similar to the effect on the anti-inflammatory activity of all test compounds. Compound **11** and **12** bearing electron-donating groups showed moderate analgesic activity and compound **9** was the least active agent. Interestingly, compound **13** was also the most active analgesic ligand among the whole synthesized series compared to the used standard analgesic diclofenac. The effects of the drug probes on the acetic acid abdominal writhing assay are summarized in **Fig. 4**.

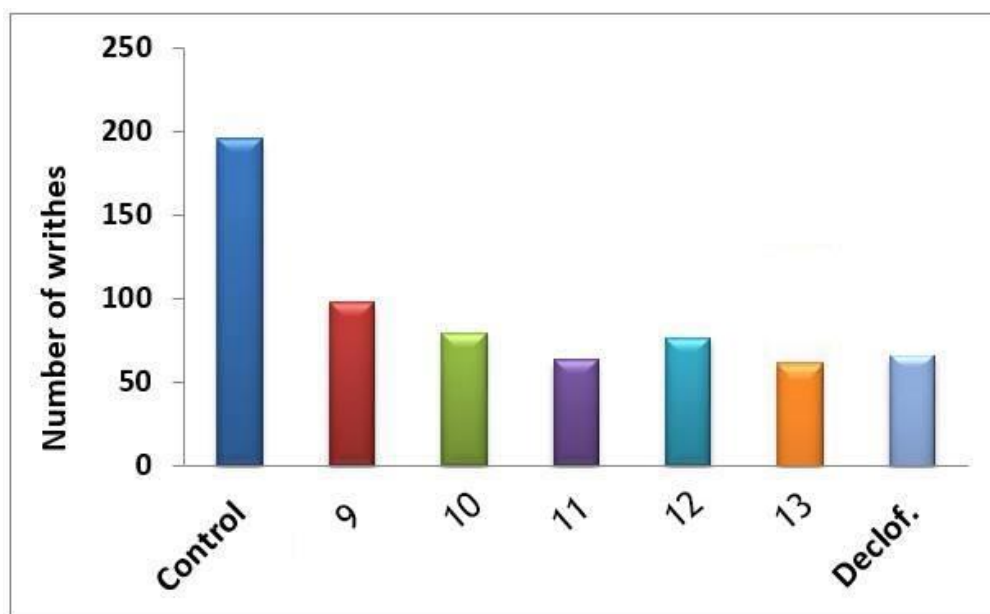


Fig.4: Graphical representation of analgesic activity of compounds against diclofenac as standard using acetic acid induced writhing test

CONCLUSION

In conclusion, we herein described the synthesis of novel set of diphenylthiazole-thiazolidin-4-one-based derivatives using fragment based drug design approach and their *in vivo* analgesic/anti-inflammatory activities were evaluated. Most of the compounds have displayed good to moderate activities compared to diclofenac as reference drug. Interestingly, compounds **13** bearing the furan moiety exhibited the most potent activities in both assays.

Acknowledgements

The authors are thankful to Dr. Ahmed Mansour, lecturer of Pharmacology, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt for his kind help in performing the *in vivo* studies.

REFERENCES

- [1] SE Wenzel. *Pharmacotherapy*, **1997**, 17, 3S-12S.
- [2] SK Natarajan; O Moreno; TJ Graddis; DF Duncan; R Laus; F Chen. **2013**, *US Patent No.* 0190296 A1.
- [3] G Dannhardt; W Kiefer. *Eur. J. Med. Chem.*, **2001**, 36,109-126.

- [4] P Gund; TY Shen. *J. Med. Chem.* **1977**, 20, 1146-1152.
- [5] U Salgin-Goksen; N Gokhan-Kelekci; OGoktas; YKoysal; E Kılıc; GAktay; MOZalp. *Bioorg. Med. Chem.*, **2007**, 15, 5738–5751.
- [6] MC Allison; AG Howatson; GJ Torrance; FD Lee; RI Russell. *J. Med.*, **1992**, 327, 749-754.
- [7] S Ghosh; M Karin. *Cell*, **2002**, 109, S81–S96.
- [8] MM Wolfe; DR Lightenstein; G Singh. *N. Engl. J. Med.*, **1999**, 340, 1888-1899.
- [9] RM wright; S Lane; W Linley; C Mcquade; F Thompson; N Maskrey. *Aliment. Pharmacol. Ther.*, **2003**, 17, 321–332.
- [10] JS Dileep Kumar; MM Ho; JM Leung. *Adv. Synth. Catal.*, **2002**, 344, 1146.
- [11] K Hiroki; Z Xiaoxia; O Hiroyuki; S Yukio; S Kenji; T Akihiro. *J. Med. Chem.*, **2008**, 51, 2400–2411.
- [12] AP Liesen; TM de Aquino; CS Carvalho; VT Lima; JM de Araújo; JG de Lima; AR de Faria; EJ de Melo; AJ Alves; EW Alves ; AQ Alves; AJ Góes. *Eur. J. Med. Chem.*, **2010**, 45, 3685-3691.
- [13] S Chandrappa; CV Kavitha; MS Shahabuddin; K Vinaya; CSKumar; SR Ranganatha; SC Raghavan; KS Rangappa. *Bioorg. Med. Chem.*, **2009**, 17, 2576–2584.
- [14] A Kumar; CS Rajput; SK Bhati. *Bioorg. Med. Chem.*, **2007**, 15, 3089–3096.
- [15] A Agarwal; S Lata; KK Saxena; VK Srivastava; A Kumar. *Eur. J. Med. Chem.*, **2006**, 15, 3089–3096.
- [16] R Maccari; A Corso; M Giglio; R Moschini; U Mura; R Ottanà. *Bioorg. Med. Chem. Lett.*, **2011**, 21, 200–203.
- [17] MF Gross; S Beaudoin; G McNaughton-Smith; GS Amato; NA Castle; C Huang; A Zou; W Yu. *Bioorg. Med. Chem. Lett.*, **2007**, 17, 2849–2853.
- [18] A Verma; SK Saraf. *Eur. J. Med. Chem.*, **2008**, 43, 897-905.
- [19] AH Abdelazeem; SA Salama; IA Maghrabi. *Arch. Pharm. Chem. Life Sci.*, **2015**, 348, 518-530.
- [20] G Sumrell; JI Stevens; GE Goheen. *J. Org. Chem.*, **1957**, 22 (1), 39–41.
- [21] K Matsumoto; PP Ho. **1982**, *US Patent* No. 4,322,428.
- [22] A Tanaka; H Sakai; Y Motoyama; T Ishikawa; H Takasugi. *J. Med. Chem.*, **1994**, 37, 1189-1199.
- [23] AH Abdelazeem; M Habash; IA Maghrabi; MO Taha. *Med. Chem. Res.*, **2015**, 1-15. DOI 10.1007/s00044-015-1418-5.
- [24] I Vazzana; E Terranova; F Mattioli; F Sparatore. *ARKIVOC*, **2004**, 364-374.
- [25] J Ren; S-M Wang; L-FWu; Z-X Xu; B-H Dong. *Dyes and Pigments*, **2008**, 76, 310-314.
- [26] P Vicini; A Geronikaki; M Incerti; F Zani; J Dearden; M Hewitt. *Bioorg. Med. Chem.*, **2008**, 16, 3714–3724.
- [27] AH Abdelazeem; AM Gouda; HA Omar; MF Tolba. *Bioorg. Chem.*, **2014**, 57, 132–141.