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Synthesis and Three-dimensional Qualitative Structure Selectivity Relationship of 3,5-Disubstituted-2,4-Thiazolidinedione Derivatives As COX2 Inhibitors

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In our effort for synthesis of selective COX2 inhibitors, certain new 2,4-thiazolidinedione derivatives were synthesized. It necessitates preparation of potassium salt of 2,4-thiazolidinedione **2**, which condensed with intermediate **4a**. The resulting 3-[2-(4-methylphenyl)-2-oxo-1-phenylethyl]-2,4-thiazolidinedione **8** was condensed with appropriate aldehyde to afford compounds **10a**, **10i-l**, **10o** and **10p**. Compounds (**9a-l**, **10a-n**, **10p**, **11** and **12)** were obtained through the preparation of 5-arylmethylidene-2,4-thiazolidinediones **6a-p** and reaction of its potassium salt **7a-p** with compounds **4a**, **4b**, and **5**. Some compounds displayed significant analgesic activity as compared to reference standards. The anti-inflammatory activity of the synthesized compounds revealed that intermediate **8** and compounds **9c**, **10c** and **10d** showed good results. Compound **10c** produced no significant mucosal injury. HipHop methodology of Catalyst program was used to build up hypothetical model of selective COX2 inhibitors followed by fitting the synthesized compounds to this model. Compounds **10c** and **10d** were suspected to be promising selective COX2 inhibitors. Also, compounds (**6c, 8, 9a,c,d,k, 10a,c,d,k, 11 and 12)** were docked into COX1 and COX2 X-ray structures, using DOCK6 program. Docking results suggested that several of these derivatives are active COX inhibitors with a significant preference for COX2.

Key words: 3D-QSSR, 2,4-Thiazolidinediones, COX2 inhibitors, Catalyst, Dock6

INTRODUCTION

Most non-steroidal anti-inflammatory drugs (NSAIDs) act through the inhibition of prostaglandin synthase (cyclooxygenase, COX) (Vane, 1971; Lombardino, 1985). Most NSAIDs in current use belong to the class of arylacetic or arylpropionic acids, and are not selective inhibitors of both constitutive (COX1) and inducible (COX2) cyclooxygenase. The typical side effect of these NSAIDs consists of gastrointestinal damage, commonly attributed to their lack of COX2 selectivity (Dannhardt *et al.*, 2001; Carabaza *et al.*, 1996). There are many chemical-structural classes that have been proved to possess appreciable selectivity towards COX2. The first class is the sulfonated diaryl heterocycles including thiophene (Gauthier *et al.*, 1996), thiazole (Gauthier *et al.*, 1996), furan (Black *et al.* 1996a, 1996b), oxazole (Hashimoto *et al.*, 2002), isoxazole (Talley *et al.*, 1997; Habeeb *et al.*, 2001; Reddy and Bell 2003), pyrazole (Bourassa *et al.*, 2003; Sakya and Rast 2003; Pal *et al.*, 2003), imidazole (Rosales *et al.*, 2003; Almansa *et al.*, 2003), triazole (Sakya *et al.*, 2003a, 2003b), pyridine (Dube *et al.*, 2003) and pyridazinone (Li *et al.*, 2003) However, several structures have been reported in which the vicinal diaryl and/or the sulfonyl moiety at the aromatic ring were missed (Portevin *et al.*, 2000; Sui *et al.*, 2000; Dannhardt *et al.*, 2000; Barnett *et al.*, 1996) Another class of compounds was identified as non-ulcerogenic orally active anti-inflammatory agents. 4-Thiazolidinone, 4-imidazolidinone or 4-oxazolidinone moiety with 2-oxo, 2-thioxo or 2-imino function linked to di-tert-butyl phenol through methylidene bridge are the most acceptable

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feature for these compounds (Dannhardt and Kiefer, 2001; De Leval *et al.*, 2002). 2,4-Thiazolidinedione derivatives were investigated as a template for design and synthesis of novel and safe anti-inflammatory compounds (Seehra *et al.*, 1999a, 1999b; Heymans, *et al.*, 2003). Based upon the above consideration, it seemed worthy to design and synthesize certain new 2,4-thizolidinedione derivatives. Despite it was reported that N-subtitution of 2,4-thiazolidinedione nucleus by methyl function retain the anti-inflammatory activity as observed in some Meclofenamic acid and Indomethacin derivatives (Boschelli *et al.*, 1992). Also, the discovery of diaryl and aryl/heteroaryl methanone derivatives as selective COX2 inhibitors (Dannhart and Laufer, 2000; Barnett *et al.*, 1996) encouraged the incorporation of diaryl ethanone substituent at the nitrogen atom of the 2,4-thiazolidinedione nucleus. Accordingly, the substituent at both 3 and 5 positions of 2,4-thiazolidinedione nucleus of our target compounds (**6a-l** and **7a-p**) were selected so as to afford a basis for studying the variation in the electronic, lipophilic and steric properties of the synthesized compounds on the activity. The synthesized compounds were tested in vivo for their activity as analgesic, anti-inflammatory agents and for ulcerogenic liability using mice and rats models. Then we used Catalyst/HipHop program to study the qualitative three-dimension structure selectivity relationship (3D-QSSR) of the target compounds. Complementary docking studies were carried out on the same set of 2,4-thiazolidinediones derivatives. The docking results suggested that these 2,4-thiazolidinediones derivatives are active COX inhibitors with a clear preference for COX2.

MATERIALS AND METHODS

Chemistry

Materials and equipments

Melting points were determined on an electrothermal melting point apparatus (Stuart Scientific, England), and are uncorrected. Precoated silica gel plates (kiesel gel 0.25 mm, 60G F254, Merck) were used for thin layer chromatography. IR spectra (KBr disc) were recorded on IR-470 Shimadzu spectrometer, Japan. ^{1}H-NMR Spectra were scanned on a Varian EM-360 L NMR spectrometer (60 MHz) (Varian, palo Alto, CA, U.S.A.) at Faculty of Pharmacy Assiut University and Jeol, JNM LA spectrophotometer 400 MHz, FT-NMR (Jeol, Tokyo, Japan). Chemical shifts are expressed in d-values (ppm) relative to TMS as an internal standard, using $CDCl_3$ or DMSO-d_6 as a solvent. Elemental analyses were performed at the Department of Chemistry, Faculty of Science, Assiut University, Assiut, Egypt.

Synthesis of 2,4-thiazolidinedion 1 (Turkevich *et al.*, 1961)

Thiourea (36.5 g, 0.48 mol) was added to a stirred solution of chloroacetic acid (47.2 g, 0.5 mol) in HCl (80.6 ml, 36%), and the reaction mixture was heated under reflux for 14 h. On cooling the product was precipitated, filtered, dried and crystallized from water to yield 35.5 g (63.0%) of 2,4-thiazolidinedione **1**; m.p. 124-25°C (Turkevich *et al.*, 1961).

Synthesis of potassium salt of 2,4-thiazolidinedione 2 (Lo *et al.*, 1953)

A solution of potassium hydroxide (10.1 g) in methanol (300 mL) was added to a stirred hot solution of 2,4-thiazolidinedione **1** (21.1 g, 0.18 mol) in methanol (40 mL). The reaction mixture was refluxed with stirring for 2 h then cooled. The precipitated product filtered, washed with methanol and dried to give 19.0 g (68.0%) of potassium salt of 2,4-thiazolidinedione **2**; m.p. 247-50°C (Lo *et al.*, 1953).

Synthesis of 1-(4-methylphenyl)-2-phenyl-1-ethanone 3a (Buck and Ide, 1932; Fred and Frederick, 1948)

Thionyl chloride (15.4 g, 0.13 mol) was added dropwise with stirring to phenylacetic acid (13.9 g, 0.1 mol). The solution was refluxed for 2.5 h until no further fumes were evolved. The reaction mixture was cooled to room temperature and the excess thionyl chloride removed under reduced pressure. The produced phenylacetyl chloride was added to a solution of dry toluene (9.2 g, 0.1 mol) in methylene chloride (100 mL), anhydrous $AlCl_3$ (16.0 g, 0.12 mol) was added portion wise and the solution was stirred at room temperature for 24 h. The reaction mixture was poured onto ice-water mixture; HCl was added drop wise till acidic to litmus paper. The organic layer was separated, washed with saturated solution of Na_2CO_3 (3 × 25 mL), then with distilled water (2 × 25 mL), dried over anhydrous $MgSO_4$ and filtered. Methylene chloride was distilled off and the residue was crystallized from ethanol to yield 16.9 g (80.5%) of 1-(4-methyl phenyl)-2-phenyl-1-ethanone; m.p. 109-10°C (Fred and Frederick, 1948).

Synthesis of 2-bromo-1-(4-methylphenyl)-2-phenyl-1-ethanone 4a (Fred and Frederick, 1948)

1-(4-Methylphenyl)-2-phenyl-1-ethanone **3a** (21.0 g, 0.1 mol) was dissolved in 60 mL of ether : dioxane (2 : 1) mixture, bromine (17.6 g, 0.11 mol) was added dropwise over a period of 1 h at room temperature with stirring. The reaction mixture was further stirred for additional 30 minutes, water was added, the organic layer was separated, dried over anhydrous $MgSO_4$, filtered and the solvent was distilled off under reduced pressure. The crude product was crystallized from ethanol to afford 22.5 g (78.0%) of

2-bromo-1-(4-methylphenyl)-2-phenyl-1-ethanone 6; m.p. 86-87°C (Fred and Frederick, 1948).

Synthesis of 2-hydroxy-1,2-diphenyl-1-ethanone (Benzoin) 3b (Vachal *et al.*, 1997)

A solution of sodium cyanide (1.1 g, 0.02 mol) in water (10 mL) was added to a stirred solution of benzaldehyde (10.6 g, 0.1 mol) in 15 mL ethanol. The reaction mixture was refluxed gently for half an hour and cooled in an ice bath. The precipitated product was filtered, washed with cold water, dried well and crystallized from ethanol to afford 9.0 g (85.0%) of benzoin; m.p. 137°C (Vachal *et al.*, 1997).

Synthesis of 2-chloro-1,2-diphenyl-1-ethanone (desyl chloride) 4b (Ward, 1929)

Dry pyridine (10.3 g, 0.13 mol) was added drop wise to benzoin (21.2 g, 0.1 mol) with stirring, the mixture was heated up to 50-70°C until a homogenous solution was obtained and then cooled in an ice bath. Thionyl chloride (15.5 g, 0.13 mol) was added drop wise over a period of 1 h with vigorous stirring. Upon addition of water, the product was precipitated, decanted, triturated twice with water, filtered and dried over sulfuric acid. The crude product was crystallized from petroleum ether 40-60 to yield 17.1 g (74.0%) of desyl chloride 7; m. p. 63-66°C (reported 67-67.5°C) (Ward, 1929).

Synthesis of 2-bromo-1-(4-methylphenyl)-1-ethanone (4-methyl phenacyl bromide) 5 (Shevchuk and Dombrovskii, 1963)

Bromine (8 g, 0.05 mol) was added dropwise to a stirred ice-cold solution of the commercially available 4-methylacetophenone (6.7 g, 0.05 mol) in 30 mL of ether : dioxane (2:1) mixture over a period of 45 min. The reaction mixture was stirred at 50°C for further 1 h. and concentrated in vacuum. Water was added, the precipitated product was filtered, well dried and crystallized from ethanol to yield 8.3 g (78%) of 4-methyl phenacyl bromide 8; m.p. 51°C (Shevchuk and Dombrovskii, 1963).

Synthesis of 5-arylmethylidene-2,4-thiazolidinediones 6a-p (Bruno *et al.*, 2002)

An appropriate aromatic aldehyde (0.04 mol) was added to a stirred mixture of 2,4-thiazolidinedione 1 (4.7 g, 0.04 mol) in ethanol (250 mL) and piperidine (2.7 g, 0.032 mol). The reaction mixture was refluxed for 16-24 h until the starting compounds had completely disappeared as monitored by TLC using chloroform/ethyl acetate (2:1). The reaction mixture was cooled, poured into water, and acetic acid was added drop wise till acidic to litmus paper. The precipitated product filtered, washed with water, dried and crystallized from methanol to give the corresponding compounds (**6a-p**). Their yields and physicochemical data are listed in Tables I and II.

Synthesis of potassium salt of 5-arylmethylidene-2,4-thiazolidinediones 7a-p (Lo *et al.*, 1953)

A mixture of an appropriate 5-arylmethylidene-2,4-thiazolidinedione **6a-p** (0.25 mol), potassium hydroxide (15.4 g, 0.27 mol) and absolute ethanol (500 mL) was refluxed for 3 h with stirring. Ethanol was then concentrated to one half its volume and allowed to cool. The precipitated potassium salt was collected, dried and used in the next step without further purification.

Synthesis of 3-[2-(4-methylphenyl)-2-oxo-1-phenylethyl]-2,4-thiazolidinedione 8

Potassium salt of 2,4-thiazolidinedione **2** (7.7 g, 0.05 mol) was added portion wise with stirring to a warm solution of 2-bromo-1-(4-methylphenyl)-2-phenyl-1-ethanone **4a** (14.45 g, 0.05 mol) in acetone (150 mL). The reaction mixture was then refluxed for 6 h, until the disappearance of the starting materials as monitored by TLC using n-hexane/ethyl acetate (4:1). The reaction mixture was cooled, filtered and acetone was distilled off. The residue was purified by dry-column flash chromatography using n-hexane/ethyl acetate (6:1), then crystallized from isopropanol to afford 7.0 g (43.0%) of 3-[2-(4-methyl phenyl)-2-oxo-1-phenylethyl]-2,4-thiazolidinedione **8**; m.;. 95-97°C.

CHN analysis $C_{18}H_{15}NO_3S$ (325.38):

	C	H	N
Calcd:	66.44	4.65	4.30
Found:	66.11	5.09	4.20

Synthesis of 5-arylmethylidene-3-(2-oxo-1,2-diphenylethyl)-2,4-thiazolidinediones (9a-l)

Potassium salt of the appropriate 5-arylmethylidene-2,4-thiazolidinedione (0.005 mol) **7a-l** was added portion wise to a stirred solution of 2-chloro-1,2-diphenyl-1-ethanone **4b** (1.2 g, 0.005) in dry DMF (15 mL). The mixture was heated at 90-100°C for 3-4 h until the starting compounds had completely disappeared as monitored by TLC using n-hexane/ethyl acetate (6:1). The reaction mixture was cooled to room temperature, poured into water, extracted with chloroform and the organic layer was dried over anhydrous $MgSO_4$. After filtration, chloroform was distilled off and the residue was crystallized from the appropriate solvent to give the corresponding 5-arylmethylidene-3-(2-oxo-1,2-diphenylethyl)-2,4-thiazolidinediones **9a-l**. Compounds **9b-g** were purified by column chromatography using ethyl acetate/n-hexane (6:1) mixture as eluent before crystallization.

Table I.. Physicochemical data of the newly synthesized derivatives (9_{a-l})

No	R	m.p. C (Cryst. Solv.)	Yield %	Mol. Formula (Mol. Wt.)	Elemental analysies Calc. / Found		
					C	H	N
9a	H	135-38 (a)	27.5	$C_{24}H_{17}NO_3S$ (399.46)	72.16 72.28	4.29 4.78	3.51 3.51
9b	2-F	144-45 (a)	51.5	$C_{24}H_{16}FNO_3S$ (417.45)	69.05 68.60	3.86 3.65	3.36 3.32
9c	2-Cl	119-20 (a)	38.5	$C_{24}H_{16}ClNO_3S$ (433.91)	66.43 66.05	3.72 3.70	3.23 3.18
9d	2-Br	145-46 (a)	20.5	$C_{24}H_{16}BrNO_3S$ (478.36)	60.26 59.98	3.37 3.22	2.63 2.92
9e	3-F	178-80 (b)	65.0	$C_{24}H_{16}FNO_3S$ (417.45)	69.05 69.00	3.86 3.72	3.36 3.35
9f	3-Cl	188-90 (a)	59.0	$C_{24}H_{16}ClNO_3S$ (433.91)	66.43 66.25	3.72 3.61	3.23 3.23
9g	3-Br	190-93 (b)	52.5	$C_{24}H_{16}BrNO_3S$ (478.36)	60.26 60.05	3.37 3.60	2.93 2.89
9h	3-OCH3	190-93 (a)	75.0	$C_{25}H_{19}NO_4S$ (429.49)	69.91 69.59	4.46 4.53	3.26 3.25
9i	4-F	160-63 (b)	25.0	$C_{24}H_{16}FNO_3S$ (417.45)	69.05 68.81	3.86 4.08	3.36 3.35
9j	4-Cl	209-10 (b)	32.0	$C_{24}H_{16}ClNO_3S$ (433.91)	66.43 65.96	3.72 3.88	3.23 3.19
9k	4-Br	207-10 (b)	45.5	$C_{24}H_{16}BrNO_3S$ (478.36)	60.26 60.08	3.37 3.58	2.93 2.87
9l	4-OCH3	158-60 (b)	61.0	$C_{25}H_{19}NO_4S$ (429.49)	69.91 69.65	4.46 4.32	3.26 3.20

(a) acetone, (b) ethyl acetate.

Synthesis of 5-arylmethylidene-3-[2-(4-methylphenyl)-2-oxo-1-phenylethyl]-2,4-thiazolidinediones (10a-p)

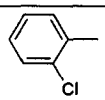
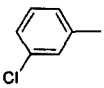
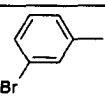
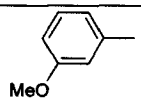
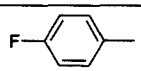
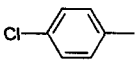
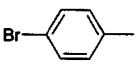
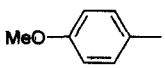
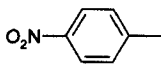
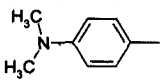
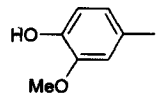
Method A (De Lima *et al.*, 1994): An appropriate aromatic aldehyde (0.005 mol) was added to a stirred mixture of 3-[2-(4-methylphenyl)-2-oxo-1-phenylethyl]-2,4-thiazolidinedione **8** (1.6 g, 0.005 mol) and piperidine (0.3 g, 0.004 mol) in ethanol (50 mL). The reaction mixture was refluxed for 7-10 h until the starting compounds had completely disappeared as monitored by TLC using n-hexane/ethyl acetate (6:1). The reaction mixture was poured into water and acetic acid was added drop wise till acidic to litmus paper. The precipitated product was filtered, washed with water, dried and crystallized from appropriate solvent to give the corresponding compounds (**10a**, **10i-l**, **10o**, and **10p**). Compound **10p** was purified by column chromatography using ethyl acetate/n-hexane (4:1) mixture as eluent before crystallization.

Method B (Bruno *et al.*, 2002): Potassium salt of the appropriate 5-arylmethylidene-2,4-thiazolidinedione **6a-n** and **6p** (0.005 mol) was added portion wise to a stirred warm solution of 2-bromo-1-(4-methylphenyl)-2-phenyl-1-ethanone (1.4 g, 0.005 mol) in acetone (50 ml). The reaction mixture was refluxed for 4-6 hrs until the starting compounds had completely disappeared as monitored by TLC using n-hexane/ethyl acetate (6:1), filtered while hot and allowed to cool. The precipitated product was filtered, dried and crystallized from appropriate solvent to afford the corresponding compounds **10a-n** and **10p**. Compounds **10b-d**, **10n** and **10p** were purified by column chromatography using different proportions of ethyl acetate/n-hexane mixture as eluent before crystallization.

Synthesis of 5-[(2-chlorophenyl)methylidene]-3-[2-(4-methylphenyl)-2-oxoethyl)-2,4-thiazolidinedione 11

Potassium salt of 5-[(2-chlorophenyl)methylidene]-2,4-thiazolidinedione (**7c**) (2.0 g, 0.008 mol) was added portion wise to a stirred warm solution of 2-bromo-1-(4-methylphenyl)-1-ethanone **4a** (1.7 g, 0.008 mol) in acetone

Table II. Physicochemical data of the newly synthesized derivatives (10_{a-p})

No	Ar	m.p. C (Cryst. Solv.)	Yield % (Method of prep.)	Mol. Formula (Mol. Wt.)	Elemental analysies Calc. / Found		
					C	H	N
10a		155-58 (a)	10.0 (A) 66.5 (B)	$C_{25}H_{19}NO_3S$ (413.49)	72.62 72.37	4.63 4.45	3.39 3.40
10b		169-72 (a)	71.5 (B)	$C_{25}H_{18}FNO_3S$ (431.48)	69.59 69.25	4.20 4.47	3.25 3.22
10c		140-42 (b)	40.0 (B)	$C_{25}H_{18}ClNO_3S$ (447.93)	67.03 67.12	4.05 3.92	3.13 3.12
10d		132-35 (a)	25.5 (B)	$C_{25}H_{18}BrNO_3S$ (492. 39)	60.98 60.93	3.68 3.84	2.84 2.80
10e		159-60 (b)	69.0	$C_{25}H_{18}FNO_3S$ (431.48)	69.59 69.33	4.20 4.12	3.25 3.20
10f		145-48 (a)	59.0 (B)	$C_{25}H_{18}ClNO_3S$ (447.93)	67.03 66.84	4.05 4.24	3.13 3.15
10g		146-47 (a)	41.0 (B)	$C_{25}H_{18}BrNO_3S$ (492. 39)	60.98 60.98	3.68 3.51	2.84 2.87
10h		160-61 (a)	70.0 (B)	$C_{26}H_{21}NO_4S$ (443.52)	70.41 70.11	4.77 5.18	3.16 3.14
10i		151-53 (b)	17.5 (A) 55.0 (B)	$C_{25}H_{18}FNO_3S$ (431.48)	69.59 69.55	4.20 4.44	3.25 3.24
10j		183-85 (b)	27.0 (A) 78.5 (B)	$C_{25}H_{18}ClNO_3S$ (447.93)	67.03 66.55	4.05 3.96	3.13 3.11
10k		188-90 (b)	36.5 (A) 83.0 (B)	$C_{25}H_{18}BrNO_3S$ (492. 39)	60.98 60.81	3.68 3.78	2.84 2.80
10l		130-31 (b)	30.0 (A) 64.0 (B)	$C_{26}H_{21}NO_4S$ (443.52)	70.41 70.13	4.77 5.10	3.16 3.15
10m		198-99 (b)	66.5 (B)	$C_{25}H_{18}N_2O_5S$ (458.49)	65.49 65.13	3.96 4.12	6.11 6.06
10n		174-76 (b)	43.0 (B)	$C_{27}H_{24}N_2O_3S$ (456.56)	71.03 70.65	5.30 5.68	6.14 6.13
10o		115-17 (c)	31.0 (A)	$C_{26}H_{21}NO_5S$ (459.51)	67.96 68.30	4.61 4.91	3.05 2.91
10p		145-46 (d)	6.0 (A) 20.0 (B)	$C_{24}H_{18}N_2O_3S$ (414.10)	69.55 69.49	4.38 4.41	6.76 6.75

(a) Acetone, (b) Et. acetate, (c) Col. Chr.(Et. acetate/n-hexane 3:1), (d) Col. Chr. (Et.acetate/n-hexane 4:1).

(60 mL). Te reaction mixture was refluxed for 1 h, until the disappearance of the starting materials as monitored by TLC using n-hexane/ethyl acetate (3:1). Acetone was distilled off under reduced pressure and the residue was crystallized from ethanol to yield 2.7 g (87.0%) of compound **11**.; m.p. 154-57°C.

CHN analysis, $C_{19}H_{14}ClNO_3S$ (371.84)

	C	H	N
Calcd:	61.37	3.79	3.7
Found:	61.17	3.99	3.78

Synthesis of 5-[(2-chlorophenyl)methylidene]-3-[2-oxopropyl)-2,4-thiazolidinedione 12

Potassium salt of 5-[(2-chlorophenyl)methylidene]-2,4-thiazolidinedione **7c** (2.0 g, 0.008 mol) was added portion wise to a stirred solution of chloroacetone (0.74 g, 0.008 mol) in dry DMF (10 mL). The reaction mixture was heated at 90-100°C for about 8 h and monitored by TLC using n-hexane/ethyl acetate (4:1) until the disappearance of the starting materials. The reaction mixture was cooled to room temperature, poured into water, extracted with chloroform and the organic layer was dried over anhydrous $MgSO_4$. After filtration, chloroform was distilled off and the residue was crystallized from ethanol to yield 1.5 g (61.0%) compound **12**; m.p. 147-50°C.

CHN analysis, $C_{13}H_{10}ClNO_3S$ (295.74):

	C	H	N
Calcld:	52.80	3.41	4.74
Found:	52.73	3.15	4.74

Biological evaluation

Analgesic activity: Hot-plate test (Valencia *et al.*, 1994)

Adult albino mice of both sexes, weighing 25-35 g, were grouped, each of six animals. A suspension of the tested compounds, Indomethacin or Rofecoxib (0.25-0.35 mL) was administered intra peritoneal to the mice groups. The control group (placebo) received an equivalent volume of the vehicle. Mice were placed for testing on the surface of a hot-plate apparatus maintained at 55 ± 0.5°C after 1, 2 and 3 h of injection. The response was assessed for each animal, as the time (seconds) elapsed until licked its hind paws (licking time) or jumped out within a plexiglass cylinder placed on the hotplate. The hotplate latency was taken as a measure of the analgesic activity, the mean licking time ± S.E. was evaluated for each group.

Anti-inflammatory activity: Carrageenin induced edema (Hernandez-Perez *et al.*, 1995)

Groups of six adult albino rats of both sexes weighing 100-120 g were used in this study. A suspension of the tested compounds, and reference standards (1-1.2 mL) was administered orally to rat groups while the control group received an equivalent volume of the vehicle. After one hour, 1% carrageenin suspension in water was injected subcutaneous in the right hind paw of each rat in a dose of 0.1 mL according to the method of (Hernandez-Perez *et al.*, 1995). The Paw volumes were assessed in right hind paw in comparison with the left one before and 3 h after carrageenin injection by means of a digital plethysmometer according to the method of (Valencia *et al.*, 1994). Assessments were carried out, by dipping of the tibiotarsal joint into the Perspex cell, and the volume displacement was measured in mL. The difference between the displaced volumes of the two paws before and after carrageenin injection for each animal was taken as a measure of edema. The percents of edema and edema inhibition by tested compounds and reference standards were calculated as a measure of the anti-inflammatory activity.

Gastric ulcerogenicity (Fadl and Omar 1998, Omar *et al.*, 1999)

Male albino rats of both sexes weighing 100-120 g were divided into groups, each of six animals. The rat groups were fasted for 12 h prior to administration of the target compounds, reference drugs and vehicle. A suspension of the tested compounds, and reference standards (1-1.2 mL) was administered orally to rat groups. The doses were repeated for four successive days and during this period rats were denied access to food while allowed free access to water. A control group was administered an equivalent oral volume of the vehicle and treated similarly. After 24 h of receiving the last dose, rats were sacrificed; the stomach was removed, opened along the greater curvature and cleaned gently by dipping in a saline solution. The mucosal damage was examined grossly under binocular magnification and the specimens were then taken and prepared for scanning in an electron microscope. Firstly, the randomly selected specimens were fixed by soaking in glutaraldehyde solution (5% in cacodylate buffer; pH 7.2) for 24 h followed by washing three times with cacodylate buffer, each for 20 min. The specimens were then treated with osmium tetraoxide (1% solution) for 2 h and washed with cacodylate buffer as shown above. The specimens were further subjected to dehydration for 30 min by treatment with 30%, 50% and 70% ethanolic solution followed by 99% ethanol for 1 h and finally absolute ethanol for 2 days. After the discharge of the alcohol, the specimens were soaked in amyl acetate solution for 2 days, dried under reduced pressure, mounted on holder and coated for scanning in the scanning electron microscope.

Catalyst COX-2 hypothetical model

General notes

All molecular modeling studies were performed on a Silicon Graphics workstation running under IRIX 64 (6.5) operating system using Catalyst software (version 4.7) (Daveu *et al.*, 1999). This modeling study was carried out at the Department of Medicinal Chemistry, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt.

Catalyst pharmacophore construction (Hypothesis generation)

Using Catalyst program, best conformational analysis was performed for training set of compounds **13-17**, Fig. 1, using a threshold of 250 conformers *per* molecule and a maximum value of 20 Kcal/mol for conformer energy. Afterwards, the emerged conformers for each compound were used to build up the hypothetical model using HipHop method of Catalyst program. HipHop tool identifies the common chemical features within the training set compounds starting from the conformers of the principle compound in the training set. Compound **13** took a value of two in the principle column and considered to be the principle one. In addition, the following parameters were loaded into the Catalyst program in order to specify the hypotheses where, MaxOmitFeat sited to be two for all compounds. Moreover, hydrogen bond acceptor (HBA), hydrogen bond donor (HBD) and hydrophobic (H) functions were specified to be the chemical features that would be considered in the generation of the hypothetical model. At the end of these procedures, Catalyst program generate 10 hypotheses, ranked according to their scores, which most likely express the common chemical features of the training set compounds **13-17**. The highest ranking-hypothesis composed of four features, three hydrophobic and one hydrogen bond acceptor, Fig. 2.

Validation of the hypothetical model (Kurogi and Guner, 2001, Hirashima et al., 1999)

The performance of the obtained hypothetical model was first evaluated by fitting the test set compounds **18-24** (Fig. 3). Fitting operations to the hypothetical model were accomplished through conformational analysis for each compound in the test set utilizing a threshold of 250 conformer *per* molecule and a maximum value of 20 Kcal/mol for conformer energy; Then, the conformers of each compound in the test set were allowed to fit to the hypothesis.

Qualitative 3D-SAR analysis

The synthesized compounds (**6a-l**, **7a-p**, **8** and **9**) were allowed to fit to the validated hypothetical model using the same fitting options, which are used in building and validation of the hypothesis. The obtained fit value for each molecule is a measure of how many and how well its functional features fit to the features of the pharmacophore model or hypothesis.

Docking study

All calculations were performed on a windows PC PIII

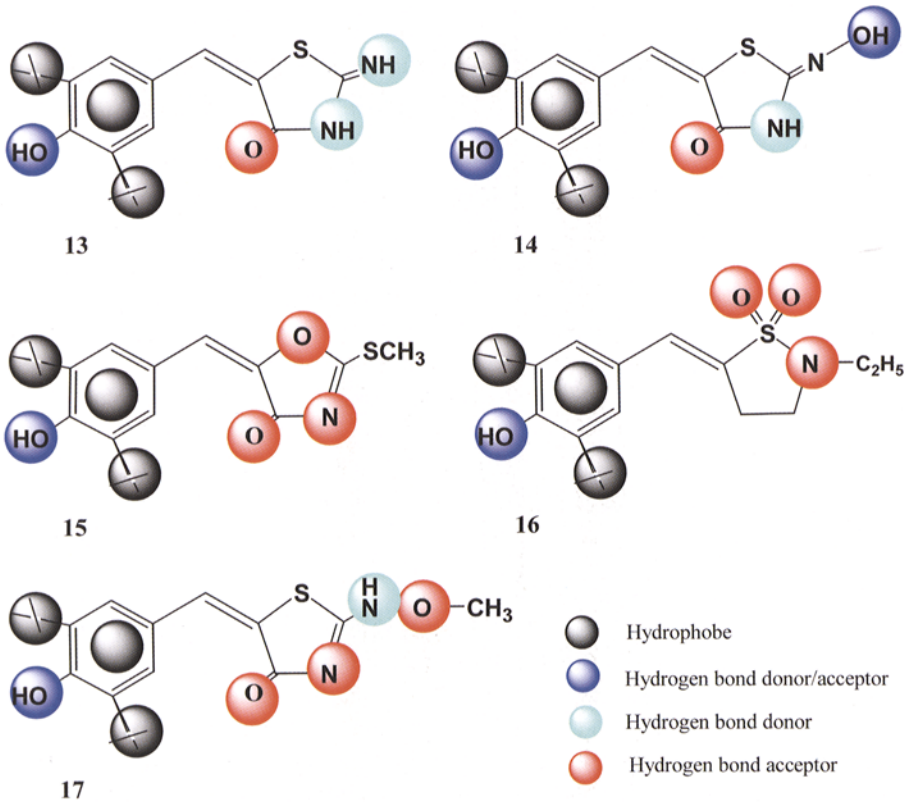


Fig. 1. Training set compounds **13-17**

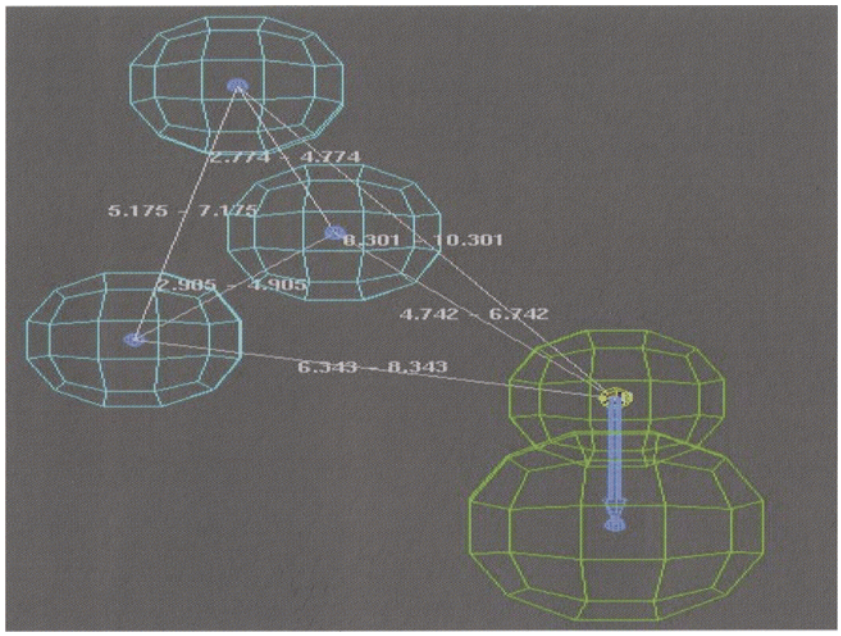


Fig. 2. The highest ranking hypothetical model, HHHA

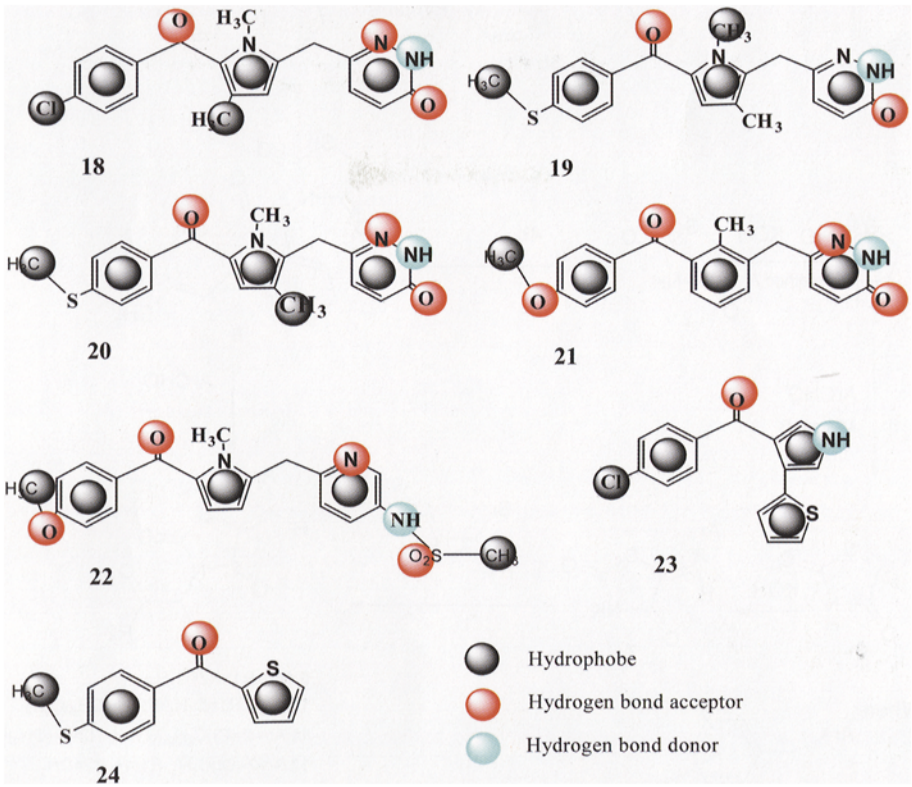


Fig. 3. Test set compounds **18-24**

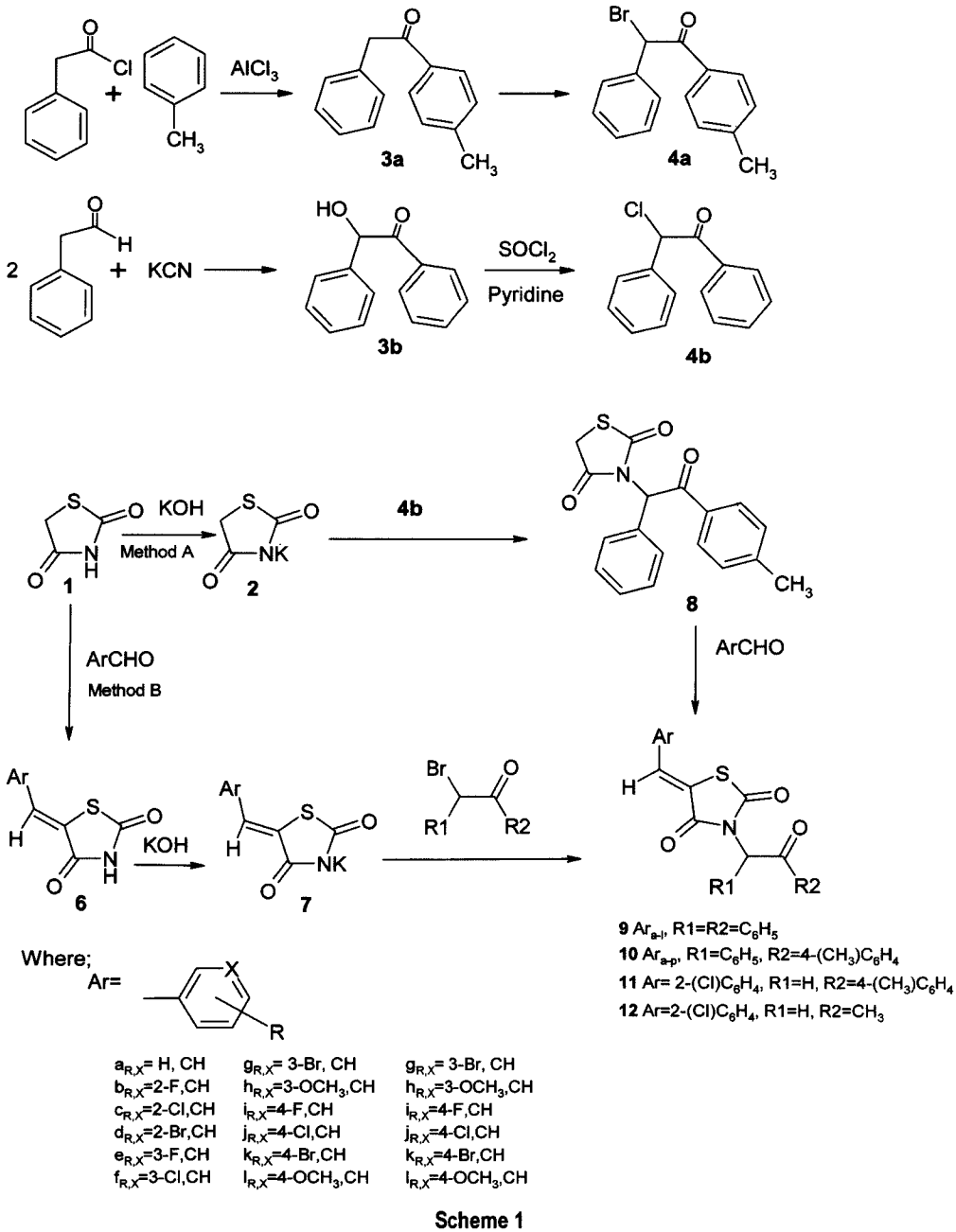
1000 MHz. The docking studies of the compounds with COX1 and COX2 were carried out using the enzyme parameters obtained from the crystallographic structures of the complexes between COX1 with flurbiprofen (1CX1.pdb) and COX2 with SC-558 (1CX2.pdb) (Berman *et al.*, 2000). Docking of the ligands was carried out using program DOCK 6.0 (Kuntz, San Fransico). Docking was performed with default settings to obtain a population of

possible conformations and orientations for the inhibitors at the binding site. A 10 Å sphere around the centre of the binding pocket was defined as binding pocket for the docking runs. All torsion angles in each compound were allowed to rotate freely.

DISCUSSION

Chemistry

The target compounds were prepared as described in the following scheme, starting from the 2,4-thiazolidinedione **1** which was prepared according to Turkevich et al procedure (Turkevich *et al.*, 1962), and condensed with the appropriate aromatic aldehydes in boiling ethanol in presence of piperidine using Bruno et al procedure (Bruno *et al.*, 2002) to yield the corresponding 5-(un)substituted arylmethylidene-2,4-thiazolidinedione derivatives (**6a-p**) having (Z) configuration (Unangst *et al.*, 1994, Lohray *et al.*, 1999) These compounds were converted into its sodium salt (**7a-p**) followed by alkylation with 2-bromo-1-(4-methyl(or unsubstituted)phenyl)-2-phenyl-1-ethanone (4a,b) in DMF (Lo and Shropshire 1957) to afford the corresponding 5-arylmethylidine-3-(2-oxo-1,2-diphenylethyl)-2,4-thiazolidinediones (**9a-l**) and 5-arylmethylidine-3-[2-(4-

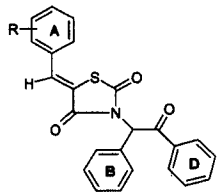


Scheme 1

methylphenyl)-2-oxo-1-phenylethyl]-2,4-thiazolidinediones (**10a-p**) derivatives. 2-bromo-1-(4-methyl(or unsubstituted) phenyl)-2-phenyl-1-ethanone (**4a**) could be synthesized through Friedel-Crafts acylation of substituted benzene with phenylacetyl chloride in presence of anhydrous $AlCl_3$ (Buck *et al.*, 1932, Bender *et al.*, 1985, Lantos *et al.*, 1984, Fred and Frederick 1948) followed by bromination of the obtained deoxybenzoin using ether : dioxane (2 : 1) as a solvent (Fred and Frederick, 1948) While **4b** was prepared through benzoin condensation of benzaldehyde by reflux with potassium cyanide in ethanol.

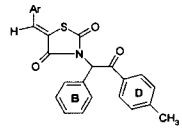
Structures of the synthesized compounds were verified on the bases of spectral and elemental methods of analyses. Characterization of the structure of compounds **1** and **6a-p** was based on m.p. as reported (Bruno *et al.*, 2002; Lo *et al.*, 1953; Giles *et al.*, 2000; Taniyama *et al.*, 1956; Robert *et al.*, 1939; Cetenko *et al.*, 1989) and spectral analyses (IR and NMR), which are consistent with literature data (Giles *et al.*, 2000) Tables I and II show the physicochemical constants of compounds (**9a-l**) and (**10a-p**) respectively. The spectral data of compounds (**9a-l**) and (**10a-p**) are shown in Tables III and IV respectively. All spectral data are in accordance with the expected structures.

Table III. IR and 1HNMR data ($CDCl_3$) (60 MHz) of 5-arylmethylidene-3-(2-oxo-1,2-diphenylethyl)-2,4-thiazolidinediones (9_{a-l})



No	R	IR (KBr, ν_{max} cm^{-1}	^{1}HNMR ($CDCl_3$, dppm)
9a	H	3040 (CH_{Ar}), 1738 (C-CO-N), 1682 (S-CO-N and C-CO-Ph), 1593 (C=C), 757 and 706 (monosubst benzene).	7.1 (1H, s, B-CHCO-D), 7.9-7.4 (13H, m, $H_{(3,5)}$ D and A and B), 8.3-8.0 (3H, m, $H_{(2,6)}$D and =CH-A
9b	2-F	3025 (CH_{Ar}), 1735 (C-CO-N), 1688 (S-CO-N and C-CO-Ph), 1612 (C=C), 769 (o-disubst benzene), 757and 706 (mono subst benzene).	7.1 (1H, s, B-CHCO-D), 7.9-7.3 (12H, m, $H_{(3,5)}$ D and A and B), 8.1-7.9 (2H, m, $H_{(2,6)}$ D and 8.3 (1H, s, =CH-A)
9c	2-Cl	3015 (CH_{Ar}), 1742 (C-CO-N), 1683 (S-CO-N and C-CO-Ph), 1604 (C=C), 777 (o-disubst benzene), 751and 701 (mono subst benzene.	7.1 (1H, s, B-CHCO-D), 7.9-7.3 (12H, m, $H_{(3,5)}$ D and A and B), 8.2-7.9 (2H, m, $H_{(2,6)}$ D and 8.4 (1H, s, =CH-A)
9d	2-Br	3015 (CH_{Ar}), 1741 (C-CO-N), 1674 (S-CO-N and C-CO-Ph), 1590 (C=C), 777 (o-disubst benzene), 754and 702 (monosubst benzene).	7.0 (1H, s, B-CHCO-D), 7.8-7.2 (12H, m, $H_{(3,5)}$ D and A and B), 8.1-7.8 (2H, m, $H_{(2,6)}$ D and 8.4 (1H, s, =CH-A)
9e	3-F	3120 (CH_{Ar}), 1735 (C-CO-N), 1681 (S-CO-N and C-CO-Ph), 1609 (C=C), 779, 674 (m-disubst benzene), 748 and 707 (monosubst benzene.	7.0 (1H, s, B-CHCO-D), 7.8-7.2 (12H, m, $H_{(3,5)}$ D and A and B), 8.2-7.9 (3H, m, $H_{(2,6)}$ D and =CH-A)
9f	3-Cl	3030 (CH_{Ar}), 1736 (C-CO-N), 1679 (S-CO-N and C-CO-Ph), 1597 (C=C), 773, 668 (m-disubst benzene), 744 and 707 (monosubst benzene.	7.0 (1H, s, B-CHCO-D), 7.8-7.3 (12H, m, $H_{(3,5)}$ D and A and B), 8.1-7.9 (3H, m, $H_{(2,6)}$ D and =CH-A)
9g	3-Br	3125 (CH_{Ar}), 1733 (C-CO-N), 1679 (S-CO-N and C-CO-Ph), 1597 (C=C), 768, 676 (m-disubst benzene), 743 and 703 (monosubst benzene).	7.1 (1H, s, B-CHCO-D), 7.9-7.4 (12H, m, $H_{(3,5)}$ D and A and B), 8.2-7.9 (3H, m, $H_{(2,6)}$ D and =CH-A)
9h	3-OCH3	3025 (CH_{Ar}), 1742 (C-CO-N), 1694 (S-CO-N and C-CO-Ph), 1602 (C=C), 1268, 1032 (C-O-C), 765, 670 (m-disubst benzene), 754 and 693 (monosubst benzene).	3.9 (3H, s, OCH3), 7.0 (1H, s, B-CHCO-D), 7.3-7.1 (3H,m, (2,4,6)A, 7.8-7.3 (9H, m, $H_{(5)}$ A, $H_{(3,5)}$ D and B), 8.1-7.9 (3H, m, $H_{(2,6)}$ D and =CH-A)
9i	4-F	3035 (CH_{Ar}), 1739 (C-CO-N), 1687 (S-CO-N and C-CO-Ph), 1608 (C=C), 829 (p-disubst benzene), 755 and 694 (monosubst benzene).	7.0 (1H, s, B-CHCO-D), 7.8-7.4 (12H, m, $H_{(3,5)}$ D and A and B), 8.1-7.9 (3H, m, $H_{(2,6)}$ D and =CH-A)
9j	4-Cl	3020 (CH_{Ar}), 1740 (C-CO-N), 1687 (S-CO-N and C-CO-Ph), 1605 (C=C), 820 (p-disubst benzene), 747 and 695 (monosubst benzene).	7.0 (1H, s, B-CHCO-D), 7.9-7.4 (12H, m, $H_{(3,5)}$ D and A and B), 8.1-7.9 (3H, m, $H_{(2,6)}$ D and =CH-A)
9k	4-Br	3030 (CH_{Ar}), 1738 (C-CO-N), 1687 (S-CO-N and C-CO-Ph), 1605 (C=C), 818 (p-disubst benzene), 745 and 695 (monosubst benzene).	7.1 (1H, s, B-CHCO-D), 7.9-7.3 (12H, m, $H_{(3,5)}$ D and A and B), 8.2-7.9 (3H, m, $H_{(2,6)}$ D and =CH-A)
9l	4-OCH3	3065 (CH_{Ar}), 1732 (C-CO-N), 1681 (S-CO-N and C-CO-Ph), 1591 (C=C), 1252, 1030 (C-O-C), 810 (p-disubst benzene), 741 and 696 (monosubst benzene.	3.9 (3H, s, OCH3), 7.0 (1H, s, B-CHCO-D), 7.2 (2H,d,J=9Hz, $H_{(3,5)}$A), 7.9-7.4 (10H, m, $H_{(2,6)}$ A, $_{(3,5)}$ D and B), 8.1-7.9 (3H, m, $H_{(2,6)}$ D and =CH-A)

Table IV. IR and 1HNMR data ($CDCl_3$) (60 MHz) of 5-arylmethylidene-3-(2-oxo-1,2-diphenylethyl)-2,4-thiazolidinediones (10_{a-p})



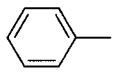
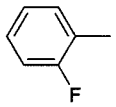
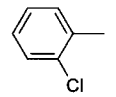
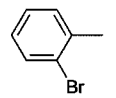
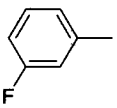
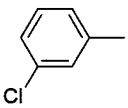
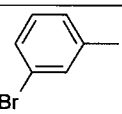
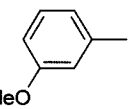
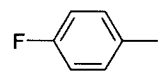
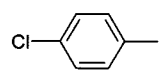
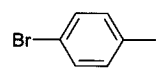
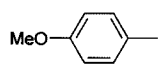
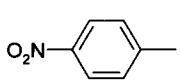
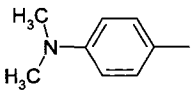
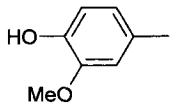
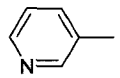
No	Ar	IR (KBr, ν_{max} cm^{-1}	^{1}H-NMR ($CDCl_3$, dppm)
10a		3040 (CH_{Ar}), 1740 (C-CO-N), 1684 (S-CO-N and C-CO-Ph), 1603 (C=C), 803 (p-disub benzene, 757 and 700 (mono subst benzene).	2.4 (3H, s, CH_3), 7.0 (1H, s, CHCO-), 7.3 (2H,d,J=8.0 Hz, $H_{(3,5)}$ D), 7.8-7.4 (10H, m, Ar and B), 7.9 (2H, d, J=8.0 Hz, $H_{(2,6)}$D), 8.1 (1H,s, =CH-Ar)
10b		3015 (CH_{Ar}), 1743 (C-CO-N), 1683 (S-CO-N and C-CO-Ph), 1603 (C=C), 801 (p-disub benzene),776 (o-disubst benzene), 751 and 701 (mono subst benzene).	2.4 (3H, s, CH_3), 7.0 (1H, s, CHCO-), 7.3 (2H,d,J=8.0 Hz, $H_{(3,5)}$ D), 7.8-7.4 (9H, m, Ar and B), 7.9 (2H, d, J=8.0 Hz, $H_{(2,6)}$D), 8.3 (1H,s, =CH-Ar)
10c		3065 (CH_{Ar}), 1741 (C-CO-N), 1682 (S-CO-N and C-CO-Ph), 1599 (C=C), 803 (p-disub benzene),762 (o-disubst benzene), 746 and 699 (mono subst benzene).	2.4 (3H, s, CH_3), 7.1 (1H, s, CHCO-), 7.3 (2H,d,J=8.0 Hz, $H_{(3,5)}$ D), 7.8-7.5 (9H, m, Ar and B), 7.9 (2H, d, J=8.0 Hz, $H_{(2,6)}$D), 8.4 (1H,s, =CH-Ar)
10d		3035 (CH_{Ar}), 1741 (C-CO-N), 1682 (S-CO-N and C-CO-Ph), 1598 (C=C), 802 (p-disub benzene),759 (o-disubst benzene), 743 and 697 (mono subst benzene).	2.5 (3H, s, CH_3), 7.1 (1H, s, CHCO-), 7.4 (2H,d,J=8.0 Hz, $H_{(3,5)}$ D), 7.9-7.5 (9H, m, Ar and B), 8.0 (2H, d, J=8.0 Hz, $H_{(2,6)}$D), 8.5 (1H,s, =CH-Ar)
10e		3070 (CH_{Ar}), 1738 (C-CO-N), 1681 (S-CO-N and C-CO-Ph), 1595 (C=C), 807 (p-disub benzene), 778, 674 (m-disubst benzene), 748 and 706 (monosubst benzene).	2.4 (3H, s, CH_3), 7.1 (1H, s, CHCO-), 7.3 (2H,d,J=8.0 Hz, $H_{(3,5)}$ D), 7.8-7.4 (9H, m, Ar and B), 7.9 (2H, d, J=8.0 Hz, $H_{(2,6)}$D), 8.0 (1H,s, =CH-Ar)
10f		3090 (CH_{Ar}), 1736 (C-CO-N), 1680 (S-CO-N and C-CO-Ph), 1601 (C=C), 807 (p-disub benzene), 771, 666 (m-disubst benzene), 734 and 703 (monosubst benzene).	2.4 (3H, s, CH_3), 7.1 (1H, s, CHCO-), 7.4 (2H,d,J=8.0 Hz, $H_{(3,5)}$ D), 7.8-7.5 (9H, m, Ar and B), 7.9 (2H, d, J=8.0 Hz, $H_{(2,6)}$D), 8.0 (1H,s, =CH-Ar)
10g		3000 (CH_{Ar}), 1734 (C-CO-N), 1679 (S-CO-N and C-CO-Ph), 1601 (C=C), 803 (p-disub benzene), 768, 666 (m-disubst benzene), 732 and 702 (monosubst benzene).	2.4 (3H, s, CH_3), 7.1 (1H, s, CHCO-), 7.4 (2H,d,J=8.0 Hz, $H_{(3,5)}$ D), 7.8-7.5 (9H, m, Ar and B), 7.9 (2H, d, J=8.0 Hz, $H_{(2,6)}$D), 8.1 (1H,s, =CH-Ar)
10h		3060 (CH_{Ar}), 1741 (C-CO-N), 1678 (S-CO-N and C-CO-Ph), 1601 (C=C), 1274, 1032 (C-O-C),793 (p-disub benzene), 766, 697 (m-disubst benzene), 732 and 673 (monosubst benzene).	2.4 (3H, s, CH_3), 3.9 (3H, s, OCH_3), 7.1 (1H, s, CHCO-), 7.5-7.2 (5H,m, $H_{(2,4,6)}$Ar and $H_{(3,5)}$D), 7.8-7.5 (6H, m, $H_{(5)}$ Ar and B), 8.0 (2H, d, J=8.0 Hz, $H_{(2,6)}$D), 8.1 (1H,s, =CH-Ar)
10i		3040 (CH_{Ar}), 1740 (C-CO-N), 1684 (S-CO-N and C-CO-Ph), 1597 (C=C), 847 (p-disub benzene), 737 and 695 (monosubst benzene).	2.4 (3H, s, CH_3), 7.1 (1H, s, CHCO-), 7.4 (2H,d,J=8.0 Hz, $H_{(3,5)}$ D), 7.8-7.5 (9H, m, Ar and B), 7.9 (2H, d, J=8.0 Hz, $H_{(2,6)}$D), 8.1 (1H,s, =CH-Ar)
10j		3065 (CH_{Ar}), 1742 (C-CO-N), 1686 (S-CO-N and C-CO-Ph), 1604 (C=C), 816 (p-disub benzene), 731 and 696 (monosubst benzene).	2.4 (3H, s, CH_3), 7.1 (1H, s, CHCO-), 7.4 (2H,d,J=8.0 Hz, $H_{(3,5)}$ D), 7.9-7.5 (9H, m, Ar and B), 8.0 (2H, d, J=8.0 Hz, $H_{(2,6)}$D), 8.1 (1H,s, =CH-Ar)
10k		3035 (CH_{Ar}), 1733 (C-CO-N), 1696 (S-CO-N), 1675 (C-CO-tolyl), 1604 (C=C), 816 (p-disub benzene), 731 and 696 (monosubst benzene).	2.4 (3H, s, CH_3), 7.1 (1H, s, CHCO-), 7.4 (2H,d,J=8.0 Hz, $H_{(3,5)}$ D), 7.9-7.5 (9H, m, Ar and B), 8.0 (2H, d, J=8.0 Hz, $H_{(2,6)}$D), 8.1 (1H,s, =CH-Ar)
10l		3000 (CH_{Ar}), 1732 (C-CO-N), 1680 (S-CO-N and C-CO-tolyl), 1591 (C=C), 1255, 1024 (C-O-C), 821 (p-disubst benzene), 742 and 695 (monosubst benzene).	2.4 (3H, s, CH_3), 3.9 (3H, s, OCH_3), 7.0 (1H, s, CHCO-), 7.1-7.3 (4H,m, $H_{(3,5)}$Ar and $H_{(3,5)}$D), 7.8-7.4 (7H, m, $H_{(2,6)}$Ar and B), 7.9 (2H, d, J=8.0 Hz, $H_{(2,6)}$D), 8.0 (1H,s, =CH-Ar)
10m		3020 (CH_{Ar}), 1746 (C-CO-N), 1688 (S-CO-N and C-CO-tolyl), 1601 (C=C), 1510, 1335 (-NO_2), 804 (p-disubst benzene), 750 and 691 (monosubst benzene).	2.4 (3H, s, CH_3), 7.0 (1H, s, CHCO-), 7.3 (2H,d,J=8.0 Hz, $H_{(3,5)}$ D), 7.6-7.4 (5H, m, B), 7.8 (2H, d, J=8.0 Hz, $H_{(2,6)}$Ar), 7.9 (2H, d, J=8.0 Hz, $H_{(2,6)}$D), 8.0 (1H,s, =CH-Ar), 8.5 (2H, d, J=8.0 Hz, $H_{(3,5)}$Ar)

Table IV. Continued

No	Ar	IR (KBr, ν_{max} cm^{-1}	^{1}HNMR ($CDCl_3$, dppm)
10n		3010 (CH_{Ar}), 1714 (C-CO-N), 1686 (S-CO-N), 1663 (C-CO-tolyl), 1603 (C=C), 803 (p-disubst benzene), 732 and 696 (monosubst benzene).	2.4 (3H, s, CH_3), 3.1 (6H, s, $N(CH_3)_2$, 6.8 (2H,d,J=9.0 Hz, $H_{(3,5)}$Ar), 7.0 (1H, s, CHCO-), 7.3 (2H,d,J=8.0 Hz, $H_{(3,5)}$ D), 7.8-7.4 (7H, m, $H_{(2,6)}$Ar and B), 7.9 (2H, d, J=8.0 Hz, $H_{(2,6)}$D), 8.0 (1H,s, =CH-Ar),
10o		3445 (OH), 3030 (CH_{Ar}), 1732 (C-CO-N), 1679 (S-CO-N and C-CO-tolyl), 1601(C=C), 1277, 1026 (C-O-C), 802 (p-disubst benzene), 735 and 696 (monosubst benzene).	2.4 (3H, s, CH_3), 4.0 (3H, s, OCH_3), 7.0 (1H, s, CHCO-), 7.3-7.1 (3H,m,$H_{(2,5,6)}$ Ar), 7.4 (2H,d,J=8.0 Hz, $H_{(3,5)}$ D), 7.8-7.5 (6H, m, OH and B), 8.0 (2H, d, J=8.0 Hz, $H_{(2,6)}$D), 8.1 (1H,s, =CH-Ar),
10p		3035 (CH_{Ar}), 1746 (C-CO-N), 1688 (S-CO-N and C-CO-tolyl), 1604(C=C), 806 (p-disubst benzene), 732 and 697 (monosubst benzene).	2.4 (3H, s, CH_3), 7.1 (1H, s, CHCO-), 7.4 (2H,d,J=8.0 Hz, $H_{(3,5)}$ D), 7.8-7.4 (6H, m, $H_{(5)}$ Ar and B), 8.2-7.8 (4H,m,$H_{(4)}$ Ar, $H_{(2,6)}$D, and =CH-Ar), 9.1-8.8 (2H, m, $H_{(2,6)}$Ar).

Biological evaluation

Analgesic activity

The analgesic activity of the synthesized thiazolidinedione derivatives and reference drugs was investigated in mice at a dose level of 10 mg/kg against thermal stimuli according to a reported method (Valencia *et al.*, 1994). Indomethacin, non-selective anti-inflammatory drug, and Rofecoxib, selective COX2 inhibitor, were used as reference standards. One-way analysis of variance (ANOVA) was used to treat the results of both control and test groups. The difference in the results was considered significant when the values of $P<0.05$ (significant difference) or $P< 0.001$ (highly significant difference) (Nojima *et al.* 1995). The results of the analgesic activity are illustrated in Table V. Seven of the investigated compounds, **9d**, **9g**, **10d**, **10j**, **10k**, **10o** and **10p**, displayed a highly significant analgesic activity ($P < 0.001$) compared with reference standards as shown in Fig. **4**. Among these derivatives compound **10d** was the most potent analog (latency ~7 minutes, 2 h after injection). In addition, the absence of 5-arylmethylidene moiety decreased the analgesic activity as shown in compound **8**. Also, decreasing the bulkiness at NH group of 2,4-thiazolidinedione ring resulted in a reduction of the analgesic activity as illustrated by 2,4-thiazolidinedione derivatives **6c**, **11** and **12** which showed no significant analgesic activity. Analogs containing halogen at ring A exhibited high activity. Replacement of phenyl ring A by pyridyl moiety did not remarkably affect the activity. In view of these results, two bulk groups are required for good activity of these compounds as analgesic agents.

Anti-inflammatory activity

The preliminary ant-inflammatory activity of the synthesized 2,4-thiazolidinones was evaluated against carragenin induced paw edema in rats, using Hernandez-Perez *et al.* method (Hernandez-Perez *et al.*, 1995). The results of the anti-inflammatory activity of the tested compounds as well

Table V. Analgesic activity (average licking latencies) of 2,4-thiazolidinediones and reference standards using the hot-plate test

Groups	Latency (sec.) after		
	1 h	2 h	3 h
Placebo	80.00 ± 0.31	83.00 ± 0.23	81.90 ± 0.22
8	202.00 ± 0.27	196.50 ± 0.21	160.75 ± 0.38
6c	97.15 ± 0.15	86.31 ± 0.29	100.98 ± 0.34
9c	196.75 ± 0.29	247.25 ± 0.34	267.67 ± 0.22
9d	232.67 ± 0.34	359.67 ± 0.11*	208.25 ± 0.23
9f	262.00 ± 0.26	239.25 ± 0.35	298.75 ± 0.34
9g	174.00 ± 0.21	159.00 ± 0.35	295.50 ± 0.12*
9h	239.30 ± 0.11	228.25 ± 0.34	282.50 ± 0.11
10a	261.25 ± 0.11	273.75 ± 0.35	298.00 ± 0.36
10b	207.25 ± 0.33	223.50 ± 0.19	187.50 ± 0.21
10c	160.75 ± 0.24	200.80 ±0.32	157.50 ± 0.12
10d	271.00 ± 0.25*	430.00 ± 0.41*	385.00 ± 0.22*
10e	212.00 ± 0.26	228.25 ± 0.31	120.50 ± 0.26
10f	158.00 ± 0.33	205.25 ± 0.26	165.50 ± 0.15
10g	147.00 ± 0.22	252.50 ± 0.30	142.75 ± 0.35
10j	176.00 ± 0.34	323.75 ± 0.50*	301.75 ± 0.38*
10k	147.00 ± 0.38	306.75 ± 0.21*	302.50 ± 0.18*
10l	180.00 ± 0.39	150.80 ± 0.33	287.30 ± 0.23
10m	177.25 ± 0.24	257.50 ± 0.25	298.75 ± 0.12
10n	144.00 ± 0.18	219.30 ± 0.35	136.75 ± 0.29
10o	228.00 ± 0.33	315.00 ± 0.25*	380.00 ± 0.33*
10p	269.25 ± 0.26*	222.25 ± 0.36	231.25 ± 0.40
11	161.00 ± 0.24	208.70 ± 0.22	153.80 ± 0.24
12	122.30 ± 0.22	121.60 ± 0.24	135.70 ± 0.36
Rofecoxib	133.00 ± 0.27	140.00 ± 0.22	121.00 ± 0.19
Indomethacin	226.00 ± 0.34	153.00 ± 0.12	137.50 ± 0.31

High significant difference ($P < 0.001$) and longer latency as compared to indomethacin and rofecoxib.

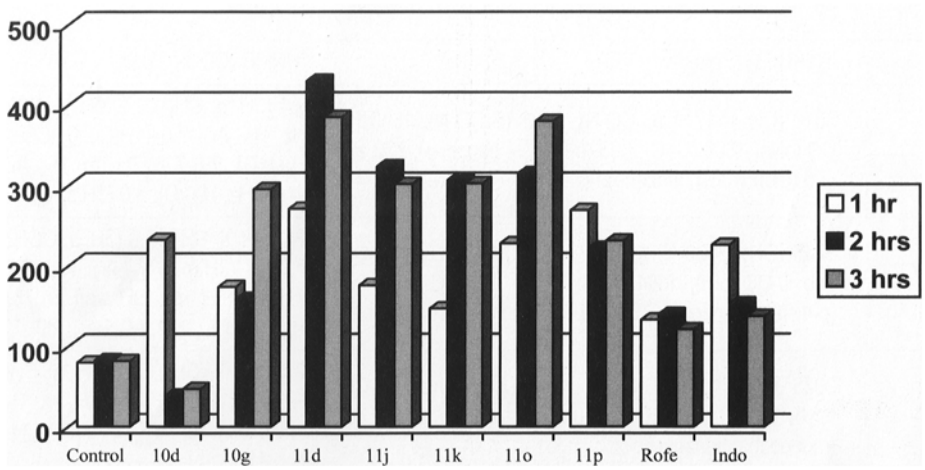


Fig. 4. Average licking latency (sec.) of thiazolidinedione derivatives, reference standards and control group at intraperitoneal dose of 10 mg/kg in mice (hot-plate test)

Table VI. Anti-inflammatory activity (% increase in edema ± S.E. and % anti-inflammatory effect)

Groups	% Increase in edema ± S.E.	% Anti-inflammatory effect
Placebo	7.7 ± 0.5	0.0
8	4.3 ± 0.22**	44.20#
6c	8.7 ± 0.34	0.00
9c	4.5 ± 0.20**	41.60#
9d	5.5 ± 0.20**	28.60#
9f	5.8 ± 0.43*	24.6*
9g	5.2 ± 0.45**	32.4*
9h	7.0 ± 0.5	9.10
10a	7.1 ± 0.3	8.0
10b	5.2 ± 0.3**	32.5#
10c	4.3 ±0.1**	44.2#
10d	4.6 ± 0.25**	40.3#
10e	5.8 ± 0.3*	24.7*
10f	7.0 ± 0.3	9.1
10g	6.7 ± 0.3*	13.0*
10j	7.3 ± 0.2	5.2
10k	5.1 ± 0.1**	33.8#
10l	5.0 ± 0.3**	35.1#
10m	5.7 ± 0.3*	26.0*
10n	6.2 ± 0.45*	19.4*
10o	6.9 ± 0.3	10.4
10p	7.0 ± 0.1	9.1
11	5.8 ± 0.35*	25.0*
12	8.0 ± 0.26	0.0
Rofecoxib	4.2 ± 0.33*	45.4*
Indomethacin	4.6 ± 0.12*	40.3*

* Significant difference (P<0.05) as compared to the control group.
# Non-significant difference as compared to indomethacin group.

as indomethacin and rofecoxib reference drugs are listed in Table VI. Fig. 5 shows the percent increase of edema and percent of edema inhibition induced by the tested compounds respectively.

Generally, it was observed that most of the tested compounds from both series (**9** and **10**) showed significant (P<0.05) inhibition. Compounds having halogen group at *ortho* position of arylmethylidene ring exhibited good activity relative to the standards. Absence of 5-arylmethylidene moiety in compound **8** resulted in most active analog (44% edema reduction). Also, decreasing or removal the bulkiness at NH group of 2,4-thiazolidinedione ring either decreased (compound **11**) or abolished (compounds **6c** and **12**) the anti-inflammatory activity. This reveals that aromatic bulk substituent at nitrogen atom of thiazolidinedione ring is important for activity.

Ulcerogenic liability

Compounds (**8**, **9c**, **9d**, **9g**, **10c**, **10d**, **10l**, **10o** and **11**) which, showed promising analgesic and anti-inflammatory profiles were tested for gastric ulcerogenic potential in rat stress model (Fadl and Omar, 1998; Omar *et al.*, 1999). Compound **10c** was found to be more safe than indomethacin and produced no significant mucosal injury similar to rofecoxib. It should be pointed out that compounds **8**, **9c**, **9d**, **9g**, **10d** and **10l** produced little mucosal damage but still much less than that produced by indomethacin. Compounds **10o** and **11** exerted complete damage of the mucous layer besides ulceration of the submucosal cells in the same pattern like indomethacin.

Catalyst COX2 hypothetical model

It was reported that the safety profile of ulcerogenic test could be considered as a measure for COX2 selectivity (Naser and Said, 2003). Accordingly, our speculations

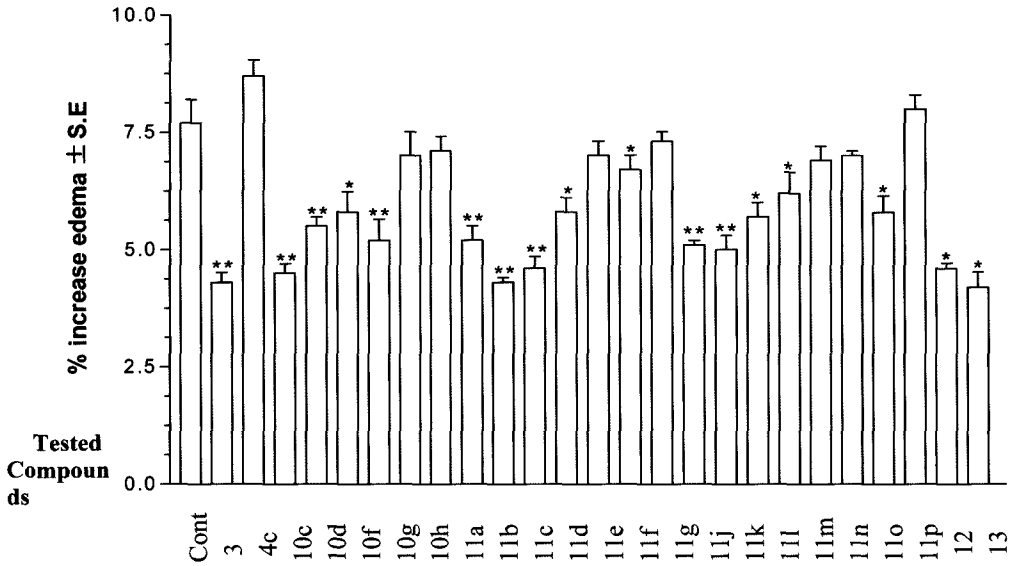


Fig. 5. (%) increase of edema induced by carrageenin in control, test compounds, Indomethacin and Rofecoxib treated groups of rats, at 10 mg/kg. *Significant difference ($P<0.05$) as compared to the control group. **Non-significant difference as compared to Indomethacin group.

were further confirmed by qualitative 3D-SSR study through Catalyst pharmacophore construction.

Catalyst pharmacophore construction (Hypothesis generation)

Training set reported as COX2 inhibitor compounds (**13-17**), Fig. 1, is used as a data set for creating common-features hypothesis by HipHop Catalyst modules (Greene *et al.*, 1994; Barnum *et al.*, 1996). Fig. 2 shows the obtained hypothetical model that was coincident with the reported data concerning the pharmacophore generation of known COX2 inhibitors (Palomer *et al.*, 2002). It is composed of four features (3H and 1HBA), the three hydrophobic features represented by blue sphere while hydrogen bond acceptor feature represented by a green sphere.

Validation of the resulting pharmacophore model (Hypothesis)

This validation was established using test set of seven compounds, Fig. 3, five of them are selective COX2 inhibitors (**18-22**) and two compounds (**23**, **24**) are non-selective (Dannhardt and Laufer, 2000; Bernard *et al.*, 1997; Edvige *et al.*, 1997). Fitting operation was performed to find best fit for one conformation of each molecule and report the fit value for each of the test set. The selective compounds **15-19** showed fit values 3.5 ~3.8 (maximum value is 4.0). However, compounds **19** and **20**, non-selective inhibitors, showed a small fit value or no fit at all.

Qualitative 3D-SSR

The target compounds (**6c**, **8**, **9a-l**, **10a-p**, **11** and **12**) were allowed to fit to the hypothetical model. In this investigation the perfect mapping will give an approximate indication of selectivity in COX2 inhibiting activity in term of a fit value, the higher the fit value, the higher expected to be selective inhibitor of COX2. Generally, compounds **10a-p** explored relatively higher fit values than their corresponding congeners **9a-l**. Thus, methyl group at *para* position of ring C (Scheme, R2 = p-tolyl) enhance the selectivity as COX2 inhibitors. Compounds containing substituted-arylmethylidene moiety are expected to be more selective than the nonsubstituted one (cf. **9a** and **10a**). The NH group of 2,4-thiazolidinedione nucleus must be substituted with a bulky group to be active hit to the hypothetical model (cf. **6c**).

Docking study

A variety of COX2-ligand crystal structures have been solved over the last years (Kurumbail *et al.*, 1996; Rowlinson *et al.*, 2003; Kiefer *et al.*, 2000). These X-ray structures provide important information about the relevant interaction possibilities at the COX2 binding pocket. The COX2 pocket is deeply buried within the protein at the end of a hydrophobic channel. The entrance of this channel is located nearby the membrane binding domain (Picot *et al.*, 1994). The X-ray structure 1CX2 with co-crystallized Celecoxib derivative SC-558 (Velázquez *et al.*, 2005 and (Anana *et al.*, 2006).) is used to show relevant protein-inhibitor interactions because docking settings reproduced the co-crystallized inhibitor correctly (rmsd 1.01 Å) (Fig. 6a). Figure 6a shows hydrogen bonding between sulphonamide, two oxygen atoms, of SC-558 with the amino group of Arg513 and imidazole NH His90 (hb distance is 2.764 Å and 3.113 Å respectively) and hydrogen bond between the sulphonamide NH_2 group of

SC-558 with the carbonyl oxygen of Gln192 and carbonyl oxygen of Leu352 (hb distance is 3.509 Å and 3.528 Å rescpectively). Also, Fig. 6b shows the COX1 X-ray structure with the co-crystallized Flurbiprofen in which the docking settings reproduced the co-crystallized inhibitor correctly (rmsd 0.76 Å). Fig. 6b shows hydrogen bonding between the carboxylate of the Flurbiprofen with the phenolic OH group of Tyr355 and amino group of Arg120 (hb distance (2.646 and 2.835 respectively).

The synthesized compounds were docked into the COX1 and COX2 binding sites using the same docking parameters as used for the co-crystallized ligand. The 2,4-thiazolidinediones derivatives show comparable interactions at COX2 as the co-crystallized SC-558. As examples the docking solutions obtained for compound **9a** at COX2 are shown in Fig. 7a in comparison with SC-558 shown in Fig. 6a. The docked compounds fit well into the binding pocket but don't show hydrogen bonding with the backbone heteroatoms. The phenylmethylidene group of 2,4-thiazolidinediones derivatives fits into the COX2 secondary pocket having the same orientation of sulfonamide group of SC-558. 2,4-Thiazolidinedione moiety adopts the position of the central pyrazole ring of SC-558 whereas the second aromatic ring can be superimposed with the bromophenyl ring of SC-558. The third aromatic ring adopts the position of the CF_3 of SC-558. The docked compound **9a** overlapped on the x-ray structure of SC-558 inside the

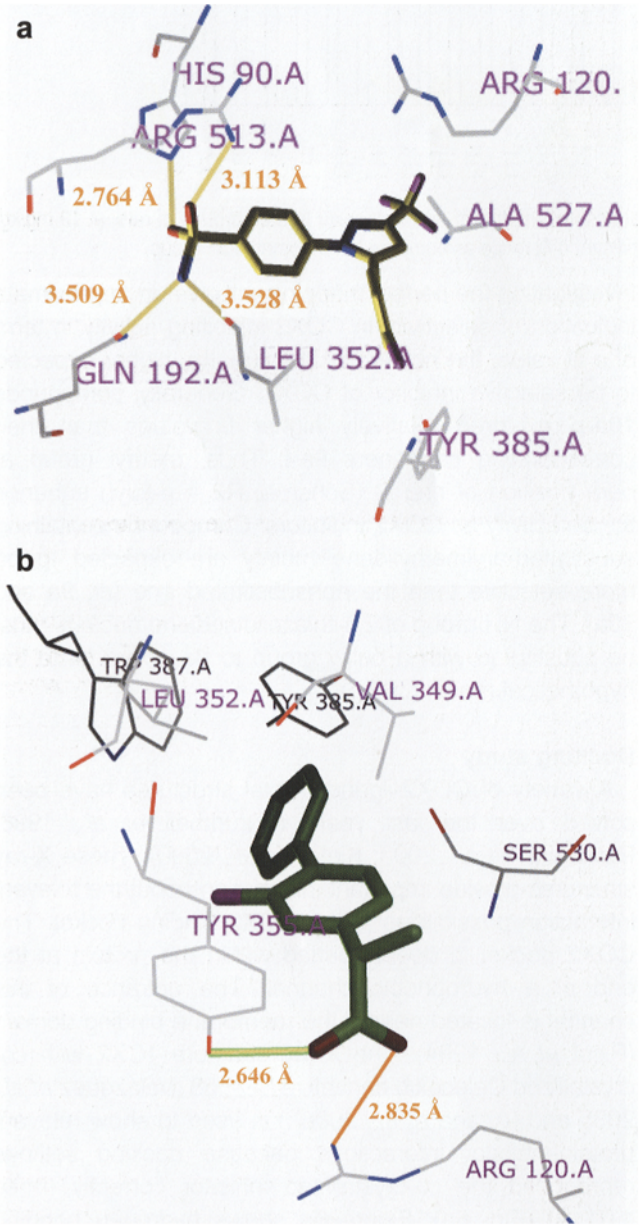


Fig. 6. (a) COX2-docked the co-crystallized Celecoxib derivative SC-558 (from 1CX2). (coloured orange), hydrogen bond (yellow cylinder). (b) COX1-docked the co-crystallized inhibitor Flurbiprofen (from 1CQE). (coloured green), hydrogen bond shown as yellow cylinder.

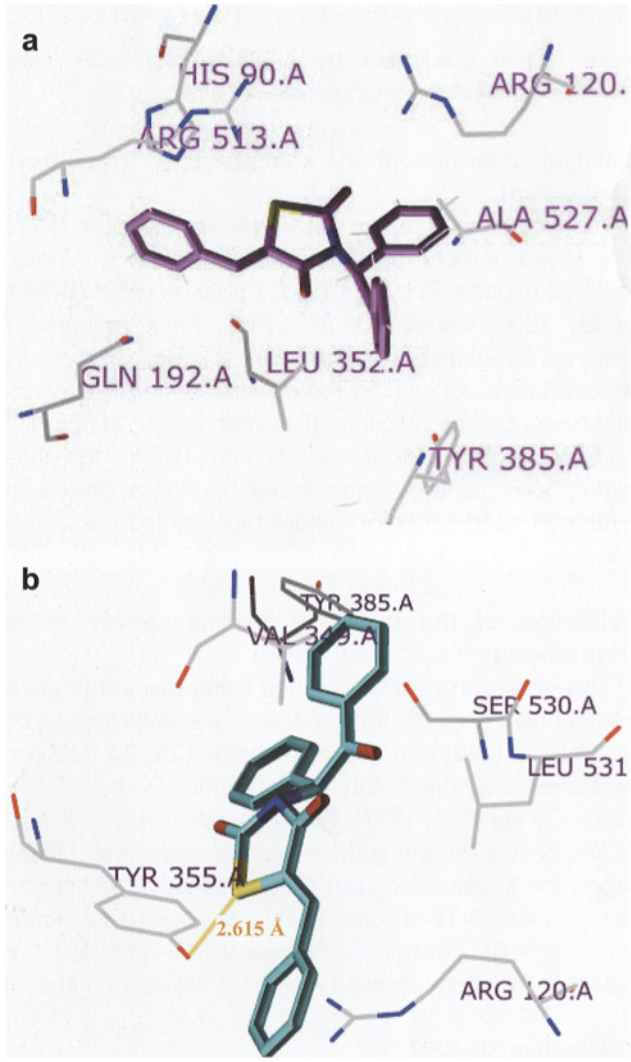


Fig. 7. (a) COX2-docked compound **9a** (coloured magenta). (b) COX1-docked compound **9a** (coloured cyan).

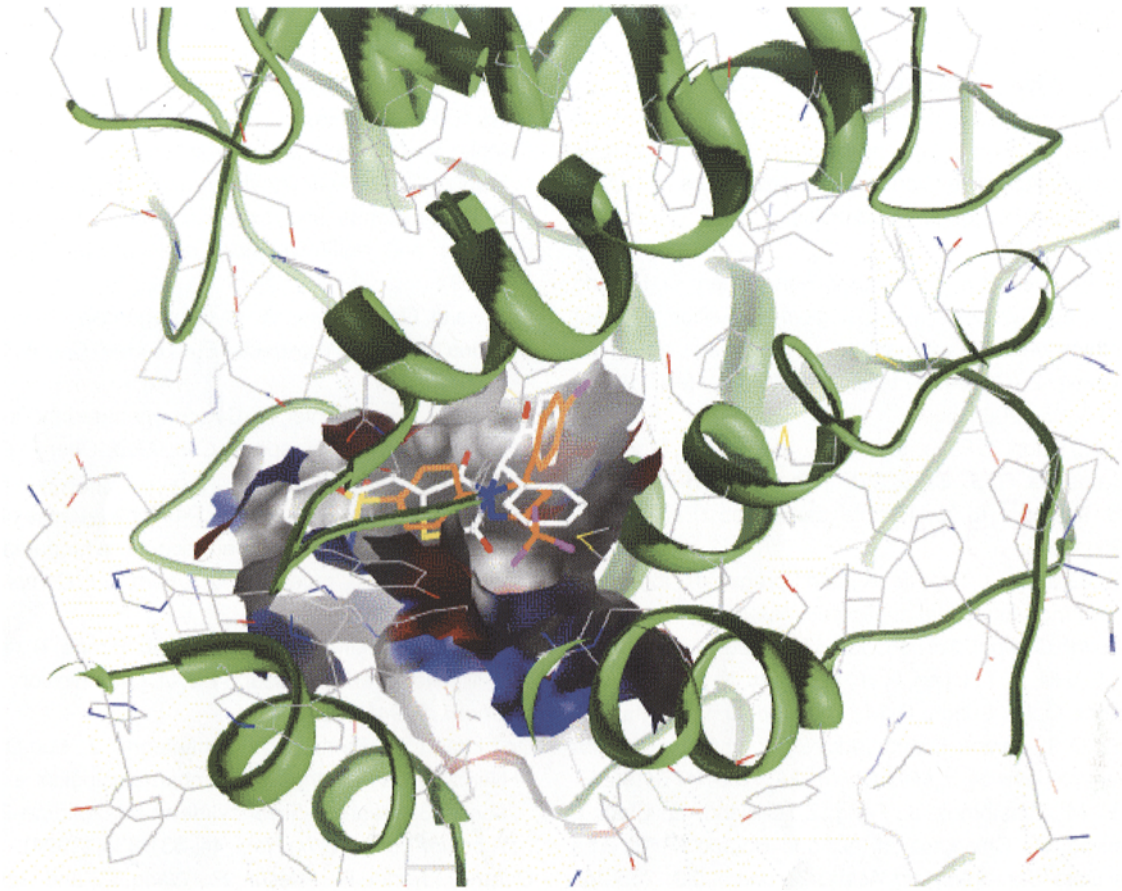


Fig. 8. COX2 superimposition of the co-crystallized Celecoxib derivative SC-558 (from 1CX2, coloured orange) and the docked compound **9a** (coloured white)

binding site of COX2 are shown in Fig. 8.

Docking into the COX1 binding pocket showed that the 2,4-thiazolidinediones derivatives show same interaction mode except the hydrogen bonding. As examples the obtained docking solutions for compound **9a** is shown in Fig. 7b in which the central 2,4-thiazolidinedione ring adopts the same position as the central aromatic ring of Flurbiprofen. The two aromatic rings of the ligands are orientated towards the hydrophobic pocket nearby Leu352. The polar substituent attached to the 2,4-thiazolidinediones derivatives is hydrogen bonded to Tyr355 as the carboxylate of the COX1 inhibitors. Due to the fact that no tricyclic inhibitor has been co-crystallized with COX1 yet, the interaction mode of the synthesized derivatives is more speculative. Comparison of docking scores that we obtained for the co-crystallized inhibitors, the training- and test set molecules yielded a preference of the 2,4-thiazolidinediones derivatives for COX2. Therefore, based on our docking results it is suggested that the synthesized 2,4-thiazolidinediones derivatives are active COX inhibitors with a clear preference for COX2.

In conclusion, the series of 3,5-disubstituted-2,4-thiazolidinedione derivatives were synthesized and tested for their analgesic and anti-inflammatory activity. The selectivity of the synthesized compounds against COX2 enzyme was investigated by studying its ulcerogenic liability. Also, molecular modeling of the compounds was utilized for studying their qualitative three dimensions structure selectivity relationship (3D-QSSR). The results indicate that, the presence of a bulk substituent at NH of 2,4-thiazolidinedione of the target compounds is essential for analgesic as well as anti-inflammatory activity. In addition ring C (scheme, R1 = phenyl) is necessary for both activities and its p-methyl substituent is preferred for the selective COX2 inhibitory activity. On the other hand, the presence of ring B (scheme, R1= H) did not attain a major effect on the analgesic and anti-inflammatory activities. Substituent like *ortho* chlorine or bromine atom at ring A of 5-arylmethylidene moiety are favored for COX2 selectivity while showed slight and variable effect on activity. Compounds **10c** and **10d** were proved to be the most promising derivatives within the synthesized compounds as analgesic and anti-inflammatory agents. Also, compounds **10c** and **10d** were suggested to have promising selectivity as COX2 inhibitors.

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