Bull. Pharm. Sci., Assiut University, Vol. 27, Part 1, June 2004, pp. 171-186.

DESIGN AND SYNTHESIS OF SOME P-SUBSTITUTED STYRYLISOXAZOLE CARBOXYLIC ACID DERIVATIVES AS ANTI-INFLAMMATORY AGENTS

Farghaly A. Omar[1], Atef A. Abdel-Hafez[1*] and Montaser S. Ahmed[2]

[1]*Department of Pharmaceutical Medicinal Chemistry, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt*
[2]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Alazhar University, Assiut, Egypt*

تم فى هذا البحث تشييد مجموعة من ٥-(مقابل-مستبدل ستايريل)-ايزوكسازول-٣ حمض الكربوكسيليك (**4a-g**) وكذلك مشتقاتها المقابلة من استرات إلايثيل (**3a-g**) والاميدات (**6-29**) بالاضافة الى مشابهات هذه الاحماض الكربوكسيلية من مجموعة حمض الهيدروكساميك (**5a-g**) وذلك بهدف اختبار فاعليتها ضد الالتهابات. ولتحضير المركبات المستهدفة المشار اليها من قبل تم اولا تكثيف مشتقات الإنشالكونات (**1a-g**) مع ثنائى ايثيل استر حمض الاوكساليك لتنتج مجموعة ٢,٤-ثنائى الأكسو-٦- أيريل هكس-٥- إينوك أسيد أثيل أستر (**2a-g**) وهى تمثل الوسيط الاساسى للحصول على مشتقات الايزوكسازول المستهدفة. ولتحقيق ذلك فقد تم تكاثف المركبات الوسيطة (**2a-g**) مع هيدروكلوريد الهيدروكسيل آمين. وفى كل هذه التفاعلات تم التحقق من التركيب البنائى للمركبات الوسيطة والمستهدفة من خلال تحاليل الاشعة دون الحمراء والرنين النووى المغناطيسى. اما درجة نقاوتها فقد امكن التاكد منها بواسطة التحليل الدقى لعناصرالكربون والهيدروجين والنيتروجين وكذلك كروماتوجرافيا الشرائح الرقيقة. الفاعلية ضد الالتهابات لواحد وعشرين مركـب [(**3a,e,f** and **g**); (**4a-g**); (**5a-g**); **22, 24** and **25**] تم اختبارهم عند جرعة ١٠٠ ملغم/كغم بواسطة إحداث التهاب فى اقدام الفئران بالكاراجنين بالمقارنة بالأندوميثازين كدواء مرجعى اختبر التاثير التقرحى للمعدة للمركبات [**3g, 4g** and **5g**] كمعبرات للمركبات الفعالة (أستر، حمض الكربوكسيليك، وحمض الهيدروكساميك) وكذلك الأندوميثازين كدواء مرجعى بواسطة الميكروسكوب الإلكترونى الماسح بعد ٢٤ ساعة من تعاطى جرعة واحدة بالفم (١٠٠ مغم/كغم) فى الفئران. أظهرت معظم المركبات المستهدفة نشاطا ملحوظا ضد الالتهابات. مشتقات مقابل-نيترو ومقابل-ميثوكس ستايريل أيزوكسازول حمض الكربوكسيليك (**4e** and **4f**) أظهرت ٧٥% تثبيط للالتهابات بعد ساعة وهذا يعكس المفعول السريع لهذه المشتقات حيث أن المفعول يمكن أن يستمر حتى ٥ ساعات كما فى حالة (**4e**). اما التأثير التقرحى للمعـدة فقـد كانت أفضل المشتقـات ٥-(مقابل-نيترو ومقابل-ثنائى الميثيل أمينوستايريل)-ايزوكسازول-٣-حمض الكربوكسيليك اثيل أستر (**3e** and **4g**) بالمقارنة بالأندوميثازين.

*A series of 5-(p-substitutedstyryl)-isoxazole-3-carboxylic acids (**4a-g**), carboxylic acid ethyl esters (**3a-g**) and their corresponding amides (**6-29**) as well as their hydroxamic acid analogues (**5a-g**) were synthesized and evaluated for their anti-inflammatory activity. The 1,3-dicarbonyl systems 2,4-dioxo-6-arylhex-5-enoic acid ethyl esters (**2a-g**) were the key intermediate for synthesis of the target isoxazole derivatives. The synthesis of these intermediate was achieved through the reaction of chalcones (**1a-g**), prepared via aldol condensation of the respective substituted benzaldehyde with acetone in alkaline medium, with diethyloxalate. The purity of the synthesized derivatives was determined by thin layer chromatography (TLC) in addition to the microanalyses and their structures were confirmed by different spectroscopic means. The anti-inflammatory activity of twenty-one compounds [(**3a,e,f** and **g**); (**4a-g**); (**5a-g**); **22; 24** and **25**] were assessed, at a dose of 100 mg/kg, by carrageenan-induced paw edema in rats in comparison to indomethacin as a reference drug. The ulcerogenicity of the compounds **3g, 4g** and **5g** as representatives of the anti-inflammatory active compounds (ester, carboxylic acid and hydroxamic acid) and indomethacin as reference drug was examined under scan*

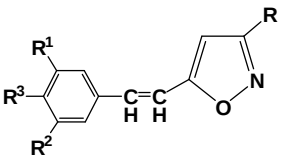
Received in 13/4/2004 & Accepted in 20/5/2004

*Corresponding author, Tel: +20-88-303006, Fax: +20-88-33-2776, E. mail: atef@acc.aun.edu.eg

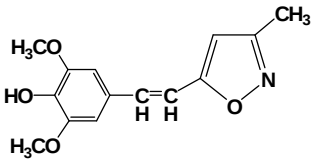
*electron microscope, after 24 hours of administration of single dose (100 mg/kg) in rats. Significant anti-inflammatory activity was displayed by most of the target derivatives. p-Nitro and p-methoxystyryl isoxazole carboxylic acid derivatives (**4e** and **4f**) revealed 75% inhibition of inflammation after 1 hr reflected the rapid onset of action of these derivatives, which may sustained up to 5 hr as in case of **4e**. The anti-inflammatory activity of 5-(p-nitro and p-dimethylaminostyryl)-isoxazole-3-carboxylic acid ethyl esters (**3e** and **3g**) being favorably comparable with indomethacin in terms of potency and ulcerogenic liability. The rational behind the synthesis of these compounds and the structure activity relationship are discussed.*

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used therapeutics, primarily for the treatment of pain and inflammation especially arthritis.[1] The Inhibition of cyclooxygenase (COX) is a hallomark feature of virtually all marketed NSAIDs currently in wide use for the treatment of rheumatoid arthritis and osteoarthritis.[2] However, NSAIDs possess certain types of mechanism-based side effects including GIT ulceration and nephrotoxicity.[3] Hence, the discovery of novel dual inhibitors of COX and 5-LO appears to be a fruitful approach towards the identification of safer second-generation NSAIDs. For a long time the presence of an acidic moiety was thought to be a pre-requisite for the classical NSAIDs. Today we know that this structural feature accounts for the formation of a salt bridge with the ARG 120 at the bottom of cyclooxygenase enzyme thus generating the COX-1 inhibiting activity.[1] Depending on their chemical structure, NSAIDs inhibit both COX-1 and COX-2 to different extents. This accounts for their anti-inflammatory and analgesic activities and their unwanted GI side effects.[1] On the other hand, hydroxamic acids were well known to form strong complexes with a variety of transition metals. This property has been exploited in the use of hydroxamates as inhibitors of several metalloenzymes.[4] Since it is generally believed that 5-lipooxygenase contains a catalytically important iron atom,[5] this enzyme is a logical candidate for inhibition by hydroxamic acid containing molecules. Styrylisoxazole derivatives (**I**) were synthesized and studied for the anti-inflammatory activity. These compounds possess anti-inflammatory activity, by dual mechanism through inhibition of 5-lipooxygenase and cyclooxygenase enzymes. Structure activity relationship study of these derivatives revealed that the styryl double bond is essential for 5-lipooxygenase inhibitory activities. Electron donating substituents on the phenyl ring lead to increase of 5-lipooxygenase-inhibition. Compound (**Ia**) was the most active one of these derivatives, it inhibits carrageenan-induced edema by 37% at 20 mg/kg po.[6] Guided by the aforementioned reports focusing on isoxazole derivatives as anti-inflammatory and the fact that isoxazole nucleus was incorporated in classical NSAIDs (isoxicam),[7] selective COX-2 inhibitors (valdecoxib),[8] dual COX/5LO inhibitors and immunomodulatory agents (Leflunomide).[9] The present work reports the design, synthesis and anti-inflammatory screening of some 5-substitutedstyryl-isoxazole-3-carboxylic acid derivatives in ongoing efforts to develop a new potent and selective anti-inflammatory candidate with minimal GIT side effects.



(I)



(Ia)

R= Me, CF_3
R^1, R^2= H, MeO, CH_3, Cl, Br, *tert*-Bu, *iso*-propyl
R^3= H, OH

MATERIALS AND METHODS

Chemistry

Melting points were determined on electrothermal melting point apparatus and were uncorrected. Precoated Silica gel plates (Kiesel gel 0.25 mm, 60G F_{254}, Merck, Germany) were used for thin layer chromatography (TLC). IR spectra were recorded as KBr discs on a Shimadzu IR 200-91527 spectrophotometer at the Faculty of Pharmacy, Assiut University, Assiut, Egypt. ^{1}H-NMR spectra were measured on Varian Em-

360L NMR Spectrophotometer (60 MHz) (Varian, USA) at Faculty of Pharmacy, Assiut University, Assiut, Egypt; JNM-LA400 FT NMR system (400 MHz) (JEOL Co., Tokyo, Japan) at the central laboratory, Assiut University, Assiut, Egypt and a JNM-GX 400 FT NMR spectrophotometer (JEOL Co., Tokyo, Japan) at the Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, Toyama, Japan. Chemical shifts were given in δ ppm relative to tetramethylsilane (TMS). Elemental micro-analyses were performed on Perkin-Elmer, 240 elemental analyzer, at the central laboratory, Assiut University, Perkin-EIMER 2400 elemental analyzer at the central laboratory, Cairo University. Anti-inflammatory activity was performed at the Department of Pharmacology, Faculty of Medicine, Assiut University, Assiut, Egypt. The stomach investigation was detected with a JEOL, JSM-4500LV Scanning Electron Microscope at Central Laboratory, Assiut University, Assiut, Egypt. Indocid® capsule was donated by kahira Pharm. and Chem. Ind. Co., Cairo, Egypt. All chemicals and solvents are of reagent grades. Adult male albino rats were obtained from animal house of the faculty of Medicine, Assiut University.

Synthesis of ethyl 2,4-dioxo-6-arylhex-5-enoate derivatives (2a-g)[10]

The appropriate chalcone derivative (**1a-g**, 0.1 mole) was added portion wise to an ice-cooled suspension of sodium ethoxide (6.8 g, 0.1 mole) in dry n-hexane (250 mL). Dithyl oxalate (14.6 g, 0.1 mole) was then added dropwise to the resulting mixture and the mixture was further stirred at room temperature overnight. The separated product obtained after acidified with dilute sulphuric acid was filtered and recrystallized from ethanol. The physical and spectral data are listed in Table 1.

Synthesis of 5-(*p*-substitutedstyryl)-isoxazole-3-carboxylic acid ethyl esters (3a-g)[10]

Glacial acetic acid (100 mL) was added to solution of appropriate ethyl hex-5-enoate derivative (**2a-g**, 0.0033 mole) in ethanol (100 mL). A mixture of hydroxylamine hydrochloride (0.5622 g, 0.0081 mole), and sodium acetate (0.5622 g, 0.0068 mole) in water was added to the former one. The reaction mixture was refluxed for 2.25-4 hours, cooled to room temperature, extracted with chloroform, washed with water and dried with anhydrous sodium sulfate. Chloroform was removed under reduced pressure and the residue was crystallized from ethanol. The physical and spectral data are listed in Table 1.

Synthesis of 5-(*p*-substitutedstyryl) - isoxazole-3-carboxylic acids (4a-f)[11]

Hydrochloric acid (30 mL) was added to the appropriate ethyl ester (**3a-f**, 0.0023 mole) dissolved in glacial acetic acid (20 mL). The mixture was refluxed for 1.5-5 hours and cooled to room temperature. The crystalline product, separated by filtration or extracted with chloroform/ether (60:40) and the organic layer was evaporated under reduced pressure, was recrystallized from suitable solvent. The physical and spectral data are listed in Table 1.

Synthesis of 5-(*p*-dimethylaminostyryl)-isoxazole-3-carboxylic acid (4g) [11]

The ethyl ester **3g** (4.0 g, 0.014 mole) was refluxed in hydrochloric acid (30 mL) for two hours. The reaction mixture was allowed to cool to room temperature. The separated crystalline product was filtered, washed with water and recrystallized from ethanol. The physical and spectral data are listed in Table 1.

Synthesis of 5-(*p*-substitutedstyryl)-isoxazole-3-hydroxamic acids (5a-g)[12]

The appropriate ethyl ester (**3a-g**, 0.004 mole) was refluxed in methanolic solution of hydroxylamine [prepared by neutralizing a solution of hydroxylamine hydrochloride (1.5 g, 0.022 mole) in methanol with a solution of potassium hydroxide (1.4 g, 0.025 mole) in methanol] for 4 hours. The reaction mixture was concentrated under reduced pressure and the separated product was filtered and washed with water. The dried product was recrystallized from an appropriate solvent. The physical and spectral data are listed in Table 1.

Table 1: The physical, IR and ^{1}H-NMR data of the newly synthesized derivatives **(2b,d; 3b,d; 4b-g; 5a-g; 6-29)**.

Compd. no.	Yield (%)	M.P./°C (Crystallization Solvent)	IR (KBr discs) cm^{-1}			^{1}H-NMR δ ppm Chemical shift	Microanalytical data (%) Cal./Found		
			OH	NH	C=O		C	H	N
2b[a**]	49	114-115 (Ehanol)	3430-3535	-	1722, 1631	1.5 (3H, t, *J* = 8 Hz, -CH_2CH_3); 4.4 (2H, q, *J* = 16 Hz, -CH_2CH_3); 6.7 (2H, s, -CH_2); 6.8 (H, d, *J* = 16 Hz, H-5); 7.4 (2H, d, *J* = 8 Hz, H-2`,6`); 7.8 (2H, d, *J* = 8 Hz, H-3`,5`); 7.9 (H, d, *J* = 16 Hz, H-6).	-	-	-
2d [a**]	62	120-121 (Ethanol)	3420-3555	-	1715, 1627	1.2 (3H, t, *J* = 8 Hz, -CH_2CH_3); 4.2 (2H, q, *J* = 16 Hz, -CH_2CH_3); 6.3 (2H, s, -CH_2); 6.5 (H, d, *J* = 16 Hz, H-5); 7.3 (5H, m, H-6 and 4-Br-C_6H_4).	-	-	-
3b[a**]	90	140-141 (Ethanol)	-	-	1725	1.5 (3H, t, *J* = 8 Hz, -CH_2-CH_3); 4.6 (2H, q, *J* = 14 Hz, -CH_2-CH_3); 6.9 (H, s, -C_4H); 7.1 (H, d, *J* = 16 Hz -H_2 vinyl); 7.4 - 7.9 (5H, m, -H_1vinyl and -4-F-C_6H_4).	64.36 64.64	4.63 4.73	5.36 5.34
3d [a**]	82	143-144 (Ethanol)	-	-	1727	1.4 (3H, t, *J* = 8 Hz, -CH_2-CH_3); 4.4 (2H, q, *J* = 14 Hz, -CH_2-CH_3); 6.6 (H, s, -C_4H); 6.8 (H, d, *J* = 16 Hz -H_2 vinyl); 7.2 - 7.6 (5H, m, -H_1 vinyl and 4-Br-C_6H_4).	54.22 54.88	3.9 3.75	4.5 4.33
4b[a*]	82	202-203 (Ethanol)	3140	-	1703	7.2 (H, s, -C_4H); 6.3 - 6.6 (4H, m, $H_{1,}H_2$ vinyl and H-2`,6`);7.9 (2H, d, *J* = 8 Hz, H-3`,5`).	61.80 61.66	3.46 3.70	6.01 6.03
4c[a*]	83	220-222 (Ethanol)	3140	-	1699	7.2 (H, s, -C_4H); 7.5 (H, d, *J* = 16Hz, -H_2 vinyl); 7.7 – 8.1 (5H, m, H_1 vinyl and 4-Cl-C_6H_4).	57.73 57.84	3.23 3.65	5.61 5.62
4d[a*]	82	224-226 (Ethanol)	3175	-	1701	7.2 (H, s, -C_4H); 7.5 (H, d, *J* = 16 Hz, -H_2 vinyl); 7.7 - 7.9 (5H, m, H_1 vinyl and 4-Br-C_6H_4).	49.01 48.82	2.74 2.98	4.76 4.75

Table 1: Continued

Compd. no.	Yield (%)	M.P./°C (Crystallization Solvent)	IR (KBr discs) cm^{-1}			^{1}H-NMR δ ppm Chemical shift	Microanalytical data (%) Cal./Found		
			OH	NH	C=O		C	H	N
4e[a*]	72	226-227 (Ethanol)	3450	-	1734	7.3 (H, s, -C_4H); 7.9 (2H, d, *J* = 16 Hz, H_1,H_2 vinyl); 8.2 (2H, d, *J* = 8 Hz, H-2`,6`); 8.5 (2H, d, *J* = 8 Hz, H-3`,5`).	55.39 55.25	3.10 2.71	10.76 10.67
4f[b*]	85	189-190 (Ethanol)	3140	-	1704	3.8 (3H, s, -OCH_3); 6.8 (H, s, -C_4H); 6.9 (2H, d, *J* = 8 Hz, H-3`,5`); 7.1 (H, d, *J* = 16 Hz, H_2vinyl). 7.4 (H, d, *J* = 16 Hz, H_1 vinyl); 7.6 (2H, d, *J* = 8 Hz, H-2`,6`); 7.9 (H, s,-OH).	63.67 63.07	4.52 4.28	5.71 5.67
4g[a*]	84	169-170 (Ethanol)	3260	-	1702	7.1 (H, s, -C_4H); 3.2 (6H, s, $(CH_3)_2$-N-); 7.3 - 7.6 (4H, m, H_1,H_2 vinyl and H-3`,5`); 7.9 (2H, d, *J* = 8 Hz, H-2`,6`).	65.10 64.95	5.46 5.76	10.85 10.72
5a[a*]	77	150-152 (Benzene)	3175	3410	1651	7.1 (H, s, -C_4-H); 7.5 – 7.9 (7H, m, H_1,H_2 vinyl and-C_6H_5).	62.60 62.63	4.38 4.10	12.17 12.14
5b[a*]	79	189-190 (Ethanol/acetic acid)	3275	3490	1639	7.7 - 8.7 (7H, m, -C_4H, H_1,H_2 vinyl and 4-F-C_6H_4).	58.06 57.83	3.65 3.92	11.28 10.89
5c[a*]	83	228-230 (Ethanol/acetic acid)	3140	3470	1607	7.0 (H, s, -C_4-H); 7.4 -7.9 (6H, m, H_1,H_2 vinyl and 4-Cl-C_6H_4).	54.46 54.36	3.43 3.54	10.58 10.40
5d[a*]	74	188-190 (Ethanol/acetic acid)	3215	3465	1636	7.1 (H, s, -C_4-H); 7.6 – 7.9 (6H, m, H_1,H_2 vinyl and 4-Br-C_6H_4).	46.63 46.20	2.93 3.41	9.06 8.80
5e[a*]	83	172-174 (Ethanol/acetic acid)	3215	3450	1654	7.3 (H, s, -C_4-H); 7.8 - 8.4 (6H, m, H_1,H_2 vinyl and 4-NO_2-C_6H_4).	52.37 51.80	3.30 3.47	15.26 15.39
5f[b*]	84	192-194 (Ethanol)	3285	3470	1610	3.9 (3H, s, -OCH_3); 6.7 (H, s, -C_4H); 7.1 (H, d, *J* = 16 Hz, -H_2); 7.2 (2H, d, *J* = 8 Hz, -H3`,5`); 7.3 (H, d, *J* = 16 Hz, -H_1); 7.6 (2H, d, *J* = 8 Hz, H2`,6`).	59.99 59.78	4.65 4.20	10.76 10.28
5g[a*]	79	>300 (Ethanol/acetic acid)	3155	3450	1669	3 (6H, s, -$N(CH_3)_2$); 6.8 (H, s, -C_4-H); 7.3 - 7.9 (6H, m, H_1,H_2 vinyl and 4-$N(CH_3)_2$ -C_6H_4).	61.53 61.69	5.53 5.80	15.37 14.95

Table 1: Continued

Compd. no.	Yield (%)	M.P./°C (Crystallization Solvent)	IR (KBr discs) cm^{-1}			^{1}H-NMR δ ppm Chemical shift	Microanalytical data (%) Cal./Found		
			OH	NH	C=O		C	H	N
6[a*]	73	163-164 (Ethanol)	-	3330	1653	2.9 (3H, d, *J* = 6 Hz, -CH_3); 7.2 (H, s, -C_4H); 7.7 - 8.1 (7H, m, -CH=CH and aromatic protons); 9.0 (H, q, *J* = 6 Hz, -NH).	68.40 68.40	5.29 4.98	12.27 12.21
7[a*]	80	210-211 (Benzene)	-	3360	1678	3.0 (3H, d, *J* = 5 Hz, -CH_3); 7.2 (H, s, -C_4H); 7.6 - 8.1 (6H, m, -CH=CH and aromatic protons); 9.0 (H, q, *J* = 5 Hz, -NH).	59.44 59.20	4.22 4.27	10.66 10.63
8[a*]	57	259-260 (Ethanol/dimethylformamide)	-	3350	1665	3.0 (3H, d, *J* = 4 Hz, -CH_3); 7.4 (H, s, -C_4H); 8.0 - 8.8 (6H, m, -CH=CH and aromatic protons); 9.1 (H, q, *J* = 4 Hz, -NH).	57.14 56.55	4.05 4.32	15.38 15.36
9[a*]	68	133-135 (Ethanol)	-	3350	1668	2.9 (3H, d, *J* = 4 Hz, -CH_3); 4 (3H, s, -OCH_3); 7.1 (H, s, -C_4-H); 7.2 - 7.9 (6H, m, -CH=CH and aromatic protons); 9.0 (H, q, *J* = 4 Hz, -NH).	65.11 64.26	5.46 5.65	10.85 10.81
10[a*]	57	229-230 (Ethanol/dimethylformamide)	-	3335	1680	7.3 (H, s, -C_4-H); 7.5 - 8.2 (11H, m, -CH=CH and aromatic protons); 11.1 (H, s, -NH).	66.57 66.48	4.03 4.54	8.62 8.66
11[a*]	73	258-260 (Ethanol/dimethylformamide)	-	3330	1658	7.4 (H, s, -C_4-H); 7.5 - 8.7 (10H, m, -CH=CH and aromatic protons); 11.2 (H, s, -NH).	64.47 64.18	3.91 4.39	12.53 12.54
12[a*]	78	185-186 (Ethanol/dimethylformamide)	-	3415	1670	4.0 (3H, s, -OCH_3); 7.1 (H, s, -C_4-H); 7.2 - 8.3 (11H, m, -CH=CH and aromatic protons); 11.1 (H, s, -NH).	71.24 71.12	5.03 5.57	8.74 8.65
13[a*]	60	202-203 (Ethanol)	-	3325	1680	7.1 (H, s, -C_4-H); 7.2 - 8.1 (11H, m, -CH=CH and aromatic protons); 11.2 (H, s, -NH).	70.12 69.73	4.25 4.25	9.09 9.09

Table 1: Continued

Compd. no.	Yield (%)	M.P./°C (Crystallization Solvent)	IR (KBr discs) cm^{-1}			^{1}H-NMR δ ppm Chemical shift	Microanalytical data (%) Cal./Found		
			OH	NH	C=O		C	H	N
14[a*]	63	224-226 (Ethanol)	-	3340	1656	7.2 (H, s, -C_4-H); 7.3 - 8.3 (10H, m, -CH=CH and aromatic protons); 11.2 (H, s, -NH).	63.08 62.94	3.53 3.93	8.17 8.22
15[a*]	72	150-152 (Ethanol/dimethylformamide)	-	3390	1662	7.4 (H, s, -C_4-H); 7.5 - 8.7 (10H, m, -CH=CH and aromatic protons); 11.3 (H, s, -NH)	61.19 61.19	3.42 3.49	11.89 11.96
16[a*]	63	240-241 (Ethanol)	-	3390	1675	4.0 (3H, s, -OCH_3); 7.1 (H, s, -C_4-H); 7.2 - 8.2 (10H, m, -CH=CH and aromatic protons); 11.2 (H, s, -NH).	67.45 67.10	4.47 4.53	8.28 8.34
17[a*]	71	212-214 (Ethanol/dimethylformamide)	-	3335	1682	7.4 (H, s, -C_4H); 7.7 - 8.3 (11H, m, -CH=CH and aromatic protons); 11.3 (H, s, -NH).	66.57 66.34	4.03 4.40	8.63 8.64
18[a*]	69	225-227 (Benzene/dimethylformamide)	-	3390	1681	7.4 (H, s, -C_4H); 7.6-8.3 (10H, m, -CH=CH and aromatic protons); 11.2 (H, s, -NH).	60.19 60.23	3.37 3.76	7.80 7.84
19[a*]	67	258-260 (Ethanol/dimethylformamide)	-	3340	1662	7.4 (H, s, -C_4H); 7.5-8.6 (10H, m, -CH=CH and aromatic protons); 11.1 (H, s, -NH).	58.47 58.15	3.27 3.56	11.36 11.32
20[a*]	64	204-206 (Ethanol/dimethylformamide)	-	3420	1676	3.9 (3H, s, -OCH_3); 7.1 (H, s, -C_4-H); 7.2 - 8.4 (10H, m, -CH=CH and aromatic protons); 11.5 (H, s, -NH)	64.32 64.18	4.26 4.72	7.89 7.77
21[a*]	57	265-266 (Ethanol/dimethylformamide)	-	3500 - 3400	1670	7.4 (H, s, -C_4H); 7.6 - 8.1 (9H, m, -CH=CH and aromatic protons).	60.59 60.34	3.73 3.31	14.13 13.90
22[a*]	61	218-220 (Ethanol/dimethylformamide)	-	3550 - 3400	1661	7.6 (H, s, -C_4-H); 7.7 - 8.6 (8H, m, -CH=CH and aromatic protons); 10.5 (H, s, NH).	57.14 57.45	3.20 3.12	13.33 13.22
23[a*]	67	274-275 (Ethanol/dimethylformamide)	-	3550 - 3375	1656	7.3 (H, s, -C_4-H); 7.5 - 8.0 (8H, m, -CH=CH and aromatic protons).	54.30 54.42	3.04 2.92	12.66 12.44

Table 1: Continued

Compd. no.	Yield (%)	M.P./°C (Crystallization Solvent)	IR (KBr discs) cm^{-1}			^{1}H-NMR δ ppm Chemical shift	Microanalytical data (%) Cal./Found		
			OH	NH	C=O		C	H	N
24[a*]	65	206-208 (Ethanol/dimethylformamide)	-	3550 - 3480	1667	7.2 (H, s, -C_4H); 7.6 - 8.6 (8H, m, -CH=CH and aromatic protons).	52.63 52.97	2.94 2.78	16.37 16.14
25[a*]	63	201-202 (Ethanol)	-	3510 - 3390	1672	4.0 (3H, s, -OCH_3); 7.0 - 8.8 (9H, m, -CH=CH and aromatic protons).	58.70 58.83	4.0 3.75	12.84 12.98
26[a*]	61	150-152 (Benzene/dimethylformamide	-	3310	1648	1.1-2.0 (11H, m, -C_6H_{11}); 7.0 (H, s, -C_4H); 7.5-7.9 (7H, m, -CH=CH and aromatic protons); 8.3 (H, d, *J* = 6 Hz, -NH).	72.95 72.69	6.8 6.58	9.45 9.49
27[a*]	66	222-224 (Benzene)	-	3315	1641	1.2-2.0 (11H, m, -C_6H_{11}); 7.0 (H, s, -C_4H); 7.3-7.8 (6H, m, -CH=CH and aromatic protons); 8.3 (H, d, *J* = 7 Hz, -NH).	65.35 65.07	5.79 6.51	8.47 8.48
28[a*]	68	249-250 (Ethanol/dimethylformamide)	-	3315	1645	1.2-1.9 (11H, m, -C_6H_{11}); 7.1 (H, s, -C_4H); 7.6-8.4 (6H, m, -CH=CH and aromatic protons); 8.5 (H, d, *J* = 5 Hz, -NH).	63.33 63.32	5.61 5.50	12.31 12.32
29[a*]	61	216-217 (Benzene)	-	3320	1646	1.4-1.9 (11H, m, -C_6H_{11}); 4 (3H, s , -OCH_3); 7.1 (H, s, -C_4-H) 7.2 - 8.0 (6H, m, -CH=CH and aromatic protons); 8.7 (H, d, *J* = 7 Hz, -NH).	69.91 69.90	6.79 6.67	8.58 8.46

a- 60 MHz b- 400 MHz *DMSO as solvent **$CDCl_3$ as solvent.

General procedure for synthesis of 5-(*p*-substitutedstyryl)-isoxazole-3-carboxamides (6-29)

The appropriate acid (0.002 mole) was refluxed for 3 hours in excess thionyl chloride (30 mL). The corresponding amine (0.005 mole) in benzene was added dropwise to the residue obtained after evaporation of excess thionyl chloride under reduced pressure. The mixture was refluxed for 2-4 hours and the separated product obtained after concentration under reduced pressure was filtered, washed with dilute hydrochloric acid followed by water, dried and recrystallized from a suitable solvent. The physical and spectral data are listed in Table 1.

Biological investigations

1- Anti-inflammatory activity

The anti-inflammatory activity of twenty-one representatives of synthesized compounds [(**3a,e,f** and **g**); (**4a-g**); (**5a-g**); **22**; **24** and **25**] was evaluated in rats by the carrageenan-induced edema method as described by Nargund *et al.*[13] in comparison to indomethacin as a reference drug. Adult male albino rats weighing (120-150 g) were divided into groups, each of four animals. Solution or suspension of the test compound or reference drug in 6% tween 80 was administrated orally into rats at dose level of 100 mg/kg. One group of animals was used for each treatment. Control animals were similarly treated with 6% tween 80. After 30 minutes 0.1 mL of freshly prepared 1% carrageenan solution in normal saline was injected into the subplantar region of the right hind paw. The right paw volume was measured by a Veriner caliper (SMIEC) directly before and at one hour intervals after administration of the test compound for five hours. The anti-inflammatory activity of the tested compound and reference drug was determined with the following formula

% Inhibition of inflammation= $(1- V_t/V_o) \times 100$

where V_t, V_o represents the mean increase in paw volume in rats treated with tested compounds, control rats respectively.

The results of anti-inflammatory evaluation of the test compounds and reference drug are listed in Table 2.

2- Ulcerogenicity[14]

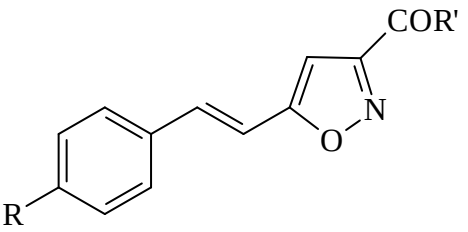
On the basis of preliminary results, three compounds (**3g, 4g, 5g**) were subjected to this investigation. Male adult albino rats (120-150 g) were divided into groups each of four animals. Animals were starved but had free access to water 24 hours prior to administration of drug. The animals were then treated orally by mean of stomach tube with solution or suspension of tested compounds and indomethacin as reference drug in tween 80 (6%) at a dose level 100 mg/kg. Control animals were treated with an equal volume of tween 80. Food was withdrawn from all groups until 24 hours after administration of drug. The rats were then sacrificed, so that the stomach could be removed, open along the greater curvature and clean gently by dipping in saline. Randomly selected specimens were then taken and prepared for scanning in an electron microscope. Specimens were fixed by soaking in glutaraldehyde solution (5% in cacodylate buffer; pH 7.2) for 24 hours followed by three washing each for 20 minutes with cacodylate buffer. The specimens then treated with osmium tetraoxide (1% solution) for 2 hours and washed with cacodylate buffer as shown above. The specimens were then subjected to dehydration by treatment for 30 minutes with each of 30%, 50% and 70% ethanolic solution followed by 90% ethanol for one hour and finally in absolute ethanol for two days. After discharge of alcohol the specimens were soaked in amyl acetate solution for two days, dried under reduced pressure, mounted on holder and coated for scanning in a scanning electron microscope (SEM).

RESULTS AND DISCUSSION

Chemistry

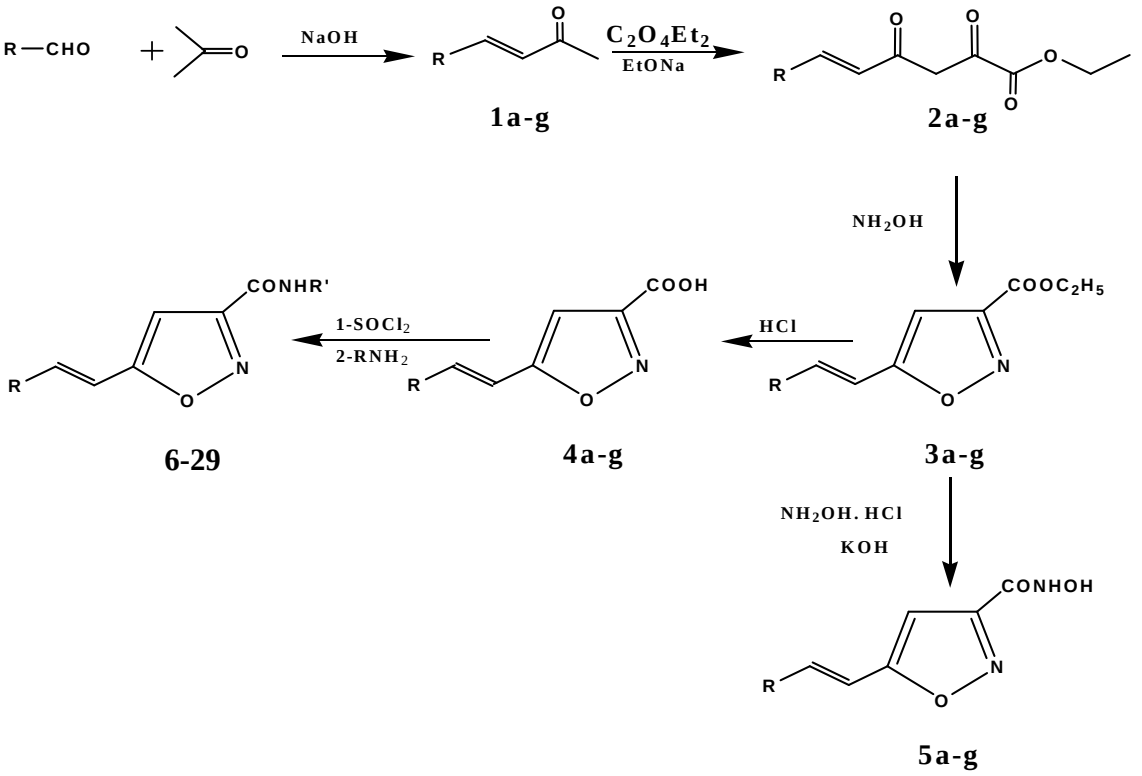
The synthetic pathways for the preparation of the target derivatives are outlined in Scheme 1. In the present work, the 3+2 route has been adopted for synthesis of the targeted isoxazole derivatives. The required three atomic component synthons were represented by 1,3-dicarbonyl system substituted by a styryl moiety which is intended to be located at C-5 of the isoxazole nucleus. Meanwhile, the planned carboxyl functionality at C-3 of the ring was introduced to the 1,3-dicarbonyl structures through interaction with diethyloxalate. The 1,3-dicarbonyl systems [2,4-dioxo-6-arylhex-5-enoic acid ethyl esters (**2a-g**)] acts as key

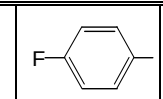
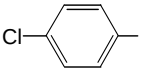
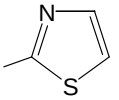
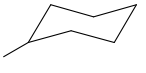
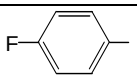
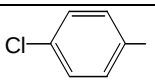
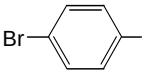
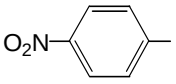
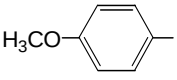
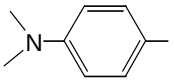
Table 2: Anti-inflammatory activity of 5-[2-(p-substitiutedphenyl)vinyl]isoxazole-3-carboxylic acid derivatives (**3b, 3e, 3f, 3g, 4a-g, 5a-g, 22, 24** and **25).**



Compd. No.	R	R'	Anti-inflammatory activity (100 mg/Kg, p.o) %Inhibition*				
			1 hr	2 hr	3 hr	4 hr	5 hr
Indomethacin			62.5	75	75	77.7	88.8
3b	F	OEt	37	50	55	68	72
3e	NO_2	OEt	37	66	88	90	90
3f	OCH_3	OEt	43	44	55	63	68
3g	$N(CH_3)_2$	OEt	50	61	72	96	90
4a	H	OH	61.25	68.75	37.5	33	33.3
4b	F	OH	75	75	75	72.5	81.5
4c	Cl	OH	50	50	37.5	44.4	44.4
4d	Br	OH	62.5	50	37.5	55.5	55.5
4e	NO_2	OH	75	62.5	62.5	66.6	55.5
4f	OCH_3	OH	75	25	33.3	22	22.2
4g	$N(CH3)_2$	OH	62.5	75	75	77.7	77.7
5a	H	NHOH	33.3	50	37.25	55.5	66.6
5b	F	NHOH	62.25	62.5	62.5	22.2	22.2
5c	Cl	NHOH	31.25	31.25	43.75	27.7	38.8
5d	Br	NHOH	50	50	37.5	44.4	44.4
5e	NO_2	NHOH	50	37.5	50	55.5	55.5
5f	OCH_3	NHOH	75	62.5	62.5	73.3	73.3
5g	$N(CH_3)_2$	NHOH	62.5	75	75	66.6	66.6
22	F	NH-thiazolyl	25	33	33	63	54.5
24	NO_2	NH-thiazolyl	37.5	44	44	54.5	36
25	OCH_3	NH-thiazolyl	12.5	44	44	54.5	63

*All results are significantly different from control at $p < 0.005$.



R \ R'	CH_3					
a 	**6**		**13**	**17**	**21**	**26**
b 					**22**	
c 	**7**	**10**	**14**	**18**	**23**	**27**
d 						
e 	**8**	**11**	**15**	**19**	**24**	**28**
f 	**9**	**12**	**16**	**20**	**25**	**29**
g 						

Scheme 1: Pathway for the synthesis of the target compounds

intermediate for synthesis of the targeted 5-(substituted) isoxazole-3-carboxylic acid derivatives. The synthesis of these intermediate was achieved through the reaction of chalcones (**1a-g**), prepared via aldol condensation of the respective substituted benzaldehyde with acetone in alkaline medium, with diethyl oxalate. The 2,4-diketoesters **2a-h** were condensed with hydroxylamine hydrochloride in presence of acetic acid/sodium acetate, to afford mainly the targeted 5-(*p*-substitutedstyryl)-isoxazole-3-carboxylic acid esters 3a-h in good yields. The regiospecificity of this reaction could be explained on basis of reported observations.[15] It is evident that the ethoxycarbonyl moiety enhances the electrophilicity of the neighboring C-atom of the 2,4-diketoester and render it preferentially susceptible for attack by NH_2OH. Moreover, control of the pH of the reaction through the use acetic acid/sodium acetate buffer (pH 4) instead of acetic acid only renders hydroxylamine in the H_2NOH form. In this case the nitrogen atom is more nucleophilic and will attack the most electrophilic C-atom (C_2=O). The suggested sequence of the condensation-cyclization reaction involved in the formation of the targeted 5-(*p*-substitutedstyryl)-isoxazole-3-carboxylic acid esters **3a-h** can be illustrated by Chart 1.

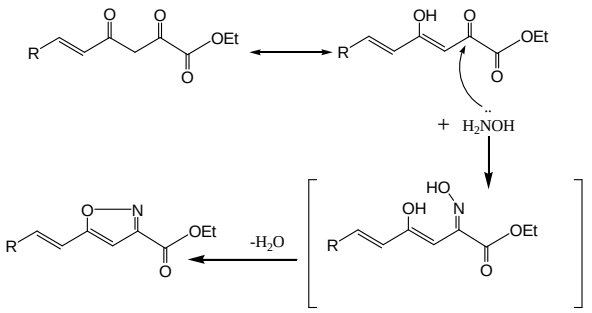


Chart 1

The resulting 5-(*p*-substitutedstyryl)-isoxazole-3-carboxylic acid esters **3a-h** were then converted to the corresponding acids **4a-h** by boiling with hydrochloric acid.[16] In this work dissolution of the isoxazole esters **3a-h** in acetic acid followed by boiling in hydrochloric acid for 2 hours afforded the isoxazole carboxylic acids **4a-h** in good yields. The physical and spectral data of the prepared compounds are agreed with reported data and that for the newly synthesized derivatives are mentioned in Table 1. The purity of the synthesized derivatives was monitored by TLC and by using microanalysis for the newly synthesized targets in addition to that compounds subjected to anti-inflammatory evaluation. The IR spectra of the esters **3a-h** revealed the characteristic C=O at δ 1730-1710 cm^{-1}, the vinyl C=C at δ 1648-1621cm^{-1} and the ester C-O-C at δ 1293-1201cm^{-1}. The ^{1}H-NMR spectral data of the esters revealed the characteristic singlet at δ 6.55-6.90 ppm that could be assigned to C_4-H of the isoxazole nucleus. This assignment is based on comparison to reported value of similar compounds.[17-20] On other hand the *J* constant value of the vinyl protons support the suggested trans configuration of these protons. The vinyl and aryl protons revealed similar splitting and chemical shift as hexenoates **2a-h**. Further structural elucidation of the formed isoxazole derivatives has been achieved through ^{13}C-NMR spectroscopy **3g** as representative of this series. The spectrum revealed the characteristic chemical shifts of isoxazole C-atoms, δ C-4 (99.73 ppm), δ C-5 (156.57 ppm), δ C-3 (160.26 ppm). In addition to ester δ C=O (171.47 ppm); the CH_2CH_3 signals at δ= 14.14 and 61.98 respectively; dimethylaminocarbons (δ= 40.15). The vinyl C-atoms appeared at δ= 107.46 for C_1, δ = 136.46 ppm for C_2. The phenyl C-atoms appeared at δ= 112.02 ppm for $C_{2`,6`}$, at δ= 128.69 ppm for $C_{3`,5`}$. The assignment of the observed chemical shifts to the respective C-atoms is based on comparison with reported value for the isoxazole nucleus,[21] as well as other related compounds such as ethyl 5-[2-(2-pyridyl)vinyl]isoxazole-3-carboxylate.[20] The mass spectra of the compound **3g** was agreed with the fact that isoxazole fragmented via formation azirine intermediate, revealed the molecular ion beak at m/z 286, and M^{-1} at m/z 285 (base peak).

The economical procedure for preparation of hydroxamic acid derivatives is the reaction of hydroxylamine hydrochloride with acid chloride or esters. In this work, the isoxazole hydroxamic acid derivatives (**5a-g**) were prepared, according to scheme 1 taking the general procedure as a guide, by refluxing the appropriate ester (**3a-g**) with hydroxylamine in slightly alkaline pH for 3-4 hours resulted in hydroxamic acids (**5a-g**) in a good yield. The reaction of esters (**3a-g**) with amines were failed to give amide and the ester was recovered unchanged where the amide

derivatives **6-29** were prepared from acid chloride. Reaction of acid chloride with solution or suspension of appropriate amine in benzene was give amide (**6-29**) in good yield. The synthesized derivatives were characterized through elemental analyses and their structures were confirmed by spectral data.

Biological investigations

5-(*p*-Substitutedstyryl)-isoxazole-3-carboxylic acid (**4a-g**), hydroxamic acid (**5a-h**), carboxylic acid ethyl ester (**3b,e-g**) and carboxylic acid amide (**22, 24, 25**) derivatives were tested for their anti-inflammatory activity. The anti-inflammatory marker was the percentage inhibition of carrageenan-induced rat paw edema. The tested compounds (100 mg/kg) were given orally, and then half an hour later, an edema was induced by subcutaneous injection of carrageenan into paw pad of the rat. Table 2 shows the results (% inhibition) of anti-inflammatory activity of the tested compounds at one-hour time interval for five hours. p-Flourophenyl and p-dimethylaminostyryl isoxazole carboxylic acid derivatives (**4b** and **4g**) showed maximum inhibition of inflammation ranging from 62~81.5%. The inhibitory effect of inflammation of **4b** and **4g** starts after 1hr and maintained or slightly increased up to 5 hr. On the other hand, p-nitrophenyl and p-methoxystyryl isoxazole carboxylic acid derivatives (**4e** and **4f**) revealed 75% inhibition of inflammation after 1 hr, which by time slightly decreased, as in case of **4e**, or dramatically decreased, as in case of **4f**. Other carboxylic acid derivatives (**4a, 4c** and **4d**) showed significant inhibitory activity (33~68%) but less than reference drug, indomethacin (62~88.8) (Fig. 1).

In the group of isoxazole hydroxamic acid derivatives (**5a-g**), p-methoxyphenylderivative (**5f**) revealed 75% inhibition of inflammation after 1hr, while other hydroxamic acid derivatives (**5a-e** and **5g**) showed inferior activity than indomethacin (Fig. 2).

Maximum inhibitory activity of inflammation was observed in isoxazole carboxylic acid ethyl esters (**3e** and **3g**) after 3 hr (72~96%) (Fig. 3). On the other hand, the anti-inflammatory activity was dramatically decreased in case of isoxazole carboxylic acid amide derivatives (**22, 24, 25**) (Fig. 4).

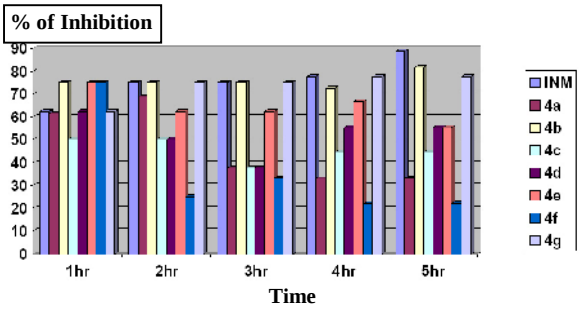


Fig. 1: Anti-inflammatory activity of 5-(p-substituted styryl)isoxazole-3-carboxylic acids **4a-g**.

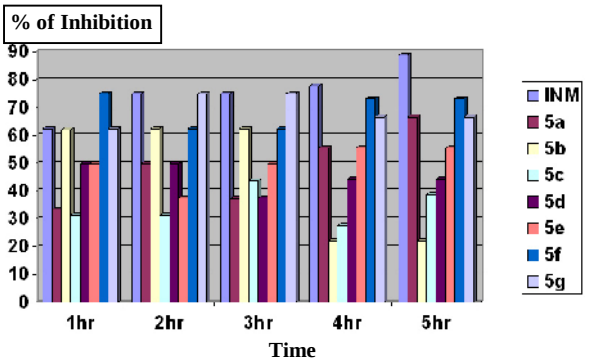


Fig. 2: Anti-inflammatory activity of 5-(p-substituted styryl)isoxazole-3-hydroxamic acids **5a-g**.

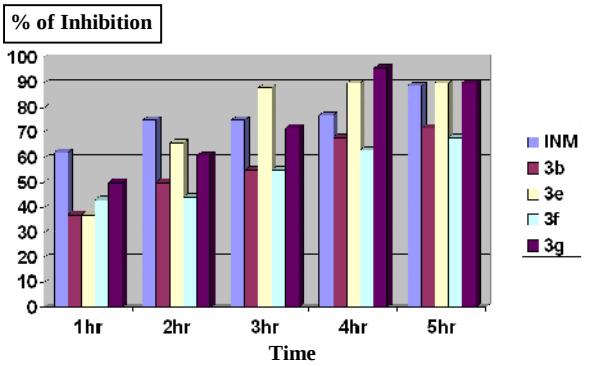


Fig. 3: Anti-inflammatory activity of 5-(p-substituted styryl)isoxazole-3-carboxylic acid ethyl esters **3b, 3e, 3f, 3g**.

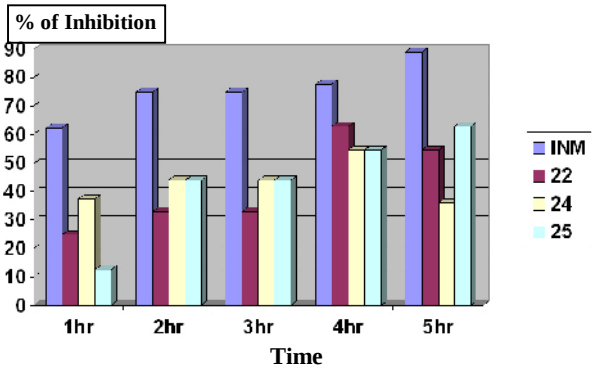


Fig. 4: Anti-inflammatory activity of 5-(p-substituted styryl)isoxazole-3-(N-substituted)carbox-amides **22, 24** and **25**.

Evaluation of the anti-inflammatory activity of p-flouoro, p-nitro and p-methoxy isoxazole carboxylic acid (**4b,e** and **f**), hydroxamic acid (**5b,e** and **f**), carboxylic acid ethyl ester (**3b,e** and **f**) and carboxylic acid amide were performed to explore the effects of derivatization of the carboxylate moiety of the target isoxazole derivatives (Fig. 5).

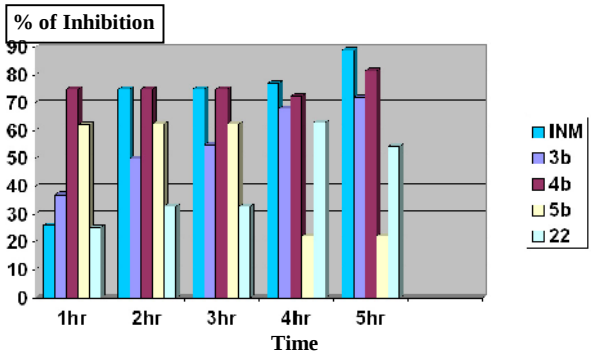


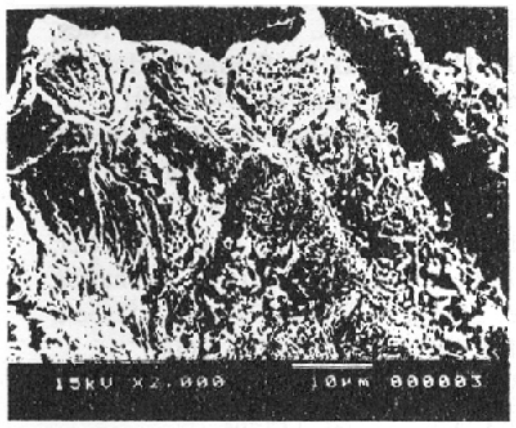
Fig. 5: Anti-inflammatory activity of 5-(p-flourostyryl)isoxazole derivatives **3b, 4b, 5b** and **22**.

Higher inhibitory activity of inflammation was noticed to isoxazole carboxylic acids (**4b** and **e**) followed by isoxazole hydroxamic acids (**5b** and **e**), while minimum inhibitory activity of inflammation was observed to isoxazole carboxylic acid amide derivatives (**22, 24** and **25**) at all time intervals. At 4 and 5 hr time intervals, 5-(*p*-nitrostyryl)-isoxazole-3-carboxylic acid ethyl ester (**3e**) exhibited higher activity than their corresponding carboxylic acid derivatives (**4e, 5e** and **24**).

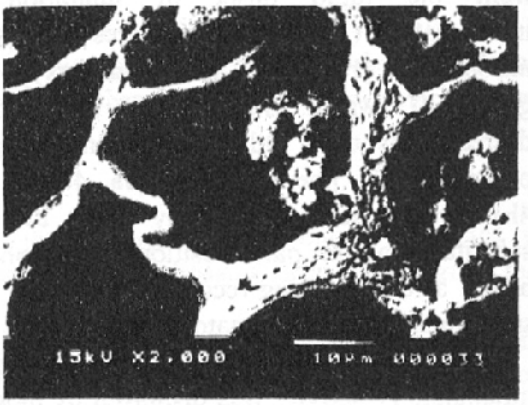
The carboxylate moiety in this series of compounds (**4b,e,f**) was transformed into carboxylic acid ethyl ester (**3b,e** and **f**), hydroxamic acids (**5b,e** and **f**) and carboxylic acid amides (**22, 24** and **25**), since a chemical derivatizations (amidation or esterification) of the carboxylate moiety in NSAIDs generates an impressive array of potent and highly selective COX-2 inhibitors. The inhibition of gastric-lesions is the indicator about such activity. Generally, isoxazole carboxylic acid derivatives showed higher activity than carboxylic acid amide derivatives, which revealed the lowest activity while hydroxamic acid derivatives were noticed in between.

Examination of The stomach specimens of the treated experimental animals under scanning electron microscope afforded a highly precise, accurate and self-explanatory description of the effects of the investigated compounds on the protective as well as the mucosal layer of the gastrointestinal tract. The ulceroginicity of the compounds **3g, 4g** and **5g** as representatives of the anti-inflammatory active compounds (ester, carboxylic acid and hydroxamic acid) and indomethacin as reference drug were examined under scan electron microscope, after 24 hours of administration of single dose (100 mg/kg) in rats. Fig. 6 illustrate scanning electro-micrographs for the stomach specimens of the rats treated with a single dose of the compounds **3g** (Fig. 6D), **4g** (Fig. 6C), **5g** (Fig. 6E), indomethacin (Fig. 6B) and control (Fig. 6A). As showed in Fig. 1B the indomethacin treated animals were characterized by complete damage of the mucous layer beside ulceration of submucosal cells. The compound **4g** treated animals showed damage of the mucosal layer but to les extent than that with indomethacin. The animals treated with hydroxamic acid (**5g**) showed the least damage of the mucosal layer. This indicates that the acid derivatives have the highest ulcerogenic effect among the tested compounds, due to the direct action of acid on mucosal, the hydroxamic acid is the least one. On the other hand, isoxazole carboxylic acid ethyl ester (**3g**) showed a matched or higher potency relative to indomethacin with a lack of ulcerogenic effect relative to indomethacin and acid derivative **4g**.

The chemical modification of the substituents on the para position of the phenyl moiety attached to the vinyl group were designed to study the effect of variation of these substituents on the electronic and the physicochemical properties of whole molecule and subsequently the anti-inflammatory activity. From the results obtained, isoxazole carboxylic acids derivatives bearing p-flourophenyl group (**4b**) and p-dimethylaminophenyl (**4g**) showed maximum inhibition of inflammation after one hour and persist up to 5 hr my indicate rapid absorption and resistance to metabolism of these compounds. Since p-fluoro is an electron withdrawing substituent while p-dimethylamino is an electron donating one and both maintain equipotency relative to indomethacin may reflect that the electronic properties of these substituents is not the key factor for the activity.



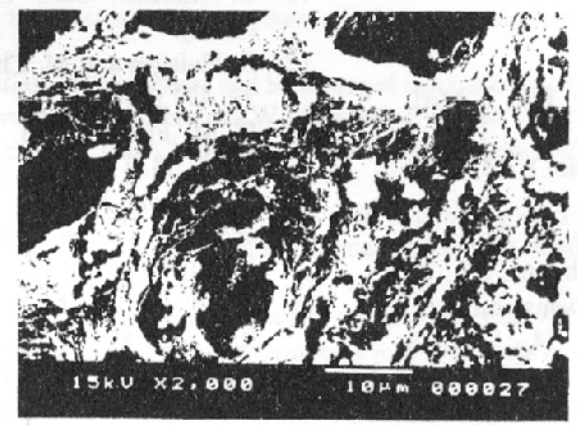
A



B

C

D



E

Fig. 6: Scanning electromicrographs of the rats stomach specimen after route dose (100 mg/kg) of: **A**: control; **B**: indomethacine; **C: 4g**; **D: 3g**; **E: 5g**.

The effects of the substituent on the para position of the phenyl moiety on the vulnerability to metabolism is clearly observed from the rapid onset of inhibition of inflammation (1 hr) for derivatives bearing p-nitrophenyl group **4e** and p-methoxyphenyl group **4f** which slightly or dramatically decreased by time. Other substituents on para position of the phenyl moiety (**4a, 4c, 4d** and **4h**) revealed less activity my prove that the substituent at the para position of the phenyl highly affect the drug-receptor interaction and subsequently the inflammatory inhibition.

In summary, a significant anti-inflammatory activity was displayed by most of the target derivatives. The anti-inflammatory activity of 5-(*p*-nitro and *p*-dimethylamino-stryryl)-isoxazole-3-carboxylic acid ethyl esters (**3e** and **3g**) being favorably comparable with indomethacin in terms of potency and ulcerogenic liability. Further structural modifications to study the structure activity relation ship for further optimization may be necessary to improve the potency and selectivity of the current derivatives. With the aim of quantification of the anti-inflammatory activity and explaining the mechanism of action of these products, further investigations are now in progress.

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