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Research article

Synthesis and anti-inflammatory activity of 2-amino-4,5-diphenyl-1-(substituted)-1*H*-pyrrole-3-carbonitrile derivatives

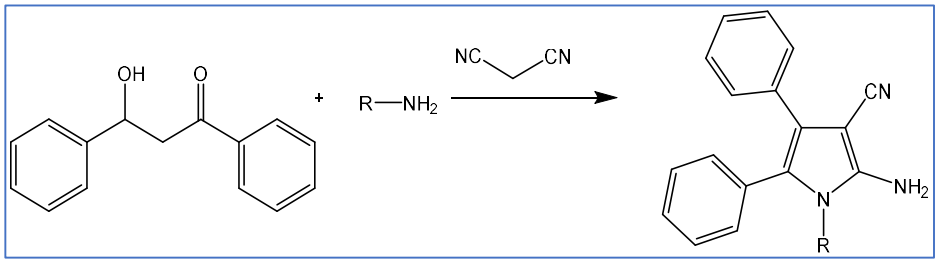
Sandip P. Dholakia*[1], Mukesh N. Kher[1], Dipen K. Sureja[1], Devang B. Sheth[1], Amitkumar J. Vyas[2]

[1] L. M. College of Pharmacy, Ahmedabad, Gujarat, India

[2] B. K Modi Pharmacy College, Rajkot, Gujarat, India

ABSTRACT

Pyrrole is privileged and active heterocycle with diverse pharmacological activities that specifically serve as a promising scaffold for anti-inflammatory, antimalarial, antimicrobial, antiviral, antitubercular, and enzyme-inhibiting drugs. In an attempt to explore this scaffold, a series of 2-amino-4,5-diphenyl-1-(substituted)-1*H*-pyrrole-3-carbonitrilewere synthesized and screened for anti-inflammatory activity. The structures of synthesized novel compounds were characterized by ^{1}H Nuclear Magnetic Resonance, Mass and Fourier Transfer Infrared spectroscopic data. All the compounds are screened for anti-inflammatory activity using the rat paw edema method. Among all, compound 1e exhibited more potent activity than the standard drug etoricoxib with the highest inhibition in paw edema at 3 h and 5 h.



Keywords: Anti-inflammatory, Etoricoxib, Pyrrole, Antiviral, Antimalarial.

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Correspondence: Sandip P Dholakia ✉ sandip.dholakia@lmcp.ac.in, **Orcid Id**: 0000-0001-7578-9067

L. M. College of Pharmacy, Ahmedabad, Gujarat, India .

INTRODUCTION

Nitrogen-containing heterocycles constitute an important class of natural and non-natural products as it plays an important role in the area of drugs and pharmaceuticals[1]. Pyrroles make considerable attention due to their synthetic and biological importance which are extensively used in drug discovery[2].Pyrrole derivatives exhibited a vital role in many pharmacological activities including anti-inflammatory[3–5], anti-microbial[6], anti-fungal[7], antiviral[8] and anti-cancer[9,10] activities. It is well known that the anti-inflammatory activity is due to the ability to inhibit the cyclooxygenase (COX) activity which mediates the production of prostaglandins from arachidonic acid. The discovery of COX-2 specific inhibitors (Coxibs), whose pharmacological properties are correlated to their capacity to reCOX-2-dependentndent prostanoid biosynthesis, provided a rationale for the development of a drug devoid of GIT disorders while maintaining clinical efficacy as an anti-inflammatory agent[11]. Prostaglandins function as mediators in the process of inflammation. The three-market removal of various coxibs, including rofecoxib (Vioxx®) and valdecoxib (Bextra®) because of their unfavourable cardiovascular side effects[12] makes it abundantly evident that other templates with COX-2 inhibitory action must be investigated and assessed. Our goal was to create NSAIDs that were derivatives of currently available, clinically utilized NSAIDs like Tolmetin and Ketorolac, well-known pyrrole compounds that operate as anti-inflammatory medicines. This research examines the synthesis of new pyrrole derivatives and assesses their anti-inflammatory potential in light of the aforementioned facts[13,14].

MATERIAL AND METHODS

Melting points of all synthesized compounds were determined in open capillaries and are uncorrected. TLC was performed on microscopic slides (2×7.5cms) coated with Silica-Gel-G and spots were visualized by exposure to iodine vapour **(Table 1).** UV

spectra were performed in methanol with double beam UV-VIS Pharm aspect 1700 Shimadzu spectrophotometer. IR spectra of all compounds were recorded in KBr (Merck) on FT-IR 8400S Shimadzu spectrophotometer. Mass spectra were performed on Shimadzu LCMS 2010 EV Mass Spectrometer. 1H NMR spectra were obtained on BRUKER Advance-II 400 MHz instrument in $CDCl_3$ as a solvent and chemical shift was measured as parts per million downfield from tetramethyl silane (TMS) as internal standard.

General method for preparation of synthesis of 2-amino-4,5-diphenyl-1-(substituted)-1*H*-pyrrole-3-carbonitrile

A mixture of benzoin (2 g, 0.01 mol), the appropriate amines [aniline (0.93 g, 0.01 mol), *o*-toluidine or *m*-toluidine or *p*-toluidine (1.17 g, 0.01 mol), or *o*-anisidine or *m*-anisidine or *p*-anisidine (1.23 g, 0.01mol), or *o*-chloroaniline, or *m*-chloroaniline, or *p*-chloroaniline (1.25 g, 0.01 mol), or *o*-nitroaniline, or *m*-nitroaniline, or *p*-nitroaniline (1.36 g, 0.01 mol)] and conc. Hydrochloric acid (8–10 drops) in toluene as solvent (50 mL) was refluxed with heating for 6 h and cooled. Malononitrile (0.66 mg, 0.01 mol) was added, followed by the addition of sodium ethoxide (2 mL) as a catalyst until a solid was formed. The solvent was evaporated under reduced pressure and the residue was recrystallized from methanol to give compounds 1a-1j respectively[15].

Anti-inflammatory activity

Animals-Albino Wistar rats (300–350 g;) were housed in a controlled environment and provided with standard rodent chow and water. Animal care complied with the CPCSEA regulations **(rkcp/med/rp/10/05).**

Carrageenan-induced rat paw edema

First, weigh the Wistar rats and assign each one a unique number for identification. Make a mark immediately beyond the tibiotarsal junction on both side hind paws so that each time the paw is dipped in the mercury column up to the set mark, the paw volume will remain consistent. Take note of the first plethysmograph mercury displacement reading for the paw volume (two sides) of each rat. Separate the animals into two groups (control and test) with five animals in each group. Give the control group a subcutaneous injection of saline while giving the second group a suspension of all the substances under test together with the regular dose of etoricoxib (50 mg/kg). Inject 0.1 ml of carrageenan (1% w/v) in both groups' left side paws' plantar area after 30 minutes. To make comparisons, the non-inflamed reference paw on the right side will be used. Take note of the paw volume on both side legs of all rats in the test and control groups at various time points beginning 60 and 120 minutes after carrageenan injection. Calculate the difference in the sizes of each animal's right and left side paws from the control, all the tested substances, and the etoricoxib-treated animals. Compare the average change in paw volume between untreated and drug-treated rats, and show the drug's percentage edema inhibition[16].

Edema volume of control (Vc) and volume of treated (Vt) were used to calculate percentage (%) inhibition and (%) edema volume by using the following formula.

% Inhibition = [1- (V_t/V_c)] × 100

% Edema volume = 100 × (Edema volume after drug treatment/Initial volume)

Gastric ulcer induced by 0.6N HCl solution

Female Wistar rats weighing 300–350 g fasted for 24 hours while being given access to water as needed to produce gastric ulcers. They were separated into four groups of five animals each and housed in cages with broad mesh bottoms to avoid coprophagia. The first group was retained as the control group, while the second group got the conventional medication etoricoxib at a dosage of 50 mg/kg in the form of sodium CMC suspension. The test group was given compound 1e at a dosage of 50 mg/kg as a sodium CMC solution. Animals were slaughtered and their guts were removed after six hours. With the use of a hand lens, the stomach was opened along the grater's curve to check for ulcers. The percentage of inhibition was measured in comparison to the ulcer control group, which was regarded as having 100% damage[16].

Ulcer index = 10/x

Where, x = (Total mucosal area / Total ulcerated area)

RESULTS AND DISCUSSION

The aim of this study to synthesize a series of novel 2-amino-4,5-diphenyl-1-(substituted)-1*h*-pyrrole-3-carbonitrile from the reaction of benzoin with respective amines, followed by treatment of *in situ* generated initially formed intermediate, 3-amino-1,3-diphenylpropan-1-one with malononitrile in the existence of sodium ethoxide as depicted in **Scheme 1.**

2-amino-1,4,5-triphenyl-1*H*-pyrrole-3-carbonitrile(1a)

Yellow crystalline solid ; Mass *m*/*z* (% abundance) 335 [M^+] (11.9 %) ; IR (cm^{-1}) 3566, 3641 (NH_2), 2202 (CN) ; ^{1}H NMR (ppm) 5.04 (br.s, 2H, NH_2, D_2O exchangeable), 7.04–7.67 (m, 15H, Ar-H)

2-amino-4,5-diphenyl-1-0-tolyl-1*H*-pyrrole-3-carbonitrile(1b)

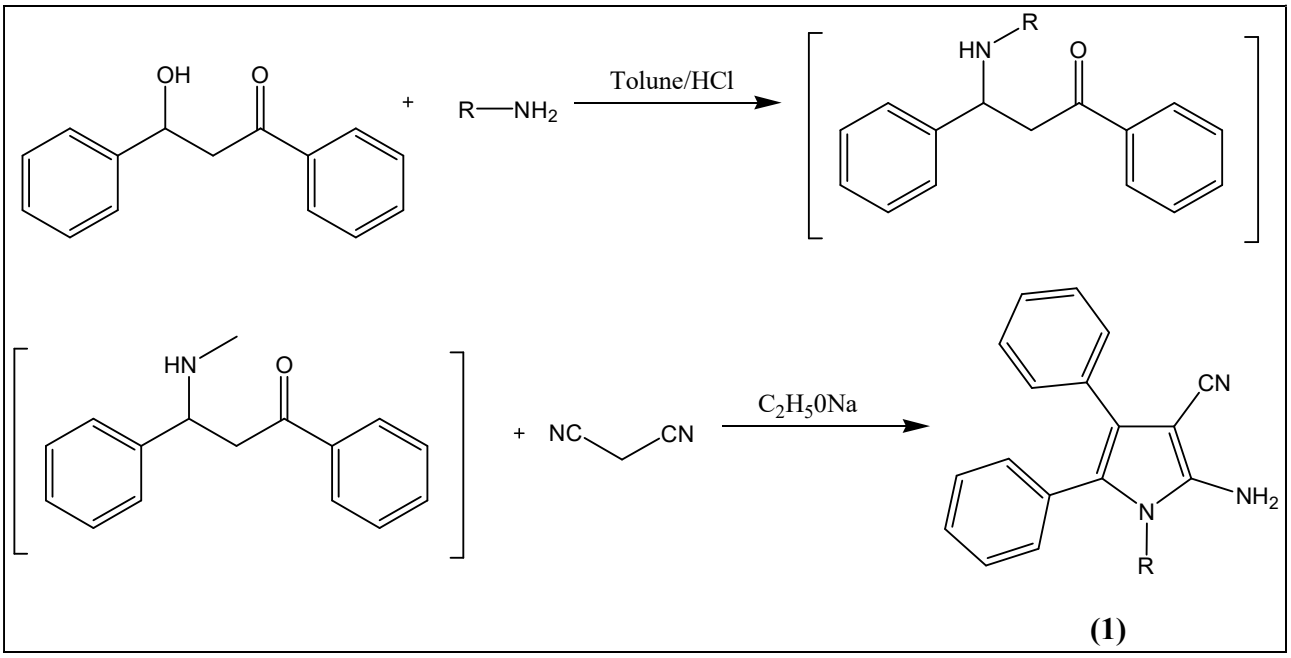
Verylight yellowish crystalline solid ; Mass *m*/*z* (% abundance) 349 [M^+] (100 %), 350 [M^++1] (34.8 %); IR (cm^{-1}) 3438, 3315 (NH_2) 2209 (CN) ; ^{1}H NMR (ppm) 2.3 (s, 3H, CH_3), 6.07 (br.s, 2H, NH_2, D_2O exchangeable), 7.04–8.03 (m, 14H, Ar-H)

2-amino-4,5-diphenyl-1-m-tolyl-1*H*-pyrrole-3-carbonitrile(1c)

Verylight yellowish crystalline solid ; Mass *m*/*z* (% abundance) 349 [M^+] (100 %), 350 [M^++1] (29.8 %); IR (cm^{-1}) 3441, 3315 (NH_2) 2210 (CN) ; ^{1}H NMR (ppm) 2.26 (s, 3H, CH_3), 5.09 (br.s, 2H, NH_2, D_2O exchangeable), 6.5–7.9 (m, 14H, Ar-H)

2-amino-4,5-diphenyl-1-p-tolyl-1*H*-pyrrole-3-carbonitrile(1d)

Light orange crystalline solid ; Mass *m/z* (% abundance) 349 [M^+] (100 %), 350 [M^++1] (26.8 %); IR (cm^{-1}) 3449, 3319 (NH_2) 2215 (CN)



Where R= a) C_6H_5NH-, b) 2-CH_3-C_6H_5-, c) 3-CH_3-C_6H_5-, d) 4-CH_3-C_6H_5-, e) 2-OCH_3-C_6H_5-, f) 3-OCH_3-C_6H_5-, g) 4-OCH_3-C_6H_5-, h) 2-Cl-C_6H_5-, i) 3-Cl-C_6H_5-, j) 4-Cl-C_6H_5-

Scheme 1: Reaction scheme for synthesis of 2-amino-4,5-diphenyl-1-(substituted)-1*H*-pyrrole-3-carbonitrile

2-amino-1-(2-methoxyphenyl)-4,5-diphenyl-1*H*-pyrrole-3-carbonitrile(1e)

Orange amorphous solid ; Mass *m/z* (% abundance) 365 [M^+] (25.3 %) ; IR (cm^{-1}) 3526, 3659 (NH_2) 2205 (CN) 1509 (C–O); ^{1}H NMR (ppm) 3.79 (s, 3H, OCH_3), 5.1 (br.s, 2H, NH_2, D_2O exchangeable), 6.6–7.8 (m, 14H, Ar-H)

2-amino-1-(3-methoxyphenyl)-4,5-diphenyl-1*H*-pyrrole-3-carbonitrile(1f)

Orange crystalline solid ; Mass *m/z* (% abundance) 365 [M^+] (29.6 %) ; IR (cm^{-1}) 3610 (NH_2) 2215 (CN) 1510 (C–O)

2-amino-1-(4-methoxyphenyl)-4,5-diphenyl-1*H*-pyrrole-3-carbonitrile(1g)

Light orange amorphous solid ; Mass *m/z* (% abundance) 365 [M^+] (5.6 %) ; IR (cm^{-1}) 3660 (NH_2) 2225 (CN) 1509 (C–O)

2-amino-1-(2-chlorophenyl)-4,5-diphenyl-1*H*-pyrrole-3-carbonitrile(1h)

Orange amorphous solid ; Mass *m/z* (% abundance) 370 [M^+] (96.3 %), 372 [M^+ + 2] (5.3 %) ; IR (cm^{-1}) 3626 (NH_2) 2190 (CN) ; ^{1}H NMR (ppm) 5.5 (br.s, 2H, NH_2, D_2O exchangeable), 6.6–7.8 (m, 14H, Ar-H)

2-amino-1-(3-chlorophenyl)-4,5-diphenyl-1*H*-pyrrole-3-carbonitrile(1i)

Light Orange amorphous solid ; Mass *m/z* (% abundance) 370 [M^+] (95.3 %), 372 [M^+ + 2] (4.3 %) ; IR (cm^{-1}) 3635 (NH_2) 2195 (CN)

2-amino-1-(4-chlorophenyl)-4,5-diphenyl-1*H*-pyrrole-3-carbonitrile(1j)

White amorphous solid; Mass *m/z* (% abundance) 370 [M^+] (93.3 %), 372 [M^+ + 2] (6.3 %) ; IR (cm^{-1}) 3610 (NH_2) 2190 (CN)

Table1: Physical properties of 2-Amino-4,5-Diphenyl-1-(Substituted)-1*H*-Pyrrole-3-Carbonitrile

Comp. Code	R	Molecular Weight	Melting Point (°C)	% Yield
1a	C_6H_5-	335.4	170-172	80
1b	2-CH_3-C_6H_5-	349.16	228-230	70
1c	3-CH_3-C_6H_5-	349.16	136-138	90
1d	4-CH_3-C_6H_5-	349.16	170-172	60
1e	2-OCH_3-C_6H_5-	365.15	174-177	70
1f	3-OCH_3-C_6H_5-	365.15	164-166	85
1g	4-OCH_3-C_6H_5-	365.15	123-125	65
1h	2-Cl-C_6H_5-	369.85	181-183	75
1i	3-Cl-C_6H_5-	369.85	160-162	70
1j	4-Cl-C_6H_5-	369.85	136-138	60

The preliminary anti-inflammatory activity of the synthesized 2-Amino-4,5-Diphenyl-1-(Substituted)-1H- Pyrrole-3-Carbonitrile were evaluated for anti-inflammatory activity by carrageenan-induced rat paw edema method.

Table 2: Anti-inflammatory Activity (% Inhibition in rat paw edema) of Synthesized Compounds

Comp. No.	R	% Inhibition (± SEM)	
		1h	3h
Control	-	-	-
1a	C_6H_5-	20.38 ± 1.76	21.69 ± 1.54
1b	2-CH_3-C_6H_5-	14.77 ± 4.48	18.04 ± 4.16
1c	3-CH_3-C_6H_5-	38.88 ± 2.35	54.19 ± 2.65
1d	4-CH_3-C_6H_5-	51.88 ± 5.29	66.26 ± 5.13
1e	2-OCH_3-C_6H_5-	59.22 ± 2.33	77.07 ± 5.61
1f	3-OCH_3-C_6H_5-	29.61 ± 3.71	40.96 ± 6.69
1g	4-OCH_3-C_6H_5-	50.00 ± 1.91	56.61 ± 6.06
1h	2-Cl-C_6H_5-	50.00 ± 4.50	63.84 ± 2.60
1i	3-Cl-C_6H_5-	14.77 ± 4.48	18.04 ± 4.16
1j	4-Cl-C_6H_5-	20.38 ± 1.76	21.69 ± 1.54
Etoricoxib	-	58.33 ± 3.67	85.53 ± 1.63

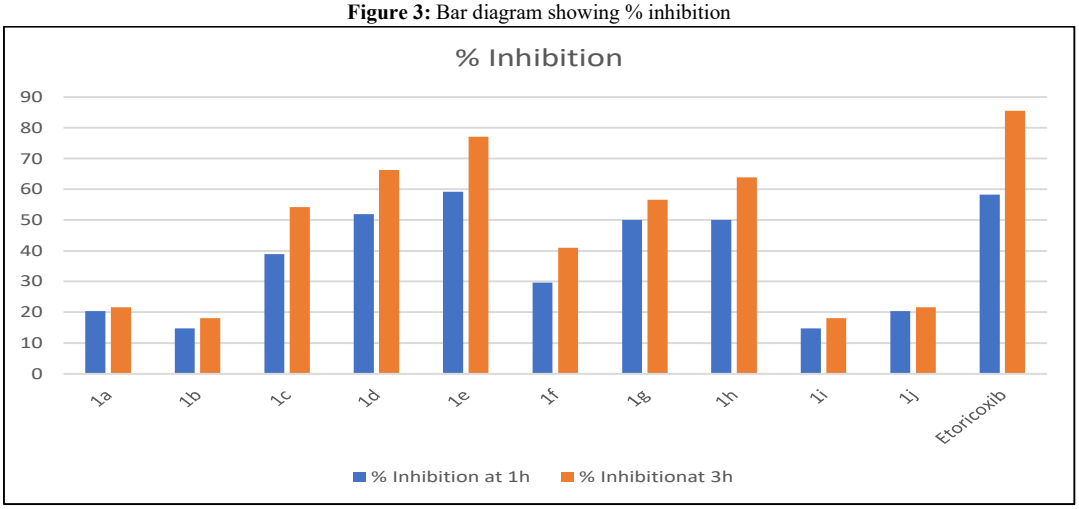
No. of animals used n = 5

Dose of the tested compounds and etoricoxib: 50mg/Kg

SEM – Standard error of the mean

Results of the anti-inflammatory activity of the tested compounds as well as Etoricoxib are shown in **Table 2**. Results showed that most of the tested compounds exhibited significant ($P < 0.05$) inhibition against carrageenan-induced rat paw edema and comparable anti-inflammatory activity relative to etoricoxib. Among these derivatives, compound 1e was found to be equally potent as Etoricoxib.

Figure 3: Bar diagram showing % inhibition



Gastric ulcerogenic effects were determined in rats[16] for representative examples of the synthesized compound 1e. Results indicated that compound 1e did not induce any ulcerogenic effect at 50 mg/kg dose which represents in **Table 3**. At higher doses, the tested compounds produced low gastric ulcerogenic compared with etoricoxib.

Table 3: Gastric ulcerogenic activity of compound 1e with respect to std etoricoxib

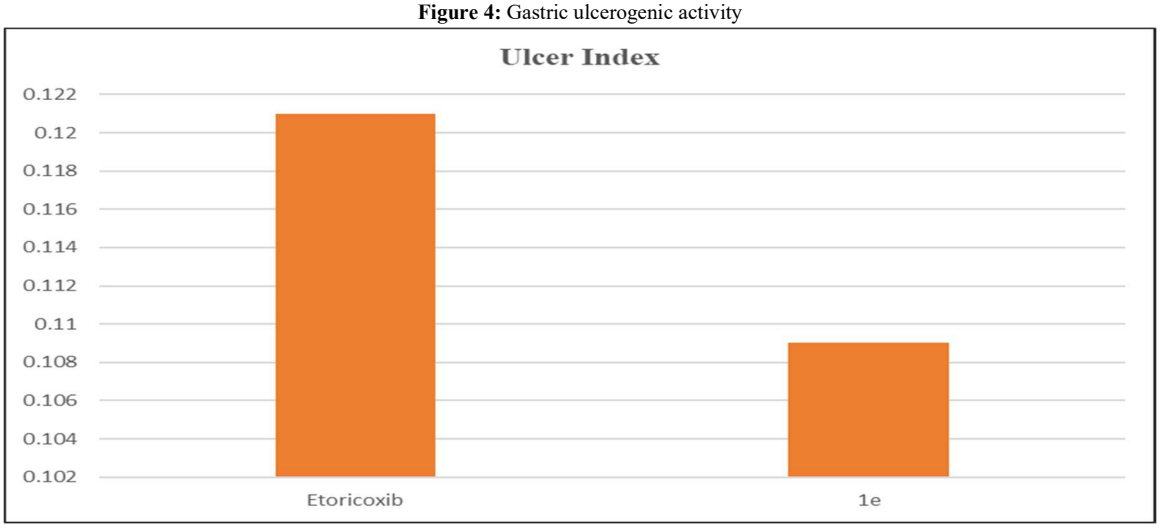
Compound	Ulcer index (± SEM)
Etoricoxib	0.121 ± 0.008
1e	0.109 ± 0.021

No. of animals used n = 5

Dose of the test compound and etoricoxib: 50mg/kg

SEM: standard error of the mean

Figure 4: Gastric ulcerogenic activity



CONCLUSION

Among all the compounds synthesized, compound 1e exhibited the most potent in the series of compounds synthesized with 77.07 % inhibition in comparison to the Standard drug etoricoxib has exhibited 85.53 % inhibition. Compound 1e was checked for ulcerogenic potential but the ulcerogenic potential of compound 1e exhibited less activity than that of Etoricoxib.

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