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ORIGINAL ARTICLE

Synthesis and pharmacological evaluation of marine bromopyrrole alkaloid-based hybrids with anti-inflammatory activity

Rajesh A. Rane [a,*,1], Mukesh Nandave [b,1], Suresh Nayak [b], Anushri Naik [c], Dhwani Shah [c], Wesam S. Alwan [a], Niteshkumar U. Sahu [c], Shital S. Naphade [d], Mahesh B. Palkar [a], Sivanandhan Karunanidhi [a], Neeta Thapliyal [a], Rajshekhar Karpoormath [a,*]

[a] *Discipline of Pharmaceutical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban 4001, South Africa*
[b] *Department of Pharmacology, SPP School of Pharmacy and Technology Management, NMIMS University, Vile Parle, Mumbai 400056, India*
[c] *Department of Pharmaceutical Chemistry, SPP School of Pharmacy and Technology Management, NMIMS University, Vile Parle, Mumbai 400056, India*
[d] *Piramal Health Care Ltd, Goregaon, Mumbai, India*

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KEYWORDS

Aroyl hydrazones;
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Rat paw edema;
Antihistaminic H_1;
Anti-serotonergic 5-HT_3

Abstract Hybrids of bromopyrrole alkaloids with aroyl hydrazone feature were synthesized and evaluated for their anti-inflammatory activity using the carrageenan induced rat paw edema method. All the tested hybrids showed good anti-inflammatory activity with 64.78–86.03% inhibition of edema. Most active hybrids **4b** and **4f** were further investigated for antihistaminic H_1 [**4b** −8.09 μg/mL (18.5 μM); **4f** −9.26 μg/mL (24.05 μM)] and anti-serotonergic 5-HT_3 [**4b** −7.01 μg/mL (16.04 μM); **4f** −9.64 μg/mL (25.04 μM)] activities. Molecule **4f** with anti-inflammatory, anti-histaminic and anti-serotonergic activities emerges as a potential anti-inflammatory lead form in this study.

1. Introduction

Inflammation is defined as a complex defensive process, in which the body responds to different injuries, and is characterized by the accumulation of local fluids and leukocytes with the objective of eliminating the noxious stimulus. In pathological conditions the evolution of persistent tissue damage by inflammatory cells is not suitably repaired (Nathan, 2002; Thorlacius and Xie, 1995). Histamine and serotonin are

* Corresponding authors.
E-mail addresses: rajeshrane_uict@yahoo.co.in (R.A. Rane), karpoormath@ukzn.ac.za (R. Karpoormath).
[1] These authors contributed equally for this manuscript.

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considered to be some of the most important mediators of inflammation. During inflammation, histamine is involved in vasodilatation, promotion of leukocyte adhesion to the vascular endothelium through the expression of P-selectin on the endothelial cell surface and sequestration of leukocytes at the inflammatory site (Thorlacius and Xie, 1995; Asako et al., 1994). Histamine-mediated inflammatory responses have long been thought to be mediated by the histamine H_1 receptor. Likewise, pro-inflammatory role of serotonin (5-HT) as a mediator of the pain has been widely investigated. Serotonin promotes the production of interferon-γ by human natural killer cells. Out of many serotonergic receptors, peripheral 5-HT_3 receptors are involved in various nociceptive pathways (Ye et al., 1997). This subtype of serotonergic receptors also plays a crucial role in spinal pain transmission as well as endogenous pain suppression. A large body of evidence implicates the efficacy of 5-HT_3 antagonists in various inflammatory conditions including rheumatic diseases (Samborski et al., 2004).

Carrageenan, a sulphated polysaccharide, produces inflammation and edema. Therefore, carrageenan-induced paw edema is a well established and broadly used model of acute inflammation for evaluation of anti-inflammatory activities of different test substances (Winter et al., 1962). Carrageenan produces inflammation and edema in two phases. The early phase is observed around 1 h where the release of histamine, serotonin, bradykinin and to some extent prostaglandins takes place. Second phase i.e. the delayed phase (after 1 h) is where polymorphonuclear (PMN) leucocyte infiltration and the continuous generation of the prostaglandin occur (Gilligan and Lovato, 1994; Di Rosa et al., 1971a, 1971b). In addition to these, release of PMN leucocyte-derived free radicals, nitric oxide (NO), tumor necrosis factor (TNF-α), and interleukin-1β (IL-1) also takes place in the delayed phase (Halici et al., 2007; Nacife et al., 2004).

A diverse set of molecules with different pharmacophores have been explored in search of novel anti-inflammatory agents using carrageenan-induced paw edema as a model for acute inflammation. Among them aroyl hydrazones are known for having anti-inflammatory activity (Bhandari et al., 2008; Rajitha et al., 2011). Aroyl hydrazones constitute an important class of privileged scaffold for new drug development. They are known to possess a diverse array of biological effects like anticonvulsant, analgesic, antidepressant, antibacterial, antifungal, antimalarial and antitumor activities (Gökçe et al., 2009).

We are currently working on the design, synthesis and evaluation of bromopyrrole alkaloid-based derivatives as antimicrobial and anticancer agents (Rane et al., 2012a,b, 2013a,b, 2014a). Bromopyrrole alkaloids are naturally occurring marine compounds found in marine sponges (Rane et al., 2014b). These alkaloids are reported to have a diverse set of pharmacological activities like antibacterial, antitubercular (Tasdemir et al., 2007), anitibiofilm (Richards et al., 2008), antineoplastic (Xiong et al., 2010; Tasdemir et al., 2002) and antiviral agents (Paul et al., 1991; Tsuda et al., 2006). Importantly these natural products also possess H1 anti-histaminic activity, where 1 and 0.1 μM response of histamine was almost completely abolished by 3 and 10 μM solution of **III** and **IV** respectively (Aiello et al., 2008; Cafieri et al., 1996; Fattorusso and Taglialatela-Scafati, 2000). **V** and **VI** were reported as having antiserotonergic (5-HT3 antagonistic) activity on isolated guinea pig ileum (Morales and Rodriguez, 1991; Cafieri et al., 1995). Similarly **VII** and **VIII** exhibited their anti-inflammatory potential by inhibition of P_2X_7 (Buchanan et al., 2007). (Fig. 1a) Looking into the pharmacological potential of these reported molecules, we indulged ourselves in the synthesis and evaluation of hybrids of bromopyrrole alkaloids with the aroyl hydrazone feature as anti-inflammatory agents using the carrageenan-induced paw edema technique. (Fig. 1b) To find out the underlying mechanism of observed anti-inflammatory effect, we screened most effective hybrids of the series for their histamine H_1 and 5-HT_3 receptor antagonistic activities by using in-vitro tissue assays.

2. Methods and materials

2.1. Chemistry

All reactions were carried out under an inert nitrogen atmosphere under anhydrous conditions and by using molecular sieves (4 Å 1/16″ pellets), ethanol and CAN (acetonitrile) were freshly distilled from $CaCl_2$. All chromatographic solvents were distilled before use. Silica gel of 60–120 mesh and 200–400 mesh was used for column and flash chromatography. Some of the starting materials were obtained from S.D. Fine Pvt. Ltd., SRL, Spectrochem/Aldrich and some of them are prepared in the laboratory and were used without further purification. All melting points (MP) were recorded on Thermomik Compbell electronics, having an oil-heating system and were uncorrected. Analytical thin layer chromatography (TLC) was carried out on precoated plates SiO_2 (silica gel 60, F 254, Merck). FTIR spectra were recorded on a Perkin Elmer RX I spectrometer using KBr pellets. All the NMR spectra were recorded on JEOL AL-300 FT-NMR spectrometer with DMSO-d_6 as solvent using tetramethyl silane (TMS) as internal reference. Mass spectra were obtained on THERMO FINNINGAN LCQ advantage max (LCMS). Compounds **1**, **2** and **3** were prepared according to the reported methods (Rane et al., 2012a,b, 2013a,b). Evaporation of final product solutions was done under vacuum with a rotary evaporator.

2.2. Synthesis of 4,5-dibromo-N′-arylidene-1H-pyrrole-2-carbohydrazide analogs (4)

Appropriate aldehydes (27.5 mmol), and **3** (6, 27.5 mmol), were stirred in 30 mL absolute ethanol in a round bottomed flask. 1–2 mL of triethylamine was added to the reaction mixture and was refluxed for 4–7 h. Most of the 4,5-dibromo-1-methyl-1H-pyrrole-2-carbohydrazide was consumed at this time (monitored by TLC), the reaction mixture was concentrated under reduced pressure and the obtained solid residue was partitioned between 50 mL water and 50 mL chloroform to extract **4** in organic layer. Column purification using flash chromatography (SiO_2, 10% MeOH /$CHCl_3$) of the crude product yielded pure 4,5-dibromo-N′-arylidene-1-methyl-1H-pyrrole-2-carbohydrazide. The obtained solid was recrystallized from MeOH.

4,5-Dibromo-N′-(furan-2-ylmethylene)-1H-pyrrole-2-carbohydrazide (**4a**) Yield: 78%; m.p.: 188–190 °C; IR (KBr): V_{max} cm^{-1} 1622 (C═C), 1648 (CH═N), 1725 (C═O), 3214 (NH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.62 (s, 1H pyrrole 3H), 6.91–7.33 (m, 3H ArH), 8.21 (s, 1H azometine H), 11.51 (s, 1H CONH), 13.00 (s, 1H pyrrole NH); ^{13}C NMR

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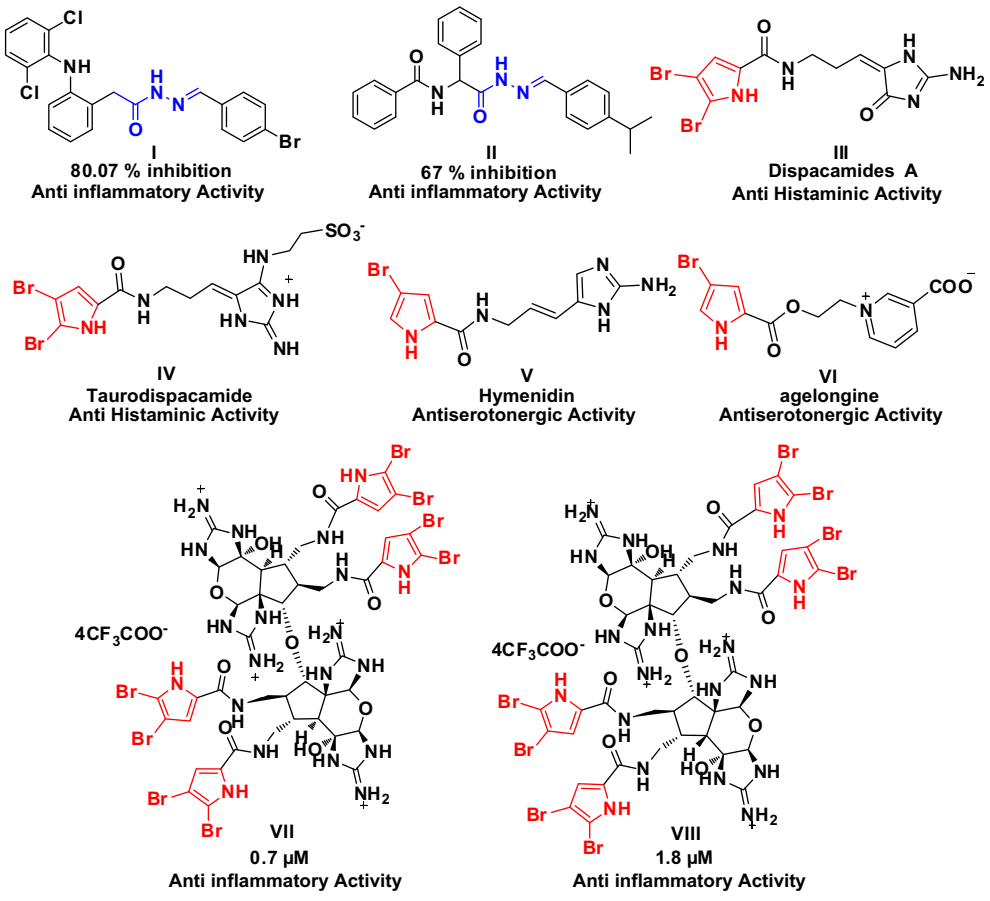

Figure 1a Reported aroyl hydrazones and bromopyrrole alkaloids with their biological activities.

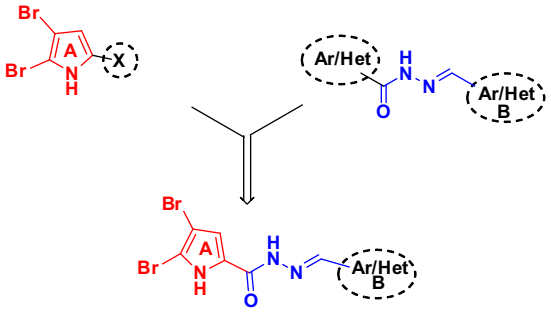

Figure 1b Design of novel N′-arylidene-4,5-dibromo-1H-pyrrole-2-carbohydrazides using the molecular hybridization approach.

(75 MHz, DMSO-d_6): δ 157.3, 149.2, 144, 135.6, 126.3, 118.2, 113.2, 112.9, 106.3, 100.2; MS *m/z*: 359.8908 $[M]^+$, 361.8911 $[M^{+2}]^+$,363.8915 $[M^{+4}]^+$.

4,5-Dibromo-N′-(2,6-dichlorobenzylidene)-1H-pyrrole-2-carbohydrazide (**4b**) (Rane et al., 2014a) Yield: 78%; m.p.: 232–234 °C; IR (KBr): V_{max} cm^{-1} 1626 (C═C), 1643 (CH═N), 1723 (C═O), 3219 (NH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.14 (s, 1H pyrrole 3H), 7.52–8.00 (m, 3H ArH), 8.68 (s, 1H azometine H), 11.84 (s, 1H CONH), 13.08 (s, 1H pyrrole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 157.1, 138.3, 133.6, 129.5, 128.2, 126.3, 114.2, 106.3, 100.2; MS *m/z*: 437.8331 $[M]^+$, 439.8335 $[M^{+2}]^+$, 441.8339 $[M^{+4}]^+$.

4,5-Dibromo-N′-(4-(dimethylamino)benzylidene)-1H-pyrrole-2-carbohydrazide (**4c**) Yield: 80%; m.p.: 216–218 °C; IR (KBr): V_{max} cm^{-1} 1626 (C═C), 1640 (CH═N), 1714 (C═O), 3214 (NH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.25–3.40 (s, 6H CH_3), 7.02 (s, 1H pyrrole 3H), 7.19–7.49 (m, 4H ArH), 8.64 (s, 1H azometine H), 11.54 (s, 1H CONH), 12.96 (s, 1H pyrrole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 157.4, 153.6, 146.3, 128.2, 126.3, 123.6, 114, 112.1, 106.3, 100.3, 41.9; MS *m/z*: 412.9536 $[M]^+$, 414.9540 $[M^{+2}]^+$, 428.9546 $[M^{+4}]^+$.

4,5-Dibromo-N′-(4-fluorobenzylidene)-1H-pyrrole-2-carbohydrazide (**4d**) (Rane et al., 2014a) Yield: 74%; m.p.: 156–158 °C; IR (KBr): V_{max} cm^{-1} 1612 (C═C), 1648 (CH═N), 1722 (C═O), 3216 (NH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.12 (s, 1H pyrrole 3H), 7.29-7.95 (m, 4H ArH), 8.73 (s, 1H azometine H), 11.59 (s, 1H CONH), 13.02 (s, 1H pyrrole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.5, 157.2, 146.3, 130.2, 129.2, 126.9, 115.4, 113.2, 106.6, 100.3; MS *m/z*: 387.9021 $[M]^+$, 389.9024 $[M^{+2}]^+$, 386.9026 $[M^{+4}]^+$.

4,5-Dibromo-N′-(4-hydroxybenzylidene)-1H-pyrrole-2-carbohydrazide (**4f**) (Rane et al., 2014a) Yield: 88%; m.p.: 212–214 °C; IR (KBr): V_{max} cm^{-1} 1624 (C═C), 1643 (CH═N), 1719 (C═O), 3222 (NH), 3452 (OH); ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.82 (s, 1H pyrrole 3H), 6.85 -7.56 (m, 4H ArH), 8.23 (s, 1H azometine H), 9.93 (s, 1H OH), 11.34 (s, 1H CONH), 12.84 (s, 1H pyrrole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 160.3, 157.6, 146.2, 130.2, 126.9, 126.3, 116.2,

114.1, 106.5, 100.3; MS *m/z*: 385.9065 $[M]^+$, 387.9067 $[M^{+2}]^+$, 389.9071 $[M^{+4}]^+$.

4,5-Dibromo-N′-(thiophen-2-ylmethylene)-1H-pyrrole-2-carbohydrazide (**4j**) Yield: 78%; m.p.: 230–232 °C; IR (KBr): V_{max} cm^{-1} 1626 (C═C), 1632 (CH═N), 1716 (C═O), 3228 (NH); ^1H NMR (300 MHz, DMSO-d_6): δ 6.47 (s, 1H pyrrole 3H), 6.90-7.47 (m, 4H ArH), 8.16 (s, 1H azometine H), 11.21 (s, 1H CONH), 12.92 (s, 1H pyrrole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 157.6, 144.5, 130.1, 128.1, 127.6, 126.2, 124.9, 113.9, 106.5, 100.1; MS *m/z*: 375.8678 $[M]^+$, 377.8681 $[M^{+2}]^+$, 379.8683 $[M^{+4}]^+$.

4,5-Dibromo-N′-(3,4-dimethoxybenzylidene)-1H-pyrrole-2-carbohydrazide (**4l**) (Rane et al., 2014a) Yield: 85%; m.p.: 134-136 °C; IR (KBr): V_{max} cm^{-1} 1622 (C═C), 1643 (CH═N), 1716 (C═O), 3234 (NH); ^1H NMR (300 MHz, DMSO-d_6): δ 3.25 (s, 3H OCH_3), 3.40 (s, 3H OCH_3), 6.76 (s, 1H pyrrole 3H), 7.07 −7.50 (m, 3H ArH), 8.19 (s, 1H azometine H), 11.26 (s, 1H CONH), 12.91 (s, 1H pyrrole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 157.6, 152.2, 149.6, 145.9, 132.2, 126.5, 122.1, 114, 112.1, 109.6, 105.9, 100.6, 56.2; MS *m/z*: 429.9329 $[M]^+$, 431.9332 $[M^{+2}]^+$, 433.9336 $[M^{+4}]^+$.

4,5-Dibromo-N′-(2,5-dihydroxybenzylidene)-1H-pyrrole-2-carbohydrazide (**4n**) Yield:80%; m.p.: 230–232 °C; IR (KBr): V_{max} cm^{-1} 1621 (C═C), 1642 (CH═N), 1719 (C═O), 3229 (NH), 3480 (OH); ^1H NMR (300 MHz, DMSO-d_6): δ 6.77 (s, 1H pyrrole 3H), 6.91–7.29 (m, 3H ArH), 8.14 (s, 1H azometine H), 9.29 (s, 1H 5-OH), 9.47 (s, 1H 2-OH), 11.33 (s, 1H CONH), 12.95 (s, 1H pyrrole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 157.2, 153.6, 151.9, 146.9, 126.5, 120.2, 118.9, 116.1, 113.6, 106.2, 100.3; MS *m/z*: 401.9018 $[M]^+$, 403.9019 $[M^{+2}]^+$, 405.9021 $[M^{+4}]^+$.

2.3. Pharmacological evaluation

2.3.1. Animal

Male Wistar rats (150 to 200 g; 10 to 12 weeks old) were used in the study. The animals were housed in polyacrylic cages (38 × 23 × 10 cm) with not more than four rats per cage and acclimatized to the laboratory conditions before randomization. They were housed in an air-conditioned room and were kept in standard laboratory conditions under natural light and dark cycles (approximately 14 h light: 10 h dark) maintained at humidity of 50 ± 10% and an ambient temperature of 25 ± 2 °C. The study protocol was reviewed and approved by the Institutional Animal Ethics Committee and conforms to the Indian National Science Academy Guidelines for the Use and Care of Experimental Animals in research.

2.3.2. Anti-inflammatory activity

This study was done according to the procedure of Winter (Winter et al., 1962). Animals were fasted with free access to water at least 12 h prior to experiments and randomly divided into groups with six animals in each group. The paw edema was induced by sub-plantar injection of 50 µL of 1% carrageenan solution in 0.9% saline. Indomethacin and the test compounds were dissolved in DMSO and were injected subcutaneously in a dose of 40 mg/kg body weight 1 h prior to carrageenan injection. The control group was prepared with 1 mL of vehicle (0.9% saline and DMSO). The paw edema volume was measured with a digital plethysmometer immediately at, 1, 2, and 4 h after injection of carrageenan. Results are reported as paw volume expressed in milliliters. Paw edema volume was compared with the vehicle control group and percent anti-inflammatory activity was calculated according to the formula as given below. The result is expressed as percentage inhibition of edema over the untreated control group. The result of anti-inflammatory analysis is reported in Table 1.

$$\text{The percentage inhibition} = 1 - (\text{Edema volume in the drug treated group} / \text{Edema volume in the control group}) \times 100.$$

2.3.3. In vitro H_1-antihistaminic and $5HT_3$-antiserotonergic activity: (Isolated guinea pig ileum method)

In vitro H1-antihistaminic and $5HT_3$-antiserotonergic activities were carried out by using the isolated guinea pig ileum method (Day and Vane, 1963). From the graphical presentation of logarithmic doses of test compounds and their corresponding antagonists, IC_{50} of the agonist response was determined. Mepyramine maleate and Ondansetron hydrochloride were used as standard antihistaminic and antiserotonergic agents, respectively.

2.4. Statistical analysis of data

Data obtained from animal experiments were expressed as mean standard error (±SEM). Statistical differences between the treatments and the control were evaluated by ANOVA and Dunnet tests. $p < 0.05$ was considered to be statistically significant. [$^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$].

3. Results and discussion

3.1. Chemistry

Aroyl hydrazones were synthesized as depicted in the Scheme 1. Compounds **1**, **2** and **3** were already reported by us in our previously communicated papers (Rane et al., 2012a,2b, 2013a,b). The aroyl hydrazone derivatives were synthesized by refluxing equimolar amounts of **3** with aryl/heteroaryl aldehydes in the presence of triethylamine in ethanol to give the desired 4,5-dibromo-N′-arylidene-1H-pyrrole-2-carbohydrazide analogs **4** (Scheme 1).

The spectral data (IR, ^1H NMR, ^{13}C NMR and MS) of the synthesized compounds were in complete agreement with the proposed structures. $[M]^+$ Molecular ion peak confirmed the molecular weight of the compounds while $[M^{+2}]^+$ and $[M^{+4}]^+$ peaks confirmed the presence of two bromine atoms in these molecules. The ^1H NMR spectra of all compounds **4a**–**4n** showed single at δ 12.8–13.0 corresponding to N–H of the pyrrole core. Proton at the third position of the pyrrole core resonates at δ 6.1–7.1 N–H proton of -CONHN═CH- group resonates in between δ 11.2 – 11.5. The azometine proton to which the aromatic ring B is attached showed a singlet at around δ 8.1–8.7. The aryl protons of ring B resonates in the range of δ 6.9–7.9.

3.2. Pharmacological activity

In present study, preliminary screening of the synthesized hybrids was carried out using carrageenan-induced paw edema as model of inflammation in rats at a dose of 40 mg/kg

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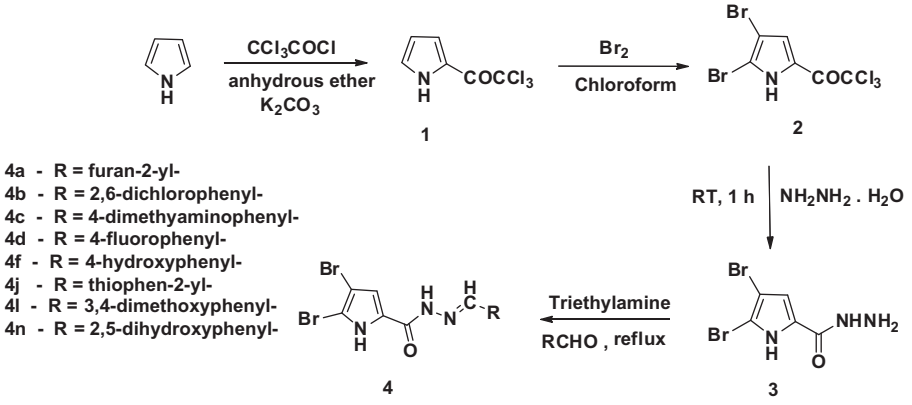
Table 1 Anti-inflammatory activity of hybrids in carrageenan-induced rat paw model.[a,b]

Hybrid code	Treatment ($n = 6$)	1 h		2 h		4 h	
	R	Swelling	% inh[c]	Swelling	% inh[c]	Swelling	% inh[c]
	Control	3.09 ± 0.10	–	3.87 ± 0.09	–	4.82 ± 0.10	–
4a	Furan-	2.63 ± 0.06*	57.14	2.80 ± 0.08*	69.00	2.35 ± 0.10*	79.57
4b	2,6-Dichlorophenyl-	2.40 ± 0.07*	55.06	2.60 ± 0.09*	60.00	2.10 ± 0.06*	85.07
4c	(4-Dimethylamino) phenyl-	2.88 ± 0.10	40.74	3.15 ± 0.12	45.07	2.71 ± 0.07*	68.89
4d	4-Fluorophenyl-	2.55 ± 0.08*	37.99	2.40 ± 0.08*	38. 01	2.70 ± 0.15*	64.78
4f	4-Hydroxyphenyl-	2.63 ± 0.09*	64.89	2.70 ± 0.10*	63.98	2.09 ± 0.11*	86.03
4j	Thiophen-	2.71 ± 0.11*	63.36	2.71 ± 0.16*	63.87	2.41 ± 0.08*	67.61
4l	3,4-Dimethoxyphenyl-	2.92 ± 0.04	52.30	3.72 ± 0.05	48.15	4.15 ± 0.08	67.14
4n	2,5-Dihydroxyphenyl-Indomethacin	2.93 ± 0.09	53.41	3.69 ± 0.06	45.15	4.29 ± 0.11	65.11
		2.48 ± 0.18*	70.2	2.42 ± 0.15*	78.30	2.29 ± 0.15*	87.26

* $p < 0.05$ versus saline control; $n = 6$.
[a] Values are expressed as mean ± SEM in carrageenan-induced rat model of paw edema.
[b] Indomethacin and test compounds were administered orally at the dose of 40 mg/kg.
[c] The percentage inhibition = 1 − (Edema volume in the drug treated group/Edema volume in the control group) × 100.



Scheme 1 Synthesis of N′-arylidene-4,5-dibromo-1H-pyrrole-2-carbohydrazides.

(Winter et al., 1962). The biological results are given in Table 1. All the synthesized hybrids exhibited moderate to good anti-inflammatory activity with 64.78–86.03% inhibition of carrageenan-induced paw edema. The hybrid with para-hydroxyl substitution at ring B (**4f**, 86.03%) has shown highest activity in the series close to standard Indomethacin (87.26%). Significant anti-inflammatory activities showed by hybrids **4c** (68.89%) and **4f** (86.03%) indicated that the presence of substituents like hydroxyl and N, N-dimethylamino at the para-position of the phenyl ring is beneficial for activity. Among the heterocyclic congener of ring B, furan substituted hybrid **4a** showed better anti-inflammatory activity (79.57%) compared to its thiophen substituted analog **4j** (67.61%). Substitution of phenyl ring at 2 and 6-positions with the chloro group is beneficial for anti-inflammatory activity (**4b**, 85.07%). Further, anti-inflammatory potential of most active hybrids **4f** was validated by evaluating its starting materials; 4-hydroxybezaldehyde and 4,5-dibromopyrrole-2-carbohydrazide for inhibition of carrageenan-induced paw edema. Both of them exhibited lower anti-inflammatory activity (edema inhibition <40%). Phenolic compounds are well known for their anti-inflammatory potential (Lee et al., 2006). High activity of **4f** may be attributed to the presence of phenolic substitution at the ring B position. Considering the antihistaminic H_1 and antiserotonergic HT_3 potentials of reported bromopyrrole alkaloid molecules and with a goal to investigate the underlying mechanism of anti-inflammatory action of synthesized hybrids, most active compounds of the series, **4b** and **4f** were evaluated for H_1-antihistaminic and $5HT_3$-antiserotonergic activities. H_1-antihistaminic activity, IC_{50} of the **4b** and **4f** derivatives were 8.09 (18.5 μM) and 9.26 μg/mL (24.05 μM), respectively, while from the $5HT_3$-antiserotonergic activity analysis, IC_{50} of the **4b** and **4f** compounds was found to be 7.01 (16.04 μM) and 9.64 μg/mL (25.04 μM), respectively. The standard drug Mepyramine displayed IC_{50} at 0.040 μM, while Ondansetron showed IC_{50} at 0.24 μM. These studies provide a novel lead molecule **4f** with anti-inflammatory, anti-histaminic and anti-serotonergic activities for further drug development.

4. Conclusions

In this work, we have prepared a series of bromopyrrole alkaloid hybrids with aroyl hydrazone feature. The synthesized hybrids were found to possess potent anti-inflammatory

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activities. Most active anti-inflammatory hybrids **4b** and **4f** were further screened for their H_1-antihistamic and 5-HT_3 antiserotonergic potentials and were found to be active (H_1-antihistamic activity: **4b** IC_{50} = 8.09 μg/mL and **4f** IC_{50} = 9.26; 5-HT_3 antiserotonergic activity: **4b** IC_{50} = 7.01 μg/mL and **4f** IC_{50} = 9.64 μg/mL). Hence from this study we can conclude that H_1-antihistamic and 5-HT_3 antiserotonergic effects may be responsible for the anti-inflammatory activities of these hybrids.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.arabjc.2014.06.004.

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