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Synthesis and evaluation of biological activity of some novel carbazole derivatives

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Natural and synthetic carbazoles contains potent activity against inflammation. Some novel carbazole derivatives have been synthesized and also checked for activity against inflammation by using Carrageenan Induced Inflammation in Rat Paw Edema Model. Structures of synthesized carbazole derivatives have been confirmed on the basis of FT-IR, ^{1}H NMR and elemental analysis. During the evaluation of biological activity, newly synthesized compounds **(4a)** and **(4f)** are found more potent against inflammation.

Keywords: Carbazole, Heterocyclic chemistry, Anti-inflammatory activity, Carrageenan induced Inflammation, Rat paw Edema model, Diclofenac sodium

Inflammation is a multi-factorial process. It reflects the reaction of the organism to a range of stimuli and is related to many disorders such as arthritis, asthma, and psoriasis which require prolonged or constant treatment. Cyclooxygenase (COX), the rate limiting enzyme of the prostanoid biosynthetic pathway, catalyzes the conversion of arachidonic acid to important anti-inflammatory mediators such as prostaglandins (PGs), prostacyclin (PGI) and thromboxane (TXA2). The COX enzyme possesses two different catalytic activities: (i) cyclooxygenase activity that catalyzes the oxidation of arachidonic acid to produce hydroperoxy endoperoxide (PGG2), and (ii) peroxidase activity that reduces the endoperoxide (PGG2) to the hydroxyl endoperoxide (PGH2). The PGH2 is malformed to a wide range of enzymatic and nonenzymatic mechanisms into the primary prostanoids.[1]

The heterocyclic ring system containing carbazole and its derivatives has attracted great interest in recent years due to a variety of biological properties such as analgesic, anti-inflammatory, anticancer, antiproliferative, antitubercular, antifungal and antimicrobial effects.[2] Carbazole is an aromatic heterocyclic organic compound. It has a tricyclic structure consisting of two six-membered benzene rings fused on both sides with the five-membered nitrogen-containing ring. The carbazole ring is present in a variety of medically active substances of natural origin, e.g. Ex. B. Carbazomycin, Oxazinocarbazole, Indolocarbazole, Oxazolinylcarbazole, etc.[3]

The propagation of heterocycles in vital restorative mixtures repeatedly determines the need for new strategies for their disposal. Carbazole subsidiaries are known to have compelling photographic physical and organic properties. Carbazole was first replaced in 1872 by Graebe and Glazer from coal tar.[4] Carbazole is a fragrant heterocyclic natural compound. It has a tricyclic structure, comprising of two six-membered benzene rings combined on either side of a five-membered nitrogen-containing ring. The compound's structure depends on the indole structure, yet in which a second benzene ring is intertwined onto the five-membered ring at the 2, 3 positions of indole (comparable to the 9a 4a twofold bond in carbazole, individually).

Experimental Details

General procedure for the synthesis of N^{9}-(chlorocetyl)-carbazole(2)

Chloroacetyl chloride (4.9 g, 0.04 mol) was added to a solution of carbazole (7 g, 0.04 mol) in 60 mL acetone. Reaction mixture was refluxed on a water bath for 8 h. The completion of reaction was monitored by TLC. The solvent was removed and washed with water. The product was recrystalized from ethanol to obtain compound. Yield:75%, Melting point: 235-238^{o}C, TLC analysis: Solvent system: Benzene: Chloroform ::9:1v/v, (R_f :0.76).

Synthesis of N^9- (hydrazinoacetyl)-carbazole(3)

Hydrazine hydrate (0.6 g, 0.012 mol) was added to a solution of N^9-(chloroacetyl)- carbazole (3 g, 0.012 mol) in 80 mL ethanol and dioxane (9:1 v/v). Reaction mixture was refluxed on a water bath for 10 h. The reaction was monitored by TLC. Reaction mixture was cooled filtered and concentrated to get a solid compound and washed with water, product was recrystallized from ethanol to obtain compound. Yield: 56%, Melting point: 248-250°C, TLC analysis: Solvent system: Benzene: Chloroform :9:1v/v, (R_f: 0.84).

General procedure for the synthesis of aldehydes substituted Carbazole derivatives (4a-4g)

Aromatic aldehyde (10 mmol) was added to a solution of N^9-(hydrazinoacetyl)-carbazole (10 mmol), aq. NaOH (10%, 30 mL) at room temperature, the reaction mixture was stirred for 4 h and the separated solid was filtered off, washed with water and the crude product was recrystalized from ethanol to obtain newer carbazole derivatives **(4a-4g)**.

4-((E)-2-(9H-carbazole-9-carboylimino)ethyl)phenyl formate (4a): FTIR (KBr *v,* cm^{-1}*)*: 727.16 (Aromatic, C-C stretching), 1020 (Ether, C-O-C symmetrical stretching), 1238 (Ether, C- O-C asymmetrical stretching), 1600.92 (Aromatic, C=C stretching), 1690 (Nitrile, C=N stretching), 1890 (Ketone, C=O stretching), 2700 (Aldehyde, -CHO stretching), 3051.39 (Amine, N-H stretching); ^{1}H NMR(700 MHz, CD_3OD): 2.6 (s, 2H, methylene), 6.95-7.03 (dd, 4H, Ar), 7.00 (s, 1H, hydrazine), 7.00-7.55 (m, 4H, indole), 7.1-7.6 (m, 4H, Ar), 7.50 (s, 1H, hydrazide), 9.6 (s, 1H, aldehyde); Yield:53.94%, Melting point: 225-230°C, TLC analysis: Solvent : Benzene: Chloroform ::9:1v/v, (R_f:0.83).

(E)-N'-(2-(4-formylphenyl)ethylidene)-9H-carbazole-9-carbohydrazide (4b): FTIR (KBr v, cm^{-1}): 842.89 (Aromatic, C-C stretching), 1450.47 (Aromatic, C=C stretching), 100.92 (Aromatic, C-H stretching), 1700 (Nitrile, C=N stretching), 2700 (Aldehyde, -CHO stretching), 2900 (Aromatic, C-H stretching); ^{1}H NMR (700 MHz, CD_3OD): 2.6 (s, 2H, methylene), 7.00-7.55 (m, 4H, indole), 7.0 (s, 1H, hydrazide), 7.1-7.6 (m, 4H, Ar), 7.50 (s, 1H, hydrazine), 7.25- 7.69 (dd, 4H, Ar), 9.7 (s, 1H, aldehyde); Yield:60.58%, Melting point: 225-228 °C, TLC analysis: Solvent: Benzene: Chloroform::9:1 v/v,(R_f :0.87).

N'-(2-(3-chloro-4-formylphenyl)ethylidene)-9H-carbazole-9-carbohydrazide(4c): FTIR (KBr v, cm^{-1}): 727.16 (Chlorine, C-Cl stretching), 927.76 (Aromatic, C-C stretching), 1600.92 (Aromatic, C=C stretching), 1624.06 (Nitrile, C=N stretching), 1892.17 (Ketone, C=O stretching), 2700 (Aldehyde, -CHO stretching), 3028.24 (Aromatic, C-H stretching), 3419.79 (Amine, N-H stretching); ^{1}H NMR (700 MHz, CD_3OD): 2.6 (s, 2H, methylene), 7.0-7.6 (m, 4H, Ar), 7.00- 7.04 (m, 4H, indole), 7.00 (s, 1H, hydrazide), s, 1H, hydrazine), 7.13- 7.63 (m, 3H, Ar), 10.24 (s, 1H, aldehyde); Yield:58.50%, Melting point: 238-240°C, TLC analysis: Solvent: Benzene: Chloroform ::9:1 v/v, (R_f:0.69).

(E)-N'-(2-(2-chloro-4-formylphenyl)ethylidene)-9H-carbazole-9-carbohydrazide(4d): FTIR (KBr v, cm^{-1}): 724.09 (Chlorine, C-Cl stretching), 919.71 (Aromatic, C-C stretching), 1598.99 (Aromatic, C=C stretching), 1627.04 (Nitrile, C=N stretching), 1887.17 (Ketone, C=O stretching), 2730 (Aldehyde, -CHO stretching), 3120.24 (Aromatic, C-H stretching), 3317.61 (Amine, N-H stretching); ^{1}H NMR (700 MHz, CD_3OD): 2.6 (s, 2H, methylene), 7.00- 7.55 (m, 4H, indole), 7.00- 7.60 (m, 4H, Ar), 7.0 (s, 1H, hydrazide), 7.50 (s, 1H, hydrazine), 7.19- 7.70 (m, 3H, Ar), 9.87 (s, 1H, aldehyde); Yield:58.92%, Melting point: 230-235°C, TLC analysis: Solvent : Benzene:Chloroform :: 9:1 v/v, (R_f:0.69).

(E)-N'-(2-(4-formyl-2-nitrophenyl)ethylidene)-9H-carbazole-9-carbohydrazide(4e): FTIR (KBr v, cm^{-1}): 729.11(Chlorine, C-Cl stretching), 923.63 (Aromatic, C-C stretching), 1604.89 (Aromatic, C=C stretching), 1617.04 (Nitrile, C=N stretching), 1897.17 (Ketone, C=O stretching), 2690 (Aldehyde, -CHO stretching), 3030.24 (Aromatic, C-H stretching), 3337.59 (Amine, N-H stretching); ^{1}H NMR (700 MHz, CD_3OD):2.6 (s, 2H, methylene), 7.0 (s, 1H, hydrazide), 7.00- 7.55 (m, 4H, indole), 7.00- 7.60 (m, 4H, Ar), 7.50 (s, 1H, hydrazine), 7.51- 8.62 (m, 3H, Ar), 9.87 (s, 1H, aldehyde).

(E)-N'-(2-(3-formyl-4-nitrophenyl)ethylidene)-9H-carbazole-9-carbohydrazide(4f): FTIR (KBr v, cm^{-1}): 858.32 (Aromatic, C-C stretching), 1330 (Nitro, O-N-O asymmetrical stretching), 1510 (Nitro, O-N-O symmetrical stretching). 1600.92 (Aromatic, C-H stretching), 1625.99 (Nitrile, C=N stretching), 1760 (Ketone, C=O stretching), 2210 (Aromatic, C-N stretching), 3051.39 (Hydrazide, N-N stretching), 3419 (Amine, N-H stretching); ^{1}H NMR (700 MHz, CD_3OD): 2.6 (s, 2H, methylene), 7.0 (s, 1H, hydrazide), 7.00- 7.55 (m, 4H, indole), 7.0- 7.6 (m, 4H, Ar), 7.50 (s, 1H, hydrazine),7.60- 8.26 (m, 3H, Ar), 10.24 (s, 1H, aldehyde); Yield:76.70%, Melting point: 237-240°C, TLC analysis: Solvent: Benzene: Chloroform::9:1 v/v, (R_f:0.58).

(E)-N'-(2-(4-chloro-3-formylphenyl)ethylidene)-9H-carbazole-9-carbohydrazide(4g): FTIR (KBr v, cm^{-1}): 900.10 (Aromatic, C-C stretching), 1075 (Ether, C-O-C stretching), 1580 (Nitrile, C=N stretching), 1600.92 (Aromatic, C=C stretching), 1900 (Ketone, C=O stretching), 3051.39 (Amine, N-H stretching) 725.13 (Chlorine, C-Cl stretching); ^{1}H NMR (700 MHz, CD_3OD): 2.6 (s, 2H, methylene),7.0 (s,1H,hydrazide), 7.50(s,1H,hydrazine), 7.00-7.55(m,4H, indole), 7.0-7.6 (m, 4H, Ar), 7.28- 7.55 (m, 3H, Ar), 10.24 (s, 1H, aldehyde); Yield:95.85%, Melting point: 200-211°C, TLC analysis: Solvent: Benzene:Chloroform ::9:1 v/v, (R_f:0.65).

Anti-inflammatory activity

Albino wistar rats of either sex (150-200 g) were divided into various groups of three animals each. Animals were deprived of food for 12 h prior to experiment and only water is given. First group was used as a control and received 1 mL of 1% w/v sodium DMSO suspension in saline, the second group received DMSO suspension of Diclofenac sodium (20 mg/kg) orally and the third group received sodium DMSO suspension of test compounds at a dose of 20 mg/kg orally. One hour after the administration of the compounds, carrageenan suspension (0.2 mL of 1% w/v suspension in 0.9% saline solution) was injected into the sub planter region of left hind paw of the animals. Immediately, the paw volume was measured using digital vernier calliper (initial paw thickness, Vc). Thereafter, the paw volume was measured after 0 h, ½ h, 1 h, 2 h and 4 h after carrageenan administration. The difference between initial and subsequent readings gave the change in edema volume for the corresponding time. The percentage of inhibition was calculated using the following equation.

$$\% \text{ inhibition} = [(Vc - Vt)/ Vc] \times 100$$

Results and Discussion

Chemistry

The desired carbazole derivatives were prepared by multistep reaction is summarized in Scheme 1. In the

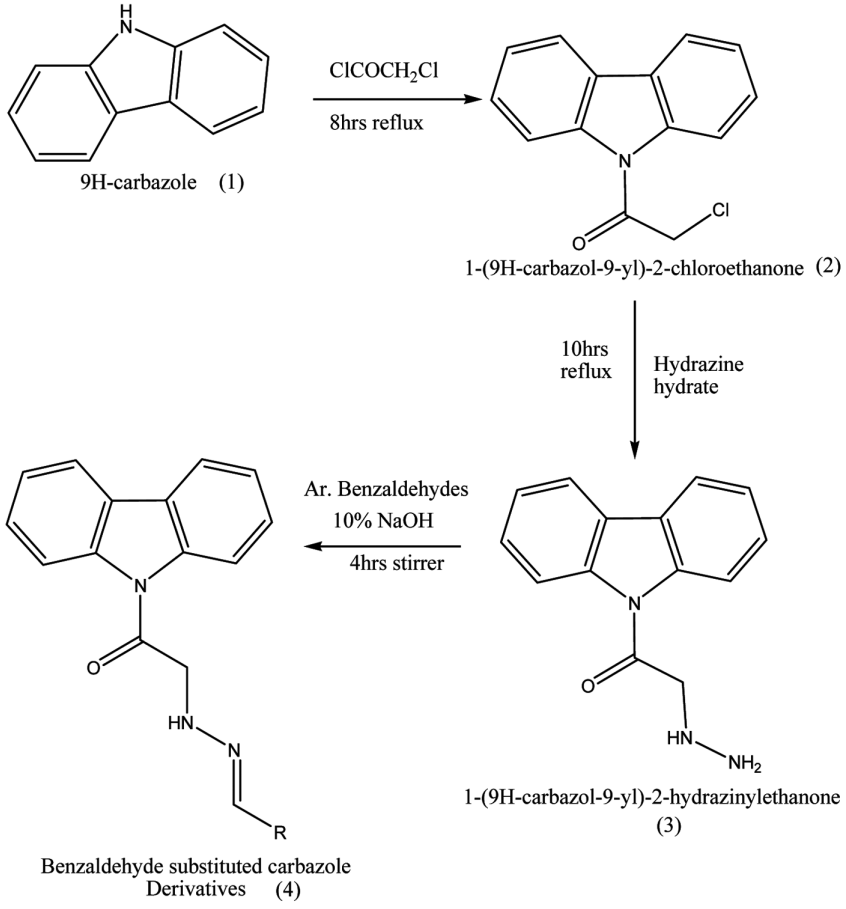

Fig. 1 — (Scheme)

Table 1 — Aromatic benzaldehydes corresponding to compounds (4a-4g)

Sr. No.	Compounds	-Aromatic Benzaldehyde
1	4a	Anisaldehyde
2	4b	Benzaldehyde
3	4c	3-chlorobenzaldehyde
4	4d	2-chlorobenzaldehyde
5	4e	3-nitrobenzaldehyde
6	4f	4-nitrobenzaldehyde
7	4g	4-chlorobenzaldehyde

Table 2 — Anti-inflammatory activity of titled compounds (Dose-20 mg/kg)

Compound	0 h	%Inhibition	1 h	%Inhibition	2 h	%Inhibition	4 h	%Inhibition
4a	2.16±0.03	2.26	2.04±0.09	12.06	1.98±0.03	21.42	1.92±0.06	30.43*
4b	2.19±0.17	0.90	2.27±0.06	2.15	2.44±0.05	3.17	2.44±0.03	11.59
4c	2.20±0.04	1.21	2.30±0.03	0.86	2.48±0.06	1.58	2.58±0.10	6.52
4d	2.19±0.12	0.90	2.12±0.07	8.62	2.08±0.17	17.46	2.06±011	25.35
4e	2.20±0.09	0.45	2.23±0.19	3.87	2.24±0.08	11.11	2.14±0.02	22.46
4f	2.18±0.09	1.35	2.08±0.12	10.34	2.02±0.09	19.8	1.99±0.02	27.89*
4g	2.20±0.06	0.45	2.27±0.03	2.15	2.34±0.23	15.07	2.32±0.07	15.94
Control	2.21±06		2.32±0.04		2.52±0.08		2.76±0.11	
Standard-Diclofenac	2.14±0.23	3.16	1.94±0.19	16.37	1.86±0.10	27.38	1.88±0.26	31.88

first step, 9H-Carbazole and acetone in presence of chloro-acetal chloride afforded the corresponding carbazole derivative (2a). Carbazole derivative was allowed to react with hydrazine hydrate in ethanol and dioxane to gives corresponding pyrazole derivative (3a). IR spectra of all final carbazole derivatives (4a-i) showed a intense peak in the region 1676 - 1600 cm^{-1} due to the C=N stretching vibration which indicate the presence of C=N in carbazole ring. A strong, characteristic band in the region 1230- 1200 cm^{-1} due to the C-N stretching vibration. Peak appeared at 869-820 cm^{-1} due to C=S stretching. Band for aromatic C-H stretching vibrations was observed at 3261-3025 cm^{-1}. Peak for aromatic C-H is generally appeared at longer wavelength than the aliphatic C-H. It is due to the higher stretching of pi electrons present in aromatic ring. The different aromatic benzaldehydes corresponding to compounds (4a-4g) are tabulated in Table 1.

Biological activity

Anti-inflammatory activity was performed by carrageenan induced inflammation in rat paw edema model. This project has been approved by the Institutional Animal Ethical Committee at Hygia Institute of Pharmaceutical Education and Research, Lucknow (Ref. No. HIPER/IAEC/19/18/04). The anti-inflammatory activity of titled compounds (Dose-20 mg/kg) is shown in Table 2.

Conclusion

Study stated that carbazole in combination with other hetero cycles ring might be used as a lead for finding the potent anti-inflammatory agents. Potency of the newly synthesized compounds was determined on the basis of their reduction in inflammation at different time interval by using carrageenan induced inflammation in rat paw edema model of three rats significantly different from standard drug diclofenac sodium. Presence of an electron releasing group on the benzene ring also increases the potency. Substituted carbazole derivative (4a and 4h) also increases the therapeutic value of carbazole toward the treatment of inflammation. It is concluded that further research on carbazole core is needed for the discovery of a potent anti-inflammatory agent. Thus, we observed that carbazole in combination with other heterocyclic might be used as a lead for further study in developing such compounds as a good lead molecules with better pharmacological profile.

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