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Synthesis Of Substituted (4(1H-Indol-3-Yl)Butanoyl)-2-(1H-4-Indol-4-Ylidene)-N-(4hydroxybenzyl)Hydrazine-1-Carboxamide And It's Anti-Inflammatory Studies

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Abstract:
Based on the provided context, a series of new indole-hydrazone derivatives were synthesized and their structures were determined through elemental and spectral studies. These synthesized compounds were then tested for their anti-inflammatory activity using carrageenan-induced oedema in albino rats, with indomethacin as the standard drug. The synthesized compounds exhibited a reduction in oedema formation ranging from 72.3% to 89.3%, surpassing the inhibition observed with indomethacin (46%). Additionally, the compounds were evaluated for their anticancer activity against the MCF-7 breast cancer cell line at a dose of 100µg/ml. Compounds 2f and 2j demonstrated mild anticancer activity with 61% and 68% inhibition, respectively, compared to doxorubicin. Furthermore, molecular docking studies were conducted using the Molego virtual docker program, which revealed that all prepared compounds exhibited high docking scores against both cox-1 and cox-2. The docking scores and binding energy correlated well with the pharmacological results. Lastly, the drug likeness and bioactivity of the compounds were predicted using Molinspiration software, indicating that all new compounds possess favorable drug likeness and bioactivity scores.

Keywords: indole Substituted hydrazonederivatives, hydrazides and anti-inflammatory activity

Introduction

The hydrazone moiety plays a significant role in synthetic and medicinal chemistry. It consists of an "azomethine" R-C(H)=N-N(H)-Ar group, which can be derived from aldehydes and ketones by replacing the oxygen atom with the =NNH2 group.[1] Hydrazones have been widely studied in the literature and have shown diverse biological and pharmacological properties, including anticonvulsant, antimycobacterial, antidepressant, anticancer, analgesic, anti-inflammatory, antiviral, antiplatelet, antimalarial, antimicrobial, cardio

protective/vasodilator, anti-HIV, antihelmintic, antidiabetic, antiprotozoal, anti-trypanosomal, and antischistosomiasis activities.[1-6] Chemists have been studying hydrazones for years due to their synthetic flexibility, selectivity, and sensitivity towards transition metal ions.[7] The metal complexes of hydrazones have potential applications as catalysts, luminescent probes, and molecular sensors.[8-10] Combining hydrazones with other functional groups leads to compounds with unique physical and chemical characteristics.[11] The chemotherapeutic value of hydrazones in developing novel pharmacologically active compounds has garnered significant attention from synthetic chemists in recent decades, and research in this area is ongoing. This review aims to explore the synthetic and chemotherapeutic potential of hydrazones in biology and medicine.

Experimental section:

In the study, an Electrothermal melting point instrument (Electrothermal 9100) was utilized to determine the uncorrected melting points of the compounds. The infrared spectra of KBr discs were recorded using a Pye Unicam SP-1000 spectrophotometer. The 1H-NMR and 13C-NMR spectra were obtained using a Varian EM-390-200 MHz instrument, with CDCl3 serving as the solvent and TMS as the internal standard. These spectra provided information on the chemical changes occurring in the compounds. The analytical data, including microanalytical data, was provided by Mysore University's Microanalytical Data Unit

1. General procedure for the Synthesis of compound (3)

The chemical solution DCM (10 mL/g) was combined with 2.0 g of indole-3-butyric acid (0.0098 mol). The agitated solution was then cooled to 0 °C, and more EDCI (2.26 g, 0.0118 mmol) and Et3N (2.05 mL, 0.0147 mol) were added while the temperature was being maintained. HOBt (1.50 g, 0.0098 mol) was added to the reaction mixture and stirred for ten minutes. Next, tyrosine methyl ester hydrochloride (2.27 g, 0.0098 mmol) and Et3N (2.05 mL, 0.0147 mol) were progressively added in DCM (10 mL/g) medium. TLC was utilised to track the reaction's development after it was finished. After adding Et3N, keep the stirring condition overnight at room temperature and maintained at a pH of 8 ,to get a Compound . methyl-2-(4(1H-indol-3-yl)-butanamido-3-(4-hydroxyphenyl)propanoate

2. General procedure for the Synthesis of compound.(4)

The methyl 2,4(1H-indol-3-yl)butanamido-3(4-hydroxyphenyl)propanoate (3) (3.4 g, 0.0079mol) is blended with the hydrazine hydrate (3.80 mL, 0.0799 mol) in a 30 mL ethanol medium. The reaction mixture was refluxed for 16 hours in an alcoholic medium, and TLC was used to track the reaction's progress. The solvent was extracted at decreased pressure, and the precipitate was chilled by adding ice-cold water. It was then filtered, cleaned with cold water, and recrystallized to produce the desired product. N-(3(4-hydroxyphenyl)-1hydrazinyl)1H-indol-3-yl-1-oxopropan-2-yl-4However, butanamide.(4)

3. General procedure for the compound.(5)

Indole substituted aldehyde (146 mg, 0.525 mmol) was added to butanamide (200 mg, 0.525 mmol) in ethanol (10 mL/g of the compound). The reaction was refluxed for 7–8 hours in an acetic acid medium, and TLC was used to track its progression. The solvent was removed under reduced pressure, chilled, and then precipitate was obtained by adding ice-cold water. The precipitate was then filtered, cleaned with water, and separated from the ethanol.

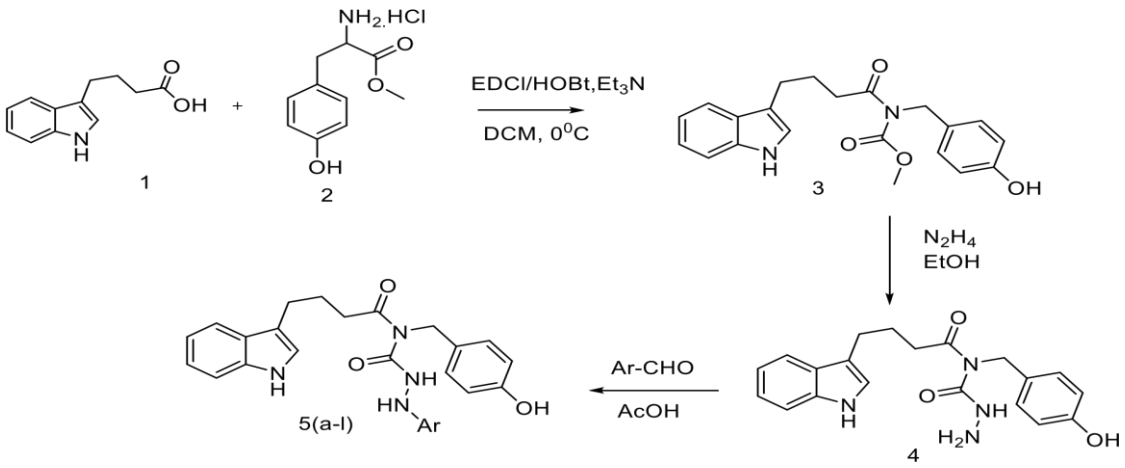
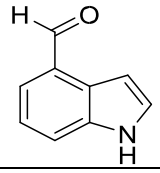
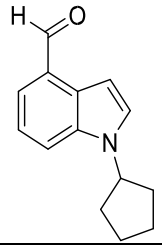
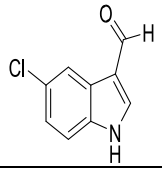
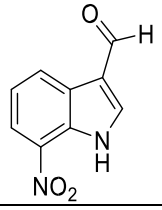
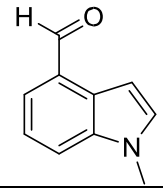
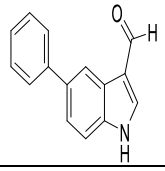
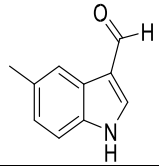
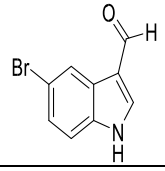
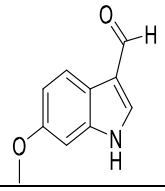
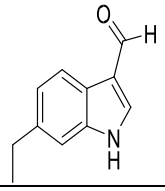


Table-01: Physicochemical characteristics of synthesized Substituted (4(1Hindol-3-yl)butanoyl)-2-(1H-4-indol-4-ylidene)-N-(4hydroxybenzyl)hydrazine-1-carboxamide derivatives

Entry	Aldehyde (Ar)	Yield (%)	MP (^{O}C)	Elemental Analysis of product 5(a-l)			Rf value
				C	H	N	
01		77	142	69.98 (69.96)	5.45 (5.44)	14.57 (14.55)	0.82
02		70	145	72.24 (72.22)	6.25 (6.23)	12.76 (12.74)	0.75
03		69	146	65.30 (65.29)	4.89 (4.88)	13.60 (13.59)	0.73
04		75	139	63.99 (63.98)	4.80 (4.79)	15.99 (15.98)	0.69
05		80	138	70.43 (70.42)	5.71 (5.69)	14.16 (14.14)	0.83
06		90	137	73.36 (73.34)	5.43 (5.42)	12.58 (12.56)	0.76
07		85	132	70.43 (70.41)	5.71 (5.70)	14.16 (14.14)	0.74
08		80	120	60.11 (60.09)	4.50 (4.49)	12.52 (12.51)	No Spot

09		78	135	68.22 (68.20)	5.53 (5.52)	13.72 (13.71)	No Spot
10		77	136	70.85 (70.83)	5.95 (5.94)	13.77 (13.76)	0.70

Spectral data of Synthesised Compounds

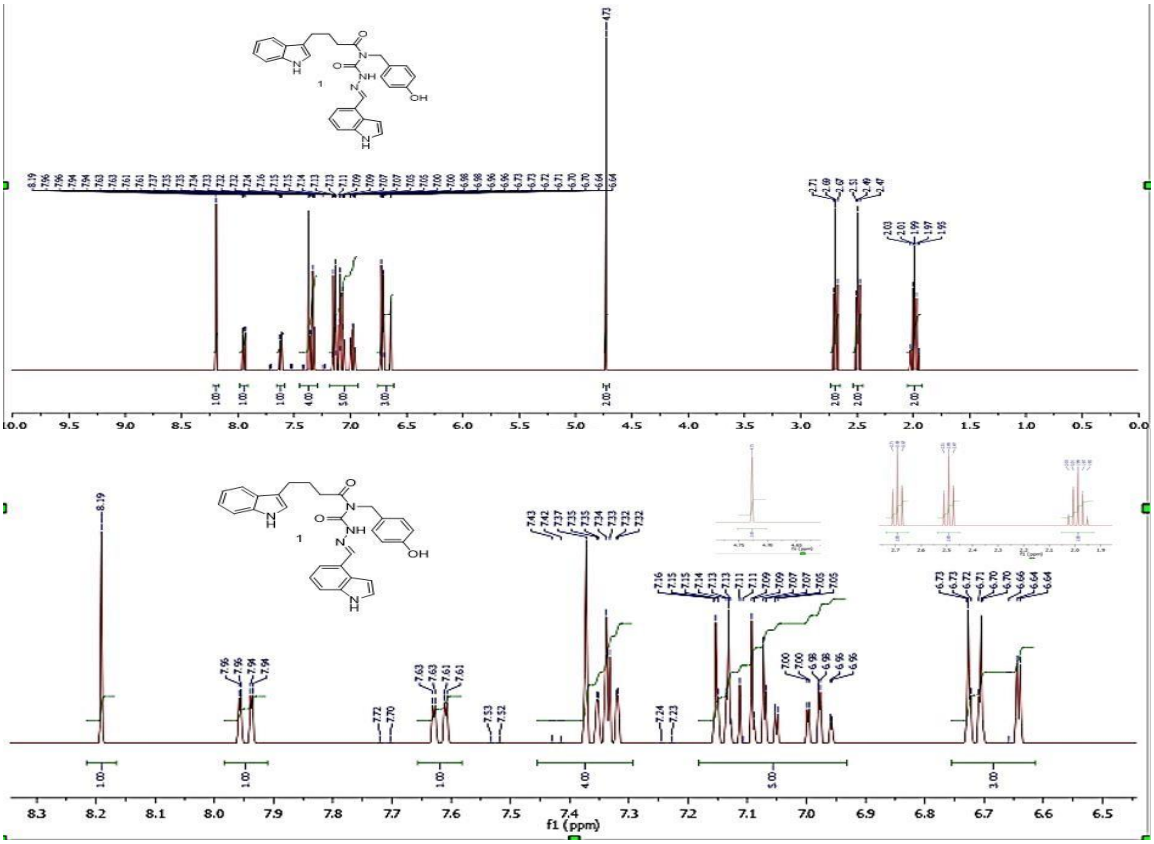
Compound 1

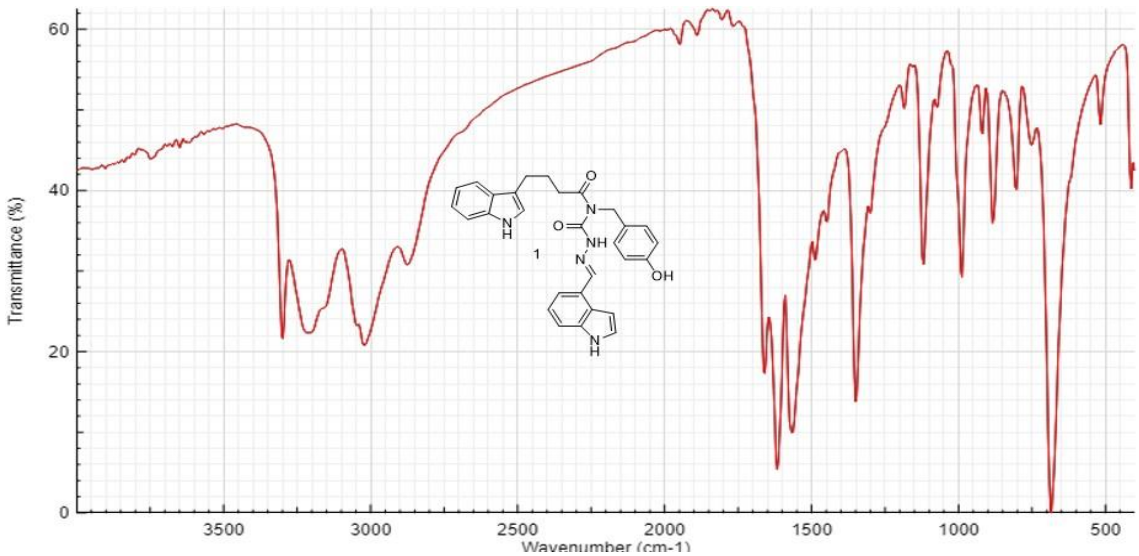
^{1}H NMR: δ 1.99 (2H, quint, *J* = 7.4 Hz), 2.49 (2H, t, *J* = 7.4 Hz), 2.69 (2H, t, *J* = 7.4 Hz), 4.73 (2H, s), 6.59-6.78 (3H, 6.64 (d, *J* = 2.2Hz), 6.72 (d, *J* = 8.2Hz)), 6.91-7.20 (5H, 6.98 (d, *J* = 8.0, Hz), 7.07 (d, *J* = 8.0Hz), 7.09 (d, *J* = 8.2 Hz), 7.14 (d, *J* = 8.2Hz)), 7.27-7.43 (4H, 7.33 (t, *J* = 8.0Hz), 7.33 (d, *J* = 2.2 Hz), 7.36 (t, *J* = 8.2Hz), 7.37 (t, *J* = 0.5 Hz)), 7.62 (1H, t, *J* = 8.0Hz), 7.95 (1H, d, *J* = 7.9Hz), 8.19 (1H, s).

^{13}C NMR: δ 26.2 (1C, s), 30.4 (1C, s), 34.3 (1C, s), 44.4 (1C, s), 101.7 (1C, s), 111.3-111.3 (2C, 111.3 (s), 111.3 (s)), 113.5 (1C, s), 115.7 (2C, s), 118.7 (1C, s), 123.2 (1C, s), 124.7 (1C, s), 127.3 (1C, s), 128.2 (1C, s), 128.3-128.6 (4C, 128.4 (s), 128.4 (s), 128.5 (s)), 128.9-129.2 (3C, 129.0 (s), 129.1 (s), 129.1 (s)), 135.6 (1C, s), 136.4 (1C, s), 136.6 (1C, s), 146.5 (1C, s), 155.2 (1C, s), 157.4 (1C, s), 169.8 (1C, s).

Mass(m/z):493

IR(KBr): 3522-3332 (NH), 3057 (CH aromatic), 2222 (CN), 1690 (CO), 1645 (C=C), 1637 (C=N).





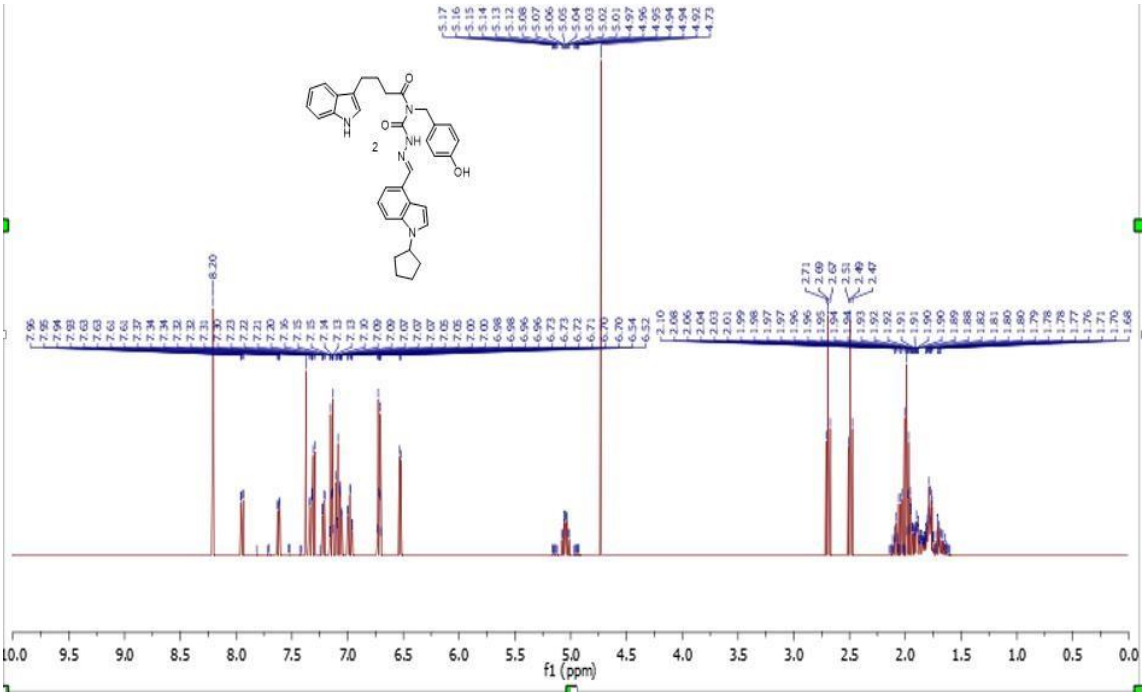
Compound 02

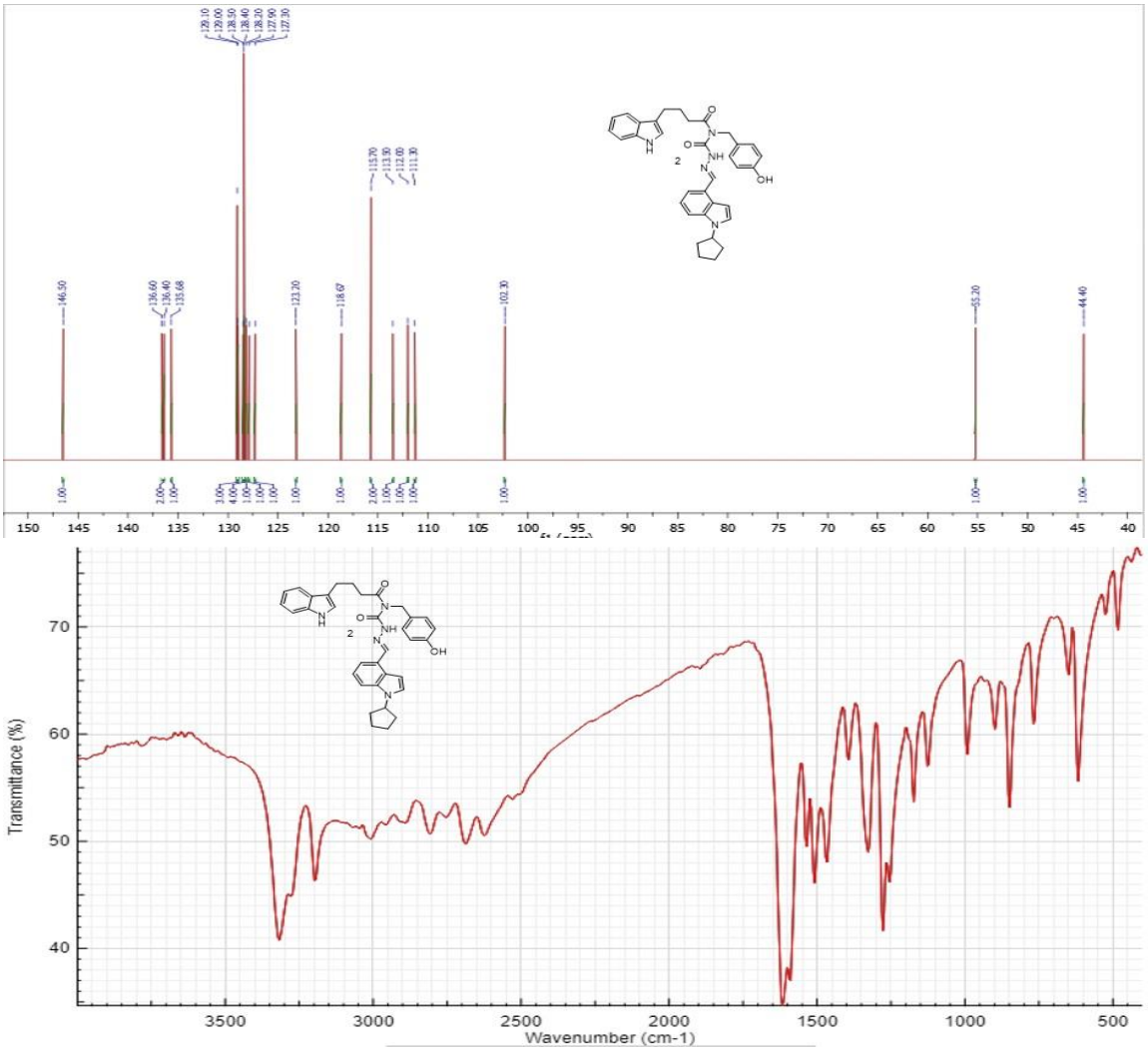
^{1}H NMR: δ 1.64-2.12 (10H, 1.73 (d, *J* = 13.0, Hz), 1.80 (d, *J* = 13.0 Hz), 1.92 (d, *J* = 13.7Hz), 2.02 (d, *J* = 13.7Hz), 1.99 (q, *J* = 7.4 Hz)), 2.49 (2H, t, *J* = 7.4 Hz), 2.69 (2H, t, *J* = 7.4 Hz), 4.73 (2H, s), 5.05 (1H, d, *J* = 8.1Hz), 6.53 (1H, t, *J* = 6.2 Hz), 6.72 (2H, d, *J* = 8.2, Hz), 6.917.42 (9H), 6.98 (d, *J* = 8.0 Hz), 7.07 (d, *J* = 8.0 Hz), 7.09 (d, *J* = 7.8 Hz), 7.14 (d, *J* = 8.2 Hz), 7.21 (d, *J* = 7.8 Hz), 7.30 (d, *J* = 6.2 Hz), 7.33 (t, *J* = 8.0 Hz), 7.37 (t, *J* = 0.5 Hz)), 7.62 (1H, d, *J* = 8.0 Hz), 7.95 (1H, d, *J* = 7.5, Hz), 8.21 (1H, s).

^{13}C NMR: δ 23.1 (2C, s), 26.2 (1C, s), 30.4 (1C, s), 32.8 (2C, s), 34.3 (1C, s), 44.4 (1C, s), 55.2 (1C, s), 102.3 (1C, s), 111.3 (1C, s), 112.0 (1C, s), 113.5 (1C, s), 115.7 (2C, s), 118.7 (1C, s), 123.2 (1C, s), 127.3 (1C, s), 127.9 (1C, s), 128.2 (1C, s), 128.3-128.6 (4C, 128.4 (s), 128.4 (s), 128.5 (s)), 128.9-129.2 (3C, 129.0 (s), 129.1 (s), 129.1 (s)), 135.7 (1C, s), 136.4 (1C, s), 136.6 (1C, s), 146.5 (1C, s), 155.2 (1C, s), 157.4 (1C, s), 169.8 (1C, s)

Mass(m/z):561

IR(KBr): υ/cm^{-1} = 3477-3328 (NH_2, NH), 3055 (CH aromatic), 2220 (CN), 1686 (2 CO), 1636 (C=C), 1625 (C=N).





Compound 03

^{1}H NMR: δ 1.99 (2H, quint, *J* = 7.4 Hz), 2.49 (2H, t, *J* = 7.4 Hz), 2.69 (2H, t, *J* = 7.4 Hz), 4.73 (2H, s), 6.72 (2H, d, *J* = 8.2Hz), 6.91-7.20 (5H, 6.98 (d, *J* = 8.0 Hz), 7.07 (d *J* = 8.0 Hz), 7.11 (dd, *J* = 8.0 Hz), 7.14 (d, *J* = 8.2Hz)), 7.27-7.42 (3H, 7.33 (t, *J* = 8.0 Hz), 7.36 (d, *J* = 0.5 Hz), 7.37 (t, *J* = 0.5 Hz)), 7.49 (1H, d, *J* = 8.0 Hz), 7.62 (1H, t, *J* = 8.0 Hz), 7.99 (1H, d, *J* = 7.8Hz),
8.22 (1H, s).

^{1}C NMR: δ 26.2 (1C, s), 30.4 (1C, s), 34.3 (1C, s), 44.4 (1C, s), 111.3-111.3 (2C, 111.3 (s), 111.3 (s)), 113.5 (1C, s), 113.9 (1C, s), 115.7 (2C, s), 118.7 (1C, s), 122.8 (1C, s), 123.2 (1C, s), 127.3 (1C, s), 128.0 (1C, s), 128.2 (1C, s), 128.3-128.6 (4C, 128.4 (s), 128.4 (s), 128.5 (s)), 128.9-129.2 (2C, 129.0 (s), 129.1 (s)), 136.3-136.5 (2C, 136.3 (s), 136.4 (s)), 136.6 (1C, s), 146.5 (1C, s), 155.2 (1C, s), 157.4 (1C, s), 169.8 (1C, s).
Mass(m/z):528
IR(KBr): υ/cm^{-1} = 3467-3338 (NH_2, NH), 3059 (CH aromatic), 1689 (CO), 1632 (C=C), 1620 (C=N).

Compound 04

^{1}H NMR: δ 1.99 (2H, quint, *J* = 7.4 Hz), 2.49 (2H, t, *J* = 7.4 Hz), 2.69 (2H, t, *J* = 7.4 Hz), 4.73 (2H, s), 6.37 (1H, d, *J* = 2.2Hz), 6.72 (2H, d, *J* = 8.2, Hz), 6.85-7.42 (8H), 6.91 (d, *J* = 7.7 Hz),
6.98 (d, *J* = 8.0,Hz), 7.07 (d, *J* = 8.0, Hz), 7.14 (d, *J* = 8.2 Hz), 7.24 (d, *J* = 2.2 Hz), 7.33 (d, *J* = 8.0 Hz), 7.37 (t, *J* = 0.5 Hz)), 7.55-7.68 (2H, 7.61 (d, *J* = 7.7, Hz), 7.62 (t, *J* = 8.0, Hz)), 8.23 (1H, s).

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[1] C NMR: δ 26.2 (1C, s), 30.4 (1C, s), 34.3 (1C, s), 44.4 (1C, s), 101.7 (1C, s), 111.3 (1C, s), 113.5 (1C, s), 115.7 (2C, s), 116.9 (1C, s), 118.7 (1C, s), 123.2 (1C, s), 124.7 (1C, s), 127.3 (1C, s), 127.8 (1C, s), 128.2 (1C, s), 128.3-128.6 (4C, 128.4 (s), 128.4 (s), 128.5 (s)), 129.1 (1C, s), 136.4 (1C, s), 136.6 (1C, s), 136.9 (1C, s), 139.5 (1C, s), 146.5 (1C, s), 155.2 (1C, s), 157.4 (1C, s), 169.8 (1C, s).

Mass(m/z):538 IR(KBr): υ/cm^{-2} = 3438-3322 (NH_2, NH), 3052 (CH aromatic), 1685 (CO), 1630 (C=C), 1628 (C=N).

Compound 05

^{1}H NMR: δ 1.99 (2H, quint, *J* = 7.4 Hz), 2.49 (2H, t, *J* = 7.4 Hz), 2.69 (2H, t, *J* = 7.4 Hz), 3.70 (3H, s), 4.73 (2H, s), 6.54 (1H, t, *J* = 6.4 Hz), 6.72 (2H, d, *J* = 8.2, Hz), 6.91-7.42 (9H, 6.98 (d, *J* = 8.0 Hz), 7.07 (d, *J* = 8.0 Hz), 7.07 (d, *J* = 7.8, Hz), 7.14 (d, *J* = 8.2,Hz), 7.21 (t, *J* = 7.8 Hz), 7.26 (d, *J* = 6.4, Hz), 7.33 (d, *J* = 8.0, Hz), 7.37 (t, *J* = 0.5 Hz)), 7.62 (1H, t, *J* = 8.0, Hz),
7.94 (1H, d, *J* = 7.5, Hz), 8.20 (1H, s)
^{13}C NMR: δ 26.2 (1C, s), 30.4 (1C, s), 32.8 (1C, s), 34.3 (1C, s), 44.4 (1C, s), 102.3 (1C, s),
111.3 (1C, s), 112.0 (1C, s), 113.5 (1C, s), 115.7 (2C, s), 118.7 (1C, s), 123.2 (1C, s), 127.3 (1C, s), 128.2 (1C, s), 128.3-128.7 (5C, 128.4 (s), 128.4 (s), 128.5 (s), 128.6 (s)), 128.9-129.2 (3C, 129.0 (s), 129.1 (s), 129.1 (s)), 135.7 (1C, s), 136.4 (1C, s), 136.6 (1C, s), 146.5 (1C, s), 155.2 (1C, s), 157.4 (1C, s), 169.8 (1C, s) Mass(m/z):507
IR(KBr): υ/cm^{-1} = 3459-3331 (NH_2, 3NH), 3056 (CH aromatic), 1689 (CO), 1631 (C=C), 1623 (C=N)

Compound 06

^{1}H NMR: δ 1.49-1.67 (4H, 1.54 (quint, *J* = 7.3 Hz), 1.54 (quint, *J* = 7.3 Hz), 1.61 (td, *J* = 7.3, 3.4 Hz), 1.61 (t, *J* = 7.3 Hz)), 2.38-2.50 (2H, 2.44 (t, *J* = 7.4 Hz), 2.44 (t, *J* = 7.4 Hz)), 3.343.55 (3H, 3.41 (d, *J* = 13.3Hz), 3.44 (d, *J* = 13.3 Hz), 3.48 (t, *J* = 8.1, Hz)), 4.67-4.77 (2H, 4.72 (s), 4.72 (s)), 6.65-7.01 (6H, 6.72 (d, *J* = 8.2, Hz), 6.78 (d, *J* = 7.9, Hz), 6.83 (d, *J* = 8.0Hz),
6.86 (d, *J* = 7.9 Hz), 6.94 (d, *J* = 8.0 Hz)), 7.14 (2H, d, *J* = 8.2, Hz), 7.30 (1H, d, *J* = 8.4 Hz), 7.39-7.74 (8H, 7.46 (d, *J* = 7.7 Hz), 7.47 (d, *J* = 8.0 Hz), 7.49 (d, *J* = 8.4 Hz), 7.55 (d, *J* = 7.8, Hz), 7.63 (d, *J* = 8.0, Hz), 7.69 (d, *J* = 0.4 Hz)), 8.16 (1H, s).
^{13}C NMR: δ 26.2 (1C, s), 26.6 (1C, s), 34.3 (1C, s), 38.9 (1C, s), 44.4 (1C, s), 49.1 (1C, s),
110.0 (1C, s), 111.3 (1C, s), 115.7 (2C, s), 123.3 (1C, s), 123.8 (1C, s), 125.9 (1C, s), 127.3
(1C, s), 127.5 (1C, s), 127.7-127.8 (3C, 127.7 (s), 127.8 (s)), 128.2 (1C, s), 128.3-128.5 (5C,
128.4 (s), 128.4 (s), 128.4 (s)), 129.0 (1C, s), 132.6 (1C, s), 133.8 (1C, s), 134.1 (1C, s), 136.6
(1C, s), 139.2 (1C, s), 143.2 (1C, s), 148.4 (1C, s), 155.2 (1C, s), 157.4 (1C, s), 169.8 (1C, s). Mass(m/z):569
IR(KBr): υ/cm^{-1} = 3446-3323 (NH_2, 3NH), 3051 (CH aromatic), 1686 (CO), 1630 (C=C), 1626 (C=N).

Compound 07

8.4 Hz), 7.39-7.74 (8H, 7.46 (d, *J* = 7.7, Hz), 7.47 (d, *J* = 8.0, Hz), 7.49 (d, *J* = 8.4, Hz), 7.55 (d, *J* = 7.8 Hz), 7.63 (d, *J* = 8.0 Hz), 7.69 (d, *J* = 0.4 Hz)), 8.16 (1H, s).
^{33}C NMR: δ 26.2 (1C, s), 26.6 (1C, s), 34.3 (1C, s), 38.9 (1C, s), 44.4 (1C, s), 49.1 (1C, s),
110.0 (1C, s), 111.3 (1C, s), 115.7 (2C, s), 123.3 (1C, s), 123.8 (1C, s), 125.9 (1C, s), 127.3
(1C, s), 127.5 (1C, s), 127.7-127.8 (3C, 127.7 (s), 127.8 (s)), 128.2 (1C, s), 128.3-128.5 (5C,
128.4 (s), 128.4 (s), 128.4 (s)), 129.0 (1C, s), 132.6 (1C, s), 133.8 (1C, s), 134.1 (1C, s), 136.6
(1C, s), 139.2 (1C, s), 143.2 (1C, s), 148.4 (1C, s), 155.2 (1C, s), 157.4 (1C, s), 169.8 (1C, s). Mass(m/z):507
IR(KBr): υ/cm^{-1} = 3560-3328 (4NH), 3046 (CH aromatic), 2227, 2220 (2CN), 1691 (CO), 1633 (C=C), 1626 (C=N)

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[2] H NMR: δ 1.49-1.67 (4H, 1.54 (quint, *J* = 7.3 Hz), 1.54 (quint, *J* = 7.3 Hz), 1.61 (td, *J* = 7.3, 3.4 Hz), 1.61 (td, *J* = 7.3, 3.4 Hz)), 2.38-2.50 (2H, 2.44 (t, *J* = 7.4 Hz), 2.44 (t, *J* = 7.4 Hz)), 3.34-3.55 (3H, 3.41 (d, *J* = 13.3, 4.2 Hz), 3.44 (d, *J* = 13.3Hz), 3.48 (t, *J* = 8.1Hz)), 4.67-4.77 (2H, 4.72 (s), 4.72 (s)), 6.65-7.01 (6H, 6.72 (d, *J* = 8.2Hz), 6.78 (d, *J* = 7.9, Hz), 6.83 (d, *J* = 8.0, Hz), 6.86 (d, *J* = 7.9, Hz), 6.94 (d, *J* = 8.0, Hz)), 7.14 (2H, d, *J* = 8.2, Hz), 7.30 (1H, d, *J* =

[3] H NMR: δ 1.49-1.67 (4H, 1.54 (quint, *J* = 7.3 Hz), 1.54 (quint, *J* = 7.3 Hz), 1.61 (td, *J* = 7.3, Hz), 1.61 (t, *J* = 7.3 Hz)), 2.38-2.50 (2H, 2.44 (t, *J* = 7.4 Hz), 2.44 (t, *J* = 7.4 Hz)), 3.34-3.55 (3H, 3.41 (d, *J* = 13.3, Hz), 3.44 (d, *J* = 13.3 Hz), 3.48 (t, *J* = 8.1, Hz)), 4.67-4.77 (2H, 4.72 (s), 4.72 (s)), 6.65-7.01 (6H), 6.72 (d, *J* = 8.2 Hz), 6.78 (d, *J* = 7.9, Hz), 6.83 (d, *J* = 8.0, Hz), 6.86 (d, *J* = 7.9, Hz), 6.94 (d, *J* = 8.0, Hz)), 7.08-7.34 (4H, 7.14 (d, *J* = 8.2, Hz), 7.18 (d, *J* = 8.4, Hz), 7.28 (t, *J* = 8.4Hz)), 7.50-7.61 (2H, 7.56 (t, *J* = 1.6 Hz), 7.56 (t, *J* = 0.5 Hz)), 8.22 (1H, s).

Compound 08

^{1}H NMR: δ 1.49-1.67 (4H, 1.54 (quint, *J* = 7.3 Hz), 1.54 (quint, *J* = 7.3 Hz), 1.61 (t, *J* = 7.3, 3.4 Hz), 1.61 (t, *J* = 7.3Hz)), 2.16 (3H, s), 2.38-2.50 (2H, 2.44 (t, *J* = 7.4 Hz), 2.44 (t, *J* = 7.4 Hz)), 3.34-3.55 (3H), 3.41 (d, *J* = 13.3 Hz), 3.44 (d, *J* = 13.3 Hz), 3.48 (t, *J* = 8.1 Hz)), 4.674.77 (2H, 4.72 (s), 4.72 (s)), 6.65-7.04 (7H, 6.72 (d, *J* = 8.2 Hz), 6.78 (d, *J* = 7.9, Hz), 6.83 (d, *J* = 8.0 Hz), 6.86 (d, *J* = 7.9 Hz), 6.94 (d, *J* = 8.0, Hz), 6.98 (d, *J* = 8.2Hz)), 7.14 (2H, d, *J* = 8.2Hz), 7.28-7.40 (2H, 7.34 (t, *J* = 8.2Hz), 7.34 (t, *J* = 1.6 Hz)), 7.49 (1H, t, *J* = 0.5 Hz), 8.21 (1H, s)

^{4}C NMR: δ 21.3 (1C, s), 26.2 (1C, s), 26.6 (1C, s), 34.3 (1C, s), 38.9 (1C, s), 44.4 (1C, s), 49.1 (1C, s), 110.0 (1C, s), 111.4 (1C, s), 115.7 (2C, s), 119.9 (1C, s), 123.3 (1C, s), 123.8 (1C, s), 127.3-127.3 (2C, 127.3 (s), 127.3 (s)), 128.2 (1C, s), 128.3-128.5 (3C, 128.4 (s), 128.4 (s)), 129.6 (1C, s), 132.6 (1C, s), 134.8 (1C, s), 136.4 (1C, s), 136.6 (1C, s), 143.2 (1C, s), 148.4 (1C, s), 155.2 (1C, s), 157.4 (1C, s), 169.8 (1C, s) Mass(m/z):572

IR(KBr): υ/cm^{-1} = 3487-3335 (2NH), 3055 (CH aromatic), 2225 (CN), 1685 (CO), 1630 (C=C), 1623 (C=N).

Compound 09

IR(KBr): υ/cm^{-1} = 3592-3330 (OH, 2NH), 3058 (CH aromatic), 2223 (CN), 1688 (CO), 1633 (C=C), 1620 (C=N) Compound 10:

^{1}H NMR: δ 1.49-1.67 (4H, 1.54 (quint, *J* = 7.3 Hz), 1.54 (quint, *J* = 7.3 Hz), 1.61 (t, *J* = 7.3 Hz), 1.61 (td, *J* = 7.3, Hz)), 2.38-2.50 (2H, 2.44 (t, *J* = 7.4 Hz), 2.44 (t, *J* = 7.4 Hz)), 3.34-3.55 (3H, 3.41 (d, *J* = 13.3 Hz), 3.44 (d, *J* = 13.3 Hz), 3.48 (t, *J* = 8.1Hz)), 3.66 (3H, s), 4.67-4.77 (2H, 4.72 (s), 4.72 (s)), 6.29 (1H, d, *J* = 8.3 Hz), 6.65-7.20 (9H, 6.72 (d, *J* = 8.2 Hz), 6.78 (d *J* = 7.9 Hz), 6.83 (d, *J* = 8.0 Hz), 6.86 (d, *J* = 7.9 Hz), 6.94 (d, *J* = 8.0 Hz), 7.03 (t, *J* = 1.7, Hz), 7.14 (d, *J* = 8.2, Hz)), 7.40 (1H, t, *J* = 0.5 Hz), 8.11-8.24 (2H, 8.17 (t, *J* = 8.3, Hz), 8.19 (s)

^{13}C NMR: δ 26.2 (1C, s), 26.6 (1C, s), 34.3 (1C, s), 38.9 (1C, s), 44.4 (1C, s), 49.1 (1C, s), 56.0 (1C, s), 94.8 (1C, s), 110.0 (1C, s), 114.3 (1C, s), 115.7 (2C, s), 120.2 (1C, s), 123.3 (1C, s), 123.8 (1C, s), 127.3-127.3 (2C, 127.3 (s), 127.3 (s)), 128.2 (1C, s), 128.3-128.5 (3C, 128.4 (s), 128.4 (s)), 132.5-132.7 (2C, 132.6 (s), 132.6 (s)), 136.6 (1C, s), 143.2 (1C, s), 148.4 (1C, s), 155.2 (1C, s), 157.4 (1C, s), 158.1 (1C, s), 169.8 (1C, s).

Mass(m/z):521

IR(KBr): υ/cm^{-1} = 3487-3335 (2NH), 3058 (CH aromatic), 2228 (CN), 1689 (CO), 1633 (C=C), 1622 (C=N).

Anti-Inflammatory Activity

In the study, ten synthesized compounds (5a–l) and diclofenac as a reference drug were evaluated for their in vivo anti-inflammatory activity using a modified carrageenan-induced rat paw edema model, as described by Winter et al. [12]. The results of the anti-inflammatory potential of these compounds are presented in Table 02.

Table 02. Anti-inflammatory activity of synthesized compounds on carrageenan-induced paw edema in rats .

Compound	**Dose (mg/kg) p.o**	**Edema 1h (mL) (%inhib.)**	**Edema 2 h (mL) (%inhib.)**	**Edema 3 h (mL) (%inhib.)**	**Edema 4 h (mL (%inhib.)**
Negative control (vehicle)		1.38 ± 0.15	2.22 ± 0.24	3.05 ± 0.18	3.20 ± 0.25
5a		1.33 ± 0.05 (3.50)	1.98 ± 0.20 (10.79)	2.52 ± 0.19 (17.63)	2.42 ± 0.24 * (24.40)
5b		1.09 ± 0.26 (20.87)	1.95 ± 0.07 (12.37)	2.45 ± 0.15 * (19.92)	2.38 ± 0.20 * (25.60)
5c		1.00 ± 0.14 (27.50)	**1.52 ± 0.14 *** (31.63)	**2.29 ± 0.04 *** (24.95)	**2.32 ± 0.16 *** (27.53)
5d		1.21 ± 0.14 (12.55)	1.95 ± 0.07 (12.29)	2.82 ± 0.04 (7.81)	2.84 ± 0.10 (11.05)

[4] C NMR: δ 26.2 (1C, s), 26.6 (1C, s), 34.3 (1C, s), 38.9 (1C, s), 44.4 (1C, s), 49.1 (1C, s), 110.0 (1C, s), 112.8 (1C, s), 115.7 (2C, s), 118.4 (1C, s), 121.1 (1C, s), 123.3 (1C, s), 123.8 (1C, s), 127.3-127.3 (2C, 127.3 (s), 127.3 (s)), 128.2 (1C, s), 128.3-128.5 (3C, 128.4 (s), 128.4 (s)), 131.7 (1C, s), 132.6 (1C, s), 136.4 (1C, s), 136.6 (1C, s), 143.2 (1C, s), 148.4 (1C, s), 155.2 (1C, s), 157.4 (1C, s), 169.8 (1C, s).

Mass(m/z):523

5e	50	0.86 ± 0.14 (37.88)	1.85 ± 0.12 (16.72)	2.69 ± 0.13 (11.90)	2.64 ± 0.18 (17.41)
5f		1.18 ± 0.18 (14.60)	1.93 ± 0.20 (13.42)	2.67 ± 0.26 (12.66)	2.66 ± 0.20 (16.84)
5g		1.23 ± 0.12 (10.86)	1.76 ± 0.15 (20.84)	2.66 ± 0.14 (12.99)	2.58 ± 0.15 (19.45)
5h		0.92 ± 0.17 (33.78)	2.09 ± 0.18 (6.00)	2.63 ± 0.14 (13.86)	**2.03 ± 0.17** * (28.05)
5i		1.01 ± 0.16 (26.90)	1.97 ± 0.16 (11.39)	2.62 ± 0.26 (14.19)	**2.35 ± 0.25** * (26.59)
5j		0.95 ± 0.15 (31.10)	1.81 ± 0.14 (18.59)	2.67 ± 0.19 (12.66)	**2.32 ± 0.20** (27.53)
5k		1.27 ± 0.11 (8.08)	2.07 ± 0.09 (7.05)	2.59 ± 0.10 (15.34)	2.60 ± 0.19 (18.61)
5l		1.10 ± 0.07 (20.63)	1.99 ± 0.08 (10.64)	2.57 ± 0.06 (15.99)	2.74 ± 0.15 (14.23)
Diclofenac (reference drug)		1.21 ± 0.17 (12.30)	**1.46 ± 0.16** * (34.33)	**1.95 ± 0.12** * (36.03)	**2.34 ± 0.10** * (26.96)

*Statistically significant

According to the provided information, when compared to the control negative group, all the synthesized compounds (5a–l) administered at a dose of 50 mg/kg bw showed a reduction in edema volume. The group treated with diclofenac (20 mg/kg bw), a non-selective NSAID, exhibited a significant reduction in edema volume, particularly at 2, 3, and 4 hours after the administration of λ-carrageenan. One hour after inducing inflammation, compounds 5b (20.87%), 5c (27.50%), 5e (37.88%), 5h (33.78%), 5i (26.90%), 5j (31.10%), and 5l (20.63%) demonstrated better anti-inflammatory activity than the diclofenac group, although the results were not statistically significant. At 2 and 3 hours after the onset of inflammation, compounds 5c (31.63%, 24.95%) and 5b (19.92%) significantly reduced paw edema. Moreover, compounds 5a (24.40%), 5b (25.60%), 5c (27.53%), 5h (28.05%), 5i (26.59%), and 5j (27.53%) exhibited significant edema inhibition at 4 hours after inflammation occurred, with compounds 5c, 5h, and 5j demonstrating better anti-inflammatory activity than diclofenac (26.96%)

Chemistry

In the synthesis of (4(1H-indol-3-yl)butanoyl)-2-(1H-4-indol-4-ylidene)-N-(4hydroxybenzyl)hydrazine-1-carboxamide 5a–l, as depicted in Scheme 1, the condensation of hydrazides 4 was carried out under reflux conditions using substituted aldehydes in the presence of an alcohol medium. The resulting compounds were purified and subjected to physico-chemical characterization, including melting points (m.p.), infrared spectra (IR), nuclear magnetic resonance (1H-NMR, 13C-NMR, and 19F-NMR), and mass spectrometry (MS). The synthetic procedures, physico-chemical analysis, and spectral data of the synthesized compounds are provided in the Materials and Methods section. The compounds 5(a-l) were characterized using physicochemical and spectral data. The IR spectra of the synthesized compounds 5a–l exhibited characteristic =CH- stretching bands in the range of 3120–2920 cm-1.

Result and Discussion

Based on the design of the molecules, the synthesized compounds (5a–l) were screened for their anti-inflammatory activity. The structures of these compounds were confirmed through spectral analysis, including IR, 1H-NMR, 13C-NMR, and MS. In the IR spectrum, the absence of NH2 bands around 3200 ppm indicated the establishment of the synthesized structures. The presence of characteristic signals of indole proton in the 1H-NMR spectra further confirmed the formation of the desired compounds. The 13C-NMR spectra showed the presence of signals corresponding to the carbon atoms of the aromatic rings in the aromatic region, as well as aliphatic signals corresponding to the carbon atoms. The MS spectra supported the structures of the synthesized compounds through the presence of characteristic molecular peaks. In vivo evaluations were conducted to assess the anti-inflammatory, analgesic, and ulcerogenic potential of the synthesized compounds. Additionally, in silico molecular modeling was performed to study their interaction with cyclooxygenases. The administration of carrageenan into the hind paw of rats induced inflammation, characterized by edema, erythema, and hyperalgesia. The compounds' mechanism of action was attributed to the inhibition of histamine, 5-hydroxytriptamine, and bradykinin release in the initial edematous phase, followed by the inhibition of prostaglandin production in the damaged tissue. During the first phase of edema development (1 hour after inflammation), compounds 5b, 5c, 5d, 5e, 5f, 5h, 5i, 5j, and 5l exhibited increased edema inhibition compared to the negative control group and performed better than diclofenac. At 4 hours after inflammation, compounds 5a, 5b, 5c, 5h, 5i, and 5j showed significant reduction in inflammation compared to the negative control group.

Notably, compounds 5b, 5j, 5h, 5c, and the combination of 5h, 5i, and 5j demonstrated the most pronounced reduction in edema volume. Compound 5c exhibited the most significant edema inhibition at 2, 3, and 4 hours after inflammation induction compared to the negative control group.
Structure-activity relationship studies indicated that the presence of an unsubstituted phenyl group inhibited both COX-1 and COX-2, while the presence of methoxy and halogen groups at the 4th position of the 6-phenyl ring contributed to selective COX-2 inhibitory activity.

Conclusions

The present study focused on the synthesis, characterization, and evaluation of the antiinflammatory and analgesic properties of a series of (4(1H-indol-3-yl)butanoyl)-2-(1H-4indol-4-ylidene)-N-(4-hydroxybenzyl)hydrazine-1-carboxamide derivatives labeled as 5a–l. Various spectroscopic techniques, including IR, 1H-NMR, 13C-NMR, 19F-NMR, and MS, were employed to confirm the structural integrity of the synthesized compounds.The antiinflammatory activity of these derivatives was assessed in vivo using a rat model of acute inflammation induced by λ-carrageenan. Additionally, molecular docking studies were conducted to gain insights into the potential mechanism of anti-inflammatory activity at the molecular level.The results of the study indicated that the in vivo anti-inflammatory findings were consistent with the in silico (molecular docking) predictions. Among the synthesized compounds, 5c exhibited the most promising anti-inflammatory activity. This suggests that compound 5c may have a potential therapeutic application in the treatment of pain and inflammatory diseases.The conclusion drawn from this study is that these 2,6-diarylimidazo[2,1-b][1,3,4]thiadiazole derivatives, particularly compound 5c, warrant further investigation and development for their potential use in therapy. Future studies could explore their efficacy, safety profile, and mechanism of action in more detail, with the ultimate goal of developing novel treatments for conditions characterized by pain and inflammation .

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