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Synthesis and characterization of some new cinnoline derivatives for its biological iterest

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Abstract
A series of pyrazolo[4,3-c]cinnoline derivatives was synthesized, characterized and evaluated for anti-inflammatory and antibacterial activity. Test compounds that exhibited good anti-inflammatory activity were further screened for their ulcerogenic and lipid peroxidation activity. Compounds 4d and 4l showed promising anti-inflammatory activity with reduced ulcerogenic and lipid peroxidation activity when compared to naproxen. Docking results of these two compounds with COX-2 (PDB ID: 1CX2) also exhibited a strong binding profile. Among the test derivatives, compound 4i displayed significant antibacterial property against gram-negative (Escherichia coli and Pseudomonas aeruginosa) and gram-positive (Staphylococcus aureus) bacteria. However, compound 4b emerged as the best dual anti inflammatory antibacterial agent in the present study.

Keywords: Cinnoline, docking, anti-inflammatory, antibacterial activity, pyrazolo etc.

Introduction
Non-steroidal anti-inflammatory drugs (NSAIDs) are used to treat inflammation because of their immanent capability of inhibiting cyclooxygenases (COXs), the key enzymes involved in the biosynthesis of prostaglandin from arachidonic acid. However, the chronic use of NSAIDs may elicit marked gastrointestinal (GI) irritation and ulceration due to their unwanted inhibition of the COX-1 enzyme and the desired blockade of the COX-2 enzyme. The recognition of COX-2 as a potential target has influenced the development of drugs that do not cause GI disorders but retain their clinical efficacy as anti-inflammatory agents [1]. However, all the selective COX-2 inhibitors are associated with an increased risk of cardiovascular events. Some recent studies suggest that, among the NSAIDs, naproxen is safe in terms of cardiovascular toxicity [2, 3]. Markedly, inflammation and infection are not equal, even in the case where infection is the primary cause of the inflammation. Furthermore, the inflammatory response elicited by an invading organism can result in host damage, increase the availability of nutrients and mediate access to host tissues. In addition, inflammation may cause accumulation of fluid in the injured area, whichmay promote bacterial growth [4]. Other reports disclosed that NSAIDs may enhance the progression of bacterial infection [5, 6]. Hence, a dual anti-inflammatory antibacterial agent with an improved safety profile is required for improved therapeutic benefits and better patient compliance. Pyridazine-containing compounds have been reported to exhibit anti-inflammatory [7e, 10] and antimicrobial activity [11]. Cinnoline, a benzfused pyridazine, has been utilized for the development of potent anti-inflammatory [12, 13] and antibacterial agents [14], such as cinnopentazon and cinoxacin, respectively. Dual effects have also been reported as a property of cinnoline derivativees [15]. Furthermore, pyrazole-containing compounds have also been documented as anti-inflammatory antibacterial agents [16e, 18]. These findings prompted us to construct a new molecular framework that contains both the cinnoline and pyrazole ring systems in the same matrix with the hope of developing a compound that possesses both anti-inflammatory and antibacterial activity.

Material and Methods
Chemistry
Melting points, which are uncorrected, were determined using open capillary tubes on an electrical melting point apparatus.Required reagents and chemicals were purchased from E Merck (India) Ltd., S. D. Fine (India) and Qualigens (India). Silica gel 60e120 mesh LR (25049 K05) was used for column chromatography. IR (KBr) spectra were recorded on a Shimadzu spectrometer (vmax in cm1).

1 H NMR and 13C NMR spectra were recorded in DMSO/CDCl3 on a Bruker 300 MHz and 75 MHz spectrometer, respectively, using tetramethylsilane (TMS) as the internal reference (chemical shift was measured in d ppm). Mass spectra (DART-MS) were measured on a JEOL-AccuTOF JMS-T100LS mass spectrometer with a DART (Direct Analysis in Real Time) source. The progress of the reaction and the purity of the synthesized compounds were verified on ascending thin layer chromatography (TLC) plates coated with silica gel G (Merck). An iodine chamber and UV lamp were used for the visualization of the TLC spots.

Ethyl-2-[2-(3-chloro-4-fluorophenyl)hydrazinylidene]-3-oxobutanoate (2):
Compound 2 was synthesized from 3-chloro-4-fluoroaniline (1) according to the procedure reported in the literature [19, 20].
Pale yellow; m.p. 88 C; IR (KBr) cm1: 3448 (NeH), 1742 (C]O), 1690 (C]O), 1526 (C]C), 1198 (CeF), 744 (CeCl). 1 H NMR (300 MHz, CDCl3): d (ppm) 1.28e1.31 (t, 3H, CH3 ester, J ¼ 12 Hz), 2.56 (s, 3H, COCH3), 4.36e4.39 (q, 2H, CH2 ester, J ¼ 9 Hz), 7.42e7.55 (m, 3H, CHeAr), 14.2 (s, 1H, NH).

3-acetyl-7-chloro-6-fluorocinnolin-4(1H)-one (3)
Powdered anhydrous AlCl3 (5 g, 37 mmol) was added to a solution of compound 2 (4 g, 13 mmol) in chlorobenzene (30 ml); the reaction mixture was kept cool during this addition. The reaction mixture was then refluxed for 16 h under anhydrous conditions. It was then cooled and dilute HCl (10% w/w, 200 ml) was added. The resulting mixture was then heated on a water bath and the excess chlorobenzene was removed by steam distillation. The separated solid was filtered, washed with 5% NaOH solution and then with water. The air dried product was again washed with benzene:ethanol (1:1) and re-crystallized with acetic acid:methanol (4:6) to obtain a pale yellow colored crystalline product.
Pale yellow; m.p.: 192 C; IR (KBr) cm1: 3436 (NeH), 2925, 2840 (CeH), 1712 (C]O), 1659 (C]O), 1522 (C]C), 1215 (CeF), 746 (CeCl). 1 H NMR (300 MHz, CDCl3) d: 2.61 (s, 3H, CH3), 7.29 (s, 1H, C5eHCinnoline), 7.54 (s, 1H, C8eHCinnoline), 13.8 (s, 1H, NH, D2OExchangeable). 13C NMR (75 MHz, CDCl3) d: 24.0 (CH3), 115.8, 117.1, 126.3, 129.3, 129.6, 151.7, 159.5, 176.8 (C]O), 194.6 (C]O). DART-MS m/z: 240.18 (Mþ), 242.18 (Mþ þ 2). Anal. Calcd for C10H6ClFN2O2: C, 49.92, H, 2.51, N, 11.64; Found: C, 49.79, H, 2.52, N, 11.60.

General procedure for the synthesis of compounds (4a-m):
Equimolar quantities of compound 3 (0.5 g, 2 mmol) and aromatic acid hydrazide (benzoic acid hydrazide, phenyl acetic acid hydrazide, phenoxy acetic acid hydrazide, p-methyl benzoic acid hydrazide, o,p-dichloro benzoic acid hydrazide, o,p-dichlorophenoxyacetic acid hydrazide, p-chloro benzoic acid hydrazide, p-fluoro benzoic acid hydrazide, p-nitro benzoic acid hydrazide, pamino benzoic acid hydrazide, m,p-dimethoxy benzoic acid hydrazide, p-methoxy benzoic acid hydrazide, 1-naphthalyl acetic acid hydrazide) were mixed on a magnetic stirrer with continuous stirring for 0.5 h, in anhydrous 1,4-dioxane (25 ml) containing 0.3 ml of concentrated hydrochloric acid. The resulting mixture was boiled under reflux for 12e18 h. After completion of reaction (monitored by TLC) the reaction mixture was allowed to cool, concentrated under reduced pressure and poured in to ice-cold water with constant stirring. The product obtained was filtered, dried and then purified through column chromatography (Hexane/ AcOEt 4:1) to afford solid product. The molecular weight and percentage yield of final compounds are presented in Table1.

Table 1: Physicochemical constants of the synthesized compounds (4a-m)

Compd. no.	R	X	Molecular formula	m.p., °C	Yield[a], %	Rf.[b]
4a	Phenyl–	–	$C_{17}H_{10}ClFN_4O$	212–213	61	0.44
4b	Phenyl–	CH_2	$C_{18}H_{12}ClFN_4O$	185–187	66	0.50
4c	Phenyl–	OCH_2	$C_{18}H_{12}ClFN_4O_2$	159	52	0.46
4d	*p*-methylphenyl–	–	$C_{18}H_{12}ClFN_4O$	197–198	69	0.52
4e	*o,p*-dichlorophenyl–	–	$C_{17}H_8Cl_3FN_4O$	226–228	65	0.61
4f	*o,p*-dichlorophenyl–	OCH_2	$C_{18}H_{10}Cl_3FN_4O_2$	150–152	58	0.56
4g	*p*-chlorophenyl–	–	$C_{17}H_8Cl_2FN_4O$	202	64	0.58
4h	*p*-fluorophenyl–	–	$C_{17}H_9ClF_2N_4O$	167–169	57	0.60
4i	*p*-nitrophenyl–	–	$C_{17}H_9ClFN_5O_3$	239	62	0.58
4j	*p*-aminophenyl–	–	$C_{17}H_{11}ClFN_5O$	180–182	49	0.62
4k	*m,p*-dimethoxyphenyl–	–	$C_{19}H_{14}ClFN_4O_3$	206	65	0.68
4l	*p*-methoxyphenyl–	–	$C_{18}H_{12}ClFN_4O_2$	221–222	60	0.64
4m	1-naphthalyl–	CH_2	$C_{22}H_{14}ClFN_4O$	165	68	0.56

[a] After recovery from hexane:AcOEt (4:1).
[b] Toluene:ethylacetate:formic acid (6:3.5:0.5).

(7-chloro-8-fluoro-3-methyl-1H-pyrazolo[4,3-c]cinnolin1-yl)(phenyl)methanone (4a): Pale yellow. IR (KBr) cm1: 1650 (C]O),1619 (C]N),1534 (C]C), 1506 (N]N), 1249 (CeF), 752 (CeCl). 1 H NMR (300 MHz, CDCl3): d (ppm) 2.27 (s, 3H, CH3), 7.14e7.20 (m, 5H, HPhenyl), 7.22 (s, 1H, C5e HCinnoline), 7.49 (s, 1H, C8eHCinnoline). 13C NMR (75 MHz, CDCl3): d (ppm) 12.2 (CH3), 115.3, 117.1, 126.3, 128.8, 129.2, 131.3, 135.4, 138.7, 139.5, 151.5, 159.4, 169.2 (C]O). Anal. Calcd for C17H10ClFN4O: C, 59.92; H, 2.96; N, 16.44. Found: C, 60.10; H, 2.95; N, 16.40. DART-MS m/z: 340.12 (Mþ), 342.12 (Mþ þ 2).

1-(7-chloro-8-fluoro-3-methyl-1H-pyrazolo[4,3-c] cinnolin-1-yl)-2-phenylethanone (4b): Pale yellow. IR (KBr) cm1: 1659 (C]O),1622 (C]N),1528 (C]C), 1511 (N]N), 1226 (CeF), 744 (CeCl). 1 H NMR (300 MHz, CDCl3): d (ppm) 2.35 (s, 3H, CH3), 4.27 (s, 2H, CH2), 7.18e7.25 (m, 5H, HPhenyl), 7.29 (s, 1H, C5eHCinnoline), 7.51 (s, 1H, C8eHCinnoline). 13C NMR (75 MHz, CDCl3): d (ppm) 12.9 (CH3), 39.6, 115.4, 117.2, 126.7, 128.2, 129.5, 131.1, 135.1, 138.6, 139.7, 151.3, 159.6, 168.8 (C]O). Anal. Calcd for C18H12ClFN4O: C, 60.94; H, 3.41; N,15.79. Found: C, 61.14; H, 3.43; N, 15.72. DART-MS m/z: 354.14 (Mþ), 356.14 (Mþ þ 2).

1-(7-chloro-8-fluoro-3-methyl-1H-pyrazolo[4,3-c]cinnolin-1-yl)-2-phenoxyethanone (4c): Fine yellow. IR (KBr) cm1: 1664 (C]O), 1604 (C]N), 1530 (C]C), 1494 (N]N), 1219 (CeF), 1046 (CeOeC), 750 (CeCl). 1 H NMR (300 MHz, CDCl3): d (ppm) 2.37 (s, 3H, CH3), 4.67 (s, 2H, CH2), 6.94e7.10 (m, 5H, HPhenyl), 7.33 (s, 1H, C5eHCinnoline), 7.54 (s, 1H, C8eHCinnoline). 13C NMR (75 MHz, CDCl3): d (ppm) 12.4 (CH3), 61.6, 114.8, 116.9, 127.0, 127.8, 129.6, 130.8, 131.0, 135.3, 137.9, 139.4, 151.7, 159.4, 168.3 (C]O). Anal. Calcd for C18H12ClFN4O2: C, 58.31; H, 3.26; N, 15.11. Found: C, 58.42; H, 3.25; N, 15.13. DARTMS m/z: 370.26 (Mþ), 372.26 (Mþ þ 2).

(7-chloro-8-fluoro-3-methyl-1H-pyrazolo[4,3-c]cinnolin1-yl)(4-methylphenyl)methanone (4d): Greenish yellow. IR (KBr) cm1: 1652 (C]O), 1624 (C]N), 1533 (C]C), 1509 (N]N), 1238 (CeF), 743 (CeCl). 1 H NMR (300 MHz, CDCl3): d (ppm) 2.38 (s, 3H, CH3), 3.49 (s, 3H, CH3), 7.32 (s, 1H, C5eHCinnoline), 7.51 (s, 1H, C8eHCinnoline), 7.74e7.77 (d, 2H, J ¼ 7.2 Hz, C3,5eHPhenyl), 8.58e8.61 (d, 2H, J ¼ 7.5 Hz, C2,6eHPhenyl). 13C NMR (75 MHz, CDCl3): d (ppm) 12.6 (CH3), 24.4, 115.3, 117.5, 126.1, 129.4, 129.6, 131.3, 135.7, 136.0, 139.3, 151.4, 159.2, 168.2 (C]O). Anal. Calcd for C18H12ClFN4O: C, 60.94; H, 3.41; N, 15.79. Found: C, 61.15; H, 3.42; N, 15.76. DART-MS m/z: 354.10 (Mþ), 356.10 (Mþ þ 2).

(7-chloro-8-fluoro-3-methyl-1H-pyrazolo[4,3-c]cinnolin1-yl)(2,4dichlorophenyl) methanone (4e): Pale yellow. IR (KBr) cm1: 1650 (C]O), 1612 (C]N), 1537 (C]C), 1504 (N]N), 1230 (CeF), 742 (CeCl). 1H NMR (300 MHz, CDCl3): d (ppm) 2.34 (s, 3H, CH3), 6.85e6.87 (d, 1H, HPhenyl, J ¼ 6.3 Hz), 7.16e7.24 (m, 2H, HPhenyl), 7.38 (s, 1H, C5eHCinnoline), 7.59 (1H, C8eHCinnoline). 13C NMR (75 MHz, CDCl3): d (ppm) 12.1 (CH3), 115.2, 117.4, 126.5, 128.6, 129.2, 129.5, 130.8, 135.2, 136.5, 139.4, 151.7, 159.3, 168.8 (C]O). Anal. Calcd for C17H8Cl3FN4O: C, 49.85; H, 1.97; N, 13.68. Found: C, 49.92; H, 1.98; N, 13.71. DARTMS m/z: 409.16 (Mþ), 411.16 (Mþ þ 2), 415.16 (Mþ þ 6).

1-(7-chloro-8-fluoro-3-methyl-1H-pyrazolo[4,3-c] cinnolin-1-yl)-2-(2,4-dichlorophenoxy) ethanone (4f): Pale yellow. IR (KBr) cm1: 1666 (C]O), 1604 (C]N), 1539 (C]C), 1498 (N]N), 1226 (CeF), 1058 (CeOeC), 755 (CeCl). 1 H NMR (300 MHz, CDCl3): d (ppm) 2.37 (s, 3H, CH3), 5.24 (s, 2H, CH2), 6.88e6.90 (d, 1H, HPhenyl, J ¼ 6.6 Hz), d 7.19e7.26 (m, 2H, HPhenyl), 7.40 (s, 1H, C5eHCinnoline), 7.58 (s, 1H, C8eHCinnoline). 13C NMR (75 MHz, CDCl3): d (ppm) 12.6 (CH3), 61.5, 114.9, 116.9, 127.1, 127.6, 129.5, 129.7, 130.8, 135.6, 136.2, 139.1, 151.9, 159.3, 168.7 (C]O). Anal. Calcd for C18H10Cl3FN4O2: C, 49.17; H, 2.29; N, 12.74. Found: C, 49.33; H, 2.31; N, 12.69. DART-MS m/z: 439.17 (Mþ), 441.16 (Mþ þ 2), 445.15 (Mþ þ 6).

(7-chloro-8-fluoro-3-methyl-1H-pyrazolo[4,3-c]cinnolin1-yl)(4-chlorophenyl)methanone (4g): Pale Yellow. IR (KBr) cm1: 1661 (C]O),1620 (C]N),1538 (C]C), 1511 (N]N), 1235 (CeF), 744 (CeCl). 1 H NMR (300 MHz, CDCl3): d (ppm) 2.37 (s, 3H, CH3), 7.38 (s, 1H, C5eHCinnoline), 7.45e7.47 (d, 2H, J ¼ 6.9 Hz, C3,5eHPhenyl), 7.52 (s, 1H, C8eHCinnoline), 7.82e7.84 (d, 2H, J ¼ 7.8 Hz, C2,6eHPhenyl). 13C NMR (75 MHz, CDCl3): d (ppm) 12.8 (CH3), 115.2, 118.1, 126.9, 128.6, 129.4, 129.7, 129.9, 131.7, 133.2, 137.1, 139.3, 152.3, 159.7, 169.4 (C]O). Anal. Calcd for C17H9Cl2FN4O: C, 54.42; H, 2.42; N, 14.93. Found: C, 54.59; H, 2.44; N, 14.90. DART-MS m/z: 375.06 (Mþ), 377.06 (Mþ þ 2), 379.06 (Mþ þ 4).

(7-chloro-8-fluoro-3-methyl-1H-pyrazolo[4,3-c]cinnolin1-yl)(4-fluorophenyl)methanone (4h): Greenish yellow. IR (KBr) cm1: 1650 (C]O), 1615 (C]N), 1532 (C]C), 1510 (N]N), 1222 (CeF), 752 (CeCl). 1 H NMR (300 MHz, CDCl3): d (ppm) 2.37 (s, 3H, CH3), 7.34 (s, 1H, C5eHCinnoline), 7.51 (s, 1H, C8eHCinnoline), 7.66e7.68 (d, 2H, J ¼ 7.2 Hz, C3,5eHPhenyl), 7.90e 7.93 (d, 2H, J ¼ 9.3 Hz, C2,6eHPhenyl). 13C NMR (75 MHz, CDCl3): d (ppm) 12.8 (CH3), 115.2, 117.4, 126.2, 129.6, 130.7, 131.3, 132.1, 135.3, 139.2, 151.0, 159.4, 168.1 (C]O). Anal. Calcd for C17H9ClF2N4O: C, 56.92; H, 2.53; N, 15.62. Found: C, 57.11; H, 2.55; N, 15.57. DART-MS m/z: 358.22 (Mþ), 360.22 (Mþ þ 2).

(7-chloro-8-fluoro-3-methyl-1H-pyrazolo[4,3-c]cinnolin1-yl) (4-nitrophenyl)methanone (4i): Pale yellow. IR (KBr) cm1: 1649 (C]O),1625 (C]N),1532 (C]C), 1506 (N]N), 1218 (CeF), 740 (CeCl). 1 H NMR (300 MHz, CDCl3): d (ppm) 2.30 (s, 3H, CH3), 7.31e7.33 (m, 2H, J ¼ 6.9 Hz, C2,6eHPhenyl), 7.47 (s,1H, C5eHCinnoline), 7.61 (s,1H, C8eHCinnoline), 7.86e7.88 (d, 2H, J ¼ 7.8 Hz, C3,5eHPhenyl). 13C NMR (75 MHz, CDCl3): d (ppm) 12.4 (CH3), 115.4, 116.7, 126.0, 129.1, 129.5, 131.4, 135.3, 139.4, 142.6, 151.3, 159.4,168.3 (C]O). Anal. Calcd for C17H9ClFN5O3: C, 52.93; H, 2.35; N, 18.16. Found: C, 53.13; H, 2.33; N, 18.20. DART-MS m/z: 385.29 (Mþ), 387.29 (Mþ þ 2).

(4-aminophenyl)(7-chloro-8-fluoro-3-methyl-1H-pyrazolo[4,3-c]cinnolin-1-yl) methanone (4j): Pale yellow. IR (KBr) cm1: 1654 (C]O),1618 (C]N),1527 (C]C), 1502 (N]N), 1221 (CeF), 749 (CeCl). 1 H NMR (300 MHz, CDCl3):d (ppm) 2.36 (s, 3H, CH3), 4.62 (s, 2H, NH2), 7.19e7.21 (d, 2H, J ¼ 6.3 Hz, C3,5eHPhenyl), 7.39 (s, 1H, C5eHCinnoline), 7.53 (s, 1H, C8e HCinnoline), 7.94e7.97 (d, 2H, J ¼ 7.8 Hz, C2,6eHPhenyl). 13C NMR (75 MHz, CDCl3): d (ppm) 12.2 (CH3), 115.8, 116.9, 126.5, 128.8, 129.3, 129.7, 131.5, 139.7, 149.8, 151.6, 159.0, 167.9 (C]O). Anal. Calcd for C17H11ClFN5O: C, 57.39; H, 3.12; N, 19.69. Found: C, 57.51; H, 3.13; N, 19.64. DART-MS m/z: 355.24 (Mþ), 357.24 (Mþ þ 2).

(7-chloro-8-fluoro-3-methyl-1H-pyrazolo[4,3-c]cinnolin1-yl)(3,4dimethoxyphenyl) methanone (4k): Pale yellow. IR (KBr) cm1: 1647 (C]O),1611 (C]N),1538 (C]C), 1497 (N]N), 1229 (CeF), 741 (CeCl). 1 H NMR (300 MHz, CDCl3): d (ppm) 2.39 (s, 3H, CH3), 3.78 (s, 6H, 2 OCH3), 7.15e7.17 (d, 1H, HPhenyl, J ¼ 6), 7.41 (s, 1H, C5eHCinnoline), 7.59 (s, 1H, C8eHCinnoline), 7.63e7.68 (m, 2H, HPhenyl). 13C NMR (75 MHz, CDCl3): d (ppm) 12.7 (CH3), 57.5, 115.4, 117.2, 117.6, 126.5, 128.4, 129.2, 130.3, 131.2, 134.8, 139.3, 152.6,

158.9, 168.2 (C]O). Anal. Calcd for C19H14ClFN4O3: C, 56.94; H, 3.52; N, 13.98. Found: C, 57.14; H, 3.51; N, 14.02. DART-MS m/z: 400.28 (Mþ), 402.28 (Mþ þ 2).

(7-chloro-8-fluoro-3-methyl-1H-pyrazolo[4,3-c]cinnolin1-yl)(4-methoxyphenyl) methanone (4l): Fine yellow. IR (KBr) cm1: 1644 (C]O),1623 (C]N),1536 (C]C), 1492 (N]N), 1226 (CeF), 752 (CeCl). 1 H NMR (300 MHz, CDCl3): d (ppm) 2.33 (s, 3H, CH3), 3.69 (s, 3H, OCH3), 6.96e7.04 (m, 2H, C3,5e HPhenyl), 7.44 (s, 1H, C5eHCinnoline), 7.64 (s, 1H, C8eHCinnoline), 7.85e 7.91 (m, 2H, C2,6eHPhenyl). 13C NMR (75 MHz, CDCl3): d (ppm) 12.3 (CH3), 58.3, 114.7, 117.6, 118.2, 126.2, 128.4, 129.5, 129.8, 132.0, 133.6, 138.5, 153.2, 159.6, 167.4 (C]O). Anal. Calcd for C18H12ClFN4O2: C, 58.31; H, 3.26; N,15.11. Found: C, 58.49; H, 3.27; N,15.14. DART-MS m/ z: 370.25 (Mþ), 372.25 (Mþ þ 2).

1-(7-chloro-8-fluoro-3-methyl-1H-pyrazolo[4,3-c]cinnolin1-yl)-2-(naphthalen-1-yl) ethanone (4m): Brownish yellow. IR (KBr) cm1: 1658 (C]O), 1620 (C]N), 1536 (C]C), 1492 (N]N), 1218 (CeF), 746 (CeCl). 1 H NMR (300 MHz, CDCl3): d (ppm) 2.39 (s, 3H, CH3), 4.06 (s, 2H, CH2), 7.14e7.22 (m, 7H, HNaphthalyl), 7.47 (s, 1H, C5eHCinnoline), 7.68 (s, 1H, C8eHCinnoline). 13C NMR (75 MHz, CDCl3): d (ppm) 12.5 (CH3), 44.6, 112.8, 115.5, 117.4, 121.3, 121.7, 123.3, 124.2, 124.8, 126.1, 129.5, 130.8, 134.6, 136.8, 138.2, 152.0, 159.4, 168.5 (C]O). Anal. Calcd for C22H14ClFN4O: C, 65.27; H, 3.49; N, 13.84. Found: C, 65.44; H, 3.47; N, 13.81. DART-MS m/z: 404.31 (Mþ), 406.31 (Mþ þ 2).

Biological activity

Animals: Adult Wistar albino rats (150e200 g) were used to measure anti-inflammatory, ulcerogenic and lipid peroxidation activity. The animals were organized randomly in groups of six and each animal was distinctly marked within its group. The animals were housed properly and provided food and water ad libitum except during the experiments. Standard drug and test compounds were administered p.o. as a suspension in a 0.5% w/v solution of carboxymethyl cellulose (CMC). The procedure and treatment schedule used for the animal experiments were approved by the Institutional Animal Ethics Committee (IAEC).

Anti-inflammatory activity

Anti-inflammatory activity was determined by the carrageenaninduced rat paw edema method [21]. Group I, which served as the control, received only 0.5% w/v carboxymethyl cellulose (CMC). Group II and the other groups received naproxen and the test compounds, respectively, at a dose level of 45 mg kg1.

The hind paw edema was induced in each rat by the sub-planter injection of 0.1 ml of 1% carrageenan solution in saline 1 h after the administration of the test compounds and standard drug. The volume of the paw edema (ml) was determined by a digital Plethysmometer (Panlab LE7500) before and after 3 and 4 h the carrageenan injection. The percent edema inhibition was calculated according to the following formula:

$$\text{Percent edema inhibition} = Vc - Vt/Vc \times 100$$

where Vc is the mean increase in paw volume in the absence of test compound (control) and Vt is the mean increase of paw volume after treatment with the test compound and standard drug. Results are presented as mean (+/-) S.E.M. (standard error of mean) of six rats in Table 2.

Acute ulcerogenicity study

Acute ulcerogenicity was determined according to the Cioli *et al*'s. method [22]. Animals were treated orally at 0 and 12 h with two equal doses of either naproxen or the test compounds (45 mg kg1 /dose) except the control group, which received only 0.5% CMC. After the drug treatment, the rats were fed a normal diet for 17 h and then sacrificed. The stomach was removed and opened along the greater curvature. It was examined with the aid of a microscope with a 4× magnifying lens. The mucosal damage in each stomach was assessed according to the following scoring system.

[Half (0.5) for redness; One (1.0) for spot ulcers; One and half (1.5) for hemorrhagic streaks; Two (2.0) for ulcers more than 3 but less than 5; Three (3.0) for more than five ulcers. The mean score of each treated group minus the mean score of control group was regarded as severity index of gastric mucosal damage.]

Lipid peroxidation study

The amount of lipid peroxidation was determined according to the Ohkawa *et al*'s. method [23]. After screening for ulcerogenic activity, the gastric mucosa was scraped with two glass slides, weighed (100 mg) and homogenized in 1.8 ml of a 1.15% ice-cold potassium chloride (KCl) solution. The homogenate was supplemented with 0.2 ml of 8.1% sodium dodecyl sulphate (SDS), 1.5 ml of acetate buffer (pH 3.5) and 1.5 ml of 0.8% thiobarbituric acid (TBA). The mixture was then heated at 95 C for 60 min. After cooling, the reactants were extracted with 5 ml of a mixture of n-butanol and pyridine (15:1 v/v), shaken vigorously for 1 min and centrifuged for10 min at 4000 rpm. The absorbance at 532 nm of the supernatant organic layer was measured on a UV spectrophotometer and the results were expressed as nmol MDA/100 mg tissue.

Acute toxicity study

The oral acute toxicity and approximate lethal dose (ALD50) of the promising compounds were determined in rat using a method previously described [24, 25]. Six animals were kept in each group. The compounds were administered orally in doses of 200, 300, 400 and 500 mg/kg. The toxic symptoms and mortality rates in each group were recorded 24 h after drug administration.

Assays for cyclooxygenase-2

Recombinant human COX-2 has been expressed in insect cell expression system. These enzymes have been purified by employing conventional chromatographic techniques. Enzymatic activities of COX-2 were measured according to the method of Copeland *et al.* (1994) [26], with slight modifications using a chromogenic assay based on the oxidation of N,N,N,N,-tetramethyl-p-phenylenediamine (TMPD) during the reduction of PGG2 to PGH2 (Egan *et al*., Pagels *et al*., 1983) [27]. Briefly, the assay mixture contained Trise HCl buffer (100 mM, pH 8.0), hematin (15 mM), EDTA (3 mM), enzyme (100 mg COX-2) and the test compound. The mixture was pre-incubated at 25 C for 1 min and then the reaction was initiated by the addition of arachidonic acid and TMPD, in total volume of 1 ml. The enzyme activity was determined by estimating the velocity of TMPD oxidation for the first 25 s of the reaction by following the increase in absorbance at 603 nm. A low rate of nonenzymatic oxidation

observed in the absence of COX-2 was subtracted from the experimental value while calculating the percent inhibition.

Antibacterial activity

All the test compounds were evaluated *in vitro* against Escherichia coli (NCTC 10418), Staphylococcus aureus (NCTC 65710) and Pseudomonas aeruginosa (NCTC 10662) using the cup plate method [28] with nutrient agar as the culture medium. A suspension of the bacterial spore for lawning was prepared with Tween 80 (0.01%) in normal saline. Liquid agar medium was poured (50 ml) into each Petri dish (15 cm diameter). The solid agar medium was inoculated with the bacterial suspension and the plates were then dried in an incubator at 37 C for 1 h. Wells were made by an agar punch on these seeded agar plates and solutions of the test compounds in DMSO were added into each well, which were labeled previously. DMSO was used as control. This procedure was repeated for each bacterial strain and the plates were then incubated at 37 C for 18e 24 h. Preliminary screening of the test compounds was carried out at a concentration of 100 mg/ml by measuring the diameter in millimeters of the zone of growth inhibition formed around each well. The minimum inhibitory concentration (MIC) was then determined. This was the lowest concentration at which there was no visible growth of the microorganism when using two-fold dilutions (50, 25, 12.5, 6.25, 3.12 mg/ml) for those compounds which showed a good antibacterial profile in the preliminary screening. The antibacterial activity of each compound (4aem) was compared against standard ciprofloxacin and the results are summarized in Table 5.

Table 2: Anti-inflammatory activity of the synthesized compounds.

Compd. number	Paw volume (mL) ± SEM			% inhibition ± SEM	
	0 h	3 h	4 h	3 h	4 h
4a	0.54 ± 0.042	1.08 ± 0.057	1.12 ± 0.058	61.31 ± 2.47	64.40 ± 2.55*
4b	0.55 ± 0.039	1.03 ± 0.035	1.07 ± 0.053	65.37 ± 2.16	67.94 ± 1.95*
4c	0.55 ± 0.038	1.32 ± 0.064	1.38 ± 0.055	44.58 ± 3.54	48.65 ± 4.70*
4d	0.56 ± 0.046	0.94 ± 0.044	0.97 ± 0.057	72.34 ± 3.51	74.67 ± 1.56
4e	0.54 ± 0.027	1.39 ± 0.075	1.49 ± 0.055	39.30 ± 3.53	41.18 ± 2.46*
4f	0.56 ± 0.039	1.64 ± 0.055	1.79 ± 0.049	22.64 ± 1.61	24.02 ± 4.14*
4g	0.54 ± 0.036	1.42 ± 0.053	1.55 ± 0.039	37.19 ± 2.55	37.95 ± 3.31*
4h	0.52 ± 0.038	0.98 ± 0.052	1.03 ± 0.052	67.36 ± 2.03	68.46 ± 2.50
4i	0.56 ± 0.046	1.63 ± 0.082	1.77 ± 0.068	24.01 ± 3.45	25.47 ± 2.54*
4j	0.53 ± 0.042	1.10 ± 0.053	1.15 ± 0.047	59.20 ± 2.32	61.52 ± 2.75*
4k	0.53 ± 0.027	1.06 ± 0.078	1.09 ± 0.059	62.49 ± 3.72	66.25 ± 2.18*
4l	0.54 ± 0.028	0.84 ± 0.028	0.86 ± 0.036	78.35 ± 2.02	80.01 ± 2.41
4m	0.52 ± 0.034	1.63 ± 0.053	1.78 ± 0.044	20.51 ± 2.93	22.13 ± 2.07*
Naproxen	0.54 ± 0.15	0.84 ± 0.018	0.85 ± 0.020	78.84 ± 1.39	81.23 ± 1.19
Control	0.55 ± 0.006	1.95 ± 0.015	2.17 ± 0.018	–	–

Data were given in mean + SEM and analyzed by ANOVA followed by Dunnert's multiple compaeison test, (n-6)
*P<0.01 Compared to standard during.

Table 3: The ulcer genic activity, lipid peroxidation and toxicity of test compounds.

Compound	Ulcerogenic activity S.I[a] ± SEM[b]	nmols of MDA content ± SEM[b]/ 100 mg tissue	Toxicity (mg/kg)
Control	0.0	3.29 ± 0.19	–
Naproxen	1.75 ± 0.35	7.83 ± 0.39	–
4a	0.82 ± 0.39	5.92 ± 0.21**	ND
4b	0.75 ± 0.28*	5.84 ± 0.30**	ND
4d	0.25 ± 0.11**	4.48 ± 0.20**	>300
4h	0.66 ± 0.16*	5.27 ± 0.13**	ND
4j	0.99 ± 0.30	6.81 ± 0.23	ND
4k	0.41 ± 0.15**	4.63 ± 0.18**	ND
4l	0.16 ± 0.10**	4.03 ± 0.32**	>300

ND denotes not determined
[A] Severity index (S.I.) mean score of each treated group minus the mean score of the control group.
[b] Relative to standard and data were analyzed by ANOVA followed by dunnet't's multiple comparison test for n-6: **P< 0.01. *P< 0.05.

Docking simulations:

Docking studies were performed using the Glide module in the Schrodinger 9.1 program. The crystal structure of COX-2 (1CX2) was obtained from the protein data bank and prepared with the protein preparation wizard module in Schrodinger 9.1 [29]. The binding site was generated by keeping the co-crystallized ligand at the center of a rectangular box drawn in the receptor. Defining this binding site is known as receptor grid generation. A 20 Å grid space was defined for the co-crystallized ligand using the glide grid module of the software. The LigPrep module was used to produce low-energy conformers and to correct the chirality of all the ligands. Ligands were kept flexible by producing the ring conformations and by penalizing nonpolar amide bond conformations, whereas the receptor was kept rigid throughout the docking studies. All other parameters of the Glide module were maintained at their default values. The lowest energy conformation was selected and the ligand interactions (hydrogen bonding and hydrophobic interaction) with the active sites of COX-2 were determined.

Table 4: COX -2 inhibition data for selective compounds

Compound	COX-2 inhibition (at 10 µM conc.), %	COX-2 IC_{50} (µM)
4b	5.82	ND
4d	39.15	12.8
4h	14.88	ND
4k	17.79	ND
4l	49.83	10.0

ND denotes not determined.

Results and Discussion

Chemistry

Syntheses of the target compounds were carried out according to the sequence of reactions outlined in Scheme 1. The key intermediate, 3-acetyl-7-chloro-6-fluorocinnolin-4(1H)-one 3, was synthesized in excellent yield through the cyclization of ethyl-2-[2- (3-chloro-4-fluorophenyl)hydrazinylidene]-3-oxobutanoate 2 in the presence of anhydrous AlCl3 and chlorobenzene. Literature revealed that reactions of aryl hydrazines with 1,3-dicabonyl compounds in acidic conditions preferentially resulted in one regioisomer [30, 31]. The structures of the newly synthesized compounds were identified using IR, 1 H NMR, 13C NMR and mass spectral data. Elemental analysis of these synthesized compounds was also in complete agreement with the proposed structures. The IR spectrum of 3 showed bands at 3436 cm1, which indicates NeH stretching, and at 1712 cm1 and 1659 cm1, indicating two carbonyl groups. The absence of the triplet for eCH3 and the quartet for eCH2 of eCH2CH3 in the 1 H NMR spectrum of 3, which was observed in the 1 H NMR spectrum of 2, confirms the cyclization. Compound 3 served as a source of 1,3-diketone and its subsequent condensation with acid hydrazides, in a polar aprotic solvent (1,4- dioxane) containing a catalytic amount of concentrated HCl, furnished the final compounds 4aem. After the pyrazole formation, the characteristic singlet of the cinnolineeNH proton at d 13.8 disappeared and the singlet of the methyl group shifted upfield to d 2.33, as observed in the 1 H NMR spectrum of compound 4l. Its 13C NMR spectrum indicated the absence of signals for the carbonyl groups at d 176.8 and d 194.6, which were seen in the 13C spectrum of 3, and showed signals at d 12.3 and at d 167.4, which represent the methyl group at C3 and the carbonyl group at C1, respectively. It was also supported by the mass spectrum of 4l, which showed a molecular ion peak at m/z 370.25.

Furthermore, an appropriate 1D Nuclear Overhausher Effect (NOE) experiment of 4l was also carried out for further structural confirmation of the synthesized compounds. The NOE difference spectrum of 4l showed that, when 4-methoxy substituted phenyl H-2 was used for irradiation, H-9 (at the cinnoline ring) achieves more enhancement than the eCH3 group (at the pyrazole ring), which is only possible if compound 4l has structure I, in which the 4-methoxy substituted phenyl and methyl groups are in the 1 and 3 positions to each other, as described in Fig. 2.

Biological Activity

Anti-inflammatory activity

The carrageenan-induced rat paw edema bioassay was used for the evaluation of the anti-inflammatory activity of the test compounds 4aem; the results are summarized in Table 2. Most of the test compounds showed appreciable inhibition of the edema size in comparisonwith naproxen. The compounds (7-chloro-8-fluoro-3- methyl-1H-pyrazolo[4,3-c]cinnolin-1-yl)(4-methylphenyl) methanone (4d) and (7-chloro-8-fluoro-3-methyl-1 H-pyrazolo [4,3-c] cinnolin-1-yl)(4-methoxyphenyl)methanone (4l) showed excellent protection against inflammation (74.67 and 80.01% inhibition, respectively), whereas compounds 4f, 4i and 4mwere found to have the least inhibitory effect. Compounds 4a, 4b, 4h, 4j and 4k also showed good systemic anti-inflammatory activity with a percent inhibition of 64.40, 67.94, 68.46, 61.52 and 66.25%, respectively, in comparison to naproxen, which has a percent inhibition of 81.23%. The rest of the compounds exhibited weak to moderate anti-inflammatory activity. Introduction of methylene (eCH2e) as a spacer group between the substituted phenyl ring and the carbonyl carbon (]C]O) increases the anti-inflammatory activity of thecompound, as in the case of 4b. On the other hand, a considerable decrease in activity was observed in compounds containing the eOe CH2e spacer group, as in the case of 4f. Furthermore, it was found that the compounds with an electron donating substituent on the benzoyl ring showed a higher anti-inflammatory activity than those compounds with a benzoyl ring substituent that has an electron withdrawing group.

Table 5: Antibacterial activity of test compounds

Compound	Diameter of zone of inhibition (mm): Mean[a] ± S.D.[b]			MIC[c] (µg/mL)		
	E. coli (NCTC 10418)	*S. aureus* (NCTC 65710)	*P. aeruginosa* (NCTC 10662)	*E. coli*	*S. aureus*	*P. aeruginosa*
4a	12.17 ± 0.99	10.13 ± 0.35	11.06 ± 0.63	25	50	50
4b	13.28 ± 1.04	13.52 ± 0.75	12.25 ± 0.73	25	25	25
4c	11.31 ± 0.39	11.15 ± 0.43	10.33 ± 0.53	25	50	50
4d	9.23 ± 1.18	8.11 ± 0.78	8.36 ± 0.38	Nt	Nt	Nt
4e	15.34 ± 0.90	12.06 ± 0.62	14.05 ± 0.53	12.5	25	12.5
4f	10.09 ± 1.05	7.41 ± 1.13	8.32 ± 0.98	Nt	Nt	Nt
4g	9.57 ± 1.26	8.18 ± 0.37	9.1 ± 0.44	Nt	Nt	Nt
4h	11.49 ± 0.61	10.22 ± 0.64	10.94 ± 0.48	25	50	50
4i	16.35 ± 0.85	13.33 ± 0.87	14.18 ± 0.59	12.5	25	12.5
4j	6.05 ± 0.76	–	–	Nt	Nt	Nt
4k	9.84 ± 0.71	8.27 ± 1.31	9.29 ± 0.89	Nt	Nt	Nt
4l	9.23 ± 0.59	9.14 ± 0.95	7.16 ± 0.51	Nt	Nt	Nt
4m	6.26 ± 0.48	–	5.11 ± 0.87	Nt	Nt	Nt
Ciprofloxacin	25.32 ± 0.52	23.57 ± 0.58	22.65 ± 0.61	3.12	3.12	3.12
DMSO	00	00	00	–	–	–

Nt denotes not tested.

[a] Mean value of measured diameters of zones of inhibition at 100 µg/ml.

[b] S.D denotes the standard deviation

[c] MIC denotes minimum inhibitory concentration

Acute ulcerogenicity study

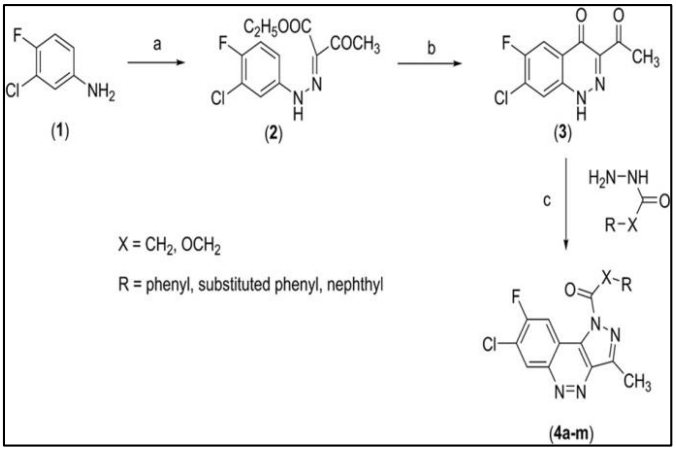
The test compounds that exhibited notable anti-inflammatory profiles were further evaluated for their ulcerogenic potential. The results, which are shown in Table 3, revealed that all the tested compounds showed a superior GI safety profile (S.I. values 0.16e 0.99) in Wistar rats compared to the standard drug naproxen (S.I. value 1.75) at an oral dose of 90 mg/day. Compounds 4d and 4l showed a severity index of 0.25 (+/-) 0.11 and 0.16 (+/-) 0.10, respectively, which is less than one-sixth (1/6) the value obtained with the standard drug. These findings further support the statement that 4d and 4l may be considered safer in terms of gastric ulcerogenicity than the conventional drug naproxen.

Lipid peroxidation

The lipid peroxidation profile of the selected compounds was also determined in order to validate the ulcerogenic activity results, as shown in Table 3. It was observed that 4d and 4l, which exhibited the least ulcerogenic effect, also showed marked reduction in lipid peroxidation. It is noteworthy that naproxen, the reference standard, exhibited the highest lipid peroxidation, 7.83 (+/-) 0.39 nmol MDA/100 mg tissue, whereas the control group showed a lipid peroxidation of 3.29(+/-)0.19 nmol MDA/100 mg tissue under the same experimental conditions. These results further suggest that the protective effect of the tested compounds might be related to the inhibition of lipid peroxidation in the gastric mucosal wall. It is important to mention here that a cinnoline derivative [32] was recently isolated by Chen *et al*. from an extract of *Cichorium endivia* L. and has been found to have antioxidant properties.

Acute toxicity study

The active compounds 4d and 4l were further evaluated to determine their acute oral toxicity. The results (Table 3) indicatedthat the tested compounds were found to be non-toxic at the normal dose and were well tolerated by the experimental animals up to 300 mg $^{kg-1}$. In addition, no mortality was observed 24 h post-administration, even at the highest dose of 500 mg/kg body weight, which suggests a wide margin of safety.



Scheme 1: Synthesis of the intermediate and target compounds: (a) 1. $NaNO_2$/HCl, 2. Ethyl acetoacetate/sodium acetate, 84%. (b) 1. $AlCl_3$, Cl-benzene 2. Dil. HCl, Dil. NaOH, reflux, 16 h, 76%. (c) Anhydrous 1,4-dioxane, Conc. HCl, 12e18 h, 105 ○C.

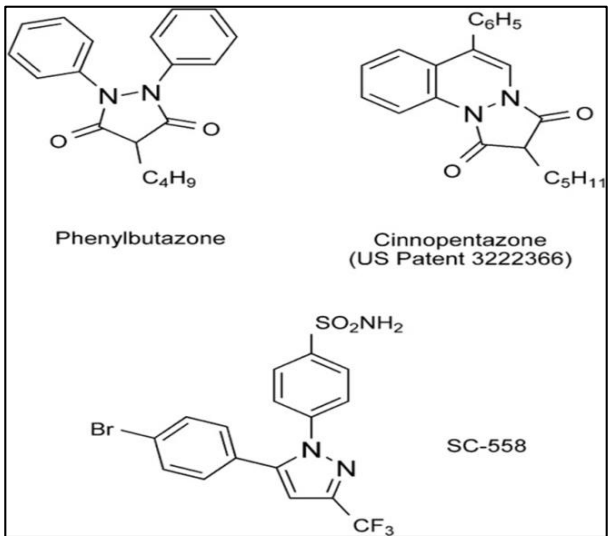


Fig 1: Structures of phenylbutazone, cinnopentazone, and reference ligand (SC-558).

COX-2 inhibition

Five synthesized compounds that demonstrated the best *in vivo* results were evaluated for their anti-inflammatory activity by measuring their *in vitro* biochemical COX-2 inhibitory activity. The results of the COX-2 inhibition are given in Table 4 and Fig. 3. Compound 4d (IC50:12.8 mM) and compound 4l (IC50:10 mM) showed promising COX-2 inhibition.

Docking studies of the compounds were performed on the Glide [33] module of Schrodinger 9.0 to rationalize their antiinflammatory results. A well-reported three-dimensional cocrystal structure of COX-2 (PDB ID: 1CX2) with a selective inhibitor, SC-558 (Fig. 1), was used as the template for the docking studies. Figs. 4 and 5 show the binding interactions of most potent compounds 4d and 4l, respectively, to the active site of COX- 2; these are similar to those observed with SC-558. Compound 4d (IC50:12.8 mM) displayed hydrogen bond interactions with Tyr 355 and Leu 359 (Fig. 4) and hydrophobic interactions with Phe 381, Ile517, Tyr 348, Leu 531, Leu 359, Trp 387, Tyr 385 and Leu 384. Compound 4l (IC50:10 mM) showed hydrogen bond acceptor interactions with His 90, Tyr 355 and Leu 359 (Fig. 5), in addition to hydrophobic interactions with Val 523, Ala 527, Val 349, Phe 518, Ile 517 and Leu 352. Moreover both the compounds have methyl group (eCH3 in 4d and eOCH3 in 4l) at the para position of phenyl ring, which is well occupied in the active site volume and could be responsible for their higher potency.

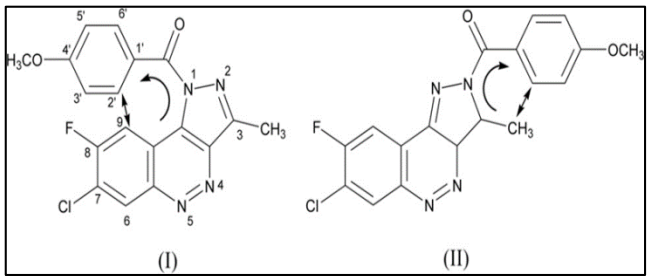


Fig 2: Diagnostic NOE in compound 4l.

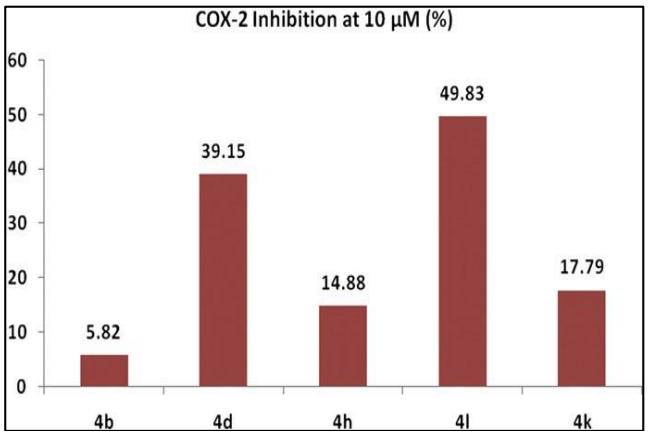


Fig 3: COX-2 inhibition data for selected compounds

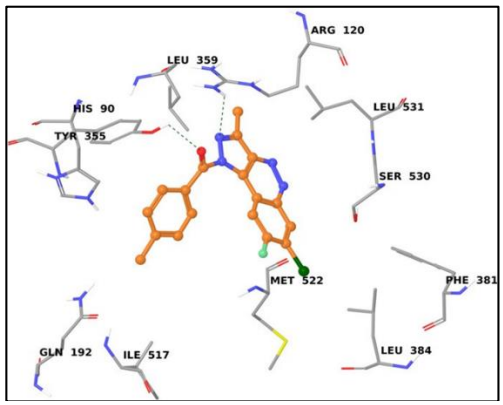


Fig 4: 3D view from a molecular modeling study, showing minimum energy structure of the complex of 4d docked in Cox-2 (PDB ID: 1CX2).

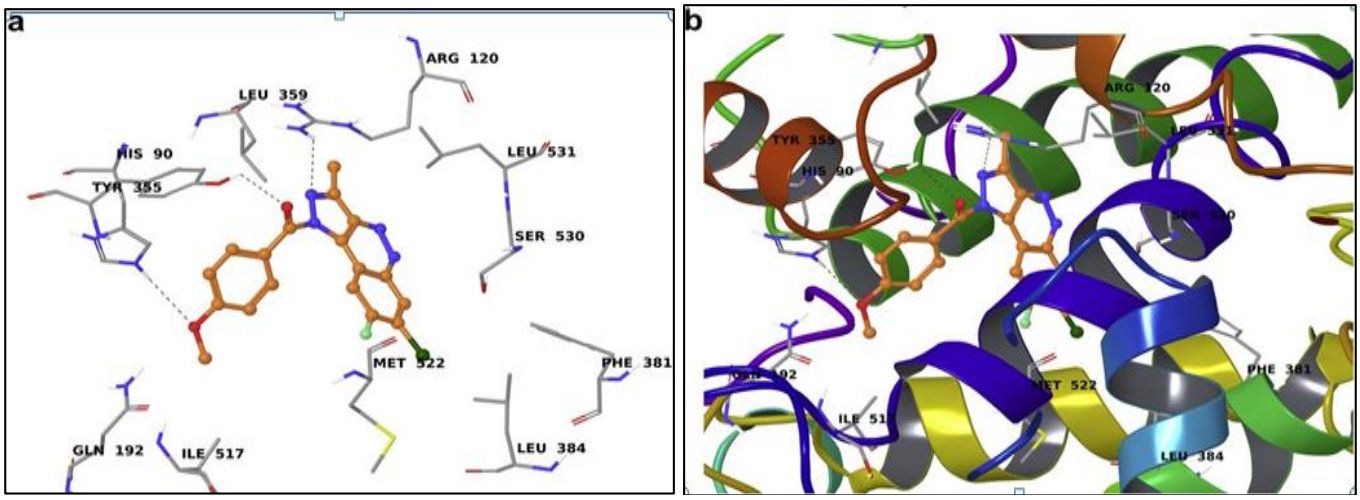


Fig 5: (a,b) 3D view from a molecular modeling study, showing minimum energy structure of the complex of 4l docked in Cox-2 (PDB ID: 1CX2).

Antibacterial activity

The minimum inhibitory concentration (MIC) was also determined for the compounds that showed a good antibacterial profile in the preliminary screening against E. coli (a gram-negative & facultative anaerobic bacteria), S. aureus (a gram-positive & facultative anaerobic bacteria) and P. aeruginosa (a gram-negative & aerobic bacteria). The measured zone of inhibition (Table 5) at 100 mg/ml indicated that almost all of the newly synthesized compounds were found to be active against E. coli and some of them exhibited appreciable antibacterial activity against the other bacterial strains. Compounds 4e and 4i emerged as the most potent antibacterial agents against E. coli (MIC = 12.5 and 12.5 mg/ml, respectively), S. aureus (MIC = 25 and 25 mg/ml, respectively) and P. aeruginosa (MIC =12.5 and 12.5 mg/ml, respectively). This high antibacterial activity may be attributed to the presence of the pharmacologically active 2,4-dichloro (4e) or 4-nitro (4l) substituents attached to the benzoyl group at position 1 of the pyrazolo [4,3-c]cinnoline rings of these compounds. It was interesting to note that compound 4b, which has an unsubstituted phenyl ring with a methylene spacer (eCH2e), showed significant antibacterial activity against all the tested strains. Furthermore, the result also showed that the tested compounds exhibited better activity againstE. coli and P. aeruginosa than S. aureus. Cinnoxacin, a cinnoline derivative exerts its antibacterial action through the inhibition of the bacterial DNA-gyrase enzyme [34]. Therefore, the antibacterial action of the designed compounds might be due to their ability to inhibit DNA-gyrase.

Conclusion

A new series of pyrazolo[4,3-c]cinnoline derivatives were synthesized with the objective of developing a new antiinflammatoryeantibacterial agent with minimum gastric irritation and a good safety margin. Compounds 4d and 4l were found to be potent anti-inflammatory agents with a percent inhibition after 4 h of 74.67 (+/-) 1.56 and 80.01(+/-) 2.41, respectively. Compound 4l displayed stronger binding interactions with the active site of the human COX-2 enzyme than compound 4d. However, both of these derivatives did not show specific antibacterial activity against all of the tested strains. Compounds 4e and 4i were found to be the most active antibacterial agents in the present study. Moreover, 4b emerged as a potential dual anti-inflammatoryeantibacterial agent with low ulcerogenic effect.

Therefore, it can be safely concluded that compound 4b would constitute a usefulmodel for furtherinvestigationin the development of a new class of dual non-acidic anti-inflammatory eantibacterial agents.

References

1. Masferrer JL, Zweifel BS, Manning PT, Hauser SD, Leahy KM, Smith WG *et al*. Seibert, Selective inhibition of inducible cyclooxygenase-2 *in vivo* is anti-inflammatory and nonulcerogenic, Proc. Natl. Acad. Sci. U S A 1994;91:3228e-3232.

2. Ray WA, Lorenzo CV, Chung CP, Castellsague J, Murray KT, Stein CM *et al*., Cardiovascular risks of nonsteroidal anti-inflammatory drugs in patients after hospitalization for serious coronary heart disease, Circ. Cardiovasc. Qual. Outcomes 2009;2:155e163.
3. Trelle S, Reichenbach S, Wandel S, Hildebrand P, Tschannen B, Villiger PM *et al*., Cardiovascular safety of non-steroidal anti-inflammatory drugs: network meta-analysis, BMJ 2011;342:c7086.
4. Titilayo Umaru O, Charles Nwamba, Ibrahim Kolo, *et al*., Antimicrobial activity of non-steroidal anti-inflammatory drugs with respect to immunological response: diclofenac sodium as a case study, Afr. J Biotechnol 2009;8:7332e7339.
5. Weng TC, Chen CC, Toh HS, Tang HJ. Ibuprofen worsens Streptococcus pyogenes soft tissue infections in mice J. Microbiol. Immunol. Infect 2011;44:418e423.
6. Leroy S, Marc E, Bavoux F, Treluyer JM, Gendrel D, Breart G *et al*., Hospitalization for severe bacterial infections in children after exposure to NSAIDs: a prospective adverse drug reaction reporting study, Clin. Drug Invest 2010;30:179e185.
7. Li CS, Brideau C, Chan CC, Savoie C, Claveau D, Charleson S *et al*., Pyridazinones as selective cyclooxygenase-2 inhibitors, Bioorg. Med. Chem. Lett. 2003;13:597e600.
8. Khaled AM, bouzid ENA, Khalil EEM, Ahmed EHA, Abd El-Latif EME *et al*., Structure-based molecular design, synthesis, and *in vivo* anti-inflammatory activity of pyridazinone derivatives as nonclassic COX-2 inhibitors, Med. Chem. Res 2010;19:629e642.
9. Sukoroglu M, Ergun BA, Sahin MF, Kupeli E, Yesilada E, Banoglu E. Synthesis, analgesic and anti-inflammatory activities of [6-(3,5-dimethyl-4- chloropyrazole-l-yl)-3(2H)-pyridazinon-2-yl]acetamides, Arch. Pharm. Res. 2005;28:509e517.
10. Refaat HM, Khalil OM, Kadry HH, Synthesis and anti-inflammatory activity of certain piperazinylthienyl pyridazine derivatives, Arch. Pharm. Res 2007;30:803e811.
11. Sheu JY, Chen YL, Fang KC, Wang TC, Tzeng CC, Peng CF. Synthesis and antibacterial activity of 1-(substituted-benzyl)-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acids and their 6,8-difluoro analogs, J Heterocyclic. Chem 1998;35:955e964.
12. Wagner-Jauregg T, Schatz F, Jahn U. 1,2-malonyl-1,2-dihydrocinnoline derivatives and process for preparation thereof. US Patent, 1965, 3222366.
13. Schatz F. Th. Wagner-Jauregg, Synthesis of substituted 1, 2-malonyl-1,2- dihydro cinnoline (1,3-dioxo-2,3-dihydro-1H-pyrazolo [1, 2-a] cinnoline) with anti-inflammatory properties, Helv. Chim. Acta 1968;51:1919e1931.
14. Conrad RA, White WA. US 4379929, Chem. Abstr. 1983;99:22481.
15. Nargund LVG, Badiger VV, Yarnal SM. Synthesis, antimicrobial and antiinflammatory activities of substituted 2-mercapto-3-(n-aryl)pyrimido[5,4-c] cinnolin-4-(3H)-ones, J Pharm. Sci 1992;81:365e366.
16. Bekhit AA, Ashour HMA, Bekhit A, El-Din A, Abdel-Rahman HM, Bekhit SA. Synthesis of some pyrazolyl benzenesulfonamide derivatives as dual anti-inflammatory antimicrobial agents, J. Enzyme Inhib. Med. Chem 2009;24:296e309.
17. Bekhit AA, Abdel-Aziem T, Design, synthesis and biological evaluation of some pyrazole derivatives as anti-inflammatory-antimicrobial agents, Bioorg. Med. Chem 2004;12:1935e1945.
18. Bekhit AA, Fahmy HTY, Rostoma SAF, Bekhit A El-Din A. Synthesis and biological evaluation of some thiazolylpyrazole derivatives as dual antiinflammatory antimicrobial agents, Eur. J Med. Chem 2010;45:6027e6038.
19. Abbady MS, Radwan M, Bakhite EA. Synthesis and antimicrobial activity of some cinnoline derivatives containing sulphonamido group, Indian J Chem 1993;32B:1281e1284.
20. Pattan SR, Ali MS, Pattan JS, Reddy VVK. Synthesis of some fluorocinnoline derivatives and evaluation for their antifungal and antibacterial activities, Ind. J. Heter. Chem 2004;14:157e158.
21. Winter CA, Risley EA, Nuss GW. Carrageenan-induced edema in hind paws of the rat as an assay for anti-inflammatory drugs, Proc. Soc. Exp. Biol. Med 1962;111:544e547.
22. Cioli V, Putzolu S, Rossi V, Barcellona SP, Corradino C. The role of direct tissue contact in the production of gastrointestinal ulcers by antiinflammatory drugs in rats, Toxicol. Appl. Pharmacol 1979;50:283e289.
23. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Anal. Biochem 1979;95:351e358.
24. Verma M, Tripathi M, Saxena AK, Shanker K, Antiinflammatory activity of novel indole derivatives, Eur. J Med. Chem 1994;29:941e946.
25. AKhila JS, Shaymjith Deepa MC. Alwar, Acute toxicity studies and determination of median lethal dose, Curr. Sci 2007;93:917e920.
26. Copeland RA, Williams JM, Giannaras J, Nurnberg S, Covington M, Pinto D. Mechanism of selective inhibition of the inducible isoform of prostaglandin G/H synthase, Proc. Natl. Acad. Sci. U S A 1994;91:11202e11206.
27. Pagels WR, Sachs RJ, Marnett LJ, Dewitt DL, Day JS, Smith WL. Immunochemical evidence for the involvement of prostaglandin H synthase in hydroperoxide-dependent oxidations by ram seminal vesicle microsomes, J Biol. Chem 1983;258:6517e6523;
28. Egan RW, Paxton Jr J., Kuehl FA. Mechanism for irreversible selfdeactivation of prostaglandin synthetase, J Biol. Chem 1976;251:7329e-7335.
29. Barry AL. The Antimicrobial Susceptibility Test: Principle and Practice, Illus Lea and Febiger, Philadelphia, 1976, 180; [Biol. Abstr. 1977, 64, 25183].
30. Schrodinger Suite 2009 Protein Preparation Wizard: Epik Version 2.0, Schrodinger, LLC, New York, NY, 2009.
31. Li JJ, Corey EJ. Name Reactions in Heterocyclic Chemistry, first ed., John Wiley & Sons, Inc., Hoboken, New Jersey, 2004.
32. Gosselin F, O'Shea PD, Webster RA, Reamer RA, Tillyer RD, Grabowski EJJ. Highly regioselective synthesis of 1-aryl-3,4,5-substituted pyrazoles, Synlett 2006;19:3267e3270.
33. Chen CJ, Deng A, Jun Deng, Chang Liu, Rui Shi, Hai-Lin Qin, Ai-Ping Wang, Hepatoprotective activity of Cichorium endivia L. Extract and its chemical constituents, Molecules 2011;16:9049e9066.
34. Glide Version 5.5, Schrodinger, LLC, New York, NY, 2009.http://www. schrodinger.com.
35. Jones RN, Fuchs PC. *In vitro* antimicrobial activity of cinoxacin against 2,968 clinical bacterial isolates, Antimicrob. Agents Chemother 1976;10:146e149.