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Full Length Research Paper

Pyrazole incorporated 1,2-diazanaphthalene derivatives: Synthesis and *in-vivo* pharmacological screening as anti-inflammatory and analgesic agents

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In the present study, a new series of compounds (3 to 22) containing pyrazole moiety clubbed with 1,2-diazanaphthalene ring, structurally related to celecoxib and naproxen was synthesized. The compounds were evaluated for anti-inflammatory activity using carrageenan induced rat paw edema bioassay. Compounds showing marked anti-inflammatory activity were further tested for their analgesic, ulcerogenic and lipid peroxidation effect using naproxen as reference drug. The results showed that compounds 6, 13 and 14 exhibited good anti-inflammatory and analgesic activity with minimum gastric irritation. Compounds 13 and 14 emerged as potent anti-inflammatory (with percentage inhibition of 79.23 and 76) and analgesic agent (with percentage protection of 68.72 and 67.68) in the present study.

Key words: Cinnoline, pyrazole, anti-inflammatory activity, analgesic activity, lipid peroxidation.

INTRODUCTION

Almost all the nonsteroidal anti-inflammatory drugs (NSAIDs) except cyclooxygenase-2 (COX-2) selective are associated with a common side effect of gastro-intestinal (GI) irritation due to their unwanted selectivity towards COX-1 enzyme. Several efforts have been made to improve the pharmacological profile of NSAIDs, but ulcerogenicity remains the most limiting problem in their clinical use.

A major breakthrough in anti-inflammatory research occurred after recognition of inducible isoform COX-2 which further provided the hypothesis for the development of COX-2 selective anti-inflammatory drugs devoid of GI disorders. However, the concern regarding their adverse cardiovascular effect has intensified since the removal of rofecoxib and valdecoxib from the market. A short term therapy with celecoxib (selective cyclooxygenase-2 inhibitor) has been recommended for the treatment of arthritis and osteoarthritis upon oral administration (Shakeel et al., 2008). Some recent reports also disclosed that among all NSAIDs, only naproxen has been found to be safe in terms of cardiovascular toxicity (Trelle et al., 2011; Ray et al., 2009). Therefore, development of novel compounds having anti-inflammatory and analgesic activity with an improved safety profile is still required. Extensive literature survey revealed that compounds containing 1,2-diazanaphthalene nucleus possessed anti-inflammatory (Schatz et al., 1968; Wagner-Jauregg et al., 1965) and analgesic activity (Gomtsyan et al., 2005). On the other hand, pyrazole containing compounds have also been well documented as anti-inflammatory and analgesic agents (Gawad et al., 2011; Bhandari et al., 2009; Chowdhury et al., 2009; Khode et al., 2009; Aal et al., 2002).

Prompted by the aforementioned findings, we designed and synthesized new compounds having pyrazolo[4,3-*c*]cinnoline as core nucleus linked with different substituted aryl/heteroaryl functionalities. The anti-inflammatory-analgesic acumen of titled compounds has been rationalized on the basis of their structural resemblance to celecoxib and naproxen as shown in Figure 1. Ring A represents a pyrazole ring substituted at 1, 3 and 5 positions. Ring B represents an aryl or a heteroaryl moiety

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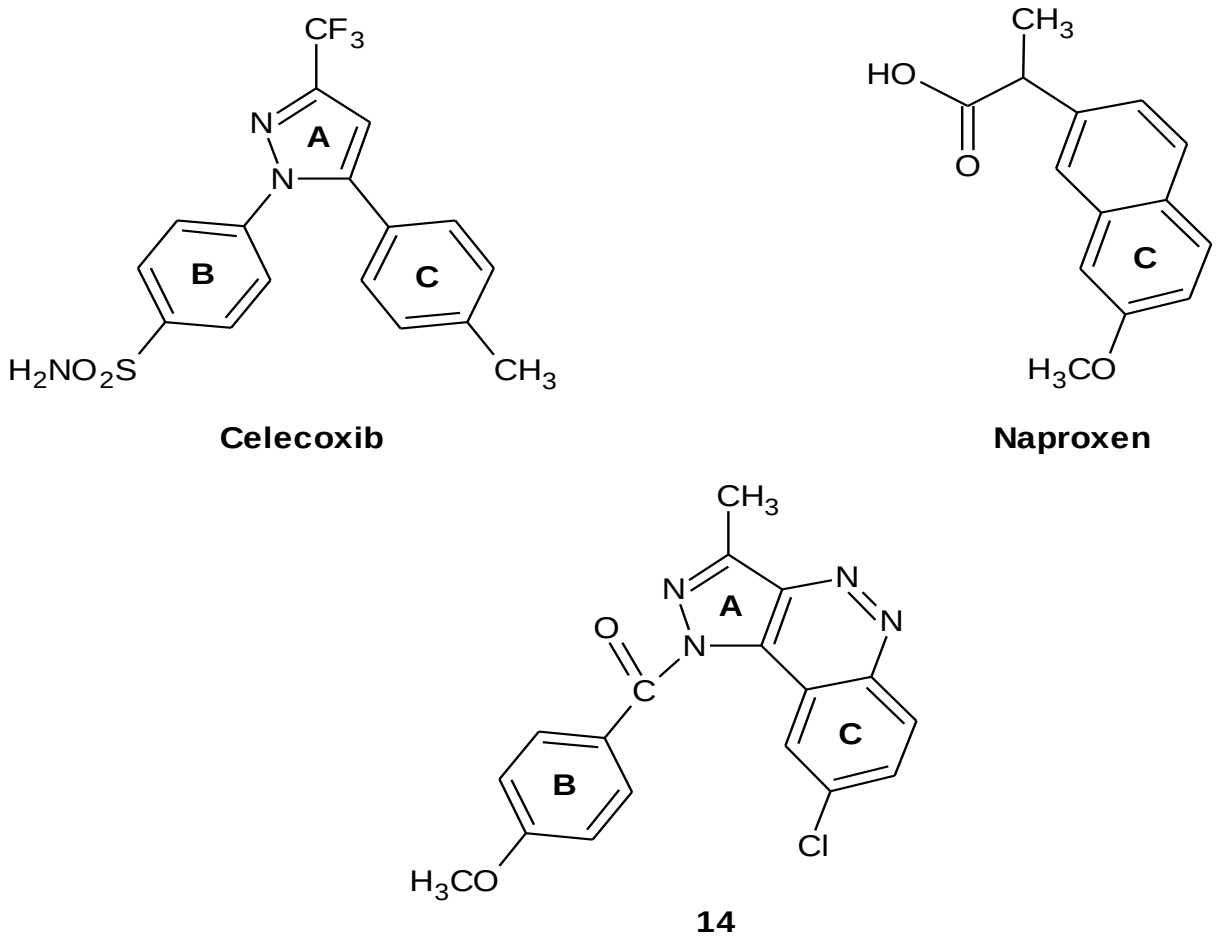


Figure 1. Structures of celecoxib, naproxen and our synthesized compound (14).

linked with ring A at position 1 either directly or through a linker group (-CO-, -CH_2-CO-). Ring C represents a phenyl ring (as shown in celecoxib), a naphthalene ring in naproxen and a 1,2-diazanaphthalene ring in compound 14.

MATERIALS AND METHODS

All the reagents and solvents used were purchased from E Merck (India) Ltd., S. D. Fine (India) and Qualigens (India). Silica gel 60 to 120 mesh LR (25049 K05) was used for column chromatography. Melting points were determined using open capillary tubes on electrical melting point apparatus and are uncorrected. IR (KBr) spectra were recorded on a Shimadzu, Fourier transform infrared (FTIR) spectrometer (ν_{max} in cm^{-1}). ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$/DMSO on a Bruker 300 MHz spectrometer using tetramethylsilane (TMS) as internal reference (Chemical shift in δ ppm). Mass spectra (DART-MS) were recorded on a JEOL-AccuTOF JMS-T100LS mass spectrometer having a direct analysis in real time (DART) source. Progress of reaction and purity of the synthesized compounds were checked on ascending thin layer chromatography (TLC) plates coated with silica gel G (Merck). Iodine chamber and ultraviolet (UV) lamp were used for visualization of thin layer chromatographic (TLC) spots. Elemental analysis was performed on the CHNOS-Elementar analyzer (Vario EL III) using sulphanilic acid as standard and tungsten (VI) oxide as a combusting agent. Sample for analysis was prepared in Tin boats of dimensions 6 × 6 × 12 mm.

Chemistry

Compound 2 was synthesized using the literature method (Abbady et al., 1993; Pattan et al., 2004). The physicochemical data of all the final compounds (3 to 22) have been reported in Table 1.

8-Chloro-3-methyl-1*H*-pyrazolo[4,3-*c*]cinnoline (3)

Compound 2 (0.8 g, 3 mmol) was dissolved in a mixture of hydrazine hydrate (0.6 ml) and absolute ethanol (25 ml). Then, the reaction mixture was refluxed on water bath for 8 h. After refluxing the contents of flask were cooled, filtered and washed thrice with distilled water to get crude product. It was then recrystallized from methanol to give amorphous compound.

IR (KBr) cm^{-1}: 3438 (N-H), 1660 (C=N), 1560 (C=C), 1446 (N=N), 752 (C-Cl). ^1H NMR (300 MHz, DMSO): δ (ppm) 2.31 (s, 3H, CH_3), 7.25-7.37 (m, 2H, $H_{Cinnoline}$), 7.62-7.65 (d, 1H, $H_{Cinnoline}$, J = 8.1 Hz), 11.49 (s, 1H, $NH_{Pyrazole}$, D_2O-Exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 12.4 (CH_3), 116.4, 124.3, 127.2, 127.8, 132.5, 134.6, 138.4, 149.8, 151.2. DART-MS m/z: 218.16 (M^+),

Table 1. Physicochemical constants of the newly synthesized compounds (**3-22)**

Compound Number	R_1	R_2	R_3	M.P. °C	Yield.[a] %	Rf.[b,c]
3	-Cl	-H	---	196-197	54	0.48[b]
4	-F	-Cl	---	184-186	70	0.57[b]
5	-Cl	-H	Phenyl-	152-153	62	0.54[b]
6	-F	-Cl	Phenyl-	165-167	64	0.56[b]
7	-Cl	-H	*o, p*-Dinitro phenyl-	210-212	66	0.61[b]
8	-F	-Cl	*o, p*-Dinitro phenyl-	234-235	67	0.59[b]
9	-Cl	-H	*o*-Nitro phenyl-	190-191	69	0.57[b]
10	-F	-Cl	1-Phthazinyl-	177-179	61	0.65[b]
11	-Cl	-H	1-Phthazinyl-	229-230	67	0.63[b]
12	-Cl	-H	Phenyl-	162-164	58[a]	0.65[c]
13	-Cl	-H	*o*-Hydroxy phenyl-	205-207	56[a]	0.68[c]
14	-Cl	-H	*p*-methoxy phenyl-	238-239	66[a]	0.62[c]
15	-Cl	-H	*p*-methyl phenyl-	171-173	57[a]	0.60[c]
16	-Cl	-H	*o*-Methyl phenyl-	168-169	53[a]	0.64[c]
17	-Cl	-H	*m*-Methyl phenyl-	157-158	55[a]	0.66[c]
18	-Cl	-H	*o*-Bromo phenyl-	201-202	51[a]	0.57[c]
19	-F	-Cl	*o*-Chloro phenyl-CH_2-	223-225	65[a]	0.68[c]
20	-Cl	-H	Phenyl-CH_2-	175-176	64[a]	0.66[c]
21	-Cl	-H	*o*-Acetoxy phenyl-	194-196	66[a]	0.72[c]
22	-Cl	-H	3-Indolyl-CH_2-	189-191	54[a]	0.65[c]

[a] After recovery from Hexane : AcOEt (4:1), [b] Benzene : Acetone (8:2), [c]Toluene : Ethylacetate : Formic acid (6 : 3.5 : 0.5).

220.16 (M+2). Anal. Calcd for $C_{10}H_7ClN_4$: C, 54.93; H, 3.23; N, 25.62. Found: C, 54.82; H, 3.24; N, 25.69.

(7-Chloro-8-fluoro-3-methyl-1*H*-pyrazolo[4,3-*c*]cinnolin-1-yl)(pyridin-4-yl)methanone (4)

Compound 2 (1.0 g, 4 mmol) was dissolved in absolute ethanol (30 ml) and isonicotinic acid hydrazide (0.68 g, 4 mmol) was added. After 15 min, concentrated sulphuric acid (0.4 ml) was added and the reaction mixture was refluxed for 6 h. Then, excess ethanol was distilled off and the mixture was allowed to cool to room temperature. The resulting solution was poured into 150 ml of cold water. The precipitate obtained was filtered, air dried and recrystallised from methanol.

IR (KBr) cm^{-1}: 2924 (C-H), 1690 (C=O), 1625 (C=N), 1524 (C=C), 1230 (C-F), 756 (C-Cl). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 2.37 (s, 3H, CH_3), 7.20 (s, 1H, $H_{Cinnoline}$), 7.52 (s, 1H, $H_{Cinnoline}$), 7.67-7.69 (d, 2H, $H_{Pyridine}$, J = 6 Hz), 8.80-8.82 (d, 2H, $H_{Pyridine}$, J = 6 Hz). DART-MS m/z: 341.16 (M^+), 343.16 (M+2). Anal. Calcd for $C_{16}H_9ClFN_5O$: C, 56.24; H, 2.65; N, 20.49. Found: C, 56.39; H, 2.64; N, 20.54. Pale yellow amorphous solid.

General procedure for the synthesis of compounds (5 to 11)

To a well stirred solution of compound 2 (3 mmol) in absolute alcohol (25 ml), an equimolar quantity of substituted phenyl/heteroaryl hydrazine (3 mmol) and glacial AcOH (0.2 ml) was added. The resulting solution was refluxed on water bath for 8 to 10 h. After completion of the 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 recrystallized from methanol to give amorphous product.

8-Chloro-3-methyl-1-phenyl-1*H*-pyrazolo[4,3-*c*]cinnoline (5)

IR (KBr) cm^{-1}: 1654 (C=N), 1562 (C=C), 1449 (N=N), 752 (C-Cl). ^{1}H NMR (300 MHz, DMSO): δ (ppm) 2.34 (s, 3H, CH_3), 7.18-7.26 (m, 5H, H_{Phenyl}), 7.51-7.57 (m, 2H, $H_{Cinnoline}$), 7.66-7.69 (d, 1H, $H_{Cinnoline}$, J = 8.1 Hz). DART-MS m/z: 294.10 (M^+), 296.11 (M+2). Anal. Calcd for $C_{16}H_{11}ClN_4$: C, 65.20; H, 3.76; N, 19.01. Found: C, 65.38; H, 3.75; N, 19.04. Brick red

7-Chloro-8-fluoro-3-methyl-1-phenyl-1*H*-pyrazolo[4,3-*c*]cinnoline (6)

IR (KBr) cm^{-1}: 1658 (C=N), 1556 (C=C), 1444 (N=N), 1237 (C-F), 758 (C-Cl). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 2.39 (s, 3H, CH_3), 7.14-7.24 (m, 5H, H_{Phenyl}), 7.24 (s, 1H, $H_{Cinnoline}$), 7.51 (s, 1H, $H_{Cinnoline}$). Anal. Calcd for $C_{16}H_{10}ClFN_4$: C, 61.45; H, 3.22; N, 17.92. Found: C, 61.58; H, 3.23; N, 17.96. Pale yellow.

8-Chloro-1-(2,4-dinitrophenyl)-3-methyl-1*H*-pyrazolo[4,3-*c*]cinnoline (7)

IR (KBr) cm^{-1}: 1654 (C=N), 1559 (C=C), 1444 (N=N), 756 (C-Cl). ^{1}H NMR (300 MHz, DMSO): δ (ppm) 2.37 (s, 3H, CH_3), 7.16-7.19 (d, 1H, H_{Phenyl}, J = 7.2 Hz), 7.32-7.40 (m, 2H, $H_{Cinnoline}$), 7.65-7.67 (d, 1H, $H_{Cinnoline}$, J = 8.1 Hz), 7.79-7.92 (m, 2H, H_{Phenyl}). DART-MS m/z: 384.04 (M^+), 386.04 (M+2). Anal. Calcd for $C_{16}H_9ClN_6O_4$: C, 49.95; H, 2.36; N, 21.84 Found: C, 50.12; H, 2.34; N, 21.78. Dark brown.

7-Chloro-1-(2,4-dinitrophenyl)-8-fluoro-3-methyl-1*H*-pyrazolo[4,3-*c*]cinnoline (8)

IR (KBr) cm^{-1}: 1652 (C=N), 1548 (C=C), 1446 (N=N), 1241 (C-F), 762 (C-Cl). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 2.39 (s, 3H, CH_3), 7.20-7.22 (d, 1H, H_{Phenyl}, *J* = 6.8 Hz), 7.28 (s, 1H, $H_{Cinnoline}$), 7.53 (s, 1H, $H_{Cinnoline}$), 7.65-7.76 (m, 2H, H_{Phenyl}). Anal. Calcd for $C_{16}H_8ClFN_6O_4$: C, 47.72; H, 2.00; N, 20.87. Found: C, 47.84; H, 2.06; N, 20.92. Dark brown.

8-Chloro-3-methyl-1-(2-nitrophenyl)-1*H*-pyrazolo[4,3-*c*]cinnoline (9)

IR (KBr) cm^{-1}: 1659 (C=N), 1564 (C=C), 1448 (N=N), 760 (C-Cl). ^1H NMR (300 MHz, DMSO): δ (ppm) 2.36 (s, 3H, CH_3), 6.95-7.22 (m, 4H, H_{Phenyl}), 7.32-7.46-7.49 (d, 2H, $H_{Cinnoline}$, *J* = 8.7 Hz), 7.52-7.54 (d, 1H, $H_{Cinnoline}$, *J* = 8.1 Hz). DART-MS m/z: 339.13 (M^+), 341.13 (M+2). Anal. Calcd for $C_{16}H_{10}ClN_5O_2$: C, 56.56; H, 2.97; N, 20.61. Found: C, 56.74; H, 2.94; N, 20.55. Brown solid.

7-Chloro-8-fluoro-3-methyl-1-(phthalazin-1-yl)-1*H*-pyrazolo[4,3-*c*]cinnoline (10)

IR (KBr) cm^{-1}: 1644(C=N), 1552 (C=C), 1449 (N=N), 1246 (C-F), 760 (C-Cl). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 2.32 (s, 3H, CH_3), 7.36 (s, 1H, $H_{Cinnoline}$), 7.55 (s, 1H, $H_{Cinnoline}$), 7.92-8.14 (m, 4H, $H_{Phthalazine}$), 8.56 (s, 1H, $H_{Phthalazine}$). DART-MS m/z: 364.06 (M^+), 366.06 (M+2). Anal. Calcd for $C_{18}H_{10}ClFN_6$: C, 59.27; H, 2.76; N, 23.04. Found: C, 59.44; H, 2.78; N, 22.97. Pale yellow.

8-Chloro-3-methyl-1-(phthalazin-1-yl)-1*H*-pyrazolo[4,3-*c*]cinnoline (11)

IR (KBr) cm^{-1}: 1660 (C=N), 1548 (C=C), 1452 (N=N), 766 (C-Cl). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 2.34 (s, 3H, CH_3), 7.48 (br s, 3H, $H_{Cinnoline}$), 8.12-8.22 (m, 4H, $H_{Phthalazine}$), 8.61 (s, 1H, $H_{Phthalazine}$). Anal. Calcd for $C_{18}H_{11}ClN_6$: C, 62.34; H, 3.20; N, 24.23. Found: C, 62.54; H, 3.24; N, 24.17. Pale yellow.

General procedure for the synthesis of compounds (12 to 22)

Equimolar quantities of compound 2 (3 mmol) and substituted phenyl/heteroaryl acid hydrazides (3 mmol) were mixed on a magnetic stirrer for 0.5 h in anhydrous 1,4-dioxane (30 ml) containing 0.4 ml of concentrated hydrochloric acid. The resulting mixture was boiled under reflux for 14 to 16 h. After completion of the reaction (monitored by TLC), the reaction mixture was allowed to cool, concentrated under reduced pressure and poured into ice-cold water with constant stirring. The product obtained was filtered, dried and then purified through column chromatography (Hexane/AcOEt, 4:1) to afford final compounds (12 to 22). The physicochemical data of the compounds has been presented in Table 1.

(8-Chloro-3-methyl-1*H*-pyrazolo[4,3-*c*]cinnolin-1-yl)(phenyl)methanone (12)

IR (KBr) cm^{-1}: 1642 (C=O), 1566 (C=N), 1528 (C=C), 1458 (N=N), 752 (C-Cl). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 2.32 (s, 3H, CH_3), 7.16-7.21 (m, 5H, H_{Phenyl}), 7.44 (br. s, 3H, $H_{Cinnoline}$). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 12.2 (CH_3), 115.3, 117.1, 127.3, 128.6, 129.2, 131.4, 134.4, 135.4, 138.7, 139.5, 151.5, 169.2 (C=O). DART-MS m/z: 322.16 (M^+), 324.16 (M+2). Anal. Calcd for $C_{17}H_{11}ClN_4O$: C, 63.26; H, 3.44; N, 17.36. Found: C, 63.42; H, 3.45; N, 17.39. Light pale yellow powder.

(8-Chloro-3-methyl-1*H*-pyrazolo[4,3-*c*]cinnolin-1-yl)(2-hydroxyphenyl)methanone (13)

IR (KBr) cm^{-1}: 1634 (C=O), 1556 (C=N), 1512 (C=C), 1450 (N=N), 762 (C-Cl). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 2.30 (s, 3H, CH_3), 6.85-7.16 (m, 4H, H_{Phenyl}), 7.39 (br. s, 3H, $H_{Cinnoline}$), 9.87 (br. s, 1H, OH). DART-MS m/z: 338.06 (M^+), 340.06 (M+2). Anal. Calcd for $C_{17}H_{11}ClN_4O_2$: C, 60.28; H, 3.27; N, 16.54. Found: C, 60.43; H, 3.26; N, 16.49. Faint orange yellow amorphous solid.

(8-Chloro-3-methyl-1*H*-pyrazolo[4,3-*c*]cinnolin-1-yl)(4-methoxyphenyl)methanone (14)

IR (KBr) cm^{-1}: 1636 (C=O), 1563 (C=N), 1526 (C=C), 1447 (N=N), 759 (C-Cl). ^1H NMR (300 MHz, DMSO): δ (ppm) 2.31 (s, 3H, CH_3), 3.71 (s, 3H, OCH_3), 7.01-7.04 (d, 2H, H_{Phenyl}, *J* = 7.8 Hz), 7.36-7.46 (m, 2H, $H_{Cinnoline}$), 7.62-7.65 (d, 1H, $H_{Cinnoline}$, *J* = 8.1 Hz), 7.88-7.91 (d, 2H, H_{Phenyl}, *J* = 8.1 Hz). DART-MS m/z: 352.11 (M^+), 354.11 (M+2). Anal. Calcd for $C_{18}H_{13}ClN_4O_2$: C, 61.28; H, 3.71; N, 15.88. Found: C, 61.48; H, 3.73; N, 15.84. Fine yellow amorphous solid

(8-Chloro-3-methyl-1*H*-pyrazolo[4,3-*c*]cinnolin-1-yl)(4-methylphenyl)methanone (15)

IR (KBr) cm^{-1}: 1644 (C=O), 1560 (C=N), 1524 (C=C), 1452 (N=N), 758 (C-Cl). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 2.38 (s, 3H, CH_3), 3.49 (s, 3H, CH_3), 7.46 (br. s, 3H, $H_{Cinnoline}$), 7.74-7.76 (d, 2H, H_{Phenyl}, *J* = 7.2 Hz), 8.52-8.54 (d, 2H, H_{Phenyl}, *J* = 7.5 Hz). DART-MS m/z: 336.06 (M^+), 338.06 (M+2). Anal. Calcd for $C_{18}H_{13}ClN_4O$: C, 64.19; H, 3.89; N, 16.64. Found: C, 64.45; H, 3.88; N, 16.60. Yellow amorphous solid.

(8-Chloro-3-methyl-1*H*-pyrazolo[4,3-*c*]cinnolin-1-yl)(2-methylphenyl)methanone (16)

IR (KBr) cm^{-1}: 1647 (C=O), 1561 (C=N), 1528 (C=C), 1456 (N=N), 759 (C-Cl). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 2.36 (s, 3H, CH_3), 3.44 (s, 3H, CH_3), 7.16-7.23 (m, 4H, H_{Phenyl}), 7.41 (br. s, 3H, $H_{Cinnoline}$). Anal. Calcd for $C_{18}H_{13}ClN_4O$: C, 64.19; H, 3.89; N, 16.64. Found: C, 64.40; H, 3.92; N, 16.66. Yellow amorphous solid.

(8-Chloro-3-methyl-1*H*-pyrazolo[4,3-*c*]cinnolin-1-yl)(3-methylphenyl)methanone (17)

IR (KBr) cm^{-1}: 1645 (C=O), 1564 (C=N), 1529 (C=C), 1456 (N=N), 757 (C-Cl). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 2.34 (s, 3H, CH_3), 3.41 (s, 3H, CH_3), 7.14-7.21 (m, 4H, H_{Phenyl}), 7.42 (br. s, 3H, $H_{Cinnoline}$). Anal. Calcd for $C_{18}H_{13}ClN_4O$: C, 64.19; H, 3.89; N, 16.64. Found: C, 64.42; H, 3.91; N, 16.62. Pale yellow amorphous solid.

(2-Bromophenyl)(8-chloro-3-methyl-1*H*-pyrazolo[4,3-*c*]cinnolin-1-yl)methanone (18)

IR (KBr) cm^{-1}: 1648 (C=O), 1560 (C=N), 1512 (C=C), 1454 (N=N), 824 (C-Br), 748 (C-Cl). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 2.31 (s, 3H, CH_3), 6.92-7.13 (m, 4H, H_{Phenyl}), 7.48 (br. s, 3H, $H_{Cinnoline}$). Anal. Calcd for $C_{17}H_{10}BrClN_4O$: C, 50.84; H, 2.51; N, 13.95. Found: C, 50.96; H, 2.48; N, 13.92. Faint yellow powder.

1-(7-Chloro-8-fluoro-3-methyl-1*H*-pyrazolo[4,3-*c*]cinnolin-1-yl)-2-(2-chlorophenyl) ethanone (19)

IR (KBr) cm^{-1}: 1659 (C=O), 1564 (C=N), 1522 (C=C), 1449 (N=N), 1236 (C-F), 758 (C-Cl). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 2.27 (s, 3H, CH_3), 4.32 (s, 2H, CH_2), 7.28-7.32 (m, 4H, H_{Phenyl}), 7.41 (s, 1H, $H_{Cinnoline}$), 7.58 (s, 1H, $H_{Cinnoline}$). 389.12 (M^+), 391.12 (M+2), 393.12 (M+4). Anal. Calcd for $C_{18}H_{11}Cl_2FN_4O$: C, 55.55; H, 2.85; N, 14.39. Found: C, 55.69; H, 2.87; N, 14.35. Pale yellow amorphous solid

1-(8-Chloro-3-methyl-1*H*-pyrazolo[4,3-*c*]cinnolin-1-yl)-2-phenylethanone (20)

IR (KBr) cm^{-1}: 1662 (C=O), 1559 (C=N), 1516 (C=C), 1460 (N=N), 756 (C-Cl). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 2.33 (s, 3H, CH_3), 4.29 (s, 2H, CH_2), 7.18-7.25 (m, 5H, H_{Phenyl}), 7.42 (br. s, 3H, $H_{Cinnoline}$). DART-MS m/z: 336.12 (M^+), 338.12 (M+2). Anal. Calcd for $C_{18}H_{13}ClN_4O$: C, 64.19; H, 3.89; N, 16.64. Found: C, 64.41; H, 3.87; N, 16.60. Fine yellow amorphous solid.

2-[(8-Chloro-3-methyl-1*H*-pyrazolo[4,3-*c*]cinnolin-1-yl)carbonyl]phenyl acetate (21)

IR (KBr) cm^{-1}: 1644 (C=O), 1556 (C=N), 1529 (C=C), 1448 (N=N), 760 (C-Cl). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.54 (s, 3H, $OCOCH_3$), 2.36 (s, 3H, CH_3), 7.27-7.34 (m, 4H, H_{Phenyl}), 7.47 (br. s, 3H, $H_{Cinnoline}$). Anal. Calcd for $C_{19}H_{13}ClN_4O_3$: C, 59.93; H, 3.44; N, 14.71. Found: C, 60.11; H, 3.41; N, 14.68. Light pale yellow amorphous solid.

1-(8-Chloro-3-methyl-1*H*-pyrazolo[4,3-*c*]cinnolin-1-yl)-2-(1H-indol-3-yl)ethanone (22)

IR (KBr) cm^{-1}: 3310 (N-H), 1662 (C=O), 1556 (C=N), 1534 (C=C), 1448 (N=N), 756 (C-Cl). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 2.35 (s, 3H, CH_3), 4.24 (s, 2H, CH_2), 7.16-7.31. (m, 5H, $H_{Indolyl}$), 7.44 (br. s, 3H, $H_{Cinnoline}$) δ 11.37 (s, 1H, NH). DART-MS m/z: 375.10 (M^+), 377.10 (M+2). Anal. Calcd for $C_{20}H_{14}ClN_5O$: C, 63.92; H, 3.75; N, 18.64. Found: C, 64.13; H, 3.76; N, 18.68. Pale yellow amorphous solid.

Anti-inflammatory activity

Anti-inflammatory activity was determined in Wistar albino rats of either sex weighing 150 to 200 g using carrageenan induced rat paw edema method (Winter et al., 1962). The animals were randomly divided into groups of six rats each. The standard drug (naproxen) and test compounds (45 mg kg^{-1}) were administered *p.o.* as a suspension in carboxymethyl cellulose (CMC) (0.5% w/v solution), 1 h prior to carrageenan injection. The control group received only 0.5% w/v solution of CMC. The right hind paw edema was induced by sub-planter injection of 0.1 ml of 1% carrageenan solution in saline (0.9%). The volume of paw edema (ml) was determined by means of digital plethysmometer (Panlab LE 7500) before and after 3 and 4 h of carrageenan injection. The percentage of edema inhibition was calculated according to the following equation:

Edema inhibition (%) = Vc - Vt/Vc × 100

where Vc represents the mean increase in paw volume in the absence of test compound (control) and Vt represents the mean increase in paw volume after treatment with test compounds and standard drugs. The anti-inflammatory activity of the test compounds compounds relative to that of naproxen was also determined (Table 2).

Analgesic activity

The method used to determine analgesic activity was based on acetic acid induced writhings in mice (Koster et al., 1959). Swiss albino mice (35 to 40 g) were divided in different groups and each group comprised of six animals. A homogeneous suspension of standard drug and test compounds were prepared in aqueous solution of carboxymethyl cellulose (0.5% w/v) and were administered orally at a dose of 45 mg kg^{-1}. The control group was treated orally with vehicle (0.5% w/v CMC solution). After 1 h, the animals were given an intraperitoneal injection of 0.6% v/v solution of acetic acid (1 ml per 100 g body weight) as writhing-inducing agent.

The number of writhings were counted for 10 min for each animal of a group (n = 6) after acetic acid injection, and the results are expressed as mean ± standard error of mean (SEM) in Table 3. Analgesic activity was measured as percentage decrease in writhings (% protection) in comparison to control. The percent protection was calculated using following formula:

Protection (%) = {1- (number of writhings in test/number of writhings in control)} × 100

Ulcerogenic effect

Acute ulcerogenicity was determined by using the reported method of Cioli et al. (1979). Male Wistar albino rats were allocated into different groups consisting of six animals in each group. They were fasted for 12 h before administration of the test and standard drug with free access to water. Then, they were treated orally with two equal doses of naproxen or test compounds (namely, 45 mg kg^{-1}/dose) at 0 and 12 h, except control group which received only 0.5% CMC. After the drug treatment, the rats were fed with normal diet for 17 h and then, they were sacrificed. The stomach was removed and opened along the greater curvature. It was examined with the aid of microscope with 4× magnifying lens. The mean score of each treated group minus the mean score of control group was regarded as severity index of gastric mucosal damage (Table 3).

Lipid peroxidation

Lipid peroxidation was determined according to the method of Ohkawa et al. (1979). After screening for ulcerogenic activity, the gastric mucosa was scraped with two glass slides, weighed (100 mg) and homogenized in 1.8 ml of 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 heated at 95°C for 60 min. After cooling, the reactants were extracted with 5 ml of the mixture of *n*-butanol and pyridine (15:1 v/v), shaken vigorously for 1 min and centrifuged for 10 min at 4000 rpm. The supernatant organic layer was used to measure the absorbance at 532 nm on UV spectrophotometer. The results (Table 3) were expressed as nmol MDA/100 mg tissue.

RESULTS AND DISCUSSION

Chemistry

The synthetic procedure involved condensation of

Table 2. *In vivo* anti-inflammatory activity of synthesized compounds and naproxen in carrageenan-induced rat paw edema assay.

Compound number	% inhibition ± SEM[a]		Activity relative to standard
	After 3 h	After 4 h	
3	35.27 ± 2.66	39.19 ± 1.62**	48.25
4	62.16 ± 2.52	65.32 ± 2.71**	80.41
5	64.97 ± 2.89	67.31 ± 2.46**	82.86
6	68.71 ± 2.34	71.47 ± 2.48**	87.99
7	32.04 ± 1.90	35.27 ± 2.32**	43.42
8	39.90 ± 0.78	42.44 ± 2.40**	52.25
9	67.76 ± 2.78	69.22 ± 2.50**	85.21
10	60.01 ± 2.93	63.07 ± 1.5**	77.64
11	53.08 ± 1.34	57.61 ± 2.47**	70.92
12	59.16 ± 1.39	61.42 ± 1.35**	75.61
13	76.23 ± 0.95	79.23 ± 1.60	97.54
14	74.89 ± 1.61	76.00 ± 2.21	93.68
15	62.69 ± 1.4	65.21 ± 2.10**	80.28
16	70.50 ± 2.12	72.43 ± 1.18	89.16
17	65.69 ± 2.12	68.13 ± 1.12**	83.87
18	55.24 ± 2.14	57.53 ± 2.91**	70.83
19	68.19 ± 2.79	69.25 ± 2.73**	85.24
20	63.40 ± 1.46	66.43 ± 3.34**	81.78
21	52.46 ± 1.05	56.05 ± 2.57**	69.01
22	57.20 ± 1.86	60.11 ± 3.12**	74.00
Naproxen	78.84 ± 1.39	81.23 ± 1.19	100

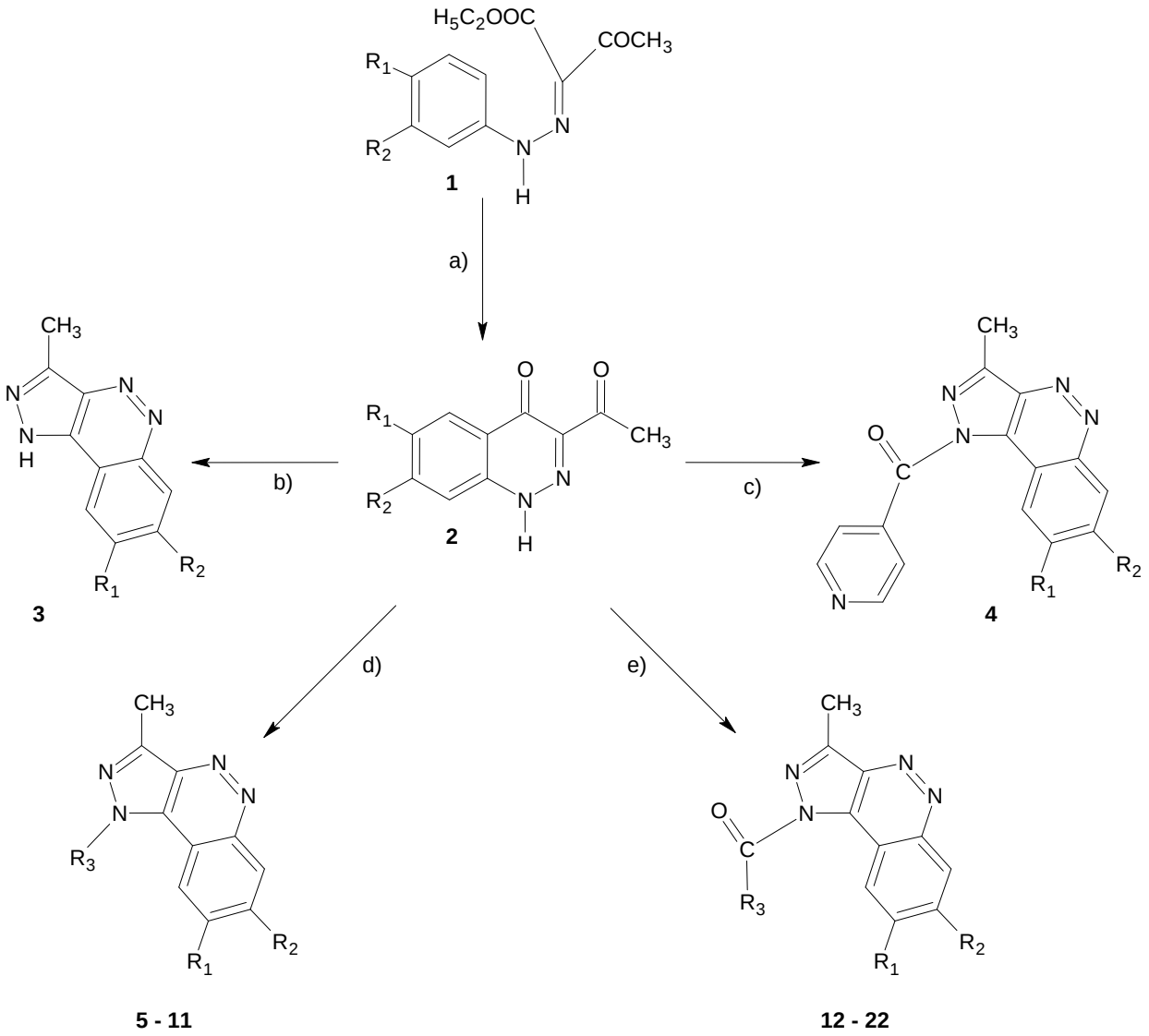
[a]SEM denotes the standard error of the mean. **P < 0.01, *P < 0.05 compared to standard, n = 6.

Table 3. The analgesic, ulcerogenic and lipid peroxidation activity of compounds.

Test compound	Analgesic activity		Ulcerogenic activity (SI[a] ± SEM[b])	nmols of MDA content ±SEM[b]/ 100 mg tissue
	No. of writhings ±SEM	Protection (%)		
5	11.5 ± 0.42	57.07	0.67 ± 0.30*	5.78 ± 0.24**
6	9.5 ± 0.67	64.28	0.58 ± 0.20*	5.27 ± 0.28**
9	12.33 ± 0.49	53.88	1.08 ± 0.32	7.02 ± 0.25
13	8.33 ± 0.49	68.72	0.41 ± 0.20**	4.57 ± 0.31**
14	8.66 ± 0.49	67.68	0.50 ± 0.18**	4.97 ± 0.23**
16	10.83 ± 0.60	59.52	0.92 ± 0.27	6.86 ± 0.26
17	12.66 ± 0.55	52.46	0.67 ± 0.16*	5.55 ± 0.29**
19	12 ± 0.36	55.30	0.75 ± 0.28	6.05 ± 0.31**
Naproxen	7.33 ± 0.49	72.74	1.75 ± 0.35	7.83 ± 0.39
Control	26.83 ± 0.60	-	00	3.29 ± 0.19

[a]Severity index (S.I.): Mean score of each treated group minus the mean score of the control group. [b,] Relative to naproxen and data were analyzed by ANOVA followed by Dunnett's multiple comparison test for n = 6; **P < 0.01, *P < 0.05.

diazonium salt of substituted aniline with ethylacetoacetate yielding ethyl-2-[2-(substitutedphenyl) hydrazinylidene]-3-oxobutanoate 1 which underwent intramolecular cyclization in the presence of anhydrous $AlCl_3$ and chlorobenzene to form the key intermediate 3-acetyl-6,7-substitutedcinnolin-4(1*H*)-one 2. Compound 2



Reagents: (a)1. $AlCl_3$, Cl-benzene 2. Dil. HCl, Dil. NaOH, 130°C , 16 h. (b) Hydrazine hydrate, Abs. EtOH, 8 h. (c) Isonicotinic acid hydrazide, Abs. EtOH, Conc. H_2SO_4, 70-80°C , 6 h . (d) Substituted phenyl/heteroaryl hydrazine, Abs. EtOH, AcOH, 8 to 10 h, 70°C (e) Substituted phenyl / heteroaryl acid hydrazide, Dry1, 4- Dioxane, Conc. HCl, 14 to 16 h, 105°C.

Figure 2. Synthesis of pyrazolo[4,3-*c*]cinnoline derivatives.

served as a source of 1, 3-diketone and its subsequent reaction with different substituted phenyl/heteroaryl/acyl hydrazines under appropriate reaction conditions as shown in Figure 2 afforded the target compounds (3 to 22).

The structures of final Compounds 3 to 22 were assigned on the basis of IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. An appropriate 1D Nuclear Overhauser Effect (NOE) experiment of Compound 4 was also carried out for further structural confirmation of synthesized compounds. Thus, from NOE difference spectrum of Compound 4, we observed that when pyridine H-3 was used for irradiation, H-9 (at the cinnoline ring) gave more enhancement than CH_3 group (at the pyrazole ring), which is only possible when Compound 4 has structure where pyridinyl and methyl groups are in positions 1 and 3. This structural assignment was also supported by the reported structure of the compound (8-Chloro-3-methyl-

1*H*-pyrazolo[4,3-*c*]cinnolin-1-yl) (pyridin-4-yl)methanone (Bawa et al., 2010). IR (KBr) spectrum of final compounds showed only one characteristic band for carbonyl group (C=O) stretching and other bands for (C=N) and (N=N) stretching which were observed at 1636, 1563 and 1447 cm^{-1}, respectively for Compound 14.

The ^{13}C NMR spectra showed two characteristic peaks, one for methyl carbon and the other for carbonyl carbon which was observed at δ 12.2 and 169.2, respectively for compound 12 along with other peaks in the aromatic region.

In the ^{l}H NMR spectra of final compounds, the characteristic peak at δ 13.90 due to cinnoline-NH proton as shown in compound 2 was missing. Moreover, a sharp singlet at δ 11.49 due to pyrazole-NH proton was observed in the ^{1}H NMR spectrum of compound 3. Further structural confirmation was accomplished by molecular ion peaks in the mass spectra. Elemental analysis of final compounds was also in complete agreement with the proposed structures.

Pharmacological screening

The anti-inflammatory results (Table 2) revealed that all the newly synthesized pyrazolo[4,3-*c*] cinnoline derivatives were active in reducing inflammation (percentage inhibition 35.27 to 79.23). Compounds 6 and 16 showed good systemic anti-inflammatory activity with percentage inhibition of 71.47 and 72.43, respectively. (8-Chloro-3-methyl-1*H*-pyrazolo[4,3-*c*] cinnolin-1-yl)(2-hydroxyphenyl)methanone 13 and (8-Chloro-3-methyl-1*H*-pyrazolo[4,3-*c*] cinnolin-1-yl) (4-methoxyphenyl)methanone 14 showed considerable protection against inflammation (percentage inhibition 79.23 and 76.00, respectively) which is slightly less than that of naproxen (percentage inhibition 81.23). The rest compounds exhibited weak to moderate anti-inflammatory activity. Compounds (12 to 22) having benzoyl ring substituted with electron donating group showed higher anti-inflammatory activity as compared to those containing benzoyl ring substituted with electron withdrawing group. On the other hand, compounds (5 to 11) having unsubstituted phenyl ring were found to be more active than the substituted ones.

Compounds 5, 6, 9, 13, 14, 16, 17 and 19 showed marked reduction in inflammation induced by carrageenan and were further evaluated for analgesic activity. The result of analgesic activity in the form of percentage protection is as shown in Table 3. These selected test compounds showed analgesic activity between 52.46 to 68.72%. Compounds 13 and 14 displayed marked 68.72 and 67.68% protection, respectively against acetic acid induced writhings as compared to naproxen (percentage protection 72.74).

Test compounds selected for analgesic activity further screened for their ulcerogenic effect. Results revealed that the tested compounds showed a superior GI safety profile (S.I. value 0.41 to 1.08) in the Wistar albino rats as compared to standard drug naproxen (S.I. value 1.75). Compounds 5, 6, 13, 14, 17 and 19 showed severity index 0.67, 0.58, 0.41, 0.50, 0.67 and 0.75, respectively, which is less than half the value of naproxen.

In order to validate the ulcerogenic activity results of selected compounds, their lipid peroxidation profile were also determined. Naproxen exhibited maximum tissue lipid peroxidation 7.83 ± 0.39, whereas control group showed 3.29 ± 0.19. It was observed that all the pyrazolo[4,3-*c*]cinnoline derivatives having less ulcerogenic activity also showed reduction in lipid peroxidation (Table 3). These studies showed that the synthesized compounds inhibit the induction of gastric mucosal lesions and the results further suggest that their protective effect might be related to the inhibition of lipid peroxidation in the gastric mucosal wall.

Thus, based on aforementioned results, it can be safely concluded that Compounds 13 and 14 would constitute a useful model for the development of a new class of non ulcerogenic anti-inflammatory analgesic agents that would be entitled to further investigation and derivatization.

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