SYNTHESIS AND BIOLOGICAL EVALUATION OF CINITAPRIDE RELATED DERIVATIVES AS POTENTIAL PROKINETIC AGENTS

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Abstract: New Cinitapride related benzimidazole derivatives are prepared from the condensation of corresponding diamines with carboxylic acids. Their anti-ulcerative activity is studied.

4-Amino-N-[1-(3-cyclohexen-1-ylmethyl)-4-piperidyl]-2-ethoxy-5-nitro benzamide (+)-tartarate (Cinitapride hydrogen tartrate) is a prokinetic benzamide derivative, a simulating gastrointestinal motility agent and is a commercially successful anti–ulcerative drug substance.[1,2] Reported general method for the preparation of this compound involved condensation of 4-amino-2-ethoxy-5-nitrobenzoic acid with 4-amino-1-(3-cyclohexen-1-ylmethyl)piperidine in the presence of triethylamine and ethyl chloroformate and subsequent salt formation with L(+) tartaric acid.[3]

Benzimidazole moiety is the common pharmocophore of potential proton pump inhibitors (anti ulcerative agents) such as Omeprazole,[4] Pantoprazole,[5] Lansoprazole[6] and Rabeprazole.[7] We have surmised that, incorporation of benzimidazole moiety into Cinitapride skeleton will have marked effect on the activity. With this objective, we have prepared various new benzimidazole derivatives and studied their anti - ulcer activity.

4, 5–Diamino-N-(piperidin-4-yl)benzamide derivatives (**2a/2b**) are identified as apt starting materials for our desired compounds. These diamines **2a/2b** are readily accessed from the catalytic hydrogenation of corresponding 4–amino-N-[1H–4-piperidinyl]–2-ethoxy–5-nitrobenzamide derivatives (**1a/1b**).

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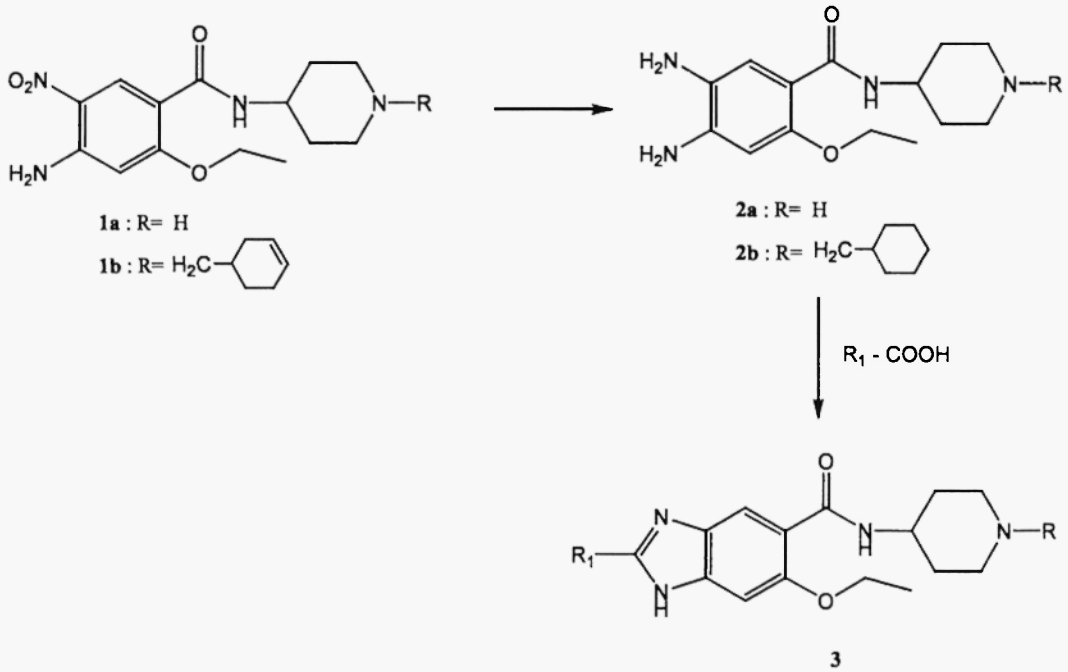
As a representive example, **2a** was refluxed in formic acid and usual workup afforded a crystalline compound, characterised as **3a.** The reaction was extended to other carboxylic acids such as acetic acid, α-hydroxy propionic acid and butyric acid. In all the cases, corresponding benzimidazole derivative was obtained in quantitative yields.

Piperidine nitrogen substituted diamine **2b** also furnished the corresponding benzimidazole derivatives **3** on reacting with carboxylic acids.

SCHEME



	3a	3b	3c	3d	3e	3f	3g	3h
R	H	H_2C-⬡	H	H_2C-⬡	H	H_2C-⬡	H	H_2C-⬡
R_1	H	H	CH_3	CH_3	CH_2-CH_2-CH_3	CH_2-CH_2-CH_3	OH HC−CH_3	OH HC−CH_3

All these benzimidazole derivatives are new and they are characterized based on their IR, H-NMR and Mass spectral data

4, 5–Diamino–2-ethoxy N- (piperidin-4-yl)benzamide (**2a**)

A solution of 4–amino–N-[1H–4-piperidinyl]–2-ethoxy–5-nitrobenzamide (**1a**, 20.0 g, 1.0 mol) in methanol (200.0 mL) was charged in hydrogen autoclave and Pd (4.0 g 5% on carbon) was added to it. Hydrogen gas was passed into autoclave and pressure was adjusted to 3.0 kg. Reaction was maintained at this pressure for 2 hr. Catalyst was filtered and washed with methanol (50.0 mL). Combined methanol fractions were distilled off at 50 °C under vacuum (Yield 18.0 g, 90.0%)

4,5-Diamino-N-(1-(cyclohexylmethyl)piperidin-4-yl)-2-ethoxybenzamide (**2b**)

A solution of 4-amino-N-(1-((cyclohex-3-enyl)methyl)piperidin-4-yl)-2-ethoxy-5-nitrobenzamide (**1b**, 20.0 g, 1.0 mol) in methanol (200.0 mL) was charged in hydrogen autoclave and Pd (5.0 g 5% on carbon) was added to it. Hydrogen gas was passed into autoclave and pressure was adjusted to 3.0 kg. Reaction was maintained at this pressure for 2hr. Catalyst was filtered and washed with methanol (50.0 mL). Combined methanol fractions were distilled off at 50 °C under vacuum (Yield 17.0 g, 91.0%)

General Procedure for the preparation of benzimidazole derivatives (**3**)
A mixture of diamine (**2a / 2b**) and carboxylic acid was heated/refluxed till the completion of reaction (vide TLC). Carboxylic acid was distilled off under vacuum and residue was recrystallised from ethylacetate. Characterization and spectral data is included in table 1 and 2.

Table –1: Characterization data of Compounds(**2a-b, 3a–h**)

Compd	Yield%	M.R. (°C)	M.Wt.	Mol formula	Found (calculated)%		
					Carbon	Hydrogen	Nitrogen
2a	90	160-162	278.3	$C_{14}H_{22}N_4O_2$	60.27 (60.41)	7.81 (7.97)	20.01 (20.13)
2b	84	272-274	374.5	$C_{21}H_{34}N_4O_2$	67.20 (67.35)	9.01 (9.15)	14.81 (14.96)
3a	82	104-106	288.3	$C_{15}H_{20}N_4O_2$	62.38 (62.48)	6.81 (6.99)	19.30 (19.43)
3b	86	164-166	384.5	$C_{22}H_{32}N_4O_2$	68.59 (68.72)	8.20 (8.39)	14.40 (14.57)
3c	85	124-126	302.3	$C_{16}H_{22}N_4O_2$	63.17 (63.55)	7.20 (7.33)	18.37 (18.53)
3d	92	242-244	398.5	$C_{23}H_{34}N_4O_2$	69.14 (69.31)	8.51 (8.60)	13.95 (14.06)
3e	88	122-124	330.4	$C_{18}H_{26}N_4O_2$	65.09 (65.43)	7.81 (7.93)	16.82 (16.96)
3f	91	157-159	426.5	$C_{25}H_{38}N_4O_2$	70.22 (70.39)	8.81 (8.98)	13.01 (13.13)
3g	63	210-212	332.4	$C_{17}H_{24}N_4O_3$	61.07 (61.43)	7.11 (7.28)	16.72 (16.86)
3h	88	230-232	428.5	$C_{24}H_{36}N_4O_3$	67.08.(67.26)	8.36 (8.47)	12.95 (13.07)

Characterization data:

Com	IR (KBr) cm^{-1}	^1H NMR , δ ppm):	Mass
2a	3358, 1620, 1562	1.39 (t, 3H, CH_3 of ethoxy), 1.41 (m, 2H, CH_2 of pipe.), 1.92 (m, 2H CH_2 of pipe.), 2.70 (m, 2H, CH_2 of pipe.), 3.01 (m, 2H, CH_2 of pipe.), 3.95(m, 1H, CH of pipe.), 4.05 (q, 2H, CH_2 of ethoxy), 5.10 (s, 2H NH of amine), 6.25 (s, 1H, Ar–H), 7.10 (s, 1H, Ar-H), 7.90 (d, 1H NH of amide)	279 (M + 1), 264, 248, 196, 141, 113
2b	3362, 1623	1.10 (t, 3H, CH_3 of ethoxy), 1.25 (m, 4H, 2CH2 of CHX), 1.33 (m, 2H CH_2 of CHX), 1.50 (m, 4H, $2CH_2$ of CHX), 1.75 (t, 2H, CH_2 of pipe.), 1.83(t, 2H, CH_2 of pipe.), 1.98(m, 1H, CH of CHX), 2.34 (d, 2H, CH_2 of N-CH_2), 3.00 (dd, 2H of CH_2 of pipe), 3.26 (dd, 2H, CH_2 of pipe), 3.63 (m, 1H, CH of pipe), 4.18 (q, 2H CH_2 of Ethoxy), 4.92 (s, 2H NH of Amine), 6.50 (s, 1H, Ar-H), 7.46 (s, 1H Ar-H).	375 (M + 1)
3a	3360, 1602, 1550, 1380	1.44 (t, 3H CH_3 of ethoxy), 1.55 (m, 2H CH_2 of pipe.), 1.95 (m, 2H, CH_2 of pipe.), 2.90 (m, 2H CH_2 of pipe.), 3.05 (m, 2H CH_2 of pipe.), 3.95 (m, 1H, CH of pipe), 4.10 (q, 2H, CH_2 of ethoxy), 7.20 (S, 1H Ar – H), 7.95 (S, 1H, Ar – H), 8.23 (d, 1H, CONH), 8.40 (s, 1H, NH of benzimidazole).	289(M+1), 232 206, 206, 189, 161, 133 and 105
3b	1648, 1618	1.02 (t, 3H, CH_3 of ethoxy), 1.11 (m, 4H, $2CH_2$ of CHX), 1.30 (m, 2H,CH_2 of CHX), 1.55 (m, 4H, $2CH_2$ of CHX), 1.62 (m, 2H, CH_2 of pipe.), 1.85 (m, 2H CH_2 of pipe.), 2.05(m, 1H, CH of CHX), 2.90 (m, 2H CH_2 of pipe.), 3.02(d, 2H CH_2 of N-CH_2), 3.18 (m, 2H CH_2 of pipe.), 3.95 (m, 1H, CH of pipe), 4.10 (q, 2H, CH_2 of ethoxy), 7.20 (S, 1H, Ar – H), 7.95 (S, 1H, Ar – H).	385 (M + 1), 306, 226, 97.
3c	1595, 1384, 1295, 1178	1.40 (t, 3H, CH_3 of ethoxy), 1.51 (m, 2H, CH_2 of pipe.), 1.89 (S, 3H, CH_3 of benzimidazole), 1.98 (S, 2H, CH_2 of pipe.), 2.79 (m, 2H, CH_2 of pipe.), 3.05 (m, 2H CH_2 of pipe.), 3.95 (m, 1H, CH of pipe), 4.10 (q, 2H, CH_2 of ethoxy), 7.10 (s, 1H, Ar – H), 7.95 (s, 1H, Ar – H), 8.23 (d, 1H, CONH).	303 (m+1), 289, 270 246, 149, 119
3d	1646, 1619	0.98 (t, 3H, CH_3 of ethoxy), 1.25 (t, 3H, CH_3 of Benzimidazole), 1.50 (m, 4H, $2CH_2$ of CHX) 1.68 (m, 2H CH_2 of CHX), 1.75 (m, 4H, $2CH_2$ of CHX), 1.80 (m, 1H CH of CHX), 2.08 (d, 2H, CH_2 of pipe.), 2.25 (dd 2H, CH_2 of pipe.), 2.50 (d, 2H, CH_2 of N-CH_2), 2.98 (s, 2H, CH_2 of pipe), 2.98 (dd, 2H of CH_2 of pipe), 3.92 (m, 1H, CH of pipe), 4.23 (q, 2H, CH_2 of Ethoxy), 7.09 (s 1H, Ar-H), 8.10 (s, 1H Ar-H).	399 (M + 1), 319, 220, 119
3e	3383, 1611, 1547, 1307	0.98 (t, 3H, CH_3 of propyl), 1.30 (m, 2H, CH_2 of Pipe), 1.50 (t, 3H CH_3 of ethyl), 1.79 (m, 2H, CH_2 of Propyl), 1.94 (m, 2H, CH_2 of Pipe), 2.70 (m, 2H CH_2 of Pipe), 2.80 (t, 2H, CH_2 of ethyl), 3.05 (m, 2H CH_2 of Pipe), 3.98 (m, 1H CH of Pipe), 4.20 (q, 2H, CH_2 of ethyl), 7.10 (s, 1H, Ar – H), 7.95 (s, 1H, Ar – H), 8.25 (d, 1H, - CONH).	331(M), 283, 270, 230, 161 and 124.
3f	1647, 1619	1.00 (t, 3H, CH_3 of propyl), 1.25 (m, 4H, $2CH_2$ of CHX), 1.51 (t, 3H, CH_3 of propyl), 1.70 (m, 4H, $2CH_2$ of CHX), 1.78 (m, 2H,CH_2 of propyl), 1.80 (m, 1H,CH of CHX), 1.90(m, 2H,CH_2 of CHX) 2.12 (t, 2H, CH_2 of pipe.), 2.50(d, 2H, CH_2 of N-CH_2), 2.60 (t, 2H,CH_2 of pipe), 2.95 (t, 2H,CH_2 of propyl) 3.15 (dd, 2H of CH_2 of pipe), 3.40 (dd, 2H of CH_2 of pipe), 4.04 (m, 1H, CH of pipe), 4.30 (q, 2H, CH_2 of Ethoxy), 7.14 (s, 1H, Ar-H), 8.08 (s, 1H, Ar-H)	427 (M + 1), 262, 230, 97.
3g	3370, 1626, 1557, 1164	1.42 (t, 3H, CH_3 of ethoxy), 1.45 (m, 2H, CH_2 of pipe), 1.52 (d, 3H, CH_3 of Hydroxyethyl), 1.95 (m, 2H, CH_2 of pipe), 2.80 (t, 2H, CH_2 of pipe), 3.05 (m, 2H of CH_2 of pipe), 3.98 (m, 1H, CH of pipe), 4.20 (q, 2H, CH_2 of ethoxy), 4.95 (q, 1H CH of Hydroxyethyl), 7.10 (s, 1H, Ar-H), 7.95 (s, 1H, Ar-H), 8.25 (d, 1H, CONH).	333(M+1), 315, 297, 258 and 153.
3h	3253, 1647, 1626	0.98 (t, 3H, CH_3 of ethoxy), 1.25(m, 4H, $2CH_2$ of CHX) 1.50 (t, 3H, CH_3 of ethyl), 1.36 (m, 2H, CH_2 of CHX), 1.48 (m, 1H,CH of CHX), 1.75(m, 4H, $2CH_2$ of CHX), 1.85 (d, 2H,CH_2 of pipe), 1.90(t, 2H, CH_2 of pipe.), 2.08(d, 2H,CH_2 of pipe), 2.30(d, 2H,CH_2 of N-CH_2), 2.91(d, 2H, CH_2 of pipe), 3.98 (m, 1H, CH of pipe), 4.26 (q, 2H, CH_2 of Ethoxy), 5.12 (q, 1H, CH of ethyl) 7.22 (s, 1H, Ar-H), 8.20 (s, 1H, Ar-H).	429 (M + 1), 411, 167.

Gastric emptying in mice:

Prokinetic studies were conducted in pharmacology lab at Discovery Research, Dr. Reddys laboratories Ltd., Hyderabad. The study design and the experimental protocol was approved by the institutional animal ethics committee.

Adult Swiss albino mice of either sex (20-25g) were fasted for 24 hours prior to experimentation but had free access to water. The compounds were tested at 10 mg/kg dose per oral. Phenol red meal was prepared as previously reported[8, 9] and was administered 1 hour after the drug administration. Fifteen minutes later, animals were sacrificed by cervical dislocation and stomach was immediately removed. The whole stomach content is alkalized with 0.1 N NaOH and homogenized. To this 5 ml of homogenate, 0.5 ml of trichloroaceticacid (20%w/v) was added and centrifuged at 3000 rpm for 20 min. To 1ml of supernatant, 4 ml of 0.5 N NaOH was added and absorbance was measured at 560 nm. This correlates to the concentration of phenol red in the stomach, which in turn depends on gastric emptying (GE). Cinitapride was taken as reference standard. The results were analysed by ANOVA. Anti ulcer activity of Cinitapride like compounds is measured by its prokinetic activity. Similarly anti ulcer activity of our compounds is also evaluated by their prokinetic activity.

% GE = (1-X/Y)* 100

X ---------- Absorbance of phenol red recovered after 15 min after test meal

Y ---------- Absorbance of phenol red recovered at 0 min following test meal

Cinitapride (**1b**) is taken as reference standard. Besides diamine (**2a**), benzimidazole derivatives **3a**, **3e** and **3g** showed significant activity

Comp	1b*	2a	2b	3a	3b	3c	3d	3e	3f	3g	3h	Control
%GE	91 ± 2	*82 ±2	64 ±5	*71 ±3	61 ±6	64 ±4	54 ±5	*77 ±3	64 ±4	*71 ±5	55 ±8	56 ±4

Values are expressed as Mean ± SE, n = 5

* $P<0.05$ is considered as statistically significant

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