Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 2749–2755
Bioorganic & Medicinal Chemistry Letters

Synthesis and evaluation of Novel Benzimidazole derivative [Bz-Im] and its radio/biological studies

Anjani K. Tiwari,[a,b] Anil K. Mishra,[b] Aruna Bajpai,[a] Pushpa Mishra,[b] Sweta Singh,[b] Deepa Sinha[b] and V. K. Singh[a,*]

[a]*Department of Chemistry, Lucknow University, Lucknow 226007, India*
[b]*Department of Radiopharmaceuticals, Institute of Nuclear Medicine and Allied Sciences, Timarpur, Delhi 110054, India*

Received 25 October 2006; revised 12 February 2007; accepted 27 February 2007
Available online 3 March 2007

Abstract—Two different benzimidazole analogues act as multimodal agent, first one as novel non-peptidic CCK-B receptor antagonist and similarly as potent anti-fungal agent, designated as [Bz-Im]. These compounds were synthesized and characterized by spectroscopic techniques such as FT-IR, NMR, EI-MS and also evaluated for specific radiopharmaceuticals. Preliminary radiolabeling results with ^{99m}Tc and biological evaluation studies showed promising results for further evaluation in vivo. The efficiency of labeling was more than 97% and complex was stable for about 12 h at 30 °C in the presence of serum. Both ligands showed binding to most of the organs, known to express CCK receptors in biodistribution studies. Cholecystokinin (CCK_1 andCCK_2) receptor binding affinities of these analogues are, IC_{50}, 0.942 ± 0.107 for compound C and 0.665 ± 0.211 for compound D in rat pancreatic acini. The anti-fungal activity has shown inhibitory activity against *Aspergillus flavus* and *Aspergillus niger*. These studies have provided a new template for further development of non-peptidic ligands for diagnostic and therapeutic purposes of diseases related with CCK receptors as well as anti-microbes.

Benzimidazole derivatives are reported to be physiologically and pharmacologically active and find application in the treatment of several diseases like epilepsy, diabetes, anti-fertility, etc.[1,2] Recently several researches elucidated that benzimidazole analogues can be suitably modified by the introduction of different hetero-cyclic moieties to exhibit a broad spectrum of biological activities such as potent anti-cancerous and anti-fungal drugs.[3–6] Keeping this view in our mind, we have modified benzimidazole analogues in such a way that it would bring them into preferred orientation. This might provide a more potent CCK (cholecystokinin) antagonist.

The importance of CCK has been implicated in various regulatory functions; as neurotransmitters in the brain and as regulators of various functions of the gastrointestinal tract, primarily at the level of the stomach, pancreas, and gallbladder.[7] In addition, they can act as physiological growth factors in most parts of the gastrointestinal tract.[8–10] Gastrin and CCK possess the same five amino acids at their COOH terminus which is a biologically active site. Their actions are mediated by two different receptor types, CCK_1 and CCK_2.[11,12] CCK_2 receptors are present in the gut mucosa and in the brain,[7,13,14] whereas, CCK_1 receptors are present in the gallbladder, pancreas, and brain.[7,15,16]

The importance of selective CCK antagonist as potential tools is to visualize malignant tumors.[17,18] Receptor autoradiography studies have shown that cholecystokinin CCK-B/gastrin receptors are expressed not only in more than 90% of medullary thyroid carcinomas (MTC)[19] but also in high percentage of small cell lung cancer, some ovarian cancer, astrocytomas, and potentially in a variety of adinocarcinomas, gastrointestinal tumors, and colorectal cell lines. In this work, we have synthesized multimodal analogues of benzimidazoles [Bz-Im] and its intermediate is synthesized according to previous literatures[20] and labeled with ^{99m}Tc to explore binding aspect with CCK receptor. The initial studies have shown good results for further evaluation.

Keywords: Non-peptidic; ^{99m}Tc-labeled; Spectroscopy; Biodistribution; Benzimidazole; Radiolabeling.
* Corresponding author. Tel.: +91 11 23905387; fax: +91 11 23919509; e-mail: vinayk2003@rediffmail.com

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.02.071

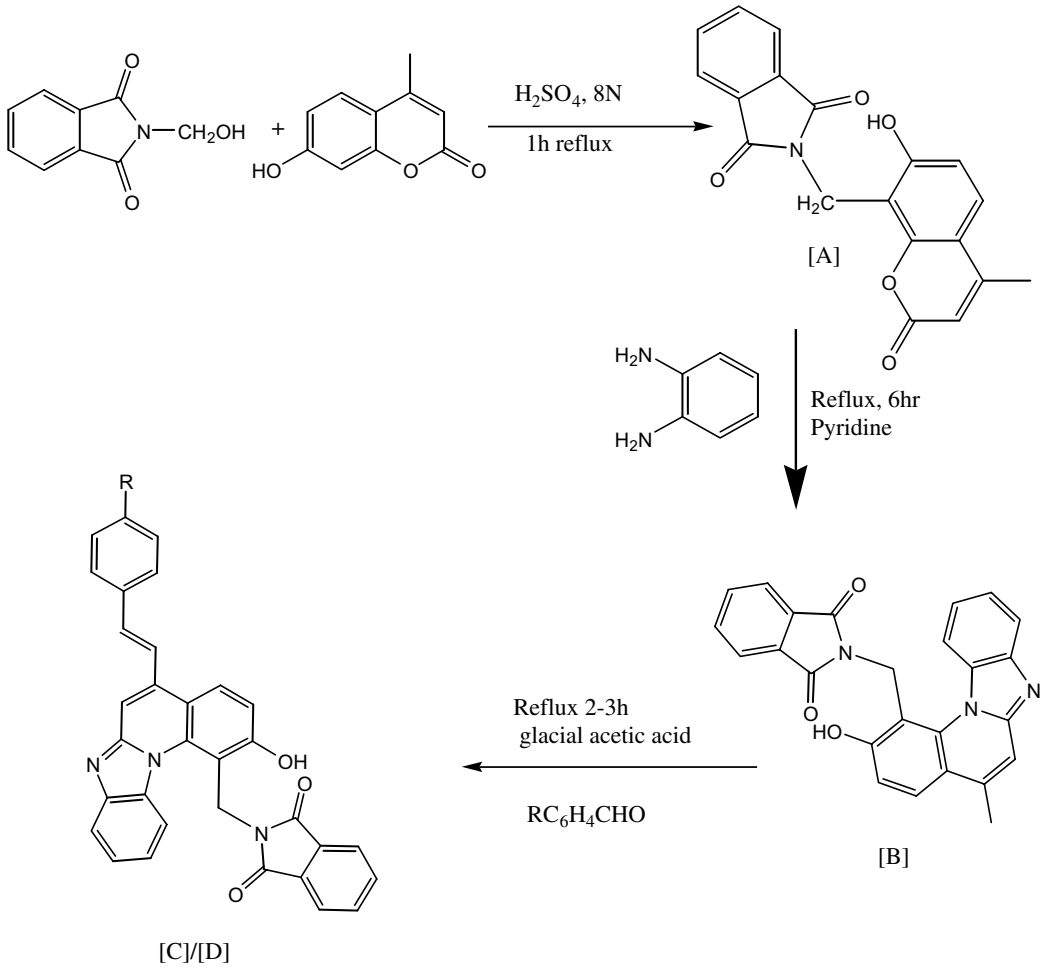
All chemicals used in the present study of analytical grade were purchased from Sigma, and all the details of materials and methods are given in reference and short notes part.[21] Synthesis of benzimidazole analogue [Bz-Im] is presented in Scheme 1 for the synthesis of [A–D]. The first two steps are performed as per,[20a] and synthesis and spectral characterization of [C] and [D] are mentioned as short notes.[22]

Compounds C and D were evaluated for their ability to displace [^{125}I] (BH)-CCK8 (sulfated) from isolated rat pancreatic acini (CCK_1) and guinea pig cerebral cortex membranes (CCK_2) according to established protocols.[23] Binding affinities expressed as IC_{50} are reported in Table 1 along with reference compound 3-ureido-1,4-benzodiazepine (Merck L-365260) and (VL-0494) without standard errors were obtained from no more than two experiments.

[^{125}I]BH-CCK-8 receptor binding assay in isolated rat pancreatic acinar cells was performed prepared by enzymatic digestion of pancreas as previously described by Makovec et al.[24] Drug displacing experiments were carried out by incubating acinar cells, [^{125}I]BH-CCK-8 (25 pM final concentration), and competitors in 0.5 mL total volume at 37 °C for 30 min, in shaking bath. At the end of incubation, 1 mL of ice-cold Hepes–Ringer buffer (10 mM Hepes, 118 mM NaCl, 1.13 mM $MgCl_2$, 1.28 mM $CaCl_2$,1% BSA, and 0.2 mg/mL soybean trypsin inhibitor, pH 7.4) was added and the tubes were centrifuged for 5 min at 12,500*g*. The supernatant was aspirated and the radioactivity associated to the pellet

Table 1. CCK receptor binding data of the target compounds

Complexes	(R)	Rat pancreatic acini (CCK_1), IC_{50} (μM)	Guinea pig brain cortex (CCK_2), IC_{50} (μM)
Ref. (VL-0494)	Ph	0.197 ± 0.107	16.40
Compound (C)	H	0.942 ± 0.141	4.32
Compound (D)	OH	0.665 ± 0.211	2.94



Scheme 1. Scheme A–D: representative scheme for the synthesis of [A–D].

measured. The non-specific binding was estimated in the presence of 1 jjM CCK-8, accounting for 15% of total binding.

[^{125}I]BH-CCK-8 receptor binding assay was performed in membranes from guinea pig cerebral cortices, prepared as previously described.[25] Protein concentration was determined by using bovine serum albumin (BSA) as standard. The binding experiments were performed in assay buffer containing 10 mM Hepes, 118 mM NaCl, 4.7 mM KCl, 5.0 mM $MgCl_2$, and 1.0 mM EGTA, pH 6.5, supplemented with 0.2 mg/mL bacitracin. The incubation of membrane suspension with labeled ligand and inhibitors was carried out in a microtiter 96-well filter plate (Multiscreen, Millipore Inc., Bedford, MA) with integral Whatman GF/B membrane filters. Aliquot of membranes (0.5 mg of protein per mL) was added to each well, containing [^{125}I]BH-CCK8 (25 pM), in a final volume of 250 jjl. The non-specific binding of iodinated peptide was determined in the presence of 1 pM CCK-8, accounting for 20% of total binding. Non-specific binding of [^{125}I]BH-CCK-8 to membrane filters (blank), measured in wells containing an equal amount of labeled ligand, but no membranes, was 0.2% of total radioligand added. After 120 min at 25 °C, the 96-well plate was placed on the vacuum filtration apparatus (Millipore Inc.).

Radiolabeling of the compounds [Bz-Im] has been done by taking 100 μl of 0.03 nM solution of the compound [Bz-Im] dissolved in DMSO in a shielded vial and 60 μl of 1×10^{-2} M $Sncl_2$. $2H_2O$ (dissolved in 1 mL of N_2 purged 10% acetic acid) was added followed by addition of (<1 h) freshly eluted saline solution of sodium pertechnetate ($NaTcO_4$) (74 MBq, 100 mL). The pH of the reaction mixture was adjusted to 6.5 with 0.1 M $NaHCO_3$ solution and the mixture was shaken to mix the contents. The vial was allowed to stand for 45 min at room temp. Labeling of the compound, radiochemical purity as well as R_f of the ^{99m}Tc (Bz-Im) complex were determined by ITLC-SG strips using 0.9% NaCl aqueous solution (saline) as developing solvent and simultaneously in acetone and PAW (pyridine, acetic acid, and water in 3:5:1.5 ratio).[24] Each TLC was cut in 0.1 cm segments and counts of each segment were taken. By this appropriate method, the percentage of complex formed between ^{99m}Tc and (Bz-Im) could be calculated as mentioned in Tables 2 and 3. The composition stability is mentioned only with compound (D) which is showing more appropriate results. The scintigraphic studies of the ^{99m}Tc-labeled [Bz-Im] in presence and absence of the CCK,the natural agonist, also confirm the nature of the labeled compound as CCK antagonist.

Table 2. R_f values

Complexes	Saline (R_f)	Acetone (R_f)	PAW (R_f)
Complex (C)	0	0.6	ND
Complex (D)	0	0.4	ND
$Na^{99m}TcO_4$	1	1	ND
Labeled complex (C)	0.3	0.3	1
Labeled complex (D)	0.1	0.1	1

Table 3. Determination of composition of the compound (Bz-Im) [D] with the ^{99m}Tc

S. No.	Molar ratio (Bz-Im ^{99m}Tc)	% Labeling
1	1:1	98.5
2	0.9:1	87.3
3	0.8:1	79.1
4	0.6:1	64.7
5	0.2:1	24.8
6	0:1	100% reduced
7	1.2:1	98.7
8	1.5:1	98.1
9	2.0:1	97.6

In vitro serum stability assay was performed in fresh human serum, which was prepared by allowing blood collected from healthy volunteers to clot for 1 h at 37 °C in a humidified incubator maintained at 5% carbon dioxide, 95% air. Then the sample was centrifuged at 400 and the serum was filtered through 0.22 μm syringe filter into sterile plastic culture tubes. The above freshly prepared technetium radiocomplex was incubated in fresh human serum under physiological conditions, that is, at 30 °C at a concentration of 100 nM/mL, and then analyzed by ITLC-SG at different time intervals to detect any dissociation of complex. Percentage of free pertechnetate at a particular time point that was estimated using saline and acetone as mobile phase represented percentage dissociation of the complex at that particular time point in serum.

The other activity analysis such as anti-fungal activity of compounds was performed by agar plate method[26] using the concentrations of 10, 20, 50, and 100 μg/mL of the test compounds. In order to perform the anti-fungal activity assay, 1 mL of each test compound was poured into a Petri dish having about 20–25 mL of molten potato dextrose agar medium. As the medium solidified, Petridishes were inoculated separately with the fungal isolates and kept at 27 °C for seven days. Percent inhibition in fungal zones was recorded after that. The solutions of the test compounds were prepared in dimethylsulfoxide (DMSO) and the required concentrations were achieved by diluting the solutions and stirring. Any turbidity if obtained was removed by quick filtration through fluted filter paper. Anti-fungal activity data are recorded in Table 4. The control is taken from amphotericin B.

In general, it has been observed that anti-fungal activity against *Aspergillus flavus* is superior to that against *Aspergillus niger* except in a few cases. Thus, compound [C] (Table 4) having R = hydrogen showed comparatively better anti-fungal activity against *A. niger* than *A. flavus* at two concentrations out of three concentrations. As well as, compound [D] having R = hydroxyl was shown the same nature.

Albino mice strain was used for the tissue distribution studies. Animal handling and experimentation was

Table 4. Anti-fungal activity data of Novel Benzimidazole derivatives [C, D]

Compound	R	Concentration (μg/mL)	*Aspergillus flavus*		*Aspergillus niger*	
			Colony diameter	Inhibition (%)	Colony diameter	Inhibition (%)
[C]	H	10	0.8	73.3	1.0	60.3
		20	0.6	76.7	0.8	76.8
		50	0.5	88.3	0.5	84.6
[D]	OH	10	1.2	60.8	0.8	60.7
		20	1.1	73.4	0.7	83.2
		50	0.7	92.1	0.7	83.6
Control (amphotericin)		20	3.0	86.4	2.0	79.9

carried out as per the guidelines of the Institutional Animal Ethics Committee. For biodistribution studies albino mice strain (A) was used as animal model and complex of ^{99m}Tc-labeled (Bz-Im) was used. An equal dose of 10 μCi of labeled test compound was injected in mice through tail vein of each animal. Similarly in another group of animals CCK-B (1 mg/kg by weight of animal model) was injected 10 min before injection of the radiolabeled test compound and biodistribution was studied under receptor saturation conditions. At different time intervals mice were sacrificed, blood was collected, and different tissue and organs were dissected and analyzed as shown in Tables 5 and 6. The radioactivity was measured in a gamma counter. The actual amount of radioactivity administered to each animal was calculated by subtracting the activity left in the tail from the activity injected. Radioactivity accumulated in each organ was expressed as percentage administered dose per gram of tissue. Total volume of the blood was calculated as 7% of the body weight.

Blood kinetics studies. The blood clearance study was performed in albino New-Zealand rabbits weighing approximately 2.5–3.0 kg after administration of 30 piCi of the ^{99m}Tc-labeled [Bz-In] in 0.3 mL via the ear vein. At different time intervals about 0.5 mL blood samples were withdrawn from the dorsal vein of the other ear and radioactivity was measured in the gamma counter. The data from the experiment are expressed as percentage of administered dose at each time interval in Figure 1.

The synthesized compound was characterized by IR (CO near 1600 cm^{-1}, OH 3200–3600, and aromatic below 900 cm^{-1}), NMR (aromatic proton in the range of 6–8.5 ppm and hetero functional group via D_2O exchange), and MASS spectroscopic studies, and also by the R_f values of the relevant ^{99m}Tc complexes (R_f values in Table 2). Labeling efficiencies were more than 97% and complex was stable about 12 h at 30 °C. Results of serum stability studies showed that the metal ion was intact under physiological conditions and unbound

Table 5. CCK-B receptor expressing organs (at the time intervals of 1, 2, 4, 6, 8 h) for compound [C]

Organs	Uptake (% ID/g)				
	After 1 h	After 2 h	After 4 h	After 6 h	After 8 h
Stomach	1	2.5	3	7	9.5
Intestine	0.5	0.5	0.2	3	3
Brain	0	0.2	0.45	1	1
Liver	18	25	18	15	15
Spleen	10	15	14	12	11
Kidney	5	7	2	5	5
Blood	3	2	2	0.5	0.5
Heart	1	0.4	0.3	0.3	0.2

Table 6. CCK-B receptor expressing organs (at the time intervals of 1, 2, 4, 6, 8 h) for compound [D]

Organs	Uptake (% ID/g)				
	After 1 h	After 2 h	After 4 h	After 6 h	After 8 h
Stomach	1	2.0	3	7	7.5
Intestine	0.5	0.5	0.2	1	1
Brain	0	0.2	0.45	1	1
Liver	16	23	14	15	15
Spleen	10	15	17	16	16
Kidney	5.5	6.0	3	4.5	4.5
Blood	2.5	2	2	2.5	2.5
Heart	0.8	0.5	0.2	0.1	0.2

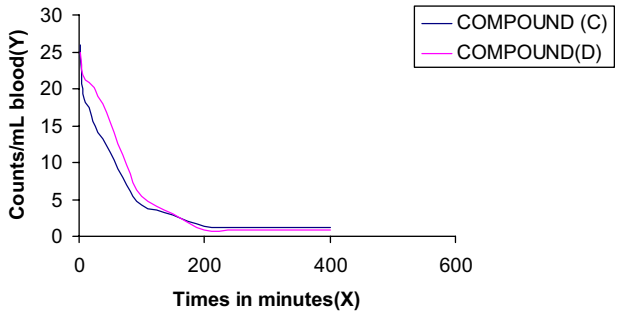


Figure 1. Blood kinetics study in rabbit.

radioactivity was less than 0.5% in 8 h. Complex formation between ^{99m}Tc and benzimidazole analogues was determined by using ITLC method. Various molar ratios were taken and ITLC was run simultaneously in two different solvent systems to determine the labeling. The data show more than 97% of labeling as the increasing ratio of (Bz-Im) is more than one, but in a very slight manner (Table 3). So 1:1 complex ratio was appropriate. The intactness of this compound is due to the fact that ^{99m}Tc molecules might occupy rest of the valency of the ^{99m}Tc left.

Biodistribution studies were performed by injecting radiolabeled compound, intravenously as a function of time, differentiating those tissues which are known or expected to express CCK receptors, organs involved in blood pool and excretion of the ligand. Higher uptake was found in liver, spleen, and stomach but retention was longer in stomach (Tables 5 and 6). Very low activity was found in brain probably due to inability of crossing the blood–brain barrier. The rapid blood clearance was also evident from the blood kinetics study.

The result of pretreatment studies of ^{99m}Tc-labeled [Bz-Im] shows that blocking with 1 mg/kg of CCK_2, 15 min before the injection of radiocomplex, reduced the accumulation in stomach, whereas the activity in the intestine was reduced Figures 2 and 3. There was increased accumulation of activity in liver, but reduction in case of rest of the organs studied.

Thus, the benzimidazole ring structure fused to pyridine ring might provide lead for new type of compounds having CCK-B receptor affinity. Some non-specific uptake in other tissues can be overcome by changing the basic skeleton by different R groups. The therapeutic potential of these complexes can be further extended by applying these in different animal models and cell lines.

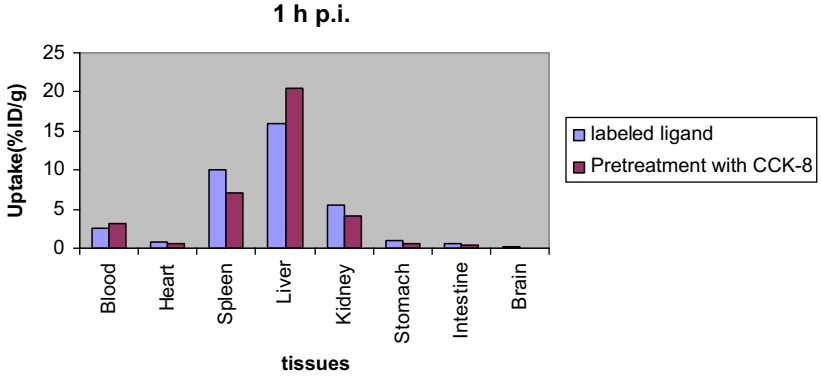


Figure 2. Pretreatment studies of ^{99m}Tc-labeled compound [C].

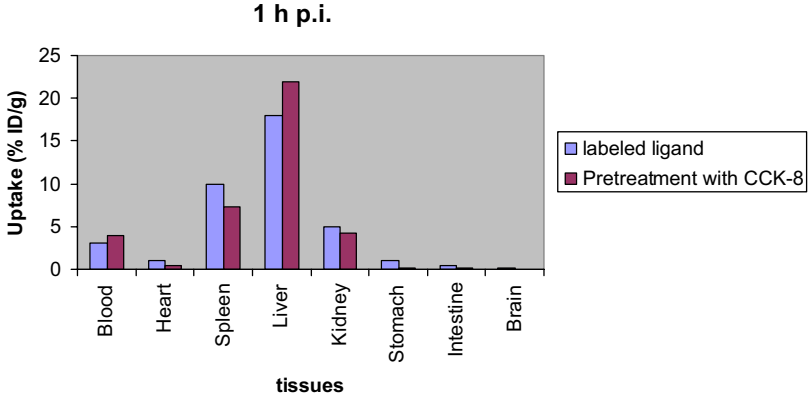


Figure 3. Pretreatment studies of ^{99m}Tc-labeled compound [D].

Acknowledgment

We thank Dr.R.P.Tripathi, Director, INMAS, for providing all the facilities and his deep interest and constant encouragement during the course of the studies.

References and notes

1. Orjales, A.; Mosquera, R.; Labeaga, L.; Rodes, R. *J. Med. Chem.* **1997**, *40*, 586.
2. Grimmett, M. *Compr. Heterocycl. Chem.* **1996**, *3*, 77.
3. Wank, S. A. *Am. J. Physiol.* **1998**, *274*, 607.
4. Turell, D. A. J., et al., 4th J. S. Ed.; London, Academic Press, 1989, pp 265–276.
5. Sullivan, O.; Solder, P. W. *Nature* **1961**, *192*, 314.
6. Iship, P. J.; Closier, M. D.; Neville, M. C. *J. Med. Chem.* **1972**, *15*, 951.
7. Walsh, J. H. Gastrin. In *Gut Peptides*; RavenPress: New York, 1994; pp 75–121.
8. Johnson, L. R. Trophic Effects of Gut Peptides. In *Handbook of Physiology Section 6*; Makhlouf, G. M., Ed.; American Physiological Society: Bethesda, MD, 1989; pp 291–310.
9. Crawley, J. N.; Corwin, R. L. *Peptides* **1994**, *15*, 731.
10. Hakanson, R.; Sundler, F. *Scand. J. Gastroenterol.* **1991**, *70*(Suppl. 180), 130.
11. Kopin, A. S.; Lee, Y.; McBride, E. W.; Miller, L. J.; Lu, M.; Lin, H. Y.; Kolakowski, L. F.; Beinborn, M. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 3605.
12. Wank, S. A.; Pisegna, J. R.; de Weerth, A. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 8691.
13. Mantyh, C. R.; Pappas, T. N.; Vigna, S. R. *Gastroenterology* **1994**, *107*, 1019.
14. Nakamura, M.; Oda, M.; Akaiwa, K. Y.; Tsukada, N.; Komatsu, H.; Tsuchiya, M. *Gastroenerology* **1988**, *94*, 968.
15. Wettstein, J. G.; Junien, J. L. *Pharmacol. Ther.* **1994**, *62*, 267.
16. Reubi, J. C. *J. Nucl. Med.* **1995**, *36*, 1825.
17. Krenning, E. P., Kwekkeboon, D. J., Pauwels, S. et al., 1995. Somatostatin receptor scintigraphy. In Freeman, L. M. (Ed.), Nucl. Med. Ann., pp 1–50.
18. Otte, A.; Mullar-Brand, J.; Dallas, S., et al. *Lancet* **1998**, *351*, 417.
19. Reubi, J. C.; Waser, B. *Cancer Res.* **1997**, *57*, 1377.
20. (a) Pandey, V. K.; Zehra, T.; Bajpai, S. K. *Indian J. Hetrocycl. Chem.* **2002**, *11*, 309; Synthesis of 4-methyl,7-hydroxyl 8[*N*-(methyl phathalamide)] benzo-[1,2,*b*] pyrane 2 one] [A] was achieved as 4 methyl-7 hydroxycoumarin (0.05 mol) and *N* methylol phthalamide (0.08 mol) were dissolved in 8 N sulfuric acid (100 mL) by stirring vigorously and carefully, while dissolving, the contents were occasionally cooled (since exothermic reaction occurred) in order to avoid the decomposition of the reactants. After refluxing one hour, the dark colored solution was left under refrigeration overnight and after that poured into cold water (250 mL). A solid separated out was filtered off and washed with water and a small portion treated with sodium bicarbonate solution (10%) to ensure the completion of reaction by ceasing of effervescence. The crude material was recrystallized by glacial acetic acid (yield 72%) mp 153 °C. IR (KBr pellets, cm^{-1}) 3385, 3290, 1640, 1715, 1196, 782;
^{1}HNMR (200 MHz, $CDCl_3$) δ ppm; 7.69–8.13(m 4H, phthalamide aryl ring), 4.89 (S, 2H, N-substituted methylene), 5.00 (S, 1H, OH exchanged with D_2O), 6.57–7.27 (m, 3H, coumarin ring), 1.71 (S, 3H, coumarin ring).Anal. Calculated for $C_{15}H_{13}NO_5$, 67.8% C (theoretical C 66.52%), 3.89% H (theoretical H 4.03%), 5.02% N (theoretical N 4.88%). Found: *m*/*z* 334[M]$^+$, 160, 105, 77; Synthesis of 4-methyl,7-hydroxy-8(*N*-methyl phthalamide) quinoline(1,5*c*) benzimidazole [B] was carried out mixing 4-methyl,7-hydroxy [*N*-(methyl phthalamide)] benzo-[1,2,*b*] pyrane 2 one [A] (0.01 mol) and *o*-phenylenediamine (0.015 mol) in dry pyridine (50 mL). The refluxing reaction mixture was stirred for 6–7 h. This reaction was monitored by TLC and on completion of reaction the solvent was removed in vacuo and the reaction mixture was cooled and then poured into ice-cold diluted HCl (50 mL). On neutralization it was purified by column chromatography [column of Sio_2 (80 g), pre-adsorption of the residue at SiO_2 with ethyl acetate, elution with petroleum ether/ethyl acetate = 60:40 (v/v)] to obtain the product. (Yield 81%) mp 101 °C. IR (KBr pellets cm^{-1}) 3461, 3355, 2988, 1657,1278,742; ^{1}H NMR (200 MHz, $CDCl_3$)δ ppm; 7.69–8.13 (m, 3H, quinoline ring). 5.02 (S, 1H, OH exchanged with D_2O), 6.4–7.39 (m, 8H, aromatic rings). Anal. Calcd for $C_{25}H_{17}N_3O_3$, 71.42% C (theoretical C 73.71%), 4.1% H (theoretical H 4.18%), 9.87% N (theoretical N 10.12). Found: *m*/*z* 404 [M]$^+$, 405, 254, 77; (b) Qun li, T. Li.; Keith, W. *Bioorg. Med. Chem. Lett.* **2006**, *15*, 2918; (c) Miyuki, Tatsuta; kataokal, M. *Bioorg. Med. Chem.Lett.* **2005**, *14*, 2265; (d) Zhang, Wei; Tempest, Paul *Tetrahedron Lett.* **2004**, *45*, 6757.
21. All the solvents were used after distillation. TLC was run on the silica gel coated aluminum sheets (silica gel 60 F_{254}, E. Merck, Germany) and visualized in UV light. Melting points were determined by using Thomas Hoover apparatus in repetitive manner. IR spectra were recorded on the FT-IR Perkin-Elmer spectrum BX spectrophotometer with KBr discs. NMR spectra were measured in $CDCl_3$ by Bruker 200 MHz apparatus with Me_4Si as an internal standard. EI-MS spectra were recorded on a JEOL SX102/DA (KV 10 mA) instrument. Elemental analysis was done on elemental analyzer GmbH variable system. Radiocomplexation and radiochemical purity were checked by instant strip chromatography (silica gel impregnated paper chromatography) with ITLC-SG (Gellman sciences, Ann Arbor, MI, USA). The gamma scintillation counting was done at ECA (Electronic Corporation of India Ltd.) with Gamma ray spectrometer K 2700 B. All the reaction steps were monitored by thin layer chromatography (TLC) [chloroform/methanol/hexane: 4:3:1]. Distilled water is used during whole of the procedure.
22. Synthesis of 4-styryl,7-hydroxy 8[(*N*-methyl phthalamide)] quinoline-(1,5*c*) benzimidazole [C] was carried out by heating equimolar quantity of (B) and benzaldehyde in glacial acetic acid (40 mL) heating under refluxing condition for 2 h. on a sand bath. Reaction mixture was poured onto crushed ice and extracted with ether. The crude material was washed by Na_2CO_3 solution and dried over Na_2SO_4. Yield (66%) mp 216–218°C. IR (KBr pellets cm^{-1}) 3461, 2988, 1754, 1576, 1306, 742 . ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm; 7.7–8.3 (m, 4H, phthalamide aryl ring). 6.4-7.1 (m, 12H, other aromatic rings). Anal. Calcd for $C_{32}H_{21}N_3O_3$, 76.21% C (theoretical C 77.56), 4.93% H (theoretical H 4.74%), 7.90% N (theoretical N 8.18%). Found: *m*/*z* 481 [M]$^+$, 335, 105, 77. Similarly synthesis of 4-(P-hydroxyl)-styryl-7-hydroxy-8[(*N*-methyl phthalamide)] quinoline-(1,5*c*) benzimidazole [D] was by taking (0.05) mole of (B) and P-hydroxy benzaldehyde in glacial acetic acid (0.09) mole as refluxing mixture and heating for 3 h. The crude material was extracted in the same manner as non-substituted styryl benzimidazole derivatives were obtained. The solvent was removed under reduced pressure. The purity of compound was confirmed by thin layer chromatography. Yield (59.6%) mp 149.8 °C.

IR (KBr pellets cm^{-1}) 1670, 3400–3672, 3363, 1660, 826. ^1H NMR (200 MHz, $CDCl_3$) δ ppm; 7.2–8.2 (m, 4H, phthalamide aryl ring). 6.9–7.4(m, 11H, other aromatic rings). Anal. Cald for $C_{32}H_{21}N_3O_4$, 73.8% C (theoretical C 75.15%), 3.9% H (theoretical H 4.01%), 13.07% N (theoretical N 12.72%). Found: *m/z* 496 $[M]^+$.

23. Giovanetti, L. C.; Mennuni, J. *Med. Chem.* **1992**, *35*, 28.
24. Makovec, F.; Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248.
25. Kumari, S.; Kalra, Neetu; Mishra, P.; Mishra, A.; Chopra, M. *Nucl. Med. Biol.* **2004**, *31*, 1087.
26. Verma, R. S.; Imam, S. A. *Ind. J. Microbiol.* **1997**, *13*, 45.