BENZIMIDAZOLE DERIVATIVES WITH POTENTIAL CYTOTOXIC ACTIVITY - SYNTHESIS AND THEIR STRUCTURE-ACTIVITY RELATIONSHIP

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Abstract

Benzimidazole is an important class of compounds with a wide spectrum of biological activity. This five membered heterocyclic moiety confer for various biological activities. In this study, we prepared some benzimidazole derivatives of 2-(2´-pyridyl) benzimidazole and investigated their cytotoxic effect. The structure of the synthesized analogs were characterized by UV, IR and ^{1}H NMR spectral data and evaluated for their cytotoxic effect.

Introduction

Medicinal Chemistry involves the design of new therapeutic molecules and their development into useful medicines (Seki, *et al*., 2000; Goncalves, *et al.,* 2000; Pathak, *et al*., 1992; Tripathi, *et al*., 1981). Clinically used drugs are not discovered directly what is more likely discovered is known as lead compound. The lead is a model compound that has the desired biological or pharmacological activity but may have many other undesirable effects. The structure of lead is then modified by synthesis to amplify the desired activity and to eradicate the unwanted properties (Veiweg, *et al*., 1988; Cheng, 1985 and Zikhareva, *et al*., 1982).

According to recent studies, modern anti-cancer drugs used to treat early stages of neoplastic diseases have a mechanism of action based on characteristic the hypoxia for cancer cells (McKeown et al., 2007). These compounds belong to the group of drugs with bioreductive mechanism of action (Albertella et al., 2008). They are activated in hypoxic conditions influenced by specific biochemical mechanisms (Denny, 2000). Derivatives containing benzimidazole ring are active as human DNA topoisomerase I inhibitors (Selcen et al., 2007; 2009; Coban et al., 2009; Singh & Tandon, 2011).

Benzimidazole compounds had proven quality exhibiting a broad range of pharmacological actions, including analgesic (Shaaban, *et al.,* 2008), anti-inflammatory (Mohamed, *et al.*, 2006), anticancer (Abdel-Monem, *et al*., 2007), antihypertensive (Serafin, *et al.,* 1989), antimicrobial (Rida *et al.*, 2006; Rohini, *et al.,* 2009), antioxidant (Ayhan-Kileigil, *et al.,* 2004), anthelmintic (Thomas, *et al.,* 1988), tranquilizing (Buu-Hoi, *et al.,* 1963), anti- paralyzing (Domino, *et al.,* 1952), immuno-suppression, antitumor and antiviral inhibition activities (Paget, *et al.,* 1969). Keeping this view in mind, 2-(2´-pyridyl)-benzimidazole has been selected as lead molecule from the heterocyclic family for the preparation of derivatives having biological potentials and their screening to find the probability of commercial exploitation.

Materials and Methods

Chemistry: 2-(2´-pyridyl) benzimidazole (**Ia)** and corresponding substituted phenacyl halides in equimolar quantities (0.01mole) were dissolved in 15-20 mL acetone separately in conical flask and mixed together in a round bottom flask. The reaction mixture was stirred on magnetic stirrer for four hrs and then refluxed on water bath for about 5 to 6 hrs. Precipitates appeared either on mixing the reactants at once or after some hours on refluxing. Completion of reaction was monitored by TLC. The resulting precipitates of products were filtered and washed with warm acetone to remove the untreated starting material. The precipitates of each product were re-crystallized. The pure compounds were dried in a vacuum desiccators over anhydrous calcium sulphate.

Biology:
Cytotoxic Activity
Brine Shrimp Lethality Bio-Assay
The brine shrimp lethality test is useful in predicting cytotoxicity (Mansoor, *et al*., 2007; Zhao, *et al.*, 1999) and for performing bioassay guided isolation (Meyer, *et al*., 1982).

The method utilizing brine shrimp (*Artemia Salina,* Leach) was proposed as a simple bioassay for natural and synthetic products in research. Brine Shrimp had been previously utilized in various bioassay systems. Among these the analysis of morphine likes compounds (Richter, *et al.,* 1970), carcinogenicity of phorpol ester, (Kinghorn., *et al.,* 1967) and toxicants in marine environments (Vanhaecke., *et al.,* 1981) were included. In a similar fashion cytotoxic pro drugs have been developed as means of anticancer therapy. The compounds were screened at 100, 10 and l µg/mL and LD_{50} values were calculated by using Finney computer program (Finney 1971).

Cell-Culture PC-3: This technique is helpful to discover the anticancer effect in case of prostate cancer. Prostate cancer is the most common malignancy in men and one of the leading causes of cancer death (Jemal, *et al*., 2006). Early stage prostate cancer is curable whereas more advanced tumors can successfully be treated only before tumor metastasis has occurred (Loberg, *et al.*, 2005; Plenta, *et al.,* 2005).

One of the most exciting discoveries from plants in recent years has been that of Taxol, which is now used clinically for the treatment of ovarian cancer (Kingston, 1993). Taxol was discovered in 1962 by Monroe Wall of the North Carolina Triangle Institute. Its chemical structure was shown to be a complex nitrogen containing diterpenoid with eleven chiral centers. As these novel derivatives also contained complex nitrogen therefore, they are expected to possess anticancer properties.

Experimental

1. *1-[2-(3´-nitrophenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1H-benzimidazol-1-ium bromide*

The characteristic UV absorption was shown at 219 nm (methanol), while the IR spectrum exhibited typical absorption for (C-H aromatic) at 3018, (C=O) at 1715 (C=N) at 1612, (C=C aromatic) 1576, (C-C aromatic) at 1339, (NO_2) at 1539 cm^{-1}.

The mass fragmentation pattern in the EI-MS (electron impact mass spectrum) of compound **1** exhibited a number of fragments and their pattern of break down helped in the structure elucidation. The spectrum showed the molecular ion peak at m/z 358 corresponding to the molecular formula $C_{20}H_{14}N_4O_3$.

In ^{1}H-NMR spectrum of compound **1** was observed in the aromatic region which includes a proton as doublet at δ 8.77 (d, *J* = 8.0 Hz) for H-12 and one proton multiplet resonated at δ 8.76 for H-20 and doublet at δ 9.01 (d, *J* = 8.0 Hz) for H-15. While down field one proton multiplet resonated at δ 8.62 for H-4'. Multiplet at δ 8.47 downfield was assigned to H-14 and down field one proton multiplet at δ 7.97 for H-5'. A three proton multiplet was observed at δ 6.94-7.71 for H-7, H-8 and H-13. Two protons set of double doublet centered at δ 7.32 (dd, *J* = 8.0, 1.5 Hz) and 7.94 (dd, *J* = 7.8, 1.4 Hz) for H-6, H-9, respectively. Two protons resonated as singlet at δ 5.00 for H-7'.

2. *1-[2-(3´4´-dihydroxyphenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1H-benzimidazol-1-ium chloride*

The characteristic UV absorption in methanol was observed at 231 nm, while the IR spectrum exhibited typical absorption for (C-H aromatic) at 3029, (C=C aromatic) at 1558, (C=N) at 1597, (C-C aromatic) at 1339, (C=O) at 1716, (OH) at 3617 cm^{-1}.

The mass fragmentation pattern in the EIMS (electron impact mass spectrum) of compound **2** exhibited a number of fragments and their pattern of break down helped in the structure elucidation. The spectrum showed the molecular ion peak at m/z 346 corresponding to the molecular formula $C_{20}H_{15}N_3O_3$.

In ^{1}H-NMR spectrum of compound **2** was observed in the aromatic region which includes two proton doublet at δ 9.27 (d, J = 7.9 Hz) for H-12 and δ 8.99 (d, J = 8.1 Hz) for H-15, Two proton resonated as multiplet at δ 8.44 for H-14 and δ 6.95 for H-13., Two protons set of double doublet observed at δ 7.47 (dd, *J* = 8.3, 1.8 Hz) and 7.50 (dd, *J* = 8.0, 1.5 Hz) for H-6, H-9, respectively. The multiplet region was assigned at δ 6.95-8.44 for H-7, H-8, H-13 and H-14. A two proton singlet was observed at δ 5.00 for H-7'.

3. *1-[2-(4´-chlorophenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1H-benzimidazol-1-ium bromide*

The characteristic UV absorption (methanol) was observed at 259 nm, while the IR spectrum exhibited typical absorption for (C-H aromatic) at 3040, (C=C aromatic) at 1554, (C=N) at 1625, (C-C Aromatic) at 1338, (C=O) at 1716, (C-Cl) at 746 cm^{-1}.

The mass fragmentation pattern in the EI-MS (electron impact mass spectrum) of compound **3** exhibited a number of fragments and their pattern of break down helped in the structure elucidation. The spectrum showed the molecular ion peak at m/z 348 corresponding to the molecular formula $C_{20}H_{14}N_3OCl$.

In ^{1}H-NMR spectrum of compound **3** was observed in the aromatic region which includes a two proton doublet at δ 9.25 (d, *J* = 7.9 Hz) and δ 8.98 (d, *J* = 8.1 Hz) for H-12 and H-15). Two protons were observed as double doublets δ 7.47 (dd, *J* = 8.0, 1.7 Hz) and 7.50 (dd, *J* = 8.0, 1.5 Hz) for H-6 and H-9, respectively. A multiplet region at δ 6.95- 8.43 were assigned to H-13, H-8, H-7 and H-14. Two protons singlet centered at δ 5.00 H-7'. The two proton signal as doublet were resonated at δ 7.70 (d, *J* = 7.5 Hz) for H-2'/H-6' and 7.76 (d, *J* = 7.5 Hz) for H-3'/5'.

4. ***1-[2-(2´-nitrophenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1H-benzimidazol-1-ium bromide***

The characteristic UV absorption in methanol was observed at 243 nm, while the IR spectrum exhibited typical absorption for (C-H aromatic) at 3029, (C=C aromatic) at 1580, (C=N) at 1629, (C-C aromatic) at 1346, (C=O) at 1720, (NO_2) at 1528 cm^{-1}.

The mass fragmentation pattern in the EIMS (electron impact mass spectrum) of compound **4** exhibited a number of fragments and their pattern of break down helped in the structure elucidation. The spectrum showed the molecular ion peak at m/z 358 corresponding to the molecular formula $C_{20}H_{14}N_4O_3$.

In ^{1}H-NMR spectrum of compound **4** was observed in the aromatic region as multiplet 6.44-8.48 for H-13, H-14, H-7, H-8, H-3', H-4' and H-5'. A pair of double doublet were assigned to H-6 and H-9 at δ 7.38 (dd, *J* = 8.0, 1.5 Hz) and 7.68 (dd, *J* = 8.0, 1.5 Hz), respectively. Another double doublet proton was observed at δ 7.68 (dd, *J* = 7.0, 1.4 Hz) for H-6'. A pair of doublet protons resonated at δ 9.00 (d, *J* = 7.9 Hz) and 8.98 (d, *J* = 8.1 Hz). A singlet of two protons H-7' was observed at δ 5.02.

5. ***1-[2-(4´-nitrophenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1H-benzimidazol-1-ium bromide***

The characteristic UV absorption in methanol was observed at 218 nm, while the IR spectrum exhibited typical absorption for(C-H aromatic) at 3025, (C=N) at 1631, (C=C aromatic) at 1570, (C-C aromatic) at 1344, (C=O) at 1706, (NO_2) at 1520 cm^{-1}.

The mass fragmentation pattern in the EI-MS (electron impact mass spectrum) of compound **5** exhibited a number of fragments and their pattern of break down helped in the structure elucidation. The spectrum showed the molecular ion peak at m/z 358 corresponding to the molecular formula $C_{20}H_{14}N_4O_3$.

In ^{1}H-NMR spectrum of compound **5** was observed in the aromatic region which include a two proton doublet at δ 8.40 (d, *J* = 7.9 Hz) for H-2'/H-6' and 8.35 (d, *J* = 7.9 Hz) for H-3'/H-5'. Similarly a pair of doublet was assigned to H-12 and H-15 at δ 9.00 (d, *J* = 9.0 Hz) and δ 8.98 (d, *J* = 8.0 Hz), respectively. The region at δ 6.44-8.47 as multiplet could be assigned to H-7, H-8, H-13 and H-14. Two double doublet were resonated at δ 7.32 (dd, *J* = 8.1, 1.5 Hz) for H-6 and δ 7.65 (dd, *J* = 8.0, 1.5 Hz) for H-9. Two proton singlet was observed at δ 5.01 for H-7'.

6. ***1-[2-(4'-fluorophenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1H-benzimidazol-1-ium bromide***

The characteristic UV absorption was observed in methanol at 248 nm, while the IR spectrum exhibited typical absorption for (C-H aromatic) at 3030, (C=C aromatic) at 1589, (C=N) at 1635, (C=O) at 1698, (C-C aromatic) at 1337 cm^{-1}.

The mass fragmentation pattern in the EIMS (electron impact mass spectrum) of compound **6** exhibited a number of fragments and their pattern of break down helped in the structure elucidation. The spectrum showed the molecular ion peak at m/z 331 corresponding to the molecular formula $C_{20}H_{14}N_3OF$.

In ^{1}H-NMR spectrum of compound **6** was observed in the aromatic region which included a two double doublet were resonated at δ 7.34 (dd, *J* = 8.0, 1.5 Hz) for H-6 and δ 7.68 (dd, *J* = 8.0, 1.7 Hz) for H-9. Two protons doublet at δ 8.19 (d, *J* = 7.7 Hz) for H-2'/H-6' and 7.19 (d, *J* = 7.7 Hz) for H-3'/H-5'. A pair of doublet were assigned to H-12 and H-15 at δ 9.00 (d, *J* = 7.8 Hz) and δ 8.96 (d, *J* = 8.0 Hz), respectively. Two proton singlet was observed at δ 5.01 for H-7'. The region at δ 6.44-8.39 as four proton multiplet could be assigned to H-7, H-8, H-13 and H-14.

7. ***1-[2-(2´,4´-difluorophenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1H-benzimidazol-1-ium bromide***

The characteristic UV absorption in methanol was observed at 252 nm, while the IR spectrum exhibited typical absorption for(C-H aromatic) at 3028, (C=C aromatic) at 1600, (C=N) at 1639, (C-C aromatic) at 1340, (C=O) at 1710, (C-F) at 910 cm^{-1}.

The mass fragmentation pattern in the EI-MS (electron impact mass spectrum) of compound **7** exhibited a number of fragments and their pattern of break down helped in the structure elucidation. The spectrum showed the molecular ion peak at m/z 349 corresponding to the molecular formula $C_{20}H_{13}N_3OF_2$.

In ^{1}H-NMR spectrum of compound **7** was observed in the aromatic region which included a four protons as doublet at δ 7.64 for H-6' (d, *J* = 7.3 Hz), δ 7.23 for H-3' (d, *J* = 1.4 Hz) δ 9.00 for H-12 (d, *J* = 7.8 Hz) and δ 8.64 for H-15 (d, *J* = 8.0 Hz). Three protons as double doublet resonated at δ 7.25 for H-5' (dd, *J* = 7.3, 1.4 Hz) δ 7.33 for H-6 (dd, *J* = 7.9, 1.5 Hz) and δ 7.68 for H-9 (dd, *J* = 8.0, 1.7 Hz). Four protons H-7, H-8, H-13 and H-14 were observed as multiplet at δ 7.21-8.40. Similarly, two protons appeared as singlet at δ 5.00 for H-7'.

8. ***1-[2-(2-oxo-2-phenyl)-ethyl]-2-(2´-pyridinyl)-1H-benzimidazol-1-ium bromide***

The characteristic UV absorption was observed at 310 nm (methanol), while at the IR spectrum exhibited typical absorption for (C-H aromatic) at 3030, (C=C aromatic) at 1520, (C=N) 1601, (C=O) 1730, (C-C aromatic) at 1339 cm^{-1}.

The mass fragmentation pattern in the EI-MS (electron impact mass spectrum) of compound **8** exhibited a number of fragments and their pattern of break down helped in the structure elucidation. The spectrum showed the molecular ion peak at m/z 313 corresponding to the molecular formula $C_{20}H_{15}N_3O$.

In ^{1}H-NMR spectrum of compound **8** was observed in the aromatic region which include a two proton doublet at δ 9.02 for H-12 (d, *J* = 7.8 Hz) and for H-15 δ at 8.53 (d, *J* = 8.0 Hz). Multiplet region for nine aromatic protons were observed at δ 7.21-8.50 were assigned to H-7, H-8, H-13, H-14, H- 2' and H- 6' Two double doublet were resonated at δ 7.31 (dd, *J* = 8.0, 1.5 Hz) and δ 7.68 (dd, *J* = 8.0, 1.9 Hz) were assigned to H-6 and H-9, respectively. Two proton were observed as singlet at δ 5.00 for H-7'.

9. ***1-(2-[1´,1´´-biphenyl]-4-yl-2-oxo-ethyl)-2-(2´-pyridinyl)-1H-benzimidazol-1-ium bromide***

The characteristic UV absorption (methanol) was observed at 215 nm, while the IR spectrum exhibited typical absorption for (C-H aromatic) at 3015, (C=C aromatic) at 1539, (C=N) at 1610, (C=O) at 1690, (C-C aromatic) at 1337 cm^{-1}.

The mass fragmentation pattern in the EI-MS (electron impact mass spectrum) of compound **9** exhibited a number of fragments and their pattern of break down helped in the structure elucidation. The spectrum showed the molecular ion peak at m/z 389 corresponding to the molecular formula $C_{26}H_{19}N_3O$.

In ^{1}H-NMR spectrum of compound **9** was observed in the aromatic region which includes a two proton doublet at δ 9.00 for H-12 (d, *J* = 7.8 Hz) and for H-15 δ 8.40 (d, *J* = 8.0 Hz). Multiplet region for five aromatic protons were resonated at δ 7.36-7.67 for H-2"-H-6". Similarly, another multiplet region for H-7, H-8, H-13 and H-14 were observed at δ 7.21-8.50. Two double doublet were resonated at δ 7.36 (dd, *J* = 8.0, 1.5 Hz) and δ 7.67 (dd, *J* = 8.0, 1.7 Hz) were assigned to H-6 and H-9, respectively. Two proton were observed as singlet at δ 5.00 for H-7'.

10. ***1-[2-(4´-methoxyphenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1H-benzimidazol-1-ium bromide***

The characteristic UV absorption (methanol) was observed at 293 nm, while the IR spectrum exhibited typical absorption for (C-H aromatic) at 3008, (C=C aromatic) 1571, (C=N) at 1620, (C=O) at 682, (C-C aromatic) at 1340, (OCH_3) at 1108 cm^{-1}.

The mass fragmentation pattern in the EI-MS (electron impact mass spectrum) of compound **10** exhibited a number of fragments and their pattern of break down helped in the structure elucidation. The spectrum showed the molecular ion peak at m/z 343 corresponding to the molecular formula $C_{21}H_{17}N_3O_2$.

In ^{1}H-NMR spectrum of compound **10** was observed in the aromatic region which includes a two proton doublet at δ 9.00 for H-12 (d, *J* = 7.8 Hz) and for H-15 δ 8.34 (d, *J* = 7.9 Hz). Multiplet region were observed at δ 7.15-8.50 for H-7, H-8, H-13 and H-14. Two double doublet were observed for H-6 at δ 7.36 (dd, *J* = 7.9, 1.5 Hz) and for H-9 at δ 7.67 (dd, *J* = 8.0, 1.5 Hz). A pair of doublet proton for H-2'/H-6' were observed at δ 7.85 (d, *J* = 7.1 Hz) and for H-3'/H-5' at δ 6.50 (d, *J* = 7.1 Hz). The three methoxy protons were resonated as singlet at δ 3.60 could be assigned to at 4' position. Two proton were observed as singlet at δ 5.01 for H-7'.

11. ***1-[2-(2´,5´dimethoxyphenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1H- benzimidazole-1- ium bromide.***

The characteristic UV absorption (methanol) was observed at 295 nm, while the IR spectrum exhibited typical absorption for(C-H aromatic) at 3010, (C=N) at 1625, (C=O) at 1683, (C-C aromatic) at 1338, (OCH_3) at 1110 cm^{-1}.

The mass fragmentation pattern in the EIMS (electron impact mass spectrum) of compound **11** exhibited a number of fragments and their pattern of break down helped in the structure elucidation. The spectrum showed the molecular ion peak at m/z 373 corresponding to the molecular formula $C_{22}H_{19}N_3O_3$.

In ^{1}H-NMR spectrum of compound **11** was observed in the aromatic region which includes a one proton singlet resonated at δ 7.31 for H-2', a double doublet at δ 7.70 (dd, *J* = 7.0, 1.4 Hz) for H-4' and a doublet at δ 7.71 (d, *J* = 7.0 Hz) for H-3'. While a set of two proton down field doublet resonated at δ 9.02 and 8.39 for H-12 and H-15 (d, *J* = 7.7 Hz) and (d, *J* = 7.9 Hz). Two double doublet were observed for H-6 at δ 7.37 (dd, *J* = 8.0, 1.5 Hz) and for H-9 at δ 7.67 (dd, *J* = 7.9, 1.6 Hz). Multiplet region were observed at δ 7.15-8.43 for H-7, H-8, H-13 and H-14. Whereas, the two methoxy protons were observed at H-2' and H-5' positions could be assigned as singlet at δ 3.75 and 4.00, respectively. Two proton were observed as singlet at δ 5.01 for H-7'.

Results and Discussion

When comparing these active derivatives, it was found that the compound containing unsubstituted phenyl moiety possessed lesser activity (LD_{50} = 0.0320 μg/mL) as compared to the substituted phenyl ring.

It was also shown that substitution of different groups at the phenyl ring imparted varying degrees of cytotoxic potentials such as addition of chloro group at *para* position to the phenyl ring made the compound more potent. Similarly, compound **4** containing nitro group at *ortho* position and compound **2** having two hydroxyl groups at *meta* and *para* positions were losing the potency as expressed by their LD_{50}=1.701 and 5.1638 µg/mL respectively. Therefore, it can be concluded that the substitution at the phenyl ring was responsible to produce highly significant and potent cytotoxic effects. The cytotoxicity in the active derivatives was in the order of chloro > phenyl > nitro > hydroxyl.

Table 1. Brine Shrimp (*Artemia Salina*) lethality bioassay results for 2-(2´-pyridyl) benzimidazole (Ia) and its phenyl derivatives (1 – 11).

S. No	IUPAC Name's	LD_{50} (µg/mL)
1	2-(2′-pyridyl)benzimidazole (Ia)	-
2	1-[2-(3'-nitrophenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1*H*-benzimidazol-1-ium bromide (1)	-
3	1-[2-(3',4'-dihydroxyphenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1*H*-benzimidazol-1-ium chloride (2)	5.1638
4	1-[2-(4'-chlorophenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1*H*-benzimidazol-1-ium bromide (3)	0.0006
5	1-[2-(2'-nitrophenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1*H*-benzimidazol-1-ium bromide (4)	1.701
6	1-[2-(4'-nitrophenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1*H*-benzimidazol-1-ium bromide (5)	-
7	1-[2-(4'-fluorophenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1*H*-benzimidazol-1-ium bromide (6)	-
8	1-[2-(2',4'-difluorophenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1*H*-benzimidazol-1-ium bromide (7)	N.T
9	1-(2-oxo-2-phenylethyl)-2-(2´-pyridinyl)-1*H*-benzimidazol-1-ium bromide (8)	0.0320
10	1-(2-[1',1"-biphenyl]-4-yl-2-oxo-ethyl)-2-(2´-pyridinyl)-1*H*-benzimidazol-1-ium bromide (9)	-
11	1-[2-(4'-methoxyphenyl)-2-oxo-ethyl]-2-(2-pyridinyl)-1*H*-benzimidazol-1-ium bromide (10)	-
12	1-[2-(2',5'-dimethoxyphenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1*H*-benzimidazol-1-ium bromide (11)	-
13	Etoposide	7.4625

Activity Key: - = no activity
NT. = not tested

Table 2. Results of *In-vitro* cytotoxic activity by Cell-Culture PC-3 of 2-(2´-pyridyl) benzimidazole (Ia) and its derivatives (1-11).

S. No	IUPAC Name's	IC_{50}±SD(µM)
1	2-(2′-pyridyl)benzimidazole (Ia)	>100
2	1-[2-(3'-nitrophenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1*H*-benzimidazol-1-ium bromide (1)	35.58±0.8
3	1-[2-(3',4'-dihydroxyphenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1*H*-benzimidazol-1-ium chloride (2)	28.42±1.21
4	1-[2-(4'-chlorophenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1*H*-benzimidazol-1-ium bromide (3)	28.16±0.46.
5	1-[2-(2'-nitrophenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1*H*-benzimidazol-1-ium bromide (4)	32.22±0.6
6	1-[2-(4'-nitrophenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1*H*-benzimidazol-1-ium bromide (5)	55.7±1.04
7	1-[2-(4'-fluorophenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1*H*-benzimidazol-1-ium bromide (6)	>100
8	1-[2-(2',4'-difluorophenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1*H*-benzimidazol-1-ium bromide (7)	>100
9	1-(2-oxo-2-phenylethyl)-2-(2´-pyridinyl)-1*H*-benzimidazol-1-ium bromide (8)	>100
10	1-(2-[1',1"-biphenyl]-4-yl-2-oxo-ethyl)-2-(2´-pyridinyl)-1*H*-benzimidazol-1-ium bromide (9)	27.79±0.3
11	1-[2-(4'-methoxyphenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1*H*-benzimidazol-1-ium bromide (10)	>100
12	1-[2-(2',5'-dimethoxyphenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1*H*-benzimidazol-1-ium bromide (11)	>100

13	Doxirubacin	0.912

During the course of searching for the cytotoxic compounds from synthetic source, we found that some of the derivatives of compound Ia showed potent cytotoxicity in the brine shrimp lethality assay and the results were displayed in Table-1.

From the table it was evident that the parent molecule, 2-(2´-pyridyl) benzimidazole showed no cytotoxicity at all three doses (100, 10 and l µg/mL) while some of its derivatives, 2, 3, 4 and 8 were proved to be highly significant cytotoxic agents. Interestingly, they also expressed more potency as compared to the standard drug (etoposide) in brine shrimp lethality bioassay.

Compound Ia, showed no cytotoxic effect on PC-3 and some of its newly synthesized derivatives were found active on prostate cancer cell. Three derivatives, 2, 3 and 9 showed moderate activity on PC-3 with IC_{50} ± SD (µM) 28.42 ± 1.21, 28.16±0.46 and 27.79 ± 0.3 respectively at the concentration of 1 mg/mL as compared to the standard drug, Doxorubicin while compounds 1, 4 and 5 showed low activity. The results were not potent. Rest of the compounds was found to exhibit no considerable cytotoxic activity against prostate cancer cell 3 as shown in the Table-2.

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