Synthesis, Leishmanicidal and anticancer activity of 4-(2-keto-1-benzimidazolinyl) piperidine and 5-chloro-1-(4-piperidyl)-2-benzimidazolinone and their derivatives

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Abstract

In the current study a series of 4-(2-keto-1-benzimidazolinyl) piperidine have been newly synthesized and activities of 5-chloro-1-(4-piperidyl)-2-benzimidazolinone derivatives that is already reported have been tested and compared. Furthermore synthesized derivatives have been characterized by physical and spectral methods (HR-EIMS, HR-FABMS, ^{1}H-NMR, ^{13}C-NMR, UV, and FT-IR). All the derivatives were tested for leishmanicidal and anticancer activities. Results demonstrated that among all the derivatives compounds **4**, **9**, and **10** exhibited significant cytotoxic effects on HeLa cells and therefore tested at 10, 50 and 100 μM concentrations. Among all the eleven derivatives eight derivatives were found having leishmanicidal activity. Compounds **3**, **4** and **10** exhibited significant activity with IC_{50} values in range of 7.06 ± 0.17 μg/mL – 84.6 ± 0.6 μg/mL. Furthermore, an attempt was made to develop a preliminary structure-activity relationship. Compound **4** found to be most active with IC_{50} = 7.06 ± 0.17 μg/mL against leishmaniasis. Compounds **4** and **10** possess both leishmanicidal and anticancer activities. From this study we identified a new class of compounds having potent leishmanicidal activity and anticancer activity.

1 Introduction

There are many similarities exist between cancerous cells and parasitic cell. It leads to the idea that drugs use to combat parasites can be used against cancer or vice versa (Klinkert & Heussler, 2006). Cancer remains one of the greatest challenges. It causes around 13% of all deaths worldwide and it is constantly increasing, particularly in the developed countries. Scientists have to explore either alternative mode of action to treat the disease or design newer and better drug entities to deal with the difficulties and sufferings of cancer patients (Elnima, Zubair, & Al-Badr, 1981; Gellis, Kovacic, Boufatah, & Vanelle, 2008). Leishmaniasis affects 12 million people around the world with an annual death rate of approximately 80,000 people. It is a widespread parasitic disease caused by protozoan parasite of the genus *Leishmania*. It causes a broad spectrum of clinical manifestations ranging from self-healing cutaneous lesions to the fatal visceral forms. Clinical manifestations depend on the *Leishmania* species involved and ranges from a life-threatening systemic infection (visceral, VL) to self-limiting or chronic skin sores (cutaneous, CL), or dreaded metastatic complications that can cause facial disfigurement (mucosal, MCL). The clinical features of VL generally include prolonged and irregular fever, often associated with rigor and chills, hepato-splenomegaly, lymphadenopathy, progressive anemia, weight loss and hyper gamma globulinemia (mainly IgG from polyclonal B cell activation) and concomitant hypo-albuminemia. African and IndianVL patients may present with a secondary form called post kala-azar dermal leishmaniasis (Saha, Mukhopadhyay, & Chatterjee, 2011). Several drugs are available for treatment of leishmaniasis, pentavalent antimonials compounds are drugs used in first line chemotherapy. As second line amphotericin B and pentamidine isothionate may be used, but current treatment is unsatisfactory due to the route of administration, unaffordable cost and toxic side-effects. That's why scientists are currently working to develop new, effective and affordable molecules as leishmanicidal agents. Fuertes *et al* reported that anticancer agents may constitute in the near future a good source of lead compounds

against leishmaniasis and other parasitic diseases. On the other hand, it is also likely that some antiparasitic drugs may be used for the treatment of certain types of tumors (Carballeira et al., 2018; Perez, Fuertes, Nguewa, Castilla, & Alonso, 2008; Santiago et al., 2012).

Benzimidazolinyl piperidine derivatives have recently attracted attention as an important class of heterocyclic compounds in the field of medicinal chemistry. Benzimidazolinyl piperidines have been potential target for synthesizing different derivatives having biological activities, *i.e.* the reason there are a number of derivatives patented by different pharmaceutical companies (Bender et al., 1996; "Benzimidazolinyl piperidines," 1965; Budzik et al., 2012; Jan & Van, 1975; Janssen, 1965; Patel, Bhatt, Bhatt, & Joshi). Study revealed novel benzophenone benzimidazole analogs exhibited*in-vivo* tumor inhibition (Croce, 2008; Ranganatha et al., 2013). Clearly, there is a need for a search for new types of drugs with high selectivity, minimum side effects and low manufacturing costs.

In the present study **11** derivatives of 4-(2-keto-1-benzimidazolinyl) piperidine and 5-chloro-1-(4-piperidyl)-2-benzimidazolinone derivatives have been synthesized and tested for leishmanicidal and anticancer activity. Numbers of analogues have showed potential for cancer activity.

2 Results And Discussion

2.1 Synthesis

We report here the synthesis of 4-(2-Keto-1-benzimidazolinyl)piperidine (KBIP) derivatives **1–6** by reaction of KBIP with a variety of acetophenones represented as R as shown in Table 1. We selected 2-Bromo – 4'-Flouroacetophenone, 2-Bromo-4'Chloroacetophenone, 2-Bromo-2',5' dimethoxy acetophenone, (1-Adamantyl bromomethyl ketone, 6-(Chloromethyl) Uracil, and 6-(Chloroacetyl)- 2H-1,4-benzoxazin-3(4H)-0ne substituents, in order to study the effect of different substituent on the (KBIP) of parent molecule. The structures of all synthesized compounds were elucidated by using different physical and spectral method (HR-EI-MS, HR-FABMS, ^{1}H-NMR, ^{13}C-NMR, UV, and FT-IR).

2.2 Anti-Proliferation Activity in Treated HeLa Cells

Anti-proliferation activity in treated HeLa cells has been carried out according to the literature protocol(Wang, Yang, Petrenko, & Torchilin, 2010). Among **1–11** synthesized derivatives few compounds exhibited anti-cancer potential at a 100 uM concentration **(Table-3)**. Concentration dependent decline in cellular viability was observed during study. The growth inhibition of HeLa cell line was observed at all concentrations after exposure to these synthesized compounds. Later the percent inhibition was calculated, which revealed greater than 50% inhibition at 100 μM concentration. The IC_{50} values of these compounds were calculated using the ED50V10 Excel add-in. The experiment was conducted three times in duplicate. Compounds **4**, **9** and **10** exhibited significant cytotoxic effect on HeLa cells and tested at 10, 50 and 100 μM concentrations **(**Table 4–5**)**. Cytotoxic effect of compounds **4**, **9** and **10** were observed after 24 h treatment showing IC_{50} of 57.619, 56.594 and 27.235 μM, respectively. The error bars represent

the SEM from the mean significantly different in treated cells compared to untreated control cells ($p < 0.05$). The cell titer blue assay was used to assess the metabolic activities of cancer cells. The viable cells showed enhanced fluorescence whereas the cells experiencing the cytotoxic effect demonstrated a reduced fluorescence. Varying concentrations of the three potent compounds were employed on HeLa cells for 24 h to achieve the IC_{50} drug response, which represented the concentration of drug necessary to induce a reduction of 50% in growth. The compounds **4**, **9** and **10** exhibited a strong cytotoxic action on human cervical cancer cells, even at the lower concentrations of 10 and 50 μM. It can be elicited that the synthesized compounds **4**, **9** and **10**demonstrated a decline in cellular functions of human cervical cancer cell as observed by a decrease in fluorescence in a concentration dependent manner (Wang et al., 2010).

2.3 Leishmanicidal Activity

Numbers of attempts have been made to discover potent antileishmanial drugs with less toxicity. The aim of present study is to investigate the *in-vitro* leishmanicidal activity of benzimidazolinyl piperidine derivatives in comparison with the standard leishmanicidal drugs amphotericin B (IC_{50} = 0.29 ± 0.05 μg/mL) and pentamidine (IC_{50} = 5.09 ± 0.09 μg/mL). All synthesized derivatives **1–11** of 4-(2-keto-1-benzimidazolinyl)piperidine (I) and 5-chloro-1-(4-piperidyl)-2-benzimidazolinone (II) were evaluated for their leishmanicidal activity, (Saied Saify et al., 2014). Results showed most of them are active againstextracellular promastigotes of *Leishmania* major. Results are presented in table-6 along with their IC_{50} values. Comparing SAR of different Benzimidazolinyl Piperidine derivatives, it can be concluded that the slight changes in structure, functional groups and their position play important role in making compound active or inactive. Among **1–11** synthesized derivatives eight compounds were found active. Compounds **3**, **4** and **10** exhibited significant activity with IC_{50} values in range of (7.06 ± 0.17 μg/mL to 84.6 ± 0.6 μg/mL), Compounds **1** and **7** showed good activities while **2**, **8** and **9** exhibited low activity. Furthermore, an attempt was made to develop a preliminary structure-activity relationship for the synthetic compounds. Compound **4** found to be most active with IC_{50} = 7.06 ± 0.17 μg/mL against leishmaniasis. Compound **10** also showed significant result with IC_{50} = 23.24 ± 0.035 μg/mL there is a 3 times reduction in leishmanicidal activity in compound **10** due to the chloro group substituent present at benzimidazolinone ring. Both the derivatives possesses adamantane ring indicated that it is responsible for significant leishmanicidal activity. Compound **3** also showed significant activity possibly due to the presence of two electron donating methoxy group substituents present at *ortho* and *meta* positions of phenyl ring. Compound **1** and **7** also showed good activity. These compounds are phenacyl halide derivative of benzimidazolinyl piperidine. They have fluorine group at *para* position of benzene ring both possesses comparable leishmanicidal activity. Compounds **8** and **9** showed weak activity. On the other hand Compound **5**, **6**, **11** and parent I showed devoid of activity against leishmaniasis. Parent molecule I found inactive while interestingly II exhibited moderate activity. Generally all newly synthesized derivatives showed promising activity. These investigations suggested that compounds **4** and **10** may serve as lead compound in search of anti-leishmanial drugs. It has been reported that anticancer agents may constitute in the near future a good source of lead compounds against leishmaniasis and other

parasitic diseases(Perez et al., 2008). On the other hand, it is also likely that some antiparasitic drugs may be used for the treatment of certain types of tumors. Due to their drug-like properties, this series of compounds can potentially serve as templates for future drug-optimization and drug-development efforts for use as therapeutic agents.

3 Experimental

3.1 General Experimental Conditions

All reagents used were purchased from Sigma Aldrich Company and organic solventswere of analytical grade. Watt man's filter paper was used for filtration. Thin layer chromatography (TLC) was performed on pre-coated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany). Chromatograms were visualized by UV at 254 and 365 nm.The synthesized derivatives were dried and stored in vacuum anhydrous condition. Melting points of products were noted on Buchi 434 melting point apparatus and are uncorrected. Hitachi U-3200 spectrophotometer used to record Ultraviolet (UV) spectra while methanol used as solvent. Infra-Red (IR) spectra were analyzed on a Jasco 302 Fourier transform FTIR spectrophotometer by making KBR disc method. Mass spectrometry were carried out on Varian Massen spectrometer MAT 311A spectrometer Varian Massen spectrometer MAT 312, MAT 113 DMASPEC system. Nuclear magnetic resonance ^{1}HNMR and ^{13}CNMR spectral analysis were carried out at Bruker AM 300, 400 and 500 MHz spectrophotometers. Solvents used were DMSO-d_6 and MeOD. Chemical shifts (δ) were recorded in parts per million (ppm) and coupling constants *J* in Hertz.

3.2 General procedure for Synthesis of 4-(2-Keto-1-benzimidazolinyl) piperidine1-6 Derivatives

4-(2-Keto-1-benzimidazolinyl)piperidine derivatives **1–6** have been synthesized by refluxing equimolar 4-(2-Keto-1-benzimidazolinyl)piperidine (I) with variety of phenacyl halides groups **(R)** dissolved in methanol in a round bottom flask. Synthesis of 5-Chloro-1-(4-piperidyl)-2-benzimidazolinone derivatives **7–11** have been reported previously by (HAIDER, SAEED, SULTANA, & KHAN, 2014). The reaction mixture was refluxed vigorously by magnetic stirring for 2 h at 25 ºC. The progress of reaction was monitored by TLC technique. The crude solid products were filtered and washed with acetone. The crude products were recrystallized using warm ethyl alcohol and diethyl ether. The pure product was dried in desiccator using anhydrous calcium sulphate.

3.2.1 [1-(2-(4-fluorophenyl)-2-oxoethyl)-4-(2-oxo-2, 3-dihydro-1H-benzo[d]imidazole-1-yl)piperidinium bromide] (1)

Yield 80.5%, White crystals, mp: 250.7 ± 1.1 ^{o}C, $C_{20}H_{21}O_2N_3F$ (354.1540); Solubility (MeOH, EtOH, DMSO); **UV$_{\lambda max}$** (MeOH) (nm): 207, 232, 249, 281; **FT-IR υ_{max}** (KBr) cm^{-1}: 700, 727, 1104, 1198, 1387, 1486, 1624,

2945, 3155, 3448, 3190; **HR-FABMS**: 354.1629 {[M + H]$^+$ $C_{20}H_{21}O_2N_3F$} (Calcd 354.1540); **^{1}H –NMR**: (d_6-DMSO, 400 MHz) δH (ppm): 1.93 (d, 2H, *J* = 12.0 Hz), 2.81 (d, 2H, *J* = 12.0 Hz), 2.81 (d, *J* = 12.0 Hz, 2H), 3.64 (d, *J* = 8.0 Hz, 4H), 4.58 (br. s, 1H), 5.07 (s, 2H), 7.02, (m, 3H), 7.47 (m, 3H), 8.10 (m, 2H), 10.9 (s, 1H); **^{13}C-NMR** (d_6-DMSO, 100 MHz) δC (ppm): 25.5 (C-3, 5), 46.4 (C-4), 52.9 (C-2, 6), 61.2 (C-7), 108.7 (C-18), 109.1 (C-15), 116.1 (C-21), 116.4 (C-23), 120.9 (C-16), 120.3 (C-17), 128.3 (C- 13), 128.5 (C-14), 130.5 (C-19), 131.3 (C-20), 131.4 (C-24), 153.5 (C-11), 167.1 (C-22), 190.2 (C-8).

3.2.2 [1-[2-(4-Chloro-phenyl)-2-oxo-ethyl]-4-(2-oxo-2, 3-dihydro-benzoimidazol-1-yl)piperidinium bromide] (2)

Yield 90.5%, White shiny crystals, mp 204 ± 0.7, $C_{20}H_{20}O_2N_3Cl$ (369.12); Solubility (MeOH, H_2O, DMSO); **$UV_{\lambda max}$** (MeOH) (nm): 206.4, 251.2, 279.6; **FT-IR υ_{max}** (KBr) cm^{-1}: 700.5, 729.1, 821.3, 932.5, 1272, 1387.5, 1485.2, 1596.4, 1624.9, 1680, 2510.5, 2638.7, 2723.2, 2816.8, 2943.7, 3071.0, 3155.6, 3190.8, 3449.5, 3660.9; **HR-EIMS** *m/z*: 369 ($C_{20}H_{20}O_2N_3Cl$ Calcd 369.12); **^{1}H –NMR** (MeOD, 300 MHz,) δH (ppm): 2.11 (d, *J* = 12.0 Hz, 2H), 4.61 (br. s, 1H), 5.00 (s, 2H), 7.10 (m, 3H), 7.33 (m, 1H), 7.62 (d, *J* = 12.0 Hz, 2H), 8.05 (d, *J* = 6.0 Hz, 2H), 10.9 (s, 1H); **^{13}C-NMR** (100 MHz, DMSO-d_6) δC (ppm): 25.5 (C3, 5), 46.4 (C-4), 52.5 (C-2, 6), 61.2 (C-7), 108.8 (C-18), 109.1 (C-15), 120.3 (C-17), 120.9 (C-16), 128.3 (C-14), 128.7 (C-13), 129.2 (C-21, 23), 130.1 (C20, 24), 132.4 (C-19), 139.7 (C-22), 153.5 (C-11), 190.7 (C-8).

3.2.3 [1-(2-(2, 5-dimethoxyphenyl)-2-oxoethyl)-4-(2-oxo-2, 3-dihydro-1H-benzo[d]imidazole-1-yl)piperidinium bromide] (3)

Yield 80.5%, Greenish white powder, mp 177.15 ± 1.55ºC, $C_{22}H_{26}N_3O_4$ (395.18); Solubility (MeOH, H_2O, DMSO); **$UV_{\lambda max}$** (MeOH) (nm): 206.4, 253.8, 279.8; **FT-IR υ_{max}** (KBr) cm^{-1}: 696, 727.2, 754.8, 966.9, 1026.3, 1168.6, 1222.7, 1280, 1309.4, 1389.7, 1497, 1583.6, 2679.7, 2831.9, 2954.9, 3183.2, 3432.7; **HR-FABMS**: 396.1029 {[M + H]$^+$ ($C_{22}H_{26}N_3O_4$} (Calcd 395.18); **^{1}H –NMR** (MeOD, 300 MHz,) δH (ppm): 1.90 (d, *J* = 12.0 Hz, 2H), 2.79 (d, *J* = 12.0 Hz, 2H), 3.62 (s, 3H), 3.77 (s, 3H), 4.54 (br. s, 1H), 4.80 (s, 2H), 7.00 (m, 3H), 7.25 (d, *J* = 8.0 Hz, 1H), 7.46 (d, *J* = 8.0 Hz, 1H), 7.34 (2H, m), 9.89 (s, 1H); **^{13}C-NMR** (100 MHz, DMSO-d_6) δC (ppm): 25.4 (C-3, 5), 46.5 (C-4), 52.5 (C-2), 55.7 (C-26), 56.5 (C-27). 65.0 (C-7), 113.3 (C-24), 114.5 (C-21), 122.6 (C-22),128.3 (C-19), 128.5 (C-13), 153.0 (C-11), 194.5 (C-8).

3.3.4 [1-(2-Adamantan-1-yl-2-oxoethyl)-4-(2-oxo-2,3-dihydro-benzoimidazol-1-yl)piperidinium bromide] (4)

Yield 90%, White crystals, mp 295 ± 1ºC, $C_{24}H_{31}O_2N_3$ (393.24); Solubility (MeOH, H_2O, DMSO); **$UV_{\lambda max}$** (MeOH) (nm): 213, 229, 282; **FT-IR υ_{max}** (KBr) cm^{-1}: 882.0, 958.1, 993.7, 1097.9, 1210.9, 1388.7, 1483.0, 1690.4, 2509, 2666.4, 2748.3, 2852.9, 2913.9, 3063.3, 3191.0, 3409.5; **HR-EIMS** *m/z*: 393 ($C_{24}H_{31}O_2N_3$ Calcd 393.24); **^{1}H-NMR** (500 MHz, DMSO-d_6) δH (ppm): 1.67 (q, *J* = 12.5, 36.5 Hz, 6H), 1.79 (d, *J* = 2.0 Hz, 6H), 1.87 (d, *J* = 10.0 Hz, 4H), 2.0 (s, 3H), 2.70 (2H, m), 3.48 (2H, m), 4.28 (s, 1H), 3.17 (m, 2H), 4.48 (s, 2H),

7.02 (m, 3H), 7.40 (d, *J* = 10.0 Hz, 1H,), 10.9 (s, 1H); **^{13}C-NMR** (100 MHz, DMSO-d_6) δC (ppm): 25.3 (C-3, 5), 27.2 (C-21, 23, 26), 35.7 (C-22, 27, 28), 37.1 (C-20, 24, 25), 44.4 (C-19), 46.4 (C-4), 52.3 (C-2, 6), 59.6 (C-7), 108.7 (C-17), 109.0 (C-15), 120.2 (C-16), 120.8 (C-18), 128.3 (C-13, 14), 153.4 (C-11), 207.4 (C-8).

3.3.5 [1-(2, 6-dioxo-1, 2, 3, 6-tetrahydropyrimidin-4-yl) methyl-4-(2-oxo-2,3-dihydro-1H-benzo[d]imidazole-1-yl)piperidinium chloride] (5)

Yield 80%, White shiny crystals, mp 189.65 ± 1.15 ºC, $C_{17}H_{19}O_3N_5$ (341.15); Solubility (MeOH, H_2O, DMSO); **$UV_{\lambda max}$** (MeOH) (nm): 206.8, 265.4; **FT-IR υ_{max}** (KBr) cm^{-1}: 737.9, 837.7, 882.0, 969.2, 1006.7, 1094.6, 1147.7, 1319.1, 1380.5, 1482.7, 1690.6, 1710.3, 2819.7, 2946, 3119.2, 3434.2; **HR-EIMS** *m/z*: 341 ($C_{17}H_{19}O_3N_5$ Calcd 341.15); **^{1}H-NMR** (300 MHz, DMSO-d_6) δH (ppm): 1.62 (d, *J* = 9.4 Hz, 2H), 2.18 (m, 2H), 2.33 (m, 2H), 2.93 (d, *J* = 10.5 Hz, 2H), 3.23 (s, 2H), 4.13 (s, 1H), 5.52 (s, 1H), 7.01 (m, 2H), 7.27 (d, *J* = 8.4 Hz, 2H), 10.94 (s, 1H); **^{13}C-NMR** (100 MHz, DMSO-d_6) δC (ppm): 28.4 (C-3, 5), 50.0 (C-4), 52.5 (C-2, 6), 98.3 (C-18),108.6 (C-13),109.8 (C-16),120.0 (C-14), 124.7 (C-15), 128.1 (C-12), 129.5 (C-11), 151.5 (C-9), 153.3 (C-21), 153.6 (C-7, C-17),164.1 (C-19).

3.3.6 [4-(2-oxo-2, 3-dihydro-1H-benzo[d]imidazole-1-yl)-1-(2-oxo-2-(3-oxo-3, 4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)ethyl) piperidinium chloride] (6)

Yield 92%, White fluffy powder, mp 251 ± 1ºC, $C_{22}H_{23}N_4O_4$ (406.16); Solubility (MeOH, H_2O, DMSO); **$UV_{\lambda max}$** (MeOH) (nm): 207.8, 243.4, 279.6; **FT-IR υ_{max}** (KBr) cm^{-1}: 927.3, 1039.6, 1095.4,1159.6, 1282.0, 1385.5,1417.1, 1499.5, 1696.8, 2771.6, 2858.2, 2954.1, 3053.1, 3213.1, 3698.4; **HR-ESI** *m/z*: 407.16 ($C_{22}H_{23}N_4O_4$ Calcd 406.16); **^{1}H-NMR** (300 MHz, DMSO-d_6) δH (ppm): 1.62 (d, *J* = 11.4 Hz, 2H), 2.26 (m, 2H), 2.31 (m, 2H), 2.60 (m, 2H), 3.0 (d, *J* = 9.3 Hz, 1H), 3.77 (s, 2H), 4.68 (s, 1H), 6.97 (m, 2H), 7.04 (d, *J* = 8.4 Hz, 1H), 7.18 (d, *J* = 4.5 Hz, 1H), 7.57 (d, *J* = 2.1 Hz, 1H), 7.68 (q, *J* = 2.1, 8.4 Hz, 2H), 10.8 (s, 1H), 10.88 (s, 1H); **^{13}C-NMR** (100 MHz, DMSO-d_6) δC (ppm): 28.5 (C-3, 5), 52.7 (C-2, 6), 59.0 (C-4), 66.7 (C-7, C-21), 108.5 (C-19), 115.9 (C-16), 116.0 (C-26), 120.3 (C-27),120.4 (C-29),124.3 (C-18), 124.7 (C-17),127.1 (C-24), 128.2 (C-14), 129.1 (C-28),130.2 (C-15),147.2 (C-11), 153.6 (C-25), 164.1 (C-22), 195.3 (C-8).

3.3 Biological Activity Evaluation

3.3.1 Cytotoxicity Assay

3.3.1.1 Protocol for Cervical (HeLa) Cancer Cell Line

HeLa cells were cultured in 75 cm^2 polystyrene culture flasks with canted neck and filtered caps (Corning Inc, NY, USA) in DMEM supplemented with 10% FBS and 1% (penstrep) agent. Cells were consistently provided a warm and humidified environment of 37°C and 5% CO_2. Cells were grown to 80% confluence

before every experiment. The cancer cells were washed with PBS and were harvested with a solution of trypsin–EDTA whilst in a logarithmic phase of growth.

The cell plating was performed in a 96-well plate, when cells were 80% confluent. After 24 h the drug treatment was performed. Next day cells were exposed to the fluorescent dye, celltiter blue (CTB), the indicator compound in the CTB is blue colored resazurin, which is oxidized by the viable cells into red colored resorufin compound. Following 2 and 4 hour of incubation at 37°C, the fluorescence is calculated in a spectro-fluorometer at 544/590 nm excitation and emission wavelength of a filter respectively (Wang et al., 2010). All experiments were repeated as a minimum of three times. Data from the experiments was assembled and the statistical significance between control and treated groups was evaluated by Student's t-test. The significance level (p-value) of $p < 0.05$ was considered significant.

3.3.2 In-Vitro Leishmanicidal Assay

For this assay *Leishmania* promastigotes were harvested in an excess quantity. Modified *NNN* biphase and RPMI 1640 mediums were used (Sigma, St. Louis, USA) supplemented with 10% heat resistant fetal calf serum (PAA Laboratories GmbH, Austria). Parasites on log phase of growth were centrifuged at 2000 rpm for 10 minutes. They were diluted and adjusted the cell density up to 1 x 10^6 cells/mL. Experiment was done in 96-well round bottom micro titer plate contains 20 µL of the synthesized compound along with medium were added and serially diluted, 100 µL of parasite culture was added in each well. Two rows of 96-well plates fixed for positive and negative control. Negative control wells comprises of medium whereas the positive control wells consist of varying concentrations of standard anti-leishmanial drugs. All samples were use as 1 mg/mL. After that 96 well plates incubated at 22-25°C for 72 hours. The culture was analyzed microscopically by using Neubaure counting chamber and IC_{50} values of anti-leishmanial activity were calculated through Software Ezfit 5.03 Perella Scientific. Experiment performed in triplicate (Choudhary, Yousaf, Ahmed, & Yasmeen, 2005; Habtemariam, 2003).

Conclusions

We have synthesized two series of **1–11** derivatives of benzimidazolinyl piperidine by taking two parent molecules one is 4-(2-keto-1-benzimidazolinyl)piperidine (I) (KBIP) and another is 5-chloro-1-(4-piperidyl)-2-benzimidazolinone (II) (CPB). Derivatives **1–6** belong to KBIP and **7–11** belongs to CPB. KBIP series synthesis is reporting for the first time while CPB synthesis has been reported by our research group previously. We selected these phenacyl halides to synthesize the derivatives, 2-Bromo – 4'-Flouroacetophenone, 2-Bromo-4'Chloroacetophenone, 2-Bromo-2',5' dimethoxy acetophenone, (1-Adamantyl bromomethyl ketone, 6-(Chloromethyl) Uracil, and 6-(Chloroacetyl)- 2H-1,4-benzoxazin-3(4H)-0ne. Derivatives have been synthesized following a single step quarternization reaction. The synthesized compounds were characterized by using different techniques such as FT-IR, UV, ^{1}H-NMR, ^{13}C-NMR and mass spectrometry.

In vivo biological potential of eleven derivatives **1–11** were evaluated by using Cervical (HeLa) Cancer cell line. Compounds **4**, **9**, and **10** exhibited significant cytotoxic effect on HeLa cell line. They exhibited significant cytotoxic action on human cervical cancer cells, even at the lower concentrations of 10 and 50

μM. It can be elicited that the synthesized compounds **4**, **9**, and **10** demonstrate a decline in cellular functions of human cervical cancer cell as observed by a decrease in fluorescence in a concentration dependent manner. Due to their promising results, they can be selected for further studies as lead molecule.

In vitro Leishmanicidal potentialof benzimidazolinyl piperidine derivatives **1–11** was evaluated using modified *NNN* biphase and RPMI 1640 mediums. Most of the synthesized compounds showed leishmanicidal potential. Compounds **3**, **4**, and **10** exhibited significant Leishmanicidal activities. These investigations suggest that compounds **4** and **10** may serve as lead compound in search of better leishmanicidal drugs.

Declarations

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Tables

Table 1. Substituents (R) of 4-(2-Keto-1-benzimidazolinyl)piperidine (I) derivatives

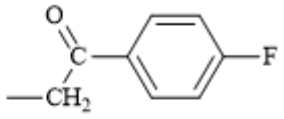
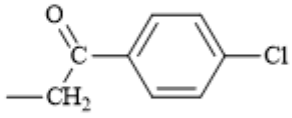
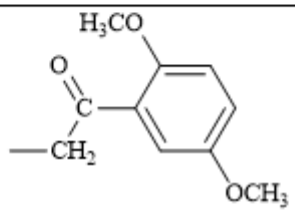
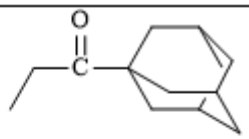
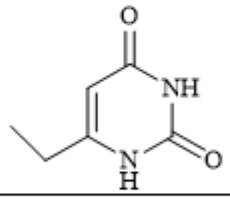
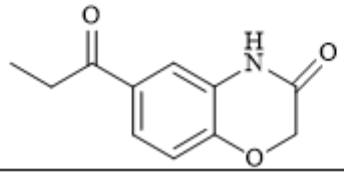
Compounds	R	X	Y
1		Br	
2		Br	
3		Br	
4		Br	
5		Cl	
6		Cl	

Table 2. Substituents (R) of 5-Chloro-1-(4-piperidyl)-2-benzimidazolinone (II) derivatives

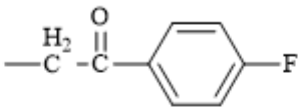
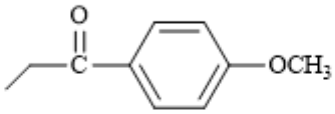
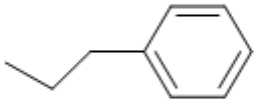
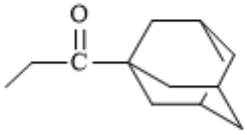
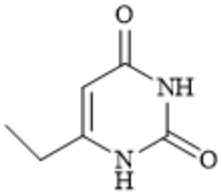
Compounds	R	X
7		B_1
8		B_1
9		B_1
10		B_1
11		Cl

Table 3. Fluorescence (R.F.U.) and cytotoxicity (%) of the human cervical cancer cells after 24 h exposure of the synthesized compounds (1-11) at 100 uM concentration.

Compound (100μM)	Avg Fluorescence (R.F.U.)	Cytotoxicity (%)
1	3631.2	-10.1
2	3929.8	-19.2
3	1838.9	44.2
4	1296.0	60.7
5	2353.0	28.6
6	3442.9	-4.4
7	**2067.7**	**37.3**
8	**3929.8**	**-19.2**
9	**1286.6**	**61.0**
10	**725.3**	**78.0**
11	**2337.3**	**29.1**
C	3929.8	-19.2

R.F.U. = Relative Fluorescence Unit; C = Control.

Table 4. Fluorescence (RFU) of the compounds 4, 9 and 10 at 10, 50 and 100 uM concentration on human cervical cancer cells after 24 h exposure.

Fluorescence (RFU)			
Compound Conc. (μM)	**4**	**9**	**10**
10	10690.994	6962.775	2729.123
50	6390.205	5094.387	2033.234
100	1413.752	1410.242	1354.971
C	8879.760	8879.760	8879.760

R.F.U. = Relative Fluorescence Unit; C = Control.

Table 5. The cytotoxicity (%) of the compounds 4, 9 and 10 at 10, 50 and 100 uM concentration on human cervical cancer cells after 24 h exposure.

% Cytotoxicity			
Compound Conc. (µM)	**4**	**9**	**10**
10	-20.397	21.588	69.266
50	28.036	42.629	77.103
100	84.079	84.118	84.741
C	0.000	0.000	0.000

C = Control.

Table 6. *In*-vitro leishmanicidal activity of benzimidazolinyl piperidine derivatives.

Compounds	IC_{50}(µg/mL) ± SEM[a]
1	43.92 ± 1.511
2	75.46 ± 0.465
3	27.41 ± 1.52
4	7.06 ± 0.17
5	NA[b]
6	NA[b]
7	46.986 ± 0.243
8	72.4 ± 1.74
9	84.6 ± 0.6
10	23.24 ± 0.035
11	NA[b]
I	NA[b]
II	51.68 ± 0.02
Amphotericin B[(st)]	0.29 ± 0.05
Pentamidine[(st)]	5.09 ± 0.09

SEM[a] = standard error of mean;NA[b] = Not active; amphotericin B[(st)] and pentamidine[(st)] = standard drugs for leishmanicidal activity.

Scheme

Scheme 1 & 2 Is Available In Supplemental Files Section.

Supplementary Files

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- 1.png
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