SYNTHESIS AND ANTICANCER ACTIVITY OF SOME NOVEL 2-SUBSTITUTED BENZIMIDAZOLE AND QUINOXALINE DERIVATIVES

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ABSTRACT

Novel 2-substituted benzimidazole derivatives were synthesized via various synthetic pathways. Among which were compounds bearing different side chains attached to the benzimidazole backbone through occupying C2 position 4-8 and 9. Also quinoxaline derivatives 10-13 were designed to be synthesized. Moreover, I have synthesized benzo[b][1,4] diazepinone derivatives 15 and 16. The newly synthesized compounds were evaluated for their in vitro anticancer activity against human liver cancer HepG2 cell line compared to the reference drug Doxorubicin.

Keywords: Benzimidazole, Quinoxaline derivatives anticancer and liver HepG2.

INTRODUCTION

The searching of new agent for the treatment of cancer is an important tool of medicinal chemistry. 2-substituted benzimidazoles have been known to act as potential anticancer1-4, antimicrobial including anti-HIV5-7, antioxidant8, antihypertensive9, antidiabetic and anticonvulsant10. Quinoxaline derivatives are valuable for their wide spectrum of biological activities viz antiviral, anticancer12, antimicrobial13, anti-depressant14, antiamoebic15, anticonvulsant16, antimalarial17, anti-inflammatory, antioxidant18, antiprotozoal activity19 and activity as kinase inhibitors20.

MATERIALS AND METHODS

Melting points were taken on Gallenkamp melting apparatus and are uncorrected. Infrared were obtained on Nexus 470-670. 1H NMR spectra and mass spectra were recorded on Ms-δ 5988 operating at 70eV.

Condensation of o-phenylenediamine with cyclopentanone

To a solution of (0.1 mol) of o-phenylenediamine and in 300 mL of boiling benzene, (0.1 mol) of cyclopentanone. Boiling continued until no more water was removed(2hr.). The cooled solution was washed with 10% sodium bicarbonate and water, dried over magnesium sulfate crystals which separate compound 2 was filtered and washed with ethanol. Distillation of the filtrate gave compound 1.

Compound 1

Crystallized from benzene/pet. ether 40-60; yield 30%; m.p. 55-57°C; IR (cm⁻¹) 3310 (2NH), 3055 (CH–Ar), 2938-2781 (CH aliph.); 1H NMR (DMSO-d₆,δppm) 1.82-1.72 (m, 4H, 2CH₂), 2.28-2.23 (m, 4H, 2CH₂), 5.53 (s, 2H, 2NH, D₂O exchangeable), 7.80-7.22 (m, 4H, Ar–H).

Compound 2, crystallized from ethanol; yield 50%; m.p. 103-105°C; IR (cm⁻¹) 3430-3370(NH₂), 3010(CH–Ar), 2900-2730 (CH aliph.), 1644 (C=N); 1H NMR (DMSO-d₆,δppm), 1.90-1.82 (m, 4H, 2CH₂), 2.28-2.23 (m, 4H, 2CH₂), 4.80 (s, 2H, NH₂, D₂O exchangeable), 7.52-7.22 (m, 4H, Ar–H).
N-Cyclopentane-benz-1,2-diamine. 3
To a solution of (0.1 mol) of 1 or 2 in 100 mL of methanol at 35-40°C was added drop wise over a period of 1h., (0.2 mol) of sodium borohydride. The reaction was then stirred for 6h. and 20 mL of water added and undissolved filter was filtered and crystallized from ethanol, Black powder; yield 55%. m.p. 183-185°C. IR (cm−1) 3361-3268 (NH, NH2) 3055 (CHAr), 2938-2739 (CH aliph.); 1H NMR (DMSO-d6-δ ppm) 1.65-1.41 (m, 4H, 2CH2), 2.52-2.37 (m, 4H, 2CH2), 5.43 (s, 2H, NH2, D2O exchangeable), 6.00 (s, 1H, –CH) 6.44-7.62 (m, 4H, Ar–H), 7.61 (d, 1H, NH, D2O exchangeable); MS m/z 176.11 (6.13%).

Ethyl-1-cyclopentane-1H-benzo[d] imidazol-2-carboxylate. 4
An equimolar mixture of 3 (1.76g, 1 mol) and diethyl oxalate (0.15 mL, 1 mol) was fused at 190°C for 5h. the reaction mixture was allowed to cool, triturated with ethanol. The obtained solid product was filtered, dried and crystallized from DMF to yield 4.
Brown powder, yield, 62%; m.p. > 360°C; IR (cm−1) 3034 (CH–Ar), 2980-2782 (CH aliph.), 1720 (C=O), 1644 (C=N); 1H NMR (DMSO-d6-δ ppm) 1.15 (t, 3H, CH3CH2), J= 5.15 Hz), 1.87-2.07 (m, 4H, 2CH2), 2.65-2.31 (m, 4H, 2CH2), 4.30 (q, 2H, CH2CH3, J= 7.00 Hz), 6.70 (s, 1H, –CH), 7.22-7.02 (m, 4H, Ar–H); MS m/z 258.00 (7.6%).

Ethyl-2-(1-cyclopentane-1H-benzo[d] imidazole-2-yl) acetate. 5
To a well stirred solution of 3 (1.76 g, 1 mol) in ethanolic sodium ethoxide [prepared by dissolving (0.05g, 2 mol) of sodium metal in 5 mL ethanol] was added diethyl malonate (0.16 g, 0.15 mL, 1 mol). The reaction mixture was heated under reflux for 12h, then allowed to cool. The formed solid was filtered off, dissolved in water and the aqueous solution was acidified with diluted hydrochloric acid. The separated solid was collected by filtration, washed with water, dried and crystallized from benzene/ethanol mixture (6: 4) to yield compound 5.
Brown powder; yield 47% ; m.p. > 360°C. IR (cm−1) 3010 (CH–Ar), 2980-2723 (CH aliph.), 1710 (C=O), 1640 (C=N); 1H NMR (DMSO-d6-δ ppm) 1.25 (t, 3H, CH3CH2), J= 5.10 Hz) 1.21-1.14 (m, 4H, 2CH2), 2.31-2.14 (m, 4H, 2CH2), 3.50 (s, 2H, CH2COO), 4.14 (q, 2H, CH2CH3, J= 4.33 Hz) 6.11 (s, 1H, –CH), 7.77-7.15 (m, 4H, Ar–H); MS m/z 272.10 (22.1%).

1- Cyclopentane-1H-benzo[d] imidazole-2(3H) one. 6
Compound 3 (1.76g, 1 mol) was treated under reflux in excess of ethylchloroformate for 10 hours. The reaction mixture was then allowed to cool, triturated with ethanol and obtained precipitate was filtered, washed with ethanol, dried and recrystallized from ethanol to yield 6. Black crystals; yield 46%, m.p. 120-122°C; IR (cm−1) 3381, 3230 (OH, NH), 3033 (CH–Ar), 2940-2899 (CH aliph.), 1680 (C=O), 1H NMR (DMSO-d6-δ ppm) 1.58-1.55 (m, 4H, 2CH2) 2.33 – 2.24 (m, 4H, 2CH2), 5.33 (s, 1H, –CH), 7.25-7.10 (m, 4H, Ar–H), 7.84 (s, 1H, NH, D2O exchangeable); MS m/z 202 (10.51%).

1-Cyclopentane–N–phenyl-1H-benzo[d] imidazole-2-amine . 7
An equimolar mixture of 3 (1.76 g, 1 mol) and phenylisothiocyanate (0.12 mL, 1 mol) in pyridine (10 mL) was heated under reflux for 12 hours. The reaction was allowed to cool, poured on to ice cold water and the precipitate formed was then filtered, dried and crystallized from THF to yield 7.
Black powder; yield 70%; m.p. > 360°C; IR (cm−1) 3270 (NH), 3050 (CH–Ar), 2940-2730 (CH aliph.), 1644 (C=N), 1H NMR (DMSO-d6-δ ppm) 1.90-1.22 (m, 4H, 2CH2), 2.65–2.30 (m, 4H, 2CH2), 5.76 (s, 1H, –CH), 7.44-6.95(m, 9H, Ar–H), 8.74(s, 1H, NH, D2O exchangeable); MS m/z 277.15 (3.18%).

2-(4-Chlorophenyl)-1-cyclopentane-1H-benzo[d] imidazole. 8
A mixture of 3 (1.76 g, 1 mol), the appropriate 4-chlorobenzaldehyde (1 mol) and anhydrous sodium acetate (0.02 mol) was stirred under reflux in glacial acetic acid (30 mL) for 9 hours. The reaction mixture was allowed to cool to room temperature, the reaction mixture was then poured onto crushed ice to yield precipitate which filter, wash with water, dried and recrystallized from AcOH. Brown crystals, yield 60%, m.p. > 360°C; IR (cm−1) 3010 (CH–Ar), 2970-2780 (CH aliph.), 1650 (C=N); 1H NMR (DMSO-d6-δ ppm) 1.63-1.19 (m, 4H, 2CH2) 2.84-2.30 (m, 4H, 2CH2), 6.10 (s, 1H, –CH), 7.92-7.27 (m, 8H, CH–Ar); MS m/z 296.00 (7.0%).

1-Cyclopentane-1H-benzo[d] imidazole. 9
A mixture of 3 (1.76g, 1 mol), formic acid (10 mL) and 2 mL of concentrated hydrochloric acid was heated under reflux for 6h. the reaction mixture was allowed to cool to room temperature and poured onto water (100 mL). The formed solid was collected by filtration, washed with ethanol (20 mL), dried and crystallized from ethanol to give 9. Brown crystals; yield 52%; m.p. 100-102°C IR (cm−1) 3088
General procedure for the synthesis of compounds 10,11,12
A mixture of 3 (1.76g, 1 mol) and chloroacetonitrile or ethyl chloroacetate (1 mol) was refluxed in ethanol (30 mL) containing sodium ethoxide (0.23g, 0.01 mol) for 10 hr. The reaction mixture was then cooled and poured onto ice cold water, the solid separated was recrystallize from proper solvent yield 10,11,12.

Method B to prepare compound 12
To a well stirred solution of 3 (1.76 g, 1 mol) in dimethyl formamide (15 mL), chloroacetyl chloride (0.11g, 0.1 mL, 1 mol), was added dropwise while stirring. The reaction mixture was heated under reflux for 6 hr. The reaction mixture was allowed to cool and poured onto crushed ice to afford a solid precipitate that was filtered, washed with ethanol, dried and recrystallized from DMF to yield compound 12 (mixture m.p. was not depressed).

1- Cyclopentane-1,4-dihydro-3-methylquinoxaline-10
Black crystals; yield 80%; m.p. 280°C; IR (cm⁻¹) 3270 (NH), 3010 (CH–Ar), 2913-2880 (CH aliph.); ¹H NMR (DMSO-d6- δ ppm) 0.96 (s, 3H, CH3), 1.20-1.00 (m, 4H, 2CH2), 2.64-2.31 (m, 4H, 2CH2), 6.70 (s, 1H, –CH), 6.18 (s, 1H, –CH), 6.78 (s, 1H, CH (quinoxaline ring), 7.99-7.26 (m, 4H, Ar–H), 4.20 (s, 1H, NH (quinoxaline ring, D2O exchangeable), MS m/z 214.11 (6.04%).

1- Cyclopentane-1,4-dihydroquinoxalin-2-amine. 11
Brown powder; recrystallized from DMF; yield 55%; m.p.> 360°C. IR (cm⁻¹) 3331, 3290 (NH2, NH), 3054 (CH–Ar), 2981-2881 (CH aliph.), ¹H NMR (DMSO-d6 – δ ppm) 1.15-1.13 (m, 4H, 2CH2), 2.65-2.31 (m, 4H, 2CH2), 4.21 (s,1H, NH (quinoxaline ring), D2O exchangeable), 3.80 (s, 2H, NH2), 5.87 (s, 1H, –CH), 7.50-7.41 (m 5H, Ar–H); MS m/z 215.11 (6.04%).

1- Cyclopentan-3,4-dihydroquinoxalin-2-(1H)-one 12.
Black powder; crystallized from dioxane; yield 60%; m.p.>360°C. IR (cm⁻¹) 3380, 3270 (OH, NH), 3011 (CH–Ar), 2980-2720 (CH aliph.) 1670 (C=O), ¹H NMR (DMSO-d6- δ ppm) 1.91-1.72 (m, 4H, 2CH2), 2.33-2.28(m,4H,2CH2)4.00 (s, 2H, CH2), 7.62-7.22 (m, 4H, Ar–H), 8.18 (s, 1H, NH, D2O exchangeable). MS m/z 216.12 (6.13%).

1- Cyclopentan-3-methylquinoxalin-2(1H)-one 13.
An equimolar mixture of 3 (1.76 g, 1 mol), pyruvic acid (0.87g, 0.23 mL, 1 mol) was heated in an oil bath at 190°C for 4hr. The reaction mixture was added to cool, triturated with ethanol, the formed precipitate was filtered wash with ethanol, dried and crystallized from ethanol to yield 13. Black crystals; yield 80%; m.p. 280-282°C IR (cm⁻¹) 3011 (CH–Ar), 2910-2881 (CH aliph.), 1670 (C=O), 1644 (C=N); ¹H NMR (DMSO-d6- δ ppm), 0.99 (s, 3H, CH3), 1.08-1.02 (m, 4H, 2CH2), 2.20-2.19 (m, 4H, 2CH2), 6.13 (s, 1H, –CH), 7.30-7.00 (m, 4H, Ar–H); MS m/z 228.11 (6.60%).

N-(2-(Cyclopentan-3-phenyl) amino) phenyl (Cinnamamide. 14
A solution of cinnamoyl chloride (0.33 g, 2 mol) in benzene (10 mL) was added to a solution of 3 (2.52 g, 2 mol) in pyridine (10 mL). The reaction mixture was then heated under reflux for 12h., concentrated, then left to cool, and the precipitated product was filtered, washed with ethanol and dried and recrystallized from dioxane to yield two compounds 14 and 15. Compound 14 insoluble during recrystallization in ethanol but compound 15, soluble on hot ethanol.

Compound 14, black crystals; crystallized from dioxane yield 52%; m.p. > 360°C; IR (cm⁻¹) 3330, 3270 (OH, 2NH) 3013 (CH–Ar), 2980-2720 (CH aliph.), 1682 (C=O), ¹H NMR (DMSO-d6- δ ppm) 1.03-0.99 (m 4H, 2CH2) 2.31-2.23 (m, 4H, 2CH2), 3.84 (s, 1H, NH, D2O exchangeable); 6.56 (br, 3H, HC=CH, –CH), 7.22–7.78 (m, 8H, Ar–H), 8.35 (s, 1H, NH, D2O exchangeable).

5- Cyclopentan-4-methyl-1H-benzo[b][1,4]diazepin-2(3H)-one 15.
Brown powder; crystallized from benzene; yield 48%; m.p. 100-102°C; IR (cm⁻¹) 3350 (NH), 3011 (CH–Ar), 2990-2730 (CH aliph.), 1684 (C=O); ¹H NMR (DMSO-d6- δ ppm) 1.96-1.92 (m, 2H, CH2,
6- Cyclopentane-4-methyl-1H-benzo[b][1,4] diazepin-2(3H)-one 16.
An equimolar mixture of compound 3 (1.76 g, 1 mol) and ethyl acetate (0.13 g, 0.12 mL, 1 mol) was heated in an oil bath at 190°C for 12 hr. The reaction mixture was allowed to cool, triturated with ethanol then, the formed precipitate was filtered washed with ethanol, dried and crystallized from DMF, to yield 16. Brown crystals; yield 60%; m.p.> 360°C IR (cm⁻¹) 3310 (OH, NH), 1680 (C=O); ¹H NMR (DMSO-d₆ ppm) 1.00 (s, 3H, CH₃), 1.90-1.21 (m, 4H, 2CH₂), 2.65-2.48 (m 4H, 2CH₂); 6.11 (s, 1H, CH), 6.62 (s, 1H, CH), 7.17-7.00 (m, 4H, Ar–H); 8.11 (s, 1H, NH, D₂O exchangeable) MS m/z 242.11 (3.30%)

Biological, results and structure activity correlation
Antitumor activity (in vitro – study)
Mammalian cell lines: HepG-2 cells (human Hepatocellular cancer cell line), were obtained from VACSERA Tissue Culture Unit.

Chemicals Used
Dimethyl sulfoxide (DMSO), crystal violet and trypan blue dye were purchased from Sigma (St. Louis, Mo., USA).
Fetal Bovine serum.OMEM, RPMI-1640. HEPES buffer solution, L-glutamine, gentamycin and 0.25% Trypsin-EDTA were purchased from Lonza.

Crystal violet stain (1%)
It composed of 0.5% (w/v) crystal violet and 50% methanol then made up to volume with ddH₂O and filtered through a Whatman No. 1 filter paper.

Cell line Propagation
The cells were propagated in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50ug/ml gentamycin. All cells were maintained at 37°C in a humidified atmosphere with 5% CO₂ and were subcultured two times a week.

Cytotoxicity evaluation using viability assay
For cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of 1x10⁴ cells per well in 100μl of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37°C in a humidified incubator with 5% CO₂ for a period of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for at 37°C, various concentrations of sample were added, and the incubation was continued for 24 h and viable cells yield was determined by a colorimetric method. In brief, after the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 minutes. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates were measured after gently shaken on Microplate reader (TECAN, Inc.), using a test wavelength of 490 run. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated. The optical density was measured with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [(ODt/ODc)]x100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve m decide the percentage of viable cells. The 50% inhibitory concentration (IC₅₀), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. using Graphpad Prism software (San Diego, CA, USA)⁴¹,⁴².
Evaluation of cytotoxicity activity against the human liver cancer (HePG2) cell line
The antitumor activities of compounds were assessed against HePG2 cancer cell line in comparison to the tradition anticancer drug (Doxorubicin) on the basis of monitoring the inhibition of the growth of human cancer cells, a series of synthesized compounds possessing a broader spectrum of antitumor activity. Compounds 4-11 and 13-16 were subjected to a screening system indicated that most of the compounds showed inhibition activity against the tested cell line but varying intensity in comparison to the known anticancer drug (Doxorubicin) (see table 1) [cytopenta-1H-benzo [d] imidazole-2 (3H) one 6 exhibited highly potent anticancer activity against HePG2 (Figure 3) cell line showing IC$_{50}$ value 4.79 (µg/ml); respectively which represent comparable activity to the reference drug doxorubicin (IC$_{50}$0.46 (µg/ml). the choice of benzimidazole ring as a focus for investigation is based on literature finding that this structural is associated with good biological activity 23,24.

Table 1: Eight dosage growth inhibition percent and IC$_{50}$ value of the tested compounds against HePG2 cell line

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>Viability %</th>
<th>IC$_{50}$ (µg/ml)$^a$</th>
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<tr>
<td></td>
<td>500 250 125 62.5 31.25 15.6 7.8 3.9</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>32.46 48.59 73.22 84.18 91.06 98.54 100 100</td>
<td>243.0</td>
</tr>
<tr>
<td>5</td>
<td>37.86 57.43 71.87 85.34 94.18 98.63 100 100</td>
<td>345.0</td>
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<td>6</td>
<td>4.65 7.08 10.74 16.53 23.41 34.12 40.93 52.67</td>
<td>4.79</td>
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<tr>
<td>7</td>
<td>43.62 88.79 96.84 100 100 100 100 100</td>
<td>465.0</td>
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<tr>
<td>8</td>
<td>18.31 26.64 36.78 46.09 71.54 89.62 95.87 99.46</td>
<td>57.7</td>
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<tr>
<td>9</td>
<td>18.34 27.21 38.42 59.87 81.25 90.16 98.73 100</td>
<td>90.9</td>
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<tr>
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<td>6.74 14.92 21.88 30.46 41.13 65.36 74.08 82.91</td>
<td>25.5</td>
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<tr>
<td>11</td>
<td>41.30 76.02 85.61 94.86 99.52 100 100 100</td>
<td>437.0</td>
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<tr>
<td>12</td>
<td>6.23 14.56 25.38 36.89 49.72 63.21 78.34 86.23</td>
<td>30.9</td>
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<tr>
<td>13</td>
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<td>160.0</td>
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<td>14</td>
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<tr>
<td>16</td>
<td>4.91 8.81 14.83 16.16 25.28 34.64 36.64 38.14</td>
<td>0.46</td>
</tr>
</tbody>
</table>

$^a$IC$_{50}$, compound concentration required to inhibit tumor cell proliferation by 50% $^b$Human liver cell line (HePG2) $^c$positive control.

RESULTS AND DISCUSSION
Condensation of o-phenylenediamine with cyclopentanone gave two types of compound$^{25}$ namely the spirocycle1 and the Schiff's base 2. Hydrogenation of either 1 or 2 with sodium borohydride gave the substituted o-phenylenediamine3. The structures of compounds 1,2 and 3 were confirmed based on their spectral data (see experimental).

![Scheme 1](image-url)
Ethyl-1-cyclopentane-1H-benzo[d]imidazole-2-carboxylate 4 was obtained by fusion of equivalent amount of diethyl oxalate and compound 3. The IR spectrum of 4 lacked the absorption band due to NH and NH₂ groups and showed a strong band at 1720 cm⁻¹ corresponding to carbonyl function. ¹H NMR of 4 showed two singlets at δ 1.15 and 4.30 ppm to ethyl ester group. N,Cyclopentane-benzene-1,2-diamine 3 was fused with diethyl malonate to afford compound 5. The reaction proceed through reaction of amino group with diethyl malonate with elimination of water molecule followed by intramolecular cyclization with elimination of an ethanol molecule. IR was showed absorption band due to carbonyl function at 1710 cm⁻¹. ¹H NMR of 5 revealed two singlet due to ethyl ester protons.

[Diagram showing reaction schemes]

Compound 3 was refluxed with excess ethyl chloroformate to yield 1-cyclopentane-1H-benzo[d]imidazole-2(3H) one 6 that is expected to obtained through intramolecular nucleophilic attack NH on the ester moiety of the open chain with subsequent elimination of an ethanol molecule. IR of compound 6 showed absorption bands due to carbonyl group at 1680 cm⁻¹ and absorption bands at 3230 cm⁻¹ corresponding to NH group. ¹H NMR of 6 revealed deuterium oxide exchangeable singlet at δ 7.84 ppm corresponding to NH proton.

N-cyclopentane-benzen-1,2-diamine 3 was refluxed with phenyl isothiocyanate in pyridine to yield cyclic 1-cyclopentane-N-phenyl-1H-benzo[d] imidazole-2-amine 7. IR spectra of 7 exhibited absorption band at 3270 cm⁻¹ corresponding to NHPh function and ¹H NMR revealed a deuterium oxide exchangeable singlet at δ 8.74 ppm. The interaction of 3 with 4-chlorobenzaldehyde using acetic acid as medium caused cyclization to give 2-(4-chlorophenyl)-1-cyclopentane-1H-benzo[d] imidazole 8. The reaction included elimination of HCl and H₂O to give 8.
1-Cyclopentane-1H-benzo[d] imidazole 9 was obtained through a one-pot reaction by condensation compound 3 with formic acid. IR spectrum of 9 showed absence of absorption bands respect to NH and NH$_2$ functions indicates the formation cyclic compound. In addition to revealed absorption band at 1644 cm$^{-1}$ corresponding to (C=N).

Compound 3 was refluxed with chloroacetone or chloroacetonitrile or ethyl chloroacetate in sodium ethoxide to yield cyclic compounds 10, 11 and 12.
IR spectra of compound 10 lacked the absorption band due to NH₂ but revealed absorption band due to NH quinoxaline ring. IR spectra of compound 11 showed absorption band at 3331, 3290 cm⁻¹ due to NH and C=O groups and ¹H NMR revealed deuterium oxide exchangeable singlet at δ 4.21 and 3.80 ppm. IR spectra of compound 12 showed absorption bands at 3270 and 1670 cm⁻¹ due to NH and C=O groups and ¹H NMR revealed deuterium oxide exchangeable singlet at δ 8.18 ppm. 1-cyclopentane-3-methylquinoxalin-2-(1H)-one 13 was synthesized via fusion of compound 3 with pyruvic acid. IR spectrum of 13 showed absorption band at 1670 cm⁻¹ due to C=O group and ¹H NMR revealed singlet at δ 0.99 ppm corresponding to CH₃ group.

The reflux of compound 3 with cinnamoyl chloride in benzene/pyridine mixture is suggested to proceed first through elimination of hydrochloride molecule to yield α,β-unsaturated carbonyl compound 14 followed by intermolecular addition of NH function on the double bond of the α,β-unsaturated carbonyl side chain to yield the product 15. The IR spectrum of 14 showed absorption band at 1682 cm⁻¹ corresponding to carbonyl function and ¹H NMR revealed two singlet at δ 3.84 and 8.35 ppm corresponding to 2NH protons. IR spectrum of 15 showed absorption band at 1684 cm⁻¹ corresponding to carbonyl function. The most information proof for the structure of compound 15 was ¹H NMR spectrum; as it revealed two singlet at δ 2.91 and 5.45 ppm corresponding to diazepine CH₂ and -CH-C₆H₅ protons.

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\text{NH}_2
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\text{NH}
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\text{NH}
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\text{C=O}
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\text{HC=CHPh}
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\text{NH}
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\text{C=O}
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\text{HC=CHPh}
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\text{Ph-HC=CH-COCl}
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\text{Ph}
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\text{C=O}
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\text{HC=CHPh}
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Scheme 5:

5-Cyclopentane-4-methyl-1H-benzo[b][1,4]diazepin-2 (3H)-one 16 was obtained through fusion of equivalent amount of ethyl acetoacetate and compound 3. The IR spectrum of 16 lacked absorption band due to NH₂ groups and showed a strong band at 1680 cm⁻¹ corresponding to carbonyl function. ¹H NMR revealed singlet signals at δ 1.00, 6.11 and 8.11 ppm corresponding to CH₃, =CH and NH groups.
Fig. 1: The inhibitory effect of compound 4 concentration on HepG-2 cell activity

Fig. 2: The inhibitory effect of compound 5 concentration on HepG-2 cell activity

Fig. 3: The inhibitory effect of compound 6 concentration on HepG-2 cell activity

Fig. 4: The inhibitory effect of compound 7 concentration on HepG-2 cell activity

Fig. 5: The inhibitory effect of compound 8 concentration on HepG-2 cell activity

Fig. 6: The inhibitory effect of compound 9 concentration on HepG-2 cell activity
Fig. 7: The inhibitory effect of compound 10 concentration on HepG-2 cell activity

Fig. 8: The inhibitory effect of compound 11 concentration on HepG-2 cell activity

Fig. 9: The inhibitory effect of compound 13 concentration on HepG-2 cell activity

Fig. 10: The inhibitory effect of compound 14 concentration on HepG-2 cell activity

Fig. 11: The inhibitory effect of compound 15 concentration on HepG-2 cell activity

Fig. 12: The inhibitory effect of compound 16 concentration on HepG-2 cell activity
CONCLUSION
In the present work, the synthesis of 2-substituted benzimidazole, quinoxalline and benzo[b][1,4] diazepinone derivatives is reported. All spectroscopic analysis confirmed the proposed structures of these compounds. Anticancer activity data of the prepared compounds showed that some fused cyclic rings good anticancer activity.

REFERENCES
12. Corona P, Vital G, Loriga M, Paglietti G and Paola M. Quinoxaline chemistry part 11.3-phenyl-2-[phenoxy-and phenoxy methyl]-6(7) or 6,8-substituted or 6 & dissubstituted-3-phenylquinoxalin-2-yl) hydroxyl or hydroxymethyl benzoylglutamates. Synth and evaluation of in vitro


