Novel Benzimidazole/Hydrazone Derivatives as Promising Anticancer Lead Compounds: Design, Synthesis and Molecular Docking Study

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Abstract
A novel series of benzimidazole-hydrazone derivatives was designed and synthesized as a potential anti-cancer agents. The structure of the target compounds was assured using different spectroscopic methods such as IR, ¹H NMR, ¹³C NMR and elemental analysis. These new compounds were examined and evaluated for their antiproliferative activities against 60 different cell lines. Combination of benzimidazole scaffold with hydrazone group exhibited promising anti-cancer activity. Compounds 3a and 3b showed significant activity against different cancer cells lines between 50-84 % of growth inhibition. A molecular modeling study for compounds 3a and 3b into VEGFR-2 active site was performed with reasonable docking scores.

Key words
Benzimidazole, Hydrazone, Anti-cancer, Molecular docking

1. Introduction
Cancer is one of the most tremendous illness in the world which is considered as the 1st life threatening disease [1]. Chemotherapy is the main cancer treatment strategy while patients administered the available chemotherapeutics are suffering undesirable side effects [2] hence, new research studies are stressed by scientists in order to discover new anticancer promising agents. Because of the structure similarity to the naturally occurring purines, some benzimidazole derivatives have proved biological importance; [3] such as: anticancer [4-6] (Figure 1), antimicrobial [7-10], antifungal [11-15], antiviral [18-20], antihistaminic [21-22], antihypertensive [23-27], antitubercular [28-31], antilulcer activity [32], anti-oxidant [34-36].

Imine group (-C=N-) included in hydrazones is considered an essential moiety of fictional group plays essential role in biological system such as mechanism of transformation reaction and racemization (Figure 2) [37].

The promising anticancer activity of benzimidazole and hydrazones compounds encouraged us to merge between these two scaffolds and design new benzimidazole compounds bearing hydrazone moiety (Figure 3). Consequently, we aimed to synthesize novel benzimidazole-hydrazone derivatives with different substituent as electron withdrawing and electron donating groups (Figure 3).

2. Experimental
2.1. Chemistry

2.1.1. Synthesis of methyl 4-(5-methyl-1H-benzimidazol-2-yl) benzoate 1
Mixture of 5-methyl-1,2-phenylenediamine (2.0 mmol, 0.25 g) and methyl 4-formyl benzoate (2.0 mmol, 0.33 g) in presence of Na₂S₂O₃ (2.4 mmol, 0.5 g) in DMF (30 mL). The reaction mixture was poured into ice-water; the formed precipitate was filtered off and re-crystallized from ethanol to afford 1 [38].

2.1.2. Synthesis of 4-(5-methyl-1H-benzimidazol-2-yl) benzohydrazide 2
Mixture of Compound 1 (2 mmol, 0.5 g) and hydrazine hydrate (2 mmol, 5 mL) in ethanol (15 mL) were heated under reflux for 7 h. The mixture was cooled and poured into iced-water. The obtained crude solid was filtered off, washed with water, dried and recrystallized from absolute ethanol to afford 2 [11].

Dark grey (77 % yield), m.p. 218-220 °C, IR (KBr, νmax cm⁻¹): 3484, 3475 (forked NH₂), 3316 (-NH), 3022 (CH aromatic), 1631 (C=O), 1567 (C=C), 1441 (C=N); ¹H NMR (DMSO-d₆, 400 MHz, δ ppm): δ (ppm) 2.43 (s, 3H, CH₃), 7.35 (m, 2H, Ar-H), 7.98 (dd, 2H, J=12 Hz, Ar-H), 8.23 (dd, 2H, J=12 Hz, Ar-H), 9.90 (s, 1H, NH amide, D₂O exchangeable), 7.05 (d, 2H, J=12 Hz, Ar-H), 7.35 (m, 2H, Ar-2H), 7.98 (dd, 2H, J=12 Hz, Ar-2H), 8.23 (dd, 2H, J=12 Hz, Ar-2H), 12.88 (brs, 1H, NH benzimidazole, D₂O exchangeable).

2.1.3. General method for synthesis of N’-benzylidene-4-(5-methyl-1H-benzimidazol-2-yl) benzohydrazide derivatives [3a-3b]

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Figure (1): Chemical structure of biologically active benzimidazole derivatives.

Figure (2): Chemical structure of compounds containing imine pharmacophore with anti cancer activity.

Figure (3): Benzimidazoles having hydrazone pharmacophore with breast cancer and design of target compounds.
Equimolar quantities (2 mmol, 0.5 g) of compound [2] and appropriate different substituted benzaldehydes in 25 mL of ethanol were refluxed for 6 h in the presence of catalytic amount of glacial acetic acid. The resulting solid was filtered and recrystallized from absolute ethanol [37].

2.1.3.1. Synthesis of N’-(2,4-di-chlorobenzylidene)-4-(5-methyl-1H-benimidazol-2-yl)benzohydrazide [3a].

Light green, 85% yield, m.p. 240-242 °C, IR (KBr, νmax cm⁻¹): 3489-3423 (NH benzimidazole, NH hydrazone), 3046 (CH aromatic), 2922 (CH aliphatic), 1660 (C═O), 1614 (C═C), 1470 (C≡N); ¹H NMR (DMSO-d₆, 400 MHz, δ ppm): 2.44 (s, 3H, CH₃), 7.08 (d, 1H, J=8.00 Hz, Ar-H), 7.42 (s, 1H, Ar-H), 7.53 (dd, 2H, J=8.00 Hz, Ar-2H), 7.72 (s, 1H, Ar-H), 8.05 (d, 1H, J=8.00 Hz, Ar-H), 8.12 (dd, 2H, J=8.00 Hz, Ar-2H), 8.31 (d, 2H, J=8.00 Hz, 2Ar-H), 8.85 (s, 1H, CH), 12.24 (s, 1H, NH hydrazone, D₂O exchangeable); ¹³C NMR (CDCl₃-d₆, 100 MHz, δ ppm): 21.81 (CH₃), 124.67, 126.77, 128.51, 128.60, 128.85, 129.85, 130.38, 131.11, 132.51, 133.56, 134.05, 134.40, 135.65, 143.35, 145.02, 151.22, 152.08 (CH=N), 172.48 (C=O); Elemental analysis calculated for C₂₂H₁₈Cl₂N₆O (M=Wt. 444.49): C, 67.55; H, 5.44; N, 12.60; Found C, 67.31; H, 5.24; N, 12.38.

2.1.3.2. Synthesis of N’-(3, 4, 5 tri-methoxybenzylidene)-4-(5-methyl-1H-benimidazol-2-yl)benzohydrazide [3b].

Canary yellow, 92% yield, m.p. 243-245 °C, IR (KBr, νmax cm⁻¹): 3448-3412 (NH benzimidazole, NH hydrazone), 3042 (CH aromatic), 2923 (CH aliphatic), 1644 (C═O), 1604 (C═C), 1463 (C≡N); ¹H NMR (DMSO-d₆, 400 MHz, δ ppm): 2.45 (s, 3H, CH₃), 3.77 (s, 3H, OCH₃), 3.86 (s, 6H, 2OCH₃), 7.07 (m, 3H, Ar-3H), 7.42 (s, 1H, Ar-H), 7.53 (d, 1H, J=8.00 Hz, Ar-H), 8.09 (dd, 2H, J=8.00 Hz, Ar-2H), 8.30 (dd, 2H, J=8.00 Hz, Ar-2H), 8.43 (s, 1H, CH), 11.79 (s, 1H, NH hydrazone, D₂O exchangeable); ¹³C NMR (CDCl₃-d₆, 100 MHz, δ ppm): 21.81 (CH₃), 56.45, 60.61 (OCH₃), 104.86, 124.57, 126.72, 128.78, 130.26, 132.39, 133.46, 134.53, 139.79, 148.56, 150.32, 153.69, 163.04 (CH=N), 172.03 (C=O); Elemental analysis calculated for C₂₅H₂₃N₉O₄ (M=Wt. 444.49): C, 67.55; H, 5.44; N, 12.60; Found C, 67.31; H, 5.22; N, 12.38.

2.2. Screening of anticancer activity

According to the protocol of Drug Evaluation Branch of the National Cancer Institute (NCI), Bethesda, USA. These studies were carried out using cells derived from NCI 60 cell line panels (six cell lines of Leukemia, nine cell lines of Lung cancer, six cell lines of CNS cancer, seven cell lines of Colon cancer, eight cell lines of Melanoma, six cell lines of Ovarian cancer, eight cell lines of Renal cancer, two cell lines of Prostate cancer and eight cell lines of breast cancer) representing on full nine human systems as Leukemia, Melanoma and cancers of Lung, Colon, Brain, Breast, Ovary, Kidney and Prostate.

2.2.1. In vitro Anticancer Screening Procedure:

NCI screening is evaluated of all compounds against the 60 cell lines at a single dose of 1 x 10⁻³ M (molar) or 15 µg/mL. Growth percentages of cells treated with the compound is expressed to anticancer activity for each of the examined compound as compared with untreated control ones. Sorafenib (SRB) solution (100 µL) at 0.4 % (w/v) in 1 % acetic acid added to each well and plates incubated for 10 min at RT. Percentages of growth were measured spectrophotometrically [39].

2.3 Molecular modeling and docking

The nature of the interaction of the most active synthesized compounds and VEGFR-2 active site can be clarified, a molecular docking study was performed from protein data bank using crystal structure data for VEGFR-2 (PDB: ID 2OH4) active site. Molecular modeling of compounds 3a, 3b and co-crystallized ligand, Sorafenib (Figure 4) using MOE 2019 modeling software.

Co-crystallized ligand was removed to prepare the enzyme for docking then the enzyme was 3D protonated, in which hydrogen atoms were added to their standard geometry. The conformers generated were docked into the VEGFR-2 receptor with MOE-dock using the triangle matcher placement method and the London dG scoring function. A molecular mechanics force field refinement was carried out on the top 30 poses generated. Sorafenib was redocked as a reference into the active site of VEGFR-2 to validate the docking protocol. The hydrogen bond lengths and amino acid interactions were summarized in (Table 2).

3. Result and discussion

3.1. Chemistry

In the present study, some N’-(substitutedbenzylidene)-4-(5-methyl-1H-benimidazol-2-yl)benzohydrazide derivatives (3a-3b) were synthesized according to (Scheme 1). Aimed compounds were acquired at three steps. At the beginning, Equimolar mixture of 5-methyl-1,2-phenylenediamine and methyl-4-formyl benzoate in presence of Na₂SO₄ in DMF to afford 1. Mixture of compound 1 and hydrazine hydrate in ethanol were heated till reflux for 7 h to obtain compound 2. Condensation of hydrazide with variously substituted benzaldehydes gave the aimed compounds 3a-3b.

Scheme (1): Synthesis of compounds 3a and 3b

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Figure (4): 2D binding of Sorafenib with active site of VEGFR-2.

Table 1: Percentage growth inhibition (GI %) of tumor cell lines at 10 mM concentration for compounds 3a and 3b.

| Subpanel cancer cell Lines | % Growth Inhibition (GI %) |
|----------------------------|-----------------------------|
|                            | 3a                          | 3b                          |
| Leukemia                   |                             |                             |
| CCRF-CEM                   | 47.86                       | 38.77                       |
| RPMI-8226                  | 10.02                       | 52.78                       |
| HL-60(TB)                  | NT                          | 6.85                        |
| K-562                      | NT                          | 56.75                       |
| MOLT-4                     | NT                          | 25.36                       |
| Non-Small Cell Lung Cancer |                             |                             |
| A549/ATCC                  | NA                          | 16.22                       |
| EKVX                       | NA                          | 32.47                       |
| HOP-62                     | 14.99                       | 25.18                       |
| HOP-92                     | 13.66                       | 53.66                       |
| NCI-H226                   | 7.24                        | 50.00                       |
| NCI-H23                    | 12.53                       | 29.93                       |
| NCI-H322M                  | 27.90                       | 7.44                        |
| NCI-H460                   | 3.37                        | 35.49                       |
| NCI-H522                   | 21.63                       | 26.92                       |
| Colon Cancer               |                             |                             |
| COLO 205                   | 14.58                       | 73.01                       |
| HCC-2998                   | 6.09                        | 13.26                       |
| HCT-116                    | 56.60                       | 56.09                       |
| HCT-15                     | 29.23                       | 5.19                        |
| HT29                       | NA                          | 24.96                       |
| KM12                       | 29.43                       | 38.72                       |
| SW-620                     | 52.55                       | 14.75                       |
| CNS Cancer                 |                             |                             |
| SF-268                     | 9.51                        | 13.45                       |
| SF-295                     | NA                          | 25.12                       |
| SF-539                     | 2.34                        | 36.80                       |
| SNB-19                     | 5.38                        | 28.74                       |
| SNB-75                     | 8.71                        | 29.44                       |
| U251                       | 4.38                        | 31.20                       |
Table 1: Percentage growth inhibition (GI %) of tumor cell lines at 10 mM concentration for compounds 3a and 3b (cont.).

| Subpanel cancer cell Lines | % Growth Inhibition (GI %) |
|----------------------------|----------------------------|
|                            | 3a            | 3b            |
| Melanoma                   |               |               |
| LOX IMVI                   | 14.02         | 12.90         |
| MALME-3M                   | 4.35          | 80.33         |
| M14                        | 26.52         | 54.28         |
| MDA-MB-435                 | 6.02          | 61.08         |
| SK-MEL-2                   | NA            | 27.93         |
| SK-MEL-28                  | NA            | 52.16         |
| SK-MEL-5                   | 0.82          | 70.24         |
| UACC-257                   | NA            | 44.40         |
| UACC-62                    | 4.19          | 55.78         |
| Ovarian Cancer             |               |               |
| IGROV1                     | 9.02          | 24.06         |
| OVCAR-3                    | 19.90         | 3.54          |
| OVCAR-4                    | 79.76         | 14.13         |
| OVCAR-5                    | 2.26          | 14.89         |
| OVCAR-8                    | 28.37         | 22.94         |
| NCI/ADR-RES                | 7.81          | 1.35          |
| SK-OV-3                    | NA            | 36.87         |
| Renal Cancer               |               |               |
| 786-0                      | 33.22         | 49.01         |
| A498                       | 15.30         | 34.06         |
| ACHN                       | 0.04          | 27.49         |
| CAKI-1                     | 12.44         | 38.81         |
| RXF 393                    | NA            | 10.90         |
| SN12C                      | 5.82          | 13.17         |
| TK-10                      | 65.51         | 60.20         |
| UO-31                      | 19.61         | 29.19         |
| Prostate Cancer            |               |               |
| PC-3                       | 30.57         | 5.82          |
| DU-145                     | 13.78         | 22.40         |
| Breast Cancer              |               |               |
| MCF7                       | 30.51         | 84.33         |
| MDA-MB-231/ATCC            | 25.34         | 15.29         |
| HS 578T                    | 10.35         | 45.91         |
| BT-549                     | 2.11          | 18.05         |
| T-47D                      | 30.2          | 54.24         |
| MDA-MB-468                 | NA            | 4.05          |
| NT: not tested             |               |               |
| NA: GI% ≤ zero             |               |               |

Table 2: Molecular docking data for compounds 3a, 3b and Sorafenib in VEGFR-2 active site (PDB ID: 2OH4).

| Compound No. | Affinity kcal/mol | Distance (in Å) from main residue | Amino acids residue | Functional group | Type of interactions |
|--------------|------------------|----------------------------------|--------------------|-----------------|---------------------|
| Sorafenib    | -19.10           | 3.02                             | Glu 883            | N12             | H-donor             |
|              |                  | 2.93                             | Glu 883            | N15             | H-donor             |
|              |                  | 2.80                             | HOH 167            | N26             | H-donor             |
|              |                  | 3.03                             | Cys 917            | N28             | H-acceptor          |
|              |                  | 2.84                             | Asp 1044           | O14             | H-acceptor          |
|              |                  | 4.29                             | Leu 838            | 5-ring          | pi-H                |
| 3a           | -14.67           | 3.23                             | Glu 883            | Cl              | H-donor             |
| 3b           | -20.75           | 2.97                             | Cys 917            | N10             | H-donor             |
|              |                  | 4.42                             | Leu 838            | 5-ring          | pi-H                |
Structure elucidations of target compounds were carried out with IR, $^1$H-NMR, $^{13}$C-NMR, and elemental analyses. IR spectra showed, stretching absorptions that were appeared at 3489-3423 cm$^{-1}$ proved N-H bond of the hydrazide group and benzimidazole. Furthermore, carbonyl (C=O) function was observed at 1660–1644 cm$^{-1}$. The stretching absorption at about 1614–1604 cm$^{-1}$ were observed for C═C and 1470–1463 C═N. $^1$H-NMR spectra of the compounds, Schiff base protons (=CONHN$\equiv$CH=) were determined at about 8.43–8.85 and 11.79–12.24 ppm, respectively. The NH proton of the benzimidazole ring was not appeared due to its acidity and rotatable proton [40]. The aromatic protons assigned at range from 6-8 ppm. In the $^{13}$C-NMR spectra, characteristic hydrazone (=CONHN$\equiv$CH=) signal at 163.08–174.04 due to carbonyl (C=O) group. Compounds 3a and 3b provided acceptable elemental analyses results.

3.2 Screening of anticancer activity

The screening results were reported (Table 1) as the percent growth of inhibition compared to untreated control cells. Compound 3b which possessing tri-methoxy substituent as electron donating group is more active than compound 3a which possessing di-chloro substituent as electron withdrawing group, which reflects a positive influence of substituent on the antiproliferative activity. Compound 3b showed promising activities against a majority of the tested cancer cell lines such as leukemia (GI%═65.75), non-small cell lung cancer (GI%═53), colon cancer (GI%═56.09), melanoma (GI%═80.33), renal cancer (GI%═60.20) and breast cancer (GI%═84.33). While compound 3a showed moderate inhibitory effect against leukemia (GI%═47.86), colon cancer (GI%═56.60), ovarian cancer (GI%═79.76) and renal cancer (GI%═65.51).

3.3 Molecular modeling

Molecular Operating Environment MOE version 2019 is used to perform molecular modeling study. Structures of 3a and 3b where built in MOE database. The X-ray crystal structure of Sorafenib bound to the VEGFR-2 enzyme binding site (PDB: ID 2OH4) active site was obtained from protein data bank at research collaboration for Structural Bioinformatics (RSCB) protein database [PDB][3].

Hydrazone derivatives 3a and 3b were fully fitted within VEGFR-2 active site with high affinity (-14.67 and -20.75 Kcal/mol, respectively) in comparison with Sorafenib (-19.10 kcal/mol). Compound 3a showed binding through the Cl atom which is an electron donor, in addition to an extra binding with secondary amino group to Glu 883. In compound 3b, the binding was observed throughout the imidazole ring via hydrophobic interaction and electron donor interaction, respectively which bound with amino groups to Cys 917 and Leu 838 (Figure 5-9). Thus, the molecular docking results ensure that compounds 3a and 3b bind to VEGFR-2 active site with the same manner of Sorafenib. Nevertheless, 3b is more active than 3b according to binding with active site.
In this work, benzimidazole bearing hydrazone moiety based compounds were designed and synthesized to afford compounds 3a and 3b. Molecular docking ensured that compound 3b which has an electron donating group (tri-methoxy substituent) can bind with the active site of VEGFR-2 with reasonable docking scores than compound 3a which as electron withdrawing group (di-chloro substituent). Type of substituent on the hydrazone moiety indicated deep impact on the binding mode, where electronic effect contributes to the interaction with the conserved amino acids as well as the reference Sorafenib. We aimed for further investigation of action mechanism.

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