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Synthesis, Cytotoxic Activity Evaluation and Molecular Docking Studies of Some Benzimidazole Derivatives

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Research Article

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ABSTRACT

In this study, the synthesis of 2-(2-acetyl-1*H*-benzimidazol-1-yl)-1-arylethanone (3a-3d) and 1-methyl-3-phenyl-benzo[4,5]imidazo[1,2-a]pyrazine derivatives (4a-4d) and to investigate their cytotoxic activity were aimed. APCI, IR, ^{1}HNMR, and ^{13}CNMR spectra were utilized to determine the structure of the synthesized compounds. The cytotoxic activity of selected compounds were detected in A549 (human lung carcinoma) and NIH3T3 (mouse embryonic fibroblasts) cell lines. Compounds 4c and 4d were found to be selectively cytotoxic against A549 and NIH3T3 cell lines. Molecular docking studies were performed using the data retrieved from the Protein Data Bank server (PDBID: 4QTX).

Keywords: Benzimidazole, Pyrazinobenzimidazole, Anticancer, Molecular docking.

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Introduction

Cancer is one of the diseases with the highest mortality and morbidity rates throughout history. According to the research statistics from the American Cancer Society, although the incidence of cancer has decreased since the beginning of the 21st century, the incidence of breast cancer, lung cancer, and liver cancer has increased [1]. Cancer is the second leading cause of death worldwide after cardiovascular disease [2].

The disease, which is observed as a result of uncontrolled division and development of abnormal cells, can develop under the influence of environmental factors as well as hereditary. Although there is no radical treatment for cancer, the progression of the disease is tried to be prevented with chemotherapy, radiotherapy, and surgical interventions. Due to the development of drug resistance to existing anticancer drugs and the side effects of these drugs, which negatively affect patient compliance, there is a need for researchers to synthesise new anticancer compounds. Benzimidazole and pyrazine ring structures are frequently encountered in anticancer compound syntheses [3–5].

The benzimidazole ring (1*H*-benzimidazole, 1,3-benzodiazole), which is a heterocyclic compound, is found both in the structure of compounds that occur naturally in the human body and in the structure of many compounds that have therapeutic effects today [6].

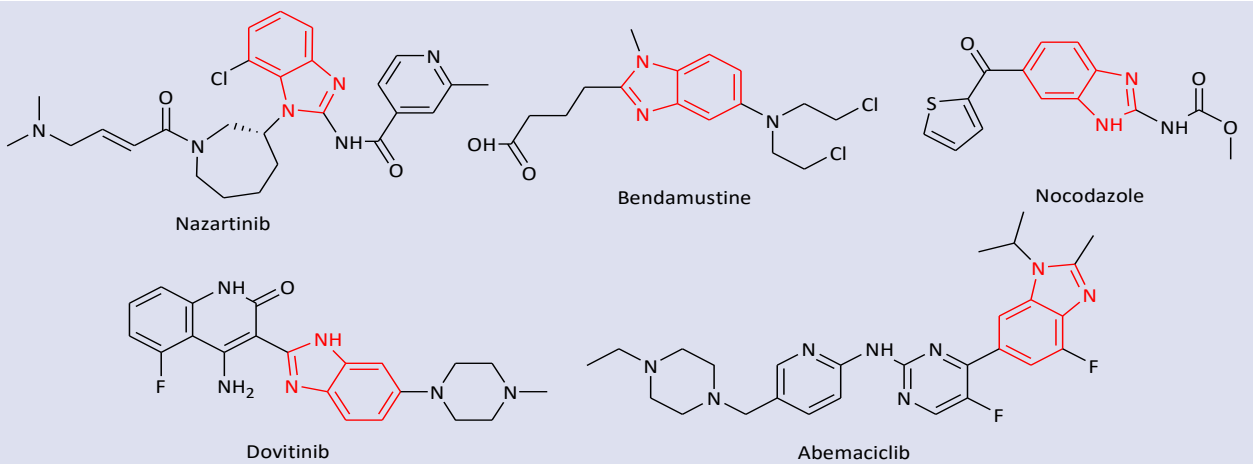


Figure 1. Benzimidazole ring contaning anticancer drugs.

Compounds containing the benzimidazole ring structure show a wide range of activities such as antiulcer, antiviral, antibacterial, anti-inflammatory, and anticancer [7]. Compounds containing benzimidazole ring have been reported in many studies to have anticancer effects [8,9]. EGRF inhibitor nazartinib, bendamustine used in the treatment of chronic myeloid leukaemia [10], dovitinib, tyrosine kinase inhibitor [11], nocodazole effective in microtubule polymerization [12], abemaciclib used in the treatment of advanced and metastatic breast cancer [13] can be given as examples of anticancer drugs containing benzimidazole ring (Figure 1).

The pyrazine ring (1,4-diazine, p-diazine) is also found in the structure of drugs with different therapeutic effects such as anti-viral, antibacterial, antituberculosis, and anticancer. A variety of anticancer drugs contain the pyrazine ring, i.e. bortezomib (used to treat multiple myeloma), barrenazine A/B, botryllazine A, cephalostatin (anti-cancer drug) (Figure 2) [14].

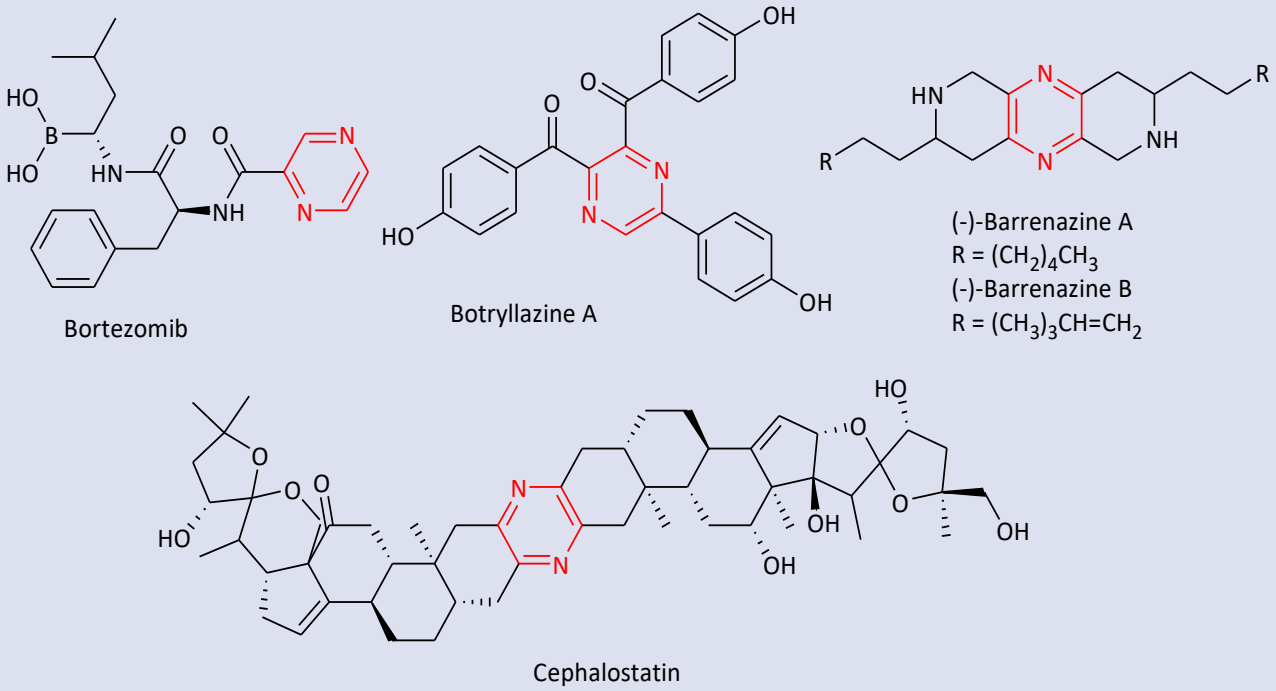


Figure 2. Pyrazine ring containing anticancer drugs.

The aim of this study is to extend the findings of our previous study [15] and to synthesize new compounds in the light of these findings and to examine the cytotoxic activities of these compounds using the A549 and NIH3T3 cell lines.

Experimental Section

Chemistry

All chemicals were purchased from Merck Chemicals (Merck KGaA, Germany) or Sigma-Aldrich Chemical (Poole, UK). The progress of the reactions was monitored by thin layer chromatography (TLC) on pre-coated silica gel 60 F_{254} sheets (acquired from Merck, Germany). The melting points of the compounds were determined by MP90 digital melting point device (Mettler Toledo, USA). Structural analyze of all compounds were obtained with ^{1}H-NMR (300 MHz and 400 MHz) and ^{13}C-NMR (75 MHz and 100 MHz) spectra. Bruker 300 MHz and 400 MHz digital FT-NMR spectrometer (Bruker Bioscience, USA) in DMSO-d_6 was used for NMR, and LC/MS-IT-TOF system (Shimadzu, Japan) and compact mass spectrometer APCI (Advion, ABD) were used for HRMS.

2-(1-Hydroxyethyl)benzimidazole (1)

A mixture of the *o*-phenylenediamine (100 mmol) and lactic acid (100 mmol) was stirred and refluxed in 4 N HCl solution (100 mL) for 8 hours. The excess water in the Dean-stark apparatus was drained off from time to time. At the end of the reaction, the cooled mixture poured into crushed ice-water and neutralized with ammonia solution. The precipitate was filtered and crystallised from ethanol–water. Yield: 71% m.p. 178–180°C (ref. 178.5–179.5°C) [16].

2-Acetylbenzimidazole (2)

2-(1-Hydroxyethyl)benzimidazole (10 mmol) was resolved in 100 mL acetic acid and the mixture was heated to 90°C. The solution of chromium trioxide (7.5 mmol) in 15 mL water was slowly added drop by drop to this mixture and the temperature was fixed at 90°C. After addition, the cooled mixture was poured into its volume of water, waited for one hour and the precipitate formed was filtered off. The substance extracted with chloroform was recrystallised from toluene after the solvent was evaporated to dryness at reduced pressure.

Yield: 76% m.p. 188–190°C (ref. 188–189°C)[26]. IR(KBr) ν_{max} (cm^{-1}): 3288–2400 (–N–H), 1674 (–C = O), 1600–1420 (–C = C, –C = N). ^{1}H-NMR (DMSO-d_6): (ppm) 2.71 (3H, s,–

$COCH_3$), 7.36–7.48 (2H, m, Ar-H), 7.86–8.14 (2H, m, Ar-H), 12.4 (1H, brs, N–H).

2-(2-Acetyl-1H-benzimidazol-1-yl)-1-arylethanone derivatives (3a–3d)

The appropriate 2-bromoacetophenone (5 mmol), 2-acetylbenzimidazole (5 mmol), and potassium carbonate (5 mmol) were stirred in acetone (50 mL) at room temperature. Thin-layer chromatography was used to determine the completion of the reaction (4–6 h). The solvent was vaporized at low pressure, and the precipitate was washed with water and ethanol. The product was recrystallized from ethanol.

2-(2-Acetyl-1H-benzimidazol-1-yl)-1-phenylethanone (3a)

Yield 75%. m.p. 167°C (ref. 166–168°C) [18]. IR (KBr) ν_{max} (cm^{-1}): 3132–3061 (aromatic –C–H), 2937–2850 (aliphatic –C–H), 1686 and 1676 (–C = O), 1593–1446 (–C = C, –C = N). ^{1}H-NMR (DMSO-d_6): (ppm) 2.71 (3H, s, –$COCH_3$), 6.23 (2H, s, –CH_2CO), 7.36–7.48 (2H, m, Ar-H), 7.60 (1H, m, Ar-H), 7.72–7.82 (2H, m, Ar-H), 7.92 (2H, d, *J*: 7.95 Hz Ar-H), 8.13 (2H, d, *J*: 8.09 Hz, Ar-H). ^{13}C NMR (125 MHz, DMSO-d_6): 27.56, 52.13, 112.39, 123.03, 124.57, 126.42, 129.43, 130.36, 134.04, 135.21, 140.12, 143.36, 147.02, 193.82 and 194.10. MS: m/z 279 (M + 1). APCI-MS (-m/z): $[M+H]^+$: 279.6.

2-(2-Acetyl-1H-benzimidazol-1-yl)-1-(4-methoxyphenyl) ethanone (3b)

Yield 65%. m.p. 141–142°C (ref. 141–142°C) [17]. IR (KBr) ν_{max} (cm^{-1}): 3052–3016 (Aromatic –C–H), 2983–2851 (Aliphatic –C–H), 1689 and 1674 (–C = O), 1641–1463 (–C = C, –C = N). ^{1}H-NMR (DMSO-d_6): (ppm) 2.71 (3H, s, –$COCH_3$), 3.72 (3H, s, –$COCH_3$), 6.24 (2H, s, –CH_2CO), 7.15–7.24 (4H, m, Ar-H), 7.58 (2H, d, *J*: 8.15 Hz, Ar-H), 7.92 (2H, d, *J*:8.14 Hz, Ar-H). ^{13}C NMR (125 MHz, DMSO-d_6): 27.68, 52.94, 56.06, 113.08, 123.44, 124.52, 127.29, 130.07, 132.14, 139.52, 140.63, 142.45, 147.53, 150.12, 193.69 and 194.55. MS: m/z 309 (M + 1). APCI-MS (-m/z): $[M+H]^+$: 309.7.

2-(2-Acetyl-1H-benzimidazol-1-yl)-1-(3-chlorophenyl) ethanone (3c)

Yield 72%. m.p. 151–153°C. IR (KBr) ν_{max} (cm^{-1}): 3068–3020 (Aromatic –C–H), 2993–2931 (Aliphatic –C–H), 1695 and 1675 (–C = O), 1620–1452 (–C = C, –C = N). ^{1}H-NMR (DMSO-d_6): (ppm) 2.71 (3H, s, –$COCH_3$), 6.24 (2H, s, –CH_2CO), 7.39–7.42 (1H, m, Ar-H), 7.45–7.49 (1H, m, Ar-H), 7.69 (1H, t, *J*: 7.88 Hz, Ar-H), 7.82 (1H, d, *J*: 8.24 Hz, Ar-H), 7.84–7.87 (1H, m, Ar-H), 7.91 (1H, d, *J*: 8.13 Hz, Ar-H), 8.06–8.09 (1H, m, Ar-H), 8.17 (1H, t, *J*: 1.82 Hz, *J*: 1.77 Hz, *J*: 1.81 Hz, Ar-H). ^{13}C NMR (125 MHz, DMSO-d_6): 27.98, 52.78, 112.66, 122.53, 124.90, 127.44, 128.02, 129.15, 132.28, 135.03, 135.17, 137.68, 138.19, 142.35, 147.21, 194.02 and 194.30. MS: m/z 312.9 (M + 1). APCI-MS (-m/z): $[M+H]^+$: 313.7.

2-(2-Acetyl-1H-benzimidazol-1-yl)-1-(4-chlorophenyl) ethanone (3d)

Yield 71%. m.p. 161–162°C (ref. 161–162°C) [18]. IR (KBr) ν_{max} (cm^{-1}): 3045–3025 (Aromatic –C–H), 2933–2833 (Aliphatic –C–H), 1695 and 1674 (–C = O), 1587–1452 (–C = C, –C = N). ^{1}H-NMR (DMSO-d_6): (ppm) 2.73 (3H, s, –$COCH_3$), 6.24 (2H, s, –CH_2CO), 7.39–7.44 (2H, m, Ar-H), 7.70 (2H, d, *J*: 6.12 Hz, ArH), 7.96–7.97 (1H, m, Ar-H), 8.10 (2H, d, *J*: 8.15 Hz, Ar-H), 8.22 (1H, d, *J*: 7.75 Hz, Ar-H). ^{13}C NMR (125 MHz, DMSO-d_6): 27.99, 52.62, 112.68, 122.52, 124.87, 127.10, 130.37, 131.35, 134.54, 138.22, 140.29, 142.36, 147.24, 193.97 and 194.31. MS: m/z 313.1 (M + 1). APCI-MS (-m/z): $[M+H]^+$: 313.7.

1-Methyl-3-phenyl-benzo[4,5]imidazo[1,2-a]pyrazine derivatives (4a-4d)

A mixture of the appropriate 2-(2-acetyl-1*H*-benzimidazol-1-yl)-1-arylethanone (3a-3d) (1 mmol) and ammonium acetate (10 mmol) was refluxed in acetic acid (50 ml). The solution was cooled and poured into distilled water. The precipitate was filtered and crystallised from ethanol.

1-Methyl-3-phenyl-benzo[4,5]imidazo[1,2-a]pyrazine (4a)

Yield 85%. m.p. 157°C. IR (KBr) ν_{max} (cm^{-1}): 688 (Aromatic -C-H), 739-764 (Aromatic -C-H), 1206-1296 (Aromatic -C-N), 1458-1506 (Aromatic -C=C). ^{1}H-NMR (DMSO-d_6): (ppm) 2.95 (3H, s, CH_3), 7.42 (1H, t, *J*: 6.75 Hz, *J*: 7.42 Hz, Ar-H), 7.54 (3H, t, *J*: 7.37 Hz, *J*: 7.26 Hz, Ar-H), 7.63 (1H, t, *J*: 7.90 Hz, *J*: 7.26 Hz, Ar-H), 7.99 (1H, d, *J*: 8.21 Hz, Ar-H), 8.18 (2H, d, *J*: 7.79 Hz, Ar-H), 8.51 (1H, d, *J*: 8.21 Hz, Ar-H), 9.56 (1H, s, Aromatic -C-H). ^{13}C NMR (125 MHz, DMSO-d_6): 21.55, 113.78, 114.69, 121.02, 123.13, 126.15, 127.06, 128.64, 129.23, 129.50, 136.78 and 152.75. APCI-MS (-m/z): $[M+H]^+$: 260.5.

3-(4-Methoxy-phenyl)-1-methyl-benzo[4,5]imidazo [1,2-a]pyrazine (4b)

Yield 80%. m.p. 160°C. IR (KBr) ν_{max} (cm^{-1}): 736 (Aromatic -C-H), 829 (Aromatic -C-H), 1026-1246 (Aromatic -C-N), 1458-1514 (Aromatic -C=C), 1606-1724 (Aromatic -C=N), 2839-2914 (Aliphatic -C-H). ^{1}H-NMR (DMSO-d_6): (ppm) 2.92 (3H, s, CH_3), 3.83 (3H, s, OCH_3), 7.08 (2H, d, *J*: 8.88 Hz, Ar-H), 7.48-7.63 (2H, m, Ar-H), 7.96 (1H, d, *J*: 8.13 Hz, Ar-H), 8.07-8.11 (2H, m, Ar-H), 8.48 (1H, d, *J*: 8.13 Hz, Ar-H), 9.46 (1H, s, Aromatic -C-H). ^{13}C NMR (125 MHz, DMSO-d_6): 21.55, 55.68, 55.71, 113.38, 113.74, 114.64, 120.96, 122.96, 126.95, 127.45, 129.19, 129.42, 135.47, 141.23, 144.16, 152.56 and 159.90. APCI-MS (-m/z): $[M+H]^+$: 290.7.

3-(3-Chloro-phenyl)-1-methyl-benzo[4,5]imidazo [1,2-a]pyrazine (4c)

Yield 82%. m.p. 168°C. IR (KBr) ν_{max} (cm^{-1}): 684 (Aromatic -C-H), 740-758 (Aromatic -C-H), 1080-1203 (Aromatic -C-N), 1456-1568 (Aromatic -C=C), 1595-1681 (Aromatic -C=N). ^{1}H-NMR (DMSO-d_6): (ppm) 2.93 (3H, s,

CH_3), 7.44-7.52 (1H, m, Ar-H), 7.53-7.58 (2H, m, Ar-H), 7.60-7.65 (1H, m, Ar-H), 7.98 (1H, d, *J*: 8.14 Hz, Ar-H), 8.15 (1H, d, J: 7.62 Hz, Ar-H), 8.23 (1H, s, Ar-H), 8.49 (1H, d, *J*:8.16 Hz, Ar-H), 9.67 (1H, s, Aromatic C-H). ^{13}C NMR (125 MHz, DMSO-d_6): 21.53, 113.79, 115.61, 121.07, 123.31, 124.59, 125.58, 127.18, 128.33, 129.53, 131.13, 133.70, 134.25, 138.97, 141.41, 144.22 and 152.91. APCI-MS (-m/z): $[M+H]^+$: 294.7.

3-(4-Chloro-phenyl)-1-methyl-benzo[4,5]imidazo [1,2-a]pyrazine (4d)

Yield 90%. m.p. 217°C. IR (KBr) ν_{max} (cm^{-1}): 735-756 (Aromatic -C-H), 812 (Aromatic -C-H), 1009-1204 (Aromatic -C-N), 1458-1477 (Aromatic -C=N), 1682 (Aromatic -C=N), 3022-3084 (Aliphatic -C-H). ^{1}HNMR (DMSO-d_6): (ppm) 2.51 (3H, s, CH_3), 7.52-7.61 (4H, m, Ar-H), 7.98 (1H, d, *J*: 8.05 Hz, Ar-H), 8.20 (2H, d, *J*: 7.86 Hz, Ar-H), 8.49 (1H, d, *J*: 8.05 Hz, Ar-H), 9.62 (1H, s, Aromatic -C-H). ^{13}C NMR (125 MHz, DMSO-d_6): 21.52, 113.73, 115.03, 121.03, 123.23, 127.12, 127.72, 129.23, 129.48, 133.26, 134.12, 135.64, 141.31, 144.20, 152.83 and 172.48. APCI-MS (-m/z): $[M+H]^+$: 294.6.

Cytotoxicity

The MTT assay based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium salt to the formazan product is used to determine the metabolic activity of living cells. Cell viability can be determined spectrometrically using the formazan salt, which turns purple at the end of the incubation period [19]. The anti-cancer activities of the compounds were determined using the 24-hour MTT assay. MTT assays were performed on NIH3T3 and A549 cells. NIH3T3 cells were grown in DMEM medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with fetal calf serum, penicillin (100 IU/ml), streptomycin (100 mg/ml) and 7.5% $NaHCO_3$ at 37°C in a humidified atmosphere of 95% air and 5% CO_2. A549 cells were grown in RPMI medium (Hyclone, Thermo Scientific, USA) supplemented with fetal calf serum, penicillin (100 IU/ml), streptomycin (100 mg/ml) and 7.5% $NaHCO_3$ at 37°C in a humidified atmosphere of 95% air and 5% CO_2. NIH3T3 and A549 cell lines were seeded in 96-well plates at 1 × 104 cell density. The culture media were removed, and the test compounds were added at concentrations of 0.000316 - 1 mM after 24 h of incubation. After 24 h incubation, colorimetric measurements were performed using a microplate reader (Biotek, USA) at 540 nm. The percentage of inhibition at all concentrations was determined using the formula below, and the IC_{50} values were calculated from a dose-response curve obtained by plotting the percentage of inhibition versus the log concentration with the use of Microsoft Excel 2013. The results were expressed as the mean value ± standard deviation (SD). As positive control, doxorubicin was used. The inhibition of cell proliferation for the cell lines A549 (human lung carcinoma) and NIH3T3 (mouse embryonic fibroblasts) is presented in Table 1. The cytotoxicity test of compounds 3a-3d and 4a-4d was screened according to MTT experiments. Doxorubicin is used as a reference drug. MTT assays were performed as previously described [20–22].

% inhibition = 100 – (mean sample x 100/mean solvent)

Molecular Docking Studies

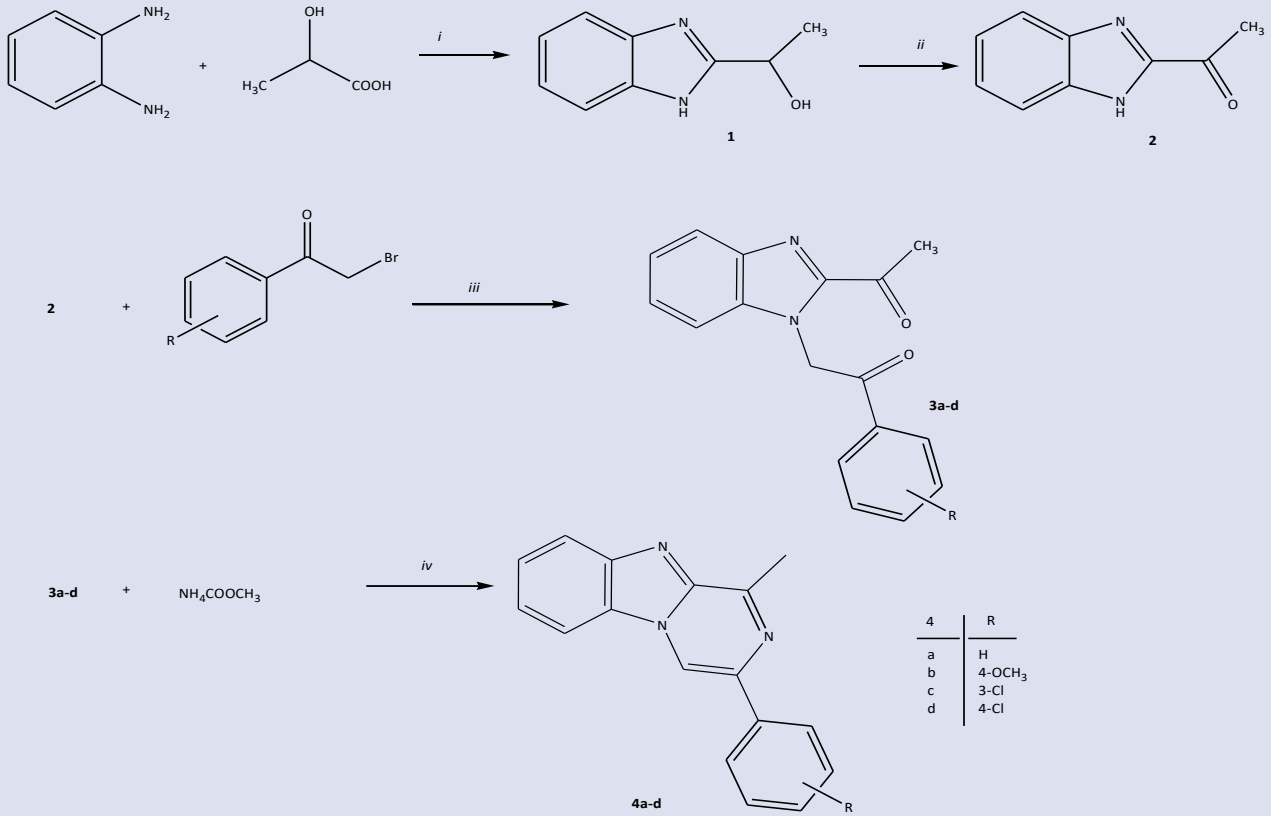
Molecular docking studies were performed using an *in-silico* procedure as previously described [3,23]. The crystal data were retrieved from the Protein Data Bank server (PDBID: 4QTX) [24]. The Schrödinger Maestro [25] interface was used for the molecular docking study, and the enzyme crystal was processed using the Protein Preparation Wizard protocol of the Schrödinger Suite 2020. Active molecules (4c and 4d) were prepared using LigPrep module [26].

The molecular docking study for compounds 4c and 4d were run according to the previously published procedures [27]. For that, ligands atoms correctly assigned the protonation states (pH=7.4±1.0) as well using LigPrep module. Bond orders were assigned, and hydrogen atoms were added to the structures. The grid generation was formed using the Glide module [28]. These grids were built up using the native ligand and its binding regions around 20 Å. After the generation of the grid maps, all of them were used in the docking runs. The docking runs were performed in extra precision docking mode (XP), and the best docking poses were determined among all outputs.

Results and Discussions

Chemistry

In this study, 2-(2-acetyl-1*H*-benzimidazol-1-yl)-1-arylethanone (3a-3d) and 1-methyl-3-phenyl-benzo[4,5]imidazo[1,2-a]pyrazine derivatives (4a-4d) were synthesized as shown in Scheme 1. Primarily *o*-phenylenediamine was reacted with lactic acid as a result compound 1 formed via Phillips reaction. Then the solution of chromium trioxide and compound 1 were heated in acetic acid for oxidation reaction. The resulting compound (2) was stirred with 2-bromoacetophenone derivatives at room temperature. Ultimately 2-(2-acetyl-1H-benzimidazol-1-yl)-1-arylethanone derivatives (3a-3d) formed. APCI, IR, ^{1}HNMR, and ^{13}CNMR spectra were utilized to determine the structure of the synthesized compounds. In the IR spectrum of the compounds 3a-3d, carbonyl bands were spotted at 1695-1674 cm^{-1} and methylene protons were spotted at 6.23-6.24 ppm. At the last step, 2-(2-acetyl-1H-benzimidazol-1-yl)-1-(substituted phenyl)ethanone derivatives (3a–3d) and sufficient amount of ammonium acetate were refluxed in acetic acid. Thus the resulting products, 1-methyl-3-phenyl-benzo[4,5]imidazo[1,2-a]pyrazine derivatives (4a-4d), were synthesized. In the IR spectrum of the final products, all bands, except aliphatic -C-H bands, were observed at the fingerprint area as expected. ^{1}HNMR spectra have shown aliphatic -C-H protons' peaks at 2.51-2.95 ppm. The singlet peaks were spotted at 9.56-9.67 ppm belonging to pyrazine proton. The other aromatic protons were pursued at the expected area, approximately 7.08-8.51 ppm. M+1 values were measured with the APCI instrument and the values were similar to the calculated molecular weights of the final compounds.



Scheme 1. The synthesis diagram of the compounds 3a-3d and 4a-4d. Reagents and conditions: (i) 4N HCl, reflux, 8h, 71%; (ii) CrO_3, CH_3COOH, 90°C during the addition of chromium trioxide solution addition then cooled, 76%; (iii) K_2CO_3, acetone, room temperature, 4–6 h, 65–75%; (iv) CH_3COOH, reflux, 10 h, 80–90%.

Cytotoxic Activity Evaluation

Table 1. IC_{50} (µM) values of synthesized compounds (3a-d and 4a-d)

Compounds	A549	NIH3T3	Compounds	A549	NIH3T3
3a	40.667±0.343	104.731±2.492	4a	32.965±0.866	254.997±1.416
3b	111.118±4.619	194.657±1.634	4b	41.267±1.439	206.877±2.036
3c	48.191±1.576	260.762±1.369	4c	26.469±0.313	130.455±0.964
3d	33.883±0.499	151.411±2.748	4d	20.137±0.311	169.542±1.069
Doxorubicin	3.001±0.071	>1000			

The test results were expressed as means of quartet assays

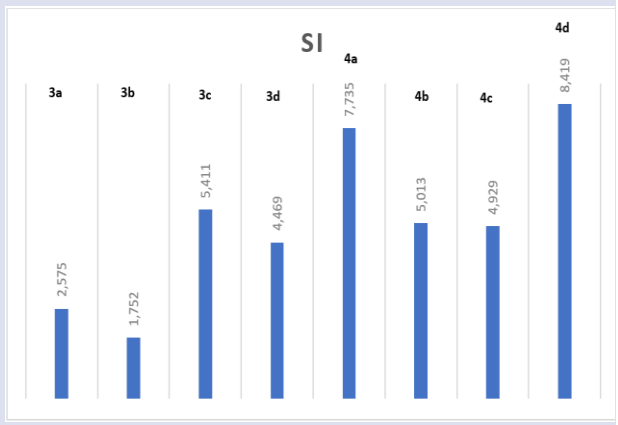


Figure 3. Selectivity indexes (SI) of obtained compounds (3a-3d and 4a-4d)

Final compounds 4a-4d and compounds 3a-3d were subjected to MTT assay to investigate their cytotoxic activity. Doxorubicin was chosen as the standard drug to perform the MTT test. IC_{50} value of doxorubicin against A549 cancer cell line is 3.001 µM and against NIH3T3 is over 1000 µM. IC_{50} value of compound 4c and 4d detected as 26.469 µM and 20.137 µM, respectively. Although compounds 4c and 4d were not found as effective as the standard drug doxorubicin, they have a selective toxic effect against cancer cells. Compounds that are selective for cancer cells are toxic to the cancer cells but have no effect on healthy cells. This makes it possible to treat the cancer without harming the healthy cells inside.

In our previous study [11], compounds containing benzimidazole ring and diketone structure (3a-3d) were found to be more active than compounds containing methylene-carrying pyrazino[1,2-a]benzimidazole. In this study 3-chloro and 4-chloro substituted pyrazinobenzimidazole ring containing compounds (4c and 4d) found more effective than compounds 3a-3d.

Molecular Docking Study

To estimate the binding modes of the two active (4c and 4d) compounds, they docked to the active site of the caspase enzyme. The 4QTX crystal form of the enzyme was chosen to dock the compounds. According to the given data, compound 4c interacted with Arg64, Gln161, and Arg207 via halogen bonds, with Hie121 via π–π

stacking, aromatic H-bond and water-mediated H-bond, with Ser120 via aromatic H-bond. On the other hand, 4d interacted with Arg64 and Gln161 via halogen bonds, with Thr62 via water-mediated H-bond, with Hie121 via aromatic H-bond.

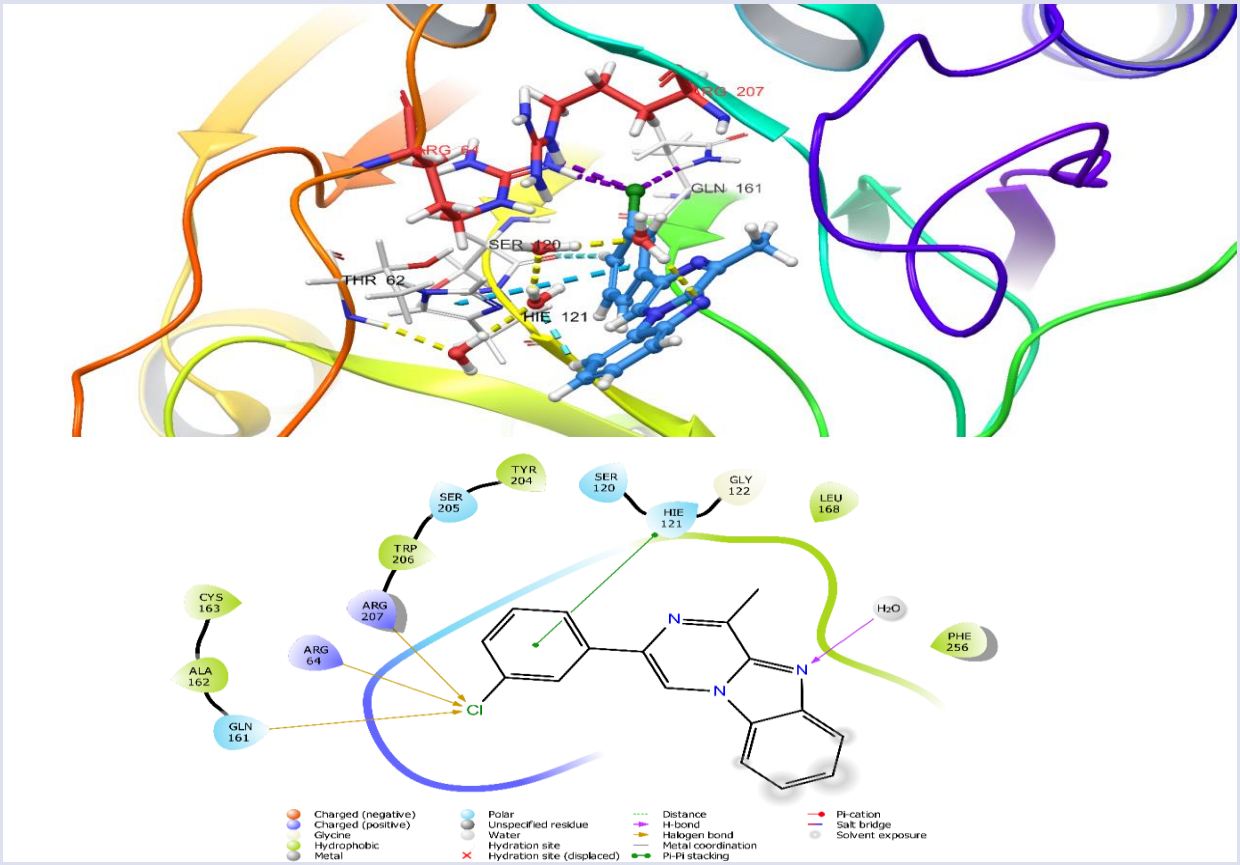


Figure 4. 3D (top) and 2D (bottom) docking poses of compound caspase-3-4c enzyme complex (PDBID: 4QTX).

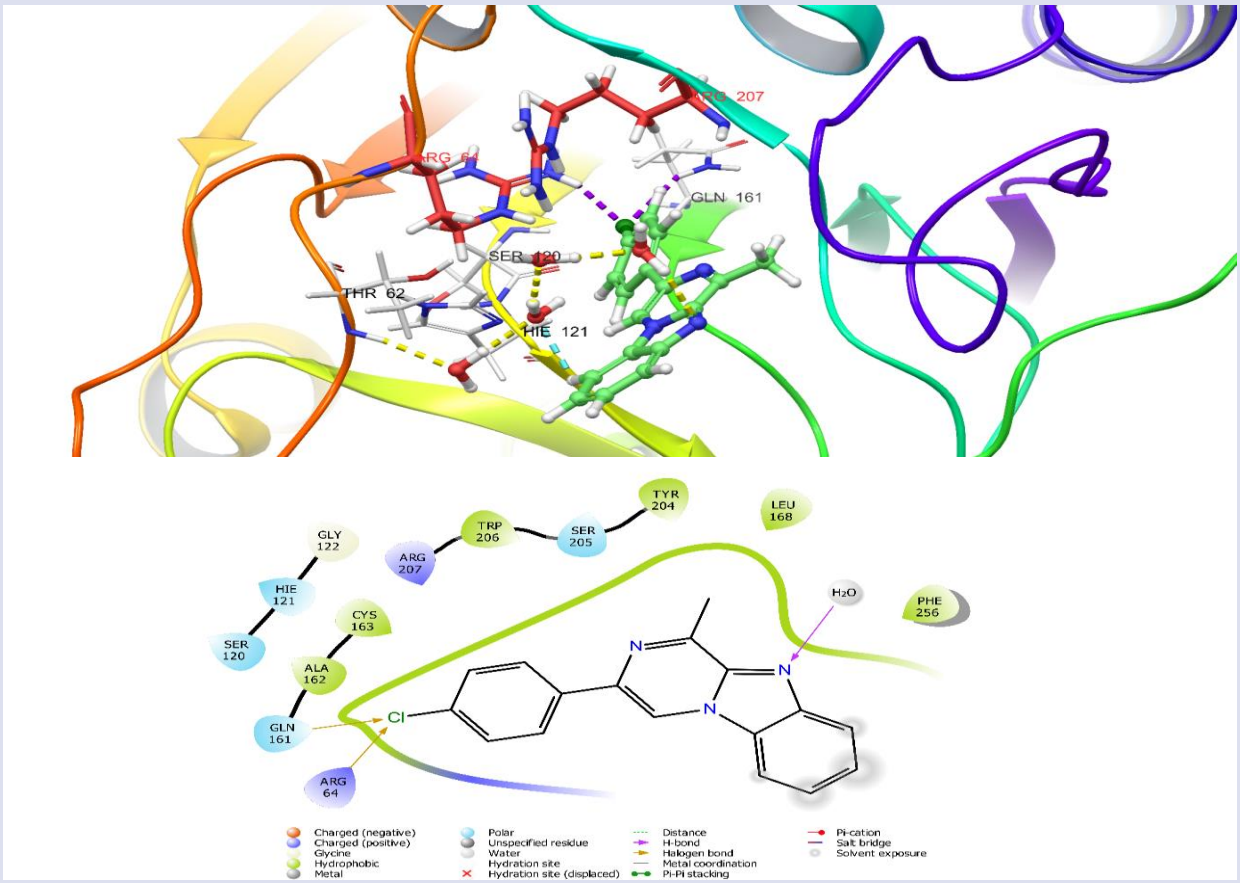


Figure 5. 3D (top) and 2D (bottom) docking poses of compound caspase-3-4d enzyme complex (PDBID: 4QTX).

Briefly, interactions with arginine residues (Arg64 and Arg207) were identified as key points in the allosteric activation of caspase-3 enzyme, and both compounds formed halogen bonds. In addition to that, both compounds showed affinity to Hie121 residue, which is this residue is a member of the loop region, thus, it has an important role in allosteric activation of the enzyme. In a result, the *in-silico* study suggests that both active compounds may be anticancer agents because they prompt the induction of the apoptotic pathway.

Conclusions

In this study, we designed and synthesized 2-(2-acetyl-1*H*-benzimidazol-1-yl)-1-arylethanone (3a-3d) and 1-methyl-3-phenyl-benzo[4,5]imidazo[1,2-a]pyrazine derivatives (4a-4d). Spectroscopic methods were used to determine the structure of the final compounds. Compounds 4c and 4d showed selective cytotoxic activity against the A549 cell lines. In the molecular docking study, compounds 4c and 4d were observed interact with arginine and histamine residues, which are key amino acids of the caspase enzyme. As a result of our study, the compounds we synthesized contain benzimidazole ring structure, which is known to have anticancer activity, and it was determined that they did not show toxic activity. Our study has been submitted to the literature for further studies for the design of anticancer drugs containing benzimidazole ring.

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Conflicts of interest

The authors declare no conflicts of interest.

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