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Cytotoxic activity evaluation of naphthalene substituted benzimidazole derivatives

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Abstract: Compounds bearing naphthalene and benzimidazole pharmacophores have been reported to possess excellent anticancer activity. In view of this, we designed, synthesized and characterized a series of naphthalene substituted benzimidazole derivatives (**11**–**19**), and further evaluated them for antiproliferative activities by employing MTT method. Among the nine investigated molecules, compounds (**11** and **13**) showed good antiproliferation of the tested cancer cell lines with IC_{50} values ranging from 0.078 to 0.625 µM. In addition, compound (**18**) exhibited selective cytotoxicity against HepG2 cell lines with high safety to normal cell (HEK293). Furthermore, cytotoxicity studies of these compounds against normal Human embryonic kidney cells (HEK293) revealed that the target molecules were less selective against HEK293 as compared to methotrexate (positive control). The high potency and selective cytotoxicity suggested that compound **18** could be a starting point for further optimization to develop novel antitumor agents towards liver cancer.

Keywords: Benzimidazole; naphthalene; anticancer; liver cancer.

1. Introduction

Cancer has now become the disease most urgently to be solved because of its incidence and high mortality rate round the globe.[1] Recent reports of the World Health Organization (WHO) stated that about one in six death cases globally is mainly due to cancer. Hence, the development of efficacious drugs with novel mechanisms is necessary for various cancer types.[2]

Benzimidazole is the most prominent heterocyclic having good cytotoxic properties against different types of cancer cell lines.[3] A number of marketed anticancer drugs comprise benzimidazole moiety, Nocodazole, Bendamustine and Veliparib are major examples (Figure 1). Nocodazole is a common inhibitor of various cancer-related kinases which includes Abelson (ABL), c-KIT receptor tyrosine kinase, serine/threonine kinase (BRAF), dual threonine and tyrosine recognition kinase (MEK-1 and MEK-2) and MET receptor tyrosine kinase.[4] Bendamustine is an alkylating agent that has clinical activity against various human cancers.[5,6] Veliparib (ABT-888), a potent anticancer drug acting as a poly(ADP ribose) polymerase (PARP) inhibitor which is targeted against breast cancer cell lines.[7]

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Development of resistance and toxicity to normal rapidly growing cells are the major limitations of existing anticancer drugs, also majority of the drugs in the market that are not specific.[8] Therefore, the development of new drugs with antiproliferative activity is a priority task.

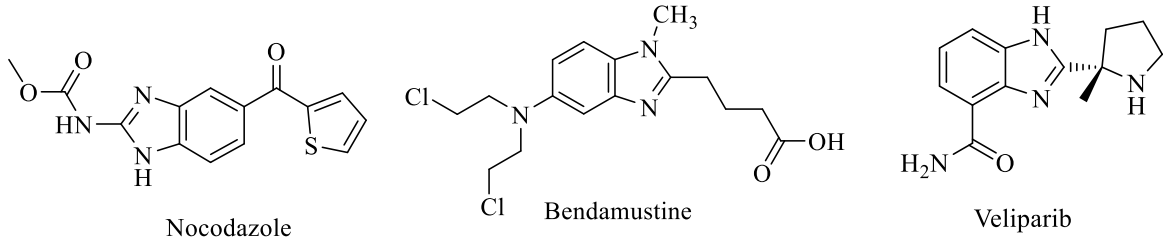


Figure 1. Benzimidazole scaffold-containing anticancer drugs

Multiple previous reports have suggested benzimidazole derivatives with an aromatic moiety at the C-2 and/or a functional group at -5/-6 positions show increased anticancer activity.[9,10] Azam et al. reported that 2-phenylbenzimidazole analogues Figure 2 (a), possess potent anti-cancer activity against THP-1, MCF-7, PC-3 and A-549 cancer cell lines.[11] Racané et al. synthesized some 2-aryl substituted benzimidazole derivatives Figure 2 (b), which emerged out as potent antiproliferative agents against HCC827 cancer cell line.[12] Reddy et al. illustrated that 2-(1,3-diphenyl-1H-pyrazol-4-yl)benzimidazole derivatives Figure 2 (c), act as anticancer agent against lung-A549, MCF-7, HeLa and human keratinocyte cells-HaCaT.[13]

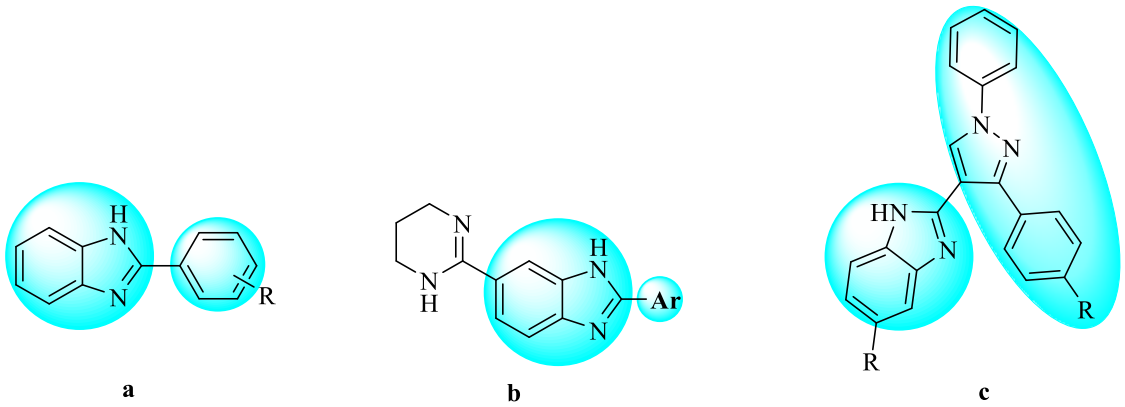


Figure 2. 2-Aryl benzimidazole derivatives acting as anticancer agents

Moreover, naphthalene containing analogs have been reported as potent anticancer agents.[14-17] Daurinol Figure 3 (d), a natural aryl naphthalene lactone, anti-cancer activities of daurinol against various human cancer cells have been reported. Recent studies have demonstrated its chemotherapeutic efficacy in colon,[18] ovarian,[19] and lung cancers.[20] Also, Xie et al. reported that aryl naphthalene derivative Figure 3 (e), act as anticancer agents by inhibiting the cytoplasmic protein-tyrosine phosphatase.[21]

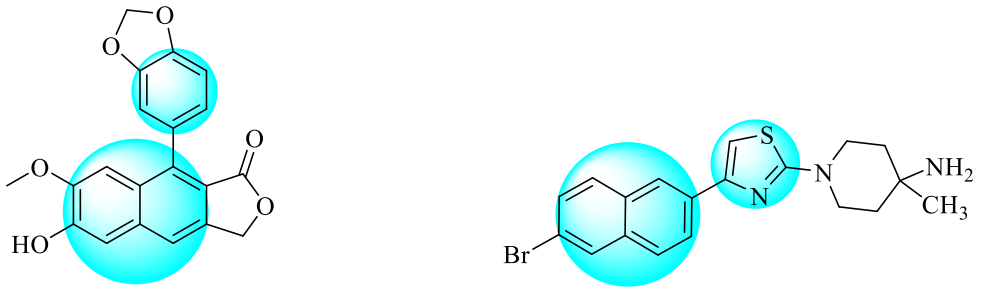


Figure 3. Aryl naphthalene derivatives acting as anticancer agents

Taking into account that both benzimidazole and naphthalene are among the privileged aromatic subunits and important building blocks in medicinal chemistry. Additionally, since the aryl group has a positive influence on anticancer activity of both benzimidazole and naphthalene, and because of our compounds are both 2-aryl benzimidazole and aryl naphthalene derivatives (Figure 4), we decided to study the antiproliferative activity of our previously synthesized 2-naphtylbenzimidazole derivatives (Figure 5).

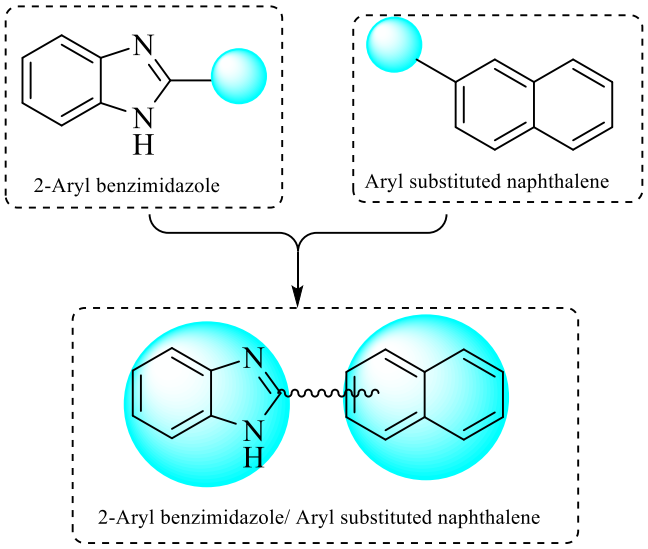


Figure 4. 2-Aryl benzimidazole and Aryl substituted naphthalene derivatives

2. Experimental

2.1. Chemistry

2.1.1. General Synthesis Procedure

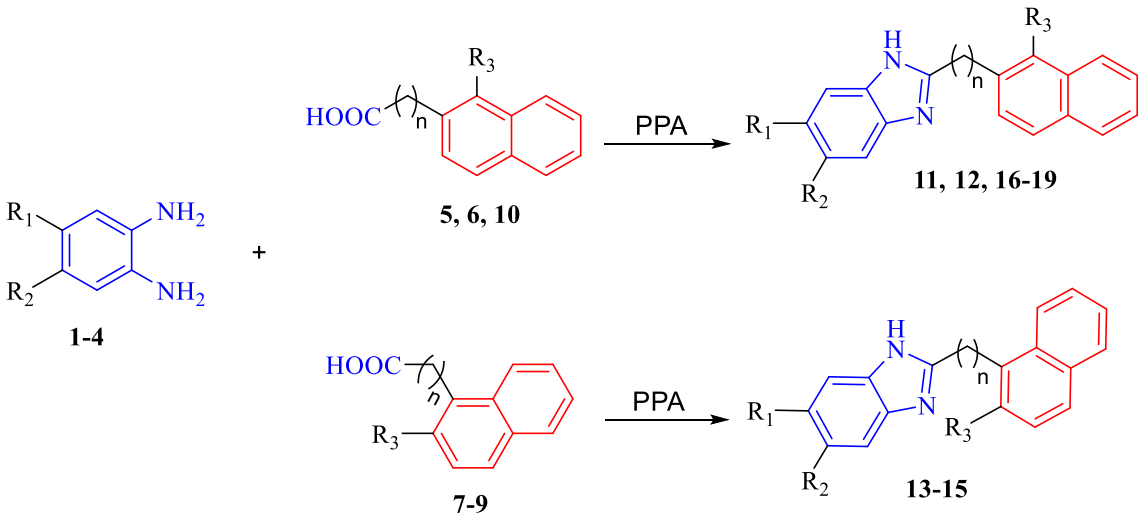
The synthetic route of the compounds is shown in Scheme 1. All compounds were synthesized by using polyphosphoric acid by microwave irradiation and conventional methods. All synthesis procedures and results are detailed in our previous study.[22]

2.1.1.2. Conventional Heating Method

o-Phenylenediamine derivatives (**1**–**4**) (1 eq) and the corresponding naphthalene carboxylic acid derivatives (**5**–**10**) (1.1 eq) in PPA (5 mL) were placed in a 25 mL flask. The reaction mixture was heated at 180 °C for 13–15 hrs. After the reaction was monitored by TLC, the mixture was poured into ice water and neutralized by 5 M NaOH until it reached a slightly basic pH (8–9) to obtain a precipitate. The resulting precipitate was filtered off, washed with cold water, and recrystallized with a suitable solvent (H_2O, EtOH, EtOH-H_2O). The resulting crystalline compounds were filtered, and the vacuumed product was dried.

2.1.1.3. Microwave Irradiation Method

In the presence of PPA, the mixture of *o*-phenylenediamine derivatives (**1**–**4**) (1 eq) and the corresponding naphthalene carboxylic acid derivatives (5–10) (1.1 eq) was stirred and irradiated in MW (7–12 mins, 100–150 W). After monitoring of the reaction with TLC, the mixture was poured into ice water and neutralized with 5 M NaOH until it reached a slightly basic pH (8–9) to obtain the precipitate. The resulting precipitate was filtered off, washed with cold water, and recrystallized with a suitable solvent (H_2O, EtOH, EtOH-H_2O). The resulting crystalline compounds were filtered, and the vacuumed product was dried.



Scheme 1. Synthesis of 2-naphthylbenzimidazole compounds (**11**–**19**)

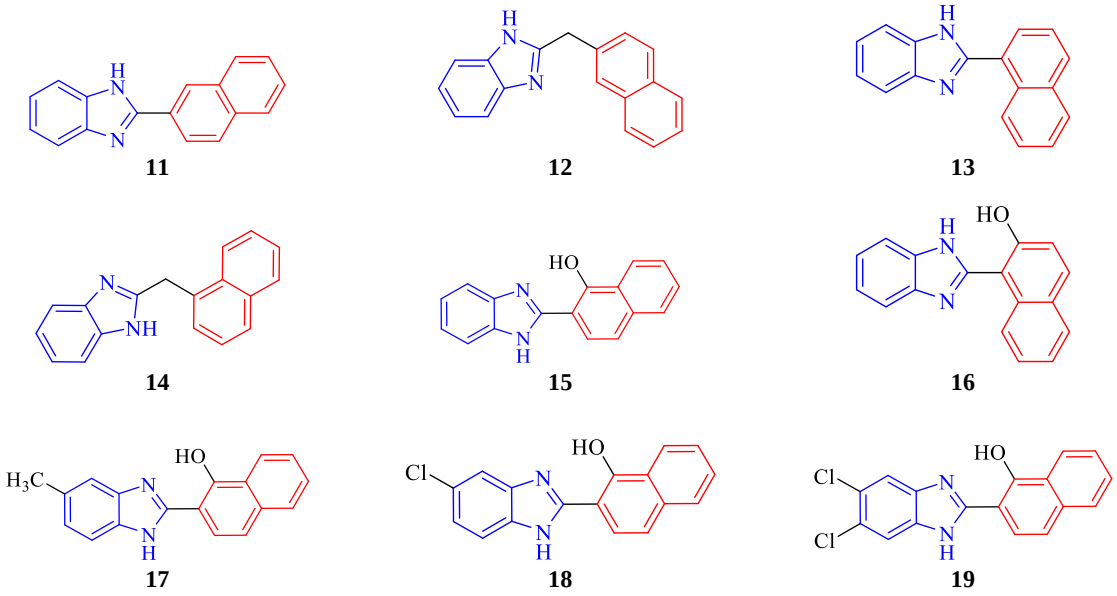


Figure 5. Structures of the 2-naphthylbenzimidazole derivatives (**11-19**)

2.2. Biological Evaluation

2.2.1. Antiproliferative Activity

The synthesized naphthalene substituted benzimidazole derivatives (11-19) were tested *in vitro* for their cytotoxic properties against tumor cell line panels—which consisted of A549 (a human lung cancer cell line), A498 (a human chronic myeloid leukemia cell line), HeLa (a human acute monocytic myeloid leukemia cell line), HepG2 (a human liver cancer cell line), A375 (human malignant melanoma) and HEK293 (Human embryonic kidney cells) by using the MTT assay Mosmann's method. The MTT assay is based on reducing the soluble MTT (0.5 mg/ mL, 100 µL) into a blue-purple formazan product, mainly by the mitochondrial reductase activity occurring inside living cells.[23] The cells used in the cytotoxicity assay were cultured in RPMI 1640 medium and were supplemented with 10% fetal calf serum, penicillin, and streptomycin at 37 °C and humidified at 5% CO_2. The cells were then briefly placed on 96-well plates at 100 mL total volume, with a density of 1–2.5 x10^4 cells per mL; they were then allowed to adhere for 24 h before being treated with tested drugs in a DMSO solution (10^{-5}, 10^{-6}, 10^{-7} mol L^{-1} final concentration). The triplicate wells were treated with media and agents, and the cell viability was assayed after 96 hrs of continuous drug exposure with a tetrazolium compound. The supernatant medium was removed, and 150 mL of DMSO solution was added to each well. The plates were gently agitated using a mechanical plate mixer until the color reaction was uniform and the OD_{570} was determined using a microplate reader. The 50% inhibitory concentration (IC_{50}) was defined as the concentration that reduced the absorbance of the untreated wells by 50% of the vehicle in the MTT assay. Assays were performed in triplicate on three independent experiments. The results had good reproducibility between the replicate wells, with standard errors below 10%.

3. Results and Discussion

All of the synthesized compounds (**11–19**) were evaluated for their *in vitro* antiproliferative activity against five human cancer cell lines by comparing the results with the standard antiproliferative drug methotrexate. *In vitro* antiproliferative screenings of the titled compounds against the lung cancer cell line (A549), the kidney cancer cell line (A498), the cervical cancer cell line (HeLa), the human malignant melanoma (A375) and the liver cancer cell line (HepG2) were performed using MTT assay.[23] The obtained results of the *in vitro* antiproliferative activities are summarized in Table 1.

Table 1. *In vitro* antiproliferative activity of synthesized naphthalene substituted benzimidazole derivatives studied using MTT assay (96 hrs).

No	HEK293	A549	A498	HeLa	A375	HepG2	Specificity					cLog*P*#
	IC_{50} (µM)						A549	A498	HeLa	A375	HepG2	
11	1.25	0.625	0.312	0.625	0.625	0.078	2.00	4.00	2.00	2.00	16.00	4.837
12	2.5	1.25	0.625	5	5	0.312	2.00	4.00	0.5	0.5	8.00	4.806
13	0.625	0.312	0.312	0.162	0.078	0.156	2.00	2.00	3.85	8.00	4.00	4.837
14	1.25	0.625	0.312	5	5	0.625	2.00	4.00	0.25	0.25	2.00	4.576
15	2.5	1.25	0.625	5	5	0.625	2.00	4.00	0.5	0.5	4.00	4.060
16	1.25	0.625	0.312	0.625	1.25	0.078	2.00	4.00	2.00	1.00	16.00	4.060
17	5	2.5	1.25	2.5	2.5	0.625	2.00	4.00	2.00	2.00	8.00	4.559
18	2.5	1.25	0.625	2.5	10	0.078	2.00	4.00	1.00	0.25	32.00	4.851
19	0.625	0.312	0.312	1.25	2.5	0.625	2.00	2.00	0.5	0.25	1.00	5.471
MTX	0.022	0.022	0.022	0.022	0.022	0.022	1.00	1.00	1.00	1.00	1.00	0.94

#: *cLogP* value of the synthesized compounds (calculated from ChemBioDrawUltra 12.0.3). MTX: Methotrexate

Cytotoxic activity evaluation of naphthalene substituted benzimidazole derivatives

The synthesized compounds (**11–19**) also evaluated to study their cytotoxic nature against HEK293. This study helps to understand the selectivity of these active compounds toward cancerous cells. If the compound is more selective toward cancerous cells, then it must be less toxic to the normal cells. From Table 1, we can conclude that all of the synthesized compounds of the series have shown good antiproliferative activities against the tested human cancer cell lines. Generally, all the compounds displayed good antiproliferative activities against HepG2 and A498 cancer cell lines, with high selectivity when compared with standard methotrexate.

Among the synthesized compounds, non-substituted naphthyl-benzimidazole derivatives (**11** and **13**) showed promising antiproliferative activity against all the tested cancer cell lines. From the Table 1, compound **11** was observed as a potent antiproliferative agent against the A498 and HepG2 Cancer cell lines, with IC_{50} values of 0.312 μM and 0.078 μM, respectively. Also, the compound **11** was found to be selective for the cancer cell lines, with an IC_{50} value of 1.25 μM against HEK293 cell line. This means that it shows 4 and 16 times more selectivity toward the A498 and HepG2 cancer cell lines, respectively. Similarly, the compound **13** shows 2, 4 and 8 times more selectivity toward the A549, HeLa and A375 cancer cell lines, respectively; as it displays IC_{50} values of 0.625 μM against HEK293 cell line, and 0.312 μM, 0.162 μM and 0.078 μM against A549, HeLa and A375 cancer cell lines (Table 1).

Among the 1-/2-(non-substituted benzimidazolyl) naphthalene derivatives (**11-16**), 2-(benzimidazolyl) naphthalene derivatives (**11** and **16**) had high selectivity and good antiproliferative activity with IC_{50} values of 0.078 μM and 1.25 μM against HepG2 and HEK293 cell lines, respectively, which means that the compounds show 16 times more selectivity toward the HepG2 cancer cell lines.

The presence of methyl linker between naphthalene and benzimidazole moieties appeared to decrease the cytotoxic effect of the compounds (**12** and **14**) against the tested cancer cell lines. Similarly, moving the hydroxyl group from β-position of naphthalene (**15**) to α-position of naphthalene (**16**) decreased the cytotoxic effects.

Among the 2-(5-substituted benzimidazolyl) naphthalene derivatives (**17-19**), 5-chlorobenzimidazole derivative (**18**) was observed as the most potent antiproliferative agent against HepG2 cancer cell lines, with an IC_{50} value of 0.078 μM; Also, the compound **18** was found to be selective for the cancer cell lines, with an IC_{50} value of 2.5 μM against HEK293 cell line. This means that it shows 32 times more selectivity toward the HepG2 cancer cell lines.

4. Conclusion

In the present work, a series of 2-naphtylbenzimidazole derivatives (**11-19**) was evaluated as potential antiproliferative agents towards HepG2 cancer cell line. Since 2-naphtylbenzimidazole derivatives are polycyclic aromatic compounds, the antiproliferative activity depend on naphthalene ring.

Minor structural modifications/changes such as extending the linker between naphthalene and benzimidazole moiety, altering the position of attachment of the naphthalene moiety (α vs β) to the benzimidazole head, adding the hydroxyl group on the naphthalene moiety or adding substitution group at C-5/−6 positions of benzimidazole moiety were evaluated.

The presence of methyl linker between naphthalene moiety and benzimidazole moiety did not significantly improve the activities against cancer cell lines. We did not observe any correlation between obtained log*P* values and antiproliferative activity of synthesized compounds. However, β-benzimidazolyl substituted naphthalene derivatives (**11, 16**) demonstrated increased antiproliferative activity and the selectivity toward HepG2 cancer cell line compared to α-benzimidazolyl substituted naphthalene derivative (**13, 15**).

Mono-substituted benzimidazole derivatives (**17**, **18**) showed a better selectivity toward HepG2 cancer cell line compared to di-substituted benzimidazole derivative (**19**), especially, chloro-substituted compound (**18**) exhibited a high selectivity to be 32 times more selective against HepG2 cancer cell line. Our results show that these 2-naphtylbenzimidazole derivatives as a class are good candidates for further study as therapeutic agents for the treatment of liver cancer.

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