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In vitro antiproliferative activity of some fluoro-substituted benzimidazole-based scaffolds

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Abstract: In the present work, a series of fluoro-substituted benzimidazole derivatives were designed and synthesized as antiproliferative agents. The antiproliferative activity of these compounds was investigated using MTT assay. Fluoro-substituted benzimidazole derivatives showed significant antiproliferative activity against all the tested cancer cell lines. All the derivatives were found to be less toxic as compared to methotrexate (positive control) in human cells, indicating selective and efficient antiproliferative activity of these benzimidazole derivatives. These findings suggest that compounds **ORT14** and **ORT15** among this series are most effective and have potential for detailed investigations.

Keywords: Benzimidazole; fluorine; antiproliferative; anticancer; SAR.

1. Introduction

Cancer, occurring by abnormal division and spread of cells, is recognized as the main global health problem and is one of the major causes of death.[1] Therefore, the development of new drugs with antiproliferative activity is a priority task, which will shorten the current chemotherapy. Benzimidazoles are the most prominent heterocycles with diverse biological functions.[2-5] Multiple previous reports have suggested that benzimidazoles to be very good cytotoxic agents against different types of cancer cell lines,[6] and exert their anticancer activity by acting on various targets such as topoisomerases inhibitors,[7] DNA-alkylating agents,[8] tubulin polymerization inhibitors,[9] and antiangiogenic agents.[10]

Due to the drug-like character and considerable range of structural diversity, large collections or libraries of diverse benzimidazoles are routinely employed in high-throughput screening at the early stages of drug discovery programs. As per the literature, after nitrogen, fluorine occupies the position

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of second favorite hetero-element in life science-oriented research. In fact, more than 20% of pharmaceuticals are prescribed or administered in the clinic, and more than 30% of the leading blockbuster drugs contain fluorine atoms in their molecules.[11]

Fluorine-containing benzimidazoles, which are showing biological activities, are well documented in the literature.[12-16] A series of benzimidazole analogues was designed by Reddy et al. The synthesized compounds were evaluated against human tumor cell lines. Structure activity relationship (SAR) studies of benzimidazole derivatives concluded that compound **1** with a fluorine appendage showed potent cytotoxicity against tested cancer cell lines.[17] Kamal et al. synthesized benzimidazole-oxindole compounds and evaluated them against human breast cancer cell line (MCF-7). The compounds with mono-fluoro, difluoro, or trifluoromethyl moieties show considerable antiproliferative activities. Their finding implies that compound **2** with a difluoro moiety at positions 3 and 5 on phenyl ring showed significant cytotoxicity against breast cancer cell line (MCF-7) with an IC_{50} value of 1.59 µM.[18] Also, Singh et al. have been reported that the presence of flour atoms as substituents at 2-position of benzimidazole enhanced the cytotoxic activity of compound **3** against human cancer cell lines, with IC_{50} values of 5.5 and 1.5 µM against MCF7 and HeLa respectively.[19]

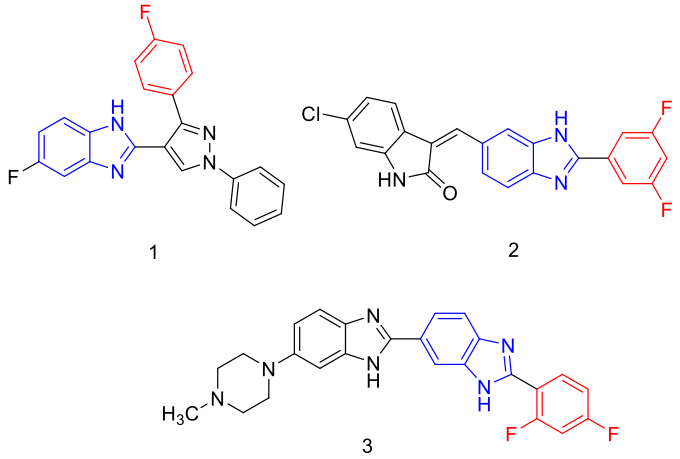


Figure 1. Benzimidazole derivatives incorporating fluorine with antiproliferative activity.

In continuation of our efforts on the design and synthesis of benzazole (benzimidazole, benzoxazole, and benzothiazole) derivatives with different biological activity and keeping in mind the medicinal importance of benzazole moieties,[20-30] we have synthesized some derivatives of 2-phenylbenzimidazole which hold fluorine atom in the *ortho-*, *meta-* or *para-* positions of phenyl ring side chain of benzimidazole in order to examine theirs *in vitro* antiproliferative property against five different cancer cell lines. Additionally, we determined their cytotoxic activity against HEK293 (Human embryonic kidney cells).

2. Experimental

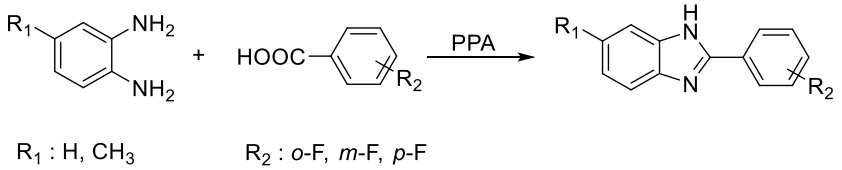
2.1. Chemistry

Commercial grade reagents and solvents were used without further purification. The purity of all of the compounds was judged by TLC analysis (single-spot/two-solvent systems) using a UV lamp. ^{1}H NMR spectra were taken on a 400 MHz spectrometer with tetramethylsilane (TMS) as an internal standard, and chemical shifts were recorded in ppm values. The IR spectra were obtained on a Perkin Elmer Spectrum One FT-IR spectrometer.

2.1.1. General Synthesis Procedure

A mixture of 1,2-Phenylenediamine derivatives (1 eq.) and the corresponding carboxylic acid derivatives (1.1 eq.) was heated for a period of 13–18 h in polyphosphoric acid (PPA) at 120-150 °C (Scheme 1).[26-30] The reaction mixture was poured onto ice water and neutralized by mixing it with 5 M

NaOH until a slightly basic pH (8–9) was obtained to get the precipitate. The resulting precipitate was filtered off, washed with cold water, and recrystallized with a suitable solvent. The resulting crystalline compounds were filtered, and the vacuumed product was dried (Fig. 2).



Scheme 1. Synthesis of 2-(fluorophenyl)-benzimidazole derivatives

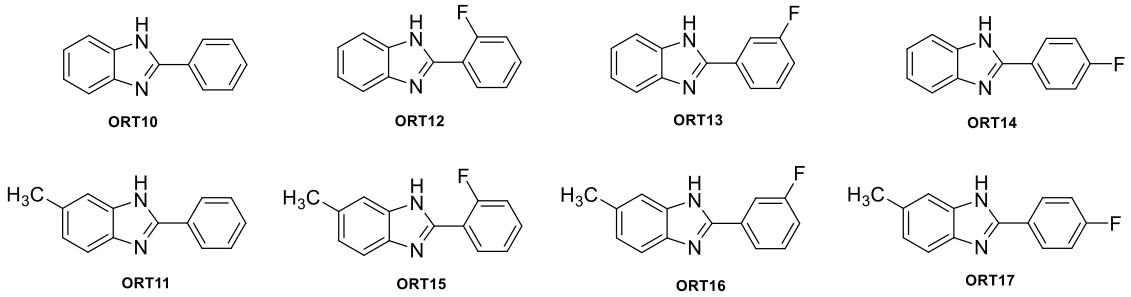


Figure 2. Structures of the 2-(fluorophenyl)-benzimidazole derivatives (**ORT10-17**)

*2-Phenyl-1H-benzo[d]imidazole (**ORT10**)*[31]: Light yellow crystalline; 66%; R_f ($CHCl_3$/MeOH 95:05) = 0.50; mp = 290-294 °C; IR (KBr, cm^{-1}) ν_{max} 3411, 1540, 1450, 1408, 967, 774; ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.18 (d, *J* = 7.5 Hz, 2H, Ar-H), 7.60 (m, 2H, Ar-H), 7.57-7.50 (m, 3H, Ar-H), 7.22-7.19 (m, 2H, Ar-H).

*2-(2-Fluorophenyl)-1H-benzo[d]imidazole (**ORT12**)*[32]: Yellow crystalline; 66%; R_f ($CHCl_3$/MeOH 95:05) = 0.62; mp = 210-215 °C; IR (KBr, cm^{-1}) ν_{max} 3320, 3048, 1585, 1444, 109.5, 743; ^{1}H NMR (400 MHz, d_6-DMSO) δ 12.55 (br s, 1H, NH), 8.23 (m, 2H, Ar-H), 7.61-7.53 (m, 2H, Ar-H), 7.47-7.37 (m, 2H, Ar-H), 7.27-7.21 (m, 2H, Ar-H).

*2-(3-Fluorophenyl)-1H-benzo[d]imidazole (**ORT13**)*[33]: yellow crystalline; 54%; R_f ($CHCl_3$/MeOH 95:05) = 0.51; mp= 253-257 °C; IR (KBr, cm^{-1}) ν_{max} 3307, 3021, 1535, 1404, 1075, 733; ^{1}H NMR (400 MHz, CD_3OD) δ 7.90–7.79 (m, 2H, Ar-H), 7.62–7.49 (m, 3H, Ar-H), 7.28–7.18 (m, 3H, Ar-H).

*2-(4-Fluorophenyl)-1H-benzo[d]imidazole (**ORT14**)*[31]: Beige crystalline; 42%; R_f ($CHCl_3$/MeOH 95:05) = 0.58; mp= 250-255 °C; IR (KBr, cm^{-1}) ν_{max} 3429, 3060, 1604, 1440, 964, 743; ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.23-8.21 (m, 2H, Ar-H), 7.59 (d, *J* = 2.7 Hz, 2H, Ar-H), 7.38 (d, *J* = 7.5 Hz, 2H, Ar-H), 7.22-7.18 (m, 2H, Ar-H).

*6-Methyl-2-phenyl-1H-benzo[d]imidazole (**ORT11**)*[34]: Red crystalline; 45%; R_f ($CHCl_3$/MeOH 95:05) = 0.54; mp = 250-255 °C; IR (KBr, cm^{-1}) ν_{max} 3375, 3052, 2919, 1504, 1420, 944, 723; ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.10 (s, 1H, NH), 8.68 (d, *J* = 1.8 Hz, 1H, Ar-H), 8.54 (d, *J* = 8.0 Hz, 1H, Ar-H), 8.40 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.50 – 7.57 (m, 2H, Ar-H), 7.46 (s, 1H, Ar-H), 7.14 (d, *J* = 8.0 Hz, 1H, Ar-H), 2.50 (s, 3H, CH_3).

*2-(2-Fluorophenyl)-6-methyl-1H-benzo[d]imidazole (**ORT15**)*[34]: Beige crystalline; 53%; R_f ($CHCl_3$/MeOH 95:05) = 0.48; mp = 263-265 °C; IR (KBr, cm^{-1}) ν_{max} 3302, 3042, 2965, 1554, 1470, 974, 833; ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.59 (s, 1H, NH), 8.45 (t, *J* = 8.0 Hz, 1H, Ar-H), 7.54 (ddd, *J* = 4.0, 8.0, 3.5 Hz, 2H, Ar-H), 7.47 (s, 1H, Ar-H), 7.32 – 7.42 (m, 2H, Ar-H), 7.10 (d, *J* = 8.0 Hz, 1H, Ar-H). 2.47 (s, 3H, CH_3).

*2-(3-Fluorophenyl)-6-methyl-1H-benzo[d]imidazole (**ORT16**)* (CAS 940703-07-1): Yellow crystalline; 54%; R_f ($CHCl_3$/MeOH 95:05) = 0.53; mp = 272-275 °C; IR (KBr, cm^{-1}) ν_{max} 3307, 3021, 1545, 1407, 1077, 736; ^{1}H NMR (400 MHz, CD_3OD) δ 7.90–7.79 (m, 3H, Ar-H), 7.62–7.49 (m, 3H, Ar-H), 7.32 (s, 1H, Ar-H), 2.40 (s, 3H, CH_3).

*2-(4-Fluorophenyl)-6-methyl-1H-benzo[d]imidazole (**ORT17**)*[35]: White crystalline; 63%; R_f ($CHCl_3$/MeOH 95:05) = 0.44; mp = 233-235 °C; IR (KBr, cm^{-1}) ν_{max} 3354, 3035, 2945, 1506, 1423, 944, 743; ^{1}H NMR (400 MHz, d_6-DMSO) δ 12.95 (s, 1H, NH), 8.05 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.56 (s, 1H, Ar-H), 7.36 (d, *J* = 7.9 Hz, 3H, Ar-H), 7.11−6.96 (m, 1H, Ar-H), 2.37 (s, 3H, CH_3).

2.2. Biological Evaluation

2.2.1. Antiproliferative Activity

The synthesized 2-(fluorophenyl)-1*H*-benzimidazole derivatives (**ORT10-17**) 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), A375 (a human colon cancer 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^{-1}, 100 µL) into a blue-purple formazan product, mainly by the mitochondrial reductase activity occurring inside living cells.[36] 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 h 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

3.1. Chemistry

The 2-(fluorophenyl)-1*H*-benzimidazole derivatives (**ORT10-17**) were synthesized using one-step synthetic pathways as illustrated in Scheme 1. The polyphosphoric acid (PPA) method was used for the synthesis of desired compounds (**ORT10-17**).

The structure of the 2-(fluorophenyl)-1*H*-benzimidazole derivatives was confirmed based on spectral data. Thus, the infrared (IR) spectra of all of the compounds appeared at their expected positions. The IR spectra of compounds (**ORT10-17**) exhibited the presence of absorption bands for -NH- of benzimidazole between 3302-3429 cm^{-1}; for =C-H of benzene between 3021–3060 cm^{-1}; for -C-H of aliphatic linkers between 2919-2965 cm^{-1}; for -C=N of benzimidazole between 1504 and 1604 cm^{-1}.

The ^{1}H NMR spectrum of 2-(fluorophenyl)-1*H*-benzimidazole derivatives indicated the presence of a singlet signal integrated by one proton at δ 11.10–12.95 ppm assigned to -NH- protons of benzimidazole rings. The equivalence of -CH_3 protons of methyl-substituted compounds was established by the presence of a singlet signal integrated by three protons at δ 2.37–2.50 ppm for the

Ar-CH_3 group. The signal of aromatic protons of benzimidazole and phenyl rings is observed as (s, d, ddd, t, and m) with chemical shift values 6.96–8.68 ppm.

3.2. Antiproliferative Activity

The in vitro antiproliferative activities of all the synthesized compounds (**ORT10-17**) were evaluated in five human cancer cell lines, A549, A498, HeLa, HepG2, and A375 using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT assay).[36]

Antiproliferative drug methotrexate (MTX) was used as the standard for comparison. The results of the MTT assay are summarized in Table 1. The antiproliferative activity of each analog was presented as the concentration of the compound that led to a 50% inhibition (IC_{50}) of cancer cell growth. These compounds were further screened for their cytotoxic nature using HEK293. This was used to establish the selectivity of these active compounds toward cancerous cells. A potent anticancer compound should be more selective toward cancer cells and less toxic toward the normal cells.

From Table 1, we can conclude that all of the synthesized compounds of the series have shown significant antiproliferative activity against the tested human cancer cell lines, with high selectivity when compared with standard methotrexate.

A visual inspection envisaged that the presence of the fluorine atom as a substituent on phenyl side chains directly attached to benzimidazole moiety is mainly influencing the inhibitory potential of compounds (**ORT12-17**) while keeping the phenyl unsubstituted (**ORT10-11**). Notable observations were that the presence of a methyl substitution group at 5 position of benzimidazole ring lead to enhancement of the antiproliferative activity of the compounds.

From Table 1, among the 5-unsubstituted benzimidazole derivatives, compound **ORT14** with *para*- fluoro substitution group on the phenyl ring of the side chain, proved to be the most potent antiproliferative agent among the current series against A549, A498, and A375 with an IC_{50} = 0.377 µM, and against HeLa and HepG2 with an IC_{50} = 0.188 µM; it also had a high selectivity, with an IC_{50} value of 9.424 µM, against HEK293. This means that it shows twenty-five times more selectivity toward A549, A498, and A375 cancer cell lines, and fifty times more selectivity toward HeLa and HepG2 cancer cell lines. Similarly, the compound **ORT12** with *ortho*- fluoro substitution group on the phenyl ring of the side chain, shows twelve and twenty-five times more selectivity toward HeLa and A498 cancer cell lines respectively, as it displays IC_{50} values of 4.712 µM against HEK293 and 0.377 µM and 0.188 µM against HeLa and HepG2 respectively (Table 1).

Table 1. *In vitro* antiproliferative activity of synthesized 2-(fluorophenyl)-benzimidazole derivatives studied using MTT assay

	HEK293	A549	A498	HeLa	A375	HepG2	Specificity					cLogP#
Compound No	IC_{50} (µM)						**A549**	**A498**	**HeLa**	**A375**	**HepG2**	
ORT10	4.056	1.622	3.245	1.622	1.622	1.622	2.50	1.25	2.50	2.50	2.50	3.663
ORT11	4.802	0.768	0.768	0.768	0.768	1.537	6.25	6.25	6.25	6.25	3.12	4.162
ORT12	4.712	1.508	0.754	0.377	0.754	0.188	3.13	6.25	12.5	6.25	25.00	3.826
ORT13	4.712	0.754	3.769	0.942	3.769	1.885	6.25	1.25	5.00	1.25	2.50	3.826
ORT14	9.424	0.377	0.377	0.188	0.377	0.188	25.00	25.00	50.00	25.00	50.00	3.826
ORT15	1.768	0.354	0.354	0.354	0.177	0.177	5.00	5.00	5.00	10.00	10.00	4.325
ORT16	0.884	0.177	1.768	0.707	0.707	0.707	5.00	0.50	1.25	1.25	1.25	4.325
ORT17	4.420	0.707	0.707	0.354	0.354	0.707	6.25	6.25	12.50	12.50	6.25	4.325
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: Methotraxate

Among 5-methyl substituted benzimidazole derivatives, compound **ORT15** with *ortho*- fluoro substitution group on the phenyl ring of the side chain displayed a good selectivity and promising antiproliferative activity against A549, A498 and HeLa cell lines with an IC_{50} = 0.354 µM, against A375, and HepG2 cancer cell lines with an IC_{50} = 0.177 µM, and against HEK293 cell line with an

IC_{50} = 1.768 µM, which means that it shows five times more selectivity toward A549, A498, and HeLa and ten times more selectivity toward A375 and HepG2 cancer cell lines (Table 1). Also, we found that the compound **ORT16** with *meta*- fluoro substitution group on the phenyl ring of the side chain exhibited potent antiproliferative activity against A549 cell line with an IC_{50} = 0.177 µM with good selectivity. Similarly, compound **ORT17** with *para*- fluoro substitution group on the phenyl ring of the side chain displayed potent antiproliferative activity against HeLa and A375 cancer cell lines with an IC_{50} = 0.354 µM and showed IC_{50} value of 4.420 µM against HEK293. Thus, **ORT17** was found to be twelve times more selective toward HeLa and A375 cancer cell lines (Table 1).

Generally, *ortho*- and *para*- fluoro substituted compounds are more active than their *meta*-fluoro counterparts. However, it seems difficult to predict the factors governing the inhibition potentials of these ligands.

4. Conclusion

A series of 2-(fluorophenyl)-1*H*-benzimidazole derivatives (**ORT10-17**) have been designed and synthesized which showed significant *in vitro* antiproliferative activity.

This study has reinforced our speculation about the presence of fluorine atom as a substituent on the phenyl side chain of benzimidazole. Generally, *ortho*- and *para*- fluoro substituted compounds are more active than their *meta*- fluoro counterparts. Compounds **ORT14** (*para*-fluoro) and **ORT15** (*ortho*-fluoro) displayed the highest antiproliferative activity with low IC_{50} (µM) values against all cancer cell lines. Generally, the presence of a methyl substitution group at 5 position of benzimidazole enhanced the antiproliferative activity, except for compound **ORT14**. Also, our results suggested that the synthesized series were characterized by more selectivity toward cancerous cells and very less toxicity toward the normal cells compared to MTX.

However, most of the derivatives are comparatively less potent but more selective than MTX; nevertheless, slight structural modification of these active derivatives may yield as prospective anticancer drugs with high selectivity. Based on the present results, it is worthy to mention fluoro substituted benzimidazole derivatives serve as a useful template for the further development of the anticancer agents.

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References

[1] World Health Organization, "Cancer," https://www.who.int/en/news-room/fact-sheets/detail/cancer (accessed April 24, 2021).

[2] Carcanague, D.; Shue, Y. K.; Wuonola, M. A.; Uria-Nickelsen, M.; Joubran, C.; Abedi, J. K.; Kühler, T. C. Novel structures derived from 2-[[(2-pyridyl) methyl] thio]-1 *h*-benzimidazole as anti-helicobacter p ylori agents, Part 2. *J. Med. Chem.* **2002**, *45*(*19*), 4300-4309.

[3] Algul, O.; Duran, N. Activity of bisbenzimidazoles derivatives to *Staphylococcus epidermidis*. *Asian J. Chem.* **2007,** *19*(*4*), 3145-3151.

[4] Shrivastava, N.; Naim, M. J.; Alam, M. J.; Nawaz, F.; Ahmed, S.; Alam, O. Benzimidazole scaffold as anticancer agent: synthetic approaches and structure–activity relationship. *Arch. der Pharm.* **201**7, *350*(*6*), e201700040.

[5] Kanwal, A.; Ahmad, M.; Aslam, S.; Naqvi, S. A. R.; Saif, M. J. Recent advances in antiviral benzimidazole derivatives: a mini review. *Pharm. Chem. J.* **2019**, *53*(*3*), 179-187.

[6] Bansal, Y.; Minhas, R.; Singhal, A.; Arora, R. K.; Bansal, G. Benzimidazole: a multifacted nucelus for anticancer agents. *Curr. Org. Chem.* **2021**, *25*(*6*), 669-694.

[7] Alper, S.; Arpaci, Ö. T.; Aki, E. Ş.; Yalçin, I. Some new bi-and ter-benzimidazole derivatives as topoisomerase I inhibitors. *II Farmaco* **2003**, *58*(*7*), 497-507.

[8] Smaill, J. B.; Fan, J. Y.; Denny, W. A. DNA minor groove targeted alkylating agents based on bisbenzimidazole carriers: synthesis, cytotoxicity and sequence-specificity of DNA alkylation. *Anti-Cancer Drug Des.* **1998**, *13*(*8*), 857-880.

[9] Kamal, A.; Reddy, M. K.; Shaik, T. B.; Srikanth, Y. V. V.; Reddy, V. S.; Kumar, G. B.; Kalivendi, S. V. Synthesis of terphenyl benzimidazoles as tubulin polymerization inhibitors. *Eur. J. Med. Chem.* **2012**, *50*, 9-17.

[10] Saulnier, M. G.; Frennesson, D. B.; Wittman, M. D.; Zimmermann, K.; Velaparthi, U.; Langley, D. R.; Vyas, D. 2-(1*H*-Imidazol-4-yl) ethanamine and 2-(1*H*-pyrazol-1-yl) ethanamine side chain variants of the IGF-1R inhibitor BMS-536924. *Bioorg. Med. Chem. Lett.* **2008**, *18*(*5*), 1702-1707.

[11] O'Hagan, D. Fluorine in health care: Organofluorine containing blockbuster drugs. *J. Fluorine Chem.* **2010**, *131*(*11*), 1071-1081.

[12] Al-Harthy, T.; Zoghaib, W.; Abdel-Jalil, R. Importance of fluorine in benzazole compounds. *Molecules* **2020**, *25*(*2*0), 4677.

[13] Bobinger, T.; Manaenko, A.; Burkardt, P.; Beuscher, V.; Sprügel, M. I.; Roeder, S. S.; Huttner, H. B. Siponimod (baf-312) attenuates perihemorrhagic edema and improves survival in experimental intracerebral hemorrhage. *Stroke* 2**019**, *50*(*11*), 3246-3254.

[14] Tonelli, M.; Novelli, F.; Tasso, B.; Vazzana, I.; Sparatore, A.; Boido, V.; Sparatore, F.; La Colla, P.; Sanna, G.; Giliberti, G.; et al. Antiviral activity of benzimidazole derivatives. III. Novel anti-CVB-5, anti-RSV and anti-Sb-1 agents. *Bioorg. Med. Chem.* **2014**, *22*, 4893–4909.

[15] Zhang, H.-Z.; Damu, G.L.V.; Cai, G.-X.; Zhou, C.-H. Design, synthesis and antimicrobial evaluation of novel benzimidazole type of fluconazole analogues and their synergistic effects with chloromycin, norfloxacin and fluconazole. *Eur. J. Med. Chem.* **2013**, *64*, 329–344.

[16] Zawawi, N.K.N.A.; Taha,M.; Ahmat, N.; Wadood, A.; Ismail, N.H.; Rahim, F.; Ali,M.; Abdullah, N.; Khan, K.M. Novel 2,5-disubtituted-1,3,4-oxadiazoles with benzimidazole backbone: A new class of b-glucuronidase inhibitors and in silico studies. *Bioorg. Med. Chem*. **2015**, *23*, 3119–3125.

[17] Reddy, T.S.; Kulhari, H.; Reddy, G.; Bansal, V.; Kamal, A.; Shukla, R. Design, synthesis and biological evaluation of 1,3-diphenyl-1*H*-pyrazole derivatives containing benzimidazole skeleton as potential anticancer and apoptosis inducing agents. *Eur. J. Med. Chem*. **2015**, *101*, 790–805

[18] Kamal, A.; Nagaseshadri, B.; Nayak, V.L.; Srinivasulu, V.; Sathish, M.; Kapure, J.S.; Reddy, S. Synthesis and biological evaluation of benzimidazole–oxindole conjugates as microtubule-targeting agents. *Bioorg. Chem.* **2015**, *63*, 72–84

[19] Singh, M.; Tandon, V. Synthesis and biological activity of novel inhibitors of topoisomerase I: 2-arylsubstituted 2-bis-1*H*-benzimidazoles. *Eur. J. Med. Chem.* **2011**, *46*, 659–669

[20] Ayaz, F.; Kheeree, R.; Isse, Q. A.; Ersan, R. H.; Algul, O. DNA base bioisosteres, bis-benzoxazoles, exert anti-proliferative effect on human prostate and breast cancer cells. *J. Turkish Chem. Soc. Section A: Chem.* **2018**, *5*(*3*), 1145-1152.

[21] Ayaz, F.; Isse, Q. A.; Kheeree, R.; Ersan, R. H.; Algul, O. Immunomodulatory and immunostimulatory effects of some bisbenzoxazole derivatives on lipopolysaccharide stimulated mammalian macrophages. *J. Pharm. Res.* **2019**, *23*(5), 944-951. https://doi.org/10.35333/jrp.2019.41

[22] Ayaz, F.; Kheeree, R.; Isse, Q. A.; Ersan, R. H.; Algul, O. Bisbenzoxazole derivatives had an antiinflammatory effect on in vitro stimulated macrophages. *Turk. J. Chem.* **2019**, *43*(*3*), 963-971.

[23] Ayaz, F.; Isse, Q. A.; Kheeree, R.; Ersan, R. H.; Algul, O. Bisbenzoxazole derivatives had anti-proliferative effect on human cancer cells. *Eskişehir Tek. Üniv. Bilim Teknol. Derg. C Yaşam Bilim. Biyoteknol.* **2019**, *8*(*2*), 203-211.

[24] Ayaz, F.; Ersan, R. H.; Kuzu, B.; Algul, O. New-generation benzimidazole-based plasmid delivery reagents with high transfection efficiencies on the mammalian cells. *In Vitro Cell. Dev. Biol. Animal* **2020**, 1-8.

[25] Ayaz, F.; Ersan, R. H.; Algul, O. Symmetric bis-benzoxazole-based chemicals exerted anti-inflammatory effect on danger signal LPS-stimulated macrophages. *Monatsh. Chem.* **2019**, *150*(*6*), 1137-1146

[26] Ersan, R. H.; Yuksel, A.; Ertan-Bolelli, T.; Dogen, A.; Burmaoglu, S.; Algul, O. One-pot synthesis of novel benzimidazoles with anaphthalene moiety as antimicrobial agents andmolecular docking studies. *J. Chin. Chem. Soc.* **2020**, 1-10.

[27] Algul, O.; Ersan, R. H.; Alagoz, M. A.; Duran, N.; Burmaoglu, S. An efficient synthesis of novel di-heterocyclic benzazole derivatives and evaluation of their antiproliferative activities. *J. Biomol. Struct. Dyn.* **2020**, (13 pages). https://doi.org/10.1080/07391102.2020.1803966

[28] Ersan, R. H.; Alagoz, M. A.; Ertan-Bolelli, T.; Duran, N.; Burmaoglu, S.; Algul, O. Head-to-head bisbenzazole derivatives as antiproliferative agents: design, synthesis, in vitro activity, and SAR analysis. *Mol. Divers*. **2020**, (13 pages). https://doi.org/10.1007/s11030-020-10115-0

[29] Ersan, R. H.; Alagoz, M. A.; Dogen, A.; Duran, N.; Burmaoglu, S.; Algul, O. Bisbenzoxazole derivatives: design, synthesis, in vitro antimicrobial, antiproliferative activity, and molecular docking studies. *Polycycl. Aromat. Comp.* **2020**, (20 pages). https://doi.org/10.1080/10406638.2020.1852589

[30] Ersan, R. H.; Bolelli, K.; Gonca, S.; Dogen, A.; Burmaoglu, S.; Algul, O. Bisbenzimidazole Derivatives as Potential Antimicrobial Agents: Design, Synthesis, Biological Evaluation and Pharmacophore Analysis. *Pharm. Chem. J.* **2021,** (10 pages). https://doi.org/10.1007/s11094-021-02389-x

[31] Nguyen, T. T.; Nguyen, X. T. T.; Nguyen, T. L. H.; Tran, P. H. Synthesis of benzoxazoles, benzimidazoles, and benzothiazoles using a Brønsted acidic ionic liquid gel as an efficient heterogeneous catalyst under a solvent-free condition. *ACS Omega* **2019**, *4*(*1*), 368-373.

[32] Weires, N. A.; Boster, J.; Magolan, J. Combined Pd/C and montmorillonite catalysis for one-pot synthesis of benzimidazoles. *Eur. J. Org. Chem.* ***2012***, *33*, 6508-6512.

[33] Nguyen, T. B.; Ermolenko, L.; Al-Mourabit, A. Sodium sulfide: a sustainable solution for unbalanced redox condensation reaction between o-nitroanilines and alcohols catalyzed by an iron–sulfur system. *Synthesis* **2015**, *47*(*12*), 1741-1748.

[34] Ke, F.; Zhang, P.; Xu, Y.; Lin, X.; Lin, J.; Lin, C.; Xu, J. Microwave-assisted nickel-catalyzed synthesis of benzimidazoles: ammonia as a cheap and nontoxic nitrogen source. *Synlett* **2018**, *29*(*20*), 2722-2726.

[35] Ravi, O.; Shaikh, A.; Upare, A.; Singarapu, K. K.; Bathula, S. R. Benzimidazoles from aryl alkyl ketones and 2-amino anilines by an iodine catalyzed oxidative C (CO)–C (alkyl) bond cleavage. *J. Org. Chem.* **2017**, *82*(*8*), 4422-4428.

[36] Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *6*5, 55–63.

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