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Synthesis of benzimidazole Schiff base derivatives and cytotoxic effects on colon and cervix cancer cell lines

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ABSTRACT

During the last few years the potential of benzimidazole derivatives in agrochemical and medicinal properties have been subjected to investigation. benzimidazole derivatives have been received central attention due to their significant antimicrobial, antibacterial, antifungal, anti-HIV, anticancer, and a wide array of other biological activities. The formation of 2-aryl-substituted benzimidazoles, by the polyphosphoric acid-catalyzed condensation of a carboxylic acid, with an o-amino-arylamine is described. The condensations proceed in good yield to give products which, in certain instances, are not readily attainable by conventional condensation techniques. The study of Benzimidazole containing Schiff bases is also of interest as some of them have shown the ability to anticancer. The structures of the compounds were identified by FT-IR, ^{1}H NMR, ^{13}C NMR and elemental analysis. Cytotoxic effects of benzimidazole derivatives on human cervical and colon cancer cell lines HeLa and SW-620 was determined spectrophotometrically by using Alamar Blue. Benzimidazole derivatives selectively inhibited proliferation of cancerous cell lines. None of the benzimidazole Schiff base derivatives inhibits proliferation of SW-620 while 4-APbzl*H*, 4SAPbz1*H* and 3SAPbz1*H* dose dependently inhibit proliferation of HeLa cells with IC50 of 230 µM, 110.6 µM and 101.3 µM, respectively.

Keywords: *Benzimidazole, Schiff base, cytotoxicity, anticancer, HeLa, SW-620.*

1. INTRODUCTION

Benzimidazoles have been known to be a very important class of heterocyclic organic compounds [1,2]. Theirs contain a phenyl ring fused to an imidazole ring. Benzimidazole and their derivatives have diverse applications in coordination chemistry, photophysics, photochemistry and bioinorganic chemistry [3-7]. In recent years, this class of organic compounds has garnered a lot of attention, especially due to their applications in various biological studies [8]. Some noteworthy because of their widespread pharmaceutical importance and biological activities of benzimidazoles are against several viruses including antimicrobial agents [9,11], anti-inflammatory [12], potential anti-tumor agents [13], anti-parasitic agents [14], antibacterial [15], anthelmintic [16], antifungal [17], anti-inflamatory [18], antiviral [19] and analgesic [20] properties.

The compounds with azomethine group in its structure are known as Schiff bases, which are synthesized by the condensation reaction of primary amines and active carbonyl groups [21]. Schiff bases of benzimidazole have been reported with remarkable antibacterial [22], antimicrobial [23] and antiproliferative [24] activities. Traditionally, benzimidazoles have most commonly been prepared from the reaction of o-phenylenediamine with carboxylic acids under harsh dehydrating reactions, utilizing strong acids [25,26]. However, though the use of milder reagents, particularly Lewis acids [27], inorganic clay [28] or mineral acids [29] has improved both the yield and purity of this reaction. The imidazole family of compounds plays an important role in biological and chemical systems. A variety of drugs contain an imidazole ring, and include the antifungal azoles, inhibitory activity, nitro imidazole and the sedative, midazolam [30,31]. Benzimidazoles derivatives have been chosen as target molecules; benzimidazole derivatives have several medicinal uses, such as antivirals, anticancer, antihypertensive, antihistamines and antiulcer [32].

The present study reports a method for achieving benzimidazole Schiff base systems formed by reaction 2-(4-Aminophenyl)-1*H*-benzimidazole [33] and 3-hydroxybenzaldehyde, [34,35] 4-hydroxybenzaldehyde. Herein, we reported the synthesis of aldehydes and its Schiff base as a new template. (Fig. 1), The aldehydes are then reacted with 2-(4-Aminophenyl)-1*H*-benzimidazole to afford the corresponding benzimidazole Schiff bases [34,38].

2. EXPERIMENTAL SECTION

2.1. Materials and Methods. All the chemicals were purchased from Aldrich. 2-(4-Aminophenyl)-1*H*-benzimidazole was prepared according to literature procedures [33]. Melting points were measured using an Optimelt Automated Melting Point System (Digital Image Processing Technology) SRS apparatus. Elemental analyses (C,H,N) were performed using a Leco, CHNS-932 model analyzer. ^{1}H NMR spectra were recorded at room temperature with a Varian, 400 MHz spectrometer using TMS as

an internal standard. FT-IR spectra were recorded with a Perkin-Elmer Spectrum 100 with Universal ATR Polarization Accessory. The pH values were measured with a WTW pH 537 pH meters.

2.2. Synthesis of 2-(4-Aminophenyl)-1H-benzimidazole(4-APbzlH). The 2-(4-Aminophenyl)-1*H*-benzimidazole (4-APbzl*H*) was prepared according to the literature method [33].

2.3. Synthesis of 2-((4-Aminophenyl)-1H-benzimidazole)-N-(3-hydroxybenzylidine) (3SAPbz1H) and 2-((4-Aminophenyl)-1H-benzimidazole)-N-(4-hydroxybenzylidine) (4SAPbz1H). The Schiff base, 3SAPbz1*H* and 4SAPbz1*H* were obtained as a solid by refluxing a mixture of 4-APbzl*H* (5.0 g 24 mmol) and 3-hydroxybenzaldehyde (3.54 mL; 29 mmol) or 4-hydroxybenzaldehyde (3.54 mL; 29 mmol) in ethanol (35 mL) for about 6 h, followed by evaporation of the solvent to a small volume. The solid was washed with ether and recrystallised from ethanol and dried in a vacuum. **(3SAPbz1*H*)** Yield: (65%); m.p.: 285 °C; The CHN analyses of the compound corresponded to the molecular formula $C_{20}H_{15}N_3O$. Elemental analysis Found: C, 76.63; H, 4.84; N, 13.43%). Calc.: C, 76.66; H, 4.82; N, 13.41; %). FT-IR(cm^{-1}): 2952(OH), 2616 (NH), 1702 (CH=N), 1609 (C=C), 1556 (C=N), 1270 (C-$O_{Phenolic}$). ^{1}H NMR (400 MHz, DMSO-d_6) (δ: ppm): 12.91 (s, H, NH), 12.47 (s, H, OH), 9.08 (s, H, CH=N), 8.30-8.28 (d, 2H, CH_{arom}), 7.11 (t, H, CH_{arom}), 7.87 (t, H, $CH_{arom.}$),7.61-7.63 (d, 2H, $CH_{arom.}$), 6.69-6.67 (d, 2H, $CH_{arom.}$), 7.43-7.71 (m, 4H, $CH_{arom.}$). ^{13}C NMR (100 MHz, DMSO–d_6, 25°C) (δ: ppm): 191.36, 161.39, 153.59, 153.05, 151.60, 151.04, 132.57, 131.38, 128.22, 122.01, 117.69, 116.19, 114.01. **(4SAPbz1*H*)** Yield: (70%); m.p.:305 °C; The CHN analyses of the compound corresponded to the molecular formula $C_{20}H_{15}N_3O$, Found: C, 76.64; H, 4.84; N, 13.43%). Calc.: C, 76.66; H, 4.82; N, 13.41; %). FT-IR(cm^{-1}): 3055 (OH), 3320 (NH), 1673 (CH=N), 1600 (C=C), 1538 (C=N), 1281 (C-$O_{Phenolic}$). ^{1}H NMR (400 MHz, DMSO-d_6): (δ: ppm): 10.09 (s, H, NH), 9.76 (s, H, OH), 8.39 (s, H, CH=N), 8.20 (dd, 2H, CH_{arom}), 8.18 (dd, 2H, CH_{arom}), 7.74-7.72 (d, 2H, $CH_{arom.}$), 7.80 (t, H, $CH_{arom.}$), 7.20 (t, H, $CH_{arom.}$), 6.91-6.89 (d, 2H, $CH_{arom.}$), 6.64-6.62 (d, 2H, $CH_{arom.}$). ^{13}C NMR (100 MHz, DMSO–d_6, 25°C) (δ: ppm): 185,17, 165,87, 158.06, 151.17, 152.18, 154.27, 155.18, 138.57, 133.48, 130.12, 129.27, 123.69, 119.14, 117.01, 112.57.

2.4. Determination of Benzimidazole Schiff base Derivatives on Human Cancerous Cell Lines. (*Cell Culture and Transfection).* The human cervical cancer cell line, HeLa (CCL-2™) and colon cancer cell line, SW-620 (CCL-227™) were provided from ATCC (American Type Culture Collection, ATCC, Rockville, MD, USA). Cell lines were routinely cultured in Eagle's Minimum Essential Medium (EMEM) and Leibovitz's L-15 Medium, respectively supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin, and incubated at 37 °C in a humidified atmosphere containing 5% CO_2 or 100% air. The grown cells were harvested by trypsinization and serially subculture. The growth media were also used for dilution of benzimidazole Schiff base derivatives.

2.5. Determination the Effects of benzimidazole Schiff base derivatives on Cell proliferation of HeLa and SW-620 Cell lines. The human cervical and colon cancer lines HeLa and SW-620 were seeded into 96-well plates as 6 replicates to determine effects of benzimidazole Schiff base derivatives on proliferation of cancer cells and IC_{50} value (Concentration of molecules that inhibits half of the viability of cancer cell). 4 x10^4 cells were seeded into96-well plate and incubated for 24 h at 37 °C in 5% CO_2/100% air. Following one-night incubation, cells were transfected with various concentrations of benzimidazole Schiff base derivatives, ranging from 1 µM to 200 µM. Differences in cell growth was monitored by alamar blue method in which conversion of resazurin (blue color) to resorufin (pink color) measured by spectrophotometrically.

3. RESULTS SECTION

2-(4-Aminophenyl)-1*H*-benzimidazole (4-APbzlH) [33], was prepared by the reaction of 1,2-phenylendiamin with 4-aminobenzoic acid (Fig. 1). The structural formulate of the 2-(4-aminophenyl)-1*H*-benzimidazole (4-APbzlH) was verified by elemental analysis, ^{1}H NMR and FT-IR [33]. Condensation of 2-((4-Aminophenyl)-1*H*-benzimidazole)-N-(3-hydroxybenzylidine) (3SAPbz1*H*) and 2-((4-Aminophenyl)-1*H*-benzimidazole)-N-(4-hydroxybenzylidine) (4SAPbz1*H*) with 3-aminophenol and 4-aminophenol readily furnishes the corresponding "benzimidazole-Schiff bases" [34-38] (Fig.1).

These compounds were characterized by their elemental analysis, ^{1}H NMR, ^{13}C NMR and FT-IR [34-43]. As a result of replacement of the carbonyl by the imines groups the band at 1665 cm^{-1} for the C=O stretch in the FT-IR spectrum disappeared, and appeared band at 1702-1673 cm^{-1} for azomethine C=N group (3SAPbz1*H*) and (3SAPbz1*H*) respectively, In the ligands2-((4-Aminophenyl)-1*H*-benzimidazole)-N-(3-hydroxybenzylidine) (3SAPbz1*H*) and 2-((4-Aminophenyl)-1*H*-benzimidazole)-N-(4-hydroxybenzylidine) (4SAPbz1*H*)the band at 2952, 3055 cm^{-1} can be assigned to the -OH group vibrations, respectively [34,39].To identify the structures of the (3SAPbz1*H*) and (4SAPbz1*H*) , the ^{1}H NMR spectra were recorded in DMSO-d_6. ^{1}H NMR spectra were also in good correlation with the structures of the synthesized compounds. The ^{1}H NMR spectrum signals of ligands (3SAPbz1*H*) and (4SAPbz1*H*) at δ (ppm), 12.91-10.09 ppm and 12.47-9.76, correspond to the –NH and -OH proton resonances, respectively.

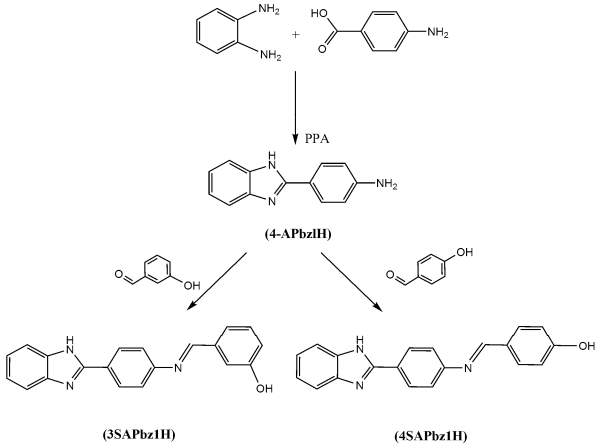

Figure 1. Proposed structures of the benzimidazole Schiff bases.

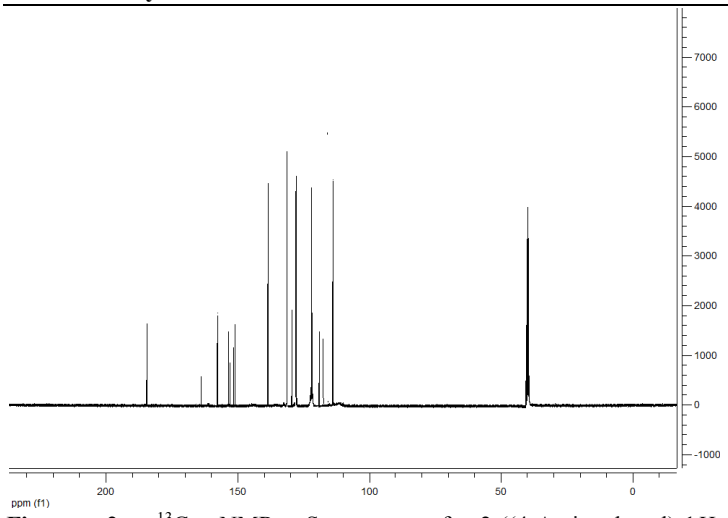

Figure 2. ^{13}C NMR Spectrum of 2-((4-Aminophenyl)-1*H*-benzimidazole)-N-(3-hydroxybenzylidine) **(3SAPbz1*H*).**

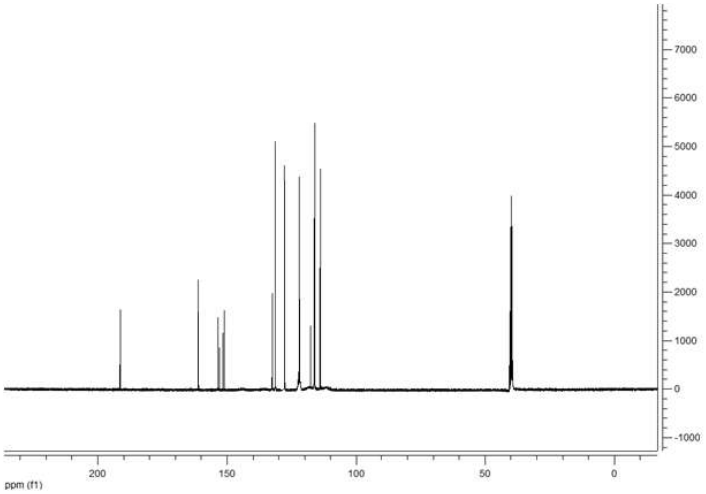

Figure 3. ^{13}C NMR Spectrum of 2-((4-Aminophenyl)-1*H*-benzimidazole)-N-(4-hydroxybenzylidine) **(4SAPbz1*H*).**

The signal for HC=O group disappeared, and the signal at higher field for the -CH=N and –OH proton in the ^{1}H NMR spectrum. The ^{13}C NMR spectrum signals of ligands results (Fig. 2 and Fig. 3) are in a good agreement with the proposed formula of the ligands. The "benzimidazole Schiff bases" prepared in this way is obtained in nearly quantitative yield and is of high purity [34-43].

Alamar blue assay [44] was conducted to investigate cytotoxic effect of benzimidazole Schiff base derivatives on HeLa and SW-620 cell lines. As shown in Fig.5, benzimidazole Schiff base dose dependently inhibited proliferation of HeLa cells while there is no cytotoxicity was observed on colon cancer cell line, SW-620.4-APbzlHslightly inhibited HeLa cells with IC50 of 230 µM while 4SAPbz1Hand 3SAPbz1*H* significantly inhibited proliferation of HeLa cells with IC50 of 110.6 µM and 101.3 µM, respectively.

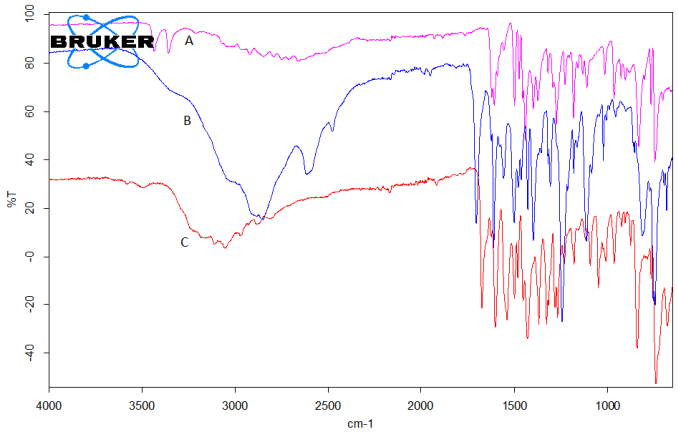

Figure 4. FT-IR spectra of **A***2-(4-Aminophenyl)-1H-benzimidazole**(4-APbzlH))*, **B***2-((4-Aminophenyl)-1H-benzimidazole)-N-(3-hydroxybenzylidine)* ***(3SAPbz1H)***, **C***2-((4-Aminophenyl)-1H-benzimidazole)-N-(4-hydroxybenzylidine)* ***(4SAPbz1H).***

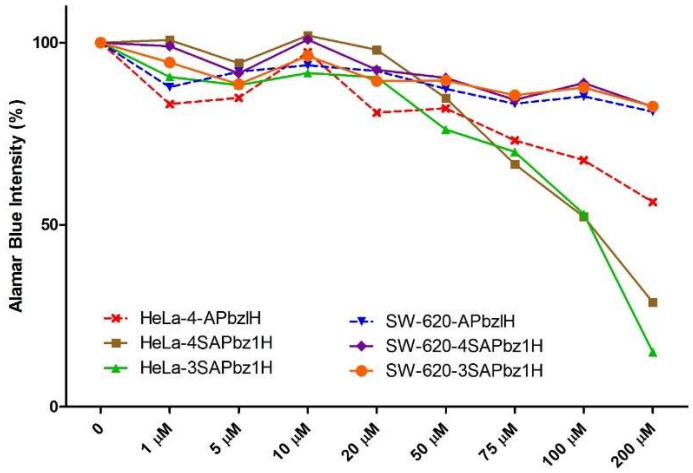

Figure 5. Effects of benzimidazole Schiff base derivative son viability and proliferation of HeLa and SW-620 cells. Viability of cervical and colon cancer cells after treatment with different concentration of benzimidazole Schiff base derivatives measured by Alamar Blue assay. Benzimidazole Schiff base derivatives dose-dependently inhibit viability of HeLa cells while no cytotoxicity was observed with SW-620cell. IC_{50} value of benzimidazole Schiff base derivative son Hela cells was determined by using sigmoidal plot of Log concentration vs. cell viability (%) with Graph Pad Prism 5.0.

4. CONCLUSIONS

In this study, 2-((4-Aminophenyl)-1H-benzimidazole)-N-(3-hydroxybenzylidine) (3SAPbz1H) and 2-((4-Aminophenyl)-1H-benzimidazole)-N-(4-hydroxybenzylidine) (4SAPbz1H) were synthesized by the reaction of 2-(4-Aminophenyl)-1H-benzimidazole (4-APbzlH), 3-hydoxybenzaldehyde and 4-hydoxybenzaldehyde according to the literature. Compounds 3SAPbz1H and 4-SAPbzlHwere originally synthesized. The structures of the compounds were identified by FT-IR, ^{1}H NMR, ^{13}C NMR and elemental analysis. Benzimidazole Schiff base derivatives selectively inhibited proliferation of human cancerous cell lines in which proliferation of HeLa cells significantly inhibited while there is no cytotoxicity was observed on SW-620 cells.

5. REFERENCES

[1] Dey M., Deb K., Dhar S.S., $VO(acac)_2$ catalyzed condensation of o-phenylenediamine with aromatic carboxylic acids/aldehydes under microwave radiation affording benzimidazoles, *Chinese Chem. Lett*., 22, 296–299, **2011**.

[2] Maiti D.K., Halder S., Pandit P., Chatterjee N., Joarder D., Pramanik N., Saima Y., Patra A., Maiti P.K., Synthesis of glycal-based chiral benzimidazoles by $VO(acac)_2$-$CeCl_3$ combo catalyst and their self-aggregated nanostructure, *Materials J. Org. Chem*., 74, 8086–8097, **2009**.

[3] Rehman M., Imran M., Arif M., Farooq M., Metal-based antimicrobial agents: synthesis, characterization and biological studies of mannich base derivatives of benzimidazole and their metal complexes, *Sci. J. Chem*., 1, (5), 56-66, **2013**.

[4] Huang X.C., Zhang J.P., Chen X.M., A new route to supramolecular isomers via molecular templating: nanosized molecular polygons of copper(I) 2-methylimidazolates, *J. Am. Chem. Soc*., 126, 13218-13219, **2004**.

[5] Cardwell T.J., Edwards A.J., Hartshorn R.M., Holmes R.J., McFadyen W.D., Structural and electrochemical studies of some cobalt(III) complexes of 2-aminomethylbenzimidazole and 2-(N-methylaminomethyl) benzimidazole, *Aust. J. Chem.,* 50, 1009-1015, **1997**.

[6] Tong Y.P., Zheng S.L., Synthesis, structure, spectroscopic properties, DFT and TDDFT investigations of copper(II) complex with 2-(2-hydroxyphenyl)benzimidazole, *J. Mol. Struct*., 841, 34-40, **2007**.

[7] Tong Y.P., Zheng S.L., Chen X.M., Syntheses, structures, photoluminescence and theoretical studies of two dimeric Zn(II) compounds with aromatic-*N*,*O*-chelate phenolic ligands, *J. Mol. Struct*., 826, 104-112, **2007**.

[8] Keri R.S., Hosamani K.M., Reddy H.R.S., Shingalapur R.V., Wellsdawson heteropolyacid: an efficient recyclable catalyst for the synthesis of benzimidazoles under microwave condition, *Catal. Lett*., 131, 552-559, **2009**.

[9] Fonseca T., Gigante B., Gilchrist T.L., A short synthesis of phenanthro[2,3-*d*]imidazoles from dehydroabietic acid. Application of the methodology as a convenient route to benzimidazoles, *Tetrahedron*, 57, 1793-1799, **2001**.

[10] Koc Z.E., Bingol H., Saf A.O., Torlak E., Coskun A., Synthesis of novel tripodal-benzimidazole from 2,4,6-tris(p-formylphenoxy)-1,3,5-triazine, *J. Hazard. Mater*., 183, 251-255, **2010**.

[11] Tunal Y., Karaca H., Isıkdag I., Özkay Y., Antimicrobial activity of a new series of benzimidazole derivatives, *Arch Pharm Res*., 34, 1427-1435, **2011**.

[12] Pabba C., Wang H.J., Mulligan S.R., Chen Z.J., Stark T.M., Gregg B.T., Microwave-assisted synthesis of 1-aryl-1*H*-indazoles via one-pot two-step Cu-catalyzed intramolecular-*N*-arylation of arylhydrazones, *Tetrahedron Lett*., 46, 7553-7557, **2005**.

[13] Denny W.A., Rewcastle G.W., Baguley B.C., Potential antitumor agents. 59. Structure-activity relationships for 2-phenylbenzimidazole-4-carboxamides, a new class of minimal DNA-intercalating agents which may not act via topoisomerase II*, J. Med. Chem*. 33, 814-819, **1990**.

[14] Villanueva J.P., Santos R., Campos A.H., Giulianotti M.A., Castillo R., Franco J.L.M., Structure–activity relationships of benzimidazole derivatives as antiparasitic agents, *Med. Chem. Commun*., 2, 44-49, **2011**.

[15] Al-Mohammed N.N., Alias Y., Abdullah Z., Shakir R.M., Taha E.M., Hamid A.A., Synthesis and antibacterial evaluation of some novel imidazole and benzimidazole sulfonamides, *Molecules,* 18, 11978-11995, **2013**.

[16] Murugesan S., Sathiyamoorthy S., Synthesis, spectral analysis and biological activity of mannich bases of 2-substituted benzimidazole, *J. Pharm. Res.,* 4, 2679-2681, **2011**.

[17] Patel K.V., Arun S., Synthesis, Characterization and chelating properties of Benzimidazole-salicyclic acid combined molecule, *Eur. J. Chem.,* 6, 281-288, **2009**.

[18] Reddy B.A., Synthesis, Characterization and biological evaluation of 1,2-disubstituted benzimidazole derivatives using Mannich bases, *Eur. J. Chem.,* 7, 222-226, **2010**.

[19] Koc Z.E., Aladag M.O., Uysal A., Synthesis of novel dopamine derived multidirectional ligands from cyanuric chloride: structural and antimicrobial studies, *EXCLI Journal,* 12, 396-403, **2013**.

[20] Jesudason E.P., Sridhar S.K., Malar E.J.P., Shanmugapandiyan P., Inayathullah M., Arul V., Selvaraj D., Jayakumar R., Synthesis, pharmacological screening, quantum chemical and *in vitro* permeability studies of *N*-Mannich bases of benzimidazoles through bovine cornea, *Eur. J. Med. Chem.,* 44, 2307-2312, **2009**.

[21] Arif M., Qurashi M.M.R., Shad M.A., Metal-based antibacterial agents: synthesis, characterization, and in vitro biological evaluation of cefixime-derived Schiff bases and their complexes with Zn(II), Cu(II), Ni(II), and Co(II), *J. Coord. Chem.,* 64, 1914-1930, **2011**.

[22] Pragathi J., Mounika K., Padmaja M., Lakshmi M., Gyanakumari C., Synthesis, characterization and antibacterial studies of some transition metal complexes of a Schiff base derived from 2-(aminomethyl)-benzimidazole and thiophene-2-carbaxaldehyde, *E. J. Chem.,* 8, 1662-1669, **2011**.

[23] Chhonker Y.S., Veenu B., Hasim S.R., Kaushik N., Kumar D., Kumar P., Synthesis and pharmacological evaluation of some new 2-phenyl benzimidazoles derivatives and their Schiff's bases, *E. J. Chem,.* 6, S342-S346, **2009**.

[24] Hranjec M., Starčević K., Pavelić S.K., Lučin P., Pavelić K., Zamola G.K., Marijana H., Kristina S., Sandra K., Synthesis, spectroscopic characterization and antiproliferative evaluation *in vitro* of novel Schiff bases related to benzimidazoles, *Eur. J. Med. Chem.,* 46, 2274-2279, **2011**.

[25] Kus C., Kılcıgil A., Tunçbilek G., Altanlar M., Çoban N. T., Can-Eke B., Can M., Synthesis, antimicrobial and antioxidant activities of some benzimidazole derivatives, *Lett. Drug Des. & Discov.,* 6, 1-5, **2009**.

[26] Bahrami K., Khodaei M.M., Naali F., Mild and highly efficient method for the synthesis of 2-arylbenzimidazoles and 2-arylbenzothiazoles, *J. Org. Chem.,* 73, 6835-6837, **2008**.

[27] Nagawade R.R., Shinde D.B., $BF_3.OEt_2$ promoted solvent free synthesis of benzimidazole derivatives, *Chinese Chem. Lett.,* 17, 453-456, **2006**.

[28] Bougrin K., Loupy A., Petit A., Daou,B., Soufiaoui M., Nouvelle voie de synthèse des 2-trifluorométhylarylimidazoles sur montmorillonite K10 en 'milieu sec' sous micro-onde Original, *Tetrahhedron,* 57, 163-168, **2001**.

[29] Rastogi R., Sharma S., 2-Aminobenzimidazole in organic syntheses, *Synthesis,* 11, 86-882, **1983**.

[30] Sivan S.K., Vangala R., Manga V., Molecular docking guided structure based design of symmetrical N,N0-disubstituted urea/thiourea as HIV-1 gp120–CD4 binding inhibitors, *Bioorg. Med. Chem.,* 21, 4591–4599, **2013**.

[31] Arya R., Sharma A.K., Bioremediation of Carbendazim by Streptomyces albogriseolus, *Biointerface Res. Appl. Chem.,* 4, 804-807, **2014.**

[32] Gorepatil P.B., Mane Y.D., Ingle V.S., Zirconyl (IV) nitrate as efficient and reusable solid lewis acid catalyst for the synthesis of benzimidazole derivatives, *Hindawi Pub. Corp. J. Chemistry*, 2013, 1-7, **2013**.

[33] Hein D.W., Alheim R.J., Leavitt J.J., The use of polyphosphoric acid in the synthesis of &aryl- and 2-alkyl-substituted benzimidazoles, benzoxazoles and benzothiazoles, *J. Am. Chem. Soc.,* 79, 427–429, **1957**.

[34] Chandrakalaa M., Sheshadria B.S., Nanjegowdaa N.M., Murthya K.G.S., Nagasundarab K.R., Synthesis and spectral studies of 2-((4-aminophenyl)-1H-benzimidazole)-n-(3-hydroxybenzylidine) and its reaction with divalent Zn, Cd and Hg: crystal structure of the cadmium bromide complex, *J. Chem. Res.,* 576, 576–580, **2010**.

[35] Chandrakala M., NanjeGowda N.M., Murthya K.G.S., Nagasundarab K.R., Activation of –N=CH– bond in a Schiff base by divalent nickel monitored by NMR evidence, *Magn. Reson. Chem.,* 50, 335–340, **2012**.

[36] Desai N.C., Dodiya A.M., Makwana A.H., Antimicrobial screening of novel synthesized benzimidazole nucleus containing 4-oxo-thiazolidine derivatives, *Med. Chem. Res.,* 21, 2320–2328, **2012**.

[37] Adnan S., Hassan K., Thamer H., Synthesis and Identification some of heterocyclic compounds from imidazole derivatives, *Iraqi Nati. J. Chem.,* 51, 327-337, **2013**.

[38] Aljahdali M., Synthesis, characterization and equilibrium studies of some potential antimicrobial and antitumor complexes of Cu(II), Ni(II), Zn(II) and Cd(II) ions involving 2-aminomethylbenzimidazole and glycine, *Spectrochim. Acta A.,* 112, 364–376, **2013**.

[39] Abd El-All A.S., Ragab F.A.F., Magd El-Din A.A., Abdalla M.M., El-Hefnawi M.M., El-Rashedy A.A., Design, Synthesis and anticancer evaluation of some selected Schiff bases derived from benzimidazole derivative, *Global J. Pharm*., 7, 143-152, **2013**.

[40] Zawadzka A., Karakas A., Płóciennik P., Szatkowski J., Łukasiak Z., Kapceoglu A., Ceylan Y., Sahraoui B., Optical and structural characterization of thin films containing metallophthalocyanine chlorides, *Dyes and Pigments,* 112, 116–126, **2015**.

[41] Koc Z.E., Uysal A., Investigation of novel monopodal and dipodal oxy-Schiff base triazine from cyanuric chloride: Structural and antimicrobial studies, *Sci. Pure Appl. Chem.,* 53, 111–115, **2016**.

[42] Ozaytekin I., Karatas I., Synthesis and characterization of thermally stable polymers (polybenzimidazoles), *J. Appl. Polym. Sci.,* 109, 1861-1870, **2008**.

[43] Uysal S., Koc Z.E., Celikbilek S., Synthesis of Star-Shaped Macromolecular Schiff Base Complexes Having Melamine Cores and Their Magnetic and Thermal Behaviors*, Synth. Commun.,* 42, 1033-1044, **2012**.

[44] Hamid R., Rotshteyn Y., Rabadi L., Parikh R., Bullock P., Comparison of Alamar Blue and MTT assays for high through-put screening, *Toxicol in vitro,* 18, 703-710, **2004**.

6. ACKNOWLEDGEMENTS

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7. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest. Compliance with ethical standards.