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Design, Synthesis and Anticancer Evaluation of Some Selected Schiff Bases Derived from Benzimidazole Derivative

[1]Amira S. Abd El-All, [2]Fatma A.F. Ragab, [1]Asmaa A. Magd El-Din,
[3]Mohamed M. Abdalla, [4]Mahmoude M. El-Hefnawi and [1]Ahmed A. El-Rashedy

[1]Department of Natural and Microbial Product,
Research Division of Pharmaceutical and Drug Industries, National Research Centre, Giza, Egypt
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Cairo University, Egypt
[3]Research Unit, Saco Pharm. Co. 6 October city 11632, Egypt
[4]Biomedical Informatics and Chemoinformatics Group,
Center of Excellence for Advanced Sciences, National Research Centre, Dokki, Cairo 12622, Egypt

Abstract: This research deals with an efficient green chemistry for synthesizing a series of Schiff bases 3a-m incorporating 4-1*H*-benzo[*d*] imidazole moiety, by microwave technique and heating conventional procedures which are used for their preparation. The newly synthesized Schiff bases are obtained by the reaction of 4-(1*H*-benzo[*d*]imidazol-2yl) aniline 1 with a series of different aromatic aldehydes 2a-m. This work aims to make a comparison between conventional and microwave irradiation methods. The design of selected newly Schiff bases is defined by molecular modeling. The evaluation of anticancer activities of synthesized Schiff bases are investigated against human cancer cell lines; Co rectal cancer cell line HCT116, human liver cancer cell line HepG2 and human ovarian cancer cell line A2780, the results show that compounds 3c,3f,3g have more activity than the comparing drug CK0106023. All the synthesized compounds are characterized by their elemental analysis, IR, ^{1}H-NMR and Mass spectral studies.

Key words: Schiff Bases · Conventional Heating Procedure · Microwave Irradiation · Anticancer Activity · Molecular Modeling

INTRODUCTION

Literature survey shows that benzimidazole derivatives play a vital role in biological activities such as anti-diabetic [1], antimicrobial [2,3], antifungal [4], antiviral [5, 6], antispasmodic [7], anticancer [8, 9], anti-tumor [10], anti-hepatitis-C-virus [11], kinase inhibitor [12,13], analgesic [14], antipsychotic [15], antidepressant [16], anti-anxiety [17], antihypertensive [18], antiulcer [19] and anti-inflammatory [20]. On the other hand Schiff bases have an efficient antimicrobial [21] and antifungal activities [22]. Benzimidazoles can be prepared by the acid catalyzed reaction of aldehyde or ketone with amines [23]. Recent advances in technology considers microwave irradiation energy as the most efficient means of heating reactions for chemical transformations that can be accomplished in a minutes. Microwave irradiation assists organic synthesis (MAOS) [24]. MAOS has emerged as frontier in pharmaceutical research for synthesis of new drugs. MAOS not only help in implementing green chemistry but also led to progress in organic synthesis. Microwave irradiation is well known to promote the synthesis of a variety of organic compounds where the chemical reactions are accelerated due to the selective absorption of microwave irradiation by polar molecules.

In this work *o*-phenylenediamine is treated with *p*-amino benzoic acid in the presence of *o*-phosphoric acid as a catalyst to yield 4-(1*H*-benzo [*d*] imidazol-2yl) aniline 1. Schiff bases 3a-m bearing 4-1*H*-benzo [*d*] imidazole moiety are synthesized by the reaction of compound 1 with a series of different aromatic aldehydes (2-chlorobenzaldehyde, 3-chlorobenzaldehyde,

Corresponding Author: Amira S. Abd El-All. Department of Natural and Microbial Product,
Research Division of Pharmaceutical and Drug Industries, National Research Centre,
Dokki, Giza 12622, Egypt.

4-chlorobenzaldehyde, 2, 4-dichloro benzaldehyde, 2-bromo benzaldehyde, 4-floro benzaldehyde, 4-nitro benzaldehyde, 2-methoxy benzaldehyde, 2,4-dimethoy benzaldehyde, 2,5-dimethoybenzaldehyde, 3-methoxy 4-hydroxy benzaldhyde (vanillin), 3,4,5-trimethoy benzaldehyde and 4-(dimethylamino)benzaldehyde) 2a-m.

The preparations of the newly synthesized Schiff bases proceed via both conventional and microwave irradiation methods. The aim of using microwave irradiation technique is to study the efficiency of Green Chemistry for synthesizing Schiff bases 3a-m, also decreasing the reaction time and increasing the yield. Anticancer evaluation *in vitro* have been screened for the newly synthesized products against human cancer cell lines ; [Co rectal cancer cell line HCT116, human liver cancer cell line HepG2 and human Ovarian cancer cell line A2780], the results showed that the compounds 3f, 3c, 3g are more potent comparing with the standard drug CK0106023. The molecular docking is performed and analyzed with Molecular Operating Environment program (MOE). The synthesized compounds are characterized by their elemental analysis, IR, ^{1}H-NMR and Mass spectral data.

MATERIALS AND METHODS

Experimental and Procedures: The microwave irradiation is carried out on Domestic Microwave Oven (model : MW83Z-G). All melting points are uncorrected and are measured on a Gallenkamp melting point apparatus. The IR spectra are recorded on a Perkin Elmer 1650 FT-IR instrument using (KBr, ν /cm^{-1}). ^{1}H NMR spectra are recorded on a Varian spectrometer 500 MHz using DMSO-d_6 as a solvent and TMS as an internal standard. Chemical shifts are reported in δ ppm. Mass spectra are recorded on a Varian MAT 112 spectrometer at 70 eV. Elemental analyses are carried out at The Micro analytical Data Center at Cairo University, Egypt. Fetal bovine serum (FBS) and L-glutamine, are obtained from Gibco Invitrogen Company (Scotland, UK). Dulbecco's modified Eagle's (DMEM) medium is provided from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), CK0106023, penicillin, streptomycin and sulforhodamine B (SRB) are obtained from Sigma Chemical Company. (Saint Louis, MO, USA)

Synthesis of 4-(1*H*-benzo[*d*]imidazol-2-yl) aniline 1 [25, 26]: A mixture of *o*-phenylenediamine (0.1mol) and *p*-amino benzoic acid (0.1mol) in 25 ml *o*-phosphoric acid, the reaction mixture was refluxed for 4 hrs allow to cool then poured over cold water and 10% ammonium hydroxide solution was added slowly to the reaction mixture with constant stirring until just alkaline. The crude product was filtered off, washed with ice-cold water, the resulting product was re-crystallized from ethanol as buff powder, yield 89%, m.p. 209-211°C. ^{1}H-NMR (500 MHz, DMSO-d_6) (δ- ppm): 6.32-6.33 (m, 2H, NH_2, exchangeable with D_2O), 6.43-7.79 (m, 8H, aromatic); 12.41 (b, 1H, NH benzimidazole, exchangeable with D_2O). IR (KBr, ν /cm^{-1}): ν (NH,) 3153, ν (NH_2) 3037 s, ν (C=N ring) 1585. Anal. calcd. for $C_{13}H_{11}N_3$(%) M. W. 209.25: C, 74,62; H, 5.30; N, 20.08. Found: C, 74.84; H, 5.25; N, 20.00.

General Procedure for Preparation of Compounds 3a-m

Method A: Conventional *Heating Procedure:* A mixture of equimolar quantities (0.01mol) of aromatic aldehydes [2-chlorobenzaldehyde, 3-chlorobenzaldehyde, 4-chlorobenzaldehyde, 2,4-dichlorobenzaldehyde, 2-bromobenzaldehyde, 4-florobenzaldehyde, 4-nitro benzaldehyde, 2-methoxybenzaldehyde, 2,4-dimethoy benzaldehyde, 2,5-dimethoybenzaldehyde, 3-methoxy4-hydroxybenzaldhyde (vanillin), 3,4,5-trimethoy benzaldehyde, 4-(dimethylamino)benzaldehyde] and 4-(1*H*-benzo [*d*] imidazol-2-yl) (0.01mol) in acetic acid (25ml) was refluxed for appropriate time with continuous stirring. Progress of the reaction was monitored by TLC. After completion of the reaction the reaction mixture was cooled. The heavy precipitate thus obtained was filtered off and purified by crystallization with ethanol (Table 1)

Method B: Microwave Irradiation Procedure: The equimolar(1:1)ratio of4-(1*H*-benzo[*d*]imidazol-2-yl)aniline and a series of heterocyclic aromatic aldehydes namely, (2-chlorobenzaldehyde, 3-chlorobenzaldehyde, 4-chloro benzaldehyde, 2,4-dichlorobenzaldehyde, 2-bromo benzaldehyde, 4-florobenzaldehyde, 4-nitrobenzaldehyde, 2-methoxybenzaldehyde, 2,4-dimethoybenzaldehyde, 2,5-dimethoy benzaldehyde, 3-methoxy4-hydroxybenzaldhyde (vanillin), 3,4,5-trimethoybenzaldehyde and 4-(dimethy lamino) benzaldehyde) was mixed thoroughly in the grinder. The reaction mixture was then irradiated in microwave oven and taking dimethyl formamide (DMF) as a solvent. The reaction was completed in a short time (15-48 min) with higher yield. The resulting products were cooled, then poured into crushed ice, filtered off, washed, dried and re-crystallized from ethanol (Table 1).

(*E*)-4-(1*h*-benzo [*D*] imidazol-2-yl)-*N*- (2-chlorobenzy lidene) aniline *3a:* Pale green powder, m. p. 289-290°C. ^{1}H-NMR (500 MHz, DMSO-d_6) (δ- ppm): 6.61-7.58 (m, 8H, aromatic); 7.70 (m, 2H, H5 and H6 benzimidazole); 7.72 (m, 2H, H4 and H7 benzimidazole); 10.31 (s, 1H, azomethine proton); 12.71(br, 1H, NH exchangeable D_2O). IR (KBr, ν /cm^{-1}): ν (NH) 3317 br. ν (C=N azomethine) 1677, ν (C=N benzimidazole ring) 1606, ν (C=C) 1545, ν (C-Cl) 528. Anal. calcd. for $C_{20}H_{14}ClN_3$ (%) M.W. 331.80: C, 72.40; H, 4.25; N, 12.66. Found: C, 72.62; H, 4.07; N, 12.47.

(*E*)-4-(1*H*-benzo[d]imidazol-2-yl)-*N*- (3-chloro benzylidene) aniline 3b: Pale blue powder, m. p. 295-297°C. ^{1}H-NMR (500 MHz, DMSO-d_6) (δ- ppm): 8.06 (m, 2H, H5 and H6 benzimidazole); 7.71 (m, 2H, H4 and H7 benzimidazole); 7.08-7.51 (m, 8H, aromatic); 10.16 (s, 1H, azomethine proton); 12.79 (br,1H, NH exchangeable D_2O). MS: m/z 331 (M, 18 %), 249 (m_1, 75%), 208 (m_2, 100%). IR (KBr, ν /cm^{-1}): ν (NH) 3317 br. ν (C=N azomethine) 1676, ν (C=N benzimidazole ring) 1608, ν (C=C) 1544, ν (C-Cl) 519. Anal. calcd. for $C_{20}H_{14}ClN_3$ (%) M.W. 331.80: C, 72.40; H, 4.25; N, 12.66. Found: C, 72.65; H, 4.07; N, 12.45.

(*E*)-4-(1*H*-benzo[d]imidazol-2-yl)-*N*- (4-chlorobenzylidene) aniline 3c: Pale blue powder, m. p. 215-217 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) (δ- ppm): 6.61-7.59 (m, 8H, aromatic); 7.70 (m, 2H, H4 and H7 benzimidazole); 8.04 (m, 2H, H5 and H6 benzimidazole); 10.16 (br, 1H, azomethine proton); 12.48 (s, 1H, NH exchangeable D_2O). MS: m/z 331 (M, 0.70 %), 228 (m1, 86%), 209 (m2, 0.99%), 193 (m3, 17.57%), 63 (m_4, 100%). IR (Kbr, ν /cm^{-1}): ν (NH) 3312 br. ν (C=N azomethine) 1677, ν (C=N benzimidazole ring) 1604, ν (C-Cl) 525. Anal. calcd. for $C_{20}H_{14}ClN_3$ (%) M.W. 331.80: C, 72.40; H, 4.25; N, 12.66. Found: C, 72.60; H, 4.06; N, 12.49.

(*E*)-4-(1*H*-benzo [*d*] imidazol-2-yl)-*N*- (2,4-dichloro benzylidene) aniline 3d : Gray powder, m. p. > 300 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) (δ- ppm): 6.63-7.52 (m, 7H, aromatic); 7.71 (m, 2H, H4 and H7 benzimidazole); 8.06 (m, 2H, H5 and H6 benzimidazole); 10.16 (s, 1H, azomethine proton); 12.77 (br, 1H, NH exchangeable D_2O). MS: m/z 368 (M^{+2}, 35%), 299 (M^{+2} -2HCl, 15%), 291(m_1, 78%), 159 (m_2, 100%). IR (KBr, ν /cm^{-1}): ν (NH) 3310 br. ν (C=N azomethine) 1675, ν (C=N benzimidazole ring) 1604, ν (2C-Cl) 525, 1008. Anal. calcd. for $C_{20}H_{13}Cl_2N_3$ (%) M.W. 366.24: C, 65.59; H, 3.58; N, 11.47. Found: C, 65.77; H, 3.38; N, 11.42.

(*E*)-4-(1*H*-benzo[d]imidazol-2-yl)-*N*- (2-bromobenzylidene) aniline 3e: Beige powder, m. p. 258-260 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) (δ- ppm): 6.54-7.52 (m, 8H, aromatic); 7.70 (m, 2H, H4 and H7 benzimidazole); 8.06 (m, 2H, H5 and H6 benzimidazole); 10.15 (s, 1H, azomethine proton), 12.70 (br, 1H, NH exchangeable D_2O). IR (KBr, ν /cm^{-1}): ν (NH) 3313 br. ν (C=N azomethine) 1677, ν (C=N benzimidazole ring) 1605, ν (C-Br) 748. MS: m/z 374 (M^{-1}, 11.41%), 370(m_1, 43%), 209 (m_2, 73%), 169 (m_3, 100%) Anal. calcd. for $C_{20}H_{14}BrN_3$ (%) M.W. 375.04: C, 63.84; H, 3.75; N, 11.17. Found: C, 64.03; H, 3.57; N, 10.97.

(*E*)-4-(1*H*-benzo[d]imidazol-2-yl)-*N*-(4-florobenzylidene) aniline 3f: Coffee powder, m. p. 291-293°C. ^{1}H-NMR (500 MHz, DMSO-d_6): (δ- ppm) 7.07-7.70 (m, 8H, aromatic); 7.72 (m, 2H, H4 and H7 benzimidazole); 8.06 (m, 2H, H5 and H6 benzimidazole); l0.18 (s, 1H, azomethine proton), 12.77 (br, 1H, NH exchangeable D_2O). IR (KBr, ν /cm^{-1}): ν (NH) 3262 br. ν (C=N azomethine) 1673, ν (C=N benzimidazole ring) 1603, ν (C-F) 1226. MS: m/z 315 (M, 1.09%), 294(m_1, 18.9%), 271 (m_2, 15.15%), 208 (m_3, 6.12%), 165 (m_4, 13.46%). Anal. calcd. for $C_{20}H_{14}FN_3$ (%) M.W. 315.34: C, 76.18; H, 4.47; F, 6.02; N, 13.33. Found: C, 76.37; H, 4.27; F,5.90; N, 13.07.

(*E*)-4-(1*H*-benzo[d]imidazol-2-yl)-*N*-(4-nitrobenzylidene) aniline 3g: Yellow powder, m. p. 265-267 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) (δ- ppm): 6.62-7.69 (m, 8H, aromatic); 8.13 (m, 2H, H4 and H7 benzimidazole); 8.38 (m, 2H, H5 and H6 benzimidazole); l0.17 (s, 1H, azomethine proton), 13.31 (Br, 1H, NH exchangeable D_2O). IR (KBr, ν /cm^{-1}): ν (NH) 3263 br. ν (C=N azomethine) 1674, ν (C=N benzimidazole ring) 1604. MS: m/z 342 (M, 33.69%), 251 (m_1, 33.69%), 209 (m_2, 100%). Anal. calcd. for $C_{20}H_{14}N_4O_2$ (%) M.W. 342.35: C, 70.17; H, 4.12; N, 16.37. Found: C, 70.37; H, 3.97; N, 16.18.

(*E*)-4-(1*H*-Benzo[*d*]imidazol-2-yl)-*N*-(2-methoxy enzylidene) aniline *3h:* Pale blue powder, m. p. > 300 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) (δ- ppm): 3.99 (s, 3H, OCH_3); 6.61-7.57 (m, 8H, aromatic); 7.71 (m, 2H, H4 and H7 benzimidazole); 8.06 (m, 2H, H5 and H6 benzimidazole); l0.17 (s, 1H, azomethine proton), 12.75 (br, 1H, NH exchangeable D_2O). IR (KBr, ν /cm^{-1}): ν (NH) 3314 br. ν (C=N azomethine) 1677, ν (C=N benzimidazole ring) 1605. MS: m/z 329 (M^{+2}, 2 . 2%), 251 (m_1, 59%), 209 (m_2, 100%). Anal. calcd. for $C_{21}H_{17}N_3O$ (%) M.W. 327.38: C, 77.04; H, 5.23; N, 12.84. Found: C, 77.23; H, 5.05; N, 12.68.

(*E*)-4-(1*H*-benzo[d]imidazol-2-yl)-*N*-(2,4-dimethoxy benzylidene) aniline *3i:* Pale blue powder, m. p. 289-290 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) (δ- ppm): 4.39, 3.82 (2s, 6H, $2OCH_3$); 6.63-7.53 (m, 7H, aromatic); 7.70 (m, 2H, H4 and H7 benzimidazole); 8.06 (m, 2H, H5 and H6 benzimidazole); l0.16 (s, 1H, azomethine proton), 12.84 (br, 1H, NH exchangeable D_2O). IR (KBr, ν /cm^{-1}): ν (NH) 3267 br. ν (C=N azomethine) 1675, ν (C=N benzimidazole ring) 1605. MS: m/z 357 (8%), 250 (m_1, 100%), 209 (m_2, 100%). Anal. calcd. for $C_{22}H_{19}N_3O_2$ (%) M.W. 357.41: C, 73.93; H , 5.36; N, 11.76. Found: C, 74.13; H, 5.15; N, 11.58.

(*E*)-4-(1*H*-benzo[d]imidazol-2-yl)-*N*- (2,5-dimethoxy benzylidene) aniline *3j:* Pale blue powder, m. p. >300 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) (δ- ppm): 3.82, 4.44 (2s, 6H, $2OCH_3$), 6.62-7.52 (m, 7H, aromatic); 7.70 (m, 2H, H4 and H7 benzimidazole); 8.06 (m, 2H, H5 and H6 benzimidazole); l0.15 (s, 1H, azomethine proton), 12.77 (br, 1H, NH exchangeable D2O). IR (KBr, ν /cm^{-1}): ν (NH) 3310 br. ν (C=N azomethine) 1677, ν (C=N benzimidazole ring) 1605. MS: m/z 357 (M, 2.4%), 341(m_1, 4.48), 251 (m_2, 12.80%), 209 (m_3, 68%), 63(m_4, 100). Anal. calcd. for $C_{22}H_{19}N_3O_2$ (%) M.W. 357.41: C, 73.93; H, 5.36; N, 11.76. Found: C, 74.14; H, 5.16; N, 11.54.

(*E*)-4-(((4-(1*H*-benzo[d]imidazol-2-yl) phenyl) imino) methyl) -2-methoxyphenol *3k*: Buff powder, m. p. > 300 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) (δ- ppm): 3.85 (s, 3H, OCH_3); 7.13-7.70 (m, 7H, aromatic); 7.71 (m, 2H, H4 and H7 benzimidazole); 8.08 (m, 2H, H5 and H6 benzimidazole); 9.73 (s, 1H, OH exchangeable D_2O). l0.17 (s, 1H, azomethine proton), 12.86 (br, 1H, NH exchangeable D_2O); IR (KBr, ν /cm^{-1}): ν (OH-NH) 3265 br. ν (C=N azomethine) 1674, ν (C=N benzimidazole ring) 1605 MS: m/z 343 (M, 0.15%), 250 (m_1, 43%), 209 (m_2, 69%), 63 (m_3, 80). Anal. calcd. for $C_{21}H_{17}N_3O_2$ (%) M.W. 343.38: C, 73.45; H, 4.99; N, 12.24. Found: C, 73.67; H, 4.77; N, 12.05.

(*E*)-4-(1*H*-benzo[d]imidazol-2-yl)-*N*- (3,4,5-trimethoxy benzylidene)aniline *3l:* Gray powder, m. p. 294-296 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) (δ- ppm): 4.39, 3.82 (s, 9H, $3OCH_3$); 6.63-7.53 (m, 6H, aromatic); 7.70 (m, 2H, H4 and H7 benzimidazole); 8.06 (m, 2H, H5 and H6 benzimidazole); l0.16 (s, 1H, azomethine proton), 12.84 (br, 1H, NH exchangeable D_2O). IR (KBr, ν /cm^{-1}): ν (NH) 3262 br. ν (C=N azomethine) 1675, ν (C=N benzimidazole ring) 1604. MS: m/z 387 (M, 7%), 332.7(m_1, 12%), 341(m_2, 4.48), 266 (m_3, 8.80%), 101.3 (m_4, 100%). Anal. calcd. for $C_{23}H_{21}N_3O_3$ (%) M.W. 387.43: C, 71.30; H, 5.46; N, 10.85. Found: C, 71.52 ; H, 5.28; N, 10.63.

(*E*)-4-(((4-(1*H*-benzo[d]imidazol-2-yl) phenylimino) methyl)-*N*, *N*-dimethyaniline 3m : Yellow powder, m. p. >300 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) (δ- ppm): 2.47 (s, 6H, $2CH_3$), 6.60-7.57 (m, 8H, aromatic); 7.71 (m, 2H, H4 and H7 benzimidazole); 8.05 (m, 2H, H5 and H6 benzimidazole); l0.15 (s, 1H, azomethine proton), 12.79 (br, 1H, NH exchangeable D_2O). IR (KBr, ν /cm^{-1}): ν (NH) 3314 br. ν (C=N azomethine) 1677, ν (C=N benzimidazole ring) 1604. MS: m/z 342 (M^{+2}, 7%), 327 (m_1, 4.30%), 251 (m_2, 12. 42%), 209 (m_3, 68%), 43(m_5, 100). Anal. calcd. for $C_{23}H_{21}N_3O_3$ (%) M.W. 340.42: C, 77.62; H, 5.92; N, 16.46. Found: C, 77.80; H, 5.70; N, 16.23.

Biological Assay

***In vitro* Cytotoxicity**

Cell Lines and Culturing: Anticancer activity screening for the tested compounds utilizing three different human tumor cell lines including human tumor cell lines as Co rectal Cancer cell Line HCT116, human Liver Cancer Cell Line HepG2 and human ovarian Cancer Cell Line A2780 are obtained from the American Type Culture Collection (Rockville, MD, USA). The tumor cells are maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (w/v) heat inactivated fetal calf serum (GIBCO), penicillin (100 U/ml) and streptomycin (100µg/ml) at 37°C in humidified atmosphere containing 5% (v/v)CO_2. Cells at a concentration of 0.50 x 10^6 µM are grown in a 25 ml flask containing 5 ml complete culture medium.

The antiproliferative activity is measured *in vitro* using the Sulfo-Rhodamine-B stain (SRB) assay according to the previous reported standard procedure [27]. Cells are inoculated in 96-well microtiter plate (104 cells/ well) for 24 hrs before treatment with the tested compounds to allow attachment of cells to the wall of the plate. Tested compounds are dissolved in DMSO (1 mg/ml) immediately before use and diluted to the appropriate volume just before addition to the cell culture. Different concentrations of the compounds under test were added to the cells. Triplicate wells are prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 hrs at 37°C and in atmosphere of 5% (v/v) CO_2. After 48 hrs cells are fixed, washed and stained for 30 min with 0.4% (w/v) SRB dissolved in 1%(w/v) acetic acid. Unbound dye is removed by four washes with 1%(w/v) acetic acid and attached stain was recovered with Tris-EDTA buffer. Color intensity is measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve for each cell line after the specified time. The concentration required for 50% inhibition of cell viability (IC_{50}) is

calculated and the results are given in Table (2). The results were compared to the anti proliferative effects of the reference control CK0106023 [28]. The results are represented in one independent experiment run in triplicates.

Molecular Docking Study: The molecular docking is performed and analyzed with the MOE program.JAK2, a member of the Janus kinase (JAK) family of protein tyrosine kinases (PTKs), is an important intracellular mediator of cytokine signaling. Mutations of the JAK2 gene are associated with hematologic cancers accordingly; the development of JAK2-specific inhibitors has tremendous clinical relevance.

The synthesized compounds 3c, 3f, 3g are investigated for the binding affinity of Janus kinase tyrosine kinases receptor (pdb: 2B7A) [29]. This purpose of lead optimization and to find out the interaction between compounds 3c, 3f, 3g and the tyrosine receptor.

Molecular modeling calculations and local docking are done by using MOE (molecular modeling environment) to evaluate the binding free energies of these inhibitors into the target tyrosine kinase receptor.

Validation of the Docking Performance and Accuracy: To validate the docking accuracy of the program used, docking of the native co-crystallized IZA ligand 2-tert - butyl-9-fluoro-3,6-dihydro-7H-benz [H]-imidaz [4,5-F]isoquinolone-7-one is done into its binding site of receptor. The docked ligand is exactly superimposed on the native co-crystallized one with RMSD being 0.2975 Å and binding free energies of (-15.675 kcal/mol).

RESULTS AND DISCUSSION

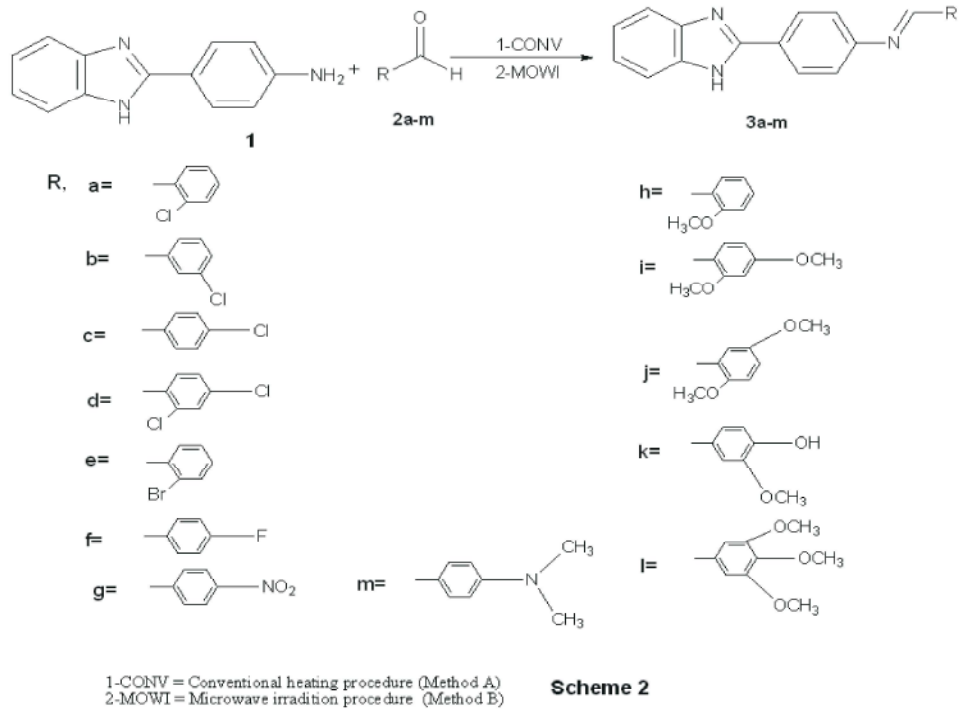
Chemistry: Recently, it has been reported the synthesis of *4-(1H-benzo[d]imidazol-2-yl)aniline* 1 [25, 26]. New method of *4-(1H-benzo[d]imidazol-2-yl)aniline* preparation 1 is achieved by the condensation of *o*-phenylendiamine and *p*-aminobenzoic acid under reflux in the presence of *o*-phosphoric acid as a catalyst, then the reaction mixture is allowed to cool, then poured over cold water and 10% NH_4OH solution was add.

The present work deals with the preparation of some selected Schiff bases (*E*)-4-(1*H*-benzo[*d*]imidazol-2-yl)-*N*-(substitutedbenzylidene)aniline (3a-m), by the reaction of *4-(1H-benzo[d]imidazol-2-yl)aniline* 1 with different aldehydes (2a-m), (2-chlorobenzaldehyde, 3-chloro benzaldehyde, 4-chloro benzaldehyde, 2,4-dichloro benzaldehyde, 2-bromo benzaldehyde, 4-floro benzaldehyde, 4-nitro benzaldehyde, 2-methoxy benzaldehyde, 2,4-dimethoy benzaldehyde, 2,5-dimethoy benzaldehyde, 3-methoxy4-hydroxybenzaldhyde (vanillin), 3,4,5-trimethoybenzaldehyde and 4-(dimethylamino) benzaldehyde) (c.f. Sheme2), using both conventional and microwave irradiation methods. In the conventional method the previous reaction mixtures are refluxed in the presence of acetic acid, while in microwave irradiation technique we used DMF (Dimethylformamide) as a solvent. Compatible elemental and spectroscopic measurements are in a good accord with the resulted assigned structures. The IR spectrum for the newly synthesized Schiff bases 3a-m show a new bands of azomethine group at the region 1673- 1677 cm^{-1} which are proved by ^{1}H-NMR spectra that shows singlets at 10.15-10.31 δ ppm indicating the presence of azomethine (-CH=N) protons. As a result of using the microwave-assisted synthesis, it is observed that the reaction is completed in a short time with higher yields compared with the conventional method (Table 1).

Biological Assay: The *in vitro* anticancer activity of the newly synthesized compounds are evaluated against human cancer cell lines Corectal Cancer Cell Line HCT116, human Liver Cancer Cell Line HepG2 and human ovarian Cancer Cell Line A2780 according to Sulfo-Rhodamine- â stain (SRB) assay method using CK0106023 as a reference drug. The results are presented in (Table 2). IC_{50} values and based on dose-response curves (IC_{50} values, defined as the concentration corresponding to 50% growth inhibition). The data in Table 2 disclosed that some of the compounds exhibited excellent activity against tumor cells. The compounds 3c, 3f, 3g exhibited the most cytotoxic activity towards all the cancer cell lines. The other compounds afforded moderate to good anticancer activity against three different cell lines are not selective towards any particular cell line (Table 2).

1

Scheme 1



1-CONV = Conventional heating procedure (Method A)
2-MOWI = Microwave irradition procedure (Method B)

Scheme 2

Table 1: Preparation of substituted 4-(1H-benzo [d] imidazol-2-yl)-N-benzylidene analine products

	Time		Yield (%)		
Product	Conv. (hr)	MWI (min)	Conv.	MWI	M.P (°C)
3a	14	11	62	92	289-290
3b	19	16	68	85	295-297
3c	16	28	62	88	215-217
3d	30	16	40	94	>300
3e	17	20	58	96	258-260
3f	16	28	70	88	291-293
3g	37	18	45	93	265-267
3h	36	15	64	96	>300
3i	28	17	66	98	289-290
3j	10	14	62	97	>300
3k	25	14	68	97	>300
3l	29	28	57	95	294-296
3m	49	30	42	89	>300

Table 2: Anti proliferative activity IC_{50} (µg/ml) of the newly synthesized Schiff bases on human cancer cell lines

	IC_{50}		
Compound	HCT116	A2780	HepG2
3c	8.07	35.72	17.29
3f	8.88	40.37	19.21
3g	9.77	45.61	21.34
3k	10.75	51.54	23.70
3e	11.82	58.24	26.33
3b	13.00	65.81	29.25
3d	14.30	74.37	32.50
3k	15.73	84.04	36.10
3m	17.31	94.96	40.10
3h	19.04	107.31	44.55
3L	20.94	121.26	49.49
3a	23.03	137.02	54.98
CK0106023	25.34	154.84	61.08

Molecular Docking Study: The docking studies are carried out using Molecular Operating Environment (MOE) 2008.10 (Moe source: Chemical Computing Group Inc. Quebec, Canada, 2008). First, of all a Gaussian Contact surface around the binding site is drawn. The surface surrounds the van der Waals surface of a molecule (filling in solvent inaccessible gaps). Then docking studies are carried out to evaluate the binding free energy of the inhibitors within the macromolecules. The Dock scoring in MOE software is done using London dG scoring function and has been enhanced by using two different refinement methods, the Force-field and Grid-Min pose have been updated to ensure that refined poses satisfy the specified conformations. We allowed rotatable bonds; the best 10 poses are retained and analyzed for the binding poses best score. The database browser is used in MOE to compare the docking poses to the ligand in the co-crystallized structure and to get RMSD (Root Mean Square Deviation) of the docking pose compared to the co crystal ligand position.

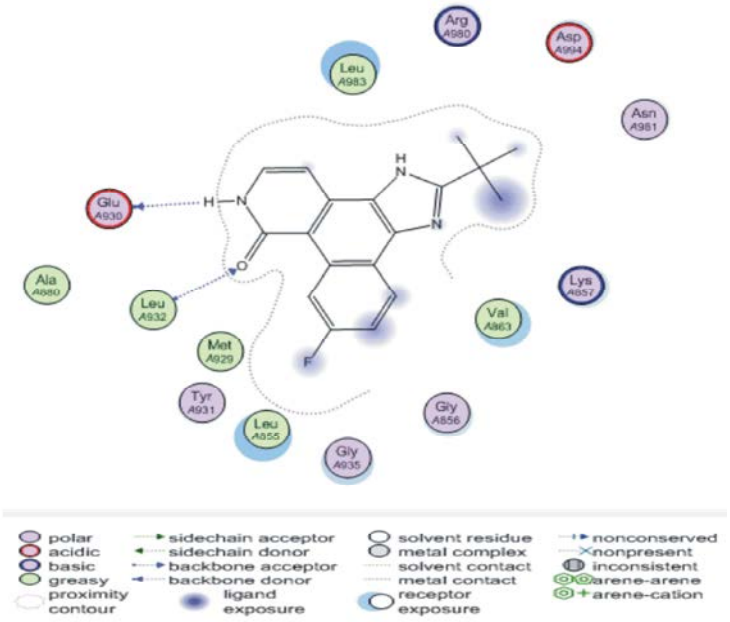


Fig. 1: The ligand interaction and the binding mode of the native ligand IZA (2-tert-butyl-9-fluoro-3,6-dihydro-7H-benz[H]-imidaz[4,5-F]isoquinolone -7-one) and exhibited one H-bond donor with GLU 930 and at distance 1.98 and one H-bond acceptor with LEU 932 at distance 2.77 and it is score -15.675 kcal/mol.

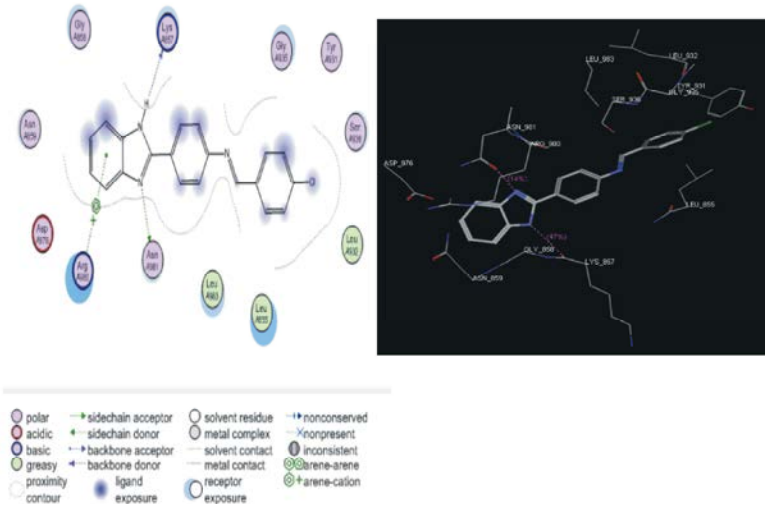


Fig. 2: Ligand interaction and the binding mode of compound 3c with Janus kinase receptor, exhibited one H-bond donor with LYS 857 at distance 1.83 and one H-bond acceptor with ASN 981 at distance 2.25, it is give score -20.05 kcal/mol.

Preparation of Ligands and Target Janus Kinase Receptor: The compounds involved in this study as ligands are 3c, 3f, 3g which are studied for their binding affinity into Janus kinase receptor. The Molecule Builder tool in MOE was used to construct a three-dimensional model of the structures. Energy minimization is done through Force-field MMFF94x Optimization using gradient of 0.0001 for determining the lower energy conformations with the most favorable (lowest energy) geometry. The crystal structures of Janus kinase receptor in complex with IZA (2-tert-butyl-9-fluoro-3,6-dihydro-7H-benz[H]-imidaz[4,5-F]isoquinolone -7-one) were obtained from the Protein Data Bank (PDB) http://www. rcsb. org/ pdb/ explore/explore.do?structureId=2B7A (PDB code: 2B7A). Hydrogen atoms and partial charges were added to the protein with the protonation 3D application in

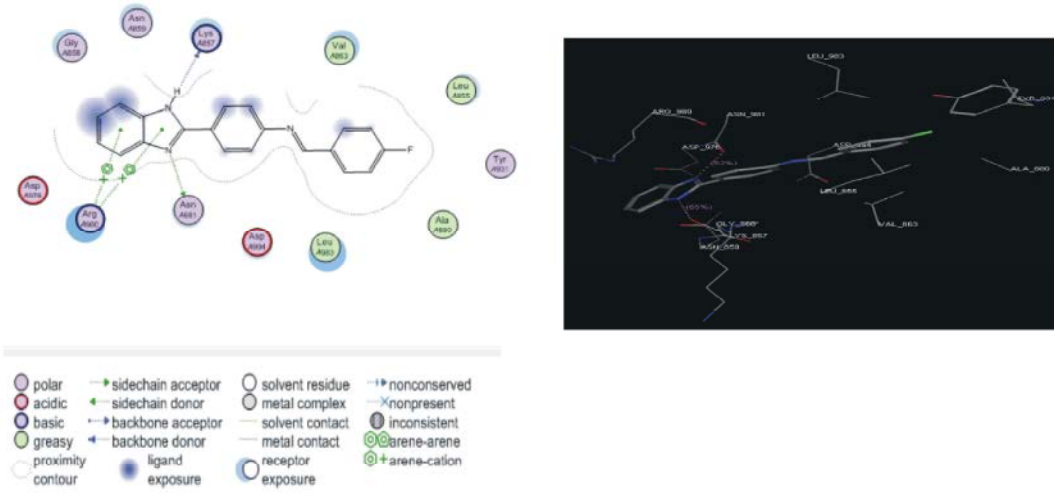


Fig. 3: Ligand interaction and the binding mode of compound 3f with Janus kinase receptor, exhibited one H-bond donor with LYS 857 at distance 1.75 and one H-bond acceptor with ASN 981 at distance 2.50, it is give score -19.909 kcal/mol.

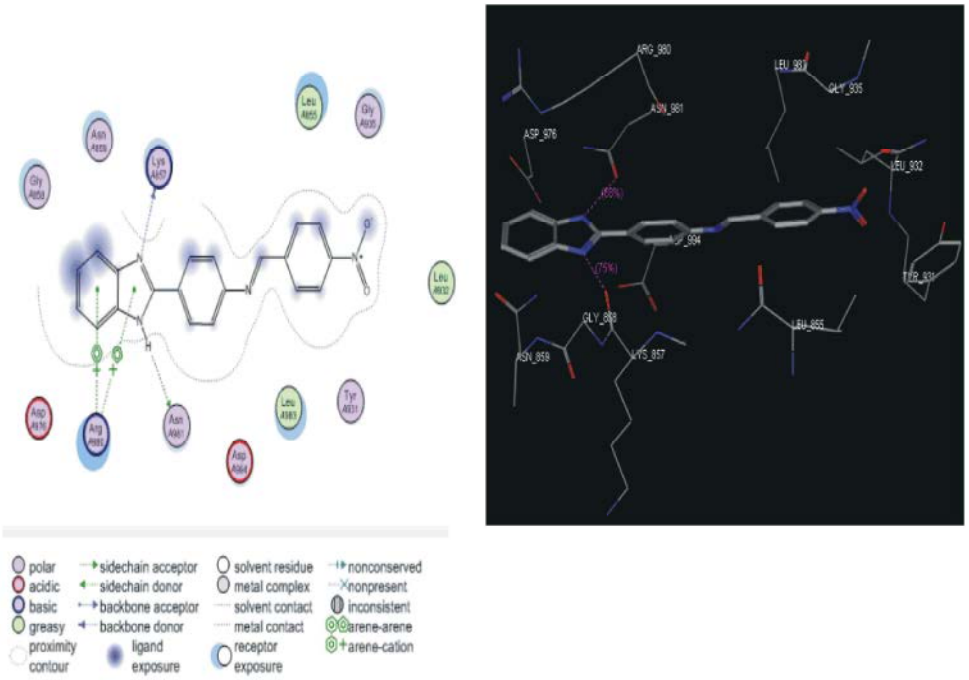


Fig. 4: Ligand interaction and the binding mode of compound 3g with Janus kinase receptor, exhibited one H-bond donor with LYS 857 at distance 2.60 and one H-bond acceptor with ASN 981 at distance 1.83, it is give score -20.5246 kcal/mol.

MOE. This application is performed to assign ionization states and position hydrogen atoms in the macromolecular structure. As most of protein structures obtained from the Protein Data Bank contain little or no hydrogen coordinate data due to limited resolution. Yet, the hydrogen bond network and ionization states can have a dramatic effect on simulations results.

Molecular Modeling and Analysis of the Docked Results: The binding free energy was used to rank the binding affinity of the synthesized compounds to Janus kinase receptor. Also, Hydrogen bonds between the ligand and amino acids in Janus kinase receptor were used in the ranking of the compounds. Evaluation of the hydrogen bonds was done by measuring the hydrogen bond length

which doesn't exceed 3°A. RMSD of the docking pose compared to the co-crystal ligand position was used in the ranking. The mode of interaction of the native ligand (IZA) within the crystal structure of Janus kinase receptor was used as a standard docked model as well as for RMSD calculation.

CONCLUSION

In conclusion, the newly synthesized benzimidazole derivatives are evaluated for their *in vitro* anticancer activity . Compounds 3c, 3f, 3g exhibit a significant anticancer activity compared with the standard drug CK0106023. Aiming for Compounds 3c, 3f, 3g to be considered as a future promising anticancer drugs.

REFERENCES

1. Vinodkumar, R., S.D. Vaidya, B.V. Siva Kumar, U.N. Bhise, S.B. Bhirud and U.C. Mashelkar, 2008. Synthesis, anti-bacterial, anti-asthmatic and anti-diabetic activities of novel N-substituted 2-(4-styrylphenyl)-1H-benzimidazole and N- substituted-3[4-(1H-benzimidazole-2-yl)-phenyl]-acrylic acid tert-butyl ester. ARKIVOC. xiv: 37-49.
2. Sutha, S., S. Shobana, J. Dharmaraja and S. Selvaraj, 2013. Mixed ligand complexation of some transition metal ions in solution and solid state: Spectral characterization, antimicrobial, antioxidant, DNA cleavage activities and molecular modeling,. Spectrochim Acta A Mol Biomol. Spectrosc., 107C : 117-132.
3. Reddy, V.B., R.K. Singla, V. Bhat and G. Shenoy, 2009. Synthesis and Antimicrobial Studies Of Some Novel Benzimidazole Derivatives, Asian J. Research Chem., 2: 162-167.
4. Micco, I., A. Nencini, J. Quinn, H. Bothmann, C. Ghiron, A. Padova and S. Papini, 2008. Parallel synthesis of a series of potentially brain penetrant aminoalkyl benzoimidazoles. Bioorg. Med. Chem., 16: 2313-2328.
5. Shadia, A.G., A.S. Abd El-All, M.M. Abdallah and H.I. El-Diwani, 2009. Synthesis of potent antitumor and antiviral benzofuran derivatives. Bioorg. Med. Chem. Lett., 19: 2420-2428.
6. Shadia, A.G., A.S. Abd El-All, K.H. Hegab, A.A. Magd-El-Din, N.S. Youssef and H.I. El-Diwani, 2010. Novel antiviral benzofuran-transition metal complexes, Eur. J. Med. Chem., 45: 3035-3046.
7. Saydin, A., K.D. Beis and D. Can, 2003. Analgesic and antispasmodic activities of 2-(2-nitro-phenyl)-1H-benzimidazole 5-carboxylic acid: evidence for the importance of the 2-(o-substituted phenyl) group, Pharmazie. 58: 405-408.
8. Rubbiani, R., I. Kitanovic, A. Hamed, S. Can, A. Kitanovic, L. Onambele, M. Stefanooulou, Y. Goldmacher, W. Sheldrick, G. Wolber, A. Pork, S. Welof and I. Ott, 2010. Benzimidazol-2-ylidene gold(I) complexes are thioredoxin reductase inhibitors with multiple antitumor properties. J. Med. Chem., 53: 8608-8618.
9. Kumar, D., M.R. Jacob, M.B. Reynolds and S.M. Kerwin, 2002. Synthesis and evaluation of anticancer benzoxazoles and benzimidazoles related to UK-1, Bioorg. Med. Chem., 10: 3997-4004.
10. Suthakaran, R., G. Nagarajan, V. Balasubramaniam, K. Suganthi, M. Kannan and G. Velrajan, 2005. synthesis and anti-tumor activity of 4-aryl/alkyl -2-D aldosugar, disubstituted Bis-benzimidazoles. Indian J. Heter. Chem., 15: 173-176.
11. Ishida, T., T. Suzuki, S. Hirashima, K. MIzutani, T. Adachi and H. Hashimoto, 2006. Benzimidazole inhibitors of hepatitis C virus NS5B polymerase: identification of 2-[(4-diarylmethoxy)phenyl]-benzimidazole. Bioorg Med. Chem. Lett., 16: 1859-1863.
12. Neef, D.K., A. Lee-Dutra, J.M. Blevitt, F.U. Axe, M.D. Hack, J.C. Buma, R.Rynberg, A. Brunmark, L. Karlsson and G. Breitenbucher, 2007. 2-Aryl benzimidazoles featuring alkyl-linked pendant alcohols and amines as inhibitors of checkpoint kinase Chk2. Bioorg. Med. Chem. Lett., 17: 6467-6471.
13. Arienti, K.L., A. Brunmark, F.U. Axe, K. Mcclure, A. Lee, J. Blevitt, D. Neef, L. Hung, S. Crawford, C.R. Pandit, L. Karlsson and J.G. Breitenbucher, 2005. Checkpoint kinase inhibitors: SAR and radioprotective properties of a series of 2- aryl benzimidazoles. J. Med. Chem., 48: 1873-1885.
14. Joseph, E.P., C. Bonnefous, K.T. Symons, P.M. Nguyen, M. Sablad, N. Rozenkrants, Y. Zhang, L. Wang, N. Yazdani, A. K. Shiau, S. A. Noble, P. Rix, T.S. Rao, C.A. Hassig and N.D. Smith, 2010. Discovery of dual inducible/neuronal nitric oxide synthase (iNOS/nNOS) inhibitor development candidate 4-((2-cyclobutyl-1H-imidazo[4,5-b]pyrazin-1-yl)methyl)-7,8-difluoroquinolin-2(1H)-one (KD7332) part 2: identification of a novel, potent and selective series of benzimidazole-quinolinone iNOS/nNOS dimerization inhibitors that are orally active in pain models,J. Med. Chem., 53: 7739-55.

15. Ingle R.G. and D.D. Magar, 2011. Heterocyclic chemistry of benzimidazoles and potential activities of derivatives, IJDRT, 1: 26-32.
16. Scates, A.C. and P.M. Doraiswamy, 2000. Reboxetine: a selective norepinephrine reuptake inhibitor for the treatment of depression, Ann Pharmacother, 34: 1302-1312.
17. David Baldwin., C. Buis and A. Mayers, 2002. Selective serotonin reuptake inhibitors in the treatment of generalized anxiety disorder, Expert Rev. Neurother, 2: 717-724.
18. Sharma, M.C., D.V. Kohli, S. Sharma and A.D. Sharma, 2010. Synthesis and antihypertensive activity of some new benzimidazole derivatives of 4'-(6-methoxy-2-substituted-benzimidazole-1-ylmethyl)-biphenyl-2-carboxylic acid in the presences of BF_3•OEt_2, Der Pharmacia Sinica, 1: 104-115
19. Bharat, S., D. Bhatta, P.K. Panda, P. Mishra and D. Pradhan, 2008. Synthesis and Antiulcer Activity Studies of 2-(1'-Iminothioimido Subs titu ted)-1'-Substituted Phenyl benzoic acids, Indian J. Pharm. Sci., 70: 381-383
20. Rajasekaran, S., R. Gopalkrishna and A. Chatterjee, 2012. Synthesis, Anti-Inflammatory and Anti-oxidant activity of some substituted Benzimidazole Derivatives, IJDDR, 4: 303-309.
21. Naik, B.K. and K.R. Desai, 2004. Synthesis of some heterocyclic Schiff base and azetidinone compounds and their antibacterial activity, Asian J. Chem., 16: 1749-1752.
22. Warad, D.U., C.D. Satish, V.H. Kulkarni and C.S. Bajgur, 2000. Synthesis, structure and reactivity of zircordum(IV), vanadium(IV), cobalt(II), nickel(II) and copper(II) complexes derived from carbohydrazide schiff base ligands, Indian J. Chem., 39A: 415-420.
23. Desai, S., P.B. Desai and K.R. Desai, 1999. Synthesis of new heterocyclic thiazolidinone and azetidinone compounds and their anticancer activity, Heterocycl Commun., 5: 385-387.
24. Varma, R.S., 2002. Microwaves in Organic Synthesis, Ed. A. Loupy : Wiley
25. Furniss, B.S., A.J. Hannaford, P.W.G. Smith and A.R. Tatchell, 1989. Vogel's.
26. Gülgün, A.K. and N. Altanlar, 2003. Synthesis and antimicrobial activities of Some.
27. Philip, S., R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H, Bokesch, S. Kenney and M.R. Boyd, 1990. Synthesis and antimicrobial activities of some new benzimidazole derivatives, J. Natl. Cancer Inst., 82: 1107-1112.
28. Li, L.H. and F.L. Yu, 1993.Transcriptional specificities of adriamycin, Biochem. Mol. Biol. Int., 31: 879-887
29. Isabelle, S.L., E. Fantino, M. Styles, R. Bamert, O. Patel, S.E. Broughton, M. Walter, C.J. Burns, H. Treutlein, A.F. Wilks and J. Rossjohn, 2006. The structural basis of Janus kinase 2 inhibition by a potent and specific pan-Janus kinase inhibitor, Blood, 107: 176-183.