SYNTHESIS, CHARACTERIZATION AND ANTI-PROLIFERATION STUDY OF SOME BENZIMIDAZOLE DERIVATIVES

by

MOHAMMED HADI SAEED AL–DOUH

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DECLARATION

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LIST OF ABBREVIATIONS

PPA = Polyphosphoric acid

DMF = Dimethyl formamide

DMA = *N*,*N*-Dimethyl acetamide

AcOH = Acetic acid

EtOH = Ethanol

MeOH = Methanol

CAN = Ceric ammonium nitrate

DCM = Dichloromethane

NMP = *N*-Methyl-2-pyrrolidone

MW = Microwave irradiation

Py. = Pyridine

MS = Molecular sieves

K-10 clay = montmorillonite

HATU = *N*,*N*,*N*`,*N*`-Tetramethyl-O-(7-azabenzotriazole-1-yl) uranium hexafluoro-phosphate

DIPEA = *N*,*N*-Di*iso*propylethylamine

MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

TBAI = Tetra-*n*-butylammonium iodide

DMAP = *N*,*N*`-4-Dimethyl aminopyridine

PTC = Phase-transfer catalysts

TBAB = Tetra-*n*-butylammonium bromide

equiv. = equivalent

Temp. = Temperature

TLC = Thin layer chromatography

R_f = Retention factor

hr = hour

min = minute

mol = mole

Δ = heat

rt = room temperature

FTIR = Fourier-Transform Infrared

as = asymmetric

sy = symmetric

ν = stretching band

δ = bending band

EIMS = Electron-Ionization Mass Spectrum

HRMS = High-Resolution Mass Spectrum

NMR = Nuclear Magnetic Resonance

δ = chemical shift

ppm = part per million

TMS = tetramethylsilane

J = coupling constant

Hz = Hertz

MHz = Megahertz

s = singlet

d = doublet

t = triplet

q = quartet

m = multiplet

dd = double doublet

dq = double quartet

td = triple doublet

br = broad

APT = Attached Proton Test

DEPT = Distortionless Enhancement by Polarization Transfer

COSY = Correlation Spectroscopy

HMQC = Heteronuclear Multiple Quantum Coherence

HMBC = Heteronuclear Multiple Bond Coherence

^{o} = degree

Å = angstrom unit ($\equiv 10^{-10}$ m)

μ = absorption coefficient

θ = scattering angle

λ = X–ray wavelength

σ = standard error

hkl = Miller indices

F = structure factor

F_o = observed structure factor

I = reflection intensity

R = conventional residual, calculated from F_o-data

S = sign of a structure factor

T = temperature

V = volume

w = weight of a structure factor

wR = weighted residual, calculated from F_o-data

wR_2 = weighted residual, calculated from F^2_o-data

Z = number of formula units per unit cell

MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

DMEM = Dulbecco's Modified Eagle Medium

PBS = Phosphate Buffer Saline

HIFCS = Heat inactivated fetal calf serum

OD = optical density

IC_{50} = 50% inhibitory concentration

SD = Standard deviation

MCF–7 = Breast cancer cell line

HCT–116 = Colon cancer cell line

HT–29 = Colon cancer cell line

MDA–MB–231 = Human Breast cancer cell line

T–47D = Human Breast cancer cell line (Human breast ductal carcinoma cell)

HepG2 = Liver cancer cell line (Human hepatocellular carcinoma)

K562 = Leukaemia cell line (Human erythromyeloblastoid leukaemia cell line)

U937 = Leukaemia cell line (Human leukemic monocyte lymphoma cell line)

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SINTESIS, PENCIRIAN DAN KAJIAN ANTI-PROLIFERASI TERBITAN BENZIMIDAZOL

ABSTRAK

Empat jenis sebatian organik telah disintesis dan dicirikan. Sintesis sebatian ini melibatkan tindak balas pembenzilan, penambahan penyingkiran dan pensiklikan. Struktur sebatian yang disintesis dipastikan secara takat lebur, spektroskopi FTIR, HRMS, NMR 1D dan 2D dan kristalografi sinar-X. Sebatian jenis pertama adalah 2-benziloksi-3-metoksibenzaldehid **75**, yang telah disintesis dengan tindak balas *o*-vanilin dengan benzil bromida dalam aseton sebagai pelarut dan K_2CO_3 sebagai bes dengan kehadiran tetra-*n*-butilammonium iodida (TBAI) sebagai mangkin. Sebatian jenis kedua yang dikaji adalah 2-amino-*N*-benzilidin benzenamina **76** dan **82**, yang disintesis dengan tindak balas *o*-fenilenadiamina dengan *o*-vanilin atau **75** dalam diklorometana dalam keadaan sejuk. Kedua-dua eksperimen NMR ^1H–^1H COSY dan HMBC dijalankan untuk mengesahkan kedudukan jalur sebatian dalam $CDCl_3$, CD_2Cl_2 dan aseton-d_6. Sebahagian kesan daripada gelang enam- dan lima- *illusory* terhasil daripada pembentukan intramolekul ikatan hidrogen antara N–H dalam gelang amino dan atom N dalam ikatan N=C–H dan O–H dengan atom N pada N=C–H pada **76** atau antara N–H dalam gelang amino dan atom N dalam ikatan N=C–H dan ikatan C–H metina pada **82** dengan atom oksigen dalam gelang benziloksi. Struktur kristal kedua **76** dan **82** disahkan dengan menggunakan analisis kristalografi sinar-X. Sebatian jenis ketiga yang dikaji adalah bes bis-Schiff **79**, **80** and **81**, yang dihasilkan dengan tindak balas antara *o*-vanillin dengan *o*-, *m*- dan *p*-fenilenadiamina, masing-masing, oleh mekanisme tetrahedra atau mekanisme penambahan penyingkiran. Struktur kristal

bes bis-Schiff disahkan gelang enam- *illusory*, yang dihasilkan daripada pembentukan intramolekul ikatan hydrogen antara ikatan N=C–H dan kumpulan O–H pada gelang tritertukarganti yang disahkan dengan menggunakan analisis kristalografi sinar-X. Sebatian jenis keempat adalah benzimidazol **77**, **78**, **83** dan **84**, yang telah dihasilkan daripada amino benzenamina **76** and **82**, masing-masing, dalam media berakues atau berbes dengan mekanisme penambahan penyingkiran diikuti oleh mekanisme pensiklikan. Eksperimen NMR ^{1}H, ^{13}C, ^{1}H–^{1}H COSY, HMQC dan HMBC dijalankan untuk mengesahkan kedudukan jalur pada **77**, **78**, **83** dan **84**. Struktur kristal benzimidazol **77**, **78** dan **83** disahkan dengan menggunakan analisis kristalografi sinar-X. Semua benzimidazol telah dinilaikan terhadap titisan sel kanser payudara MCF–7 dan titisan sel kanser kolon HCT–116 menggunakan kaedah MTT. Keputusan benzimidazol **84** menunjukkan aktiviti sitotoksik yang tinggi terhadap titisan sel *MCF–7* dengan IC_{50} = 8.86 ± 1.10 μg/mL, dan aktiviti sitotoksik yang sederhana terhadap titisan sel *HCT–116* dengan IC_{50} = 24.08 ± 0.31 μg/mL. Manakala benzimidazol **78** menunjukkan IC_{50} rendah bernilai 16.18 ± 3.85 μg/mL terhadap titisan sel *HCT–116*. Kedua benzimidazol **77** dan **78** menunjukkan aktiviti sitotoksik yang sederhana terhadap titisan sel *MCF–7*, manakala benzimidazol **83** tidak menunjukkan sebarang aktiviti terhadap titisan sel *MCF–7* dan *HCT–116*.

SYNTHESIS, CHARACTERIZATION AND ANTI-PROLIFERATION STUDY OF SOME BENZIMIDAZOLE DERIVATIVES

ABSTRACT

Four different types of compounds were successfully synthesized and characterized. The syntheses of these compounds involved benzylation, addition elimination and cyclization reactions. The structures of the synthesized compounds were confirmed by melting points, FTIR, HRMS, 1D and 2D NMR spectroscopy and X–ray crystallography. The first type of these compounds is 2-benzyloxy-3-methoxybenzaldehyde **75**, which was synthesized by the reaction of *o*-vanillin with benzyl bromide in acetone as the solvent and K_2CO_3 as a base in the presence of tetra-*n*-butylammonium iodide (TBAI) as catalyst. The second type of the studied compounds is 2-amino-*N*-benzylidene benzeneamines **76** and **82**, which were synthesized from the reaction of *o*-phenylenediamine with *o*-vanillin or **75** in dichloromethane at cold conditions. Both ^1H–^1H COSY and HMBC NMR experiments were performed to further confirm the assigned peaks of compounds in $CDCl_3$, CD_2Cl_2 and acetone-d_6. Some effect from those six– and five–membered illusory rings, which were formed from the intramolecular hydrogen bonding between N–H in the amino ring and the N atom in N=C–H bond and O–H with N atom of N=C–H of **76** or between N–H in the amino ring and the N atom in N=C–H bond and the bond of the methine C–H of **82** with oxygen atom in the benzyloxy ring. The crystal structures of both **76** and **82** confirmed the hydrogen bonds by using the X–ray crystallographic analysis. The third type of the studied compounds is bis-Schiff bases **79**, **80** and **81**, which are formed from the reaction between *o*-vanillin with *o*-, *m*- and *p*-phenylenediamines, respectively, by a tetrahedral mechanism or

addition/elimination mechanism. The crystal structures of those bis-Schiff bases confirmed six–membered illusory rings, which formed from the intramolecular hydrogen bonding between N=C–H bonds and O–H groups of the trisubstituted rings by using the X–ray crystallographic analysis. The fourth type of the studied compounds is benzimidazoles **77**, **78**, **83** and **84**, which are converted from amino benzeneamines **76** and **82**, respectively, in aqua or basic media by addition/elimination mechanism followed by cyclization. Those benzimidazoles have been evaluated against both breast cancer cell line MCF–7 and colon cancer cell line HCT–116 using MTT assay. The results of benzimidazole **84** showed high cytotoxic activity against MCF–7 cell lines with IC_{50} = 8.86 ± 1.10 μg/mL, and moderate cytotoxic activity against HCT–116 cell lines with IC_{50} = 24.08 ± 0.31 μg/mL. Benzimidazole **78** showed the lowest IC_{50} value at 16.18 ± 3.85 μg/mL against HCT–116 cell lines. Both benzimidazoles **77** and **78** showed moderate cytotoxic activity against MCF–7 cell lines, while benzimidazole **83** showed no cytotoxic effect with both MCF–7 and HCT–116 cell lines.

CHAPTER ONE

INTRODUCTION

1.1 Schiff bases

Schiff bases or imines are compounds formed from the condensation reaction between aldehydes or ketones and primary amines or amino acids in the presence of an acid catalyst (Figure 1.1). The product containing an azomethine, C=N linkage was discovered by Hugo Joseph Schiff (Layer, 1963; Cozzi, 2004). Many nomenclatures are used to describe the formation of Schiff bases, for example: aldimines, anils, benzanils and ketimines, derived from aldehydes, aniline, aromatic aldehydes and ketones, respectively (Layer, 1963; El–Bayoumi *et al*., 1971*a*,*b*).

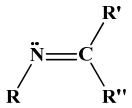


Figure 1.1: The general formula of Schiff base or imine.

These compounds are ranked among the most versatile synthetic organic intermediates, which are important for the synthesis of biologically important compounds. They are widely used as medical, pharmaceutical and industrial materials and were reported to show a variety of biological activities including as antifungal (Singh and Dash, 1988; More *et al*., 2001), antibacterial (Baseer *et al*., 2000; El-Masry *et al*., 2000; Kabeer *et al*., 2001) and anticancer (Kuz'min *et al*., 2000; Desai *et al*., 2001), among others. They also showed moderate activity against *Staphylococcus aureus* and *Bacillus subtilis* (Jarrahpour *et al*., 2004).

They have also been used extensively in inorganic and coordination chemistry fields for the synthesis of new organometallic compounds. The imines formed from the reaction of the aldehydes and amines also proved to be the source of versatile compounds for many transition metals where they act as donor groups to bind the metal ions (Vigato and Tamburini, 2004). Besides that, Schiff bases are also used to produce new azo dyes (Jarrahpour and Zarei, 2004, Naeimi *et al*., 2007). In another application, So *et al*. (2007) synthesized and characterized a series of Schiff base derivatives, which exhibited liquid crystal properties.

Bis-Schiff bases are produced from two equivalents of aldehyde or ketone with a diamine. The bases can be either symmetric or unsymmetric (asymmetric) structures, depending on the reacted aldehydes and diamines. Figure 1.2 shows examples of symmetric and unsymmetric bis-Schiff bases **1-4** (Lopez *et al*., 1998*a,b*). Compounds **1** and **2** started off with symmetrical aromatic diamine ring, but the aldehyde rings are different. Compound **3** has symmetrical cyclic diamine ring with symmetrical aldehyde rings, while both diamine and aldehyde in compound **4** are unsymmetric.

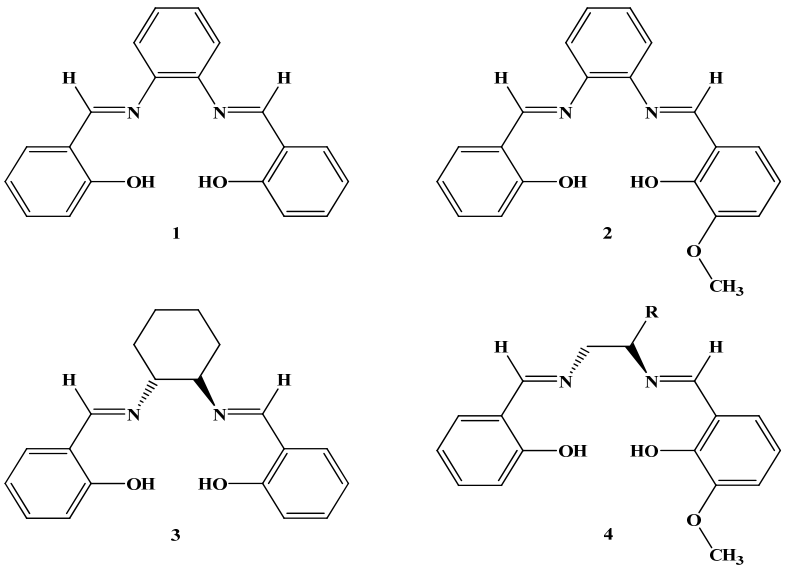


Figure 1.2: Some symmetrical and unsymmetrical bis-Schiff bases.

Both symmetrical and unsymmetrical bis-Schiff bases are interesting compounds particularly when they coordinate around the central metal ion to form transition metal ion complexes. For example, the symmetric bis-Schiff base complexes are normally used as macrocyclic models, while the unsymmetric complexes are used as irregular binding model with peptides (Atkins *et al*., 1985). However, the unsymmetrical compounds are more advanced than their symmetrical counterparts in the explaining of the composition and geometry of metal ion binding sites in metalloproteins, leading to the enzymatic selectivity of the natural systems with synthetic materials (Bu *et al*., 1997). Intercalation of other derivatives of symmetrical bis-Schiff bases with DNA by UV spectroscopy has also been studied (Parra *et al*., 2007).

Various molecular structural studies on bis-Schiff bases and their complexes with different metal ions have been reported. Bis-Schiff bases and others were found to form suitable inner coordination sphere between tin atom with O and N atoms as quadridentate chelates (Teoh *et al*., 1997). Ruthenium complexes of bis-Schiff bases derived from *o*-vanillin and salicyldehyde however, showed dibasic tetradentate chelates (Viswanathamurthi *et al*., 1998). An intramolecular hydrogen bond between the O and N atoms in Schiff bases is one of the important factors leading to the formation of metal complexes (Cohen *et al*., 1964). Kabak *et al.* (2000) prepared the derivatives of bis-Schiff bases and studied their photochromic conformational properties. The intramolecular hydrogen bond causes a six–membered illusory ring to be formed between the O–H group at *ortho* position of the aldehydic moiety and N atom of the aminic moiety (Kawasaki *et al*., 1999; Fukuda *et al*., 2003); (Figure 1.3).

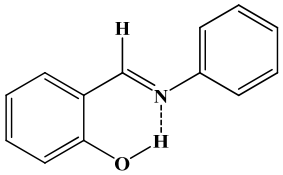


Figure 1.3: Intramolecular hydrogen bond.

1.2 Benzimidazoles

Benzimidazole (Figure 1.4) is a heterocyclic compound containing an imidazole ring fused at the 4,5-position with benzene ring. Hobrecker prepared the first benzimidazole in 1872 when he reduced 2-nitro-4-methylacetanilide, **5** to obtain the tautomers 2,5 (or 2,6)-dimethyl benzimidazole (**6**), (White, 1951; Hofmann, 1953); (Scheme 1.1).

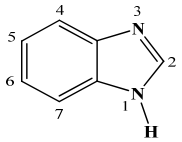
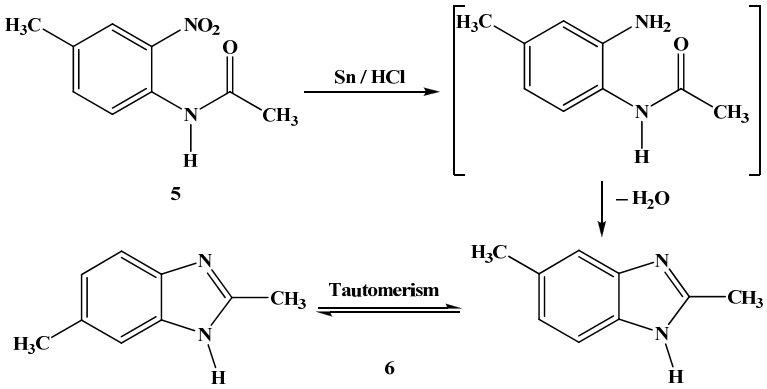


Figure 1.4: The benzimidazole ring.



Scheme 1.1: First benzimidazole **6**, prepared by Hobrecker in 1872 (White, 1951).

Since benzimidazoles have similar structures to purines, whose derivatives play important roles in biological systems; substituted benzimidazoles also showed interesting biological activities. For example, the ring of the benzimidazoles is found

as an integral part in the chemical structure of vitamin B_{12}, **7** (Bonnett, 1963; Preston, 1974); (Figure 1.5).

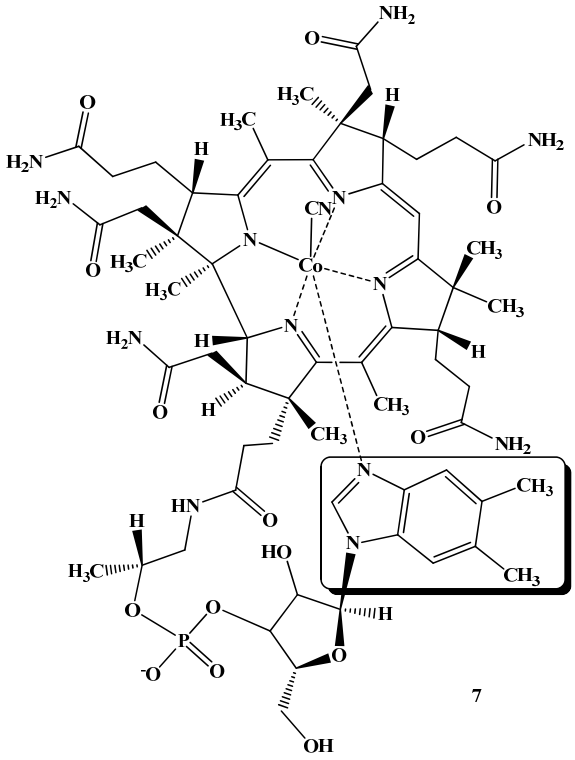


Figure 1.5: The benzimidazole ring in the chemical structure of vitamin B_{12}, **7**.

Many benzimidazoles are pharmaceutical agents and used widely in biological system applications (Townsend and Revankav; 1970; Trivedi *et al*., 2006). Some derivatives of benzimidazoles were reported and used as antiviral agents (**8-9**, Gudmundsson *et al*., 2000; Cheng *et al.*, 2005), topoisomerase I inhibitors (**10-11**, Kim *et al*., 1996, 1997; Rangarajan *et al*., 2000; Mekapati and Hansch, 2001) and as antiproliferative agent (**12**, Hong *et al.*, 2004), (Figure 1.6).

Some of 4,5,6,7-tetrahalo-1*H*-benzimidazoles (**13**) were synthesised and showed antiprotozoal activity against *Acanthamoeba castellanii* (Kopanska *et al*., 2004), antimycobacterial activity against *Mycobacterioum* strains (**14**, Kazimierczuk *et al*., 2005). In other studies, they can also act as antibacterial agents (**15-16**, Ozden

et al., 2005; Nezhad *et al*., 2005), and showed anthelmintic activity against *Trichinella spiralis* (Mavrova *et al*., 2005), anti-inflammatory and analgesic activities (**17**, Sondhi *et al*., 2006) and as inhibitors for hepatitis B (Li *et al.*, 2006) and C viruses (**18**, Beaulieu *et al.*, 2004). Some benzimidazoles were also tested as anti-HIV (**19**, Roth *et al*., 1997; Smith *et al.*, 2003) and anticancer agents (**20-21**, Craigo *et al*., 1999; Rida *et al*., 2006).

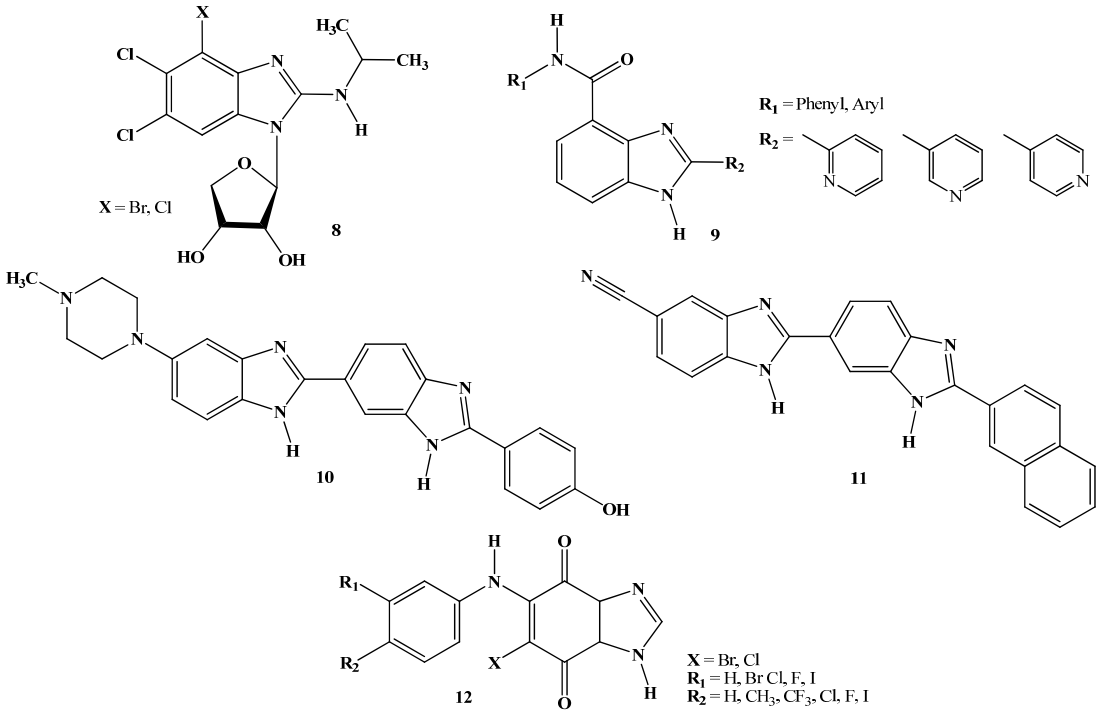


Figure 1.6: The chemical structures of some benzimidazole derivatives evaluated medicinally.

Recent publication also reported the use of phenolic and anisolic benzimidazole derivatives in vasodilator and antihypertensive studies (Soto *et al*., 2006), while other alkyloxyaryl benzimidazole derivatives have been tested for the spasmolytic activity (Vazquez *et al*., 2006). Figure 1.7 shows the chemical structures of many useful benzimidazole derivatives evaluated for biological activities.

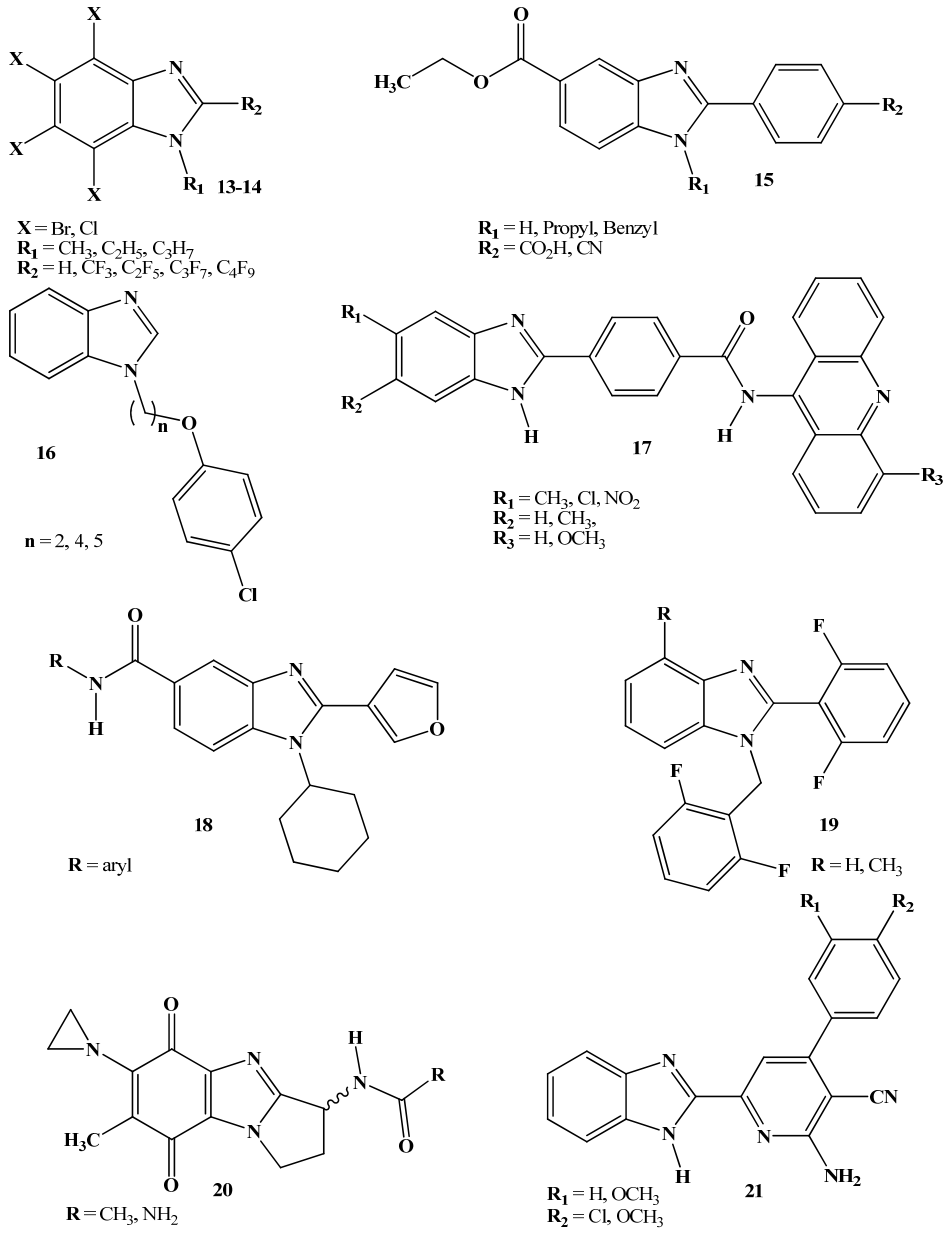


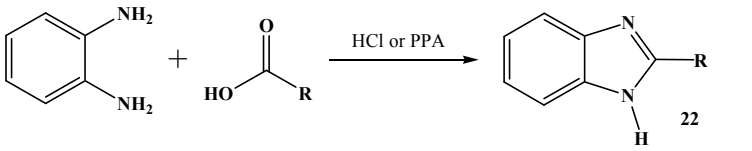
Figure 1.7: Some benzimidazole derivatives evaluated for biological activity.

The preparation of benzimidazole derivatives is well established. Monosubstitution occurs mainly at position 2, while the disubstitution at positions 1 and 2.

1.2.1 Substitution at position 2

1.2.1.1 Reaction of *o*-arylene diamines with carboxylic acids

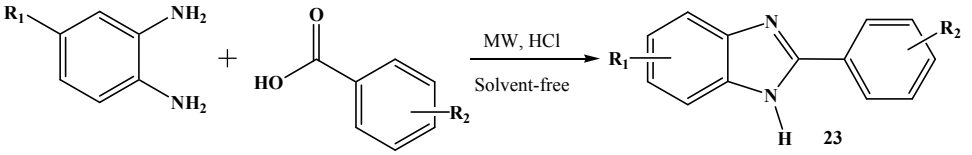
The Philips-type method follows the reaction of *o*-arylene diamines with carboxylic acids or their derivatives using hydrochloric acid or polyphosphoric acid (PPA) as a catalyst to produce the respective benzimidazoles **22**, (Scheme 1.2). The benzimidazoles can also be produced from both reactants at 18 ^{o}C for 120 hr at pH 0.5 (Haley and Maitland, 1951).



R = H, CH_3, $CHCl_2$, CCl_3, CF_3, Ph, CH_2Ph

Scheme 1.2: The Phillips-type to prepare the benzimidazole.

In solvent-free conditions with 7-10 mol % 8N HCl as a catalyst, a mixture of *o*-arylene diamines with carboxylic acids or their derivatives reacted in a domestic microwave oven at 600 W for 10 min to produce benzimidazoles **23** (Wang *et al*., 2007; Scheme 1.3).

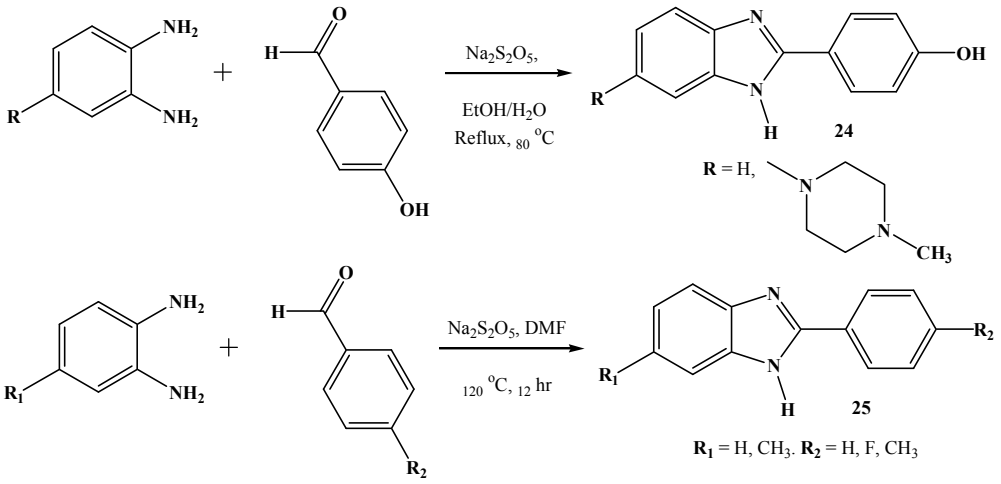


R_1 = H, CH_3
R_2 = H, 2-CH_3, 2-Cl, 3-Cl, 4-CH_3, 4-Cl, 4-OCH_3

Scheme 1.3: Preparation of benzimidazoles using the method of Wang *et al*.

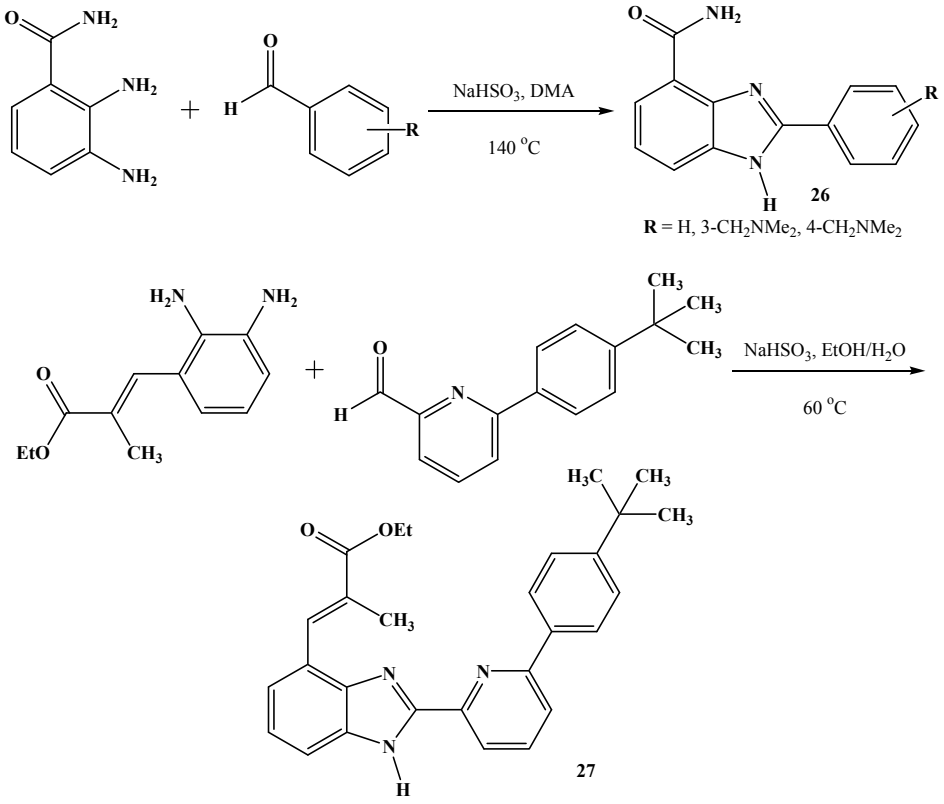
1.2.1.2 Reaction of *o*-arylene diamines with aldehydes

Kamal *et al*. (2004) prepared benzimidazoles **24** by reacting *o*-arylene diamines with 4-hydroxybenzaldehydes in ethanolic solution under reflux in the presence of $Na_2S_2O_5$ as oxidizing reagent, while Falco *et al*. (2006) used the same oxidizing reagent to prepared benzimidazoles **25** in dimethylformamide (DMF) under reflux for 12 hr, (Scheme 1.4).



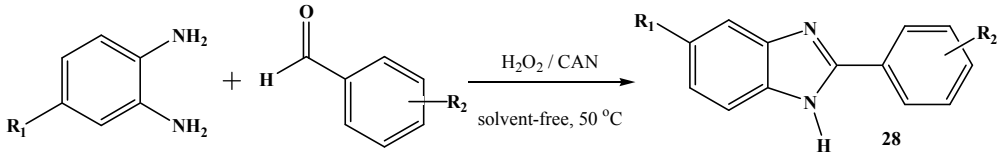
Scheme 1.4: Benzimidazoles preparation in the presence of $Na_2S_2O_5$.

Other benzimidazole derivatives **26-27** were yielded by refluxing *o*-arylene diamines with benzaldehydes in the presence of $NaHSO_3$ as oxidizing reagent at 140 ^oC in *N*,*N*-dimethylacetamide (DMA) or in $EtOH/H_2O$ at 60 ^oC, respectively (White *et al*., 2004; Safonov *et al*., 2006); (Scheme 1.5).



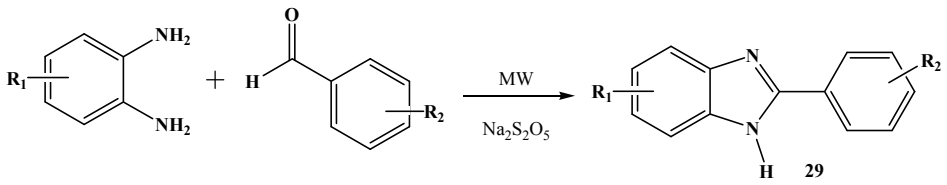
Scheme 1.5: Benzimidazoles preparation in the presence of $NaHSO_3$.

Meanwhile, Bahrami *et al*. (2008) described a new convenient method for the synthesis of benzimidazole derivatives **28** by the reaction of substituted *o*-arylene diamines with aromatic aldehydes in the presence of ceric ammonium nitrate (CAN) as a catalytic redox reagent and H_2O_2 in free solvent for 10-70 min at 50 ^oC; (Scheme 1.6). Under microwave irradiation at 1000 W, the reaction between aldehydes and *o*-arylene diamines afforded the benzimidazole derivatives **29** (Soto *et al*., 2006; Scheme 1.7).



R_1 = H, CH_3, NO_2
R_2 = H, 2-OH, 2-Cl, 3-NO_2, 4-CH_3, 4-OCH_3, 4-OH, 4-Cl, 4-F

Scheme 1.6: Preparation of benzimidazoles using the method of Bahrami *et al*.

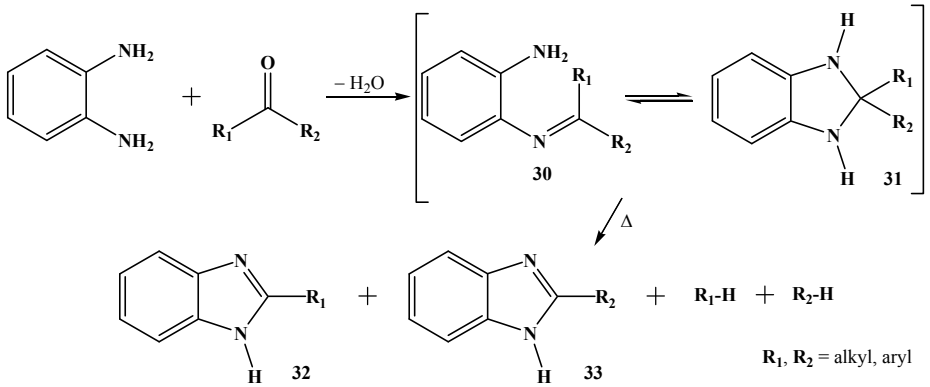


R_1 = H, CH_3, NO_2, CF_3
R_2 = 2-OH, 4-OCH_3

Scheme 1.7: The preparation of the benzimidazoles from MW irradiation.

1.2.1.3 Reaction of *o*-arylene diamines with ketones

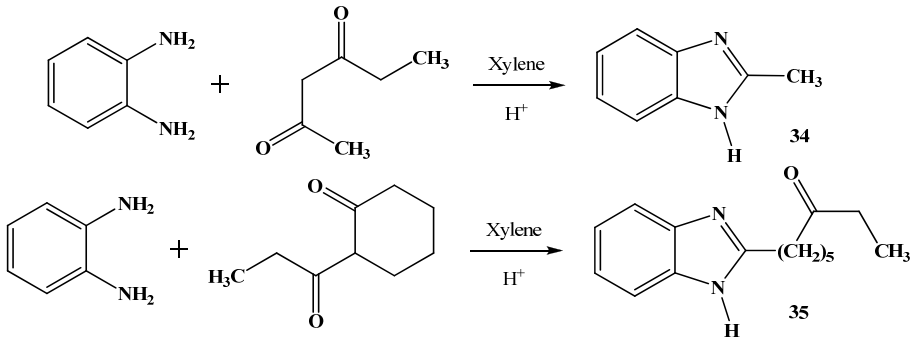
2-Disubstituted benzimidazoline **31** was formed from the reaction between *o*-arylene diamines and ketones by elimination of water, which decomposed under heat to produce 2-substituted benzimidazoles, **32-33** and hydrocarbons (Elderfield and Kreysa, 1948); (Scheme 1.8). The intermediate **31** was isolated with its tautomer **30** (Elderfield and Meyer, 1954*a*,*b*).



Scheme 1.8: Preparation of benzimidazoles using the method of Elderfield and Kreysa.

1.2.1.4 Benzimidazole derivatives from *β*-diketone

Benzimidazole derivatives can also be synthesized from *β*-diketone. This was described by Rossi *et al*. (1960) when *o*-phenylenediamine was heated with *β*-diketone derivatives in xylene with acidic media to furnish good yields of **34-35** (Scheme 1.9).

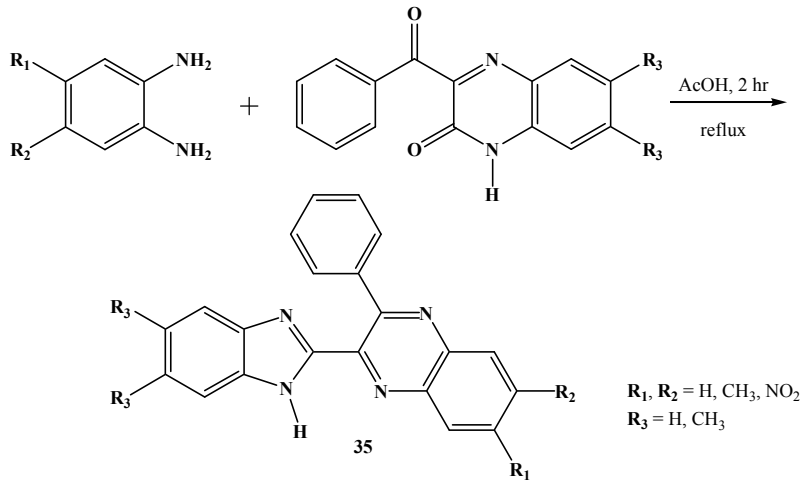


Scheme 1.9: Benzimidazoles from *β*-diketones.

1.2.1.5 Benzimidazoles from the reaction of *o*-arylene diamines with quinoxalin-2-one derivatives

Novel benzimidazoles were obtained from the reaction between *o*-arylene diamines and quinoxalin-2-one derivatives under reflux in acetic acid for 2 hr to afford 2-benzimidazolylquinoxaline derivatives, **35**. Good yield of **35** was obtained,

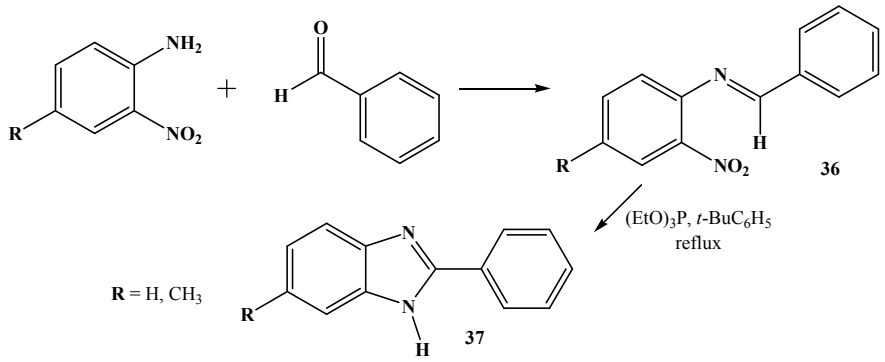
which depends on the position of R in *o*-phenylenediamine (Mamedov *et al*., 2008); (Scheme 1.10).



Scheme 1.10: Preparation of benzimidazoles using the method of Mamedov *et al*.

1.2.1.6 Benzimidazoles from *o*-nitroarylamines

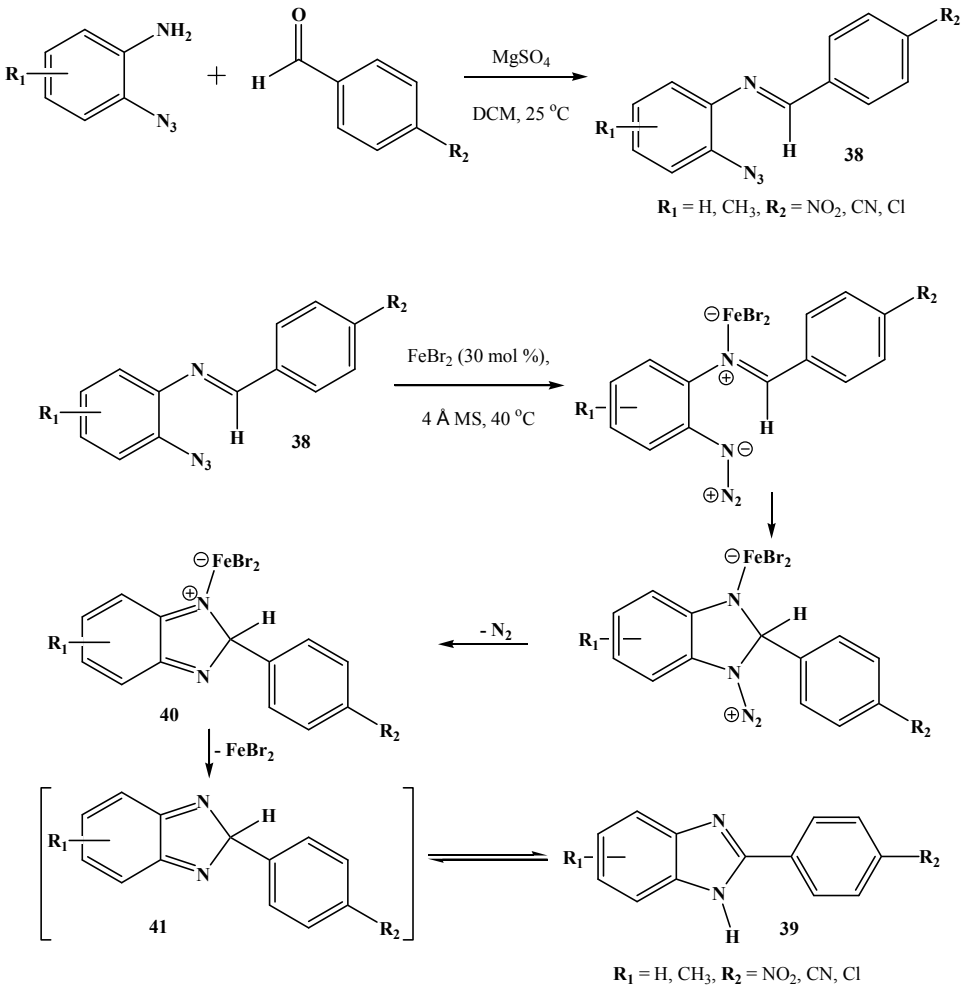
The reaction between *o*-nitroarylamines and aldehydes forms *N*-benzylidene-2-nitroaniline derivatives **36**, which was converted to 2-substituted benzimidazole derivatives **37** by the reductive cyclization using trialkyl phosphite (Smith and Suschitzky, 1961; Scheme 1.11). The trialkyl phosphite which acts as a reducing agent reduced the nitro group, followed by intramolecular cyclization process to produce **37** (Grimmett, 1997).



Scheme 1.11: Benzimidazoles from *o*-nitroarylamines.

1.2.1.7 Benzimidazoles from *o*-azidoanilines

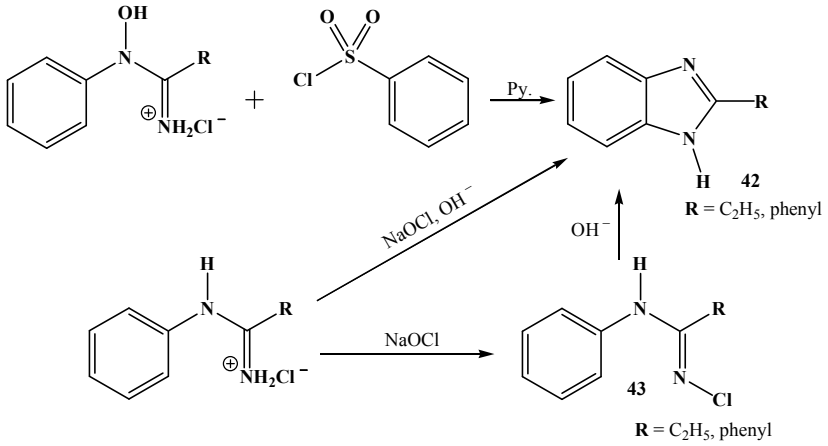
Thermolysis method is used to react *o*-azidoarylamines with aldehydes to produce good yields of *N*-benzylidene-2-azidoaniline derivatives, **38**, which were converted to 2-substituted benzimidazole derivatives, **39** (Krbechek and Takimoto, 1964; Hall and Kamm, 1965). Recently, Shen and Driver (2008) synthesized benzimidazoles from *o*-azidoanilines in two steps; using $MgSO_4$ in dichloromethane (DCM) at room temperature for 36 hr, followed by the addition of 30 mol % $FeBr_2$ as a catalyst with 4 *Å* molecular sieves in DCM at 40 ^oC for 12 hr (Scheme 1.12). Ferrous bromide, a Lewis acid was coordinated to the imine nitrogen to increase the electronegativity of **38**, followed by the reduction to generate **40**, which dissociated from iron catalyst to produce **41**. The tautomerisation of **41** gave **39**.



Scheme 1.12: Benzimidazoles from *o*-azidoarylamines.

1.2.1.8 Benzimidazoles from amidines

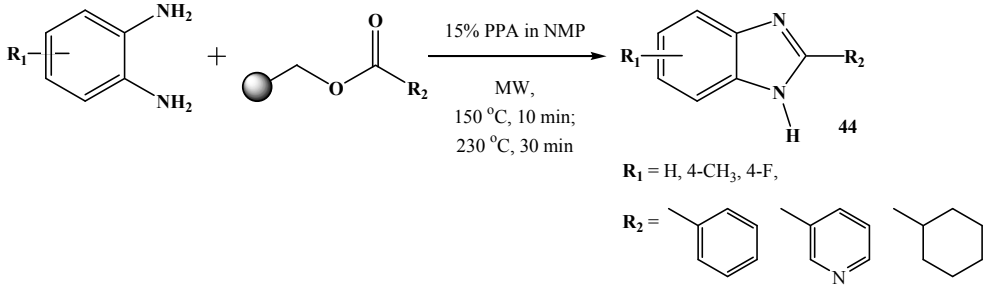
There are two ways of synthesizing benzimidazole derivatives **42** from amidines; i) by the reaction of *N*-arylamidines with benzenesulfonyl chloride in a base under anhydrous conditions (Preston, 1974), ii) by the oxidation of the amidines using sodium hypochlorite in basic media *via N*-chloroamidines **43** (Grenda *et al*., 1965); (Scheme 1.13).



Scheme 1.13: Benzimidazoles from amidines.

1.2.1.9 Benzimidazoles from resin-bound esters

Lim *et al*. (2008) prepared 2-substituted benzimidazoles **44** from the condensation process between *o*-arylene diamines and resin-bound esters under microwave irradiation condition in the presence of PPA and *N*-Methyl-2-pyrrolidone (NMP) at 150 ^{o}C for 10 min and then 230 ^{o}C for 30 min to give an excellent yield (Scheme 1.14).

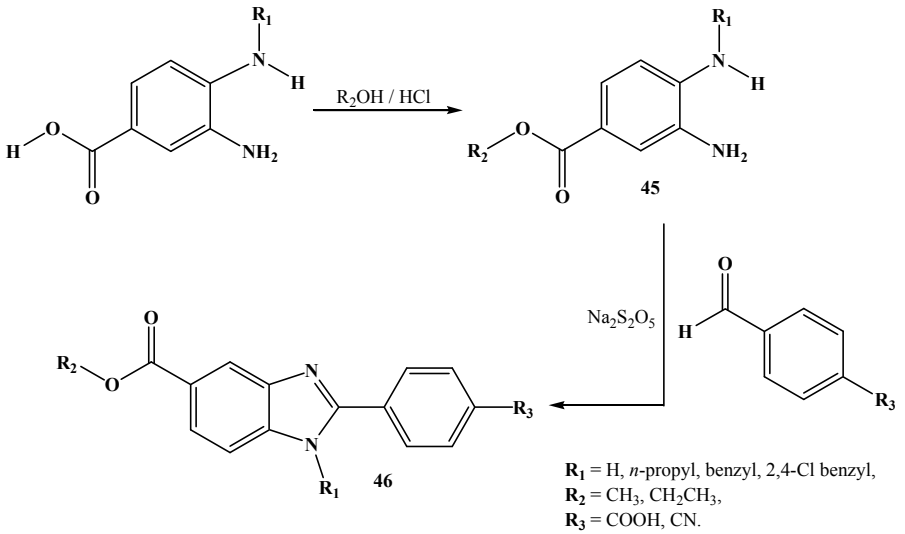


Scheme 1.14: Synthesize benzimidazoles by MW assay using the method of Lim *et al*.

1.2.2 1,2-Disubstitution of benzimidazoles

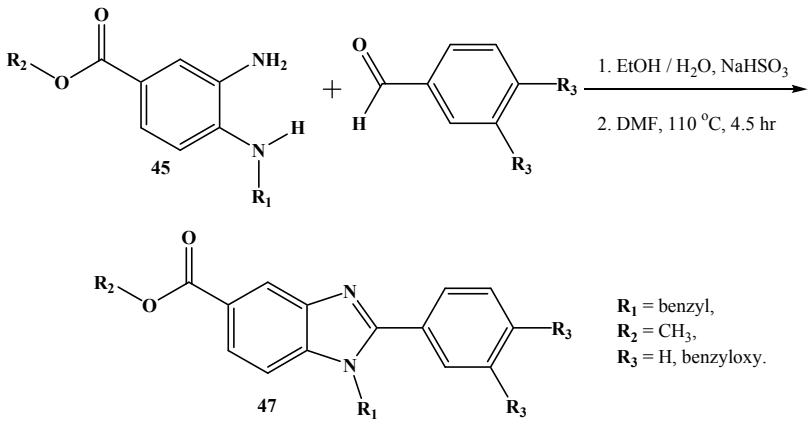
1.2.2.1 Benzimidazoles from *o*-arylene amines

The protection of one of the amino groups in *o*-arylene amines produced a derivative of *N*-substituted *o*-arylenediamine **45**. A cyclization of **45** with the second molecule of aldehyde derivatives in the presence of $Na_2S_2O_5$ as an oxidizing reagent produced the benzimidazole derivative **46** (Ozden *et al*., 2005); (Scheme 1.15).

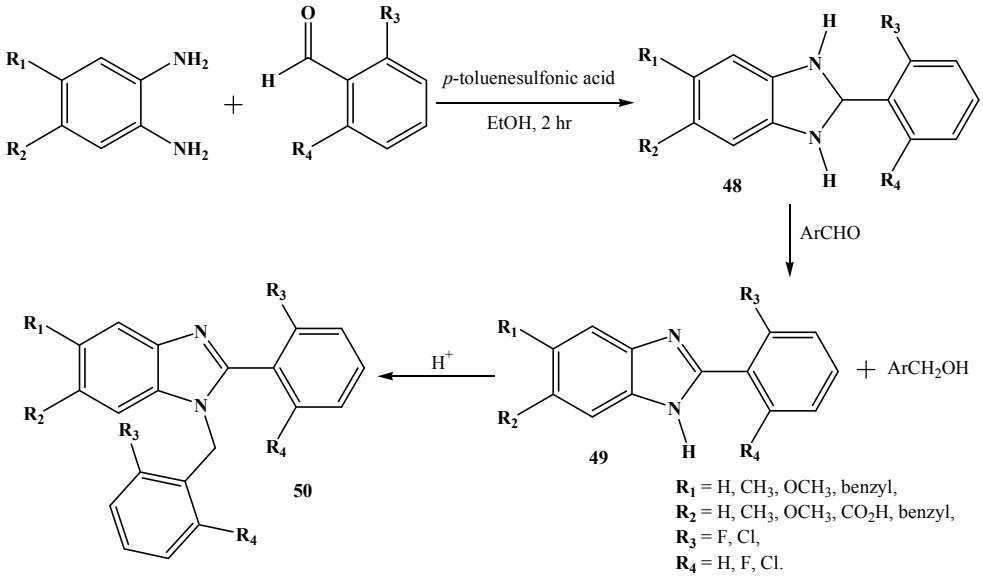


Scheme 1.15: Synthesize benzimidazoles with $Na_2S_2O_5$ using the method of Ozden *et al*.

Kilcigil *et al*. (1999, 2003) used oxidative condition by adding $NaHSO_3$ in DMF at 110 ^oC for 4.5 hr to prepare the benzimidazoles **47** from **45** (Scheme 1.16). Both **46** and **47** were evaluated as having antimicrobial activity against for *Candida albicans*. The reaction of two moles of aldehyde derivatives with one mole of *o*-arylene amine in the presence of a catalytic amount of *p*-toluenesulfonic acid produces 2-arylbenzimidazoline **48**, which is converted to alcohols and 2-arylbenzimidazole derivative **49** by redox process. Benzimidazole derivative **50** is generated when **49** is heated in ethanolic solution. The product was then tested as an anti-HIV agent (Rao *et al*., 2002); (Scheme 1.17).

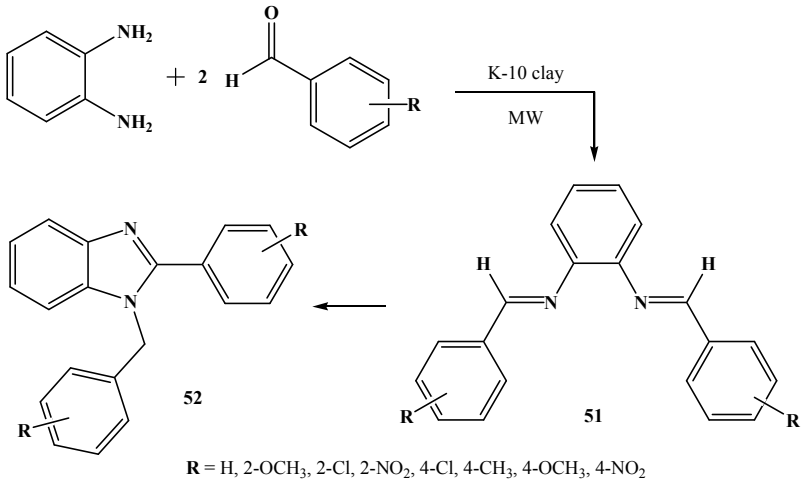


Scheme 1.16: Synthesize benzimidazoles with $NaHSO_3$ using the method of Kilcigil *et al*.



Scheme 1.17: Synthesize benzimidazoles with *p*-toluenesulfonic acid using the method of Rao *et al*.

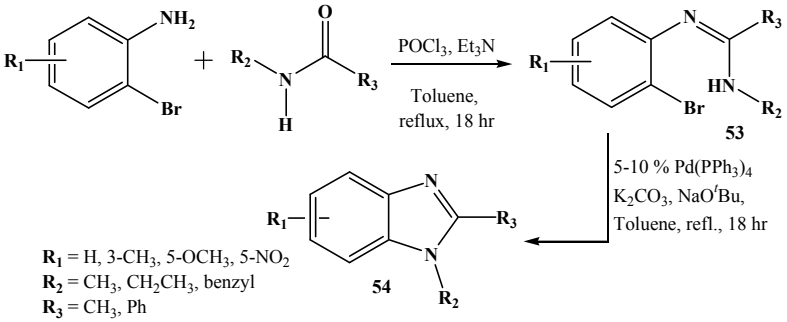
In another method, Perumal *et al*. (2006) reacted two moles of various aldehydes with *o*-phenylenediamines in the presence of montmorillonite K-10 under microwave irradiation and in the absence of solvent to produce bis-Schiff bases **51**, which is cyclized thermally to benzimidazole derivatives **52**; (Scheme 1.18).



Scheme 1.18: Benzimidazoles from MW irradiation by K-10 clay.

1.2.2.2 Benzimidazoles from *o*-haloarylamines

The amidation of *o*-haloarylamine to **53** occurred by heating a mixture of 2-bromoanilines with the amides in the presence of $POCl_3$ in toluene. It was then treated with 5–10% $Pd(PPh_3)_4$ catalyst mixed with NaO^tBu and K_2CO_3 in toluene for 18 hr under reflux to yield benzimidazole **54** (Brain and Brunton, 2002); (Scheme 1.19).

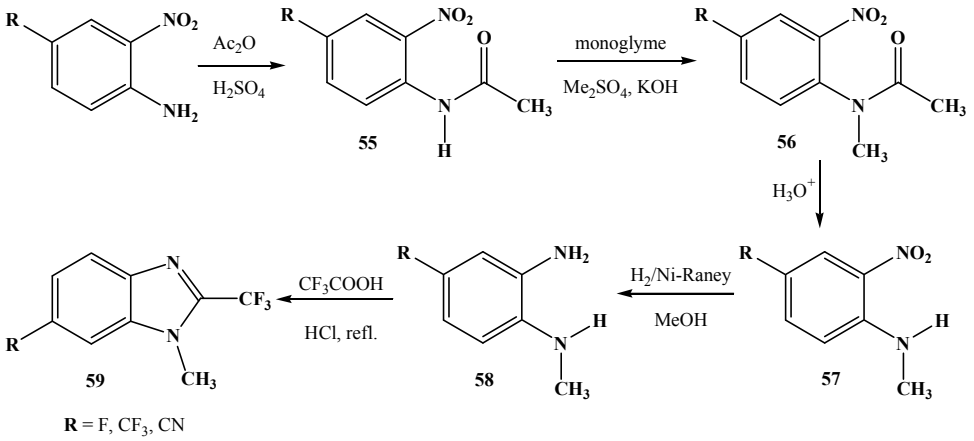


Scheme 1.19: Synthesize benzimidazoles with 5-10% $Pd(PPh_3)_4$ using the method of Brain and Brunton.

1.2.2.3 Benzimidazoles from *o*-nitroarylamines

The acetylation of *o*-nitroarylamines with Ac_2O using H_2SO_4 as catalyst produced the acetanilide **55**, which was treated with Me_2SO_4 and KOH to form *N*-methylated acetamide **56**. Addition of H_2SO_4, hydrolyzed **56** to *N*-methyl-2-

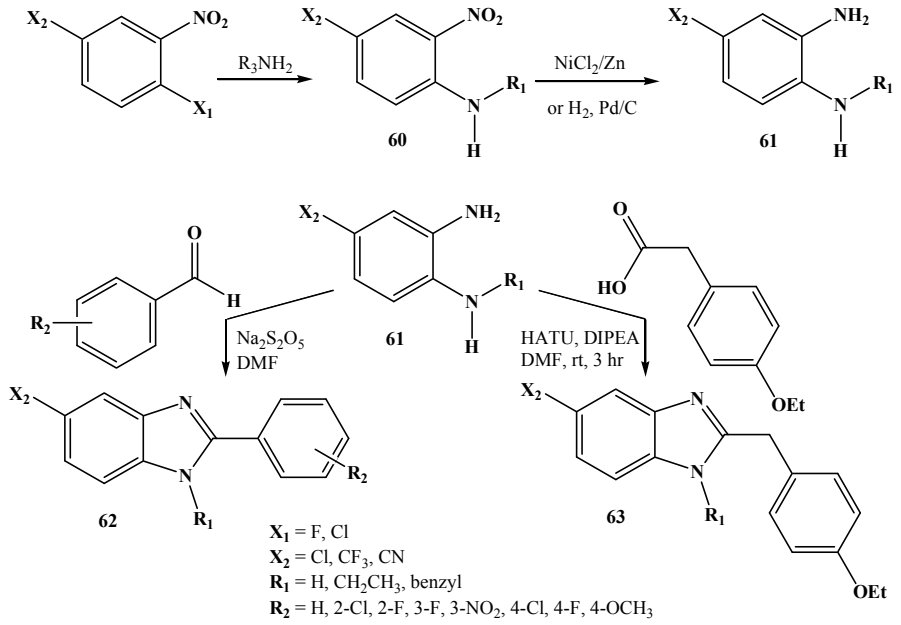
nitroaniline, **57**, which was reduced by H_2 and Ni-Raney to afford *o*-phenylenediamine **58**. The benzimidazole **59** was produced by refluxing **58** with CF_3COOH. Compound **59** showed antiparasitic activity against *Giardia intestinalis*, *Trichomonas vaginalis* and *Plasmodium falciparum* (Vazquez *et al*., 2006); (Scheme 1.20).



Scheme 1.20: Synthesize benzimidazoles using the method of Vazquez *et al*.

1.2.2.4 Benzimidazoles from *o*-halonitrobenzenes

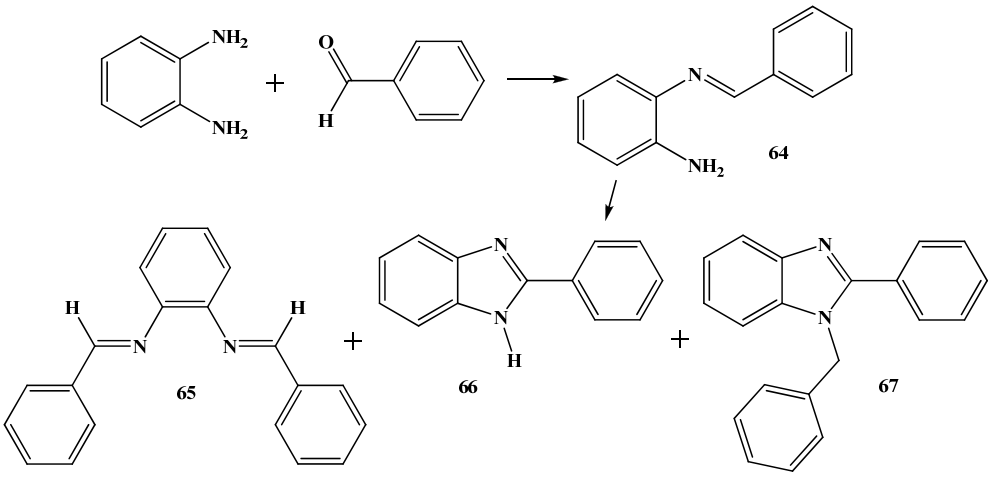
Many researchers have reported the methods to synthesize benzimidazoles **62** and **63** starting from *o*-halonitrobenzene derivatives, which was then aminated to produce *o*-nitroarylamine **60**. The reduction of **60** with $NiCl_2$/Zn or with H_2 and Pd/C afforded *o*-phenylenediamine derivative **61**. The cyclization of **61** with corresponding benzaldehydes in the presence of $Cu(AcO)_2/H_2S$, or in the presence of $Na_2S_2O_5$ in DMF gave benzimidazoles **62** and **63** (Goker *et al*., 1998, 2002). However, using carboxylic acid derivatives in the presence of *N*,*N*,*N*`,*N*`-tetramethyl-*O*-(7-azabenzotriazole-1-yl) uranium hexafluorophosphate (HATU) as a coupling reagent and a base *N*,*N*-diisopropylethylamine (DIPEA) or Hunig`s base in DMF under acidic condition, afforded benzimidazole **63** (Page *et al*., 2008; Scheme 1.21).



Scheme 1.21: Synthesize benzimidazoles using the method of Goker *et al*. and Page *et al*.

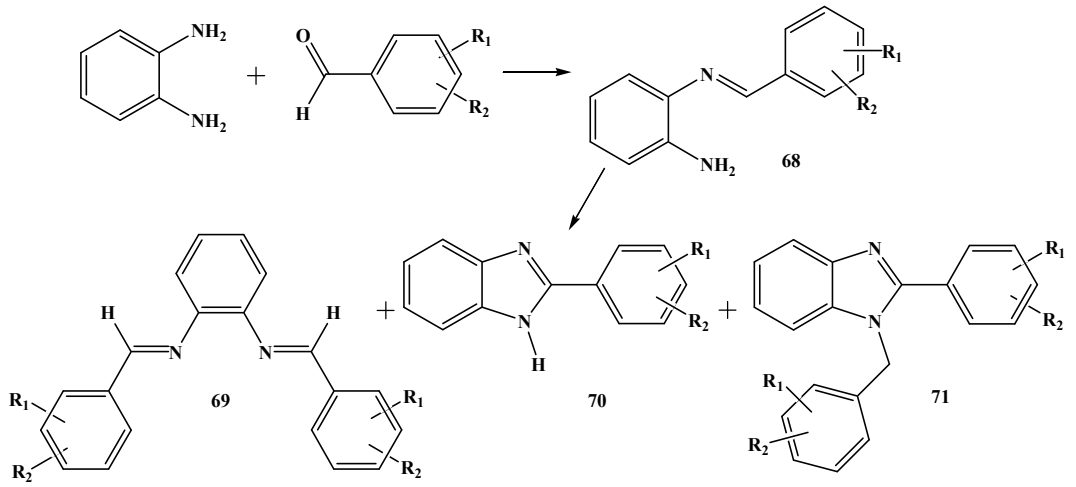
1.2.3 Synthesis leading to the formation of both Schiff base and benzimidazoles

Smith and Ho (1971) described the reaction of *o*-phenylenediamine with benzaldehyde by oxidative process in their preparation of 2-amino-*N*-benzylidene aniline, **64**, *N*,*N*`-dibenzal-*o*-phenylenediamine, **65**, 2-phenylbenzimidazole, **66** and 1-benzyl-2-phenylbenzimidazole, **67** (Scheme 1.22).



Scheme 1.22: Synthesize Schiff bases and benzimidazoles using the method of Smith and Ho.

Latif *et al*. (1983) reported in great detail the reaction of some phenolic aldehydes with *o*-phenylenediamine in boiling ethanolic solution, and they isolated the intermediates, **68**. Bis-Schiff bases **69** and benzimidazoles **70**-**71** produced from the reaction in most cases depended largely on the substituents in the phenyl ring of the aldehyde and the solvent used in the reflux process (Scheme 1.23).



Scheme 1.23: Synthesize Schiff bases and benzimidazoles using the method of Latif *et al*.

Both methods resulted in the formation of intermediates **64** and **68** from the reaction of one molecule of aldehyde derivatives with *o*-phenylenediamine under low temperature. However, the bis-Schiff bases **65** and **69**, including the benzimidazoles **66**, **67**, **70** and **71** were formed when **64** or **68** reacted with the second molecule of aldehyde derivative under reflux with nitrobenzene or 1-butanol.

Benzimidazole **73** is an analogue of the natural products **72**, which were synthesized from benzoic acid in multiple steps by Kumar *et al*. (2002) which produced moderate yields. Other benzimidazole derivative **74** was also prepared from the condensation of benzoic acid with *o*-phenylenediamine derivative. The natural products **72** were isolated from acetone extract of *Streptomyces* sp. 517-02

(Shibata *et al*., 1993; Ueki *et al.*, 1993; Ueki and Taniguchi, 1997) and from *Streptomyces* sp. AJ956 (Sato *et al.*, 2001, Figure 1.8). Both **73** and **74** lacked of cytotoxity activity against a range of cancer cell lines (Kumar *et al*., 2002; Huang *et al*., 2006).

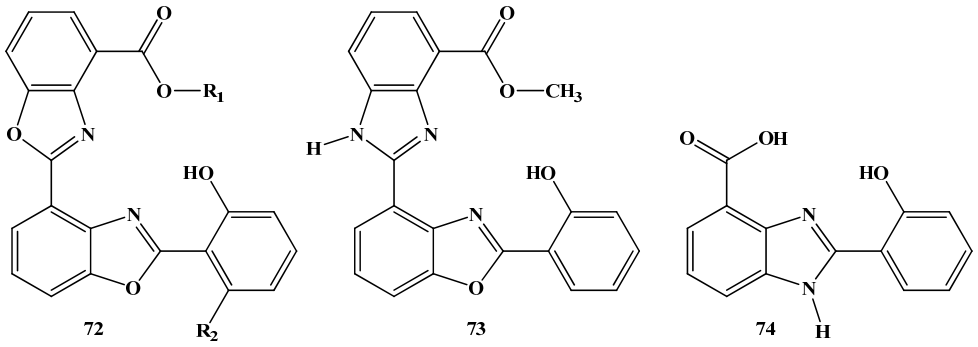
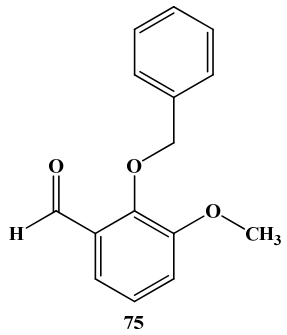


Figure 1.8: Benzimidazole derivatives evaluated against cancer cell lines.

1.3 Scope of this work

In the current project, four types of different structures were targeted. 2-Benzyloxy-3-methoxy-benzaldehyde or benzyl *o*-vanillin **75**, two of 2-amino-*N*-benzylidene benzeneamines **76** and **82**, three of bis-Schiff bases **79**, **80** and **81** and four of benzimidazoles **77**, **78**, **83** and **84** were synthesized with modified methods and characterized by FTIR, HRMS, 1D and 2D NMR spectroscopy and X–ray crystallography (Figures 1.9-1.12).

Benzyl *o*-vanillin **75** was prepared to protect the hydroxyl group of *o*-vanillin, which was benzylated using modified preparation method. The comparison of the *o*-vanillin products and its derivative **75** reactions with *o*-phenylenediamine was studied. Both aldehydes *o*-vanillin and **75** were used to prepare benzimidazole derivatives **77**, **78**, **83** and **84** *via* amino benzeneamines intermediates **76** and **82**, respectively.



75

Figure 1.9: Structure of benzyl *o*-vanillin **75**.

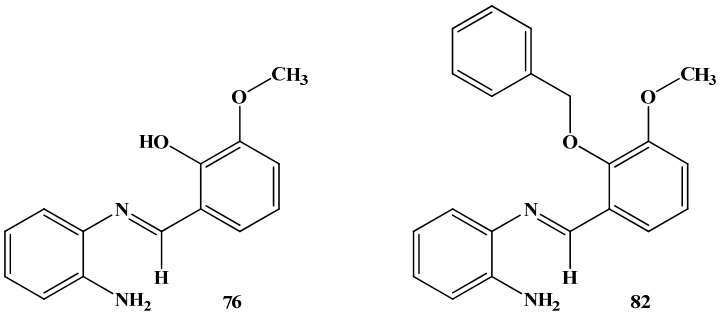


Figure 1.10: Structures of 2-amino-*N*-benzylidene benzeneamines **76** and **82**.

Bis-Schiff base **79** was also found as a main product from the reaction between *o*-vanillin with *o*-phenylenediamine, while both bis-Schiff bases **80** and **81** were prepared from the reaction of *o*-vanillin with *m*-phenylenediamine and *p*-phenylenediamine, respectively. The benzimidazoles **77**, **78**, **83** and **84** were evaluated as anti-proliferation with MTT assay.

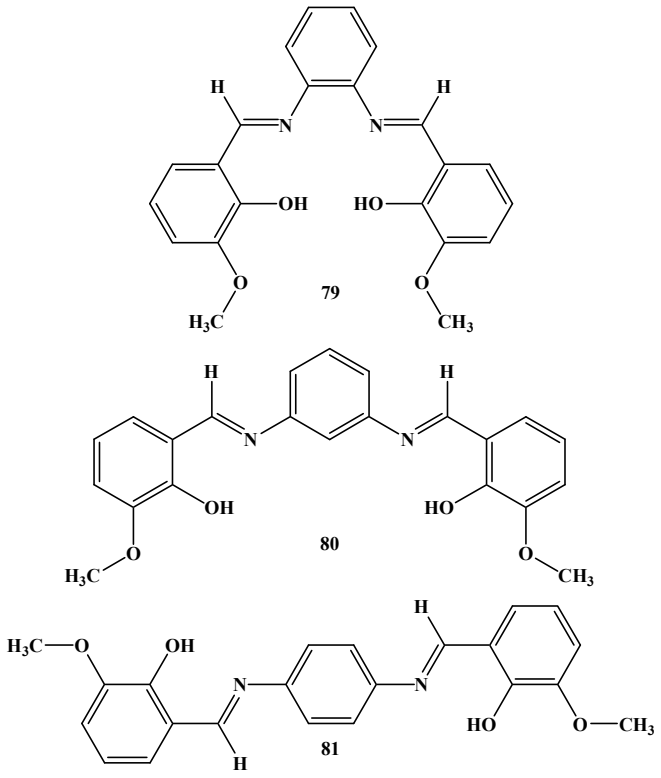


Figure 1.11: Structures of bis-Schiff bases **79**, **80** and **81**.

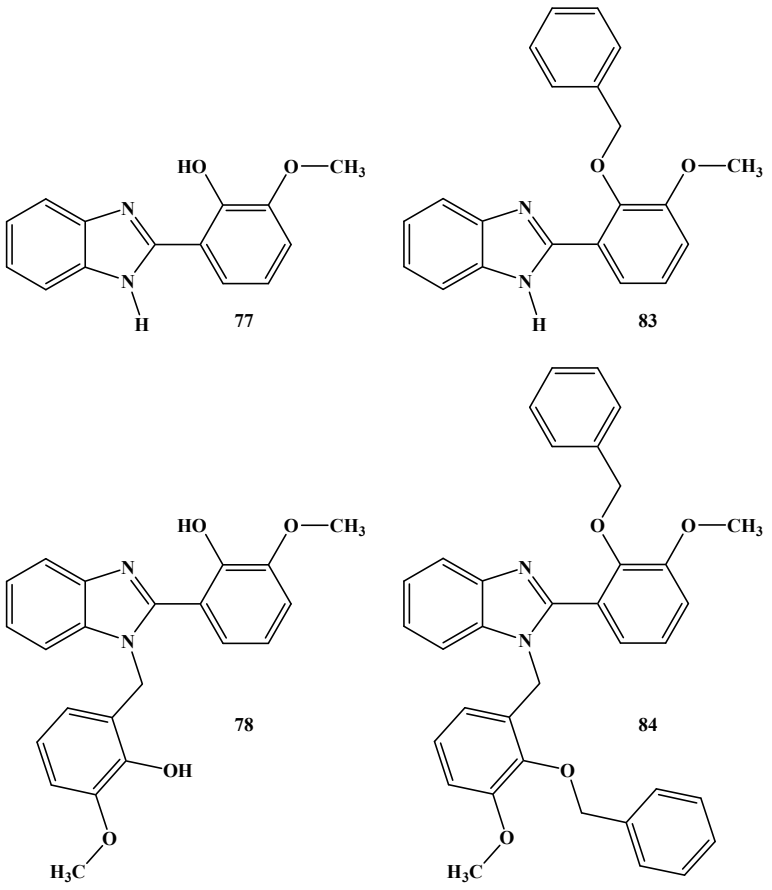


Figure 1.12: Structures of benzimidazoles **77**, **78**, **83** and **84**.

1.3.1 The objectives

The objectives of this work are listed as follows:

(a) To synthesize 2-benzyloxy-3-methoxybenzaldehyde, **75**.

(b) To synthesize both 2-amino-*N*-benzylidene benzeneamines **76** and **82**.

(c) To synthesize bis-Schiff bases, **79**, **80** and **81**.

(d) To synthesize the benzimidazole derivatives, **77**, **78**, **83** and **84**.

(e) To elucidate the structures of the synthesized compounds using FTIR, HRMS, NMR and X–ray crystallography techniques (if applicable).

(f) To evaluate the benzimidazoles **77**, **78**, **83** and **84** as anti-proliferation with MTT assay.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Chemicals

The chemicals that were used throughout the project are listed as follows, which were used without further purification:

Acetone (AR grade)	Riedel-de Haën, Germany.
Acetone-D6 (acetone-d_6) contains 0.03 v/v % TMS (for NMR spectroscopy)	Sigma-Aldrich, USA.
Anhydrous Magnesium sulfate	Fluka Chemika, Switzerland.
Benzyl bromide	Fluka Chemika, Switzerland.
Chloroform ($CHCl_3$), (AR grade)	Riedel-de Haën, Germany.
Chloroform-D ($CDCl_3$) contains 0.03 v/v % TMS (for NMR spectroscopy)	Sigma-Aldrich, USA.
Dichloromethane (DCM), (AR grade)	Riedel-de Haën, Germany.
Dichloromethane-D2 (CD_2Cl_2) contains 0.03 v/v % TMS (for NMR spectroscopy)	Sigma-Aldrich, USA.
Diethyl ether	Riedel-de Haën, Germany.
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich, USA.
Dulbecco's Modified Eagle Medium (DMEM)	Gibco, Life technology, UK.
Ethanol	Riedel-de Haën, Germany.
HCT–116	ATCC, Rockville, MD, USA.
Heat inactivated fetal calf serum (HIFCS)	Sigma-Aldrich, Germany.
n-Hexane	Riedel-de Haën, Germany.
2-Hydroxy-3-methoxybenzaldehyde (*o*-vanillin)	Sigma-Aldrich, USA.
McCoy’s (growth medium)	Gibco, Life technology, UK.