Faculty of Resource Science and Technology

SYNTHESIS OF BENZALDEHYDE DERIVATIVES AS ANTI-CANCER AGENT (LUNG CANCER) USING PHARMACOPHORE MODELLING AND IN VITRO BRINE SHRIMP ASSAY AS PRELIMINARY IDENTIFICATION OF CYTOTOXIC PROPERTIES

Nor Izzati Binti Nadir (24416)

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(Resource Chemistry)
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Nor Izzati Binti Nadir

This Final Year Project is submitted in partial fulfillment of the degree of Bachelor of Science with Honours

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DECLARATION

I hereby declare that no portion of this dissertation has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.

…….

Nor Izzati Binti Nadir

Resource Chemistry Programme

Department of Chemistry

Faculty of Resource Science and Technology

University Malaysia Sarawak (UNIMAS)

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List of Abbreviations

Small cell lung cancer	SCLC
Non-small cell lung cancer	NSCLC
Fourier Transform Infrared Spectroscopy	FTIR
Structure Based Drug Design	SBDD
Thin Layer Chromatography	TLC
Retention factor	Rf
Potassium Bromide	KBr
Hydrogen Bond Acceptor	HBA
Hydrogen Bond Donor	HBD
Hydrophobic	HY
Negative Ionisable	NI
Positive Ionisable	PI

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Synthesis of Benzaldehyde Derivatives as Anti-Cancer Agent (Lung Cancer) Using Pharmacophore Modelling and In Vitro Brine Shrimp Assay as Preliminary Identification of Cytotoxic Properties

Nor Izzati Binti Nadir

Resource Chemistry Programme
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak

ABSTRACT

Cancer is categorized as one of the dangerous diseases globally since cancer cell is rapidly developed or mutates compare to the normal cell. Rapid proliferation of cells may cause it to invade other tissues if not being removed immediately. Thus, it is necessary to find appropriate treatment for cancer patients. There are different types of cancer and this project focus on lung cancer. This project was carried out to synthesize anti cancer agent (lung cancer) from benzaldehyde and amine derivatives *via* Schiff base reaction. Compound that has been synthesized was then characterized by Fourier Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance ^{1}H NMR, elemental analysis, and Gas Chromatography-Mass Spectrometer (GC-MS). This project was assist by pharmacophore modelling in order to facilitate search of anti cancer agent. Pharmacophore model was developed using LigandScout 3.03 software. Six training set from established database search of anti-cancer (lung) drugs was selected to develop the pharmacophore model. The best hypothesis of the pharmacophore model consists of several chemical features including five hydrogen bond acceptor (HBA), one aromatic ring (AR) and one hydrophobic (HY). This best model was further validated with twenty test set from the synthesized compound. From the validation, the most active compound, (Compound 9) shows the highest fit value. The chemical feature of both compounds is such as hydrogen bond acceptor (HBA), hydrophobic (HY), and aromatic ring (AR). Cytotoxicity test using brine shrimp (*Artemia salina)* was also carried out to determine the activity of the compounds synthesized. From the result of cytotoxicity test, the lethal concentrations LC_{50} were calculated.

Keywords: Lung cancer, pharmacophore, training set, amine derivatives, *Artemia salina*

ABSTRAK

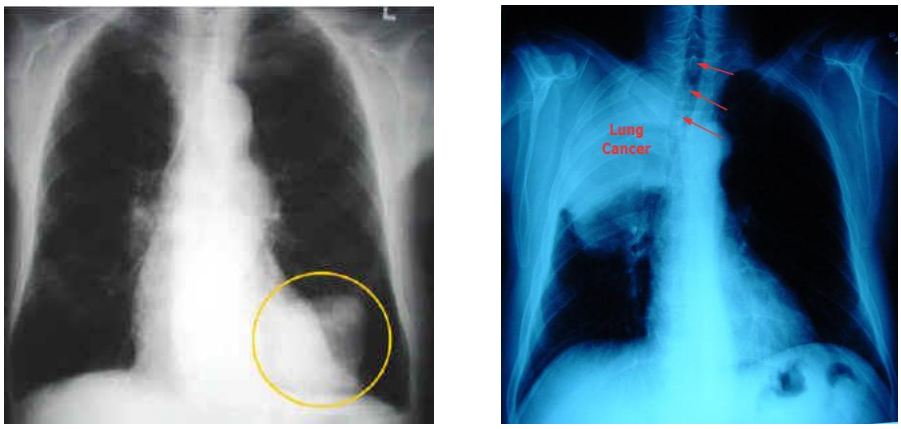
*Kanser dikategorikan sebagai salah satu penyakit paling berbahaya di dunia lantaran sel-sel kanser mengalami mutasi yang pantas. Sel yang terbentuk dengan pantas menyebabkan sel kanser merebak ke bahagian tisu-tisu yang lain sekiranya tidak segera dibuang. Oleh itu, rawatan yang tepat adalah penting bagi penghidap kanser. Projek ini memfokuskan terhadap kanser paru-paru. Projek dijalankan dengan mensintesis agen anti kanser (kanser paru-paru) daripada benzaldehyde dan dua puluh amine melalui tindakbalas Schiff base. Kompound yang telah disentesis kemudiannya diuji melalui Fourier Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance (^{1}H NMR), elemental analysis, dan Gas Chromatography-Mass Spectrometer (GC-MS). Projek ini turut mengaplikasikan pharmacophore modelling menggunakan perisian LigandScout 3.03. Enam training set tediri daripada ubatan yang telah dipasarkan dipilih untuk membentuk model pharmacophore. Hipotesis terbaik yang telah dipiih mengandungi beberapa ciri kimia seperti lima hydrogen bond acceptor (HBA) dan satu kumpulan hydrophobik (HY). Model ini kemudiannya diuji dengan dua puluh test set yang terdiri daripada kompound-kompound yang telah disentesis. Kompound paling aktif adalah kompound **9** yang mempunyai nilai yang tertinggi. Ujian kesitotoksikan turut dijalankan bagi melihat kadar aktiviti kompound yang telah disentesis. Kemudiannya, LC_{50} iaitu kadar kematian sel sebanyak 50 peratus telah ditentukan.*

Kata kunci: *Kanser paru-paru, model pharmacophore, training set, terbitan amine, Artemia salina*

1.0 Introduction

Cancer is well known as one of the dangerous diseases over the world and also second leading cause of human death after cardiovascular diseases in developing countries (Babasaheb *et al*., 2010). It is manifested by the abnormal cell proliferated rapidly. The genetic material or known as DNA (deoxyribonucleic acid) found in cell of cancer patients were damaged or altered which finally affected the normal cell growth. If the abnormal cell or tumour were not removed, it may invade the surrounding tissue of human body. Various type of cancer exists where lung cancer is one of it. Lung cancer occurs because of uncontrolled proliferation of cell in the lung. There are two types of lung cancer that are non small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) (Nisa *et al*., 2011). Some of this tumour can be removed by therapy or surgery but it might cause other side effect. Radiation therapy is one of the treatments but some side effect such as skin irritation, hair loss and cough might occur. As for chemotherapy treatment, it might cause hearing changes which occurs after the ingestion of substances toxic to the hair cells of the cochlea (Andrade *et al*., 2009). The invention of drugs also assists the cancer treatment. In recent years, some approved drugs for non small cell lung cancer are bevazicumab (avastin), cisplatin, carboplatin, erlotinib , and gefitinib.

Gefitinib was approved as monotherapy for patients that are previously treated with advanced NSCLC in Japan and USA (Onn *et al*., 2004) while cisplatin currently being one of the potent anti – tumour drugs. However, due to its nephrotoxic side effect, usage of cisplatin has been restricted. In vivo experimental studies have shown acute cytotoxic effects following cisplatin treatment, mostly affecting tubular epithelial cells (Razzaque., 2007). Therefore, this is an opportunity to design or synthesis anti- cancer agent besides using the existing treatment. Figure 1 shows images of X-Ray for lung cancer.



(a) **(b)**

Figure 1 : Images of lung cancer (a) and the image of right lung cancer with deviation of trachea (b)

1.1 Schiff Base Compounds

Synthesis of anti-cancer agent in this project was done using Schiff base reaction involving benzaldehyde with twenty primary amine. The synthesized compound were than characterized by using Fourier Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance (^{1}H NMR), elemental analysis, and Gas Chromatography-Mass Spectrometer (GC-MS).

1.2 Pharmacophore Modelling

Structure Based Drug Design (SBDD) with computational method shows that it assists to enhance progress in discovery and refinement of therapeutic agents (Marrone *et al*., 1997). Pharmacophore modelling is one of the applications of SBDD where it used training set from literature and database search to generate pharmacophore model. Pharmacophore modelling was carried out using LigandScout 3.03 software. The chemical features considered in the pharmacophore model generation are Hydrogen Bond Donor (HBD), H-bond acceptor (HBA), hydrophobic aromatic (HY), negative ionisable (NI) and positive ionisable (PI) feature (Dayam *et al*., 2008).

1.3 Objective

This project is carried out to design and synthesis anti-cancer agent (lung cancer) through Schiff base reaction and pharmacophore modelling based on training set of established drugs.

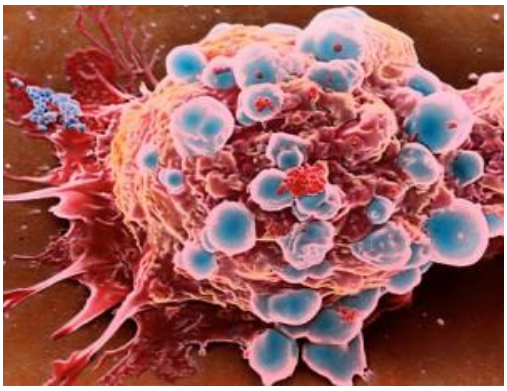
The objectives of this project are:

1. To design anti cancer agent from Schiff base containing benzaldehyde using pharmacophore modelling.
2. To synthesize the design compounds *via* Schiff base reaction based on modelling simulation outcomes.
3. To validate the synthesize compounds *via* biological evaluation (*in vitro*) cytotoxicity test using brine shrimp (*Artemia salina)* in order to determine the potential anti-cancer agents.

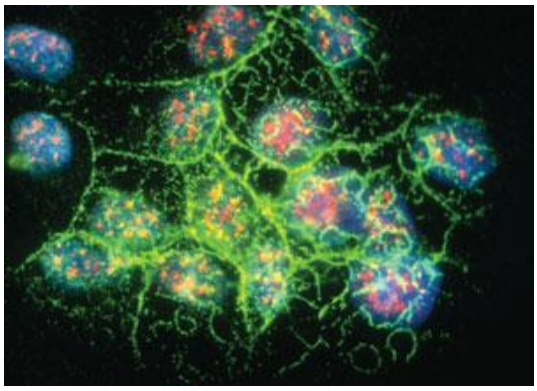
2.0 Literature Review

2.1 Lung Cancer

Lung cancer is one of the most common malignancies occurs in developed countries and it cause millions of deaths worldwide (Onn *et al*., 2004). 90 percent of lung cancer cases that occurs in the world was cause by cigarette smoking (Minna *et al*., 2002). According to Farley, in 2000, the annual incidence of NSCLC, which comprises 80% of all lung cancer cases, was 991 089 and the worldwide mortality was 882 495. To date, chemotherapy and radiotherapy are the priority treatment for patients suffering from lung cancer. Chemotherapeutic agent should only destroy the target cancer cells, however anticancer drugs with such a sparing effect on normal tissues are not yet available, thus some damage to normal tissues is inevitable, especially in part where rapid cell division normally occurs such as hair and skin (Ilgenli *et al*., 2001). Invention of anti cancer drug are also used for cancer treatment including drug such as cisplatin. However, cisplatin resistance has become major problem for its application in cancer treatment (Ghazizadeh., 2003). This resistance occurs due to detoxification of reactive oxygen species and intracellular inactivation of cisplatin. Therefore, search for new potent anti cancer drug is necessary to provide safer treatment for the patients. Figure 2a and 2b shows images of proliferation of cancer cells.



(a)



(b)

Figure 2 : Proliferation of cancer cells

2.2 Schiff Base Reaction

In this project, synthesis process was done via Schiff Base mechanism. To date, Schiff bases are widely used since it is applicable in analytical determination, using reactions of condensation of primary amines and carbonyl compounds in which the azomethine bond is formed (Ibrahim *et al.*, 2007). Due to their properties such as good solubility in common solvent and remarkable versatility, Schiff base becomes among the most widely used ligands. (Sedaghat., 2008). Schiff bases derived from aromatic amines and aromatic aldehydes have various applications, for example they are known to show potent anti bacterial and anti inflammatory properties. They also exhibit pharmacologically useful activities like anticancer (Pandeya *et al.*, 1999). Figure 3 shows the mechanism of Schiff base reaction.

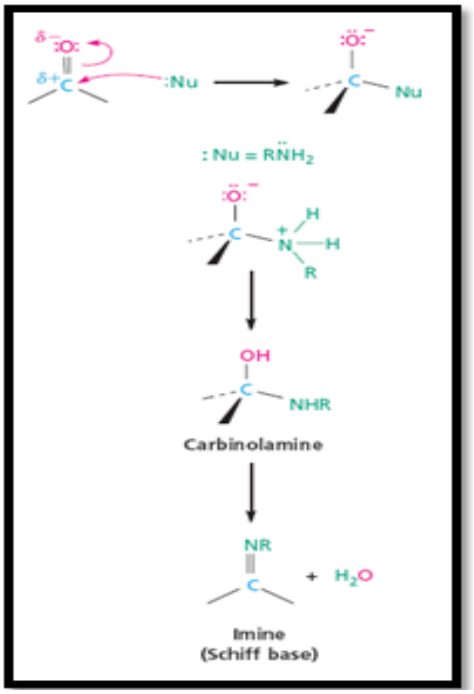


Figure 3: Mechanism of Schiff base reaction taken from (Mcmurry., 2005)

2.3 Pharmacophore Modelling

Besides synthesizing compound, pharmacaphore modelling is also used in this project in order to facilitate the design of anti cancer agent. Pharmacophore model can be considered as the ensemble of steric and electrostatic features of different compounds which are necessary to ensure optimal supramolecular interactions with a specific biological target structure and to trigger biological response (Wermuth *et al.*, 1993).

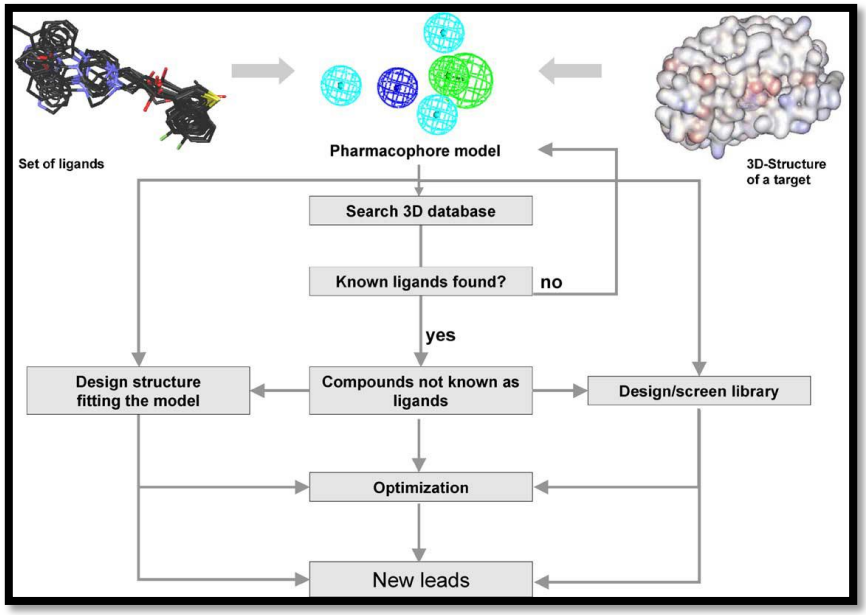


Figure 4 : Workflow of typical pharmacophore based from (Wolber & Langer., 2004)

Pharmacophore model can be established either in a ligand based manner or in a structure-based manner. Pharmacophore approaches have been used extensively in virtual screening, de novo design and other applications such as lead optimization and multitarget drug design (Sheng., 2010). Modelling is widely used since applications of 3D-pharmacophore-based has shown successful result in medicinal chemistry, thus demonstrate its utility in modern drug chemistry (Dror *et al.*, 2004 ; Lyne *et al.*, 2004). Besides that, if using the typical steps in drug discovery cycle, the duration from lead identification until clinical trials may take to 14 years and is also cost consuming. (Chun *et al.*, 2009).

Pharmacophore will generate hypothesis generation, and from this hypothesis generation, the fit value will be determined. Fit value will indicate how well the features in the pharmacophore overlap the chemical features in the molecule (Suresh *et al*., 2010). The top rank of the pharmacophore hypothesis will be selected where it will be used for database search (Mustapha *et al*., 2011). The first model (Model 1) with the highest fit value will be choosen as the best pharmacophore hypothesis. The pharmacophore model generated will be validated using test set in order to predict the activity of the molecules accurately. Figure 5a shows image of the best pharmacophore model that has several chemimal features such as Hydrogen Bond Acceptor and Hydrogen Bond Donor while figure 5b indicate the mapping of compound onto best pharmacophore model.

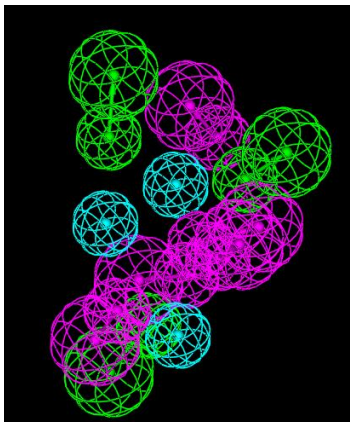


Figure 5a: Image of best pharmacophore model taken from (El Moghazy *et al*., 2011)

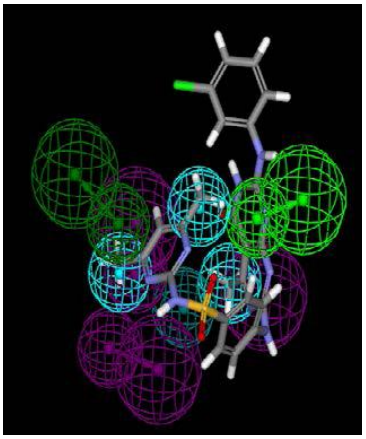


Figure 5b: Mapping of compound onto best pharmacophore model from (El Moghazy *et al*., 2011)

3.0 Materials and Method

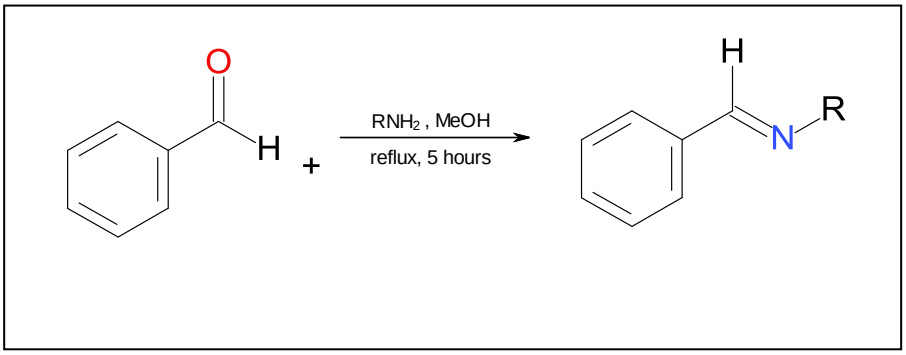
3.1 General Procedure

The syntheses of compound were done at Bioassay Laboratory of Faculty of Resource Science and Technology, Universiti Malaysia Sarawak (UNIMAS). Chemical used in this project were purchased from Fluka, Aldrich, and Merck. All synthesized compounds were characterized by Fourier Transform Infrared Spectroscopy of model Perkin Elmer Spectrum GX Fourier Transform Spectrophotometer using potassium bromide (KBR) disc , while ^{1}H NMR were run on JEOL 500 MHz using DMSO-d_6, acetone-d_4, chloroform and methanol-d_3 as solvent. The molecular weights of compound were recorded using Shimadzu Mass Spectrophotometer. Then, the elemental analysis was done using Flesh EA 1112. Thin Layer Chromatography Technique (TLC) was used to separate the mixture presence in each compound synthesized by applied different solvent polarity. The mobile phases uses were ethyl acetate and hexane as solvent system while stationary phase comprises of silica gel. The retention factor or R_f value were then calculated. Melting point of each compound synthesized was recorded on Stuart MP3. Then, cytotoxicity test was also carried out using *Artemia Salina* (Hong Da, Hai Xing country, Li Da, China).

3.1.1 Synthesis

Synthesis of compounds was done according to Schiff base reaction using solvent of analytical grade. The starting material used was benzaldehyde. Methanolic solution of 0.02 mol amine derivatives was added into methanolic solution (20 mL) of benzaldehyde 0.02 mol. The mixture was refluxed and stirs using magnetic stirrer for five hours and was then cool down. Precipitate form was collected by filtration and washed several times with methanol and then recrystallized from methanol. The reaction was monitored by TLC.

Scheme 1 shows the schematic diagram of general reaction involving Schiff base containing benzaldehyde.



Scheme 1 : Schematic diagram of Schiff base reaction

3.1.2 Synthesized of Schiff base compound

A series of benzaldehyde derivatives were synthesized via Schiff base reaction using as starting material as shown in Scheme 1 to Scheme 20.

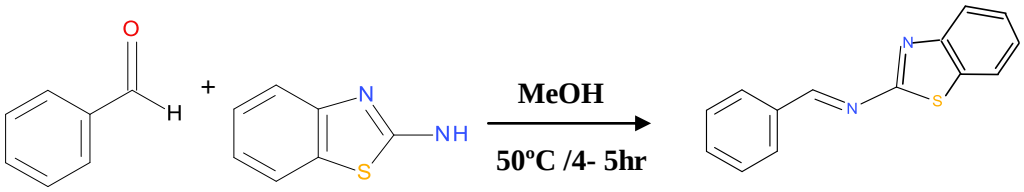
Synthesis of N-[(E)-benzylideneamino]acetamide (**1**)

Benzaldehyde Acetohydrazide (**1**)

Scheme 2: Schiff base reaction of Compound (**1**)

Methanolic solution (20 mL) of 0.01 mol acetohydrazide was added into methanolic solution (20 mL) of benzaldehyde 0.01 mol and yield N-[(E)-benzylideneamino]acetamide (**1**). The reaction yield 66.85 % and melting point was recorded at 144-147 ° C. The IR, ^{1}H NMR , GC-MS spectra and elemental analysis was displayed in appendices.

Synthesis of N-(1, 3-benzothiazol-2-yl) 1-phenyl-methanimine (**2**)

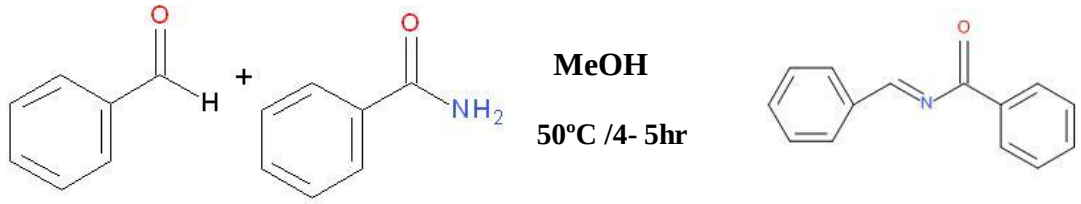


Benzaldehyde 2-aminobenzothiazole (**2**)

Scheme 3: Schiff base reaction of Compound (**2**)

Methanolic solution (20 mL) of 0.02 mol 2-aminobenzothiazole was added into methanolic solution (20 mL) of benzaldehyde 0.02 mol. The reaction yield N-(1, 3-benzothiazol-2-yl) 1-phenyl-methanimine (**2**) with percentage yield of 50.63%. Melting point was recorded at 126-129 ° C.

Synthesis of (NE)-N-benzylidenebenzamide (**3**)



Benzaldehyde Benzamide (**3**)

Scheme 4: Schiff base reaction of Compound (**3**)