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The Synthesis A Series Of 2 – Anilinophenylacetic Acid Derivativs For Their Antimicrobial Activity

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ABSTRACT

The synthesis of a series of 2-anilinophenylacetic acids, close analogue of diclofenac, is synthesized and tested for their anti-microbial activity by disk diffusion method. Each 2-anilinophenylacetic acid derivative will be screened for their anti-microbial activity. Syntheses have been carried out following simple methodology in excellent isolated yields. It has been found that compounds AB_{11}, AB_{12}, AB_{14}, AB_{21}, AB_{24}, AB_{25}, AB_{32}, AB_{34}, AB_{35}, AB_{44}, AB_{45} and AB_{54} showed the good activity against both of strain comparable to ampicillin and ciprofloxacin was taken as standard at the same concentration whereas all the other compound has showed mild to moderate anti-microbial activity as compared to standard drug. The structure and purity of the original compounds were confirmed by Melting Point, IR, TLC and elemental analysis. Each 2-anilinophenylacetic acid derivative will be screened for their anti-microbial activity. These preliminary results indicate that some of compounds are exhibiting good activity.

Keywords: 2-anilinophenylacetic acids, diclofenac, Synthesis, anti-microbial activity, disk diffusion method, Chemotherapeutic Science International

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INTRODUCTION

The main objective of medicinal chemistry is the design and the production of compounds that can be used as medicine for the prevention, treatment and cure of human or animal diseases. Taken in the retrospective sense, medicinal chemistry also includes the study of already existing drugs, of their pharmacological properties, and their structure– activity relationships. An official definition of medicinal chemistry was given by an IUPAC specialized commission. "Medicinal chemistry concerns the discovery, the development, the identification and the interpretation of the mode of action of biologically active compounds at the molecular level. Emphasis is put on drugs, but the interest of the medicinal chemistry is also concerned with the study, identification, and synthesis of the metabolic products of drugs and related compounds"[1]. Thus, medicinal chemistry covers three critical steps[2].

A discovery step, consisting of the choice of the therapeutic target (receptor, enzyme, transport group, cellular or in vivo model) and the identification (or discovery) and production of new active substances interacting with the selected target. Such compounds are usually called lead compounds; they can originate from synthetic organic chemistry, from natural sources, or from biotechnological processes.

An optimization step, that deals with the improvement of the lead structure. The optimization process takes primarily into account the increase in potency, selectivity and toxicity. Its characteristics are the establishment and analysis of structure–activity relationships, in an ideal context to enable the understanding of the molecular mode of action. However, an assessment of the pharmacokinetic parameters such as absorption, distribution, metabolism, excretion and oral bioavailability is almost systematically practiced at an early stage of the development in order to eliminate unsatisfactory candidates.

A development step, whose purpose is the continuation of the improvement of the pharmacokinetic properties and the fine tuning of the pharmaceutical properties (chemical formulation) of the active substances in order to render them suitable for clinical use. This chemical formulation process can consist in the preparation of better absorbed compounds, of sustained release formulations, of water-soluble derivatives or in the elimination of properties related to the patient's compliance (causticity, irritation, painful injections, and undesirable organoleptic properties).

Medicinal chemistry is an interdisciplinary science. It has been stated that 'medicinal chemistry concerns the discovery, the development, the identification and interpretation of the mode of action of biologically active compounds at the molecular level.' Evidently it touches all branches

of chemistry and biology. Medicinal chemistry is also treated under the term's pharmaceutical chemistry, molecular pharmacology and bio-organic chemistry.

The focus is mainly on organic medicinal substances. The organic drugs may be of natural or synthetic origin. Among the drugs from natural sources are several alkaloids, glycosides, vitamins, hormones and antibiotics. Medicinal chemistry remains a challenging science which provides profound satisfaction to its practitioner. It intrigues those of us who like to solve problems posed by nature. It verges increasingly on biochemistry and on all the physical, genetic and chemical riddles in animal physiology which bear on medicine. Medicinal chemists have a chance to participate in the fundamentals of prevention, therapy and understanding of diseases and thereby to contribute to healthier and happier life. Medicinal chemistry as an important science started less than 100yrs ago. The active principles of plants mostly alkaloids were isolated and were the starting point for synthesis.

In medicinal chemistry, the chemist attempts to design and synthesize a medicine or a pharmaceutical agent which will benefit humanity. Such a compound could also be called a "drug", but this is a word which many scientists dislike since society views the term with suspicion.[1] source may be prepared synthetically (example-: vitamins and hormones). Medicinal chemistry covers the following stages-:[2]

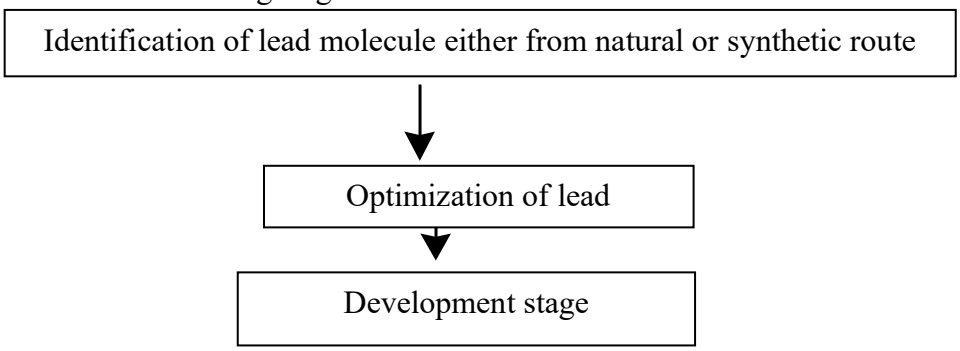


Figure 1: (Different stages of medicinal chemistry)

Drug Design:[3]

'Drug design' or 'tailor-made compound' aims at developing a drug with high degree of chemotherapeutic index and specific action.

Drug design seeks to explain:

- Effects of biological compound on the basis of molecular interaction in terms of molecular structures or precisely the physico-chemical properties of the molecules involved.
- Various processes by which the drug usually produce their pharmacological effects.

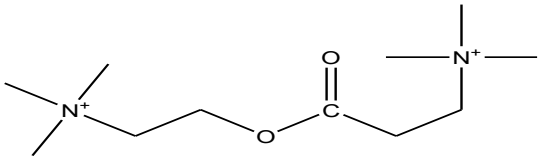
- How the drug specifically reacts with the protoplasm to elicit a particular pharmacological response.
- How the drugs usually get modified or detoxicated, metabolized or eliminated by the organism.
- Probable relationship between biological activity with chemical structure.

METHODS OF DRUG DESIGN:

Drug Design Through Conjunction Method

In this method a new drug molecule is developed from a biologically active prototype. This is known as the systemic formulation of analogues of a prototype agent, to word structurally complex produces, which may be viewed as structures embodying in a general or specific way. Certain or all of the features of the prototype. In this type of the drug design, the main principle involved is the principle of mixed moieties. A drug molecule is essentially made up with two or more pharmacophoric moieties embedded in to a single molecule or conjunction of two or more different types pharmacophoric moieties within a single molecule.

Example; Hexamethonium, promptly suggests the following design, thus embodying the ganglionic moiety and the muscarinic moiety in to a single molecule by using the structure of acetyl choline.



HEXAMETHONIUM

The internitrogen distance essentially constitutes an important facter in many series constitutes an Important factor in many series of bisquaternary salts that possess ganglionic blocking activity. The actual synthesis and pharmacological evaluation of the above hexamethyl analogue reveal the presence of both muscarine stimulant and ganglionic blocking action.

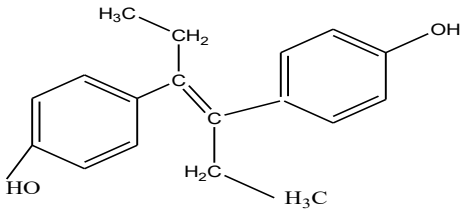
Now by using conjunction method we may take the different derivatives of coumarin. In which two pharmacophoric moieties are used. These are 4-methyl-7-hydroxy coumarin N-acyl –N-aryl thiourea attached by s- triazine.

Drug Design Through Disjunction Method:

Disjunction comes in where there is the systematic formulation of analogues of a prototype agent, in general, to ward structurally simpler products, as partial or quasi-replicas of the prototype agent. Disconjunction method usually employed in three different manners, namely:

Unjoining of certain bond Substitution of aromatic cyclic system for saturated bond Diminution of the size of the hydrocarbon portion of the parent molecule

EXAMPLE: The estrogenic activity of estradiol via drug design through disjunction ultimately rewarded in the crowning success of the synthesis and evaluation of trans-diethylstilbestrol.



trans-Diethylstilbesterul

Methods For The Design Of A New Drug[6]

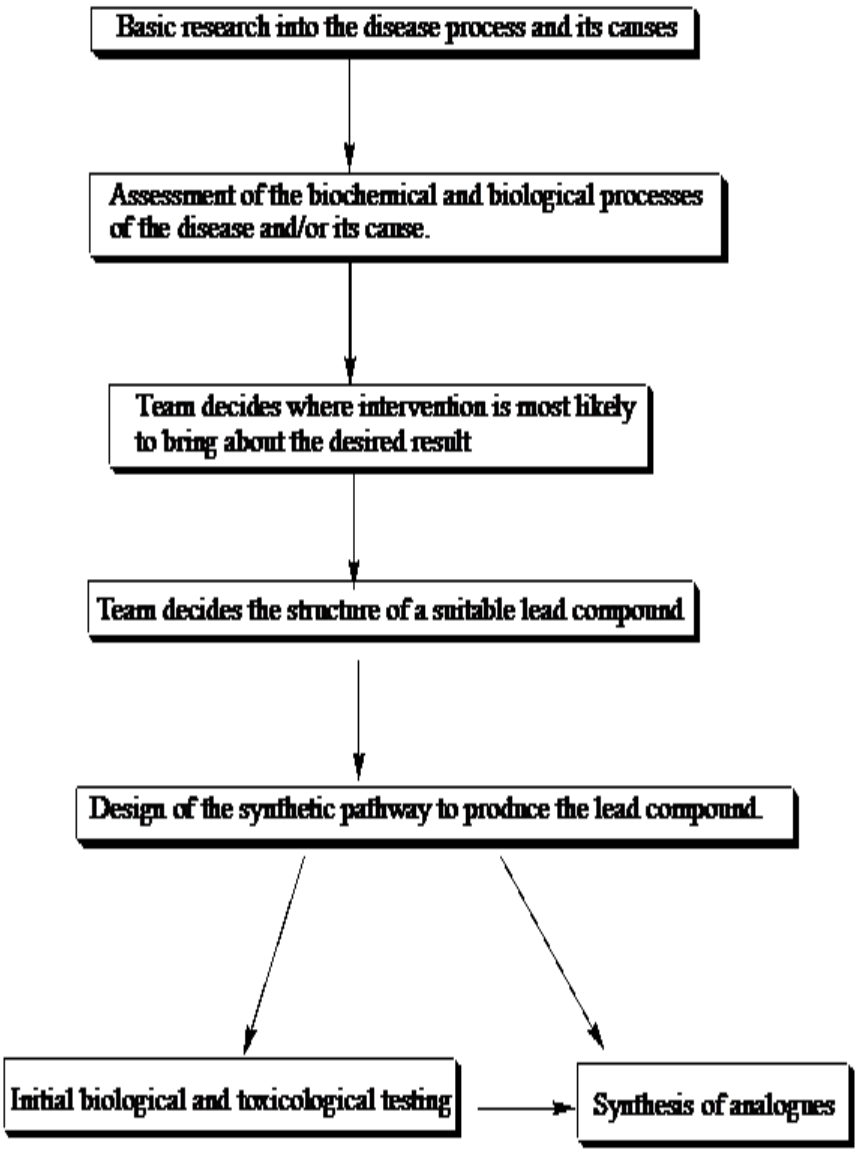


Figure:2 Methods for The Design Of A New Drug

More random approach to discovering a lead is the combinatorial chemistry approach. This uses a simultaneous multiple synthesis technique to produce large numbers of potential lead.

Antimicrobial Agents;[6]

An antimicrobial is a substance that kills or inhibits the growth of microbes such as bacteria, fungi, or viruses. it is a general term that refers to a group of drugs that includes antibiotics, antifungals, antiprotozoals and antivirals and used to treat a microbial infection. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbistatic).

Antimicrobial agents are.

Antibacterial:

Antibacterial antibiotics normally act by either making the plasma membrane of bacteria more permeable to essential ions and other small molecules by ionophoric action or by inhibiting cell wall synthesis. Those compounds that act on the plasma membrane also have the ability to penetrate the cell wall structure. In both cases, the net result is a loss in the integrity of the bacterial cell envelope, which leads to irreversible cell damage and death. Ionophoric antibiotic action Ionophores are substances that can penetrate a membrane and increase its permeability to ions. They transport ions in both directions across a membrane.

Consequently, they will only reduce the concentration of a specific ion until its concentration is the same on both sides of a membrane. This reduction in the concentration of essential cell components of a microorganism is often sufficient to lead to the destruction of the organism.

Ionophores are classified as either channel or carrier ionophores. Channel ionophores form channels across the membrane through which ions can diffuse down a concentration gradient. The nature of the channel depends on the ionophore, for example, gramicidin A channels are formed by two gramicidin molecules, N-terminus to N-terminus, each molecule forming a left-handed helix. Carrier ionophores pick up an ion on one side of the membrane, transport it across, and release it into the fluid on the other side of the membrane. They usually transport specific ions. For example, valinomycin transports Kþ but not Naþ Liþ ions.

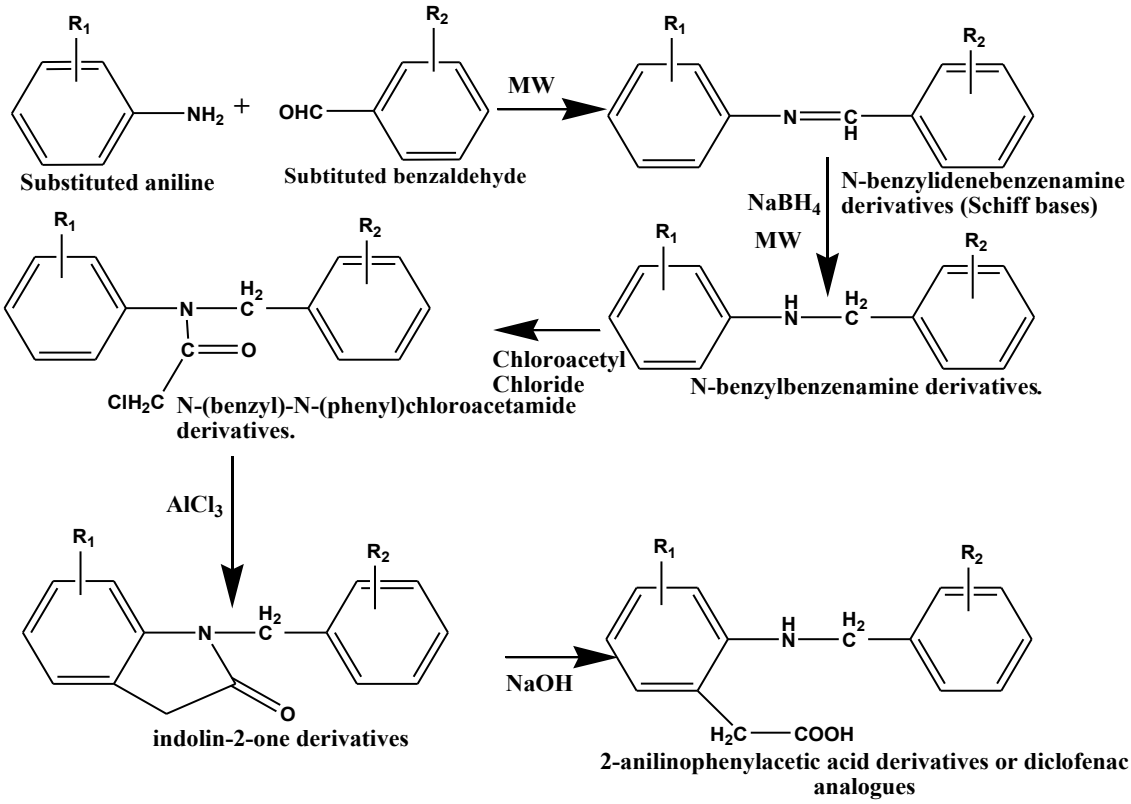
The activity of the penicillin's and Cephalosporins is believed to be due to the B-lactam ring. Bacterial resistance to these drugs is thought to be mainly due to inactivation of the drug by hydrolysis of the B-Lactum ring by the B-lactamases produced by the bacteria. Both Gram-positive and Gram-negative bacteria produce B-lactamases. In the former case, the enzyme is liberated into the medium surrounding the bacteria, which results in the hydrolysis of the B -Lactum ring before the drug reaches the bacteria.

EXPERIMENTAL SECTION

General Synthetic Scheme:

The synthesis of a series of 2-anilinophenylacetic acids, close analogue of diclofenac, is described. These syntheses scheme was proceed in five step reactions. The products of these five step reaction are as such.

1. 2-anilinophenylacetic acid derivatives or diclofenac analogues.
2. 2- indolone derivatives.
3. N-(benzyl)-N-(phenyl)chloroacetamide derivatives.
4. N-benzylbenzenamine derivatives.
5. N-benzylidenebenzenamine (Schiff bases) derivatives.



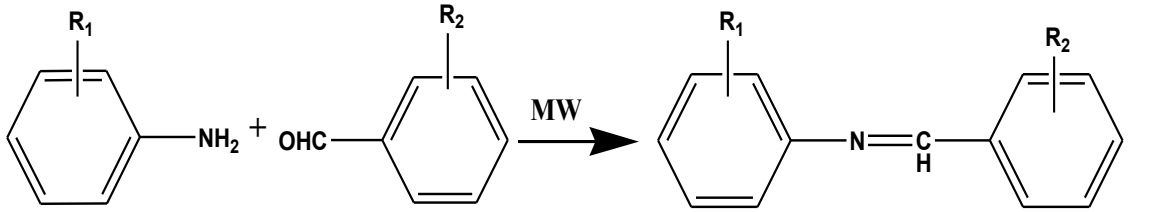
All of these, four compounds are having pharmacological and synthetic interest so the literature is review for 2-anilinophenylacetic acid, 2-indolone, N-benzylbenzenamine, N-benzylidenebenzenamine (Schiff Bases) derivatives. Therefore, we are able to get more pharmacological active compounds.

General method for synthesis:

The synthesis of a series of 2-anilinophenylacetic acids was preceded in five step reactions. The steps of reaction are as follows.

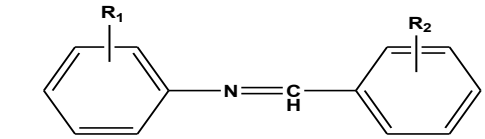
Synthesis of N-benzylidenebenzenamine derivatives-:

N-benzylidenebenzenamine derivatives are the product of substituted benzaldehyde and anilines. This reaction was done in solvent less condition by the help of microwave. In These procedures, Power and radiation time was optimized with the help of thin layer chromatography. All Shiff bases were recrystallized in methanol and checked for melting point and R_f value.

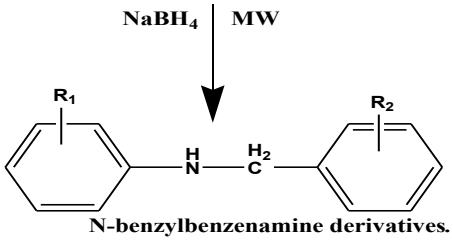


Substituted aniline Subtituted benzaldehyde N-benzylidenebenzenamine derivatives (Schiff bases)

Synthesis of N-benzylbenzenamine derivatives -: N-benzylbenzenamine derivatives are reduced products of N-benzylidenebenzenamine derivatives. This reaction was microwave assisted solid phase synthesis. In this synthesis scheme, $NaBH_4$-wet silica gel [The reagent, 10% $NaBH_4$-silica, is prepared by mixing $NaBH_4$ (0.5 g) with silica gel 60- 120 mesh (4.5 g) in solid state using a pestle and mortar] was used as solid phase as well as reducing agent. $NaBH_4$-wet silica gel was used for reductive amination of carbonyl compounds, because $NaBH_4$-wet clay has been reported as best material for reductive amination. Writer justified that clay gave best result because Clay not only behaves as an acid but also provides water from its inter layers that is responsible for the acceleration of the reducing ability of $NaBH_4$ abhi project sodium. Silica gel also contains all these properties remington which accelerate the reaction. A Silica bath (Silica gel 60- 120 mesh: 50 g, Petri dish 6.5 cm diameter) was used as a heat sink inside the MW oven to irradiate the reaction mixtures in all experiments. In these procedures, Power and radiation time was optimized with the help of thin layer chromatography. All reduced products were extracted with methanol for three successive times. The methanol was evaporated and solid reduced product was obtained. This crude product contains sodium borohydride as an impurity. That's why this crude was treated with water and reduced product was extracted with Di ethyl ether. The ether was evaporated and reduced product was remained in vessel. All reduced products were recrystallized (Except Ab_{22}) in methanol and checked for melting point (Except Ab_{22}) and R_f value.

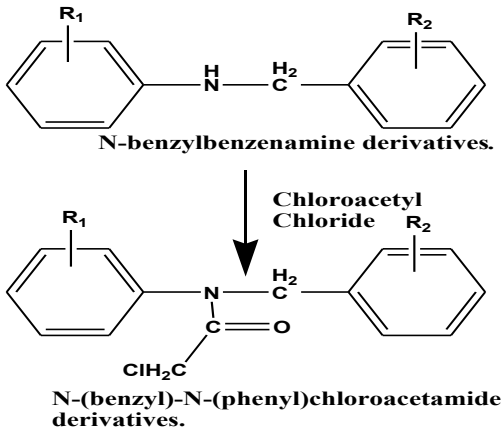


N-benzylidenebenzenamine derivatives (Schiff bases)



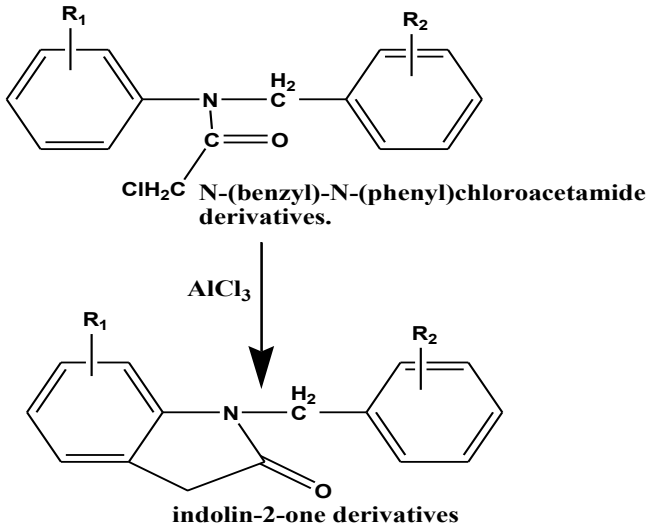
N-benzylbenzenamine derivatives.

Synthesis of N-(benzyl)-N-(phenyl)chloroacetamide derivatives-: N-(benzyl)-N-(phenyl)chloroacetamide derivatives are chloroacetylated products of N-benzylbenzenamine derivatives. This rection was done in three necked round bottom (RBF) flask fitted with two dropping funnels and a mechanical stirrer. N-benzylbenzenamine derivative was dissolved in ethyl methyl ketone (MEK) and placed in to the RBF. The chloroacetyl chloride was dissolved in MEK and placed in a fitted dropping funnel. A 10% solution of sodium carbonate was placed in to another dropping funnel. Reaction was done in cool condition at 5-10ºC. Chloroacetyl chloride was mixed in to reaction vessel with constant steering. pH of reaction mixture was maintained at 8-10 with the help of sodium carbonate solution. After completion of addition ice bath was removed and reaction vessel was placed at room temperature with stirring for 2 hours. After completion of reaction, reaction mixture was placed in to a separating funnel. Product containing MEK layer was separated. This material was washed with water. And clean MEK solution was placed in anhydrous potassium carbonate containing iodine flask. After 24 hours, MEK solution was decant off and remaining MEK solution was placed on water bath for evaporating the MEK. After completion of evaporation, 1 ml diethyl ether was poured in to the flask and solid product was obtained. Product was recrystallized with acetone and checked for melting point and R_f value.



N-(benzyl)-N-(phenyl)chloroacetamide derivatives.

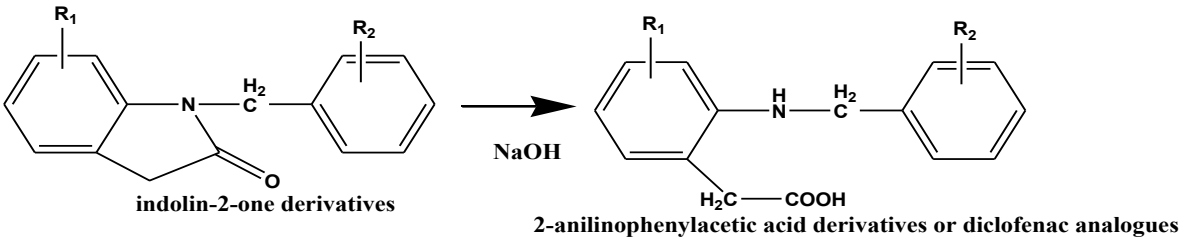
Synthesis of 2- indolone derivatives-: 2- indolone derivatives are friedalcraft cyclized products of N-(benzyl)-N-(phenyl)chloroacetamide derivatives. This reaction was done in presence of anhydrous $AlCl_3$ in anhydrous condition. For the Frieda craft cyclization chloroacetylated product was dissolved in nitrobenzene in two necked round bottom flasks. A magnetic bead was placed in to RBF. This assembly was fixed over magnetic stirrer. $AlCl_3$ was mixed in reaction mixture with successive addition. After completion of $AlCl_3$ mixing, this reaction mixture with an air condenser was placed over water bath for one hour. Then this reaction vessel placed over oil bath at 170 ºC. Reaction time was varied derivative to derivative. After cyclization, 20 g of acidified crushed ice was added in to RBF for decomposition of $AlCl_3$. After 30 min. this reaction mixture was assembled for steam distillation to remove nitrobenzene. After removal of nitrobenzene, reaction vessel contained the solid material of 2- indolone derivative. 2- indolone derivative was separated by filtration (In some condition it was extracted by diethyl ether). Filtrate was washed with water. Then recrystallized by diethyl ether and checked for melting point and R_f value.



N-(benzyl)-N-(phenyl)chloroacetamide derivatives.

indolin-2-one derivatives

2-anilinophenylacetic acid derivatives or diclofenac analogues-: 2-anilinophenylacetic acid derivatives are hydrolyzed products of 2- indolone derivatives. For hydrolysis of 2- indolone derivatives 5N NaOH solution was used for ring breaking. For completion of reaction 2-indolone derivative was dissolved in ethanol and 10 ml 5N NaOH solution was mixed in it. It was refluxed for 3 hours. Then ethanol was removed by distillation. Remaining aqueous solution was filtered then acidified with dilute HCl. After achieving acidic pH 2-anilinophenylacetic acid derivative was precipitated out. It was filtered then filtrate was recrystallized by diethyl ether and checked for melting point and R_f value.

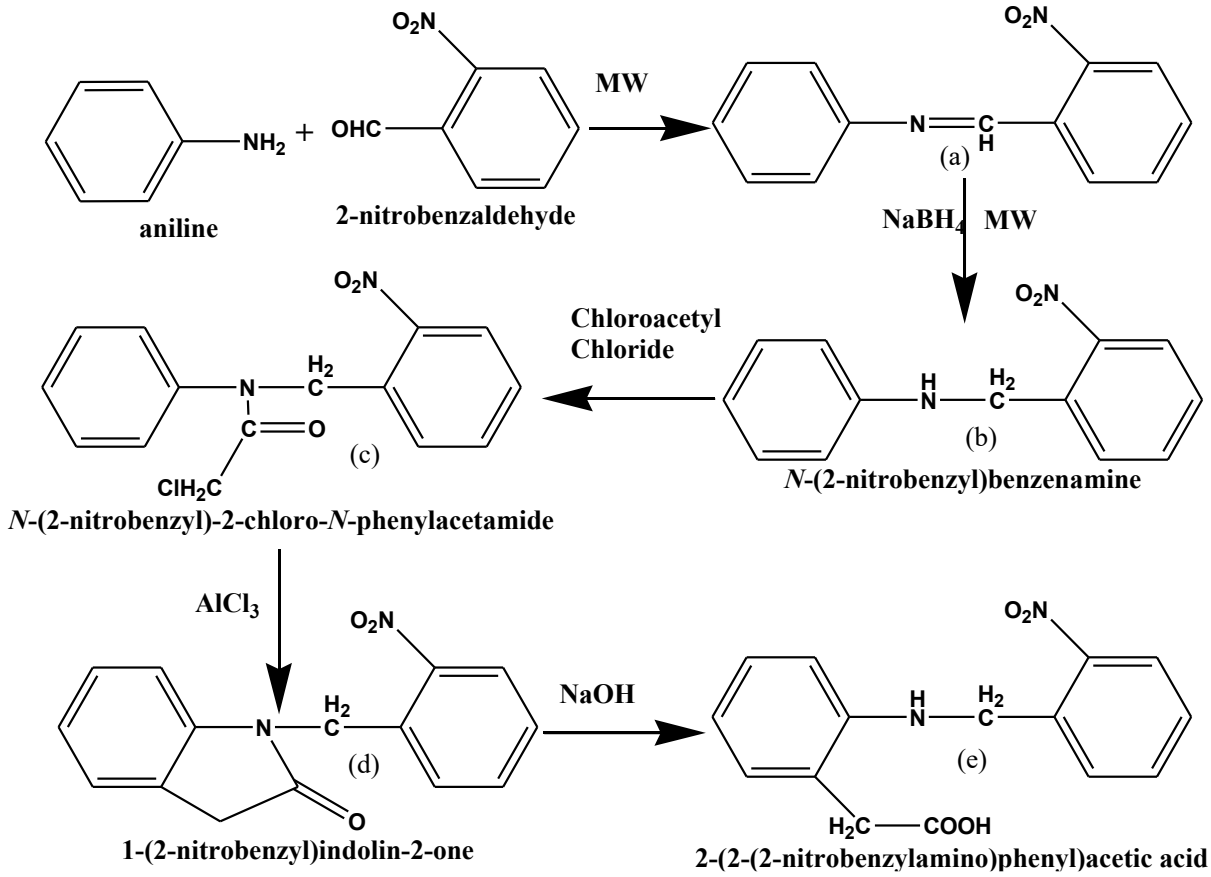


indolin-2-one derivatives

2-anilinophenylacetic acid derivatives or diclofenac analogues

Synthesis of 2-anilinophenylacetic acid derivatives

Synthesis of 2-(2-(2-nitrobenzylamino) phenyl) acetic acid (AB_{15})



aniline

2-nitrobenzaldehyde

N-(2-nitrobenzyl)benzenamine

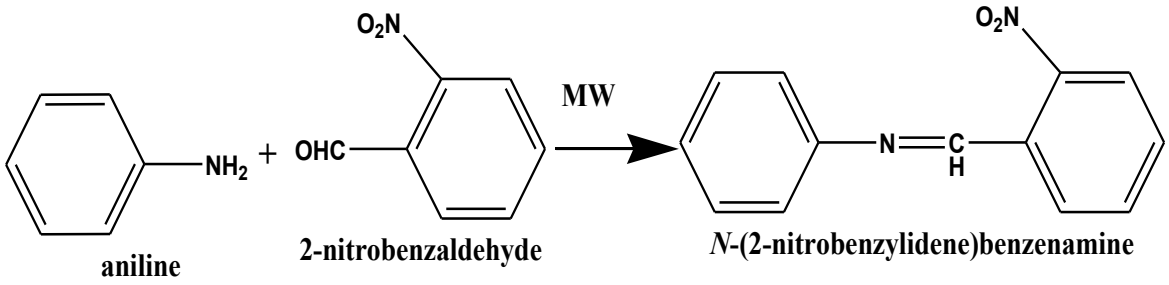
N-(2-nitrobenzyl)-2-chloro-*N*-phenylacetamide

1-(2-nitrobenzyl)indolin-2-one

2-(2-(2-nitrobenzylamino)phenyl)acetic acid

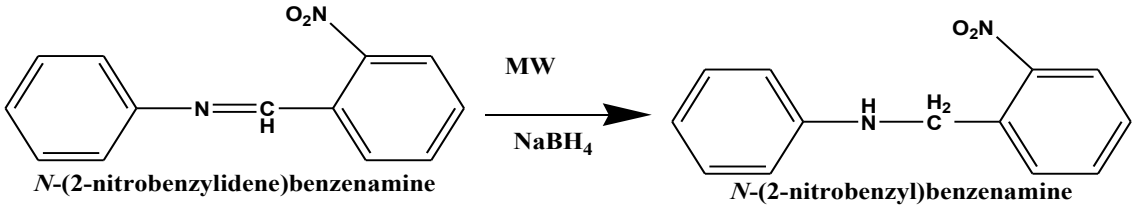
(a) Synthesis of N-(2-nitrobenzylidene) benzenamine (AB_{11}): 453.4 mg (3 mmol) of 2 nitro benzaldehyde and 279.9 mg or 1.023 ml (3 mmol) of aniline was taken in 25 ml in an erlenmeyer flask. A funnel was placed over the mouth of the Erlenmeyer flask. This reaction mixture was placed in to microwave oven at 180 W for 2.5 min. After completion of radiation time reaction mixture was placed a side for getting room temperature. After attaining room temperature small amount of methanol was mixed in it for inducing recrystallization. Crude Schiff base was recrystallized by methanol.

Mol. Formula	Mol. Weight	Melting point	R_f value (Hexane 5, Ethyl acetate 2)	Percentage Yield
$C_{13}H_{10}N_2O_2$	226.31	50-52°C	0.88	90%



Synthesis of N-(2-nitrobenzyl) benzenamine (AB_{12}): 452.6 mg (2 mmol) of synthesized N-(2-nitrobenzylidene) benzenamine was mixed in $NaBH_4$-wet silica gel in a mortar and pestle. After mixing this reaction mixture was poured in to an erlenmeyer flask. This erlenmeyer flask was placed in to microwave oven at 600 W for 4 min. After completion of radiation time reaction mixture was placed a side for getting room temperature. After attaining room temperature small amount of methanol was mixed in it for extraction of crude N-(2-nitrobenzyl) benzenamine. This extraction procedure was done for three times by methanol. After completion of extaraction methanol was evaporated and crude N-(2-nitrobenzyl) benzenamine was remained. This crude N-(2-nitrobenzyl) benzenamine was treated with water to decompose the remaining sodium borohydridel. N-(2-nitrobenzyl) benzenamine was extracted by die ethyl ether. After completion of extraction Die ethyl ether was evaporated and remaining N-(2-nitrobenzyl) benzenamine was obtained. It was recrystallized by methanol.

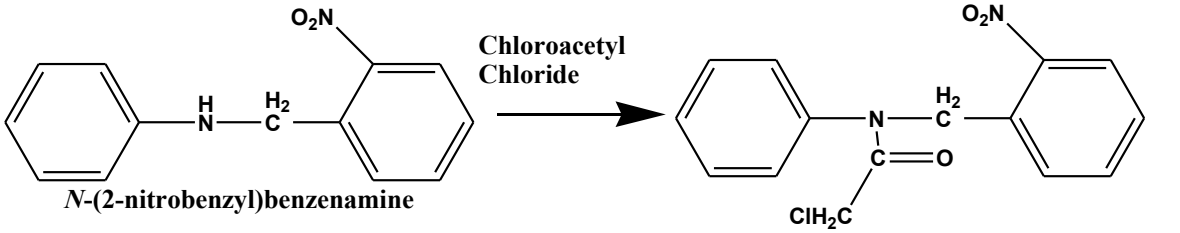
Mol. Formula	Mol. Weight	Melting point	R_f value (Hexane 5, Ethyl acetate 2)	Percentage Yield
$C_{13}H_{12}N_2O_2$	228.247	38-41°C	0.625	78.95%



Synthesis of N-(2-nitrobenzyl)-2-chloro-N-phenylacetamide (AB_{13}): 6.9 g (0.03026 mol) of N-(2-nitrobenzyl) benzenamine was dissolved in ethyl methyl ketone (MEK) and placed in to a three naked RBF. Three naked RBF was pre fitted with two dropping funnels and a mechanical stirrer. 3.417 g (2.41 ml, 0.03026 mol) of Chloroacetyl chloride was dissolved in MEK and placed in a fitted dropping funnel. A 10% solution of sodium carbonate was placed in to another dropping funnel. Reaction was done in cool condition at 5-10ºC. Chloroacetyl chloride was mixed in to reaction vessel with constant steering. pH of reaction mixture was maintained at 8-10 with the help of sodium carbonate solution. After completion of addition ice bath was removed and reaction vessel was placed at room temperature with stirring for 2 hours. After completion of

reaction, reaction mixture was placed in to a separating funnel. MEK layer was separated. This material was washed with water. And clean MEK solution was placed in anhydrous potassium carbonate containing iodine flask. After 24 hours, MEK solution was decant off and remaining MEK solution was placed on water bath for evaporating the MEK. After completion of evaporation, 1 ml diethyl ether was poured in to the flask and solid product was obtained. N-(2-nitrobenzyl)-2-chloro-N-phenylacetamide was recrystallized with acetone.

Mol. Formula	Mol. Weight	Melting point	R_f value (Hexane 5, Ethyl acetate 2)	Percentage Yield
$C_{15}H_{13}ClN_2O_3$	304.728	62-63°C	0.564	82%

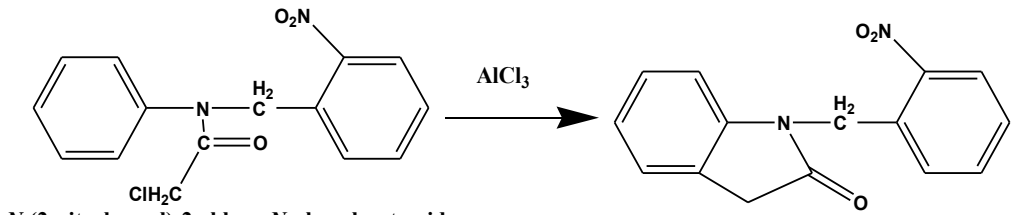


N-(2-nitrobenzyl)benzenamine

N-(2-nitrobenzyl)-2-chloro-*N*-phenylacetamide

Synthesis of 1-(2-nitrobenzyl) indolin-2-one (AB_{14})-: 6.2 g (0.02036 mol) of N-(2-nitrobenzyl)-2-chloro-N-phenylacetamide was weighed and dissolved in nitrobenzene in a two necked round bottom flask. A magnetic bead was placed in to the RBF. This assembly was fixed over magnetic stirrer. 2.71 g (0.02036 mol) anhydrous $AlCl_3$ was mixed in reaction mixture with successive addition. After completion of $AlCl_3$ mixing, this reaction mixture with an air condenser was placed over water bath for one hour. Then this reaction vessel placed over oil bath at 170 ºC for 2 hours. After completion of cyclization, 20 g of acidified crushed ice was added in to RBF for decomposition of $AlCl_3$. After 30 min, this reaction mixture was assembled for steam distillation to remove nitrobenzene. After removal of nitrobenzene, reaction vessel contained 1-(2-nitrobenzyl) indolin-2-one. This was separated by filtration. Filtrate was washed with water. Than recrystallized by diethyl ether.

Mol. Formula	Mol. Weight	Melting point	R_f value (Hexane 5, Ethyl acetate 2)	Percentage Yield
$C_{15}H_{12}N_2O_3$	268.267	74-76°C	0.52	60.58%

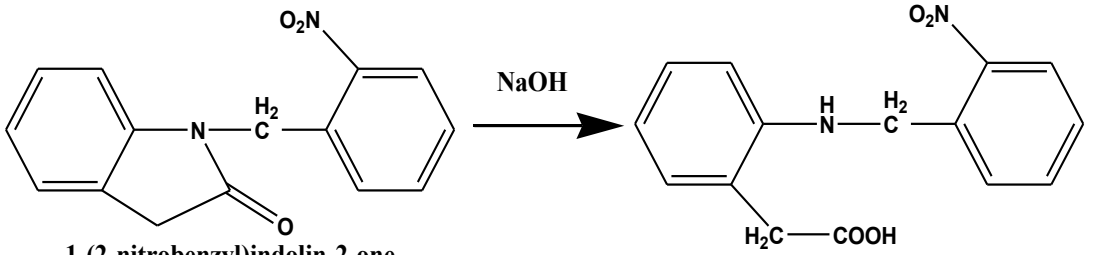


N-(2-nitrobenzyl)-2-chloro-*N*-phenylacetamide

1-(2-nitrobenzyl)indolin-2-one

Synthesis of 2-(2-(2-nitrobenzylamino) phenyl) acetic acid (AB_{15}): 2.68 g (0.01 mol) 1-(2-nitrobenzyl) indolin-2-one was dissolved in ethanol and 10 ml 5N NaOH solution was mixed in it. It was refluxed for 3 hours. Then ethanol was removed by distillation. Remaining aqueous solution was filtered then acidified with dilute HCl. After achieving acidic pH was2-(2-(2-nitrobenzylamino) phenyl) acetic acid precipitated out. Synthesized 2-(2-(2-nitrobenzylamino) phenyl) acetic acid was recrystallized by diethyl ether.

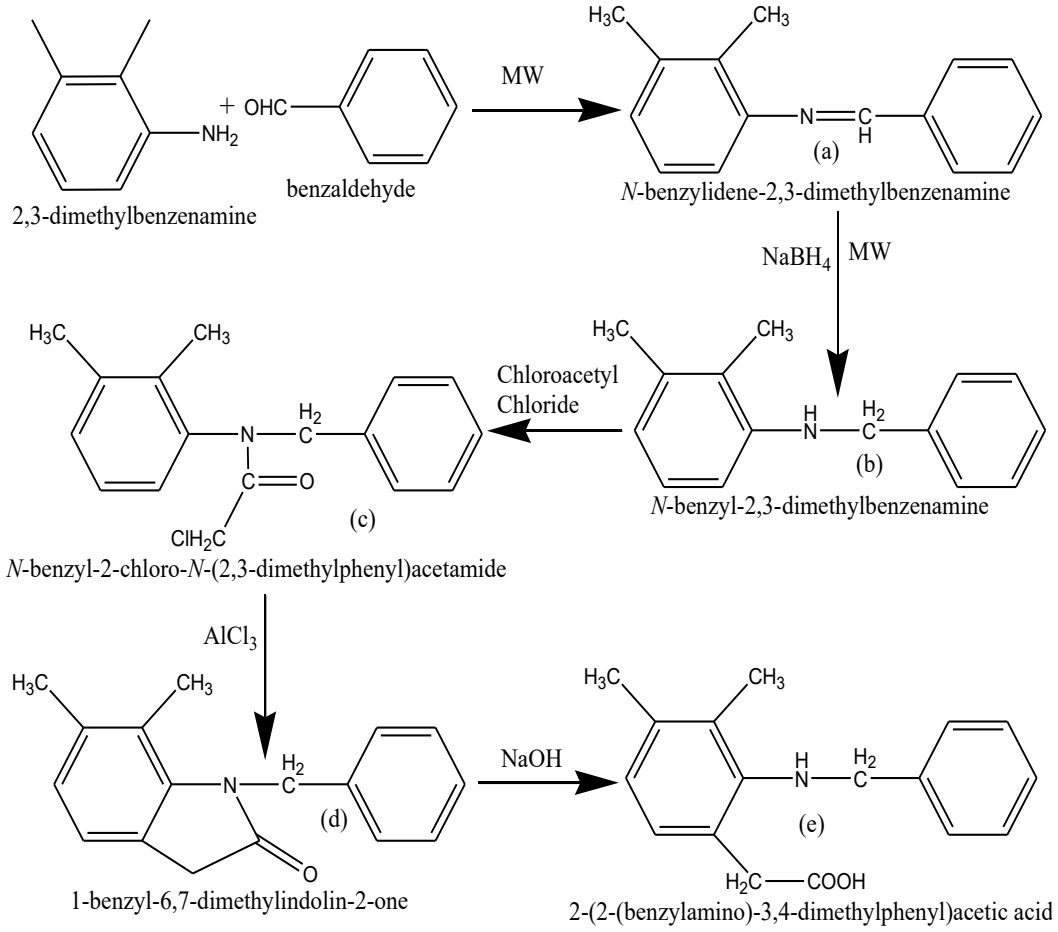
Mol. Formula	Mol. Weight	Melting point	R_f value (Hexane 5, Ethyl acetate 2)	Percentage Yield
$C_{15}H_{14}N_2O_4$	286.283	68-72°C	0.781	48.19%



1-(2-nitrobenzyl)indolin-2-one

2-(2-(2-nitrobenzylamino)phenyl)acetic acid

5.3.2 Synthesis of 2-(2-(benzylamino)-3,4-dimethylphenyl) acetic acid (AB_{25})



2,3-dimethylbenzenamine

benzaldehyde

N-benzylidene-2,3-dimethylbenzenamine

N-benzyl-2,3-dimethylbenzenamine

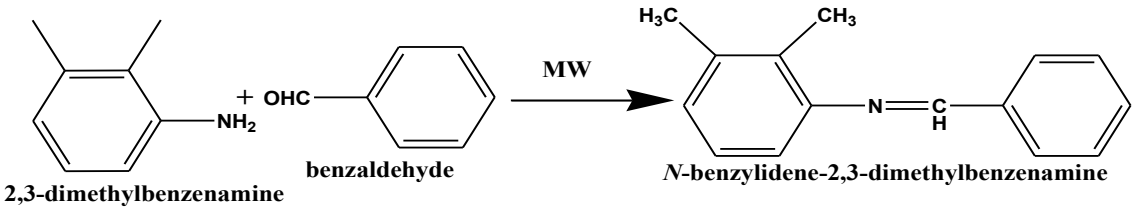
N-benzyl-2-chloro-*N*-(2,3-dimethylphenyl)acetamide

1-benzyl-6,7-dimethylindolin-2-one

2-(2-(benzylamino)-3,4-dimethylphenyl)acetic acid

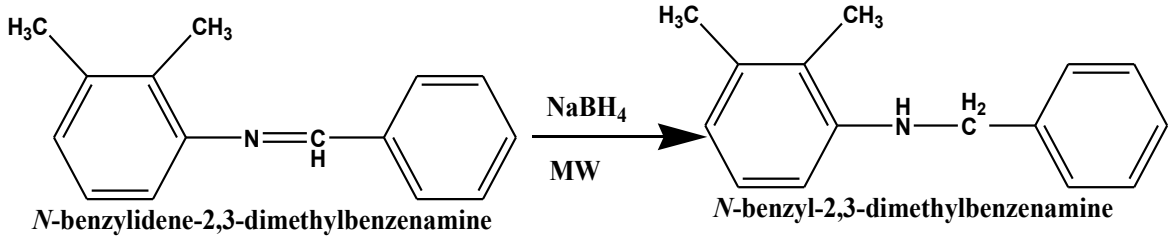
Synthesis of N-benzylidene-2,3-dimethylbenzenamine (AB_{21}): 318 mg (3 mmol) of benzaldehyde and 363.54 mg or 0.36 ml (3 mmol) of 2,3-dimethyl aniline was taken in 25 ml in an Erlenmeyer flask. A funnel was placed over the mouth of the Erlenmeyer flask. This reaction mixture was placed in to microwave oven at 180 W for 3 min. After completion of radiation time reaction mixture was placed a side for getting room temperature. After attaining room temperature small amount of methanol was mixed in it for inducing recrystallization. Crude Schiff base was recrystallized by methanol.

Mol. Formula	Mol. Weight	Melting point	R_f value (Methanol)	Percentage Yield
$C_{15}H_{15}N$	209.286	38°C	0.56	92%



Synthesis of N-benzyl-2,3-dimethylbenzenamine (AB_{22}): 418.6 mg (2 mmol) of synthesized N-benzylidene-2,3-dimethylbenzenamine was mixed in $NaBH_4$-wet silica gel in a mortar and pestle. After mixing this reaction mixture was poured in to an Erlenmeyer flask. This Erlenmeyer flask was placed in to microwave oven at 600 W for 2.5 min. After completion of radiation time reaction mixture was placed a side for getting room temperature. After attaining room temperature small amount of methanol was mixed in it for extraction of crude N-(2-nitrobenzyl) benzenamine. This extraction procedure was done for three times by methanol. After completion of extraction methanol was evaporated and crude N-(2-nitrobenzyl) benzenamine was remained. This crude N-benzyl-2,3-dimethylbenzenamine was treated with water to decompose the remaining sodium borohydride. N-(2-nitrobenzyl) benzenamine was extracted by diethyl ether. After completion of extraction diethyl ether was evaporated and remaining N-(2-nitrobenzyl) benzenamine was obtained.

Mol. Formula	Mol. Weight	Boiling point	R_f value (Methanol)	Percentage Yield
$C_{15}H_{17}N$	211.302	260°C	0.62	64.17%

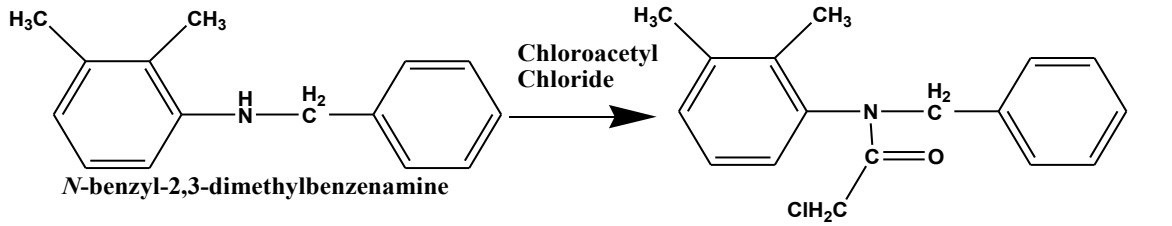


N-benzylidene-2,3-dimethylbenzenamine

N-benzyl-2,3-dimethylbenzenamine

Synthesis of N-benzyl-2-chloro-N-(2,3-dimethylphenyl) acetamide (AB_{23}): 12 g (0.056 mol) of N-benzyl-2,3-dimethylbenzenamine was dissolved in ethyl methyl ketone (MEK) and placed in to a three naked RBF. Three naked RBF was pre fitted with two dropping funnels and a mechanical stirrer. 7.22 g or 5.1 ml (0.056 mol) of Chloroacetyl chloride was dissolved in MEK and placed in a fitted dropping funnel. A 10% solution of sodium carbonate was placed in to another dropping funnel. Reaction was done in cool condition at 5-10ºC. Chloroacetyl chloride was mixed in to reaction vessel with constant steering. pH of reaction mixture was maintained at 8-10 with the help of sodium carbonate solution. After completion of addition ice bath was removed and reaction vessel was placed at room temperature with stirring for 2 hours. After completion of reaction, reaction mixture was placed in to a separating funnel. MEK layer was separated. This material was washed with water. And clean MEK solution was placed in anhydrous potassium carbonate containing iodine flask. After 24 hours, MEK solution was decant off and remaining MEK solution was placed on water bath for evaporating the MEK. After completion of evaporation, 1 ml diethyl ether was poured in to the flask and solid product was obtained. N-benzyl-2-chloro-N-(2,3-dimethylphenyl) acetamide was recrystallized with acetone.

Mol. Formula	Mol. Weight	Melting point	R_f value (Methanol)	Percentage Yield
$C_{17}H_{18}ClNO$	287.784	54-58°C	0.80	78%



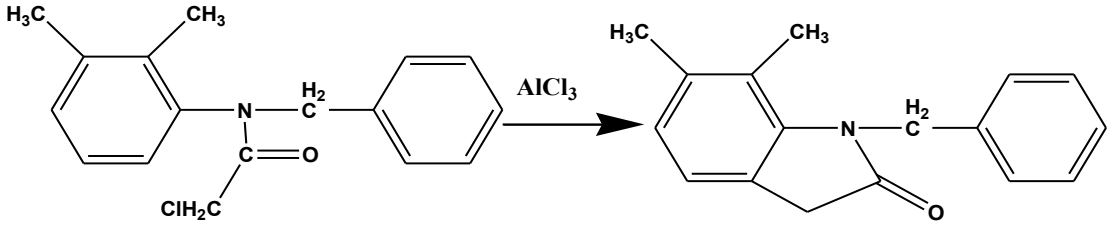
N-benzyl-2,3-dimethylbenzenamine

N-benzyl-2-chloro-*N*-(2,3-dimethylphenyl)acetamide

Synthesis of 1-benzyl-6,7-dimethylindolin-2-one (AB_{24}): 4 g (0.0139 mol) of N-benzyl-2-chloro-N-(2,3-dimethylphenyl) acetamide was weighed and dissolved in nitrobenzene in a two necked round bottom flask. A magnetic bead was placed in to the RBF. This assembly was fixed over magnetic stirrer. 1.86 g (0.0139 mol) anhydrous $AlCl_3$ was mixed in reaction mixture with

successive addition. After completion of $AlCl_3$ mixing, this reaction mixture with an air condenser was placed over water bath for one hour. Then this reaction vessel placed over oil bath at 170 ºC for 2 hours. After completion of cyclization, 20 g of acidified crushed ice was added in to RBF for decomposition of $AlCl_3$. After 30 min, this reaction mixture was assembled for steam distillation to remove nitrobenzene. After removal of nitrobenzene, reaction vessel contained 1-benzyl-6,7-dimethylindolin-2-one. This was separated by filtration. Filtrate was washed with water. Then recrystallized by diethyl ether.

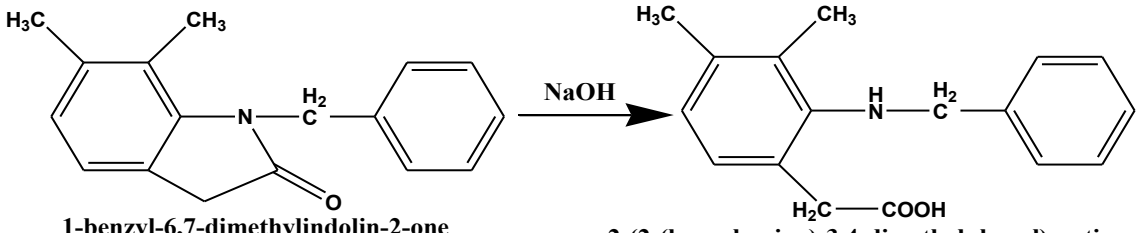
Mol. Formula	Mol. Weight	Melting point	R_f value (Methanol)	Percentage Yield
$C_{17}H_{17}NO$	251.323	64-70°C	0.674	62.61%



***N*-benzyl-2-chloro-*N*-(2,3-dimethylphenyl)acetamide** **1-benzyl-6,7-dimethylindolin-2-one**

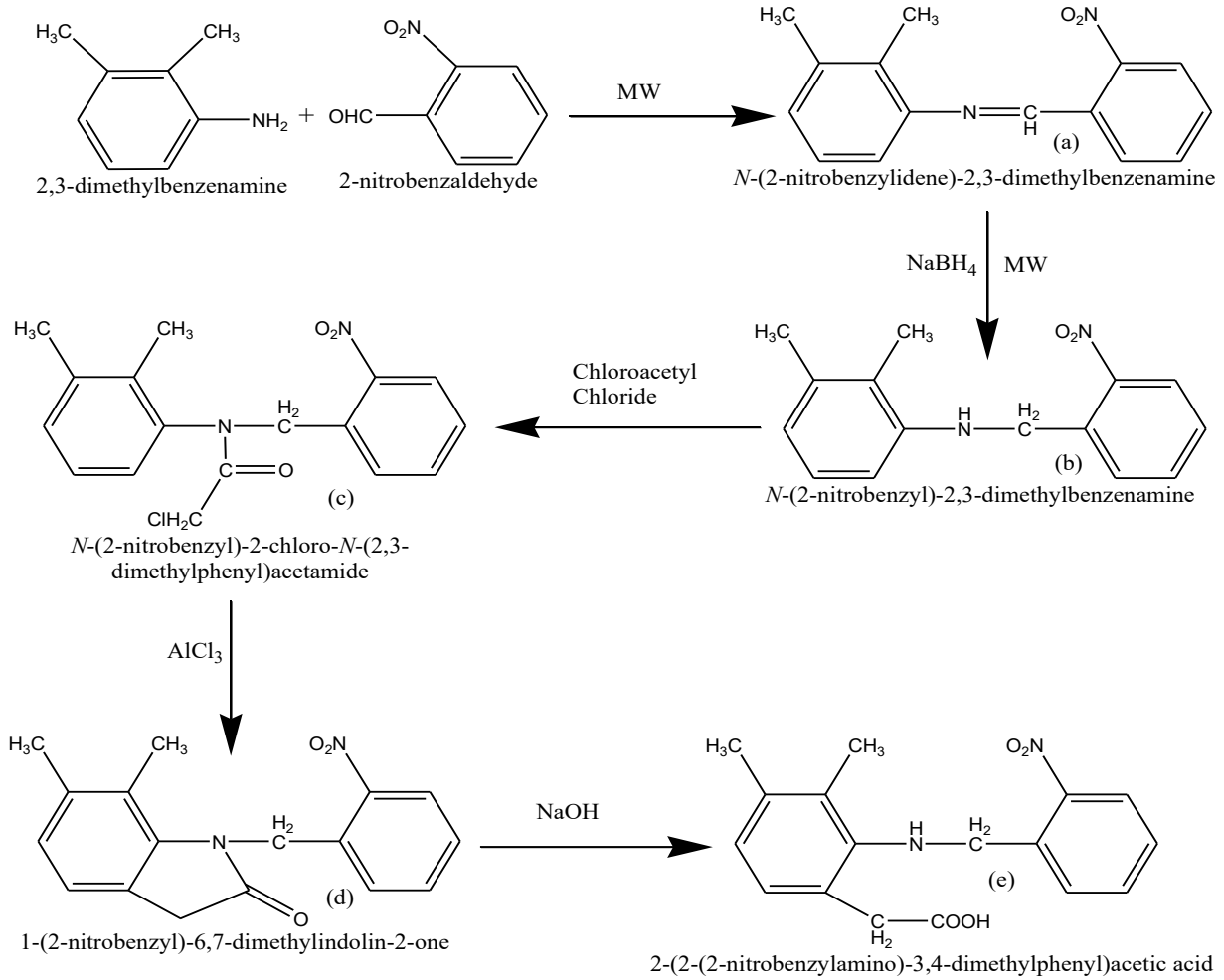
Synthesis of 2-(2-(benzylamino)-3, 4-dimethylphenyl) acetic acid (AB_{25}): 1 g (0.00397 mol) of 1-benzyl-6,7-dimethylindolin-2-one was dissolved in ethanol and 10 ml 5N NaOH solution was mixed in it. It was refluxed for 3 hours. Then ethanol was removed by distillation. Remaining aqueous solution was filtered then acidified with dilute HCl. After achieving acidic pH 2-(2-(benzylamino)-3,4-dimethylphenyl) acetic acid was precipitated out. Synthesized 2-(2-(benzylamino)-3,4-dimethylphenyl) acetic acid was recrystallized by diethyl ether.

Mol. Formula	Mol. Weight	Melting point	R_f value (Methanol)	Percentage Yield
$C_{17}H_{19}NO_2$	269.338	76-78°C	0.583	40%



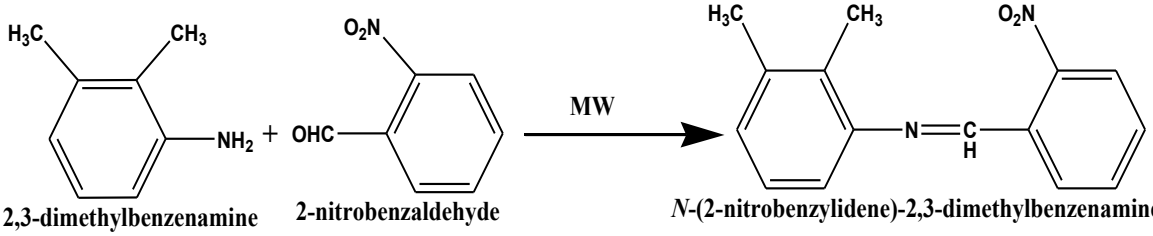
1-benzyl-6,7-dimethylindolin-2-one **2-(2-(benzylamino)-3,4-dimethylphenyl)acetic acid**

Synthesis of 2-(2-(benzylamino)-3,4-dimethylphenyl) acetic acid (AB_{35})



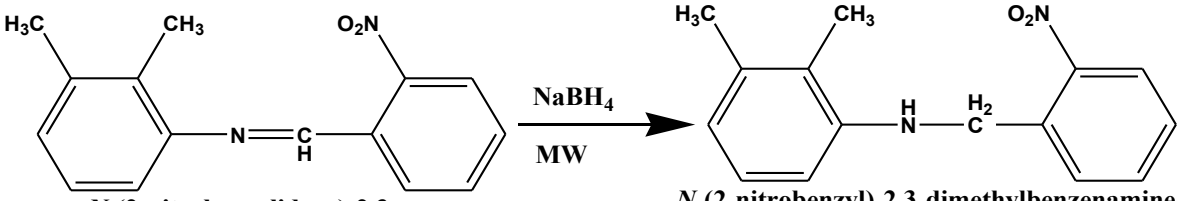
Synthesis of N-(2-nitrobenzylidene)-2,3-dimethylbenzenamine (AB_{31}): 453.4 mg (3 mmol) of 2-nitro benzaldehyde and 363.54 mg or 0.36 ml (3 mmol) of 2,3-dimethyl aniline was taken in 25 ml in an Erlenmeyer flask. A funnel was placed over the mouth of the Erlenmeyer flask. This reaction mixture was placed in to microwave oven at 180 W for 3 min. After completion of radiation time reaction mixture was placed a side for getting room temperature. After attaining room temperature small amount of methanol was mixed in it for inducing recrystallization. Crude Schiff base was recrystallized by methanol.

Mol. Formula	Mol. Weight	Melting point	R_f value (Methanol)	Percentage Yield
$C_{15}H_{14}N_2O_2$	254.284	80-84 °C	0.6	92.11%



Synthesis of N-(2-nitrobenzyl)-2,3-dimethylbenzenamine (AB_{32}): 508.6 mg (2 mmol) of N-(2-nitrobenzylidene)-2,3-dimethylbenzenamine was mixed in $NaBH_4$-wet silica gel in a mortar and pestle. After mixing this reaction mixture was poured in to an Erlenmeyer flask. This Erlenmeyer flask was placed in to microwave oven at 600 W for 3 min. It was irradiated more at 750 W for 1 min. After completion of radiation time reaction mixture was placed a side for getting room temperature. After attaining room temperature small amount of methanol was mixed in it for extraction of crude N-(2-nitrobenzyl) benzenamine. This extraction procedure was done for three times by methanol. After completion of extraction methanol was evaporated and crude N-(2-nitrobenzyl)-2,3-dimethylbenzenamine was remained. This crude N-(2-nitrobenzyl)-2,3-dimethylbenzenamine was treated with water to decompose the remaining sodium borohydridel. N-(2-nitrobenzyl)-2,3-dimethylbenzenamine was extracted by die ethyl ether. After completion of extraction diethyl ether was evaporated and remaining N-(2-nitrobenzyl)-2,3-dimethylbenzenamine was obtained. N-(2-nitrobenzyl)-2,3-dimethyl benzenamine was recrystallized with methanol.

Mol. Formula	Mol. Weight	Melting point	R_f value (Methanol)	Percentage Yield
$C_{15}H_{16}N_2O_2$	256.284	60-64°C	0.674	85.17%



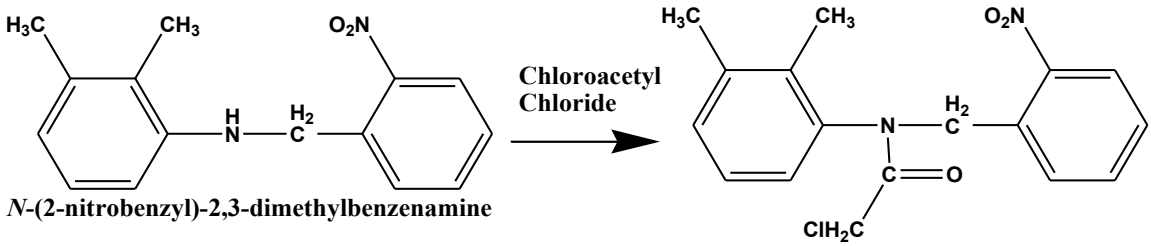
N-(2-nitrobenzylidene)-2,3-dimethylbenzenamine

N-(2-nitrobenzyl)-2,3-dimethylbenzenamine

Synthesis of N-(2-nitrobenzyl)-2-chloro-N-(2,3-dimethylphenyl) acetamide (AB_{33}): 5g (0.0195 mol) of N-(2-nitrobenzyl)-2,3-dimethylbenzenamine was dissolved in ethyl methyl ketone (MEK) and placed in to a three naked RBF. Three naked RBF was pre fitted with two dropping funnels and a mechanical stirrer. 2.203 g or 1.56 ml (0.0195 mol) of Chloroacetyl chloride was dissolved in MEK and placed in a fitted dropping funnel. A 10% solution of sodium carbonate was placed in to another dropping funnel. Reaction was done in cool condition at 5-10ºC. Chloroacetyl chloride was mixed in to reaction vessel with constant steering. pH of reaction mixture was maintained at 8-10 with the help of sodium carbonate solution. After completion of addition ice bath was removed and reaction vessel was placed at room temperature with stirring for 2 hours. After completion of reaction, reaction mixture was placed in to a separating funnel. MEK layer was separated. This material was washed with water. And clean MEK solution was

placed in anhydrous potassium carbonate containing iodine flask. After 24 hours, MEK solution was decant off and remaining MEK solution was placed on water bath for evaporating the MEK. After completion of evaporation, 1 ml diethyl ether was poured in to the flask and solid product was obtained. N-(2-nitrobenzyl)-2-chloro-N-(2,3-dimethylphenyl) acetamide was recrystallized with acetone.

Mol. Formula	Mol. Weight	Melting point	R_f value (Methanol)	Percentage Yield
$C_{17}H_{17}ClN_2O_3$	332.781	86-88°C	0.861	80%

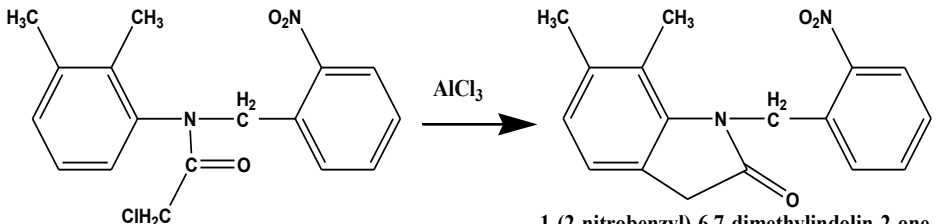


N-(2-nitrobenzyl)-2,3-dimethylbenzenamine

N-(2-nitrobenzyl)-2-chloro-*N*-(2,3-dimethylphenyl)acetamide

Synthesis of 1-(2-nitrobenzyl)-6,7-dimethylindolin-2-one (AB_{34}): 3.1 g (0.0093 mol) of N-(2-nitrobenzyl)-2-chloro-N-(2,3-dimethylphenyl) acetamide was weighed and dissolved in nitrobenzene in a two naked round bottom flask. A magnetic bead was placed in to the RBF. This assembly was fixed over magnetic stirrer. 1.24 g (0.0093 mol) anhydrous $AlCl_3$ was mixed in reaction mixture with successive addition. After completion of $AlCl_3$ mixing, this reaction mixture with an air condenser was placed over water bath for one hour. Then this reaction vessel placed over oil bath at 170 ºC for 2 hours. After completion of cyclization, 20 g of acidified crushed ice was added in to RBF for decomposition of $AlCl_3$. After 30 min, this reaction mixture was assembled for steam distillation to remove nitrobenzene. After removal of nitrobenzene, reaction vessel contained 1-(2-nitrobenzyl)-6,7-dimethylindolin-2-one. This was separated by filtration. Filtrate was washed with water. Then recrystallized by diethyl ether.

Mol. Formula	Mol. Weight	Melting point	R_f value (Methanol)	Percentage Yield
$C_{17}H_{16}N_2O_3$	296.321	78-82°C	0.72	60.73%

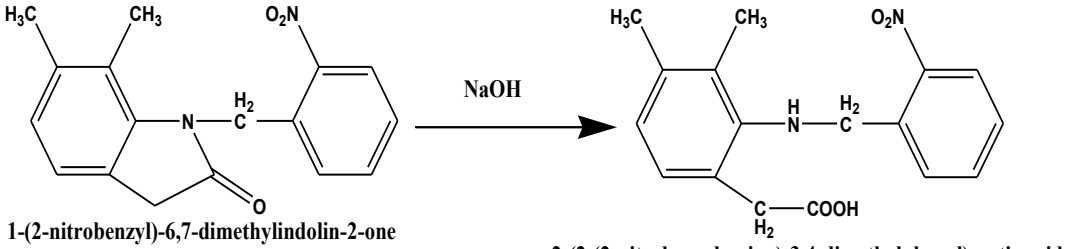


N-(2-nitrobenzyl)-2-chloro-*N*-(2,3-dimethylphenyl)acetamide

1-(2-nitrobenzyl)-6,7-dimethylindolin-2-one

Synthesis of 2-(2-(2-nitrobenzylamino)-3,4-dimethylphenyl) acetic acid (AB_{35}): 1 g (0.00337 mol) of 1-(2-nitrobenzyl)-6,7-dimethylindolin-2-one was dissolved in ethanol and 10 ml 5N NaOH solution was mixed in it. It was refluxed for 3 hours. Then ethanol was removed by distillation. Remaining aqueous solution was filtered then acidified with dilute HCl. After achieving acidic pH 2-(2-(2-nitrobenzylamino)-3,4-dimethylphenyl) acetic acid was precipitated out. Synthesized 2-(2-(benzylamino)-3,4-dimethylphenyl) acetic acid was recrystallized by diethyl ether.

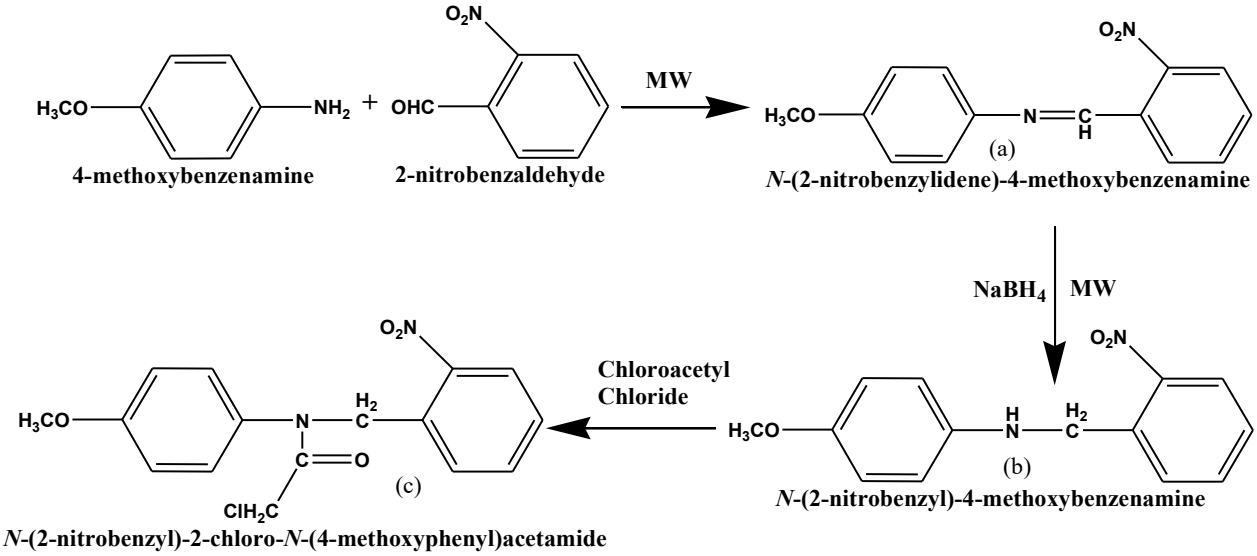
Mol. Formula	Mol. Weight	Melting point	R_f value (Methanol)	Percentage Yield
$C_{17}H_{18}N_2O_4$	314.336	72-74°C	0.545	50.45%



1-(2-nitrobenzyl)-6,7-dimethylindolin-2-one

2-(2-(2-nitrobenzylamino)-3,4-dimethylphenyl)acetic acid

5.3.4 Synthesis of 2-(2-(2-nitrobenzylamino)-5-methoxyphenyl) acetic acid (AB_{45})



4-methoxybenzenamine

2-nitrobenzaldehyde

(a)

N-(2-nitrobenzylidene)-4-methoxybenzenamine

(b)

N-(2-nitrobenzyl)-4-methoxybenzenamine

(c)

N-(2-nitrobenzyl)-2-chloro-*N*-(4-methoxyphenyl)acetamide

(d)

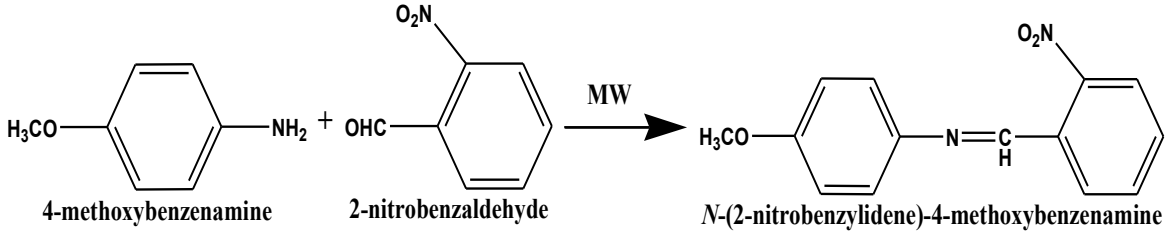
1-(2-nitrobenzyl)-5-methoxyindolin-2-one

(e)

2-(2-(2-nitrobenzylamino)-5-methoxyphenyl)acetic acid

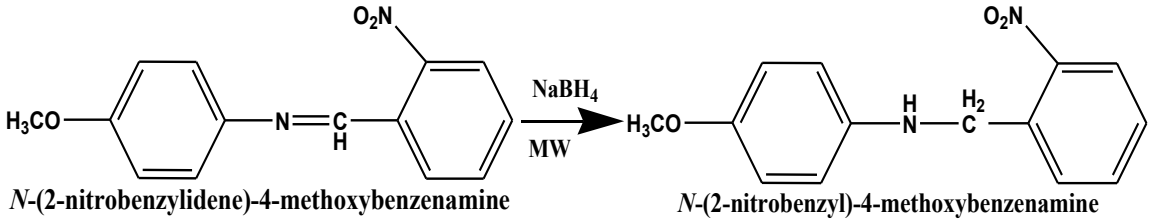
Synthesis of N-(2-nitrobenzylidene)-4-methoxybenzenamine (AB_{41}): 453.36 mg (3 mmol) of 2-nitro benzaldehyde and 369 mg (3 mmol) of 4-methoxybenzenamine was taken in 25 ml in an Erlenmeyer flask. A funnel was placed over the mouth of the Erlenmeyer flask. This reaction mixture was placed in to microwave oven at 180 W for 3 min. After completion of radiation time reaction mixture was placed a side for getting room temperature. After attaining room temperature small amount of methanol was mixed in it for inducing recrystallization. Crude Schiff base was recrystallized by methanol.

Mol. Formula	Mol. Weight	Melting point	R_f value (Hexane 5, Ethyl acetate 0.5, Ethanol 0.5)	Percentage Yield
$C_{14}H_{12}N_2O_3$	256.257	66-68°C	0.95	92%



Synthesis of N-(2-nitrobenzyl)-4-methoxybenzenamine (AB_{42}): 512.5 mg (2 mmol) of N-(2-nitrobenzylidene)-4-methoxybenzenamine was mixed in $NaBH_4$-wet silica gel in a mortar and pestle. After mixing this reaction mixture was poured in to an Erlenmeyer flask. This erlenmeyer flask was placed in to microwave oven at 600 W for 3.5 min. After completion of radiation time reaction mixture was placed a side for getting room temperature. After attaining room temperature small amount of methanol was mixed in it for extraction of crude N-(2-nitrobenzyl) benzenamine. This extraction procedure was done for three times by methanol. After completion of extraction methanol was evaporated and crude N-(2-nitrobenzyl)-4-methoxybenzenamine was remained. This crude N-(2-nitrobenzyl)-4-methoxybenzenamine was treated with water to decompose the remaining sodium borohydride. N-(2-nitrobenzyl)-4-methoxybenzenamine was extracted by die ethyl ether. After completion of extraction diethyl ether was evaporated and remaining N-(2-nitrobenzyl)-4-methoxybenzenamine was obtained. N-(2-nitrobenzyl)-4-methoxy benzenamine was recrystallized with methanol.

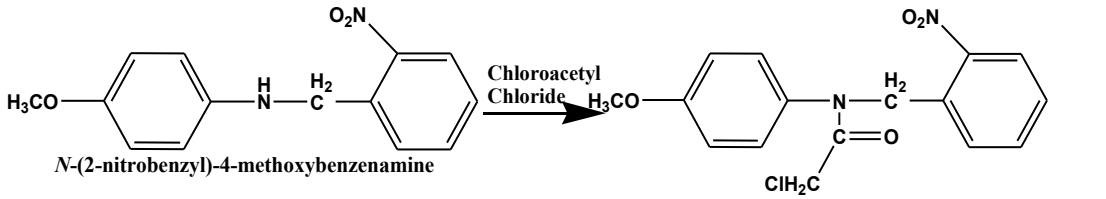
Mol. Formula	Mol. Weight	Melting point	R_f value (Hexane 5, Ethyl acetate 0.5, Ethanol 0.5)	Percentage Yield
$C_{14}H_{14}N_2O_3$	258.273	58-60°C	0.909	68.1%



N-(2-nitrobenzylidene)-4-methoxybenzenamine — *N*-(2-nitrobenzyl)-4-methoxybenzenamine

Synthesis of N-(2-nitrobenzyl)-2-chloro-N-(4-methoxyphenyl) acetamide (AB_{43}): 5 g (0.0193 mol) of N-(2-nitrobenzyl)-4-methoxybenzenamine was dissolved in ethyl methyl ketone (MEK) and placed in to a three naked RBF. Three naked RBF was pre fitted with two dropping funnels and a mechanical stirrer. 2.18 g or 1.54 ml (0.0193 mol) of Chloroacetyl chloride was dissolved in MEK and placed in a fitted dropping funnel. A 10% solution of sodium carbonate was placed in to another dropping funnel. Reaction was done in cool condition at 5-10ºC. Chloroacetyl chloride was mixed in to reaction vessel with constant steering. pH of reaction mixture was maintained at 8-10 with the help of sodium carbonate solution. After completion of addition ice bath was removed and reaction vessel was placed at room temperature with stirring for 2 hours. After completion of reaction, reaction mixture was placed in to a separating funnel. MEK layer was separated. This material was washed with water. And clean MEK solution was placed in anhydrous potassium carbonate containing iodine flask. After 24 hours, MEK solution was decant off and remaining MEK solution was placed on water bath for evaporating the MEK. After completion of evaporation, 1 ml diethyl ether was poured in to the flask and solid product was obtained. N-(2-nitrobenzyl)-2-chloro-N-(4-methoxyphenyl) acetamide was recrystallized with acetone.

Mol. Formula	Mol. Weight	Melting point	R_f value (Hexane 5, Ethyl acetate 0.5, Ethanol 0.5)	Percentage Yield
$C_{16}H_{15}ClN_2O_4$	334.754	62-64°C	0.568	82.57%

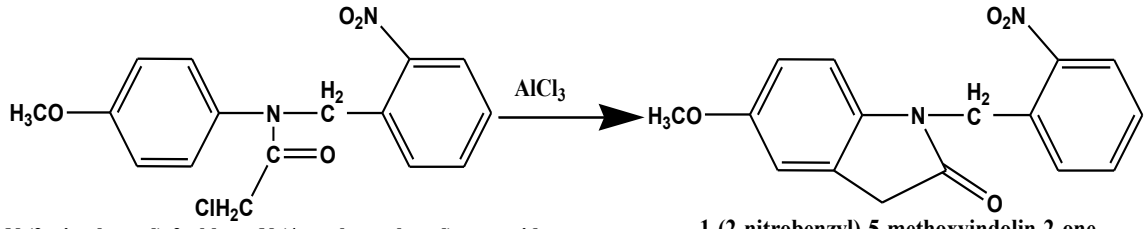


N-(2-nitrobenzyl)-4-methoxybenzenamine — *N*-(2-nitrobenzyl)-2-chloro-*N*-(4-methoxyphenyl)acetamide

Synthesis of 1-(2-nitrobenzyl)-5-methoxyindolin-2-one (AB_{44}): 3.67 g (0.011 mol) of N-(2-nitrobenzyl)-2-chloro-N-(4-methoxyphenyl) acetamide was weighed and dissolved in nitrobenzene in a two naked round bottom flask. A magnetic bead was placed in to the RBF. This assembly was fixed over magnetic stirrer. 1.468 g of anhydrous $AlCl_3$ was mixed in reaction mixture with successive addition. After completion of $AlCl_3$ mixing, this reaction mixture with an air condenser was placed over water bath for one hour. Then this reaction vessel

placed over oil bath at 170 ºC for 2 hours. After completion of cyclization, 20 g of acidified crushed ice was added in to RBF for decomposition of $AlCl_3$. After 30 min, this reaction mixture was assembled for steam distillation to remove nitrobenzene. After removal of nitrobenzene, reaction vessel contained 1-(2-nitrobenzyl)-5-methoxyindolin-2-one. This 1-(2-nitrobenzyl)-5-methoxyindolin-2-one was extracted by diethyl ether. Diethyl ether was evaporated by heating. Remaining solid was collected and recrystallized by diethyl ether.

Mol. Formula	Mol. Weight	Melting point	R_f value (Hexane 5, Ethyl acetate 0.5, Ethanol 0.5)	Percentage Yield
$C_{16}H_{14}N_2O_4$	298.293	54-58°C	0.5	58.35%

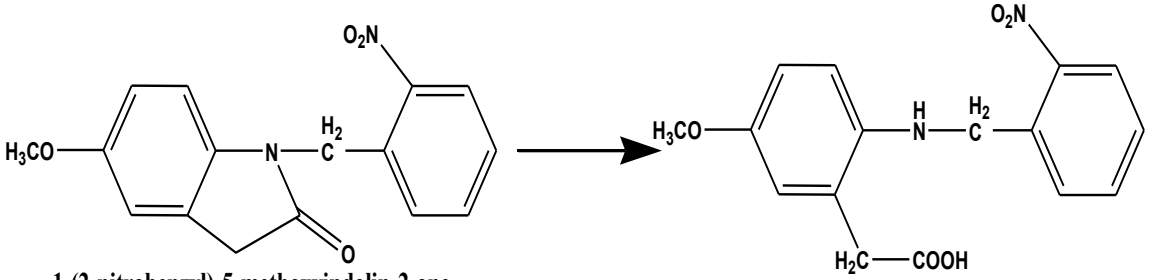


N-(2-nitrobenzyl)-2-chloro-*N*-(4-methoxyphenyl)acetamide

1-(2-nitrobenzyl)-5-methoxyindolin-2-one

Synthesis of 2-(2-(2-nitrobenzylamino)-5-methoxyphenyl) acetic acid (AB_{45}): 1 g (0.00335 mol) of 1-(2-nitrobenzyl)-5-methoxyindolin-2-one was dissolved in ethanol and 10 ml 5N NaOH solution was mixed in it. It was refluxed for 3 hours. Then ethanol was removed by distillation. Remaining aqueous solution was filtered then acidified with dilute HCl. After achieving acidic pH 2-(2-(2-nitrobenzylamino)-5-methoxyphenyl) acetic acid was precipitated out. Synthesized 2-(2-(2-nitrobenzylamino)-5-methoxyphenyl) acetic acid was recrystallized by diethyl ether.

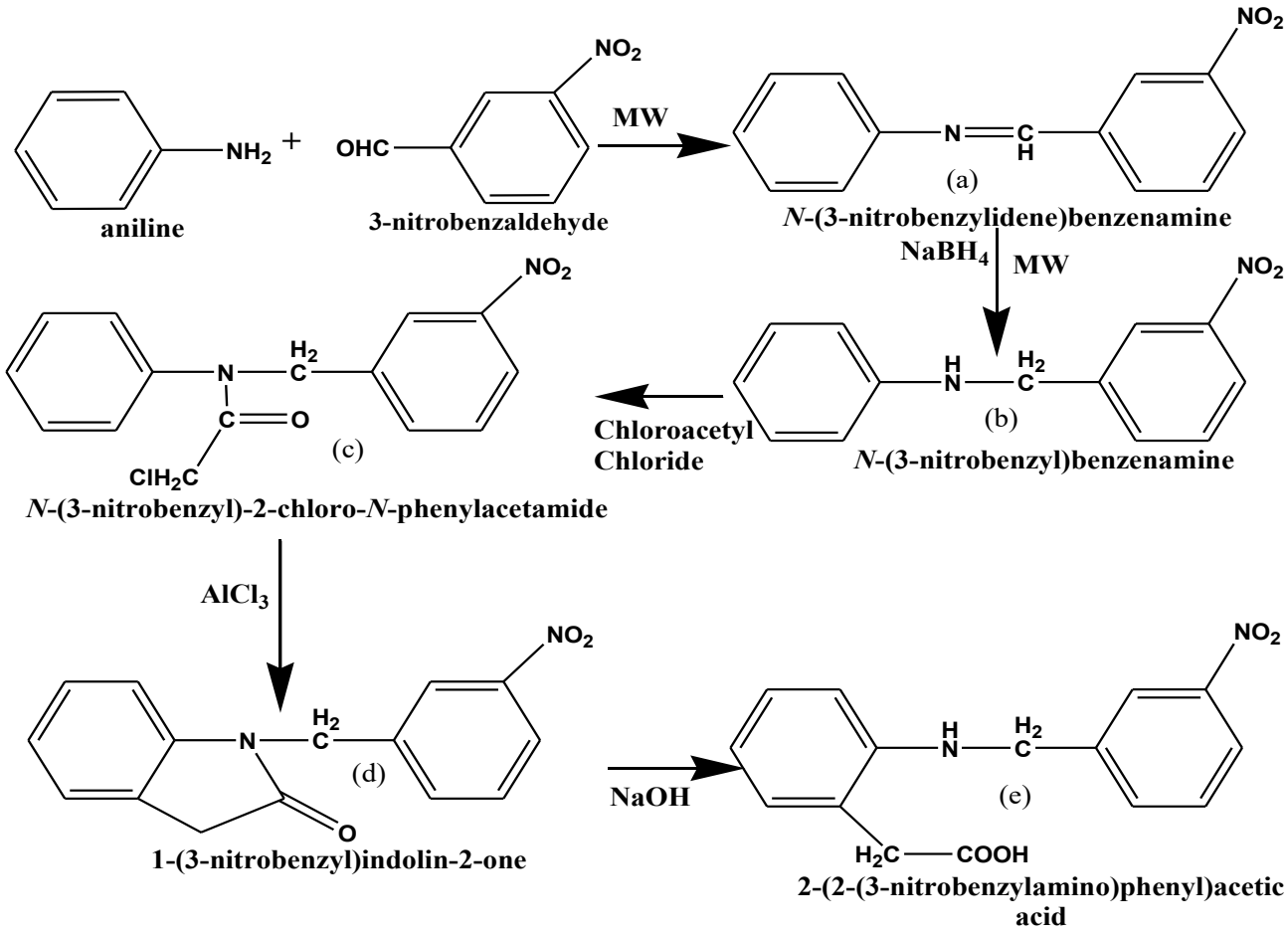
Mol. Formula	Mol. Weight	Melting point	R_f value (Hexane 5, Ethyl acetate 0.5, Ethanol 0.5)	Percentage Yield
$C_{16}H_{16}N_2O_5$	316.309	82-84°C	0.25	40.59%



1-(2-nitrobenzyl)-5-methoxyindolin-2-one

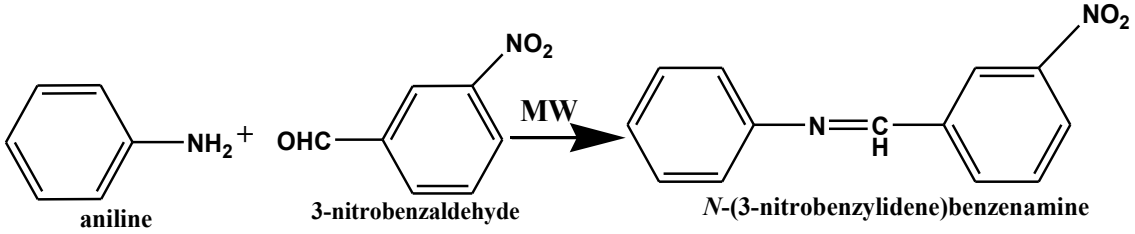
2-(2-(2-nitrobenzylamino)-5-methoxyphenyl)acetic acid

Synthesis of 2-(2-(3-nitrobenzylamino) phenyl) acetic acid (AB_{55})



Synthesis of N-(3-nitrobenzylidene) benzenamine (AB_{51}): 453.36 mg (3 mmol) of 3-nitro benzaldehyde and 279.9 mg or 0.27 ml (3 mmol) of aniline was taken in 25 ml in an Erlenmeyer flask. A funnel was placed over the mouth of the Erlenmeyer flask. This reaction mixture was placed in to microwave oven at 180 W for 4 min. After completion of radiation time reaction mixture was placed a side for getting room temperature. After attaining room temperature small amount of methanol was mixed in it for inducing recrystallization. Crude Schiff base was recrystallized by methanol.

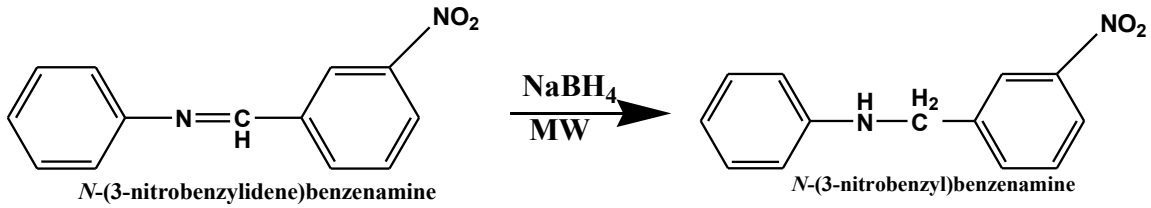
Mol. Formula	Mol. Weight	Melting point	R_f value (Methanol 2.5, Hexane 2.5)	Percentage Yield
$C_{13}H_{10}N_2O_2$	226.231		0.88	94.21%



Synthesis of N-(3-nitrobenzyl) benzenamine (AB_{52}): 452.5 mg (2 mmol) of N-(3-nitrobenzylidene) benzenamine was mixed in $NaBH_4$-wet silica gel in a mortar and pestle. After

mixing this reaction mixture was poured in to an Erlenmeyer flask. This Erlenmeyer flask was placed in to microwave oven at 600 W for 2.5 min. After completion of radiation time reaction mixture was placed a side for getting room temperature. After attaining room temperature small amount of methanol was mixed in it for extraction of crude N-(2-nitrobenzyl) benzenamine. This extraction procedure was done for three times by methnol. After completion of extraction methanol was evaporated and crude N-(3-nitrobenzyl) benzenamine was remained. This crude N-(3-nitrobenzyl) benzenamine was treated with water to decompose the remaining sodium borohydride. N-(3-nitrobenzyl) benzenamine was extracted by die ethyl ether. After completion of extraction diethyl ether was evaporated and remaining N-(3-nitrobenzyl) benzenamine was obtained. N-(3-nitrobenzyl) benzenamine was recrystallized with methanol.

Mol. Formula	Mol. Weight	Melting point	R_f value (Methanol 2.5, Hexane 2.5)	Percentage Yield
$C_{13}H_{12}N_2O_2$	228.23		0.56	56%

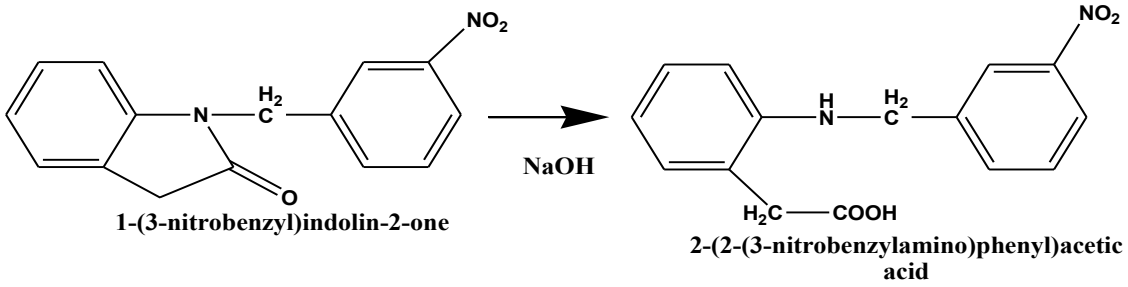


N-(3-nitrobenzylidene)benzenamine

N-(3-nitrobenzyl)benzenamine

Synthesis of N-(3-nitrobenzyl)-2-chloro-N-phenylacetamide (AB_{53}): 4.56 g (0.02 mol) of N-(3-nitrobenzyl) benzenamine was dissolved in ethyl methyl ketone (MEK) and placed in to a three naked RBF. Three naked RBF was pre fitted with two dropping funnels and a mechanical stirrer. 2.58 g or 1.83 ml (0.02 mol) of Chloroacetyl chloride was dissolved in MEK and placed in a fitted dropping funnel. A 10% solution of sodium carbonate was placed in to another dropping funnel. Reaction was done in cool condition at 5-10ºC. Chloroacetyl chloride was mixed in to reaction vessel with constant steering. pH of reaction mixture was maintained at 8-10 with the help of sodium carbonate solution. After completion of addition ice bath was removed and reaction vessel was placed at room temperature with stirring for 2 hours. After completion of reaction, reaction mixture was placed in to a separating funnel. MEK layer was separated. This material was washed with water. And clean MEK solution was placed in anhydrous potassium carbonate containing iodine flask. After 24 hours, MEK solution was decant off and remaining MEK solution was placed on water bath for evaporating the MEK. After completion of evaporation, 1 ml diethyl ether was poured in to the flask and solid product was obtained. N-(3-nitrobenzyl)-2-chloro-N-phenylacetamide was recrystallized with acetone.

Mol. Formula	Mol. Weight	Melting point	R_f value (Methanol 2.5, Hexane 2.5)	Percentage Yield
$C_{15}H_{13}ClN_2O_3$	304.728	62-64°C	0.510	76.58%

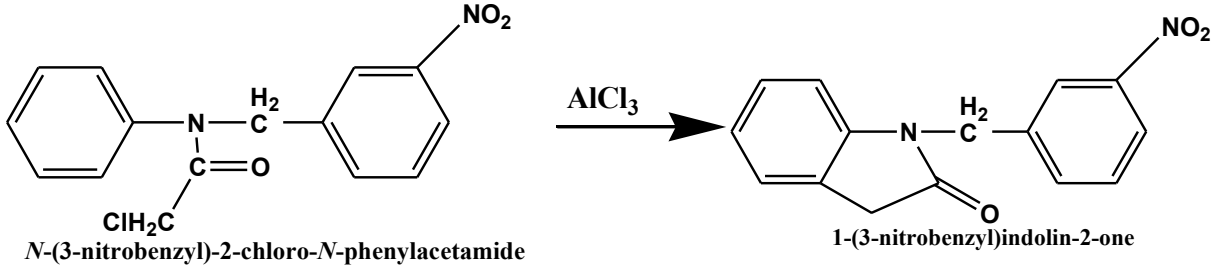


1-(3-nitrobenzyl)indolin-2-one

2-(2-(3-nitrobenzylamino)phenyl)acetic acid

(a) Synthesis of 1-(3-nitrobenzyl) indolin-2-one (AB_{54}): 3.045g (0.01 mol) of N-(3-nitrobenzyl)-2-chloro-N-phenylacetamide was weighed and dissolved in nitrobenzene in a two naked round bottom flask. A magnetic bead was placed in to the RBF. This assembly was fixed over magnetic stirrer. 1.34 g (0.01 mol) of anhydrous $AlCl_3$ was mixed in reaction mixture with successive addition. After completion of $AlCl_3$ mixing, this reaction mixture with an air condenser was placed over water bath for one hour. Then this reaction vessel placed over oil bath at 170 ºC for 2 hours. After completion of Cyclization, 20 g of acidified crushed ice was added in to RBF for decomposition of $AlCl_3$. After 30 min, this reaction mixture was assembled for steam distillation to remove nitrobenzene. After removal of nitrobenzene, reaction vessel contained 1-(3-nitrobenzyl) indolin-2-one. This 1-(3-nitrobenzyl) indolin-2-one was separated out by filtration. The product was collected and recrystallized by diethyl ether.

Mol. Formula	Mol. Weight	Melting point	R_f value (Hexane 5, Ethyl acetate 3)	Percentage Yield
$C_{15}H_{12}N_2O_3$	268.267	68-70°C	0.759	56.32%

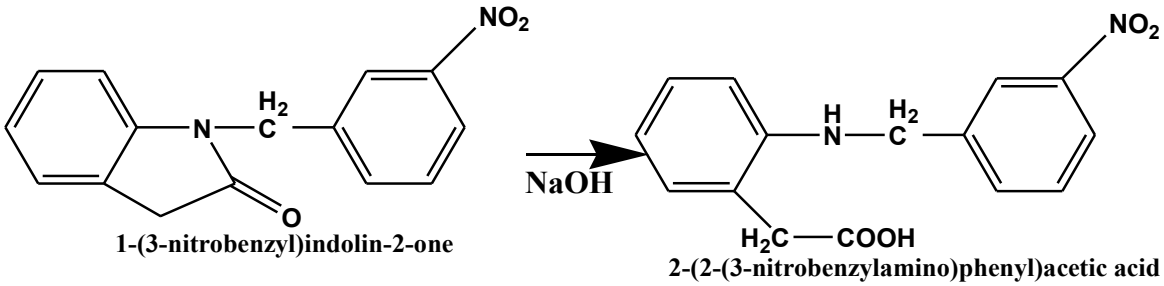


N-(3-nitrobenzyl)-2-chloro-*N*-phenylacetamide

1-(3-nitrobenzyl)indolin-2-one

(b) Synthesis of 2-(2-(3-nitrobenzylamino) phenyl) acetic acid (AB_{55}): 1 g (0.00372 mol) of 1-(3-nitrobenzyl) indolin-2-one was dissolved in ethanol and 10 ml 5N NaOH solution was mixed in it. It was refluxed for 3 hours. Then ethanol was removed by distillation. Remaining aqueous solution was filtered then acidified with dilute HCl. After achieving acidic pH 2-(2-(3-nitrobenzylamino) phenyl) acetic acid was precipitated out. Synthesized 2-(2-(3-nitrobenzylamino) phenyl) acetic acid was recrystallized by diethyl ether.

Mol. Formula	Mol. Weight	Melting point	R_f value (Hexane 5, Ethyl acetate 3)	Percentage Yield
$C_{15}H_{14}N_2O_4$	286.283	74-76°C	0.78	40.56%



1-(3-nitrobenzyl)indolin-2-one

2-(2-(3-nitrobenzylamino)phenyl)acetic acid

Pharmacological Screening

Antimicrobial activity[43, 45, 44, 45, 46, 47]

Introduction

A drug is considered to have antibacterial (bacteriostatic) or antifungal activity when it inhibits the growth or multiplication of bacteria or fungi respectively and is considered as bactericidal or fungicidal when it actually kills bacteria or fungi. Drugs that are bactericidal under certain circumstances may have an apparent bacteriostatic effect at other times. Important factors for the antimicrobial activity are size of the inoculum, metabolic state of organisms, pH, temperature, duration of interaction, concentration of the inhibitor and presence of interfering substance.

In vitro tests are used as screening procedure for new agents and for testing the susceptibility of individual isolates from infections to determine which of the available drugs might be useful therapeutically. In general, minimum inhibitory concentration (MIC) and sensitivity tests are used to express the effectiveness of a compound as an antimicrobial agent. MIC is the smallest concentration of the substance required to inhibit the growth of a test organism under specified conditions. MIC can be determined by tube dilution technique. Sensitivity testing is done to determine the range of microorganisms that are susceptible to the compound under specified conditions. It can be done by zone inhibition method. This method is suitable for the organisms that grow well overnight such as most of the common aerobes and facultative anaerobes and rapidly growing fungi.

Determination of Antibacterial activity

Preparation of the solutions of synthesized compounds

Accurately weighed 50 mg of each synthesized compound was transferred to different 100 ml volumetric flasks. These compounds were dissolved in to DMF and volume in each flask was made up to 100 ml with DMF. These solutions (each having conc. 500 µg/ml) were used as stock solutions. Two ml of stock solutions were transferred in to 10 ml volumetric flasks and further

dilutions were made to 10 ml mark with DMF. The final concentrations of prepared solutions were 100 µg per ml.

Preparation of stock solution of standard drug (Ampicillin & Ciprofloxacin)

Ampicillin (50 mg) was accurately weighed and transferred to 100 ml volumetric flask. The drug was dissolved and diluted up to 100 ml with DMF. The final solution contained 500 µg/ml of the ampicillin. This solution in volume of 2 ml was transferred in to a 10 ml volumetric flask and further dilutions were made to 10 ml mark with DMF. The final solutions contained 100 µg ampicillin per ml of the solution.

Same procedure was followed for ciprofloxacin.

Used Glassware's

Petridishes, pipettes and culture tubes were washed with distilled water, dried in oven, packed in autoclaved at 120 lb /inch^2 pressure (121^0 C) for 15 min.

Preparation of reagent and media

All reagent and media were prepared in distilled autoclaved water.

Preparation of Nutrient broth (pH 7.03± 0.1)

S. No.	Ingredients	Quantity
1.	Peptone	10 g
2.	Beef Extract	10 g
3.	NaCl	5 g
4.	Distilled water	Up to 1000 ml

Procedure

5 g Peptone, 3 g beef extract and 3 g sodium chloride (all of biological grades) were weighed and dissolved in 800 ml of distilled water in a 1000 ml volumetric flask. The volume was made up to 1000 ml with distilled water. This nutrient broth was sterilized in autoclave at 120 lb/inch^2 pressure (121^0 C) for 15 minutes.

Preparation of nutrient agar media (2%, pH 6.8±0.2):

Composition

S. No.	Ingredients	Quantity
1.	Peptone	10g
2.	Beef Extract	10 g
3.	NaCl	5 g
4.	Agar	20 g
5.	Distilled water	Up to 1000 ml

Procedure:

Peptone (5 g) beef extract (3 g) and sodium chloride (3g) (all of biological grades) were weighed and dissolved in 400 ml of distilled water in a 500 ml volumetric flask and warmed. 20 g agar

was dissolved in 50 ml of warm distilled water. The two solutions were mixed and the volume in volumetric flask was made up to 500 ml with warm distilled water. This nutrient agar media was sterilized in an autoclave at 120 lb /inch^2 pressure (121^0 C) for 15 min.

Preparation of inoculum:

Vial containing lactose dilution (dehydrated powder) of *Escherichia coli* was broken using sterile scalpal knife in aseptic condition in a conical flask containing 100 ml of nutrient broth. This flask was incubated for 24 hours at 37^0 C in Biological Oxygen Demand (BOD) incubator. After 24 hours, a turbid solution was obtained.

Inoculum of *Bacillus subtilis* was also prepared by the same method.

Procedure for determination of anti-bacterial activity: -

Following steps were followed for determination of antibacterial activity of synthesized compounds:

- Laminar airflow bench was swapped with 70 % alcohol and UV lamp was switched on. After 30 min, the UV lamp was switched off.
- All the reagents, media, inoculum and glassware were placed in laminar airflow bench observing all aseptic conditions.
- Inoculum was mixed in to nutrient agar medium solution. This inoculated nutrient agar medium was placed in to petridishes before its conversion in to gel form. After solidification of agar plates, gel was punched to prepare wells (diameter 10 mm) using sterile corn borer. Drug solutions were added to these wells with the help of micropipette. Plates were kept at room temperature for one hour to facilitate diffusion and later were transferred to an incubator. After inoculation at 37 ^{0}C for 48 hours. The zone of inhibition was measured using mm scale.
- Negative controlled plate- In this plate, only nutrient agar medium was poured i.e. it did not contain drug dilution and inoculum.
- Positive controlled plate 1 ml and 2 ml DMF was poured in to inoculated petridishes.

Results of antibacterial activity

The result of antibacterial activity observed, are reported in Table

Table: Antibacterial activity data of synthesized compounds

Compound (100 µg)	Zone of inhibition in mm		Compound (100 µg)	Zone of inhibition in mm	
	B. Subtilis (MTCC-441)	*E. Coli (ATCC-11775)*		*B. Subtilis (MTCC-441)*	*E. Coli (ATCC-11775)*
Ampicillin	27		AB_{32}	-	-
Ciprofloxacin		25	AB_{34}	14	17
DMF	-	-	AB_{35}	15	13

AB_{11}	21	12	AB_{41}	-	-
AB_{12}	15	16	AB_{42}	-	-
AB_{14}	31	26	AB_{44}	15	17
AB_{15}	-	-	AB_{45}	13	21
AB_{21}	27	12	AB_{51}	-	-
AB_{22}	-	-	AB_{52}	-	-
AB_{24}	17	15	AB_{54}	18	25
AB_{25}	27	24	AB_{55}	-	-
AB_{31}	-	-			

(-) Indicates no activity

Preliminary antibacterial studies were conducted on synthesized compounds (AB_{11}, AB_{12}, AB_{14}, AB_{15}, AB_{21}, AB_{22}, AB_{24}, AB_{25}, AB_{31}, AB_{32}, AB_{34}, AB_{35}, AB_{41}, AB_{42}, AB_{44}, AB_{45}, AB_{51}, AB_{52}, AB_{54}, AB_{55}) using *Bacillus subtilis* (MTCC-441) and *E. coli* (ATCC-*11775*), at concentration of 100 µg/ml by agar plate method. The zone of inhibition of each strain recorded for comparison, Ampicillin was used as standard for gram +ve and ciprofloxacin was used for gram -ve strains.

From the antibacterial activity data, it was found that most of compounds exhibited significant antibacterial activity against microorganisms.

It has been found that compounds AB_{11}, AB_{12}, AB_{14}, AB_{21}, AB_{24}, AB_{25}, AB_{34}, AB_{35}, AB_{44}, AB_{45} and AB_{54} showed the good activity against both of strain comparable to ampicillin and ciprofloxacin was taken as standard at the same concentration.

CONCLUSION

This research work comprises of the synthesis of 2-anilinophenylacetic acids derivatives. The synthesis of a series of 2-anilinophenylacetic acids is synthesized as per scheme in five steps. Syntheses have been carried out following simple methodology in excellent isolated yields. A series of 2-anilinophenylacetic acids were synthesized are identity and confirmed on the basis of their sharp melting point determination, thin layer chromatography and elemental analysis.

All the synthesized compounds have been tested for anti-microbial activities by “Cup – Plat Method”.

Each 2-anilinophenylacetic acid derivative will be screened for their anti-microbial activities. It was also found that the synthesized compound It has been found that compounds AB_{11}, AB_{12}, AB_{14}, AB_{21}, AB_{24}, AB_{25}, AB_{34}, AB_{35}, AB_{44}, AB_{45} and AB_{54} showed the good activity against both of strain comparable to ampicillin and ciprofloxacin was taken as standard at the same concentration. Whereas all the other compound has showed mild to moderate anti-microbial activities activity as compared to standard drug. These preliminary results indicate that some of compounds are exhibiting good activity.

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