IJMPR 2024, 8(7), 136-157

International Journal of Modern Pharmaceutical Research
www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Review Article
SJIF Impact Factor: 6.669

THE SYNTHESIS A SERIES OF 2 – ANILINOPHENYLACETIC ACID DERIVATIVS FOR THEIR ANTI-INFLAMMATORY ACTIVITY

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Article Received on: 01/06/2024
Article Revised on: 20/06/2024
Article Accepted on: 01/07/2024

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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-inflammatory activity by Carrageenin Induced Paw edema method. Each 2-anilinophenylacetic acid derivative will be screened for their anti-inflammatory activity. It was also found that the synthesized compound YS-1,YS-4, YS-5, *and YS-6* has shown significant anti-inflammatory activity whereas all the other compound have showed mild to moderate anti-inflammatory activity as compared to standard drug. Syntheses have been carried out following simple methodology in excellent isolated yields. The structure and purity of the original compounds were confirmed by melting point, IR, TLC, and elemental analysis. These preliminary results indicate that some of compounds are exhibiting good activity.

KEYWORDS: 2-anilinophenylacetic acids, diclofenac, Synthesis, anti-inflammatory activity, Carrageenin Induced Paw edema method, Chemotherapeutic Science International.

INTRODUCTION

Medicinal chemistry is an interdisciplinary science covering a particularly wide domain situated at the interface of organic chemistry with life sciences, such as biochemistry, pharmacology, molecular biology, immunology, pharmacokinetics and toxicology on one side, and chemistry- based disciplines such as physical chemistry, crystallography, spectroscopy and computer-based information technologies on the other. The knowledge of the molecular targets (enzymes, receptors, nucleic acids), has benefitted from the progress made in molecular biology, genetic engineering and structural biology. For an increasing number of targets the three-dimensional structure and the precise location of the active site are known. The design of new active substances is therefore more and more based on results obtained from ligand–receptor modeling studies ie the COX receptor consists of two noncoplanor hydrophobic regions with a cationic center. This receptor consists essentially of a large flat area, a trough to accommodate an out-of-plane group (such as an aryl ring acting possible by a charge-transfer type of interaction), and a cationic site to accommodate an acidic anion.[3] By seeing this phenomenon a medicinal chemist will able to synthesize a molecule which satisfies all steric parameters to bind COX receptor.

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for treatment of pain, fever and inflammations, particularly arthritis. Among the most widely used NSAIDs, diclofenac is most widely used NSAIDs in the world. It is available in 120 different countries and ranked 30th among top 200 drugs with respect to new prescription drug.[4] There are two basic approaches to develop new synthetic drugs:
(a) Synthesis of analogues, modifications, or derivatives of existing compounds for shortening and improving treatments and (b) searching for novel structure, which has never been synthesised. Discovery of newer and more potent analogs of molecules with already established activities form a key part of research in the pharmaceutical field. Bringing about modifications in the parent compound serves to enhance the activity of the compound and also in most cases, eliminates adverse effects or toxicity associated with the parent drug. To pursue the goal to develop a novel anti-inflammatory agent, our research efforts are directed to synthesize novel analogue of the well-known anti-inflammatory compound diclofenac.

1.1 Inflammation

Inflammation is a normal, protective response to tissue injury caused by physical trauma, noxious chemicals, or microbiologic agents. Inflammation is the body's effort to inactivate or destroy invading organisms, remove irritants, and set the stage for tissue repair. When healing is complete, the inflammatory process usually subsides. However, inflammation is sometimes inappropriately triggered by an innocuous agent, such as pollen, or by an autoimmune response, as in asthma or rheumatoid arthritis. In such cases, the defense reactions themselves may cause progressive tissue injury, and anti-inflammatory or immuno-suppressive drugs may be required to modulate the inflammatory process.

Inflammation is triggered by the release of chemical mediators from injured tissues and migrating cells. The specific chemical mediators vary with the type of inflammatory process and include amines, such as histamine and 5-hydroxytryptamine; lipids, such as the prostaglandins; small peptides, such as bradykinin; and larger peptides, such as interleukin-l. Discovery of the wide variation among chemical mediators has clarified the apparent paradox that an anti-inflammatory drug may interfere with the action of a particular mediator important in one type of inflammation but be without effect in inflammatory processes not involving the drug's target mediator.

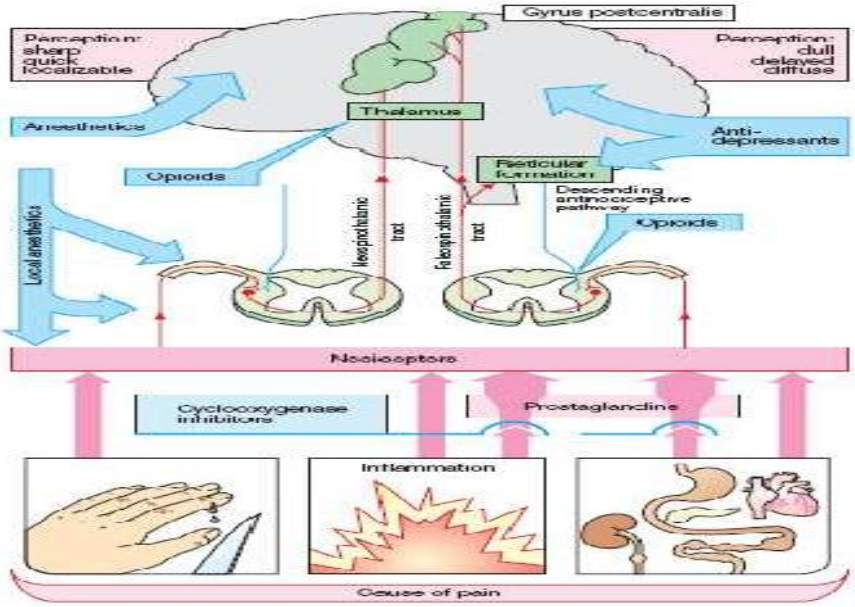


Fig. 1: Pain mechanisms and pathways (Colour atlas of pharmacology).

Eicosanoids

Eicosanoids, unlike histamine, are not found preformed in the tissues; they are generated de novo from phospholipids. They are implicated in the control of many physiological processes and are among the most important mediators and modulators of the inflammatory reaction.

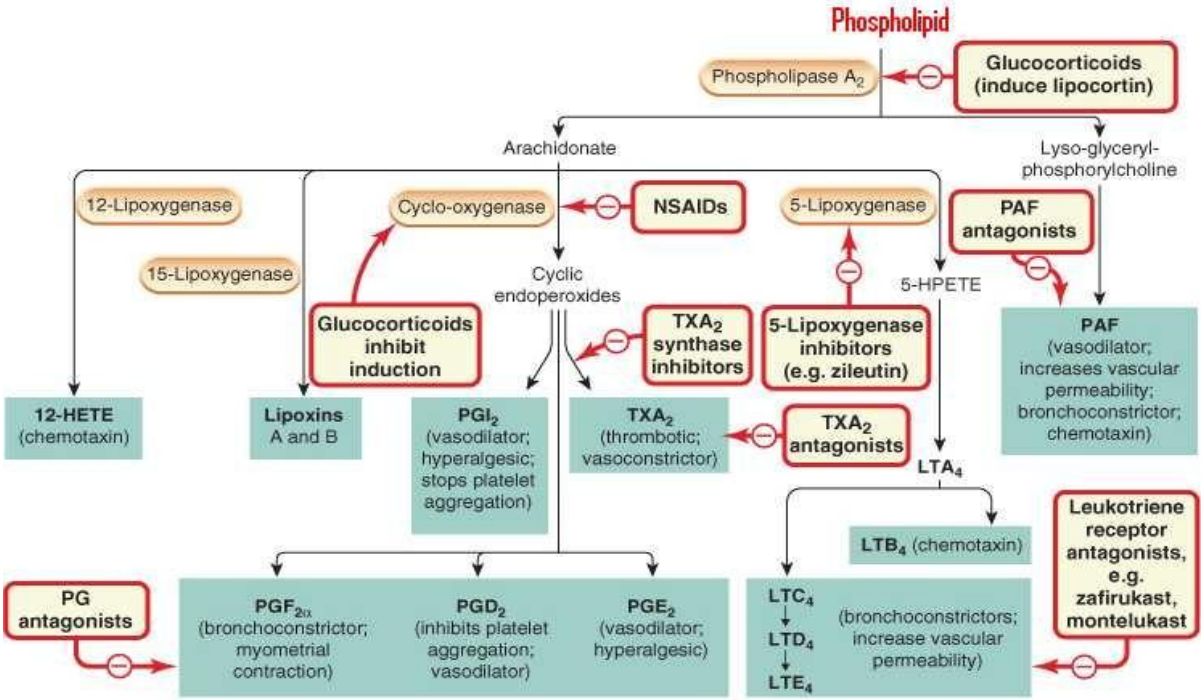


Fig. 2: Summary diagram of the inflammatory mediators derived from phospholipids with an outline of their actions, and the sites of action of anti-inflammatory drugs.

The anti-inflammatory action of the non-steroidal anti-inflammatory drugs (NSAIDs) results mainly from the fact that they inhibit the action of the fatty acid COXs.

Prostanoids

COX exists in two forms COX-1 and COX-2. COX-1 is found in most cells as a constitutive enzyme (i.e. it is always present) and it is thought that the prostanoids it produces are involved in normal homeostasis (e.g. regulating vascular responses). COX-2 is induced in inflammatory cells by an inflammatory stimulus. Subsequent steps in arachidonate metabolism differ in different cells. In platelets, the pathway leads to thromboxane A_2 synthesis, in vascular endothelium it leads to PGI_2 synthesis and in macrophages it leads mainly to synthesis of PGI_2. Mast cells synthesise PGI_2. The nomenclature of the eicosanoids derives from the fact that the names of the first two prostaglandins were based on the separation procedure PGE partitioned into ether, and PGF into the phosphate buffer. PGA and PGB were so-called because of their stability or otherwise in acids and bases. Thereafter other letters of the alphabet were filled in rather randomly. The subscripts refer to the number of double bonds; thus PGF_2 has two double bonds. The Greek letter subscript, the α in $PGE_{2\alpha}$, refers to the orientation of the hydroxyl above or below the plane of the ring. PGE_2, PGI_2, PGD_2, $PGF_{2\alpha}$ and thromboxane A_2 are the most important products of the COX pathway. If the COX acts on eicosatrienoic acid instead of arachidonic acid, the resulting prostanoids have only a single double bond, for example PGE_1.

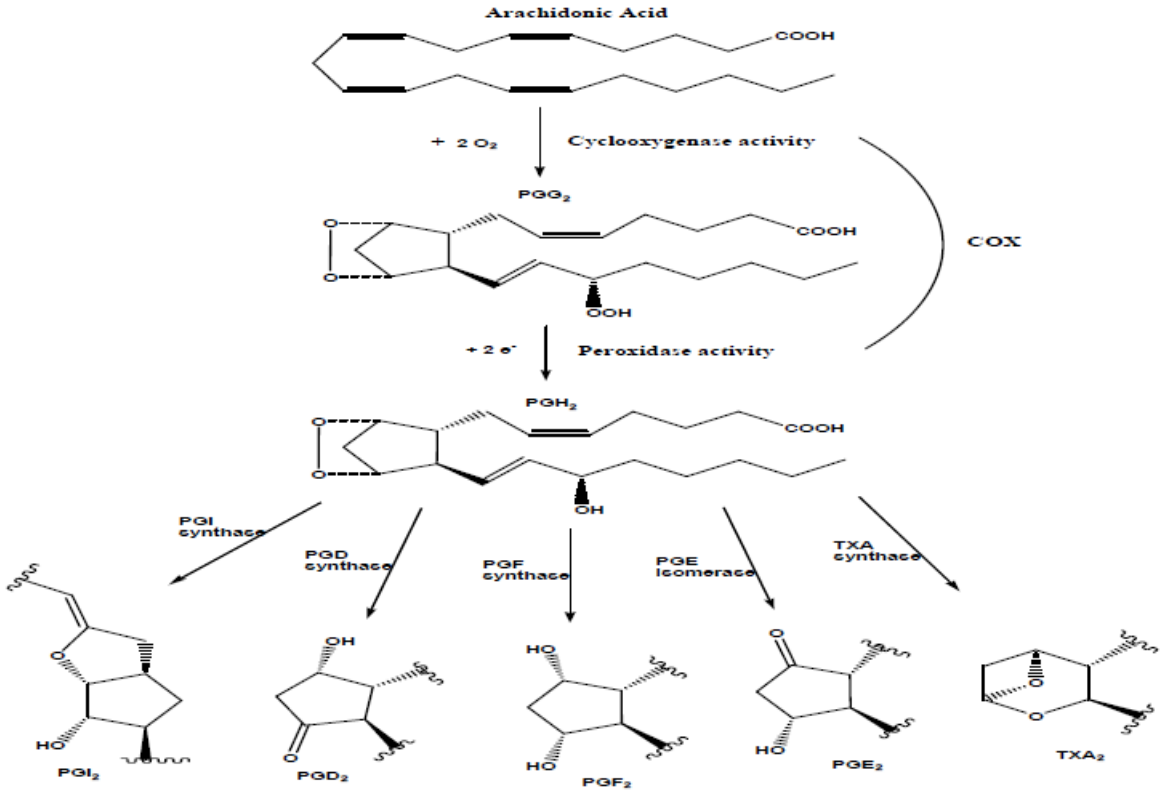


Fig. 3: The Cyclooxygenase Pathway.

1.2 Non-steroidal anti-inflammatory Drugs.

Table 1: Showing anti-inflammatory Drugs Detail's.

Drug	Plasma half-life (hours)	Comments
Non-selective COX inhibitors		
Aspirin	3-5	Old anti-inflammatory drug, now used in cardiovascular disorders, colonic and rectal cancer, Alzheimer's disease and radiation-induced diarrhoea.
Diflunisal	8-13	Less GIT irritation than aspirin; long acting (is related to aspirin).
Ibuprofen	2	First-choice drug; lowest incidence of unwanted effects.
Fenbufen	10	A pro-drug, activated in the liver; less risk of GIT, reactions, more risk of skin reactions.
Naproxen	14	The same chemical class as ibuprofen but rather more potent; reasonable efficacy, moderate risk of adverse reactions.
Mefenamic acid	4	Only moderate anti-inflammatory action; diarrhoea likely; haemolytic anaemia

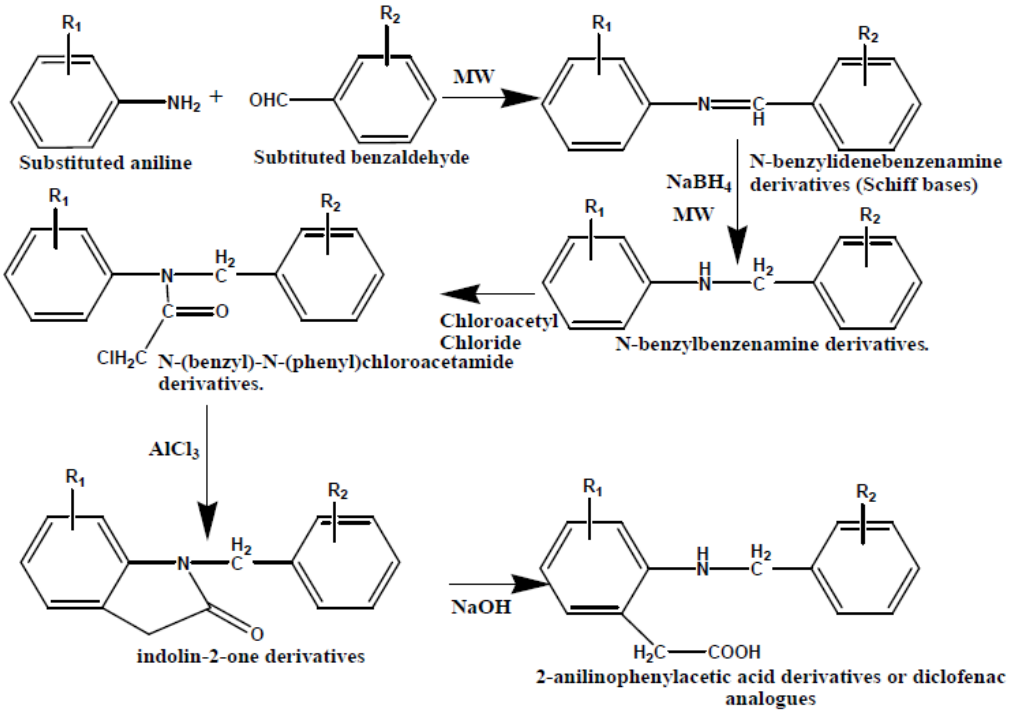
		has been reported; possible interaction with warfarin; skin reactions can occur.
Nabumetone	24[a]	A pro-drug, activated in the liver; adverse effects less marked than with aspirin, antipyretic action more marked.
Paracetamol	2-4	Paracetamol has potent analgesic and antipyretic actions but rather weaker anti-inflammatory effects.
Diclofenac	1-2	Moderate potency; moderate risk of adverse GIT effects.
Sulindac	7 (18)[a]	A pro-drug interconvertible with active sulfide metabolite; moderate risk of side-effects; chemically related to indometacin but less potent.
Indometacin	2	Potent inhibitor of COX in vitro; high incidence of non-GIT side- effects;[b] headache, dizziness, etc.
Tolmetin	5	Efficacy as for ibuprofen; moderate risk of adverse effects.
Piroxicam	45	GIT irritation in 20% of patients; tinnitus; rashes
Tenoxicam	72	Steady-state plasma concentration only after 2 weeks
Etodalac	7	Possibly fewer GIT effects than other non-selective NSAIDs
Meloxicam	20	Possibly fewer GIT effects than other non-selective NSAIDs
COX-2 inhibitors		
Celecoxib	11	New compound; markedly less GIT toxicity
Rofecoxib	17	New compound; markedly less GIT toxicity
Valdecoxib	11	New compound; markedly less GIT toxicity
Etoricoxib	22	New compound; markedly less GIT toxicity

EXPERIMENTAL SECTION

1. 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.

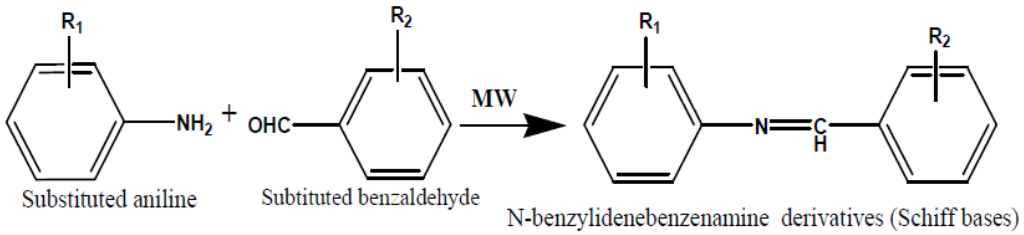
1. 2-anilinophenylacetic acid derivatives or diclofenac analogues.
2. 2- indolone derivatives.
3. N-(benzyl)-N-(phenyl)chloroacetamide derivatives.
4. N-benzylbenzenamine derivatives.
5. N-benzylidenebenzenamine (Schiff bases) derivatives.



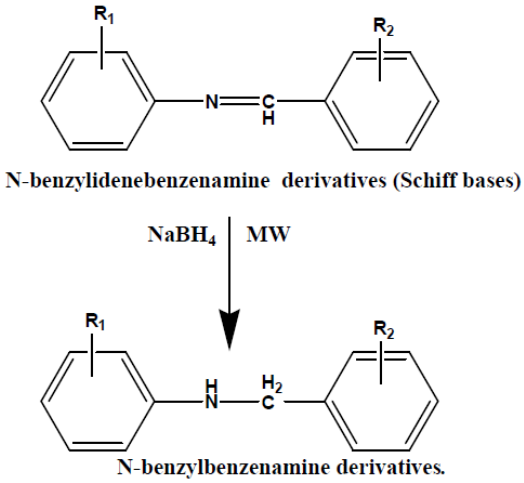
All of these, four compounds are having pharmacological and synthetic interest so the literature is review for 2-anilinophenylacetic acid, 2-indolone, N-benzylbenzenamine, N-benzylidenebenzenamine (Schiff Bases) derivatives. Therefore we are able to get more pharmacological active compounds.

2. **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.

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

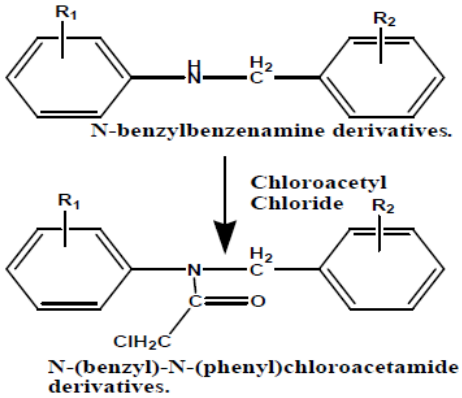


2. Synthesis of N-benzylbenzenamine derivatives -: N-benzylbenzenamine derivatives are reduced products of N-benzylidenebenzenamine derivatives. This reaction was microwave assisted solid phase synthesis. In this synthesis scheme, $NaBH_4$-wet silica gel [The reagent, 10% $NaBH_4$-silica, is prepared 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 optimised 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.

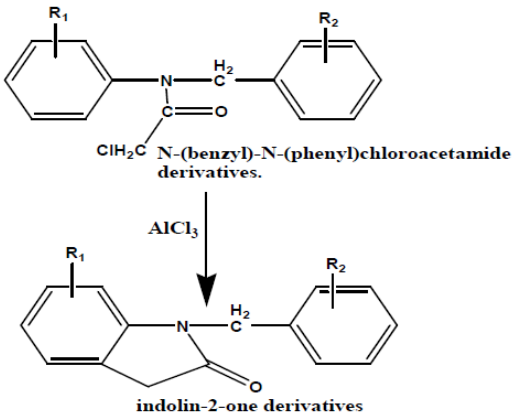


3. Synthesis of N-(benzyl)-N-(phenyl)chloroacetamide derivatives-: N-(benzyl)-N-(phenyl)chloroacetamide derivatives are chloroacetylated products of N-benzylbenzenamine derivatives. This rection was done in three necked round bottom (RBF) flask fitted with two dropping funnels and a mechanical stirrer. 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

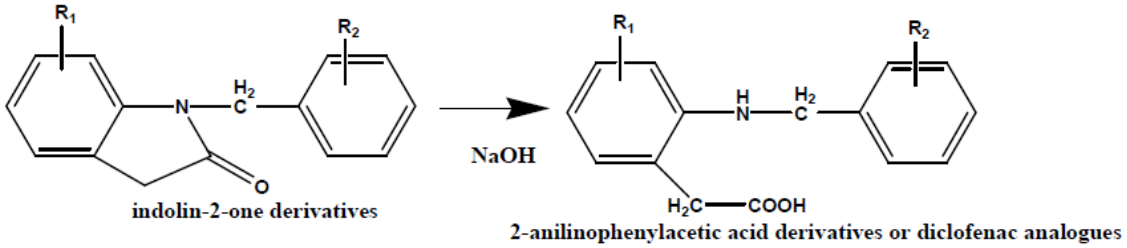
the help of sodium carbonate solution. After completion of addition ice bath was removed and reaction vessel was placed at room temperature with stirring for 2 hours. After completion of reaction, reaction mixture was placed in to a separating funnel. Product containing MEK layer was separated. This 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.



4. 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 friedalcraft cyclization chloroacetylated product was dissolved in nitrobenzene in two necked round bottom flask. 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. Than 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. Than recrystallized by diethyl ether and checked for melting point and R_f value.

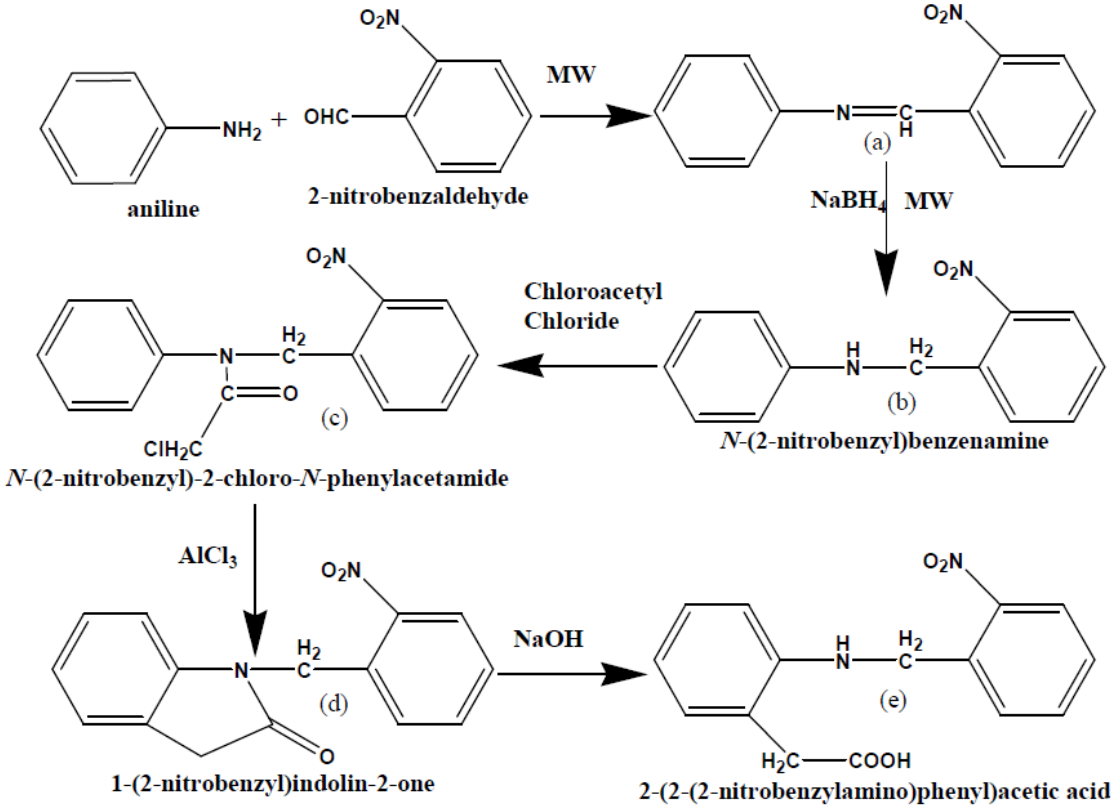


5. 2-anilinophenylacetic acid derivatives or diclofenac analogues-: 2-anilinophenylacetic acid derivatives are hydrolyzed products of 2- indolone derivatives. For hydrolysis of 2- indolone derivatives 5N NaOH solution was used for ring breaking. For completion of reaction 2- indolone derivative was dissolved in ethanol and 10 ml 5N NaOH solution was mixed in it. It was refluxed for 3 hours. Than 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.



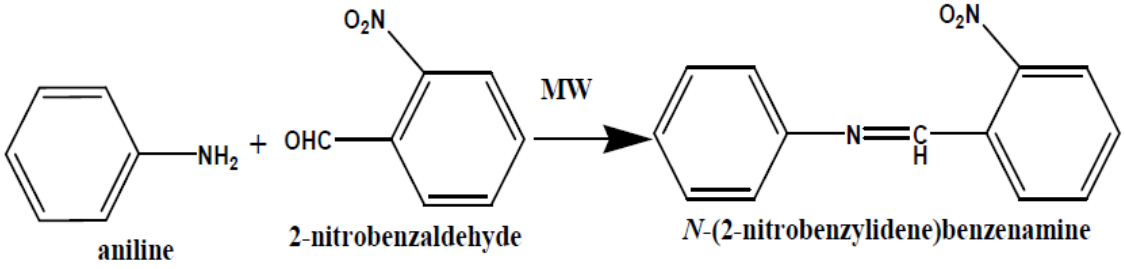
5.3. Synthesis of 2-anilinophenylacetic acid derivatives

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



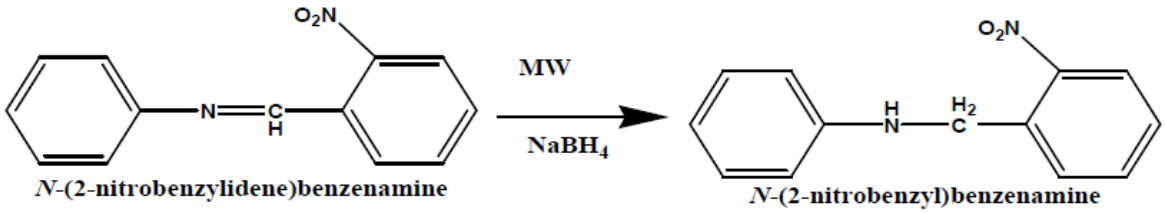
(a) Synthesis of N-(2-nitrobenzylidene) benzenamine (AB_{11}): 453.4 mg (3 mmol) of 2 nitro benzaldehyde and 279.9 mg or 1.023 ml (3 mmol) of aniline was taken in 25 ml in an erlenmeyer flask. A funnel was placed over the mouth of the erlenmeyer flask. This reaction mixture was placed in to microwave 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%



(b) 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 methnol. 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.

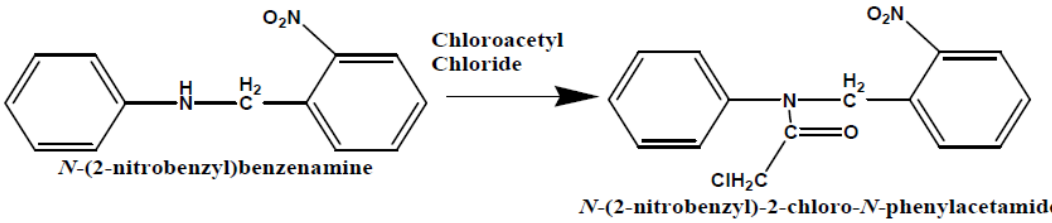
Mol. Formula	Mol. Weight	Melting point	R_f value (Hexane 5, Ethyl acetate 2)	Percentage Yield
$C_{13}H_{12}N_2O_2$	228.247	38-41°C	0.625	78.95%



N-(2-nitrobenzylidene)benzenamine — *N*-(2-nitrobenzyl)benzenamine

(c) 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
C15H13ClN2O3	304.728	62-63°C	0.564	82%

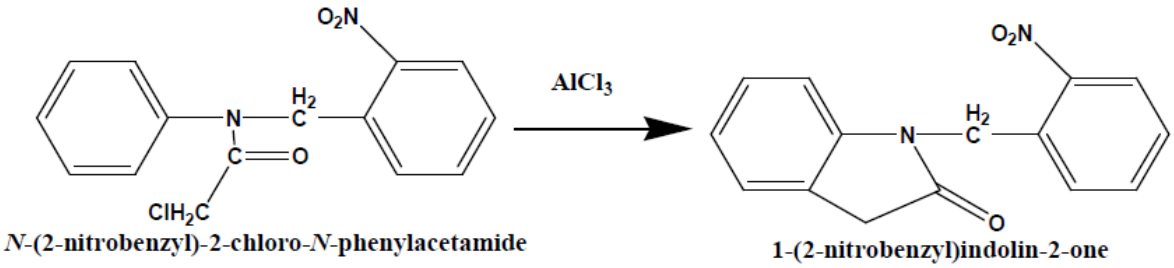


N-(2-nitrobenzyl)benzenamine — *N*-(2-nitrobenzyl)-2-chloro-*N*-phenylacetamide

(d) 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. Than 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%



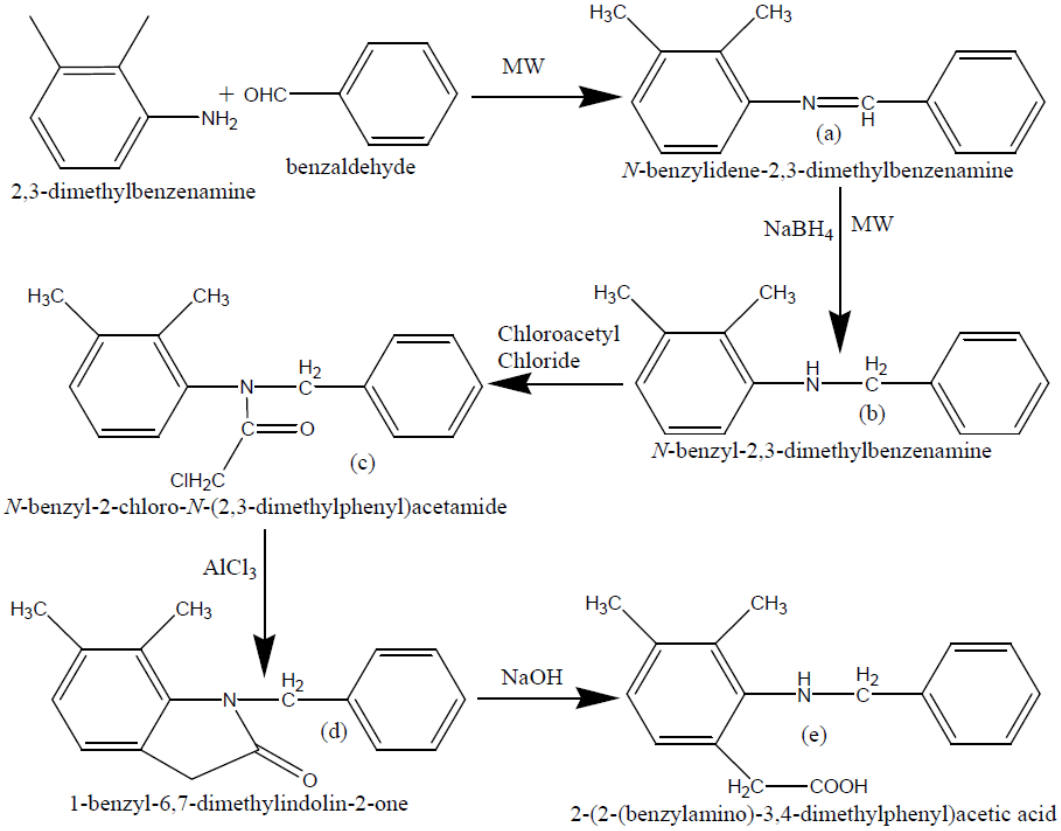
N-(2-nitrobenzyl)-2-chloro-*N*-phenylacetamide — 1-(2-nitrobenzyl)indolin-2-one

(e) 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. Than 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. Synthesised 2-(2-(2-nitrobenzylamino) phenyl)acetic acid was recrystallized by diethyl ether.

(f)

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%

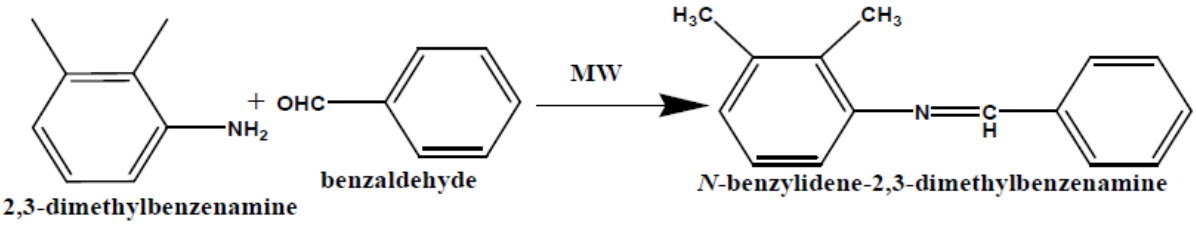
5.3.2 Synthesis of 2-(2-(benzylamino)-3,4-dimethylphenyl)acetic acid (AB_{25})



2,3-dimethylbenzenamine — *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

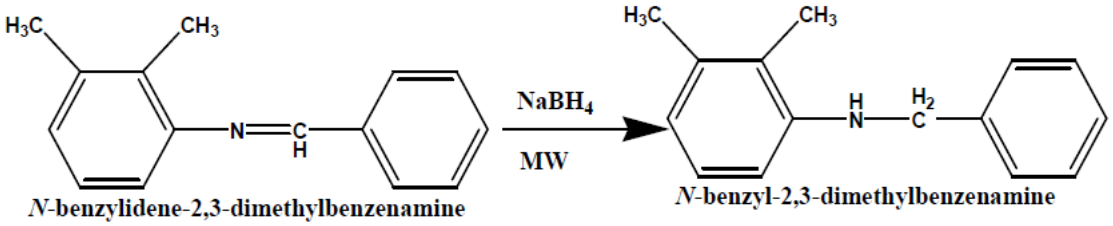
(a) Synthesis of N-benzylidene-2,3-dimethylbenzenamine (AB_{21}): 318 mg (3 mmol) of benzaldehyde and 363.54 mg or 0.36 ml (3 mmol) of 2,3-dimethyl aniline was taken in 25 ml in an erlenmeyer flask. A funnel was placed over the mouth of the erlenmeyer flask. This reaction mixture was placed in to microwave 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%



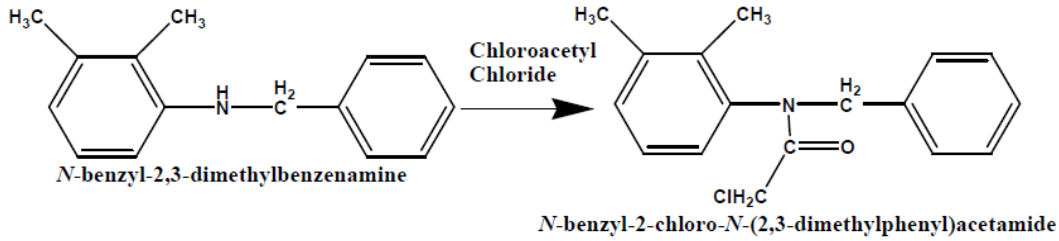
(b) Synthesis of N-benzyl-2,3-dimethylbenzenamine (AB_{22}): 418.6 mg (2 mmol) of synthesized N-benzylidene-2,3-dimethylbenzenamine was mixed in $NaBH_4$-wet silica gel in a mortar and pestle. After mixing this reaction mixture was poured in to an erlenmeyer flask. This erlenmeyer flask was placed in 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 methnol. After completion of extaraction 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%



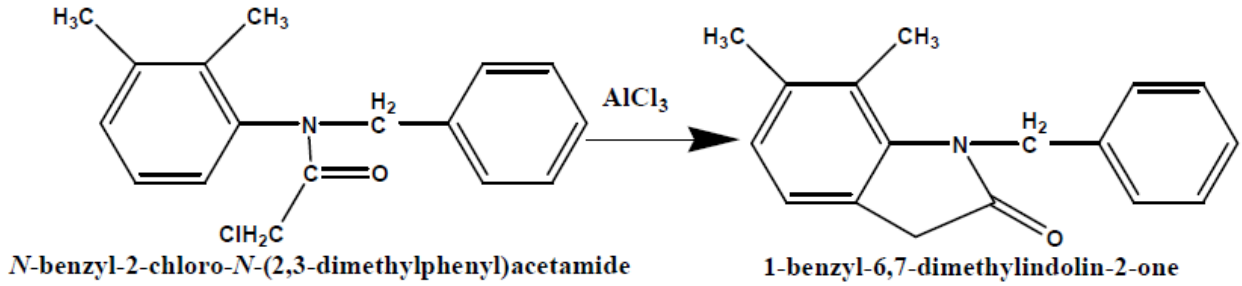
(c) Synthesis of N-benzyl-2-chloro-N-(2,3-dimethylphenyl)acetamide (AB_{23}): 12 g (0.056 mol) of N-benzyl-2,3-dimethylbenzenamine was dissolved in ethyl methyl ketone (MEK) and placed in to a three naked RBF. Three naked RBF was pre fitted with two dropping funnels and a mechanical stirrer. 7.22 g or 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%



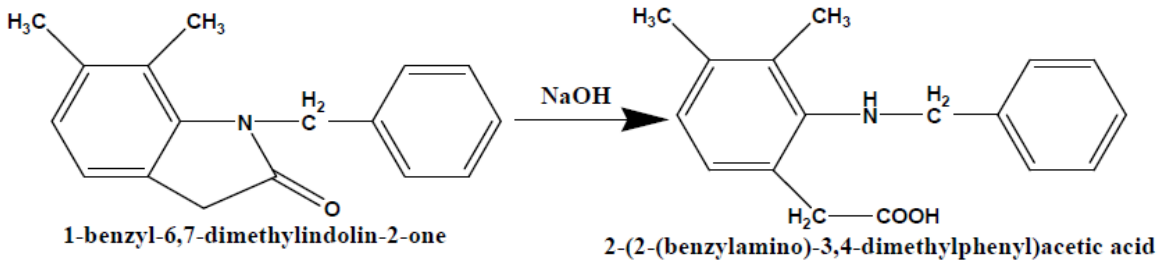
(d) Synthesis of 1-benzyl-6,7-dimethylindolin-2-one (AB_{24}): 4 g (0.0139 mol) of N-benzyl-2-chloro- N-(2,3-dimethylphenyl)acetamide was weighed and dissolved in nitrobenzene in a two necked round bottom flask. A magnetic bead was placed in to the RBF. This assembly was fixed over magnetic stirrer.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

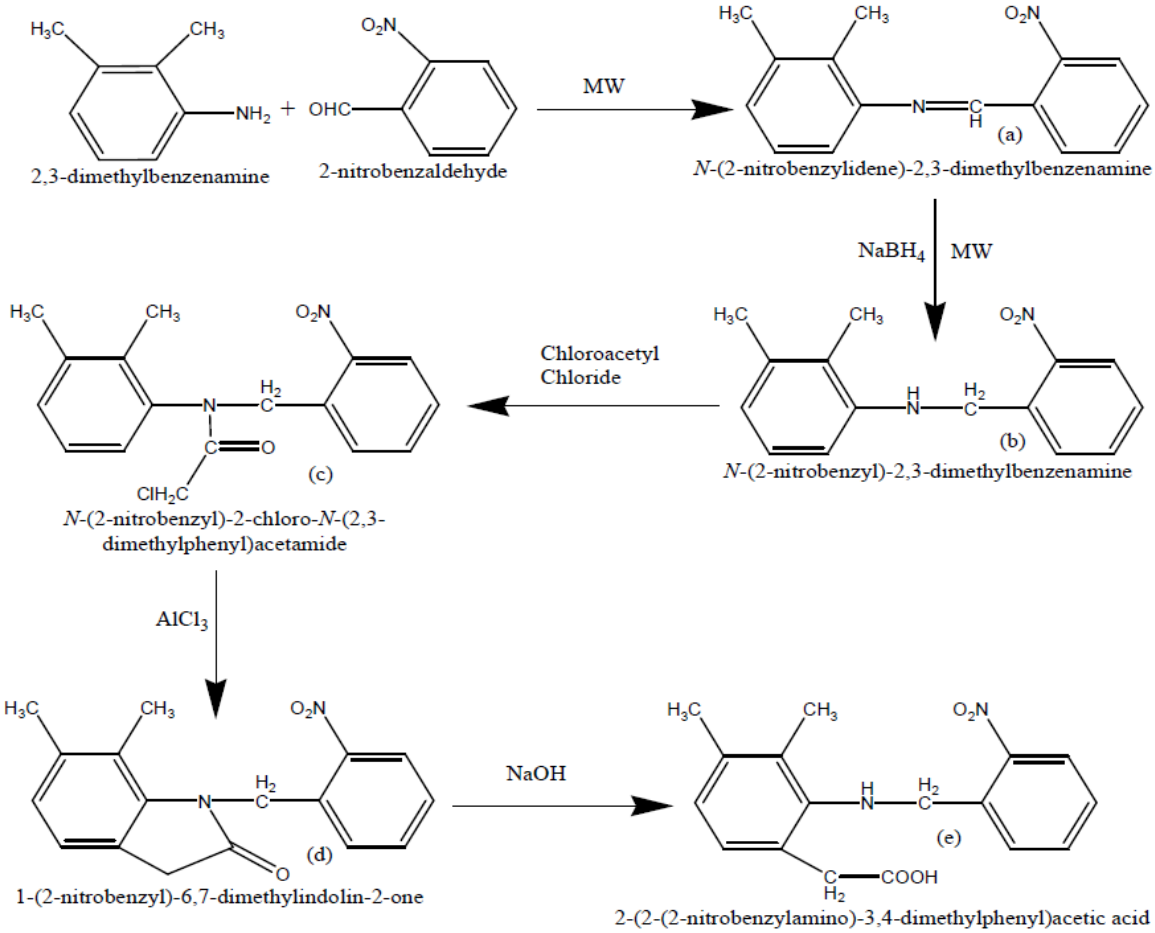


(e) Synthesis of 2-(2-(benzylamino)-3, 4-dimethylphenyl)acetic acid (AB_{25}): 1 g (0.00397 mol) of 1- benzyl-6,7-dimethylindolin-2-one was dissolved in ethanol and 10 ml 5N NaOH solution was mixed in it. It was refluxed for 3 hours. Than ethanol was removed by distillation. Remaining aqueous solution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

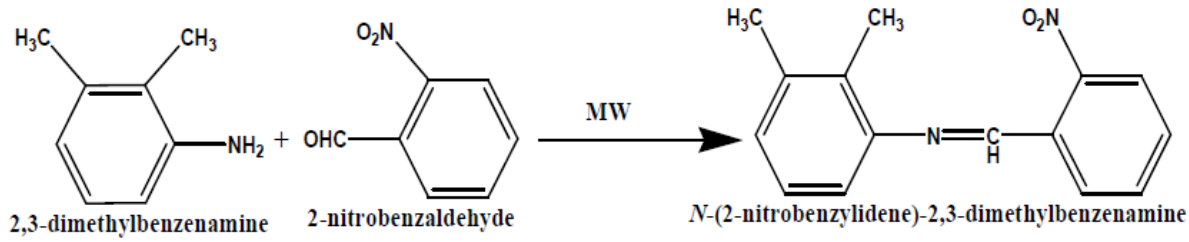


5.3.3 Synthesis of 2-(2-(benzylamino)-3,4-dimethylphenyl)acetic acid (AB_{35})



(a) 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%



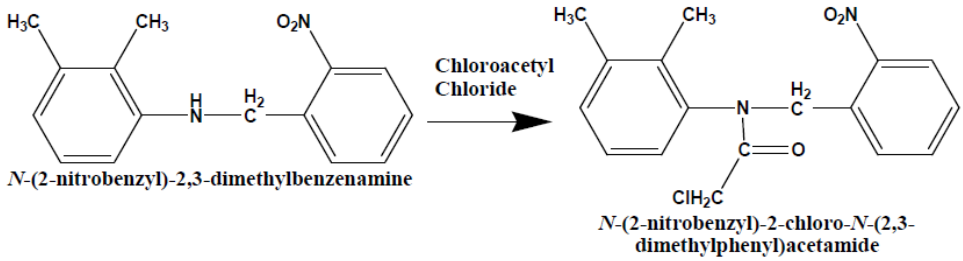
(b) Synthesis of N-(2-nitrobenzyl)-2,3-dimethylbenzenamine (AB_{32}): 508.6 mg (2 mmol) of N-(2- nitrobenzylidene)-2,3-dimethylbenzenamine was mixed in $NaBH_4$-wet silica gel in a mortar and pestle. After mixing this reaction mixture was poured in to an erlenmeyer flask. This erlenmeyer flask was placed 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 methnol. 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 benzenaminewas 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%

(c) 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%

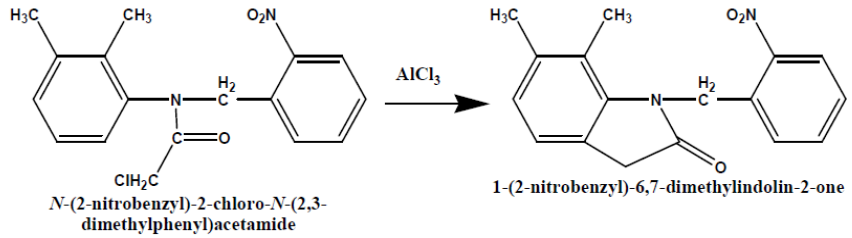


N-(2-nitrobenzyl)-2,3-dimethylbenzenamine

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

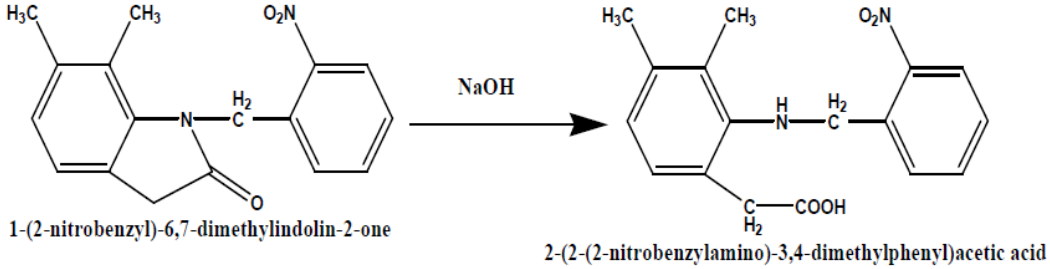
(d) Synthesis of 1-(2-nitrobenzyl)-6,7-dimethylindolin-2-one (AB_{34}): 3.1 g (0.0093 mol) of N-(2-nitrobenzyl)-2-chloro-N-(2,3-dimethylphenyl)acetamide was weighed and dissolved in nitrobenzene in a two naked round bottom flask. A magnetic bead was placed in to the RBF. This assembly was fixed over 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. Than 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. Than 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%

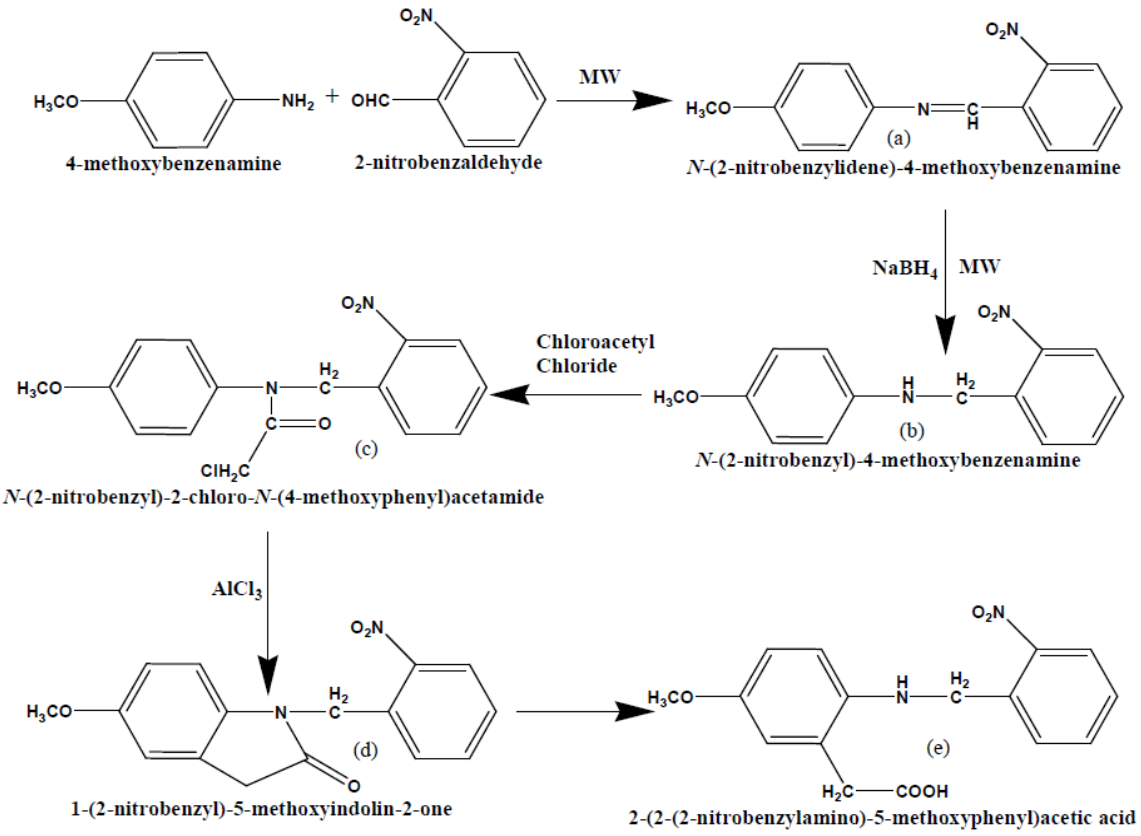


(e) Synthesis of 2-(2-(2-nitrobenzylamino)-3,4-dimethylphenyl)acetic acid (AB_{35}): 1 g (0.00337 mol) of 1-(2-nitrobenzyl)-6,7-dimethylindolin-2-one was dissolved in ethanol and 10 ml 5N NaOH solution was mixed in it. It was refluxed for 3 hours. Than ethanol was removed by distillation. Remaining aqueous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

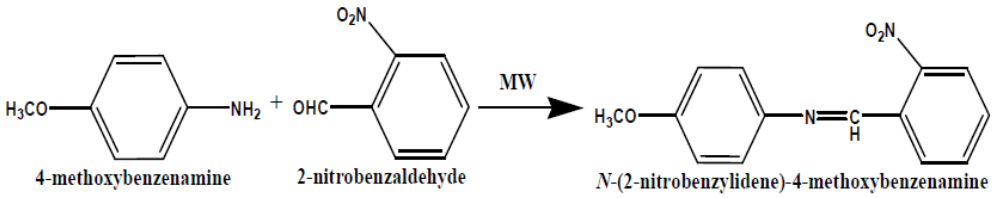


5.3.4 Synthesis of 2-(2-(2-nitrobenzylamino)-5-methoxyphenyl)acetic acid (AB_{45})



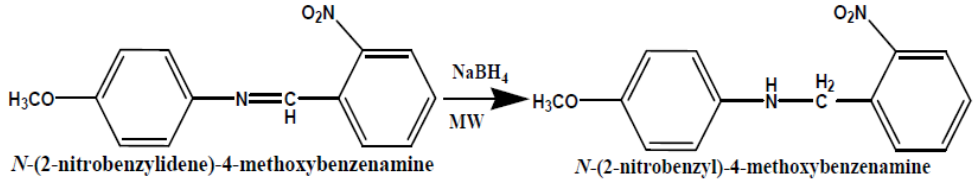
(a) Synthesis of N-(2-nitrobenzylidene)-4-methoxybenzenamine (AB_{41}): 453.36 mg (3 mmol) of 2- nitro benzaldehyde and 369 mg (3 mmol) of 4-methoxybenzenamine was taken in 25 ml in an erlenmeyer flask. A funnel was placed over the mouth of the erlenmeyer flask. This reaction mixture was placed in to 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%



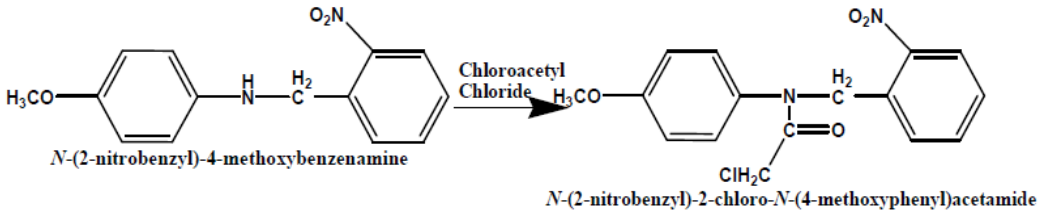
(b) Synthesis of N-(2-nitrobenzyl)-4-methoxybenzenamine (AB_{42}): 512.5 mg (2 mmol) of N-(2- nitrobenzylidene)-4-methoxybenzenamine was mixed in $NaBH_4$-wet silica gel in a mortar and pestle. After mixing this reaction mixture was poured in to an erlenmeyer flask. This erlenmeyer flask was placed in 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 methnol. 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%



(c) 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%

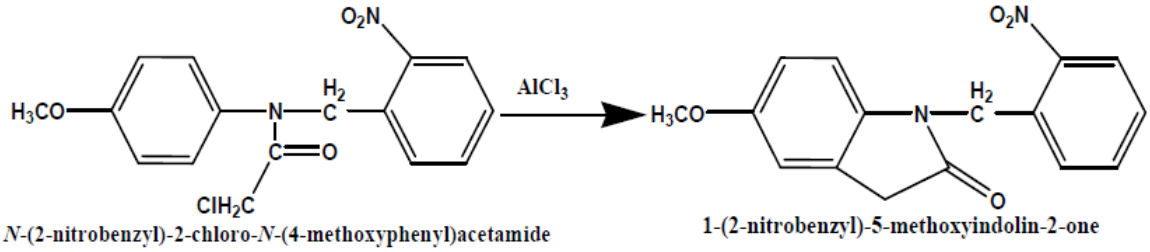


N-(2-nitrobenzyl)-4-methoxybenzenamine

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

(d) 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. Than 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%

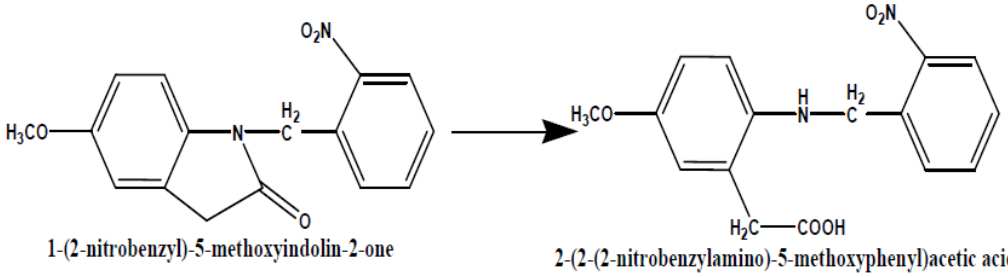


N-(2-nitrobenzyl)-2-chloro-*N*-(4-methoxyphenyl)acetamide

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

(e) 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. Than 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. Synthesised 2- (2-(2-nitrobenzylamino)-5-methoxyphenyl) acetic acid was recrystallized by diethyl ether.

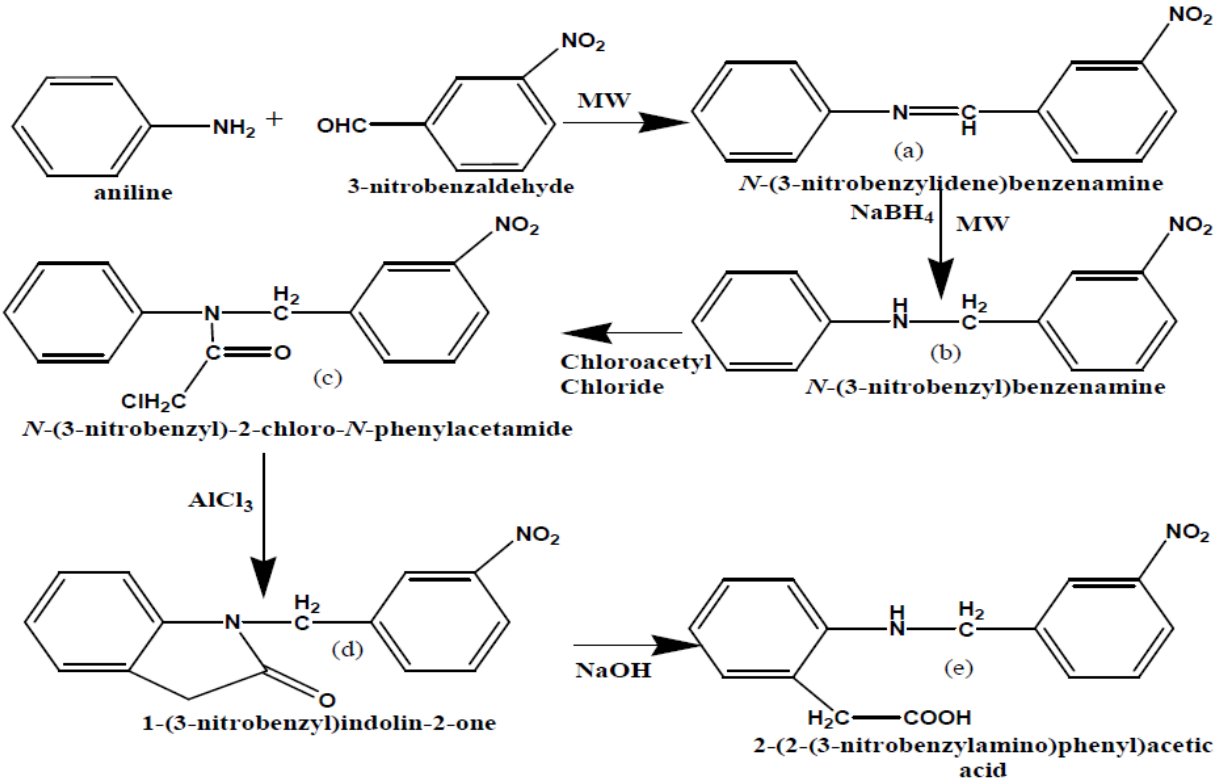
Mol. Formula	Mol. Weight	Melting point	R_f value (Hexane 5, Ethyl acetate 0.5, Ethanol 0.5)	Percentage Yield
$C_{16}H_{16}N_2O_5$	316.309	82-84°C	0.25	40.59%



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

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

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



(a) 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%

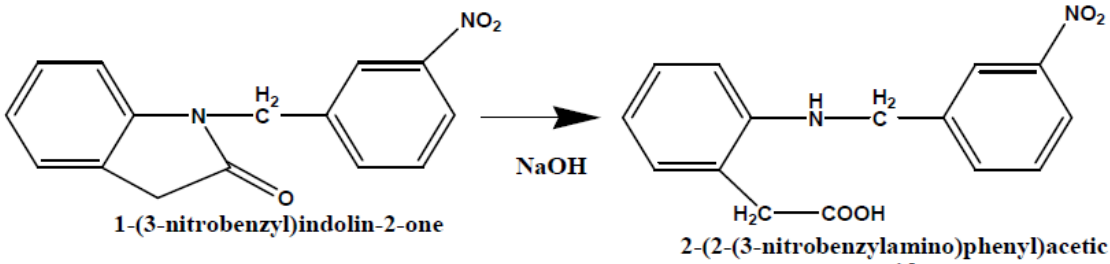
(b) Synthesis of N-(3-nitrobenzyl) benzenamine (AB_{52}): 452.5 mg (2 mmol) of N-(3- nitrobenzylidene) benzenamine was mixed in $NaBH_4$-wet silica gel in a mortar and pestle. After mixing this reaction mixture was poured in to an erlenmeyer flask. This erlenmeyer flask was placed in to microwave oven 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 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%

(c) 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%

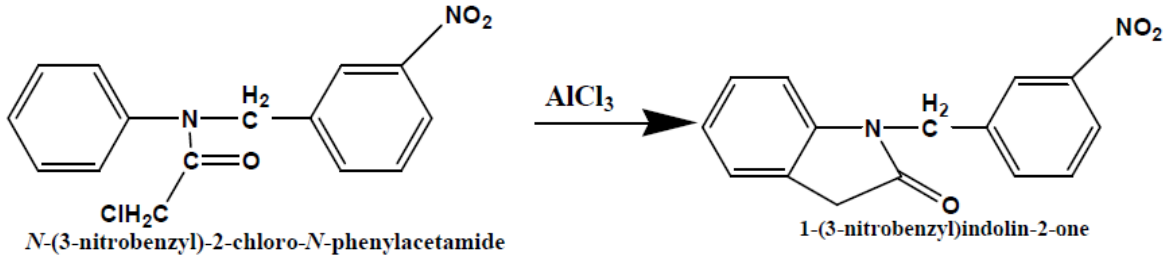


1-(3-nitrobenzyl)indolin-2-one

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

(d) 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. Than 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

(e) 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. Than 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. Synthesised 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%

Pharmacological Screening

Anti-inflammatory activity[yash] [14,48,49,50,51,52,53]

Among the many methods used for screening of anti-inflammatory drugs, one of the most commonly employed techniques is based upon the ability of such agents to inhibit the edema produced in the hind paw of the rat after injection of a phlogistic agent. Many phlogistic agents (irritants) have been used, such as brewer's yeast, formaldehyde, dextran, egg albumin, kaolin, aerosil, sulfated polysaccharides like carrageenan or naphthoylheparamine. The effect can be measured in several ways. The hind limb can be dissected at the talocrural joint and weighed. Usually, the volume of the injected paw is measured before and after application of the irritant and the paw volume of the treated animals is compared to the controls. Many methods have been are being used to measure the paw volume by simple and less accurate and by more sophisticated electronically devised methods. The value of the assessment is less dependent on the apparatus but much more on the irritant being chosen. Some irritants induce only a short lasting inflammation whereas other irritants cause the paw edema to continue over more than 24 h. In this research work we are using Winter *et al's* Carrageenin induced paw edema method. In this method paw volume was measured with plethysmograph using mercury immersion technique.[Vogel 760]

1. **Animal*:*** Albino rats of either sex, weighing 200-300 g, were used for the study. They were obtained from institute animal house. The animals were fed with standard chow diet and water ad libitium. They were housed in poly propylene cages maintained under standard condition (12 hour light / 12 hour dark cycle).The animals were deprived of food for 24 hour before experiment but allowed free access to drinking water throughout. The experimental protocol was approved from institutional animal ethical committee.
2. **Procedure for preparation of control vehicle (5% CMC solution):** Accurately weighed 5 gm of carboxy methyl cellulose was transferred to 100 ml volumetric flask. It was dissolved in 10 ml distilled water and made-up the volume to 100 ml by distilled water.
3. **Procedure for preparation of standard drug solution (Diclofenac sodium, dose- 10 mg/ kg, body weight of animal):** Synthesized compounds were diclofenac derivatives therefore diclofenac was taken as standard drug. Diclotol (Blue cross laboratories) injection was procured for using standard drug solution. It contained 25 mg/ml of diclofenac sodium therefore dose was calculated and drug was directly injected to the animal as per its body weight.
4. **Preparation of dosing solution of synthesized compounds:** 100 mg of synthesized compounds were weighed and transferred to 25 ml beaker. It was suspended in 10 ml, 5% cmc solution.
5. **Procedure for preparation of 1% Carrageenin solution:** Accurately weighed 1 gm of carrageenin was transferred to 100 ml volumetric flask. It was dissolved in 10 ml of normal saline solution and made-up volume up to 100 ml with normal saline solution.

Procedure for carrageenin Induced Paw edema Method: The Synthesized compounds were evaluated for its anti-inflammatory activity by carrageenin induced paw edema method. The albino rats (200-300 gm) were divided into seven groups of six animals each. One group named as Control (Group-I), another was Standard (Group-II) and remaining four groups were used for test groups, named as Test Group I, Test group II, Test group III Test group IV and Test group V. A mark was made on left hind paw of each rat just beyond tibio-tarsal junction, so that every time the paw could be dipped in the column up to the fixed mark to ensure constant paw volume. The initial paw volume of each rat was noted by mercury displacement method. The Group-I, serving as control, was administered 5% cmc solution in a volume of 1 ml/100g, body weight, orally. Group-II, serving as standard, was administered diclofenac sodium (10 mg/kg, body weight, orally). Test Group I, Test group II, Test group III and Test group IV, serving as test was administered synthesized compounds in the dose of 100 mg/kg body weight, orally. Thirty minutes later, the rats were challenged by a subcutaneous injection of 0.05 ml of 1% solution of carrageenan into the plantar side of the left hind paw. The volume of the paw was measured by a plethysmometer in 1, 2, 3 and 4 hour after carrageenin suspension injection. The percentage increase in paw volume in animals treated with standard, synthesized compounds were compared with the increase paw volume of animals of control group after 1, 2, 3 and 4 hour. The percent inhibition was calculated by following formula.

$$\% \text{ Inhibition} = (1 - Vt / Vc) * 100$$

Where, Vt and Vc are the mean change in paw volume of treated and control rats respectively.

Results: Results were expressed as Mean ± SEM and evaluated by Dunnett test. Values of $P < 0.05$ were considered statistically significant. The results of anti-inflammatory effect of synthesized compounds are given in the table 2.

Table 2: The anti-inflammatory effect of synthesized compounds on Carrageenin Induced Paw edema.

Treatment	Dose mg/Kg	Mean changes in paw edema (ml) (Mean ± SEM) , % Inhibition				
		0 hr(Initial)	1 hr	2 hr	3 hr	4 hr
Control(5% Acacia solution)		0.70 ± 0.04	0.77 ± 0.02	0.60 ± 0.00	0.37±0.02	0.33±0.05
Standard (Indomethacin)	10 mg/Kg,	0.53±0.05 (24.29)	0.50±0.04* (35.06)	0.50 ± 0.04 (16.67)	0.27±0.05 (27.02)	0.13±0.02* (60.61)
Test Group I (AB_{15})	100 mg/Kg,	0.57 ± 0.06 (18.57)	0.60 ± 0.08 (22.00)	0.47 ± 0.08 (21.67)	0.23±0.06* (37.83)	0.20±0.04* (39.39)
Test Group II (AB_{25})		0.50±0.04 (28.14)	0.33±0.06* (57.14)	0.30±0.07* (50.00)	0.23±0.05 (37.83)	0.17±0.06* (48.48)
Test Group III (AB_{35})		0.57 ± 0.02 (18.57)	0.53±0.08* (31.17)	0.47 ± 0.06 (21.67)	0.27±0.05 (27.03)	0.13±0.06* (60.61)
Test Group IV (AB_{45})		0.63 ± 0.05 (10.00)	0.57±0.08 (25.97)	0.43 ± 0.06 (28.33)	0.17±0.02* (54.05)	0.10±0.04* (69.69)
Test Group V (AB_{55})		0.63 ± 0.05 (10.00)	0.57±0.08 (25.97)	0.43 ± 0.06 (28.33)	0.17±0.02* (54.05)	0.10±0.04* (69.69)

Each value represents the Mean ± SEM (n = 6).
* ($P < 0.05$) control vs. treated group
Figures in parenthesis indicate % of inhibition
From the results of anti-inflammatory effects it can be concluded that the synthesized compound YS-1,YS-4, YS-5 *and YS-6* has shown significant activity ($P < 0.05$) at 2, 3 and 4 hrs when compared to the control group.

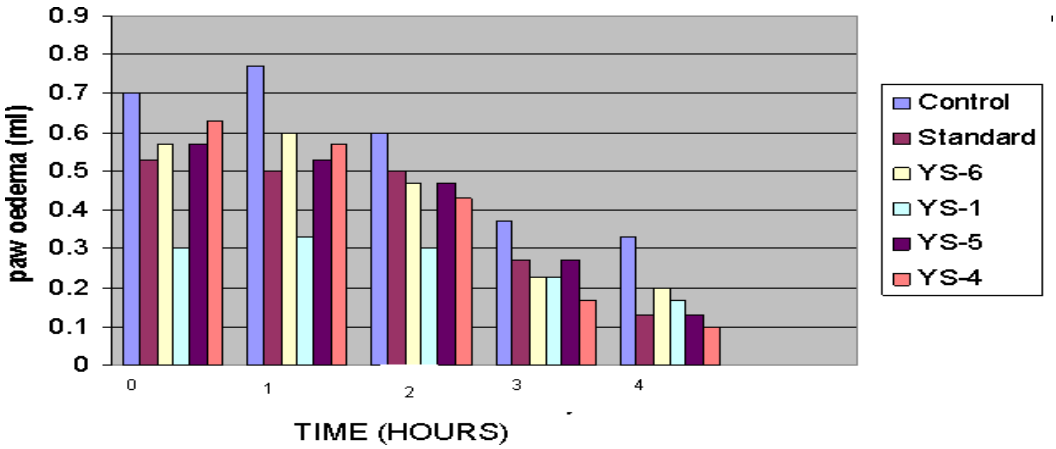


Fig. 4: Graph of anti-inflammatory activity responses.

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-inflammatory activities by "Carrageenin Induced Paw edema Method".

Each 2-anilinophenylacetic acid derivative will be screened for their anti-inflammatory activity. It was also found that the synthesized compound YS-1,YS-4, YS-5, *and YS-6* has shown significant anti- inflammatory activity whereas all the other compound have showed mild to moderate anti-inflammatory activity as compared to standard drug. These preliminary results indicate that some of compounds are exhibiting good activity.

ACKNOWLEDGEMENT

Author thanks to thanks to Dr. G. Jeyabalan, Principal, Alwar College of Pharmacy, IET Campus, Alwar and Dr. (Prof.) Yogendra Singh, Dean Academics, Director – IQAC, MVN University, Palwal (Haryana) India.

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