Deccan J. Medicinal Chemistry 1(1): Jan.-Mar. 2010 **ISSN 0976 – 1373**

DECCAN PHARMA JOURNAL SERIES

ARMS Online Publications

www.deccanpharmajournals.com

SYNTHESIS AND MICROBIOLOGICAL EVALUATION OF ISONIAZID AMIDES

Asif Husain[a]*, M. Mujeeb[b], Aftab Ahmed[c], Ausaf Ahmad[d], M. Rashid[a]

[a]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard (Hamdard University), New Delhi- 110 062, India*

[b]*Department of Pharmacognosy & Phytochemistry, Faculty of Pharmacy, Jamia Hamdard (Hamdard University), New Delhi- 110 062, India*

[c]*Health Information Technology Department, Jeddah Community College, King Abdul-Aziz University, Jeddah-21589, KSA*

[d]*Department of Biochemistry and Biophysics, University of Rochester Medical Center, Rochester, NY 14642, USA*

Abstract

In the present study, a series of amide derivatives of isoniazid has been synthesized through one-pot method by condensing isoniazid with appropriate 4-oxo-4-(4-substituted phenyl)butanoic acid moiety. The structures of the newly synthesized compounds were established on the basis of elemental analysis, ^{1}H NMR and Mass spectral data. The amides have been evaluated for their antibacterial activity (*MIC*) against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Two compounds, **10** and **13**, were found to have significant antibacterial activity.

Key words: Isoniazid, amide, butanoic acid, antibacterial.

Introduction

There is an increasing demand for the preparation of new antimicrobial agents due to the developing resistance towards conventional antibiotics[1]. Designing of new compounds, which would combine a non specific activity against a wide variety of bacteria and low toxicity, seems to be a promising way to overcome that problem. Isoniazid is an important antibacterial drug and to increase its usefulness various derivatives have been synthesized with encouraging results[2,3]. On the other hand, the derivatives of 4-oxo-4-(substituted phenyl)butanoic acids also show significant antimicrobial activities[4]. In view of these points, It was considered worthwhile to study various amide derivatives of isoniazid with 4-oxo-4-(substituted phenyl)butanoic acids with a view to obtain potential antimicrobial agents. Therefore, eight different 4-oxo-4-(substituted phenyl)butanoic acids were condensed with isoniazid through ***one-pot method*** (**Scheme 1**). The title compounds were evaluated for their antibacterial activities (*MIC*) against some selected microbes.

Material and Methods

SYNTHESIS

Melting points were determined in open capillary tubes and are uncorrected. ^{1}H-NMR spectra were recorded on DPX-300 NMR spectrometer. The splitting pattern abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Mass spectra were recorded on a Jeol JMS-D 300 instrument at 70 eV. Microanalyses of the compounds were found within ±0.4% of the theoretical values. All solvents were distilled prior use. The progress of the reactions was monitored on silica gel G plates using iodine vapors as visualizing agent.

Step-1: 4-Oxo-4-(substituted phenyl)butanoic acids (**1-8**) were synthesized according to the literature method[4].

Step-2: The title amides (**9-16**) were synthesized by dissolving 4-oxo-4-(substituted phenyl)butanoic acid (**1-8**) (0.001 mol) and isoniazid (0.001 mol) in minimum quantity of dry pyridine separately. The two solutions were then mixed together and stirred magnetically followed by the addition of phosphorous oxychloride (0.9mL) drop wise while maintaining the temperature below 5°. The contents were stirred for another half-hour and left overnight. The reaction mixture was then poured into ice cold water and a solid mass, which separated out, was filtered, washed, dried and crystallized from ethanol to give **9-16** (Table 1).

ANTIBACTERIAL STUDY

All the newly synthesized compounds were screened for their antibacterial activity against *Staphylococcus aureus* (ATCC-29737), *Escherichia coli* (ATCC-8739) and *Pseudomonas aeruginosa* (NCLM-2035) at a concentration of 100 µg/mL turbidity method[5]. Compounds inhibiting growth of one or more of the above microorganisms were further tested for minimum inhibitory concentration (*MIC*) by broth dilution technique. Solvent (DMF) and growth controls were kept. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls were inoculated with approximately $5x10^5$ c.f.u. of actively dividing bacteria cells. The cultures were incubated for 24 h at 37°C and the growth was monitored visually and spectrophotometrically. Ciprofloxacin was used as standard drug for comparison. The lowest concentration (highest dilution) required to arrest the growth of bacteria was considered as *MIC*.

Results and Discussion

The synthesis of the title compounds was performed in a one-pot reaction method and is presented in Scheme 1. The desired amides (**9-16**) were synthesized by reacting 4-oxo-4-(substituted phenyl)butanoic acid (**1-8**) with isoniazid in dry pyridine in presence of phosphorous oxychloride as condensing agent and obtained in appreciable yields (50-61%). The purity of the compounds was controlled by TLC in solvent system toluene:ethyl acetate:formic acid (5:4:1). Spectral data and microanalysis data were in agreement with the proposed structures (**Table-1**).

The spectra (^{1}H NMR; δ ppm) showed two triplets at around δ 2.8 & 3.3 (-CH_2-CH_2-); signals in the region δ 7.5-8.6 (aryl protons). The mass spectra showed molecular ion peaks in reasonable intensities supporting the structure. There was splitting of Ar-$COCH_2CH_2$-CON-bond resulting in formation of Ar-$COCH_2CH_2$-C≡O^+ (fragment-1) or [Ar-$COCH_2CH$=C=O]$^+$ (fragment-2) and/or C_5H_4N-C≡O^+. These fragments provided important clue for successful formation of the product. Fragment-1/2 further splitted to Ar-C≡O^+ and to Ar^+ and then to $C_6H_5^+$ (m/z=77). The fragment C_5H_4N-C≡O^+ further splitted to $C_5H_4N^+$ (**Chart 1**).

The antibacterial activity (minimum inhibitory concentration; *MIC*) of the compounds was evaluated against *S. aureus*, *E. coli* and *P. aeruginosa*. Ciprofloxacin was used as standard drug for comparison, which showed *MIC*-6.25 µg/mL. The compound **10** showed very good activity against *S. aureus* and *E. coli* with *MIC*-12.5 µg/mL and good activity against *P. aeruginosa* with *MIC*-25 µg/mL concentration. Another compound, **13**, showed significant activity against *S. aureus* and *E. coli* with *MIC*-25.0 µg/mL. Rest of the compounds were moderate in their action. From the antibacterial results, it was observed that the compound having chloro function (**10**) was most active among the synthesized compounds.

Conclusion

In conclusion, eight new compounds (**9-16**) were successfully synthesized from isoniazid. Among these, one compound **10** exhibited very good activity against *S. aureus* and *E. coli* with *MIC* 12.5 µg/mL. These results confirmed the importance of exploring old drugs to obtain compounds of potential pharmaceutically interest.

Acknowledgement One of the authors (AH) is thankful to the Department of Science & Technology (DST), New Delhi, for the financial support under the SERC-fast track proposal for young scientists.

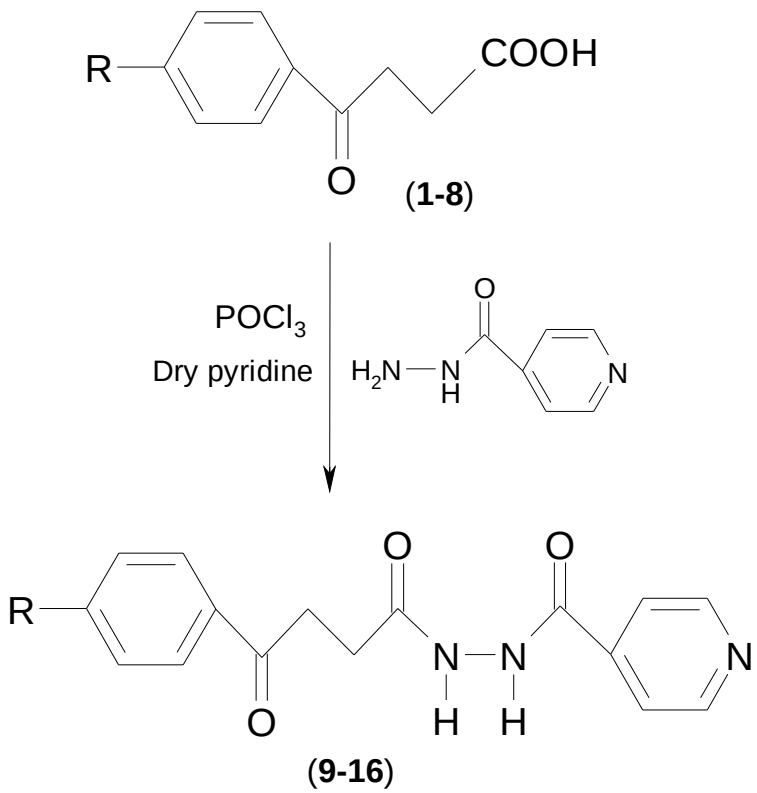
References

1. Khan, M. S. Y. et al., *Scientia Pharmaceutica* 70 (2002) 277.
2. Hearn M. J., Cynamon M. H., *J. Antimicrob. Chemother.*. 53 (2004) 185.
3. Husain, A., *Acta Pola Pharm. (Drug Research)* 54 (2009) 152.
4. Husain, A., Ahuja, P., Sarafroz, *Ind. J. Pharm. Sci.*, 71 (2009) 62.
5. Cruickshank R. et al., Medical Microbiology, vol. 2, Churchill-Livingstone, Edinburge, London (1975).

Table-1. Physical and spectral data of the amide derivatives of isoniazid (**2a-e**).

Compd	R	M.p.; Yield (%)	Mol. formula; Mass spectral data (m/z)	^{1}H NMR spectral data (δ ppm)	Antibacterial activity (*MIC*)		
					S. aureus	*E. coli*	*P. aeruginosa*
9	H–	128-130 58	$C_{16}H_{15}N_3O_3$ 297(M^+), 279, 160, 105, 78, 77	2.77 & 3.43 (t, each, 2x -CH_2), 7.56 (m, 3H, H-3,4,5, phenyl), 7.89 (m, 2H, H-2,6, phenyl), 8.25 & 8.73 (d, each, A_2B_2, 4H, 4-pyridyl ring), 9.16 & 9.83 (s, each, 2x -NH-).	50.0	>100	>100
10	Cl–	172-174 55	$C_{16}H_{14}ClN_3O_3$ 331(M^+), 332 (M^++1),193, 139, 111,53	2.78 & 3.31 (t, each, 2x -CH_2-), 7.43 & 7.67 (d, each, A_2B_2, 4H, *p*-chlorophenyl), 8.06 & 8.77 (d, each, A_2B_2, 4H, 4-pyridyl ring), 9.28 & 9.65 (s, each, 2x -NH-).	12.5	12.5	25.0
11	CH_3–	140-142 50	$C_{17}H_{17}N_3O_3$ 311(M^+), 119, 91, 78, 77	2.39 (s, 3H, –CH_3), 2.63 & 3.26 (t, each, 2x -CH_2-), 7.28 & 7.88 (d, each, A_2B_2, 4H, *p*-tolyl), 7.81 & 8.79 (d, each, A_2B_2, 4H, 4-pyridyl ring), 9.11 & 9.36 (s, each, 2x -NH-).	25.0	50.0	50.0
12	C_2H_5–	156-158 56	$C_{18}H_{19}N_3O_3$ 325(M^+), 307, 133, 105, 91, 77	1.25 (t, 3H, –CH_3), 2.68 (q, 2H, –CH_2–), 2.73 & 3.34 (t, each, 2x -CH_2), 7.29 & 7.83 (d, each, A_2B_2, 4H, *p*-ethylphenyl), 7.81 & 8.71 (d, each, A_2B_2, 4H, 4-pyridyl ring), 9.08 & 9.19 (s, each, 2x -NH-).	>100	50.0	>100
13	CH_3O–	148-150 61	$C_{17}H_{17}N_3O_4$ 327(M^+), 191, 189, 135, 78	3.82 (s, 3H, –OCH_3), 2.78 & 3.30 (t, each, 2x -CH_2-), 6.96 & 7.56 (d, each, A_2B_2, 4H, *p*-methoxyphenyl), 7.91 & 8.83 (d, each, 4H, A_2B_2, 4-pyridyl ring), 9.23 & 10.11 (s, each, 2x -NH-).	25.0	25.0	50

14	$C_6H_5CH_2$–	120-122 60	$C_{23}H_{21}N_3O_3$ 387(M^+), 237, 181, 153	2.79 & 3.28 (t, each, 2x -CH_2-), 4.52 (m, 2H, -CH_2-), 6.97 & 7.48 (d, each, A_2B_2, 4H, *p*-substituted phenyl), 7.13 (m, 2H, H-2,6, phenyl), 7.24 (m, 1H, phenyl), 7.44 (m, 2H, H-3,5, phenyl), 7.87 & 8.81 (d, each, 4H, A_2B_2, 4-pyridyl ring), 9.18 & 9.65 (s, each, 2x -NH-).	50.0	50.0	>100
15	$C_{10}H_8$–	162-164 52	$C_{20}H_{17}N_3O_3$ 347(M^+), 211, 155, 127	2.57 & 3.26 (t, each, 2x -CH_2), 7.43-7.86 (complex m, 7H, naphthyl), 8.05 & 8.22 (d, each, A_2B_2, 4H, 4-pyridyl ring), 9.06 & 9.54 (s, each, 2x -NH-).	>100	50.0	>100
16	$(CH_3)_2CHCH_2$-	174-176 57	$C_{20}H_{23}N_3O_3$ 353(M^+), 217, 91, 77	0.86, d, 6H, 2xCH_3; 1.82, m, 1H, CH; 2.53, d, 2H, CH_2), 2.61 & 3.23 (t, each, 2x -CH_2-), 7.22 & 7.58 (d, each, A_2B_2, 4H, *p*-isobutylphenyl), 7.86 & 8.73 (d, each, A_2B_2, 4H, 4-pyridyl ring), 9.24 & 9.78 (s, each, 2x -NH-).	>100	>100	>100
	Ciprofloxacin				6.25	6.25	6.25
	Control				-	-	-



9; R= H, **10**; R= Cl, **11**; R= CH_3, **12**; R= C_2H_5, **13**; R= OCH_3, **14**; R= $CH_2C_6H_5$, **15**; R= $C_{10}H_8$, **16**; R= $CH_2CH(CH_3)_2$

Scheme 1: Protocol for synthesis of isoniazid amides

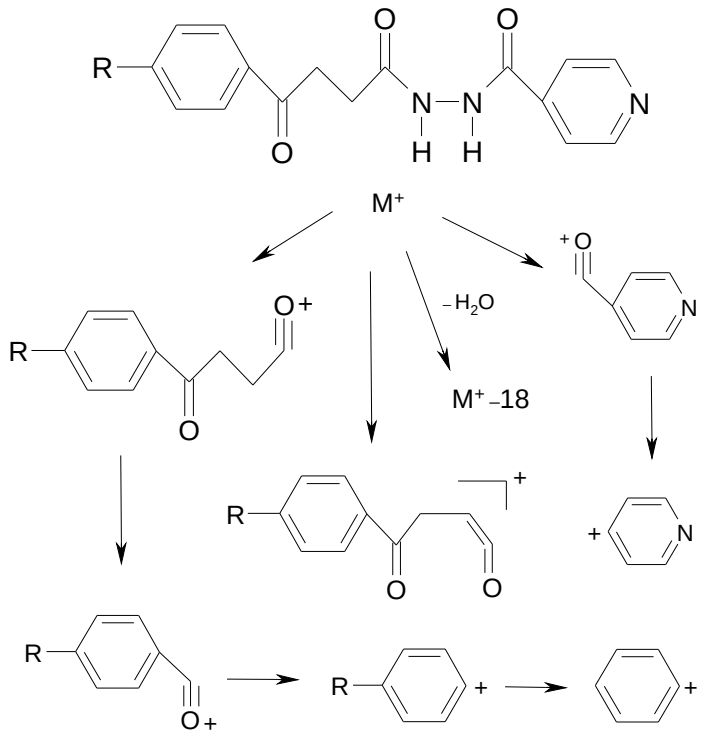


Chart 1: Mass fragmentation pattern of the amides (**9-16**)

Deccan J. Medicinal Chemistry 1(1): Jan.-Mar. 2010 **ISSN 0976 – 1373**

DECCAN PHARMA JOURNAL SERIES

ARMS Online Publications

www.deccanpharmajournals.com

SYNTHESIS OF NOVEL 2,4-THAIZOLIDINEDIONES

Dr. A. Roy, S. T. Khandagale*, A. E. Kandekar

Department of Pharmaceutical Chemistry, Sharadchandra Pawar College of Pharmacy, Otur, pune, (MH.), India.

Abstract : Some 2,4-Thiazolidinediones derivatives have been prepared by hydrolysis of para substituted bromoacetophenone and substituted 2-amino-4-phenyl thiazole. Structurally and Structure Activity Relationship of some synthesized novel 2,4-Thiazolidinediones well related with reputed Antidiabetic compounds e.g.Pioglitazone and Rosiglitazone.

Keywords : TZD , PPARγ, Hypoglycemic.

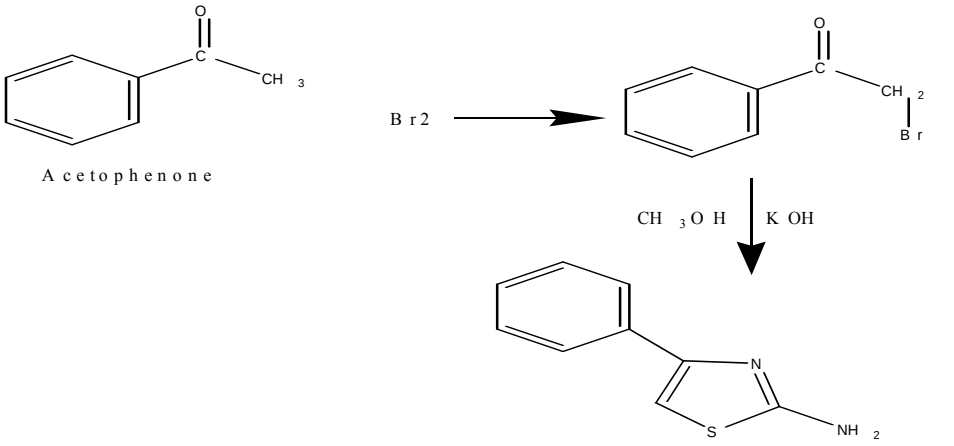
Introduction

TZDs (Thiazolidinediones) is based on binding to the peroxisome proliferator activated receptor (PPARγ). PPARγ belongs to the group of nuclear transcription factors. Transcription factors affect the level of expression and thus the activity of various genes. By making some genes more and others less active, transcription factors affect cellular function. In humans, Thiazolidinediones are highly selective agonists for the PPARγ, which is responsible for improving glycemic control. These agents normalize glucose metabolism and reduce the amount of insulin needed to achieve glycemic control[1,2].

Thiazolidinediones as a hypoglycemic agents composed of an acidic head group connected to a lipophilic tail by a phenoxyalkyl linker.This appears to be important, given that removal of the acidic function by N-methylation leads to loss of antihyperglycemic (Hypoglycemic)activity.Thatswhy synthesis of any new TZDs have

acidic group as it is important for activity.A phenoxyethyl group as the central phenoxyalkyl linker is commonly found to yield highly active compounds in SAR studies of hypoglycemic thiazolidinediones. Often shorter chain lengths or inclusion of phenoxyethyl group into heterocyclic ring also leads to active compounds[3,4,5].

Scheme for synthesis of 2-amino-4-phenylthizole and derivatives[6].

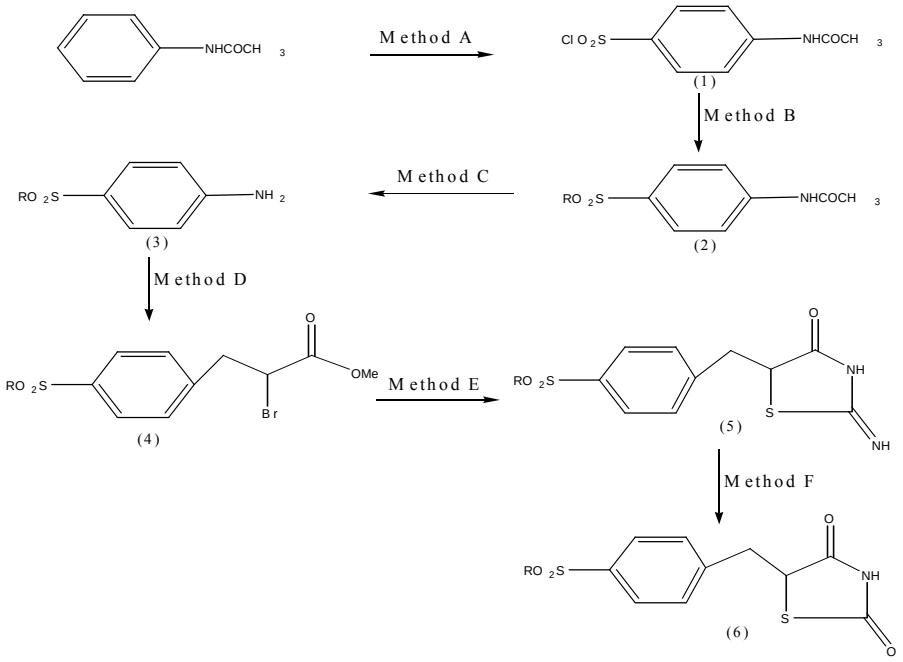


Acetophenone

2-amino-4-phenylthiazole (15)

Reaction Scheme followed for the synthesis of compounds [7,8,9].

Synthesis of substituted 5-[4-(sulphonyl)benzyl]-2,4-thiazolidinediones.



Method A: acetanilide, chlorosulphonic acid; Method B: dry pyridine, acetone, R [substituted amines]; Method C: concentrated HCl, ethanol; Method D: NaNO2, HBr, Cuprous oxide, methyl acrylate at 0-5ºc; Method E: Thiourea, sodium acetate; Method F: 2N HCl, ethanol.

Chemistry of synthesis of thiazolidine-2,4-diones (TZDs)-

A common strategy was adopted to synthesize 4-substituted sulphonyl benzyl thiazolidinediones as shown in the scheme 1. Appropriately substituted anilines were diazotized using the method given in literature and the diazonium salt formed was treated with methyl acrylate at room temperature to obtain the corresponding bromoesters in good yield (60 to 90%). The corresponding bromo esters were then reacted with thiourea, sodium acetate to get 5-substituted-2-iminothiazolidine-4-ones (compound 5), which on oxidation with 2N HCl give the corresponding thiazolidinediones, the target compounds.

Appropriately substituted anilines (3) which were used in the above reactions were obtained by appropriate ethanolic acidic (15ml.HCl+75ml.ethanol) hydrolysis of the precursor, 4-substituted acetanilide (2). The yield of the reaction is good (50 to 92%) and the time of the reaction is also very short involving heating the reaction mixture at water bath for 20 minutes.2-amino-4-phenylthiazole was synthesized by using acetophenone reacted with bromine in cold condition with stirring gives brominated acetophenone which then reacted with thiourea with solvent methanol require 3-4 hours give target compounds.

Experimental

Preparation of p-(acetamidobenzene) sulphonyl chloride (1)

Frequently shaked a solution of dry acetanilide (20gm, 0.148 mol) and chlorosulphonic acid (50ml, 90gm,0.77 mol) was heated at 100^0C for 1 hour, cooled, poured into ice – water.The reaction mixture was filtered to afford the title compound as a granular white solid.

Preparation of p-(Substituted)sulphonyl acetanilide (2)

Substituted amine (0.05 mol) was dissolved in mixture of anhydrous acetone (40 ml) and dry pyridine (6 ml) and added 0.05 mol) pure p-acetamidobenzene sulphonyl chloride. The reaction mixture was set aside overnight and filtered the product.

Preparation of p-(substituted sulphonyl) aniline (3)

The substituted sulphonyl acetanilide was hydrolyzed by ethanol (75 ml) and concentrated hydrochloric acid (15 ml) for 20 minutes. The cooled solution was diluted with concentrated ammonia solution to afford to afford the title compound.

Preparation of Methyl-2-bromo-3-(4-(para substituted)phenyl) Propionate (4)

A solution of sodium nitrite ($NaNO_2$) (4.2 gm, 0.06 mol) in water (7.5 ml) was added dropwise to a stirred and ice cold mixture of appropriate p-sustituted aniline.(0.055 mol), aqueous hydrobromic acid (47%, 37.6gm, 0.22mol), methanol (50 ml) and acetone (125 ml) below 5^0c. The whole reaction mixture was stirred at 5^0c for 30 minutes and then, methyl acrylate (30 ml, 0.33mol) was added and the temperature was slowly raised to 38^0c. Powdered cuprous oxide (0.5 gm) was added.After nitrogen evolution has ceased, the reaction mixture was concentrated in vacuo. The residue was diluted with water, made alkaline with concentrated ammonia and extracted with ethyl acetate and dried over anhydrous magnesium sulphate and concentrated in vacuo to afford the title compound.

Preparation of 2-imino-5-(4-(substituted) benzyl)-4-thiazolidinone (5)

A mixture of methyl [2-bromo-3-{4-(para-substituted) phenyl} propionate, a crude oil (21mmol), (14 or21), thiourea (2.1gm,21mmol) ,ethanol (80ml) & sodium acetate (2.3 gm,0.328 mol) was stirred under reflux for 3 hour and concentrated in vacuo. The residue was neutralized with aqueous sodium bicarbonate and extracted with ethyl acetate. The ethyl acetate extract was concentrated in vacuo to afford the title compound.

Preparation of 5-{4-(substituted) benzyl}-thiazolidine-2,4-diones(6)

A mixture of 2-imino-5-[4-(substituted) benzyl}-4-thiazolidinone(10 mmol) , 2N hydrochloric acid (50ml) was stirred under reflux for 12 hour. The reaction mixture was concentrated in vacuo. The residue was diluted with water, neutralized with saturated

aqueous sodium bicarbonate, extracted with chloroform and dried over anhydrous magnesium sulphate, and then concentrated in vacuo to afford the title compound.

Preparation of para substituted bromoacetophenone and substituted 2-amino-4-phenyl thiazole(15)

Add a mixture of substituted acetophenone (0.015 mol) and glacial acetic acid(24) to a solution of bromine (6ml) and glacial acetic acid (12ml) at 3^0C , dumped in water give substituted bromoacetophenone and which reflux for 3 hour with thiourea, methanol and potassium hydroxide,dumped in ice-water give substituted 2-amino-4-phenyl thiazole.

Melting Points were determined on a Gallenkamp melting point apparatus and are uncorrected PMR data is reported in.ppm and IR in cm^{-1}

5-[4-{(4-pheny thaizolyl-2-amino)sulfonyl}benzyl]-2,4-thiazolidinedione .

4-pheny thaizolyl-2-amine (0.05 mol) hydrolysed with p-(acetamidobenzene) sulphonyl chloride in step 2 and synthesize of compounds following above reaction method.Final compound synthesized by using oxidation reaction having yield 79.29% Mol.formula - $C_{19}H_{15}N_3S_3O_4$ Mol.Wgt. 445 M.P.:164-165^{0}C Rf value:.38 (Chloroform : Ethyl acetate, 2:8) Recrystalization solvent: Chloroform. Solubility: Highly soluble in methanol, ethanol, ethyl acetate, benzene, DMF and DMSO, moderately soluble in acetone, slightly soluble in chloroform and insoluble in water.IR(KBr) (cm^{-1}) 2910, 3072 1360, 1632, 1292,1167,1725,1749, 3433,1616,820 678,749

5-[4-{(p-hydroxy-4-phenythaizole-amino)sulfonyl}benzyl]-2,4-thiazolidinedione

Para hydroxy-4-pheny thaizolyl-2-amine (0.05 mol) hydrolysed with p-(acetamidobenzene) sulphonyl chloride in step 2 and synthesize of compound following above reaction method.Final compound synthesized by using oxidation reaction having yield 64.87% Mol.formula - $C_{19}H_{15}N_3S_5O_3$ Mol.Wgt. 447 M.P.:158-160^{0}C. Solubility: Highly soluble in methanol, ethanol, DMF and DMSO, moderately soluble in acetone and insoluble in water. IR(KBr) (cm^{-1}) 2923,3033, 1362, 1640, 1290, 1168, 1732, 1751,

3426,1609, 3662, 1168,1412, 834, 669,751 ^{1}H NMR: 8.80 (s, 1H).,6.60(d, 2H),6.70d,2H),6.08(s,1H),10.00(s,1H),7.51d,2H),7.40(d,2H),5.10(dd,1H),3.51(dd,1H),3.48(dd,1H), 10.70(s,1H).^{3}CNMR(DMSO)175.11,173.167.58,157.28,143.72,136.25,129.69,125.64,116.75,103.84,56.06

5-[4-{(p-methoxy-4-phenythaizole-2-amino)sulfonyl} benzyl]-2,4-thiazolidinedione.

Para methoxy- 4-pheny thaizolyl-2-amine (0.05 mol) hydrolysed with p-(acetamidobenzene) sulphonyl chloride in step 2 and synthesize of compound following above reaction method.Final compound synthesized by using oxidation reaction having yield 69.71%.Molecular formula – $C_{20}H_{17}N_3S_3O_5$ Mol.Wgt. 491 M.P.: 168-170^{0}C. Solubility: Highly soluble in methanol, ethanol, ethyl acetate, benzene, DMF and DMSO, moderately soluble in acetone, slightly soluble in chloroform and insoluble in water. IR(KBr) (cm^{-1}) 2913,3072,1360,1632,1290,1167,1724,1747,3431,1616,825,678,749.

5-[4-{(2,5-dihydroxy-4-phenylthaizole-2-amino)sulfonyl}benzyl]-2,4-thiazolidinedione

2,5-dihydroxy-4-pheny thaizolyl-2-amine (0.05 mol) hydrolysed with p-(acetamidobenzene) sulphonyl chloride in step 2 and synthesize of compound following above reaction method.Final compound synthesized by using oxidation reaction having yield 65.29%.Molecular formula – $C_{19}H_{16}N_3S_3O_6$ Mol.Wgt. 494 M.P.:172-174^{0}C. Solubility: Highly soluble in methanol, ethanol, ethyl acetate, benzene, DMF and DMSO, moderately soluble in acetone, slightly soluble in chloroform and insoluble in water. IR(KBr) (cm^{-1}) 2925, 1367,1653,1296,1167,1724,1746,3440,3651,3668, 1746,3440,3651,3668.

Conclusion

In the present research work, syntheses of some novel subtituted 5-[4-sulfonyl benzyl]-2,4-thiazolidinediones were designed and successfully performed to evaluate their oral anti-hyperglycemic agents.Currently, rosiglitazone and pioglitazone are there in the market, however, these drugs have also been occasionally linked to liver, cardiovascular,

and hematological toxicity, as well as body weight gain, require restriction or close monitoring of side effects by clinicians. Therefore, improvement of the TZDs class of anti diabetic agents is still worth pursuing and it is anticipate that this type of modification will yield potent thiazolidinediones than available ones.

References

1.Herman W.H., et al.,**Diabetes Care**, 1984,**7**,367.

2.National Diabetes Data Group In Diabetes in America, 1985;National Institute of Health: Bethesda, **MD**, 1985.

3.Donald J. Abraham (Ed.), **Burger's, Medicinal chemistry and Drug Discovery,** Vol 4, (Autocoids, Diagnostics and Drug from New biology), 6th Edition, A John Wiley and Sons publication, 2003, pp. 1-39.

4.Rami H.,et al.; Non thiazolidinedione antihyperglycaemic agents.2: α- carbon substituted β-phenylpropanoic acids, **Bioorganic & Medicinal Chemistry Letters,** 1996, **6**, 17, 2127-30.

5.Miyachi H., et al.; (3, Substituted Benzyl) thiazolidine-2, 4-diones as Structurally New Antihyperglycemic Agents, **Bioorganic & Medicinal Chemistry Letters,** 1999, **9**, 533-

50.Zierath J.,et al., **Endocrinology***,* 1998, **139,** 5034.

6. N.Agarwal, et al; Synthesis and biological evaluation of some 2-amino-4-arylthiazole and their thiazolylamides, **Indian Journal Of Heterocyclic Chemistry**, 1997,**6**, 291-294.

7. Shoda T.,et al.; Novel Thiazolidinediones Derivatives as Potent Hypoglycemic and Hypolipidemic Agents.,**J. Med. Chem**., 1992, **35**, 2617-2626.

8. Lohray, et al.; Novel Euglycemic and Hypolipidemic Agents. **J. Med. Chem**. 1998, **41**, 1626-1630.

9.Furniss, B.S., et al.; Reaction of Diazonium salts, Vogel's **Text book of Practical Organic Chemistry**, 5th edition, Longman Singapore Publishers Pvt. Ltd., Pp. 889 -890.

Deccan J. Medicinal Chemistry 1(1): Jan.-Mar. 2010 **ISSN 0976 – 1373**

DECCAN PHARMA JOURNAL SERIES

ARMS Online Publications

www.deccanpharmajournals.com

SYNTHESIS AND ANTIBACTERIAL ACTIVITY EVALUATION OF SOME NOVEL 7-CHLORO-4-AMINOQUINOLINE DERIVATIVES

M. Rudrapal[*] and D. Chetia[1]

[*]Koringa College of Pharmacy, Korangi, Tallarevu [M], East Godavari Dist. A.P- 533 461, India

[1]Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh-786 004, India

Abstract

Some new 7-chloro-4-aminoquinoline derivatives were prepared by modification at C-2 position of six-membered 1,3-thiazinan-4-one ring system attached at the terminal propyl side chain of 7-chloro-4-aminoquinoline nucleus. The synthesized compounds were characterized by their physical, analytical (CHN) and spectral data (UV-Visible, IR, ^{1}H NMR, ^{13}C NMR and MS).

In addition to evaluation of antimalarial activity, the synthesized compounds were evaluated for antibacterial activity against six different strains of Gram positive (*Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*) and Gram negative bacteria (*Eschericia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*) at two different tested doses viz. 25 µg/disc and 50 µg/disc by disc diffusion method. All the compounds were found to be active against the tested organisms, but were less active as compared to standard drug ofloxacin (5 µg/disc). The compounds with aromatic bulky substituents such as 2-fluorophenyl, 3-hydroxyphenyl, furan-2-yl at C-2 position of 1,3-thiazinan ring system showed better antibacterial activity than that of the compounds with aliphatic alkyl (ethyl) substituent. It indicates that aromatic bulky substituents have greater contributing effect to the antibacterial activity of the 7-chloro-4-aminoquinoline derivatives as compared to aliphatic non-bulky group.

Key words: 7-chloro-4-aminoquinoline, Antibacterial activity, Bulky substituents.

Introduction

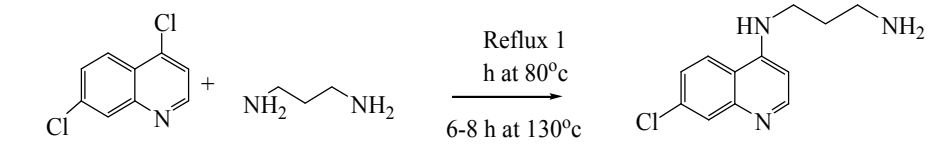
The incidence of microbial infections has been increasing worldwide over the past two decades because of widespread emergence of bacterial resistance to the currently available beta-lactam antibiotics, quinolones, macrolids etc [1]. A matter of concern in the treatment of microbial infection is the limited number of efficacious antimicrobial agents, which clearly highlights the urgent need of novel antimicrobial agents.

A large variety of synthetic compounds having therapeutic use in some other ailments possess antibacterial activity. The medicinal properties of imidazole drugs include antibacterial [2], antifungal [3], alongwith antimalarial [4]. On the basis of this premise, few 7-chloro-4-aminoquinoline analogues synthesized and tested for antimalarial activity were also subjected to antibacterial activity screening. The present study was aimed at evaluation of antibacterial activity of 7-chloro-4-aminoquinoline analogues with substituted heterocyclic ring at the side chain using 3-(3-(7-chloroquinolin-4-ylamino)propyl)-1,3-thiazinan-4-one as lead compound in the development of a new series of antibacterial agents.

Materials and Methods

All chemicals and reagents were procured from Sigma-Aldrich Corporation (USA), Merck (Germany) or Spectrochem Pvt. Ltd. (India) and were used without further purification unless otherwise stated. 4,7-dichloroquinoline was obtained from M/s. Mangalam Drug & Organics, Mumbai, India. Melting points (mp) were taken in open capillaries on a Veego-MPI melting point apparatus and are uncorrected. The progress of reactions and purity of synthesized compounds were checked on silica gel-G TLC plate using various solvent combinations of different polarity. The spots were detected with iodine vapors on UV-light (254 nm). The UV-visible spectra (λ_{max}, nm) of synthesized compounds were obtained on *Shimadzu UV-1700* UV-visible spectrophotometer. Infrared (IR) spectra were recorded on a FT-IR *Perkin-Elmer Spectrum RX-I* spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a *Bruker AC-F 300* FT-NMR spectrometer using $CDCl_3$ as solvent. Chemical shifts (δ in ppm) are reported with tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained with a LC-MS *Water 4000 ZQ* instrument using atmospheric pressure ionization (API). Elemental microanalyses (CHN) were performed on a *Perkin Elmer 2400 Series II* CHNS/O analyzer and values were within the acceptable limits of the calculated values.

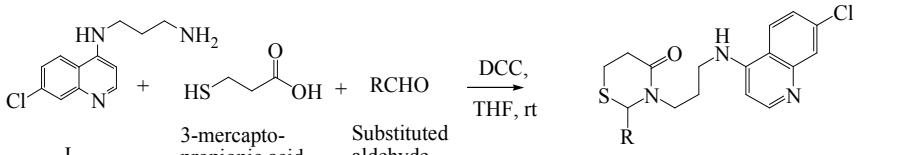
The intermediate reaction product N^1-(7-chloroquinolin-4-yl)-propane-1,3-diamine, I (Scheme 1) was prepared according to the method reported by Madrid *et al.* [5,6]. 3-(3-(7-chloroquinolin-4-ylamino)propyl)-1,3-thiazinan-4-one derivatives, II $_{A-D}$ (Scheme 2) were prepared as per the method designed by Solomon *et al.* [7,8].



4,7-dichloroquinoline 1,3-Diaminopropane

N^1-(7-chloroquinolin-4-yl)propane-1,3diamine (I)

Scheme 1



I 3-mercapto-propionic acid Substituted aldehyde

3-(3-(7-chloroquinolin-4-ylamino)propyl)-2-substituted-1,3-thiazinan-4-one (II_{A-D})

.

Scheme 2

N^1-(7-chloroquinolin-4-yl)propane-1,3-diamine (I)

Yellowish white solid, 86% yield, mp: 96-98°C, R_f: 0.25 (chloroform : methanol=1 :1); UV-visible spectrum (chloroform), λ_{max} (nm) : 270, 366, 412.5. IR (KBR), ν, cm^{-1} : 3422, 3382 (N-H str., -NH_2); 3312 (N-H str., >NH); 1352, 1286 (C-N str.); 1078 (Ar. C-Cl str). ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 1.82–1.86 (t, J=9.6 Hz, 2H, CH_2); 2.71–2.81 (t, J=19.2 Hz, 2H, CH_2); 3.20–3.32 (dd, J=9.6, 25.2 Hz, 2H, CH_2); 6.54–6.55 (d, J=5.6 Hz, 1H, quinoline-H_3); 7.25 (bs, 2H, NH_2), 7.51 (s, 1H, NH); 7.56–7.77 (dd, J=18.0, 18.0 Hz, quinoline-H_6); 7.89–7.90 (d, J=6.0 Hz, 1H, quinoline–H_5); 8.02–8.04 (d, J=9.2 Hz, 1H, quinoline–H_8); 8.22–8.29 (dd, J=5.2, 6.4 Hz, IH, quinoline- H_2). ^{13}C NMR (100MHz, $CDCl_3$), δ (ppm): 27.65 (CH_2), 39.38 (CH_2), 44.67 (CH_2); 109.54 (C-3, quinoline), 117.72 (C-4, quinoline); 124.48 (C-5, quinoline), 127.59 (C-6, quinoline); 134.26 (C-8, quinoline), 136.79 (C-7, quinoline C-Cl), 146.89 (C-8a, quinoline), 152.72 (C-2, quinoline), 151.53 (C-4, quinoline). MS (API), m/z (%): 236.72 (100), $[M+H]^+$. Anal. cacld. (%) for C_{12} H_{14} N_3Cl: C, 61.15; H, 5.99; N, 17.83; found (%): C, 61.42; H, 6.36; N, 12.99.

3-(3-(7-chloroquinolin-4-ylamino)propyl)-2-(2-fluorophenyl)-1,3-thiazinan-4-one (II_A)

Light yellow gummy solid, 73% yield; R_f: 0.55 (chloroform : methanol=3:1); UV-visible spectrum (chloroform), λ_{max} (nm) : 262.0, 364.5, 428.0. IR spectrum (chloroform), υ, cm^{-1}: 3340 (N-H str., >NH); 1698 (C=O str.); 1371, 1275 (C-N str.); 1097 (Ar. C-Cl str.). ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 1.73–1.85 (t, 2H, J=17.4 Hz, CH_2), 2.57–2.61 (t, 2H, J=6.0 Hz, CH_2); 2.67–2.84 (m, 2H, CH_2); 3.16–3.37 (m, 2H, CH_2); 5.70 (s, 1H, NH), 6.22–6.23 (d, 1H, J=4.5 Hz, quinoline-H_3); 6.82–7.11 (m, 4H, C_6H_4–); 7.49–7.55 (dd 1H, J=8.7 Hz, 5.1 Hz, quinoline-H_6); 7.73–7.79 (dd 1H, J=7.5, 4.8 Hz, quinoline-H_5); 7.98–

8.00 (d, 1H, J=6.9 Hz, quinoline-H_8); 8.23–8.24 (d, 1H, J=4.5 Hz, 2H quinoline). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 25.74 (CH_2), 29.59 (CH_2), 34.37 (CH_2), 39.85 (CH_2), 44.78 (CH_2), 55.73 (CH), 115.85 (C-2, quinoline), 116.58 (C-4a, quinoline), 123.07 (C-5, quinoline), 124.08, 124.64, 125.95, 126.63 (Ar-C), 127.63 (C-6, quinoline) 128.68 (Ar-C), 130.30 (C-8, quinoline), 132.67 (Ar-C), 136.37 (C-7, quinoline, C-Cl), 148.16 (C-8a, quinoline), 151.94 (C-2, quinoline), 158.28 (C-4, quinoline), 160.76 (C-F), 170.70 (C=O). MS (API), m/z (%): 430.2 (100), $[M+H]^+$; 431.1 (25.67), 432.1 (36.45), 433.2 (10.35). Anal. cacld. (%) for $C_{22}H_{21}N_3OSClF$: C, 61.46; H, 4.92; N, 9.77; found (%): C, 58.59 ; H, 5.65; N, 5.28.

3-(3-(7-chloroquinolin-4-ylamino)propyl)-2-(3-hydroxyphenyl)-1,3-thiazinan- 4-one (II_B)

Light yellow gummy solid, 62% yield; R_f: 0.51 (chloroform : methanol=3:1); UV-visible spectrum (chloroform), λ_{max} (nm): 264.5, 388.0, 416.0. IR spectrum (chloroform), υ, cm^{-1}: 3530 (O-H str., bonded OH); 3430 (N-H str., >NH); 1310, 1283, (C-N str.), 1215 (C-O str.); 1083 (Ar. C-Cl.str.). ^1H NMR (300 MHz, $CDCl_3$, δ (ppm): 1.73–1.81 (m, 2H, CH_2), 2.63–2.66 (t, 2H, J=48 Hz, CH_2), 2.67–2.77 (m, 2H, CH_2), 3.62-3.66 (t, 2H, J=72 Hz, CH_2), 5.35 (s, 1H, NH), 6.20-6.21 (d, 1H, J=4.5 Hz, quinoline-H_3); 6.57–6.59 (d, 1H, J=5.7 Hz, C_6H_4–), 6.75–6.77 (d, 1H, J=6.0 Hz, C_6H_4–), 7.20–7.22 (d, 1H, J=3.9 Hz, quinoline-H_6), 7.78 (s, 1H, OH), 7.88–7.90 (d, 1H, J=6.6 Hz, quinoline-H_5), 8.19–8.20 (d, IH, J=4.2 Hz, quinoline-H_8), 8.46 (bs, 1H, quinoline-H_2). ^{13}C NMR (100 MHz, $CDCl_3$, δ (ppm): 24.85 (CH_2), 29.66 (CH_2), 33.74 (CH_2), 39.96 (CH_2), 45.13 (CH_2), 62.25 (CH_2), 113.86 (C-3, quinoline); 115.47 (2C), 116.58 (C-4a, quinoline) 121.75 (Ar-C), 122.48 (C-5, quinoline), 126.29 (C-5, quinoline), 129.90 (C-8, quinoline), 130.10 (Ar-C), 136.94 (C-7, quinoline, C-Cl), 139.80 (Ar-C), 147.58 (C-8a, quinoline), 152.41 (C-2, quinoline), 153.76 (C-4, quinoline), 158,14 (Ar-C), 171.25 (C=O). MS (API), m/z (%): 428.2 (100), $[M+H]^+$; 429.2 (27.45%), 433.2 (40.50), 433.1 (10.75). Anal. cacld. (%) for $C_{22}H_{21}N_3O_2SCl$: C, 61.74; H, 5.18; N, 9.82; found (%): C, 62.67; H, 7.16; N, 4.43.

3-(3-(7-chloroquinolin-4-ylamino)propyl)-2-ethyl-1,3-thiazinan- 4-one (II_C)

Light yellow gummy solid in 74% yield; R_f: 0.49 (chloroform : methanol=3:1); UV-visible spectrum (chloroform), λ_{max} (nm): 255.0, 350.0, 419.0. IR spectrum (chloroform), υ, cm^{-1}: 3422 (N-H str., >NH); 1719 (C= O str.); 1398, 1283 (C-N str.); 1074 (Ar. C-Cl str.). ^1H NMR (300 MHz, $CDCl_3$), δ (ppm): 0.96–0.98 (t, 3H, J=2.1 Hz, CH_3), 1.75–1.79 (m, 2H, CH_2), 1.91-1.96 (dd, 2H, J=5.1, 4.8 Hz, CH_2), 2.54–2.59 (m, 2H, CH_2), 2.60–2.77 (m, 2H, CH_2), 3.07–3.12 (dd, 2H, J=4.8, 4.8 Hz, CH_2), 3.65–3.68 (t, 2H, J=4.8 Hz, CH_2), 4.26–4.30 (t, 1H, J=5.4 Hz, CH), 6.35–6.37 (d, 1H, J=5.1 Hz, quinoline-H_3); 6.77 (bs, 1H, NH), 7.24–7.32 (dd, 1H, J=6.6, 17.7 Hz, quinoline-H_6); 7.88 (d, 1H, J=0.9 Hz, quinoline-H_5), 8.06 –8.09 (d, 1H, J=6.9 Hz, quinoline-H_8); 8.28–8.30 (d, 1H, J=4.8 Hz, quinoline-H_2). ^{13}C NMR (100 MHz, $CDCl_3$, δ (ppm): 11.45 (CH_3), 25.54 (CH_2), 27.78 (CH_2), 30.85 (CH_2), 36.07 (CH_2), 39.61 (CH_2), 40.56 (CH_2), 67.89(CH_2), 115.58 (C-3, quinoline), 120.87 (C-4a, quinoline), 123.90 (C-5, quinoline), 127.16 (C-6, quinoline), 138.62 (C-8, quinoline), 139.92 (C-7, quinoline, C-Cl), 143.97 (C-8a, quinoline), 154.52 (C-2, quinoline), 157.58 (C-4, quinoline), 170.41 (C=O). MS (API), m/z (%): 364.1 (100), $[M+H]^+$; 365.1 (22.95), 366.1 (41.25), 367.0 (8.72). Anal. cacld. (%) for $C_{18}H_{22}N_3OSCl$: C, 59.41; H, 6.09; N, 11.55; found (%): C, 52.13; H, 7371; N, 4.13.

3-(3-(7-chloroquinolin-4-ylamino)propyl)-2-(2-furyl)-1,3-thiazinan-4-one(II_D)

Light yellow gummy solid, 65% yield; R_f: 0.54 (chloroform : methanol=3:1); UV-visible spectrum (chloroform), λ_{max} (nm): 257.0, 364.0, 426.0. IR spectrum (chloroform), υ, cm^{-1}: 3432 (N-H str., >NH); (C=O str.); 1375,1305, (C-N str.); 1091 (Ar. C-Cl str.). ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm): 1.75–1.87 (m, 2H, CH_2), 2.52–2.55 (t, 2H, J=4.8 Hz, CH_2), 2.70–2.73 (m, 2H, CH_2), 3.64–3.78 (m, 2H, CH_2), 5.49 (s, 1H, NH), 6.18 (bs, 1H, CH), 6.09-6.12 (m, 2H, furan-2-yl); 6.22–623 (d, 1H, J=1.2 Hz, quinoline-H_3); 7.25 (bs, 1H, furan–2–yl), 7.29–7.32 (d, 1H, J=8.4 Hz, quinoline-H_6), 7.60 -7.62 (d, 1H, J=7.2 Hz, quinoline-H_5), 7.97–8.04 (d, 1H, J=18.3 Hz, quinoline-H_8); (bs, 1H, quinoline-H_2). ^{13}C NMR (100 MHz, $CDCl_3$, δ (ppm): 25.47 (CH_2), 27.15 (CH_2), 35.47 (CH_2), 39.39 (CH_2), 40.19 (CH_2), 67.78 (CH_2), 106.99 (C_3, furan-2-yl), 110.60 (C_4, furan-2-yl), 112.67 (C-3, quinoline); 119.78 (C-4a, quinoline), 121.70 (C-5, quinoline), 127.17 (C-6, quinoline), 138.66 (C-7, quinoline, C-Cl), 142.33 (C_5, furan-2-yl), 148.31 (C-8a, quinoline), 151.69 (C-2, quinoline), 152.25 (C_2, furan-2-yl), 154.95 (C-4, quinoline), 170.67 (C=O). MS (API), m/z (%): 402.1 (100), $[M+H]^+$; 403.1 (24.30), 404.1 (41.40), 405.1 (9.00). Anal. cacld. (%) for $C_{20}H_{20}N_3O_2SCl$: C, 59.77; H, 5.02; N, 10.46; found: C, 51.63; H, 6.11; N, 3.06.

Antibacterial activity testing:

All the synthesized compounds were screened for antibacterial activity by Kirby-Bauer disc diffusion method [9-11] against six different strains of Gram positive and Gram negative bacteria at two different tested doses viz. 25 μg/disc and 50 μg/disc. Three strains of Gram positive bacteria; *Bacillus subtilis* [(ATCC 11774), *Bacillus cereus* (ATCC 10876), *Staphylococcus aureus* (ATCC BAA 1026); and three strains of Gram negative bacteria [*Eschericia coli* (ATCC 10536)], *Klebsiella pneumoniae* (ATCC 33495), *Pseudomonas aeruginosa* (ATCC 10662)] were used for the study. All the bacterial strains were obtained from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (Council of Scientific & Industrial Research), Pune, India. The cultures of bacteria were maintained in their appropriate agar slants at 4^{o}C throughout the study and used as stock cultures. Ofloxacin was used as reference standard drug. Studies were performed in triplicate; mean values with standard deviation were calculated

Table 1: Antibacterial activity data

Compd.	Strength (μg/disc)	Diameter of zone of inhibition (mm ± SD)*					
		Bacillus subtilis ATCC 11774	*Bacillus cereus* ATCC 10876	*Staphylococcus aureus* ATCC BAA 1026	***Escherichia coli*** ATCC 10586	*Klebsiella pneumoniae* ATCC 33495	*Pseudomonas aeruginosa* ATCC 10662
II_A	50	23.66±0.57	24.26±0.28	23.93±0.11	24.36±0.30	2416±0.11	2480±0.20
	25	18.83±0.28	14.26±0.28	14.16±0.15	14.33±0.23	14.33±0.30	14.06±0.11
II_B	50	23.30±0.50	24.40±0.20	24.06±0.11	24.40±0.20	24.20±0.20	24.06±0.11
	25	14.16±0.28	1433±0.11	14.13±0.11	14.23±0.05	14.06±0.11	14.33±0.11
III_C	50	23.16±0.28	23.60±0.20	23.06±0.11	23.41±0.10	23.13±0.23	23.06±0.11
	25	13.06±0.11	13.53±0.11	12.93±0.11	13.66±0.11	13.23±0.05	13.46±0.11
IV_D	50	23.83±0.28	23.66±0.28	24.06±0.11	24.33±0.11	24.20±2.10	23.90±0.36
	25	13.83±0.28	14.06±0.11	14.26±0.23	13.93±0.11	14.00±0.20	13.86±0.11
Ofloxacin[#]	5	20.66±0.25	20.33±0.57	21.66±0.57	21.50±0.50	20.33±0.57	20.83±0.28
DMSO[@]	-	Nil	Nil	Nil	Nil	Nil	Nil

*Values are mean inhibition zone (mm±SD) of three replicates

[#] Ofloxacin disc 5 μg used as a positive reference standard.

[@] DMSO was used as vehicle control.

Results and Discussion

In this study, some new 3-(3-(7-chloroquinolin-4-ylamino)propyl)-1,3-thiazinan-4-one derivatives were synthesized. The intermediate (I) and all the products (II_{A-D}) were obtained in good yield and purity. The analytical and spectral data confirms the structures of the Purified compounds.

All the compounds in chloroform exhibited three characteristic absorption maxima (λ_{max}, nm) in the range between 220- 450nm. The shift of λ_{max} towards longer wavelength indicate the presence of strong chromophoric group such as quinoline structure, and C=O group in the molecule. The maxima in the lower wavelength range between 220-280 nm is due to the presence of substituted phenyl ring like 2-fluorophenyl (II_A), and hetero-aromatic ring system like furan-2-yl (II_D). The infrared spectral data as depicted in experimental section showed characteristic absorption bands for >NH (3340-3435 cm^{-1}); C=O (1693-1734 cm^{-1}); C-N (1275-1398 cm^{-1}); C-Cl (1074-1097 cm^{-1}); $>CH_2$ (υ_{as}: 2976-2930 cm^{-1} & υ_s: 2819-1863 cm^{-1}), and aromatic C=C (1432-1657 cm^{-1}) stretching which confirms the anticipated structure of the synthesized compounds, II_A-II_D. The assignment of protons is fully supported by the characteristic chemical shift values for the 4-aminoquinoline nucleus as discussed in experimental section. The assignment of ^{13}C resonance for different carbon atoms of quinoline nucleus, $>CH_2$ group of side chain and C=O of 1,3-thiazinan-4-one ring system is in close agreement with the structures of the synthesized compounds. The prominent molecular ion peaks, $[M+H]^+$ for all the compounds are in accordance with the anticipated mass of II_{A-D}. The results of CHN analyses were within the acceptable limits of the calculated values. The elemental analyses data are shown in experimental section.

The results depicted in Table 1 clearly revealed that all the compounds at the tested dose showed antibacterial activity and were equally active with some degree of variations, but were less active as compared to standard drug ofloxacin (5 μg/disc). However, it is interesting to note that no obvious difference in susceptibility was found between gram positive and gram negative bacterial strains for all the test compounds. Among the synthesized compounds, compounds with aromatic bulky substituents such as

2-fluorophenyl (II_A), 3-hydroxyphenyl (II_B), furan-2-yl (II_D) is more active than that of compound with aliphatic alkyl substituent (ethyl in II_C) at C-2 position of 1,3-thiazinan ring system.

Conclusion

All the 7-chloro-4-aminoquinoline derivatives with substituted heterocyclic ring at the side chain possess antibacterial activity. It has also been observed that aromatic bulky substituents have greater contributing effect to the antibacterial activity of the new series of prepared derivatives as compared to aliphatic non-bulky group.

References

[1] D. Sharma *et al.*, *Eur. J. Med. Chem.,* 2009, 44, 2347.

[2] K. Bhandari *et al*., *Eur. J. Med. Chem*., in press.

[3] S. Emami *et al*., *Biorg. Med. Chem.Lett.*, 2008, 18, 141-146.

[4] J. Z. Valhhakis *et. al*., *Biorg. Med. Chem.Lett.*, 2006, 16, 2396-2406.

[5] P. B. Madrid, N. T. Wilson, J. L. DeRisi, R. K. Guy, *J. Comb. Chem*., 2004*,* 6, 437-442.

[6] V. R. Solomon, W. Haq, K. Srivastava, S. K. Puri, S. B. Katti, *Bioorg. Med. Chem*., 2005,

13, 2157-2165.

[7] V. R. Solomon, S. K. Puri, K. Srivastava, S. B. Katti, *J. Med. Chem.*, 2007, 50, 394-398.

[8] T. Srivastava, *Tetrahedron*, 2002, 58, 7619-7624.

[9] A. W. Bauer *et al*., *Am. J. Clin. Pathol.,* 1996, 45, 493.

[10] J. G. Collee *et al*., *Practical Medical Microbiology*, 1989, 13th Edn, Churchill Livingstone; 163-165.

[11] W. Hewitt, The agar diffusion assay-Its quantitative basis & choice of experimental design, In: *Microbiological assay for Pharmaceutical analysis: A rational approach*, 2004, New York, 183-205.

Deccan J. Medicinal Chemistry 1(1): Jan.-Mar. 2010 **ISSN 0976 – 1373**

DECCAN PHARMA JOURNAL SERIES

ARMS Online Publications

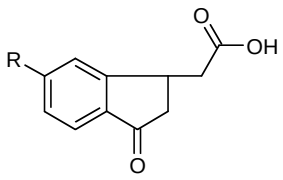
www.deccanpharmajournals.com

PHARMACOLOGICAL STUDY OF BIOTRANSFORMATION OF SUBSTITUTED AND UNSUBSTITUTED INDANONE ACETIC ACID ADDUCT WITH PYRAZOLONE RING FOR ANALGESIC ACTIVITY *IN-VIVO*

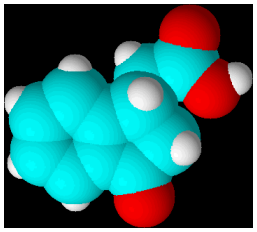
Nirali A. Passi*, Yatri R Shah, Prof Dr Dhrubo Jyoti Sen and Prof Dr Indermeet Singh Anand

Department of Pharmaceutical Chemistry and Pharmacology, Shri Sarvajanik Pharmacy College, Hemchandracharya North Gujarat University, Arvind Baug, Mehsana-384001, Gujarat, India

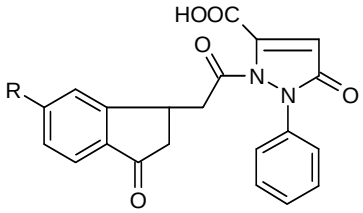
DESIRED PRODUCTS



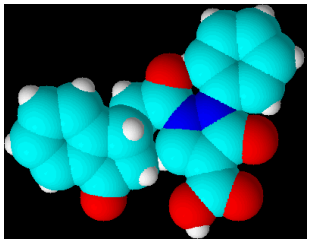
Indanone acetic acid



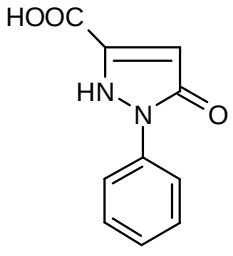
3D-View



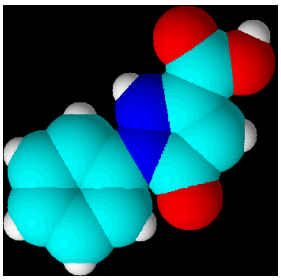
Indanone-pyrazolone adduct



3D-View

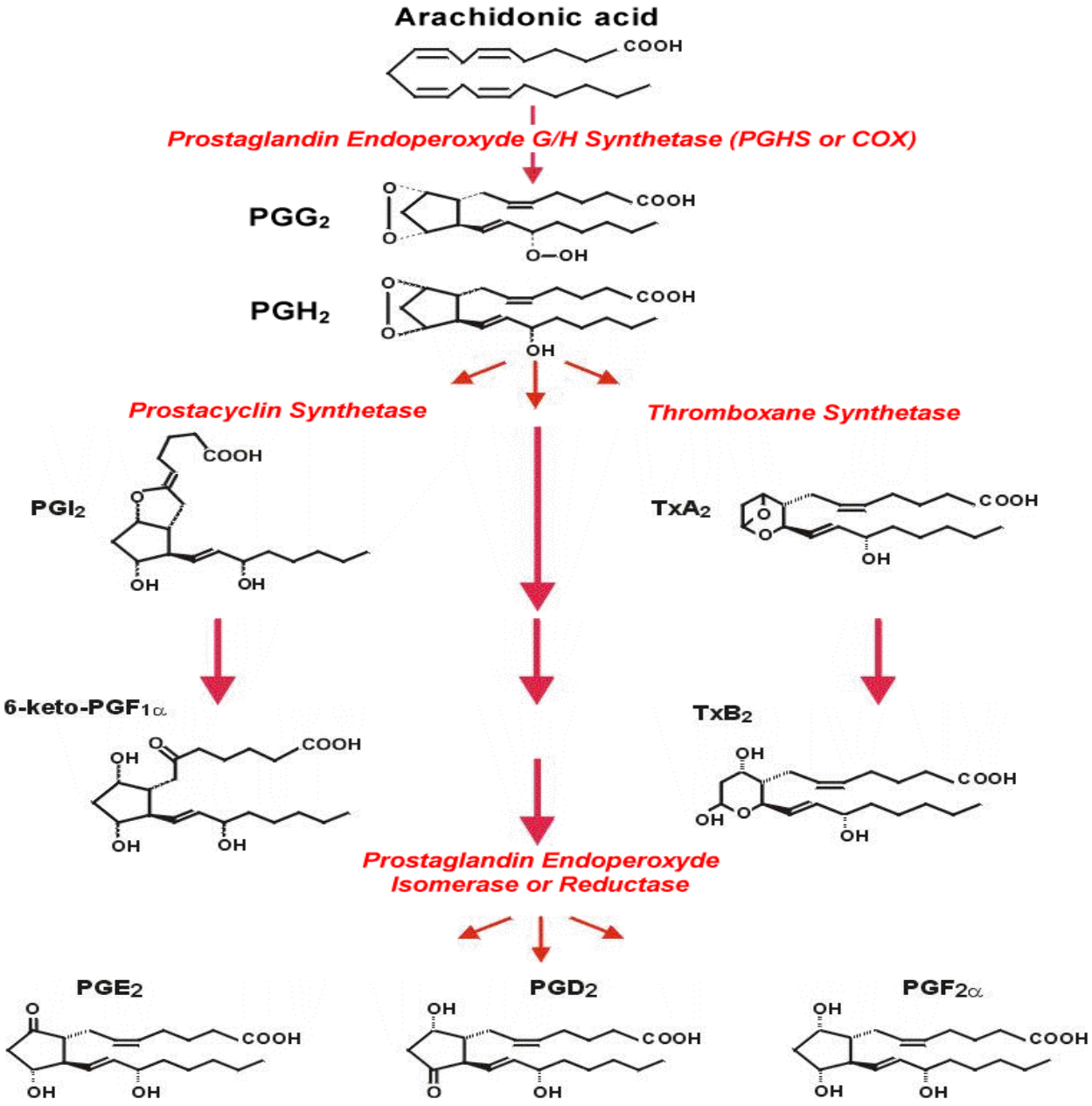


Carboxy pyrazolone

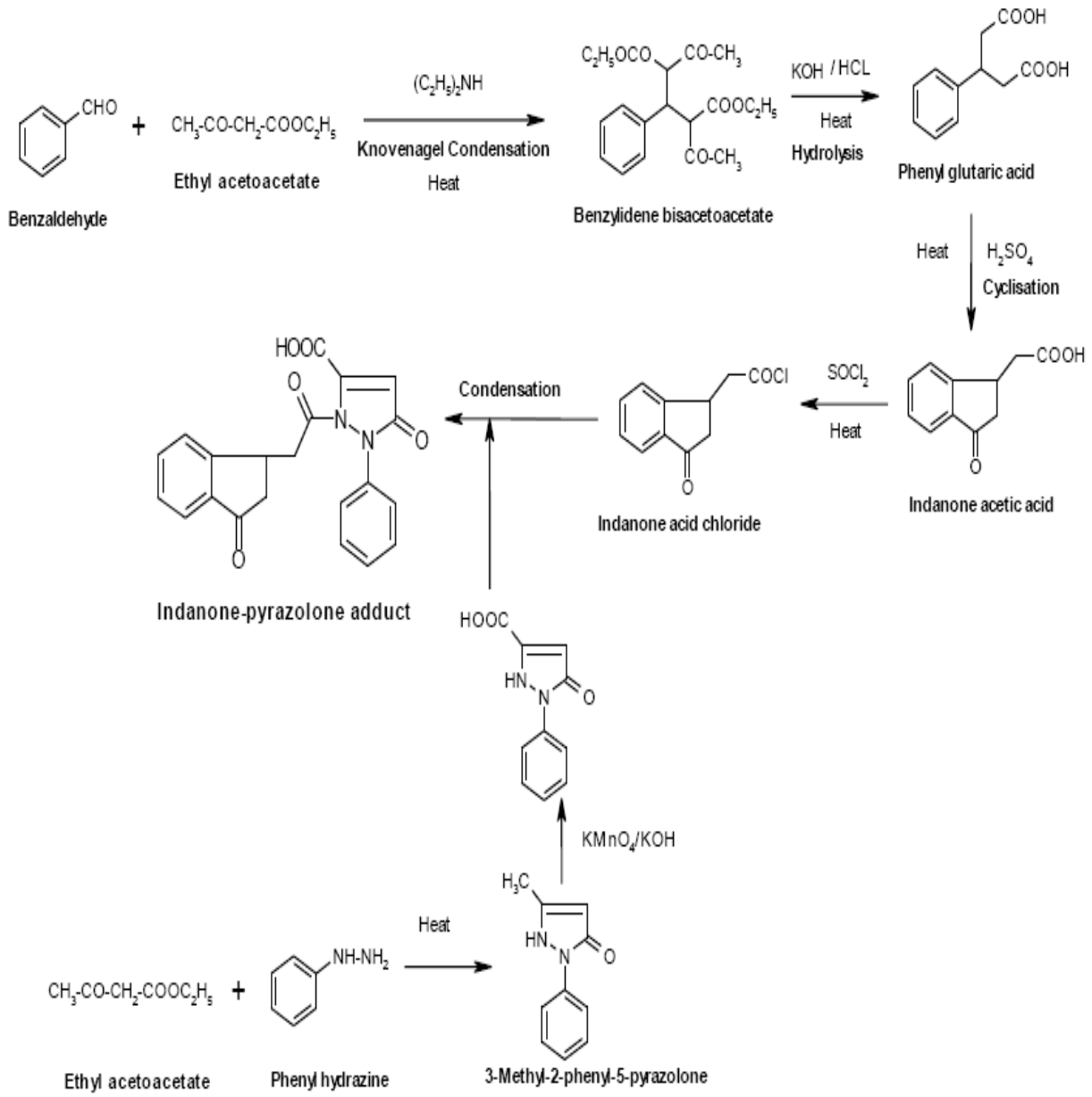


3D-View

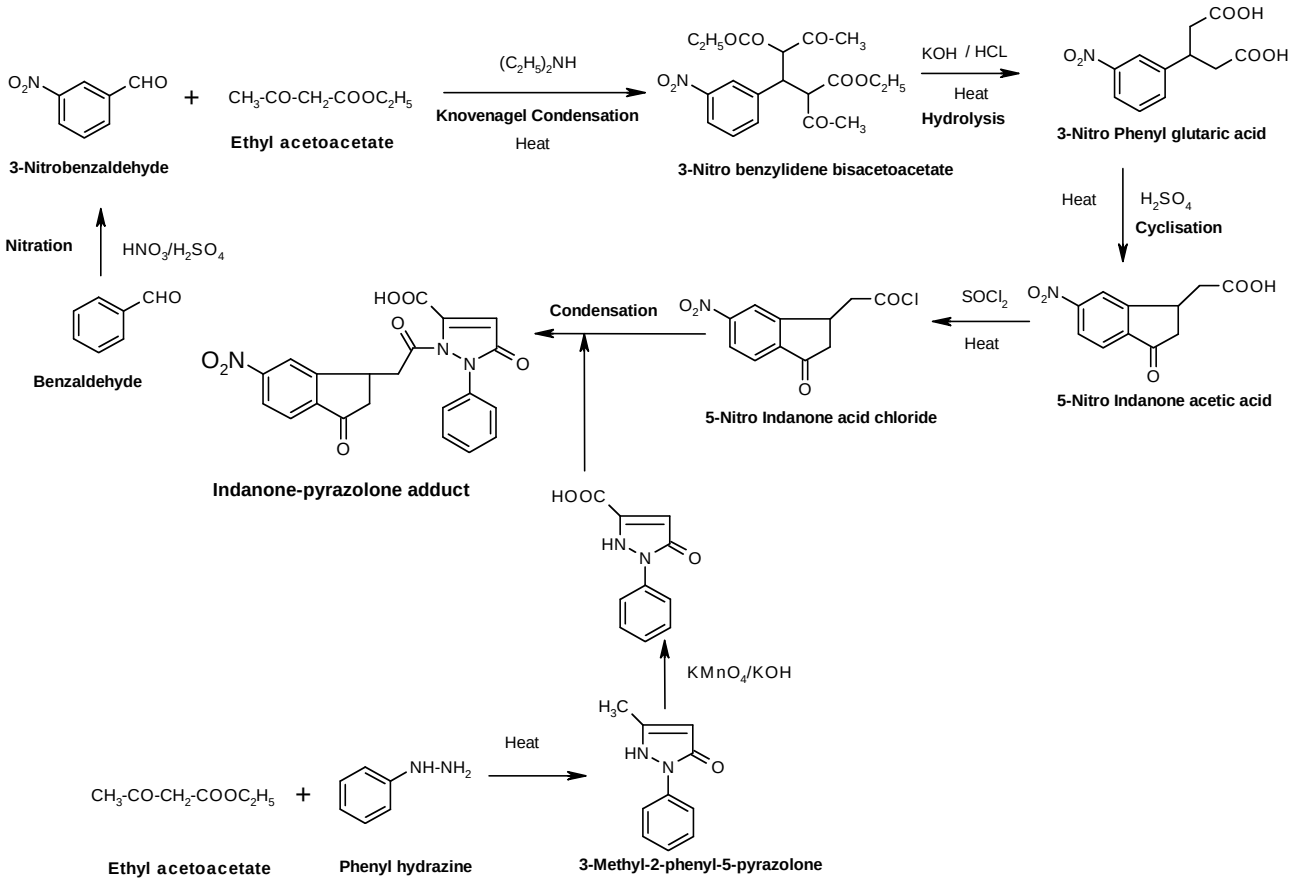
CYCLOOXYGENASE PATHWAY



SYNTHESIS SCHEME-I



SCHEME-II



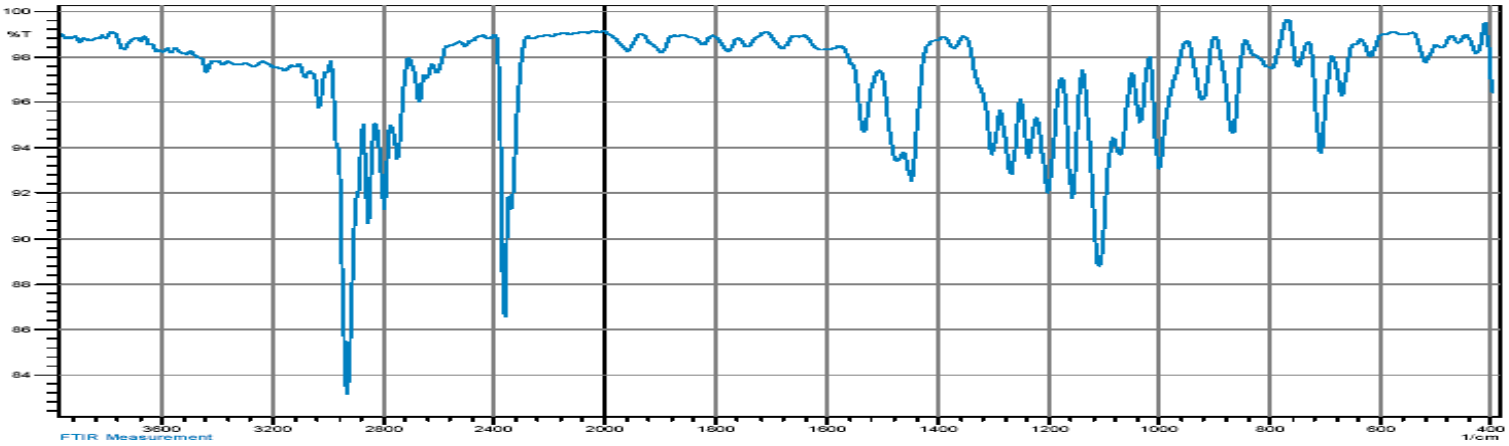
CHEMISTRY

Substituted and unsubstituted indanone acetic acids has been synthesized; where R=NO_2 and R=H. Substituted and unsubstituted aromatic aldehyde followed the Knovenagel condensation by ethyl acetoacetate as β-ketoester in presence of secondary amine to form a bisacetoacetate, which on alkaline hydrolysis and subsequent ring cyclisation by dehydrating agent produced the Indanone acetic acid (R=NO_2: 5-Nitro indanone acetic acid and R=H: Indanone acetic acid)[1].

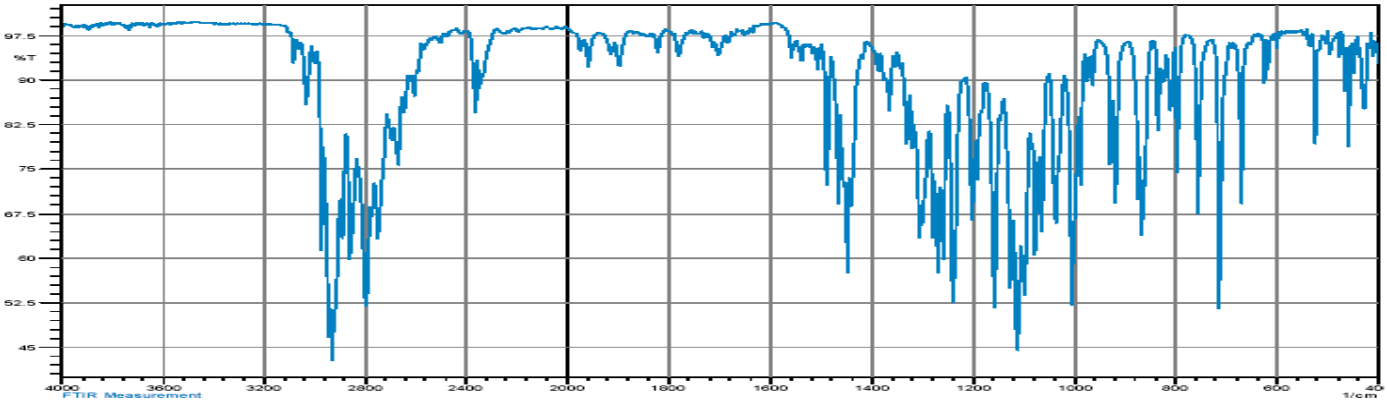
Condensation of phenyl hydrazine with ethyl acetoacetate as β-ketoester produced methyl substituted pyrazolone and oxidizing the methyl group to carboxylic acid by alkaline potassium hydroxide produced Carboxy pyrazolone. The free carboxy group of indanone acetic acids (R= NO_2 and H) have been converted into acid chloride and condensing with free imino group of carboxy pyrazolone to achieve the desired product Indanone-pyrazolone adduct[2]. All the three different components (Indanone acetic acids: R=NO_2 and R=H, Indanone-pyrazolone adduct and Carboxy pyrazolone) were characterized by spectroscopy and N%[3].

INFRARED SPECTROSCOPY OF SYNTHESISED COMPOUNDS

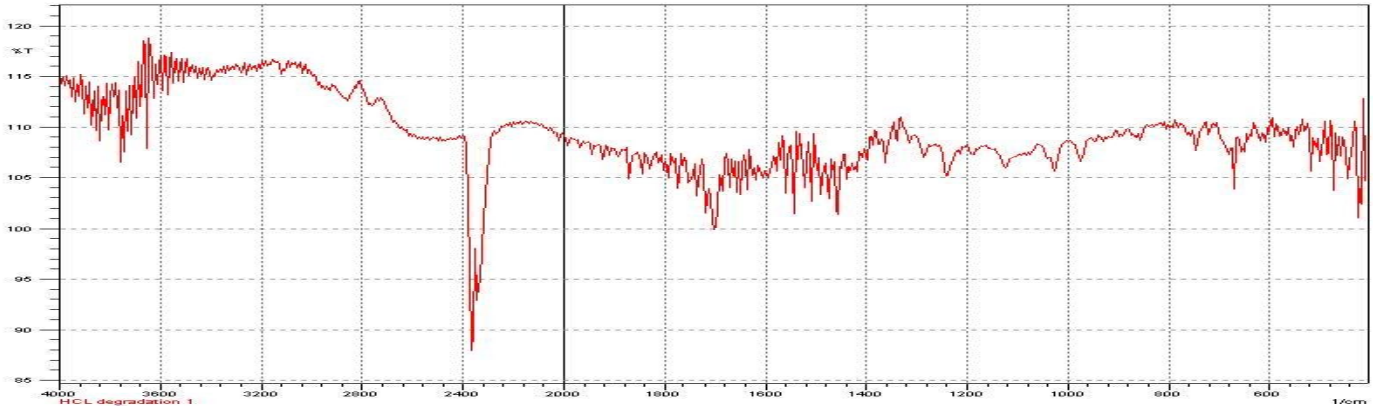
INDANONE ACETIC ACID



INDANONE-PYRAZOLONE ADDUCT

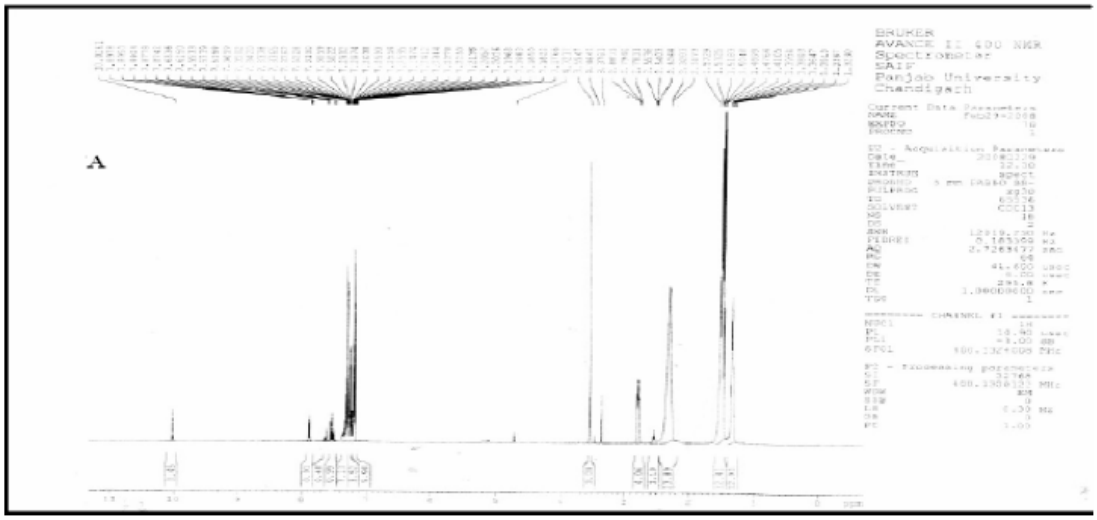


PYRAZOLONE CARBOXYLIC ACID

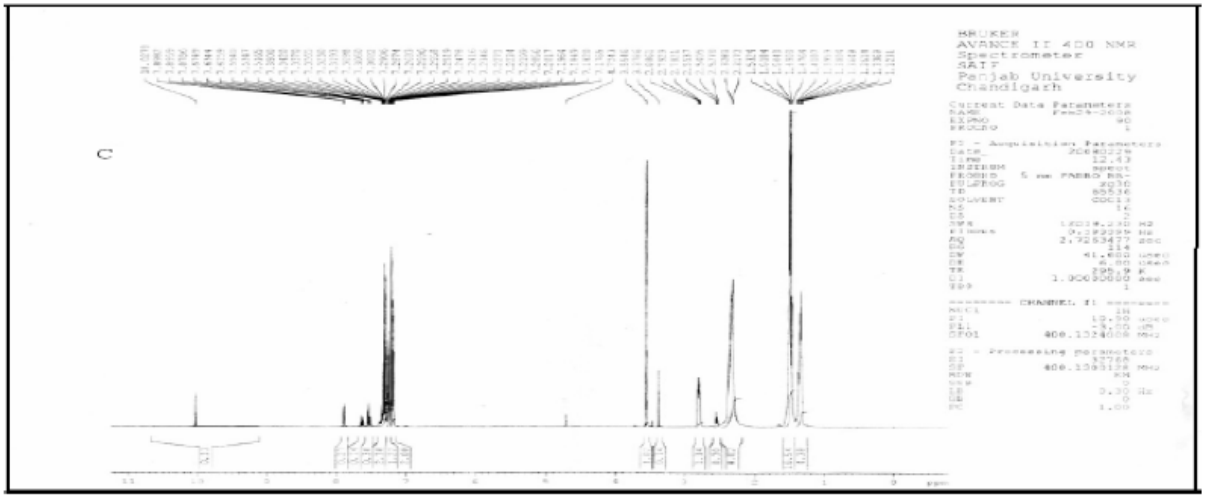


NMR OF SYNTHESISED PRODUCTS

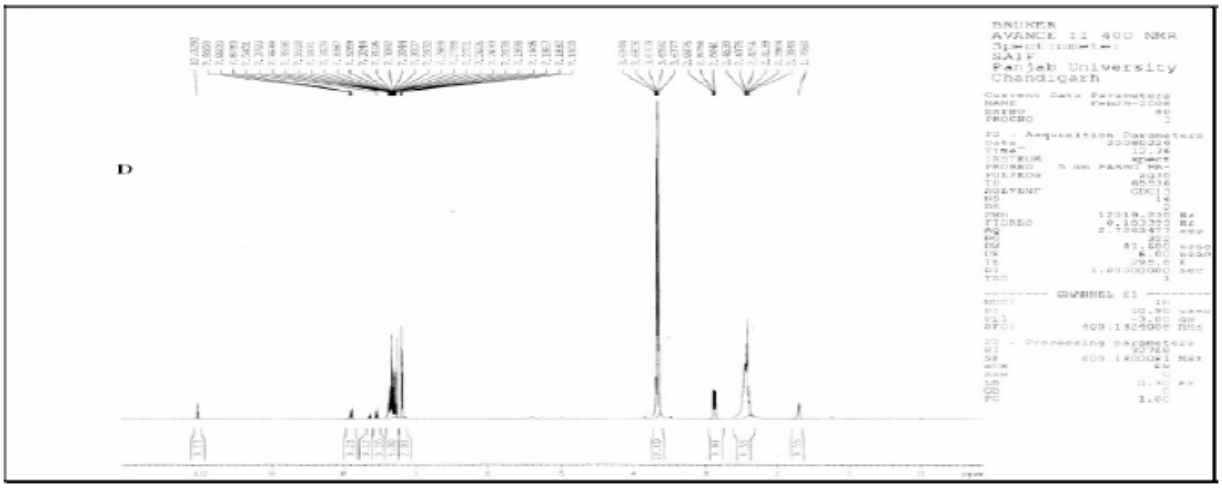
INDANONE ACETIC ACID



INDANONE-PYRAZOLONE ADDUCT



PYRAZOLONE CARBOXYLIC ACID

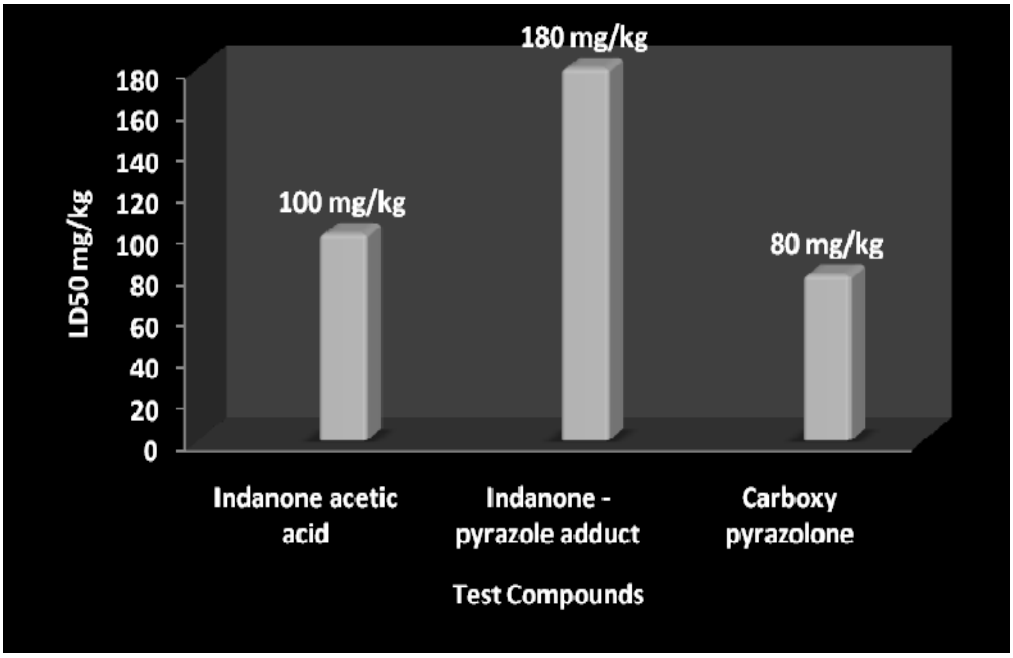


PHYSICOCHEMICAL PARAMETERS

COMPOUNDS	% YIELD	M.P.	POLARITY	MOL. FORMULA	N% CALCD	N% FOUND
Indanone acetic acid	54	134	Semipolar	$C_{11}H_{10}O_3$	Nil	Nil
5-Nitro Indanone acetic acid	48	142	Nonpolar	$C_{11}H_9O_5N$	5.95	6.12
Indanone-pyrazolone adduct	62	156	Nonpolar	$C_{21}H_{16}O_5N_2$	7.44	7.52
5-Nitro Indanone-pyrazolone adduct	58	176	Nonpolar	$C_{21}H_{15}O_7N_3$	9.97	10.12
Pyrazolone carboxylic acid	74	120	Semipolar	$C_{10}H_8O_3N$	7.36	7.48

PHARMACOLOGY

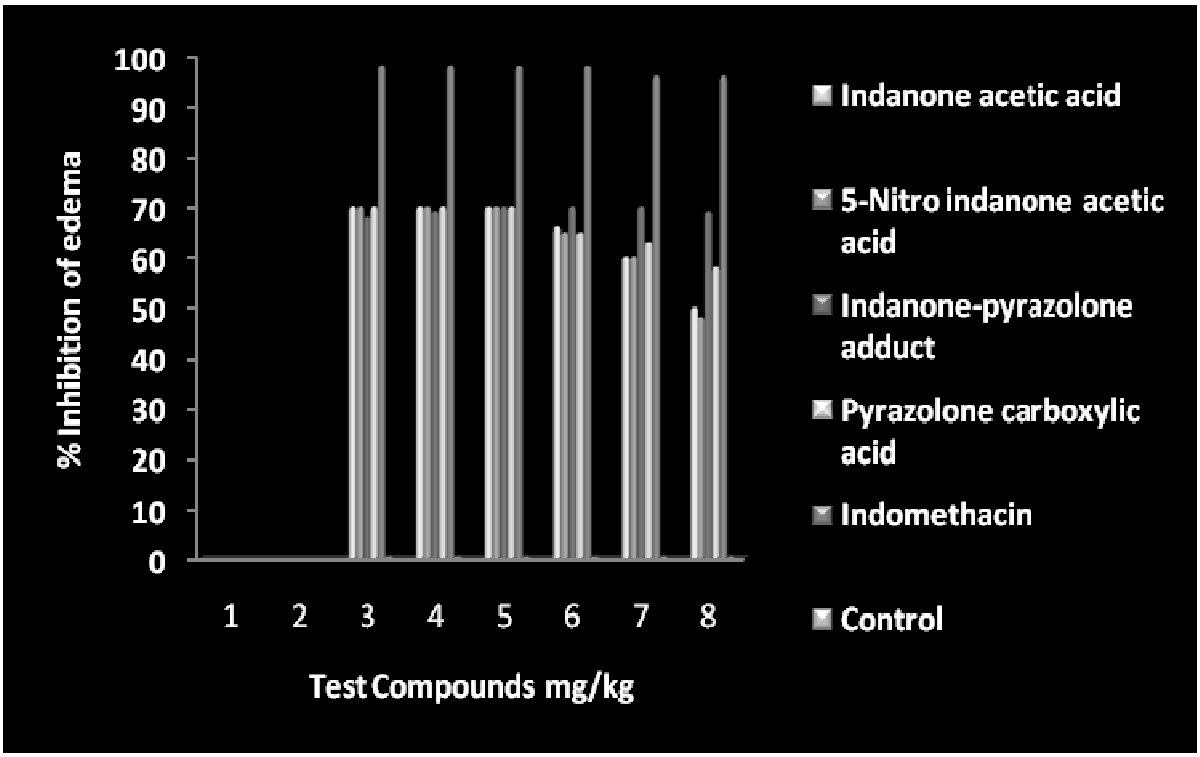
Acute toxicity screening:



The anti-inflammatory screening of the all compounds were performed on rat by plethismometer using rat-paw edema method by treating Indanone acetic acid/5-Nitro Indanone acetic acid to the first group and Indanone-pyrazolone carboxylic acid to the second group having four animals each at 20mg/kg dose intraperitoneally[4]. Next group of four animals received the same compounds with indomethacin at the same dose and % inhibition of paw edema has been noted at one-hour interval for six hours. Indomethacin and the vehicle propylene glycol have been used for the two sets of animal for standard and control group.

The analgesic activity of all the test compounds has been screened intraperitoneally by carrageenan induced rat paw edema method with Indomethacin as standard[5]. The % edema has been calculated by %=[(Control-Test)÷Control]×100. All the observations were calculated with statistical parameters by following Student's-t test[6].

Compounds	Hours					
	1st Hour	2nd Hour	3rd Hour	4th Hour	5th Hour	6th Hour
Indanone acetic acid	70%	70%	70%	66%	60%	50%
5-Nitro Indanone acetic acid	70%	70%	70%	65%	60%	48%
Indanone Pyrazolone Adduct	68%	69%	70%	70%	70%	69%
Pyrazolone carboxylic acid	70%	70%	70%	65%	63%	58%
Indomethacin	98%	98%	98%	98%	96%	96%
Control	00%	00%	00%	00%	00%	00%



CONCLUSION

It has been observed that all the test compounds have anti-inflammatory activity and 70% inhibition of edema persists for 3 hours in the case of Indanone acetic acids and for Carboxy pyrazolone and 60-70% inhibition of edema persists for 5 hours in the case of Indanone-pyrazolone adduct. Indanone has non-heterocyclic fused ring having six membered benzene ring and five membered cyclopentane ring where as pyrazolone has five membered heterocyclic ring substituted with six membered benzene ring. The adduct of indanone acetic acid and carboxy pyrazolone joins with amide linkage which has free carboxy group which is present in the both Indanone acetic acid and Carboxy pyrazolone. Indanone-pyrazolone adduct shows high lipid solubility by partition coefficient rather than the other two compounds which shows the same % inhibition of edema and

longer duration of analgesic activity which is equal to the other test compounds[7]. It proves that the Indanone-pyrazolone adduct shows activity for a certain period and then releases the two factors Indanone acetic acid and Carboxy pyrazolone after hydrolysis of amide linkage by biotransformation *in-vivo* and the activity becomes prolonged but the % inhibition remains the same due to the *in-vivo* synergism appears and the combination activity of the three shows more prolonged action with longer duration by competitive inhibition of arachidonic acid pathway which has also free carboxylic acid group.

Acknowledgement: The author **Nirali A Passi** is thankful to the research group of Shri Sarvajanik Pharmacy College, Mehsana, Gujarat, India for providing the necessary equipments, chemicals, instruments, animals and library facility for performing this project successfully under the guidance of **Prof. Dr. Dhrubo Jyoti Sen** who has guided nicely these synthetic as well as pharmacological experiments under his expertise. The author is also thankful to Panjab University, Chandigarh for NMR and Mass Spectras of the synthesised compounds. This project work has been presented by the author in **42nd IUPAC Congress, 2-7 August, SECC, Glasgow, United Kingdom sponsored by the Royal Society of Chemistry of Great Britain**.

References:

1. S.C. Lahiri and J.K. Gupta***, J.Indian Chem.Soc.,*** 53, 1041 (1976).
2. A. I. Vogel; Vogel's Textbook of Practical Organic Chemistry; 3rd edition, Longmans, Green and Co., Ltd, London, 998-999, (1956).
3. A. Roy et al., ***Indian J.Physiol. Pharmac.,*** 24, 369-372 (1980).
4. D.J. Sen, PhD Thesis, **Jadavpur University**, Kolkata, (2000).
5. S.N. Abramson and P.B. Molinoff, ***Molecular Pharmacology,*** 31, 103-111(1987).
6. M.L. Capone, S. Tacconelli *et al*; ***Circulation.*** 30; 109(12): 1468-71, (2004).
7. C.A. Winter, et al., ***J.Pharmac.Exp.Ther,*** 141, 369-376 (1963).