

SYNTHESIS AND IN-VITRO PROTEIN DENATURATION SCREENING OF NOVEL SUBSTITUTED ISOXAZOLE/PYRAZOLE DERIVATIVES

M.Banerjee^{1,*}, H.K.Sundepp Kumar¹, S.K.Sahu¹, A. Das¹ and P.Parasar²

¹ Medicinal Chemistry Research Lab, Institute of Pharmacy & Technology, Salipur,
Cuttack, Odissa-754202

²School of Pharmaceutical Sciences, SOA University, Bhubaneswar, Odissa-751003

*E-mail: mrityunjaybanerjee@pharmacy@gmail.com

ABSTRACT

A series of novel 2-(5-phenyl-4, 5-dihydroisoxazol-3-yl) benzoic acids (2a-b) / 2-(5-phenyl-4, 5-dihydro-1H-pyrazol-3-yl) benzoic acids (2c-d) were synthesized and tested for their In-vitro protein denaturation activity. Compound 2d was found to be promising and was more potent than the Acetylsalicylic acid (NSAID) in the inhibition of Bovine serum albumin denaturation. The purity of the compounds was checked by TLC and elemental analyses. Both analytical and spectral data (1H NMR, IR and MS) of all the synthesized compounds were in full agreement with the proposed structures.

Keywords: 2-(5-phenyl-4, 5-dihydroisoxazol-3-yl) benzoic acid, 2-(5-phenyl-4, 5-dihydro-1H-pyrazol-3-yl) benzoic acid, 1H NMR, IR and MS

© 2011 RASĀYAN. All rights reserved.

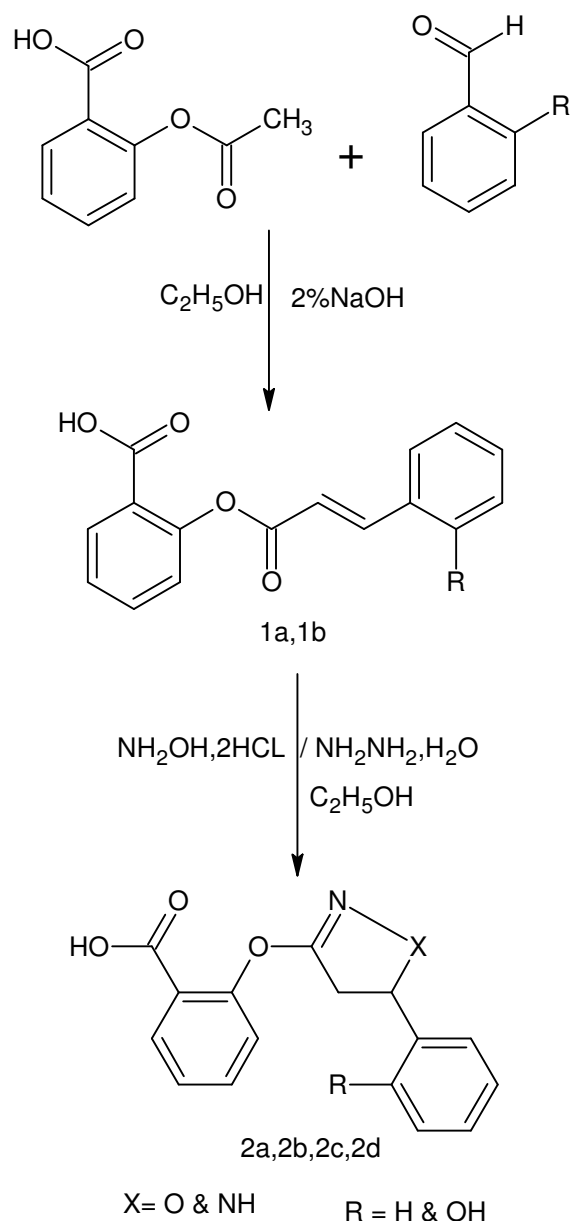
INTRODUCTION

Five member Nitrogen containing heterocyclic compounds possessing broad-spectrum of biological activity¹. Isoxazole²⁻⁴ and Pyrazole⁵⁻⁶ have proven record of biological activities, which contains two Nitrogen atoms. Acetylsalicylic acid (Aspirin) is very useful units in the fields of medicinal and pharmaceutical chemistry and has been reported to exhibit a variety of biological activities. Chalcones and their analogies having α , β - Unsaturated carbonyl system are very versatile substrates for the evolution of various reactions and physiologically active compounds⁷. The reaction of the $\text{NH}_2\text{OH}\cdot 2\text{HCl}/\text{NH}_2\text{NH}_2$, H_2O with the different chalcones of acetylsalicylic acid derivative resulting 2-(5-phenyl-4,5-dihydroisoxazol-3-yl)benzoic acids (2a-b) / 2-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)benzoic acids (2c-d). In view of the pharmacological profiles of these two chemical moieties as described above, we considered it interesting to synthesized two chemically different but pharmacologically compatible molecules with the aim of obtaining some novel heterocyclic systems with potentially enhanced biological properties. In the present investigation a new series of novel 2-(5-phenyl-4,5-dihydroisoxazol-3-yl)benzoic acids (2a-b) / 2-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)benzoic acids (2c-d). were synthesized as per Scheme 1 and evaluated for their In-vitro protein denaturation activity.

EXPERIMENTAL

Melting points were determined on a Tempstar apparatus and are uncorrected. Electronoc spectra were recorded in double beam uv-vis spectrophotometry (V-600,Jasco),Infrared spectra were recorded on a Jasco (410) FT-infrared spectrophotometer, measured as KBr disks.¹H NMR were recorded on a Bruker DPX-300 MHz spectrometer in deuteriochloroform with trimethylsilane as internal standard (chemical shift in δ ppm). The mass spectral data were obtained with a Perkin-Elmer Hitachi RMU-6L MS-30 spectrometer at 70 ev and a 90 °C inlet temperature. Purity of all the compounds was checked on silica gel plates and spots were located in iodine vapours. Elemental analysis was performed on EURO EA (Italy)

analyzer and the results were within $\pm 0.4\%$ of calculated values. Physical data of the synthesized compounds are presented in Table 1.



Scheme-1

General method for the preparation of 2-{-3-phenylprop-2-enoyloxy} benzoic acid (1a)

Equimolar quantity of acetylsalicylic acid (0.01m) and benzaldehyde (0.01m) were added in ethanol. 10ml of 2% sodium hydroxide solution were added drop wise with constant stirring in a magnetic stirrer over a period of 30 mins. The reaction mixture was stirred for 17hrs and then refluxed for 6 hrs. then the reaction mixture was poured into ice cold water. The separated solid was filtered, washed with aqueous ethanol, and recrystallized from ethanol : water (50:50). Compound **1b** was prepared similarly.

2-{-3-phenylprop-2-enoyloxy} benzoic acid (1a)

IR (KBr): 3076(Ar-CH), 1732 (C=O), 1670 (C=O), 1609 (C=C), MS: (m/z) 252(M⁺); ¹H NMR (CDCl₃) δ : 11.69 (s, 1H, COOH), 7.61(d,CH,CH=CH), 7.68(d,CH,CH=CH),8.06 (m, 9H, ArH), 6.02-7.58 (m, 4H, ArH);

2-[3-(2-hydroxyphenyl)prop-2-enyl]benzoic acid (1b)

IR (KBr): 3470 (OH), 3072(Ar-CH), 1730 (C=O), 1672 (C=O), 1602 (C=C), MS: (m/z) 268(M⁺); ¹H NMR (CDCl₃) δ : 11.68 (s, 1H, COOH), 7.66(d,1H,CH=CH), 7.74(d,1H,CH=CH),8.06 (m, 9H, ArH), 6.02-7.58 (m, 4H, ArH),5.69(s,1H,OH);

General method for the preparation of 2-(5-phenyl-4,5-dihydroisoxazol-3-yl)benzoic acid (2a)

Equimolers quantity of 1a (0.1M) and hydroxylamine HCL(0.1M) were mixed in ethanol. The reaction mixture was refluxed for 6 hrs. Then the reaction mixture was poured into ice cold water. The separated solid was filtered, washed with aqueous ethanol, and recrystallized from ethanol: water (50:50), Compound **2b** was prepared similarly.

2-(5-phenyl-4,5-dihydroisoxazol-3-yl)benzoic acid (2a)

IR (KBr): 3075(Ar-CH), 1730(C=O), 1681 (C=O), 1611 (C=N),1046(C-O-C) MS: (m/z) 267 (M⁺); ¹H NMR (CDCl₃) δ : 11.71 (s, 1H, COOH), 5.63(t,1H,CH), 3.25(d,2H,CH₂), 6.02-8.29 (m, 4H, ArH);

2-[5-(2-hydroxyphenyl)-4,5-dihydroisoxazol-3-yl]benzoic acid (2b)

IR (KBr): 3471 (OH),3076(Ar-CH), 1734 (C=O), 1686 (C=O), 1614 (C=N), 1045(C-O-C) MS: (m/z) 283 (M⁺); ¹H NMR (CDCl₃) δ : 11.72 (s, 1H, COOH), 5.68(t,1H,CH), 3.23(d,2H,CH₂), 6.02-8.28 (m, 4H, ArH),5.24(s,1H,OH);

General method for the preparation of 2-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)benzoic acid (2c)

Equimolers quantity of 1a (0.1M) and hydrazine hydrate(0.1M) were mixed in ethanol. The reaction mixture was refluxed for 6 hrs. Then the reaction mixture was poured into ice cold water. The separated solid was filtered, washed with aqueous ethanol, and recrystallized from ethanol: water (50:50), Compound **2d** was prepared similarly.

2-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)benzoic acid (2c)

IR (KBr): 3342(NH),3075(Ar-CH), 1731(C=O), 1687 (C=O), 1609 (C=N), MS: (m/z) 266 (M⁺); ¹H NMR (CDCl₃)δ : 11.70 (s, 1H, COOH), 9.56(d,1H,NH),5.69(t,1H,CH), 3.28(d,2H,CH₂), 6.08-8.17 (m, 4H, ArH);

2-[5-(2-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl]benzoic acid (2d)

IR (KBr): 3470 (OH), 3341(NH),3076(Ar-CH), 1729 (C=O), 1688 (C=O), 1615 (C=N), MS: (m/z) 282(M⁺); ¹H NMR (CDCl₃) δ : 11.72 (s, 1H, COOH), 9.56(d,1H,NH),5.61(t,1H,CH), 3.31(d,2H,CH₂), 6.08-8.27 (m, 4H, ArH),5.3(s,1H,OH);

Table-1: Physical data of synthesized compounds 1a-b & 2a-d

Compd.	R	Molecular Formula	m.p (C ⁰)	Yield (%)	% Analysis Calc.(Found)		
					C	H	N
1a	H	C ₁₆ H ₁₂ O ₃	105	70	76.18 (76.22)	4.79 (4.76)	-
1b	OH	C ₁₆ H ₁₂ O ₄	98	68	71.64 (71.53)	4.51 (4.48)	-
2a	H	C ₁₆ H ₁₃ NO ₃	160	68	71.90 (71.94)	4.90 (4.88)	5.24 (5.26)
2b	OH	C ₁₆ H ₁₃ NO ₄	157	70	67.84 (67.87)	4.63 (4.62)	4.94 (4.89)
2c	H	C ₁₆ H ₁₄ N ₂ O ₂	154	71	72.16 (72.17)	5.30 (5.33)	10.52 (10.48)
2d	OH	C ₁₆ H ₁₄ N ₂ O ₃	152	69	68.07 (68.03)	5.00 (4.98)	9.92 (9.96)

Inhibition of protein denaturation

The reaction mixtures (0.5ml) consisted of 0.45ml bovine serum albumin (5% aqueous solution) and 0.05ml of test compound (100 and 250 mg/ml of final volume). pH was adjusted at 6.3 using a small amount of IN HCl. The samples were incubated at 37⁰C for 3 min. After cooling the samples 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660nm. For control tests 0.05ml distilled water was used instead of synthesized product control tests lacked bovine serum albumin. The percentage inhibition of protein denaturation was calculated as follows-

$$100 - \frac{(O.D. \text{ of test} - O.D. \text{ of product control})}{O.D. \text{ of control}} \times 100$$

Table -2: In vitro anti-inflammatory screening of synthesized compounds by bovine serum albumin denaturation

Compound	Absorbance value (660nm)*	Protein denaturation Mean (%)
2a	0.194	25
2b	0.185	34
2c	0.180	38
2d	0.160	56
Aspirin	0.149	66
Control	0.111	00

*Average of Three readings

RESULTS AND DISCUSSION

Chemical synthesis

2-{3-phenylprop-2-enoyloxy} benzoic acid (1a) and 2-[3-(2-hydroxyphenyl)prop-2-enoyl]benzoic acid 1b were prepared by reacting with aspirin and substituted benzaldehyde. Compounds 1a,b were condensed with hydroxylamine dihydrochloride or hydrazine hydrate in ethanol to afford the corresponding isoxazole (2a,2b) or pyrazole (2c,2d) derivatives in 68-71 % yields (Table 1). All the synthesized compounds were characterized by their elemental analysis, FT-IR, ¹H NMR, and mass spectroscopy. For example, the IR spectrum of 1a shows an absorption band at 1732 cm⁻¹ corresponding to the stretching vibration of COOH group, while band at 1670 cm⁻¹ correspond to the characteristic keto group of chalcone. The IR spectrum of 2a shows an absorption band at 1046 and 1607cm⁻¹ corresponding to the amino(C-O-C) and Imino(C=N) groups, respectively present at Isoxazole moiety and 2c shows an absorption band at 3342 and 1609 cm⁻¹ corresponding to the amino(NH) and Imino(C=N) groups, respectively present at Pyrazole moiety.

The ¹H NMR of 2a showed the absence of the signal for the CH=CH group, while the pyrazole (2c) NH signal appeared as a singlet at 9.56 ppm. Diagnostically important signals in the nuclear magnetic resonance (¹H NMR) spectrum of 2a were two singlet at 5.63 and 3.24 ppm attributed to the CH and CH₂ groups, respectively. Aromatic protons of isoxazole and pyrazole moieties all appeared at the expected chemical shifts. The structures of 1a and 2a were also confirmed by its mass spectrum that shows molecular ion peaks (M⁺) at m/z 252 and m/z 267.

Biological activity

Various 2-(5-phenyl-4, 5-dihydroisoxazol-3-yl) benzoic acids (2a-b) / 2-(5-phenyl-4, 5-dihydro-1H-pyrazol-3-yl) benzoic acids (2c-d) were tested for their Inhibition of protein denaturation activity by BSA assay method⁸ using Bovine Serum Albumin. Percent protein denaturation activity of the compounds are reported in Table 2 with standard drug Acetyl Salicylic acid(Aspirin) for comparison. Among the 4 compounds synthesized two compounds exhibited significant activity Hence, we can conclude that size of hydroxyl substituents at position of isoxazole/pyrazole moiety is important for In vitro anti-inflammatory activity.

Protein Denaturation activity

Denaturation of proteins as one of the causes of RA is well documented⁷. Production of auto antigens in certain rheumatic diseases may be due to in-vitro denaturation of proteins. The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide

boarding. From the results of the present study it can be stated that synthesized oxopyrimidine analogs is capable of controlling the production of auto antigens due to in-vivo denaturation of proteins in rheumatic disorder. This finding justifies the usefulness of this product in the management and treatment of inflammation associated diseases like arthritis, from the results obtained in the present studies, it may be concluded that novel 2-(5-phenyl-4, 5-dihydroisoxazol-3-yl) benzoic acids (2a-b) / 2-(5-phenyl-4, 5-dihydro-1H-pyrazol-3-yl) benzoic acids (2c-d) acid possesses significant anti-arthritis and anti-inflammatory action, which is comparable to synthetic anti-inflammatory agents. All the synthesized compounds were screened for their antiarthritis activity and the effect of all the test compounds of scheme-I, was found to be very good. Further clinical studied are needed to establish its safety and usefulness in arthritic patient.

Among the newer derivatives, compound **2d** showed a promising activity in the test. It is conceivable that these derivatives showing Invitro Protein Denaturation activity can be further modified to achieve NSAID agents with antiarthritis activity. SAR studies and evaluation of more potent analogues are continuing.

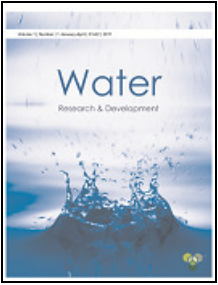
ACKNOWLEDGEMENTS

The authors are grateful to the Principal and management of IPT,SALIPUR & S.P.S., SOA University for providing necessary facilities to carry out this research work.

REFERENCE

1. F.E. Goda, A.R. Maarouf and E.L. Bendary, *S. Pharm. J.* **111**, 11 (2003).
2. S. K .Sahu, S. K. Mishra, M. Banerjee, P. K. Panda and P. K. Mishro, *J.Indian Chem.Soc.*, **725**,83 (2006).
3. G. A. Habeeb, P. N. P. Rao and E. E. Kanus, *J. Med. Chem.*, **2921**, 44 (2001)
4. G. A. Habeeb P. N. P.,Rao and E. E. Kanus, *Drug Dev.Res.* **273**,51 (2000)
5. T. Akihiko, O .Yoshihiro, O. Keiko, T. Hideo, K. Motoji, W. Masaaki and Y .Jun-ichi. *Bioorg. Med. Chem Letters*; **4299**,15 (2005)
6. S.K.Sahu, M.Banerjee, S. K.Mishra, R. K.Mohanta, P. K.Panda and P.K. Mishro. *Acta Poloniae Pharmaceutica-Drug Research*, **121**,64 (2007)
7. M.Amir and S.Kumar, *Ind. J. Chem.*, **2532**,44B (2005)
8. S. Chatterjee and S.N. Das, *Indian J. Pharmacology*, **116**,28(1996)

[RJC-719/2011]

<p>New Journal WaterR&D ISSN: 2249-2003</p>  <p>[April, August and December] All articles will be peer-reviewed.</p>	<p>Scope and Coverage: Water: Research & Development [Water R&D] is an international Research Journal, dedicated to 'Water'. It is a truly interdisciplinary journal on water science and technology. It'll showcase the latest research related to Water in the field of chemistry, physics, biology, agricultural, food, pharmaceutical science, and environmental, oceanographic, and atmospheric science. It includes publication of reviews, regular research papers, case studies, communications and short notes.</p> <p>Its Coverage area is: Water Pollution; Ecology of water resources, including groundwater; Monitoring, remediation and conservation of water resources; Rain Water Harvesting; Absorption and Remediation; Aquatic and Marine life ; Corrosion ; Industrial Effluent treatments; Physics, Chemistry and Biology of water; Water, as a Green solvent/ Reaction Medium; Management of water resources and water provision; Wastewater and water treatment; Water related Rules, Policies, Laws; Dyes and Pigments; Water and Health; Sustainable use of water; Policies and Regulations about water; Degradation of aquatic ecosystem; Water Footprints and Virtual water calculations.</p> <p>All submissions are addressed to the Editor-in-Chief at Editorial office address or by e-mail to: water.editor@yahoo.co.in</p>
<p><i>*Note: If you think you may be a potential reviewer for water related manuscripts, please send your CV and Photo to the Editor.</i></p>	<p>WaterR&D 23 'Anukampa', Janakpuri, Opp. Heerapura Power Stn., Ajmer Road, Jaipur-302024 (India) E-mail : water.editor@yahoo.co.in ; Phone : 0141-2810628(Off.), 07597925412(Mob.)</p>