AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Synthesis and Biological Evaluation of Amino acid Derivatives of Salicylic Acid As Analgesic and Anti-inflammatory Agents

Kamlesh. R. Ahire[*1], Sampada. S. Jangam[1] , Rishikesh. V. Antre[1], Prafulla P. Adkar[1], Hemlata M. Nimje[1],

1. JSPM'S Charak College of Pharmacy and Research, Wagholi, Pune-412 207, India

ABSTRACT

Inflammation is a complex biological response of vascular tissues to harmful stimuli. The stimulus may be thermal (heat or cold), chemical (foreign substances, foreign organisms, drugs), or mechanical (trauma). Anti-inflammatory agents are the agents which relieves the inflammation. Amino acids L-tyrosine was refluxed in presence of thionyl chloride and methanol for 8 hrs to form its methyl ester hydrochloride(a). Compound (a) and taurine were refluxed with Salicylic acid in 30 % NaOH solution for 2 hrs and neutralized with conc. hydrochloric acid to precipitate target compounds (b, c). These compounds were characterized on basis of melting point, TLC (R_f value), IR and ^1H NMR spectra and evaluated for analgesic and anti-inflammatory activity. Target compound (b) was found to be most active with 96.3 % protection for analgesic and anti-inflammatory activity.

Keywords: Salicylic acid, Amino acids, Analgesic, Anti-inflammatory.

*Corresponding Author Email: kamleshahire13@gmail.com

Received 07 November 2012, Accepted 26 December 2012

Please cite this article in press as Ahire KR *et al.*, Synthesis and Biological Evaluation of Amino acid Derivatives of Salicylic Acid As Analgesic and Anti-inflammatory Agents. American Journal of PharmTech Research 2013.

INTRODUCTION

Inflammation is a complex biological response of vascular tissues to harmful stimuli.[1] The stimulus may be thermal (heat or cold), chemical (foreign substances, foreign organisms, drugs), or mechanical (trauma). The most important activator of the inflammatory response is the mast cell, which initiates inflammation by releasing biochemical mediators (e.g. histamine, chemotactic factors) from preformed cytoplasmic granules and synthesizing other mediators (prostaglandins, leukotrienes) in response to stimulus.[2] The classical signs of an inflammatory process are *Rubor* (redness), *Tumour* (swelling), *Calor* (heat), *Dolor (pain)* and *function laesa* (loss of function).[3] The process of inflammation is not undesirable; it is protective mechanism essential for survival. Analgesics are the drugs which relieves the pain. Anti-inflammatory agents are the agents which relieves the inflammation. Anti-inflammatory agents are broadly classified into Steroidal and NSAIDs (Non-steroidal anti-inflammatory agents). In market various analgesic and anti-inflammatory formulations and dosage forms are available of aspirin, paracetamol, ibuprofen etc. Generally most of the NSAIDs are associated with common adverse effects like platelet dysfunction, acute renal failure, sodium and water retention, edema, gastritis and peptic ulceration with bleeding etc. Since present NSAIDS suffer from these common side effects, the efforts are underway to come up with safer and better NSAIDs. One of the attempt is the synthesis of potential anti TNF-α agents. [4] Another approach is to synthesize various L-Amino acid derivatives of these NSAIDs in order to overcome the common adverse effects associated with it. The salient features of the usefulness of conjugation of amino acids are i) Amino acids are normal dietary constituents and they are non-toxic in moderate doses as compared to other promoieties, ii) A drug with free carboxyl group can be derivatized into corresponding esters and amides of amino acids, so as to alter the physical properties of a parent drug with one more of hydrolases enzymes serving as the *in-vivo* reconversion site(s), iii) Being a nutritional substance, the use of amino acids as a derivatizing group might also permit more specific targeting site for enzymes involved in the terminal phase of digestion, iv) By using different types of amino acids like polar, non-polar, acidic and basic, the drug molecule can be made more or less polar, or more or less soluble in given solvent.[5,6] Salicylic acid, *2-hydroxybenzoic acid*[1], is a salicylate drug often used as an analgesic to relieve minor aches and pains, as antipyretic to reduce fever and as an anti-inflammatory medication by inhibiting cyclooxygenase enzyme. It also has an antiplatelet effect by inhibiting the production of thromboxane, which under normal circumstances binds platelet molecules together to create a

patch over damaged walls of blood vessels. The main undesirable side effects of Salicylic acid are gastrointestinal ulcers, stomach bleeding and tinnitus especially in higher doses.[7] Its chemical structure consist of free carboxylic acid functional group which is responsible for gastric irritation. Therefore, attempts have been made to synthesize amino acid derivatives of aspirin by using L-tyrosine[2] and Taurine[3] amino acid. Taurine is useful in the treatment inflammation and epilepsy. It is polar amino acid. It is neuroregulator and nerve cell growth factor. It is having anticonvulsant activity by potentiating GABAergic inhibition.[8, 9, 10] L-tyrosine is a polar amino acid. It is useful during condition of stress, cold, fatigue and improvements in cognitive and physical performance.

MATERIAL AND METHOD:

All the chemicals used for synthesis were of LR (Laboratory Reagent) and AR (Analytical Reagent) grade. To monitor the reactions as well as to establish the identity and purity of reactants and products, TLC (Thin Layer Chromatography) was performed on microscopic glass slides (2 x 7.5 cm) coated with silica gel-G, using Chloroform : Carbon Tetrachloride : Ethyl acetate as a solvent system and the spots were visualized by exposure to iodine vapours or under Ultra-violet (UV) light. The melting points were determined in open capillary on Veego (Model : VMP-D) electronic apparatus and uncorrected. The IR spectra of synthesized compounds were recorded on Schimadzu 8400-S FTIR spectrophotometer using potassium bromide. The ^{1}H NMR was recorded in DMSO-D6 using NMR Varian –Mercury 300 MHz spectrometer and chemical shifts are given in parts per million, downfield from tetra methyl silane (TMS) as an internal standard from University of Pune.

STEP-I :Synthesis of L-tyrosine methyl ester hydrochloride (a)[11]:

Freshly distilled thionyl chloride 0.468 ml (0.005 mol+30% extra) was slowly added to methanol (20 ml) contained in a 50 ml R.B.F. with cooling. To this solution 1.81 gm (0.01 mol) L-tyrosine was added and the mixture was refluxed for 7 hours at 60-70^{0}C with continuous stirring. Excess of thionyl chloride and solvent were removed under reduced pressure giving crude tryptophan methyl ester hydrochloride. The crude product was triturated with 20 ml portions of cold ether at 0^{0}C until excess of dimethyl sulfide was removed. The resulting solid product was collected and dried under high vacuum.

STEP-II :Synthesis of (1-methyl)-2-(2-hydroxy-phenyl-carboxamido)-3-(4-hydroxy phenyl)propanoate. (b)

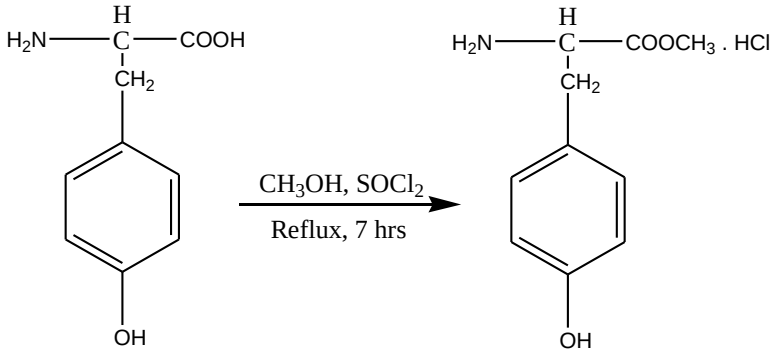
Salicylic acid 0.414 gm (0.003mol) and methyl ester of L-tyrosine **0.6 gm** was placed in R.B.F.

This mixture was refluxed in aqueous sodium hydroxide solution (30% w/v) for 2 hrs. The resulting solution was neutralized by using conc. Hydrochloric acid solution. The precipitate so obtained was dried in air.

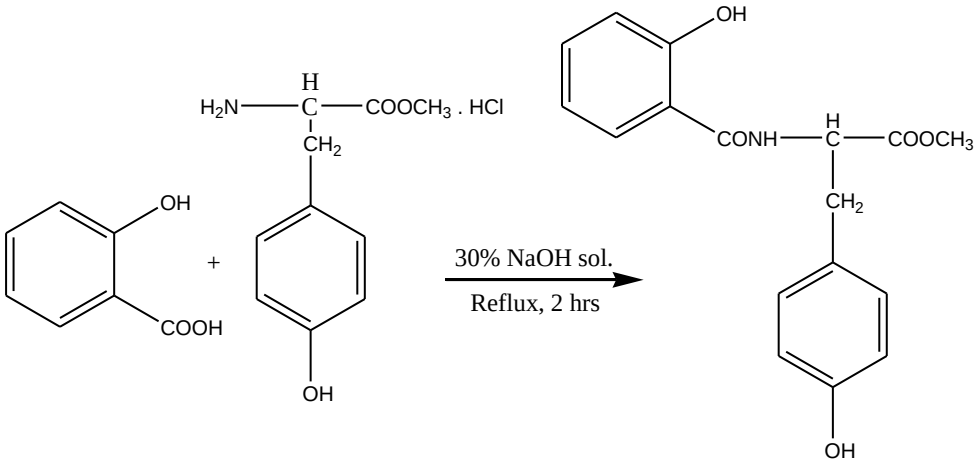
STEP-III: Synthesis of 2-(2-hydroxy-phenyl-carboxamido)ethanesulfonic acid. (c)

2 gm (0.016 mol) of taurine, 2.208gm (0.016 mol) of salicylic acid was placed in R.B.F 40 ml of methanol was added as a solvent .Resulting solution was refluxed for 30 min. 0.896gm (0.016mol) of KOH was added and reflux was continued for next 4.30 hours. Methanol present in filtrate was recovered by distillation and the crude product obtained was recrystallized by using ethanol.

SCHEME FOR SYNTHESIS:

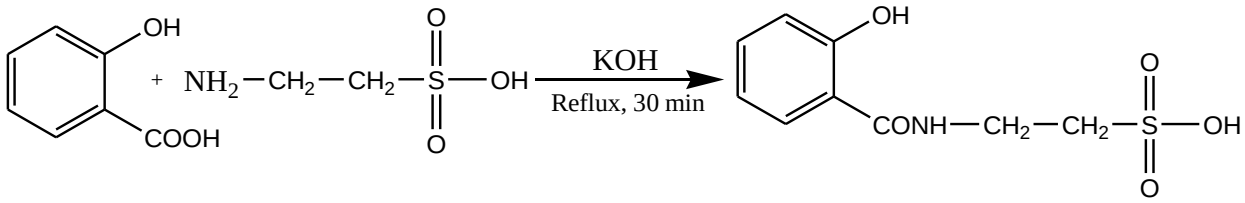


L-tyrosine **L-tyrosine methyl ester hydrochloride (a)**



Salicylic acid(a) **(1-methyl)-2-(2-hydroxyphenyl-carboxamido)-3-(4-hydroxy phenyl)propanoate(b)**

Scheme 1



Salicylic acid Taurine 2-(2-hydroxy-phenyl-carboxamido) ethanesulfonic acid **(c)**

Scheme 2

The purity of the synthesized compounds (a,b,c) was established by TLC using silica gel G as stationary phase and Chloroform:Ethyl acetate:Carbon tetrachloride, (6.5:2.5:1) as mobile phase.

BIOLOGICAL EVALUATION[12] :

Acetic acid induced writhing in mice (Analgesic and anti-inflammatory activity):

Painful reaction in animals may produced by chemicals. Intraperitoneal injections of bradykinin or acetic acid produce a pain reaction which is characterized as a writhing response. Constriction of abdomen, turning of trunk (twist) and extension of hind legs are taken as a reaction to chemically induced pain. Analgesics, both narcotic and non-narcotic type, inhibit writhing response.

Procedure

The Swiss albino mice of either sex weighing 25-30 gm were used for the test. They divided into 6 groups each containing 4 mice. Saline water used for the preparation of suspension of target compounds. Control group was injected by acetic acid 0.1% per kg (i.p.) and Diclofenac Sodium 5 mg/kg was used as a standard. Test group was treated Intraperitoneal with 1 mg/kg of both target compounds. After 30 minutes, the mice were injected with 0.1% per kg acetic acid i.p. The time until occurrence of writhing and death is recorded during a 10 min. Result were expressed as a mean ± S.E.M. Statistical was tested using ANOVA dunnet t-test. The difference was to be statistically significant at *P<0.5, **P<0.1, ***P<0.001.

Hot Plate Method (Analgesic activity):

In this method heat is used as a source of pain. Animals are individually placed on a hot plate maintained at a constant temperature (55^0 C) and the reaction of animals, such as paw licking or jump response is taken as the endpoint. Analgesics increase the reaction time.

Procedure:

The Swiss albino mice of either sex weighing 25-30 gm were used for the test. They were divided into six groups each containing 4 mice. Saline water used for the preparation of

suspension of both target compounds. Control group was injected with saline water (i.p.) and Diclofenac Sodium 5mg/kg, was used as a standard. Test group was treated Intraperitoneal with 1mg/kg of both target compounds. The animal placed on hot plate maintained at a 55^0 C. After 15 sec. animal removed from hot plate because to avoid injury to the paws. The time until occurrence of paw licking and paw jumping response is recorded during a 15 sec. Result were expressed as mean ± S.E. M. Statistical was tested using ANOVA dunnett t-test. The difference was to be statistically significant at *P<0.5, **P<0.01, ***P<0.001.

RESULTS AND DISCUSSION

Physical characterization data (a,b,c) are presented in Table 1 and spectral data (a,b,c) are presented in Table 2.

Table 1: Physical data for Synthesized compounds (a,b,c):

Compound	Molecular Formula	M.W gm/mol	Melting Point	% Yield	R_f Value
a	$C_{10}H_{13}O_3N$. HCI	231.5	255-260^0 C	30.92	0.6
b	$C_{17}H_{17}O_5N$	315	285-290^0 C	33.97%	0.93
c	$C_9H_{11}O5NS$	245	245-250^0 C	59.18%	0.29

Table 2: Spectral data for Synthesized compounds (a,b,c):

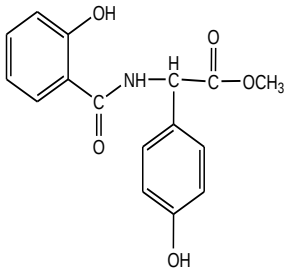
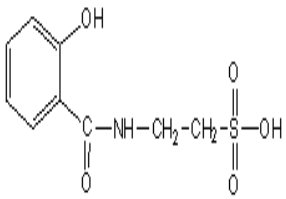
Compound	Structure	IR (KBr) cm^{-1}	^1H NMR(δ ppm) (DMSO-D6)
a		1592 (C=O of amide), 3205.11 (-NH.), 1701 (O-C=O of ester), 2954-2749(CH_2-CH), 3509.81(-OH), 3205.11(N-H).	2.500(s,3H,CH_3), 2.69-3.05(m,3H,CH-CH_2), 9.21(s,1H,NH), 7.02-7.04(m,7H,Ar)
2		3640.95 (-OH), 748-813(CH=CH), 1589(C=O), 2622.72(CH_2-CH_2), 1145(S=O), 3286.11(-NH)	16.59(s,1H,OH), 16.56(s,1H,OH(Ar)), 6.56(s,1H,NH), 7.08-7.66(m,4H,Ar), 2.50-3.36(m,4H,CH_2-CH_2)

Table 3: Data of Acetic acid induced writhing method in mice:

Groups	Treatment	Acetic acid induced	
		No. of writhing	% protection
Control	Acetic acid	32.17 ± 2.30	00.00
Standard	Acetic acid + Diclofenac Sodium	00.00	100
Compound b	Acetic acid + Compound b	14.91 ± 1.89**	70.06
Compound c	Acetic acid + Compound c	9.63 ±2.23	53.65

Table 4: Data of Hot Plate Method in mice:

Treatment	Basal reaction time (Sec.)		Reaction time (Sec) after drug administration		% protection
	Paw licking	Paw jumping	Paw licking	Paw jumping	
Control (WFI)	4.32±0.13	5.72±3.20	-	-	0.00
Standard (Diclofenac Sodium)	3.69±1.80	4.30±1.97	14.38±0.84	15***	100
Compound b	2.99±1.22	6.50±1.09	12.20±1.02*	14.98±0.20	96.30
Compound c	3.72±0.39	8.80±0.92	14.72±1.33**	15***	87.69

The synthesized compounds were confirmed by the spectral and analytical data, After this activity compounds shows the significant action.

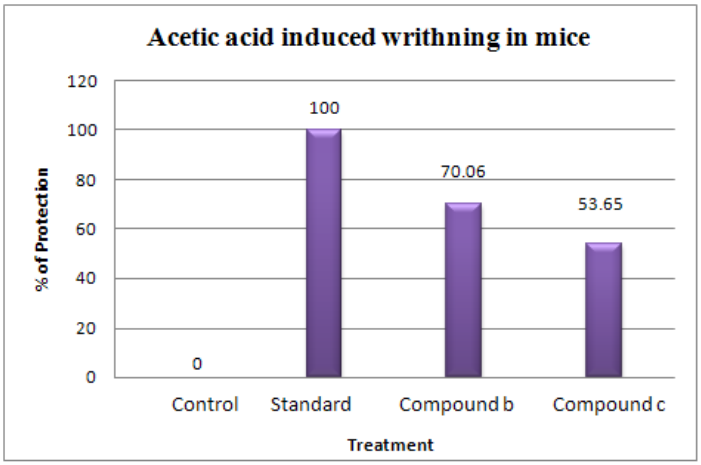


Figure 1: **Acetic acid induced writhing in mice**

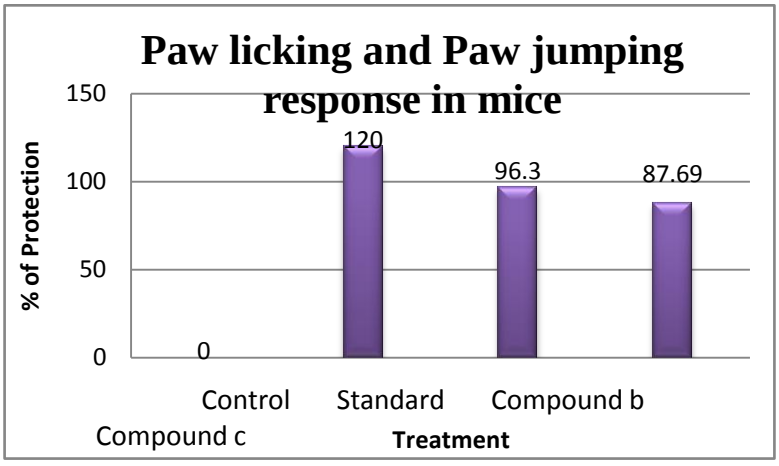


Figure 2: Paw licking and Paw jumping response in mice

CONCLUSION:

The yield of the products ranged from 30-60%. The structures of the newly synthesized compounds (a,b,c) are confirmed by spectral data through, IR, 1H NMR. All the synthesized final compounds were screened for their Analgesic activity.

ACKNOWLEDGEMENT:

Authors gratefully acknowledge the University of Pune for providing instrumental facility for completion of research.

REFERENCES:

1. Colquhoun D. Shelley C Anti-inflammatory Analgesics In Burger's Medicinal chemistry Drug Discovery; Abraham D. J. Eds.; John willey & sons: New York, 2003:281-367.
2. Foreman JC. Mast cells and basophil leucocytes. In textbook of immunopharmacology; Eds,; Blackwell Scientific: Oxford, 1994:21-34.
3. Chandrasoma P. Taylor C. R. The acute Inflammatory Response. In Concise pathology; Eds; McGraw-Hill: New York, 2005:629-632.
4. Rang H.P., Dale M.M., In Pharmacology, Churchill Livingstone, London: 1995; 246-265.
5. Rooseboom M, Commandeur JNM, Vermeulen NPE. Enzyme-Catalyzed Activation of Anticancer Prodrugs, Pharmacol. Rev., 2004, 56:53
6. Mishra A, Veerasamy R, Jain PK, Dixit VK and Agrawal RK: Synthesis, characterization and pharmacological evaluation of amide prodrugs of Flurbiprofen. J. Braz. Chem. Soc. 2008; 19:89-100
7. Ferrero-Miliani L, Nielson OH, Anderson PS, Girardin SE. Inflammation: Importance of NOD 2 and NALP3 in interleukin-1beta generation. Clin. Exp. Immunol. 2007;147(2):227-35.
8. A. Barbeaub in The Brain, The Heart and Taurine. Can. J. Neurol. Sci.1975:2(4):346.
9. M. Ashinara, in Immunohistochemical Study of The Human Olfactory System. Nippon Jibiinkoka Gakkai Kaiho, 1992;985(6): 851-9.
10. G. Chapman, C. Greenwood in Taurine in Nutrition and Brain Development. Nutri. Res. 1988;8:955-68.
11. Dhaneshwar SS, Gairola N, Kandpal M, Vadnerkar G, Bhatt L. Colon-specific, mutual azo prodrug of 5-aminosalicylic acid with L-tryptophan: synthesis, kinetic studies and evaluation of its mitigating effect in trinitrobenzenesulfonic acid-induced colitis in rats. Bioorg. Med. Chem. 2007:15(14): 4903-4909.
11. S. K. Kulkarni, Experimental Pharmacology and Toxicology, Vallabh Publication, Third Edition.28-29.