J. Biol. Chem. Chron. **2019**, **5***(3)*, 131-136
ISSN (Print): 2454 – 7468
ISSN (Online): 2454 - 7476
www.eresearchco/jbcc/

Amino acids Prodrugs of Aceclofenac

Sabale Prafulla, Potey Lata and Chaudhari Aishwarya

Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur-440033 (M.S.), INDIA

* *Correspondence: E-mail: prafullasable@yahoo.com*

(Received 10 Dec, 2018; Accepted 11 Jan, 2019; Published 18 Jan, 2019)

ABSTRACT: (Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly used medications in the world, due to their Analgesic, Anti-inflammatory and Antipyretic properties. However, the use of NSAIDs results in serious upper gastrointestinal (GI) adverse effects. Prodrug designing is an important and productive area of drug research. The prodrug approach able to improve the safety and efficiency of existing NSAIDs. The present research work was attempted to develop synthetic scheme for the novel amide acids prodrugs of NSAIDs such as Aceclofenac and evaluated their capabilities to improve site specificity and reduce gastrointestinal toxicity by maintaining the desired pharmacological properties for better efficacy. All the prodrug derivatives were characterized by UV, IR and ^{1}H NMR Spectroscopy along with solubility and partition coefficient study also has been carried out to discuss the lipophilicity of novel prodrugs. The biological evaluation of all the compounds was carried out by *in-vitro* hydrolysis in simulated gastric fluid (SGF) and hydrolysis in simulated intestinal fluid (SIF), in-vivo Anti-inflammatory, Analgesic activity and Ulcerative index. Prodrugs of Aceclofenac showed better anti-inflammatory and Analgesic activity than the parent drug. The ulcerative index of all the prodrug was lesser which revels the superiority of prodrugs than the parent drugs, concluded that the prodrug approach could successfully attained the goal of minimization of gastrointestinal toxicity by improving the desired Anti-inflammatory and Analgesic activities.

Keywords: NSAIDs; Prodrug; Aceclofenac; Anti-inflammatory; Analgesic; Ulcerative index.

INTRODUCTION: Non-steroidal anti-inflammatory drugs (NSAIDs) are used primarily to treat inflammation, mild to moderate pain and fever. The diverse uses of NSAIDs comprise the treatment of Headache, Arthritis, Gout, Inflammatory Arthropathies, Dysmenorrhoea, Sports injuries, Migraine, Post-operative pain, Tissue injury, Sciatica and Rheumatism (Ashuthosh, 2007). NSAIDs structurally consist of an acidic moiety which is represented by a carboxylic acid group, an enolic group, a hydroxamic acid group and a sulphonamide or tetrazole ring. The centre of acidity is attached to a planar aromatic or hetero aromatic ring of NSAIDs. The anti-inflammatory activity depends on the acidic centre attached to the planar aromatic or hetero aromatic ring. The lipophilicity of NSAIDs is due to the formation of alkyl chain or additional aromatic ring attached to the planar moiety. NSAIDs act by inhibiting the biosynthesis of prostaglandin (PG), which is the basic cause behind fever, pain and inflammatory conditions. The biosynthesis of PG involves the release of arachidonic acid (AA) from damaged cell membranes by the action of phospholipase. AA is metabolized by cyclooxygenases (COX) into prostanoids and by lipoxygenase into leukotrienes respectively. Cyclooxygenase (COX) is divided into two isoforms, COX-1 and COX-2. COX-1 and COX-2 are both increase the concentration of prostaglandins (PGs) in various regions of the body during inflammation, pain and fever. NSAIDs inhibit both COX-1 and COX-2 and thus reduce pain and inflammation. NSAIDs blocks the pain induced by PGs, they block the production of pyrogens and inhibits the synthesis of PGs at the site of inflammation. Thus NSAIDs are used as mild analgesics, antipyretics, and anti-inflammatory respectively. (Asif Husain, 2016)

NSAIDs give the major side effects like GI toxicity and renal toxicity in the body (Graham, 2002). By using prodrug approach these side effects may be overcome. Prodrug designing is a concept of retro-metabolic drug design that considers targeting, metabolism, duration of action, biological action, side effect, physico-chemical properties etc. into the drug design process. Prodrug designing is very important area of medicinal chemistry research. Generally, in a prodrug, an inert or non toxic carrier group or promoiety is

used, whereas the selection of promoiety or carrier group is primarily depends on the objectives to be achieved in prodrug designing. In case of mutual prodrug, the carrier group may be another drug. Mainly the mutual prodrug is approaches for the synergistic effects. Generally, they may improve biological, pharmacokinetics and pharmacodynamic properties with or without less side effects. (Rautio, 2008)

In this paper the main prodrug approach is to development of prodrugs to mask the acidic group of NSAIDs temporarily has been regarded as a promising approach to reduce their GI toxicity.

MATERIALS AND METHODS: Melting point was determined by capillary fusion method and is uncorrected. The infrared spectra were recorded on IR spectrophotometer (Shimadzu 8201 PC) in KBr phase. 1H NMR and spectra were recorded in NMR spectrophotometer (BRUKER AVANCE II 400 MHz) in Panjab University, Chandigarh. The UV spectra were recorded on Shimadzu UV spectrophotometer at a range of 200-800 nm and their λmax was determined.[10] The purity of aceclofenac, and their prodrugs were monitored by thin layer chromatography (TLC) on precoated silica G plates using iodine vapour as detecting agent. (Sherma J., 1996)

Experimental:

Methyl ester of amino acid: Freshly distilled (0.05 M, 6 ml) of thionyl chloride was slowly added to methanol (100 ml) maintained at 0 ^{O}C and amino acid (0.1 M) was added to it. The resulting mixture was refluxed for 6-8 hours at ambient temperature (60^{0}C) with continuous stirring on magnetic stirrer. Excess thionyl chloride and solvent was removed under reduced pressure obtain crude amino acid methyl ester hydrochloride. It was treated with 20 ml portion of cold ether at 0ºC until the excess of dimethyl sulphate was removed. It was recrystallized from hot methanol by slow addition of ether followed by cooling at 0ºC. (Brenner M., 1953)

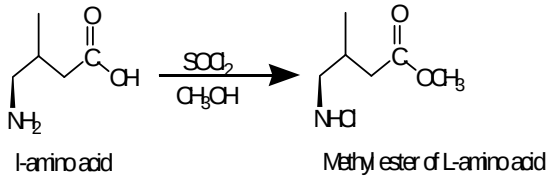

Figure 1: Synthesis of methyl ester hydrochloride of amino acid.

Acid chloride of Aceclofenac: Aceclofenac (0.01 mol, 3.54 g) was refluxed with excess of redistilled thionyl chloride (0.1 mol, 11.8 ml) for 2 hours. Progress of reaction was monitored by checking TLC. After completion of reaction, excess of thionyl chloride was removed under reduced pressure. The crude Aceclofenac acid chloride was used for further condensation with methyl ester of amino acid. (Furniss BS., 1989)

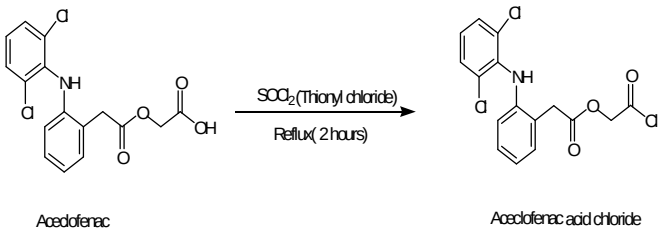

Figure 2: Synthesis of acid chloride of Aceclofenac.

Acylation of amino acid methyl ester with acid chloride of Aceclofenac using Schotton –Baumann Reaction: Methyl ester of amino acid (0.05 M) was dissolved in chilled solution of NaOH (5%, 100 ml) with stirring for about 30 minutes at room temperature. Crude Aceclofenac acyl chloride was added in small portion to reaction mixture with constant stirring at 10 ^{O}C. After complete addition of Aceclofenac acid chloride, reaction mixture was further stirred for 7-8 hours at 8-10 ^{O}C. After completion of reaction, solid product separated out. The solid product was filtered off, dried and recrystallized from methanol. **(**Christian, 2005**)**

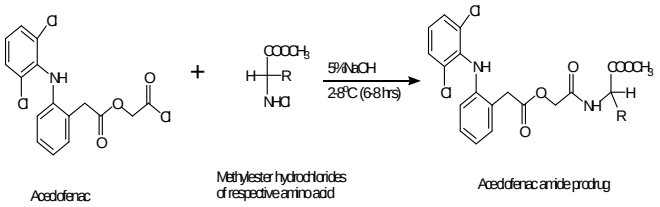

Figure 3: Acylation of methyl ester of amino acid with Aceclofenac acid chloride.

Acylation of Aceclofenac acid chloride with methyl ester of Glycine (AC1): Methyl ester of Glycine (4.45g) was dissolved in chilled solution of NaOH (5%, 100 ml) with stirring for about 30 minutes at room temperature. Crude Aceclofenac acyl chloride was added in small portion to reaction mixture with constant stirring at 10 ^{O}C. After complete addition of Aceclofenac acid chloride, reaction mixture was further stirred for 7-8 hours at 8-10 ºC. After completion of reaction, solid product separated out. The solid product was filtered off, dried and recrystallized from methanol.

Yield (%): 65, M.P. (^{O}C): 196-197, Rf: 0.56 (Toluene: MeOH, 7:3), λ_{max} (nm): 287 (MeOH), IR (KBr, cm^{-1}): 3395, 3050, 1692, 1450, 700

Acylation of Aceclofenac acid chloride with methyl ester of Asparazine (AC2): Methyl ester of Asparazine (8.05g) was dissolved in chilled solution of NaOH (5%, 100 ml) with stirring for about 30 minutes at room temperature. Crude Aceclofenac acyl chloride was added in small portion to reaction mixture with constant stirring at 10 ^{O}C. After complete addition of Aceclofenac acid chloride, reaction mixture was further stirred for 7-8 hours at 8-10 ^{O}C. After completion of reaction, solid product separated out. The solid product was filtered off, dried and recrystallized from methanol.

Yield (%): 59, M.P. (^{O}C): 296-298, Rf: 0.61 (Toluene: MeOH, 7:3), λ_{max} (nm): 304 (MeOH), IR (KBr, cm^{-1}): 3395, 3046, 2842, 1679, 1465,700. NMR (δ,ppm): 6.2 (t,3H;aromatic), 2.5 (d,2H;-CH2), 3.4 (d,2H;-CH), 6.880-6.992 (m,4H;Ar-H), 10.39 (1H,NH), 11.52 (d,2H;NH2).

Acylation of Aceclofenac acid chloride with methyl ester of Cysteine (AC3): Methyl ester of Cystein (6.75g) was dissolved in chilled solution of NaOH (5%, 100 ml) with stirring for about 30 minutes at room temperature. Crude Aceclofenac acyl chloride was added in small portion to reaction mixture with constant stirring at 10 ^{O}C. After complete addition of Aceclofenac acid chloride, reaction mixture was further stirred for 7-8 hours at 8-10 ^{O}C. After completion of reaction, solid product separated out. The solid product was filtered off, dried and recrystallized from methanol.

Yield (%):61**,** M.P. (^{O}C): 270-272**,** R_f: 0.58 (Toluene:MeOH,7:3)**,** λ_{max} (nm): 297 (MeOH)**,** IR (KBr, cm^{-1}): 3395, 3050, 1679, 1475, 700.

Acylation of Aceclofenac acid chloride with methyl ester of Alanine (AC4): Methyl ester of Alanin (5.15g) was dissolved in chilled solution of NaOH (5%, 100 ml) with stirring for about 30 minutes at room temperature. Crude Aceclofenac acyl chloride was added in small portion to reaction mixture with constant stirring at 10 ^{O}C. After complete addition of Aceclofenac acid chloride, reaction mixture was further stirred for 7-8 hours at 8-10 ^{O}C. After completion of reaction, solid product separated out. The solid product was filtered off, dried and recrystallized from methanol.

Yield (%): 68, M.P. (^{O}C): 210-212, Rf: 0.63 (Toluene:MeOH, 7:3), λ_{max} (nm): 302 (MeOH), IR (KBr, cm^{-1}) :3395, 3050, 2842, 1712, 1604, 1473. NMR (δ,ppm):1.2(s,1H;-CH), 1,6 (m,6H;-CH3), 4.2 (d,2H;-CH2), 6.880-6.992 (m,6H;Ar-H), 7.416 (m,4H;Ar-H), 10.2 (s,1H;N-H).

Acylation of Aceclofenac acid chloride with methyl ester of Proline (AC5): Methyl ester of Proline (6.45g) was dissolved in chilled solution of NaOH (5%, 100 ml) with stirring for about 30 minutes at room temperature. Crude Aceclofenac acyl chloride was added in small portion to reaction mixture with constant stirring at 10 ^{O}C. After complete addition of Aceclofenac acid chloride, reaction mixture was further stirred for 7-8 hours at 8-10 ^{O}C. After completion of reaction, solid product separated out. The solid product was filtered off, dried and recrystallized from methanol.

Yield (%): 47, M.P. (^{O}C): 214-216, Rf: 0.56 (Toluene:MeOH, 7:3), λ_{max} (nm): 277 (MeOH), IR (KBr, cm^{-1}): 3450, 3050, 2992, 1691, 1600, 1161.

Acylation of Aceclofenac acid chloride with methyl ester of Leucine (AC6): Methyl ester of Leucine (7.26g) was dissolved in chilled solution of NaOH (5%, 100 ml) with stirring for about 30 minutes at room temperature. Crude Aceclofenac acyl chloride was added in small portion to reaction mixture with constant stirring at 10 ^{O}C. After complete addition of Aceclofenac acid chloride, reaction mixture was further stirred for 7-8 hours at 8-10 ^{O}C. After completion of reaction, solid product separated out. The solid product was filtered off, dried and recrystallized from methanol.

Yield (%): 60, M.P. (^{O}C): 245-247, Rf: 0.59 (Toluene: MeOH, 7:3), λ_{max} (nm): 285 (MeOH), IR (KBr, cm^{-1}): 3495, 3050, 1697, 1608, 1562, 1473.

Acylation of Aceclofenac acid chloride with methyl ester of Tryptophan (AC7): Methyl ester of Tryptophan (1.23g) was dissolved in chilled solution of NaOH (5%, 100 ml) with stirring for about 30 minutes at room temperature. Crude Aceclofenac acyl chloride was added in small portion to reaction mixture with constant stirring at 10 ^{O}C. After complete addition of Aceclofenac acid chloride, reaction mixture was further stirred for 7-8 hours at 8-10 ^{O}C. After completion of reaction, solid product separated out. The solid product was filtered off, dried and recrystallized from methanol.

Yield (%): 53, M.P. (^{O}C): 302-304, Rf: 0.68 (Toluene: MeOH, 7:3), λ_{max} (nm): 283 (MeOH), IR (KBr, cm^{-1}): 3395, 3050, 2850, 1695, 1353.

Acylation of Aceclofenac acid chloride with methyl ester of Serine (AC8): Methyl ester of Serine (5.95g) was dissolved in chilled solution of NaOH (5%, 100 ml) with stirring for about 30 minutes at room temperature. Crude Aceclofenac acyl chloride was added in small portion to reaction mixture with constant

stirring at 10 ^{O}C. After complete addition of Aceclofenac acid chloride, reaction mixture was further stirred for 7-8 hours at 8-10 ^{O}C. After completion of reaction, solid product separated out. The solid product was filtered off, dried and recrystallized from methanol.

Yield (%): 64, M.P. (^{O}C): 224-226, Rf: 0.57 (Toluene: MeOH, 7:3), λ_{max} (nm): 294 (MeOH), IR (KBr, cm^{-1}): 3395, 3050, 2916, 1697, 1600, 1475

Biological Evaluation:

Anti-inflammatory activity: Anti-inflammatory activity was determined by using hind paw oedema method using carrageenan (0.1 ml, 1 % w/v) as phlogistic agent 224. All animal experiments were carried out according to the guidelines of the Committee for the Purpose of Control and supervision on Experiments on Animals. (Reg. No. IAEC/UDPS/2015-31) Wistar albino rats (150-200 g) were divided into six groups, each comprising of six animals, including a control and a standard group. The initial volume of right hind paw of rat was measured by plethysmometer without administration of drug. A 1 % sodium carboxy methyl cellulose (CMC) suspension containing drug (100 mg) was prepared and a volume of this suspension containing an equivalent dose (AC-100 mg/kg) was administered orally to the standard groups. Similarly equivalent quantity of each prodrug was administered to the test groups. After 30 min of administration of the drug and prodrugs, carrageenan solution in normal saline was injected into the planter surface of right hind paw of each animal. The volume of swelling of right hind paw of each rat was measured after 2, 4 and 6 h. The mean increase in the volume of the right hind paw of rats was compared with control and standard. (Winter, 1962; Kellett DN., 1965)

The percent inhibition of paw oedema was calculated as:

$$\text{Percentage inhibition} = (1\text{-}Vt/Vc) \text{ X } 100$$

Where **Vt** - mean relative change in paw oedema volume in test group, **Vc** – mean relative change in paw edema volume in control group.

Analgesic activity: The analgesic activity of synthesized prodrug is investigated by using the hot plate, which is commercially available, consists of electrically heated surface. The temperature is controlled for 55° to 56 °C. This can be a copper plate or a heated glass surface. The animals are placed on the hot plate and the time until either licking or jumping occurs is recorded by a stop-watch.

Swiss albino mice weighing between 20-30 g were used for evaluation of analgesic activity; in each group six albino mice were kept. A solution of Aceclofenac (dose-100mg/kg/10ml) was prepared in normal saline water. Wistar albino mice of either sex were divided into six different groups each containing six animals, the animals were marked individually. Food was withdrawn 12 hours prior to drug administration till completion of experiment. The animals were weighed and numbered appropriately. The animals were placed on hot plate and initial response time was recorded. The cut-off time was set to 15 second. The test and standard drugs were given orally. After 2 hours, the animals were placed on the hot plate and the observations were recorded. (Kazunaga, 1980; Bianchi, 1954)

Ulcerogenic activity: Gastrointestinal toxicity of the drugs and prodrugs was measured and compared with the parent drug by measuring mean ulcer index. Wistar albino rats (100-130 g) were divided into six groups, each comprising six animals, including a control and standard group. The control group was administered orally by 1 % CMC suspension. Test compounds and standard were administered orally (at 10 times higher dose) as a suspension with 1 % CMC daily for 5 days. The rats were fasted after the administration of last dose, thereafter they were sacrificed by decapitation and the stomach was removed, opened and washed with distilled water. The lesions on the gastric mucosa were counted by visual examination using a binocular magnifier. (Coili, 1979; Brodie, 1970)

Ulcer index = {1x(number of lesions of score 1)+2x(number of lesions of score 2)+3x(number of lesions of score 3)}/10

Score 0 = No Ulcer, Score 1 = Spot Ulcer

Score 2 = Deep ulcer, Score 3 = Perforation

RESULTS AND DISCUSSION: The amide prodrugs of aceclofenac were synthesized and outlined in figure 3. All derivatives are found to possess good yield. The anti-inflammatory activity obtained after of administration of standard drug AC was found to decrease while all prodrugs showed an increase in percentage anti-inflammatory activity. Maximum anti-inflammatory activity was observed at 6 h. The results shown in Table 1 revealed that the increase in the anti-inflammatory activity of prodrugs with time is due to their higher bioavailability compared to the parent drug. Evaluation of analgesic activity was performed by Hot Plate method. Reaction time was found more in prodrug as compared to Aceclofenac. The antiulcer

activity in rats and histopathological studies of rat stomach. The gross observation of the stomach revealed obvious wide spread haemorrhagic spots in the parent drug treated animals compared to the prodrug treated animals. Thus, in all the cases, the ulcer index of prodrugs treated animals showed lower value than the drug treated animals. The minimized side effect obtained by the prodrugs might be due to the inhibition of direct contact of carboxylic acid group of the drug to the gastric mucosa, which is mainly responsible for the damage. These findings revealed better gastro protective activity of prodrugs. Following table shows the mean ulcer index of standard drug and synthesized prodrugs.

Table 1: %age inhibition of paw edema.

Compound	% Inhibition		
	2 h	4 h	6 h
Std.	43.3	47.1	42.0
AC1	73.3	74.2	67.0
AC2	53.3	61.4	67.0
AC3	63.3	65.7	72.0
AC4	60.0	71.4	73.0
AC5	50.0	62.8	63.0
AC6	60.0	58.5	68.0
AC7	43.3	58.5	55.0
AC8	56.6	65.7	70.0

Table 2: Reaction time before and after drug administration.

Compound	Reaction time before drug administration (sec)	Reaction time after drug administration (sec)
Control	7.38±010	8.01±0.134
Std.	8.24±0.211	13.43±0.216
AC1	6.83±0.323	15.09±0.265
AC2	8.78±0.254	16.32±0.321
AC3	7.21±0.369	15.83±0.269
AC4	8.90±0.658	17.21±0.365
AC5	8.23±0.356	16.38±0.398
AC6	7.07±0.211	18.50±0.367
AC7	9.01±0.129	16.43±0.327
AC8	8.21±0.385	15.48±0.178

Table 3: Mean ulcer index.

Compound	Mean ulcer index
Std	0.575±0.170
AC1	0.225±0.125
AC2	0.275±0.095
AC3	0.175±0.050
AC4	0.200±0.081
AC5	0.300±0.141
AC6	0.281±0.080
AC7	0.323±0.101
AC8	0.250±0.060

CONCLUSION: The prodrugs were successfully synthesized as per the synthetic protocol. All the physicochemical studies (TLC, Melting point, Partition coefficient and solubility study) revealed that increased lipophilicity of prodrugs as compared to parent drug and thus increases the absorption as well as bioavailability of synthesized prodrugs. All the prodrugs exhibited a better anti-inflammatory and analgesic activity than the parent drug indicating that the prodrugs had better activity than the parent drugs. The ulcer index of prodrugs was lower than the parent drugs revealing better gastro protective activity of prodrugs.

ACKNOWLEDGEMENT: The authors are thankful to Head, Department of Pharmaceutical Sciences, Rastrasant Tukdoji Maharaj Nagpur University, Nagpur for providing necessary facilities and other technical supports during the preparation of this research article

REFERENCES:

1. Ashuthosh Kar. Medicinal chemistry, New Age International Publishers, New Delhi, India, 2007; 522-580.
2. McAdam B. F., Lawson C. F., Mardini I. A. (1999) Systemic biosynthesis of prostacyclin by Cyclooxygenase (COX)-2: the human pharmacology of a selective inhibitor of COX-2, *Proc. Natl. Acad. Sci. USA*, 96, 272–277.

3. Vane J. R. (1996) Introduction: Mechanism of action of NSAIDs, *Br. J. Rheumat.*, 35, (S-1), 1-3.

4. Asif Husain, Aftab Ahmad, and Shah A. Khan, (2016), Synthesis and biological evaluation of a mutual prodrug of norfloxacin and fenbufen, *Journal of Taibah University Medical Sciences*, 1-5.

5. Graham D. Y., Agrawal N. M., Campbell D. R. (2002) Ulcer prevention in long-term users of non-steroidal anti-inflammatory drugs, *Arch. Intern. Med.*, 162,169-175.

6. Rautio J., Kumpulainen H., Heimbach T., Oliyai R., Oh D., Jarvinen T., Savolainen J. (2008) Prodrugs: design and clinical applications, *Nat. Rev.*, 7, 255-270.

7. Brenner M. Huber W. (1953) Synthesis of methyl ester of amino acid, *Helvchim acta*, 36, 1109.

8. Furniss BS, Hannaford A. J., Vogel' textbook of practical organic chemistry, 5th edition, 1989; 5:1261-1262.

9. Christian AGN, Falque V. (2005) Amide bond formation and peptide coupling, *Tetrahedron*, 61, 10827–10852.

10. Sherma J., Fried B., Dekker M. (1996) Handbook of thin-layer chromatography.1st edition, New York, 1-1104.

11. Furniss B. S., Hannaford A. J., Vogel's textbook of practical organic chemistry, 5th edition, 1989, 5, 236-238.

12. Silverstein R.M., Webster F. X. (1998) Spectrophotometric identification of organic compounds, *John Wiley and Sons Inc., New York*, 1-502.

13. Winter C. A., Risely E. A., Nuss G. W. (1962) Carregeenan induced edema in hind paw of the rat as assay for anti-inflammatory drugs, *Proc. Soc. Exp. Biol. Med.*, 111, 544-547.

14. Kellett D. N. (1965) On the anti-inflammatory activity of protamine sulphate and of hexadimethrine bromide, inhibitors of plasma kinin formation, *Br. J. Pharmacol. Chemother.*, 24,705-713.

15. Kazunaga F., Osamu K., Morihide H., Noriyuki M., Seiichi O., Yoshikazu H. A. (1980) method for evaluating analgesic agents in rats, *J. Pharmacol. Methods*, 4, 251-259.

16. Bianchi C. Franceschini J. (1954) Experimental observations on Haffner's method for testing analgesic drugs, *Br. J. Pharmacol. Chemother.*, 9, 280–284.

17. Coili V., Putzolu S., Rossi V., Barcellona P. S., Corradino C. (1979) The role of direct tissue contact in the production of gastrointestinal ulcers by anti-inflammatory drugs in rats, Toxicol. *Appl. Pharmacol.*, 50, 283-289.

18. Brodie D. A., Cook P. G. , Bauer B. J., Dagle G. E., (1970) Indomethacin-induced intestinal lesions in the rat, *Toxicol. Applied Pharmacol.*, 17, 615-624.