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Synthesis and microwave assisted transformation of ethyl-4,5,6,7-tetrahydro benzo (b) thiophene-2-amino-3-carboxylate into potential anti–inflammatory agents–novel mono and diacids

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ABSTRACT

Alkaline hydrolysis of ethyl-4,5,6,7-tetrahydro benzo (b) thiophene-2-amino-3-carboxylate by ethanolic sodium hydroxide resulted into the corresponding 3-carboxylic acid which upon microwave irradiation with succinic and maleic anhydrides yielded amic acids. The anti-inflammatory activity of these compounds was evaluated by rat hind paw edema method on albino rats. An in-vitro COX-1 and COX-2 enzyme inhibition assay was also performed. The results confirmed the synthesized derivatives as potential anti-inflammatory drugs and support the prototype model of the arachidonic acid binding site of cyclooxygenase enzyme proposed by Peter Gund and T. Y. Shen. The novel derivatives have been characterized by ^{1}H NMR, mass, and IR spectroscopic studies.

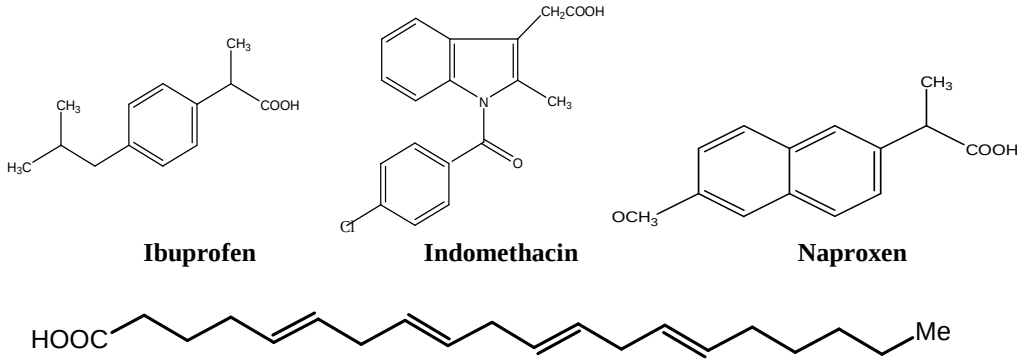
Key words: succinamic acid, maleiamic acid, anti–inflammatory activity.

INTRODUCTION

Non–steroidal anti-inflammatory drugs (NSAIDs) are known to inhibit the arachidonic acid binding site of cyclooxygenase enzyme and suppress the biosynthesis of prostaglandins and other pro-inflammatory agents. This is believed to be their mode of action. Peter Gund and T. Y. Shen[1] have proposed a prototype model of the arachidonic acid binding site of cyclooxygenase enzyme which consists of an anionic binding site, a flat region–presumably the binding area for a flat hydrophobic moiety and regions for the double bonds of the arachidonic acid to bind.

Widely prescribed NSAIDs like ibuprofen, indomethacin, naproxen etc. are characterized by an aromatic group, an acid group or a functional group which gets converted to an acidic group in-vivo and a chain linking the two moieties. A C-C chain or a C-C-C chain links the hydrophobic moiety (aromatic group) with the acidic group. We felt that the chain, if elongated, furthermore mimicks the unsaturated fatty acids in general and arachidonic acid, the precursor for the biosynthesis of pro–inflammatory agents in the body in particular. We anticipated that such a structure would competitively antagonize arachidonic acid at the arachidonic acid binding site of cyclooxygenase enzyme.

It is well known that the acid anhydrides react with amino group to form amic acids. In such a structure, the chain linking the hydrophobic moiety to the carboxylic acid group begins with a nitrogen atom. The electronegative nitrogen atom exerts negative inductive effect on the carboxylic acid group at the other end. In other words, binding of such a molecule to the arachidonic acid binding site would be facilitated. Moreover, such classes of compounds have not been reported as potential anti–inflammatory agents. Hence, we have treated succinic anyhydride and maleic anhydride with 2-amino-4,5,6,7-tetrahydro benzo (b) thiophene-3-carboxylic acid to obtain corresponding novel succinamic and maleiamic acids. The novel acids were tested for anti–inflammatory activity to evaluate the designed model. The starting material ethyl-4,5,6,7-tetrahydro benzo (b) thiophene-2-amino-3-carboxylate was prepared by the application of Gewald[2] reaction.



Ibuprofen **Indomethacin** **Naproxen**

Fig.1. Structures of ibuprofen, indomethacin, naproxen and arachidonic acid

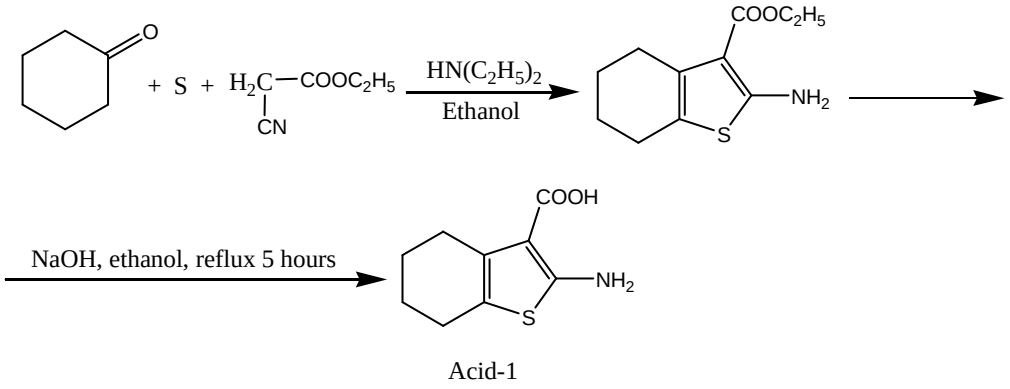
MATERIALS AND METHODS

Chemicals and solvents were of reagent grade and used without further purification. Melting points were determined on a capillary melting point apparatus and are uncorrected. IR spectra were recorded using SHIMADZU FT-IR spectrometer in the range of 400-4000cm^{-1}. ^{1}H NMR spectra were recorded in DMSO at room temperature.

Synthesis of 2-amino-4, 5, 6, 7-tetrahydro-benzo (b) thiophene-3-carboxylic acid

A mixture of previously prepared ethyl-4,5,6,7-tetrahydro benzo (b) thiophene-2-amino-3-carboxylate (0.01mol) (prepared by a three hours stirring of 0.08mol each of cyclohexanone, ethylcyanoacetate, pulverized sulphur and 24mL of absolute ethanol) and ethanolic sodium hydroxide was refluxed for 5 hours. The resulting solid product was mixed with ice cold water followed by adjustment of pH to 7.0 with dilute acetic acid

to get a buff colored crude product. The latter was worked up with ethanol to get a pure amorphous product (Acid-1 in Scheme-1).

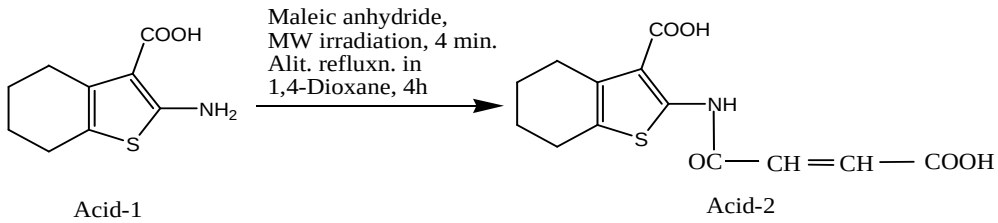


Scheme 1. Synthesis of 2-amino-4,5,6,7-tetrahydro benzo (b) thiophene-3-carboxylic acid

Microwave assisted synthesis of N-[2'-(4,5,6,7-tetrahydro-benzo (b) thiophene-2-amino-3-carboxylic acid)]-maleiamic acid

A mixture of maleic anhydride (0.01 mol) and previously prepared Acid – 1 (0.01 mol) in a 100mL conical flask was subjected to microwave irradiation in a domestic microwave oven for four minutes and then cooled to get crude maleiamic acid (Acid – 2) as a pale yellow product. It was crystallized from ethanol (Acid – 2 in Scheme – 2).

On the other hand, the conventional method required usage of freshly distilled 1,4 – dioxane as solvent and four hours of refluxation.

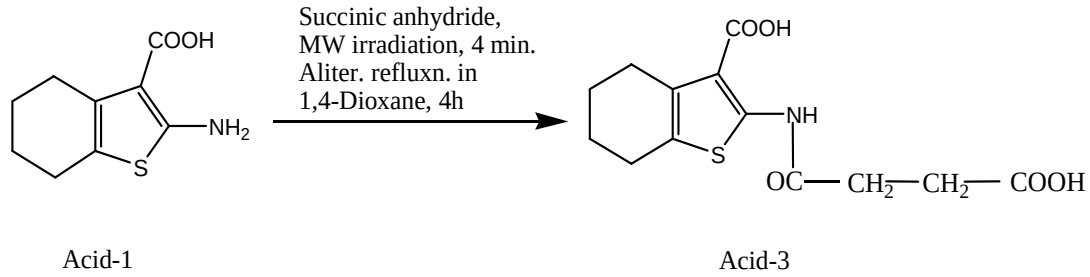


Scheme 2. Synthesis of N-[2'-(4,5,6,7-tetrahydro benzo (b) thiophene-2-amino-3-carboxylic acid)]-maleiamic acid

Synthesis of N-[2'-(4, 5, 6, 7-tetrahydro-benzo (b) thiophene-2-amino-3-carboxylic acid)]-succinamic acid

A mixture of succinic anhydride (0.01 mol) and previously prepared Acid – 1 (0.01 mol) in a 100mL conical flask was subjected to microwave irradiation for four minutes and then cooled to get crude succinamic acid (Acid – 3) as a white product. It was crystallized from ethanol (Acid – 3 of Scheme – 3).

On the other hand, the conventional method required usage of freshly distilled 1,4 – dioxane as solvent and four hours of refluxation.



Acid-1 Acid-3

Scheme 3. Synthesis of N-[2'-(4,5,6,7-tetrahydro benzo (b) thiophene-2-amino-3-carboxylic acid)]-succinamic acid

Pharmacology

The carrageenan induced rat hind–paw oedema method[3-5] illustrated below by taking the novel compound, 3a was employed. Plethysmograph was used to measure the volume of paw edema. The standard was an aqueous suspension of Ibuprofen prepared by using Tween 80 as the suspending agent. Ibuprofen was sourced from Dr. Reddy's Laboratories. A 1%w/v saline solution of carrageenan was employed as inflammation inducer. A 1%w/v saline solution was used to inject the animals marked as control group. Route of administration was oral for test, standard and control. The inflammation inducer was injected under the plantar region of left hind paw of animals.

Method of screening: Albino rats (18 in number) of either sex, previously fasted for 24 hours were divided into 3 groups – 6 each for standard and control and 6 for the test drug, 3a. Different identification marks were given to them. The animals of different group were orally administered with respective drug/chemical/saline. After 30 minutes, carrageenan was injected beneath the plantar region of the left hind paw. The paw volume of both legs of rats of control, test and standard groups were measured by mercury dipping method. This served as zero hour reading. The paw volumes were recorded at 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 6^{th} and 24^{th} hour. It was noted that at 24^{th} hour the paws had come to their original volume.

The percentage inhibition of oedema volume was calculated using the formula,

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

Where, Vt = Mean oedema volume of group treated with test drug/standard
Vc = Mean oedema volume of control group

The anti–inflammatory activity studies were made in one sets. The results and statistical analysis of anti–inflammatory activity of ibuprofen and the compounds tested are shown in Table 1.

The acids formed by succinylating the aromatic amine were found to be much more active than the parent amine. Compound 3 showed maximum activity amongst the screened ones.

Acids 1, 2 and 3 were tested for their ability to inhibit COX-1 and COX-2. Cyclooxygenase activity of ovine COX-1 and COX-2 was assayed using COX inhibitory screening assay kit (Cayman chemicals) by method of Gierse et al. [6] All assays were conducted in duplicate at a concentration of 0.05µm of test compounds (Acid – 1,2,3). Acid – 2 and Acid – 3 inhibit both the COX-1 and COX-2 whereas, Acid – 1 remain a poor inhibitor (Table 5).

RESULTS AND DISCUSSION

The parent compound 2-amino-4,5,6,7-tetrahydro-benzo (b) thiophene-3-carboxylic acid showed only negligible anti–inflammatory activity (7.6%) when compared to the standard drug (ibuprofen, 94.7%). However, its conversion into the corresponding succinamic and maleiamic acids showed significant anti–inflammatory activities (79.8% for succinamic acid whereas 77.7% for maleiamic acid). The acids were found to be more potent anti-inflammatory agents than the parent amines. The proposed model which contains a benzothiophene ring as hydrophobic moiety separated from the carboxylic acid group by N – C – C – C chain is thus justified.

Table 1. Chemical data of 2-amino-4, 5, 6, 7-tetrahydro-benzo (b) thiophene-3-carboxylic acid

Molecular Formula	Melting point	Yield	IR (KBr, cm^{-1})	^1H NMR (DMSO, d6, δ)	Mass
$C_9H_{11}NO_2S$	125°C	47.6%	3230 (NH); 2980 br (COOH); 1690-1680 C=O); 1550 (Ar)	2.60 – 2.85, 8H, Complex (4x CH_2) 11.45, s, 1H (COOH)	m/e 198.5

Table 2. Chemical data of N-[2'-(4, 5, 6, 7-tetrahydro-benzo (b) thiophene-2-amino-3-carboxylic acid)]-maleiamic acid

Molecular Formula	Melting point	Yield	IR (KBr, cm^{-1})	^1H NMR (DMSO, d6, δ)	Mass
$C_{13}H_{13}NO_5S$	65°C	35.6%	3230 (NH); 2980 br (COOH); 1690-1680 C=O); 1550 (Ar)	2.65 – 2.90, 8H, Complex (4x CH_2) 7.30, 1H (NH) 11.40, s, 1H (COOH)	m/e 296.5

Table 3. Chemical data of N-[2'-(4, 5, 6, 7-tetrahydro-benzo (b) thiophene-2-amino-3-carboxylic acid)]-succinamic acid

Molecular Formula	Melting point	Yield	IR (KBr, cm^{-1})	^1H NMR	Mass
$C_{13}H_{15}NO_5S$	169°C	50.6%	3230 (NH); 2980 br (COOH); 1690-1680 C=O); 1550 (Ar)	2.65 – 2.90, 8H, Complex (4x CH_2), 1.80, br, 4H (CH_2 – CH_2), 7.39, 1H (NH), 11.65, s, 1H (COOH) 12.05, s, 1H (COOH)	m/e 298.4

Table 4. Anti-inflammatory activity of Acid-1, Acid-2 and Acid-3

Compound	Mean volume of oedema ± S.E.	Percentage inhibition at 3rd hour	't' value	Level of significance
Acid-1	0.87 ± 0.0791	7.6	6.180	P<0.001
Acid-2	0.21 ± 0.0708	77.7	6.265	P<0.001
Acid-3	0.19 ± 0.0708	79.8	6.197	P<0.001
Ibuprofen (Standard)	0.05 ± 0.0129	94.7	11.291	P<0.001
Control	0.941 ± 0.057	…	…	…

Table 5: In vitro COX – 1 and COX – 2 enzyme inhibition assay data for test compounds Acids 1, 2, 3

Test compounds	**Percentage inhibition (0.05 µm)**	
	COX-1	COX-2
Acid – 1 (AI activity 7.6%)	5.34	7.07
Acid – 2 (AI activity 77.7%)	54.95	51.20
Acid – 3 (AI activity 79.8%)	57.23	45.71

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REFERENCES

[1] P. Gund, T. Y. Shen. *J. Med. Chem.* **(1977)**; 20:1146-1152.
[2] K. Gewald, E. Shinke and H. Bottcher, *Chem. Ber.*, **(1966)** 99, 94 .
[3] Seigler., E. P. and Moyer., H. J., *Animal and Pharmacological Techniques in Drug Evaluation,* **(1967)**, 740–8.
[4] F. R. Dower, Animal Experiments in Pharmacological Analysis, **(1971)** 273–274.
[5] Kulkarni, S. K. Handbook of Experimental Pharmacology, Delhi, **(1993)**, 2nd revised and enlarged edition, 78-80.
[6] J. K. Gierse, C. M. Koboldt, M. C. Walker, K. Siebert, P. C. Isakson, *Biochem. J.* **(1999)**, 339:607–614.