Analgesic and Anti-Inflammatory Activity Studies of Some novel Benzothiazole Schiff's bases on Experimental Rats

Padmavathi P.Prabhu, [1*] C.S.Shastry[2], Padmashree[3], Sushant Pande[1].

[1]Department of Pharmaceutical chemistry
[3]Department of Pharmacology
Srinivas College of Pharmacy, Valachil
Post- Parangepete, Mangalore- 574 143,
Karnataka, India.
[2]Department of Pharmacology, NGSMIPS, Paneer, Deralakatte, Mangalore

Corresponding Author: [*]Tel: +91-824-2274722, Fax: +91-824-2274725
Email: padmapprabhu@gmail.com

Summary

The present study is based on the investigation of analgesic and anti-inflammatory activity studies of some synthesized novel benzothiazole derivatives on albino rats. A series of Schiff's base of several benzothiazole derivatives have been synthesized. Para-nitro benzothiazole carboxylic acid was synthesized by Jacobson synthesis[1]. It was then reduced to para amino benzothiazole carboxylic acid with ammonium chloride and iron metal. The resulting product was then condensed with various aromatic or heterocyclic aldehydes in the presence of concentrated sulphuric acid as a catalyst using ethanol as solvent to yield different Schiff bases. The structure of synthesized compounds was characterized by IR, ^{1}H NMR and Mass spectral data. Purity of the individual compound was confirmed by TLC. In the present study we have used tail flick method using analgesiometer. The ability of a compound to reduce the local edema induced in a rat paw by various irritants is the most widely used method to screen the new anti-inflammatory agents. Compounds like formalin, carragenin, kaolin, yeast and dextran have been used as irritants to produce edema. On the basis of this we have screened all the newly synthesized compounds during the present investigation for their anti inflammatory activity. The synthesis of benzothiazole schiff's bases by the described method resulted in products with good yield. Spectral analysis revealed the successful formation of schiff's bases of benzothiazole derivatives.All the synthesized benzothiazole derivatives have shown analgesic activity. When compared to standard drug (pentazocin) all the compounds were found to be slightly active among which P5d, P5e, P5g, and P5h showed significant analgesic activity. However the activity was less than that of standard drug. All the synthesized benzothiazole derivatives have shown anti inflammatory activity in suppressing carrageenan induced edema in rats. When compared to standard drug (indomethacine), all the compounds were found to be moderately active, among which P5e, P5g, and P5h showed significant anti-inflammatory activity. However the activity was less than that of the standard drug.Analgesic and anti-inflammatory study of the synthesized compounds showed mild to moderate activity.

Keywords: benzothiazole schiff's bases, tail-flick method, analgesiometer Analgesic and anti-inflammatory activity, Seizures, rats.

Introduction

Benzothiazoles are bicyclic ring system with multiple applications. The small and simple benzothiazole nucleus is present in compounds involved in research aimed at evaluating new products that possess biological activities such as analgesic[2], anti-inflammatory[3-5], anti-tumor[6], anti-microbial[7], anti-convulsant[8], antifungal[9] and antitubercular[10]. Present study focuses on the analgesic and anti-inflammatory activities of the newly synthesized benzothiazole Schiff's bases on albino rats.

Materials and Methods

All the reactions were carried out under prescribed laboratory conditions. The products were purified by recrystallisation and melting points were determined by melting point apparatus. All the final compounds were characterized by their IR spectra, mass spectra, and PMR spectra.

SHIMADZU PERKIN-ELMER 8201 PC, IR Spectrophotometer using a thin film supported on KBr pellets recorded FT-IR spectra. PMR spectra were recorded on BRUKER AC 300 (300 MHz), NMR Spectrometer using TMS as internal standard; FAB mass spectra were recorded on JEOL SX 102 (DA-6000 mass Spectrometer) Data system using Argon Xenon (6KV.10MA) as the FAB gas. The purity of the compounds was checked on silica gel coated plates (Merck).

A series of Schiff bases of several benzothiazole derivatives have been synthesized. Para nitro benzothiazole carboxylic acid is synthesized by Jacobson synthesis[1]. It is then reduced to para amino benzothiazole carboxylic acid and then condensed with various aromatic or heterocyclic aldehydes in ethanol in the presence of concentrated sulphuric acid as a catalyst to yield the Schiff bases. The structure of synthesized compounds has been established on the basis of their spectral (IR, ^{1}H NMR and Mass) data. The purity of compounds was confirmed by TLC. The molecules were synthesized as per the procedure and the outline is described in the scheme as given in scheme-1. Physical data of final products is given in table 1.

PROCEDURE:

1. Synthesis of N-(4-Carboxy phenyl)-4- nitro phenyl benzamide: To a solution of Para amino benzoic acid (11.5 g, 62.6 mmol) in dichloro methane (100 ml) was added Para nitro benzoyl chloride (8 ml, 68.9 mmol), toluene (30 ml), and pyridine (40 ml). The mixture was heated at reflux for 5 hrs, after which time it was concentrated, extracted with dichloromethane and rinsed with 1M HCl (200 ml) followed by a saturated aqueous solution of sodium bi carbonate ($NaHCO_3$) (100 ml). The organic layer was then dried (Na_2SO_4) and concentrated to produce benzamide derivative as a purple crystalline solid. (15.3g, 86 %). The compound was recrystallized from dichloromethane and hexane.

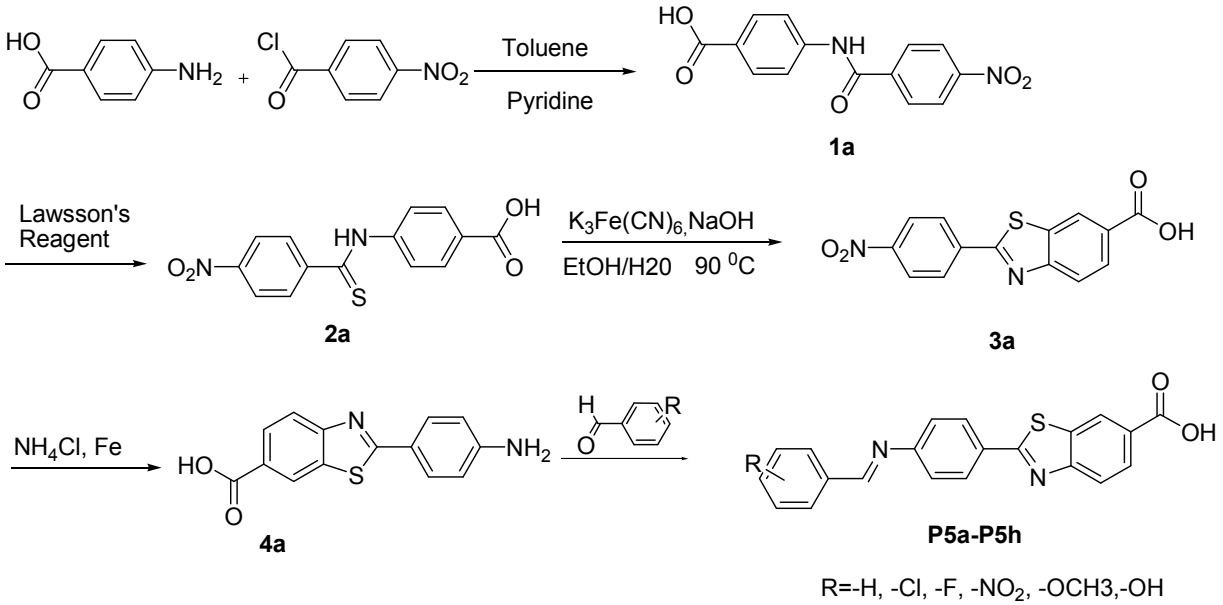
2. Synthesis of N-(4-Carboxy phenyl)-4- nitrophenyl thio benzamide: To a solution of benzamide (1.0 gm) in dry toluene (40 ml) was added lawesson's reagent (0.6 molar eq). The mixture was heated under an atmosphere of nitrogen at reflux for 2 hrs, after which it was concentrated and purified by column chromatography or recrystallized from methanol or ethyl acetate-hexane to give yellow crystals.

3. Synthesis of 6-carboxy-2-(P-nitrophenyl) benzothiazole: To the thiobenzamide (0.1g) in ethanol (0.5 ml) was added 1.5 M NaOH (7 ml). The solution was cooled in an ice water bath and freshly prepared aqueous potassium ferricyanide (2-3 molar equivalents) added. The mixture was stirred at room temperature for one day; the mixture is neutralized with 1M HCl and extracted with ethyl acetate. The organic layer was dried (Na_2SO_4), the solvent removed in vacuum and the residue is purified by column chromatography or recrystallisation from ethanol or ethyl acetate-hexanes to give white needles.

4. Synthesis of 6-carboxy-2-(P-aminophenyl) benzothiazole: 6-carboxy-2-(P-nitrophenyl) benzothiazole (42.2 mmol) in ethanol and water were added iron powder (127mmol) (325 mesh) and ammonium chloride (25.2 mmol). The reaction was stirred at 85^{o}C for one hr, cooled to room temperature, and filtered through celite. The filter cake was then washed with toluene and the filtrate was concentrated to a low volume (50 ml), diluted with toluene and washed with water (2X100 ml). The organic extracts were dried over $MgSO_4$, filtered and concentrated to a solid that was triturated with hexane (25 ml). The solid was filtered.

5. Synthesis of Schiff's bases of benzothiazole: 6-carboxy-2-(P-aminophenyl) benzothiazole (0.025 moles) was dissolved in 20 ml ethanol, followed by dropwise addition of substituted aromatic aldehyde (0.030 moles) dissolved in 10ml ethanol at room temperature. The reaction mixture was stirred for 24 Hr at room temperature. Then, ethanol was evaporated and product was recrystallized from ethyl acetate: hexane mixture.

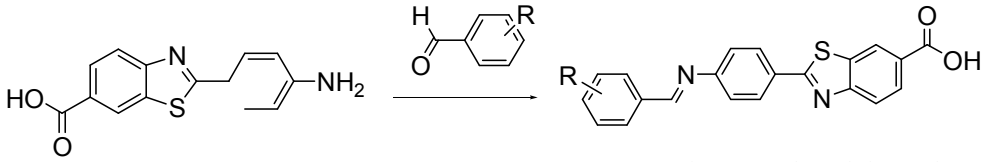
Chemistry: Scheme I



Results and Discussions

IR spectra of all synthesized aryl- benzothiazole derivatives s revealed the important functional groups. ^{1}H NMR spectra of the products indicate the formation of the benzothiazole schiff's bases.The mass spectral data indicated stable molecular ion peak for all the synthesized final products. All the synthesized compounds gave satisfactory elemental data

Table 1: Physical data of compounds prepared.



R=-H, -Cl, -F, -NO_2, -OCH3,-OH

Sr No.	Code	-R	Physical nature	% Yield	M.P.
1	P5a	-H	Light orange crystals	71	230-232
2	P5b	*para*-Cl	Yellow-orange crystals	67	245-258
3	P5c	*meta*-F	Light orange crystals	70	260-263
4	P5d	*para*-NO_2	Orange crystals	68	235-238
5	P5e	*para*-OCH_3	Yellow crystals	65	285-288
6	P5f	4-F, 3-OCH_3	Yellow-white crystals	75	305-307
7	P5g	*para*-CH_3	Yellow-white crystals	74	265-268
8	P5h	*para*-OH	Yellow crystals	78	250-252

BIOLOGICAL ACTIVITY

Analgesic activity:

All the compounds were tested for their analgesic activity using Analgesiometer. Rats of either sex weighing between 150-200g were used for the experiment. The animals were weighed and divided into different groups (control, standard and the test groups) of five rats each. Reaction time is noted at an intervals of 30, 60, 90 min after the administration of drug. Values are expressed as mean±SEM, by one way ANOVA analysis followed by dunnet's-t-test.

Table 2

compound	Tail flick latency in seconds			
	0 min	30 min	60 min	90 min
Control	3.45 ± 0.05	3.5±0.06	3.34± 0.06	3.32± 0.06
Pentazocine	3.4 ± 0.11	6.60±0.25**	7.24± 0.11**	7.57± 0.06**
P5a	3.35 ± 0.05	3.72±0.12 ns	4.11± 0.11 ns	4.22± 0.08 ns
P5b	3.35±0.11	3.62±0.11 ns	4.21± 0.09 ns	4.42± 0.15*
P5c	3.17±0.12	3.27±0.05 ns	3.37± 0.10 ns	3.42± 0.09 ns
P5d	3.25 ± 0.05	3.89±0.06**	5.42± 0.11**	6.21± 0.16**
P5e	3.37 ± 0.13	5.47±0.05**	5.97± 0.11**	6.05± 0.12**
P5f	3.45 ± 0.06	3.52±0.04 ns	3.92± 0.12 ns	4.43± 0.11*
P5g	3.45 ± 0.12	5.40±0.08**	6.30± 0.28**	5.87± 0.08**
P5h	3.52 ± 0.10	3.57±0.12 ns	4.37± 0.08*	4.44± 0.09**

Values are expressed as mean±SEM, *P<0.05, **P<0.01, ***P<0.001 and ns statistically not significant.

All the synthesized benzothiazole derivatives have shown significant analgesic activity except P5a and P5c. When compared to standard drug (pentazocine) all the compounds were found to be slightly active among which P5d,P5e,P5g and P5h compounds showed very significant analgesic activity. However the activity was less than that of standard drug

ANTI-INFLAMMATORY ACTIVITY

All the synthesized compounds were evaluated for their anti-inflammatory activity against carrageenan-induced acute paw oedema rats (Wistar strain) weighing 150-200 g. The animals were weighed and divided into different groups (control, standard and the test groups) of five rats each. The first groups of rats were treated with 1mL of 1% gum acacia suspension orally (control), second group was administered with a dose of 20 mg/Kg of the Indomethacine (standard) and third group was treated with 20 mg/Kg of the suspension of test compounds. After 30 min the animal were injected with 0.1 ml of 1 % carrageenan in normal saline subcutaneously to the sub-planar region of right hind paw. The paw volume was measured immediately (0 h) and after 30 min, 60 min and 120 min by using Plethysmometer. Values are expressed as mean, by one way ANOVA analysis followed by dunnet's-t-test.

Table 3

compounds	Edema volume in ml			Percentage reduction (%)		
	1/2h	1 h	2 h	1/2h	1 h	2h
Control	0.74	0.76	0.77			
Indomethacine	0.24	0.25	0.23	67.56**	67.10**	70.13**
P5a	0.69	0.68	0.70	6.75**	10.53*	9.09ns
P5b	0.68	0.69	0.69	8.10*	9.21 ns	10.39 ns
P5c	0.71	0.70	0.71	4.05 ns	7.99 ns	7.79 ns
P5d	0.42	0.49	0.45	43.24**	35.52**	41.55**
P5e	0.49	0.47	0.48	33.78**	38.15**	37.66**
P5f	0.67	0.64	0.65	9.45*	15.79*	15.58*
P5g	0.36	0.35	0.32	51.35**	53.94**	58.44**
P5h	0.65	0.59	0.44	12.16*	22.37**	42.86**

Values are expressed as mean, *P<0.05, **P<0.01, ***P<0.001 and ns statistically non significant.

All the synthesized benzothiazole derivatives have shown anti inflammatory activity in suppressing carageenan induced edema in rats. When compared to standard drug (indomethacine), all the compounds were found to be moderatively active, among which P5e, P5g and P5h compounds showed very significant anti-inflammatory activity. However the activity was less than that of the standard drug.

Conclusions

Various benzothiazole schiff's bases were synthesized with a view of enhancing the biological activity. The structure of newly synthesized compounds was confirmed by IR, ^{1}H NMR, MASS spectra and elemental analysis. Further evaluation of analgesic and anti-inflammatory activity was carried out. The synthesis of 1, 3-thiazolidin-4-ones by the described method resulted in products with good yield. Analgesic and anti-inflammatory study of the synthesized compounds showed good to moderate activity.

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