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MICROWAVE ASSISTED SYNTHESIS AND EVALUATION OF SOME FLUORO, CHLORO 2-N (SUBSTITUTED SCHIFF'S BASES) AMINO BENZOTHIAZOLES DERIVATIVES FOR THEIR ANTIINFLAMMATORY ACTIVITY.

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ABSTRACT
The present research work is aimed to synthesize a series of various substituted benzothiazole derivatives containing 7-chloro-6-fluoro-N (substituted hydrozones) - benzothiazole.Structures of compounds has been established by means of IR, ^{1}H-NMR and elemental analysis. The compounds $MIII_c$, $MIII_f$ and $MIII_J$ at dose 5 mg/kg and 10mg/kg body.wt were evaluated for anti-inflammatory activity using carragennan induced paw edema method. The selected compounds have shown significant anti-inflammatory activity as compared to the standard drug. Compound $MIII_J$ (10 mg/kg body weight) has shown more significant result when compared with standard drug.

KEYWORDS: Microwave synthesis, Anti-inflammatory activity, Benzothiazole.

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INTRODUCTION

Benzothiazoles play a vital role in the field of medicinal chemistry. Benzothiazole moiety is an important pharmacophore and exhibits outstanding biological activities[1-5]. Day by day Schiff bases are more frequently applied for the treatment of human welfare. Though extensive research work has been reported on benzothiazole with Schiff base, but relatively very little is known so far about substituted benzothiazole with Schiff's base.

Microwave–assisted organic synthesis (MAOS) has been widely employed to enable and expedite the synthesis of diverse heterocycles[6]. Microwave irradiation has been shown not only to reduce reaction times, but also often to provide higher yields of the desired products as compared to traditional heating methods.

The compound 2-aminobenzothiazole is a versatile material for a number of syntheses. 7-chloro-6-fluorobenzothiazole-2-yl amine **(MI)** was synthesized from 3-chloro-4-fluoro phenylamine by reacting with potassium thiocynate and bromine solution in glacial acetic acid according to the literature. The obtained 7-chloro-6-fluoro benzothiazole-2-yl amine was made to react with hydrazine hydrate in the presence of concentrated HCl to give 7-chloro-6-fluoro-benzothiazole-2-yl hydrazine **(MII)**. Different derivatives were synthesized by reacting various substituted aromatic aldehydes, and forming the Schiff's base **MIII (a-j)**.

MATERIAL AND METHODS

Edema was produced by using type IV lambada carrageenan from sigma laboratories. Foot volumes were measured in plethysmometer by mercury displacement[7,8].

The instrument was calibrated before performing the experimental using standard calibrated probe number and standard drug used Diclofenac sodium.

Carrageenan Induced Rat Hind Paw Edema

Anti-inflammatory activity was determined by carrageenan induced rat hind paw method of winter et al. Wistar rats (120-150 g) were used for the experiment. The drugs were prepared as a suspension by triturating with water and 0.5% sodium CMC. The standard group received 40 mg/kg body weight of Diclofenac sodium; test group received 5 mg/kg body weight and 10 mg/kg body weight of synthesized compounds and the control group received 0.5% w/v of sodium CMC. All the drugs were administered orally 60 mins before the carrageenan injection.

The difference between '0' hour reading and one of the subsequent reading provide the actual edema volume at that time. The mean paw edema at different time was calculated and compared with the control. The percentage inhibition of inflammation was calculated by using the formula.

Experimental

Melting points were determined in open capillaries and are uncorrected. The IR spectra were recorded on a Jasco FT\ IR-460 spectrophotometer using KBr disc method. ^{1}H NMR spectra were scanned on a bruker ultraspec 500MHZ/ AMX400MHZ spectrometer using Dimethyl Sulfoxide d_6 as solvent and tetramethylsilane as internal standard. The reactions were carried in domestic microwave oven.

A] Synthesis of 7-chloro-6-fluorobenzothiaozol-2-yl-amine (M1)

To glacial acetic acid (40 ml) precooled to 5°C were added 40 g (0.416 mol) of potassium thiocyanate and 7.25g (.05 mol) of 3-chloro-4-fluoroaniline. The mixture was placed in freezing mixture of ice and salt and mechanically stirred while 6 ml of bromine in 24 ml of glacial acetic acid was added from a dropping funnel at such a rate that the temperature does not rise beyond 0°C. After all the bromine has been added (105min), the solution was stirred for an additional 2 hour at 0°C and at room temperature for 10 hours. It was allowed to stand overnight, during which an orange precipitate settled at the bottom, water (30 ml) was added quickly and slurry was heated at 85°C on a steam bath and filtered hot. The orange residue was placed in

a reaction flask and treated with 10 ml of glacial acetic acid, heated again to 85°C and filtered hot. The combined filtrate was cooled and neutralized with concentrated ammonia solution to pH 6 when a dark yellow precipitate was collected. Recrystallization from ethanol and water mixture, compound (MI) was obtained as colorless powder (85%); m.p. 189-191°C.

B] Synthesis of 7-chloro-6-fluorobenzothiaozol-2-yl-hydrazine. (MII)

Concentrated HCl (10 ml) was added drop wise with stirring to hydrazine hydrate (10 ml) at 5-10°C; to it ethylene glycol (22 ml) and 7-chloro-6-fluoro-benzothiazol-2-ylamine (0.01 mol) were added and charged in a modified microwave for 6 minutes. On cooling solid separated out, this was filtered and washed with water and recrystallized from ethanol and water mixture. By conventional method the time required for the synthesis is 3-4 hrs. Compound (MII) was obtained as colorless crystals (65%); m.p. 218-220°C.

C] General procedure for the microwave assisted synthesis of 7-chloro-6-fluoro-N (substituted hydrozones)- benzothiazole.MIII (a-j)

Substituted benzaldehyde (0.002 Mol) and 7-chloro- 6- Fluoro- benzothiazol- 2- yl hydrazine (0.002 Mol) were added with suitable solvent (DMF, DMSO, Ethanol) and charged in microwave for 5 minutes, on cooling solid separated out, which was filtered and re-crystallized with ethanol to give (MIIIa-j). Analytical data given in the table.

STATISTICS

The readings were calculated by one way ANOVA followed by Dunnet's t test.

RESULT AND DISCUSSION

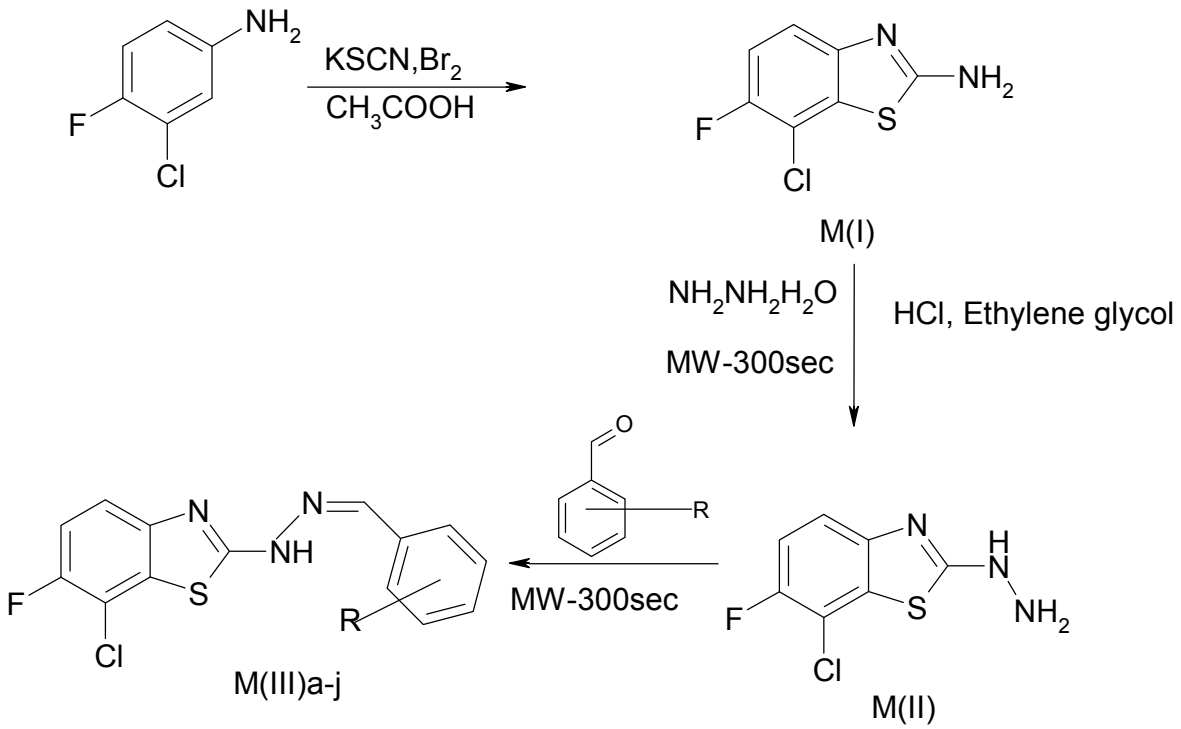
In the present research work a series of various substituted benzothiazole derivatives containing 7-chloro-6-fluoro-N (substituted hydrozones)- benzothiazole were synthesized as mentioned in the scheme and experimental work.

All these compounds were tested for their purity by TLC and melting point. The structures of these compounds were confirmed by IR, NMR and CNH analysis. All these were found to be satisfactory.

The selected synthesized compounds $MIII_c$, $MIII_f$ and $MIII_J$ were evaluated for anti-inflammatory activity by paw edema method using carragennan. The inflammation was measured by plethysmometer and synthesized compounds were given by oral route. There was significant reduction in the inflammation and compound $MIII_J$ (10mg/kg body wt) has shown more significant anti-Inflammatory activity.

With the suitable molecular modification of these compounds can prove as potent anti-inflammatory agents in future.

SCHEME



Codes and corresponding R of different derivatives

CODE	ORTHO	META	PARA
MIII(a)	H	H	H
MIII(b)	OH	H	H
MIII(c)	H	OCH_3	OH
MIII(d)	H	H	OH
MIII(e)	Cl	H	H
MIII(f)	H	NO_2	H
MIII(g)	H	OH	H
MIII(h)	H	H	NO_2
MIII(i)	Cl	H	Cl
MIII(j)	OCH_3	OCH_3	OCH_3

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Table 1: Analytical data of the synthesized compounds

Comp	Mol. Formula	Mol. Wt	M.P° C	Yield%	Time Taken in Microwave	Elementalanalysis Calculated		
						C	H	N
MI	$C_7H_4ClFN_2S$	203	189-191	95	-	41.37	1.97	13.79
MII	$C_7H_5ClFN_3S$	218	218-220	90	6 mins	38.53	2.29	19.26
MIII (a)	$C_{14}H_9ClFN_3S$	306	258-260	60	4 min 50 sec	54.90	2.94	13.72
MIII (b)	$C_{14}H_9ClFN_3OS$	322	190-192	70	5 min 50 sec	52.17	2.79	13.04
MIII(c)	$C_{15}H_{11}ClFN_3O_2S$	352	180-182	60	4 min 10 sec	51.13	3.12	11.93
MIII (d)	$C_{14}H_9ClFN_3OS$	322	240-242	72	4 min 40 sec	52.17	2.79	13.04
MIII (e)	$C_{14}H_8Cl_2FN_3S$	340	250-252	65	5 min 50 sec	49.41	2.35	12.35
MIII (f)	$C_{14}H_8ClFN_4O_2S$	351	219-221	70	4 min 50 sec	47.86	2.27	15.95
MIII (g)	$C_{14}H_9ClFN_3OS$	322	185-187	80	4 min 30 sec	52.17	2.79	13.04
MIII (h)	$C_{14}H_8ClFN_4O_2S$	351	245-247	75	4 min 45 sec	47.86	2.27	15.95
MIII (i)	$C_{15}H_9Cl_3FN_3OS$	404	256-257	80	6 min 35 sec	44.55	2.22	10.39
MIII (j)	$C_{17}H_{15}ClFN_3O_3S$	396	205-207	75	4 min 30 sec	51.51	3.78	10.60

Table 2: Spectral data of synthesized compounds

Compounds	IR Bands (cm^{-1})	Types of Vibration	ppm	Proton nature
MI	3477,3290,3089, 1648,1216,686	Ar-NH_2 Sym, asym, -C-H Ar str C=N str, C-F str, C-Cl str	5.32 7.35 7.45	2H-NH_2 1H, Ar-H 1H, Ar-H
MII	3317,3200,3067, 1202,687	Ar-NH_2 , N-H str, -C-H Ar str, C-F str, C-Cl str	5.05 7.40 9.19	2H-NH_2 2H, Ar-H 1H, -NH
MIII (a)	3200,3084,1488	N-H str, -C-H Ar str, C=N str	7.69-7.38 8.03 11.92	7H, Ar-H 1H –C-H 1H, -NH
MIII (b)	3250,3216, 3072, 1455	N-H str, O-H str -C-H Ar str, C=N str	7.67-8.65 10.10 12.98	6H, Ar-H 1H,-NH 1H-OH
MIII (c)	3366,3068,2850, 1434	N-H str, -C-H Ar str, -O-CH_3 C=N str	3.87 6.75-7.99 8.76 9.60 12.68	3H-CH3 5H, Ar-H 1H-C-H 1H, -NH 1H-OH
MIII (d)	3366,3087,1454	N-H str, -C-H Ar str, C=N str	_	_
MIII (e)	3271,2917,1454	N-H str, -C-H Ar str, C=N str	7.39-8.48 8.98 12.74	6H, Ar-H 1H –C-H 1H, -NH
MIII (f)	3235,2958,1514, 1456	N-H str, -C-H Ar str, -NO_2 C=N str	6.88-7.34 9.01 12.74	6H, Ar-H 1H –C-H 1H, -NH
MIII (g)	3250,3200,3063, 1455	-O-H str, N-H str, -C-H Ar str, C=N str	_	_
MIII (h)	3102,3087,1550, 1456	N-H str, -C-H Ar str, -NO_2 C=N str	_	_
MIII (i)	3300, 2927, 1452	N-H str, -C-H Ar str, C=N str	_	_
MIII (j)	3328, 3088,2835, 1446	N-H str, -C-H Ar str, -O-CH_3 C=N str	_	_

Table 3: Anti- inflammatory activity of selected synthesized compounds

S.NO.	Experimental Groups.	Mean ± SEM (Paw Volume in ml) followed % inhibition.			
		1 hr	2 hr	3 hr	5 hr
01	Control	0.041 ± 0.001	0.042±0.0012	0.043 ± 0.0014	0.045±0.001
02	Standard Diclofenac 40mg/kg	0.034 ± 00007 (17.07 %) [b]	0.027± 0.00/2 (35. 7.1%) [a]	0.021±0.001 (51.16%) [a]	0.019±0.008 (57.77%) [b]
03	Test Drug $MIII_c$ (5mg/kg)	0.036 ± 0.0005 (12.19%) [c]	0.029 ± 0.005 (30.95%) [b]	0.023 ± 0.0005 (46.51%) [a]	0.023±0.0007 (46.51%) [c]
04	Test Drug $MIII_c$ (10mg/kg)	0.033 ±0.0007 (19.51) [b]	0.028± 0.0009 (33.33%) [b]	0.022 ±0.0007 (48.88 %) [a]	0.021±0.0009 (51.1%) [b]
05	Test Drug $MIII_f$ (5mg/kg)	0.035 ±0.007 (14.63 %) [c]	0.030± 0.0005 (28 . 57 %) [b]	0.026 ± 0.0011 (39.53%) [b]	0.024 ± 0.009 (46.66%) [c]
06	Test Drug $MIII_f$ (10mg/kg)	0.034 ± 0.0008 (17.07%) [b]	0.031± 0.0007 (26.19%) [b]	0.022 ± 0.0008 (40.83 %) [a]	0.024±0.0009 (55.56%) [b]
07	Test Drug $MIII_J$ (5mg/kg)	0.034±0.0007 (17.07 %) [b]	0.029 ± 0.001 (30.95%) [a]	0.023 ± 0.0005 (46.51%) [a]	0.020±0.0011 (55.55%) [b]
08	Test Drug $MIII_J$ (10mg/kg)	0.033± 0.0009 (19.51 %) [b]	0.029± 0.00.1 (30.95%) [a]	0.023 ± 0.0005 (46.51%) [a]	0.019±0.0005 (57.77%) [b]

a < 0.0001 (extremely significant), b < 0.01 (very significant), c < 0.05 (significant) Vs Standard.

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