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Journal of Advanced Scientific Research

Available online through http://www.sciensage.info/jasr

ISSN
0976-9595
Research Article

SYNTHESIS AND BIOLOGICAL EVALUATION OF 2-SUBSTITUTED HYDRAZINO-6-FLUORO-1, 3-BENZOTHIAZOLES AS ANTI-INFLAMMATORY AGENTS

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ABSTRACT

Benzothiazole moiety is expanding its pharmaceutical importance and has been studied frequently for the exploration of pharmacological assistance in varied pharmacological circumstances. In the present investigation a series of 2-substituted hydrazino-6-fluoro-1,3-benzothiazole derivatives was synthesized via the synthesis of 2-hydrazino-6-fluorobenzothiazole and subsequent condensation with substituted acetophenones. The synthesized compounds were evaluated for their possible anti-inflammatory activity. The structures of few representative compounds were elucidated by IR and NMR. For the present work efficacy of compounds was determined by carageenan induced paw oedema method which was comparable with that of Indomethacin (10mg/kg/ml). The results of the study demonstrated moderate to good inhibitory impact of the synthesized compounds on induced inflammation.

Keywords: Fluorobenzothiazole, Anti-inflammatory, Acetophenones

1. INTRODUCTION

The derivatives of benzothiazoles have beneficial effects on inflammatory disorders, microbacterial infection, COX-2 mediatory responses and on DNA topoisomerases [1-7]. The contributing physical chemical properties for their therapeutic efficacy need to be established by QSAR studies, which may also provide imminent to the essential structural modifications to this class of compounds. The scrutiny have been guiding for development of benzothiazole nucleus, which results in a lead compound for future development of new drug to be used against variety of ailments.

Benzothiazole derivatives have attracted a great deal of interest due to their biological and commercial importance. They have been found to have antiviral [8], antibacterial [9], antimicrobial [10], and fungicidal activities [11]. They are also useful as antiallergic [12], antidiabetic [13], antitumor [14], anti-inflammatory [15], anthelmintic [16], and anti HIV agents [17]. The present study is aiming at the synthesis of heterocyclic systems containing the substituted benzothiazole moiety that would be expected to have anti-inflammatory activity.

In the present study 6-fluoro-1, 3-benzothiazol-2-Amine was synthesized from 4-fluoro aniline. This has been condensed with hydrazine hydrate to form 2-hydrazino-6-fluorobenzothiazole. Then the derivatives (D1-D4) were synthesized by condensation of 2-hydrazino-6-fluorobenzothiazole with different substituted acetophenones. Chromatographic analysis (TLC) of these compounds, used to check the completion of reaction. The structures of the compounds were stabilized on the basis of their IR and ^{1}H-NMR data. In IR spectra some significant stretching bands due to C=N between a range of 1600-1750 cm^{-1}, N-H at 3400-3100 cm^{-1}, C-F~ 1100 cm^{-1}, C-H Ar~3080 cm^{-1}, C-N~1281 cm^{-1}, thiazole~1441 cm^{-1}were found.

In the ^{1}H-NMR spectra, the signal due to NH proton were observed at 9.0-11.0 ppm as broad peak, while the signals due to aromatic protons were observed at 6.5-7.5ppm.

All the synthesized compounds were screened for their anti-inflammatory activity using the Indomethacin as reference standard.

2. EXPERIMENTAL

All melting points were determined in open capillaries and are uncorrected. The progress of the reaction and the purity of compounds were checked by TLC on percolated silica gel plates using chloroform-ethyl acetate mixture (7:3). Detection of compounds was made by treatment with iodine vapours.

IR spectra of compounds were recorded on FT/IR 4100 type A spectrophotometer and HNMR spectra (DMSO) on Bruker FTAC spectrometer with TMS as internal standard.

2.1. Synthetic Scheme

2.1.1. Synthesis of 6-Fluoro-1, 3-benzothiazol-2-amine (S1)

Glacial acetic acid (20 ml) was cooled to 5°C and added to a mixture of potassium thiocyanate (8 gm) and 4-substituted aniline (0.01 mol). To this, bromine solution was added (1.6 ml bromine in 6 ml glacial acetic acid) drop wise during the

stirring. The solution was stirred for 2 hrs. at 0-10°C, then at room temperature for 10 hrs. and then allowed to stand for overnight at room temperature. To this 10 ml of water was added and the mixture was heated on water bath and filtered while hot. Treated the filtrate with glacial acetic acid 10 ml and heated again on water bath and filtered. Combined and cooled the hot filtrate and neutralized with conc. ammonia to pH 6. The resulting ppt. was dried and crystallized with absolute ethanol.

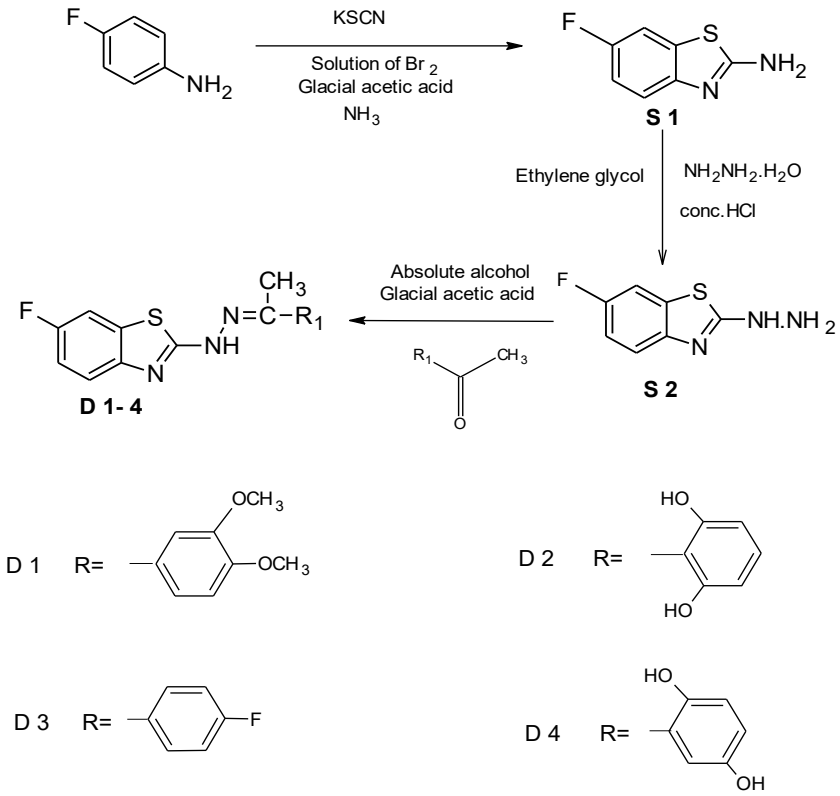

Fig. 1: Synthetic Scheme

2.1.2. Synthesis of 2-hydrazino-6-fluorobenzothiazole (S2)

Conc. HCl (6 ml) was added drop wise with stirring to hydrazine hydrate (6 ml) at 5-10°C. To it ethylene glycol (24 ml) and Benzothiazolamine (0.03 mol) were added and refluxed for 3 hours. Upon cooling separated solid was filtered, washed with water and recrystallized from ethanol.

2.1.3. General procedure for synthesis of derivatives (D1-D4)

2-hydrazino-6-fluorobenzothiazole (1.5mmol) and appropriate substituted acetophenone (2.2 mmol) and glacial acetic acid (2-3 drops) were taken in absolute ethanol (20 ml) and refluxed on water bath for 10-14 hours, till a different spot on TLC may appear. Upon cooling separated solid was filtered, washed with little water and recrystallized from ethanol.

2.1.4. Synthesis of 6-fluoro-1, 3-benzothiazol- 2- Amine (S1)

Compound S1was found as light brown solid. Yield = 90%, mp 205-210°C. IR (KBr, cm^{-1}) υ; 3452 (1°N-H), 3080 & 813 (C-H Ar), 1281 (C-N), 1630 (C=N), 1529 (C=CAr), 1441 (thiazole), 712 (C-F). ^{1}H NMR (400.13 MHz, DMSO) δ; 6.9 (S, 2H, NH), 7.0-7.5 (m, 3H, Ar-H). .Anal. for $C_7H_5N_2SF$, Calcd (%) C 49.99, H 3.00, F 11.30, N 16.66, S 19.06. Found (%) C 50.12, H 3.17, F 11.02, N 16.30, S 19.39.

2.1.5. Synthesis of 2-hydrazino-6-fluorobenzothiazole (S2)

Compound S2 was found as white solid. Yield = 91%, mp 192-198°C. IR (KBr, cm^{-1}) υ; 3200 & 1595(1° & 2° N-H), 3060 & 857 (C-H Ar), 1262 (C-N), 1645 (C=N), 1550 (C=C Ar), 1451 (thiazole), 1046 (C-F). ^{1}H NMR (400.13 MHz, DMSO) δ; 9.0 (S, NH).7.0-7.5(S, Ar- H). Anal. for $C_7H_6N_3SF$, Calcd (%) C 45.89, H 3.30, F 10.37, N 22.94, S 17.50. Found (%) C 45.71, H 3.77, F 10.52, N 23.30, S 16.70.

2.1.6. Synthesis of 2-{(2Z)-2-[1-(3,4-dimethoxyphenyl) ethylidene]hydrazinyl}-6-fluoro-1,3-benzothiazole (D1)

Compound D1was found as white solid. Yield = 94%, mp 210-217°C. IR (KBr, cm^{-1}) υ; 3080 AND 1519 (1° and 2° NH), 2841 & 802 (C-H Ar), 1272 (C-N), 1605 (C=N), 1519 (C=C Ar), 1022 (C-F), (1253) R-OCH_3, (1340) C-CH_3). ^{1}H NMR (400.13 MHz, DMSO) δ; 6.9-7.7 (Ar-H), 2.3-3.3 (CH_3), 3.7 (OCH_3), 11.5 (1S, 1H, NH). Anal. for $C_{17}H_{16}N_3O_2SF$, Calcd (%) C 59.12, H 4.67, N 12.17, O 9.26,

S 9.28, F 5.50. Found (%) C 60.10, H 5.12, N 11.30, O 8.71, S 9.14, F 5.63.

2.1.7. Synthesis of 2-{(1Z)-1-[2-(6-fluoro-1,3-benzothiazol-2-yl)hydrazinylidene]ethyl} benzene-1,3-diol (D2)

Compound was found as brown solid. Yield = 92%, mp 197-210 °C. IR (KBr, cm^{-1}) υ; 3461 & 1566 (1° and 2°NH), 825 (C-H Ar), 1222 (C-N), 1613 (C=N), 1454 (C=C Ar), 1042 (C-F), 1326 (C-OH), 1368 (C-OH), 1253 (C-CH_3). ^{1}H NMR (400.13 MHz, DMSO) δ; 7.0-7.6 (Ar-H), 2.4-3.3 (CH_3), 3.3 (OCH_3), 11.7 (1S,1H,NH). Anal. for $C_{15}H_{12}N_3O_2SF$, Calcd (%) C 56.77, H 3.81, N 13.24, O 10.08, S 10.11, F 5.99. Found (%) C 56.06, H 4.09, N 13.46, O 10.86, S 9.70, F 5.83.

2.1.8. Synthesis of 6-fluoro-2-{(2Z)-2-[1-(4-fluorophenyl) ethylidene]hydrazinyl}-1,3-benzothiazole (D3)

Compound was found as white solid. Yield = 90%, mp 200-210 °C. IR (KBr, cm^{-1}) υ; 3346 & 1509 (1° and 2°NH), 832 (C-H Ar), 1232 (C-N), 1573 (C=N), 1459 (C=C Ar), 1342 (C-CH_3), 1459 (Thiazole), 1071 (C-F), 1157 (C-F). ^{1}H NMR (400.13 MHz, DMSO) δ; 2.3-3.3 (1S, 3H, CH_3), 11.7 (1S, 1H, NH), 7.1-7.8 (Ar-H). Anal. for $C_{15}H_{11}N_3SF_2$, Calcd (%) C 59.39, H 3.66, N 13.85, S 10.57, F 12.53. Found (%) C 59.06, H 3.89, N 13.49, S 9.70, F 13.86.

2.1.9. Synthesis of 2-{(1Z)-1-[2-(6-fluoro-1,3-benzothiazol-2-yl)hydrazinylidene]ethyl}benzene-1,4-diol (D4)

Compound was found as brown solid. Yield = 94%, mp 198-201 °C. IR (KBr, cm^{-1}) υ; 3267 & 1567 (1° and 2°NH), 842 (C-H Ar), 1239 (C-N), 1517 (C=N), 1464 (Thiazole), 1106 (C-F), 1376 (Alkane), 1567 (C=C Ar), 1288 (C-OH), 1348 (C-OH). ^{1}H NMR (400.13 MHz, DMSO) δ; 2.4-3.3 (Ar-OH), 6.8-7.9 (Ar-H), 3.3 (CH_3), 10.1 (1S, 2H, NH), 10.31 (S, 2H, NH). Anal. for $C_{15}H_{12}N_3O_2SF$, Calcd (%)C 56.77, H 3.81, N 13.24, O 10.08, S 10.11, F 5.99. Found (%) C 57.06, H 3.76, N 13.49, O 9.87, S 9.70, F 6.12.

2.2. Biological Activity

Male wistar strain rats, weighing about 200-250 g were used for the study. All animals were kept and maintained under laboratory conditions of temperature (22±2°C), humidity (45±5°C) and 12 hr day: 12 hr night cycle as per CPCSEA guidelines [8]. Animals were allowed free access to food (standard pellet diet) and water *ad libitum*.

2.2.1. Determination of Acute Toxicity

The acute toxicity of synthesized derivatives was determined by using male wistar strain rats (200-250gm) and maintained under standard experimental conditions and divided into three groups of six animals each.

These animals were fasted overnight prior to the experiment and acute oral toxicity (as per OECD guideline no.423) was determined.

Animals were administered with different doses (50 mg/kg, 100 mg /kg and 250 mg/kg, 500 mg/kg and 1000mg/kg) of test compounds and observed individually & critically for a period of 14 days at every pre-determined fragment of time. During this period the mortality and/or the moribund status of the animal were noted.

2.2.2. Carrageenan induced hind paw oedema

The animals were divided into six groups of five animals each and were fasted for a period of 24 h prior to the study. Group 1 was treated as control; Group 2 received indomethacin 10 mg/kg/ml, suspended in 1% sodium carboxymethyl cellulose. Group 3 and 6 were treated with 100 mg/kg of synthesized derivatives suspended in carboxymethyl cellulose. Oedema was induced by injecting 0.1 ml of a 1% solution of carrageenan in saline into the subplantar region of the right hind paw of the rats. The vehicle, derivatives and the standard drugs were administered 60 min. prior to the injection of the phlogestic agent. The volumes of oedema of the injected and the contralateral paws were measured at 30, 60, 120, 180 min after the induction of inflammation using a plethysmometer to calculate the percentage of paw oedema inhibition [10].

The percent inhibition was calculated using the relation, % inhibition = 1-(Vt /Vc)×100, where, vt and vc are the mean relative changes in the volume of paw edema in the test and control, respectively.

2.3. Statistical Analysis

The results were presented as mean ±SEM of the five observations. One way analysis of variance (ANOVA) followed by Dunnett's *t*-test for multiple comparisons were used for statistical evaluation. *p* values ≤0.05 were considered as significant.

3. RESULTS AND DISCUSSION

All the 6-fluoro-1,3-benzothiazole derivatives were obtained in good yield and has the melting points in the range of 192-210 0C. In IR spectra some significant stretching bands due to C=N between a range of 1600- 1750 cm-1, N-H~3400-3100 cm-1, C-F~ 1100 cm-1, CH Ar~3080 cm-1, C-N~1281 cm-1, thiazole~1441 cm-1 were observed. In the 1H-NMR spectra, the signal due to NH proton were observed at 9.0-11.0 ppm as broad peak, while the signals due to aromatic protons were observed at 6.5-7.5ppm. All the newly synthesized compounds showed good anti-inflammatory activity in carageenan induced inflammatory model. Compound D1 and D2 exhibited good activity with maximum inhibition in oedema 67.14% and 67.34% respectively after two hours. All other compounds displayed satisfactory activity comparable to standard drug Indomethacin.

Table 1: Anti-inflammatory activity of substituted benzothiazoles

S. No	Group	Edema volume (ml), % inhibition			
		30 Min	60 min	120 min	180 min
1	Control (1% CMC)	1.14± 0.0293	1.24±0.053	1.47±0.032	1.30±0.032
2	Standard (Indomethacin)	0.67±0.011** (41.22)	0.67±0.052 (45.96)	0.52±0.080** (64.62)	0.44±0.085** (66.15)
3	D1	0.698±0.040** (38.77)	0.43±0.078 (65.32)	0.483±0.060** (67.14)	0.493+0.062** (62.07)
4	D2	0.72±0.06108** (36.84)	0.51±0.07055** (58.87)	0.48±0.08449** (67.34)	0.55±0.07079 (57.69)
5	D3	0.75±0.063** (34.21)	0.57±0.075089** (54.03)	0.50±0.079** (65.98)	0.52±0.072** (60)
6	D4	0.67±0.011** (41.22)	0.58±0.057** (53.22)	0.52±0.0395** (64.62)	0.49±0.077** (62.30)

*Each value expressed as Mean + SEM (n= 6) in the carrageenan paw oedema method using Dunnet's t test followed by one way ANOVA, **P<0.01,*

4. CONCLUSION

Benzothiazole moiety is expanding their pharmaceutical importance and has been studied frequently for the exploration of their pharmacological assistance in varied pharmacological circumstances. The present work establishes a novel synthetic route and substituted analogs of benzoxazole moiety whereby all the synthesized derivatives were found to be in full consignment with the assigned structures. The anti-inflammatory potential of the compounds were encouraging and opens the possibility of existence of some other pharmacological prospective of the said molecules.

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