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Research Article

SYNTHESIS OF SOME DERIVATIVES OF 2-MERCAPTOBENZOTHIAZOLE AND THEIR EVALUATION AS ANTI-INFLAMMATORY AGENTS

PRATIUSH SAXENA*[1], D.C.P. SINGH[1], ABID ALI[1], VIJAY SHARMA[2]

[1]College of Pharmacy, IFTM, Moradabad, U.P., India, [2]SRMS, Dept. of Pharmacy, Bareilly, U.P. India. Email: pratiush.pharma@rediffmail.com,

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ABSTRACT

Benzothiazole is known to be pharmacologically active moiety having a potential as an anti-inflammatory agent. So, the present work has been done in the search of some potentially active derivatives of 2-mercaptobenzothiazole. Two series of derivatives of 2-mercaptobenzothiazole were synthesized having a nitrobenzene and methylene groups as linking moieties between mercaptobenzothiazole and N-substituted amides and cyclohexyl amides. In scheme 1 compounds were synthesized in three steps firstly in dry acetone 2-mercaptobenzothiazole and 4-chloro-2-nitro benzoic acid were refluxed for 4 hr in the presence of anhydrous potassium carbonate and potassium iodide to yield 4-(benzo[d]thiazole-2-ylthio)-2-nitrobenzoic acid which was made to react with thionyl chloride to effort 4-(benzo[d]thiazole-2-ylthio)-2-nitrobenzoyl chloride. Lastly, final derivatives were synthesized by adding different substituted aromatic amines and cyclohexyl amine either in the presence of potassium bicarbonate or toluene. In another scheme various substitued aromatic amines were made to react with chloroacetyl chloride to yield 2-chloro-N-substituted acetamide which were then separately refluxed with 2-mercaptobenzothiazole to yield final compound. The synthesized compounds were purified by recrystallization from appropriate solvent and their structures were elucidated with the help of IR and ^{1}HNMR spectroscopy. The synthesized compounds were screened for anti-inflammatory activity. The results of the anti-inflammatory activity showed variable inhibition with compound 3 (e) being least effective member in the series of newly synthesized compounds. The method for the synthesis of N-substitudes amides and cyclohexyl amide derivatives of 2-mercaptobenzothiazole was established with mild to moderate anti-inflammatory activity.

Keywords: 2-Mercaptobenzothiazole, N-substituted amides, Cyclohexyl amides, Anti-inflammatory.

INTRODUCTION

The derivatives of benzothiazole have been studied extensively and have been reported to exhibit antitumor[1], vasodilator[2], antitubercular[3], antifungal[4], CNS[5], anti-inflammatory[6] and antidiabetic[7]. 2-mercaptobenzothiazole has also been found to be effective against acute and chronic cholangites, cholecystites, gallstone and lambliosis of bile tract and gallbladder [8]. In the present work we are reporting the synthesis of some derivatives of 2-mercaptobenzothiazole under two schemes to obtain pharmacological more potent derivatives.

Chemistry

All recorded melting points were determined on a laboratory melting point apparatus by open capillary tubes and are uncorrected. The IR spectra (KBr) were recorded on Shimadzu 8201PC FT IR spectrophotometer and ^{1}H NMR were scanned on MODEL AV-300 BROKE JEOL at 300MHz spectrophotometer (ppm) using TMS as an internal standard. Purity of the compound was checked by TLC using silica gel G. All compounds showed satisfactory analytical results.

MATERIAL AND METHODS

Scheme 1:

Synthesis of 4-(benzo[d]thiazole-2-ylthio)-2-nitrobenzoic acid (2a)

A mixture of equimolar quantity of 2-mercaptobenzothiozoleic (0.05 M) and 4-chloro-2-nitro benzoic acid (0.05 M) in dry acetone was refluxed on water bath in the presence of anhydrous potassium carbonate and catalytic amount of potassium iodide for 4 hours. The mixture was filtered, acetone was removed by distillation and hot water was added gradually to dissolve the product. The solution was acidified with dil. HCl to precipitate the product; the crude product was filtered, washed and purified by recrystallization from acetone. Yield (83%), m.p. 179^{o}C.

Synthesis of 4-(benzo[d]thiazole-2-ylthio)-2-nitrobenzoyl chloride (2 b)

The compound 2 a (0.01 M) was placed in the reflux assembly with guard tube on water bath in a fuming hood. Thionyl chloride (0.02 M) was added slowly by dropping funnel and refluxed for one and half hour. The excess of thionly chloride was removed by distillation under reduced pressure. The product was cooled to afford the brown colored crystals. Yield (72 %), m.p. 96^o C.

Synthesis of 4-(benzo[d]thiazole-2-ylthio)-2-nitro-N-substituted benzamines. (2 c-g)

The synthesis of amides was carried out by the two general methods:

Method 1: In a 100ml conical flask solution of 0.02M $NaHCO_3$ in 2ml of water was prepared by gentle heating. In another conical ethanol (15 ml) was taken and an equimolar quantity of the aromatic substituted amines were added in it and mixed to form a solution. The solutions of aromatic amines were transferred to 100 ml conical flask containg $NaHCO_3$solution. This flask was cooled in ice bath and in the fuming hood the synthesized 4-(benzo[d]thiazole-2-ylthio)-2-nitrobenzoyl chloride was added while the flask remains in ice-bath with swirriling. The solution in the conical flask was checked and was made alkaline if required by adding more of sodium bicarbonate. If required the reaction mixture was heated gently on water bath. The reaction mixture was left overnight and observed for the precipitate formation[8]. The precipitate formed was filtered, washed with water several times and dried. The products were purified by recrystallization from appropriate solvents. Derivatives 2c and 2f were synthesized by this method.

Method 2: In a 100ml conical flask 0.0013M of substituted aromatic amines were taken in 1.3ml of toluene. The mixture was heated to 40^{o}C to form a solution. 0.0013M of sodium bicarbonate and 4-5 drops of triethylamine were added. The slurry thus formed was cooled to 25^{o}C and 0.0013M of 4-(benzo[d]thiazole-2-ylthio)-2-nitrobenzoyl chloride was added very slowly to keep reaction temperature at 25^{o}C. After addition it was warmed to 45^{o}C and was left overnight. The solution was cooled to 8°C, the solid obtained was filtered, washed well with petroleum ether dried and again washed with water to remove any alkalinity, dried and recrystallized from appropriate solvent[9]. Derivatives 2d, 2e and 2g were synthesized by this method.

The physical constants of the synthesized compounds areshown in table 1.

Scheme 2:

Synthesis of 2-chloro-N-substituted acetamide (1 a-e; General Method)

Method 1: In a clean and dry conical flask (0.01 M) of substituted aromatic amine was dissolved in sufficient amount of glacial acetic acid to form a clear solution. The amount of the glacial acetic acid consumed was recorded. 0.15 M of chloro acetyl chloride was added drop wise in fuming hood with vigorous shaking. The mixture was warmed on water bath for 15 min. with swirling, the mixture was then removed and sufficient amount of anhydrous sodium acetate solution in water was added to get the precipitate. The mixture was cooled in ice bath for few minutes, filtered, washed with water to completely remove the odour of glacial acetic acid. The product was recrystallized from methanol, dried. Intermediates of compounds 3b and 3e were synthesized by this method.

Method 2: In a conical flask containing 10% NaOH solution, (0.01 M) of substituted aromatic amine was added with continuous shaking. The conical flask was cooled on ice bath in fuming hood and (0.015 M) of chloro acetyl chloride was added drop wise by dropping funnel. The addition of chloro acetyl chloride was stopped till the fumes from the reaction mixture ceased completely. After complete addition of chloro acetyl chloride the reaction mixture was allowed to cool for some time[9], the products which separated were filtered, washed with water, dried, recrystallized from methanol or ethanol. Intermediates of compounds 3a, 3c and 3d were synthesized by this method.

Synthesis of 2-(benzo[d]thiazol-2-ylthio)-N-substituted acetamide (3 a-e)

The equimolar quantities of synthesized 2-chloro-N-substituted acetamide (0.01M) and 2-mercaptobenzothialzole (0.01M) were refluxed in the presence of potassium carbonate and potassium iodide in complete anhydrous conditions in dry acetone. The TLC was taken to confirm the completion of the reaction. The acetone was removed from the reaction mixture by simple distillation and the residue thus obtained was washed several times with water and dried. The dried product was then recrystallized with methanol or ethanol.

The reaction involved in the synthesis of new derivatives of 2-mercaptobenzothiazole is shown in Fig. 1.

The physical constants of the synthesized compounds are shown in table 2.

Spectral Data

1. 4-(benzo[d]thiazol-2-ylthio)-2-nitro-N-o-tolylbenzamide (2c)

IR. cm^{-1} (KBr): 3367 (N-H), 2575 (C-S), 1680 (C=0), 1444 ($-CH_3$)

^1H NMR ($CDCl_3$): 8.01 (s,1H, NH), 6.89-8.29 (m, 11H, Ar-H), 2.37 (s, 3H, CH_3),

2. 4-(benzo[d]thiazol-2-ylthio)-N-(4-chlorophenyl)-2-nitrobenzamide (2d)

IR cm^{-1} (KBr): 3356 (N-H), 1680 (C=O), 620 (C-Cl),

^1H NMR ($CDCl_3$), 8.01 (s, 1H, N-H), 6.36-8.33 (m, 11H, ArH)

3. 4-(benzo[d]thiazol-2-ylthio)-N-(2,3-dimethylphenyl)-2-nitrobenzamide (2e)

IR cm^{-1} (KBr): 2940 (C-H str), 725 (m-subst.), 1444 ($-CH_3$),

^1H NMR ($CDCl_3$): 8.01 (s, 1H, NH), δ 6.692-8.324 (m, 10H, C-H, aromatic), δ 2.347 (s, 6H, C-H, CH_3)

4. 4-(benzo[d]thiazol-2-ylthio)-2-nitro-N-p-tolylbenzamide (2f)

IR cm^{-1} (KBr): 3367 (N-H str), 2940 (C-H str), 1680 (C=O str), 1444 ($-CH_3$),

^1H NMR ($CDCl_3$): δ 8.017 (s, 1H, N-H), δ 7.066-8.298 (m, 11H, C-H, aromatic), δ 2.371 (s, 3H, C-H, methyl group)

5. 4-(benzo[d]thiazol-2-ylthio)-N-cyclohexyl-2-nitrobenzamide (2g)

IR cm^{-1} (KBr): 3367 (NH), 1688 (C=O), 2853 (C-H str in cyclohexane)

^{1} H NMR ($CDCl_3$), 7.53-8.29 (m, 10H, Ar-H), 3.57 (t, 1H, C-H), 1.34-1.834 (m, 10H, CH_2 cyclohexane)

6. 2-(benzo[d]thiazol-2-ylthio)-N-o-tolylacetamide (3a)

IR cm^{-1}(KBr): 3308 (N-H), 2592 (C-S), 1453 ($-CH_3$), 758 (o- Ar subst),

^1H NMR ($CDCl_3$): 8.01 (s, 1H, N-H), 7.03-8.25 (s, 8H, ArH). 4.31 (s, 2H, CH_2), 2.37 (s, 3H, CH_3)

7. 2-(benzo[d]thiazol-2-ylthio)-N-(4-chlorophenyl)acetamide (3b)

IR cm^{-1} (KBr): 3352 (N-H), 2576 (C-S), 2840 ($-CH_2-$), 1662 (C=O), 607 (C-Cl),

^1H NMR ($CDCl_3$): 8.00 (N-H), 7.22-8.24 (m, 8H, Ar-H), 4.21 (s, 2H, CH_2).

8. 2-(benzo[d]thiazol-2-ylthio)-N-(2,3-dimethylphenyl) acetamide (3c)

IR cm^{-1} (KBr): 3060 (Ar-H str), 2921 (C-H str), 2628 (C-S), 1655 (C=O str)

^1H NMR ($CDCl_3$): δ 8.002 (s, 1H, N-H), δ 6.692-8.246 (m,7H, Ar-H), δ 4.231 (m, 2H, CH_2),δ 2.347 (s, 6H, C-H, methyl group),

9. 2-(benzo[d]thiazol-2-ylthio)-N-p-tolyl acetamide (3d)

IR cm^{-1} (KBr): 3344 (N-H str), 3055 (Ar-H str), 2905 (C-H str (CH_3), 2860 ($-CH_2-$), 1654 (C=O str), 830(p-subst.)

^1H NMR ($CDCl_3$): δ 8.002 (s, 1H, N-H), δ 6.692-8.246 (m, 7H, - Ar-H), δ 4.231 (s, 2H, CH_2) δ 2.347 (s, 3H, C-H, methyl group)

10. 2-(benzo[d]thiazol-2-ylthio)-N-cyclohexylacetamide (3e)

IR cm^{-1} (KBr): 3352 (N-H), 3000 (C-H cyclohexane), 2740 ($-CH_2-$), 2575 (C-S), 1662 (C=O)

^1H NMR ($CDCl_3$): 8.00 (s, 1H, N-H), 7.47-8.23 (, m, 4H,Ar-H), 4.23 (, s, 2H,CH_2), 3.57 (t,1H, C-H cyclohexane), 1.34- 1.80 (m,10H, C-H cyclohexane)

Pharmacological activity

All the newly synthesized compounds were evaluated for their anti-inflammatory activity and compared with the reference drug Indomethacin.

Anti-inflammatory activity

The activity was performed by carrageenan induced rat paw edema method[11].The albino rats (200-250 gm) were divided into groups (one for control, one for standard drug and ten groups for test drugs) having six rats in each group. The rats were marked on their right hind paw just beyond tibio-tarsal junction, so that while taking reading every time the paw is dipped in the column up to the fixed mark to ensure constant paw volume. The initial paw volumes of all the rats of each group were taken.

The percent inhibition was calculated by following Newbould formula[12]

$$\% \text{ Inhibition} = (1\text{-}Vt / Vc)100$$

Where, Vt and Vc are the mean change in paw volume of treated and control rats respectively.

Statistical Analysis

The results are expressed as mean ± SEM. Statistical analysis of the results in the test group was carried out by comparison with the results in the control group, by one-way ANOVA (Jandel Sigmastat version 2.0). The statistical level of significance was fixed at $p<0.05$.

The results of anti-inflammatory activity are shown in Table 3 and Fig 2.

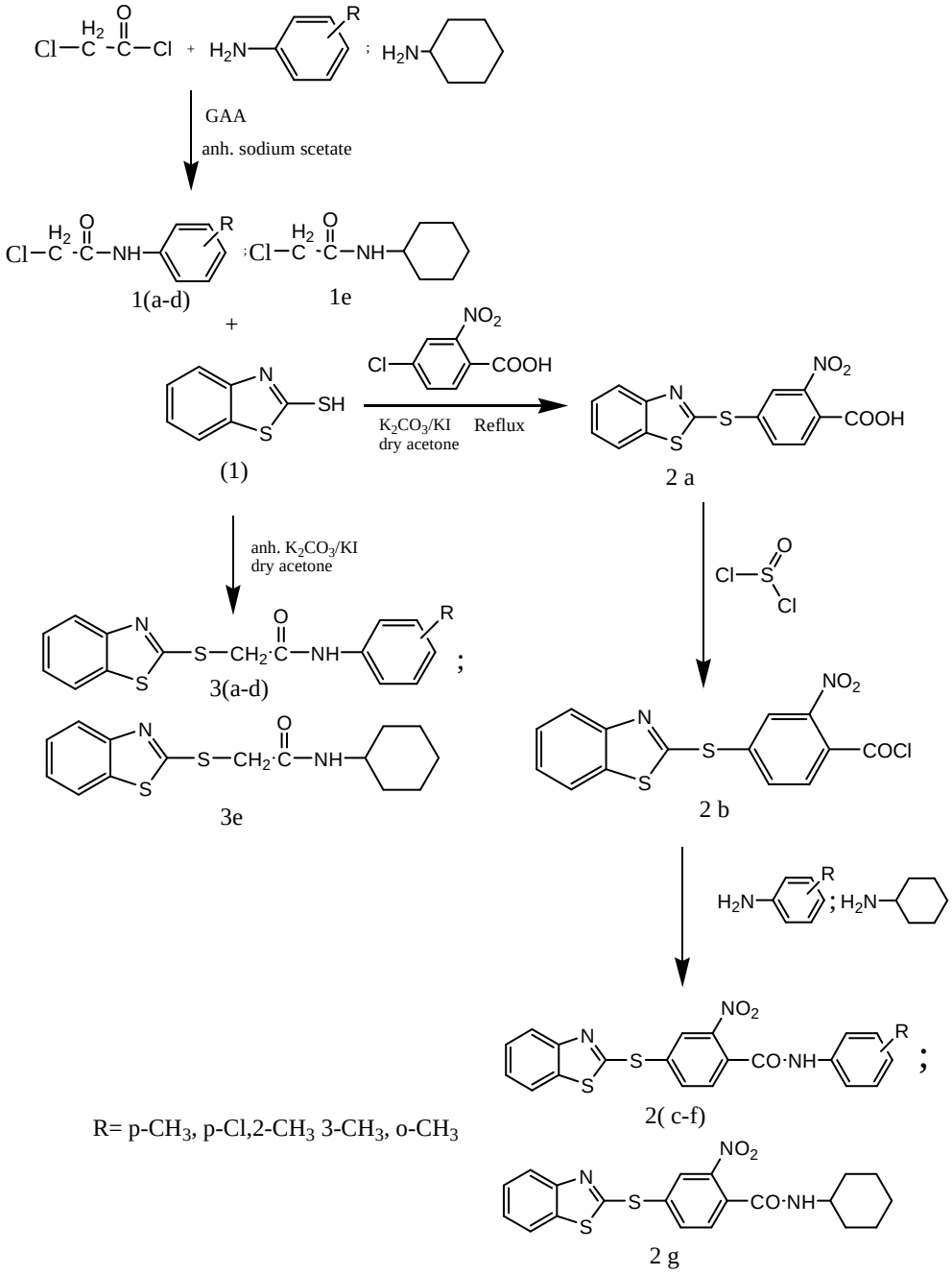


Fig. 1: Synthesis of the different derivatives of 2-mercaptobenzothiazole

Table 1: Physical Constants of derivatives (2c-g)

Compound	Mol. Wt.	M.P. (°C)	Yield (%)	Mob. Ph.	R_f
2c	403	120-121	22%	Hex: Et Ac	0.62
2d	423.5	158-159	19%	Hex: Et Ac	0.75
2e	417	188-200	27%	Hex: Et Ac	0.59
2f	403	106-107	32%	Hex: Et Ac	0.65
2g	394	88	28%	Hex: Et Ac	0.72

Table 2: Physical Constants of derivatives (3 a-e)

Compound	Mol. Wt.	M.P. (°C)	Yield (%)	Mob. Ph.	R_f
3a	314	145-147°C	51%	Hex.: Et. Ac.	0.76
3b	334.5	152-155°C	64%	Hex.: Et. Ac.	0.70
3c	328	149-152°C	54%	Hex.: Et. Ac.	0.41
3d	314	130-132°C	71%	Hex.: Et.Ac.	0.65
3e	305	116-117°C	46%	Hex.: Et.Ac.	0.81

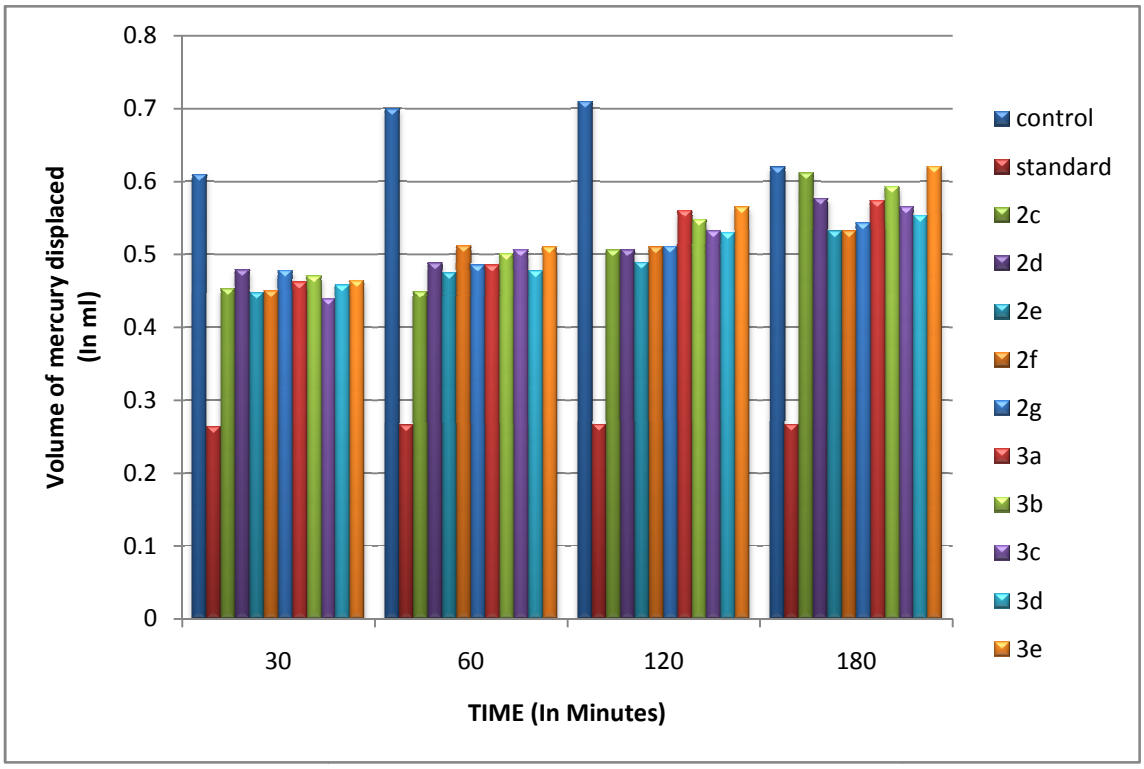
Table 3: Results of anti-inflammatory activity of derivatives of 2-Mercaptobenzothaiazole (2 c-g; 3 a-e)

Treatment	Dose mg/Kg	Mean changes in paw edema (ml) (Mean ± SEM) (% Inhibition)			
		30min	1hr	2hr	3hr
Group-I, Control(2% TW-80 solution)	-	0.61±0.032	0.70±0.033	0.71±0.028	0.62±0.037
Group-II, Standard (Indomethacin)	10 mg/kg	0.264±0.012 (56.79)	0.266±0.016 (61.89)	0.267±0.017 (63.52)	0.267±0.016 (57.00)
Group-III **2c**	100 mg/kg	0.453±0.016 (25.73)	0.449±0.018 (35.86)	0.506±0.031 (28.73)	0.612±0.010 (1.29)
Group-VI **2d**	100mg/kg	0.479±0.025 (21.47)	0.488±0.024 (30.28)	0.506±0.024 (28.73)	0.577±0.017 (6.94)
Group-V **2e**	100 mg/kg	0.448±0.010 (26.56)	0.475±0.016 (32.14)	0.489±0.016 (31.02)	0.532±0.024 (14.19)
Group-VI **2f**	100 mg/kg	0.450±0.025 (26.23)	0.512±0.032 (26.86)	0.510±0.028 (28.06)	0.532±0.0243 (14.19)
Group-VII **2g**	100 mg/kg	0.478±0.021 (21.64)	0.485±0.029 (30.71)	0.511±0.028 (21.02)	0.543±0.032 (12.42)
Group-VIII **3a**	100 mg/kg	0.462±0.027 (24.26)	0.485±0,024 (30.71)	0.56±0.024 (21.02)	0.574±0.029 (7.41)
Group-IX **3b**	100 mg/kg	0.470±0.021 (22.95)	0.501±0.026 (28.43)	0.548±0.028 (22.17)	0.593±0.025 (4.35)
Group-X **3c**	100 mg/kg	0.439±0.020 (28.03)	0.506±0.024 (27.17)	0.532±0.017 (24.96)	0.565±0.015 (8.87)
Group-XI **3d**	100 mg/kg	0.458±0.025 (24.91)	0.477±0.026 (31.86)	0.530±0.028 (25.25)	0.553±0.021 (10.81)
Group-XII **3e**	100 mg/kg	0.464±0.030 (23.93)	0.510±0.028 (27.14)	0.565±0.023 (20.13)	0.621±0.028 (0.16)

Each value represents the Mean ± SEM (n = 6).

($P < 0.05$) control vs. treated group

Figures in parenthesis indicate % of inhibition



RESULTS AND DISCUSSION

The reaction was initiated by refluxing a mixture of 2-mercaptobenzothiazole**(1)** and 4-chloro 2- nitro benzoic acid in dry acetone, with anhydrous potassium carbonate and catalytic amount of potassium iodide to yield 4-(benzo[d]thiazole-2-ylthio)-2-nitrobenzoic acid**(2a).** The compound (2a) was treated with thionyl chloride to synthesize 4-(benzo[d]thiazole-2-ylthio)-2-nitrobenzoyl chloride **(2b),** which was finally reacted with substituted aromatic amines and cyclohexyl amine to afford the synthesis of respective amides (2c-f and 2g).

In another scheme 2-chloro-N-substituted acetamides 1(a-d) and 1e were refluxed separately with 2-mercaptobenothiazole(1) in a

complete anhydrous condition in dry acetone in the presence of anhydrous potassium carbonate and potassium iodide to afford the synthesis of 2-(benzo[d]thiazol-2-ylthio)-N-substituted acetamides (3a-d) and 3e. The completion of reaction was monitored by TLC .The compounds were purified by recrystallization from appropriate solvent. The structure of the compounds was elucidated with the help of IR and ^{1}HNMR spectroscopy.

The appearance of absorption bands at 3367 cm^{-1} and 1680 cm^{-1} due to N-H str and CONH respectively, confirmed the structure. The ^{1}H NMR spectra showed a broad singlet in the range of δ 8-9 confirmed the presence of –NH and multiplets in the range of δ 7-9 confirmed for the aromatic protons in the structure. In compound 4 (a-d) infrared spectrums showed band at 1444 cm^{-1} which indicates the presence of methyl group while a band at 2575 cm^{-1} indicates the presence of thio linkage in the compound. The ^{1}H NMR spectra showed a singlet at δ 2.37 for three protons of methyl group.

In compound 1(c) infrared spectrum showed vibrations at 1453 cm^{-1} which indicates the presence of methyl group while a vibration at 2592 cm^{-1} indicates the presence of thio linkage in the compound. The ^{1}H NMR spectra showed a singlet at δ 2.37 for three protons of methyl group.

Synthesized compound were screened for anti-inflammatory activity by carrageenan induced rat paw edema model. The paw edema was employed as a model of acute inflammation. The compound was tested on a group of 6 albino rats of either sex weighing 200-250 gm. Each test compound was dosed orally (at 100 mg/kg) one hour prior to the induction of inflammation in the right hind paw of the animal by carrageenan injection. The anti-inflammatory activity was then recoded from 0.5-3h and the data is presented in the Table 3. A comparative study of the ten compounds synthesized under two schemes with the standard drug Indomethacin at different time intervals reveals the following information: the compounds of both the series showed mild to moderate anti-inflammatory activity at 0.5 h. Compound 2(c) has shown the maximum activity among all after 1h (35.86% inhibition) but the activity soon diminishes after 3 h of carrageenan induction for all the compounds with a max. of 14.19 % inhibition by compound 2(e) and 2(f). Among compounds of second scheme compound 3(d) has shown maximum activity (10.81) after 3h of carrageenan induction.

CONCLUSION

The series of derivatives of 2-mercaptobenzothiazole were systhesized and evaluated for anti-inflammatory activity. At 0.5 h. compounds of both the series showed mild to moderate anti-inflammatory activity, after one hour compound 2 (c) showed maximum activity among the other members of series. The activity of all derivatives decrease considerably after 3 hrs with maximum activity of 14.19% inhibition by compounds 2 (e) and 2 (f). In second scheme compounds showed inferior activity than scheme first compound with a maximum inhibition of 10.18% by compound 3 (d).

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