International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3 Suppl 3, 2011

Research Article

SCREENING OF *IN-VITRO* ANTI-INFLAMMATORY ACTIVITY OF SOME NEWLY SYNTHESIZED FLUORINATED BENZOTHIAZOLO IMIDAZOLE COMPOUNDS

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Received: 24 March 2011, Revised and Accepted: 22 April 2011

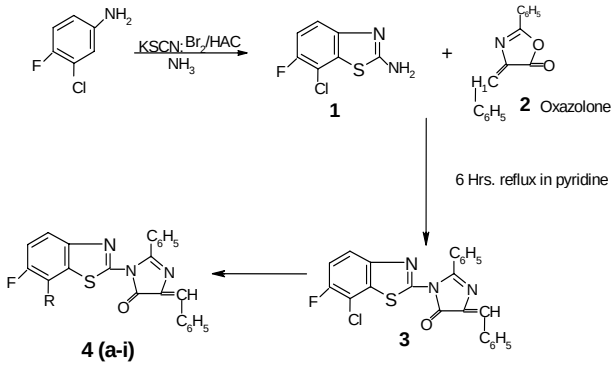
ABSTRACT

4-Fluoro-3-chloroanilline treated with Potassium thiocyanate in presence of Glacial acetic acid and bromine was converted into 2-amino-6-fluoro-7-chlorobenzothiazole, resulting into 2-amino benzothiazole. The synthesized compound in presence of 2-phenyl-4-benzylidine-5-oxazolinone refluxed in pyridine to obtained 2-(2-Phenyl-4-benzylidenyl-5-oxo-imidazolin-1-yl amino)-6-fluoro-7-substituted(1,3)benzothiazoles. The above said compound was treated with ortho, meta and para nitroanillines, ortho, meta, para chloroanillines, morpholino, Piperazine, diphenylamine in the presence of DMF to obtain different derivatives. Some compounds showed promising anti-microbial activity.

Keywords: Flourine, Benzothiazole, Oxazalinone, Imidazoline, Anti-Inflammatory

INTRODUCTION

Fluorobenzothiazoles and Imidazoles exhibit the broad range of antibacterial[1], antifungal[2], anthelmintic[3], anti-inflammatory[4] and antitubercular[5] activity. In the recent years, the chemistry of oxazolones[6] has received much attention due to their use as intermediates for synthesis of some heterocyclic systems. In the present study we made an attempt to link[7-8] fluorobenzothiazoles with imidazoles for generating various derivatives, screened for anti-inflammatory activity. Benzylidine derivatives were found to possess MAO Inhibitory activity, therefore in the present work we have treated oxazolones benzothiazole ring to get potent anti-inflammatory[9, 10,11] compounds.



MAERIALS AND METHODS

Purity of compounds was checked by TLC. Melting points were determined by open capillaries method and uncorrected. IR spectra (NaCl) are recorded on FTIR (Schimadzu-8300) spectrophotometer using nujol mull technique. ^{1}HNMR spectra are recorded on a spectrophotometer (Bruker AMX) at 500MHz, using TMS as internal reference.

General procedure

2-amino-6-fluoro-7-chloro-(1,3)benzothiazole (1)

To the glacial acetic acid (20ml) which is cooled below room temperature, 8gm (0.08mol) of potassium thiocyanate and 1.45g (0.01mol) of fluorochloroaniline was added. The mixture was placed in freezing mixture of ice and salt, mechanically stirred while 1.6ml of bromine in 6ml of glacial acetic acid was added, from a dropping funnel at such a rate that the temperature never rose beyond room temperature. After all the bromine was added (105min), the solution was stirred for 2 hours below room temperature and at room temperature for 10 hours, it was then allowed to stand over night, during which period an orange precipitate settle at the bottom, water (6ml) was added quickly and slurry was heated at 85^{0}c on a steam bath and filtered hot. The orange residue was placed in a reaction flask and treated with 10ml of glacial acetic acid heated again to 85^{0}c and filtered hot. The combined filtrate was cooled and neutralized with concentrated ammonia solution to p^H 6. A dark yellow precipitate was collected. Recrystallised from benzene, ethanol of (1:1) after treatment with animal charcoal gave yellow plates of 2-amino-6-fluoro-7-chloro-(1,3) benzothiazole. After drying in an oven at 80^{0}c, the dry material (1gm 51.02%) melted at 210-212^{0}c. UV 307.4, 269nm, IR 1542cm^{-1}(aromatic C=C) and 3475cm^{-1} (NH_2); 1456 cm^{-1}(thiazole), 1215 cm^{-1}(aromatic-F), 712 cm^{-1}(aromatic-Cl). ^{1}HNMR protons of aromatic NH_2 appear as a hump at 4.6 delta. Aromatic protons appeared as a cluster at7.2 to 7.9 delta. Mass spectra M$^+$ion peak- M/Z peak -202, 204, (M$^+$HCN) – M/Z-175,177, (M$^+$HCN-Cl)-M/Z-140, [(M$^+$HCN)-C HCN]-M/Z-148,150

2-Phenyl- 4-benzylidine-5-oxazol-5-one (oxazolone) (2)

Redistilled benzaldehyde was treated with benzoyl glycine (Hippuric acid) in presence of acetic anhydride (dry acetic acid) and anhydrous sodium acetate to get 4-benzylidene-2-phenyl-oxazol-5-one(oxazolone).Upon washing with ice cold alcohol and then with boiling water (Yield 80%),melted at 165-166^{0}C, IR (NaCl) 1790 cm^{-1}(Lactone carbonyl) and another bond at 1650 cm^{-1}(C=N stretching).

2[2'- Phenyl -4'- benzidinyl- 5'- oxo- imidazoline- 1yl- amino] -6 fluoro- 7- chloro (1,3) benzothiazoles (3)

A mixture of 0.01 mol. of 2-amino-6-fluoro-7-chloro-(1,3)benzothiazole (1) with 2-Phenyl- 4-benzylidine-5-oxazol-5-one (oxazolone)(2) refluxed in pyridine for 6-8 hours. Excess of pyridine was distilled off and resulting mass was poured on to crushed ice and neutralized with dil HCl, filtered and product was recrystallised from ethanol. The dry material melted at 110-112^{0}c (72%). IR (NaCl) 3452 cm^{-1}(-NH stretching), 121 cm^{-1}(C-F), 677 cm^{-1}(C-Cl stretching),3091 cm^{-1}(C=C stretching),1601 cm^{-1}(C=O stretching).

Preparation of various derivatives (4a-i)

2[2'- Phenyl -4'- benzidinyl- 5'- oxo- imidazoline- 1yl- amino] -6 fluoro- 7- chloro (1,3) benzothiazole (3) was treated with various aromatic amines. Refluxed for 2 hrs. in presence of DMF (dimethyl formamide) yields various 2[2'- Phenyl -4'- benzidinyl- 5'- oxo-imidazoline- 1yl- amino] -6 fluoro- 7- chloro (1,3) benzothiazole derivatives(4a-i).The solid separated was cooled and poured on to crushed ice. The solid separated was filtered off, dried and recrystallised from benzene and super dry alcohol (1;1). Newly prepared derivatives showed ^{1}HNMR protons (Ha) 2 gives doublet at 8.06 delta have J = 6.6Hz, protons (H) 13 appeared as multiplet at 7.0 to 7.7 delta, Poton (Hb) 2 appeared as doublet at 6.61 delta, J= 6.6Hz, Secondary amine proton -NH appeared at 4.5 delta.

Table 1: Analytical data of the synthesized compounds (4a-i)

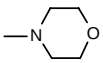
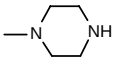
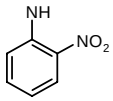
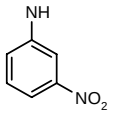
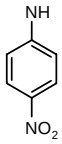
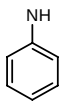
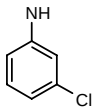
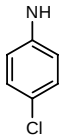
Comp No	R	M.P. ºC	Yield %	M. F.	Elemental Analysis %			
						C	H	N
4a		117	80	$C_{27}H_{17}N_4O_2SF$	Found Calc.	68.00 67.50	3.56 3.54	12.00 11.66
4b		128	79	$C_{27}H_{18}N_4OSF$	Found Calc.	70.01 69.67	3.90 3.87	12.13 12.04
4c	-N-$(C_6H_5)_2$	117	74	C35H23N4OSF	Found Calc.	74.90 74.20	4.15 4.06	10.09 9.89
4d		116	66	C29H18N5O3SF	Found Calc.	65.09 65.04	3.56 3.36	13.89 13.08
4e		126	68	C29H18N5O3SF	Found Calc.	66.00 65.04	3.89 3.36	14.02 13.08
4f		122	70	C29H18N5O3SF	Found Calc.	66.09 65.04	3.45 3.36	12.80 13.08
4g		111	72	$C_{29}H_{19}N_4OSF$	Found Calc.	71.09 71.02	3.90 3.87	12.56 11.42
4h		85	77	$C_{29}H_{18}N_4OSFCl$	Found Calc.	67.77 66.28	3.89 3.42	10.89 10.66
4i		115	70	$C_{29}H_{18}N_4OSFCl$	Found Calc.	67.05 66.28	3.67 3.42	11.09 10.66

Table 2: IR spectral data of the synthesized compounds (4a-i)

Comp. code	NH cm^{-1}	Imidazoline ring carbonyl cm^{-1}	C=N stretching cm^{-1}	C=C stretching cm^{-1}	NO_2 cm^{-1}	C-F cm^{-1}	C-Cl cm^{-1}
4a	3353	1640	1612	1485	---	1167	---
4b	3200	1640	1673	1460	---	1161	---
4c	3200	1630	1600	1490	---	1163	---
4d	3300	1640	1600	1490	802	1167	---
4e	3350	1639	1613	1487	799	1163	---
4f	3400	1630	1640	1400	890	1167	---
4g	3351	1641	1610	1460	---	1164	---
4h	3350	1643	1600	1462	---	1169	714
4i	3349	1637	1653	1493	---	1160	714

In-vitro anti-inflammatory activity:[9,10,11]

The synthesized compounds were screened for anti-inflammatory activity using inhibition of albumin denaturation technique which was studied according to Mizushima and Kobayashi with slight modification. The standard drug and test compounds were dissolved in minimum quantity of dimethyl formamide (DMF) and diluted with phosphate buffer (0.2 M, pH 7.4).

Final concentration of DMF in all solution was less than 2.5%. Test solution (1ml) containing different concentrations of drug was mixed with 1 ml of 1mM albumin solution in phosphate buffer and incubated at 27° + 1° C in BOD incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 60° + 1° C in water bath for 10 min.

After cooling, the turbidity was measured at 660 nm (UV-Visible Spectrophotometer SL-159, Elico India Ltd.). Percentage of inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average is taken. The Ibuprofen was used as standard drug.

The percentage inhibition of denaturation was calculated by using following formula.

% of Inhibition = 100 X { Vt / Vc – 1 }
Where, Vt = Mean absorbance of test sample.
Vc = Mean absorbance of control

Table 3: Screening of *in-vitro* anti-inflammatory activity

Compounds	Absorbance value (Mean + SE)	Inhibition of denaturation (in %)
Control	0.098 + 0.009	----
4a	0.159 + 0.004	62.92
4b	0.118+ 0.004	20.40
4c	0.147+ 0.003.	50.00
4d	0.138 + 0.002	40.80
4e	0.170 + 0.002	73.80
4f	0.164 + 0.002	68.02
4g	0.121 +0.001	23.46
4h	0.176+ 0.004	79.93
4i	0.170 + 0.003	73.80
IBUPROFEN	0.190 +0.002	93.87

CONCLUSION

All the newly synthesized fluorinated benzothiazole imidazole compounds have given appreciable yield with satisfactory elemental analysis. It is inferred from the Table 3 that synthesized compounds (4a-i), have shown significant anti-inflammatory activity. However, animal study and other studies are necessary for its activity and also there is a need to elucidate its mechanism/s of anti– inflammatory action.

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