SYNTHESIS AND CHARECTERIZATION OF 2-BENZENE SULPHONAMIDO-N-(2'-BENZOTHIAZOLYL 6'-FLUORO-7'-SUBSTITUTED) BENZAMIDE AND 2-(2-PHENYL-4-BENZYLIDENYL-5-OXO-IMIDAZOLIN-1-YL) N-(2'-AMINO (1', 3') BENZOTHIAZOLYL 6'-FLUORO 7'-SUBSTITUTED) BENZAMIDE

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ABSTRACT

Various substituted 2-benzene sulphonamido-N-(2'-benzothiazolyl 6'-fluoro-7'-substituted) benzamide or 2-(2-phenyl-4-benzylidenyl-5-oxo-imidazolin-1-yl) N-(2'-amino(1',3') benzothiazolyl 6'-fluoro 7'- substituted) benzamide have been synthesized by condensing the compound 1 with anthranilic acids in dry pyridine (Scheme-I) or with oxazolone refluxed in pyridine (Scheme-II). The identities of compounds were confirmed on the basis of their spectral UV-Visible, IR, ^{1}HNMR and Mass spectral data. Further, they have been screened for their antibacterial, antifungal, anti-inflammatory (in vivo & in vitro) and anticonvulsant activities.

Keywords: Imidazole, Anthranilic acid, Pyridine

INTRODUCTION

The reduction products, named as are other rings of five atoms, are imidazoline and imidazolidine and their derivatives structure given below, Imidazole[1-8] exhibit diverse biological properties. Hence synthesis of new imidazolinones is of considerable interest.

- Planar, five membered heteroaromatic molecule having two nitrogen.
- Named first as gluoxaline (first synthesis with glyoxal and ammonia).
- Amphoteric nature, susceptible to electrophilic and nucleophilic attack.
- High stability to thermal, acid, base, oxidation and reduction conditions.
- Extensive intramolecular hydrogen bonding.

In the recent years the chemistry of sulphonamides imidazolinones has received much attention due to their use as intermediates for the synthesis of some heterocyclic systems. Some biological properties like antibacterial, antifungal, anticancer,anti-hiv, treatment of hypoxic tumour cells, analgesic, anti-inflammatory, CNS depressants, Antiviral, Antitubercular and Antihistamine.

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EXPERIMENTAL

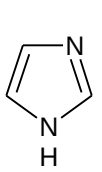
Synthesis of 2-amino-N-(2'-benzothiazolyl 6'-fluoro-7'-chloro) benzamides:

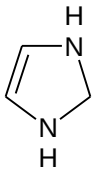
Anthranilic acid (4.0 g, 0.029 mol) and 2-amino-benzothiazole (5.22 g, 0.026 mol), were dissolved in dry pyridine (20 ml, 0.25 mol). The solution was refluxed for 8 hr. The solution was cooled and pured in water. The separated mass was filtered, washed with water and dried. The product was recrystallized using ethanol

Preparation of p-acetamido benzene sulphonyl chloride:

A 500 ml two necked flask was equipped with a dropping funnel and a reflux condenser, attached the top of the later to calcium guard tube for the absorption of hydrogen chloride. 20g (0.148 mol) of dry acetanilide was placed in the flask and 50 ml (90g, 0.77 mol) of a good grade of chlorosulphonic acid in the dropping funnel. A calcium guard tube was inserted to the later. Chlorosulphonic acid was added in small portions and the contents of flask were shaken from time to time to ensure thorough mixing.

When the addition has been made the reaction mixture was heated on a water bath for 1 hr in order to complete the reaction. It was allowed to cool and the oily mixture was poured with stirring into 300g of crushed ice contained in a 1 litre beaker. This operation was carefully carried out in the fume cupboard since the excess of chloro-sulphonic acid


Imidazole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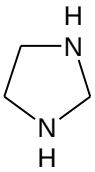
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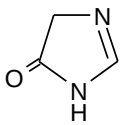
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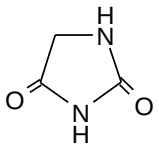

Imidazolidinedione

reacts vigorously with the water. The flask was rinsed with a little ice water and rinsing was added to the contents of the beaker. The mixture was stirred for several minutes, the solid lump material was broken to obtain even suspension of the granular white solid. The obtained solid *p*-acetamido benzene sulphonyl chloride was filtered at the pump and washed with cold water. It was pressed and drained well, kept for drying.

Synthesis of 6-fluoro-7-chloro-2-(p-acetamido benzene sulphonamido) (1,3)- benzothiazole:

2-amino-6-fluoro-7-chloro (1,3) benzothiazole (0.013 mol) pyridine (4 ml) and acetic anhydride (20 ml), has been added with *p*-acetamido benzene sulphonyl chloride (0.01 mol), the mixture were refluxed in water bath for 2 hrs. then reaction mixture poured into 20 ml of ice cold water. The solid obtained was filtered and recrystallized from dil ethanol (80%) to get titled compound 6-fluoro-7-chloro-2-(*p*-acetamido benzene sulphonamido) (1,3)- benzothiazole.

Synthesis of 2-benzene sulphonamido-N-(2'-benzothiazolyl 6'-fluoro-7'-substituted) benzamide:

The 0.0075 mol (2.7 gm) of 6-fluoro-7-chloro-2-(*p*-acetamido benzene sulphonamido) (1,3)-benzothiazole was treated with 0.008 mol of various substituted aromatic amines, PABA, morpholine, piperazine, diphenylamine o-toluidine, N-methyl piperazine and refluxed for 2 hrs in presence of DMF (dimethyl formamide) then the mixture was cooled and poured into crushed ice.The solid separated was filtered, dried and recrystallized from super dry alcohol.

Synthesis of 2-hydrazino-6-fluoro-7-chloro-(1,3)-benzothiazole:

Hydrazinehydrate (0.2 mol) added dropwise with constant stirring to Concentrated hydrochloric acid (HCl) 10 ml by placed in ice cold water (5-10°C) followed by ethylene glycol (40 ml).

To the above solution, 2-amino-6-fluoro-7-chloro benzothiazole (0.01 mol) was added in portions and resultant mixture was refluxed for 6-7 hrs and cooled and poured onto crushed ice. The solid separated was filtered and recrystallized from ethanol (Yield 76%).

Synthesis of 2-Amino N-(2'-amino(1,3) benzothiazolyl 6'-fluoro 7'-chloro) benzamide:

Antranilic acid 4 gm (0.029 mol) and 2-hydrazino-6-fluoro-7-chloro (1,3) benzothiazole 5.6 gm (0.026 mol) were dissolved in dry pyridine (30 ml). The solution was refluxed for 8 hrs. The reaction mixture was cooled and poured into ice water. The separated mass was filtered, washed with water and dried. The product was recrystallized from ethanol.

Preparation of 4-benzylidene-2-phenyl-oxazol-5-one (oxazolone):

A mixture of 27 g (26 ml, 0.25 ml) of redistilled benzaldehyde, 45 g (0.25 mol) of benzoylglycine, 77 g (71.5 ml, 0.75 mol) of acetic anhydride and 20.5 gm (0.25 mol) of anhydrous sodium acetate was placed in a 500 ml conical flask and heated on an hot plate with constant shaking. As soon as the mixture has liquefied completely, the flask was transferred to water bath and refluxed for 2 hrs. 100 ml of ethanol was added slowly to the contents of the flask. The mixture was allowed to stand overnight. The crystalline product obtained was filtered by suction. It was washed with two 25 ml portions of boiling water and dried at 100°C.

The yield of pure oxazolone is 40 g (64%) m.p 165 - 166°C. Recrystallisation from benzene raised the m.p. to 167-168°C.

Synthesis of 2-(2-phenyl-4-benzylidenyl-5-oxo-imidazolin-1-yl) N-(2'-amino(1',3') benzothiazolyl 6'-fluoro 7'-chloro) benzamide:

2-Amino N-(2'-amino(1,3) benzothiazolyl 6'-fluoro 7'-chloro) benzamide. and oxazolone refluxed in pyridine for 6-8 hours. excess of pyridine was distilled off and resulting mass was poured on to crushed ice and

neutralised with dil HCl, filtered and product was recrystallised from ethanol gave 2-(2-phenyl-4-benzylidenyl-5-oxo-imidazolin-1-yl) N-(2'-amino(1',3') benzothiazolyl 6'-fluoro 7'-chloro) benzamide

Synthesis of 2-(2-phenyl-4-benzylidenyl-5-oxo-imidazolin-1-yl) N-(2'-amino(1',3') benzothiazolyl 6'-fluoro 7'-substituted) benzamide:

The 0.007 mol 2-(2-phenyl-4-benzylidenyl-5-oxo-imidazolin-1-yl) N-(2'-amino(1',3') benzothiazolyl 6'-fluoro 7'-chloro) benzamide was treated with equimolar quantity (0.0075 mol) of various substituted aromatic amines, PABA, morpholine, piperazine, dimethyl amine, diphenylamine were refluxed for 2 hrs. in presence of DMF (dimethyl formamide) then the mixture was cooled and poured into crushed ice. The solid separated was filtered, dried and recrystallised from benzene and super dry alcohol (1:1).

BIOLOGICAL ACTIVITY

Antimicrobial activit:

2-benzene sulphonamido-N-(2'-benzothiazolyl 6'-fluoro-7'-substituted) benzamide and 2-(2-phenyl-4-benzylidenyl-5-oxo-imidazolin-1-yl) N-(2'-amino(1',3') benzothiazolyl 6'-fluoro 7'-substituted) benzamide. The compounds prepared in the course of present investigation are screened for antibacterial activity against the following bacteria and antifungal activity against fungi [9-21].

- *Staphylococus aureus (Gram +ve).*
- *Escherichia coli (Gram -ve).*
- *Bacillas subtilis (Gram +ve).*
- *Pseudomonas aureus (Gram -ve).*
- *Candida albicans*.
- *Aspergillus niger*.

Antifungal activity:

The synthesized compounds are screened against two selected fungal strains *Candida albicans* and *Aspergillus niger* by using diffusion method. The 48 hours old fungal culture inoculated into nutrient broth by following aseptic techniques and incubated for 48 hours at $37^0 \pm 2^0$C in an incubator. This culture mixed with Potato-dextrose agar media (20%) and poured into petriplates. After solidification five bores are made at equal distance by using sterile steel cork borer (8 mm in diameter). Into these cups different concentrations of standard drug and synthesized compounds along with control (Dimethyl formamide) introduced. After introduction of standard drug and compounds, these plates are placed in a refrigerator at 8^0-10^0C for two hours for proper diffusion of the drugs. After 2 hours of cold incubation, the petriplates are transferred to incubator and maintained at $37^0 \pm 2^0$C for 24-36 hours. After the incubation period, the plates were observed for zone of inhibition by using verniar scale. Results evaluated by comparing the zone of inhibition shown by the synthesized compounds with standard drug. The results are the mean value of zone of inhibition measured in millimeter of two sets. The results are tabulated in the table. The standard drug and synthesized compounds were dissolved in minimum quantity of DMF and adjusted, to make up the volume with distilled water to get 50µg/ml and 100µg/ml concentrations. The griseoflavin used as a standard drug.

Anti-inflammatory activity by carrageenin induced rat hind paw edema method (in-vivo model):

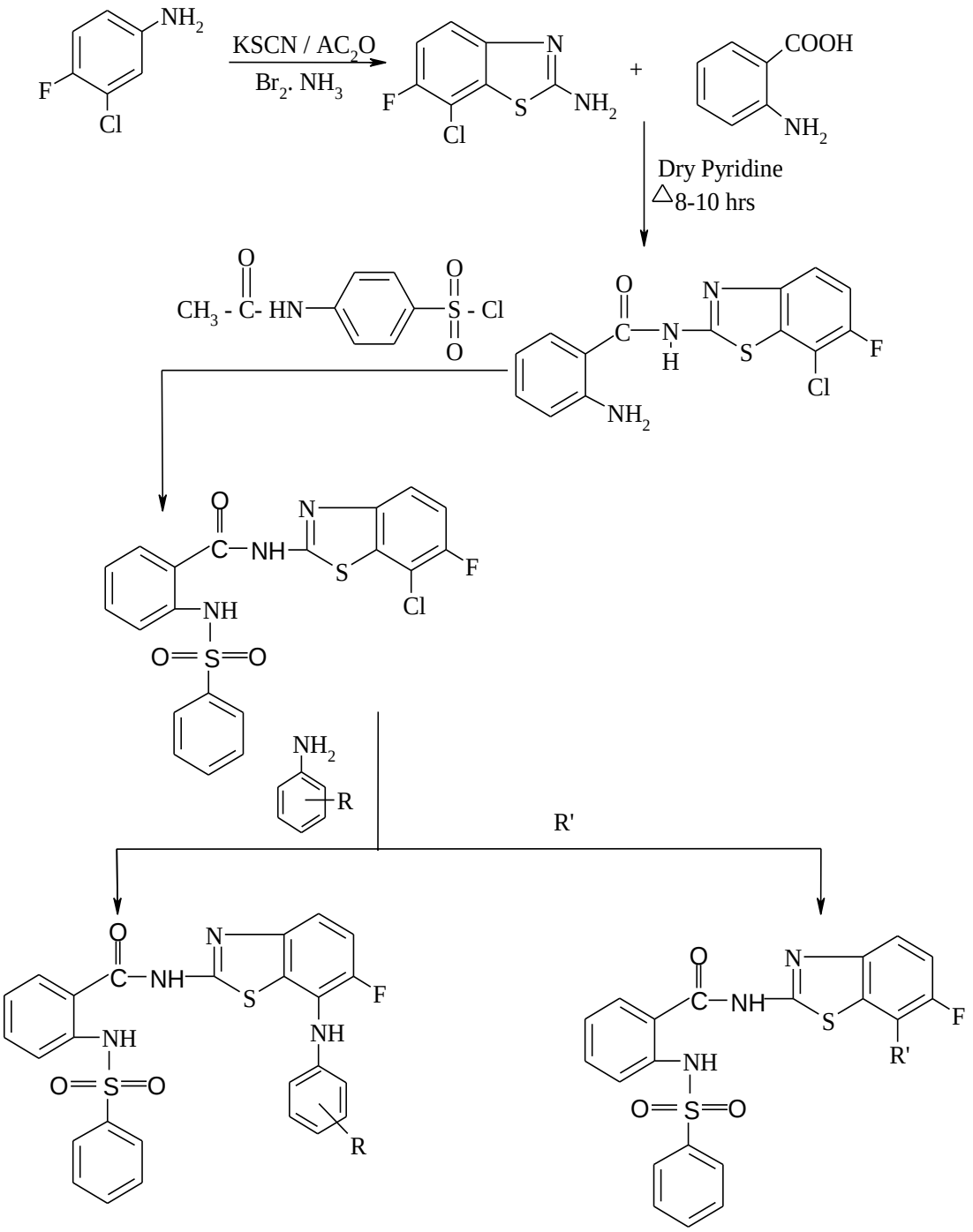
Animals were divided into control, standard, different test groups comprising of five animals in each group. They were fasted overnight with free access to water before experiment. In all groups, acute inflammation was produced by subplanter injection of 0.1 ml of freshly prepared 1% suspension of carrageenin in the right hind paw of the rats and paw of the rats and paw volume was measured plethysmometrically at 0 hr and 3 hrs after carrageenin injection. The test compounds (50 mg/kg) was administered orally, standard group was treated with diclofenac (50 mg/kg) orally 1 hr. before by injection and control group received only vehicle[22-25]. Mean difference in paw volume was measured and percentage inhibition was calculated by following formula.

$$\% \text{ inhibition of edema} = \left[\frac{Vc - Vt}{Vc} \right] \times 100$$

Where, Vt = mean paw volume of test group.

Vc = mean paw volume of control group.

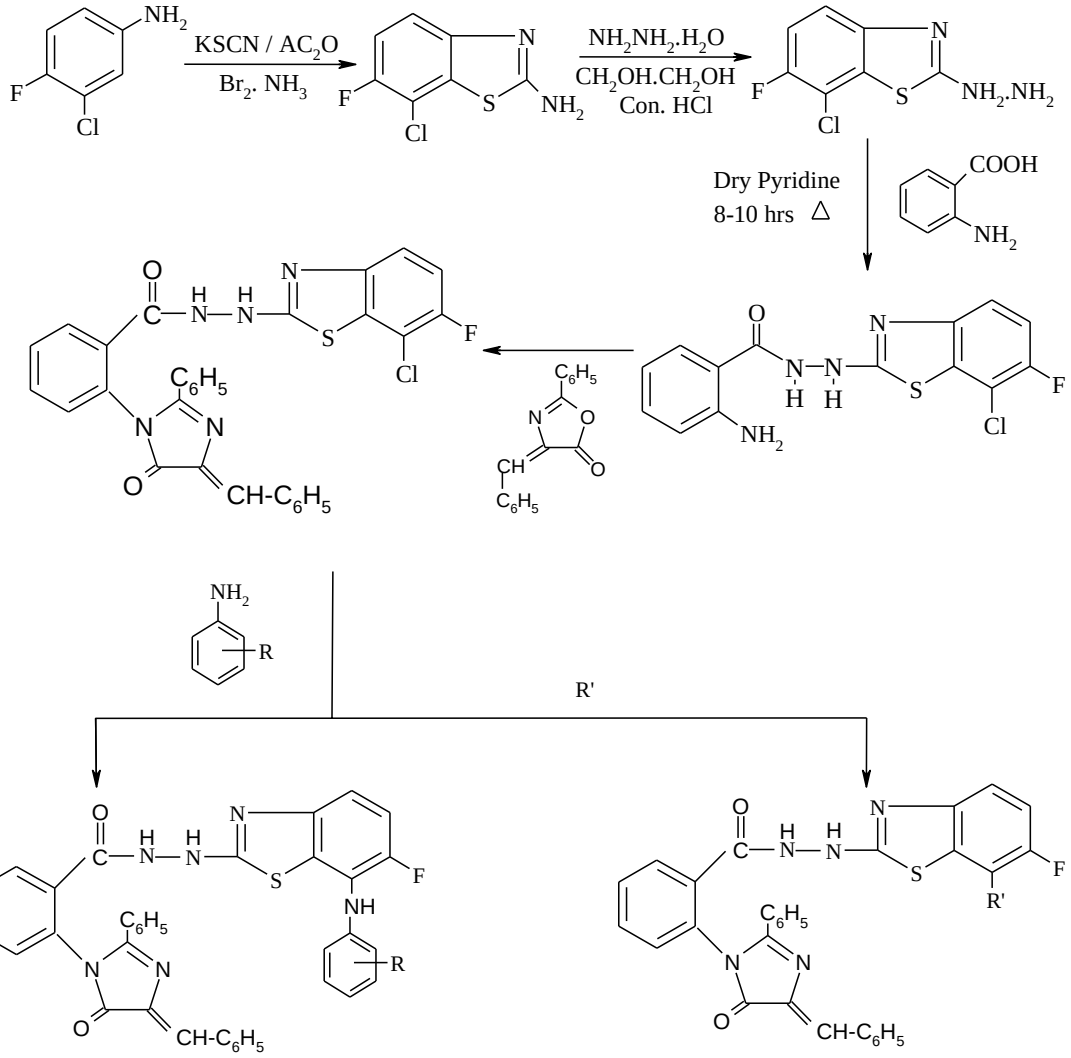
SCHEME-I



R = o, m, p- nitro aniline
= o, m, p- chloro aniline
= N- phenyl
= o-methyl, p-carboxyl

R' = morpholine, piperazine
= N-methyl piperazine

SCHEME-II



R = o, m, p- nitro aniline
= o, m, p- chloro aniline
= p-toluidine

R' = morpholine, piperazine
= N-methyl piperazine
= PABA, diphenyl amine

Anti-inflammatory activity (in-vitro models):

The synthesized compounds are screened for anti-inflammatory activity by using inhibition of albumin denaturation technique which was studied according to Muzushima and Kabayashi with slight modification.[26-29]

The standard drug and test compounds were dissolved in minimum amount of dimethyl formamide (DMF) and diluted with phosphate buffer (0.2 M, pH 7.4). Final concentration of DMF in all solutions was less than 2.0%. Test solution (1 ml) containing different concentrations of drug was mixed with 1 ml of 1% mM albumin solution in phosphate buffer and incubated at $27^0 \pm 1^0$C in BOD incubator for 15 min.

Denaturation was induced by keeping the reaction mixture at $60^0 \pm 1^0$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 was taken. The diclofenac sodium was used as standard drug. Results are tabulated in table.

$$\% \text{ of inhibition} = 100 \times \left(\frac{Vt}{Vc} \right) - 1$$

Anticonvulsant activity:

In the present study the mice of either sex, weighing between 20-25 g were selected and divided into control, test and standard.

Before experiment the animal were fasted for 24 hrs with only water *ad-libitum*. Control group received only 0.5 ml DMF as vehicle. Standard group animals were received diazepam (4 mg/kg b.w.) oral test group animals were received the synthesized derivatives at 4 mg/kg b.w. oral in DMF.

Now for the animals of control group pentylene tetrazole (PTZ) 1ml/100 g b.w. was administered and actions like stratubs tail, jerky movements of whole body and convulsions were observed.

For animals of standard test group PTZ was injected (1 ml/100 g body weight). After 30 min animals of standard and test received diazepam and synthesized derivatives respectively.[30-32]

RESULTS AND DISCUSSION

Anti-bacterial activity:

2-benzene sulphonamido-N-(2'-benzothiazolyl 6'-fluoro-7'-substituted) benzamide and 2-(2-phenyl-4-benzylidenyl-5-oxo-imidazolin-1-yl) N-(2'-amino(1',3') benzothiazolyl 6'-fluoro 7'-substituted) benzamide were tested for the antibacterial activity against following bacteria;

i) *Staphylococcus aureus*

ii) *Bacillus substillis (gram +ve)*

iii) *Escherichia coli*

iv) *Pseudomonas aureus (gram –ve).*

The compounds S_1, S_4, S_{10}, S_{11}, S_{12}, R_2, R_3, R_4, and R_8, showed better antibacterial activity against *Staphylococcus aureus* (gm +ve) at lower and higher concentration the compounds S_2, S_4, S_5, S_6, S_{11}, S_{12}, R_1, R_2, R_6, and R_{11}, showed better antibacterial activity against *Bacillus substillis* (gm +ve) at lower and higher concentration. Procaine penicillin used as standard for *Staphylococcus aureus* and *Bacillus substillis*.

The compounds S_3, S_5, S_6, S_{12}, S_{14}, R_2, R_4, R_7, R_8, and R_{12}, showed better antibacterial activity against *Escherichia coli* gm –ve, streptomycin used as standard.

The compounds S_5, S_6, S_8, S_9, S_{10}, R_2, R_3 R_4 R_9 and R_{10}, showed better antibacterial activity against *Pseudomonas aureus* gm –ve, streptomycin used as standard.

Anti-fungal activity:

The above synthesized compounds were tested for antifungal activity against *Candida albicans* and *Aspergillus niger,* using griseofulvin as standard.

The compounds S_9, S_{13}, S_{14}, R_1, R_2, R_3, R_5, R_6, R_7, R_9 and R_{12}, showed significant anti-fungal activity (*Candida albicans*) when compare to standard drug.

The compounds S_4, S_5, S_9, S_{10}, S_{11}, R_4, R_5, R_6, R_7, R_8 and R_{11}, showed significant anti-fungal activity (*Aspergillus niger*) when compare to standard drug.

Anti-inflammatory activity (in vivo models):

The above synthesized compounds were tested for anti-inflammatory activity by in vivo method using Diclofenac Sodium (80.00%) as standard drug.

The compounds S_2, S_3, S_4, S_{14}, R_1, R_2, R_4, R_6, and R_7, showed significant anti-inflammatory activity.

Anti-inflammatory activity (in vitro models):

The above synthesized compounds were tested for anti-inflammatory activity by in vitro method using Diclofenac Sodium (83.30%) as standard drug.

The compounds S_1, S_3, S_4, S_5, S_7, S_9, S_{12}, S_{14} R_2, and R_4, showed significant anti-inflammatory activity.

Anti-convulsant activity:

The above synthesized compounds were tested for anticonvulsant activity by PTZ (pentylene tetrazole) induced method, used diazepam as standard.

The anticonvulsant activity conform these compounds S_1, S_6, S_8, S_9, S_{12}, S_{13}, R_4, R_6 R_7 R_9, R_{10} and R_{12}, shown significant anti-convulsant activity compare to standard. The other compounds S_2, S_3, S_4, S_5, S_{10}, S_{11}, R_2, R_3, R_5, R_8, and R_{11}, showed mild anticonvulsant activity.

SUMMARY AND CONCLUSION

Scheme-I

In present work, Fluorochloro aniline was treated with KSCN in presence of bromine in glacial acetic acid and ammonia to get 2-amino-6-fluoro-7-chloro (1,3)-benzothiazole, which was condensed with anthranilic acid in presence of dry pyridine to get 2-amino-N-(2'-benzothiazolyl 6'-fluoro-7'-chloro) benzamides. To the above product refluxed with p-acetamidobenzenesulphonylchloride in presence of pyridine to get 6-fluoro-7-chloro-2-(*p*-acetamido benzene sulphonamido) (1,3)- benzothiazole. The said above product treated with different aromatic amines, PABA, piperzine, diphenylamine, N-Methyl piperzine, o-toluidine in presence of DMF to get newly synthesized titled compound.

Scheme-II

In present work, Flurochloro aniline was treated with KSCN in presence of bromine in glacial acetic acid and ammonia to get 2-amino-6-fluoro-7-chloro (1,3)-benzothiazole, which was treated with hydrazine hydrate (99%) in the presence of concentrate HCl using ethylene glycol as solvent to get 2-hydrazino-6-fluoro-7-chloro(1,3) benzothiazoles, then the product refluxed with anthranilic acid in presence of dry pyridine to get 2-Amino N-(2'-amino(1,3) benzothiazolyl 6'-fluoro 7'-chloro) benzamide. The above titled product was treated with oxazolone in the presence of pyridine to get 2-(2-phenyl-4-benzylidenyl-5-oxo-imidazolin-1-yl) N-(2'-amino(1',3') benzothiazolyl 6'-fluoro 7'-chloro) benzamide. The obtained product treated further with different aromatic amines, PABA, piperzine, diphenylamine, N-Methyl piperzine, o-toluidine in presence of DMF gave targeted titled compounds.

The lead molecules of scheme I & II were characterised by solubility, TLC, analytical data, UV-Visible, IR and ^{1}HNMR and Mass spectral studies.

The compounds were screened for antibacterial, antifungal, anti-inflammatory (invivo and invitro) and anticonvulsant activity

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