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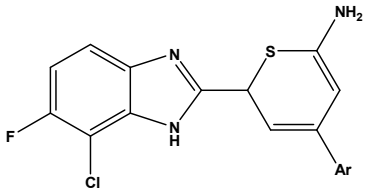
Microwave Assisted Synthesis of Fluoro, Chloro 2-Substituted Benzimidazole Thiazine Derivatives for Antibacterial and Analgesic Activities

Gayathri Banda[1], S.M.Hipparagi[1], Ramjith.U.S[2*], Cyril Mathews Jacob[3]

[1]KLE University's College of Pharmacy, Rajajinagar, Bangalore-10, Pin-560010, Karnataka.
[2]Crescent College of Pharmaceutical Sciences, Payangadi, Kannur, Kerala, Pin-670538
[3]Nandha College of Pharmacy, Erode, Tamil Nadu

ABSTRACT

The flouro, chloro 2-acetyl benzimidazole were prepared by the microwave induced reaction between 3-chloro-4-fluorobenzene-1, 2- diamine and lactic acid, followed by oxidation with potassium permanganate and aluminum oxide. The chalcone derivative of fluoro,chloro-2-acetyl benzimidazole were prepared by the condensation with different aldehydes and the resulting compounds were cyclized with thiourea, to get the thiazine derivatives of fluoro, chloro benzimidazole. The synthesized compounds have been characterized and confirmed by TLC, elemental analysis, IR, and ^{1}H NMR spectroscopy and screened for their antibacterial and analgesic activity. Compounds containing electron withdrawing groups in the substituted Benzimidazole thiazine were found to show potent analgesic and antibacterial activities.



Ar = Aromatic aldehydes

Keywords: Benzimidazole, microwave, ^{1}H NMR, antibacterial activity, analgesic activity.

*** Corresponding author**
Ramjith.U.S
Lecturer,
Crescent College of Pharmaceutical Sciences,
Payangadi, Kannur, Kerala, Pin-670538
Phone No: 04742714306, 09037176285
E-mail:-ramjithganesh143@gmail.com

INTRODUCTION

Benzimidazole is a heterocyclic aromatic organic compound. Owing to the immense importance and varied bioactivities exhibited by benzimidazoles, efforts have been made from time to time to generate libraries of these compounds and screen them for potential biological activities. These observations have encouraged us to synthesize some new products containing the benzimidazole moiety hoping to obtain new compounds with potential biological activity. The benzimidazole has been an important pharmacophore and privileged structure in medicinal chemistry. Encompassing a plethora of useful biological activities such as antimicrobial, antifungal, antitubercular, anticancer, antitumour, antihepatitis-c-virus, antiallergic, antiHIV, analgesic, antipsychotic, antidepression, antianxiety, antiviral, antihypertensive, antiulcer, anti-inflammatory, topoisomerase inhibitor, thromboxaneA_2 receptor antagonist, $5HT_3$ antagonist, cardiovascular and antihistaminics[1-2].

Compounds containing Benzimidazole rings have been used extensively for pharmaceutical purposes since 1960. Almost all benzimidazole derivatives with their two ring systems bear different functional substituents and this leads to essential modifications of the physic-chemical, metabolic and pharmacokinetic properties of drugs[3].

The widespread interest in benzimidazole containing structures has prompted extensive studies for their synthesis.

There are two general methods for the synthesis of 2-substituted benzimidazoles.

1. By coupling of ortho phenylenediamines and carboxylic acids or their derivatives (nitriles, imidates, or ortho esters), which often requires strong acidic conditions and sometimes combines with very high temperatures or the use of microwave irradiation.

2. The other way involves a two-step procedure that includes the oxidative cyclodehydrogenation of aniline Schiff's bases, which are often generated in situ from the condensation of ortho phenylenediamines and aldehydes.

On the basis of these reports microwave assisted synthesis of novel moieties of benzimidazole were performed and the compounds were screened for antibacterial and analgesic activities.

EXPERIMENTAL SECTION

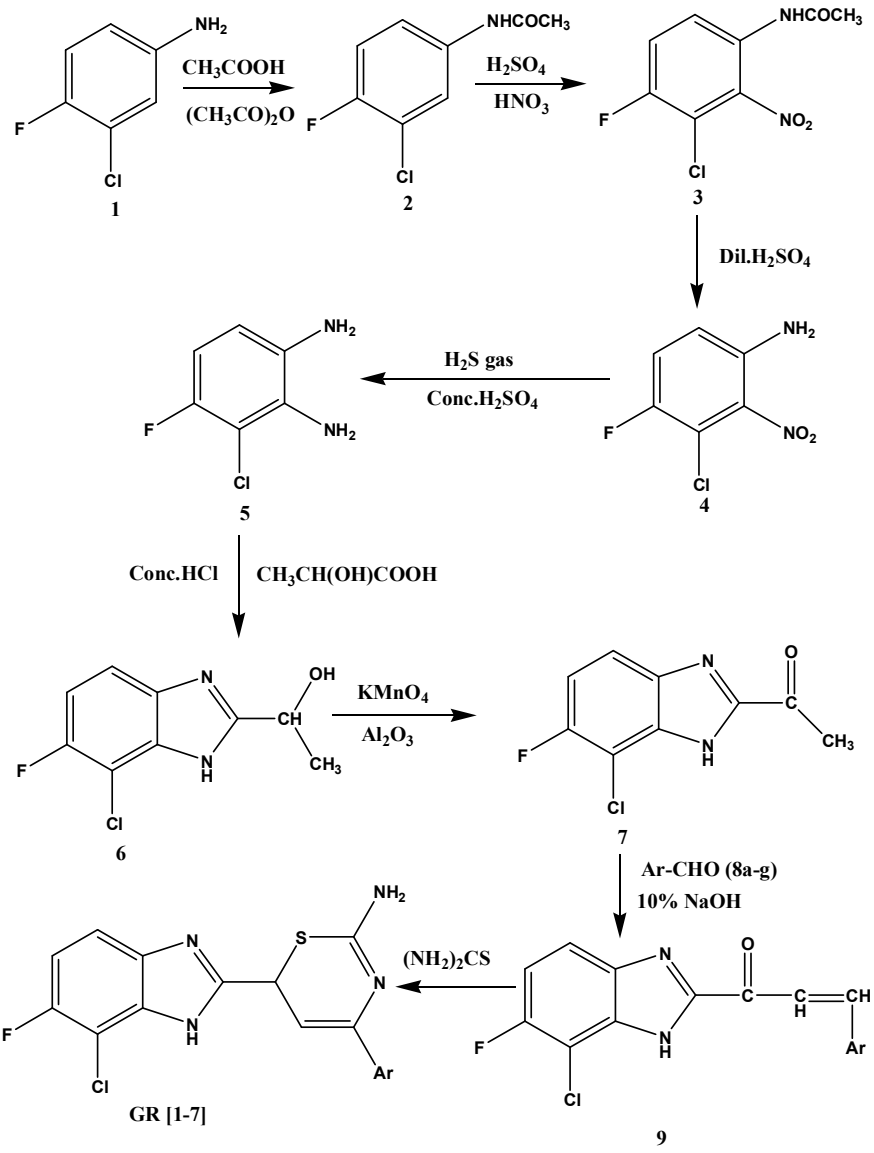


Fig 1: Preparation of derivatives of novel fluoro, chloro 2-substituted benzimidazole thiazine derivatives (GR [1-7]) from 3-chloro-4-fluoro-aniline (1)

General Procedure

Synthesis of N-(3-chloro-4-fluorophenyl) acetamide(2)[4]

10 g of 3-chloro-4-fluoro-aniline **(1)** was dissolved in 25 ml of glacial acetic acid in a

250 ml round bottom flask. To this solution, added 12.5 ml of acetic anhydride and mixed well by swirling and heat at a gentle reflux for thirty minutes.

After reflux added 10 ml of cold water through the top of the condenser into the reaction mixture. Boiled the solution for an additional five minutes so as to hydrolyze any unreacted acetic anhydride .After boiling for five minutes, allowed to cool slightly and then poured it slowly with stirring into 75 ml of ice cold water, allowed the mixture to stand for 15 minutes with occasional stirring, collected the precipitate. Washed the solid crystals with 25 ml of cold water, dried and Recrystallized from ethanol. The melting point was found to be 95°C. The yield of acetanilide was 9 g (90%).

Synthesis of N-(3-chloro-4-fluoro-2-nitrophenyl) acetamide(3)[5]

9g of finely powdered 3-chloro-4-fluorophenylacetamide (**2**) was dissolved in3.12g (18 ml) of glacial acetic acid contained in 500 ml beaker. When all the material has dissolved, cooled the solution in an ice bath. Crystals may begin to form then after added 4.5 ml of concentrated ice cold sulfuric acid slowly and kept the reaction mixture under ice bath to cool to 5 °C.In a separate 25 ml flask, prepared a nitrating mixture by adding 5.58g (3.96ml) of concentrated nitric acid to 4.5g (2.5 ml) of ice cold sulfuric acid. The addition reaction is exothermic. Cooled the nitrating mixture in ice for several minutes.

Added this nitrating mixture to the compound. After adding the nitrating mixture, the flask was removed from the ice and allowed it to stay at room temperature for 5-8 min with occasional stirring followed by addition of ice water with stirring. Collected the product by suction pump using Buchner funnel. Washed it thoroughly with cold water until free from acids and drained well. Recrystallized the pale yellow product from ethanol. The yield of o-nitro acetanilide was 8 g (88.8%), M.P-78°C.

Synthesis of 3-chloro-4-fluoro-2-nitro aniline(4)[6]

A mixture of 8g of 3-chloro-4-fluorophenylacetamide (**3**) and 39.9 ml of 70% w/w sulphuric acid were subjected to in microwave radiation at 320 watts for 5 minutes. The nitro aniline was present in the liquid as the sulphate. The clear hot solut ion was poured in to 200 ml of cold water and the nitro aniline was precipitated by adding excess of 10% NaOH solution. Filtered the yellow crystalline precipitate at the pump, washed it well with water and drained thoroughly. Recrystalised from ethanol. The yield of nitro aniline was 7.5 g (93.75%). Melting point was found to be 125°C. The development of TLC was done by using toluene and chloroform (3:2).

Synthesis of 3-chloro-4-fluorobenzene-1, 2-diamine(5)[6]

7.5 g of 3-chloro-4-fluoro-2-nitro aniline, 77.5 ml of ethanol and 38 ml of ammonia solution were taken in a 250 ml of round bottom flask. The mixture was heated to 45°C and hydrogen sulphide gas was passed. The gas is passed continuously in to the solution until the yellow particles of 3-chloro-4-fluoro-2-nitro aniline was dissolved. The flask was kept in ice-bath for 17-18 hours. The deep red colored crystals of 3-chloro, 4- flouro orthophenylene diamine was collected on Buchner funnel and wash with cold water. Recrystalised from hot water containing HCl. Filtered and the filtrate was treated with ammonia solution. Cooled and collected the crystals. The yield obtained was 5 g (66.66%). The melting point was found to be 97°C.

Synthesis of 1-(7-chloro-6-fluoro-1*H*-benzo[d]imidazol-2-yl) ethanol(6)[7]

This is prepared by both conventional and non-conventional methods.

Nonconventional Method:

Prepared by the reaction of 0.01 mole (1.65g) of 3-chloro-4-fluoro-orthophenylene diamine (**5**) with 1.3 equivalent of lactic acid and 25 ml of dilute 4N HCl. The mixture was refluxed with condenser in synthetic microwave oven at 160 Watts for 40 minutes. The mixture was cooled and added 10% NaOH solution till the reaction mixture was neutralized. Then the precipitate was filtered, washed with cold water, dried and recrystalised from methanol. The yield of compound was 1.50g (91%). The melting point was found to be 235°C.

Conventional Method:

Prepared by the reaction of 0.01 mole of 3-chloro-4-fluoro-orthophenylene diamine(**5**) with 1.3 equivalent of lactic acid and 25 ml of dilute 4N HCl. The mixture was refluxed at 80 ºC for 34 hours and the mixture was cooled to room temperature and added 10% NaOH solution till the reaction mixture was neutralized. Then the precipitate was filtered, washed with cold water, dried and recrystalised from methanol. The yield of compound was 0.9g (54.5%).

Synthesis of 1-(7-chloro-6-fluoro-1*H*-benzo[d]imidazol-2-yl) ethanone(7)[8]

The alumina supported permanganate was prepared by mixing solid potassium permanganate (2 g, 12.65 moles) and solid alumina (2.5 g) in a mortar and ground with a pestle until a fine homogenous purple powder was obtained. Later benzimidazole (5 m moles) was added to above

and mixed well at room temperature. Acetone (20 ml) was added to reaction mixture and after vigorous stirring a mixture was evaporated to obtain a crude residue. Extracted with chloroform (15 ml) and washed with water (30 ml) to remove any occluded. The organic matter was dried over anhydrous sodium sulfate. The crude product thus obtained was recrystalised from hot hexane to get pure product. The percentage yield was 72%. The melting point was found to be 245°C.

Synthesis of 1-(7-chloro-6-fluoro-1*H*-benzo[d]imidazol-2-yl)-3-arylprop-2-en-1-one(9)[9]

Microwave Method:

Dissolved the 2-acetyl benzimidazole in ethanol (30 ml) and various aromatic aldehydes (0.01 mole) were taken and then an aqueous solution of NaOH (10%, 10 ml) added to it. The reaction mixture refluxed in microwave oven at 210 Watts for 10-20 min and then the excess solvent was removed by distillation and then it was poured in to crushed ice and acidified with dilute HCl. The solid separated was filtered and recrystalised from ethanol.

Conventional method:

Dissolved the 2-acetyl benzimidazole in ethanol (30 ml) and various aromatic aldehydes (8a-g) (0.01 mole) were taken and then an aqueous solution of NaOH (10%, 10 ml) added to it. The reaction mixture refluxed for 12-18 hours at 60ºC and then the excess solvent was removed by distillation and then it was poured in to crushed ice and acidified with dilute HCl. The solid separated was filtered and recrystalised from ethanol.

Synthesis of 6-(7-chloro-6-fluoro-1*H*-benzo[d]imidazol-2-yl)-4-aryl-6*H*-1, 3-thiazin-2-amine([GR 1-7])[10]

A mixture of compound 1-(7-chloro-6-fluoro-1*H*-benzo[d]imidazol-2-yl)-3-arylprop-2- en-1-one (0.02 mol) and thiourea (0.02 mol) were dissolved in ethanolic NaOH (10 ml), stirred for 3 hrs using a magnetic stirrer and then poured in to 200 ml of cold water with continuous stirring. This was kept in a cold condition in refrigerator for 24 hrs. The precipitate obtained was filtered, washed and recrystalised from ethanol.

SPECTRAL ANALYSIS

[GR-1]6-(7-chloro-6-fluoro-1H-benzimidazol-2-yl)-4-phenyl-6H-1, 3-thiazin-2-amine

IR (KBr) 3344-3460cm^{-1}(NH_2), 1589-1679cm^{-1}(C=N), 2192-2298 cm^{-1} (C-S-C).

^{1}HNMR δ:4.52(1H,s,C-H),6.29(1H,s,C-H),6.93-7.58(7H,m,Ar-H).Mass Spectroscopy (MS) m/z: 358.08 (M^+).

[GR-2]6-(7-chloro-6-fluoro-1H-benzimidazol-2-yl)-4-(4-chlorophenyl-(6H-1, 3-thiazin-2-amine

IR (KBr) 3347-3458cm^{-1}(NH_2), 1590-1670cm^{-1}(C=N), 2190-2293 cm^{-1} (C-S-C).

^{1}HNMR δ: 4.52(1H, s, C-H), 6.94-7.26(6H, m, Ar-H).

Mass Spectroscopy (MS) m/z: 392.03 (M^+).

[GR-3]4-(2-amino-6-(7-chloro-6-fluoro-1H-benzimidazol-2-yl)-6H-1,3-thiazin-4-yl) phenol

IR (KBr) 3341-3459cm^{-1}(NH_2), 1568-1660cm^{-1}(C=N), 2191-2290 cm^{-1} (C-S-C).

^{1}HNMR δ: 4.52(1H, s, C-H), 6.93-7.58(7H, m, Ar-H).

Mass Spectroscopy (MS) m/z: 374.06 (M^+).

[GR-4] 6-(7-chloro-6-fluoro-1H-benzimidazol-2-yl)-4-(4-methoxyphenyl)-6H-1, 3 thiazin-2-amine

IR (KBr) 3341-3459cm^{-1}(NH_2), 1568-1660cm^{-1}(C=N), 2191-2290 cm^{-1} (C-S-C).

^{1}HNMR δ: 3.73(3H, s,-OCH_3), 4.50(1H, s, C-H), 6.91-7.56(6H, m, Ar-H).

Mass Spectroscopy (MS) m/z: 388.07 (M^+).

[GR-5] 6-(7-chloro-6-fluoro-1H-benzimidazol-2-yl)-4-4-p-tolyl--6H-1, 3 thiazin-2-amine

IR (KBr) 3340-3450cm^{-1}(NH_2), 1565-1661cm^{-1}(C=N), 2193-2294 cm^{-1} (C-S-C).

^{1}HNMR δ: 2.35(3H,s, CH_3),6.91-7.56(6H, m, Ar-H).

Mass Spectroscopy (MS) m/z: 372.06 (M^+).

[GR-6] 6-(7-chloro-6-fluoro-1H-benzimidazol-2-yl)-4-(3-nitrophenyl)-6H-1, 3 thiazin-2-amine

IR (KBr) 3340-3450cm^{-1}(NH_2), 1565-1661cm^{-1}(C=N), 2193-2294 cm^{-1} (C-S-C).

^{1}HNMR δ: 6.92-8.23 (6H, m, Ar-H).

Mass Spectroscopy (MS) m/z: 403.04 (M^+).

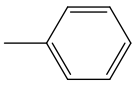
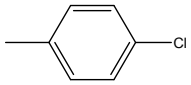
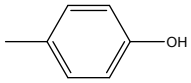
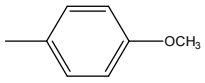
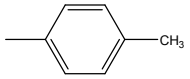
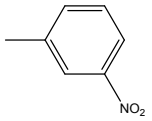
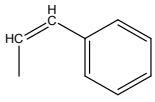
GR-7] 6-(7-chloro-6-fluoro-1H-benzimidazol-2-yl)-4-styryl)-6H-1, 3 thiazin-2-amine

IR (KBr) 3342-3450cm^{-1}(NH_2), 1565-1660cm^{-1}(C=N), 2193-2294 cm^{-1} (C-S-C).

^{1}HNMR δ: 6.81(2H, d, HC=CH), 6.96-7.58 (7H, m, Ar-H).

Mass Spectroscopy (MS) m/z: 386.07 (M+2).

Table-I: Characterization data of synthesized compounds

Compound Code	Ar	Melting point (M.P) (^{O}C)	Yield (%)	Solvent for Crystallisation
GR-1		145	76	Ethanol
GR-2		134	87	Ethanol
GR-3		160	56	Ethanol
GR-4		139	78	Ethanol
GR-5		159	89	Ethanol
GR-6		186	49	Ethanol
GR-7		175	65	Ethanol

PHARMACOLOGICAL EVALUATION

The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) of Nandha College of Pharmacy, Erode – 638 052, Tamil Nadu with CPCSEA Registration No No: 688/02/c/CPCSEA.

Animals

Albino mice of either sex weighing 20-25g were used for performing acute toxicity studies and analgesic activity. Animals were kept in Nandha College of Pharmacy, Erode, Tamil Nadu, housed individually in polypropylene cages, maintained under standard conditions of alternating 12hr light dark cycles at a constant temperature (25±2^{o}c) and 40-60% room humidity).Animals were fed with standard rat pellet and water and libitum.

Acute Toxicity

The acute toxicity test was carried out according to OECD guidelines[11] to establish the effective dose of test compounds after obtaining ethical clearance from animal ethics committee. Albino mice of either sex weighing between 25-35g were grouped into 10 groups of six animals each, starved for 24h with water *ad libitum* prior to test. The experiment animals were administered with different compounds of increasing dose of 10,20,50,100,200,1000,1500,2000,2500mg/kg orally. The animals were observed continuously for 3h for general behavioural,neurological,autonomic profiles and then every 30min for next 3hr and finally for 24hr or till death.

Analgesic Activity

Acetic acid induced writing model in mice[12].

Twenty four hours prior to actual testing a large number of mice (25-35mg) received intraperitoneal 10ml/kg of 0.6% glacial acetic acid. Animals were observed for writing movements. Only these showing one or other type of writhing movements (positive responders) were chosen for the test on the next day. On the test day the responders received synthesized compounds (GR-1 to GR-7) half an hour prior to glacial acetic acid challenge. Synthesized compounds GR-1 to GR-7 were administered orally at a dose of 40mg/kg as a suspension in 0.5 sodium carboxyl methyl cellulose (CMC).Each mouse was then observed for total number of stretching episodes or writing for 15 minutes following glacial acetic acid

injection. The standard employed was Indomethacin (5mg/kg).Percentage of writing was calculated using the relation.

Inhibition of writing (%) =100 X (1-b/a).

Where, a= mean writhing number of control mice.

b= mean writhing number of treated mice.

Antimicrobial activity[13-14]

Table-II: Screening for anti-bacterial activity of synthesized compounds for Gram positive strains (MIC in µg/ml).

S.No	Compound Code	Microorganisms		
		Bacillus cereus	***Micrococcus luteus***	***Staphylococcus Albus***
1	GR-1	125	-	250
2	GR-2	50	65	200
3	GR-3	100	-	80
4	GR-4	-	250	-
5	GR-5	-	-	-
6	GR-6	70	125	-
7	GR-7	300	-	-
8	Standard Ciprofloxacin	≥2.5	≥2.5	≥2.5

The minimal inhibitory concentration (MIC) was defined for each chemical as the lowest dilution associated with at least a 99% reduction in the number of viable colonies. In the present study three gram-positive organisms (*Bacillus lentus*, *Micrococcus luteus, and Staphylococcus albus)* and three gram negative (*Escherichia coli, Salmonella paratyphi, Proteus vulgaris)*. The strain was confirmed for its purity and identity by the gram staining method and it was further characterized by chemical reaction. The bacterial strains were grown overnight in Mueller-Hinton broth test inocula were prepared diluting the overnight suspension to a density of 104 microorganisms per millimeter. The MIC determinations were performed by the agar dilution method.; Mueller-Hinton agar were used for bacterial strains to prepare quadrant plates with serial dimethyl sulfoxide two fold dilutions of the different chemical tested. A 20µl sample of each 10^{-4} ml microbial suspension was inoculated on to each chemical containing quadrant. Control plates consisted of Mueller-Hinton agar alone,

culture medium with dimethyloxide and culture medium with known antimicrobial drugs like Ciprofloxacin (the reference antibacterial drugs) for bacterial strain. All the plates were then incubated at 37°c overnight. The observed data on antibacterial activity of the compound (GR-1 to 7) and control drugs are given in tables II and III.

Table-III screening for anti-bacterial activity of synthesized compounds for Gram Negative strains (MIC in µg/ml).

Sl.No	Compound Code	Microorganisms		
		Escherichia Coli	***Salmonella paratyphoid***	***Proteus vulgaris***
1	GR-1	-	250	-
2	GR-2	125	125	-
3	GR-3	150	-	-
4	GR-4	180	-	300
5	GR-5	130	165	250
6	GR-6	-	300	-
7	GR-7	-	250	300
8	Standard Ciprofloxacin	≥2.5	≥2.5	≥2.5

Table IV: Analgesic Activity of Synthesized compounds by acetic acid induced writhing method

Compound Code	%Protection
GR-1	21.2
GR-2	65.3
GR-3	60.4
GR-4	48.3
GR-5	30.7
GR-6	63.1
GR-7	19.8
Standard Indomethacin	74.08

Dose of test compounds-50mg/kg

Indomethacin-5mg/kg

RESULTS AND DISCUSSION

Chemistry

Prepared 3-chloro-4-fluoro aniline was made to undergo four step reaction involving acetylation, nitration, hydrolysis, and reduction for preparation of 3- chloro-4-fluoro phenylene diamine. 3-chloro-4-fluoro phenylene diamine was starting material to synthesize various benzimidazole chalcone derivatives. The key intermediate 1-(7-chloro-6-fluoro-1*H*-benzimidazol-2-yl) ethanone [2-acetyl benzimidazole] required for the synthesis of the title compounds was prepared by the oxidation of 1-(7-chloro-6-fluoro-1*H*-benzimidazol-2-yl) ethanol with $KMnO_4$ in presence of Al_2O_3.The condensation of 2-acetylbenzimidazole with various aromatic aldehydes which produce various benzimidazolyl chalcone derivatives. A mixture of chalcone derivatives and thiourea were dissolved in ethanolic NaOH stirred for 3hrs using magnetic stirrer and then poured in to cold water with continuous stirring. This was kept in a refrigerator for 24 hrs. The precipitate obtained was fluoro, chloro 2-substituted benzimidazole thiazine derivatives.

The synthesized compounds were recrystalised from ethanol and characterized by thin layer chromatography (TLC), Melting point, FT-IR, ^1H NMR and Mass spectral studies.

The synthesized compounds were screened for antimicrobial and analgesic activity.

The compound GR-2 showed potent antibacterial activity against gram positive and moderate activity against gram negative organism when compared to standard ciprofloxacin while compounds GR-3 and GR-6 showed potent antibacterial activity against Gram positive organism. The compounds GR-2, GR-6, GR-3, and GR-4 showed potent analgesic activity when compared to standard Indomethacin.

CONCLUSION

The compound containing electron withdrawing (Cl, OH) groups in para position or meta position (NO_2) of the phenyl ring present in the Substituted Benzimidazole thiazine were found to show potent analgesic and antibacterial activities. Further studies are required to establish the exact mechanism of action.

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