SAR Journal of Medical Biochemistry

Abbreviated Key Title: *SAR J Med Biochem*
Home page: https://sarpublication.com/journal/sarjmb/home
DOI: 10.36346/sarjmb.2022.v04i01.002

ISSN 2707-7721 (P)
ISSN 2709-6882 (O)

Original Reserach Article

Synthesis and Biological Screening of Novel Derivatives of Benzothiazol as Anticonvulsant Agents

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Article History: | Received: 09.03.2023 | Accepted: 26.04.2023 | Published: 30.04.2023 |

Abstract: The Benzothiazole ring system belongs to a much studied class of compound. In the last few decades, the chemistry of benzothiazole and their fused heterocyclic derivatives have received considerable attention owing to their significant and effective biological activity. The present study aimed to design and synthesize novel derivatives of bezothiazole obtained from 3-chloro-4-flouro aniline treated with potassium thiocynide with chloro acetyl chloride gives (7-chloro-6-fluro-1,3-benzothiazol-2-yl) acetyl chloride which is converted into hydrazide and yields the resultant compound derivatives of 2-(7-chloro-6-fluro-1,3-benzothiazol-2-yl) –N'-[(Z)-phenyl methylidene] acetohydrazide (SMVB-IIIA-IIIG).Title compound were synthesized and the structures of newly synthesized compounds were confirmed by IR, Mass and ^{1}H-NMR spectroscopy All the compounds synthesized were confirmed by spectral data and evaluated for their anticonvulsant activity. The Compounds SMVB-IIIC, SMVB-IIIEand SMVB-IIIF showed maximal activity whereas remaining compounds showed good activity.

Keywords: Benzothiazole, 3-Chloro-4-Flouro Aniline, Anticonvulsant Activity.

INTRODUCTION

Benzothiazole ring system is present in various marine and terrestrial natural compounds, which have useful biological activities [1] and important class of heterocyclic compounds that exhibit a wide range of biological properties in medicinal [2] 2-(4-Aminophenyl) benzothiazole structure is known with high antitumor activity since 1996 [3]. Benzothiazole derivatives have been synthesised and claimed to have significant analgesic and anti- inflammatory activity [4], antimicrobial, anticancer, antidiabetic [5], antifungal activity[6]. Benzothiazole are bicyclic ring system ring and made from thiazole ring fused with benzene ring. Thiazole ring is a five-member ring consists of one nitrogen and one sulphur atom in the ring was shown in Fig 1. The newly synthesized compounds of Derivatives of 2-(7-chloro-6-fluro-1,3-benzothiazol-2-yl) –N'-[(Z)-phenyl methylidene] acetohydrazidewere evaluated for their anticonvulsant activity by MES method.

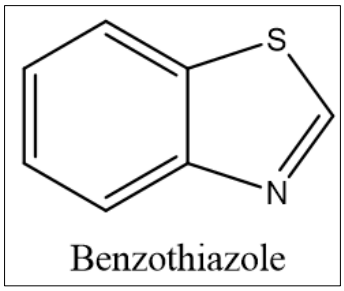


Figure 1: Structure of Benzothiazole

MATERIALS AND METHODS

The entire all chemicals used were procured from Lobachemie Pvt. Ltd., Mumbai. Purity of starting materials used for reaction was confirmed by checking their melting point and by thin layer chromatography. All the reactions were monitored using thin layer chromatography. The FT-IR spectrum of the synthesized compounds has been obtained from oxygen health care and research center Pvt, Ltd Ahmadabad, Gujarat. The IR spectra were carried out by FT-IR (KBr Press Pellet) spectra were recorded on SHIMADZU Spectrophotometer (λ_{max}).

METHODOLOGY

Method of Preparation of Synthesis of (7-chloro-6-fluro-1,3-benzothiazol-2-yl) acetyl chloride

The (7-chloro-6-fluro-1,3-benzothiazol-2-yl) acetyl chloride was prepared by the condensation of glacial acetic acid (20ml) with potassiumthiocyanateand1.45g(0.01mol)of 3-fluoro 4-chloroaniline by refluxing for20-30mins.Theproductobtainedwas isolated dried overnight, which was confirmed through TLC and recrystallized with ethanol.

Method of Preparation of Synthesis of 2-(7-chloro-6-fluro-1,3-benzothiazol-2-yl) acetohydrazide

Take 10 ml of concentrated hydrochloric acid in round bottom flask add 12ml of hydrazine hydrate drop wise cool the mixture and add 20.2gm (0.1 mol) of (7-chloro-6-fluro-1,3-benzothiazol-2-yl) acetyl chloride add 40ml of ethylene glycol refluxed for 8hrs the reaction mixture was monitored by TLC, then in hotcondition poured into crushed ice. Filter and dry the product and recrystallize formalcohol.Scheme for the synthesis of 2-(7-chloro-6-fluro-1,3-benzothiazol-2-yl) –N'-[(Z)-phenyl methylidene] acetohydrazide (SMVB-IIIA-IIIG) shown in Figure 2.

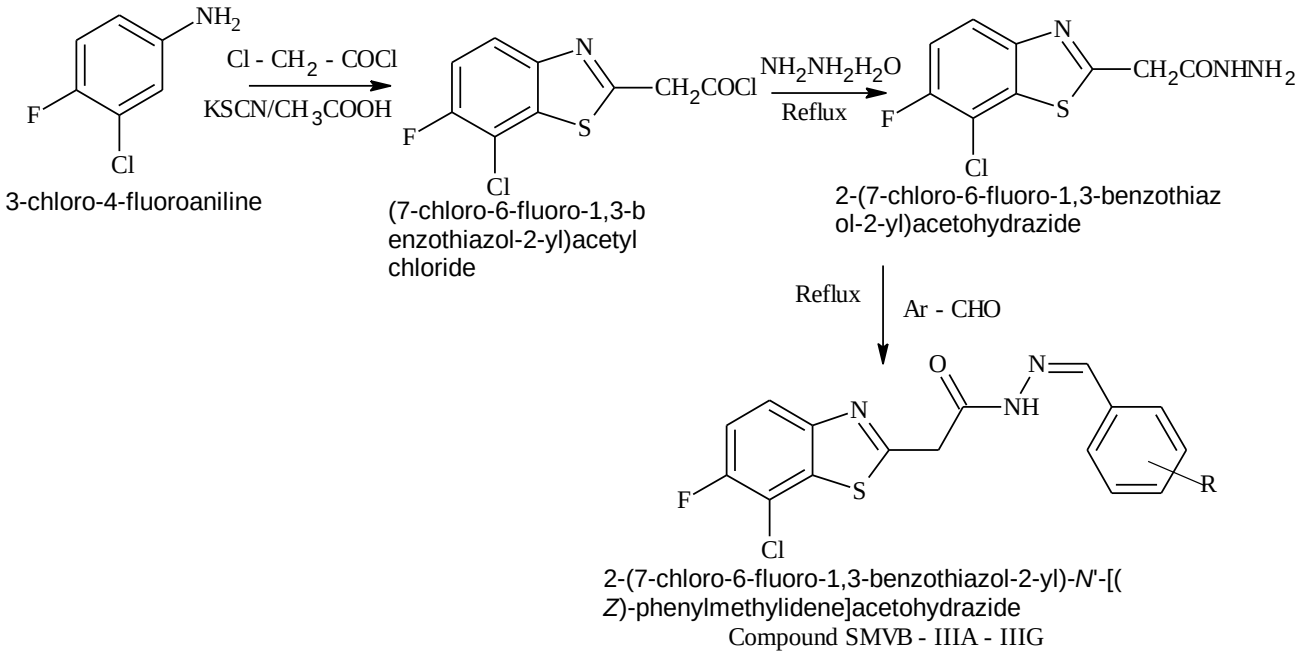
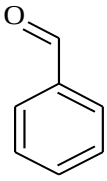
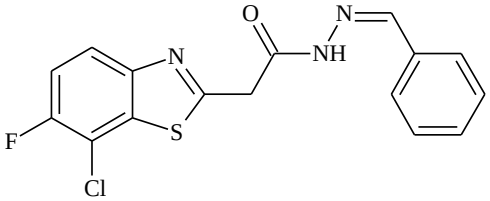
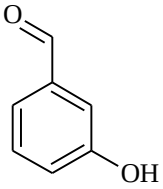
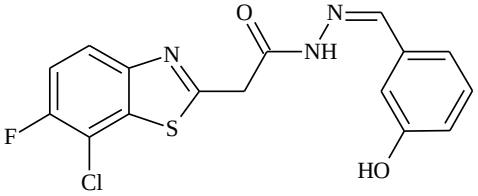
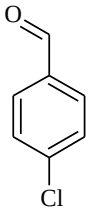
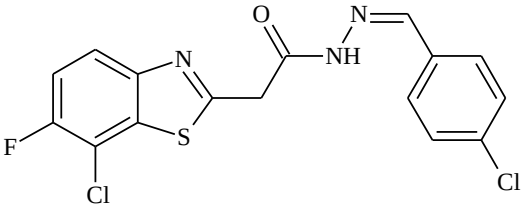
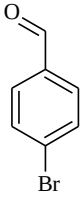
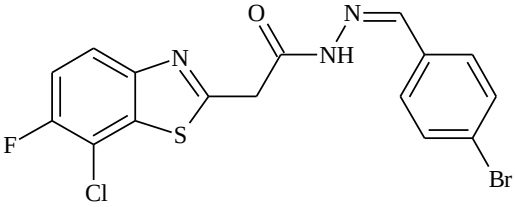
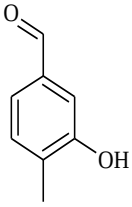
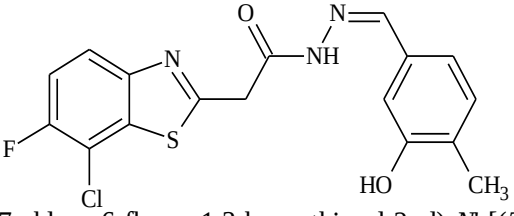
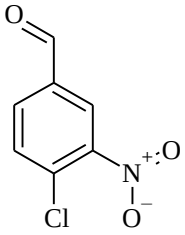
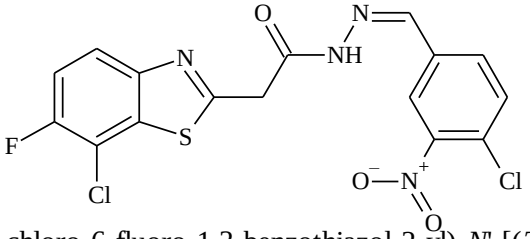


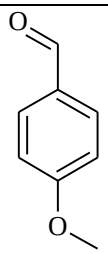
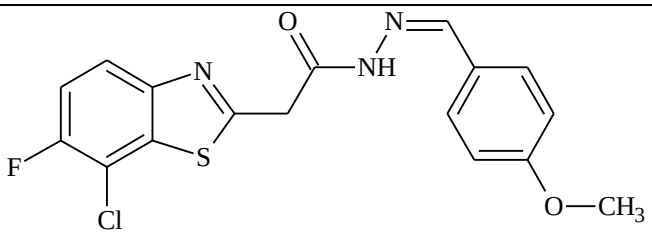
Figure 2: Representative Scheme for the synthesis of 2-(7-chloro-6-fluro-1,3-benzothiazol-2-yl) –N'-[(Z)-phenyl methylidene] acetohydrazide

Method of Preparation of Derivatives of 2-(7-chloro-6-fluro-1,3-benzothiazol-2-yl) –N'-[(Z)-phenyl methylidene] acetohydrazide (SMVB-IIIA-IIIG)

Toamixture of 2-(7-chloro-6-fluro-1,3-benzothiazol-2-yl) acetohydrazide [0.01mole] and substituted aromaticaldehydes [0.01mole], 10ml of ethanol was added with stirring. The mixture was keptin ice bath for 15-20 minutes and freshly prepared 10ml of 60% aqueous potassiumhydroxide solution was added drop wise to the above reaction mixture with constantstirring. The reaction mixture was then stirred for 4 hours. It was then acidified withdilute HCl and the precipitate formed was filtered, washed with cold water, dried andrecrystallized from ethanol. Derivatives of 2-(7-chloro-6-fluro-1,3-benzothiazol-2-yl) –N'-[(Z)-phenyl methylidene] acetohydrazide (SMVB-IIIA-IIIG)was shown in Table 1.

Table 1: Derivatives of 2-(7-chloro-6-fluro-1,3-benzothiazol-2-yl) –N'-[(Z)-phenyl methylidene] acetohydrazide (SMVB-IIIA-IIIG)

Compound Code	Substituted Name With Structure	Derivatives of 3-(5-sulfanyl-1,3,4-oxadiazol-2-yl) -2H-chromen-2-one (VBS-IVA-IVF)
SMVB-IIIA	 benzaldehyde	 2-(7-chloro-6-fluoro-1,3-benzothiazol-2-yl)-*N*'-[(*Z*)-phenylmethylidene]acetohydrazide
SMVB-IIIB	 3-hydroxybenzaldehyde	 2-(7-chloro-6-fluoro-1,3-benzothiazol-2-yl)-*N*'-[(*Z*) -(3-hydroxyphenyl)methylidene]acetohydrazide
SMVB-IIIC	 4-chlorobenzaldehyde	 2-(7-chloro-6-fluoro-1,3-benzothiazol-2-yl)-*N*'-[(*Z*)-(4-chlorophenyl)methylidene]acetohydrazide
SMVB-IIID	 4-bromobenzaldehyde	 *N*'-[(*Z*)-(4-bromophenyl)methylidene]-2-(7-chlor o-6-fluoro-1,3-benzothiazol-2-yl)acetohydrazide
SMVB-IIIE	 3-hydroxy-4-methylbenzaldehyde	 2-(7-chloro-6-fluoro-1,3-benzothiazol-2-yl)-*N*'-[(*Z*)-(3-h ydroxy-4-methylphenyl)methylidene]acetohydrazide
SMVB-IIIF	 4-chloro-3-nitrobenzaldehyde	 2-(7-chloro-6-fluoro-1,3-benzothiazol-2-yl)-*N*'-[(*Z*)-(4 -chloro-3-nitrophenyl)methylidene]acetohydrazide

SMVB-IIIG	 4-methoxybenzaldehyde	 2-(7-chloro-6-fluoro-1,3-benzothiazol-2-yl)-*N*'-[(*Z*)-(4-methoxyphenyl)methylidene]acetohydrazide

BIOLOGICAL EVALVATION

The animals used in the examination were sheltered in analogy of the Maratha Mandal'sNathajirao G Halgekar Institute of Dental Sciences and Research Centre Belgaum animal house, which follows the guidelines and regulation set by the committee for the control and administration of experiments on animals (CPCSEA), Ministry of social justice and empowerment, Government of India. The studies were attempted with previous approval from the Institutional Animal Ethics committee (IAEC) and ultimate care was taken to establish that the animals were handling in the most kind and satisfactory manner. Wister rats and albino mice of either sex, weighing 150-200 gm and 20-25 gm, respectively, were used. Pregnant females were eliminated.

ANTICONVULSANT ACTIVITY

Maximal Electro Shock Model

The MES test, developed by Toman and collaborators more than 60 years ago, is probably the best-validated preclinical test that predicts drugs effective against generalized seizures of the tonic–clonic (grand mal) type. It permits evaluation of the ability of a substance to prevent seizure spread through neural tissue. In the MES test, mice or rats receive an electrical stimulus of sufficient intensity to induce maximal seizures of their hind limbs, with tonic extension as the endpoint of the test. MES-induced seizures was defined as the absence of tonic extension of the hind leg a. After 0.5 and 4.0 h of drug administration, the activities were evaluated in MES test.

Maximal Electro Shock Model: For the assessment of anticonvulsant activity, the Swiss albino mice (25-30gm) of either sex were used. The animals were obtained from animal house animals were divided into five groups of five animals each Swiss albino mice.

Group I received Normal saline

Group II received Phenytoin

Group III received 25 mg/kg of derivatives of 1,3,4-oxadiazoles

Corneal electrodes were used for bilateral delivery of electrical stimulus. Electroconvulsive shock (50 mA for 0.2 sec) was delivered through corneal electrode to induce Hind Limb Tonic Extensor (HLTE) phase in mice. There are five phases observed in mice after giving maximal electroshock. The five phases are (i) Flexor (ii) Extensor (iii) Convulsion (iv) Stupor and (v) Recovery or Death are noted and also the time spent by mice in each phase. Prior to delivery, the current output was checked by using millimeter. The orientation for the anticonvulsant affect was abolition of HLTE within 10 sec after delivery of the electroshock statical analysis.

RESULTS AND DISCUSSION

Thenovel derivatives of benzothiazole obtained from 3-chloro-4-flouro aniline treated with potassium thiocynide with chloro acetyl chloride gives (7-chloro-6-fluro-1,3-benzothiazol-2-yl) acetyl chloride which is converted into hydrazide and yields the resultant compound derivatives of 2-(7-chloro-6-fluro-1,3-benzothiazol-2-yl) –N'-[(Z)-phenyl methylidene] acetohydrazide (SMVB-IIIA-IIIG).

A synthesized compound SMVB-IIIC i.e. 2-(7-chloro-6-fluoro-1,3-benzothiazol-2-yl)-N'-[(Z)-(4-chlorophenyl)methylidene]acetohydrazide was confirm by IR spectracharacteristic peak of -NH stretching at 3190-3210cm-1, Aromatic CH stretching at 2930-3180cm-1, Aliphatic CH at 2400-2550cm-1, C=O absorption at 1650 cm-1, -Halogens stretching at 750-850 cm-1. An IR spectrum was shown in Figure 3.

A synthesized compound SMVB-IIIE i.e. 2-(7-chloro-6-fluoro-1,3-benzothiazol-2-yl)-*N*'-[(*Z*)-(3-hydroxy-4-methylphenyl)methylidene]acetohydrazide was confirm by IR spectra characteristic peak of -NH stretching at 3290-3250cm^{-1}, AromaticCHstretching at 3150-3210cm^{-1}, AliphaticCH at 2420-2550cm^{-1}, C=Ostretching at 1620 cm^{-1}, -Halogens stretching at 730-820 cm^{-1}, Alkyl Group $–CH_3$ at 910-1050 cm^{-1}. An IR spectrum was shown in Figure 4.

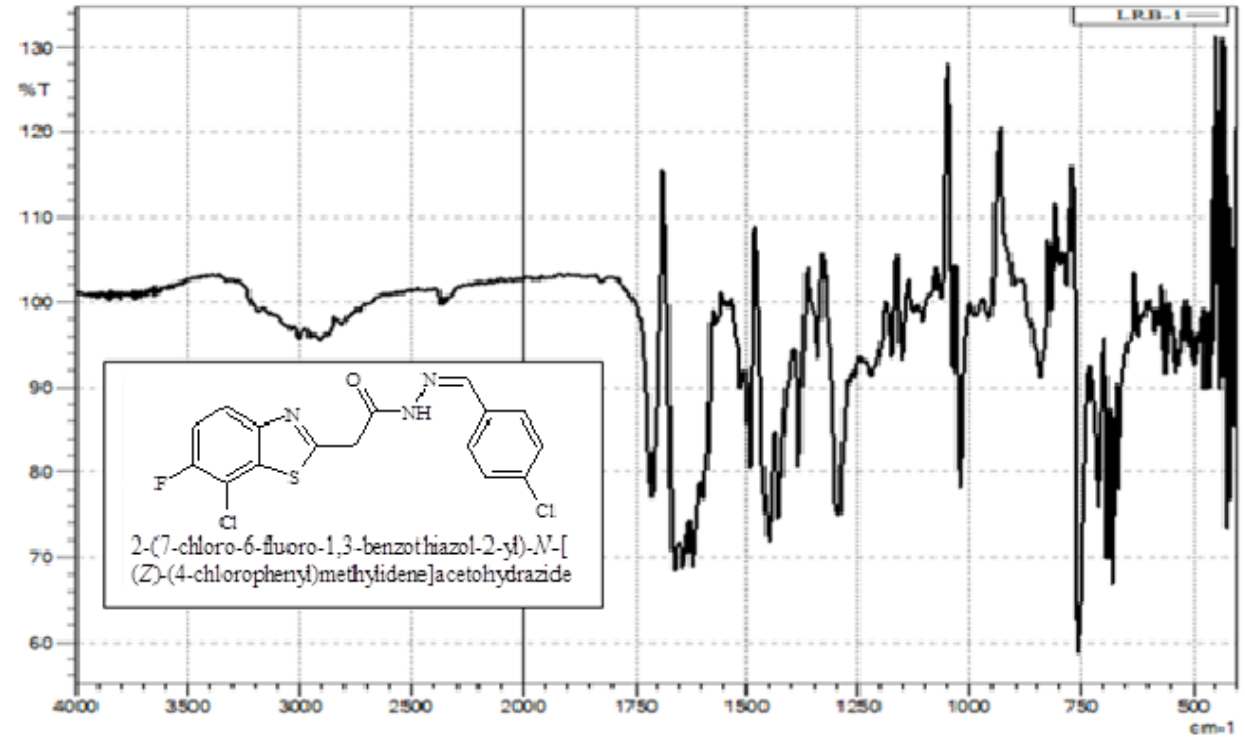


Figure 3: FT-IR Spectrum of Compound SMVB-IIIC

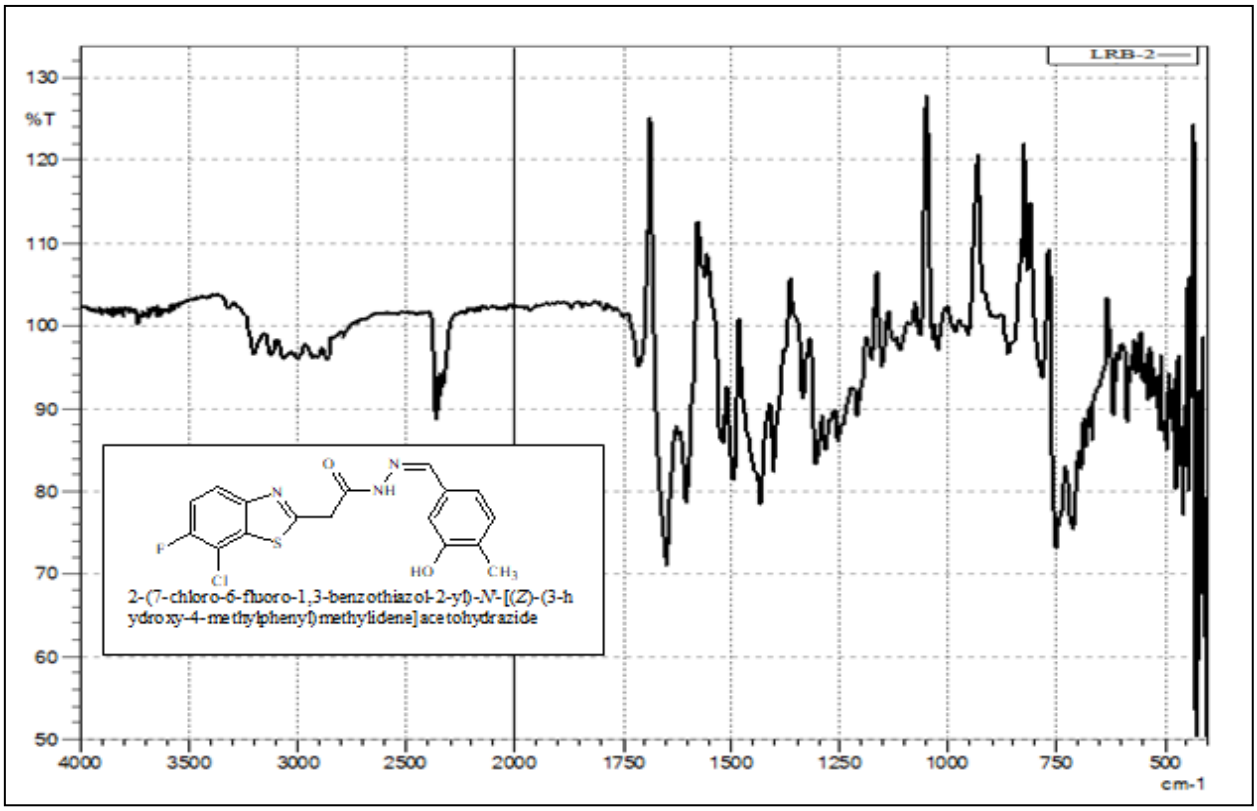


Figure 4: FT-IR Spectrum of Compound SMVB-IIIE

A synthesized compound SMVB-IIIEconfirm by 1H-NMR spectra characteristic peak of δ 7.00 (-NH), δ 7.20-7.55 (Ar-H multiplate).1H-NMR spectrum was shown in Figure 5.

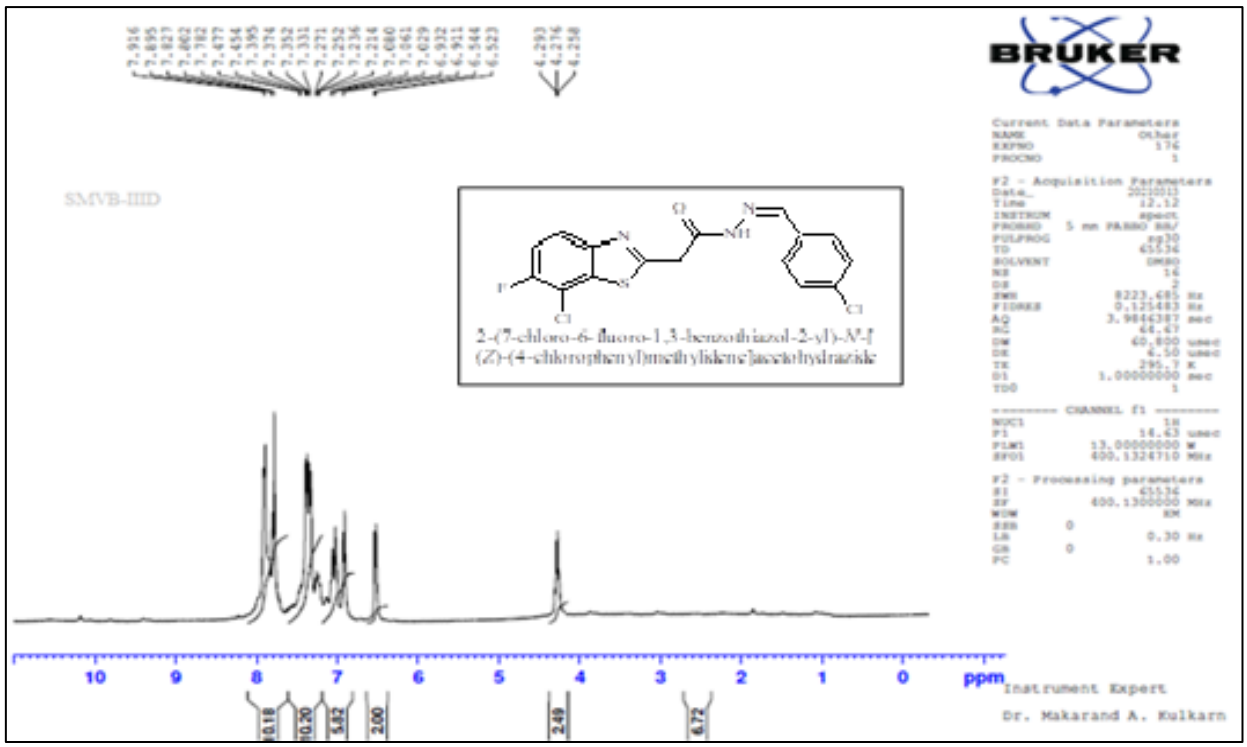


Figure 5: 1H-NMR Spectrum of Compound SMVB-IIIC

A synthesized compound SMVB-IIIE confirm by 1H-NMR spectra characteristic peak of δ 2.00-3.00 (-CH3), δ 10.844 (-OH hydroxy) δ 6.4-8.2 (Ar-H multiplate),δ 4.00-5.00 (-NH). 1H-NMR spectrum was shown in Figure 6.

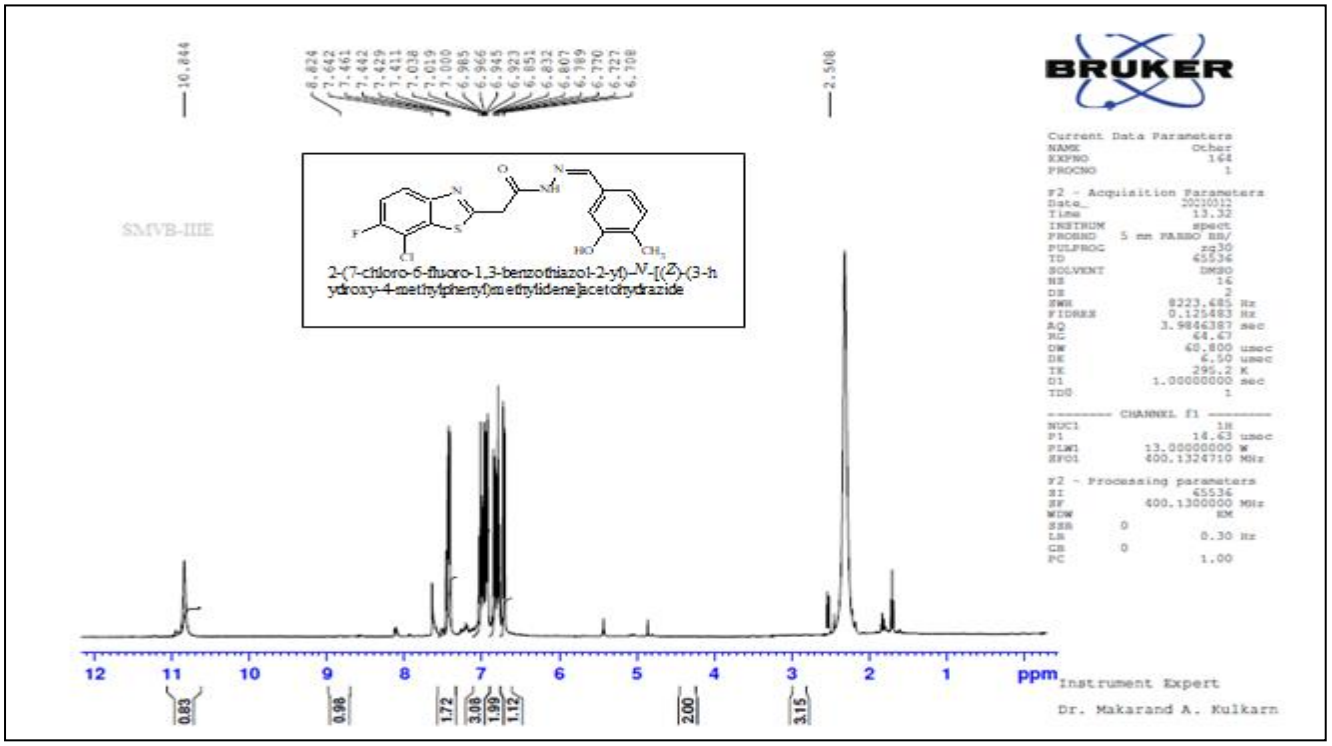


Figure 6: 1H-NMR Spectrum of Compound SMVB-IIIE

Synthesized compounds SMVB-IIIC confirm by mass spectra of M^+ Peaks (Mass Peak) at m/z 382.99 and Base Peak is 380.99and Molecular weight of compound SMVB-IIIC is 382.99mass spectrums was shown in Figure 7.

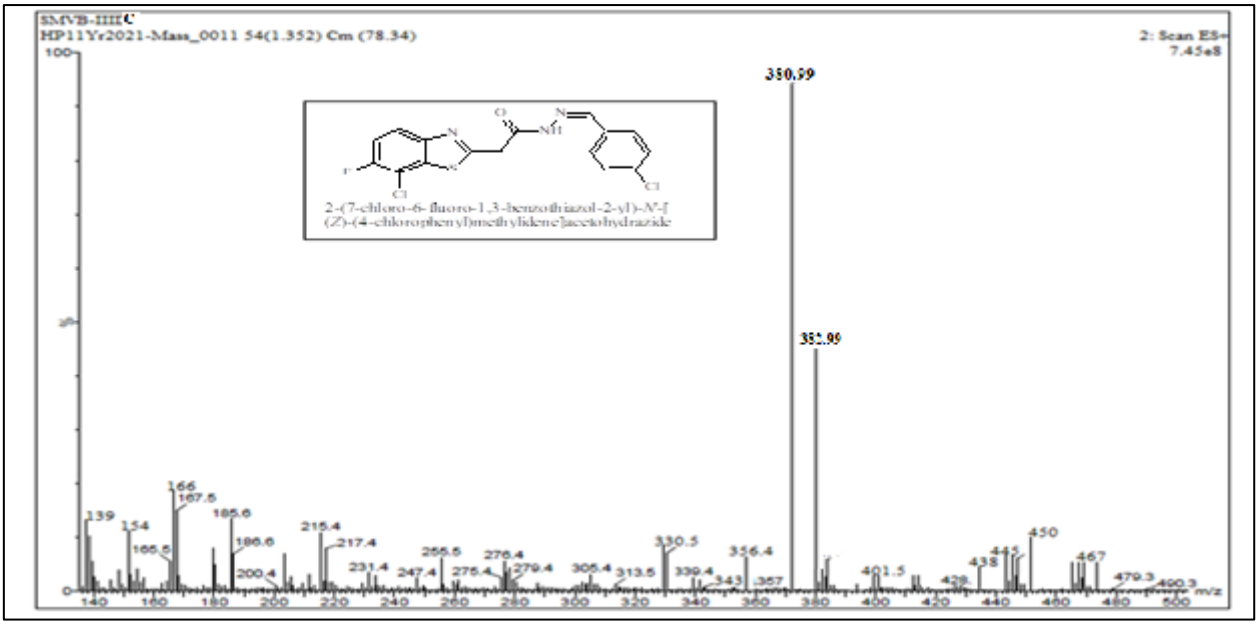


Figure 7: Mass Spectrum of Compound SMVB-IIIC

Synthesized compounds SMVB-IIIE confirm by Mass spectra of M^+ Peaks (Mass Peak)at m/z 427 and Base Peak is 418.5 and molecular weight of compound SMVB-IIIE is 427mass spectrums was shown in Figure 8.

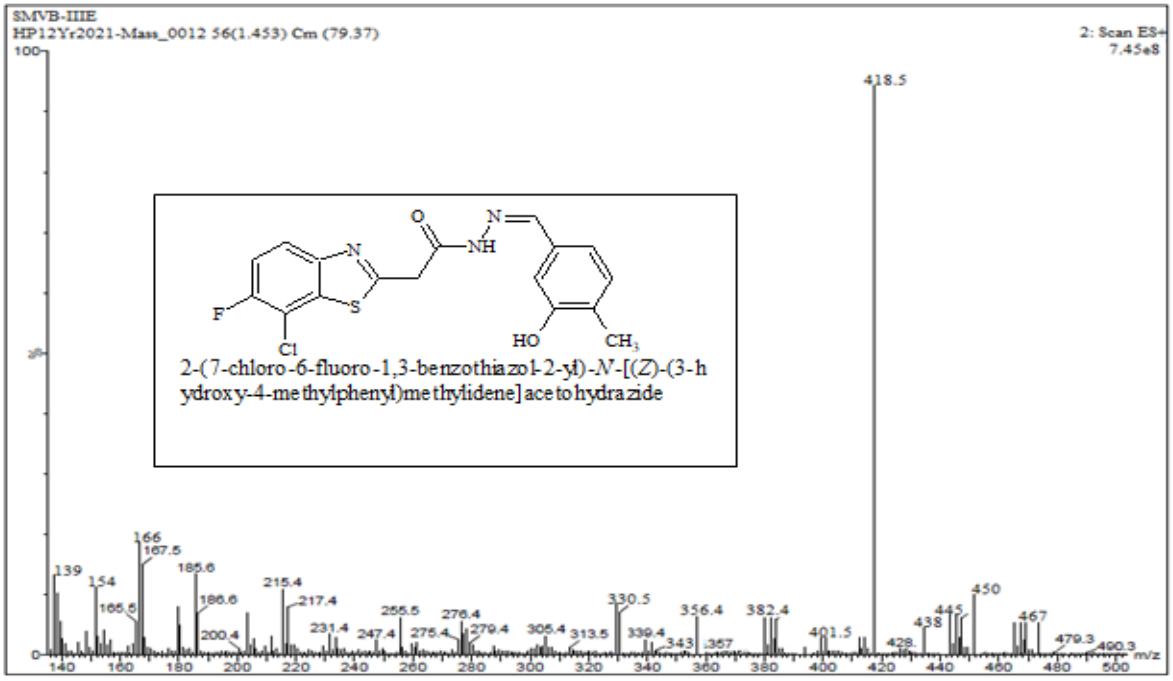


Figure 8: Mass Spectrum of Compound SMVB-IIIE

In-vivo Anticonvulsant Activity

The derivatives of 2-(7-chloro-6-fluro-1,3-benzothiazol-2-yl) –N'-[(Z)-phenyl methylidene] acetohydrazide (SMVB-IIIA-IIIG) have shown a anticonvulsant activity by MES method. Results are tabulated in table no.2 and graph was shown in Figure 9.

All the compounds showed activity in the range of 44-70% in comparison to Phenytoin which completely inhibited the convulsions produced by electroconvulsometer in albino mice. Compounds SMVB-IIIC, SMVB-IIIEand SMVB-IIIF showed maximal activity whereas remaining compounds showed good activity.

Table 2: Data showing *In-vivo* Anticonvulsant Activity (MES) % Protection of Derivatives of 2-(7-chloro-6-fluro-1,3-benzothiazol-2-yl) –N'-[(Z)-phenyl methylidene] acetohydrazide (SMVB-IIIA-IIIG)

Comp code	Dose mg/kg	Anticonvulsant Activity(MES) % Protection
SMVB-IIIA	25	60.20
SMVB-IIIB	25	58.34
SMVB-IIIC	25	70.87
SMVB-IIID	25	48.33
SMVB-IIIE	25	68.48
SMVB-IIIF	25	69.45
SMVB-IIIG	25	44.56
Phenytoin Sodium	25	100

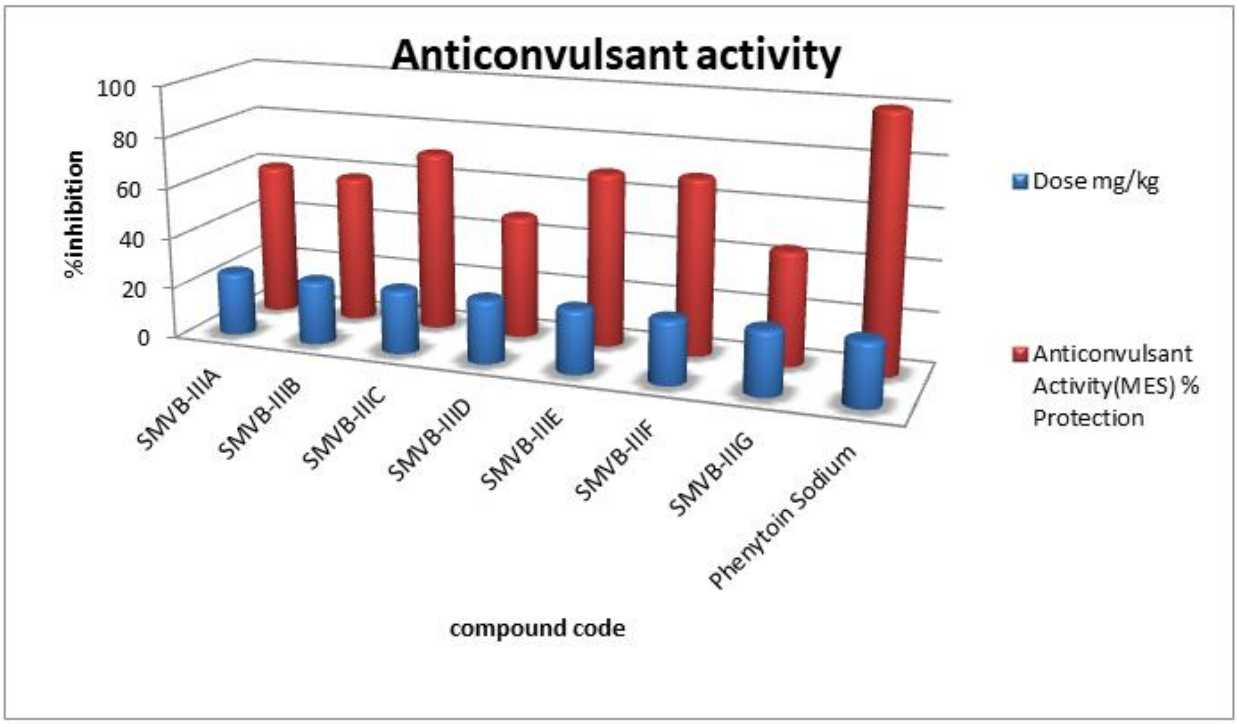


Figure 9: *In-vivo* Anticonvulsant Activity (MES) of Derivatives of 2-(7-chloro-6-fluro-1,3-benzothiazol-2-yl) –N'-[(Z)-phenyl methylidene] acetohydrazide (SMVB-IIIA-IIIG)

CONCLUSION

Duringthepresentinvestigation,thenovel derivatives of benzothiazole obtained from 3-chloro-4-flouro aniline treated with potassium thiocynide with chloro acetyl chloride gives (7-chloro-6-fluro-1,3-benzothiazol-2-yl) acetyl chloride which is converted into hydrazide and yields the resultant compound derivatives of 2-(7-chloro-6-fluro-1,3-benzothiazol-2-yl) –N'-[(Z)-phenyl methylidene] acetohydrazide (SMVB-IIIA-IIIG).All the compounds synthesized were confirmed by spectral data and evaluated for their anticonvulsant activity.

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