FINAL REPORT OF WORK DONE ON THE MINOR RESEARCH PROJECT

Title

SYNTHESIS OF 1-(5-BROMO-2-CHLOROPYRIMIDIN-4-YL) HYDRAZINE DERIVATIVES AND THEIR ANTI-CONVULSANT ACTIVITY

Ref: 2177-MRP/15-16/KAMY013/UGC-SWRO

Submitted to
Joint Secretary
University Grants Commission (UGC)
SOUTH WESTERN REGIONAL OFFICE
P.K Block, Palace Road, Gandhinagar
Banglore-560 009

Submitted by
Dr. L. MALLESHA
Principal Investigator
Assistant Professor & HOD
PG Department of Chemistry
JSS College of Arts Commerce & Science
B.N. Road, Mysuru-25

UNIVERSITY GRANTS COMMISSION

FINAL REPORT OF THE WORK DONE ON THE MINOR RESEARCH PROJECT

1. Project reference No: **Final Report**

2. UGC Reference No: **2177-MRP/15-16/KAMY013/UGC-SWRO**

3. Period of report: **From March 2016 to March 2018**

4. Title of research project: **SYNTHESIS OF 1-(5-BROMO-2-CHLOROPYRIMIDIN-4-YL) HYDRAZINE DERIVATIVES AND THEIR *IN VIVO* ANTI-CONVULSANT ACTIVITY**

5. (a) Name of the Principal Investigator: **Dr. L. Mallesha**

(b) Department and University/College where work has progressed:
PG Department of Chemistry, JSS College of Arts Commerce & Science, B N Road, Mysuru-25

6. Effective date of starting of the project **31-03-16**

7. Grant approved and expenditure incurred during the period of the report:

a. Total amount approved **Rs.4,90,000/-**
(1^{st} sanctioned amount: Rs. 3,55,000/-, 2^{nd} sanctioned amount: Rs. 1,02,122/-)

b. Total expenditure **Rs. 4,89,980/-**

c. Report of the work done:

i. Brief objective of the project

- To encourage the development of research in the synthesis of pyrimidine analoges in the field of reducing inflammation in India with a view to manufacture and encourage such products in developing countries at affordable costs.
- To further studies on the basic mechanisms of anti-convulsant activity of heterocyclic derivatives.
- To grow and support standardized practices and research in the field of epileptic seizures diseases in India.

- To promote continuing education in the field of epileptic seizures, organizing, special lectures and publishing journals on academic matters pertaining to the society.
- To do every anti-convulsant activity to achieve the above objectives and carry out all actions necessary for harmonious and cogent execution of the above objectives.

ii Work done so far

A mixture of 5-bromo-2,4-dichloropyrimidine (1) (0.01 mol) in methanol was taken and cooled to 0–5 °C in an ice bath. Trietheylamine (0.01 mol) was added to the cold reaction mixture and then hydrazine hydrate (0.012 mol) was added slowly at 5-10 °C. The reaction mass was allowed to stir at room temperature for 1h. The solid thus obtained was filtered, washed with chilled water and dried to afford 1-(5-bromo-2-chloropyrimidin-4-yl)hydrazine. Compound, 1-(5-bromo-2-chloropyrimidin-4-yl)hydrazine was dissolved in ethanol and aryl aldehyde was added to it. The content were refluxed on a water bath for 1 h and allowed to stand at room temperature. The crystalline solid thus obtained, was filtered, washed with ethanol and dried to afford compounds aryl-(5-bromo-2-chloro-pyrimidine-4-yl)hydrazone.

The title compounds of pyrimidines analogues so synthesized and characterized by different spectral studies. The newly synthesized compounds were screened for their anticonvulsant activity against maximal electroshock seizure (MES) model in male wistar rats and compared with the standard drug phenytoin. The neurotoxic effects were determined by rotorod test by using mice.

iii. Has the progress been according to original plan of work and towards achieving the objective. if not, state reasons:

Yes, work done has fulfilled the objective.

iv. Please indicate the difficulties, if any, experienced in implementing the project: **No**

v. If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet: **Completed**

vi. If the project has been completed, please enclose a summary of the findings of the study. Two bound copies of the final report of work done may also be sent to the Commission:
Report Copy enclosed

vii. Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as (a) Manpower trained (b) Ph. D. awarded (c) Publication of results (d) other impact, if any

Sl. No.	**Particulars**	
a	Manpower trained	I have trained one M.Sc. Student
b	Ph. D. awarded	One Student Registered under University of Mysore
c	Publication of results	01Paper-Accepted (Asian Journal of Research in Chemistry, UGC ID: 4008). 01Paper-Communicated
d	Project papers presented in Conferences	Presented 02- Project papers in national Conference and International Symposium.

ISAM - 2017

ONE DAY INTERNATIONAL SYMPOSIUM
on
ADVANCED MATERIALS

Department of Chemistry
Sri Jayachamarajendra College of Engineering
JSS Science & Technology University
Mysuru - 570 006

Certificate

This is to certify that Mr / Mrs / Dr .....L. Mallesha.....
of ...J.S.S. College of Arts, Commerce and Science, Mysuru.
participated in One Day International Symposium on Advanced Materials (ISAM-17) held on 27th December 2017 organized by Department Of Chemistry, Sri Jayachamarajendra College of Engineering, JSS Science & Technology University , Mysuru, Karnataka, India.

He/she has presented Oral / Poster presentation and has been awarded Best Paper Presentation.

P. Mall
Dr. P. Mallu
Organizing Secretary

Dr. T.N. Nagabushana
Principal

PP-02

Synthesis of 1,2,4-triazole derivatives and their anticonvulsant activity

G.Vinay[1], D. M. Gurudatt[2], L. Mallesha[1,*] and B. Veeresh[3]

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[2]Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore 570 006, India
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A series of 1,2,4-triazole derivatives **(5a-g)** have been accomplished with excellent yields by the oxidation of pyrimidinylhydrazines of various aryl aldehydes with iodobenzene diacetate. The chemical structures of the synthesized compounds were confirmed by FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectral studies. All the compounds were screened for their anticonvulsant activity against maximal electroshock (MES) seizure method and their neurotoxic effects were determined by rotorod test. Compound **5f** was found to be the most potent of this series. The same compound showed no neurotoxicity at the maximum dose administered (100 mg/kg).

PP-03

Extracellular Synthesis of Gold Nanoparticles by *Azospirillum brasilense* and Promising Antibacterial and Anti-inflammatory Agent with Good Biocompatibility

H. P. Spoorthy[a,b], C. S. Karthik[c] and S. Satish[a,*]

[a] DOS in Microbiology, University of Mysore, Mysuru-06, Karnataka, India
[b] Department of Microbiology, JSS College, B. N. Road, Mysuru-25, Karnataka, India
[c] Department of Chemistry, Sri Jayachamarajendra College of Engineering, Mysuru-06, Karnataka, India.
**Corresponding Author E-mail: satish.micro@gmail.com*

Gold nanoparticles (AgNPs) were biosynthesized by using bacteria, *Azospirillum brasilense (Ab)*. The extracellular synthesis of gold nanoparticles is fast and lead to the development of an easy bioprocess. The characterization of nanoparticles was carried out using various spectroscopic techniques such as UV-visible, SEM, XRD, EDAX, DLS and FT-IR Spectroscopy.

KSTA

UNIVERSITY
Bengaluru, India

Certificate

This is to certify that

Dr/Mr/Ms L. MALLESHA

of PG Chemistry, JSS College, Mysore - 25 has presented a poster in

10th Annual Conference of
KARNATAKA SCIENCE AND TECHNOLOGY ACADEMY 2018

jointly organized by Karnataka Science and Technology Academy and REVA University

held during 18 - 19 January 2018

Poster Title Anticonvulsant activity of 5-bromo-2,4-dichloropyriminide derivatives: Synthesis and Characterization.

Dr. H. Honne Gowda
CEO & Member Secretary
KSTA

Dr. M. Dhanamjaya
Registrar
REVA University

ethanol production in *Zymomonas mobilis* NCIM 2915 (44.97±3.21 g/L) and *Schizosaccharomyces pombe*NCIM 3457 (42.60±3.0 g/L), average ethanol production in *Saccharomyces cerevisiae* NCIM 3095 (33.13±1.96 g/L) and very low ethanol production in *Candida shehatae* NCIM 3500 (25.24±2.30 g/L). In simultaneous saccharification and fermentation process, it showed the higher ethanol production in *Zymomonas mobilis* NCIM 2915 (47.34±3.22 g/L) and *Saccharomyces uvarum* NCIM 3455 (44.18±2.67 g/L), average ethanol production in *Pichia stipitis* NCIM 3498 (34.71±1.89 g/L), and very low ethanol production in *Saccharomyces cerevisiae* NCIM 3095 (26.82±2.63 g/L) was monitored after the fermentation process. Structural changes of areca nut husk before and after acid pre-treatment were further investigated through Scanning electron microscopy (SEM) and Fourier transformed infrared spectroscopy (FTIR). Hence, acid and enzymatic pre-treatment is more effective for ethanol production. Areca nut husk was revealed as a suitable substrate for ethanol production.

Key words: Acid pretreatment, Enzymatic hydrolysis, Laccase, Areca nut husk, Yeasts, *Zymomonas mobilis* NCIM 2915, Bioethanol.

CLS-53: Synthesis and biological activity of metal nanoparticles via biological entity

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[a]*DOS in Microbiology, Manasagangotri, Mysuru-570006, Karnataka, India*
[b]*PG Department of Biotechnology, JSS College,Ooty Road, Mysuru-25, India*

Abstract: Biosynthesis of metal nanoparticles using fungi *Claviceps paspali* by varying composition of media, pH and temperature. The synthesized nanoparticles were characterized by UV-visible spectroscopy, SEM and FT-IR Spectroscopy. Antimicrobial property of the nanoparticles was carried out against tested strains. Nanoparticles were also tested for their anti-angiogenic properties. This work revealed that metal nanoparticles can be easily produced by fungi and may be a feasible, low-cost, environmentally friendly method for generating stable nanoparticles.

CLS: 54: Anticonvulsant activity of 5-bromo-2,4-dichloropyrimidine derivatives: Synthesis and characterization

G.Vinay[1], L. Mallesha[1,*] and Bantal Veeresh[2]
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Abstract: A series of new 5-bromo-2,4-dichloropyrimidine derivatives, **3(a-f)** were synthesized by using different aryl aldehydes. The synthesized compounds were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectral studies. The newly synthesized compounds were screened for their anticonvulsant activity against maximal electroshock seizure (MES) model in male wistar rats and compared with the standard drug phenytoin. The neurotoxic effects were determined by rotorod test by using mice. Compound **3b** was found to be most potent of this series. The same compound showed no neurotoxicity at the maximum dose administered (100 mg/kg). The compound **3b** showed 68.15% protection in comparison to phenytoin which completely inhibited the convulsions produced by electro-convulsometer, Similarly, compounds **3e** and **3a** showed moderate protective effects and a significant difference in protectiveness were observed when compared to standard group.

Keywords: Pyrimidine, Aldehyde, Characterization, MES, Neurotoxicity.

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RESEARCH ARTICLE

Anticonvulsant activity of 5-bromo-2,4-dichloropyrimidine derivatives: Synthesis and characterization

Lingappa Mallesha[1*], Guruswamy Vinay[1], Bantal Veeresh[2]

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*Corresponding Author E-mail: **mallesha83@gmail.com**

ABSTRACT:

A series of new 5-bromo-2,4-dichloropyrimidine derivatives, **3(a-f)** were synthesized by using different aryl aldehydes. The synthesized compounds were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectral studies. The newly synthesized compounds were screened for their anticonvulsant activity against maximal electroshock seizure (MES) model in male wistar rats and compared with the standard drug phenytoin. The neurotoxic effects were determined by rotorod test by using mice. Compound **3b** was found to be most potent of this series. The same compound showed no neurotoxicity at the maximum dose administered (100 mg/kg). The compound **3b** showed 68.15% protection in comparison to phenytoin which completely inhibited the convulsions produced by electro-convulsometer. Similarly, compounds **3e** and **3a** showed moderate protective effects and a significant difference in protectiveness were observed when compared to standard group.

KEYWORDS: Pyrimidine, Aldehyde, Characterization, MES, Neurotoxicity.

INTRODUCTION:

Epilepsy is not a singular disease entity but a variety of disorders reflecting underlying brain dysfunction that may result from many different causes.[1] Therefore, there is continuing demand for new anticonvulsant agents. So, there is an urgent requirement for the discovery and development of some novel anticonvulsant agents with more selective activity and lower toxicity for the effective treatment of epilepsy. Several five-member aromatic systems having three heteroatom at symmetrical positions such as thiadiazoles have been studied extensively owing to their interesting pharmacological activities.

The word epilepsy usually describes a group of common chronic neurological disorders characterized by recurrent unprovoked seizures due to synchronous neuronal activity in the brain. Several new drugs have appeared on the market, the development of novel agents, particularly compounds effective against complex partial seizures remains a major focus of antiepileptic drug research.[2] A review on new structural entities having anticonvulsant activity has recently appeared.[3] The anticonvulsants are a diverse group of pharmaceuticals used in the treatment of epileptic seizures. The goal of an anticonvulsant is to suppress the rapid and excessive firing of neurons that start a seizure.

The structural diversity and biological importance of nitrogen containing heterocycles have made them attractive targets for synthesis over many years. They are found in various natural products and have been identified as products of chemical and biological importance. Pyrimidine is considered to be a resonance

LIST OF RESEARCH PUBLICATIONS (Published/Communicated): 02

1. **L. Mallesha,** Vinay G. and B. Veeresh. Anticonvulsant activity of 5-bromo-2,4-dichloropyrimidine derivatives: Synthesis and characterization. **Asian Journal of Research in Chemistry** (Accepted, 2018). ICI, 4008. ISSN: 0974-4150, A&V Publications.

2. **L. Mallesha**,Vinay G., D. M. Gurudattand B. Veeresh. Synthesis of 1,2,4-triazole derivatives and their anticonvulsant activity (Communicated).

WORKSHOP/CONFERENCE/SEMINAR-PAPER PRESENTED: 02

1. Two days 10th Annual Conference of Karnataka Science and Technology Academy (KSTA) held at **Revauniversity, Bengaluru** on **18-19th January, 2018**. **Title:** Anticonvulsant activity of 5-bromo-2,4-dichloropyrimidine derivatives: Synthesis and characterization.

2. One day International Symposium on Advanced Materials held at **SJCE, JSS Science and Technology University, Mysuru** on **27thDecember 2017**. **Title:** Synthesis of 1,2,4-triazole derivatives and their anticonvulsant activity.

SYNTHESIS OF 1-(5-BROMO-2-CHLOROPYRIMIDIN-4-YL) HYDRAZINE DERIVATIVES AND THEIR *IN VIVO* ANTI-CONVULSANT ACTIVITY

Introduction

Epilepsy is not a singular disease entity but a variety of disorders reflecting underlying brain dysfunction that may result from many different causes.[1] Therefore, there is continuing demand for new anticonvulsant agents. So, there is an urgent requirement for the discovery and development of some novel anticonvulsant agents with more selective activity and lower toxicity for the effective treatment of epilepsy. Several five-member aromatic systems having three heteroatom at symmetrical positions such as thiadiazoles have been studied extensively owing to their interesting pharmacological activities. The word epilepsy usually describes a group of common chronic neurological disorders characterized by recurrent unprovoked seizures due to synchronous neuronal activity in the brain. Several new drugs have appeared on the market, the development of novel agents, particularly compounds effective against complex partial seizures remains a major focus of antiepileptic drug research.[2] A review on new structural entities having anticonvulsant activity has recently appeared.[3] The anticonvulsants are a diverse group of pharmaceuticals used in the treatment of epileptic seizures. The goal of an anticonvulsant is to suppress the rapid and excessive firing of neurons that start a seizure.

The structural diversity and biological importance of nitrogen containing heterocycles have made them attractive targets for synthesis over many years. They are found in various natural products and have been identified as products of chemical and biological importance. Pyrimidine is considered to be a resonance hybrid of the charged and uncharged cannonical structures; its resonance energy has been found to be less than benzene or pyridine. The pyrimidine moiety is a versatile lead molecule in pharmaceutical development and has a wide range of biological activities. In the past few years, the therapeutic interest of pyrimidine derivatives in pharmaceutical and medicinal field has been given a great attention to the medicinal chemist. Pyrimidine ring is fused to various heterocycles, that represent an important class of heterocyclic compounds having wide range of applications.[4,5] The existing methods for the preparation of triazolopyrimidines are based on heterocyclic hydrazones. Pyrimidines and their derivatives are considered to be important for drugs and agricultural chemicals. A large

number of pyrimidine derivatives are reported to exhibit antimycobacterial,[6] antitumor,[7] antiviral,[8] anticancer,[9] anti-inflammatory[10] and antimicrobial[11] activities. We have reported the synthesis and biological activity of 1-(5-bromo-2-chloropyrimidin-4-yl)hydrazine derivatives.[12] In the present study, a series of new pyrimidine analogues, **3(a-f)** have been synthesized and their anticonvulsant activity were determined.

Materials and methods

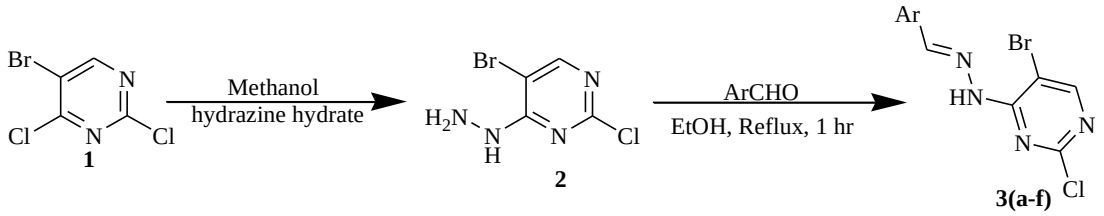
All solvents and reagents were purchased from Sigma Aldrich Chemicals Pvt Ltd/SD Fine Chemicals. Melting range was determined by GLNR SELEC apparatus. The new compounds were analyzed with FT-IR spectrophotometer (Agilent FT-IR ATR Cary 630) in the range of 7000-350 cm^{-1}. ^1H NMR spectra were recorded on Bruker DRX -500 spectrometer at 400 MHz using d_6-DMSO as solvent and TMS as an internal standard. The mass spectra of the samples were recorded using the instrument LC-MSD-Trap-XCT.

Synthesis of 1-(5-bromo-2-chloropyrimidin-4-yl)hydrazine (2)

A mixture of 5-bromo-2,4-dichloropyrimidine (**1**) (0.01 mol) in methanol was taken and cooled to 0–5 °C in an ice bath. Trietheylamine (0.01 mol) was added to the cold reaction mixture and then hydrazine hydrate (0.012 mol) was added slowly at 5-10 °C. The reaction mass was allowed to stir at room temperature for 1h. The solid thus obtained was filtered, washed with chilled water and dried to afford compound **2** (Yellow solid). Yield- 83 %. ^1H NMR (DMSO-d_6, 400 MHz) δ: 8.05 (s, 1H, NH), 7.80 (s, 1H, py-H), 4.30 (s, 2H, NH_2).

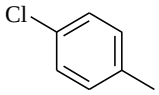
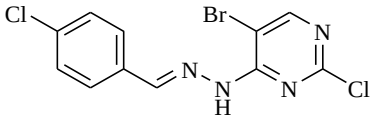
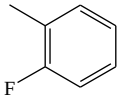
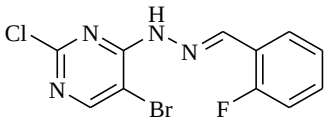
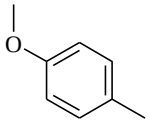
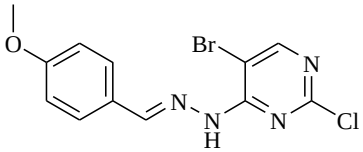
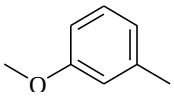
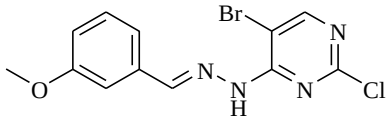
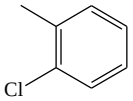
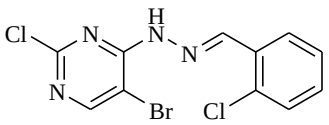
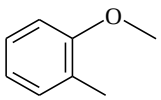
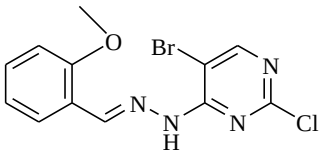
*General procedures for the synthesis aryl-(5-bromo-2-chloro-pyrimidine-4-yl)hydrazone **3(a-f)***

Compound **2** was dissolved in ethanol and aryl aldehyde was added to it. The content were refluxed on a water bath for 1 h and allowed to stand at room temperature. The crystalline solid thus obtained, was filtered, washed with ethanol and dried to afford compound **3(a-f)**. New pyrimidine derivatives, **3(a-f)** were synthesized according to Scheme 1. The chemical structures of all the synthesized compounds are tabulated in Table 1. Readily available starting materials and simple synthesizing procedures make this method is very attractive and convenient for the synthesis of various pyrimidines.



Scheme 1

Table 1: Chemical structure of pyrimidine derivatives **3(a-f)**.

Compound	Ar	Structure
3a		
3b		
3c		
3d		
3e		
3f		

1-(4-Chlorobenzylidene)-2-(5-bromo-2-chloropyrimidin-4-yl)hydrazine (3a)

The product obtained from (**2**) (0.01 mol) and 4-chlorobenzaldehyde (0.02 mol). Brown solid, Yield: 84 %, M.p.: 155-157 °C. FT-IR (KBr, cm^{-1}): 3435 (NH), 2950 (C-H), 1610 (C=N), 1375 (C-N), 715 (C-Cl), 520 (C-Br). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.38 (s, 1H, NH), 8.51 (s, 1H, Py-H), 8.48 (s, 1H, CH), 7.91 (d, 1H, J = 8.20 Hz, Ar-H), 7.87 (d, 1H, J = 8.10 Hz, Ar-H), 7.72 (s, 1H, Ar-H). MS (ESI) *m/z*: 381.56.

1-(2-Fluorobenzylidene)-2-(5-bromo-2-chloropyrimidin-4-yl)hydrazine (3b)

The product obtained from (**2**) (0.01 mol) and 2-fluorobenzaldehyde (0.012 mol). Yellow solid, Yield: 88 %, M.p.: 174-176 °C. FT-IR (KBr, cm^{-1}): 3490 (NH), 2935 (C-H), 1613 (C=N), 1370 (C-N), 1090 (C-F), 725 (C-Cl). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.74 (s, 1H, NH), 8.29 (s, 1H, Py-H), 8.26 (s, 1H, CH), 7.20 (d, 1H, J = 7.42 Hz, Ar-H), 7.03 (d, 1H, J = 7.15 Hz, Ar-H). MS (ESI) *m/z*: 373.1.

1-(4-Methoxybenzylidene)-2-(5-bromo-2-chloropyrimidin-4-yl)hydrazine (3c)

The product obtained from (**2**) (0.01 mol) and 4-methoxyenzaldehyde (0.012 mol). White solid, Yield: 85 %, M.p.: 151-153 °C. FT-IR (KBr, cm^{-1}): 3447 (NH), 2930 (C-H), 1612 (C=N), 1375 (C-N), 720 (C-Cl). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.21 (s, 1H, NH), 8.52 (s, 1H, Py-H), 8.47 (s, 1H, CH), 7.40 (t, 1H, J = 7.84 Hz, Ar-H), 7.31 (d, 1H, J = 7.64 Hz, Ar-H), 7.25 (s, 1H, Ar-H), 7.06 (d, 1H, J = 7.61 Hz, Ar-H), 3.81 (s, 3H, OCH_3). MS (ESI) *m/z*: 342.7.

1-(3-Methoxybenzylidene)-2-(5-bromo-2-chloropyrimidin-4-yl)hydrazine (3d)

The product obtained from (**2**) (0.01 mol) and 3-methoxybenzaldehyde (0.012 mol). White solid, Yield: 92 %, M.p.: 141-143 °C. FT-IR (KBr, cm^{-1}): 3349 (NH), 2928 (C-H), 1618 (C=N), 1375 (C-N), 725 (C-Cl). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.31 (s, 1H, NH), 8.74 (s, 1H, Py-H), 8.49 (s, 1H, CH), 7.51-7.50 (m, 2H, Ar-H), 7.20 (t, 1H, J = 6.60 Hz, Ar-H). MS (ESI) *m/z*: 350.0.

1-(2-Chlorobenzylidene)-2-(5-bromo-2-chloropyrimidin-4-yl)hydrazine (3e)

The product obtained from (**2**) (0.01 mol) and 2-chlorobenzaldehyde (0.012 mol). White solid, Yield: 92 %, M.p.: 143-145 °C. FT-IR (KBr, cm^{-1}): 3345 (NH), 2930 (C-H), 1615 (C=N), 1380 (C-N), 725 (C-Cl). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.31 (s, 1H, NH), 8.74 (s, 1H, Py-H), 8.49 (s, 1H, CH), 7.51-7.50 (m, 2H, Ar-H), 7.20 (t, 1H, J = 6.60 Hz, Ar-H). MS (ESI) *m/z*: 350.0.

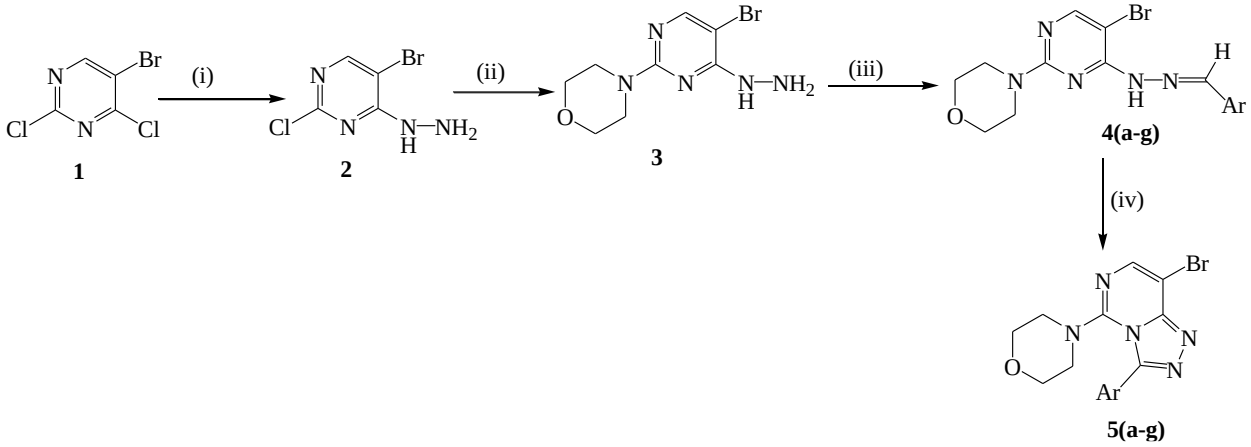
1-(2-Methoxybenzylidene)-2-(5-bromo-2-chloropyrimidin-4-yl)hydrazine (3f)

The product obtained from (**2**) (0.01 mol) and 2-methoxybenzaldehyde (0.02 mol). White solid, Yield: 85 %, M.p.: 155-157 °C. FT-IR (KBr, cm^{-1}): 3440 (NH), 2945 (C-H), 1614 (C=N), 1372

(C-N), 715 (C-Cl). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.38 (s, 1H, NH), 8.51 (s, 1H, Py-H), 8.48 (s, 1H, CH), 7.91 (d, 1H, J = 8.20 Hz, Ar-H), 7.87 (d, 1H, J = 8.10 Hz, Ar-H), 7.72 (s, 1H, Ar-H). MS (ESI) *m/z*: 381.56.

*2.1. General procedures for the synthesis of (**5a-g**)*

Initially 1-(5-bromo-2-chloropyrimidin-4-yl)hydrazine (**2**) was synthesized by the reaction of 5-bromo-2,4-dichloropyrimidine (**1**) in MeOH and hydrazine hydrate, and the mixture was stirred for about 1 h at r.t. to afforded desired product **2** with high yield. The reaction of **2** with morpholine was carried out in the presence of EtOAc to yield 1-(5-bromo-2-morpholinopyrimidin-4-yl)hydrazine (**3**) and the yield was 75–85 %. The hydrazines (**4a-g**) were obtained by condensation of **3** (0.01 mol) with different aldehydes (0.01 mol) in EtOH. Compounds (**5a-g**) were obtained in good yield by the reaction of (**4a-g**) (0.01 mol) with IBD (0.012 mol) in MeOH and stirred for about 2 h at 15-20 °C. The synthesized molecules (**5a-g**) were structurally characterized by mass, ^{13}C NMR, ^{1}H NMR and FT-IR spectral studies. The synthetic route for the synthesis of (**5a-g**) is summarized in Scheme 1. Chemical structures and physical data of the synthesized compounds are given in Table 1

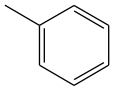
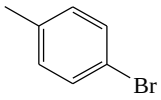
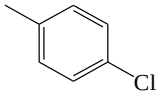
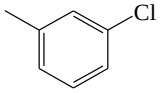
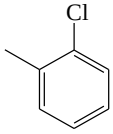
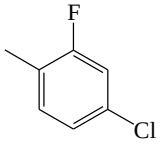
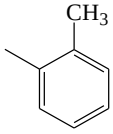


1 2 3

Scheme 2

Reagents and conditions:(i) $NH_2NH_2.H_2O$, MeOH, TEA, 5-10 °C, 1 h. (ii) morpholine, EA, r.t., 1 h. (iii) ArCHO, EtOH, r.t., 1 h. (iv) IBD, MeOH, 15-20 °C, 2 h.

Table 2. Chemical structures and physical data of **5a-g**

Compound	Ar	Yield (%)	m. p. (°C)	Solvent for recrystallization
5a		76	124-126	Methanol
5b		74	160-163	Ethanol
5c		81	170-172	Ethanol
5d		80	179-181	Ethanol
5e		79	200-202	Methanol
5f		81	164-166	Methanol
5g		80	137-140	Ethanol

Synthesis of 1-(5-bromo-2-chloropyrimidin-4-yl)hydrazine (2)

A solution of 5-bromo-2,4-dichloropyrimidine (**1**) (0.01 mol) in EtOH was taken and cooled to 0–5 °C in an ice bath. Triethylamine (0.01 mol) was added to the cold reaction mixture and then hydrazine hydrate (0.02 mol) was added slowly at 5-10 °C. The reaction mass was allowed to stir at room temperature for 1h. The solid thus obtained was filtered, washed with chilled water and dried to afford compound **2**. ^{1}H NMR (DMSO-d_6) δ: 8.06 (s, 1H, NH), 7.85 (s, 1H, py-H), 4.34 (s, 2H, NH_2).

Synthesis of 1-(5-bromo-2-morpholinopyrimidin-4-yl)hydrazine (3)

A solution of 1-(5-bromo-2-chloropyrimidin-4-yl)hydrazine (**2**) (0.01 mol) in EtOAc (50 ml) was taken and morpholine (0.021 mol) was added to it. The contents were refluxed on a water bath for 1 h. The solvent was evaporated on a steam bath, water was added into crude mass and stirred for 15 min. The solid thus obtained was filtered, washed with chilled water and dried to afford compound **3**. ^{1}H NMR (DMSO-d_6) δ: 8.06 (s, 1H, py-H), 7.85 (s, 1H, NH), 4.38 (s, 2H, NH_2), 3.63-3.60 (m, 8H, $4CH_2$).

2-Benzylidene-1-(5-bromo-2-morpholinopyrimidin-4-yl)hydrazine (4a)

Compound **4a** was obtained from **3** (2.74 g, 0.01 mol) and benzaldehyde (1.06 g, 0.01 mol). ^{1}H NMR (DMSO-d_6) δ: 10.46 (s, 1H, NH), 8.43 (s, 1H, P y-H), 8.11 (s, 1H, C H), 7.69-7.66 (d, 2H, Ar-H, J=9.0 Hz), 7.45-7.37 (m, 3H, Ar-H), 3.66-3.64 (m, 8H, $4CH_2$).

2-(4-Bromobenzylidene)-1-(5-bromo-2-morpholinopyrimidin-4-yl)hydrazine (4b)

Compound **4b** was obtained from **3** (2.74 g, 0.01 mol) and 4-bromobenzaldehyde (1.85 g, 0.01 mol). ^{1}H NMR (DMSO-d_6) δ: 10.53 (s, 1H, NH), 8.40(s, 1H, Py-H), 8.11 (s, 1H, CH), 7.61 (s, 4H, Ar-H), 3.66-3.63 (m, 8H, $4CH_2$).

2-(4-Chlorobenzylidene)-1-(5-bromo-2-morpholinopyrimidin-4-yl)hydrazine (4c)

Compound **4c** was obtained from **3** (2.74 g, 0.01 mol) and 4-chlorobenzaldehyde (1.40 g, 0.01 mol). ^{1}H NMR (DMSO-d_6) δ: 10.53 (s, 1H, NH), 8.41 (s, 1H, Py-H), 8.12 (s, 1H, CH), 7.70-7.67 (d, 2H, Ar-H, J=9.0 Hz), 7.50-7.47 (d, 2H, Ar-H), 3.66-3.64 (m, 8H, $4CH_2$).

2-(3-Chlorobenzylidene)-1-(5-bromo-2-morpholinopyrimidin-4-yl)hydrazine (4d)

Compound **4d** was obtained from **3** (2.74 g, 0.01 mol) and 3-chlorobenzaldehyde (1.40 g, 0.01 mol). ^{1}H NMR (DMSO-d_6) δ: 10.52 (s, 1H, NH), 8.41 (s, 1H, P y-H), 8.11 (s, 1H, CH), 7.70 (d, 2H, Ar-H, J=9.0 Hz), 7.49 (d, 2H, Ar-H, J=9.0 Hz), 3.66-3.65 (m, 8H, $4CH_2$).

2-(2-Chlorobenzylidene)-1-(5-bromo-2-morpholinopyrimidin-4-yl)hydrazine (4e)

Compound **4e** was obtained from **3** (2.74 g, 0.01 mol) and 2-chlorobenzaldehyde (1.40 g, 0.01 mol). ^{1}H NMR (DMSO-d_6) δ: 10.82 (s, 1H, NH), 8.86 (s, 1H, Py-H), 8.13 (s, 1H, CH), 8.02 (d, 1H, Ar-H, J=9.0 Hz), 7.52 (d, 1H, Ar-H, J=9.0 Hz), 7.40-7.37 (t, 2H, Ar-H), 3.66-3.63 (m, 8H, $4CH_2$).

2-(4-Chloro -2-fluorobenzylidene)-1-(5-bromo-2-morpholinopyrimidin-4-yl)hydrazine (4f)

Compound **4f** was obtained from **3** (2.74 g, 0.01 mol) and 4-chloro-2-fluorobenzaldehyde (1.58 g, 0.01 mol). ^{1}H NMR (DMSO-d_6) δ: 10.68 (s, 1H, NH), 8.40 (s, 1H, P y-H), 8.13 (s, 1H, CH), 7.67 (d, 2H, Ar-H, J=9.0 Hz), 7.54 (s, 1H, Ar-H), 3.66-3.64 (m, 8H, 4CH_2).

2-(2-Methylbenzylidene)-1-(5-bromo-2-morpholinopyrimidin-4-yl)hydrazine (4g)

Compound **4g** was obtained from **3** (2.74 g, 0.01 mol) and 2-methylbenzaldehyde (1.20 g, 0.01 mol). ^{1}H NMR (DMSO-d_6) δ: 10.44 (s, 1H, NH), 8.72 (s, 1H, P y-H), 8.10 (s, 1H, CH), 7.78 (d, 1H, Ar-H, J=8.0 Hz), 7.25 (d, 1H, Ar-H, J=8.0 Hz), 7.23-7.21 (t, 2H, Ar-H), 3.65-3.62 (m, 8H, 4CH_2).

8-Bromo-5-morpholino-3-phenyl-[1,2,4]triazolo[4,3-f]pyrimidine (5a)

The product obtained from 4a (3.62 g) and iodobenzene diacetate (IBD, 3.86 g). FT-IR (KBr, cm^{-1}) ν: 2936 (C-H), 1640 (C=N), 1472 (C=C), 1376 (C-N), 521 (C-Br). ^{1}H NMR (DMSO-d_6) δ: 7.87 (s, 1H, Py-H), 7.73-7.71 (d, 2H, Ar-H), 7.60-7.52 (m, 3H, Ar-H), 3.25-3.05 (m, 8H, 4CH_2). ^{13}C NMR (DMSO-d_6) δ: 152.2, 144.8, 129.0, 65.0, 49.3. MS: *m/z*, M^+ 359, 360 (M+2).

8-Bromo-3-(4-bromophenyl)-5-morpholino-[1,2,4]triazolo[4,3-f]pyrimidine (5b)

The product obtained from **4b** (4.41 g) and IBD (3.86 g). FT-IR (KBr, cm^{-1}) ν: 2930 (C-H), 1638 (C=N), 1460 (C=C), 1375 (C-N), 520 (C-Br). ^{1}H NMR (DMSO-d_6) δ: 7.88 (s, 1H, Py-H), 7.71-7.68 (d, 2H, Ar-H), 7.63-7.61 (d, 2H, Ar-H), 3.35-3.04 (m, 8H, 4CH_2).^{13}C NMR (DMSO-d_6) δ: 152.2, 144.3, 129.0, 65.7, 49.3, 21.0. MS: *m/z*, M^+ 436, 438 (M+2), 440 (M+4).

8-Bromo-3-(4-chlorophenyl)-5-morpholino-[1,2,4]triazolo[4,3-f]pyrimidine (5c)

The product obtained from **4c** (3.96 g) and IBD (3.86 g). FT-IR (KBr, cm^{-1}) ν: 2945 (C-H), 1645 (C=N), 1470 (C=C), 1375 (C-N), 721 (C-Cl), 520 (C-Br). ^{1}H NMR (DMSO-d_6) δ: 7.88 (s, 1H, Py-H), 7.70-7.68 (d, 2H, Ar-H), 7.55-7.53 (d, 2H, Ar-H), 3.34-3.04 (m, 8H, 4CH_2). ^{13}C NMR (DMSO-d_6) δ: 150.7, 145.8, 140.8, 128.0, 64.4, 49.5. MS: *m/z*, M^+ 393, 395 (M+2), 396 (M+3).

8-Bromo-3-(3-chlorophenyl)-5-morpholino-[1,2,4]triazolo[4,3-f]pyrimidine (5d)

The product obtained from **4d** (3.96 g) and IBD (3.86 g). FT-IR (KBr, cm^{-1}) ν: 2930 (C-H), 1644 (C=N), 1470 (C=C), 1375 (C-N), 720 (C-Cl), 525 (C-Br). ^{1}H NMR (DMSO-d_6) δ: 7.91 (s, 1H, Py-H), 7.79 (d, 1H, Ar-H, J= 8.0 Hz), 7.50 (s, 1H, Ar- H), 7.48 (d, 1H, Ar-H, J= 8.0 Hz), 7.46 (t, 1H, Ar-H), 3.31-3.00 (m, 8H, 4CH_2). ^{13}C NMR (DMSO-d_6) δ: 152.3, 144.8, 129.0, 65.7, 49.3. MS: *m/z*, M^+ 393, 395 (M+2), 396 (M+3).

8-Bromo-3-(2-chlorophenyl)-5-morpholino-[1,2,4]triazolo[4,3-f]pyrimidine (5e)

The product obtained from **4e** (3.96 g) and IBD (3.86 g). FT-IR (KBr, cm^{-1}) ν: 2935 (C-H), 1645 (C=N), 1478 (C=C), 1375 (C-N), 721 (C-Cl), 520 (C-Br). ^{1}H NMR (DMSO-d_6) δ: 7.89 (s, 1H, Py-H), 7.74-7.69 (d, 2H, Ar-H), 7.58-7.47 (t, 2H, Ar- H), 3.33-3.04 (m, 8H, $4CH_2$). ^{13}C NMR (DMSO-d_6) δ: 151.1, 147.1, 141.2, 125.9, 64.8, 49.9. MS: *m/z*, M^+ 393, 395 (M+2), 396 (M+3).

8-Bromo-3-(4-chloro-2-fluorophenyl)-5-morpholino-[1,2,4]triazolo[4,3-f]pyrimidine (5f)

The product obtained from **4f** (4.14 g) and IBD (3.86 g). FT-IR (KBr, cm^{-1}) ν: 2930 (C-H), 1640 (C=N), 1475 (C=C), 1376 (C-N), 1299 (C–F), 720 (C-Cl), 520 (C-Br). ^{1}H NMR (DMSO-d_6) δ: 8.10 (s, 1H, Py-H), 7.94-7.92 (d, 1H, Ar-H, J= 8.0 Hz), 7.86 (s, 1H, Ar-H), 7.72-7.70 (d, 1H, Ar-H, J = 8.0 Hz), 3.33-2.97 (m, 8H, $4CH_2$). 13C NMR (DMSO-d_6) δ: 152.2, 144.8, 126.8, 65.7, 49.3. MS: *m/z*, M^+ 410, 412 (M+2), 414 (M+4).

8-Bromo-5-morpholino-3-o-tolyl-[1,2,4]triazolo[4,3-f]pyrimidine (5g)

The product obtained from **4g** (3.76 g) and IBD (3.86 g). FT-IR (KBr, cm^{-1}) ν: 2935 (C-H), 1645 (C=N), 1476 (C=C), 1370 (C-N), 520 (C-Br). ^{1}H NMR (DMSO-d_6) δ: 7.85 (s, 1H, Py-H), 7.50-7.45 (D, 2H, Ar-H), 7.37-7.26 (t, 2H, Ar-H), 3.10-2.95 (m, 8H, $4CH_2$), 2.18 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ: 150.2, 144.3, 129.1, 65.7, 49.4. MS: *m/z*, M^+ 373, 375 (M+2).

Anticonvulsant activity

Animals: Male wistar rats procured from National Institute of Nutrition, Hyderabad (190-220 g) were used in the present study. The animals were kept in individual cages for one week to acclimatize for the laboratory conditions. They were allowed to free access of water and food.

All the experimental procedures were carried out in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. The study was reviewed and approved by the Institutional Animal Ethics Committee, G Pulla Reddy College of Pharmacy, Hyderabad, India.

Maximal Electroshock Seizure Model (MES): Maximal electroshock seizure model was used in the present study to evaluate the anticonvulsant activity of the compounds on male wistar rats. Seizures were induced in rats by delivering electro shock of 150 mA for 0.2 s by means of a convulsiometer through a pair of ear clip electrodes. The test compounds (100 mg/kg) were administered by oral route in the form of solution (The compounds were dissolved in 1% sodium

carboxymethyl cellulose), 30 minutes before the maximal electroshock seizure test. The animals were observed closely for 2 minutes. The percentage of inhibition of seizure relative to control was recorded and calculated.[15] Phenytoin (100 mg/kg) was used as a standard drug.

Neurotoxicity screening: The minimal motor impairment was measured in mice by the rotorod test. Acute neurological toxicity in mice was evaluated by rotorod test.[15] The mice were trained to stay on the accelerating rotorod that rotates at 10 revolutions per minute. The rod diameter was 3.2 cm. Trained animals were administered with the test compounds at dose of 100 mg/kg. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least one minute in each of the three trails. Phenytoin was used as a standard drug.

Results and Discussion

Spectral studies

Hydrazinopyrimidine (**1**) was synthesized according to the reported procedure.[13] The reaction of 5-bromo-2,4-dichloropyrimidine with hydrazine hydrate in methanol to afford the corresponding hydrazino-pyrimidine (**2**). Hydrazino-pyrimidine, **2** which was reacted with substituted benzaldehyde to afford aryl-(5-bromo-2-chloropyrimidine-4-yl)hydrazone **3**(**a-f**). A series of new 1,2,4-triazole derivatives (**5a-g**) have been synthesized by the reaction of aryl aldehydes with one equivalent of iodobenzene diacetate (IBD) in MeOH. The synthesized molecules (**5a-g**) were structurally characterized by mass, ^{13}C NMR, ^{1}H NMR and FT-IR spectral studies. The absence of N-H absorption bands in the IR spectra confirmed that the synthesized compounds. The appearance of a medium to strong absorption band from 1460 to 1478 cm^{-1} is due to the stretching vibration of C=C bond formation in the synthesized compounds. The ^{1}H NMR spectrum of compound (**5a**) exhibited a singlet in the region δ 7.87 due to pyrimidine proton. The aromatic doublet and multiplet protons appeared between δ 7.73-7.52 (Ar-H). A multiplet was observed between δ 3.25-3.05 due to methylene proton of morpholine moiety. The spectra of ^{13}C NMR present the correct number of carbon atoms at the appropriate chemical shift values. The mass spectra of all the synthesised compounds showed molecular ion peaks, which are in agreement with their molecular formula.

Anticonvulsant activity

Antiepileptic drug research has for several decades focused on identifying new potential drugs based on their anticonvulsant activity against single acute seizures induced by various stimulators, usually in mice and rats. All established antiepileptic drugs have anticonvulsant activity in at least MES model.[14] In the present study, the anticonvulsant activity of the synthesized compounds **3(a-f)** was evaluated by MES model at the dose of 100 mg/kg and the results are summarized in Table 3.

Formation of products was confirmed by recording their ^{1}H NMR, FT-IR and mass spectra. The absence of N-H absorption bands in the IR spectra confirmed that the synthesized compounds. The appearance of a medium to strong absorption band from 1610 to 1618 cm^{-1} is due to the stretching vibration of C=N bond formation in the synthesized compounds. The ^{1}H NMR data of **3(a-f)** showed, singlet in the region of δ, 8.74-8.29 (pyrimidine ring) and 8.49-8.26 (CH group), respectively. The mass spectra of all the synthesized compounds showed molecular ion peaks, which are in agreement with their molecular formula. The mass spectra of all the synthesized compounds showed molecular ion peaks, which are in agreement with their molecular formula. Compound **3b** was shown good protective effect on MES induced seizure, and the effect was nearer to that of standard (phenytoin). Similarly, compounds **3e** and **3a** showed moderate protective effects and a significant difference in protectiveness were observed when compared to standard group. All the compounds were examined for their neurotoxicity using rotorod method given in the dose of 100 mg/kg.

Table 3: Effect of compounds in the maximal electroshock seizure test.

Treatment	E/F	% Protection
3a	5.49	42.61
3b	2.07	68.15
3c	6.25	41.32
3d	6.25	41.34
3e	6.53	44.76
3f	6.25	41.35

Standard	1.96	75.85
Control (Vehicle)	8.20	-

E/F = Extension/Flexion [Decrease in ratio of extension phase (in seconds)/flexion phase (in seconds].

None of the compounds showed neurotoxicity (Table 4). The compound **3b** showed 68.15% protection in comparison to phenytoin which completely inhibited the convulsions produced by electro-convulsometer, which having electron withdrawing groups showed excellent anticonvulsant activity.

Table 4: Neurotoxicity screening of the compounds.

Compound	Neurotoxicity Screen			
	0.5 h	1h	2h	4h
3a	0/4	0/4	0/4	0/4
3b	0/4	0/4	0/4	0/4
3c	0/4	0/4	0/4	0/4
3d	0/4	0/4	0/4	0/4
3f	0/4	0/4	0/4	0/4
Standard	0/4	0/4	0/4	0/4

The data in the table represent ratio between the numbers of the animals that exhibited neurotoxicity against the number of tested animals.

All the synthesized triazole analogs were screened for their anticonvulsant potential through MES model in the dose of 100 mg/kg. Antiepileptic drug research has, for several decades, focused on identifying new potential drugs based on their anticonvulsant activity against single acute seizures induced by various stimulators, usually in mice and rats. All established antiepileptic drugs have anticonvulsant activity in at least MES model. Compounds **5b**, **5c**, **5d 5e** and **5f** did not exhibit toxicity, whereas compounds **5a** and **5g** showed 25% toxicity compared to standard at 2 h of oral administration (Table 6).

Table 5. *In vivo* anticonvulsant activity of compounds **5a-g**.

Compound	E/F	% Protection
5a	3.98	22.31
5b	3.13	31.10
5c	2.20	33.95
5d	2.18	34.00
5e	2.10	34.21
5f	1.65	66.45
5g	3.85	22.40
Standard	1.61	75.60
Control (Vehicle)	4.71	–

Values are expressed as mean ± SE. n = 6 animals in each group E/F = Extension/Flexion [Decrease in ratio of extension phase (in seconds)/flexion phase (in seconds)] , % Protection = (Control-test)/(Control) x 100

Table 6. Neurotoxicity screening of the compounds

Compound	Neurotoxicity screen			
	0.5 h	1 h	2 h	4 h
5a	0/4	0/4	1/4	1/4
5b	0/4	0/4	0/4	0/4
5c	0/4	0/4	0/4	0/4
5d	0/4	0/4	0/4	0/4
5e	0/4	0/4	0/4	0/4
5f	0/4	0/4	0/4	0/4
5g	0/4	0/4	0/4	0/4
Standard	0/4	0/4	1/4	1/4

The data in the table represent ratio between the numbers of the animals that exhibited neurotoxicity against the number of tested animals

Conclusion

In conclusion, a series of new 1,2,4-triazole derivatives (**5a-g**) were synthesized in good yield, characterized by spectral data and their anticonvulsant activity have been evaluated. Compound **5f** demonstrated good anticonvulsant activity with no neurological toxicity. The substituent on phenyl ring is responsible for the anticonvulsant activity of these classes of agents. Compound **5f** was shown to have significant protective effect on MES-induced seizure, and the effect was similar to that of standard (phenytoin). Similarly, compounds **5b**, **5c**, **5d** and **5e** showed moderate protective effect and a significant difference in protectiveness was observed when compared to standard group (Table 5). Compounds **5a–g** was examined for their neurotoxicity using rotorod method given in dose 100 mg/kg. The additional modification and diversification of functional groups in order to improve the activity is currently in progress.

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