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Synthesis, Molecular Docking, Anticonvulsant and Antimicrobial Activity of 1, 3, 5-Trisubstituted-2-Pyrazolines Linked Benzimidazole Derivatives

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Abstract: ***A series of 1-(3-(4-(2-methyl-5-(substituted)-1H-benzomidazole-1-yl)phenyl)-5-(4-(substituted)phenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (SMa-f [A,B,C]) are synthesized and evaluated their antiepileptic and anti-microbial activity. The formation of benzimidazole proceeds through following mechanism of Phillips reaction. Preparation of chalcones proceeds through following Claisen-Schmidt condensation reaction. Pyrazolines are proceeds through following mechanism of Fischer and Knoevenagel condensation reactions. Antiepileptic screening of the title compounds was performed using pentylenetetrazole seizures test. Compound SMb-B showed significant antiepileptic activity compared to diazepam as standard. Antimicrobial activity against two gram positive and two gram negative bacteria using by serial dilution and agar well diffusion method. The newly synthesized compounds SMa-C and SMb-A have showed excellent antimicrobial activity against tested microorganisms. In correlation to antiepileptic activity, selected compounds were subjected for molecular docking studies and the compounds SMb-A, SMb-B, SMc-B, SMd-A and SMe-A have emerged maximum binding affinity with standard drug using calculated Autodock 4.2.1 with GABA-A receptor (PDB ID:6D6T). The drug likeness properties of evaluated the compounds were also showed the satisfactory results. Compounds were characterized by spectroscopic techniques such as UV, ^{1}HNMR, ^{13}CNMR, IR and mass spectroscopy.***

Keywords: 2-methyl benzimidazole, 1,3,5-trisubstituted pyrazolines, anticonvulsant activity, antimicrobial activity, molecular docking, ADME properties, 6D6T

1. Introduction

Epilepsy refers to a disorder of brain function characterized by the periodic and unpredictable occurrence of seizures. Antiseizure drug–enhances Na+ channel inactivation. Some antiseizure drugs prolong the inactivation of the Na+ channels, thereby reducing the ability of neurons to fire at high frequencies. Some antiseizure drugs act by reducing the metabolism of GABA. Others act at the GABA receptor, enhancing Cl^- influx in response to GABA [1]. It is estimated that there are more than 10 million peoples with epilepsy in India. The annual economic burden of epilepsy in India is 88.2% of the gross national product (GNP) per capita and 0.5% of the GNP. Epilepsy is a chronic non communicable disease of the brain that affects around 50 million people worldwide [2].

Antimicrobial drugs may be bactericidal or bacteriostatic. A bactericidal drug kills bacteria, whereas a bacteriostatic drug inhibits the growth of bacteria. Bactericidal drugs are very much useful in life- threatening situations, endocarditis, patients with low polymorph nuclear count and conditions in which bacteriostatic drugs do not cause a cure [3]. As per analysis every year 7, 00,000 people losing battle to anti-microbial resistant (AMR) per year and another 10 million projected to die from it by 2050, AMR would decrease gross domestic product by 2-3.5% with a fall in livestock by 3-8%, costing USD100 trillion to the world [4]. Methicillin-resistant *S. aureus* was 12.11% and that for E. coli resistant to third generation Cephalosporin's was 36.0% [5].

Medicinal chemistry and pharmaceutical chemistry are disciplines at the intersection of chemistry, especially synthetic organic chemistry, pharmacology and various other biological specialties, which is involved with design, chemical synthesis and development of pharmaceutical agents or bio-active molecules (drugs) [6].

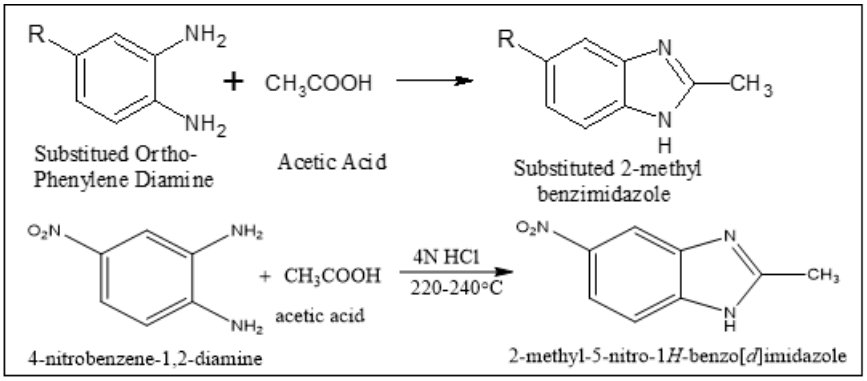
Heterocyclic compounds containing nitrogen, oxygen and sulphur in which one or more of the ring carbons are replaced by another atom. The non-carbon atoms in such rings are referred to as hetero atoms. The hetero atoms are boron, phosphorous, or silicon can also be members of heterocyclic rings [7]. Genetic material DNA is also composed of heterocyclic bases-pyrimidine and purines a large number of heterocyclic compounds, both synthetic and natural, are pharmacologically active and are in clinical use [8].

The benzimidazole contains a phenyl ring fused to an imidazole ring, as indicated in the structure of benzimidazole [9]. Five-membered aromatic systems with two nitrogen atoms are known as diazoles, and the isomer in which these nitrogens are adjacent is pyrazole [10][11]. Pyrazolines widely occur in nature in the form of vitamins, alkaloids, pigments and as constituents of plant and animal cell. The N–N bond linkage of pyrazoline ring is considered to be the key factor in their biological action [12].

As a part of on-going research work reported as 1-(3-(4-(2-methyl-5-(substituted)-1H-benzomidazole-1-yl)phenyl)-5-(4-(substituted) phenyl)-4,5-dihydro-1H-pyrazol-1-yl) ethan-1-one (SMa-f [A, B, C]) new hybrid of pyrazoline linked benzimidazole derivatives.

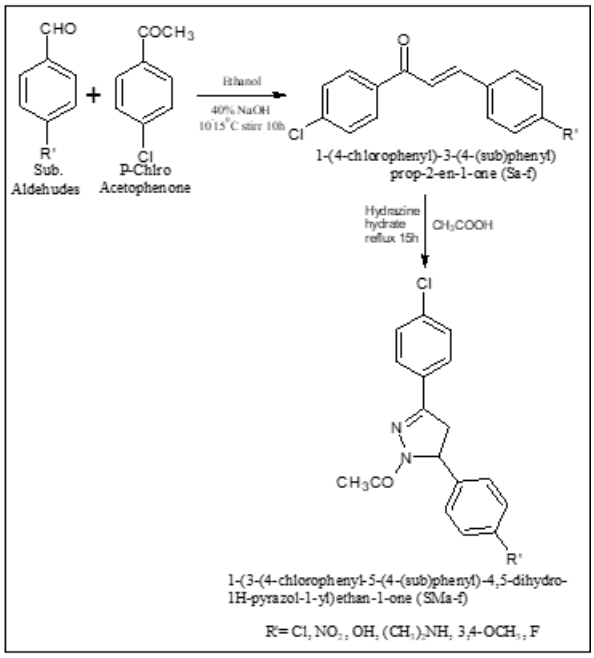
2. Results and Discussion

A series of benzimidazoles (A, B, C) were synthesized, ortho phenylene diamine reacted with acetic acid to give good yields in illustrated in scheme-I. IR spectrum of benzimidazoles (A, B, C) in between the range of NH-Imidazole stretching 3187-3253 cm^{-1}. The formation of benzimidazoles proceeds through the following mechanism. Initially one of the amine groups is acylated with the organic acid in presence of mineral acid to furnish an N-acylated compound. In the next step, the other nitrogen is also acylated by making bond with the carbonyl carbon of the first acyl group leading to ring closure [13].



Scheme I: Synthesis of substituted 2-methyl benzimidazole compounds (A, B, C)

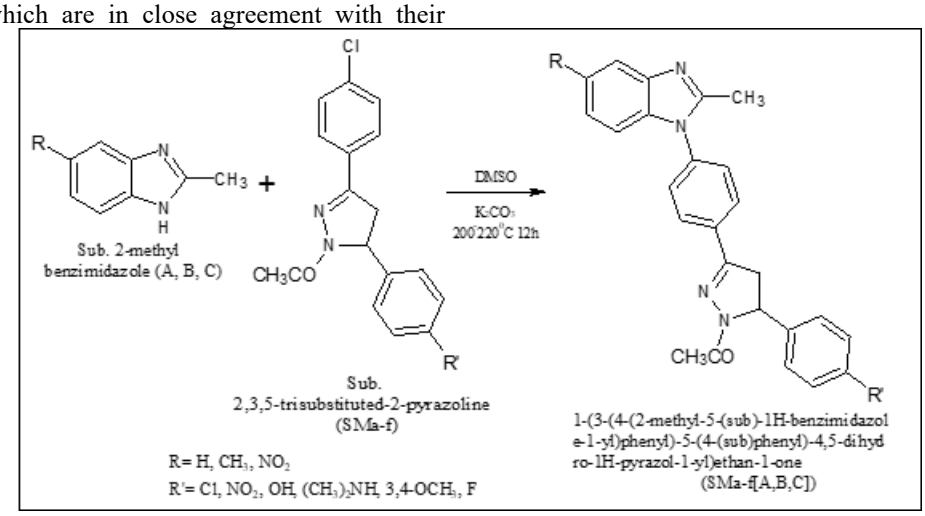
Chalcones (Sa-f) were synthesized according to Claisen-Schimdt reaction by condensing various substituted aldehydes with p-chloro acetophenone in presence of 40% NaOH in good to admirable yields in illustrated to scheme-II. The presence of aromatic aldehyde hinders the base catalysed reaction (though some of them are also catalysed to acids). Decrease the activity of the aldehyde component because of stabilization of the resultant anion by delocalization [14]. In the aldol reaction α carbon of one aldehyde or ketone molecule adds to the carbonyl carbon atom. The base most often used is OH^-, though strongest base [15]. The following mechanism in Claisen-Schimdt condensation reaction of aldehydes are treated to acetophenone in the following through intermediate reaction to the dehydrated of water molecule to formation of α-β unsaturated ketone of chalcones are present [16]. Chalcones (Sa-f) in their IR spectra showed characteristic α-β unsaturated C=O stretching at 1655-1579 cm^{-1} and C=C stretching frequencies at 1587-1511 cm^{-1} among other frequencies. The chalcones (Sa-f) were allowed to react with hydrazine hydrate in acetic acid by Fischer and Knoevenagel condensation to good prospective yields to illustrate the scheme II. The IR spectra of compounds (SMa-f) C=N stretching at 1515-1434 cm^{-1}, C=O stretching at 1662-1642 cm^{-1}, aromatic C-H stretching at 3120-3005 cm^{-1}, aliphatic C-H stretching at 2980-2900 cm^{-1}. In ^{1}HNMR spectra of 4th and 5th position of pyrazoline showed a doublet of doublet in most instances suggesting pyrazoline ring protons are diastereotopic. In ^{1}HNMR spectra, the two methylene protons of C-4 pyrazoline ring in compounds (SMa-f) resonated as two doublets in the range of δ 3.74. The OH protons and OCH_3 protons are resonated at δ 9.31 and δ 2.25. In ^{13}CNMR of pyrazolines C4 and C5 signals appeared at δ 44.41 and δ 64.70. C=O and C=OCH_3 signals at appeared at δ 172.82 and δ 20.15. In CH-Cl signals at appeared at δ 142.07. The mass spectra of compound SMc showed at M^+ and M^+-1. The spectral data of compounds listed in Table 3. The formation of pyrazolines proceeds through the following mechanism of Fischer Knoevenagel condensation. Pyrazoline derivatives are cyclization and acylation of aryl chalcones with the hydrazine hydrate and acetic acid. Hydrazine moiety is attacked from strong ketone moiety of aryl chalcones through following the intermediate reaction to attacked from hydroxonium ion from through cyclization to formation of pyrazoline ring [17].



Scheme II: Synthesis of 1, 3, 5-trisubstituted-2-pyrazoline compounds (SMa-f)

The pyrazolines (SMa-f) react with benzimidazoles (A, B, C) in presence of DMSO and Potassium bromide to give the pyrazole linked benzimidazole derivatives [SMa-f (A, B, C)]. The physicochemical parameters of compounds [SMa-f (A, B, C)] are presented in Table 4. The compounds [SMa-f (A, B, C)] in their UV spectra exhibited λmax in the range of 314nm to 203.50nm. The IR spectra of compounds [SMa-f (A, B, C)] C=N stretching 1510-1410 cm^{-1} and C-N stretching at 1091-1013 cm^{-1}. In ^{1}HNMR spectra of 5th position of 1H pyrazoline range at δ 5.39-3.35, 4th position of 2H pyrazoline range at δ 3.78-3.23 and OCH_3 of 3H range at δ 2.42-1.42. The compounds [SMa-f (A, B, C)] showed

M^+, $M^+ + 1$, $M^+ + 2$ and $M^+ -1$ in their respective m/z values in mass spectra which are in close agreement with their molecular weights.



Scheme III: Synthesis of title compounds (SMa-f[A,B,C])

Antiepileptic Activity:

For the identification of antiepileptic activity in mice, test compounds were administered *i.p.* and challenged by the subcutaneous pentylenetetrazole seizure (*sc*PTZ) test. Compounds found to be active in these seizure challenges are generally regarded to be significantly useful candidates in treatment of partial, generalized and even absence seizures [18]. The data regarding the antiepileptic screening of all the compounds are reported as table 1. Pentylenetetrazole has been reported to produce seizures by inhibiting γ-amino butyric acid (GABA) neurotransmission. GABA is the main inhibitory neurotransmitter substance in the brain, and is widely implicated in epilepsy. Inhibition of GABAergic neurotransmission or activity has been shown to promote and facilitate seizures, while enhancement of GABA-A neurotransmission is known to inhibit or attenuate seizures. At a dose of 200mg/kg compounds SMb-A, SMb-B, SMc-B, SMd-A and SMe-A showed protection after 0.5 h. These compounds continued to show the activity after 4.0 h indicating the rapid onset as well as long duration of action of these compounds. Results obtained in the PTZ induced seizure model have shown that the compound SMb-B is more potent among all the other synthesized compounds, probably because of nitro group is strongly electron withdrawing group due to the resonance delocalization. All other compounds have doesn't shown the antiepileptic activity [19]. Graphically represented at the actions agents are figure 1.

Antimicrobial Activity:

Ten title compounds [SMa-f (A, B, C)] were screened for their *in vitro* antimicrobial activity by agar streak dilution and serial dilution method. To control the sensitivity of the test organisms, the MICs of Ciprofloxacin were determined in parallel experiments. The MICs values determined as the lowest concentration that totally inhibited visible growth of the microorganisms.

From the results of agar well diffusion method compound SMa-c have shown good activity for *S. aureus, E. fecalis, E. coli* organism compared with standard drug. Compound SMb-A, SMb-C and SMe-A have shown good activity for *S. aureus, E fecalis, E. coli, P. aeruginosa* compared with standard drug. Compound SMb-B has shown good activity for *P. aeruginosa* compared with standard drug as Ciprofloxacin. The results, shows that compounds SMa-C and SMb-A (MIC; <12.5µg/ml) SMe-A (MIC: 100µg/ml) displayed comparable activity like Ciprofloxacin against *S. aureus*. Compounds SMa-C (MIC: 100µg/ml) SMb-A and SMe-A (MIC: 50µg/ml) displayed comparable activity like Ciprofloxacin against *E. fecalis*. Compounds SMa-C and SMb-A (MIC: 25µg/ml) SMe-A (MIC: 100µg/ml) exhibited comparable activity like Ciprofloxacin against *E. coli*. Compounds SMb-A (MIC: 50µg/ml) SMe-A (MIC: 100µg/ml) showed equivalent activity like Ciprofloxacin against *P. aeruginosa*. All other seven compounds have doesn't shown the antimicrobial activity of serial dilution method. The MICs and Agar well diffusion method of the test compounds [SMa-f (A, B, C)] and standard drug is efficiently presented in Table 2.

Table 1: Anticonvulsant Activity of Compounds [SMa-f (A, B, C)] by PTZ Method

Compound name	Number of animals	Onset of action	Straub tail	Jerking	Clonic	Tonic	Jumping	Recovery or Death
Control	6	41.65±5.35	56.57±7.265	43.17±5.244	116.8±5.036	232.2±11.33	83±7.385	Death
Standard (Diazepam)	6	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	Recovery
SMb-A	6	199.8±4.586***	213.2±4.206***	201.3±4.379***	280.3±5.875***	353.7±14.48***	265.2±10.98***	Death
SMb-B	6	238.7±3.383***	252±7.24***	239.0±3.416***	305.2±5.594***	0.00±0.00	0.00±0.00	Recovery
SMc-B	6	73.33±2.246***	89.50±3.547***	74.67±2.362***	139.7±4.931**	464.5±20.04***	127.0±8.77***	Death
SMd-A	6	175.7±7.492***	177.8±7.319***	177.2±4.68***	250.7±9.752***	324.5±13.26***	201.3±6.723***	Death
SMe-A	6	206.5±10.15***	207.3±10.39***	208.2±9.850***	300.2±17.09***	344.7±13.03***	229.2±10.39***	Death

Values represent means ±S.E.M (n=6)

*** Values are significantly different at P<0.0001 vs control mice by One way ANOVA followed by Dennett's multiple comparison test
** P<0.001 vs control highly significant

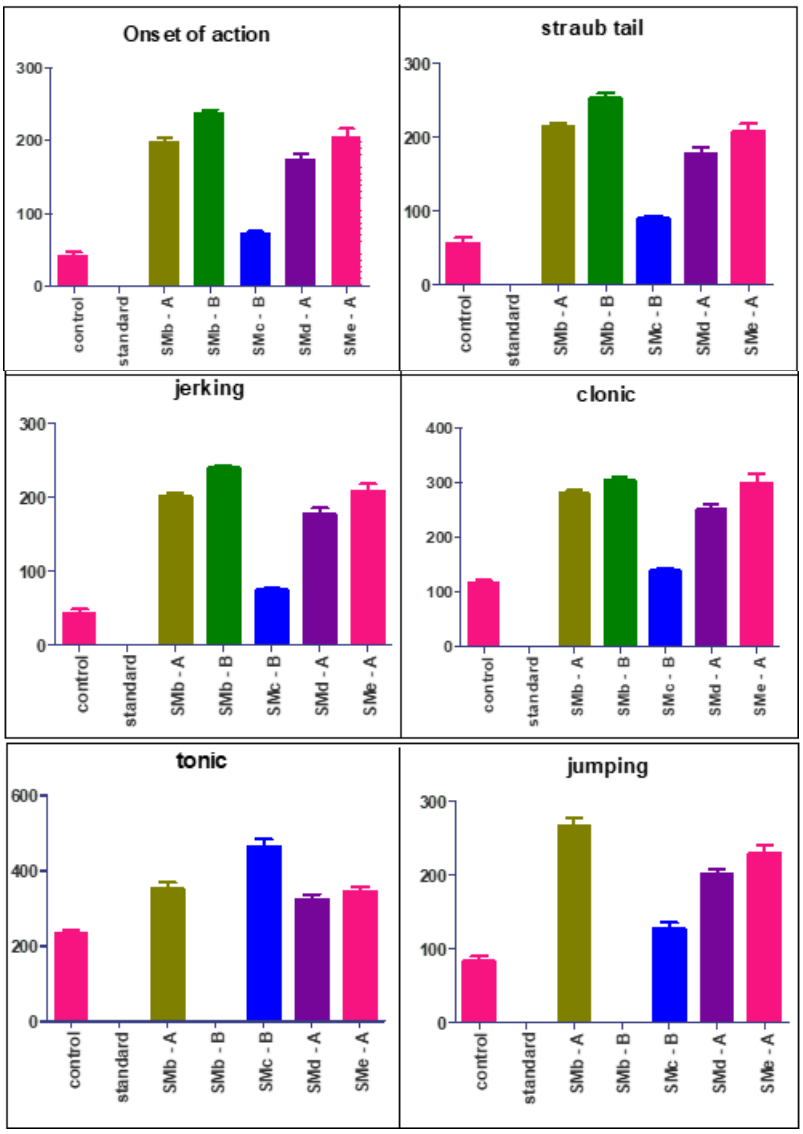


Figure 1: Anticonvulsant Activity of Compounds [SMa-f (A, B, C)]

Table 2: Antimicrobial Activity of Compounds [SMa-f (A, B, C)] by Agar Well Diffusion and Serial Dilution Method

Compounds	MIC (µg/ml)				Agar well Diffusion with 200 µg/ml			
	SA	EF	EC	PS	SA	EF	EC	PS
SMa-C	<12.5	100	25	-	+	+	+	-
SMb-A	<12.5	50	25	50	+	+	+	+
SMb-B	-	-	-	-	-	-	-	+
SMb-C	-	-	-	-	+	+	+	+
SMc-A	-	-	-	-	-	-	-	-
SMc-B	-	-	-	-	-	-	-	-
SMc-C	-	-	-	-	+	-	-	-
SMe-A	100	50	100	100	+	+	+	+
SMe-C	-	-	-	-	-	-	-	-
SMf-C	-	-	-	-	-	-	-	-
Ciorpfloxacin	0.12-0.5	0.25-2	0.004-0.0016	0.12-1	+	+	+	+

SA: *Staphylococcus aureus* (ATCC-25923) EF: *Enterococcus faecalis* (ATCC-29212)
EC: *Escherichia coli* (ATCC-25922) PS: *Pseudomonas aeruginosa* (ATCC-29212)
+ = Active - = Inactive

Molecular Docking of Antiepileptic Activity:

In correlation to anticonvulsant activity, it was thought worth wile to carry out in silico studies to support the activity. The molecular docking of ligand molecules SMb-A, SMb-B, SMc-B, SMd-A and SMe-A with GABA-A receptor revelled that the tested compounds have exhibited the

binding with amino acids. All the docked ligand molecules showed encouraging ligand affinity. Amongst the docked molecules with GABA-A receptors best dock conformation with maximum binding affinity (-6.96 kcal/mol, -6.39 kcal/mol, -6.0 kcal/mol, -5.82 kcal/mol and -5.08 kcal/mol) compared with standard drug as Diazepam (-4.01 kcal/mol). Standard drug Diazepam establishes a conventional hydrogen bond with LEU231 amino acid, pi-sigma LEU235 amino acid, pi-pi stacked PHE298 amino acid, Alkyl chain VAL238, LEU301 amino acid bind with the ligand molecule. Compound SMb-A recognized a conventional hydrogen bond interaction with SER231 amino acid, pi-sigma interaction with LEU235 amino acid, pi-pi stacked interaction withPHE298 amino acid, pi-alkyl interaction with LEU231, ILE234, PRO228 amino acid bind with the ligand molecule. Amino acids interaction with conventional hydrogen bond LYS222, pi-pi stacked TRP341, Alkyl ILE235, LEU232, pi-alkyl VAL227, LEU345, ILE223 bind with SMb-B compound. Compound SMc-B established a interaction with pi-sigma ILE232, LEU235, Alkyl LEU301, VAL260, pi-alkyl ILE271, PRO228, VAL263 amino acid are bind with ligand molecule. Carbon-hydrogen bond LEU223, pi-sigma LEU235, Alkyl MET227, pi-alkyl VAL260, VAL263, LEU231, PRO228 amino acids are binding to the ligand molecule of SMd-A compound. Compound SMe-A established a interaction with pi-sigma LEU235, Alkyl ILE234, LEU231, ILE271, PRO226, pi-alkyl ILE264, LEU231, PRO226 amino acids are bind with ligand molecule.

Root-mean-square deviation (RMSD) has often been used to measure the quality of reproduction of a known binding pose by molecules with ligands. All docked molecules have zero RMSD values; this indicates that the true binding pose of molecules with protein. The docking results for ligand molecules against GABA-A receptor (PDB ID 6d6t) [20] are given in table 3.

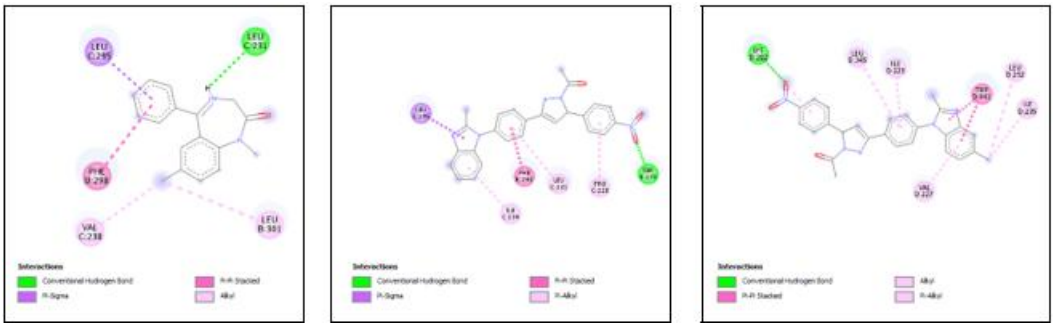
Ligand protein interaction of compounds and the standard drug Diazepam 2D represented as seen in Figure 2. Figure 3 represents the 3D interaction of the same set of molecules using the of Biovia discovery studio Client 2021 [21]. The amino acids are represented as different colours.

Table 3: Molecular docking analysis results for pyrazole linked benzimidazole derivatives of anticonvulsant activity by GABA-A receptor [SMa-f (A, B, C)]

Compounds	Anthelmintic Binding Affinity (kcal/mol)	Anticonvulsant Binding Affinity (kcal/mol)		Anticonvulsant		
			RMSD	PDB File GABA-A Receptor	Hydrophobic Interaction	Hydrogen bond interaction
SMa-C	-6.86	-	0	6D6T		
SMb-A	-7.65	-6.96	0	6D6T	LEU235, LEU231	SER231
SMb-B	-6.53	-6.39	0	6D6T	TRP341	LYS222
SMb-C	-6.93	-	0	6D6T		
SMc-A	-6.51	-	0	6D6T		
SMc-B	-7.23	-6.0	0	6D6T	LEU235	
SMc-C	-7.27	-	0	6D6T		
SMd-A	-7.58	-5.82	0	6D6T	LEU235, LEU231	LEU223
SMd-C	-6.03	-	0	6D6T		
SMe-A	-6.44	-5.08	0	6D6T	LEU235, LEU231	
SMe-C	-5.64	-	0	6D6T		
SMf-C	-6.62	-	0	6D6T		
Albendazole	-5.88	-	0	6D6T		
Diazepam	-	-4.01	0	6D6T	LEU235	LEU231

ADME Properties of Antiepileptic Activity:

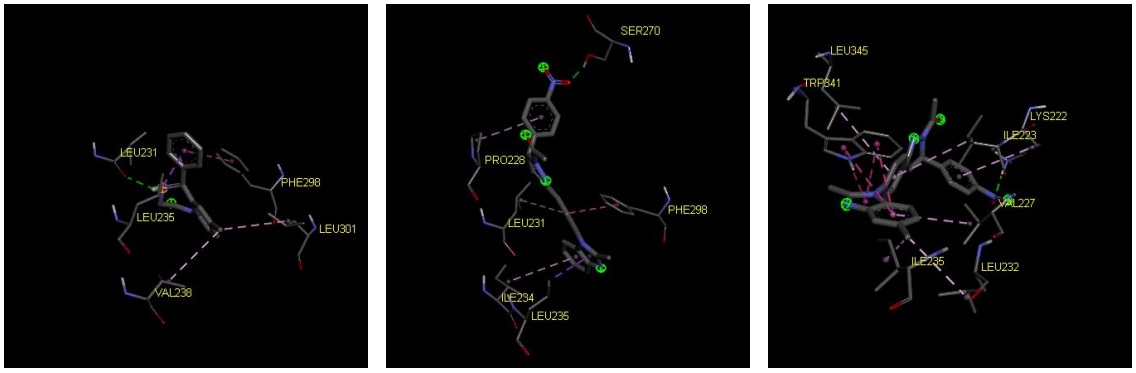
Drug likeness and toxicity risk of the synthesized compounds **[SMa-f (A, B, C)]** was analyzed using various parameters including molecular weight (MW), hydrogen bond donors (HBD) count, hydrogen bond acceptors (HBA) count**,** partition coefficient, skin permeation and bioavailability score by manual application of Lipinski rule of Five and pharmacokinetics. According to the latter, an orally administered drug should have molecular weight MW $\leq$ 500, number of hydrogen bond acceptors HBA $\leq$ 10, number of hydrogen bond donors HBD $\leq$ 5, skin permeation log KP $\leq$ 10, bioavailability score F $\leq$ 0.55. Its ClogP value should be $\leq$5. None of the synthesized compounds **[SMa-f (A, B, C)]** violated the Lipinski rule of five and toxicity effect are suitable for the administration [22]. Drug likeness of synthesized compounds results are followed by table 4 and toxicity risk results are followed by table 5.



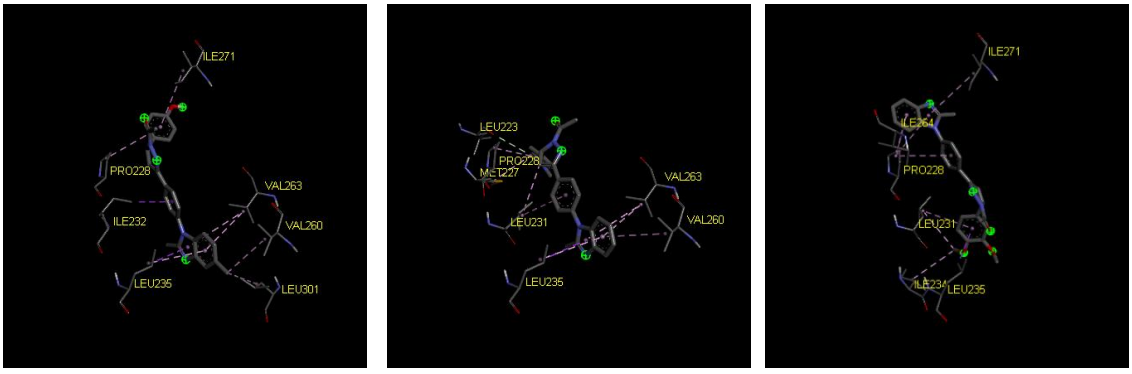
Diazepam | Compound SMb-A | Compound SMb-b

Compound SMc-B | Compound SMd-A | Compound SMe-A

Figure 2: 2D Representation of the Interaction of the Synthesized Molecules [SMa-f (A, B, C)] and Standard Diazepam with GABA-A Receptor



Diazepam | Compound SMb-A | Compound SMb-B



Compound SMc-B | Compound SMd-A | Compound SMe-A

Figure 3: 3D Representation of the Interaction of the Synthesized Molecules [SMa-f (A, B, C)] and Standard Diazepam with GABA-A Receptor

Table 4: Analysis of Lipinski rule of 5 for the Pyrazole linked Benzimidazole Derivatives [SMa-f (A, B, C)]

Compound Code	Molecular Weight	No. of Hba	No. of Hbd	ClogP	No. of Rotatable Bonds	n Violations
SMa-C	473.91	5	0	3.83	5	0
SMb-A	439.47	5	0	3.3	5	0
SMb-B	453.49	5	0	3.65	5	0
SMb-C	484.46	7	0	2.57	6	1
SMc-A	410.47	4	1	3.64	4	0
SMc-B	424.49	4	1	3.97	4	0
SMc-C	455.47	6	1	2.92	5	0
SMd-A	437.54	3	0	4.05	5	0
SMd-C	482.53	5	0	3.33	6	0
SMe-A	454.52	5	0	4.01	6	0
SMe-C	499.52	7	0	3.27	7	0
SMf-C	457.46	6	0	3.26	5	0
Diazepam	284.74	2	0	2.97	1	0
Ciprofloxacin	331.34	5	2	1.1	3	0

HBA: Hydrogen Bond Accepter, HBD: Hydrogen Bond Donor, ClogP: Consensus log p

Table 5: Analysis of Pharmacokinetic Studies of Pyrazole linked Benzimidazole Derivatives [SMa-f (A, B, C)]

Compound Code	Skin permeation Log KP (cm/s)	Bioavailability Score (F)
SMa-C	-5.83	0.55
SMb-A	-6.06	0.55
SMb-B	-5.89	0.55
SMb-C	-6.46	0.55
SMc-A	-6.02	0.55
SMc-B	-5.84	0.55
SMc-C	-6.42	0.55
SMd-A	-5.84	0.55
SMd-C	-6.24	0.55
SMe-A	-6.08	0.55
SMe-C	-6.47	0.55
SMf-C	-6.1	0.55
Diazepam	-5.91	0.55
Ciprofloxacin	-9.09	0.55

Log KP: Skin permeation

3. Materials and Methods

Chemistry

All the chemicals using the work to brought from SDFCL, Sisco research laboratories, Sigma Aldrich, Loba chemie, Spectro chemical pvt ltd, Nice chemi, Indico remidies, A. A, Pharma and Wellona Pharma were laboratory reagents drugs and analytical reagents. Melting points were determined by the using open capillary tubes (Veego VMP-DS, Mumbai, India). Compounds were monitored for their purity on precoated aluminium sheets with silica gel 60 F254 (Merck, Darmstadt, Germany) and different solvents used as mobile phase are n-hexane : ethyl acetate- 3:7, methanol : chloroform- 1:9, n-hexane: ethyl acetate- 4:1; iodine chamber and UV lamp (Dolphin, Mumbai) were used for visualization of TLC. Synthesized and characterization of the compounds were carried out by the UV spectra (UV 1601 Shimadzu, Kyoto, Japan). The studies of vibration frequency are stretching and bending of infrared spectra (PerkinElmer FT-IR C94012) was taken by KBr disc method. ^{1}H NMR chemical shifts were measured on δ scale in ppm downfield to TMS. Peak multiplicities were indicated as s (singlet), d (doublet), dd (doublet of doublet), t (triplet) and m (multiplet) were recorded in DMSO-*d6* on (Agilent 400 MHz, California, USA) and also ^{13}C NMR was recorded at 50 MHz. Mass spectra were recorded on (Waters-SynaptG2, Massachusetts, USA) by LCMS technique. *in vitro* antimicrobial activity tubes were incubated (Kemi Incubator) aerobically at 37^{0}C for compounds.

General procedure for 2-methyl benzimidazoles A-C

Synthesis of 2-methyl benzimidazole and 2, 5-dimethyl benzimidazole (A and B)

In a 500ml round bottom flask (RBF) 0.1mol of o-phenylene diamines (OPD) was treated with 11.61ml (0.2mol) of acetic acid. The mixture was heated in a water bath at 100^{0}C for 15-18hrs. After cooling, 10% sodium hydroxide solution was added slowly with through mixing by rotation of the flask until the mixture was just alkaline to litmus. The crude Benzimidazole was collected with suction in a Buchner funnel. Ice cold water is used to rinse all solid out of the reaction flask. The crude precipitate is pressed thoroughly on the filter washed with cold water and then recrystallized with water [23]. TLC is monitored by using solvent system is n-hexane: ethyl acetate (3:7).

Synthesis of 5-nitro-2-methyl benzimidazole (C)

A mixture of 5-nitro OPD 6.12g (0.04mol) and acetic acid 2.4ml (0.04mol) and 4N HCl (200ml) was refluxed for 12-15hr at 220-240^{0}C. The progress of the reaction was monitored by TLC analysis. After completion of the reaction, the mixture was cooled to room temperature and neutralized using aq. Ammonia solution to pH ≥ 8.0. The separated solid was filtered, washed with ice cold water to remove any salts present and dried to obtain crude product. The latter was recrystallized using water as solvent to obtain pure product [24]. TLC is monitored by using solvent system is methanol: chloroform (1:9).

2-methyl-1H-benzo[d]imidazole (A)

Yield 94.67%, m.p. 174^{0}C, R_f 0.22, solid white colour. IR (KBr, cm^{-1}) 3385 (N-H) 3026 (Ar-CH) 1462 (C=N) 1273 (C-N).

2,5-dimethyl-1H-benzo[d]imidazole (B)

Yield 94.89%, m.p. 204^{0}C, R_f 0.24, solid dark red colour. IR (KBr, cm^{-1}) 3487 (N-H) 3037 (Ar-CH) 2980-2974 (Ali-CH) 1450 (C=N) 1280 (C-N).

2-methyl-5-nitro-1H-benzo[d]imidazole (C)

Yield 91.66%, m.p. 224^{0}C, R_f 0.63, solid pink colour. IR (KBr, cm^{-1}) 3353 (N-H) 3093 (Ar-CH) 2922 (Ali-CH) 1451 (C=N) 1331 (C-NO_2) 1272 (C-N).

General procedure for synthesis of chalcones (Sa-f)

Substituted aldehydes (0.1mol) and p-chloro acetophenone 13ml (0.1mol) were dissolved in ethanol (35ml). The reaction mixture was cooled in an ice bath to 10-15^{0}c and 10ml aqueous solution of 40% sodium hydroxide was added drop wise with continuous stirring for 10h and then left overnight. The reaction mixture was poured into crushed ice and neutralised with dilute hydrochloric acid if necessary. The solid obtain was filtered and washed thoroughly with ice-cold water, dried and recrystallized with alcohol [25]. TLC is monitored by using solvent system is n-hexane: ethyl acetate (4:1).

(E)-1,3-bis(4-chlorophenyl)prop-2-en-1-one (Sa)

Yield 98.86%, m.p. 128-130^{0}C, R_f 0.62, solid pale yellow colour. IR (KBr, cm^{-1}) 3030 (Ar-CH) 1698 (C=O) 1585 (C=C) 814 (C-Cl) 798 (C-Cl).

(E)-1-(4-chlorophenyl)-3-(4-nitrophenyl)prop-2-en-1-one (Sb)

Yield 77.43%, m.p. 158^{0}C, R_f 0.43, solid wooden colour. IR (KBr, cm^{-1}) 3027 (Ar-CH) 1694 (C=O) 1534 (C=C) 1346 (C-NO_2) 822 (C-Cl).

(E)-1-(4-chlorophenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (Sc)

Yield 89.14%, m.p. 84-86^{0}C, R_f 0.34, solid dark yellow colour. IR (KBr, cm^{-1}) 3450 (C-OH) 3100 (Ar-CH) 1735 (C=O) 1587 (C=C) 813 (C-Cl).

(E)-1-(4-chlorophenyl)-3-(4-(dimethylamino)phenyl)prop-2-en-1-one (Sd)

Yield 92.63%, m.p. 138^{0}C, R_f 0.6, solid orange colour. IR (KBr, cm^{-1}) 3050 (Ar-CH) 2900 (Ali-CH) 1713 (C=O) 1548 (C=C) 808 (C-Cl).

(E)-1-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (Se)

Yield 98.84%, m.p. 98^{0}C, R_f 0.3, solid pale yellow colour. IR (KBr, cm^{-1}) 3100 (Ar-CH) 2930-2890 (Ali-CH) 1690 (C=O) 1581 (C=C) 801 (C-Cl).

(E)-1-(4-chlorophenyl)-3-(4-fluorophenyl)prop-2-en-1-one (Sf)

Yield 97.57%, m.p. 132-134^{0}C, R_f 0.71, solid sky blue colour. IR (KBr, cm^{-1}) 3034 (Ar-CH) 1726 (C=O) 1586 (C=C) 1208 (C-F) 814 (C-Cl).

General procedure for synthesis of 1, 3, 5-trisubstituted 2-pyrazoline derivatives (SMa-f)

Chalcones (0.07mol) and hydrazine hydrate 10.5ml (0.21mol) were taken into 30ml glacial acetic acid and the reaction mixture was refluxed for 15h. The resulting mixture was allowed to stand overnight and excess solvent was removed by distillation. The reaction mixture was poured into ice-cold water and neutralised with dilute sodium bicarbonate solution. The solid obtained was filtered and washed thoroughly with ice-cold water and recrystallized with aqueous alcohol [25]. TLC is monitored by using solvent system is n-hexane: ethyl acetate (4:1).

1-(3,5-bis(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (SMa)

Yield 59.90%, m.p. 88-90^{0}C, R_f 0.27, solid white colour. IR (KBr, cm^{-1}) 3020 (Ar-CH) 2950 (Ali-CH) 1709 (C=O) 1513 (C=N) 872 (C-Cl) 825 (C-Cl).

1-(3-(4-chlorophenyl)-5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (SMb)

Yield 92.74%, m.p. 156-158^{0}C, R_f 0.26, solid brown colour. IR (KBr, cm^{-1}) 3090 (Ar-CH) 2910 (Ali-CH) 1693 (C=O) 1515(C=N) 1399 (C-NO_2) 821 (C-Cl).

1-(3-(4-chlorophenyl)-5-(4-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (SMc)

Yield 84.62%, m.p. 216-218^{0}C, R_f 0.26, solid beige colour. IR (KBr, cm^{-1}) 3580 (C-OH) 3120 (Ar-CH) 2980 (Ali-CH) 1732 (C=O) 1443 (C=N) 826 (C-Cl). ^{1}HNMR (DMSO-d6, 400MHz) δppm: 9.31 (s, 1H, OH) 7.76 (d, 2H, 2',6', Ar-H, J=8.4Hz) 7.49 (d, 2H, 3',5', Ar-H, J=8Hz) 6.97 (d, 2H, 2'6', Ar-H, J=8.8Hz) 6.69 (d, 2H, Ar-H, J=8.4Hz) 5.44 (dd, 1H, pyra 5th, J=4, 4Hz) 3.79 (dd, 1H, pyra 4th, J=12, 12Hz) 3.10 (dd, 1H, pyra 4th, J=4.4, 4Hz) 2.25 (s, 3H, $COCH_3$). ^{13}CNMR (DMSO-d6, 50MHz) δppm: 172.82 (C=O) 161.46 (C-OH) 156.58 (C, pyra) 142.07 (CH-Cl) 135.62 (C, 4 & 1') 132.26 (Ar-C, 2',6' & 3',5')120.76 (Ar-C, 2,6 & 3,5) 64.70 (CH-pyra) 44.41 (CH_2-pyra) 20.15 ($COCH_3$). TOF ES-MS m/z: 314.97 (M^+) 312.98 (M^--1).

1-(3-(4-chlorophenyl)-5-(4-(dimethylamino)phenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (SMd)

Yield 71.15%, m.p. 148-150^{0}C, R_f 0.15, solid pale yellow colour. IR (KBr, cm^{-1}) 3020 (Ar-CH) 2900 (Ali-CH) 1696 (C=O) 1434 (C=N) 824 (C-Cl).

1-(3-(4-chlorophenyl)-5-(3,4-dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (SMe)

Yield 90.58%, m.p. 144^{0}C, R_f 0.63, solid pale yellow colour. IR (KBr, cm^{-1}) 3005 (Ar-CH) 2943 (Ali-CH) 1709 (C=O) 1514 (C=N) 828 (C-Cl).

1-(3-(4-chlorophenyl)-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (SMf)

Yield 92.52%, m.p. 78-80^{0}C, R_f 0.22, solid pale yellow colour. IR (KBr, cm^{-1}) 3052 (Ar-CH) 2980 (Ali-CH) 1721 (C=O) 1506 (C=N) 1091 (C-F) 846 (C-Cl).

General procedure for synthesis of pyrazole containing benzimidazole derivatives [SMa-f (A, B, C)]

Pyrazole containing benzimidazole derivatives was synthesized by substituted Pyrazolines (0.02mol) and substituted benzimidazoles (0.02mol) were taken into potassium carbonate (0.02mol) with 10ml of DMSO and the reaction mixture was refluxed for 12h. Excess solvent of DMSO was removed by distillation. The reaction mixture was poured into cold alcohol and stirring for 30min and neutralized with dil. HCl solution. The compound stickiness removed from 10% sodium hydroxide (NaOH) solution. The solid obtained was filtered and washed thoroughly with cold alcohol. Compounds are not recrystallized because of soluble in DMSO solvents. DMSO is an organic polar aprotic molecule with an amphipathic nature that is ideal for dissolving poorly soluble polar and non-polar molecules. TLC is monitored by using solvent system is n-hexane: ethyl acetate (3:7).

1-(5-(4-chlorophenyl)-3-(4-(2-methyl-5-nitro-1H-benzo[d]8midazole-1-yl)phenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (SMa-C)

Yield 65.77%, m.p. 98-100^{0}C, R_f 0.31, solid black colour, λmax; 295.00nm. IR (KBr, cm^{-1}) 3100 (Ar-CH) 2900 (Ali-

CH) 1700 (C=O) 1591 (C=N) 1491 (C-NO_2) 1090 (C-N) 818 (C-Cl). ^{1}HNMR (DMSO-d6, 400MHz) δ ppm: 8.39 to 7.81 (m, 11H, Ar-H) 4.9 (dd, 1H, pyr, J= 31.8Hz & 9.6Hz) 3.94 to 3.69 (dd, 2H, pyr, J=82Hz & 10.5Hz) 2.62 (s, 3H, imidazole CH_3) 1.96 (s, 3H, $COCH_3$). ^{13}CNMR (DMSO-d6, 50MHz) δppm: 168.5 (C=O) 151.7 (C_3, pyra) 145.6 to 112.9 (Ar-C) 132.3 (CH-Cl) 65.9 (C_5-pyra) 39.9 (C_4-pyra) 23.4 ($COCH_3$). TOF, ES-MS m/z: 473.32 (M^+) 474.83 (M^++1).

1-(3-(4-(2-methyl-1H-benzo[d]9midazole-1-yl)phenyl)-5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (SMb-A)

Yield 77.22%, m.p. 114-116^{0}C, R_f 0.34, solid chocalate colour, λmax 297.00nm. IR (KBr, cm^{-1}) 3050 (Ar-CH) 2924 (Ali-CH) 1716 (C=O) 1546 (C=N) 1446 (C-NO_2) 1013 (C-N). ^{1}HNMR (DMSO-d6, 400MHz) δ ppm: 8.73 to 6.93 (m, 12H, Ar-H) 5.3 (dd, 1H, pyr, J= 21.5Hz & 10.4Hz) 3.91 to 3.57 (dd, 2H, pyr, J=63Hz & 11.8Hz) 2.74 (s, 3H, imidazole CH_3) 1.83 (s, 3H, $COCH_3$). ^{13}CNMR (DMSO-d6, 50MHz) δppm: 162.4 (C=O) 155.9 (C_3, pyra) 143.6 to 119.9 (Ar-C) 142.3 (C-NO_2) 61.8 (C_5-pyra) 33.5 (C_4-pyra) 29.8 ($COCH_3$). TOF, ES-MS m/z: 441.15 (M^++2).

1-(3-(4-(2,5-dimethyl-1H-benzo[d]9midazole-1-yl)phenyl)-5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (SMb-B)

Yield 74.69%, m.p. 160-162^{0}C, R_f 0.34, solid wooden colour, λmax 297.00nm. IR (KBr, cm^{-1}) 3050 (Ar-CH) 2910 (Ali-CH) 1650 (C=O) 1510 (C=N) 1290 (C-NO_2) 1060 (C-N). ^{1}HNMR (DMSO-d6, 400MHz) δ ppm: 8.91 to 7.23 (m, 11H, Ar-H) 5.24 (dd, 1H, pyr, J= 24.4Hz & 9.6Hz) 3.85 to 3.63 (dd, 2H, pyr, J=58Hz & 10.4Hz) 2.86 (s, 3H, imidazole CH_3) 1.95 (s, 3H, $COCH_3$). ^{13}CNMR (DMSO-d6, 50MHz) δppm: 168.2 (C=O) 155.2 (C_3, pyra) 145.3 (C-NO_2) 141.8 to 123.6 (Ar-C) 65.8 (C_5-pyra) 37.9 (C_4-pyra) 27.5 ($COCH_3$). TOF ES-MS m/z: 455.21 (M^++2).

1-(3-(4-(2-methyl-5-nitro-1H-benzo[d]9midazole-1-yl)phenyl)-5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (SMb-C)

Yield 76.61%, m.p. 234^{0}C, R_f 0.53, solid dark brown colour, λmax 298.50nm. IR (KBr, cm^{-1}) 3100 (Ar-CH) 2900 (Ali-CH) 1719 (C=O) 1491 (C=N) 1492 (C=N) 1451 (C-NO_2) 1331 (C-NO_2) 1024 (C-N). ^{1}HNMR (DMSO-d6, 400MHz) δ ppm: 8.83 to 7.25 (m, 11H, Ar-H) 5.15 (dd, 1H, pyr, J= 22.6Hz & 10.8Hz) 3.94 to 3.62 (dd, 2H, pyr, J=54Hz & 12.6Hz) 2.80 (s, 3H, imidazole CH_3) 1.92 (s, 3H, $COCH_3$). ^{13}CNMR (DMSO-d6, 50MHz) δppm: 166.6 (C=O) 154.6 (C_3, pyra) 145.3 (C-NO_2) 141.2 to 115.6 (Ar-C) 62.6 (C_5-pyra) 32.4 (C_4-pyra) 26.4 ($COCH_3$). TOF, ES-MS m/z: 485.21 (M^++1).

1-(5-(4-hydroxyphenyl)-3-(4-(2-methyl-1H-benzo[d]9midazole-1-yl)phenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (SMc-A)

Yield 88.53%, m.p. 124^{0}C, R_f 0.37, solid brown colour, λmax 300.50nm. IR (KBr, cm^{-1}) 3600 (C-OH) 3005 (Ar-CH) 2970 (Ali-CH) 1725 (C=O) 1491 (C=N) 1451 (C=N) 1054 (C-N). ^{1}HNMR (DMSO-d6, 400MHz) δ ppm: 9.25 (s, 1H, OH) 6.69 to 7.92 (m, 12H, Ar-H) 5.39 (dd, 1H, pyr, J= 36.4Hz & 11.6Hz) 3.7 (dd, 2H, pyr, J=76Hz & 9.2Hz) 2.55 (s, 3H, imidazole CH_3) 1.42 (s, 3H, $COCH_3$). ^{13}CNMR (DMSO-d6, 50MHz) δppm: 162.4 (C=O) 158.2 (C_3, pyra) 148.6 to 118.2 (Ar-C) 144.3 (C-OH) 61.3 (C_5-pyra) 39.8 (C_4-pyra) 22.4 ($COCH_3$). TOF, ES-MS m/z: 408.94 (M^+-1).

1-(3-(4-(2,5-dimethyl-1H-benzo[d]9midazole-1-yl)phenyl)-5-(4-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (SMc-B)

Yield 85.37%, m.p. 68^{0}C, R_f 0.62, solid red colour, λmax 294.50nm. IR (KBr, cm^{-1}) 3350 (C-OH) 3086 (Ar-CH) 2920 (Ali-CH) 1710 (C=O) 1490 (C=N) 1436 (C=N) 1091 (C-).^{1}HNMR (DMSO-d6, 400MHz) δ ppm: 9.16 (s, 1H, OH) 8.83 to 7.05 (m, 11H, Ar-H) 5.08 (dd, 1H, pyr, J= 22.2Hz & 10.8Hz) 3.83 to 3.62 (dd, 2H, pyr, J=60Hz & 12.4Hz) 2.83 (s, 3H, imidazole CH_3) 1.75 (s, 3H, $COCH_3$). ^{13}CNMR (DMSO-d6, 50MHz) δppm: 166.7 (C=O) 152.6 (C_3, pyra) 142.4 to 123.6 (Ar-C) 138.5 (C-OH) 65.32 (C_5-pyra) 38.4 (C_4-pyra) 30.5 ($COCH_3$). TOF, ES-MS m/z: 424.32 (M^+).

1-(5-(4-hydroxyphenyl)-3-(4-(2-methyl-5-nitro-1H-benzo[d]9midazole-1-yl)phenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (SMc-C)

Yield 83.51%, m.p. 196-198^{0}C, R_f 0.5, solid brown colour, λmax 241.50nm. IR (KBr, cm^{-1}) 3680 (C-OH) 3025 (Ar-CH) 2960 (Ali-CH) 1723 (C=O) 1500 (C=N) 1430 (C=N) 1290 (C-NO_2) 1013 (C-N). ^{1}HNMR (DMSO-d6, 400MHz) δ ppm: 8.2 (s, 1H, OH) 7.95 (m, 11H, Ar-H) 5.30 (dd, 1H, pyr J= 5.2Hz & 46Hz) 3.78 (dd, 2H, pyr J= 25.8Hz & 148Hz) 2.55 (s, 3H, imidazole CH_3) 2.18 (s, 3H, $COCH_3$). ^{13}CNMR (DMSO-d6, 50MHz) δppm: 161.7 (C=O) 152.8 (C_3, pyra) 142.4 (C-OH) 138.6 to 108.4 (Ar-C) 65.3 (C_5-pyra) 33.5 (C_4-pyra) 27.2 ($COCH_3$). TOF ES-MS m/z: 454.12 (M^+-1) 455.12 (M+).

1-(5-(4-(dimethylamino)phenyl)-3-(4-(2-methyl-1H-benzo[d]9midazole-1-yl)phenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (SMd-A)

Yield 61.53%, m.p. 112^{0}C, R_f 0.42, solid wooden colour, λmax 314.00nm. IR (KBr, cm^{-1}) 3010 (Ar-CH) 2976 (Ali-CH) 2900 (Ali-CH) 1696 (C=O) 1524 (C=N) 1440 (C=N) 1055 (C-N) 1032 (C-N). ^{1}HNMR (DMSO-d6, 400MHz) δ ppm: 8.95 to 7.65 (m, 12H, Ar-H) 5.13 (dd, 1H, pyr J= 5.4Hz & 42Hz) 3.89 to 3.54 (dd, 2H, pyr J= 24.2Hz & 142Hz) 2.34 (s, 3H, imidazole CH_3) 2.25 (s, 3H, $COCH_3$). ^{13}CNMR (DMSO-d6, 50MHz) δppm: 163.8 (C=O) 154.5 (C_3, pyra) 144.6 (C-N-$(CH_3)_2$) 142.5 to 113.7 (Ar-C) 67.6 (C_5-pyra) 37.6 (C_4-pyra) 29.7 ($COCH_3$). TOF ES-MS m/z: 439.32 (M^++2).

1-(5-(4-(dimethylamino)phenyl)-3-(4-(2-methyl-5-nitro-1H-benzo[d]9midazole-1-yl)phenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (SMd-C)

Yield 65.95%, m.p. 98^{0}C, R_f 0.62, solid brown colour, λmax 308.00nm. IR (KBr, cm^{-1}) 3030 (Ar-CH) 2910 (Ali-CH) 1714 (C=O) 1460 (C=N) 1430 (C=N) 1360 (C-NO_2) 1057 (C-N) 1040 (C-N). ^{1}HNMR (DMSO-d6, 400MHz) δ ppm: 8.56 to 7.24 (m, 11H, Ar-H) 5.12 (dd, 1H, pyr J= 6.4Hz & 43.2Hz) 3.86 (dd, 2H, pyr J= 24.6Hz & 146.8Hz) 2.55 (s, 3H, imidazole CH_3) 2.24 (s, 3H, $COCH_3$). ^{13}CNMR (DMSO-d6, 50MHz) δppm: 164.5 (C=O) 157.6 (C_3, pyra) 147.8 (C-N-$(CH_3)_2$) 146.3 to 116.32 (Ar-C) 66.4 (C_5-pyra) 35.6 (C_4-pyra) 29.8 ($COCH_3$). TOF ES-MS m/z: 482.56 (M^+) 483.82 (M^++1).

1-(5-(3,4-dimethoxyphenyl)-3-(4-(2-methyl-1H-benzo[d]9midazole-1-yl)phenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (SMe-A)

Yield 54.73%, m.p. 132-134^{0}C, R_f 0.56, solid black colour, λmax 296.50nm. IR (KBr, cm^{-1}) 3100 (Ar-CH) 2990 (Ali-CH) 1720 (C=O) 1510 (C=N) 1052 (C-N). ^{1}HNMR (DMSO-d6, 400MHz) δ ppm: 7.53 to 7.12 (m, 12H, Ar-H) 3.72 (dd, 3H, OCH_3 J= 10Hz & 0.8Hz) 3.53 (dd, 3H, OCH_3

J= 21.2Hz & 5.6Hz) 3.35(dd, 1H pyra) 3.35 to 3.12 (dd, 2H, pyra J= 9.6Hz & 15.2Hz)) 2.56 (s, 3H, imidazole) 2.42 (s, 3H, $COCH_3$). ^{13}CNMR (DMSO-d6, 50MHz) δppm: 162.5 (C=O) 157.1 (C_3, pyra) 147.5 (C-$(OCH)_2$) 143.7 to 121.3 (Ar-C) 63.5 (C_5-pyra) 36.9 (C_4-pyra) 27.9 ($COCH_3$). TOF ES-MS m/z: 454.09 (M^+) 455.10 (M^++1).

1-(5-(3,4-dimethoxyphenyl)-3-(4-(2-methyl-5-nitro-1H-benzo[d]10midazole-1-yl)phenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (SMe-C)

Yield 42.44%, m.p. 112^{0}C, R_f 0.48, solid black colour, λmax 295.50nm. IR (KBr, cm^{-1}) 3100 (Ar-CH) 2990 (Ali-CH) 1728 (C=O) 1510 (C=N) 1445 (C=N) 1410 (C-NO_2) 1024 (C-N). ^{1}HNMR (DMSO-d6, 400MHz) δ ppm: 8.95 to 7.45 (m, 11H, Ar-H) 5.23 (dd, 1H, pyr J= 5.82Hz & 42.6Hz) 3.86 to 3.42 (dd, 2H, pyr J= 25.8Hz & 148Hz) 2.42 (s, 3H, imidazole CH_3) 2.16 (s, 3H, $COCH_3$). ^{13}CNMR (DMSO-d6, 50MHz) δppm: 164.7 (C=O) 156.8 (C_3, pyra) 148.4 (C-$(OCH)_2$) 146.4 to 112.6 (Ar-C) 68.4 (C_5-pyra) 37.5 (C_4-pyra) 30.28 ($COCH_3$). TOF ES-MS m/z: 499.83 (M^++) 501.56 (M^++2).

1-(5-(4-fluorophenyl)-3-(4-(2-methyl-5-nitro-1H-benzo[d]10midazole-1-yl)phenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (SMf-C)

Yield 50.76%, m.p. 118^{0}C, R_f 0.44, solid brown colour, λmax 203.50nm. IR (KBr, cm^{-1}) 3065 (Ar-CH) 2900 (Ali-CH) 1690 (C=O) 1420 (C=N) 1400 (C=N) 1190 (C-NO_2) 1043 (C-N) 990 (C-F). ^{1}HNMR (DMSO-d6, 400MHz) δ ppm: 7.86 (m, 12H, Ar-H) 5.45 (dd, 1H, pyra J= 9.6Hz & 17.6Hz) 3.23 (dd, 2H, pyra J= 2.4Hz & 2.8Hz) 2.55 (s, 3H, imidazole) 2.22(s, 3H, $COCH_3$). ^{13}CNMR (DMSO-d6, 50MHz) δppm: 164.7 (C=O) 157.8 (C_3, pyra) 150.3 to 112.8 (Ar-C) 147.6 (C-F) 65.4 (C_5-pyra) 39.7 (C_4-pyra) 24.3 ($COCH_3$). TOF, ES-MS m/z: 459.19 (M^++2).

4. Biological Activity

Anticonvulsant activity by PTZ induced method

Animals

Adult *swiss albino* mice (30-35g) were used for this study. All the animals either sex (male or female) were obtained from the central animal house of H. S. K. College of Pharmacy and Research Centre, Bagalkot. The animals were housed in standard cages, at room temperature (22-28^{0}C), with 55 ± 5% relative humidity for 12hr dark and 12hr light cycle and given standard laboratory feed (Amruth, Sangli, Maharashtra) and water *ad libitum*. The study was approved and conducted as per the norms of the Institutional Animal Ethics Committee (IAEC/HSKCOP/March 2020/PG7).

Acute oral toxicity (LD_{50})

The acute toxicity study of synthesized pyrazole containing benzimidazole derivatives was determined by using *swiss albino* mice weighing 25-30g and regardless sex were separated in to each groups of six animals. The animals were fasted for 24hrs before experimenting and up and down method (OECD Guideline No. 425) of CPCSEA used for the acute toxicity studies. The acute toxicity is involved in the estimation of LD_{50}. A maximum dose administered up to 100-1000mg/kg has been tested for mortality. The visual observations while acute toxicity studies included skin changes, mobility, aggressiveness, sensitivity to sound and pain, excitation, tremors as well as respiratory movements and number of survivors was noted after 24hrs. Also, the animals are observed for next 14 days where their weights were recorded. The LD_{50} was then determined at the end of the experiment. From one dose selected 200mg/kg which is 1/5th of the LD_{50} value. After the test, the animal was the sole occupant of the cage with free access to food and water during the observation period of 1-2hrs and therefore at intervals. The animals were dead during the experimental work were used for *in vitro* studies in other institutional experiments to avoid the sacrificing of live animals [26].

PTZ Induced Method

The anticonvulsant activity was carried out by PTZ induced convulsion method. The scPTZ test detects the ability of test compounds to raise the seizure threshold of an animal and thus protect it from exhibiting a clonic seizure. Mice were treated with control (2% w/v Tween 80), titled compound (200mg/kg) are suspension with tween 80 and make up with normal saline given by the oral route and standard drug as diazepam (4mg/kg) given by ip route. The dose of PTZ (80mg/kg) which induces convulsion of animals is injected in to a loose fold of the neck. The animals are placed in isolation cages to minimize stress and observed for the next 30 min for the presence or absence of a seizure. An episode of clonic spasms, approximately 3-5 sec, of the fore and hind limbs, jaws, or vibrissae is taken as the endpoint. Animals which do not meet this criterion are considered protected [27].

Antimicrobial activity by agar diffusion and minimum inhibitory concentration (MIC) assay

The bacterial isolates were standardized using colony suspension method and matching the strain's suspension with 0.5 McFarland standards to give a resultant concentration of 1.5 X 108cfu/ml. The antibiotic susceptibility testing was determined using the modified Kirby-Bauer diffusion technique by swabbing the Mueller–Hinton agar (MHA) plates with the resultant saline suspension of each strain and six wells were made in the agar with aid of cork borer (No. 4, the diameter of the borer is 6mm). The wells were sealed at the bottom with molten sterilized agar, 0.1ml and 0.2ml of each of the compounds representing 10mg/ml, and 20mg/ml respectively were aseptically dispensed into the labelled wells while antibiotic disc (ciprofloxacin 5µg) used as control was placed on the agar aseptically. The plates were then incubated at 37°C for 24 hours. The zone diameters of inhibition produced by each concentration of the plants extracts and that of the antibiotic disc was measured and recorded [28].

MIC study for concentration range of 100 µg/ml, 50 µg/ml, 25 µg/ml and 12.5 µg/ml. Preparation of compound stock solution being lipid soluble were first dissolved in DMSO and later required dilutions were prepared in Brain heart infusion (BHI) broth as under 2mg compound in 1 ml DMSO of 2000 µg/ml 1/10 dilution in BHI broth of 200 µg/ml (2X compound dilution). After inoculum preparation checking identity and purity of growth, individual test organisms were grown on nutrient agar plates. 16-18hr old cultures were used and 0.5 McFarland turbidity adjusted inocula were prepared. They were further diluted 10 times to get 2X final strength of inoculum test compound (100µg/ml, 50µg/ml, 25µg/ml and 12.5µg/ml), inoculum in BHI.

Sterility control is BHI broth growth control 2X bacterial inocula were incubated and observed for increase in turbidity used as macro broth dilution assay. All the tubes were incubated aerobically at 37^0 C for 24 hours reading result the highest dilution of compounds that gave no turbidity was considered as MIC. MIC concentration range is taken for minimum 100µg/ml in compounds has showed the antimicrobial activity. More than 100µg/ml MIC concentration range in compounds has showed the antimicrobial activity in prepared that minimum concentration range for lipid soluble solution [29].

***In-silico* Molecular docking study**

The structure of synthesized molecules and the standard drugs were sketched in the ChemDraw professional 16.0 software and converted them into 3D structures by using Avogadro [30], further energies were minimized with the MMFF94s force field and saved as .mol2 files. The crystal structure of 6D6T [20] (PDB) taken in this study was received from RCSB protein data bank. The non-essential water molecules were removed and polar hydrogen's were merged. The missing residues were corrected and the complexes bound to the receptor molecule removed and charges (Kollmann charges) were added using Autodock 4.2. Grid maps [31] were generated using Autogrid 4.2.6 and it was saved as gpf files. Accept the all searching parameters and convert to Lamarckian GA (4.2) as saved that dpf files. Analyse the docking and macromolecules to play conformation then showed the binding affinity of receptor molecules.

More negative the binding affinity better the orientation of the ligand in the binding site [32]. Results can be exported to Biovia Discovery studio.

The visualization of docking simulation results were conducted using the Biovia Discovery studio. The protein PDBQT molecules are placed in Biovia discovery studio then add the ligand molecule. Copy the ligand molecule to paste the macromolecules of PDBQT file. Select undefined ligand and receptor molecules to click the ligand interaction. The interaction of macromolecule amino acids binds to the ligand molecule to convert the 2D and 3D format [21].

The ADME properties, pharmacokinetic properties and drug likeness of the compounds were investigated with the Swiss ADME webserver [33].

5. Conclusion

Understanding the growing need for anticonvulsant and antimicrobial therapeutics, a panel of compounds belonging to series of titled compounds **(SMa-f [A, B,C])** derived from sub. 2-methyl benzimidazole and substituted Pyrazoline benzimidazole architecture were successfully synthesized by a facile protocol in good yields and characterized by IR, NMR and Mass spectral data. The result of their reveled that in SMb-B compound have shown the good Anticonvulsant activity compared with Diazepam. All compounds have screened for two gram positive and gram negative bacteria. Resulting that SMa-C and SMb-A have showed excellent MIC range in compared to standard drug as Ciprofloxacin. Compounds SMa-C, SMb-A, SMb-C and SMe-A have showed significant agar well diffusion method as compared Ciprofloxacin. Compound SMb-B exhibited various degree of antiepileptic activity and also established conventional hydrogen bond and pi-pi stacked with amino acid and showed maximum binding affinity of receptor [6d6t] PDB. All the compounds are analyzed for drug likeness by manual applications of Lipinksi rule of five. Compound SMb-B showed one violations and other compounds have not showed the violations. Toxicity risk of found to suitable for the administration. The present work would be a step forward in developing more active antimicrobial, anticonvulsant and anthelmintic agents. In conclusion, the structures synthesized in the study would be a fruitful architecture deserving further investigation for the development of more potent antimicrobial, anticonvulsant and anthelmintic compounds.

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References

[1] Brunton LL, Parker KL, Blumenthal DK, Buxton ILO. Goodman and Gilman's Manual of Pharmacology and Therapeutics. The McGraw-Hill Comp, United State of America. 2008:698 and 319-21.
[2] Dixit AB, Banerjee J, Chandra PS, Tripathi M. Recent Advances in Epilepsy Research in India. Neurological Soc India. 2017;65(7):S83-92.
[3] Parija SC. Textbook of Microbiology and Immunology. A Div Reed Else, New Delhi, Ind Pvt Ltd. 2012;2:211&229.
[4] Taneja N, Sharma M. Antimicrobial Resistance in the Environment the Indian Scenario. Indian J Med Res. 2019;149(2):119-28.
[5] https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance Accessed on: 01/04/2021.
[6] file:///E:/Dissertation/Medicinal%20chemistry%20-%20Wikipedia.html Accessed on: 23/02/2021.
[7] Bhal A, Bahl BS. Advanced Organic Chemistry. S Chand and Comp Ltd, Ram Nagar, New Delhi. 2009;21:1314-5.
[8] Arora P, Arora V, Lamba HS, Wadhaw D. Importance of Heterocyclic Chemistry. Int J Pharm Sci Res. 2012;3(9):2947-54.
[9] Wright JB. The Chemistry of Benzimidazoles. Chem Rev: 1951;48(3):397-541.
[10] Rodd H. Rodd's Chemistry of Carbon Compounds. A Modern Comprehensive Treatise, Elsevier, New York. 1964;4(2):489.
[11] Ali AR, Revanasiddappa BC, Kumar MV, Kumar H. Synthesis and Anti-inflammatory Activity of Novel Pyrazoline Derivatives. Heterocycl Lett. 2018;8(2):297-302.
[12] Ahmad A, Husain A, Khan SA, Mujeeb M, Bhandari A. Synthesis, Antimicrobial and Antitubercular

Activities of Some Novel Pyrazoline Derivatives. J Saudi Chem Soc. 2016;20(5):577–84.
[13] https://www.adichemistry.com/organic/namedreactions/phillips/phillips-condensation-1.html Accessed On: 19/04/2021.
[14] Patil CB, Mahajan SK, Katti SA. Chalcone: a Versatile Molecule. J Pharm Sci Res. 2009;1(3):11-22.
[15] March J. Adavnced Organic Chemistry: Reaction, Mechanism and Structure. John Wiley Sons Daryaganj New Delhi Pvt Ltd. 2010;4:937.
[16] https://shodganga.inflibnet.ac.in/bitstream/10603/130965/5/05_part%201.pdf Accessed On: 06/05/2021.
[17] Thirunarayanan G, Sekar KG. Solvent-Free One-pot Cyclization and Acetylation of Chalcones: Synthesis of Some 1-Acetyl Pyrazoles and Spectral Correlations of 1-(3-(3,4-Dimethylphenyl)-5- (Substituted Phenyl)-4,5-Dihydro-1 *H*-Pyrazole-1-yl) Ethanones. J Saudi Chem Soc. 2016;20(6):661-72.
[18] Chikkula KV, Raja S. Design and Synthesis of Novel Substituted Benzimidazole Analogues as Potent Anticonvulsant Agents. Eur J Biomed Pharm Sci. 2017;4(8):749-58.
[19] Priyal J, Prakash KS, Harish R, Rajesh SP, Umesh KP, Pradeep KS. Design, Synthesis and Biological Evaluation of Some Novel Benzimidazole Derivatives for their Potential Anticonvulsant Activity. Arch Pharm Res. 2010;33(7):971-80.
[20] https://www.rcsb.org/structure/6d6t Accessed On: 05/07/2021
[21] Biovia DS. Discovery Studio Modelling Enviroment. Dassault Syst Emes, San Diago. 2021.
[22] Jangam SS, Wankhede SB. Synthesis, Molecular Docking, and Biological Evaluation of the New Hybrids of 4-Thiazolidinone and 4 (3 H)-Quinazolinone Against Streptozotocin Induced Diabetic Rats. Russ J Gen Chem. 2019;89(5):1029-41.
[23] Saini S, Neerupma D, Mittal A, Kumar G. Synthesis and Antioxidant Activity of the 2-Methyl Benzimidazole. J Drug Deliv Ther. 2016;6(3):100-2.
[24] Rao AM, Kumar TA, Devi BR. Synthesis of Certain Benzimidazoloquinolone Carboxylic Derivatives as Potential Antitubercular Agents. Rasayan J Chem. 2016;9(4):745-50.
[25] Deshpande SR, Nagrale SN, Patil MV, Chavan PS. Novel 3,4-Methylenedioxybenzene Scaffold Incorporated 1,3,5-trisubstituted-2-Pyrazolines: Synthesis, Characterization and Evaluation for Chemotherapeutic Activity. Ind J Pharm Sci. 2015;77(1):24-33.
[26] Neelottamma K, Chandra SS. Synthesis, Characterization and Anticonvulsant Activity of Some 1, 2, 4-triazole Derivatives. Der Pharm Lett. 2021;13(2):1-12.
[27] Pullagura MKP, Sundararjan R. Design and Synthesis of Novel 2-(5-(4-aryl)-4,5-dihydro-1H-pyrazol-3-yl)-1-(substituted aminomethyl)-1H-Benzimidazole as Potent Anticonvulsant. Med Chem Res. 2017;26(12):3158-72.
[28] Baba H, Onanuga A. Preliminary Phytochemical Screening and Antimicrobial Evaluation of Three Medicinal Plants used in Nigeria. Afr J Tradit Complement Altern Med. 2011;8(4):387-90.
[29] European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). Determination of Minimum Inhibitory Concentrations (MICs) of Antibacterial Agents by Broth Dilution. Clin Microbiol Infect. 2003;9(8):1-7.
[30] Marcus DH, Donald EC, David CL, Tim VEZ, Geoffrey RH. Avogadro: An Advanced Semantic Chemical Editor, Visualization, and Analysis Platform. J Cheminform. 2012;4(1):17.
[31] Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. Autodock4 and AutodockTools4: Automated Docking with Selective Receptor Flexibility. J Comput Chem 2009;30(16):2785-91.
[32] Trott O, Olson AJ. Software News and Update AutoDock Vina: Improving the Speed and Accuracy of Docking with a New Scoring Function, Efficient Optimization, and Multithreading. J Comput Chem. 2009;31(2):455-61.
[33] Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. 2017;7(1):1-3.