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Synthesis, characterization and anticonvulsant evaluation of new derivatives derived from 5-methoxy-2-mercapto benzimidazole

Haider J. Al-Karagully[1], Ammar A. Razzak Mahmood[2*], Nada N. Al-Shawi[3] and Ammar A. Fadhil [3]

[1]*Ministry of health, National Center of Drug Control and Research (NCDCR) Baghdad-IRAQ*
[2]*Department of Pharmaceutical Chemistry, College of Pharmacy, University of Baghdad-Baghdad-IRAQ*
[3]*Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad-Baghdad-IRAQ*

ABSTRACT

A new series of 5-methoxy-2-mercapto benzimidazole derivatives were synthesized by the reaction of 5-methoxy-2-mercaptobenzimidazole with chloroacetic acid and affords 2-((5-methoxy-1H-benzo[d]imidazol-2-yl)thio) acetic acid ***(1),****which on cyclization with acetic anhydride and pyridine gives 7- methoxybenzo[4,5]imidazo[2,1-b]thiazol-3(2H)-one****(2),*** *which on condensation with different aryl aldehydes in the presence of anhydrous sodium acetate in glacial acetic acid, furnishes a arylidene thiazolidinone. The purity of the synthesized compounds was confirmed by melting point and TLC.The structures were established by different spectral analysis such as FTIR,[1]HNMR, and CHN analysis. The newly synthesized compounds (3a-d) were in vivo evaluated for their anticonvulsant activity against yohimbine hydrochloride- induced epilepsy in comparison with 2mg/kg diazepam as a reference drug. The anticonvulsant effect of the intended compounds was assessed by their ability to delay the onset of seizure attack and by a reduction in the number of attacks. All of the synthesized compounds were found to have anticonvulsant activity.*

Keywords: anticonvulsant activity**,** clonic seizure, 5-methoxy-2-benzimidazole derivatives, number of clonic seizures, yohimbine hydrochloride.

INTRODUCTION

Thiazolidinones and their derivatives are the significant class of compounds in medicinal chemistry. They display a wide range of activities such as anticonvulsan**t** [1], antibiotics, diuretic, and tuberculostatic**,** antileukemic and antiparasitic [2.3,] Thiazolidinones are considered as doubly unsaturated five-membered ring contains one nitrogen, one sulfur and three carbon atoms including a carbonyl group**.** On the other hand**,** benzimidazole derivatives are synthetically important analogs and are associated with several biological and pharmacological properties, such as antibacterial [4], antifungal [5], analgesic[6], anti-inflammatory [7], antiviral [8], antitumor [9], and antioxidant [10] activities.

Therefore, in this study, the thiazolidinone was fused with 5-methoxy-2-mercapto benzimidazole by conversion of the 5-methoxy-2-mercapto benzimidazole to its corresponding acid **(1).** It underwent cyclization by using acetic anhydride and pyridine furnishing (2),which on condensation with different aromatic aldehydes afforded arylidene thiazolidine derivatives (3a-d), or (3a-d) can be obtained directly by refluxing of the 5-methoxy-2-mercapto benzimidazole with chloroacetic acid in the presence of different aromatic aldehydes and anhydrous sodium acetate in a mixture of acetic anhydride and glacial acetic acid, then the title compounds **(3a-d**) were evaluated for their anticonvulsant activity.

It's well known that epilepsy is the most common disabling chronic illness of the central nervous system (CNS). It is characterized by epileptic seizures, which are episodes that can vary from brief and nearly undetectable to long periods of vigorous shaking [11][12]; these episodes can result in physical injuries including occasionally broken bones [13]. Besides, epileptic seizures may tend to recur and have no immediate underlying cause [11].

It has been reported that the pathophysiology of epilepsy is not exactly known. The inhibitory neurotransmitter gamma-aminobutyric acid (GABA) in the cerebral cortex maintains the inhibitory tone that counterbalances neuronal excitation. When this balance is disturbed, seizures may happen [14]. Many investigators demonstrated that less than 70% of patients stricken with epilepsy achieve seizure control with the available antiepileptic drugs [15]. Moreover, many of the current anticonvulsants have various complications and serious side effects such as hepatotoxicity and agranulocytosis [16][17] which necessitate new drugs with more suitable margins of safety and more tolerability.

Thus, the synthesis of new compounds that may augment the activity of the inhibitory GABAergic system may be obtained; where, the newly synthesized compounds (3a-d) were *in vivo* assessed for their anticonvulsant activity against yohimbine-induced convulsion in rats by utilizing diazepam as standard drug.

MATERIALS AND METHODS

General

Starting materials and reagents were purchased from commercial suppliers. Melting points were measured in open-ended capillary tubes using an electric melting point apparatus (Thomas Hoover UK). The purity of compounds and monitoring of the reactions were checked by thin layer chromatography (TLC) on Merck silica gel 60_{F254} and visualized with UV light. IR spectra were recorded using KBR discs on a Shimadzu spectrophotometer WQF-520,Japan (υ_{max} =cm^{-1}). Proton Magnetic Resonance (^{1}H-NMR) spectra were recorded on Bruker, Germany NMR spectrometer 400 MHz, Avance III 400 spectrometer) in Central Laboratory Isfahan University-Iran). The chemical shifts are reported in δ values (parts per million, ppm) relative to tetramethylsilane (TMS) as an internal standard. Elemental analysis was recorded on microanalyzer (Euro EA 3000, Europe) in the university of Baghdad, College of Education for Pure Sciences, Ibn Al-Haitham Advisory Office the Central Service Laboratory.The results of the elemental analysis (C,H,N) were found to be in good agreement (-+ 0.5%) with the calculated values. The synthetic method is depicted in Scheme 1, and the physical data of the synthesized compounds are listed in Table1.

Synthesis of 2-(5-methoxy-1*H*-benzo[d]imidazol-2-ylthio) acetic acid (1)[18].

A mixture of 5-methoxy-1*H*-benzo[d]imidazole-2-thiol (1.802 g, 0.01 mol), chloroacetic acid (0.945g, 0.01 mol) and potassium hydroxide (2 g, 0.035mol) in ethanol (40 mL) was heated under reflux on a steam bath for 4h. The reaction mixture was cooled to room temperature, filtered to remove the insoluble impurities, diluted with water, acidified with dil. acetic acid and kept overnight. The solid, thus separated, was filtered, washed well with water and recrystallized from ethanol.

White powder, m.p: 202-204 ºC , yield: 85%, IR (cm^{-1}): 3300-2500cm^{-1} broad (OH str) of carboxylic acid, 2960cm^{-1} and 2879cm^{-1} (CH_3str), 2939cm^{-1} and 2841cm^{-1} .(CH_2str), 1718 cm^{-1} (C=O str.) of carboxylic acid , 1635cm^{-1} (C=N str), 1600cm^{-1} for (C=C str), 1265and 1016cm^{-1} (C-O str.) methyl ether, 625cm^{-1} (C-S)str.

Synthesis of 7-methoxybenzo[4,5]imidazo[2,1-b]thiazol-3(2H)-one (2)[19].

To (2.382 g, 0.01 mol) of compound **(H_6),** pyridine (3 mL) and acetic anhydride (1.0 mL) were added and the mixture was heated on a water bath for 20 min. The reaction mixture was kept overnight and the solid, thus obtained, was filtered, washed well with water and recrystallized from ethanol to give brown powder.

m.p: 100-103ºC, yield 65%, IR (cm^{-1}): 3076 cm^{-1} (Ar-H str.), 2980 cm^{-1} and 2839 cm^{-1}(CH_3-str), 2931cm^{-1}(CH_2-str), 1739cm^{-1} (C=O str.), 1616cm^{-1} (C=N str), 1591 cm^{-1} (C=C str), 1273 cm^{-1} and 1026.13cm^{-1} (C-O str.) methyl ether, 1139 cm^{-1} (C-N str); 623 cm^{-1} (C-S str); ^{1}H-NMR (DMSO-d_6 400 MHz): δ (ppm) = 7.29 -6.82 (m,3H, Ar-H -benzimidzole), 4.09(s,2H, S-CH_2)

Elemental analysis: Calcd for $C_{10}H_8N_2O_2S$: C, 54.53; H, 3.66; N, 12.72; S, 14.56% Found: C, 54.13;H, 3.82;N, 12.43;S,14.23%

General method for the synthesis of compounds (3a-d).

Route 1[20]:

A mixture of (**H_7**) (1.101 g, 0.005 mol) with different aryl aldehydes (0.005 mol, (0.530g) benzaldehyde, (0.610g)salicyaldehyde, (0.660g) cinnamaldehyde, and (0.680g) p-methoxy-benzaldehyde) and anhydrous sodium acetate

(0.412g, 0.005 mol) in glacial acetic acid (25 mL) was refluxed on a heating mantle for 3h.The colored solid that separated on cooling, was filtered, washed with water and recrystallized from ethanol to give bright colored flakes.

Route 2[21]:
A mixture of 5-methoxy-1*H*-benzo[d]imidazole-2-thiol (1.802g, 0.01mol), 2-chloroacetic acid (0.95 ml, 0.01 mol), and different appropriate aromatic aldehydes [0.012 mol,(1.273g) benzaldehyde,(1.465g) salicylaldehyde, (1.586g) cinnamaldehyde and (1.633g) *p*-methoxy-benzaldehyde] and anhydrous sodium acetate (1.64 g, 0.02 mol) were refluxed for 3h. in a mixture of acetic anhydride (5 mL) and glacial acetic acid (5 mL). The obtained powders were filtered off, washed with methanol and recrystallized with acetic acid.

3a:
Greenish –brown powder,m.p:190-192 ºC, yield: 71%,IR (cm^{-1}): 3082cm^{-1} (C-H)str.; 3032 cm^{-1} (Ar-H)str.; 2918and 2850cm^{-1} (CH_3 str).; 1728 cm^{-1} (C=O str.) thiazolidine ring ; 1650cm^{-1} (C=N str.); 1600cm^{-1} (C=C str); 1271cm^{-1} and 1022 cm^{-1} (C-O str.) methyl ether; 1139 cm^{-1} (C-N str.) ; 690cm^{-1} (C-S str); **^{1}H-NMR (DMSO-d$_6$ 400 MHz): δ (ppm)=** 8.83-6.98 (m,8H,Ar-H),3.86 (s,1H,CH), 3.83(s,3H,OCH_3); Elemental analysis: Calcd. for $C_{17}H_{12}N_2O_2S$: C,66.22; H, 3.92; N, 9.08; S, 10.14% Found: C, 65.63;H, 3.72;N, 8.96, S, 9.81%

3b:
3419cm^{-1} (OH str). of phenol; 3068. cm^{-1} (CH str.); 3030 cm^{-1} (Ar-H str.); 2958 and 2850cm^{-1} (CH_3 str.), 1732 cm^{-1} (C=O str.) thiazolidine. ring; 1630cm^{-1} (C=N str.), 1600cm^{-1} (C=C str.); 1270 cm^{-1} and 1026cm^{-1} (C-O str.) methyl ehter; 1138cm^{-1} (C-N str.),625cm^{-1}(C-S str.); **^{1}H-NMR (DMSO-d$_6$400MHz):δ(ppm)**=7.31-6.72(m,7H,Ar-H),5.40(s,1H,OH), 3.94(s,1H,CH),3.75(s,3H,OCH_3); Elemental analysis: Calcd. for $C_{17}H_{12}N_2O_3S$:
C, 62.95; H, 3.73; N,8.64; S, 9.89% Found:C,61.50; H,3.61; N, 8.32; S, 9.30%

3c:
3100cm^{-1} (CH str.); 3010cm^{-1} (Ar-H str.); 2958 cm^{-1} and 2831 cm^{-1} (CH_3 str.), 1716 cm^{-1} (C=O str.) thiazolidine; 1681 (C=N str.), 1649 cm^{-1} (C=C str.); 1271cm^{-1} and 1024.24 cm^{-1} (C-O str.) methyl ether,1136 cm^{-1} (C-Nstr), 625 cm^{-1} (C-S) str; **^{1}H-NMR (DMSO-d$_6$ 400 MHz): δ (ppm)**=7.53-7.38(m,8H,Ar-H),7.77 (dd,1H,H_x 7.43(dd,1H,Hc,J_{CE}=16Hz),6.96(dd,1H,H_e,J_{ce}=16 Hz)3.80(s,3H,OCH3),3.80(s,1H,CH);

Elemental analysis: Calcd. for $C_{19}H_{14}N_2O_2S$:C, 68.24; H, 4.22; N, 8.38; S,9.59% Found:C, 68.00; H, 4.38; N,8.04; S,9.02%

3d:
3100cm^{-1} (CH str.); 3001cm^{-1} (Ar-H)str., 2935cm^{-1} and 2839cm^{-1} . (CH_3 str.),1720 cm^{-1} (C=O str) thiazolidine , 1646cm^{-1} (C=N str.), 1593cm^{-1} (C=C str), 1267cm^{-1} and 1028 cm^{-1} (C-O str) methyl ether, 1136 cm^{-1} (C-N str.) , 667cm^{-1} and 623 cm^{-1} (C-S str.**); ^{1}H-NMR (DMSO-d$_6$ 400 MHz): δ (ppm)**= 8.05-6.88 (m,7H,Ar-H)**,** 3.86(s,1H,CH),3,80 and 3.72 (2s,6H,2OCH_3); Elemental analysis Calcd. for C_{18} $H_{14}N_2O_3S$: C,63.89; H,4.17; N, 8.28; S, 9.48% Found: C,63.60, H, 4,17; N, 8.53; S,9.10%

Anticonvulsant activity:
The anticonvulsant activity of the four-synthesized compounds **(3a-d)** was tested against yohimbine by utilizing rat model [22]. This study has been approved by the Scientific and Ethical Committees of the College of Pharmacy-Baghdad University. The test of anticonvulsant activity of the synthesized compounds was performed in the Animal House of the College of Pharmacy, Baghdad University. Twenty-four Adult Albino rats of both sexes weighing 180-200 g were utilized. They divided into 6 groups (6 animals each). The vehicle and test compounds were administered intraperitoneally (IP) 30 min prior to subcutaneous (SC) injection of 20 mg/kg yohimbine HCl. The animals were observed for the onset and number of clonic seizures for 120 min [23] [24]; as follows:

Gr I- Yohimbine-induced convulsion: I.P injection of DMSO (0.5ml/ 250 g rat) then after 30 min yohimbine in a dose of 20 mg/kg is injected subcutaneously (SC)
Gr II- Standard drug (diazepam in a dose of 2mg/kg I.P.) then after 30 min yohimbine in a dose of 20 mg/kg SC is injected.
Gr III- 3a (2mg/kg I.P). Then after 30 min yohimbine in a dose of 20mg/kg SC is injected.
Gr IV- 3b (2mg/kg I.P). Then after 30 min yohimbine in a dose of 20mg/kg SC is injected.
Gr V- 3c (2mg/kg I.P). Then after 30 min yohimbine in a dose of 20mg/kg SC is injected.
Gr VI- 3d (2mg/kg I.P). Then after 30 min yohimbine in a dose of 20mg/kg SC is injected.

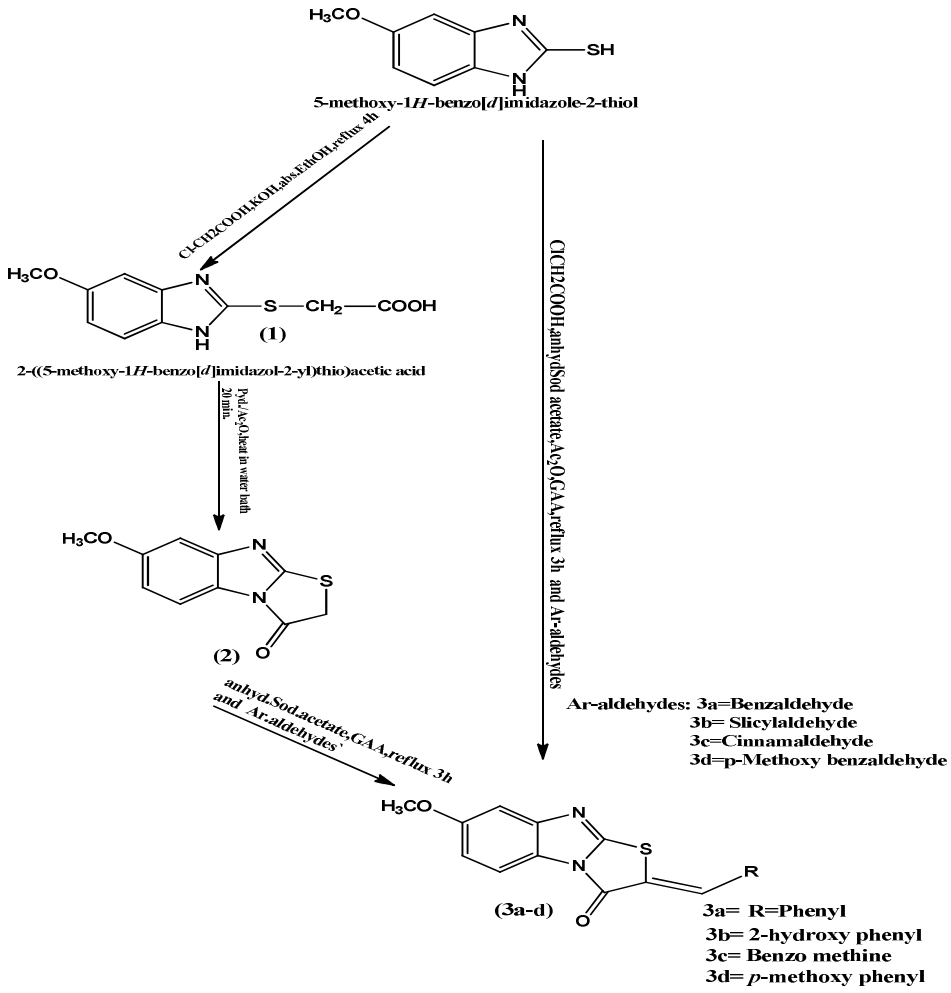
The selected dose of each test compound is comparable to the dose of the standard drug, diazepam. The animals were observed for 120 min for the onset, and number of clonic seizures. In addition to the measurement of percent

(%) change in number of clonic seizures attack induced by yohimbine HCl at 120min, according to the following equation [25]: % change: (mean of treated group - mean of control group)/control X 100

Statistical Analysis: The results were expressed as the mean ± S.E.M. The significance of differences between the mean values was calculated using unpaired Student t-test. Comparison among multiple groups was made by using analysis of variance (ANOVA). *P*-values less than 0.05 were considered significant for all data showed in the study part of anticonvulsant activity

RESULTS AND DISCUSSION

A series of new compounds were synthesized starting from the 5-metghoxy-2-mercapto benzimidazole nucleus. The chemical structures of the compounds were characterized on the basis of their R_f value, m.p, FTIR,^{1}H-NMR, and CHN-analysis, all the spectral data showed good agreements with the proposed structures.



Scheme1: Synthesis of the title compounds (3a-d)

Table 1: Characterization data for the synthesized compounds

Comp. No.	m.p. °C	%Yield	*R_f	physical appearance	Recrystallization solvent
1	202-204	85	0.72[a]	White powder	Ethanol
2	100-103	65	0.68 [b]	Brown powder.	Ethanol
3a	190-192	71	0.39 [c]	Greenish-brown powder	Ethanol for route1 and acetic acid for route 2
3b	164-167	54	0.38[d]	Yellow powder	Ethanol for route1 and acetic acid for route 2
3c	192-195	63	0.29 [c]	Yellow powder.	Ethanol for route1 and acetic acid for route 2
3d	190-192	81	0.32 [c]	Yellowish-brown powder.	Ethanol for route1 and acetic acid for route 2

R_f: solvents: [a] = $CHCl_3$: Acetone: G.AA (8.5:1.0:0.5)
[b] = n-Hexane: EtOAc (8.5:1.5)
[c]= n-Hexane: EtOAc(6:4)
[d]= $CHCl_3$: MeOH (7:3)

The anticonvulsant activity of the test compounds **3a-d** was performed against yohimbine HCl-induced clonic seizure compared to diazepam-treated and yohimbine HCl-treated rats. **Table 2** showed that each of the synthesized

compounds at dose 2mg/kg produced a significant delay in the onset of clonic seizure attack induced by yohimbine HCl compared to the reference drug (diazepam).

Table 2. The effects of newly synthesized compounds (3a-d) on the onset (min) of yohimbine hydrochloride (HCl)-induced clonic seizure in rats compared to control- and diazepam-treated groups

Group	Onset of clonic seizure attack induced by yohimbine HCl (min)
Group I- Control [Dimethylsulfoxide (DMSO) 30min prior to yohimbine HCl (20mg/kg)]	39± 2.75
Group II- Diazepam (2mg/kg) 30min prior to yohimbine HCl (20mg/kg)	67.4± 2.25*[a]
Group III - **3a** (2mg/kg) 30min prior to yohimbine HCl (20mg/kg)	111.0±5.6*[b]
Group IV - **3b** (2mg/kg) 30min prior to yohimbine HCl (20mg/kg)	112.7± 5.6*[b]
Group V - **3c** (2mg/kg) 30min prior to yohimbine HCl (20mg/kg)	116.1±5.8*[d]
Group VI - **3d** (2mg/kg) 30min prior to yohimbine HCl (20mg/kg)	108.2±5.4*[c]

- Data expressed as Mean ±SEM.
*- *P<0.05: Significant different from the control group.*
- Values with non-identical superscripts (a, b, c, and d) are considered significantly different.
- Animals number: 6/group.

Moreover, the synthesized compounds produced zero number of clonic seizure induced by yohimbine HCl at 30-60min, or 60-90min time intervals; and it is non-significantly different (P>0.05) compared to the reference anticonvulsant drug (diazepam). **Table 3**.

At 90-120 min interval, the synthesized compounds produced significant reduction in the number of clonic seizures attack induced by yohimbine HCl compared to diazepam-treated rats (P<0.05). Additionally, there were marked percent reductions in the number of clonic seizures attack induced by yohimbine HCl at 120 min. **Table 3**.

Table 3. The effects of newly synthesized compounds (3a-d) on the number of clonic seizure induced by yohimbine hydrochloride in rats at 30-60min, 60-90min, and 90-120min after yohimbine injection compared to control- and diazepam-treated groups; and the percent (%) change in number of clonic seizures attack induced by yohimbine HCl at 120min

Group	Number of Clonic seizure attack induced by yohimbine HCl at 30-60min	Number of Clonic seizure attack induced by yohimbine HCl at 60-90min	Number of Clonic seizures attack induced by yohimbine HCl at 90-120min	Percent (%) change in number of Clonic seizures attack induced by yohimbine HCl at 120min
Group I- Control [Dimethylsulfoxide (DMSO) 30min prior to yohimbine(20mg/kg)]	5.3±1.26	14.5±3.1	20.8±3.9	----------
Group II- Diazepam (2mg/kg) 30min prior to yohimbine HCL(20mg/kg)	(----------)	1.5±0.29*	6.83±0.92*[a]	-67.16
Group III - **3a** (2mg/kg) 30min prior to yohimbine HCl(20mg/kg)	(----------)	(----------)	0.6±0.067*[c]	-97.12
Group IV - **3b** (2mg/kg) 30min prior to yohimbine HCl(20mg/kg)	(----------)	(----------)	0.3±0.016*[b]	-98.55
Group V - **3c** (2mg/kg) 30min prior to yohimbine HCl(20mg/kg)	(----------)	(----------)	0.33±0.017*[b]	-98.4
Group VI - **3d** (2mg/kg) 30 min prior to yohimbine HCl(20mg/kg)	(----------)	(----------)	1.66±0.037*[d]	-92.02

- Data expressed as Mean ±SEM.
*- *P<0.05: Significant difference to control group.*
- Values with non-identical superscripts (a, b, c, d) are considered significantly different.
- Animals number: 6/group.
- Light dashed lines between brackets (-------) represent zero number of clonic seizures attack at 30-60min, or 60-90min time intervals.
- Bold dash line -------- represents no Percent (%) change in the number of clonic seizures at 120min time period.
- Negative results of percent change represent reduction value in the number of clonic seizures at 120 min. time period.

The anticonvulsant activity of the synthesized compounds was found to be much more effective than the GABA-mimetic drug (diazepam) used as reference anticonvulsant drug; this is may be due to the presence of thiazolidinone

moiety fused to the parent nucleus; where, the compound **3b** was found to possess more anticonvulsant activity than the other new series of 5-methoxy-2-mercapto benzimidazole derivatives.

CONCLUSION

In conclusion, the present work indicated that the synthesized compounds have obvious anticonvulsant activity as indicated by a significant delay in the onset, and number- of clonic seizures attack induced by yohimbine HCl in comparison with a standard drug, diazepam. Moreover, the work highlights the importance of structural features of the 5-methoxy- 2-mercaptobenzimidazol and the fused cyclized derivative condensed with different aromatic aldehydes, responsible for the anticonvulsant activity. Furthermore, many structural modifications can still be approved., and there is full scope for further research which will lead to systematic structure-activity relations ship.

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Conflict of Interest: The authors declare no conflict of interest.

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