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Azole antimicrobial pharmacophore-based tetrazoles: Synthesis and biological evaluation as potential antimicrobial and anticonvulsant agents ☆

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ABSTRACT

The azole pharmacophore is still considered a viable lead structure for the synthesis of more efficacious and broad spectrum antimicrobial agents. Potential antibacterial and antifungal activities are encountered with some tetrazoles. Therefore, this study presents the synthesis and antimicrobial evaluation of a new series of substituted tetrazoles that are structurally related to the famous antimicrobial azole pharmacophore. A detailed discussion of the structural elucidation of some of the newly synthesized compounds is also described. Antimicrobial evaluation revealed that twenty compounds were able to display variable growth inhibitory effects on the tested Gram positive and Gram negative bacteria with special efficacy against the Gram positive strains. Meanwhile, six compounds exhibited moderate antifungal activity against *Candida albicans* and *Aspergillus fumigatus*. Structurally, the antibacterial activity was encountered with tetrazoles containing a phenyl substituent, while the obtained antifungal activity was confined to the benzyl variants. Compounds **16**, **18**, **24** and **27** were proved to be the most active antibacterial members within this study with a considerable broad spectrum against all the Gram positive and negative strains tested. A distinctive anti-Gram positive activity was displayed by compound **18** against *Staphylococcus aureus* that was equipotent to ampicillin (MIC 6.25 μg/mL).
On the other hand, twelve compounds were selected to be screened for their preliminary anticonvulsant activity against subcutaneous metrazole (ScMet) and maximal electroshock (MES) induced seizures in mice. The results revealed that five compounds namely; **3**, **5**, **13**, **21**, and **24** were able to display noticeable anticonvulsant activity in both tests at 100 mg/kg dose level. Compounds **5** and **21** were proved to be the most active anticonvulsant members in this study with special high activity in the ScMet assay (% protection: 100% and 80%, respectively).

1. Introduction

Research and development of potent and effective antimicrobial agents represents one of the most important advances in therapeutics, not only in the control of serious infections, but also in the prevention and treatment of some infectious complications of other therapeutic modalities such as cancer chemotherapy and surgery. Over the past decade, fungal infection became an important complication and a major cause of morbidity and mortality in immunocompromised individuals such as those suffering from tuberculosis, cancer or AIDS and in organ transplant cases.[1] However, in recent years, much attention has been focused on addressing the problem of multi-drug resistant (MDR) bacteria and fungi resulting from the widespread use and misuse of classical antimicrobial agents.[2] Such serious global health problem demands a renewed effort seeking the development of new antimicrobial agents effective against pathogenic microorganisms resistant to currently available treatments.

Among the important pharmacophores responsible for antimicrobial activity, the azole scaffold (**A**; Fig. 1) is still considered a viable lead structure for the synthesis of more efficacious and broad spectrum antimicrobial agents. Azoles (imidazole and triazole derivatives) inhibit the synthesis of sterols in fungi by inhibiting cytochrome P450-dependent 14α-lanosterol demethylase (P-450_{14DM}), which removes the methyl group on C-14 of lanosterol, a key intermediate step in the formation of ergosterol in the fungal cell membrane.[3] Moreover, it was recently found that generation of

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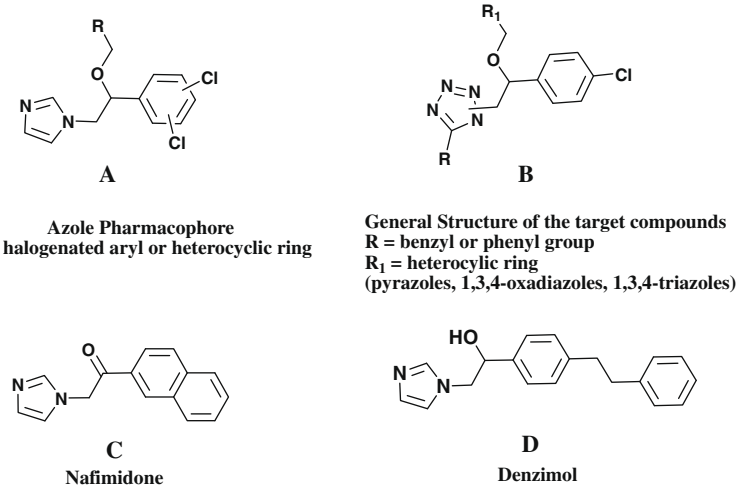


Figure 1. (A) The Azole pharmacophore, (B) general structure of the target compounds, (C) nafimidone, (D) denzimol.

reactive oxygen species (ROS) is important for the antifungal activity of miconazole, pointing to an additional mode of action for this azole.[4] Additionally, it has been reported that remarkable antibacterial activity against methicillin-resistant strains of *Staphylococcus aureus* (MRSA) was ascribed to some azoles, particularly miconazole.[5] Furthermore, some azoles were proved to be effective inhibitors of enoyl acyl carrier protein reductase (FabI), a novel antibacterial target.[6] However, the frequent use of azoles has resulted in clinically resistant strains of fungi and bacteria.[7] The primary structural requirement for the antimicrobial azole class is a weakly basic imidazole or triazole ring bonded by a nitrogen–carbon linkage to the rest of the structure. At the molecular level, the amidine nitrogen (N-3 in the imidazole or N-4 in the triazole) is believed to bind to the heme iron of enzyme-bound cytochrome P450 to prevent oxidation of steroidal substrates by the enzyme. Most of them possess aromatic rings that are believed to mimic the corresponding non-polar steroidal portion of the substrate for the enzyme. The non-polar functionality confers a high degree of lipophilicity to the antifungal azoles.[8] Various attempts have been undertaken to modify the structures of the so far effective azole drugs in order to improve their antimicrobial potency and selectivity,[9–11] however, few reports have discussed the contribution of the tetrazole moiety in such pharmacophore in spite of the potential antibacterial and antifungal activities encountered with some tetrazoles.[12–17]

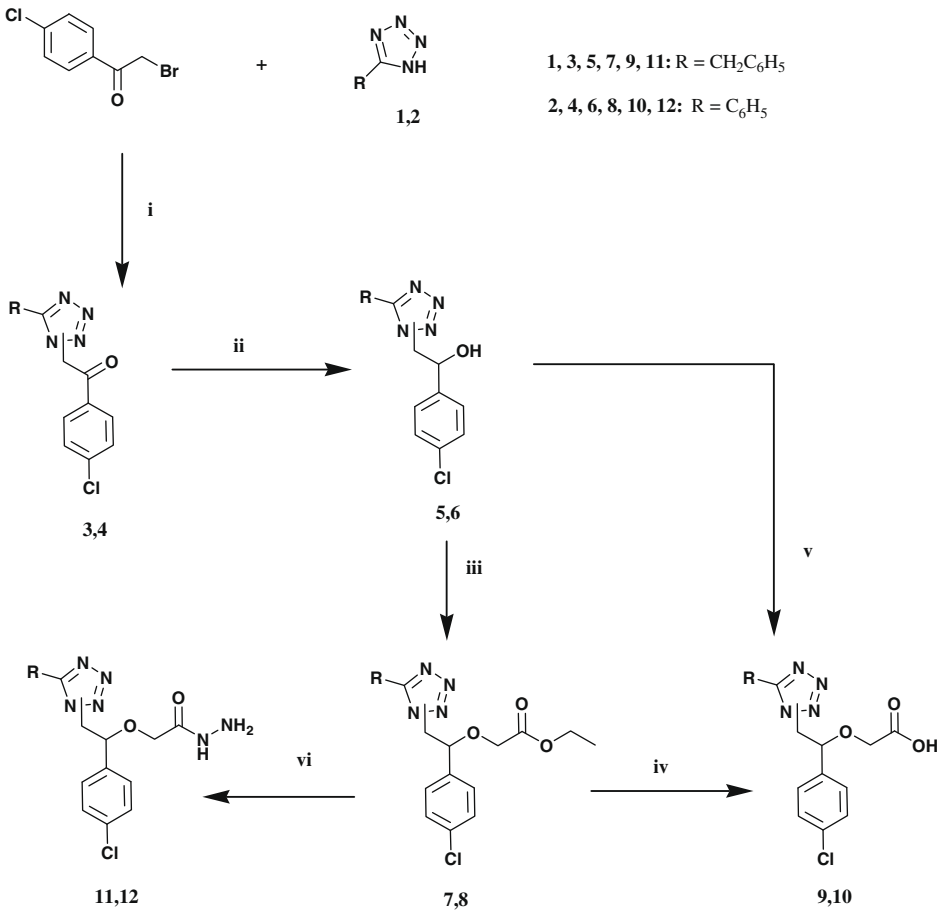
Motivated by these findings, and in continuation of our ongoing efforts endowed with the discovery of nitrogenated heterocycles with potential chemotherapeutic activities,[18–26] it was planned to synthesize and investigate the antimicrobial activity of a new series of substituted tetrazoles having the general formula (**B**) that are structurally related to the famous azole pharmacophore (**A**) (Fig. 1). The target compounds were designed so that the imidazole ring was isosterically replaced with a tetrazole one, keeping almost the same dimensions and substitution pattern of such type of compounds. Moreover, the aralkyl moiety attached to the secondary alcoholic group was varied to include other functionalities that are known to contribute to a variety of antimicrobial activities including the ester, acid, acid hydrazide and thiosemicarbazide groups. Additionally, it was considered of interest to incorporate other chemotherapeutically-active heterocyclic rings (pyrazoles, oxadiazoles and triazoles) within the structure, hoping to impart some synergism to the target compounds.

On the other hand, literature survey revealed that the newly synthesized compounds are structurally related to a distinct class of antiepileptic drugs; the aralkylimidazoles. Nafimidone (**C**) and denzimole (**D**) (Fig. 1) are two independently discovered representatives of this group and possess a profile of activity similar to that of phenytoin or carbamazepine but more distinct than barbiturates and valproic acid.[27,28] Structure–activity relationship studies revealed that anticonvulsant properties of this group are associated with the presence of an imidazole ring, a small oxygen functional group in addition to a lipophilic aryl portion facilitating penetration of the blood–brain barrier.[29] Consequently, it was considered worthwhile to investigate the anticonvulsant activity of some of the newly synthesized compounds comprising both the above-mentioned pharmacophore and the tetrazole ring which is reported to contribute to some anticonvulsant activity.[17,30]

2. Results and discussion

2.1. Chemistry

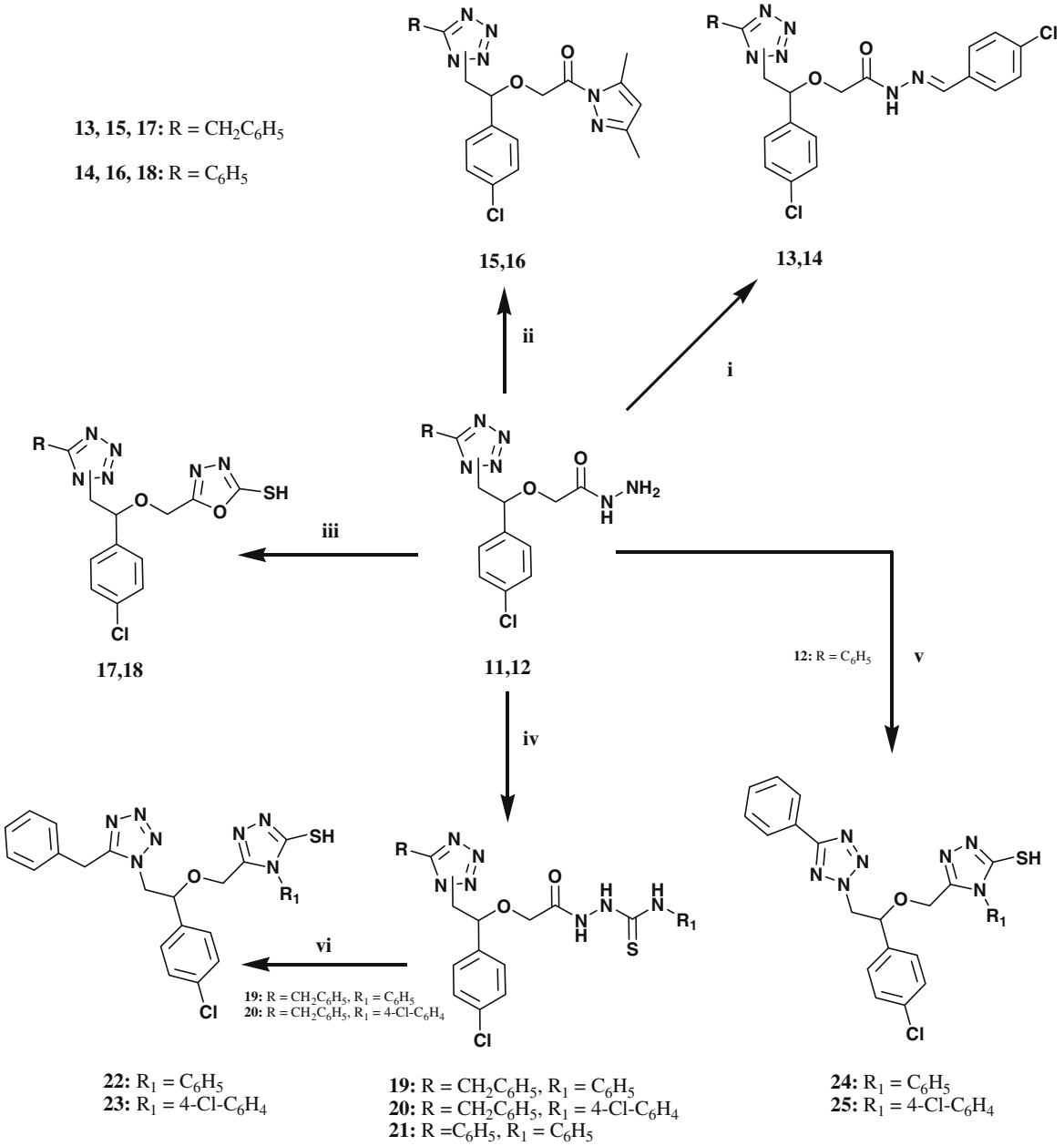
Synthesis of the intermediate and target compounds was performed according to the reactions outlined in Schemes 1–3. In Scheme 1, the starting compounds 5-(benzyl or phenyl)-1*H*-tetrazole **1**, **2** were prepared following a previously reported literature procedure.[31] Alkylation of these tetrazoles with 4-chlorophenacyl bromide in refluxing acetone containing anhydrous potassium carbonate afforded the ethanone derivatives **3**, **4** in good yields. A literature survey revealed that alkylation of 5-substituted tetrazoles affords generally mixtures of 1,5- and 2,5-regioisomers,[32,33,13] which could be separated by silica gel chromatography.[34,35] However in the present study, compounds **3** and **4** were purified by fractional crystallization from diethyl ether followed by crystallization from the proper solvent. Furthermore, ^{1}H NMR studies of disubstituted tetrazoles have shown that protons of CH_2 group attached to N_1 of 1,5-disubstituted tetrazoles are more shielded than the corresponding protons of 2,5-disubstituted tetrazoles.[36–38] Moreover, their ^{13}C NMR spectra have shown that the tetrazole-C_5 carbon atom of 1,5-disubstituted tetrazoles is more shielded than the corresponding carbon of the 2,5-disubstituted tetrazoles.[36–38] Based on these reported observations, compound **3** (R = CH_2–C_6H_5) was designated as 1,5-disubstituted tetrazole since its ^{1}H NMR spectrum displayed a signal for N–CH_2 protons at δ 6.32 ppm, while its ^{13}C NMR spectrum showed a C_5 resonance at δ 156.17 ppm. Meanwhile, compound **4** (R = C_6H_5) was designated as 2,5-disubstituted tetrazole as evidenced from its ^{1}H NMR that exhibited a signal for N–CH_2 protons at δ 6.72 ppm, whereas its ^{13}C NMR spectrum showed a signal for tetrazole-C_5 atom at δ



Scheme 1. Reagents and conditions: (i) dry acetone, K_2CO_3, reflux; (ii) $NaBH_4$, ethanol, room temperature; (iii) ethyl bromoacetate, sodium hydride, room temperature; (iv) sodium hydroxide, ethanol, reflux; (v) 2-chloroacetic acid, sodiumhydride, room temperature; (vi) hydrazine hydrate, ethanol, reflux.

164.93 ppm. In addition, more spectroscopic evidence for the regiochemical assignment of the two ethanone derivatives **3**, **4** was obtained by the aid of nuclear overhauser effect (NOE) and heteronuclear multiple bond correlation (HMBC) experiments. NOE experiment for compound **3** showed a correlation between N–CH_2 protons at δ 6.32 ppm and benzyl CH_2 protons at δ 4.25 ppm (Fig. 2). Moreover, HMBC spectrum for compound **3** revealed a strong correlation between the protons of the N–CH_2 group at 6.32 ppm and the tetrazole-C_5 carbon at 156.17 ppm, a feature that was not observed in compound **4** (R = C_6H_5). Reduction of the ethanone derivatives **3**, **4** using sodium borohydride at room temperature resulted in the formation of the corresponding ethanol derivatives **5**, **6** in excellent yields. Being chiral molecules, the geminal hydrogens of the (N–CH_2) moiety adjacent to the chiral centre (CH) experience different magnetic environments.[39] Hence, their ^{1}H NMR spectra showed both the N–CH_2 and CH methine protons as double doublets at about δ 4.43–4.94 and 4.92–5.30 ppm, respectively (see Section 4), in addition to D_2O-exchangeable signals at δ 6.04 and 5.99 ppm due to OH protons. Alkylation of **5**, **6** with ethyl bromoacetate at room temperature using sodium hydride yielded the requisite acetate derivatives **7**, **8** in moderate yields. Here, it is worth mentioning OCH_2 protons of **7** were found to be relatively more shielded than those of compound **8**, providing a substantial proof for their assigned 1,5- and 2,5-disubstituted structures. Synthesis of acetic acid derivatives **9** and **10** was accomplished either through alkylation of the ethanol derivatives **5**, **6** with chloroacetic acid using sodium hydride (method A), or by hydrolysis of the esters **7** and **8** using 5% sodium hydroxide (method B). Refluxing the esters **7**, **8** in ethanolic hydrazine hydrate gave the corresponding acetohydrazides **11**, **12**, respectively, which were employed as key intermediates for synthesis of the target compounds presented in Scheme 2.

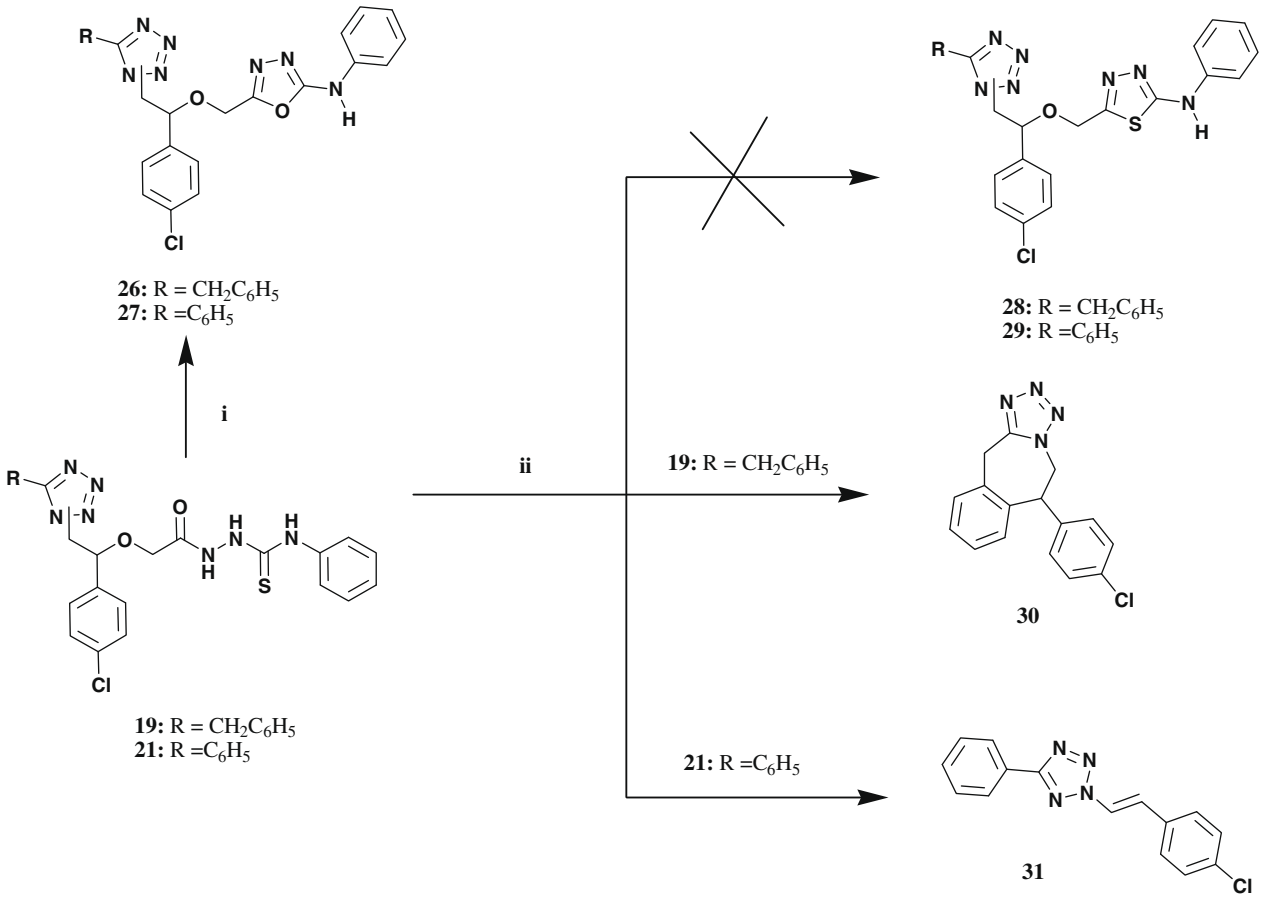
Thus, condensing the acetohydrazides **11**, **12** with 4-chlorobenzaldehyde in boiling ethanol furnished the corresponding azomethines **13**, **14**, respectively. It should be noted down here that, compounds having the arylidene–hydrazide structure may exist as *E*/*Z* geometrical isomers about C=N double bond and as *cis*/*trans* amide conformers at the CO–NH moiety (Fig. 3).[40,41] In this respect, the DMSO-d_6 ^{1}H NMR spectra of these compounds confirmed their existence as *E* geometrical isomers, which coincides with the literature findings for analogous compounds containing the imine (C=N) functionality.[41] On the other hand, further interpretation of their DMSO-d_6 ^{1}H NMR spectra revealed presence of three sets of signals at δ 3.78–3.97, 4.17–4.45, 7.84–8.13, 8.17–8.68 ppm and δ 11.12–11.20, 11.39–11.44 ppm attributed to OCH_2, N=CH and CO–NH groups of the *cis* and *trans* conformers, respectively. According to the literature, the upfield lines of the N=CH and CONH protons were assigned to the *cis* conformer of the amide structure, whereas, the downfield lines were due to the *trans* conformer.[42] Cyclocondensation of acetohydrazides **11**, **12** with acetyl acetone resulted in formation of the pyrazole derivatives **15**, **16**. Furthermore, synthesis of 1,3,4-oxadiazol-2-thiol **17**, **18** was achieved by treatment of **11**, **12** with carbon disulfide in boiling ethanol containing potassium hydroxide. Treatment of **11** (R = benzyl) and **12** (R = phenyl) with aryl isothiocyanates in ethanol at room temperature afforded the corresponding thiosemicarbazides **19**–**21**. Unexpectedly, when the phenyl analog **12** was refluxed with ary lisothiocyanates in ethanol, the 1,3,4-triazole derivatives



Scheme 2. Reagents and conditions: (i) 4-chlorobenzaldehyde, ethanol, reflux; (ii) acetyl acetone, ethanol, reflux; (iii) carbon disulfide, potassium hydroxide; (iv) aryl isothiocyanate, ethanol, room temperature; (v) aryl isothiocyanate, ethanol, reflux; (vi) 5% aq sodiumcarbonate, reflux.

24, **25** were directly obtained. On the other hand, synthesis of the 1,3,4-triazole derivatives **22**, **23** was achieved through refluxing the thiosemicarbazides **19**, **20** in 5% sodium carbonate solution.

Finally, cyclodesulfurisation of the intermediate thiosemicarbazides **19** and **21** to the 1,3,4-oxadiazole derivatives **26**, **27** was performed using freshly prepared yellow mercuric oxide in boiling dioxane (Scheme 3). At this stage, it was attempted to prepare the analogous 1,3,4-thiadiazole derivatives **28** and **29** through treatment of the thiosemicarbazides **19**, **21** with concd sulfuric acid following different reported procedures, however, all these trials were abortive. Surprisingly, two unexpected products namely; a tetrazolobenzazepine **30** and a styryl tetrazole derivative **31** were isolated instead as evidenced from their elementary analyses, ^{1}H NMR, ^{13}C NMR, HMBC, and mass spectral data. The ^{1}H NMR spectrum of compound **30** showed complete absence of signals relevant to the 1,3,4-thiadiazole structure, and appearance of signals corresponding to the N–CH_2, benzyl CH_2 and CH protons in addition to a reduction in the expected number of aromatic protons (8 instead of 9 protons). Moreover, an obvious change in the chemical shifts and splitting pattern of the previously mentioned protons was observed (see Section 4). ^{13}C NMR spectrum of the same compound displayed signals attributed to five quaternary carbons instead of the expected eight carbons and eight aromatic carbons instead of the expected thirteen. Its mass spectrum showed a molecular ion peek at *m*/*z* 296 instead of the expected 1,3,4-thiadiazole structure *m*/*z* 483. The found values of the elementary analysis of this compound were in favor of the cyclized tetrazolobenzazepine structure. Moreover, HMQC (Heteronuclear Multiple Quantum Coherence) data were in accordance with the assigned structure. More evidence to assess the new structure of **30** was obtained from HMBC experiment that showed a strong correlation between the CH carbon (δ 42.73 ppm) and the benzyl C_3–H



Scheme 3. Reagents and conditions: (i) HgO, dioxane, reflux; (ii) sulfuric acid, 0 °C, 15 min.

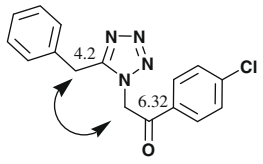


Figure 2. NOE correlation of compound **3**.

(δ 6.8 ppm) which confirms the process of internal cyclization. The reaction mechanism for such cyclization was suggested to take place through protonation of the ether oxygen followed by cleavage of the side chain leaving a secondary carbocation. The latter was then subjected to a nucleophilic attack by the benzyl ortho carbon leading to cyclization to the seven-membered azepine ring. Concerning the styryl tetrazole derivative **31**, investigation of its ^{1}H NMR spectrum revealed absence of signals relevant to the 1,3,4-thiadiazole structure as well as disappearance of signals due N–CH_2 and CH protons. Instead, two doublets at δ 7.66 and 8.50 ppm with a coupling constant of 14.6 Hz corresponding to two vinylic protons in the *E* configuration were displayed. Other aromatic protons of the phenyl and 4-chlorophenyl moieties were located at their expected chemical shifts. In addition, its ^{13}C NMR, HMBC spectral data and the found values of the elementary analysis of this compound agreed with the styryl rather than the 1,3,4-thiadiazolyl structure. The formation of such derivative was suggested to proceed via protonation of the ether oxygen followed by cleavage of the side chain leaving a secondary carbocation which loses a proton from the neighboring carbon to form the vinyl CH=CH double bond.

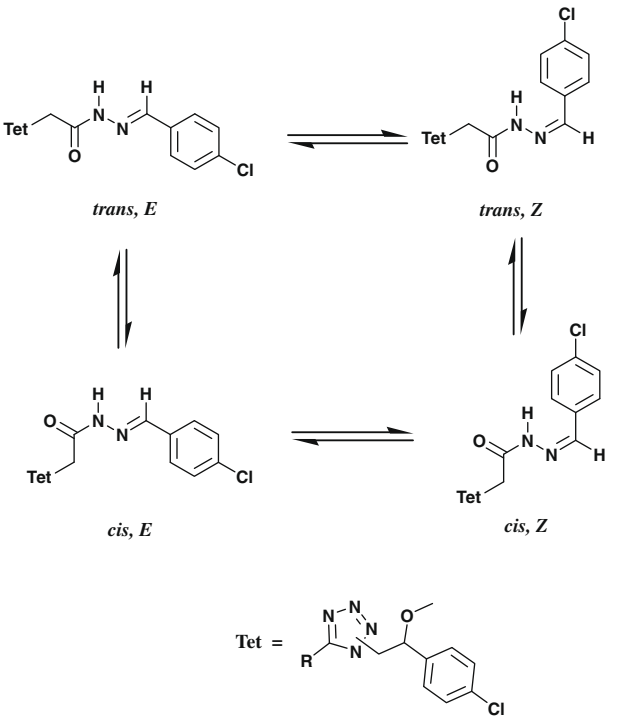


Figure 3. *E*/*Z* geometrical isomers and *cis*/*trans* conformers of compounds **13** and **14**.

2.2. Biological evaluation

2.2.1. In vitro antibacterial and antifungal activities

All the newly synthesized compounds were evaluated for their in vitro antibacterial activity against *S. aureus* (ATCC 6538), *Bacillus subtilis* (NRRL B-14819) and *Micrococcus luteus* (ATCC 21881) as examples of Gram positive bacteria and *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumonia* (clinical isolate) as examples of Gram negative bacteria. They were also evaluated for their in vitro antifungal potential against *Candida albicans*, *Candida tropicalis* and *Candida krusei* as representatives of fungi and *Aspergillus niger*, *Aspergillus fumigatus* and *Tricophyton rubrum* as examples of moulds. All the fungal and mould strains were clinical isolates, identified with conventional morphological and biochemical methods. Agar-diffusion method was used for determination of the preliminary antibacterial and antifungal activity. Ampicillin trihydrate (antibiotic), clotrimazole and miconazole (antifungals) were used as reference drugs. The results were recorded for each tested compound as the average diameter of inhibition zones (IZ) of bacterial or fungal growth around the discs in mm. The minimum inhibitory concentration (MIC) measurement was determined for compounds that showed significant growth inhibition zones ($\geqslant$14 mm) using the two-fold serial dilution method.[43] The MIC (μg/mL) values of the active compounds against the tested bacterial and fungal strains are recorded in Tables 1 and 2.

Regarding the antibacterial activity, the results revealed that 20 out of the tested 27 compounds displayed variable inhibitory effects on the growth of the tested Gram positive and Gram negative bacterial strains. In general, most of the tested compounds revealed better activity against the Gram positive rather than the Gram negative bacteria. Among the Gram positive bacteria tested, two strains namely; *S. aureus* and *B. subtilis* showed relative high sensitivity towards the tested compounds. In this view, compound **18** was equipotent to ampicillin (MIC 6.25 μg/mL) against *S. aureus,* whereas the analogs **16**, **24** and **27** (MIC 12.5 μg/mL) were 50% less active than ampicillin. Moreover, compounds **15**, **23**, **25** and **26** (MIC 25 μg/mL) showed 25% of the activity of ampicillin against the same organism. With regard to the activity against *B. subtilis*, the best activity was displayed by compounds **16** and **18** (MIC 25 μg/mL), which represented half the potency of ampicillin (MIC 12.5 μg/mL). The analogs **14**, **15**, **23**, **24**, **25**, **26** and **27** (MIC 50 μg/mL) exhibited 25% of the potency of ampicillin (MIC 12.5 μg/mL) against the same species. *M. luteus* was proved to be the least sensitive Gram positive microorganism to most of the tested compounds. Only four compounds namely; **15**, **16**, **18** and **24** (MIC 50 μg/mL) exhibited moderate growth inhibitory effect towards *M. luteus* which was 25% of the activity of ampicillin (MIC 12.5 μg/mL) (Table 1). On the other hand, investigation of antibacterial activity of the active compounds against the three tested Gram negative strains revealed that two analogs namely **18** and **27** were able to produce moderate growth inhibitory activity against *E. coli* (MIC 25 μg/mL) which was 25% of the activity of ampicillin (MIC 6.25 μg/mL). Whereas, compounds **16**, **23**, **24**, **26** and **30**, (MIC 50 μg/mL), exhibited mild activity against the same organism. Meanwhile, the tested *P. aeruginosa* and *K. pneumonia* strains were proved to be weakly sensitive to most of the tested compounds. Among these, compounds **18** and **24** showed moderate growth inhibitory profiles against these organisms (MIC 50 μg/mL), which were about 25% of the activity of ampicillin.

Concerning the antifungal activity of the tested compounds, only two organisms namely; *C. albicans* and *A. fumigatus* showed certain sensitivity against some of the tested compounds, whereas the rest of the fungal strains were totally insensitive to the same compounds. Five compounds namely; **15**, **17**, **22**, **23** and **26** exhibited moderate growth inhibitory action on *C. albicans* with MIC

Table 2
Minimal inhibitory concentrations (MIC, μg/mL) of the active newly synthesized compounds against some fungal strains

Compound no.	*Candida albicans*	*Aspergillus fumigatus*
15	100	–[a]
17	100	–
22	50	–
23	25	–
26	25	–
30	–	25
C[b]	6.25	12.5
M[c]	3.12	3.12

[a] (–): Totally inactive (MIC $\geqslant$ 200 μg/mL).
[b] C: Clotrimazole (standard broad spectrum antifungal agent).
[c] M: Miconazole (standard broad spectrum antifungal agent).

Table 1
Minimal inhibitory concentrations (MIC, μg/mL) of the active newly synthesized compounds against some Gram positive and Gram negative bacterial strains

Compound no.	*S. aureus ATCC 6538*	*B. subtilis NRRL B-14819*	*M. luteus ATCC 21881*	*E. coli ATCC 25922*	*P. aeruginosa ATCC 27853*	*K. pneumonia* (clinical isolate)
8	100	–[a]	–	–	–	–
10	100	100	–	–	–	–
12	100	–	–	–	–	–
13	100	100	100	100	–	–
14	50	50	100	100	–	–
15	25	50	50	100	–	–
16	12.5	25	50	50	100	100
17	50	100	100	–	–	–
18	6.25	25	50	25	50	100
19	100	–	100	100	–	–
20	100	–	–	–	–	–
21	50	100	–	–	–	–
22	50	100	–	100	–	–
23	25	50	100	50	–	–
24	12.5	50	50	50	100	50
25	25	50	100	100	–	100
26	25	50	–	50	–	–
27	12.5	50	100	25	100	100
30	50	–	–	50	–	–
31	100	–	–	100	–	–
A[b]	6.25	12.5	12.5	6.25	12.5	12.5

[a] (–): Totally inactive (MIC $\geqslant$ 200 μg/mL).
[b] A: Ampicillin trihydrate (standard broad spectrum antibiotic).

range of 25–100 μg/mL, when compared with clotrimazole and miconazole (MICs 6.25 and 3.12 μg/mL, respectively), the standard antifungal agents utilized in this assay. Only one compound namely; tetrazolobenzazepine **30**, was able to inhibit the growth of *A. fumigatus* at MIC of 25 μg/mL (Table 2).

A close examination of the structures of the active compounds presented in Tables 1 and 2 revealed that, their antimicrobial activity is strongly bound to the nature of the substituent at the tetrazole-C_5, together with the substituent linked to the alkoxy part of the structure. In general, it could be clearly recognized that potential antibacterial activity was encountered with tetrazoles containing a phenyl substituent, while the obtained antifungal activity was confined to those comprising the benzyl group. Moreover, the unsubstituted ethanone and ethanol derivatives **3**–**6** together with the analogs substituted with open chain counterparts like the esters, acids and acid hydrazides **7**–**12** showed weak or no antimicrobial activity (MIC $\geqslant$100 μg/mL). An exception is the azomethines **13**, **14** which showed relative better antibacterial profile when compared with the parent acid hydrazides **11**, **12**. Derivatization of the latter compounds into thiosemicarbazides **19**–**21** offered very limited improvement in the antibacterial spectrum. On the other hand, cyclization of the prementioned functionalities provided a variety of heterocycles that possess remarkable antibacterial and antifungal spectra. In this respect, cyclocondensation of the acid hydrazides **11**, **12** to the pyrazoles **15**, **16** resulted in an obvious improvement in the antimicrobial spectrum. Among these, compound **16** (R = C_6H_5) was able to exert about 50% of the activity of ampicillin against the Gram positive *S. aureus* and *B. subtilis*, whereas it showed mild activity against the Gram negative *E. coli* (MIC 50 μg/mL). On the other hand, cyclization of the thiosemicarbazides **19**–**21** resulted in a series of 1,3,4-oxadiazole and 1,3,4-triazole derivatives with significantly improved antimicrobial potentials. Regarding the 2-substituted-1,3,4-oxadiazoles structure variants **17**, **18**, **26** and **27**; the antimicrobial activity of these compounds appears to be closely related to the nature of the substituent at position-2 of the 1,3,4-oxadiazole counterpart. The 2-thiol substituent (compounds **17** and **18**) proved to be the favorite functionality and resulted in a remarkable improvement in antibacterial spectrum. In this respect, compound **18** (R = C_6H_5) showed a broad antibacterial spectrum against all the tested Gram positive and negative strains, with a unique potency against *S. aureus* that was equipotent to ampicillin (MIC 6.25 μg/mL). However, it was devoid of any antifungal activity. Referring to its benzyl analog **17**, it was noticeably less active than **18** as revealed from its activity against the three tested Gram positive strains (MIC range 50–100 μg/mL), while it was totally inactive against Gram negative bacteria. However, it showed weak antifungal activity against *C. albicans* (MIC 100 μg/mL). On the other hand, introduction of an aminophenyl moiety at position-2 of the 1,3,4-oxadiazole counterpart (compounds **26**, **27**) resulted in a noticeable change in both the antimicrobial potential and spectrum of these compounds. Thus, compound **26** (R = CH_2–C_6H_5) showed a remarkable antifungal activity against *C. albicans* (MIC 25 μg/mL) together with a moderate antibacterial profile. Replacing the benzyl group with a phenyl one as in **27** (R = C_6H_5) resulted in an obvious improvement in the antibacterial spectrum against both Gram positive and negative bacteria with special high activity against *S. aureus* (MIC 12.5 μg/mL) and *E. coli* (MIC 25 μg/mL), however, it lacked any antifungal activity. With regard to the substituted 1,3,4-triazoles **22**–**25**, the nature of the substituents seems to manipulate the antimicrobial activity. Among the benzyltetrazoles **22**, **23**, the analog **23** (R_1 = 4-Cl–C_6H_4) revealed an acceptable broad antibacterial spectrum particularly against Gram positive bacteria, with special high activity against *S. aureus* (MIC 25 μg/mL), and a remarkable antifungal potential against *C. albicans* (MIC 25 μg/mL). Replacement of the benzyl group with phenyl one (compounds **24** and **25**) enhanced both the antimicrobial spectrum and potential of these compounds. Consequently, a broad spectrum antibacterial activity was displayed by the 1,3,4-triazole derivative **24** (R_1 = C_6H_5), with a special growth inhibitory effect on *S. aureus* (MIC 12.5 μg/mL). Introduction of a chlorine atom to the structure as in compound **25** (R_1 = 4-Cl–C_6H_4) resulted in about 50% reduction in the antibacterial activity. Finally, the unexpected tetrazolobenzazepine **30** and styryltetrazole **31** derivatives revealed mild antibacterial activity against some Gram positive and Gram negative bacteria with MIC range of 50–100 μg/mL. Meanwhile, the analog **30** showed appreciable antifungal activity against *A. fumigatus* at MIC of 25 μg/mL (Table 2).

2.2.2. Preliminary anticonvulsant screening

Twelve compounds namely; **3**–**6**, **13**, **15**, **16**, **18**, **21**, **22**, **24** and **30**, were selected to be screened for their preliminary anticonvulsant properties against subcutaneous metrazole (ScMet)[44] and maximal electroshock (MES)[45] induced seizures in mice. Wister albino mice of either sex, weighing 25–30 g and 3 months old were

Table 3
Preliminary anticonvulsant screening of some representative compounds

Compound no.	Dose (mg/kg)	Anticonvulsant activity[a]			
		ScMet[b]	% Protection	Mes[c]	% Protection
3	30	0/6	0	–[d]	–
	100	3/6	50	3/6	50
	300	0/6	0	–	0
4	30	–	–	–	–
	100	0/6	0	0/6[e]	0
	300	–	–	–	–
5	30	0/6	0	–	–
	100	6/6	100	4/6	67
	300	3/6	50	–	–
6	30	–	–	–	–
	100	0/6	0	0/6	0
	300	–	–	–	–
13	30	–	–	–	–
	100	3/6	50	3/6	50
	300	–	–	–	–
15	30	–	–	–	–
	100	0/6	0	0/6	0
	300	–	–	–	–
16	30	–	–	–	–
	100	0/6	0	0/6	0
	300	–	–	–	–
18	30	–	–	–	–
	100	2/6	33	0/6	0
	300	–	–	–	–
21	30	0/6	0	–	–
	100	5/6	80	4/6	67
	300	0/6	0	–	–
22	30	–	–	–	–
	100	0/6	0	2/6	33
	300	–	–	–	–
24	30	–	–	–	–
	100	2/6	33	2/6	33
	300	–	–	–	–
30	30	–	–	–	–
	100	0/6	0	2/6[f]	33
	300	–	–	–	–

[a] Convulsions were detected within 30 min of seizure induction.
[b] Subcutaneous metrazole test (number of animals protected/number of animals tested).
[c] Maximal electroshock test (number of animals protected/number of animals tested).
[d] Not determined.
[e] Dead following tonic extension.
[f] One animal died following tonic extension.

utilized with accommodation conditions maintained at 25 °C. Dimethyl sulfoxide (DMSO) was used for dissolving pentylenetetrazole (metrazole) and the test compounds, whereas the control experiments were performed with solvent alone. The compounds were administered intraperitoneally (ip) at doses of 30, 100 and 300 mg/kg body weight, 30 min before the induction of seizures. A minimal time of 30 min subsequent to subcutaneous administration of metrazole was used for seizure detection.

The results presented in Table 3 reveal that, out of the compounds tested, five compounds namely; **3**, **5**, **13**, **21**, and **24** were able to display noticeable anticonvulsant activity in both the ScMet and MES tests with percentage protection range of 33–100%. It could be obviously recognized that the prominently active compounds **3**, **5** and **21** exerted their anticonvulsant activity at 100 mg/kg dose level, however, no activity was observed at doses of 30 or 300 mg/kg. Among these, compounds **5** (% protection: ScMet: 100 and MES: 67%) and **21** (% protection: ScMet: 80 and MES: 67%) were proved to be the most active members in this study. Furthermore, at a dose level of 100 mg/kg, compound **18** showed mild activity only in the ScMet test (33% protection), whereas, compounds **22** and **30** revealed the same activity only in the MES test (33% protection). The rest of the tested compounds namely; **4**, **6**, **15** and **16** lacked any anticonvulsant activity in both tests at the same dose levels used.

Further interpretation of the results revealed that, in spite of the observable activity of the benzyl derivatives **3** and **5** in both tests, their phenyl analogs **4** and **6** were devoid of any anticonvulsant activity. Moreover, it is clear that good anticonvulsant activity was confined to compounds including a free oxygen functionality such as carbonyl or hydroxyl groups (compounds **3** and **5**, respectively), and to those containing an alkoxy group comprising an open-chain substituted hydrazinocarbonyl group attached to the methoxy substituent at the ethylene bridge as in the azomethine **13** and the substituted thiosemicarbazide **21**. This observation is concordant with previous literature findings for the structural requirements of anticonvulsant activity for arylalkyl imidazoles represented by Nafimidone and Denzimole.[46–48] Cyclization of the alkoxy side chain to different heterocyclic ring systems as in compounds **15**, **16**, **18**, **22** and **24**, resulted in an obvious reduction in the anticonvulsant potential. In this view, while compounds comprising the 1,3,4-oxadiazolyl (**18**) and the 1,3,4-triazolyl (**22** and **24**) moieties exhibited mild anticonvulsant activity, those containing a pyrazolyl counterpart (**15** and **16**) were devoid of any activity.

3. Conclusion

The main aim of the present investigation is to synthesize and investigate the antimicrobial activity of new tetrazole-containing compounds that are structurally related to the famous antimicrobial azole pharmacophore, with the hope of discovering new structure leads serving as potential broad spectrum antimicrobial agents. The obtained results revealed that twenty compounds were able to display variable growth inhibitory effects on the tested Gram positive and Gram negative bacterial strains. Meanwhile, five compounds exhibited moderate antifungal activity against *C. albicans*, whereas, only one analog was able to inhibit the growth of *A. fumigatus*. In general, most of the tested compounds showed better activity against the Gram positive rather than the Gram negative bacteria, particularly *S. aureus* and *B. subtilis.* Structurally, the antimicrobial potential of the active compounds is dependent on the nature of the substituents: remarkable antibacterial activity was encountered with tetrazoles containing a phenyl substituent, while the obtained antifungal activity was confined to those comprising the benzyl group. Moreover, the unsubstituted ethanone and ethanol derivatives, together with the analogs substituted with open chain counterparts like the esters, acids, acid hydrazides and thiosemicarbazides showed weak or no antimicrobial activity (MIC $\geqslant$100 μg/mL). However, cyclization of the pre-mentioned functionalities provided a variety of heterocycles with improved antibacterial and antifungal spectra. Among these, the 1,3,4-oxadiazole and 1,3,4-triazole derivatives revealed significant broad spectrum antimicrobial activities. Compounds **16**, **18**, **24** and **27** proved to be the most active antibacterial members within this study with a considerable broad spectrum against all the Gram positive and negative strains tested. Among these, particular anti-Gram positive activity was displayed by compound **18** against *S. aureus* that was equipotent to ampicillin (MIC 6.25 μg/mL).

On the other hand, twelve compounds were selected to be screened for their preliminary anticonvulsant activity against subcutaneous metrazole (ScMet) and maximal electroshock (MES) induced seizures in mice. The results revealed that five compounds were able to display noticeable anticonvulsant activity in both tests at 100 mg/kg dose level. Good anticonvulsant activity was confined to compounds including a free oxygen functionality such as carbonyl or hydroxyl groups, and to those containing an alkoxy group with an open chain substituent. Cyclization of the alkoxy side chain to different heterocyclic ring systems resulted in an obvious reduction in the anticonvulsant potential. Compounds **5** and **21** were proved to be the most active anticonvulsant members in this study with special high activity in the ScMet assay (% protection: 100% and 80%, respectively). Finally, these compounds represent new structure scaffolds that could be further optimized for future development of more potent and selective antimicrobial and/or anticonvulsant agents.

4. Experimental

4.1. Chemistry

Melting points were determined in open glass capillaries on a Stuart melting point apparatus and were uncorrected. The infrared (IR) spectra were recorded on Perkin–Elmer 1430 infrared spectrophotometer using the KBr plate technique. ^{1}H NMR, ^{13}C NMR, NOE, HMQC and HMBC spectra were determined on Jeol spectrometer (500 MHz) at the Microanalytical unit, Faculty of Science, Alexandria University using tetramethylsilane (TMS) as internal standard and DMSO-d_6 as solvent (chemical shifts in δ, ppm). Splitting patterns were designated as follows: s: singlet; d: doublet; dd: doublet of doublet; t: triplet; m: multiplet. Mass spectra were carried out using a Schimadzu GCMS-QP-1000EX mass spectrometer at 70ev, Faculty of Science, Cairo University. Microanalyses were performed at the Microanalytical Unit, Faculty of Science, Cairo University and at the Central lab, Faculty of Pharmacy, Alexandria University, Egypt. The found values were within ±0.4% of the theoretical values. Silica gel 60GF 254 was used for column chromatography. Follow up of the reactions and checking the purity of the compounds were made by TLC on silica gel-protected glass plates and the spots were detected by exposure to UV-lamp at λ 254.

4.1.1. 1-(4-Chlorophenyl)-2-(5-substituted-1*H*-tetrazol-1-yl or 2*H*-tetrazol-2-yl)ethanones (3,4)

A mixture of the appropriate tetrazole **1**, **2** (75 mmol), 4-chlorophenacyl bromide (17.6 g, 75 mmol) and anhydrous potassium carbonate (10.4 g, 75 mmol) in dry acetone (120 mL) was heated under reflux with stirring for 8 h. The reaction mixture was left to cool and filtered. The filtrate was evaporated under reduced pressure and the residual solid mass was triturated with diethyl ether and the separated solid was filtered, dried and crystallized.

4.1.1.1. 2-(5-Benzyl-1*H*-tetrazol-1-yl)-1-(4-chlorophenyl)ethanone (3). White crystals, (ethyl acetate/*n*-hexane). Yield: 75%;

mp: 130–132 °C; IR (KBr, cm^{-1}): 1693 (C=O), 1589 (C=N). ^1H NMR (δ ppm): 4.25 (s, 2H, benzyl CH_2), 6.32 (s, 2H, N-CH_2), 7.15–7.26 (m, 5H, benzyl-H), 7.66, 8.0 (2d, J = 8.4 Hz, 4H, chlorophenyl $C_{3,5}$–H & $C_{2,6}$–H). ^{13}C NMR (δ ppm): 28.36 (benzyl CH_2), 53.63 (N–CH_2), 127.52 (benzyl C_4), 129.07 (benzyl $C_{3,5}$), 129.47 (chlorophenyl $C_{3,5}$), 129.60 (benzyl $C_{2,6}$), 130.83 (chlorophenyl $C_{2,6}$), 132.96 (chlorophenyl C_1), 135.50 (benzyl C_1), 139.91 (chlorophenyl C_4), 156.17 (tetrazole-C_5), 190.79 (C=O). Anal. Calcd for $C_{16}H_{13}ClN_4O$ (312.75): C, 61.44; H, 4.19; N, 17.91. Found: C, 61.32; H, 4.53; N, 17.97.

4.1.1.2. 1-(4-Chlorophenyl)-2-(5-phenyl-2*H*-tetrazol-2-yl)ethanone (4). White crystals, (ethyl acetate/*n*-hexane). Yield: 85%; mp: 159–160 °C; IR (KBr, cm^{-1}): 1691 (C=O), 1589 (C=N). ^1H NMR (δ ppm): 6.72 (s, 2H, N-CH_2), 7.50–7.57 (m, 3H, phenyl $C_{3,4,5}$–H), 7.66 (d, J = 8.4 Hz, 2H, chlorophenyl $C_{3,5}$–H), 8.07 (d, J = 8.4 Hz, 4H, phenyl $C_{2,6}$–H & chlorophenyl $C_{2,6}$–H). ^{13}C NMR (δ ppm): 59.57 (N–CH_2), 126.91 (phenyl $C_{2,6}$), 127.32 (phenyl C_4), 129.7 (chlorophenyl $C_{3,5}$), 129.84 (phenyl $C_{3,5}$), 130.84 (chlorophenyl $C_{2,6}$), 131.18 (phenyl C_1), 132.95 (chlorophenyl C_1), 140.11 (chlorophenyl C_4), 164.93 (tetrazole-C_5), 190.97 (C=O). Anal. Calcd for $C_{15}H_{11}ClN_4O$ (298.73): C, 60.31; H, 3.71; N, 18.76. Found: C, 60.92; H, 3.61; N, 19.16.

4.1.2. 1-(4-Chlorophenyl)-2-(5-substituted 1*H*-tetrazol-1-yl or 2*H*-tetrazol-2-yl)ethanols (5, 6)

To a well stirred suspension of compound **3** or **4** (30 mmol) in ethanol (80 mL), sodium borohydride (4.5 g, 120 mmol) was added portion wise over a period of 1 h. Stirring at room temperature was maintained for further 24 h and the reaction mixture was then poured onto crushed ice. The separated solid was filtered, washed with water, dried and crystallized.

4.1.2.1. 2-(5-Benzyl-1*H*-tetrazol-1-yl)-1-(4-chlorophenyl) ethanol (5). White crystals, (ethanol/water). Yield: 87%; mp: 114–115 °C; IR (KBr, cm^{-1}): 3355 (OH), 1598 (C=N); ^1H NMR (δ ppm): 4.23, 4.28 (2d, J = 16.4 Hz, 2H, benzyl CH_2), 4.43 (dd, J = 14.2, 7.8 Hz, 1H, N–CH_2), 4.52 (dd, J = 14.2, 4 Hz, 1H, N–CH_2), 4.92 (dd, J = 7.8, 4 Hz, 1H, CH), 6.04 (s, 1H, OH, D_2O exchangeable) 7.19–7.32 (m, 5H, benzyl-H), 7.35, 7.38 (2d, J = 8.4 Hz, 4H, chlorophenyl $C_{2,6}$–H & $C_{3,5}$–H). Anal. Calcd for $C_{16}H_{15}ClN_4O$ (314.77): C, 61.05; H, 4.80; N, 17.80. Found: C, 61.45; H, 4.76; N, 18.27.

4.1.2.2. 1-(4-Chlorophenyl)-2-(5-phenyl-2*H*-tetrazol-2-yl)ethanol (6). White crystals, (ethanol). Yield: 89%; mp: 120–121 °C; IR (KBr, cm^{-1}): 3320 (OH), 1592 (C=N); ^1H NMR (δ ppm): 4.85–4.94 (m, 2H, N–CH_2), 5.20–5.30 (m, 1H, CH), 5.99 (d, J = 5 Hz, 1H, OH, D_2O exchangeable), 7.39, 7.45 (2d, J = 8.4 Hz, 4H, chlorophenyl $C_{2,6}$–H & $C_{3,5}$–H), 7.50–7.56 (m, 3H, phenyl $C_{3,4,5}$–H), 8.01–8.06 (m, 2H, phenyl $C_{2,6}$–H). Anal. Calcd for $C_{15}H_{13}ClN_4O$ (300.74): C, 59.91; H, 4.36; N, 18.63. Found: C, 60.37; H, 4.31; N, 18.88.

4.1.3. Ethyl2-[1-(4-chlorophenyl)-2-(5-substituted 1*H*-tetrazol-1-yl or 2*H*-tetrazol-2-yl)ethoxy]acetates (7, 8)

To a well stirred solution of compounds **5** or **6** (10 mmol) in dry tetrahydrofuran (15 mL), sodium hydride (0.36 g, 15 mmol) was added portion wise and the mixture was stirred at room temperature for 1 h. Ethyl bromoacetate (1.67 g, 1.1 mL, 10 mmol) was added dropwise and the reaction mixture was left stirred at room temperature overnight. The reaction mixture was concentrated under reduced pressure, poured onto cold water (100 mL) and extracted with methylene chloride (2 × 50 mL). The combined organic extracts were washed with water (2 × 30 mL), dried over anhydrous sodium sulfate, concentrated under vacuum to a small volume and chromatographed on a silica gel column eluting first with a mixture of methylene chloride/petroleum ether (60/80) (20:1) then with mixtures containing decreasing amounts of petroleum ether and finally with methylene chloride alone. The separated products were then crystallized.

4.1.3.1. Ethyl2-[2-(5-benzyl-1*H*-tetrazol-1-yl)-1-(4-chlorophenyl)ethoxy]acetate (7). White crystals, (methylene chloride/petroleum ether 60/80). Yield: 40%; mp: 80–81 °C; IR (KBr, cm^{-1}): 1745 (C=O), 1600 (C=N); 1247, 1133, 1090, 1046 (C–O–C); ^1H NMR (δ ppm): 1.10 (t, J = 6.9 Hz, 3H, CH_2CH_3), 3.76, 3.90 (2d, J = 16.4 Hz, 2H, OCH_2), 3.96 (q, J = 6.9 Hz, 2H, CH_2CH_3), 4.31, 4.34 (2d, J = 16.1 Hz, 2H, benzyl CH_2), 4.60 (dd, J = 14.5, 4.2 Hz, 1H, N–CH_2), 4.65 (dd, J = 14.5, 8 Hz, 1H, N–CH_2), 4.84 (dd, J = 8, 4.2 Hz, 1H, CH), 7.21–7.32 (m, 5H, benzyl-H), 7.35, 7.43 (2d, J = 8.1 Hz, 4H, chlorophenyl $C_{2,6}$–H & $C_{3,5}$–H). Anal. Calcd for $C_{20}H_{21}ClN_4O_3$ (400.86): C, 59.92; H, 5.28; N, 13.98. Found: C, 59.67; H, 5.03; N, 13.72.

4.1.3.2. Ethyl2-[1-(4-chlorophenyl)-2-(5-phenyl-2*H*-tetrazol-2-yl)ethoxy]acetate (8). White crystals, (methylene chloride/petroleum ether 60/80). Yield: 35%; mp: 68–70 °C; IR (KBr, cm^{-1}): 1745 (C=O), 1596 (C=N); 1207, 1125, 1090, 1045 (C–O–C); ^1H NMR (δ ppm): 1.0 (t, J = 6.9 Hz, 3H, CH_2CH_3), 3.86 (d, J = 16.4 Hz, 1H, OCH_2), 3.91 (q, J = 6.9 Hz, 2H, CH_2CH_3), 4.04 (d, J = 16.4 Hz, 1H, OCH_2), 4.96 (dd, J = 13.6, 3.2 Hz, 1H, N–CH_2), 5.11 (dd, J = 13.6, 8.4 Hz, 1H, N–CH_2), 5.16 (dd, J = 8.4, 3.2 Hz, 1H, CH), 7.42, 7.45 (2d, J = 8.4 Hz, 4H, chlorophenyl $C_{2,6}$–H & $C_{3,5}$–H), 7.49–7.55 (m, 3H, phenyl $C_{3,4,5}$–H), 8.03 (d, J = 7.65 Hz, 2H, phenyl $C_{2,6}$–H). Anal. Calcd for $C_{19}H_{19}ClN_4O_3$ (386.83): C, 58.99; H, 4.95; N, 14.48. Found: C, 58.30; H, 4.87; N, 14.25.

4.1.4. 2-[1-(4-Chlorophenyl)-2-(5-substituted 1*H*-tetrazol-1-yl or 2*H*-tetrazol-2-yl)ethoxy]acetic acids (9, 10)

Method A: To a well stirred solution of compounds **5** or **6** (1.5 mmol) in dry tetrahydrofuran (5 mL), sodium hydride (0.054 g, 2.25 mmol) was added and the mixture was stirred at room temperature for 1 h. Chloroacetic acid (0.14 g, 1.5 mmol) was then added and the mixture was left stirred at room temperature overnight. The reaction mixture was evaporated to dryness under reduced pressure and the remaining residue was triturated with diethyl ether. The separated solid was filtered, dissolved in water, neutralized with dil HCl and the obtained product was filtered, dried and crystallized.

Method B: A mixture of the selected ester **7** or **8** (2.7 mmol) and 5% sodium hydroxide (2.6 ml) in ethanol (15 mL) was heated under reflux for 2 h. After evaporation of the reaction mixture to dryness, the residue was dissolved in water and acidified with dil HCl. The separated solid was filtered, washed with water, dried and crystallized.

4.1.4.1. 2-[2-(5-Benzyl-1*H*-tetrazol-1-yl)-1-(4-chlorophenyl)ethoxy]acetic acid (9). White crystals (ethanol). Yield: 35% (method A), 85% (method B); mp: 132–133 °C; IR (KBr, cm^{-1}): 3166–2625 (OH), 1730 (C=O), 1596 (C=N); 1245, 1135, 1088, 1049 (C–O–C); ^1H NMR (δ ppm): 3.70, 3.86 (2d, J = 16.4 Hz, 2H, OCH_2), 4.30, 4.36 (2d, J = 16.4 Hz, 2H, benzyl CH_2), 4.61 (dd, J = 14.89, 4.78 Hz, 1H, N–CH_2), 4.65 (dd, J = 14.89, 8.22 Hz, 1H, N–CH_2), 4.86 (dd, J = 8.22, 4.78 Hz, 1H, CH), 7.22–7.31 (m, 5H, benzyl-H), 7.33, 7.42 (2d, J = 8.4 Hz, 4H, chlorophenyl-H), 12.67 (br s, 1H, OH, D_2O exchangeable). Anal. Calcd for $C_{18}H_{17}ClN_4O_3$ (372.81): C, 57.99; H, 4.60; N, 15.03. Found: C, 58.33; H, 4.75; N, 15.04.

4.1.4.2. 2-[1-(4-Chlorophenyl)-2-(5-phenyl-2*H*-tetrazol-2-yl)ethoxy]acetic acid (10). White crystals (ethanol/water). Yield: 34% (method A), 82% (method B); mp: 126–128 °C; IR (KBr, cm^{-1}): 3166–2625 (OH), 1701 (C=O), 1593 (C=N); 1235, 1115, 1088, 1045 (C–O–C); ^1H NMR (δ ppm): 3.78, 3.96 (2d, J = 16.8 Hz, 2H, OCH_2), 4.96 (dd, J = 13.74, 4.59 Hz, 1H, N–CH_2), 5.09 (dd,

J = 13.74, 8.02 Hz, 1H, N–CH_2), 5.15 (dd, J = 8.02, 4.59 Hz, 1H, CH), 7.42 (s, 4H, chlorophenyl-H), 7.49–7.55 (m, 3H, phenyl $C_{3,4,5}$–H), 8.01 (d, J = 7.1 Hz, 2H, phenyl $C_{2,6}$–H), 12.68 (s, 1H, OH, D_2O exchangeable). Anal. Calcd for $C_{17}H_{15}ClN_4O_3$ (358.78): C, 56.91; H, 4.21; N, 15.62. Found: C, 55.60; H, 4.31; N, 15.42.

4.1.5. 2-[1-(4-Chlorophenyl)-2-(5-substituted 1*H*-tetrazol-1-yl or 2*H*-tetrazol-2-yl)ethoxy]acetohydrazides (11, 12)

To a solution of the acetate ester **7** or **8** (20 mmol) in absolute ethanol (50 mL), hydrazine hydrate 98% (8 g, 160 mmol) was added and the reaction mixture was heated under reflux for 4 h. For compound **11**, the reaction mixture was evaporated to dryness under reduced pressure and the residual mass was crystallized. For compound **12**, the reaction mixture was left overnight and the separated solid product was filtered, dried and crystallized.

4.1.5.1. 2-[2-(5-Benzyl-1*H*-tetrazol-1-yl)-1-(4-chlorophenyl)ethoxy]acetohydrazide (11). White crystals (ethanol/water). Yield: 73%; mp: 30–31 °C; IR (KBr, cm^{-1}): 3330, 3274 (NH_2, NH), 1682 (C=O), 1615 (C=N); 1200, 1104, 1040 (C–O–C); ^1H NMR (δ ppm): 3.76 (s, 2H, NH_2, D_2O exchangeable), 3.80, 3.86 (2d, J = 16.4 Hz, 2H, OCH_2), 4.33, 4.37 (2d, J = 16.3 Hz, 2H, benzyl CH_2), 4.60 (dd, J = 14.6, 4.5 Hz, 1H, N–CH_2), 4.64 (dd, J = 14.6, 7.8 Hz, 1H, N–CH_2), 4.86 (dd, J = 7.8, 4.5 Hz, 1H, CH), 7.20–7.33 (m, 5H, benzyl-H), 7.37, 7.46 (2d, J = 8.2 Hz, 4H, chlorophenyl $C_{2,6}$–H & $C_{3,5}$–H), 8.84 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{18}H_{19}ClN_6O_2{\cdot}1H_2O$ (404.86): C, 53.40; H, 5.23; N, 20.75. Found: C, 53.75; H, 4.84; N, 20.60.

4.1.5.2. 2-[1-(4-Chlorophenyl)-2-(5-phenyl-2*H*-tetrazol-2-yl)ethoxy]acetohydrazide (12). White crystals (ethanol). Yield: 75%; mp: 130–131 °C; IR (KBr, cm^{-1}): 3334–3274 (NH_2, NH), 1685 (C=O), 1620 (C=N); 1202, 1104, 1045 (C–O–C); ^1H NMR (δ ppm): 3.79 (s, 2H, NH_2, D_2O exchangeable), 4.22 (s, 2H, OCH_2), 4.94 (dd, J = 13.7, 4.6 Hz, 1H, N–CH_2), 5.12 (dd, J = 13.7, 8 Hz, 1H, N–CH_2), 5.20 (dd, J = 8, 4.6 Hz, 1H, CH), 7.45 (s, 4H, chlorophenyl-H), 7.52–7.61 (m, 3H, phenyl $C_{3,4,5}$–H), 8.0–8.1 (m, 2H, phenyl $C_{2,6}$–H), 8.92 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{17}H_{17}ClN_6O_2$ (372.81): C, 54.77; H, 4.60. N, 22.54. Found: C, 54.59; H, 4.70; N, 22.43.

4.1.6. (*E*)-2-[1-(4-Chlorophenyl)-2-(5-substituted-1*H*-tetrazol-1-yl or 2*H*-tetrazol-2-yl)ethoxy]-*N*^{1}-(4-chlorobenzylidene)acetohydrazides (13,14)

A solution of the selected acetohyrazide **11** or **12** (1 mmol) in ethanol (10 mL) was heated under reflux with an equimolar amount of 4-chlorobenzaldehyde for 2 h. The reaction mixture was left for an overnight and the precipitated solid was filtered, washed with ethanol, dried and crystallized.

4.1.6.1. (*E*)-2-[2-(5-Benzyl1*H*-tetrazol-1-yl)-1-(4-chlorophenyl)ethoxy]-*N*^{1}-(4-chlorobenzylidene)acetohydrazide (13). White crystals (ethanol). Yield: 82%; mp: 191–192 °C; IR (KBr, cm^{-1}): 3312 (NH), 1697 (C=O), 1607 (C=N); 1232, 1124, 1087, 1041 (C–O–C); ^1H NMR (δ ppm): 3.78, 3.87 (2d, J = 14.55 Hz, OCH_2, *cis* conformer), 4.17–4.38 (m, 4H, benzyl CH_2 & OCH_2; *trans* conformer), 4.64 (dd, J = 15.27, 3.82 Hz, 1H, N–CH_2), 4.72 (dd, J = 15.27, 7.85 Hz, 1H, N–CH_2), 4.89 (dd, J = 7.85, 3.82 Hz, 1H, CH), 7.20–7.28 (m, 13H, benzyl-H & two chlorophenyl-H), 8.13, 8.68 (2s, 2H, two N=CH, *cis* and *trans* conformers), 11.20, 11.44 (2s, 2H, 2CONH, *cis* and *trans* conformers), Anal. Calcd for $C_{25}H_{22}Cl_2N_6O_2$ (509.39): C, 58.95; H, 4.35; N, 16.50. Found: C, 58.58; H, 3.84; N, 15.74.

4.1.6.2. (*E*)-2-[1-(4-Chlorophenyl)-2-(5-phenyl-2*H*-tetrazol-2yl)ethoxy]-*N*^{1}-(4-chlorobenzylidene)acetohyrazide (14). White crystals (ethanol). Yield: 85%; mp: 198–199 °C; IR (KBr, cm^{-1}): 3188 (NH), 1685 (C=O), 1594 (C=N), 1232, 1121, 1086, 1011 (C–O–C); ^1H NMR (δ ppm): 3.93, 3.97 (2d, J = 15.8 Hz, 2H, OCH_2, *cis* conformer), 4.28, 4.45 (2d, J = 16.1 Hz, 2H, OCH_2; *trans* conformer), 4.8–5.4 (m, 3H, N–CH_2 & CH), 7.40–7.70 (m, 11H, phenyl $C_{3,4,5}$–H & two chlorophenyl-H), 7.84 (s, 1H, N=CH, *cis* conformer), 8.03 (m, 2H, phenyl $C_{2,6}$–H), 8.17 (s, 1H, N=CH, *trans* conformer), 11.12, 11.39 (2s, 2H, 2CONH, *cis* and *trans* conformers). Anal. Calcd for $C_{24}H_{20}Cl_2N_6O_2$ (495.36): C, 58.19; H, 4.07; N, 16.97. Found: C, 57.71; H, 4.09; N, 16.66.

4.1.7. 2-[1-(4-Chlorophenyl)-2-(5-substituted1*H*-tetrazol-1-yl or 2*H*-tetrazol-2-yl)ethoxy]-1-(3,5-dimethyl-1*H*-pyrazol-1-yl)ethanones (15, 16)

A mixture of equimolar amounts of **11** or **12** (1 mmol) and acetyl acetone (1 mmol) in ethanol (10 mL) was heated under reflux for 6 h. The reaction mixture was concentrated to approximately half of its volume and allowed to cool to room temperature. The separated solid was filtered, washed with ether, dried and crystallized.

4.1.7.1. 2-[2-(5-Benzyl-1*H*-tetrazol-1-yl)-1-(4-chlorophenyl)ethoxy]-1-(3,5-dimethyl-1*H*-pyrazol-1-yl)ethanone (15). White crystals (ethanol). Yield: 53%; mp: 152–153 °C; IR (KBr, cm^{-1}): 1740 (C=O), 1655 (C=N); 1210, 1131, 1090 (C–O–C); ^1H NMR (δ ppm): 2.05, 2.39 (2s, 6H, $2CH_3$), 4.34, 4.37 (2d, J = 16.8 Hz, 2H, benzyl CH_2), 4.49–4.70 (m, 4H, OCH_2 & N–CH_2), 4.97 (dd, J = 6.85, 4.6 Hz, 1H, CH), 6.12 (s, 1H, pyrazole C_4–H), 7.20–7.44 (m, 9H, benzyl-H & chlorophenyl-H). Anal. Calcd for $C_{23}H_{23}ClN_6O_2$ (450.92): C, 61.26; H, 5.14; N, 18.64. Found: C, 61.66; H, 4.99; N, 18.39.

4.1.7.2. 2-[1-(4-Chlorophenyl)-2-(5-phenyl-2*H*-tetrazol-2-yl)ethoxy]-1-(3,5-dimethyl-1*H*-pyrazol-1-yl)ethanone (16). White crystals (ethanol). Yield: 65%; mp: 160–161 °C; IR (KBr, cm^{-1}): 1742 (C=O), 1655 (C=N); 1201, 1137, 1090 (C–O–C); ^1H NMR (δ ppm): 2.03, 2.33 (2s, 6H, $2CH_3$), 4.59, 4.73 (2d, J = 17.5 Hz, 2H, OCH_2), 4.99 (dd, J = 13.76, 3.0 Hz, 1H, N–CH_2), 5.14 (dd, J = 13.76, 7.47 Hz, 1H, N–CH_2), 5.26 (dd, J = 7.47, 3.0 Hz, 1H, CH), 6.08 (s, 1H, pyrazole C_4–H), 7.40–7.60 (m, 7H, phenyl $C_{3,4,5}$–H & chlorophenyl-H), 8.0 (d, J = 7.1 Hz, 2H, phenyl $C_{2,6}$–H). Anal. Calcd for $C_{22}H_{21}ClN_6O_2$ (436.89): C, 60.48; H, 4.84; N, 19.24. Found: C, 60.17 H, 4.89; N, 18.92.

4.1.8. 5-{[1-(4-Chlorophenyl)-2-(5-substituted1*H*-tetrazol-1-yl or 2*H*-tetrazol-2-yl)ethoxy]methyl}-1,3,4-oxadiazole-2-thiols (17, 18)

A mixture of **11** or **12** (5 mmol), potassium hydroxide (5 mmol) and carbon disulfide (5 mL) in ethanol (50 ml) was heated under reflux for 12 h. The reaction mixture was concentrated, cooled, diluted with water and acidified with dil HCl. The separated solid was filtered, washed with ethanol, dried and crystallized.

4.1.8.1. 5-{[2-(5-Benzyl-1*H*-tetrazol-1-yl)-1-(4-chlorophenyl)ethoxy]methyl}-1,3,4-oxadiazole-2-thiol (17). White crystals (ethanol). Yield: 73%; mp: 230–231 °C; IR (KBr, cm^{-1}): 3125 (NH), 2788 (SH), 1635, 1598 (C=N), 1513, 1270, 1095, 940 (NCS), 1199, 1129, 1061 (C–O–C); ^1H NMR (δ ppm): 4.24 (s, 2H, OCH_2), 4.28, 4.32 (2d, J = 14.5 Hz, 2H, benzyl CH_2), 4.62 (dd, J = 14.5, 4 Hz, 1H, N–CH_2), 4.72 (dd, J = 14.5, 8.4 Hz, 1H, N–CH_2), 4.91 (dd, J = 8.4, 4 Hz, 1H, CH), 7.17–7.32 (m, 5H, benzyl-H), 7.34, 7.43 (2d, J = 7.25 Hz, 4H, chlorophenyl-H). 14.5 (s, 1H, SH, D_2O exchangeable). Anal. Calcd for $C_{19}H_{17}ClN_6O_2S$ (428.90): C, 53.21; H, 4.00. Found: C, 53.01; H, 4.55; N. 19.64.

4.1.8.2. 5-{[1-(4-Chlorophenyl)-2-(5-phenyl-2*H*-tetrazol-2-yl)-ethoxy]methyl}-1,3,4-oxadiazole-2-thiol (18). White crystals (ethanol). Yield: 72%; mp: 172–173 °C; IR (KBr, cm^{-1}): 3136 (NH), 2774 (SH), 1619, 1595 (C=N), 1511, 1288, 1083, 940 (NCS), 1249, 1153, 1054 (C–O–C); ^1H NMR (δ ppm): 4.43, 4.51 (2d, J = 14.2 Hz, 2H, OCH_2), 5.01 (dd, J = 17, 4.15 Hz, 1H, N–CH_2), 5.20

(dd, J = 17, 7.65 Hz, 1H, N–CH_2), 5.30 (dd, J = 7.65, 4.15 Hz, 1H, CH), 7.40–7.70 (m, 7H, phenyl $C_{3,4,5}$–H & chlorophenyl-H), 8.02–8.09 (m, 2H, phenyl $C_{2,6}$–H), 14.44 (s, 1H, SH, D_2O exchangeable). Anal. Calcd for $C_{18}H_{15}ClN_6O_2S$ (414.87): C, 52.11; H, 3.64; N, 20.26. Found: C, 51.88; H, 4.05; N, 19.93.

4.1.9. N^4-Aryl-N^1-{[1-(4-chlorophenyl)-2-(5-substituted1*H*-tetrazol-1-yl or 2*H*-tetrazol-2-yl)ethoxy]acetyl} thiosemicarbazides (19–21)

A mixture of equimolar amounts of **11** or **12** (5 mmol) and the selected aryl isothiocyanate (5 mmol) in absolute ethanol (20 mL) was stirred at room temperature for 3 h. The reaction mixture cleared then a heavy white precipitate separated out. It was filtered, washed with ether, dried and crystallized.

4.1.9.1. N^1-{[2-(5-Benzyl-1*H*-tetrazol-1-yl)-1-(4-chlorophenyl)ethoxy]acetyl}-N^4-phenylthiosemicarbazide (19). White crystals (dioxane/water). Yield: 80%; mp: 186–187 °C; IR (KBr, cm^{-1}): 3250, 3182 (NH), 1697 (C=O), 1597 (C=N), 1552, 1278, 1089, 973 (NCS), 1237, 1124, 1058, 1032 (C–O–C); ^{1}H NMR (δ ppm): 3.77, 3.86 (2d, J = 14.5 Hz, 2H, OCH_2), 4.30 (s, 2H, benzyl CH_2), 4.66 (dd, J = 15.28, 4.6 Hz, 1H, N–CH_2), 4.71 (dd, J = 15.28, 6.87 Hz, 1H, N–CH_2), 4.92 (dd, J = 6.87, 4.6 Hz, 1H, CH), 7.12–7.32 (m, 10H, benzyl-H & phenyl-H), 7.34, 7.43 (2d, J = 8.4 Hz, 4H, chlorophenyl-H), 9.58 (s, 2H, NHC=S & NH-phenyl, D_2O exchangeable), 9.93 (s, 1H, NH–C=O). Anal. Calcd for $C_{25}H_{24}ClN_7O_2S$ (522.02): C, 57.52; H, 4.63; N, 18.78. Found: C, 57.84; H, 5.00; N, 18.77.

4.1.9.2. N^1-{[2-(5-Benzyl-1*H*-tetrazol-1-yl)-1-(4-chlorophenyl)ethoxy]acetyl}-N^4-(4-chlorophenyl)thiosemicarbazide (20). White crystals (dioxane/water). Yield: 84%; mp: 179–180 °C; IR (KBr, cm^{-1}): 3255, 3182 (NH), 1700 (C=O), 1599 (C=N), 1558, 1279, 1089, 975 (NCS), 1196, 1126, 1034 (C–O–C); ^{1}H NMR (δ ppm): 3.77, 3.86 (2d, J = 14.5 Hz, 2H, OCH_2), 4.3 (s, 2H, benzyl CH_2), 4.63 (dd, J = 12.23, 6.5 Hz, 1H, N–CH_2), 4.67 (dd, J = 12.23, 5.35 Hz, 1H, N–CH_2), 4.93 (dd, J = 6.5, 5.35 Hz, 1H, CH), 7.12–7.33 (m, 9H, benzyl-H & N^4-chlorophenyl-H), 7.35, 7.43 (2d, J = 8.4 Hz, 4H, chlorophenyl-H), 9.57 (s, 2H, NHC=S & NHC_6H_4–Cl, D_2O exchangeable), 9.92 (s, 1H, NHC=O, D_2O exchangeable). Anal. Calcd for $C_{25}H_{23}Cl_2N_7O_2S$ (556.47): C, 53.96; H, 4.17; N, 17.62. Found: C, 53.70; H, 4.01; N, 17.50.

4.1.9.3. N^4-Phenyl-N^1-{[2-(5-phenyl-2*H*-tetrazol-2-yl)-1-(4-chlorophenyl)ethoxy]acetyl}thiosemicarbazide (21). White crystals (dioxane/water). Yield: 69%; mp: 175–176 °C; IR (KBr, cm^{-1}): 3347, 3322, 3221 (NH), 1727 (C=O), 1596 (C=N); 1513, 1265, 1088, 932 (NCS), 1210, 1108, 1041 (C–O–C); ^{1}H NMR (δ ppm): 3.86, 3.93 (2d, J = 14.55 Hz, 2H, OCH_2), 5.0 (dd, J = 6.85, 5.35 Hz, 1H, CH), 5.13 (dd, J = 17.5, 6.85 Hz, 1H, N–CH_2), 5.17 (dd, J = 17.5, 5.35 Hz, 1H, N–CH_2), 7.1–7.54 (m, 12H, phenyl $C_{3,4,5}$–H, N^4-phenyl-H & chlorophenyl-H), 7.95–8.02 (m, 2H, phenyl $C_{2,6}$–H), 9.51 (s, 2H, NHC=S & –NH phenyl, D_2O exchangeable). Anal. Calcd for $C_{24}H_{22}ClN_7O_2S$ (508.0): C, 56.74; H, 4.37; N, 19.30. Found: C, 56.46; H, 4.41; N, 19.06.

4.1.10. 5-{[2-(5-Benzyl-1*H*-tetrazol-1-yl)-1-(4-chlorophenyl) ethoxy]methyl}-4-substituted 4*H*-1,2,4-triazole-3-thiols (22, 23)

A suspension of the selected thiosemicarbazide **19** or **20** (1.1 mmol) in 10 mL 5% Na_2CO_3 solution was heated under reflux for 2 h. The reaction mixture was cooled, filtered and the filtrate was acidified using 2 N HCl. The obtained precipitate was filtered, washed with water, dried and crystallized.

4.1.10.1. 5-{[2-(5-Benzyl-1*H*-tetrazol-1-yl)-1-(4-chlorophenyl)-ethoxy]methyl}-4-phenyl-4*H*-1,2,4-triazole-3-thiol (22). White crystals (ethanol/water). Yield: 85%; mp: 191–192 °C; IR (KBr, cm^{-1}): 3433 (NH), 2766 (SH), 1593 (C=N), 1249, 1108, 1054 (C–O–C); ^{1}H NMR (δ ppm): 4.06 (s, 2H, OCH_2), 4.17 (s, 2H, benzyl CH_2), 4.56 (dd, J = 14.5, 4.4 Hz, 1H, N–CH_2), 4.58 (dd, J = 14.5, 6.8 Hz, 1H, N–CH_2), 4.75 (dd, J = 6.8, 4.4 Hz, 1H, CH), 7.02–7.57 (m, 14H, Ar–H), 13.98 (s, 1H, SH, D_2O exchangeable). Anal. Calcd for $C_{25}H_{22}ClN_7OS$ (504.01): C, 59.58; H, 4.40; N, 19.45. Found: C, 59.81; H, 4.80; N, 19.10.

4.1.10.2. 5-{[2-(5-Benzyl-1*H*-tetrazol-1-yl)-1-(4-chlorophenyl)-ethoxy]methyl}-4-(4-chlorophenyl)-4*H*-1,2,4-triazole-3-thiol (23). White crystals (ethanol/water). Yield: 84%; mp: 108–110 °C; IR (KBr, cm^{-1}): 3422 (NH), 2761 (SH), 1597 (C=N); 1220, 1092, 1013 (C–O–C); ^{1}H NMR (δ ppm): 3.98, 4.02 (2d, J = 12.25 Hz, 2H, OCH_2), 4.12, 4.16 (2d, J = 16.05 Hz, 2H, benzyl CH_2), 4.49 (dd, J = 14.53, 3.8 Hz, 1H, N–CH_2), 4.58 (dd, J = 14.53, 7.25 Hz, 1H, N–CH_2), 4.73 (dd, J = 7.25, 3.8 Hz, 1H, CH), 7.04–7.52 (m, 13H, Ar–H), 14.0 (s, 1H, SH, D_2O exchangeable). Anal. Calcd for $C_{25}H_{21}Cl_2N_7OS$ (538.45): C, 55.76; H, 3.93; N, 18.21. Found: C, 55.51; H, 3.62; N, 17.96.

4.1.11. 5-{[1-(4-Chlorophenyl)-2-(5-phenyl-2*H*-tetrazol-2-yl) ethoxy]methyl}-4-substituted4*H*-1,2,4-triazole-3-thiols (24, 25)

A mixture of equimolar amounts of **12** (0.75 g, 2 mmol) and the proper aryl isothiocyanate (2 mmol) in ethanol (20 mL) was heated under reflux for 3 h. The reaction mixture was left to attain room temperature and the precipitated product was filtered, washed with ethanol, dried and crystallized.

4.1.11.1. 5-{[1-(4-Chlorophenyl)-2-(5-phenyl-2*H*-tetrazol-2-yl)ethoxy]methyl}-4-phenyl-4*H*-1,2,4-triazole-3-thiol (24). White crystals (ethanol). Yield: 82%; mp: 218–219 °C; IR (KBr, cm^{-1}): 3421 (NH), 2771 (SH), 1595 (C=N); 1219, 1092, 1045 (C–O–C); ^{1}H NMR (δ ppm): 4.09 (s, 2H, OCH_2), 4.83 (dd, J = 13.75, 4 Hz, 1H, N–CH_2), 4.96 (dd, J = 13.75, 8.21 Hz, 1H, N–CH_2), 5.04 (dd, J = 8.21, 4 Hz, 1H, CH), 7.14–7.41 (m, 9H, *N*-phenyl-H & chlorophenyl-H), 7.51–7.57 (m, 3H, phenyl $C_{3,4,5}$–H), 8.01 (d, J = 7.63 Hz, 2H, phenyl $C_{2,6}$–H), 13.94 (s, 1H, SH, D_2O exchangeable). Anal. Calcd for. $C_{24}H_{20}ClN_7OS$ (489.98): C, 58.83 H, 4.11; N, 20.01. Found: C, 58.81; H, 4.80; N, 19.91

4.1.11.2. 5-{[1-(4-Chlorophenyl)-2-(5-phenyl-2*H*-tetrazol-2-yl)ethoxy]methyl}-4-(4-chlorophenyl)-4*H*-1,2,4-triazole-3-thiol (25). White crystals (ethanol). Yield: 84%; mp: 204–205 °C; IR (KBr, cm^{-1}): 3415 (NH), 2761 (SH), 1580 (C=N),1246, 1087, 1046 (C–O–C); ^{1}H NMR (δ ppm): 4.13 (s, 2H, OCH_2), 4.85 (dd, J = 13.75, 3.8 Hz, 1H, N–CH_2), 4.99 (dd, J = 13.75, 8.4 Hz, 1H, N–CH_2), 5.06 (dd, J = 8.4, 3.8 Hz, 1H, CH), 7.18, 7.21, 7.35, 7.39 (4d, J = 8.4 Hz, 8H, N-chlorophenyl-H & chlorophenyl-H), 7.50–7.57 (m, 3H, phenyl $C_{3,4,5}$–H). 8.02 (d, J = 7 Hz, 2H, phenyl $C_{2,6}$–H), 13.99 (s, 1H, SH, D_2O exchangeable). Anal. Calcd for $C_{24}H_{19}Cl_2N_7OS$ (524.43): C, 54.97; H, 3.65; N, 18.70. Found: C,54.52; H, 3.48; N, 18.01.

4.1.12. 5-Phenylamino-2-{[2-(5-substituted1*H*-tetrazol-1-yl or 2*H*-tetrazol-2-yl)ethoxy]methyl}-1,3,4-oxadiazoles (26, 27)

A mixture of the selected thiosemicarbazide **19** or **21** (1 mmol), freshly prepared yellow mercuric oxide (0.22 g, 1 mmol) in dioxane (20 mL) was heated under reflux for 4 h then filtered while hot. The filtrate was evaporated to dryness under reduced pressure and the remaining residue was triturated with diethyl ether, then filtered and crystallized.

4.1.12.1. 2-{[2-(5-Benzyl-1*H*-tetrazol-1-yl) 1-(4-chlorophenyl) ethoxy]methyl}-5-phenylamino-1,3,4-oxadiazole (26). White crystals (CH_2Cl_2/ethanol). Yield: 52%; mp: 114–116 °C; IR (KBr, cm^{-1}): 3259 (NH), 1617 (C=N), 1247, 1208, 1110, 1034 (C–O–C); ^{1}H NMR (δ ppm): 4.0–4.09 (m, 4H, OCH_2 & benzyl CH_2), 4.44 (dd, J = 14.73, 4.78 Hz, 1H, N–CH_2), 4.50 (dd, J = 14.73, 7.66 Hz, 1H, N–CH_2), 4.67 (dd, J = 7.66, 4.78 Hz, 1H, CH), 7.0–7.54 (m, 14 H, benzyl-H, phenyl-H

& chlorophenyl-H), 10.45 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for C_{25} $H_{22}ClN_7O_2$ (487.94): C, 61.54; H, 4.54; N, 20.09. Found: C, 61.24; H, 4.63; N, 20.34.

4.1.12.2. 2-{[1-(4-chlorophenyl)-2-(5-Phenyl-2*H*-tetrazol-2-yl)ethoxy]methyl}-5-phenylamino-1,3,4-oxadiazole (27). White crystals (CH_2Cl_2/ethanol). Yield: 49%; mp: 127–128 °C; IR (KBr, cm^{-1}): 3314 (NH), 1607 (C=N), 1260, 1228, 1180, 1031 (C–O–C); ^1H NMR (δ ppm): 4.14, 4.17 (2d, J = 12.58 Hz, 2H, OCH_2), 4.82 (dd, J = 13.75, 4.4 Hz, 1H, N–CH_2), 4.96 (dd, J = 13.75, 8.23 Hz, 1H, N–CH_2), 5.05(dd, J = 8.23, 4.4 Hz, 1H, CH), 7.12–7.43 (m, 9H, phenylamino-H & chlorophenyl-H), 7.48–7.58 (m, 3H, phenyl $C_{3,4,5}$–H), 8.01 (d, J = 7 Hz, 2H, phenyl $C_{2,6}$–H), 10.36 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{24}H_{20}ClN_7O_2$ (473.91): C, 60.82; H, 4.25; N, 20.69. Found: C, 60.57; H, 4.22; N, 20.78.

4.1.13. General procedure for the synthesis of 6-(4-chlorophenyl)-5,6-dihydro-11*H*-tetrazolo[1,5-a]benz[d]azepine (30) and 2-(4-chlorostyryl)-5-phenyl-2*H*-tetrazole (31)

The appropriate thiosemicarbazide **19** or **21** (1 mmol) was dissolved in ice-cold concd H_2SO_4 (4 mL) and stirred for 15 min at 0 °C followed by further stirring for 15 min at room temperature. The reaction mixture was poured onto crushed ice and the obtained precipitate was filtered, washed with water and crystallized.

4.1.13.1. 6-(4-Chlorophenyl)-5,6-dihydro-11*H*-tetrazolo[1,5-a]benz[d]azepine (30). White crystals (ethanol). Yield: 52%; mp: 169–170 °C; IR (KBr, cm^{-1}): 1617 (C=N); ^1H NMR (δ ppm): 4.26, 4.72 (2d, J = 16.8 Hz, 2H, tetrazolobenzazepine C_{11}–H), 4.91 (dd, J = 17.96, 9.16 Hz, 1H, tetrazolobenzazepine C_6–H), 5.11 (dd, J = 17.96, 9.16 Hz, 2H, tetrazolobenzazepine C_5–H), 6.83–6.92 (m, 1H, tetrazolobenzazepine C_7–H), 7.20–7.30 (m, 2H, tetrazolobenzazepine C_8–H & C_9–H), 7.31 (d, J = 7.6 Hz, 2H, chlorophenyl $C_{2,6}$–H), 7.40–7.49 (m, 3H, tetrazolobenzazepine C_{10}–H & chlorophenyl $C_{3,5}$–H). ^{13}C NMR (δ ppm): 28.43 (tetrazolobenzazepine C_{11}), 42.73 (tetrazolobenzazepine C_6), 51.07 (tetrazolobenzazepine C_5), 127.48, 127.84 (tetrazolobenzazepine C_8 & C_9), 128.58 (chlorophenyl C_3 & C_5), 128.97 (tetrazolobenzazepine C_7), 129.5 (tetrazolobenzazepine C_{10}), 129.79 (chlorophenyl C_2 & C_6), 131.88 (chlorophenyl C_4), 133.76 (tetrazolobenzazepine C_{10a}), 137.99 (chlorophenyl C_1), 139.89 (tetrazolobenzazepine C_{6a}), 151.63 (tetrazolobenzazepine-C_{11a}); MS, m/z (relative abundance%): 297 [M^++1, (17.3)], 296 [M^+, (26.9)], 240 (40.4), 179 (73.1), 178 (92.3), 131 (42.3), 91 (50), 89 (86.5), 77 (50), 63 (100), 51 (100). Anal. Calcd for $C_{16}H_{13}ClN_4$ (296.75): C, 64.76; H, 4.42; N, 18.88. Found: C, 64.65; H, 4.35; N, 18.51.

4.1.13.2. 2-(4-Chlorostyryl)-5-phenyl-2*H*-tetrazole (31). White crystals (ethanol). Yield: 48%; mp: 130–131 °C; IR (KBr, cm^{-1}): 1616 (C=N); ^1H NMR (δ ppm): 7.47 (d, J = 8 Hz, 2H, chlorophenyl $C_{2,6}$–H), 7.53–7.60 (m, 3H, phenyl $C_{3,4,5}$–H), 7.66 (d, J = 14.6 Hz, 1H, NCH=*CH*–C_6H_4–Cl), 7.78 (d, J = 8 Hz, chlorophenyl $C_{3,5}$–H), 8.06–8.17 (m, 2H, phenyl $C_{2,6}$–H), 8.50 (d, J = 14.6 Hz, 1H, N–*CH*=CH). ^{13}C NMR (δ ppm): 123.41 (NCH=*CH*), 123.65 (–N*CH*=CH), 126.26 (phenyl C_4), 126.51 (phenyl C_2 & C_6), 128.77 (chlorophenyl C_3 & C_5), 129.15 (phenyl C_3 & C_5), 129.19 (chlorophenyl C_2 & C_6), 130.77 (phenyl C_1), 131.85 (chlorophenyl C_1), 133.84 (chlorophenyl C_4), 163.87 (tetrazole-C_5). Anal. Calcd for $C_{15}H_{11}ClN_4$ (282.73): C, 63.72; H, 3.92; N, 19.82. Found: C, 63.58; H, 3.31; N, 19.86.

4.2. Biological evaluation

4.2.1. In vitro antibacterial and antifungal activities

4.2.1.1. Inhibition zone (IZ) measurement. Standard sterilized filter paper discs (5 mm diameter) impregnated with a solution of the test compound in DMSO (1 mg/mL) were placed on an agar plate seeded with the appropriate test organism in triplicates. The utilized test organisms were: *S. aureus* (ATCC 6538), *B. subtilis* (NRRL B-14819) and *M. luteus* (ATCC 21881) as examples of Gram positive bacteria and *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853) and *K. pneumonia* (clinical isolate) as examples of Gram negative bacteria. They were also evaluated for their in vitro antifungal potential against *C. albicans*, *C. tropicalis* and *C. krusei* as representatives of fungi and *A. niger*, *A. fumigatus* and *T. rubrum* as examples of moulds. All of these fungal strains were clinical isolates, identified with conventional morphological and biochemical methods. Ampicillin trihydrate (antibiotic), clotrimazole and miconazole (antifungals) were used as reference standards. DMSO alone was used as control at the same above-mentioned concentration. The plates were incubated at 37 °C for 24 h for bacteria and for 7 days for fungi. Compounds that showed significant growth inhibition zones ($\geqslant$14 mm) using the twofold serial dilution technique, were further evaluated for their minimal inhibitory concentrations (MICs).

4.2.1.2. In vitro antibacterial activity (minimal inhibitory concentration (MIC) measurement). The microdilution susceptibility test in Müller–Hinton Broth (Oxoid) was used for the determination of antibacterial activity.[43] Stock solutions of the tested compounds and ampicillin trihydrate were prepared in DMSO at concentration of 800 μg/mL followed by two-fold dilution at concentrations of (400, 200,...6.25 μg/ml). The microorganism suspensions at 10^6 CFU/ml (Colony Forming Unit/mL) concentration were inoculated to the corresponding wells. Plates were incubated at 36 °C for 24–48 h and the minimal inhibitory concentrations (MIC) were determined. Control experiments were also done. All assays were performed in duplicate and results are represented in Table 1.

4.2.1.3. In vitro antifungal susceptibility assay. MIC of the six active compounds **15**, **17**, **22**, **23**, **26** and **30** against *C. albicans* and *A. fumigatus* was determined by broth microdilution testing in accordance with the guidelines in NCCLS document M27-A and M38-P.[49,50] Briefly stock solutions were prepared in DMSO for clotrimazole, miconazole and the compounds. Serial twofold dilution of the compounds was made in RPMI1640 medium buffered to pH 7.0 with 0.165 M 4-morpholinepropanesulfonic acid (MOPS) buffer as outlined in NCCLS M27-A document. Aliquots of 0.1 mL of each compound were dispensed into the wells of plastic microdilution microtiter plates so that the final concentration of solvent did not exceed 1% in any well. An inoculum of the organisms at 10^6 CFU/mL (Colony Forming Unit/mL) concentration was prepared and 100 μL of the individual fungal inoculum was added to each well of the microtiter plate containing the reference drug or the compound. The plates were incubated at 25 °C for 72 h. After the completion of incubation, the broth microdilution wells were examined and the growth in each well was compared with that of the control. The MIC of each compound was defined as the lowest concentration that produced 80% inhibition in the growth of the organism compared with that of the control. All assays were performed in duplicate and the results are presented in Table 2.

4.2.2. Preliminary anticonvulsant screening

4.2.2.1. Subcutaneous metrazole seizure pattern test. This test was carried out following the procedure described by Chaturvedi et al.[44] Wister albino mice of either sex, weighing 25–30 g and 3 months old were utilized throughout the assay. They were kept in the animal house under standard conditions of light and temperature (25 °C) with free access to food and water. The animals were randomly divided into groups each of six rats. Dimethyl sulfoxide (DMSO) was used for dissolving metrazole and the test com-

pounds, whereas the control experiments were performed with solvent alone. The compounds were administered intraperitoneally (ip) at doses of 30, 100 and 300 mg/kg body weight. The test was conducted by administering 100 mg/kg of metrazole dissolved in DMSO into the posterior midline of mice 30 min after administration of the tested compounds. A minimal time of 30 min subsequent to subcutaneous administration of metrazole was used for seizure detection. A failure to observe even a threshold seizure (a single episode of clonic spasm of at least 5 s) was regarded as protection. The anticonvulsant activity was assessed at 30 min interval after administration. The results are presented in Table 3.

4.2.2.2. Maximal electroshock seizure test. The MES test was performed following the protocol of the 6 Hz seizure model as previously described.[45] Topical anesthetic (0.5% xylocaine hydrochloride ophthalmic solution) was applied to the cornea 30 min before corneal stimulation (0.2-ms duration pulses at 6 Hz for 3 s) administered by a constant-current device (Grass S48 stimulator; W-Warwick, RI, USA). Saline (0.9%) was used to wet the electrodes immediately before testing to ensure good electrical contact. Each animal was stimulated once at a selected current intensity in the range of 6–64 mA to determine the convulsive threshold for mice in each group. The current intensity values were chosen in which the stimulation intensity for a group of animals did or did not exhibit a seizure.[51] Mice were manually restrained during stimulation. Immediately following stimulation, mice were placed in a Plexiglas arena for behavioral observation. Seizures were characterized by a stunned or fixed posture often accompanied by rearing, forelimb clonus, and twitching. After the seizures, mice resumed normal exploratory behavior within 45s. Mice not experiencing seizures exhibited normal exploratory behavior when placed in the arena.[52] Protection in the MES test was defined as the abolition of the hind limb tonic extension component of the seizure. The anticonvulsant activity was assessed at 30 min interval after administration and the results are recorded in Table 3.

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