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Research Article

An Interactive Human Carbonic Anhydrase-II (hCA-II) Receptor – Pharmacophore Molecular Model & Anti-Convulsant Activity of the Designed and Synthesized 5-Amino-1,3,4-Thiadiazole-2-Thiol Conjugated Imine Derivatives

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New imines, derived from aromatic aldehyde, chalcones and 5-amino-1,3,4-thiadiazole-2-thiol exhibited promising anti-convulsant activity which is explained through chemo-biological interactions at receptor site producing the inhibition of human Carbonic Anhydrase-II enzyme (hCA-II) through the proposed pharmacophore model at molecular levels as basis for pharmacological activity. The compounds 5-{1-(4-Chlorophenyl)-3-[4-(methoxy-phenyl)-prop-2-en-1-ylidene]amino}-1,3,4-thiadiazole-2-thiol (2b), 5-{[1-(4-chloro-phenyl)]-3-[4-(dimethyl-amino-phenyl)-prop-2-en-1-ylidene]amino}-1,3,4-thiadiazole-2-thiol (2c) and 5-{[1-(4-chloro-phenyl)]-3-[(4-amino-phenyl)-prop-2-en-1-ylidene]amino}-1,3,4-thiadiazole-2-thiol (2f) showed 100% activity in comparison with standard Acetazolamide, a known anti-convulsant drug. The compounds 2c, 2f also passed the Rotarod and Ethanol Potentiation tests which further confirmed them to be safe in motor coordination activity and safe from generating neurological toxicity.

Key words: 5-amino-1,3,4-thiadiazole-2-thiol imines, anti-convulsant activity, human carbonic anhydrase-II, neurotoxicity test, pharmacophore model, receptor–ligand interaction

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Epilepsy, the neurological disorder affecting about 1% of worlds' population, is among one of the hard to manage diseases. The conventional anti-convulsants are widely prescribed but induce a range of side effects and, are many times ineffective. There is also a significant population of patients (approximately 30%) resistant to these drugs for controlling and treating the epileptic seizures. Therefore, in spite of the availability of a number of prescription drugs in the market and development of other novel therapies as well as new drugs with newer pharmacological mode of actions, the efficiency for treatment for convulsion has not increased significantly over decades. Today, none of the presently approved drugs is ideal in terms of biological activity levels, mode of action and limited or no neurological side effects. They are best used as part of add-on therapy and are associated with chronic and acute side effects of adverse nature. Therefore, the search for new templates and compounds is imperative and continued.

The interesting observation of anti-depressant activity at certain levels in some of the extended series (1) of 5-amino-1,3,4-thiadiazole-2-thiol imines and thiobenzyl derivatives opened the possibilities of detailed correlation between different CNS biological activities and set of molecular attributes including molecular sizes of the compounds. The observation that the compounds with comparatively smaller molecular sizes and certain set of molecular & electronic attributes exhibited negligible or no anti-depressant activity but significant anti-convulsant activity while compounds of larger molecular volumes and extended sizes and molecular attributes exhibited significant anti-depressant activity, but no anti-convulsant activity suggested a different mode and site of pharmacological action and biological approach of the extended and non-extended products from the 5-amino-1,3,4-thiadiazole-2-thiol series. This also established the versatility of 5-amino-1,3,4-thiadiazole-2-thiol synthon as synthetically extendable entity for further structural transformations to yield new bioactive compounds and provide indications for structure activity relationships in a vast bioactivity profiles with hereto ambiguous receptor interactions for different biological activities, some of which are appearing interesting in our ongoing experimentations.

The currently employed molecular modifications affected through different synthetic transformations yielded comparatively (1) smaller sized, anti-convulsant active

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compounds supporting a different receptor type interaction for anti-convulsant biological activity and thus were chosen to design, synthesize and analyse on the receptor-pharmacophore molecular model.

The carbonic anhydrase (CA) is Zinc (II) ion, (Zn^{+2}), based specific enzyme biochemically catalysing the formation of carbonic acid from water, carbon dioxide and vice-versa. It has been suggested that anti-convulsant effect in humans can be carried out away through inhibition of human carbonic anhydrase-II enzyme (hCA-II) which also causes neuro-depression by accumulation of carbon dioxide in brain (2). The CA complexes are reported (3–5) for sulphamic acid, sulfamide and other drugs (6,7) including the known (8,9) 1,3,4-thiadiazole compounds. An attempt has been made to understand the pharmacophore functioning based on these complexes where the active pharmaceutical ingredients have been envisioned as forming hydrogen bonds, ion-dipole and dipole–dipole interactions at reactive sites in the enzyme substrate. Based on this understanding, compounds were designed, synthesized and biologically tested for their pharmacological efficacy.

Methods and Materials

The infrared spectra were recorded as KBr pellets FTIR; Bruker Optics, Karlsruhe, Germany; NMR spectra were recorded on Bruker DRX-400 MHz (Karlsruhe, Germany) in DMSO-d_6 as solvent with TMS as reference, and mass spectra were recorded on JOEL SX102/DA-6000 (Peabody, MA, USA) spectrometer using Argon/Xenon gas in FAB mode with *meta*-nitro benzyl alcohol as matrix under accelerating voltage of 10 KV. The elemental analysis established 95% and above purity for all compounds.

Synthesis of substituted chalcones

5-Amino-1,3,4-thiadiazoles were synthesized from thiosemicarbazide and carbon disulphide. Their aldehyde imines and conjugated chalcone imines were synthesized according to scheme 1 and 2 (Figure 2). Briefly, a solution of aqueous sodium hydroxide (2 g, 20 mL) and ethanol (12.5 mL), both precooled at 5 °C were poured in to crushed ice containing freshly distilled acetophenone (0.04 M) and benzaldehyde (0.04 M). The mixture was vigorously stirred and allowed to come to RT with stirring continued for further 2 h. The mixture was kept overnight in the refrigerator at 0 °C. The completion of reaction was confirmed through TLC with Benzene: Acetone (9:1) as mobile phase (10,11). The chalcone product was filtered under vacuum, washed with cold water until washings were neutral. The crude product was recrystallized from 50 °C warm ethanol.

Synthesis of 5-amino-1,3,4-thiadiazole-2-thiol

Thiosemicarbazide 45.5 g (0.25 M) was suspended in absolute ethanol and anhydrous Na_2CO_3 (24 g) with carbon disulphide 0.25 mol (46 g). The mixture was warmed under stirring and refluxed for 1 h and then heated on steam bath for 4 h. Completion of the reaction was indicated by TLC with Toluene, Ethyl Acetate and aq. Formaldehyde (TEF) 5:4:1 as mobile phase. The solvent was evaporated under vacuum and residue dissolved in water (200 mL), acidified with conc. HCl to give the product (12) (54 g), 84%, m. p. 232 °C.

Syntheses of aromatic aldehyde, imine derivatives of 5-amino-1,3,4-thiadiazole-2-thiol (Scheme 1)

5-Amino-1,3,4-thiadiazole-2-thiol (0.02 M) was added to benzaldehyde (0.02 M) in 25 mL methanol, and reaction mixture refluxed till the completion of reaction (TLC, TEF 5:4:1). The mixture was concentrated in *vacuo* to one-fourth volume and kept in refrigerator overnight, crystals filtered and recrystallized from hot 95% ethanol.

5-[(2-chlorophenyl)methylene]amino-1,3,4-thiadiazole-2-thiol (1a)

Yields 55%, m. p. 217–219 °C. ^{1}H-NMR: δ 7.09–7.29 (4H, m, Ar-Bz), 9.15 (H, s, CH=N), 2.53 (H, s, SH). IR ν_{max}/cm 3390–3318 (Ar C-H_{str}, Bz), 2553 (S-H_{str}, thiol), 1610 (Ar C=C_{str}, Bz), 1540 (C=N_{str}, imine), 720 (Ar C-H_{def}, o-disubs-Bz), 680 (Ar C-Cl_{str}, Bz). MS m/z [M^+] 256, 220, 165, 138, 112. Anal., Calcd. for $C_9H_6ClN_3S_2$: C, 42.27; H, 2.36; N, 16.43. Found: C, 42.24; H, 2.35; N, 16.39.

5-[(4-chlorophenyl)methylene]amino-1,3,4-thiadiazole-2-thiol (1b)

Yields 70%, m. p. 213–214 °C. ^{1}H-NMR: δ 7.14–7.31 (4H, m, Ar-Bz), 9.72 (H, s, CH=N), 2.41 (H, s, SH). IR ν_{max}/cm 3377–3309 (Ar C-H_{str}, Bz), 2546 (S-H_{str}, thiol), 1612 (Ar C=C_{str}, Bz), 1541 (C=N_{str}, imine), 800 (Ar C-H_{def}, p-disubs-Bz), 685 (Ar C-Cl_{str}, Bz). MS m/z [M^+] 256, 219,165, 138, 111, 92. Anal., Calcd. for $C_9H_6ClN_3S_2$: C, 42.27; H, 2.36; N, 16.43. Found: C, 42.26; H, 2.34; N, 16.37.

5-[(2-nitrophenyl)methylene]amino-1,3,4-thiadiazole-2-thiol (1c)

Yields 40%, m. p. 231–233 °C. ^{1}H-NMR: δ 7.19–7.28 (4H, m, Ar-Bz), 9.12 (H, s, CH=N), 2.54 (H, s, SH). IR ν_{max}/cm 3375–3324 (Ar C-H_{str}, Bz), 2556 (S-H_{str}, thiol), 1607 (Ar C=C_{str}, Bz), 1566 (C=N_{str}, imine), 715 (Ar C-H_{def}, o-disubs-Bz). MS m/z [M^+ + 1] 268, 176, 151, 145, 92. Anal., Calcd. for $C_9H_6N_4O_2S_2$: C, 40.59; H, 2.27; N, 21.04. Found: C, 40.56; H, 2.24; N, 21.07.

5-[(4-nitrophenyl)methylene]amino-1,3,4-thiadiazole-2-thiol (1d)

Yields 68%, m. p. 195–197 °C. ^{1}H-NMR: δ 7.21–7.33 (4H, m, Ar-Bz), 9.74 (H, s, CH=N), 2.47 (H, s, SH). IR ν_{max}/cm 3379–3312 (Ar C-H_{str}, Bz), 2548 (S-H_{str}, thiol),

1619 (Ar C=C_{str}, Bz), 1548 (C=N_{str}, imine), 805 (Ar C-H_{def}, p-disubs-Bz). MS m/z [M^+] 267, 176, 151, 145, 92. Anal., Calcd. for $C_9H_6N_4O_2S_2$: C, 40.59; H, 2.27; N, 21.04. Found: C, 40.55; H, 2.23; N, 21.04.

5-[(2-florophenyl)methylene]amino-1,3,4-thiadiazole-2-thiol (1e)

Yields 52%, m. p. 186–190 °C. ^{1}H-NMR: δ 7.19–7.35 (4H, m, Ar-Bz), 9.23 (H, s, CH=N), 2.73 (H, s, SH). IR ν_{max}/cm 3389–3314 (Ar C-H_{str}, Bz), 2547 (S-H_{str}, thiol), 1611 (Ar C=C_{str}, Bz), 1539 (C=N_{str}, imine), 719 (Ar C-H_{def}, o-disubs-Bz). MS m/z [M^+ + 1] 241, 167, 145, 121, 93. Anal., Calcd. for $C_9H_6FN_3S_2$: C, 45.17; H, 2.53; N, 17.56. Found: C, 45.13; H, 2.53; N, 17.54.

5-[(4-fluorophenyl)methylene]amino-1,3,4-thiadiazole-2-thiol (1f)

Yields 70%, m. p. 182–184 °C. ^{1}H-NMR: δ 7.23–7.31 (4H, m, Ar-Bz), 9.23 (H, s, CH=N), 2.64 (H, s, SH). IR ν_{max}/cm 3374–3326 (Ar C-H_{str}, Bz), 2560 (S-H_{str}, thiol), 1610 (Ar C=C_{str}, Bz), 1574 (C=N_{str}, imine), 812 (Ar C-H_{def}, p-disubs-Bz).MS m/z [M^+ + 1] 241, 166, 145, 121, 92. Anal., Calcd. for $C_9H_6FN_3S_2$: C, 45.17; H, 2.53; N, 17.56. Found: C, 45.12; H, 2.52; N, 17.56.

Syntheses of various imines derived by different Chalcones and 5-amino-1,3,4-thiadiazole-2-thiol (Scheme 2, Compounds 2a–2g)

2-Amino-5-mercapto-1,3,4-thiadiazole was suspended (0.02 mol) in 25 mL absolute ethanol, and various chalcones (0.02 Mol, predissolved in solvent) were added. The reaction mixture was refluxed for 7 h, left overnight, solvent evaporated under vacuum and residue crystallized from methanol to give the products (1), 2a–2e, and products **2f** and **2g**.

5-{[1-(4-chlorophenyl)-3-(4-aminophenyl)-prop-2-en-1-ylidene]amino}-1,3,4-thiadiazole-2-thiol (2f)

Yields 25%, m. p. 121–124 °C. ^{1}H-NMR: δ 7.69 (1H, d, J = 15.6 Hz, trans-ene-H), 7.86 (1H, d, J = 15.6 Hz, trans-ene-H), 6.91–7.68 (m, 8H, Ar-Bz), 5.64 (2H, s, NH_2) 2.40 (H, s, SH).IR ν_{max}/cm 3395–3320 (Ar C-H_{str}, Bz), 3027 (C-$H_{str,}$ trans-ene), 2564 (S-H_{str}, thiol), 1689 (C=N_{str}), 976 (C-$H_{def,}$ trans-ene), 815 (C-$H_{def,}$ p-disubst. Bz), 712 (C-$H_{def,}$ monosubst. Bz), 610 (Ar C-Cl_{str}, Bz). MS m/z [M^+] 373, 256, 131. Anal., Calcd. for $C_{17}H_{13}ClN_4S_2$: C, 54.76; H, 3.51; N, 15.02. Found: C, 54.71; H, 3.51; N, 15.01.

5-{[1-(4-chlorophenyl)-3-(4-methylphenyl)-prop-2-en-1-ylidene]amino}-1,3,4-thiadiazole-2-thiol (2g)

Yields 56%, m. p. 71–73 °C. ^{1}H-NMR: δ 7.7 (1H, d, J = 15.6 Hz, trans-ene-H), 7.84 (1H, d, J = 15.6 Hz, trans-ene-H), 6.92–7.66 (m, 8H, Ar-Bz), 2.64 (3H, s, CH_3) 2.42 (H, s, SH). IR ν_{max}/cm 3393–3315 (Ar C-H_{str}, Bz), 3029 (C-$H_{str,}$ trans-ene), 2562 (S-H_{str}, thiol), 1686 (C=N_{str}), 977 (C-H_{def}, trans-ene), Anal., Calcd. for $C_{18}H_{14}ClN_3S_2$: C, 58.13; H, 3.79; N, 11.30. Found: C, 58.11; H, 3.78; N, 11.11.

Anti-convulsant activity

The pharmacological activity was conducted on male albino mice (25–30 g) kept under standard conditions of ambient temperature at 25 ± 2 °C in the animal house. Food and water were withdrawn prior to the experiments. The standard drug acetazolamide and test compounds were administered through intra-peritoneal route in doses of 20 mg/kg in propylene glycol. The anti-convulsant activity was evaluated by maximal electroshock seizure test (3), supra maximal electroshock of current intensity of 54 mA, 60 Hz were given to mice for 0.2 seconds. The abolition of the hind limb tonic extensor spasm was recorded as exhibition of increased anti-convulsant activity (Table 1).

Rotarod and ethanol potentiation tests for neurotoxicity

The male albino mice were placed on a rotating rod (24 rpm) and observed for 5 min. The skeletal muscle

Table 1: Anti-convulsant activity and neurotoxicity tests

	Anti-convulsant activity (% Protection)[a]				
		Post treatment			
Compound	Control 24 h Prior	After 0.5 h	After 4 h	Rotarod test[b]	Ethanol potentiation test [c]
1a	0	33	16	–	–
1b	0	66	50	–	–
1c	0	50	16	+	–
1d	0	66	16	+	–
1e	0	16	0	–	–
1f	0	66	50	–	–
2a	0	66	50	+	–
2b	0	100	16	+	–
2c	0	100	50	+	+
2d	0	83	16	–	–
2e	0	83	50	+	–
2f	0	100	66	+	+
2g	0	83	33	+	+
Ref	0	100	50	+	+

[a]Test compounds and acetazolamide (Ref = Reference) were dissolved in propylene glycol and administered (20 mg/kg body weight dose) *via* intra-peritoneal (i.p.) route, 30 min prior to test in groups (n = 6). For neurotoxicity, 20 mg/kg level dose was administered (i.p.) and neurotoxicity observed after 30 min.

[b]The (+) sign indicates 50%; group members or more passing the test.

[c]The mice were treated with test compounds and 1 h later with ethanol 2.5 g/kg (i.p.); neurotoxicity was measured after 30 min. The (+) sign indicates 50% group members or more passing the test.

relaxation induced by test compound was evaluated by testing the ability of mice to remain on the revolving rod (4) which assessed the disruptive effects of compounds on motor coordination. The dose which impaired the ability of 50% of mice to remain on the revolving rod was considered the endpoint. For ethanol potentiation test, compounds and ethanol (2.5 g/kg i.p.) after 1 h were administered to mice. This ethanol dose did not induce lateral position in control animals. The number of mice that were in the lateral position after receiving ethanol in each group was determined (3,4) (Table 1).

Results and Discussion

The imine derivatives in both the series were synthesized starting from 5-amino-1,3,4-thiadiazole-2-thiols (Figure 1). A part of the synthetic details have been reported earlier (10–12) and by us (1). The IR spectra of compounds exhibited absorption bands in the ranges of 3390–3318/cm due to C-H stretching, 2600–2550/cm due to SH, 1600–1550/cm due to aromatic (C=C), 1540–1500/cm due to imine stretching and 800–720/cm due to aromatic C-H deformations in the fingerprint region. The ^{1}H-NMR peaks were observed in the ranges of δ 6.50–8.00 for different aromatic protons, while the peaks in the of δ 8.00–10.00 range were characteristic of imine protons. The δ 10.00–13.1 signals were assigned to thiol-protons. The two doublets in the range δ 7.50–8.00 having coupling constant of 15.6 Hz were characteristic of presence of *trans*-protons of the -HC=CH- group. The spectral data and elemental analyses of all the compounds were in complete agreement with the proposed structures.

Based on previous reports (2–9) and our understanding of the pharmacophore model (Figure 2), it was indicated that thiadiazole ring, free thiol and imine group are part of the main pharmacophore responsible for exhibiting the anticonvulsant bioactivity. The two nitrogens at position 1,2 of the thiadiazole ring forms mesoionic complex with the hydrogen of the hydroxyl group of amino acids threonine (Thr), positioned as Thr-200 and Thr-199 in the polypeptide chain of the hCA-II enzyme. The imine nitrogen interacts through dipole–ion interaction with Zn^{+2} ion. The hydrogen atom of the thiol group (SH) interacts (1) with the oxygen atom of the water molecule. From the biological evaluations, it was inferred that scheme-1 compounds (Figure 1) forming the imine series from 5-amino-1,3,4-thiadiazole-2-thiol and originating from differently substituted benzaldehyde molecules, partially occupies the pharmacophore-binding site, whereas the compounds in scheme-2 completely occupies the binding site resulting in total blockade of active site of hCA-II receptor. The compounds in scheme-1 possessed chloro, fluoro and nitro electron-withdrawing groups at positions 2 and 4 of the benzyl ring and exhibited average levels of bioactivity. Notably, compounds **1b** and **1f** were active at 50% and above potentiation levels in anti-convulsant tests (Table 1). These two

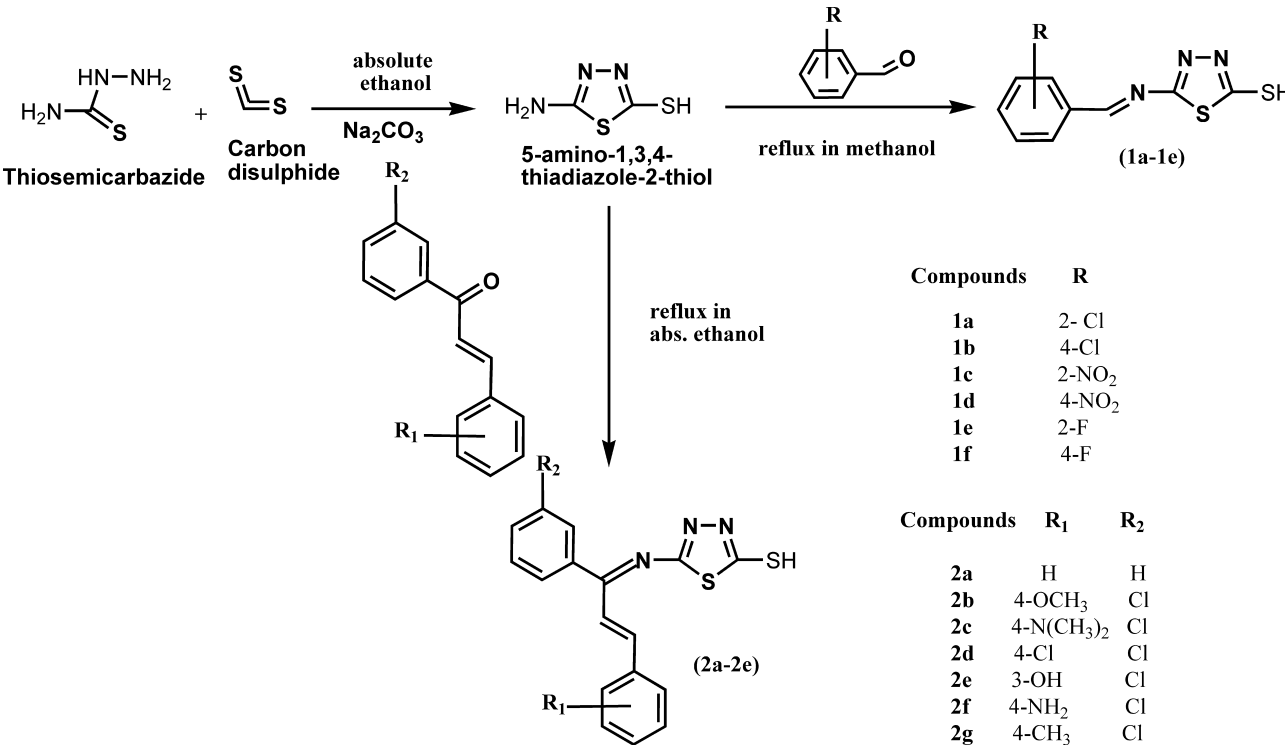

Figure 1: Synthetic scheme.

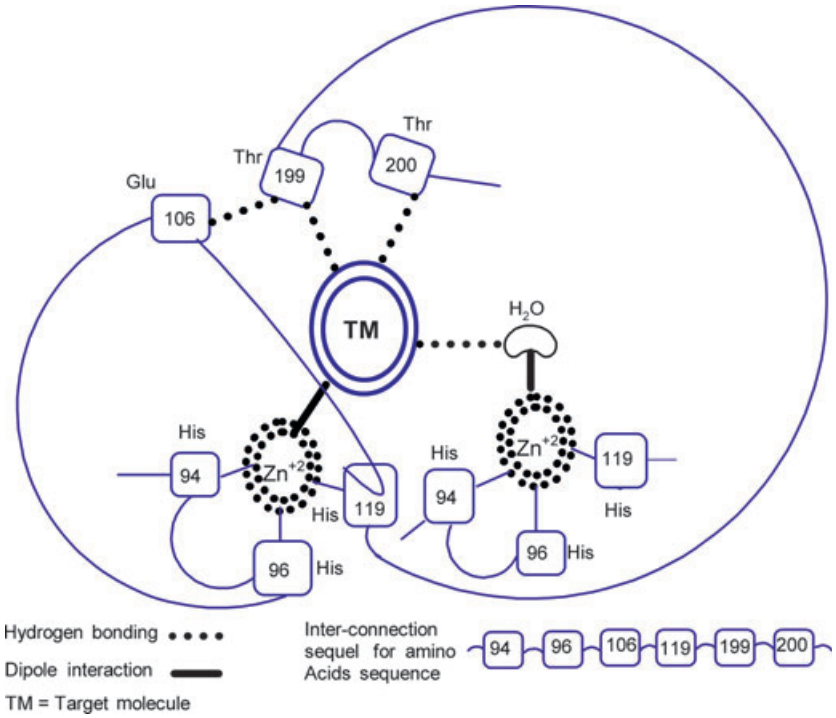

Figure 2: Pharmacophore model with active receptor sites in vicinity of target molecule (TM). The broken black lines represent Hydrogen bondings while solid lines represent dipole interaction. The dithiazole moiety is flanked by the amino acids (Glu at position 106, two adjacent Thr at 199 and, 200) taking part in Hydrogen bondings while amino acids (Histidine, His at positions 94, 96 and, 119) takes part in dipolar interactions with the Zn^{+2} ion, water and; Nitrogen at position 1 of the dithiazole group. The nearly squared boxes and, solid and, broken blue lines connecting each other represent the peptidic sequence connections while the free-ending lines represent the terminals of polypeptide chain of the hCA-II sequence. The presence of two Zn^{+2} metal ions means that two molecules of enzyme hCA-II are taking part in the biochemical activity.

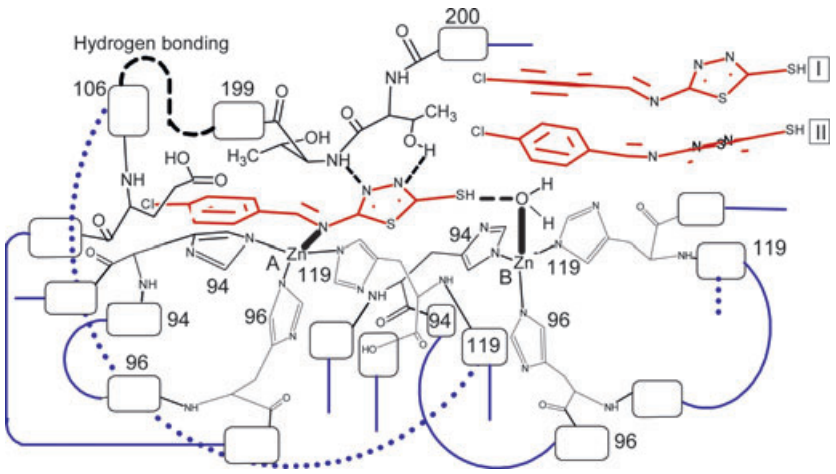

Figure 3: The detailed model showing compound **1b** at active site of the receptor cavity. The 1,2 Nitrogens of the thiadiazole ring participating in Hydrogen bondings with Thr-199 and, Thr-200 while the exocyclic nitrogen atom of the 5-amino-1,3,4-thiadiazole-2-thiol moiety is in a dipole interaction with the Zn^{+2} ion of the His moieties at 94, 96 and, 116 positions. The benzyl ring is few A0 distances away from the diazole ring of the His-94 moiety. The SH group of thiadiazole ring is participating through Hydrogen bonding with water molecule which itself is in dipole interaction with Zn^{+2} ion. The molecular orientations of compound **1b** represented as colored entity in I and, II inside the figure represents the stereo-orientation of the thiadiazole ring. The structure I shows benzyl ring to be nearly perpendicular to the thiadiazole ring while structure II shows the nearly perpendicular orientation of the thiadiazole ring. Both the structures are at their minimum energy conformation status and, are energetically at same levels. The receptor cavity requirement for Hydrogen bindings and, dipole interaction prefers the structure form I. The presence of two Zn^{+2} metal ions means that two molecules of enzyme hCA-II are taking part in this biochemical action.

compounds have 4-Cl and 4-F substitutions on the benzyl ring which indicated that the *para* substitution on the benzyl ring extends the length of the compound to the extent for anti-convulsant bioactivity generation through on-site molecular interactions with the receptor. The substituent on the benzene ring for series-1 compounds having both the electron-withdrawing as well as electron-donating groups did not affect the sustained order of activity in potentiation tests after 4 h of compounds administration to experimental animals. However, there was some moderate activity observed within half hour of the administration of some of the test compounds. The active compounds **1b** and **1f** pharmacophore model suggested that the benzene ring places itself deep under the polypeptide receptor cavity and is in nearly perpendicular orientation with respect to the thiadiazole ring. It is also orienting itself in parallel and in stacked position a few angstrom distance away from the His-94 (Histidine amino acid's residue at position 94) which itself is attached to the Zn^{+2} ion whereby the Zn^{+2} ion is taking part in dipole formation with the exocyclic exocyclic nitrogen atom of the 5-amino-1,3,4-thiadiazole-2-thiol moiety. Moreover, the products in series-1 finds themselves, than products in series-2, comparatively far away from the Zn^{+2} ion due to their adopted conformation at the binding site as observed by the minimum energy conformation of these products in the receptor cavity. A weak ion–dipole interaction is generated between exocyclic nitrogen atom of the thiadiazole moiety and the target molecule (TM) from series-1 (Figure 3). The size of the substituent atom (Cl or, F) does not seem to be effecting the bioactivity elicitation, but the electronic nature and position of the substituent groups are playing a part. The substitution by an electron-withdrawing atom/group at position 4 has favourable effects for activity generation which further indicates that the strengthening of the dipole interaction arising out of Zn^{+2} ion and slightly altered electronic environment at exocyclic nitrogen atom of 5-amino-1,3,4-thiadiazole-2-thiol moiety through its exocyclic nitrogen atom lone pair of electrons has a positive role in bioactivity generation.

The compounds in scheme-2 (Figure 1) produced anticonvulsant activity in higher order than the series-1 compounds. Compounds like **2a**, **2c**, **2e** and **2f** are notable. Some of these products exhibited bioactivities after half an hour of administration and retained active till 4 h after the administration. The compound **2b** also exhibited 100% potency levels after half an hour of administration but could not last longer beyond half an hour. The compounds **2c** and **2f** were among the most promising templates exhibiting time-retained biological activity at 50% and 66% levels after 4 h of administration. The compound **2e** followed in by exhibiting 83% and 50% potencies for anticonvulsant activity after half an hour and 4 h of administrations, respectively. The most potent products namely **2c** and **2f** had a *para*-position substitution, while some of the products do not have *para*-chloro substitution in the aromatic ring, and their activity is in ranges with the activity of

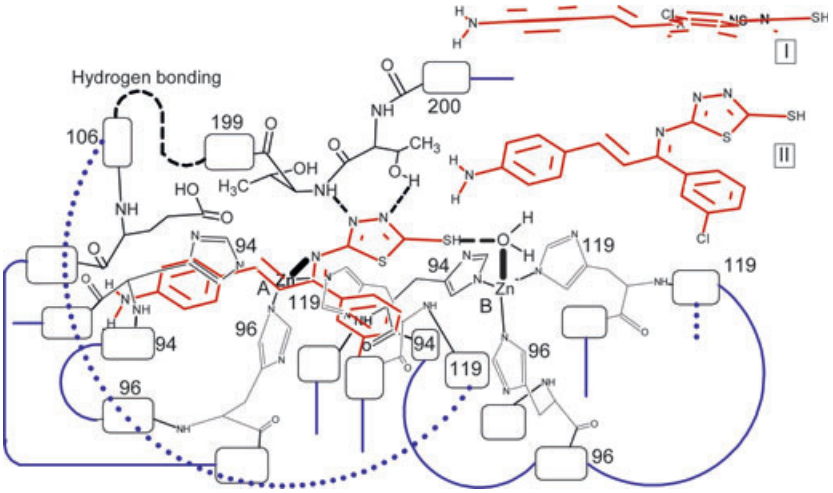

Figure 4: The detailed model showing compound **2f** at binding site of the receptor cavity. The 1,2 Nitrogens of the thiadiazole ring participating in Hydrogen bondings with Thr-199 and, Thr-200 while the exocyclic nitrogen atom of the 5-amino-1,3,4-thiadiazole-2-thiol moiety is in a dipole interaction with the Zn^{+2} ion of the His (Histidine) moieties at 94, 96 and, 116 positions. The benzyl ring is flat-planer to the dithiazole ring. At the other side of the thiadiazole ring, the -SH group is participating through the water molecule via dipole interactions with the Zn^{+2} ion. The molecular orientations of compound **2f** are represented as colored entity in I and, II which inside the figure represents the stereo-orientation of the thiadiazole ring with all the aromatic rings of the molecule. The structure I and, II shows all rings to be flatplaner to the thiadiazole ring. The structure II take part in receptor binding as shown in relation to the receptor model residues and other chemical entities. Both the structures are at their minimum energy conformations status and are energetically at same levels. The receptor cavity requirements for hydrogen bindings and dipole interaction prefer the structure form as represented in II. The inter-atomic distances of dipole and hydrogen bondings have considerable reduced in comparison to the compounds in series-1.

products in series-1. This substitution pattern and the linked aromatic ring does not makes the similar binding coordinate as depicted for series-1 compounds but is placed near the diazole ring of His-119 residue making part of the Zn^{+2} interaction coordinates in the receptor model as illustrated for series-2 compounds (Figure 4). The exocyclic nitrogen atom N^5 of the 5-amino-1,3,4-thiadiazole-2-thiol has dipole interaction with the Zn^{+2} placed at right side and makes a triangular coordinate between the N^5 nitrogen atom, Zn^{+2} ion and carbon 1' of the nearest to exocyclic nitrogen atom aromatic ring substitution in products **2c** and **2f**.

The $-N(CH_3)_2$ and NH_2 substitutions at *para*-positions in C_6 aromatic ring derived from chalcone part of the imine product **2c** and **2f** extends beyond or at the outer limits of the diazole ring of the His-94 residue. This conformation has made the comparative distance of the Zn^{+2} and exocyclic nitrogen atom a bit shorter than in series-1 binding conformation and thus facilitates the easy dipole interaction between the exocyclic N^5 nitrogen atom and Zn^{+2} ion. The other end of the compound **2c** and **2f** with thiol group has also shortened the dipolar interaction distances between the oxygen of the water molecule and Zn^{+2} ion as well as the hydrogen bonding distances between the H of SH group and oxygen of the water molecule. The complete coordination of receptor interaction of compounds **2c** and **2f** has produced a compact and full extent occupation of the receptor sites in hCA-II receptor (Figures 3 and 4). The nearly flat ligand occupies the receptor cavity and compactly binds through hydrogen bondings. The substitution groups with electron-donating and hydrogen-binding capacities in the chalcone derived C_6 aromatic ring of the series-2 compounds seem to be preferred for better receptor interactions which suggested a specific molecular framework preferably having molecular volumes between 180 and 190 A^3 wherein the substitutions is prime contributor in eliciting the biological activity. This observation in the products of series-2 is substantiated by the higher order of activity for $-N(CH_3)_2$, $-NH_2$ and OH substitutions, whereas the substitutions by H, OCH_3 and Cl groups did not exhibited comparative levels of activity as for other products in the series are concerned. The R^1 substitution at *para*-position was preferred in comparison with substitution at position 3 (*meta*) of the C_6 aromatic ring which did not exhibited enough potency levels after half an hour of administration of the compound, nor the activity was retained for other high level (100%) potent products from the series, namely product **2c** and **2f**. These observations again confirmed the *para*-position preference as well as presence of electron-donating groups for the designed product to be bioactive.

The limited SAR of tested compounds suggested the presence of electron rich and comparatively bulky group with availability of free electrons, such as OCH_3, $-N(CH_3)_2$ and $-NH_2$ in comparison with OH, CH_3 and Cl atom at position 4 of the benzene ring away from thiadiazole moiety as preferred substitutions for generating higher biological activity for series-2 compounds (Figure 1). Herein, the R^1 substitution and the ring holding this substitution is overlaid deep into the receptor cavity with the other two rings namely dithiazole and benzene ring of the sub-structure H_2N-C_6H_4-CH=CH- in a preferred flat orientation. The inter-atomic distances for dipole interactions and hydrogen bondings necessary for biological activity generation are reduced in comparison with the series-1 superiorly active compounds (Figures 3 and 4). The biological activity profile in series-1 compounds also preferred the *para*-position substitutions or, no substitution in the lone benzene ring. The *ortho* substitution seemingly interfered with the receptor sequence residues especially with His-94 imidazole ring (Figure 3). The series-2 compounds did not show any interference to His-94 residue and extended past the domain of this amino acid residue of the hCA-II sequence at the binding site. In case of R^2 substituted benzene ring as part of sub-structure H_2N-C_6H_4-CH=CH- in series-2 compounds, this substitution (NH_2) was well buried deep into the receptor cavity past the imidazole ring of His-119 residue at the binding site (Figure 4). The disposition of series-2 compounds inside the receptor cavity provided shorter distances for molecular interactions of dipole nature with Zn^{+2} ions and shortened distances for hydrogen bondings between dithiazole ring nitrogens with amino acids residue of Thr at 199 and 200 and free SH of the compounds in series-2 with a

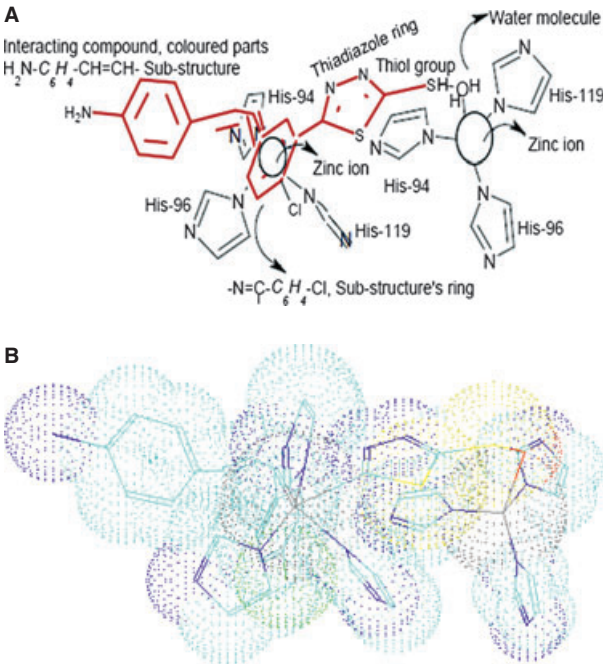

Figure 5: (A) Three-dimensional representation of the ligand-receptor interactions (Hydrogen bondings and ion-dipole interactions). (B) Three-dimensional representation of the ligand-receptor interactions (space-filling model of interacting area inside the receptors' cavity).

water molecule. The preferences for rings' R^1 substitution for *para*-position avoided the interference of the *ortho* substitutionally placed groups with amino acids residues of the receptor chain in the series-2 compounds. The receptor cavity is extending on both the right and left sides of the target molecule (TM) rather than on the upper and down sides (northern and southern parts) of the TM as represented in receptor model (Figure 2). A closer look is depicted in Figure 5 which represents the 3D view of the interaction. Thus, it was concluded that scheme-1 compounds partially acquired the binding site, whereas scheme-2 compounds perfectly acquired the binding site resulting in complete blockade of the hCA-II receptor. The scheme-1 compounds having electron-withdrawing group at R position of the benzene ring showed good anti-convulsant activity whereas, in scheme-2 compounds, the electron-donating groups at R^1 position and electron-withdrawing groups at R^2 position showed potent anti-convulsant activity.

Conclusions

The compact and full extent occupation of the receptor-binding site by compounds **2c** and **2f** is necessary and holds true for higher activity levels with both passing the Rotarod and Ethanol Potentiation tests for neurotoxicity evaluations. The fact that certain products exhibited neurotoxicity is due to alternate binding and undesired interactions in the receptor cavity. The fact that no other compounds than **2c** and **2f** passed both the primary screenings for neurotoxicity evaluations, further establishes the binding and interaction preferences as detailed with the help of the presented receptor model. Thus, the interdependent molecular descriptors for preferable molecular volume, hydrogen bondings and ion–dipole interactions feasibilities may serve as a tool in the design of new anti-convulsant agents and provide indicators for more detailed quantitative structure activity relationships (QSAR) in future.

Conflicts of interest

There are no conflicts of interest.

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