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Research paper

# Probing ferrocene-based thiosemicarbazones and their transition metal complexes as cholinesterase inhibitors

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ABSTRACT

The transition metal complexes (Cu(II) and Co(II)) of ferrocene-based thiosemicarbazones hitherto were synthesized (compounds **13–39**) by the condensation of different thiosemicarbazides with acetylferrocene in presence of catalytic amount of acetic acid, characterized and their inhibitory potential against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) was evaluated. Both the ligands and their metal complexes in general showed moderate to potent inhibition activity against the said enzymes. All the Cu(II) complexes were found more potent than their respective ligands or with complexes of other metals. Similarly, the studied Co(II) complexes also showed potent inhibition of enzymes inhibition and was found even higher than the standard "Eserine" but with slightly higher $IC_{50}$ values than that of Cu(II) complexes. *In silico* studies supported the structure activity relationship of the compounds.

## 1. Introduction

Enzymes are proteins that act as biological catalysts with ability to promote specific chemical reactions under the mild conditions prevailing in the most living organisms. In 1940 Allis and Hawes discovered two major forms of cholinesterases [1], acetylcholinesterase (AChE, EC 3.1.1.7) and the butyrylcholinesterase (BChE, EC 3.1.1.8). These two types of enzymes are present in the human body; AChE is found in erythrocytes, neuromuscular junction in the muscle tissue and in the cholinergic nerve synapses, while BChE is present in nervous system, liver, plasma and pancreas. AChE and BChE belong to the class of serine hydrolases. The differences in amino acid residues of the active sites of AChE and BChE determine the different specificities for substrates and inhibitors for these enzymes. These are key components of cholinergic brain synapses and neuromuscular junctions. The major function of AChE and BChE is to catalyze the hydrolysis of the neurotransmitter acetylcholine and termination of the nerve impulse in cholinergic synapses [2,3]. It has been found that BChE is present in significantly higher quantities in Alzheimer's plaques than in the normal age related non-dementia of brains. Cholinesterase inhibitors increase the amount of acetylcholine available for neuronal and neuromuscular transmission. Hence, the search for the new inhibitors is considered an important and ongoing strategy to introduce new drug candidates for the treatment of Alzheimer's and other related diseases [4,5] Scheme 1.

Thiosemicarbazones are Schiff bases with thiourea functionality obtained by condensing thiosemicarbazide with suitable aldehydes or ketones. Thiosemicarbazone derivatives have found application in drug discovery and development for the treatment of various disorders. They are excellent intermediates for the synthesis of pharmaceuticals and other bioactive molecules. Thiosemicarbazone derivatives are of special importance because of their versatile biological and pharmacological activities such as antimicrobial [11,6–10,22–24], antiviral [12], antiamoebic [13], antimalarial [14–15], antitumor [16,33,34,44–55], antituberculosis [17–20], antipyretic [21], anti-inflammatory [25–27], anticonvulsant [28,29], antihypertensive [30,31], local anaesthetic

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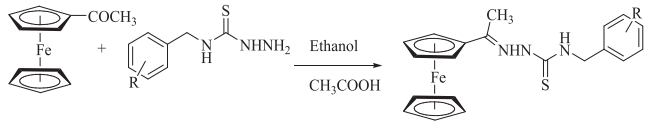


1 2-12 13-23

**(13)** R = H **(19)** R = 3-F
**(14)** R = 2-$CH_3$ **(20)** R = 4-F
**(15)** R = 3-$CH_3$ **(21)** R = 2-Cl
**(16)** R = 3-$OCH_3$ **(22)** R = 3-Cl
**(17)** R = 4-$OCH_3$ **(23)** R = 4-Cl
**(18)** R = 2-F

**Scheme 1.** Synthesis of ligands.

[32], hypoglycemic [35], anti-HIV [36] and cytotoxic activities [37–43]. Thiosemicarbazones are also reported as an antidote for metal toxicity [56,57]. Anticonvulsant activities of substituted thiosemicarbazones have been reported [58]. Citral thiosemicarbazones have exhibited inhibitory activities against leukemia cell proliferation [59]. Antitrypanosomal activity against *Trypanosoma brucei* of citral substituted thiosemicarbazones in *Cymbopogon citratus* essential oil has also been reported [60].

The main purpose of the study was to synthesize new thiosemicarbazone derivatives of ferrocene and its transition metal complexes that were characterized by XRD and other spectroscopic techniques. The compounds were subjected to the determination of their inhibitory potential against AChE and BChE wherein they showed potent to moderate activity against the said enzymes.

## 2. Result and discussion

### 2.1. Chemistry of ligands

The targeted compounds were synthesized by the condensation of thiosemicarbazides with acetylferrocene in presence of acetic acid as catalyst. The reactions were monitored by thin layer chromatography and the crude products were purified by recrystallization using ethanol as a solvent. Respective products were obtained in the range 74–86% yield.

The IR spectral data of novel compounds **(13–23)** indicated the presence of functional groups corresponding to the expected structure of the compounds. The characteristic stretching frequency of C═O group of acetylferrocene is observed at 1618 $cm^{-1}$. In the IR spectrum of synthesized compounds, the absorption band at 1618 $cm^{-1}$ disappeared and new sharp absorption bands in the range of 1521–1539 $cm^{-1}$ were observed indicating the presence of an azomethine linkage (C═N). The absorption bands observed at 3200–3349 $cm^{-1}$ and 3033–3137 $cm^{-1}$ are assigned to N−H stretching. The absorption bands for C═S stretching were observed at 1176–1209 $cm^{-1}$, respectively.

The $^{1}$HNMR spectra of these compounds showed singlet at 2.08–2.43 ppm for $CH_3C$═N. All the five protons of unsubstituted Cp ring appeared as singlet at 4.06–4.36 ppm. A multiplet at 4.30–4.52 ppm was observed for protons at $C_{13(14)}$ of Cp ring whereas protons at $C_{12(15)}$ show multiplet at 4.43–4.57 ppm. The benzylic protons appeared as doublet at 4.85–5.03 ppm. The signal for CS-NH and CN-NH protons appeared as singlet in the range 7.72–8.53 ppm and 8.20–10.07 ppm respectively. The aromatic protons appeared in their typical range.

The $^{13}$C NMR spectra of these compounds showed signal at 24.24 ppm for $CH_3C$═N. The signal for benzylic carbon appeared in the range 46.61–48.30 ppm. The signal for unsubstituted Cp ring appeared in the range 69.38–69.86 ppm, $C_{13(14)}$ of substituted Cp ring appeared in the range 69.86–70.35 ppm, whereas $C_{12(15)}$ appeared in the range 70.45–70.80 ppm. The signal for $C_{11}$ of the substituted Cp ring appeared in the range 82.12–82.28 ppm. The signal appearing in the range 148.0–148.40 ppm was because of C═S; the azomethine carbon appeared in the range 149.30–149.83 ppm.

The EIMS data of all the synthesized compounds **(13–23)** demonstrated that observed molecular ion peaks were in accordance with the calculated mass of the respective compounds. The fragmentation pattern of all these newly synthesized compounds supported the proposed structures.

### 2.2. Chemistry of complexes

The ligands were reacted with metal (II) salts like $CuCl_2$, $CoCl_2$, in absolute ethanol under refluxing conditions in molar ratio 1:2 (metal:ligand), producing a series of metal complexes. The dark brown complexes are stable for long times, insoluble in water and most organic solvents except chloroform but are freely soluble in DMF and DMSO and partially soluble in ethanol. The molar conductance of metal complexes was observed in the range 7.99–15.94-$Ohm^{-1}$ $cm^2$ $mol^{-1}$ indicating non-electrolyte nature of the complexes.

To determine the coordination behavior, infrared spectra of metal complexes were taken. The IR spectra of ligands did not display C−SH strectching in the region 2500–2600 $cm^{-1}$, indicating that in the solid state, these ligands remain in the thione form. The C═N stretching band of thiosemicarbazone in metal complexes undergoes a higher shift of compared to that of the free ligand, which a clear sign of coordination via the imine nitrogen. The increase in the stretching frequency of N−N band of thiosemicarbazone in the spectra of metal complexes is due to the increase in the bond strength, again confirming the coordination via the imine nitrogen. The thioamide stretching band, which contains considerable n(C═S) character, is less intense in the complexes and was observed at higher frequency, suggesting coordination of the metal through sulfur.

In the electronic absorption spectra the bands observed in the range 50000–22222 $cm^{-1}$ can be assigned to$\pi \rightarrow \pi *$ and n $\rightarrow \pi*$ intra-ligand transition. In the electronic spectra, free ligands exhibited two absorption bands at around 40000–33898 $cm^{-1}$ and 29154–24038 $cm^{-1}$ which can be assigned to $\pi \rightarrow \pi^*$ and n $\rightarrow \pi^*$ respectively. In metal complexes, these transitions were shifted to higher frequencies which confirmed the formation of Schiff bases metal complexes. The broad band observed in the region 12500–16666 $cm^{-1}$ with maximum absorbance at 16000–13368 $cm^{-1}$ is assigned to a merges of $^{2}B_{1g} \rightarrow {}^{2}A_{1g}$ and $^{2}B_{1g} \rightarrow {}^{2}B_{2g}$, $^{4}T_2g(F) \leftarrow {}^{4}T_1g(F)$ transitions for Cu and Co respectively in tetrahedral geometry.

The magnetic moment values of the complexes at 30 °C were found to be 4.49–4.75 (Co) and 1.96–2.85 (Cu) B.M. for $d^8$ corresponding to the values normally observed for distorted tetrahedral type geometry of the present complexes.

### 2.3. AChE inhibition of ligands (13–23)

Inhibition of cholinesterases remains at the forefront of therapeutic strategies for treatment of Alzheimer's disease. Metal based inhibitors of cholinesterases have been reported to have better inhibition and pharmacokinetic profile as compared to their parent compounds [61] hence there is a need to explore and develop further metal based cholinesterase inhibitors. All the synthesized thiosemicarbazones **(13–23)** were screened for their AChE inhibitory activity. Eserine was used as a standard inhibitor. Among the tested compounds, (E/Z)-4-(4-chlorobenzyl)-1-(1-ferrocenylethyl)thiosemicarbazone **(23)** and (E/Z)-4-(2-chlorobenzyl)-1-(1-ferrocenylethyl)thiosemicarbazone **(21)** containing 4-chloro and 2-chloro substituents on the phenyl ring, were found to be the most active inhibitors against the enzyme AChE with $IC_{50}$ values of 53.76 ± 0.06 μM and 63.54 ± 0.07 μM, respectively, relative to standard eserine ($IC_{50}$ 0.04 ± 0.0001 μM). Compounds **20** (containing 4-fluorophenyl) and **14** (2-methylphenyl) showed comparable

inhibitory activities ($IC_{50}$ = 71.23 ± 0.03 and 74.71 ± 0.12 μM, respectively). The 3-methoxyphenyl containing compound **16** showed $IC_{50}$ value of 87.91 ± 0.08 μM. Compounds **15** (3-methylphenyl), **22** (3-chlorophenyl) and **17** (4-methoxyphenyl) again showed comparable inhibitory activities ($IC_{50}$ = 95.12 ± 0.12, 95.31 ± 0.09 and 97.12 ± 0.03 μM respectively). The compounds containing 3-fluorophenyl (**19**) and 2-fluorophenyl (**18**) substituents were found to be less active in the series with $IC_{50}$ values of 112.71 ± 0.04 and 115.21 ± 0.12 respectively. Compound **13** having no substituent on the phenyl ring was least active having less than 50% inhibition. The structure activity relationship clearly shows that AChE inhibition activity increases significantly with the introduction of substituents on the phenyl ring. Generally substituent at 4 position of the phenyl ring was found to be more active than the corresponding substituent on 2 or 3 position. This data indicates that usually electron withdrawing groups showed maximum inhibition at position 4 or 2 while electron donating groups having maximum inhibition at position 3.

### 2.4. AChE inhibition of complexes (*24–39*)

The synthesized complexes **(24–34)** of Cu(II) with thiosemicarbazone ligands **(13–23)** exhibited increased inhibition profiles against AChE (Table 1). Potent inhibition activities were observed with $IC_{50}$ values ranging from 9.21 ± 0.29 μM to 24.90 ± 0.39 μM as compared to the corresponding ligands **(13–23)** which showed only moderate inhibition. The metal complexes **(24, 25)** showed better inhibition ($IC_{50}$ 9.21 ± 0.02, 9.51 ± 0.01 μM) as compared to their respective ligands **(13, 14)** ($IC_{50}$ < 250, 74.71 ± 0.12 μM respectively). Likewise, all the Cu (II) complexes were highly active against the enzyme; the order of their decrease in inhibition is as; **24 > 25 > 26 > 30 > 34 > 31 = 32 > 27 = 29 > 28 > 33.**

The metal complexes of Co(II) **(35–39)** were also found to exhibit excellent inhibition against AChE with $IC_{50}$ values ranging from 23.42 ± 0.06 μM to 29.63 ± 0.09 μM as compared to the corresponding ligands **(13–23).** The metal complex **(39)** showed much more potent inhibitory activity ($IC_{50}$ 23.42 ± 0.06 μM) compared with the respective ligand **(23)** ($IC_{50}$ 53.76 ± 0.06 μM). The order of reduction in inhibition profiles is as; **39 > 38 > 37 > 35 > 36.**

### 2.5. BChE inhibition of ligands (*13–23*)

BChE inhibitory activities of newly synthesized thiosemicarbazones **(13–23)** were also determined (Table 1). Eserine was used as standard inhibitor. Among the tested compounds, the 4-fluoro substituted compound **20** and 4-chloro substituted compound **23** showed somewhat comparable inhibition activities with $IC_{50}$ values of 63.45 ± 0.02 μM and 67.25 ± 0.09 μM respectively, followed by 3-fluoro substituted compound **19** ($IC_{50}$ = 74.89 ± 0.08 μM). Compounds **14**, **17** and **18** showed comparable, low inhibition activities with $IC_{50}$ values of 98.61 ± 0.12 μM, 98.32 ± 0.11 μM and 99.23 ± 0.16 μM respectively. The remaining compounds, **13, 15, 16, 21** and **22** exhibited less than 50% inhibition and were thus considered to be inactive in the present assay. The order of decrease in inhibition profiles is as; **20 > 23 > 19 > 17 = 14 > 18.**

### 2.6. BChE inhibition of complexes (*24–39*)

The synthesized Cu(II) complexes **(24–34)** were evaluated for their BChE inhibitory potential. The coordination of thiosemicarbazone ligands **(13–23)** to metal ions was found to cause an increase in the inhibition of BChE (Table 1). The metal complexes **(24–34)**, exhibited potent activity with $IC_{50}$ values ranging from 12.51 ± 0.01 to 32.23 ± 0.08 μM as compared to the corresponding ligands **(13–23)** which proved to be less active. Similarly, the metal complex **29** showed much enhanced activity as compared to the respective ligand **18;** ($IC_{50}$ 12.51 ± 0.01 μM vs. 99.23 ± 0.16 μM). The order of decrease in enzyme inhibition potential is as; **29 > 24 > 26 > 25 = 31 > 30 = 32 > 34 > 33 > 28 > 27.**

**Table 1**
AChE and BChE inhibition profiles of compounds **(13–39).** Data is mean ± sem, n = 3.

| Comp. No. | Groups | AChE | | BChE | |
|---|---|---|---|---|---|
| | | Inhibition (%) at 0.125 mM | $IC_{50}$ μM | Inhibition (%) at 0.125 mM | $IC_{50}$ μM |
| **13** | L = H | 41.61 ± 0.14 | < 250 | 45.21 ± 0.29 | < 250 |
| **24** | Cu | 94.36 ± 0.16 | 9.21 ± 0.02 | 91.76 ± 0.18 | 12.71 ± 0.07 |
| **35** | Co | 94.14 ± 0.12 | 28.91 ± 0.02 | 89.66 ± 0.13 | 57.42 ± 0.05 |
| **14** | L = 2-$CH_3$ | 74.23 ± 0.18 | 74.71 ± 0.12 | 59.49 ± 0.18 | 98.61 ± 0.12 |
| **25** | Cu | 93.35 ± 0.12 | 9.51 ± 0.01 | 89.43 ± 0.12 | 19.12 ± 0.02 |
| **36** | Co | 94.67 ± 0.18 | 29.63 ± 0.09 | 93.69 ± 0.18 | 54.21 ± 0.11 |
| **15** | L = 3-$CH_3$ | 67.62 ± 0.17 | 95.12 ± 0.12 | 41.46 ± 0.26 | < 250 |
| **26** | Cu | 91.26 ± 0.13 | 11.25 ± 0.04 | 89.15 ± 0.16 | 18.12 ± 0.07 |
| **16** | L = 3-$OCH_3$ | 82.22 ± 0.13 | 87.91 ± 0.08 | 49.25 ± 0.16 | < 250 |
| **27** | Cu | 85.39 ± 0.12 | 21.51 ± 0.02 | 89.72 ± 0.11 | 32.23 ± 0.08 |
| **17** | L = 4-$OCH_3$ | 81.64 ± 0.11 | 97.12 ± 0.03 | 57.17 ± 0.13 | 98.32 ± 0.11 |
| **28** | Cu | 87.73 ± 0.15 | 23.61 ± 0.06 | 91.85 ± 0.13 | 29.71 ± 0.09 |
| **37** | Co | 96.72 ± 0.11 | 26.31 ± 0.07 | 96.19 ± 0.12 | 39.21 ± 0.07 |
| **18** | L = 2-F | 57.74 ± 0.15 | 115.21 ± 0.12 | 61.37 ± 0.21 | 99.23 ± 0.16 |
| **32** | Cu | 94.43 ± 0.16 | 19.62 ± 0.05 | 93.27 ± 0.09 | 22.53 ± 0.01 |
| **20** | L = 4-F | 76.69 ± 0.12 | 71.23 ± 0.03 | 68.27 ± 0.16 | 63.45 ± 0.02 |
| **34** | Cu | 95.21 ± 0.18 | 16.51 ± 0.08 | 89.15 ± 0.13 | 26.72 ± 0.09 |
| **19** | L = 3-F | 61.71 ± 0.16 | 112.71 ± 0.04 | 67.55 ± 0.15 | 74.89 ± 0.08 |
| **33** | Cu | 93.14 ± 0.11 | 24.91 ± 0.02 | 91.51 ± 0.13 | 27.15 ± 0.07 |
| **38** | Co | 92.96 ± 0.18 | 24.11 ± 0.05 | 92.39 ± 0.11 | 66.94 ± 0.03 |
| **22** | L = 3-Cl | 78.25 ± 0.13 | 95.31 ± 0.09 | 32.35 ± 0.12 | < 250 |
| **30** | Cu | 93.13 ± 0.12 | 13.41 ± 0.03 | 89.19 ± 0.11 | 22.13 ± 0.01 |
| **21** | L = 2-Cl | 91.24 ± 0.18 | 63.54 ± 0.07 | 34.37 ± 0.23 | > 250 |
| **29** | Cu | 92.27 ± 0.11 | 21.53 ± 0.05 | 86.25 ± 0.11 | 12.51 ± 0.01 |
| **23** | L = 4-Cl | 73.67 ± 0.12 | 53.76 ± 0.06 | 74.39 ± 0.16 | 67.25 ± 0.09 |
| **31** | Cu | 91.35 ± 0.15 | 19.61 ± 0.02 | 82.61 ± 0.11 | 19.91 ± 0.06 |
| **39** | Co | 95.13 ± 0.12 | 23.42 ± 0.06 | 92.78 ± 0.13 | 73.22 ± 0.09 |
| Eserine | | 91.27 ± 1.17 | 0.04 ± 0.0001 | 82.82 ± 1.09 | 0.85 ± 0.0001 |

**Table 2**
Crystal data and structure refinement for compound 21.

| **Compound** | **21** |
|---|---|
| CCDC | **1,899,605** |
| Chemical formula | $C_{22}H_{24}ClFeN_3OS$ |
| $M_r$ | 469.80 |
| Crystal system, space group | Triclinic, $P\bar{1}$ |
| Temperature (K) | 296 |
| *a*, *b*, *c* (Å) | 10.4519 (6), 10.9195 (6), 11.3018 (8) |
| α, β, γ (°) | 77.556 (3), 62.550 (2), 74.000 (2) |
| *V* (Å$^3$) | 1094.38 (12) |
| *Z* | 2 |
| Radiation type | Mo *Kα* |
| μ(mm$^{-1}$) | 0.93 |
| Crystal size (mm) | 0.38 × 0.32 × 0.24 |
| Diffractometer | Bruker Kappa APEXII CCD |
| Absorption correction | Multi-scan (*SADABS*; Bruker, 2005) |
| $T_{min}$, $T_{max}$ | 0.721, 0.810 |
| No. of measured, independent and observed [$I > 2\sigma(I)$] reflections | 17298, 4768, 3766 |
| $R_{int}$ | 0.027 |
| (sin θ/λ)$_{max}$ (Å$^{-1}$) | 0.639 |
| $R[F^2 > 2\sigma(F^2)]$, w$R(F^2)$, *S* | 0.033, 0.084, 1.03 |
| No. of reflections | 2804 |
| No. of parameters | 200 |
| H-atom treatment | 2 |
| $\Delta_{>max}$, $\Delta_{>min}$ (e Å$^{-3}$) | 0.033, 0.084, 1.03 |

### 2.7. Structural insight by XRD study

To determine the structure of compound **21** unambiguously, the X-ray quality single crystals (Table 2) were obtained by slow evaporation of its solution in ethanol at room temperature. Compound **21** was crystallized as 1,4-dioxane solvate in the triclinic, crystal lattice with the $P\bar{1}$ space group. The molecular structure of compound **21** along with crystallographic numbering scheme is shown in Fig. 1.

The central *N*-iminothiourea moiety that is linking the ferrocenyl and 2-chlorobenzyl moiety is essentially planar in the solid state crystal structure of compound **21**. The planarity of this moiety can be attributed to the some extent to the partial double bond character of C—N bonds of thiourea moiety [N2 C13 1.352(3); N3 C13 1.332(2)] due to significant delocalization of nitrogen lone pair towards thiocarbonyl group. The slightly longer bond lengths of N(2)-C(13) indicate slightly

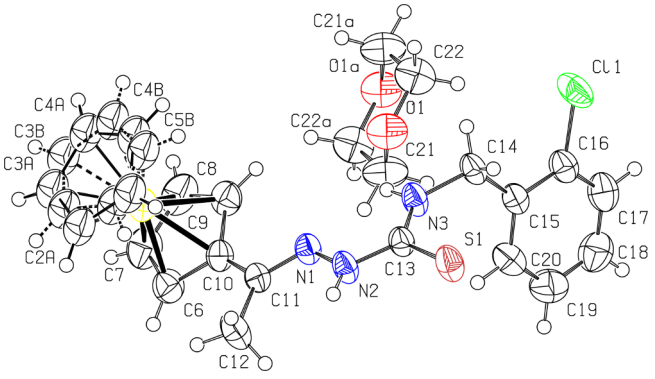


**Fig. 1.** A view of the structure of compound **21**, showing the atom-labelling scheme. Displacement ellipsoids are drawn at the 50% probability level. Selected bond lengths (Å): N1 C11 1.281(3); N1 N2 1.381(2); N2 C13 1.352(3); N3 C13 1.332(2); N3 C14 1.447(3); S1 C13 1.678(2), Selected bond angles (°):C11 N1 N2 118.39(17); C13 N2 N1 118.01(16); C13 N3 C14 124.54(18); N3 C13 S1 123.86(16); N2 C13 S1 120.28(15); N3 C14 C15 113.25(19); N3 C13 N2 115.86(18), Selected dihedral angles (°):C11 N1 N2 C13 172.5(2); N2 N1 C11 C10 179.23(18); N2 N1 C11 C12 –0.8(3); C9 C10 C11 N1 –15.6(3); C6 C10 C11 N1 166.8(2); C14 N3 C13 N2 –176.7(2); C14 N3 C13 S1 3.1(3); N1 N2 C13 N3 1.6(3); N1 N2 C13 S1 –178.14(15); C13 N3 C14 C15 95.5(3); N3 C14 C15 C16 159.17(19); N3 C14 C15 C20 –22.3(3).

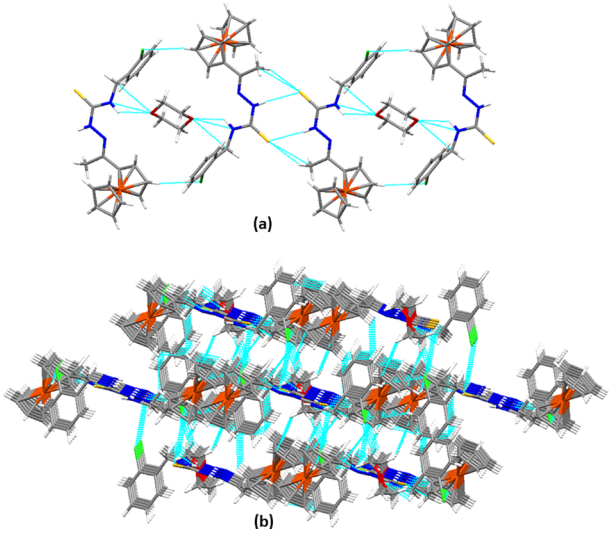


**Fig. 2.** Molecular packing of compound **21** in the solid state, a) showing 1D-supramolecular tapes along *c*-axis; b) showing 3D-network structure.

less delocalization from this side, most probably due to the attachment of sp$^2$-hybridized nitrogen atom. This observation together with the planarity of the central core allows the molecule to adopt preferably *cis, trans*-conformation in the solid state. The appearance of only one conformation can be ascribed to the relatively strong intramolecular hydrogen bond [(N(3)-H(3C)···N(1) 2.180 Å]. It is worth mentioning here that the observation of this conformation in this compound is entirely different from thiourea where there is no such internal stabilization; however this is in complete agreement with the previously described related compounds [62–65].

Owing to the *cis, trans* conformation, the crystal packing of **21** is dominated by acentrosymmetric thioamide $R_2^2(8)\{\cdots H-N-C=S\}_2$ synthon. The interesting feature in the packing of compound 21 is the formation of 1D-supramolecular tapes by mean of thisthioamide synthon [N(2)-H(2c)···S(1) 2.662 Å] and self-complementary CH-Cl [C(8)-H(8)···Cl(1) 2.95 Å], holding a 1,4-dioxane molecules in the centre by means of CH-O [N(3)-H(3c)···O(1) 2.366 Å]interactions (Fig. 2a). These 1D-tapes are connected with the neighboring tapes by means of various non-covalent interactions to form a 3D-network structure (Fig. 2b)

### 2.8. Molecular docking studies

Molecular docking studies were carried out to rationalize possible binding mode and binding site interactions of ferrocene derivatives with AChE. All compounds (**14–23**) were found to have a similar binding mode and were oriented inside the active site gorge. Table 3 shows AutoDock calculated binding free energies for compounds **14–23**. Detailed binding site interactions of most potent inhibitor in this series (**23**) are given in Fig. 3. The ferrocene moiety was found to be

**Table 3**
AutoDock calculated binding free energies (kcal/mol) of AChE inhibitors, **14–23**.

| **Compound** | **Binding Free Energy (ΔG, kcal/mol)** |
|---|---|
| **14** | −8.34 |
| **15** | −8.46 |
| **16** | −8.26 |
| **17** | −8.1 |
| **18** | −7.39 |
| **19** | −7.34 |
| **20** | −8.3 |
| **23** | −8.51 |
| **22** | −8.39 |

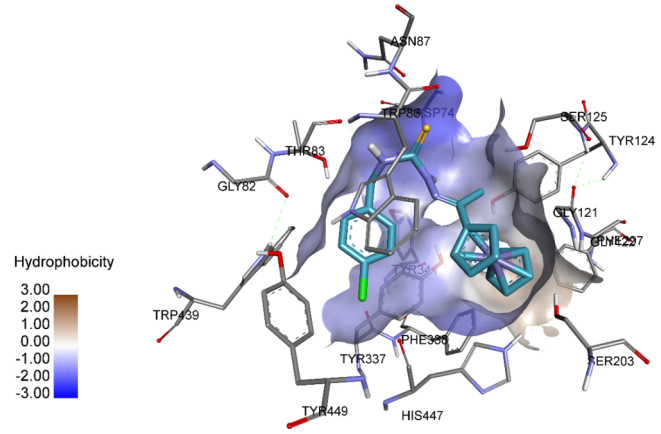


**Fig. 3.** The most probable binding site interactions of **23** inside active site of AChE.

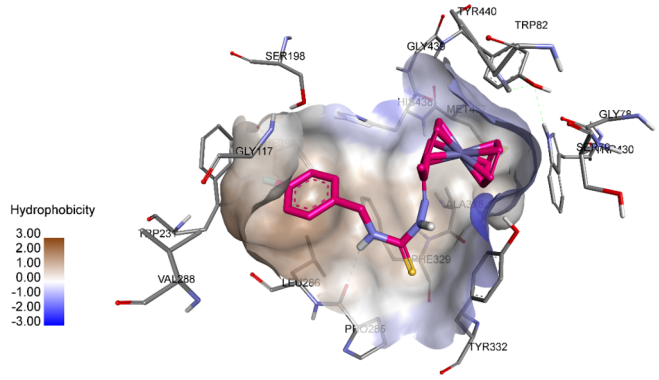


**Fig. 4.** The most probable binding site interactions of **20** inside active site of BChE.

oriented towards Gly121, Gly122, Ser125, and Phe297 amino acid residues. The NH group of thiosemicarbazide part of the molecule was found to act as a hydrogen bond donor towards carbonyl oxygen atom of Thr83 (1.94 Å). This hydrogen bond interaction seems to be an important factor contributing towards the inhibitory potential of compound **23** against AChE.

Molecular docking studies were carried out to rationalize possible binding mode and binding site interactions of most active ferrocene derivatives against BChE. All inhibitors were found to have a similar orientation inside the active site, their AutoDock calculated binding free energies are given in Table 4. Detailed binding site interactions of most active inhibitor in this series (compound **20**) are given in Fig. 4. The ferrocene moiety was found to be oriented towards the amino acid residues Trp430, Gly439, Gly78, Trp82 and Ser79. A hydrogen bond was found between the NH group of the thiosemicarbazide moiety and carbonyl oxygen of Pro285 (2.07 Å).

### 2.9. Experimental

A solution of the appropriate thiosemicarbazide (6.60 mmol) in ethanol (20 mL) mixture was added drop-wise to a stirred hot solution of acetylferrocene (6.60 mmol) in ethanol (20 mL). Acetic acid (1 mL) was added and the reaction mixture was refluxed for 3–5 h and upon cooling to room temperature, reddish-orange crystalline solids separated. The products were filtered, washed several times with cold ethanol and dried under vacuum. Single crystals suitable for X-ray analysis were obtained by recrystallization from ethanol using the slow evaporation method.

#### 2.9.1. (E/Z)-4-benzyl-1-(1-ferrocenylethyl)thiosemicarbazone (13) [65,67]

Yield, 75%; mp, 140; IR (KBr), V ($cm^{-1}$): 3342, 3222, 3067(NH), 1531(C═N), 1191(C═S). $^{1}$H NMR ($CDCl_3$), $^{\delta}$ (ppm): 4.12(5H, s, Cp-ring $C_{16}$-H), 4.3(2H, m, Cp-ring $C_{13(14)}$-H), 4.51(2H, m, Cp-ring $C_{12(15)}$-H), 2.13(3H, s, $CH_3$-C═N), 4.94(2H, d, $J$ = 4.5 Hz, $CH_2$-N), 8.53(1H, s, CS-NH), 10.09 (1H, s, *N*-NH), 7.23–7.40(5H, m, Phenyl $C_{2\text{-}6}$), EIMS, *m/z* (rel. int.): 391 ($M^+$, 83), 284(100), 227(80), 184(61), 210(19), 129(27), 106(21), 91(74), 56(17). $C_{20}H_{21}N_3S$ Fe (3 9 1); calcd.: C 60.38H 3.37 N 10.74; found: C 59.34H 2.89 N 10.25.

**Table 4**
AutoDock calculated binding free energies (kcal/mol) of BChE inhibitors.

| Compound | Binding Free Energy (ΔG, kcal/mol) |
|---|---|
| **14** | −7.34 |
| **17** | −7.08 |
| **18** | −7.17 |
| **19** | −7.28 |
| **20** | −7.59 |
| **23** | −7.23 |

#### 2.9.2. (E/Z)-4-(2-methylbenzyl)-1-(1-ferrocenylethyl)thiosemicarbazone (14)

Yield, 75%; Orange crystalline: mp, 164; IR (KBr), V ($cm^{-1}$): 3365, 3215, 3084(NH), 1531(C═N), 1205(C═S). $^{1}$H NMR ($CDCl_3$), $^{\delta}$ (ppm): 4.36 (5H, s, Cp-ring $C_{10'}$-H), 4.52 (2H, m, Cp-ring $C_{13(14)}$-H), 4.57 (d, 2H, Cp-ring $C_{12(15)}$-H), 2.12 (3H, s, $CH_3$-C═N), 4.95 (2H, d, $J$ = 5.37 Hz, $CH_2$-N), 7.72(1H, s, CS-NH), 8.4(1H, s, *N*-NH), 2.09 (3H, d, $J$ = 13.11 Hz, Phenyl $C_2$-$CH_3$), 7.29 (4H, m, Phenyl $C_{3\text{-}6}$-H)) $^{13}$C NMR $^{\delta}$ (ppm) 136.4 ($C_1$-Phenyl), 135.4 ($C_2$-Phenyl),130.5 ($C_3$-Phenyl), 128.7 ($C_6$-Phenyl), 128.3 ($C_4$-Phenyl), 126.2 ($C_5$-Phenyl), 149.3(C═N), 148.04 (C═S), 46.6(NH-$CH_2$), 24.2 ($CH_3$-C═N),19.28 ($CH_3$-Pheny), 82.2($C_{11}$-Cpring), 69.86($C_{16}$-Cpring), 70.36($C_{13(14)}$-Cpring), 70.74($C_{12(15)}$-H), EIMS, *m/z* (rel. int.): 405($M^+$, 45), 284(75), 227(100), 211(18), 185(58), 162(22), 121(35), 105(80), 56(13). $C_{21}H_{23}N_3S$ Fe (4 0 5); calcd.: C 62.22H 5.68 N 10.37; found: C 61.74H 4.79 N 10.17.

#### 2.9.3. (E/Z)-4-(3-methylbenzyl)-1-(1-ferrocenylethyl)thiosemicarbazone (15)

Yield, 76%; Orange crystalline: mp, 149; IR (KBr), V ($cm^{-1}$): 3350, 3257(NH), 1533(C═N), 1203(C═S). $^{1}$H NMR ($CDCl_3$), $^{\delta}$ (ppm): 4.12 (5H, s, Cp-ring $C_{16}$-H), 4.44 (2H, m. Cp-ring $C_{13(14)}$-H), 4.48 (2H, m, Cp-ring $C_{12(15)}$-H), 2.3 (3H, s, $CH_3$-C═N), 4.85 (2H, d, $J$ = 5.55 Hz, $CH_2$-N), 7.75(1H, s, CS-NH), 8.29 (1H, s, *N*-NH), 2.29 (3H, d, $J$ = 13.2 Hz, Phenyl $C_3$-$CH_3$), 7.65(1H, s, Phenyl $C_2$), 7.13 (3H, m, Phenyl $C_{4\text{-}6}$-H)) $^{13}$C NMR $^{\delta}$ ppm) 138.4 ($C_1$-Phenyl), 137.6 ($C_3$-Phenyl), 128.8 ($C_2$-Phenyl), 128.6 ($C_5$-Phenyl), 128.5 ($C_4$-Phenyl),124.9 ($C_6$-Phenyl), 149.4(C═N), 148.0 (C═S), 48.3 (NH-$CH_2$), 24.2 ($CH_3$-C═N), 21.4 ($CH_3$-Pheny), 82.23($C_{11}$-Cpring), 69.86($C_{16}$-Cpring), 70.35($C_{13(14)}$-H), 70.74($C_{12(15)}$-H), EIMS, *m/z* (rel. int.): 405($M^+$, 27), 284(59), 227(100), 211(18), 185(47), 162(22), 105(75), 56(10). $C_{21}H_{23}N_3S$ Fe (4 0 5); calcd.: C 62.22H 5.68 N 10.37; found: C 61.84H 5.59 N 9.87.

#### 2.9.4. (E/Z)-4-(3-methoxybenzyl)-1-(1-ferrocenylethyl)thiosemicarbazone (16) [65]

Yield, 86%; Orange crystalline: mp, 140; IR (KBr), V ($cm^{-1}$): 3338, 3238(NH), 1539(C═N), 1163(C═S). $^{1}$H NMR ($CDCl_3$), $^{\delta}$ (ppm): 4.32(5H,s, Cp-ring $C_{16}$-H), 4.40(2H, m, Cp-ring $C_{13(14)}$-H), 4.46(2H, m, Cp-ring $C_{12(15)}$-H), 2.1 (3H,s, $CH_3$-C═N), 4.86 (2H, d, $J$ = 5.52 Hz $CH_2$-N), 7.78 (1H, s, CS-NH), 8.25(1H, s, *N*-NH), 3.75(3H, s, Phenyl $C_3$-$OCH_3$), 6.91 (2H, m, Phenyl $C_{2,4}$-H), 7.20(1H, dd,$J$ = 2.28 Hz, Phenyl $C_5$-H), 6.78(1H, d, $J$ = 7.8 Hz, Phenyl $C_6$-H), $^{13}$C NMR $^{\delta}$ (ppm) 159.9($C_3$-Phenyl), 139.40($C_1$-Phenyl), 129.79($C_5$-Phenyl), 120.02($C_4$-

Phenyl), 120.24($C_2$-Phenyl), 113.63($C_6$-Phenyl), 159.93($C_3$-Phenyl), 148.04(C═S), 149.61(C═N), 48.3(NH-$CH_2$), 24.2($CH_3$-C═N), 55.31(O-$CH_3$), 82.18($C_{11}$-Cpring), 69.86($C_{16}$-Cpring), 70.37($C_{13(14)}$-H), 70.76($C_{12(15)}$-H), EIMS, *m/z* (rel. int.): 420 ($M^+$, 37), 283(93), 227(100), 185(80), 211(25), 136(30), 129(36), 121(79), 106(12), 91(19), 56(15). $C_{21}H_{23}N_3SO$ Fe (421); calcd.: C 59.86H 4.46 N 9.98; found: C 58.64H 3.69 N 9.17.

### 2.9.5. (E/Z)-4-(4-methoxybenzyl)-1-(1-ferrocenylethyl)thiosemicarbazone (17) [66]

Yield, 78%; Orange crystalline: mp, 190; IR (KBr), V (cm$^{-1}$): 3676, 3100(NH), 1531(C═N), 1176(C═S). $^1$H NMR ($CDCl_3$), $^\delta$ (ppm): 4.36(s, 5H, Cp-ring $C_{16}$-H), 4.51(m, 2H, Cp-ring $C_{13(14)}$-H), 4.52(m, 2H, Cp-ring $C_{12(15)}$-H), 2.15(s, 3H,$CH_3$-C═N), 4.89(d, 2H, $CH_2$-N), 7.77(s, 1H, CS-NH), 8.43 (s, 1H, *N*-NH), 3.81(s, 3H, Phenyl $C_4$-$OCH_3$), 7.32(d, 2H, Phenyl $C_{2,3}$-H), 6.91(d, 2H, Phenyl $C_{5,6}$-H), $^{13}$C NMR $^\delta$ (ppm) 159.16($C_4$-Phenyl), 129.82($C_1$-Phenyl), 129.45($C_3$,$C_5$-Phenyl), 114.15($C_2$,$C_6$-Phenyl), 148.0(C═S), 149.33(C═N), 47.85(NH-$CH_2$), 24.22($CH_3$-C═N), 55.33(O-$CH_3$), 82.28($C_{11}$-Cpring), 69.85($C_{16}$-Cpring), 70.34($C_{13(14)}$-H), 70.67($C_{12(15)}$-H), EIMS, *m/z* (rel. int.): 420 ($M^+$, 31), 284(53), 227(81), 210(16), 185(46), 121(100), 136(22), 91(6), 56(11). $C_{21}H_{23}N_3SO$ Fe (421); calcd.: C 59.86H 4.46 N 9.98; found: C 58.34H 4.39 N 9.57.

### 2.9.6. (E/Z)-4-(2-flourobenzyl)-1-(1-ferrocenylethyl)thiosemicarbazone (18) [65,66]

Yield, 80%; Orange crystalline: mp, 188; IR (KBr), V (cm$^{-1}$): 3355, 3274(NH), 1531(C═N), 1207(C═S). $^1$H NMR ($CDCl_3$), $^\delta$ (ppm): 4.35(s, 5H, Cp-ring $C_{16}$-H), 4.39(m, 2H, Cp-ring $C_{13(14)}$-H), 4.49(m, 2H, Cp-ring $C_{12(15)}$-H), 2.18(s, 3H,$CH_3$-C═N), 5.03(d, 2H, $CH_2$-N), 7.99(s, 1H, CS-NH), 8.27 (s, 1H, *N*-NH), 7.06–7.54(m, 4H, Phenyl $C_{3\text{-}6}$-H).$^{13}$C NMR $^\delta$ (ppm) 162.72($C_2$-Phenyl), 130.55($C_1$-Phenyl), 129.47($C_6$-Phenyl), 129.36($C_4$-Phenyl), 124.35($C_5$-Phenyl), 115.52($C_3$-Phenyl), 148.3(C═N), 159.8(C═S), 41.9(NH-$CH_2$), 24.2($CH_3$-C═N), 82.13($C_{11}$-Cpring), 69.86($C_{16}$-Cpring), 70.40($C_{13(14)}$-H), 70.75($C_{12(15)}$-H), EIMS, *m/z* (rel. int.): 409 ($M^+$, 21), 374(41), 284(22), 227(100), 211(44), 185(42), 129(15), 121(49), 109(83), 56(13), 43(84). $C_{20}H_{20}N_3S$ FeF (409); calcd.: C 58.63H 4.89 N 10.27; found: C 57.84H 4.69 N 9.72.

### 2.9.7. (E/Z)-4-(3-flourobenzyl)-1-(1-ferrocenylethyl)thiosemicarbazone (19)

Yield, 83%; Orange crystalline: mp, 150; IR (KBr), V (cm$^{-1}$): 3349, 3275 (NH), 1521(C═N), 1109(C═S). $^1$H NMR ($CDCl_3$), $^\delta$ (ppm): 4.22(5H, s, Cp-ring $C_{16}$-H), 4.42(2H, m, Cp-ring $C_{13(14)}$-H), 4.48(2H, m, Cp-ring C-$_{12(15)}$), 2.11(3H, s, $CH_3$-C═N), 4.88(2H, d, $J$ = 5.64 Hz, $CH_2$-N), 7.78(1H, s, CS-NH), 8.40(1H, s, *N*-NH), 7.74(1H, d, $J$ 14.34 Hz Phenyl $C_2$-H), 7.19–7.33(3H, m, Phenyl $C_{4\text{-}6}$-H), $^{13}$C NMR $^\delta$ (ppm) 126.07($C_5$-Phenyl), 127.95($C_4$-Phenyl), 127.79($C_6$-Phenyl), 129.99($C_2$-phenyl), 134.57($C_3$-Phenyl), 139.93($C_1$-Phenyl), 148.04(C═S), 149.7(C═N), 47.56(NH-$CH_2$), 24.26($CH_3$-C═N), 82.15($C_{11}$-Cpring), 69.38($C_{16}$-Cpring), 69.87($C_{13(14)}$-H), 70.45($C_{12(15)}$-H), EIMS, *m/z* (rel. int.): 409($M^+$, 69), 284(100), 227(79), 211(18), 185(51), 162(14), 129(21), 121(26), 109(34), 56(7). $C_{20}H_{20}N_3S$ FeF (409); calcd.: C 58.63H 4.89 N 10.27; found: C 58.34H 3.99 N 9.49.

### 2.9.8. (E/Z)-4-(4-flourobenzyl)-1-(1-ferrocenylethyl)thiosemicarbazone (20) [65]

Yield, 84%; Orange crystalline: mp, 141; IR (KBr), V (cm$^{-1}$): 3449, 3357(NH), 1531(C═N), 1191(C═S). $^1$H NMR ($CDCl_3$), $^\delta$ (ppm): 4.06(5H, s, Cp-ring $C_{16}$-H), 4.3(2H, m, Cp-ring $C_{13(14)}$-H), 4.43(2H, m, Cp-ring $C_{12(15)}$-H), 2.08(3H, s, $CH_3$-C═N), 4.85(2H, d, $J$ = 3.2 Hz, $CH_2$-NH), 7.77(1H, s, CS-NH), 8.20 (1H, s, *N*-NH), 7.25(2H, d, Phenyl $C_{3,5}$-H)), 6.98(2H, d, Phenyl $C_{2,6}$-H)) $^{13}$C NMR $^\delta$ (ppm) 163.94($C_4$-Phenyl), 133.66($C_1$-Phenyl), 129.68($C_{2,6}$-Phenyl), 115.68($C_{3,5}$-Phenyl), 149.83 (C═S), 160.68(C═N), 47.50(NH-$CH_2$), 24.26($CH_3$-C═N), 82.12($C_{11}$-Cpring), 69.86($C_{16}$-Cpring), 70.41($C_{13(14)}$-H), 70.80($C_{12(15)}$-H), EIMS, *m/z* (rel. int.): 409($M^+$, 29), 284(36), 227(90), 211(23), 185(38), 162(20), 129(15), 121(26), 109(100), 56(14). $C_{20}H_{20}N_3S$ FeF (409); calcd.: C 58.63H 4.89 N 10.27; found: C 58.34H 4.25 N 9.74

### 2.9.9. (E/Z)-4-(2-chlorobenzyl)-1-(1-ferrocenylethyl)thiosemicarbazone (21)

Yield, 76%; Orange crystalline: mp, 145; IR (KBr), V (cm$^{-1}$): 3376, 3285 (NH), 1525(C═N), 1190(C═S). $^1$H NMR ($CDCl_3$), $^\delta$ (ppm): 4.23(5H, s, Cp-ring $C_{16}$-H), 4.47(2H, m, Cp-ring $C_{13(14)}$-H), 4.49(2H, m, Cp-ring C-$_{12(15)}$), 2.09(3H, s, $CH_3$-C═N), 5.04(2H, d, $J$ = 5.70 Hz, $CH_2$-N), 7.07(1H, s, CS-NH), 8.40(1H, s, *N*-NH), 7.22–7.36(4H, m, Phenyl $C_{3\text{-}6}$-H), $^{13}$C NMR $^\delta$ (ppm) 162.72($C_2$-Phenyl), 130.55($C_1$-Phenyl), 129.47($C_6$-Phenyl), 129.36($C_4$-Phenyl), 124.35($C_5$-Phenyl), 115.52($C_3$-Phenyl), 148.3(C═N), 159.8(C═S), 41.9(NH-$CH_2$), 24.2($CH_3$-C═N), 82.13($C_{11}$-Cpring), 69.86($C_{16}$-Cpring), 70.40($C_{13(14)}$-H), 70.75($C_{12(15)}$-H), EIMS, *m/z* (rel. int.): 425($M^+$, 39), 391(20), 284(87), 227(1 0 0), 210(22), 185(86), 162(30), 125(99), 121(42), 106(22), 77(11), 56(17). $C_{20}H_{20}N_3S$ FeCl (425); calcd.: C 56.47H 4.71 N 9.88; found: C 55.34H 4.39 N 9.78.

### 2.9.10. (E/Z)-4-(3-chlorobenzyl)-1-(1-ferrocenylethyl)thiosemicarbazone (22) [65]

Yield, 74%; Orange crystalline: mp, 175; IR (KBr), V (cm$^{-1}$): 3338, 3263(NH), 1537(C═N), 1193(C═S). $^1$H NMR ($CDCl_3$), $^\delta$ (ppm): 4.22(5H, s, Cp-ring $C_{16}$-H), 4.42(2H, m, Cp-ring $C_{13(14)}$-H), 4.48(2H, m, Cp-ring C-$_{12(15)}$), 2.11(3H, s, $CH_3$-C═N), 4.88(2H, d, $J$ = 5.64 Hz, $CH_2$-N), 8.40(1H, s, CS-NH), 10.07(1H, s, *N*-NH), 7.74(1H, d, $J$ 14.34 Hz Phenyl $C_2$-H), 7.19–7.33(3H, m, Phenyl $C_{4\text{-}6}$-H), $^{13}$C NMR $^\delta$ (ppm) 126.07($C_5$-Phenyl), 127.95($C_4$-Phenyl), 127.79($C_6$-Phenyl), 129.99($C_2$-phenyl), 134.57($C_3$-Phenyl), 139.93($C_1$-Phenyl), 148.04(C═S), 149.7(C═N), 47.56(NH-$CH_2$), 24.24($CH_3$-C═N), 82.15($C_{11}$-Cpring), 69.38($C_{16}$-Cpring), 69.87($C_{13(14)}$-H), 70.45($C_{12(15)}$-H), EIMS, *m/z* (rel. int.): 425($M^+$, 66), 284(100), 227(60), 210(18), 185(57), 162(22), 140(10), 125(35), 121(35), 106(14), 56(12). $C_{20}H_{20}N_3S$ FeCl (425); calcd.: C 56.47H 4.71 N 9.88; found: C 56.24H 4.69 N 9.18.

### 2.9.11. (E/Z)-4-(4-chlorobenzyl)-1-(1-ferrocenylethyl)thiosemicarbazone (23) [67]

Yield, 81%; Orange crystalline: mp, 115; IR (KBr), V (cm$^{-1}$): 3327, 3237 (NH), 1525(C═N), 1190(C═S). $^1$H NMR ($CDCl_3$), $^\delta$ (ppm): 4.24(5H, s, Cp-ring $C_{16}$-H), 4.45(2H, m, Cp-ring $C_{13(14)}$-H), 4.49(2H, m, Cp-ring C-$_{12(15)}$), 2.08(3H, s, $CH_3$-C═N), 4.89(2H, d, $J$ = 5.68 Hz, $CH_2$-N), 7.77(1H, s, CS-NH), 8.20(1H, s, *N*-NH), 7.28(2H, d, Phenyl $C_{3,5}$-H)), 6.99(2H, d, Phenyl $C_{2,6}$-H)), EIMS, *m/z* (rel. int.): 425($M^+$, 13), 391(25), 284(33), 227(100), 210(32), 185(57), 162(21), 125(84), 121(35), 106(11), 56(13). $C_{20}H_{20}N_3S$ FeCl (425); calcd.: C 56.47H 4.71 N 9.88; found: C 56.12H 4.23 N 9.17.

## 2.10. General method for the synthesis of complexes

Solution of an appropriate ligand (0.002 mmol) in ethanol (5 mL) was added to the hot stirred solution of metal (II) chloride or acetate (0.001 mmol) in ethanol (10 mL) in 2:1 ratio, respectively. The resultant mixture was heated at 100 °C for 2 hrs. The solid mass was formed which washed with cold ethanol, desired complexes in pure form obtained.

The synthesized complexes are characterized as given below.

### 2.10.1. 4-benzyl-1-(1-ferrocenylethyl)thiosemicarbazonecopper (II) chloride (24)

Yield, 50%; mp, 182 °C(*d*); IR (KBr), V (cm$^{-1}$): 3172, 3349(NH), 1568(C═N), 1199(C═S). UV (DMSO): λmax 28409, 16000 cm$^{-1}$; Molar conductance (14.49 $O^{-1}$, $cm^2$, $mol^{-1}$); $\mu_{eff}$ = 2.07

$C_{40}H_{42}N_6S_2$ $Fe_2Cl_2Cu$ (916); calcd.: C 56.47H 4.71 N 9.17; found: C 56.34H 3.95 N 9.12

*2.10.2. 4-(2-methylbenzyl)-1-(1-ferrocenylethyl)thiosemicarbazonecopper (II) chloride **(25)***

Yield, 52%; mp, 172 °C(*d*); IR (KBr), ν (cm$^{-1}$): 3263,3338(NH), 1568(C=N), 1209(C=S). UV (DMSO): λmax 25974, 15082 cm$^{-1}$; Molar conductance (12.66 Ω$^{-1}$, cm$^2$, mol$^{-1}$); $\mu_{eff}$ = 1.98

$C_{42}H_{46}N_6S_2Fe_2Cl_2Cu$ (944); calcd.: C 53.38H 4.87 N 8.89; found: C 46.34H 3.55 N 8.45

*2.10.3. 4-(3-methylbenzyl)-1-(1-ferrocenylethyl)thiosemicarbazonecopper (II) chloride **(26)***

Yield, 50%; mp, 164 °C(*d*); IR (KBr), ν (cm$^{-1}$): 3423, 3176(NH), 1569(C=N), 1218(C=S). UV (DMSO): λmax 25316, 15244 cm$^{-1}$; Molar conductance (14.97 Ω$^{-1}$, cm$^2$, mol$^{-1}$); $\mu_{eff}$ = 1.96

$C_{42}H_{46}N_6S_2Fe_2Cl_2Cu$ (944); calcd.: C 53.38H 4.87 N 8.89; found: C 53.34H 3.95 N 8.25

*2.10.4. 4-(3-methoxybenzyl)-1-(1-ferrocenylethyl) thiosemicarbazonecopper (II) chloride **(27)***

Yield, 53%; mp, 160 °C(*d*); IR (KBr), ν (cm$^{-1}$): 3363, 3168(NH), 1560(C=N), 1175(C=S). UV (DMSO): λmax 25706, 14815 cm$^{-1}$; Molar conductance (14.29 Ω$^{-1}$, cm$^2$, mol$^{-1}$); $\mu_{eff}$ = 2.07

$C_{42}H_{46}N_6S_2O_2Fe_2Cl_2Cu$ (976); calcd.: C 51.63H 4.71 N 8.60; found: C 50.94H 3.95 N 8.45

*2.10.5. 4-(4-methoxybenzyl)-1-(1-ferrocenylethyl) thiosemicarbazonecopper (II) chloride **(28)***

Yield, 51%; mp, 143 °C(*d*); IR (KBr), ν (cm$^{-1}$): 3168, 3376(NH), 1544(C=N), 1207(C=S). UV (DMSO): λmax 24271, 15037 cm$^{-1}$; Molar conductance (9.50 Ω$^{-1}$, cm$^2$, mol$^{-1}$); $\mu_{eff}$ = 2.13

$C_{42}H_{46}N_6S_2O_2Fe_2Cl_2Cu$ (976); calcd.: C 51.63H 4.71 N 8.60; found: C 51.34H 4.55 N 8.26

*2.10.6. 4-(2-chlorobenzyl)-1-(1-ferrocenylethyl)thiosemicarbazonecopper (II) chloride **(29)***

Yield, 49%; mp, 180 °C(*d*); IR (KBr), ν (cm$^{-1}$): 3093,3250(NH), 1560(C=N), 1195(C=S). UV (DMSO): λmax 24038, 15552 cm$^{-1}$; Molar conductance (15.20 Ω$^{-1}$, cm$^2$, mol$^{-1}$); $\mu_{eff}$ = 2.34

$C_{40}H_{40}N_6S_2Cl_3Fe_2Cl_2Cu$ (984); calcd.: C48.78H 4.06 N 8.53; found: C 47.84H 3.85 N 7.94

*2.10.7. 4-(3-chlorobenzyl)-1-(1-ferrocenylethyl)thiosemicarbazonecopper (II) chloride **(30)***

Yield, 50%; mp, 168 °C(*d*); IR (KBr), ν (cm$^{-1}$): 3260, 3345 (NH), 1544(C=N), 1218(C=S). UV (DMSO): λmax 24875, 15673 cm$^{-1}$; Molar conductance (15.94 Ω$^{-1}$, cm$^2$, mol$^{-1}$); $\mu_{eff}$ = 2.6

$C_{40}H_{40}N_6S_2Cl_3Fe_2Cl_2Cu$ (984); calcd.: C 48.78H 4.06 N 8.53; found: C 48.34H 4.01 N 8.44

*2.10.8. 4-(4-chlorobenzyl)-1-(1-ferrocenylethyl)thiosemicarbazonecopper (II) chloride **(31)***

Yield, 56%; mp, 118 °C(*d*); IR (KBr), ν (cm$^{-1}$): 3099, 3398 (NH), 1560(C=N), 1199(C=S). UV (DMSO): λmax 29154, 14880 cm$^{-1}$; Molar conductance (11.51 Ω$^{-1}$, cm$^2$, mol$^{-1}$); $\mu_{eff}$ = 2.85

$C_{40}H_{40}N_6S_2Cl_3Fe_2Cl_2Cu$ (9 8 4); calcd.: C 48.78H 4.06 N 8.53; found: C 48.39H 3.05 N 8.25

*2.10.9. 4-(2-flourobenzyl)-1-(1-ferrocenylethyl)thiosemicarbazonecopper (II) chloride **(32)***

Yield, 54%; mp, 170 °C(*d*); IR (KBr), ν (cm$^{-1}$): 3195, 3370(NH), 1566(C=N), 1178(C=S). UV (DMSO): λmax 28089, 15432 cm$^{-1}$; Molar conductance (15.02 Ω$^{-1}$, cm$^2$, mol$^{-1}$); $\mu_{eff}$ = 2.15

$C_{40}H_{40}N_6S_2FCl_2Fe_2Cu$ (952); calcd.: C 50.42H 4.20 N 8.82; found: C50.34H 4.15 N 8.49

*2.10.10. 4-(3-flourobenzyl)-1-(1-ferrocenylethyl)thiosemicarbazonecopper (II) chloride **(33)***

Yield, 52%; mp, 172 °C(*d*); IR (KBr), ν (cm$^{-1}$): 3170, 3349(NH), 1560(C=N), 1215(C=S). UV (DMSO): λmax 26666, 15105 cm$^{-1}$; Molar conductance (14.69 Ω$^{-1}$, cm$^2$, mol$^{-1}$); $\mu_{eff}$ = 2.72

$C_{40}H_{40}N_6S_2FCl_2Fe_2Cu$ (952); calcd.: C 50.42H 4.20 N 8.82; found: C 50.24H 4.23 N 8.49

*2.10.11. 4-(4-flourobenzyl)-1-(1-ferrocenylethyl)thiosemicarbazonecopper (II) chloride **(34)***

Yield, 51%; mp, 166 °C(*d*); IR (KBr), ν (cm$^{-1}$): 3147, 3356(NH), 1558(C=N), 1209(C=S). UV (DMSO): λmax 25510, 14858 cm$^{-1}$; Molar conductance (14.36 Ω$^{-1}$, cm$^2$, mol$^{-1}$); $\mu_{eff}$ = 2.43

$C_{40}H_{40}N_6S_2FCl_2Fe_2Cu$ (952); calcd.: C50.42H 4.20 N 8.82; found: C50.38H 4.13 N 8.73

*2.10.12. 4-benzyl-1-(1-ferrocenylethyl)thiosemicarbazonezinc (II) acetate **(35)***

Yield, 54%; mp, 208 °C(*d*); IR (KBr), ν (cm$^{-1}$): 3135, 3336(NH), 1560(C=N), 1195(C=S). UV (DMSO): λmax32786, 25706 cm$^{-1}$; Molar conductance (9.24 Ω$^{-1}$, cm$^2$, mol$^{-1}$);

$C_{49}H_{48}N_6S_2Fe_2Zn$ (965); calcd.: C 60.93H 4.97 N 8.70; found: C60.74H 4.95 N 8.63

*2.10.13. 4-(2-methylbenzyl)-1-(1-ferrocenylethyl)thiosemicarbazonezinc (II) acetate **(36)***

Yield, 50%; mp, 212 °C(*d*); IR (KBr), ν (cm$^{-1}$): 3140,3321(NH), 1573(C=N), 1190(C=S). UV (DMSO): λmax 32258, 22421 cm$^{-1}$; Molar conductance (4.01 Ω$^{-1}$, cm$^2$, mol$^{-1}$);

$C_{51}H_{52}N_6S_2O_4Fe_2Zn$ (993); calcd.: C61.63H 5.23 N 8.45; found: C60.84H 5.15 N 7.95

*2.10.14. 4-(3-methylbenzyl)-1-(1-ferrocenylethyl)thiosemicarbazonezinc (II) acetate **(37)***

Yield, 55%; mp, 182 °C(*d*); IR (KBr), ν (cm$^{-1}$): 3082, 3364(NH), 1564(C=N), 1190(C=S). UV (DMSO): λmax 34482, 24096 cm$^{-1}$; Molar conductance (3.97 Ω$^{-1}$, cm$^2$, mol$^{-1}$);

$C_{51}H_{52}N_6S_2O_4Fe_2Zn$ (993); calcd.: C 61.63H 5.23 N 8.45; found: C 61.34H 5.55 N 7.92

*2.10.15. 4-(4-methoxybenzyl)-1-(1-ferrocenylethyl)thiosemicarbazonezinc (II) acetate **(38)***

Yield, 53%; mp, 149 °C(*d*); IR (KBr), ν (cm$^{-1}$): 3172, 3349(NH), 1568(C=N), 1199(C=S). UV (DMSO): λmax 33112, 2369 cm$^{-1}$; Molar conductance (7.70 Ω$^{-1}$, cm$^2$, mol$^{-1}$);

$C_{51}H_{52}N_6S_2O_6Fe_2Zn$ (1025); calcd.: C 59.70H 5.07 N 8.19; found: C 59.34H 4.97 N 7.95

*2.10.16. 4-(2-chlorobenzyl)-1-(1-ferrocenylethyl)thiosemicarbazonezinc (II) acetate **(39)***

Yield, 52%; mp, 221 °C(*d*); IR (KBr), ν (cm$^{-1}$): 3123, 3397(NH), 1553(C=N), 1203(C=S). UV (DMSO): λmax 32154, 22472 cm$^{-1}$; Molar conductance (9.43 Ω$^{-1}$, cm$^2$, mol$^{-1}$);

$C_{41}H_{46}N_6S_2O_4Fe_2Cl_2Zn$ (1034); calcd.: C 47.58H 4.44 N 8.12; found: C 46.34H 4.31 N 7.97

*2.11. AChE and BChE assays*

The AChE and BChE inhibition assays were performed according to the Ellman's method [68]. Total volume of 100 μL reaction mixture contained 60 μL 50 mM $Na_2HPO_4$ buffer, pH 7.7. Ten μL test compound (0.5 mM well$^{-1}$) was added, followed by the addition of 10 μL (0.005 unit well$^{-1}$ AChE, or 0.5 units well$^{-1}$ BChE, Sigma Inc.) enzyme solution. The contents were mixed, pre-incubated for 10 min at 37 °C and pre-read at 405 nm. The reaction was initiated by the addition of 10 μL of 0.5 mM well$^{-1}$ substrate (acetylthiocholine iodide or

butyrylthiocholine chloride, Sigma Inc.), followed by the addition of 10 μL DTNB from Sigma Inc. (0.5 mM well$^{-1}$). After 15 min of incubation at 37 °C, absorbance was measured using 96-well plate reader Synergy HT, Biotek, USA. All experiments were carried out with their respective controls in triplicate. Eserine (0.5 mM well$^{-1}$) was used as a positive control. The percent inhibition was calculated by the help of following equation.

Inhibition (%) = 100 − (Abs of test sample $x$ 100 / Abs of control)

Active compounds were serially diluted with HPLC grade methanol and their percentage inhibition profiles were calculated as mentioned in the method. The data obtained (percent inhibition vs concentration) was inserted in the Ez-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA) for the calculation of $IC_{50}$ values.

### 2.12. Molecular docking studies

The crystal structures of human AChE and BChE (PDB ids: 4BDT and 4BDS, respectively) [69] were selected for docking studies as the only available *Electrophorus electricus* AChE structures are of low resolutions (> 4 Å), and X-ray crystal structure of equine BChE is currently not available at the Protein Data Bank. The enzyme was prepared for docking by using DockPrep utility of Chimera [70], whereby all solvent and hetero molecules were removed, hydrogen atoms and charges were added. Using AutoDock Tools [71], a large grid of 80x80x80 was selected that was centered round the active site of the enzyme and was large enough to allow free movement of the ligand. Docking was carried out using AutoDock 4.0 [71] where the enzyme receptor is kept rigid and the inhibitor ligand is treated as flexible. Method validation was carried out by re-docking the original inhibitor that had co-crystallized with the enzyme. The docking methodology was able to reproduce the experimentally observed binding conformation with rmsd of less than 0.5 Å. Before docking the energies of the ligands were minimized using Chimera, through 100 steepest descents and 100 conjugate gradient steps using a step size of 0.02. Gasteiger charges were added using Antechamber [72] utility of Chimera. Lamarckian Genetic Algorithm (LGA) was used for docking, the number of GA runs was set to 25. Discovery Studio Visualizer 4.0 [72] was used for visualization of docked results.

### 2.13. Conclusion

In conclusion, we have synthesized metal complexes (Cu(II) and Co (II) of a series of ferrocene-based thiosemicarbazones and tested them along with their ligands for their inhibitory potential against AChE and BChE. A considerable increase in inhibitory activity especially in both Cu(II) and Co(II) complexes of ferrocene-based thiosemicarbazone ligands was found against the enzymes. The results indicate an increase in AChE and BChE inhibition profiles upon coordination with the metal ions that would surely contribute to the discovery of more potent metal-based AChE and BChE inhibitors.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ica.2020.119658.

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