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Review paper

FERROCENE-CONTAINING CHOLINESTERASE INHIBITORS

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Abstract. *Inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) represents the most established treatment strategy for Alzheimer's disease, which is marked by a deficiency of acetylcholine in the brain. The inhibitory effects of ferrocene derivatives on cholinesterases have not been as thoroughly investigated as their antimalarial and antiproliferative properties. This short review details the advancements in this area since 1962 when the first ferrocene derivative was assessed for its effects. To date, approximately 100 structurally diverse ferrocene-containing compounds have been evaluated for their inhibitory activity against AChE and/or BChE, with several demonstrating promising effects in the low micromolar or submicromolar range. The most active derivatives have emerged from the bioisosteric replacement of the aryl group with a ferrocene unit in known inhibitors or through the development of hybrids that link ferrocene with established inhibitors. It is encouraging to note that research in this field has intensified over the past five years, promising substantial progress soon.*

Key words: *ferrocene, acetylcholinesterase, butyrylcholinesterase, inhibitor, Alzheimer's disease, Parkinson's disease*

1. INTRODUCTION

One of the most prevalent neurodegenerative diseases is Alzheimer's disease, along with Parkinson's disease. It is believed that a decline in the levels of the neurotransmitter acetylcholine in the brain contributes to the pathology of Alzheimer's disease. Inhibiting the catabolism of acetylcholine through the action of the enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) can lead to increased levels of this neurotransmitter, making inhibitors of these enzymes essential medications for alleviating the symptoms of

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Alzheimer's (Marucci et al., 2021). There is also evidence suggesting that cholinesterase inhibitors may be effective in treating cognitive disorders associated with Parkinson's disease (van Laar et al., 2011). The primary treatment for Parkinson's is based on the use of inhibitors of the enzyme monoamine oxidase (MAO), specifically its isoform MAO-B. These inhibitors reduce the breakdown of the neurotransmitter dopamine in the brain, thereby replenishing dopamine levels that are known to decrease as Parkinson's disease progresses (Myburg et al., 2022).

Ferrocene is an organometallic compound consisting of two cyclopentadienyl anions coordinated to a Fe^{2+} ion. It is characterized by several unique properties, including stability, reversible valence changes, a non-benzenoid aromatic structure, and low toxicity, making it an attractive structural fragment for drug development (Patra and Gasser, 2017). In the synthesis of new potentially biologically active ferrocene derivatives, bioisosteric replacement is often employed. This approach involves replacing an aryl group in a known bioactive compound with a ferrocene unit. For instance, this method resulted in the development of ferrocifen, which is currently being evaluated in clinical trials as a potential breast cancer treatment. Ferrocifen was produced by replacing one of the phenyl groups in the drug tamoxifen with a ferrocene moiety (Aksić et al., 2023).

To date, dozens of ferrocene derivatives have been evaluated for their anti-cholinesterase activity, but to our knowledge, none have been investigated as potential MAO inhibitors. Hence, this short review aims to summarize the current understanding and recent developments in the design of ferrocene-based cholinesterase inhibitors.

2. Acetylcholinesterase and Butyrylcholinesterase: Structure and Biological Importance

In mammals, two enzymes are responsible for the hydrolysis of the neurotransmitter acetylcholine: acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). AChE is primarily located at neuromuscular junctions and in cholinergic synapses in the brain, while BChE is mainly found in plasma and the liver (Čolović et al., 2013). AChE regulates acetylcholine levels through hydrolysis, fulfilling a similar role to BChE, although the latter's natural substrate remains unidentified. Unlike AChE, BChE is involved in the metabolism of various xenobiotics (including organophosphates, carbamate pesticides, and cocaine), contributes to lipoprotein metabolism (such as the peptide hormone ghrelin), and activates certain drugs (like bambuterol and heroin). Additionally, when AChE is inhibited or has reduced activity, BChE can act as a compensatory enzyme (Bosak et al., 2018; Rosenberry et al., 2017).

AChE catalyzes the hydrolysis of acetylcholine into acetate and choline. When AChE is inhibited, acetylcholine levels increase, resulting in prolonged cholinergic effects (Li et al., 2021). The hydrolysis process starts with the transfer of the acetyl group from acetylcholine to the serine residue Ser-200 in the enzyme's active site, creating an acetylated enzyme and free choline. This is followed by a nucleophilic attack by water on the acetylated enzyme, releasing acetic acid and regenerating the enzyme for further hydrolysis reactions. The residues glutamic acid (Glu-327) and histidine (His-440) are essential for facilitating proton transfer during both steps. The mechanism of this hydrolysis reaction is illustrated in Fig. 1 (Čolović et al., 2013).

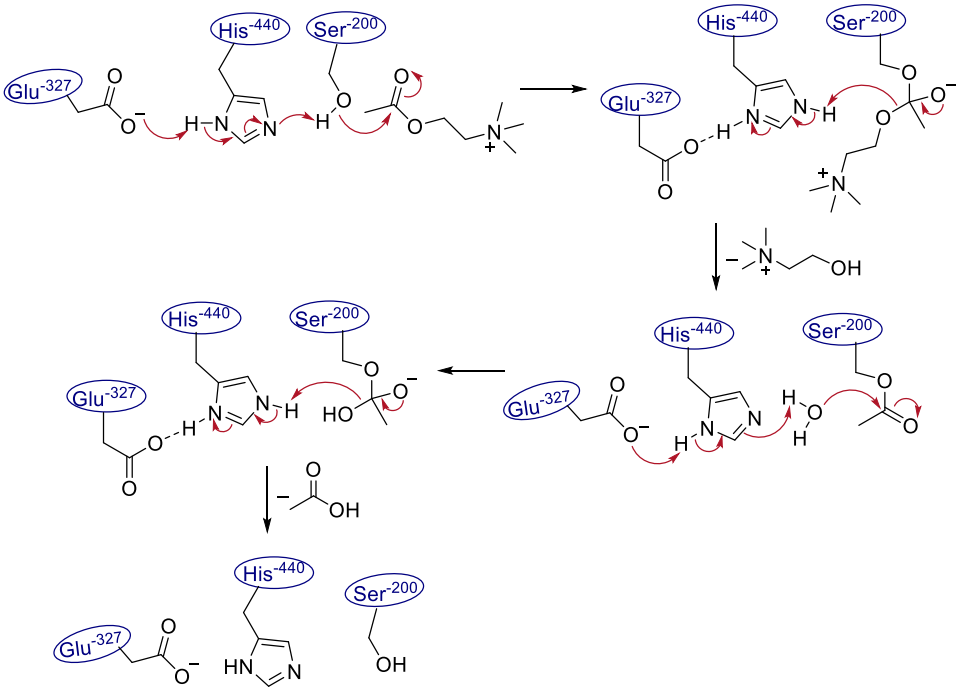


Fig. 1 Acetylcholine hydrolysis catalyzed by AChE

AChE and BChE have a similar structure, exhibiting over 50% homology in their amino acid sequences. The active site of these enzymes is located within a pocket approximately 20 Å in size. This active site can be divided into two regions: a peripheral anionic site at the entrance and a catalytic domain at the base of the pocket. Both enzymes feature the same catalytic triad (Ser-200, Glu-327, and His-440) along with an oxyanion hole; however, variations exist in their amino acid sequences. Specifically, certain aromatic amino acids in the active site of AChE are replaced by aliphatic amino acids in BChE. This variation impacts not only their selectivity for inhibitors and substrates but also their distinct stereoselectivities (Bosak et al., 2018).

These enzymes play a crucial role in the treatment of neurodegenerative diseases such as myasthenia gravis, Alzheimer's disease, and Parkinson's disease. Cholinesterase inhibitors have emerged as the most effective options for alleviating the symptoms of these conditions. In recent decades, therapeutic agents like rivastigmine and galantamine, which are dual inhibitors of AChE and BChE, as well as donepezil, a selective inhibitor of AChE, have been employed (Fig. 2). Recent research indicates that selective BChE inhibitors may offer a novel approach to treating Alzheimer's disease. Studies have shown that patients with advanced Alzheimer's symptoms exhibit a reduction in AChE levels to 55-67% of normal and an increase in BChE levels to 120% of normal values (Bosak et al., 2018; Li et al., 2021).

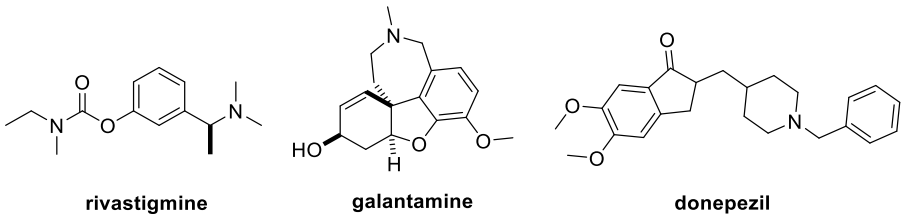


Fig. 2 Structures of cholinesterase inhibitors

3. INHIBITORS OF ACETYLCHOLINESTERASE

In 1969, Cavallito and colleagues (1969) explored various molecular modifications of a stirylpyridine prototype as AChE inhibitors, shedding light on the steric and electronic effects influencing their activity. Among the 31 synthesized analogs, one ferrocene derivative **1** was included in this study (Fig. 3). Despite its structural novelty, this ferrocene derivative, like many others in the library, exhibited relatively weak inhibitory activity, with an IC_{50} of 250 μM.

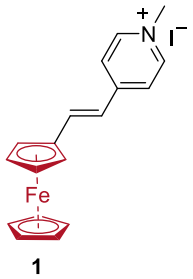
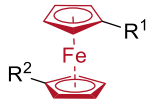


Fig. 3 Structure of ferrocenyl analog of stirylpyridine **1**

Considering the established anti-AChE activity of *N*-methyl carbamates derived from planar aromatic oximes, Hetnarski and coworkers (1980) synthesized eight novel ferrocenyl methyl carbamates (**2**-**9**) to investigate how the three-dimensional aromatic structure of the ferrocene unit impacts their activity. The general structure of these organometallic carbamates, along with the results of the inhibitory assays (IC_{50} values), is presented in Table 1.

Derivatives **2** and **3**, derived from the oximes of ferrocene carbaldehyde and acetylferrocene, displayed the lowest activity, with IC_{50} values of 100 and 180 μM, respectively. Interestingly, longer alkyl chains in the starting ketoxime were associated with enhanced activity. Furthermore, branching in the alkyl chain significantly enhanced inhibitory potency; for example, derivative **7** (IC_{50} = 2 μM), which contains an isopropyl substituent, was 7.5 times more active than its *n*-propyl counterpart **6**. Additionally, derivative **9**, featuring an additional *N*-phenyl substituent, demonstrated notable inhibitory activity with an IC_{50} of 3.5 μM. These results indicate a specific interaction between these substituents and the enzyme's active site. Similar findings were previously reported for methylcarbamates derived from acetophenone oximes (Fukuto et al., 1969).

Table 1 Inhibitory effect of ferrocenyl methyl carbamates **2**-**9** on AChE



Designation	R^1	R^2	IC_{50} [μM]
2	H C=NOC(O)NHCH$_3$	H	100
3	CH$_3$ C=NOC(O)NHCH$_3$	H	180
4	CH$_3$ C=NOC(O)NHCH$_3$	CH$_3$ C=NOC(O)NHCH$_3$	10
5	C$_2$H$_5$ C=NOC(O)NHCH$_3$	H	18
6	C$_3$H$_7$ C=NOC(O)NHCH$_3$	H	15
7	CH(CH$_3$)$_2$ C=NOC(O)NHCH$_3$	H	2
8	C$_6$H$_5$ C=NOC(O)NHCH$_3$	H	17
9	H C=NC$_6$H$_4$OC(O)NHCH$_3$-*p*	H	3.5

Heteroannular double substitution of ferrocene (as seen in compound **4**) resulted in a significant increase in anti-AChE potency, achieving an order of magnitude enhancement compared to the monosubstituted compound **3**. Moreover, the activity of derivative **9** demonstrated that substituting phenyl methylcarbamate (IC_{50} = 200 μM; Metcalf, 1962) with a ferrocenylaldimine group substantially enhanced the inhibitory effect.

Ferrocene-indole hybrids, namely 2-(3-ferrocenylphenyl)-1*H*-indole (**10**) and 2-(4-ferrocenylphenyl)-1*H*-indole (**11**), represent another class of ferrocene derivatives that were tested for their inhibitory effects on AChE (Fig. 4). The *meta*-isomer **10** displayed a weak inhibitory effect, with an IC_{50} value of 255 μM, in contrast to the positive control, physostigmine, which had an IC_{50} of 0.3 μM. For the *para*-isomer **11**, it was not possible to determine the IC_{50} value due to its lower solubility; the most concentrated tested solution (ca. 100 μg/mL in medium) did not significantly affect AChE activity (Radulović et al., 2014).

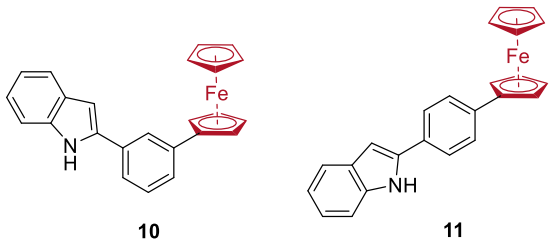


Fig. 4 Structures of ferrocene-indol hybrids **10** and **11**

4. INHIBITORS OF BUTYRYLCHOLINESTERASE

In two previous studies, the inhibitory effects of amides derived from ferrocenylanilines and various benzoic acids on the enzyme BChE were explored. Initially, Altaf and coworkers (2017) synthesized three ferrocenyl amides featuring regioisomeric methoxybenzoic acids: *N*-(4-ferrocenylphenyl)-2-methoxybenzamide (**12**), *N*-(4-ferrocenylphenyl)-3-methoxybenzamide (**13**), and *N*-(4-ferrocenylphenyl)-4-methoxybenzamide (**14**). The general structural formula for these compounds is illustrated in Fig. 5.

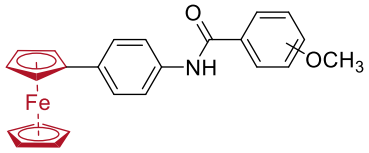
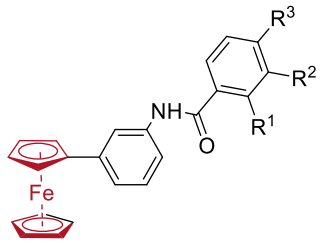


Fig. 5 General structure of ferrocenylaniline-based amides of regioisomeric methoxybenzoic acids **12**-**14**

It was found that in the solid state, the *para*-derivative **14** displayed only non-covalent interactions, while the *ortho*-derivative **12** exhibited intermolecular hydrogen bonding as well. As a result, it was expected that the *ortho*-derivative **12** would form stronger interactions with the enzyme and display better inhibitory effects compared to the other two regioisomers. In the *in vitro* BChE assay, the *para*-derivative **14** exhibited the highest activity (IC_{50} = 16 μM). The *ortho*-derivative **12** showed slightly lower activity (IC_{50} = 22 μM), while the *meta*-derivative **13** was the least effective, with an IC_{50} of 24 μM. However, all three amides exhibited significantly lower effectiveness than galantamine, which served as the positive control (IC_{50} = 8 μM). To address the discrepancy between expected and actual results, docking analysis was performed. This analysis highlighted that the formation of hydrogen bonds between the enzyme's active site and the inhibitor is essential for the inhibitory activity of these amides. The *para*-derivative forms more hydrogen bonds than the *ortho*-derivative, which contributes to its superior docking score and overall effectiveness (Altaf et al., 2017).

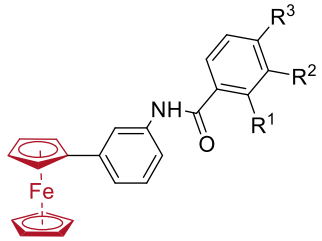
One year later the same research group synthesized additional 23 related ferrocenyl amides with the general formula C_5H_5-Fe-C_5H_4-C_6H_4-NH-CO-C_6H_4-R and assessed their inhibitory effects on the same enzyme (Altaf et al., 2018). These organometallic compounds were classified into two series based on whether they were derivatives of *meta*- or *para*-ferrocenylaniline. Their IC_{50} values are provided in Tables 2 and 3.

Table 2 Inhibitory effect of ferrocene-based anilides **15**-**27** on BChE



Designation	R^1	R^2	R^3	IC_{50} [μM]
15	H	H	H	13
16	F	H	H	9
17	H	F	H	14
18	H	H	F	14
19	Cl	H	H	15
20	H	Cl	H	15
21	H	H	Cl	14
22	CH_3	H	H	13
23	H	CH_3	H	12
24	H	H	CH_3	10
25	OCH_3	H	H	14
26	H	OCH_3	H	9
27	H	H	OCH_3	13

Table 3 Inhibitory effect of ferrocene-based anilides **28**-**40** on BChE



Designation	R^1	R^2	R^3	IC_{50} [μM]
28	H	H	H	17
29	F	H	H	15
30	H	F	H	24
31	H	H	F	21
32	Cl	H	H	16
33	H	Cl	H	25
34	H	H	Cl	21
35	CH_3	H	H	16
36	H	CH_3	H	21
37	H	H	CH_3	24
38	OCH_3	H	H	21
39	H	OCH_3	H	24
40	H	H	OCH_3	16

Solid-state studies revealed that ferrocene derivatives with *meta*-amide substituents engage in intermolecular hydrogen bonding, which stabilizes the *meta* derivatives compared to their *para* analogs. This hydrogen bonding arises as the ferrocene conformation shifts through rotation around the C–N bond, promoting interactions between adjacent molecules in the solid state. To investigate the biological implications of this hydrogen bonding, both experimental and computational studies were performed. All tested compounds inhibited BChE, with the most active derivatives **16** and **26** achieving 50% inhibition at a concentration of 9 μM, similar to the known inhibitor galantamine (IC_{50} = 8 μM).

The compounds from the first series, featuring ferrocene and amide groups in the *meta* position, consistently demonstrated slightly greater activity compared to their structural isomers. This suggests that while hydrogen bond formation contributes to stability, its impact on enhancing overall affinity for the enzyme is limited. In contrast, the positional variation of substituents on the acid side of the amide did not have a regular impact on activity. Notably, compounds **16** and **29**, which have a fluoride atom *ortho* to the amide, exhibited slightly higher activity than the other fluoride-containing isomers. However, overall, the studied compounds displayed very similar inhibitory potencies in the micromolar range against butyrylcholinesterase.

Docking studies further supported these findings, revealing that the effect of hydrogen bonding in the active site of BChE is modest, regardless of the presence of water. This highlighted the essential role of hydrophobic interactions in the binding affinity of inhibitors for this enzyme, illustrating the complexity of ligand-enzyme interactions in developing effective pharmacological agents. Additionally, the authors concluded that their results suggest ferrocenes exhibit low selectivity for BChE, indicating that minor structural modifications are unlikely to lead to significant changes in the IC_{50} values of these compounds (Altaf et al., 2018).

5. Selective or Dual Inhibitors

Organophosphorus compounds can inhibit serine hydrolases, such as AChE and BChE, through the phosphorylation of the active site serine residue. The phosphorylated enzyme may either revert to its regenerated form or shift to an 'aged' form, resulting in irreversible inhibition. In this context, Rudolf and colleagues (2010) synthesized two new ferrocenyl phosphonate derivatives, **41** and **42**, by reacting ferrocenyl methyl maleimide with dimethyl- and diphenylphosphite, respectively (Fig. 6).

Biochemical studies demonstrated that these compounds could inhibit the catalytic activity of AChE and BChE reversibly. Notably, a very slow, time-dependent inactivation of BChE by both derivatives was also observed. The ferrocenyl diphenyl complex **42** showed greater anti-AChE activity, with an IC_{50} of 10 μM and a competitive inhibition constant of 35 μM. Unlike derivative **41**, it effectively distinguished between AChE from BChE. Therefore, it seems that both the nature of the substituents on the phosphorus atom and the presence of the ferrocene unit play a crucial role in determining the affinity constant and reactivity of these compounds (Rudolf et al., 2010).

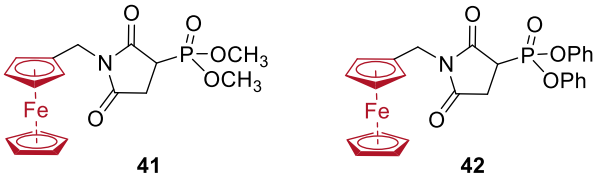
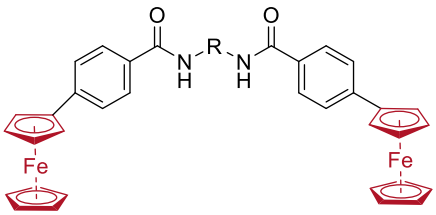
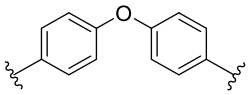
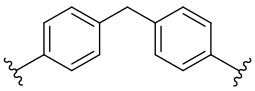
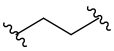
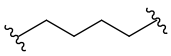
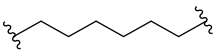
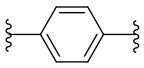


Fig. 6 Structures of ferrocenyl phosphonate derivatives **41** and **42**

Ferrocenyl amides are particularly interesting due to their ability to form supramolecular structures through non-covalent interactions, such as hydrogen bonding. Specifically, the oxidation of ferrocene to the ferrocenium ion results in the withdrawal of electron density from the neighboring N-H bond in the amide group, enhancing its capacity as a proton donor. Therefore, Waseem Abbasi et al. (2020) synthesized a series of symmetrical ferrocenyl diamides (**43**-**48**) and ferrocenyl amides (**49**-**55**) along with their phenyl analogs and assessed their anti-cholinesterase activity at a concentration of 200 ppm (see Tables 4 and 5).

Table 4 Inhibitory effect of symmetrical ferrocenyl diamides **43**-**48** on AChE and BChE at a concentration of 200 ppm

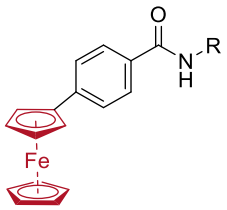


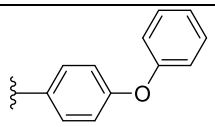
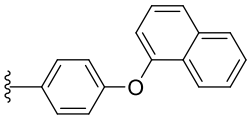
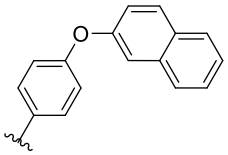
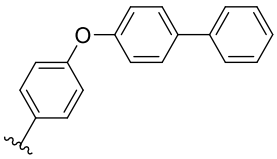
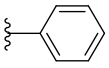
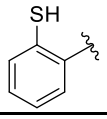
Designation	R	% of inhibition	
		AChE	BChE
43		0	5.2
44		0	2.2
45		0	11.7
46		0	8.1
47		0	1.3
48		0	2.2
Galantamine		95	91.5

All tested compounds exhibited slight to moderate reductions in enzyme activity. The phenyl analogs were identified as more effective inhibitors of AChE, while the ferrocenyl

amides demonstrated slightly better inhibitory effects on BChE. Among the ferrocene derivatives, the most significant reductions in AChE activity were noted for amides **50** (11.7%) and **52** (12.6%), while diamide **45** exhibited the highest inhibition of BChE (11.7%). In comparison, the positive control galantamine nearly completely inhibited both enzymes, achieving over 91% inhibition at the same concentration.

Table 5 Inhibitory effect of symmetrical ferrocenyl amides **49**-**55** on AChE and BChE at a concentration of 200 ppm

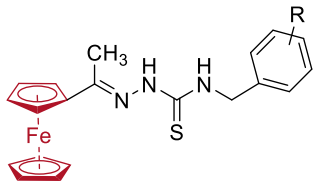


Designation	R	% of inhibition	
		AChE	BChE
49		0	2.1
50		11.7	3.4
51		0.6	3.4
52		12.6	3.9
53		0	9
54		0	2.3
55		3.4	10.5

The effect of ferrocenyl thiosemicarbazones and their complexes with divalent metal ions, specifically copper(II) and cobalt(II), on the activity of AChE and BChE was also

investigated (Jawaria et al., 2020). All synthesized ferrocenyl thiosemicarbazone ligands exhibited moderate anti-AChE effect (see Table 6). The most potent were **64** (IC_{50} = 64 μM) and **66** (IC_{50} = 54 μM), both containing a chlorine atom in the *ortho* and *para* positions on the aromatic ring, respectively. In contrast, the least active thiosemicarbasone, **56**, lacked substituents on the benzene ring, suggesting that the introduction of substituents positively influences activity. Furthermore, it was noted that most ligands with a substituent in the *para* position exhibited better inhibitory effects compared to their regioisomers. Overall, the ligands displayed slightly weaker inhibition of BChE compared to AChE. The most active thiosemicarbazone was **63** (IC_{50} = 64 μM), which features a fluorine atom in the *para* position. A similar level of activity was noted for the already mentioned derivative **66** (IC_{50} = 68 μM), which contains another halogen, chlorine atom, in the *para* position.

Table 6 Inhibitory effect ferrocenyl thiosemicarbazones **56**-**66** and their complexes on AChE and BChE



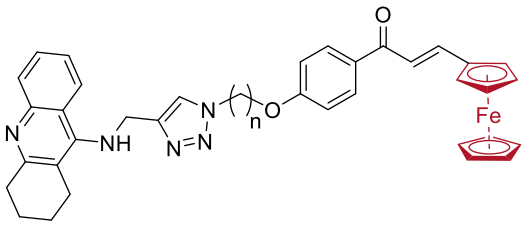
Designation	R	IC_{50} [μM]					
		AChE			BChE		
		ligand	Co^{2+} complex	Co^{2+} complex	ligand	Co^{2+} complex	Co^{2+} complex
56	H	> 250	9.2	29	> 250	13	57
57	2-CH_3	75	9.5	30	99	19	54
58	3-CH_3	95	11	n.d.[a]	> 250	18	n.d.
59	3-OCH_3	88	88	n.d.	> 250	32	n.d.
60	4-OCH_3	97	24	26	98	30	39
61	2-F	115	20	n.d.	99	22	n.d.
62	3-F	113	25	24	75	27	67
63	4-F	71	16	n.d.	64	28	n.d.
64	2-Cl	64	22	n.d.	> 250	12	n.d.
65	3-Cl	95	13	n.d.	> 250	22	n.d.
66	4-Cl	54	20	23	68	20	73
Eserine		0.04			0.85		

[a] IC_{50} value was not determined.

There was a significant increase in activity against both enzymes following the coordination of these thiosemicarbazone ligands with Cu^{2+} and Co^{2+} ions. Except for the complex involving ligand **59**, all other Cu(II) complexes demonstrated notably enhanced activity. Interestingly, the complex formed by coordinating the least active ligand **56** with the Cu^{2+} ion exhibited the strongest inhibitory effects against both enzymes among all tested compounds (IC_{50} = 9.2 and 13 μM, respectively). The Co(II) complexes showed slightly higher IC_{50} values compared to the analogous Cu(II) complexes. *In silico* studies further supported the structure-activity relationships of these compounds (Jawaria et al., 2020).

In recent years, various chalcones and tacrine derivatives have demonstrated inhibitory effects on cholinesterases. Consequently, two series of aryl and ferrocenyl tacrine-chalcone hybrids were synthesized, connecting these two pharmacophores via a 1*H*-1,2,3-triazole ring. Their *in vitro* anti-AChE activity was subsequently evaluated (Rani et al., 2021). The general formula for the ferrocenyl derivatives, along with their IC_{50} values and the IC_{50} value of the lead compound tacrine (used as the positive control), are presented in Table 7.

Table 7 Inhibitory effect of ferrocenyl tacrine-chalcone hybrids **67**-**72** on AChE



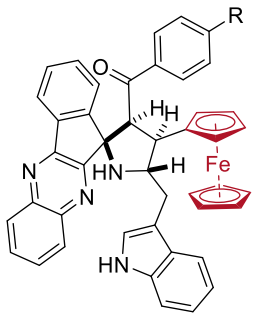
Designation	n	IC_{50} [μM]
67	2	0.73
68	3	n.d.[a]
69	4	0.67
70	5	0.93
71	6	0.92
72	8	0.33
Tacrine		0.38

[a] IC_{50} value was not determined.

In vitro screening revealed that among the ferrocenyl derivatives, only compound **72** (n = 8) demonstrated superior inhibitory activity against AChE (IC_{50} = 0.33 μM) compared to tacrine. The other analogs displayed higher IC_{50} values than tacrine, and overall, the activity decreased as the number of methylene groups in the linker increased from 2 to 6. Besides **72**, two aryl derivatives (n = 4 and n = 5), with a chlorine atom in the *para* position, also exhibited strong inhibitory effects. The anti-BChE activity of these three compounds was assessed as well, with tacrine serving as the standard inhibitor (IC_{50} = 0.21 μM). Ferrocene containing derivative **72**, at a concentration of 10 μM, did not reduce enzyme activity by 50%, indicating that it acts as a potent and selective inhibitor of AChE. Additionally, none of these three compounds showed acute toxicity (Rani et al., 2021).

In a very recent study, Almansour and coworkers (2024) synthesized a series of spiro compounds that, in addition to ferrocene, incorporate three privileged structures: pyrrolidine, quinoxaline, and indole. These structurally intriguing bicyclic compounds demonstrated notable anti-cholinesterase activity, which is summarized in Table 8.

Table 8 Inhibitory effect of spiro ferrocene derivatives **73**-**78** on AChE and BChE



Designation	R	IC_{50} [μM]	
		AChE	BChE
73	H	24	28
74	Br	9.5	22
75	Cl	8.8	20
76	F	5.1	21
77	CH_3	23	29
78	4-OCH_3	26	30
Galantamine		2.1	19

Most of these spiro derivatives were found to be effective and selective inhibitors of AChE compared to BChE, with selectivity indexes (SI) ranging from 1.15 to 4.15. In this context, three compounds (**74**, **75**, and **76**) distinguished themselves due to the presence of halogen atoms (Br, Cl, and F) in their structures. These spiro compounds inhibited AChE with IC_{50} values below 10 μM. The most active was the fluorine-containing derivative (IC_{50} = 5.1 μM), whose efficacy was comparable to that of the positive control, galantamine (IC_{50} = 2.1 μM). This derivative also displayed the highest selectivity for AChE. In contrast, derivative **78**, which contains a methoxy group, was the least active against AChE (IC_{50} = 26 μM). All derivatives exhibited very similar inhibitory effects on BChE activity (IC_{50} in the range from 20 to 30 μM; Almansour et al., 2024).

5. Prophylaxis Against Nerve Agent Poisoning

Certain ferrocene derivatives have been identified as potential prophylactic agents against acute poisoning from the nerve agent soman. Karlsson et al. (1984) initially explored the efficacy of ferrocenyl carbamates **79** and **80** as antidotes (Fig. 7), discovering that only derivative **80** demonstrated significant efficacy. Pretreatment of experimental mice with **80** at a dose of 5.5 mg/kg (representing 1/30 of its LD_{50} value) led to a sixfold reduction in soman toxicity. This ferrocene carbamate acted as a strong and reversible *in vivo* inhibitor of AChE; at the same dose, it led to a 30% decrease in AChE activity in mice 20–30 minutes post-administration, without exhibiting any toxic effects. Its high lipophilicity,

attributed to the presence of the ferrocene unit, likely facilitates its ability to easily cross the blood-brain barrier.

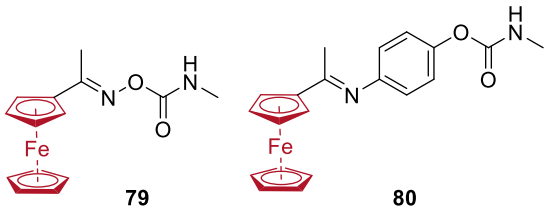


Fig. 7 Structure of ferrocenyl carbamates **79** and **80**

Karlsson and coworkers (1992) further investigated ferrocene carbamate **80** as a potential antidote for soman, this time using guinea pigs. While this ferrocene carbamate proved to be less effective than physostigmine in terms of *in vivo* inhibition of cholinesterase activity in the blood and brain, it offered better protection against soman poisoning.

6. CONCLUSION

Although research in this area commenced in 1969, the inhibitory effects of ferrocene derivatives on cholinesterase have not been as thoroughly investigated as their antimalarial and antiproliferative activities. So far, around 100 structurally diverse ferrocene-containing compounds have been evaluated for their inhibitory activity against AChE and/or BChE. Some of them exhibited promising activity in the low micromolar or submicromolar range. The most active derivatives were obtained by performing bioisosteric replacement of the aryl group with a ferrocene unit in known inhibitors, or by developing hybrids that combine ferrocene with established inhibitors. It's encouraging to see that research in this field has intensified over the past five years, promising significant advancements ahead.

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DERIVATI FEROCENA KAO INHIBITORI HOLINESTERAZA

Inhibicija enzima acetilholinesteraze (AChE) i butirilholinesteraze (BChE) predstavlja najprihvaćeniju strategiju lečenja Alchajmerove bolesti, koju karakteriše nedostak neurotransmitera acetilholina u mozgu. Inhibitorni efekti derivata ferocena na pomenute holinesteraze nisu proučavani u tolikoj meri kao njihova antimalarijska i antiproliferativna svojstva. Ovaj kratki pregledni rad detaljno opisuje napredak u ovoj oblasti od 1962. godine, kada je prvi derivat ferocena proučavan u ovom kontekstu. Do danas je inhibitorna aktivnost prema AChE i/ili BChE određena za približno 100 strukturno različitih jedinjenja koja sadrže ferocen. Neka od ovih jedinjenja su pokazala obećavajuću aktivnost i to u niskom mikromolarnom ili submikromolarnom opsegu koncentracija. Najaktivniji derivati su dobijeni bioizosternom zamenom aril-grupe ferocenom u već poznatim inhibitorima ili kroz razvoj hibrida koji povezuju ferocen sa poznatim inhibitorima. Ohrabrujuće je što je istraživanje u ovoj oblasti intenzivirano tokom proteklih pet godina, što obećava značajan napredak u bliskoj budućnosti.

Ključne reči: *ferocen, acetilholinesteraza, butirilholinesteraza, inhibitor, Alchajmerova bolest, Parkinsonova bolest*