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SYNTHESIS OF NOVEL SCHIFF BASE DERIVATIVES OF TACRINE AND INVESTIGATION OF THEIR ACETYLCHOLINESTERASE INHIBITION POTENCY

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Investigation of acetylcholinesterase (AChE) inhibition potency of some new Schiff base derivatives of tacrine (9-amino-1,2,3,4-tetrahydroacridine) was reported in this paper. Novel Schiff base derivatives of tacrine (**3a**–**g**) have been synthesized, and they have been characterized by several methods (FT-IR, ^{1}H-NMR, ^{13}C-NMR, etc.). Then, inhibition effects on AChE by the synthesized compounds have been investigated by the spectrophotometric Ellman method. IC_{50}, K_i, K_M and V_{max} values and inhibition types have been determined. It was seen that all compounds had the property of a water-soluble reversible AChE inhibitor. Structure **3a** was found to be the most potent inhibitor, with the IC_{50} value of 22.1 ± 1.11 nM (tacrine's IC_{50} value was calculated as 34.1 nM).

Key words: inhibition; acetylcholinesterase; tacrine; Schiff base; inhibition type

СИНТЕЗА НА НОВИ ДЕРИВАТИ НА ШИФОВИ БАЗИ НА ТАКРИН И ИСПИТУВАЊЕ НА НИВНАТА СПОСОБНОСТ ЗА ИНХИБИЦИЈА НА АЦЕТИЛХОЛИНЕСТЕРАЗА

Во овој труд е опишано испитувањето на способноста за инхибиција на холинестеразата (AChE) на некои деривати на Шифови бази на такрин (9-амино-1,2,3,4-тетрахидроакридин). Синтетизирани се нови деривати на такрин (**3a**–**g**) кои се карактеризирани со неколку методи (FT-IR, ^{1}H-NMR, ^{13}C-NMR, etc.). Испитани се инхибициските ефекти на синтетизираните соединенија врз AChE со спектроскопскиот метод на Ellman. Определени се вредностите на IC_{50}, K_i, K_M и V_{max}, како и типот на инхибиција. Утврдено е дека сите соединенија имаат својство на во вода растворлив реверзибилен инхибитор на AChE. Најдено е дека **3a** е најмоќен инхибитор со вредност на IC_{50} од 22.1 ± 1.11 nM (пресметано е дека вредноста IC_{50} на такрин изнесува 34.1 nM).

Клучни зборови: инхибиција; ацетилхолинестераза; Шифови бази; тип на инхибиција

1. INTRODUCTION

Alzheimer's disease (AD) is a chronic neurodegenerative disease which is characterized by memory loss, difficulty in speaking, problems with communication and reasoning [1–3]. There are many potential causes for the emergence of this disease, such as genetic factors, autoimmune reactions, and protein plaques and tangles [4, 5]. One promising explanation is the cholinergic hypothesis, that AD is caused by reduced synthesis of the neurotransmitter acetylcholine (ACh) [1]. ACh ($CH_3COOCH_2CH_2N^+(CH_3)_3$) is an alkaloid which was the first discovered neurotransmitter. It is an ester of choline and acetic acid [6, 7]. It has a great biological importance. This ester is responsible for carrying nerve impulses. It is associated with dementia, AD, and Parkinson's disease [7].

The primary function of acetylcholinesterase (AChE) is terminating cholinergic neurotransmission by rapid splitting of Ach [6]. Increasing the level of ACh with AChE inhibitors is an efficient strategy for AD therapies [8, 9]. Many inhibitors such as tacrine, donepezil, and physostigmine are used as drugs for AD treatment (Fig. 1) [10]. AChE inhibitors have the potential to alleviate neurodegenerative diseases and dementia [11].

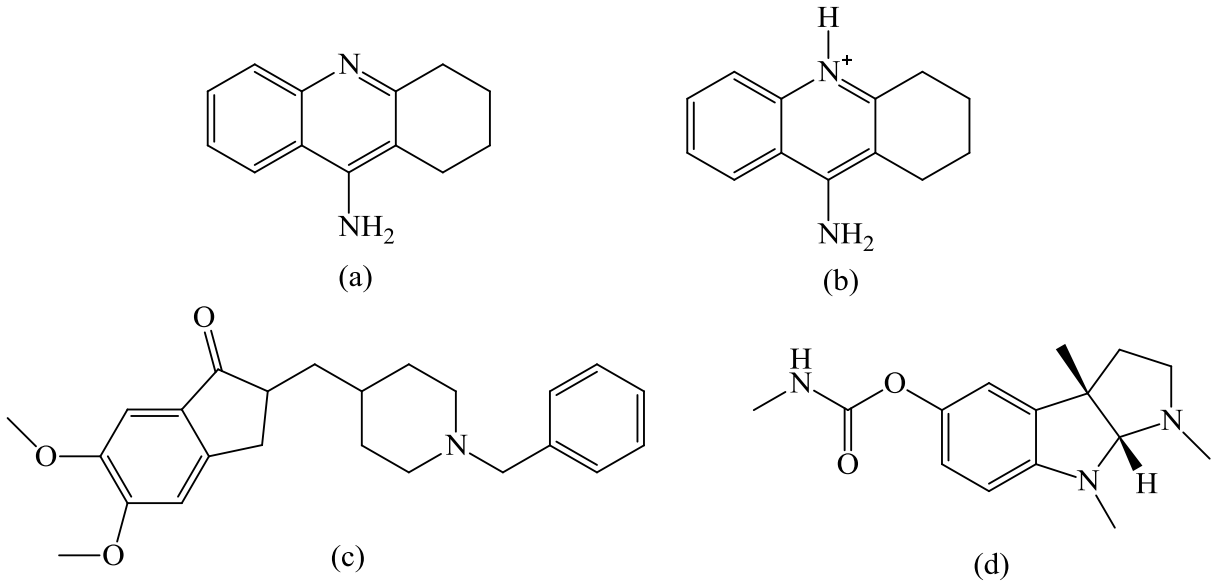


Fig. 1. (a) Tacrine. **(b)** Tacrine with protonated acridine nitrogen [12]. **(c)** Donepezil. **(d)** Physostigmine.

AChE (E.C. 3.1.1.7) is a hydrolase that hydrolyzes neurotransmitter ACh. AChE's active site comprises 2 subsites, the esteratic site and the anionic site [6]. The catalytic triad within the esteratic site, with amino acid positions, for electric eel AChE is follows: Ser 200, His 440 and Glu 327 [13–15]. The anionic site is composed of the aromatic amino acids Trp 86, Tyr 337 and Phe 338 for murine AChE [13, 16] or Trp 84, Tyr 121 and Phe 330 for electric eel AChE [13, 17, 18].

Tacrine (9-amino-1,2,3,4-tetrahydroacridine) is a reversible AChE inhibitor, and it was the first cholinesterase inhibitor approved by US FDA (Food and Drug Administration) for the treatment of AD [19–21]. It is known, as a result of investigation of X-ray crystal structure of tacrine-AChE complex that a hydrogen bond occurs between the backbone carbonyl oxygen of His 440 in the catalytic triad and the hydrogen of the protonated acridine nitrogen of tacrine (Fig. 1) [12, 22]. It is also known that there is π-π stacking of two aromatic rings of tacrine between indol ring of Trp 84 and phenyl ring of Phe 330 [12, 22].

The synthesis of different AChE inhibitor derivatives or hybrid molecules has attracted attention in recent years (for example, piperidine derivatives [23], tetrazole derivatives [3], hydroxyquinoline derivatives [24], isoquinolines [25], carbamate derivatives [26], β-lactam analogs and Schiff bases [from 2-naphtaldehyde and substituted aniline derivatives] [27], and coumarin derivatives [28]). Because tacrine is a well-known cholinesterase inhibitor for the treatment of AD, researchers have focused on the development of more active and selective ligands than unmodified tacrine. As of 2019, many tacrine derivatives have been synthesized, their AChE inhibition effects have been investigated, and most have been found more active than tacrine. For example, tacrine–melatonin hybrids [29, 30], tacrine–ferulic acid hybrids [31, 32], tacrine–lipoic acid [33] and tacrine–curcumin hybrids [34] have been investigated.

In this study, we report synthesis, characterization and AChE inhibition properties of new Schiff base derivatives of tacrine. Schiff base derivatives of tacrine (**3a-g**) were synthesized with different aldehydes (salicylaldehyde and its derivatives, **2a-g**) (Fig. 2). Then, inhibition effects on AChE of the synthesized compounds were investigated, and IC_{50} and K_i values were determined. Inhibition types of these inhibitors have been determined also.

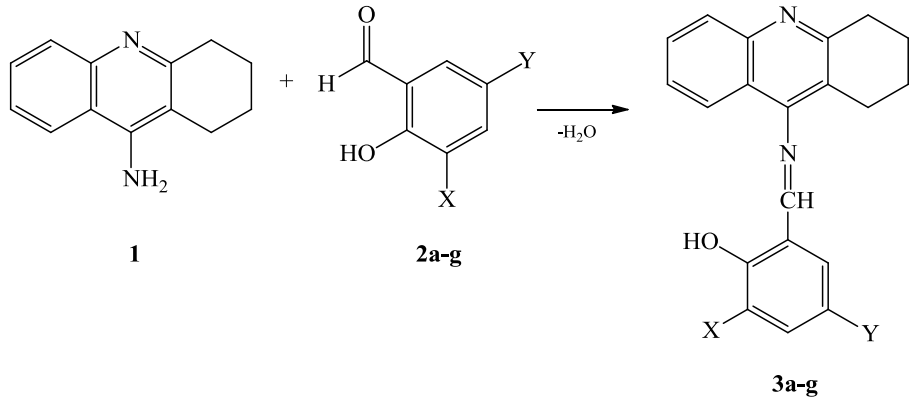


Fig. 2. General reaction scheme.
1 tacrine, **2a** 5-fluoro-3-methylsalicylaldehyde, **2b** 2-hydroxy-5-methylbenzaldehyde, **2c** 3-chloro-5-fluorosalicylaldehyde, **2d** 5-bromosalicylaldehyde, **2e** 5-chlorosalicylaldehyde, **2f** 5-fluorosalicylaldehyde, **2g** salicylaldehyde. **3a** X: -CH_3, Y: -F; **3b** X: -H, Y: -CH_3; **3c** X: -Cl, Y: -F; **3d** X: -H, Y: -Br; **3e** X: -H, Y: -Cl; **3f** X: -H, Y: -F; **3g** X: -H, Y: -H.

2. EXPERIMENTAL

2.1. *Materials and methods*

All organic solvents used in this study were purified according to standard methods. The aldehydes (salicylaldehyde, 5-bromosalicylaldehyde, 5-chlorosalicylaldehyde, 5-fluorosalicylaldehyde, 5-fluoro-3-methylsalicylaldehyde, 3-chloro-5-fluorosalicylaldehyde, 2-hydroxy-5-methylbenzaldehyde), tacrine hydrochloride (9-amino-1,2,3,4-tetrahydroacridine hydrochloride hydrate), acetylcholinesterase (E.C. 3.1.1.7, purified from *Electrophorus electricus* [electric eel] Type V-S, activity of 100 unit/ml) acetylthiocholine iodide (ATCh), and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB, Ellman's reagent) were purchased from Sigma-Aldrich. Elemental analysis for C, H, and N was carried out on a LECO 932 elemental analyzer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded employing a Bruker Ultrashield AC300 Plus 300 MHz spectrometer, with DMSO-d_6 as solvent. Chemical shifts (δ) are given in ppm relative to TMS. IR spectra were recorded on a Perkin Elmer Spectrum Two FT-IR spectrometer. Thermogravimetric analysis (TGA) was performed on a Seiko 6300 Model Thermal Analyzer in the temperature range of 0–900 ºC under nitrogen atmosphere. Melting points were determined with a Gallenkamp melting point apparatus.

2.2. *Synthesis of Schiff base derivatives of tacrine (general method)*

Schiff base derivatives were synthesized by following a general method (except **3e**). Schiff base derivatives (except **3e**) were prepared by reacting of tacrine ($1.0\cdot10^{-3}$ mol) in hot methanol (10.0 ml) with **2a-g (**$1.0\cdot10^{-3}$ mol, except **2e**) in methanol (10.0 ml) and stirring for 4 h under a reflux condenser at 70 ºC. Thus tacrine-Schiff bases were obtained (Fig. 2). After the mixtures cooled to room temperature, tacrine-Schiff base solutions were concentrated through evaporation, to half their original volumes. After storing the solutions for 2 days, the solid complexes formed were collected by filtration and then dried in a desiccator over $CaCl_2$. Compound **3e** was prepared by reacting of tacrine ($1.0\cdot10^{-3}$ mol) in hot DMSO (2.5 ml) with **2e** (5-chlorosalicylaldehyde, $1.0\cdot10^{-3}$ mol) in DMSO (10.0 ml) and stirring for 4 h under a reflux condenser at 70 ºC. After the mixture cooled to room temperature, **3e** was collected by filtration, washed with cold ethanol, and recrystallized from ethanol.

2.3. *AChE activity assay*

AChE activity measurements were performed at 30 ºC, according to the spectrophotometric assay of Ellman [35]. ATCh was used as substrate for all experiments. The reaction took place in a final volume of 3.0 ml of phosphate-buffered solution, pH 8.0, containing AChE (25.0 µl, 5.0 unit/ml), DTNB (50.0 µl, 0.01 M), ATCh (39.5 µl, $7.6\cdot10^{-3}$ M), and inhibitor solution (varying from $5.0\cdot10^{-6}$ to $2.5\cdot10^{-10}$ M), to produce the yellow anion of 5-thio-2-nitrobenzoic acid (Fig. 3). After 30 minutes' incubation, the absorbance of the mixture was measured with the spectrophotometer, at 412 nm. One sample without inhibitor was always present to yield the 100 % of AChE activity. The IC_{50} and K_i values were calculated with GraphPad Prism 6 (GraphPad Software). Inhibition types were determined in the absence and presence of inhibitor ($1.0\cdot10^{-7}$, $5.0\cdot10^{-8}$ and $1.0\cdot10^{-8}$ M) from Lineweaver–Burk plots. Each determination was repeated three times and the average values were reported.

3. RESULTS AND DISCUSSION

3.1. *Chemistry*

The route for the synthesis of Schiff base derivatives (**3a-g**) is illustrated in Figure 2. The reaction of tacrine (**1**) and different aldehydes (**2a-g**) in refluxing methanol (DMSO for **3e**) at 70 ºC gave new Schiff base derivatives (**3a-g**).

Analytical data and some of the physical properties of the Schiff base derivatives of tacrine are summarized in Table 1.

T a b l e 1

Analytical data of Schiff base derivatives of tacrine

	Compounds	Empiric formula (Formula weight, g/mol; obtained amount, g; yield %)	Analysis % Found (calcd)				M.p. (ºC) Colour
			C	H	N	S	
3a	(*Z*)-4-fluoro-2-methyl-6-(((1,2,3,4-tetrahydroacridin-9-yl)imino)methyl)phenol	$C_{21}H_{19}N_2FO{\cdot}2H_2O$ (370; 0.158; 43)	68.15 (68.04)	6.52 (6.21)	7.13 (7.56)	–	215–218 Straw yellow
3b	(*Z*)-4-methyl-2-(((1,2,3,4-tetrahydroacridin-9-yl)imino)methyl)phenol	$C_{21}H_{20}N_2O{\cdot}2H_2O$ (352; 0.135; 39)	71.84 (71.59)	6.79 (6.82)	7.63 (7.95)	–	160–162 Fawn
3c	(*Z*)-2-chloro-4-fluoro-6-(((1,2,3,4-tetrahydroacridin-9-yl)imino)methyl)phenol	$C_{20}H_{16}N_2ClFO{\cdot}2H_2O$ (390.5; 0.20; 52)	62.09 (61.56)	4.82 (5.12)	6.74 (7.17)	–	201–203 Yellow
3d	(*Z*)-4-bromo-2-(((1,2,3,4-tetrahydroacridin-9-yl)imino)methyl)phenol	$C_{20}H_{17}N_2BrO{\cdot}H_2O$ (399; 0.181; 47)	59.75 (60.15)	4.38 (4.76)	6.93 (7.02)	–	209–211 Straw yellow
3e	(*Z*)-4-chloro-2-(((1,2,3,4-tetrahydroacridin-9-yl)imino)methyl)phenol	$C_{20}H_{17}N_2ClO.0.5$ DMSO$\cdot 4H_2O$ (447.5; 0.242; 55)	52.16 (51.74)	5.59 (5.04)	6.62 (7.10)	3.58 (3.57)	225–227 Brown
3f	(*Z*)-4-fluoro-2-(((1,2,3,4-tetrahydroacridin-9-yl)imino)methyl)phenol	$C_{20}H_{17}N_2FO{\cdot}1{\cdot}5H_2O$ (347; 0.133; 39)	68.55 (69.00)	5.98 (5.76)	8.53 (8.07)	–	219–220 White
3g	(*Z*)-2-(((1,2,3,4-tetrahydroacridin-9-yl)imino)methyl)phenol	$C_{20}H_{18}N_2O{\cdot}4H_2O$ (374; 0.15; 41)	64.17 (64.25)	4.81 (5.25)	4.42 (4.01)	–	165–168 Brown

Thermal data of derivatized tacrine molecules are given in Table 2. As seen in Table 2, decomposition temperatures (T_i, T_{max}, and T_f) of the molecules are different from each other. All molecules exhibit a one-step weight change in the range of 243–370 ºC. The **3a-g** compounds are thermally stable up to 70, 70, 90, 85, 100, 70 and 108 ºC, respectively. In the decomposition process of the **3a-g** molecules, the mass losses corresponded to absorption H_2O or DMSO leaving in the first stages of the decomposition.

Characteristic IR spectral data of the Schiff bases are given at Table 3. It was concluded from TGA analysis that crystal water was found in all synthesized Schiff bases. The peaks that were observed in the range 3136–3181 cm^{-1} may be attributed to the ν(O–H), ν(H_2O) or ν(*aryl*–N–H^+) (Fig. 1). The aromatic –C–H stretching bands of all Schiff bases were predicted to be in the range 2933–2952 cm^{-1}, and the *cyclo*–C–H stretching bands of all Schiff bases were predicted to be in the range 2858–2871 cm^{-1}. The observation of bands in the range 1653–1659 cm^{-1} may be attributed to the ν(–CH=N–). The observation of bands at 1133, 1179 and 1032 cm^{-1}; 768 and 700 cm^{-1}; 592 cm^{-1}; and 784 and 705 cm^{-1} may be attributed to the ν(C–Z), where Z = F, Cl, Br and Me, respectively.

T a b l e 2

Thermal data of Schiff base derivatives of tacrine

Compound	$T_{crystalwater}$	T_i	T_{max}	T_f
3a	70–125	308	325	358
3b	70–113	250	305	318
3c	90–160	275	310	338
3d	85–138	318	345	370
3e	100–258*	305	325	365
3f	70–130	313	338	363
3g	108–110	243	273	305

T_i: Initial degradation temperature (ºC).
T_{max}: Maximum degradation temperature (ºC).
T_f: Final degradation temperature (ºC).
*This derivative has both crystal water and DMSO.

T a b l e 3

Important IR vibration frequencies (cm^{-1}) of Schiff bases

Compound	ν_{NH2}	$\nu_{-CH=N}$	$\nu_{O-H,\ H2O,\ aryl-N-H+}$	ν_{C-H} (*aromatic*) ν_{C-H} (*cyclo*)	ν_{C-Z}
Tacrine	3333 3175	–	–	2933 2871	–
3a	–	1654	3181	2952 2865	1032 705
3b	–	1653	3149	2945 2858	784
3c	–	1657	3149	2945 2861	700 1179
3d	–	1653	3174	2945 2874	592
3e	–	1630	3131	2943 2870	768
3f	–	1659	3156	2945 2881	1133
3g	–	1657	3172	2934 2865	–

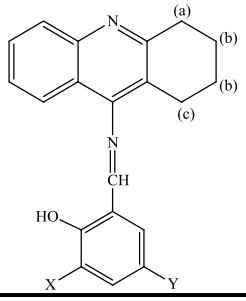
The ^{1}H-NMR data of the Schiff bases are presented in Table 4. In general, the duplets observed at 8.15–8.6 ppm were assigned to Schiff base –C<u>H</u>=N– proton. Multiplets observed at 2.4–3.8 ppm were assigned to –C<u>H</u>$_a$ and –C<u>H</u>$_c$ protons of tacrine's cyclo ring. Multiplets observed at 1.7–2.3 ppm were assigned to tacrine's –C<u>H</u>$_b$ protons. Multiplets at 7.4–8.8 ppm and 6.8–7.6 ppm were assigned to tacrine's and aldehydes' –C<u>H</u>$_{aryl}$ protons, respectively. Singlets observed at 10.2–14.0 ppm were assigned to –O<u>H</u> protons. The protons of –C<u>H</u>$_3$ of aldehyde moieties in the Schiff bases were also observed as expected.

The ^{13}C-NMR spectral data of the Schiff bases (Table 5) are also in accordance with the proposed structures. In general, the peaks observed at 155.16–162.548 ppm and 135.57–157.39 ppm were assigned to the aromatic ring carbons attached to the –CH=N– and –OH groups, respectively. Peaks corresponding to the –C–X and –C–Y residues of the Schiff bases were also observed as expected.

Table 4

^{1}H-NMR chemical shifts (ppm) of Schiff base derivatives of tacrine

Compounds	a / c	b	−C<u>H</u>(*aryl*) (Tacrine)	−C<u>H</u>(*aryl*) (Aldehyde)	−C<u>H</u>=N-	−O<u>H</u>	−X / −Y
3a	2.5–3.0 (m, 4H)	1.8–2.1 (m, 4H)	7.8–7.9 (m, 4H)	7.5–7.7 (m, 2H)	8.5 (d, 1H)	13.6 (s, 1H)	1.8–2.1 (m, 3H)
3b	3.0–3.5 (s, 4H)	1.7–1.9 (d, 4H)	7.8–7.9 (m, 4H)	7.5–7.6 (m, 3H)	8.5 (d, 1H)	13.6 (s, 1H)	2.4–2.7 (m, 3H)
3c	2.4–3.1 (m, 4H)	1.7–1.9 (m, 4H)	7.7–8.4 (m, 4H)	7.1–7.5 (m, 2H)	8.5 (d, 1H)	10.2 (s, 1H)	–
3d	2.5–3.0 (m, 4H)	1.7–2.0 (s, 4H)	7.8–8.0 (m, 4H)	7.5–7.6 (m, 3H)	8.55 (d, 1H)	14.0 (s, 1H)	–
3e	3.2–3.8 (m, 4H)	1.8–2.3 (m, 4H)	7.6–8.8 (m, 4H)	7.0–7.5 (m, 3H)	8.6 (d, 1H)	10.2 (s, 1H)	–
3f	2.5–3.0 (m, 4H)	1.8–2.0 (m, 4H)	7.8–8.0 (m, 4H)	7.5–7.6 (m, 3H)	8.5 (d, 1H)	13.9 (s, 1H)	–
3g	2.5–2.9 (m, 4H)	1.7–1.9 (m, 4H)	7.4–7.7 (m, 4H)	6.8–7.3 (m, 4H)	8.15 (d, 1H)	10.3 (s, 1H)	–



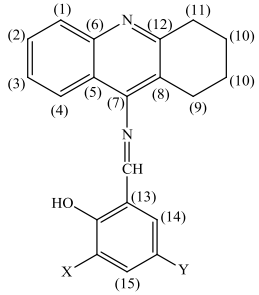
X: -H, -Cl, -CH_3

Y: -H, -Br, -Cl, -F, -CH_3

Table 5

^{13}C-NMR chemical (ppm) of Schiff base derivatives of tacrine

Compounds	$C_{1–5}$	$C_{6–9}$	C_{10} C_{10}[a]	C_{11}	$C_{12–15}$	−N=CH $C_{–OH}$	−C−X −CH_3	−C−Y −CH_3
3a	39.44–40.55	28.41–109.51	21.55 23.07	39.16	115.29–133.12	155.56 152.02	119.79 21.04	137.83 –
3b	39.43–40.54	28.19–133.30	21.50 23.02	39.15	115.20–137.49	155.82 151.80	119.46 –	109.52 20.96
3c	39.15–40.55	23.13–120.30	21.17 21.64	28.74	109.52–132.85	155.16 152.37	125.61 –	138.36 –
3d	39,14–40.54	23.04–115.19	20.96 21.51	28.19	123.91–137.55	155.71 151.75	119.46 –	109.43 –
3e	31.59–40.81	23.12–110.56	19.19 22.11	23.99	112.08–133.41	155.58 135.57	119.6 –	123.45 –
3f	39.15–40.55	23.03–109.51	20.96 21.50	28.19	115.20–133.28	155.81 151.79	119.45 –	137.49 –
3g	39.15–40.54	24.05–109.34	22.91 22.97	33.61	117.37–149.01	162.54 157.39	128.63 –	129.24 –



X: -H, -Cl, -CH_3

Y: -H, -Br, -Cl, -F, -CH_3

3.2. *AChE inhibitory activity and kinetic studies*

AChE activity of the synthesized compounds was investigated, and IC_{50}, K_i, K_M, and V_{max} values were calculated by the spectrophotometric Ellman method [35] (Fig. 3). IC_{50} (the molarity of inhibitor causing a 50 % decrease of enzyme activity, Fig. S1), K_i (inhibitor–enzyme dissociation constant), K_M (Michaelis–Menten constant), and V_{max} (maximum reaction velocity) values and inhibition types are given in Table 6. K_i values were calculated from Lineweaver–Burk graphs (Fig. S2). As seen in Table 6, all the compounds behave as inhibitors against AChE. It was seen that all compounds had the property of a water-soluble reversible AChE inhibitor. The inhibition potency of the compounds indicates an increasing inhibitory effect on AChE as follows: **3a** > **3b** > **3g** > **3c** > **3f** > **3d** > **3e**. When the inhibitory potency of compounds was compared with respective to K_i values, same ranking was observed (except **3e)** (Table 6). The best AChE inhibition potency was observed for **3a,** with IC_{50} value of 22.1 ± 1.11 nM, showing superior activity to that of tacrine reference drug. It was concluded that the synthesized compounds showed more activity than tacrine, because IC_{50} values of those compounds were lower than tacrine's IC_{50} value (34.1 nM) (except **3e**, because it was synthesized in DMSO, resulting in a structure crystallization, its increased molecular mass caused reduced water solubility, so its inhibition potency was reduced compared to other inhibitors in the series).

Luo and co-workers have reported that the hydroxyl group on the benzene ring of the molecule they synthesized, could form hydrogen bonds with residues in the binding site of cholinesterase [36, 37]. In our study, salicylaldehyde and its derivatives have a hydroxyl group on their benzene ring. So, it could be that this hydroxyl group is important for inhibitor activity.

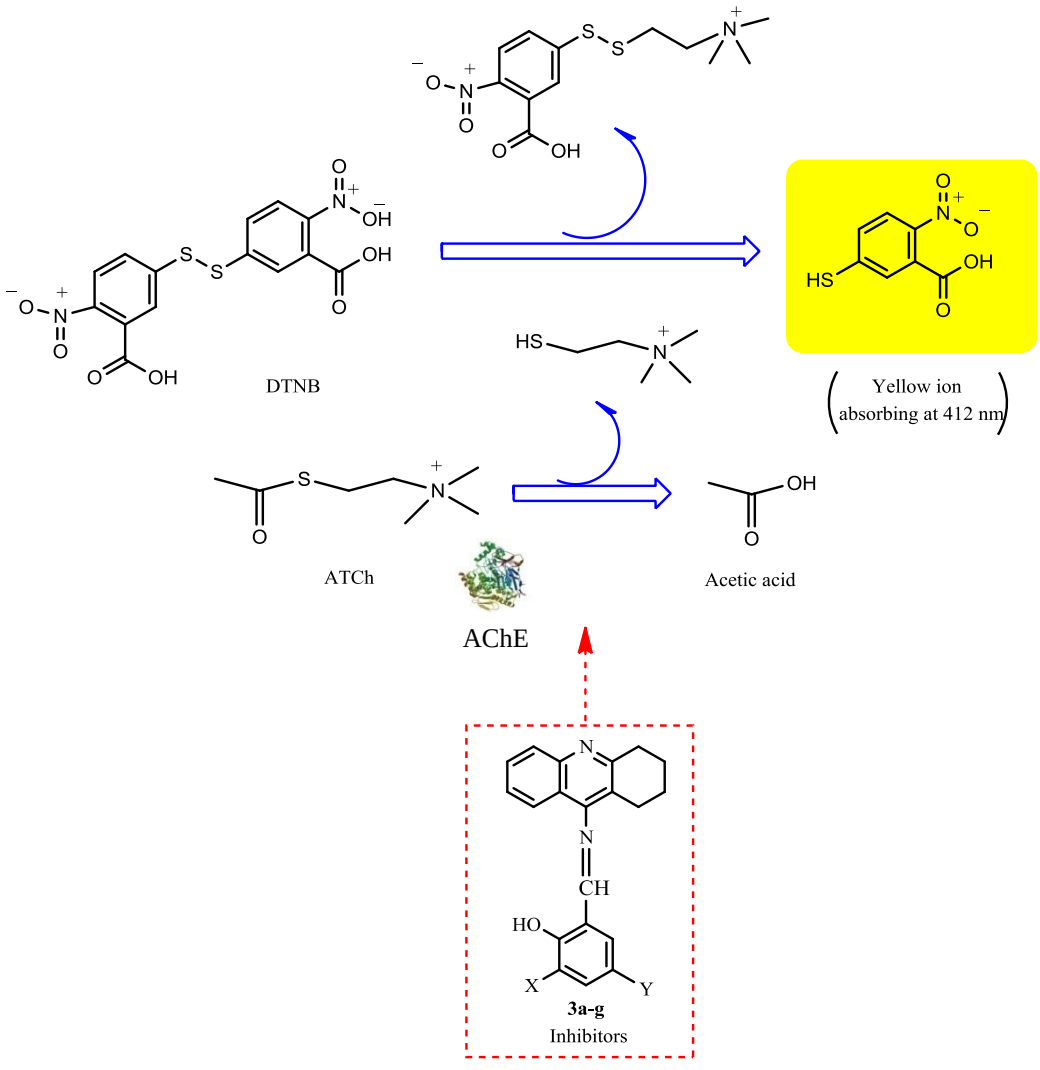


Fig. 3. AChE activity assay by Ellman method

T a b l e 6

The result of inhibition studies of tacrine derivatives on AChE

Compounds	IC_{50} (nM)	K_i (mM)	K_M (mM)	V_{max} (mM/min)	Inhibition type
3a	22.1 ± 1.11	2.14 ± 0.89	0.145 ± 0.023	0.066 ± 0.003	Mixed
3b	23.9 ± 1.10	3.77 ± 1.28	0.179 ± 0.015	0.106 ± 0.003	Mixed
3c	27.5 ± 1.10	13.6 ± 2.18	0.170 ± 0.035	0.103 ± 0.007	Noncompetitive
3d	32.1 ± 1.11	21.9 ± 2.38	0.159 ± 0.015	0.066 ± 0.002	Uncompetitive
3e	730.0 ± 1.52	14.86 ± 4.7	0.157 ± 0.057	0.059 ± 0.008	Uncompetitive
3f	30.5 ± 1.09	17.3 ± 1.34	0.202 ± 0.022	0.111 ± 0.005	Noncompetitive
3g	25.0 ± 1.32	10.2 ± 0.79	0.218 ± 0.020	0.110 ± 0.004	Uncompetitive

AChE inhibition activity is highly dependent on substituents X and Y in compounds **3a–g**. The most active compound, **3a,** has 3-methyl and 5-fluoro groups as substituents. It was seen that **3a** was 1.5-fold more active than tacrine.

When inhibitors containing only halogen substituents (X: **–**H, Y: **–**halogen substituent, **3f** < **3d** < **3e**, ranked according to IC_{50} values) were compared, no connection could be obtained between the electronegativity of substituents and inhibitor activity. The best inhibitor activity was obtained from inhibitor containing fluorine substituent (**3f)**. In the literature, an analogous result was seen in Mohsen's work [38]. When the most active inhibitors (**3a** and **3b**) were compared, the inhibitor containing -F substituent (**3a**) was more active than the inhibitor lacking -F (**3b**). When **3c** and **3e** inhibitors, containing -Cl substituents, were compared, results showed that inhibitor containing -F substituent (**3c**) was more active than inhibitor lacking -F (**3e**). It was evident from all these results that -F substituent increased the inhibitory activity.

The most active inhibitors' (**3a** and **3b**) inhibition types were determined as mixed-type. Accordingly, inhibitors can bind both free enzyme and enzyme-substrate complex, but their binding affinities are different [39].

When the series was compared within itself, the most active inhibitors were determined as **3a**. It includes a $-CH_3$ substituent. In the literature, the best inhibitor activity was seen for inhibitors containing an electron-donating $-CH_3$ substituent [40, 41].

4. CONCLUSIONS

In summary, novel tacrine derivatives have been synthesized, and their inhibitory activity against AChE enzyme was investigated. Characterizations of the synthesized compounds were made by using elemental analysis and spectroscopic methods. All the synthesized compounds behaved as inhibitors against AChE and had the property of a water-soluble reversible AChE inhibitor. These inhibitors showed more activity than tacrine (except **3e**). The greatest inhibition potency was obtained from **3a**. In the literature, there are a few studies of Schiff base derivatives of AChE inhibitors [27, 42, 43]. So, this work can contribute for synthesizing new Schiff base derivatives of AChE inhibitors and points a way to potential new therapies of AD.

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