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Extended Abstract to obtain the Master of Science Degree in Chemistry

Tacrine derivatives as potential multifunctional drugs against

Alzheimer's disease-physicochemical and biological properties

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Keywords

Alzheimer's disease

Acetylcholinesterase (AChE)

Tacrine

Beta amyloid (Aβ)

Anti oxidant activity

Metal chelation

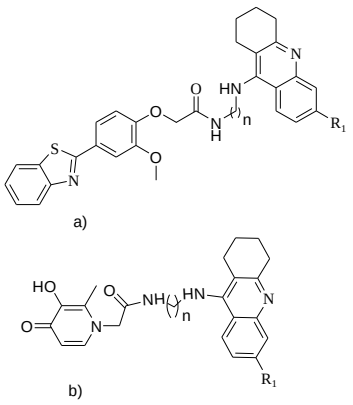
Abstract

Alzheimer's disease (AD) is a multifactorial age-dependent neurodegenerative disorder. The main hallmarks are the low levels of acetylcholine (ACh), responsible for memory loss, and the senile plaques, due to misfolding and aggregation of beta amyloid (Aβ), although disease progression is also enhanced by oxidative stress and metal (Fe, Cu, Zn) dyshomeostasis. Therefore, as multifunctional anti-AD drug candidates, two new series of tacrine derivatives were developed and evaluated, namely as inhibitors of AChE and self-induced Aβ aggregation along with the radical scavenging and metal chelation capacity. Compounds **TAC-BTA** (**RSC-1 to 6**) showed quite good AChE inhibitory capacity in the range of IC_{50} (0.04 - 0.27 μM), moderate self-induced Aβ aggregation inhibition (27-44%), but poor antioxidant activity. In case of **TAC-HP** hybrids (**TACHP-9 to 16**) are good AChE inhibitors with IC_{50} in the range of (0.64 – 1.71 μM); some compounds presented reasonably good radical scavenging capacity, EC_{50} (**TACHP-10**, 450 μM; **TACHP-12**, 399 μM; **TACHP-16,** 483 μM), but all these compounds had very high potency to inhibit self-induced Aβ aggregation i.e. in the range of (84-95%). Metal chelation studies, performed for **TACHP-12** by spectrophotometric and potentiometric techniques, showed that the chelating affinity depends on the metal ion (Fe>Cu>Zn) with pM values at the physiological pH (pFe = 21.7, pCu = 10.8, pZn = 6.9) confirming the good chelating capacity of the HP moiety. So due to their multifunctional ability, both series of tacrine derivatives appear as multi-potent agents, which could be selected for further investigation as drug candidates against AD.

1 INTRODUCTION

Alzheimer's disease (AD) is a chronic, irreversible and progressive age related disorder [1]. In the recent few years death rate due to AD have been increased by 66% in USA. Worldwide AD has become sixth leading cause of death [2]. Very initial symptom includes memory loss, dementia, change in mood and personality, trouble in understanding visual images, impaired judgment, disorientation, confusion etc [3]. Basically AD is characterised as a slow, progressive disorder which has no definite onset [3]. The hallmark of AD patient shows plenty of other features such as low levels of acetylcholine (ACh), due to acetylcholinesterase (AChE) which is responsible for the hydrolysis of acetylcholine in the synaptic clefts, neuritic plaques i.e. generated by proteolytic cleavage of amyloid precursor protein (APP), and later becomes responsible for the neuropathological features of AD [4]. Other leading factor considered responsible for the AD is the oxidative stress [5], in patient body and

brain, which results from an imbalance between the oxidant production and endogenous antioxidant capabilities of the body [3, 4]. Overproduction of reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, and also some nitrogen species, as nitric oxide and peroxynitrite, are responsible for the cell apoptosis and AD [6]. Besides this, some transition metals, such as iron, copper, aluminum and zinc, have been found in high concentrations in the brain [7], and are reported to change the normal functioning of brain and act as a catalyst for free radical formation reaction in the patient body and also responsible for the β-amyloid toxicity i.e. metal induced Aβ aggregation [8, 9]. So, all these factors imply that AD has a multifactorial nature and so it has been worldwide accepted that it can be better tackled through a complex multi-targeting pharmacological approach rather than through a single-target strategy [7, 10]. Taking into consideration the known capacity of tacrine for the inhibition of acetylcholinesterase (AChE), the multifactorial nature of AD, and also the important biological properties of benzothiazole [8, 11] and hydroxypyridinone [7, 12] molecular moieties, in present studies, two series of tacrine derivatives, i.e. tacrine-methoxyphenylbenzothiazole (TAC-BTA), and tacrine-hydroxypyridinones (TAC-HP) (see **Scheme1**.) have been synthesized and evaluated, through multiple techniques, for their most important physico-chemical and biological properties, in view of their assessment as potential drug candidates against AD.



Scheme 1. Representation of general structure of both series; a) Tacrine-benzothiazole (TAC-BTA) (n = 2, 3 and R_1 = H, Cl); b) Tacrine-hydroxypyridinone (TAC-HP) (n = 2, 3, 4 and R_1 = H, Cl)

2 EXPERIMENTAL

2.1 CHEMISTRY

2.1.1 EQUIPMENT/ REAGENT

The melting points were measured with a Leica Galen III hot stage apparatus. The ^{1}H and ^{13}C NMR spectra were recorded on Bruker AVANCE III spectrometers at 300 MHz and 400 MHz, respectively. Mass spectra (ESI-MS) were performed on a 500 MS LC Ion Trap (Varian Inc., Palo Alto, CA, USA) mass spectrometer. Analytical grade reagents were purchased from Sigma-Aldrich, Fluka and Acros and were used as supplied. Solvents were dried according to standard methods [13].The chemical reactions were monitored by TLC using alumina plates coated with silica gel 60 F254 (Merck). Column chromatography separations were performed on silica gel Merck 230-400 mesh (Geduran Si 60). Equipped with an ESI ion source, operated in the positive ion mode, elemental analyses were performed on a Fisons EA1108 CHNS/O instrument and were within the limit of ± 0.4%.

2.1.2 SYNTHESIS OF COMPOUNDS

3-(benzyloxy)-2-methyl-4*H*-pyran-4-one (2): To a solution of maltol **(1)** (30 g, 0.237 mol) in methanol (100 mL) was added NaOH solution (10.46 g in 30 mL of H_2O) drop wise with stirring. When mixture becomes clear solution then BnCl (26.99 g, 0.213 mol) was added drop wise over a period of 0.5 h and reaction mixture was heated for 12 h. The reaction mixture was cooled to room temperature, filtered to remove the inorganic salt, and the filtrate so obtained was concentrated under reduced pressure. The crude mixture was taken in CH_2Cl_2 (300 mL), and washed with 5% NaOH (2×100 mL) to remove the excess of maltol. The organic layer was washed with brine and dried over anhydrous sodium sulphate and finally evaporated to give the desired compound as an oily material with 82% yield; ^{1}H NMR (400 MHz, MeOD-d4), δ (ppm): 2.14 (s, 3H, CH_3), 5.10 (s, 2H, OCH_2Ph), 6.43 (d, 1H, *J* = 7.0 Hz, H-5, Py), 7.37–7.44 (m, 5H, Ph), 7.93 (d, 1H, *J* = 8.5 Hz, H-6, Py); ^{13}C NMR (400 MHz, MeOD-d4),δ (ppm): 13.68, 73.38, 116.14, 128.15, 128.79, 136.78, 143.46, 155.16, 161.21, 175.98; m/z (ESI MS): calculated for $C_{13}H_{12}O_3$ obtained 239.13 $(M + Na)^+$.

3-(benzyloxy)-2-methylpyridin-4(1*H*)-one (3): The solution of compound (**2**) (10 g) in ethanol (30 mL) was heated to reflux with 30 mL of 30% aqueous ammonia. After 4 h during the reflux course, an additional 10 mL of aqueous ammonia was added drop wise with the help of a dropping funnel. The reaction was monitored on TLC, and subjected to completion over a period of 18 h by adding more aqueous ammonia (additional aqueous ammonia is required to add due to its high volatile nature). On completion, the mixture was concentrated under reduced pressure to yield a light brown solid residue, which was recrystallized from ethanol/ether to afford white solid in 80% yield; m.p. 189-190 °C; ^{1}H NMR (400 MHz, MeOD-d4), δ (ppm): 2.08 (s, 3H, CH_3), 5.08 (s, 2H, OCH_2Ph), 6.47 (d, 1H, *J* = 7.1 Hz, H-5, Py), 7.31–7.40 (m, 5H, Ph), 7.54 (d, 1H, *J* = 8.0 Hz, H-6, Py); ^{13}C NMR (400 MHz, MeOD-d4), δ (ppm): 12.85, 72.97, 115.98, 127.91, 128.02, 128.72, 135.01, 137.29, 141.97, 144.82, 174.73; m/z (ESI MS): calculated for $C_{13}H_{13}NO_2$ obtained 216.05 $(M + H)^+$.

Ethyl 2-(3-(benzyloxy)-2-methyl-4-oxopyridin-1(4*H*)-yl) acetate (**4**): The mixture of 3-(benzyloxy)-2-methylpyridin-4(1H)-one (8 g, 37.2 mmol), K_2CO_3 (1 g, 7.3 mmol) in anhydrous DMF (10 mL), was treated with ethyl chloroacetate (1.2 g, 7.3 mmol) and the reaction mixture was stirred for 12 h at room temperature. Progress of reaction was analysed with the help of TLC. On completion, inorganic material was filtered and filterate so obtained was dried under reduced pressure to give a semisolid mixture, which was taken into ethyl acetate and washed with brine solution and water. Finally organic layer was dried over anhydrous sodium sulphate and then evaporated under reduced pressure. The solid material so obtained was washed with diethyl ether to give pure compound (**5)** in

63% yields; m.p. 110-112 °C; ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 1.05 (m, 3H,CH_3), 1.92 (s, 3H, CH_3), 4.00 (q, 2H, *J* = 7.0 Hz, CH_2CH_3), 4.76 (s, 2H, NCH_2), 4.89 (s, 2H, OCH_2Ph), 6.06 (d, 1H, *J* = 10.0 Hz, 5-HPy), 7.17–7.28 (m, 5H, Ph), 7.45 (d, 1H, *J* = 10.3 Hz, 6-HPy); ^{13}C NMR (400 MHz, DMSO-d6), δ (ppm): 12.42, 14.39, 54.24, 62.02, 72.43, 116.38, 128.31, 128.68, 128.89, 138.11, 140.97, 141.76, 145.50, 168.65, 172.89; m/z (ESI MS): calculated for $C_{17}H_{19}NO_4$ obtained 302.22 $(M + H)^+$.

2-(3-(benzyloxy)-2-methyl-4-oxopyridin-1(4H)-yl)acetic acid (**5**): To the solution of ethyl 2-(3-(benzyloxy)-2-methyl-4-oxopyridin-1(4H)-yl)acetate (1g) in methanol (20 mL), sodium hydroxide solution (1.05 eq, in 1 mL of water) was added and reaction was stirred at room temperature for 3-4 h. Completion of reaction was monitored on TLC. On completion, methanol was removed under reduced pressure to give a light brown solid. The solid material so obtained was then dissolved in 10 mL of ice cold water and acidified with conc. HCl (pH ≈ 1-2) to give a white solid, which was filtered and washed with 2 mL of water to give desired compound (6) with 80% yield; m.p. 186-188 °C; ^{1}H NMR (400, MHz, DMSO-d6), δ (ppm): 2.17 (s, 3H, CH_3), 4.97 (t, 2H, *J* = 8.0 Hz, CH_2N), 5.06 (s, 2H, OCH_2Ph), 6.59 (d, 1H, *J* = 8.5 Hz, 5-HPy), 7.34–7.44 (m, 5H, Ph), 7.86 (d, 1H, *J* = 10.0 Hz, 6-HPy); ^{13}C NMR (400, MHz, DMSO-d6), δ (ppm): 12.80, 55.29, 73.16, 128.51, 128.77, 128.95, 137.66, 141.88, 144.51, 144.71, 169.53, 170.40; m/z (ESI MS): calculated for $C_{15}H_{15}NO_4$ obtained 274.15 $(M + H)^+$.

6-13 got already synthesized in the lab by colleague

General procedure for synthesis of 2-(3-(benzyloxy)-2-methyl-4-oxopyridin-1(4*H*)-yl)-*N*-(3-((1,2,3,4-tetrahydroacridin-9-yl)amino)alkyl)acetamide (**14-21**):

To the mixture of 2-(3-(benzyloxy)-2-methyl-4-oxopyridin-1(4H)-yl) acetic acid (**5**) (0.5 g, 1.83 mmol) in 25 ml of dry DCM, N-methylmorpholine (3.66 mmol) was added under nitrogen. Few minutes later when reaction become clear solution propylphosphonic anhydride (T3P) solution (2.2 mmol) was added drop wise under nitrogen and reaction was stirred for half an hour. Finally, solution of different amines (**6-13**) (1.83 mmol) in dry DCM was added to it under nitrogen atmosphere and reaction is stirred at RT for overnight completion of reaction mixture was monitored with TLC. On completion of reaction DCM layer was washed with brine solution and crude reaction mixture was purified through column chromatography over silica in 3-5% MeOH-DCM system to give desired compounds (**14-21**) in 70-89% yield.

Compound (**14**) with 77% yield has following characterisation; m.p. 146-148 °C; ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 1.79 (br.s, 4H, H-2 & H-3), 1.94 (s, 3H, CH_3), 2.65 (br.s, 2H, H-1), 3.02 (br.s, 2H, H-4), 3.51-3.52 (m, 2H, H-2′), 3.99-4.01 (m, 2H, H-1′), 4.68 (s, 2H, NCH_2), 4.98 (s, 2H, OCH_2Ph), 6.98 (d, 2H, *J* = 8.5 Hz, H-5″), 7.30-7.51 (m, 5H, Ph), 7.55 (t, 2H, *J* = 7.0 Hz, H-6 & H-7), 7.82-7.86 (m, 2H, H-5 & NH), 8.04 (d, 1H, *J* = 7.3 Hz, H-8), 8.47 (d, 1H, *J* = 9.5 Hz, H-6″), 9.04 (s, 1H, NH); ^{13}C NMR (400 MHz, DMSO-d6), δ (ppm): 12.47, 20.69, 20.76, 21.89, 24.38, 28.35, 47.59, 55.55, 72.46, 111.76, 115.86, 115.95, 119.58, 125.52, 125.64, 128.24, 128.69, 132.98, 138.19, 141.36, 141.99, 145.37, 151.17, 156.27, 168.01, 172.26; m/z (ESI MS): calculated for $C_{30}H_{32}N_4O_3$ obtained 497.39 $(M + H)^+$. Remaining intermediate (**15-21**) were also characterised with ^{1}H NMR, ^{13}C NMR, m/z (ESI MS) spectroscopy, characterisation has been attached in the supplementary information.

General procedure for synthesis of 2-(3-hydroxy-2-methyl-4-oxopyridin-1(4H)-yl)-N-(2-((1,2,3,4-tetrahydroacridin-9-yl)amino)alkyl)acetamide(22-29) i.e. (TACHP-9 to 16):

The mixture of 2-(3-(benzyloxy)-2-methyl-4-oxopyridin-1(4H)-yl)-N-(3-((1,2,3,4-tetrahydroacridin-9-yl)amino)alkyl)acetamide (**14-21**) (0.95 mmol) and 10% Pd/C in 25 mL of methanol was stirred for 3 h under H_2 (2 bar). Completion of the reaction was monitored on thin layer chromatography (TLC). On completion, reaction mixture was filtered through the bed of ceilite and evaporated to dryness under reduced pressure to give the desired deprotected 2-(3-hydroxy-2-methyl-4-oxopyridin-1(4H)-yl)-N-(2-((1,2,3,4-tetrahydroacridin-9-yl)amino)alkyl)acetamide derivatives (**22-29**) i.e. **(TACHP-9** to **16)** in 90-95% yield.

Compound (**22,TACHP-11**) with 95% yield has following characterisation; m.p. 176-177 °C; ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 1.81 (br.s, 4H, H-2 & H-3), 2.00 (s, 3H, CH_3), 2.65 (br.s, 2H, H-1), 3.03 (br.s, 2H, H-4), 3.53 (m, 2H, H-2′), 4.01 (m, 2H, H-1′), 4.68 (s, 2H, NCH_2), 6.10 (d, 2H, *J* = 10.9 Hz, H-5″), 7.48 (d, 1H, *J* = 7.1 Hz, H-5), 7.55 (t, 1H, *J* = 7.5 Hz, H-7), 7.78 (s, 1H, NH (D_2O exchanged)), 7.86 (t, 1H, *J* = 7.5 Hz, H-6), 8.01 (d, 1H, *J* = 7.0 Hz, H-8), 8.46 (d, 1H, H-6″), 8.93 (s, 1H, NH (D_2O exchanged); ^{13}C NMR (400 MHz, DMSO-d6), δ (ppm): 11.80, 20.77, 21.92, 24.32, 28.48, 31.10, 47.85, 55.54, 110.73, 111.97, 116.09, 119.78, 125.52, 125.60, 129.60, 132.96, 138.54, 139.53, 145.45, 151.38, 156.30, 168.26, 169.69; m/z (ESI MS): calculated for $C_{23}H_{26}N_4O_3$ obtained 407.38 $(M+H)^+$. Remaining final (**23-29**) compounds were also have been characterised with ^{1}H NMR, ^{13}C NMR, m/z (ESI MS) spectroscopy, characterisation has been attached in the supplementary information.

2.2 BIOLOGICAL ASSAY

2.2.1 EQUIPMENT/ REAGENT

For antioxidant assay reading the solution absorbance at 517 nm, recorded on a Perkin-Elmer scan Lambda 35 UV-Vis Spectrophotometer. For AChE inhibition The initial rate of the enzymatic reaction was monitored by reading the solution absorbance at 405 nm, recorded on a Perkin-Elmer Lambda 35 UV-Vis spectrophotometer, pharmacokinetic proprieties were carried out using in silico tools, namely descriptors using QIKPROP v. 2.5 [14] provided by MAESTRO [15]. ThT fluorescence beta amyloid aggregation assay was measured using a Cary Eclipse of Varian fluorimeter. (Molecular Devices) at the following wavelengths: excitation (446 nm) and emission (486 nm). TEM assays were performed with a Hitachi H8100 transmission electron microscope, at Micro-Lab /IST. Amyloid β-peptide, (1-42) ($Aβ_{42}$), was purchased from Aldrich as a lyophilized powder. Acetylcholinesterase 500 U (extracted from *electrophorus electricus* and purchased from Sigma-Aldrich.

ANTIOXIDANT ASSAY

All synthesized ligands were investigated for their anti-oxidant activity (EC_{50}), based on reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH) a stable free radical, through Blois method [16] by reading the solution absorbance at 517 nm, recorded on a Perkin-Elmer scan Lambda 35 UV-Vis Spectrophotometer. The test compounds (200 µL-1 mL) were mixed with 2.5 mL of DPPH solution and filled up with methanol solvent to the total volume of 3.5 mL incubated for 30 minutes at RT protected from light. The absorbance was measured at 517 nm against the corresponding blank (methanol) in a visible range (300-700 nm) of spectrophotometer.

ACETYLCHOLINESTERASE ASSAY

Acetylcholinesterase stock solution was prepared by dissolving 500 U (extracted from *Electrophorus electricus*) in TRIS buffer (50 mM, pH 8) (10 mL). The enzyme was later diluted with HEPES buffer to give the final AChE concentration conditions [17]. The initial rate of the enzymatic reaction was monitored by reading the solution absorbance at 405 nm, recorded on a Perkin-Elmer Lambda 35 UV-Vis Spectrophotometer. Modified method of Ellman *et al*. [17, 18] was used for the determination of AChE activity, portions of enzyme were added to HEPES buffer and in different volume inhibitor and methanol were added to it then samples were left to incubate for 15 min at room temperature then sodium chloride and magnesium chloride, pH 8.0, containing 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) and acetylthiocholine iodide (AChI) i.e. the substrate for enzyme and source of thiol also added to it and reaction was monitored for 5 min. at 405 nm along with blank containing all the components except AChE, which was replaced by Hepes buffer.

Aβ AGGREGATION ASSAY

Aβ peptide ($_{1–42}$) was purchased from Aldrich and stored at -20 °C, were treated with 1,1,1,3,3,3-hexafluoropropan2-ol (HFIP) to avoid self-aggregation and reserved. HFIP pre-treated $Aβ_{1-42}$ samples were re-dissolved in CH_3CN/Na_2CO_3 (300 µM)/NaOH (250 µM) (48.3:48.3:4.3, v/v/v) solvent mixture in order to have a stable stock solution; this solution (500 µM) was further diluted in phosphate buffer (0.215 M, pH 8.0) to get 40 µM solution, then ligand which is dissolved in methanol was added to get final concentration 80 µM. $Aβ_{42}$ aggregation measured using Thioflavin T dye using a Cary Eclipse of Varian fluorimeter (Molecular Devices) at the following wavelengths: excitation (446 nm) and emission (486 nm).

TRANSMISSION ELECTRON MICROSCOPY (TEM)

$Aβ_{1-42}$ stock solutions were prepared by dissolving the lyophilized peptide in a mixture of acetonitrile (48 µL), 2% NH_4OH (10 µL) and NaCl 300 µM (48 µL). The peptide stock solution was diluted to a final concentration of 50 µM in a buffered solution containing 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, 50 mM, pH = 6.6). Then compounds (50 µM final concentration) were added to the sample of Aβ (25 µM final concentration) in the absence or in the presence of copper chloride (25 µM final concentration) followed by incubation for 24 h at 37 °C. Formvar/Carbon 200-mesh Cu grids (Ted Pella) were treated with amyloid-β peptide aggregated samples (10 µL) for 2 min at room temperature. Each grid incubated with uranyl acetate (1%, 10 µL, and 1 min) was stained and dried for 15 min at room temperature. Images from each sample were taken by a Hitachi H8100 TEM with a Lab 6 filament (200 kV, 10000-20000x magnification).

POTETIOMETRIC STUDY

pH-potentiometric and UV–vis spectrophotometric titrations of compound **TAC HP -12** done in a 20% w/w DMSO/H_2O medium, at T = 25.0 ± 0.1 °C and ionic strength (I) 0.1 M KCl, by using 0.1 M KOH as titrant. Both glass and Ag/AgCl reference electrodes were previously calibrated in different DMSO/H_2O mixtures of increasing DMSO % composition. The stepwise protonation constant of the ligand, K = [HL]/[L][H], and the overall metal–complex stability $\beta_{M_mH_hL_l}$ = $[M_mH_hL_l]/[M]^m[H]^h[L]^l$, were calculated by fitting the pH-potentiometric and spectrophotometric data with, respectively, Hyperquad 2008 [19] and PSEQUAD programs [20], metal hydrolysis constants were taken from values in the literature [21] determined in aqueous media and were also included in the equilibrium models. The species distribution curves were obtained with the Hyss program [19].

MOLECULAR MODELLING

Docking was performed using program GOLD, v. 5 [22]. The X-ray crystallographic structure of acetylcholinesterase *Torpedo californica* AChE (*Tc*AChE) in complexation with an inhibitor (original ligand) was taken from RCSB Protein Data Bank (PDB entry 1ODC) [23] to be used as a receptor in the docking simulations. For simulations original complex structure was treated using a MAESTRO v. 9.3 [15] 3D structures of the ligand were built with Maestro, geometry of ligand was optimized by making a random conformational search (RCS) of 1000 cycles then optimisation done by 2500 optimization steps with program GHEMICAL v. 2.0 [24]. Ligand was docked into the AChE using GOLD v. 5.1. with the default parameters of GOLD and the ASP scoring function.

3 RESULT AND DISCUSSION

3.1 MOLECULAR MODELLING

Docking study in present research work involves the study of interaction between synthesized tacrine derivatives of both series and enzyme involved in cholinergic loss i.e. AChE, in order to predict the binding mode. AChE has a catalytic triad of three amino acids, Ser200, His440 and Glu327 (sequence numbering of Torpedo californica AChE, *Tc*AChE) [17, 18], along with the catalytic anionic site (CAS) and peripheral anionic site (PAS). Three amino acids, Phe330, Trp84 and Glu199 forms CAS while PAS is formed by Trp279, Asp72 and Tyr70 [17, 18]. So design of new potential AChE

inhibitors based on the coupling of two main moieties – TAC and BTA, as well as TAC and HP unit through an alkyl spacer in such a way that one moiety can interact with CAS while at the same time other moiety can interact with the PAS and produces maximum AChE inhibition. TAC was always found well inserted in the bottom of the gorge of the enzyme, binding to the CAS by π–π stacking with the aromatic ring of Trp84 and Phe330, overlapping almost perfectly with the TAC moiety of the original ligand, and in both series alkyl spacer placed the second lipophilic moiety i.e. BTA and HP in the hydrophobic PAS to form aromatic stacking with Tyr70 and Trp279. (See **Figure 1**.) for compound **RSC-3** of TAC-BTA. and (see **Figure 2.**) for compound **TACHP-12** of TAC-HP series.

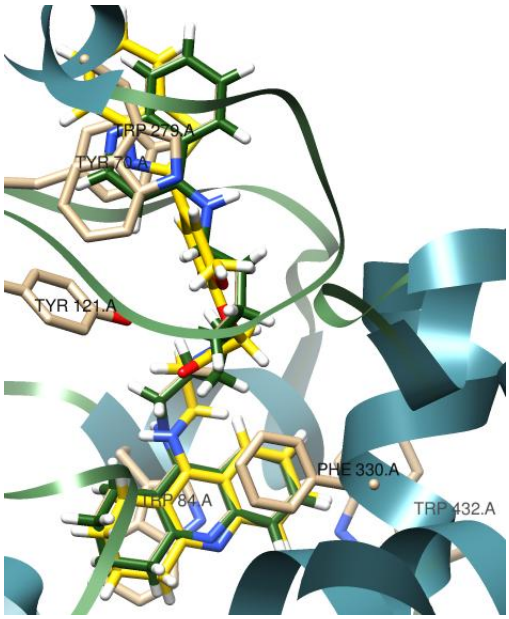


Figure 1. Docking results for the **TAC-BTA** hybrids with AChE: superimposition of **RSC-3** (yellow) with original ligand (green).

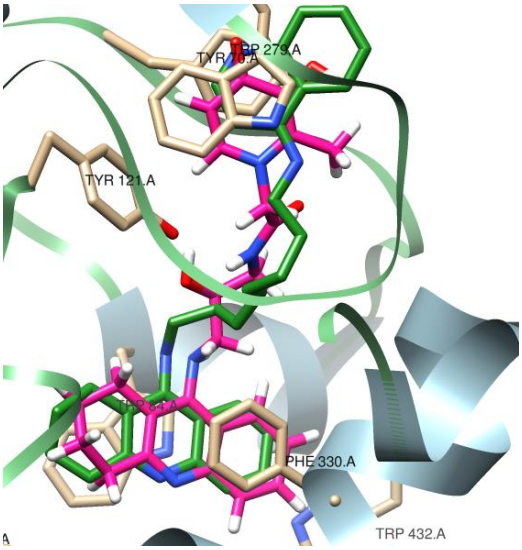


Figure 2. Docking results for the **TAC-HP** hybrids with AChE: superimposition of **TACHP-12** (pink) with original ligand (green)

3.2 CHEMISTRY

The tacrine-benzothiazole hybrids (**RSC-1** to **RSC-6**) were not synthesized under the framework of this article. The tacrine-methylhydroxypyridone (**TACHP-9** to **TACHP-16**) hybrids were synthesized according to shown in **Scheme 2.** TACHP hybrids (**TACHP-9** to **16**). It involved the previous synthesis of an *N*-carboxylic derivative of hydroxypyridinone (**5**) followed by its attachment to alkylamine-tacrine derivatives. The preparation of compound **5** involved 4 steps. Firstly, the commercial available pyrone, 3-hydroxy-2-methyl-pyran-4-one, was *O*-benzylated (**2**) by the refluxing with benzylchloride in a mixture of NaOH aqueous solution and MeOH. Afterwards, the protected pyrone (**2**) was transformed in the corresponding hydroxypyridinone (**3**) by its reflux in a mixture of NH_3 and ethanol. Then, the heterocyclic amine group of (**3**) was attached to acetyl ester through nucleophilic substitution with $ClCH_2COOEt$ and K_2CO_3 in DMF, to provide compound (**4**). Further hydrolysis of compound (**4**), in the presence of NaOH in MeOH and H_2O, afforded compound (**5**). Compound (**5**) was coupled with the previously synthesized alkylamine tacrine intermediates (**6-13**), in the presence of base (NMM) and coupling agent T3P in dry DCM, to provide (**14-21**). The benzyl groups of these pre-final intermediates were further removed by hydrogenolysis with, H_2, Pd/C in MeOH to get the final compounds (**22-29**) i.e. (**TACHP-9 to 16**).

3.3 BIOLOGICAL STUDIES

Biological studies of all new synthesized tacrine derivatives (**RSC-1**to **6**) (**TACHP-9** to **16**) has been investigated by some biological *in vitro* assays i.e. inhibition of AChE and $A\beta_{1-42}$ self aggregation, also Pharmacokinetics properties has been determined using QIKPROP v. 2.5 [14] provided by MAESTRO [15].

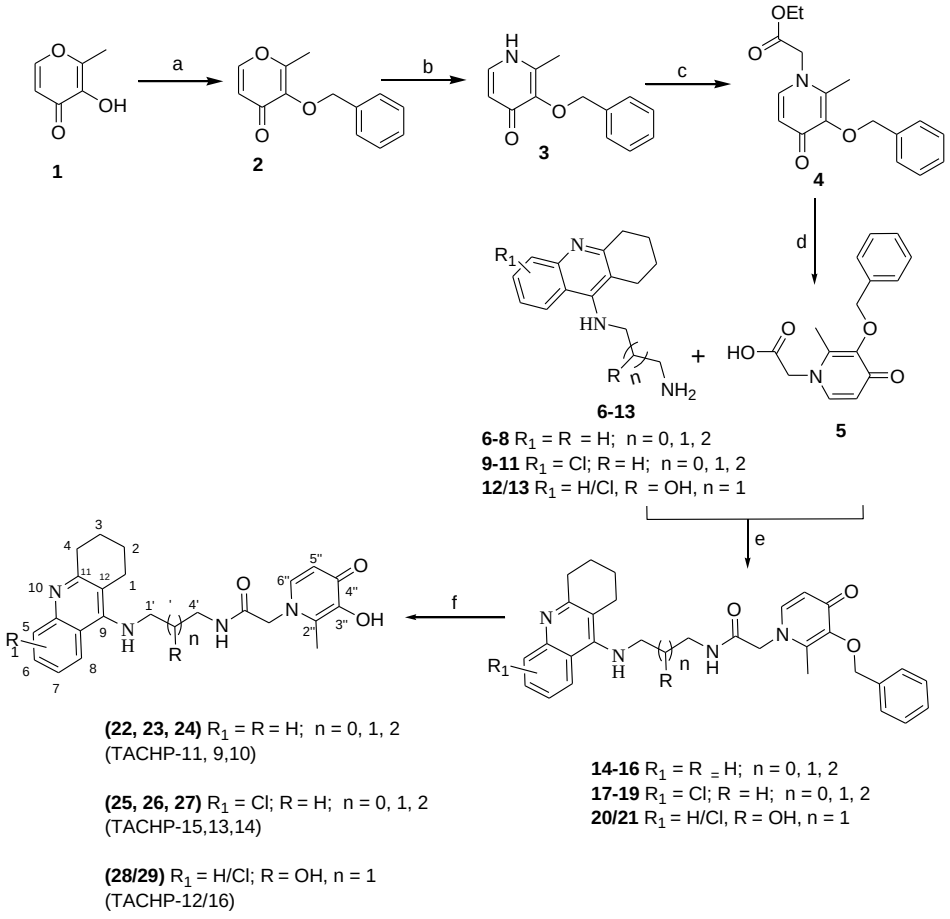
ACETYLENECHOLINESTERASE INHIBITION

The AChE inhibitory activities of all synthesized tacrine hybrids of both series were evaluated by previously described spectroscopic method by Ellman *et al* [17, 18]. Results for TAC-BTA presented in the **Table-1.** it is already explainable from the molecular modelling that all TAC-BTA compounds were good in interaction with the AChE , specially TAC moiety. Because of good interaction behaviour all the compounds of these series has good inhibitory potential in the range of (IC_{50} = 0.05-0.27 µM), among all inhibitors , compound **RSC-4** with two carbon chain linker and chloro-substituted TAC reported as best compound with (IC_{50} = 0.05 µM), further we observe a decrease in inhibition capacity for the compound **RSC-1** (IC_{50} = 0.15 µM),and **RSC-3** (IC_{50} = 0.12 µM), with non chloro-substituted tacrine with propyl and ethyl carbon chain linker respectively , compound **RSC-2** with propyl carbon linker chain with chloro-substituted tacrine has value (IC_{50} = 0.13 µM), further compound **RSC-6** with non chloro-substituted TAC moiety, and with hydroxyl group in propyl linker chain shows decrease in inhibition (IC_{50} = 0.27 µM), so compounds with chloro-substitution in tacrine were reported as good AChE inhibitors, while introduction of OH group leads to decrease in inhibition potential. In case of TAC-HP series all inhibitor shows

AChE inhibition in the range (IC_{50} = 0.64-1.71 µM), compound **TACHP-16** (IC_{50} = 0.64 µM) with three carbon chain linker with chloro and hydroxy substitution reported to show best potential among all analogue of this series, followed by this compound **TACHP-13** (IC_{50} = 0.86 µM) with three carbon linker and chloro-substitution at tacrine moiety, compound **TACHP-9** with three carbon linker and without chloro-substituted tacrine reported to show (IC_{50} = 0.90 µM), in conclusion all non chloro-substituted hybrids **TACHP-10** and **TACHP-11** shows bad inhibition (IC_{50} = 1.71 µM) and (IC_{50} = 1.07 µM), so chloro substitution at phenyl ring of tacrine enables the compound to fit in both catalytic sites of AChE , also three carbon substitution is proved necessary in order to well accommodate the inhibitor molecule in the reactive sites of AChE, although only hydroxy substitution reported to decrease the inhibition potential.

AMYLOID BETA AGGREGATION ASSAY

All the synthesized derivatives of tacrine of both series were investigated for the amyloid β_{1-42} self-aggregation inhibition potential with the approach of Thioflavin-T fluorescence method. In case of TAC-BTA series there was a solubility problem with MeOH solvent, so A-beta assay were performed with the concentration (40 µM) i.e. half of the concentration that described in the assay for ligand, and percentage inhibition reported in the range (27-44.6%) as described in **Table-1**. In case of TACHP series all the hybrids reported as very much potent inhibitor of amyloid β_{1-42} self – aggregation i.e. in the range (85.3-95.2%) (see **Table-2**.) In order to gain support for the rationalization of the obtained results in terms of the effect of the hybrids on the inhibition of $A\beta_{42}$ aggregation instead of their competition with ThT for fibril binding, independent transmission electron microscopy (TEM) assays were performed with one model compound, **TACHP-12**, results of TEM point to the role of these hybrid compounds as inhibitors of $A\beta_{42}$ aggregation, they are not clear about the role of Cu(II).



Scheme 2. Reagents and conditions: a) MeOH, NaOH (1.1eq) benzyl chloride, reflux; b) Ethanol, Ammonia (28%) reflux; c) anhydrous DMF, K_2CO_3, $ClCH_2COOEt$; d) MeOH, NaOH, H_2O; e) anhydrous DCM, NMM, T3P, RT, 7-8 h; f) MeOH, H_2 (2 bar), Pd/c.

PHARMOCOKINETICS STUDY

For TAC-BTA series, as summarized in **Table 1**, all the compounds presented clog P (octanol/water) coefficients superior to five. So this range of clog p values leads this set of compounds to have a lipophilic character; molecular weight of compounds also present in violation of Lipinski's rule [25], although Caco-2 permeability of (**RSC-1,2,3,4)** reported as very good results ranging from ca. 700-1086 nm/sec (higher than 500 nm/sec is considered good [14]. In case of TAC-HP series, as described in **Table 2**, all the compounds presented clog P (octanol/water) coefficients inferior to five, and log P values approximately between (1-3) consider being good in order to penetrate the cell membrane and maintain water solubility. All compounds also have molecular weights lower than 500, which is in accordance with Lipinski's rule, but in Caco-2 permeability all compounds exhibited very bad results ranging from ca. 66-260 indicating that the absorption through the intestinal tract to the blood would be very poor.

4 PHYSIOCHEMICAL STUDIES

ANTIOXIDANT ACTIVITY

All new designed and synthesized tacrine derivatives i.e. TAC-BTA, and TAC-HP, has been investigated for their antioxidant potential based on their interaction with 2,2-diphenyl-1-picrylhydrazyl (DPPH) a stable free radical, through Blois method [16]. As we analyse the **Table-1**. We can see that TAC-BTA hybrids i.e. **RSC-1** to **6** were concluded to show poor antioxidant capacity (EC_{50} > 1000 µM), i.e. same as or even bad (EC_{50} =1500 µM) than reference compound tacrine. From the analysis of **Table-2** we can conclude that in case of TAC-HP compounds, compound with phenolic hydroxyl substitution and with four carbon chain linker i.e. **TACHP-10** reported show good antioxidant activity (450±8 µM), followed by compound **TACHP-12** which is substituted by hydroxyl group in three carbon chain linker along with the hydroxyl substitution, two OH substituent provides best radical scavenging activity and **TACHP-16** found to exhibits significant antioxidant activities (399±5 µM), another similar compound **TACHP-16** results in lower antioxidant activity (483±1 µM) most probably due to the presence of chloro-substituted tacrine. Another compound of TAC-HP series reported to have lower antioxidant capacity because of chloro substitution on tacrine.

METAL CHELATION

TACHP-12 was the compound chosen as a model for analyzing the chelating capacity of the TACHP hybrids and the equilibrium solution studies were performed in a mixed 20% (w/w) DMSO/water medium, due to solubility reasons. Protonation constants (log K_i) were determined by pH-potentiometry and reported as phenolic hydroxyl group of the HP moiety (10.54), the TAC amine (8.59) and the pyridinic nitrogen (2.88).The chelating capacity of compound **TACHP-12** towards Cu(II) and Zn(II) was evaluated through the determination of the global formation constants of the complexes by pH-potentiometric (Hyperquad 2008 program [19]) titrations while for the Fe(III) complexes by UV–vis spectrophotometry (PSEQUAD program [20]). In case of 1:3 Fe(III)/**TACHP-12** system fully coordinated tris-chelate species FeH_3L_3 is predominant above pH ca 3.8 and at the physiological pH (7.4) (**Figure 3**.**a**), and respective calculated pM values for this complex was (pFe =21.7). For 1:2 Cu(II)/**TACHP-12** system tetradentate species CuH_2L_2 is predominant above pH ca 4 and attains almost 100% formation at the physiological pH (see **Figure 3.b**), and calculated pCu value at pH 7.4 for was (pCu = 10.8), and for 1:2 Zn(II)/ **TACHP-12** system at the physiological pH the 1:1 ZnL complex predominates, (**see Figure 3.c**) and (pZn = 6.9) have been calculated. Results showed that the chelating affinity depends on the metal ion (Fe>Cu>Zn), as demonstrated by the respective pM values at the physiological pH 7.4 (pFe =21.7, pCu = 10.8, pZn = 6.9), which are comparable to the known chelator drug DFP (pFe =20.7, pCu =10.5, pZn = 6.3) at pH 7.4 in water medium [26, 27] confirm the good chelating capacity of the HP moiety.

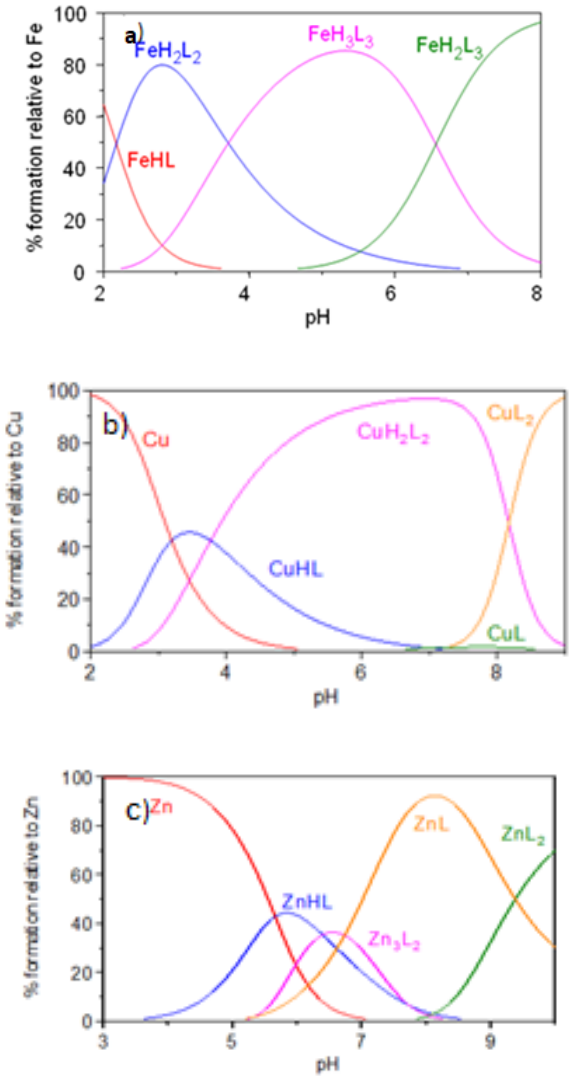
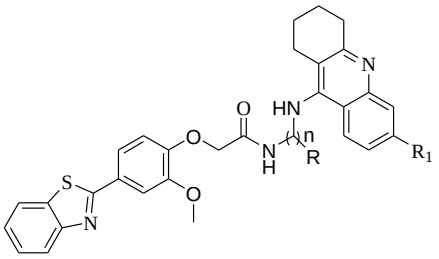


Figure 3. Species distribution curves for the a) 1:3 Fe(III)/**TACHP-12** system (C_L = 2.0 ×10^{-4} M); b)1:2 Cu(II)/**TACHP-12** system (C_L = 6.7 ×10^{-4} M); c) 1:2 Zn(II)/ **TACHP-12** system (C_L = 6.7 ×10^{-4} M).

Table 1. Summary of activities of TAC-BTA derivatives (**RSC-1** to **RSC-6**) towards radical scavenging (DPPH), inhibition of AChE and $A\beta_{1-42}$ self-aggregation and Predicted pharmacokinetic values for TAC-BTA hybrids[a]

Comp. Code	R_1	DPPH scavenging (EC_{50},μM)[b]	AChE (IC_{50},μM)[c]	Inhibition of Aβ-self-aggregation(%)[d,e]	MW	clog *P*[f]	log BB[g]	Caco-2 Permeability (nm/sec)	Violations of Lipinski's rule of 5	CNS activity
RSC-1	**H**	> 1500	0.15	33.7	552.69	6.237	-0.975	1057	2	-
RSC-2	**Cl**	> 1500	0.13	32.5	587.13	6.726	-0.739	1022	2	-
RSC-3	**H**	Nd*	0.12	44.6	538.66	6.200	-0.927	1086	2	-
RSC-4	**Cl**	Nd*	0.06	27.0	573.10	5.445	-0.914	722	2	-
RSC-5	**Cl**	Nd*	0.14	31.3	603.13	5.692	-1.268	496	2	-
RSC-6	**H**	>1500	0.27	31.1	568.60	5.222	-1.592	388	2	-
TAC	**-**	>1000	0.30	11	198.1	-	-	-	-	-

[a]Predicted values using program QikProp v. 2.5. K [14]; [b]Capacity to scavenge the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was monitored according to the Blois method [16] (EC_{50}, μM) (means of two experiments); [c]AChE from *electric eel*, IC_{50}, inhibitor concentration (means of two experiments) for 50% inactivation of AChE; [d]Inhibition of self-mediated $A\beta_{42}$ aggregation (means of two experiments). The Thioflavin-T fluorescence method was used, and the measurements were carried out in the presence of an inhibitor; [e]Assays performed with C_L = 40 μM; *Not determined due to very poor solubility in methanol; [f] Calculated octanol/water partition coefficient; [g] Brain/blood partition coefficient.



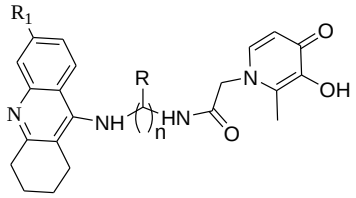
	RSC-1	RSC-2	RSC-3	RSC-4	RSC-5	RSC-6
R_1	H	Cl	H	Cl	Cl	H
n	3	3	2	2	3	3
R	H	H	H	H	OH	OH

Scheme 2. Representation of derivatives of TAC-BTA

Table 2. Activities of the TACHP derivatives (**TACHP-9** to **TACHP-16**) towards radical scavenging (DPPH), inhibition of AChE and $A\beta_{1-42}$ aggregation and predicted pharmacokinetic values for TACHP hybrids[a]

Comp. Code	R_1	DPPH scavenging (EC_{50},μM)[b]	AChE (IC_{50},μM)[c]	Inhibition of Aβself- aggregation(%)[d,e]	MW	clog *P*[f]	log BB[g]	Caco-2 Permeability (nm/sec)	Violations of Lipinski's rule of 5	CNS activity
TACHP-9	H	925±8	0.90	85.3 Cu/94.8	420.51	2.745	-1.739	154	0	--
TACHP-10	H	450±8	1.71	89.1	434.53	3.128	-1.941	139	0	--
TACHP-11	H	> 1200	1.07	88.1	406.48	2.531	-1.494	230	0	--
TACHP-12	H	399±5	0.96	86.0 Cu/54.0	436.50	1.812	-2.199	69	0	--
TACHP-13	Cl	1110±7	0.86	95.2	454.18	3.341	-1.649	161	0	--
TACHP-14	Cl	955±1	1.01	90.1	468.98	2.926	-1.562	265	0	--
TACHP-15	Cl	1085±4	0.97	89.6	440.92	2.976	-1.507	167	0	--
TACHP-16	Cl	483	0.64	87.0	470.17	2.274	-2.106	66	0	--
Tac	-	> 1000	0.30	11.0	198.12	-	-	-	-	-

[a] Predicted values using program QikProp v. 2.5 [14]; [b]Capacity to scavenge the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was monitored according to the Blois method [16] (means of two experiments)(EC_{50}, μM); [c]AChE from electric *ell*, IC_{50}, inhibitor concentration (means of two experiments) for 50% inactivation of AChE; [d]Inhibition of self-mediated $A\beta_{42}$ aggregation (means of two experiments). The Thioflavin-T fluorescence method was used, and the measurements were carried out in the presence of an inhibitor; [e]Assays performed with C_L = 80 μM; *Not determined; [f]Calculated octanol/water partition coefficient; [g] Brain/blood partition coefficient.



Comp	TACHP-9	TACHP-10	TACHP-11	TACHP-12	TACHP-13	TACHP-14	TACHP-15	TACHP-16
R_1	H	H	H	H	Cl	Cl	Cl	Cl
n	3	4	2	3	3	4	2	3
R	H	H	H	OH	H	H	H	OH

Scheme 3. Representation of TAC-HP derivatives

5 CONCLUSION

Present research work based on the strategy of conjugation of two moieties, on the basis of adequate beneficiary recent literature study regarding potential of different moieties against AD. Following this hybridization strategy two series of an active AChE inhibitors tacrine hybrids were designed on the basis of molecular modelling study, then synthesized and bio-analysed for basically three main target of AD i.e. AChE inhibition, antioxidant activity, self or metal-induced $A\beta_{42}$ aggregation inhibition and metal chelating potential, thus following a multi-target approach in order to tackle various aspects of Alzheimer's disease. Screening of present two series showed that compounds of TAC-BTA series have much better AChE inhibitory capacity even than tacrine, attributed to the good interaction of these hybrids with CAS and PAS; they also showed good self-induced $A\beta_{1-42}$ aggregation inhibition. In case of TACHP hybrids, there is a moderate antioxidant but still better than tacrine; they also presented good AChE inhibition, specifically the chloro derivatives of TAC-HP. Regarding the inhibition of beta amyloid aggregation, all compounds of TAC-HP series appeared as quite potent inhibitors of self -induced

$A\beta_{42}$ aggregation, and even few compounds were reported as quite good inhibitor of Cu metal-induced $A\beta_{1-42}$ aggregation. $A\beta_{42}$ aggregation inhibition also supported by the TEM study. Also compound **TACHP-12** of TACHP series proved as potent chelator for Fe(III) and Cu(II)), and moderate chelator for Zn(II)) based on potentiometric, UV–vis spectrophotometric techniques. Overall, TAC-BTA series shows very much promising results in AChE inhibition, while the TACHP series proved also to be promising for inhibition of self as well as metal-induced $A\beta_{42}$ aggregation, besides its potential chelator role for the control of metal dyshomeostasis in AD brains.

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Instituto Superior Técnico (September 2017)

Extended Abstract to obtain the Master of Science Degree in Chemistry

Tacrine derivatives as potential multifunctional drugs against

Alzheimer's disease-physicochemical and biological properties

Rajeshwari

1. Supplementary Information

A.) Characterization of remaining pre-final compounds (15-21):

General synthesis procedure described in article (**section 2.):**

2-(3-(Benzyloxy)-2-methyl-4-oxopyridin-1(4H)-yl)-N-(3-((1,2,3,4-tetrahydroacridin-9-yl)amino)propyl)acetamide (**15**):

Compound **15** was obtained as a yellow solid at room temperature in 81% isolated yield from the reaction of **7** with **5** by following the general procedure; m.p. 159-160 °C; ^{1}H NMR (300 MHz, DMSO-d6), δ (ppm): 1.81 (br.s, 4H, H-2 & H-3), 1.88-1.92 (m, 2H, H-2′), 2.00 (s, 3H, CH_3), 2.67 (br.s, 2H, H-1), 3.01 (br.s, 2H, H-4), 3.19 (t, 2H, H-3′), 3.88-3.90 (m, 2H, H-1′), 4.66 (s, 2H, NCH_2), 4.98 (s, 2H, OCH_2Ph), 6.14 (d, 1H, *J* = 10.5 Hz, H-5″), 7.29-7.40 (m, 5H, Ph), 7.55 (t, 2H, H-6 & H-7), 7.83-7.85 (m, 2H, H-5 & NH), 7.99 (d, 1H, *J* = 7.5 Hz, H-8), 8.44 (1H, H-6″), 8.77 (s, 1H, NH); ^{13}C NMR (300 MHz, DMSO-d6), δ (ppm): 12.55, 20.72, 21.92, 24.50, 28.40, 30.25, 36.40, 45.11, 55.51, 72.35, 111.74, 115.99, 116.06, 119.64, 125.53, 128.20, 128.65, 128.71, 132.94, 138.25, 138.35, 141.30, 141.70, 145.48, 151.14, 156.09, 167.16, 172.61; m/z (ESI MS): calculated for $C_{31}H_{34}N_4O_3$ obtained 511.40 $(M + H)^+$.

2-(3-(Benzyloxy)-2-methyl-4-oxopyridin-1(4H)-yl)-N-(4-((1,2,3,4-tetrahydroacridin-9-yl)amino)butyl)acetamide (**16**):

Compound **16** was obtained as a yellow solid at room temperature in 80% isolated yield from the reaction of **8** with **5** by following the general procedure; m.p. 84-86 °C; ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 1.45-1.46 (m, 2H, H-2′), 1.54-1.56 (m, 2H, H-3′), 1.80 (m, 4H, H-2 & H-3), 2.02 (s, 3H, CH_3), 2.71 (br.s, 2H, H-1), 2.90-2.91 (m, 2H, H-4), 3.08-3.10 (m, 2H, H-4′), 3.41-3.42 (t, 2H, *J* = 7.1 Hz, H-1′), 4.56 (s, 2H, NCH_2), 5.01 (s, 2H, OCH_2Ph), 5.45 (br.s, 1H, NH (D_2O exchanged)), 6.14 (d, 2H, *J* = 10.0 Hz, H-5″), 7.32-7.42 (m, 6H, Ph & H-7), 7.50-7.52 (m, 2H, H-6 & H-5), 7.72 (d, 1H, *J* = 7.5 Hz, H-8), 8.12 (1H, H-6″), 8.23 (s, 1H, NH (D_2O exchanged)); ^{13}C NMR (400 MHz, DMSO-d6), δ (ppm): 12.43, 22.89, 23.21, 25.58, 26.92, 28.52, 33.93, 48.06, 55.54, 72.34, 116.00, 116.34, 120.71, 123.50, 123.73, 128.20, 128.39, 128.66, 128.71, 138.36, 141.28, 141.49, 145.58, 147.26, 150.75, 158.32, 166.76, 172.63; m/z (ESI MS): calculated for $C_{32}H_{36}N_4O_3$ obtained 525.43 $(M + H)^+$.

2-(3-(Benzyloxy)-2-methyl-4-oxopyridin-1(4H)-yl)-N-(2-((6-chloro-1,2,3,4-tetrahydroacridin-9-yl)amino)ethyl)acetamide (**17**):

Compound **17** was obtained as a yellow solid at room temperature in 84% isolated yield from the reaction of **9** with **5** by following the general procedure; m.p. 118-120 °C; ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 1.79-181 (m, 4H, H-2 & H-3), 1.96 (s, 3H, CH_3), 2.68 (br.s, 2H, H-1), 2.98-2.90 (m, 2H, H-4), 3.34-3.35 (m, 2H, H-2′), 3.54-3.56 (m, 2H, H-1′), 4.56 (s, 2H, NCH_2), 4.99 (s, 2H, OCH_2Ph), 5.66 (br.s, 1H, NH (D_2O exchanged)), 6.14 (d, 2H, *J* = 10.5 Hz, H-5″), 7.31-7.42 (m, 6H, Ph & H-7), 7.47 (d, 1H, *J* = 7.1 Hz, H-8), 7.73 (s, 1H, H-5), 8.13 (d, 1H, *J* = 8.5 Hz, H-6″), 8.37 (s, 1H, NH (D_2O exchanged)); ^{13}C NMR (400 MHz, DMSO-d6), δ (ppm): 12.39, 22.67, 23.00, 25.34, 33.90, 47.91, 55.46, 72.34, 116.06, 116.51, 118.82, 124.02, 125.91, 128.20, 128.67, 128.68, 133.04, 138.34, 141.20, 141.49, 145.56, 147.91, 150.75, 159.72, 167.59, 172.64; m/z (ESI MS): calculated for $C_{30}H_{31}ClN_4O_3$ obtained 531.36 $(M + H)^+$.

2-(3-(benzyloxy)-2-methyl-4-oxopyridin-1(4H)-yl)-N-(3-((6-chloro-1,2,3,4-tetrahydroacridin-9-yl)amino)propyl)acetamide (**18**):

Compound **18** was obtained as a yellow solid at room temperature in 89% isolated yield from the reaction of **10** with **5** by following the general procedure; m.p. 118-120 °C; ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 1.70-1.76 (m, 2H, H-2′), 1.79-1.80 (m, 4H, H-2 & H-3), 2.01(s, 3H, CH_3), 2.69-2.70 (m, 2H, H-1), 2.88-2.90 (br.s, 2H, H-4), 3.16 (t, 2H, *J* = 7.1 Hz, H-3′), 3.43 (t, 2H, *J* = 7.1 Hz, H-1′), 4.58 (s, 2H, NCH_2), 5.00 (s, 2H, OCH_2Ph), 5.60 (br.s, 1H, NH (D_2O exchanged)), 6.14 (d, 2H, *J* = 10.5 Hz, H-5″), 7.30-7.37 (m, 5H, Ph), 7.42 (d, 1H, *J* = 8.5 Hz, H-7), 7.51 (d, 1H, H-8), 7.73 (d, 1H, *J* = 8.0 Hz, H-8), 8.14 (d, 1H, *J* = 8.5 Hz, H-6″), 8.27 (s, 1H, NH (D_2O exchanged)); ^{13}C NMR (400 MHz, DMSO-d6), δ (ppm): 12.45, 22.74, 23.04, 25.51, 30.92, 33.98, 36.84, 45.83, 55.48, 72.33, 116.03, 116.86, 119.21, 124.03, 125.77, 127.20, 128.19, 128.65, 128.70, 132.96, 138.33, 141.26, 141.50, 145.56, 148.00, 150.86, 159.91, 167.09, 172.63; m/z (ESI MS): calculated for $C_{31}H_{33}ClN_4O_3$ obtained 545.41 $(M + H)^+$.

2-(3-(Benzyloxy)-2-methyl-4-oxopyridin-1(4H)-yl)-N-(4-((6-chloro-1,2,3,4-tetrahydroacridin-9-yl)amino)butyl)acetamide (**19**):

Compound **19** was obtained as a yellow solid at room temperature in 83% isolated yield from the reaction of **11** with **5** by following the general procedure; m.p. 118-120 °C; ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 1.41-1.46 (m, 2H, H-2′), 1.54-1.57 (m, 2H, H-3′), 1.78-1.80 (m, 4H, H-2 & H-3), 2.01 (s, 3H, CH_3), 2.67-2.68 (br.s, 2H, H-1), 2.87-2.90 (m, 2H, H-4), 3.07-3.08 (m, 2H, H-4′), 3.56 (t, 2H, *J* = 7.0, H-1′), 4.55 (s, 2H, NCH_2), 4.99 (s, 2H, OCH_2Ph), 5.76 (br.s, 1H, NH (D_2O exchanged)), 6.14 (d, 2H, *J* = 10.5 Hz, H-5″), 7.30-7.37 (m, 5H, Ph), 7.41 (d, 1H, *J* = 8.5 Hz, H-7), 7.50 (d, 1H, *J* = 8.0 Hz, H-8), 7.73 (d, 1H, H-8), 8.16 (d, 1H, H-6″), 8.23 (t, 1H, *J* = 4.0 Hz, NH (D_2O exchanged)); ^{13}C NMR (400 MHz, DMSO-d6), δ (ppm): 12.42, 22.55, 22.94, 25.40, 26.83, 28.36, 33.93, 47.93, 55.54, 72.34, 115.99, 116.13, 118.72, 124.04, 126.04, 128.21, 128.66, 128.73, 133.28, 138.29, 141.29, 141.57, 145.54, 147.33, 151.27, 159.23, 166.76, 172.65; m/z (ESI MS): calculated for $C_{32}H_{35}ClN_4O_3$ obtained 559.57 $(M + H)^+$.

2-(3-(Benzyloxy)-2-methyl-4-oxopyridin-1(4*H*)-yl)-*N*-(2-hydroxy-3-((1,2,3,4-tetrahydroacridin-9-yl)amino)propyl)acetamide (20):

Compound **20** was obtained as a yellow solid at room temperature in 74% isolated yield from the reaction of **12** with **5** by following the general procedure; m.p. 134-136 °C; ^{1}H NMR (300 MHz, DMSO-d6), δ (ppm): 1.81 (br.s, 4H, H-2 & H-3), 2.00 (s, 3H, CH_3), 2.71 (br.s, 2H, H-1), 2.93 (br.s, 2H, H-4), 3.21 (br.s, 2H, H-3′), 3.43-3.59 (m, 2H, H-1′), 3.77 (br.s, 1H, H-2′), 4.62 (s, 2H, NCH_2), 4.98 (s, 2H, OCH_2Ph), 5.41 (br.s, 1H, NH (D_2O exchanged)), 5.98 (br.s, 1H, OH (D_2O exchanged)), 6.13 (d, 2H, *J* = 10.1 Hz, H-5″), 7.30-7.42 (m, 6H, Ph & H-7), 7.51 (d, 1H, *J* = 8.0 Hz, H-5), 7.62 (t, 1H, *J* = 8.5 Hz, H-6), 7.78 (d, 1H, H-8), 8.20 (d, 1H, *J* = 8.0 Hz, H-6″), 8.44 (br.s, 1H, NH (D_2O exchanged)); ^{13}C NMR (300 MHz, DMSO-d6), δ (ppm): 12.52, 22.20, 22.74, 24.83, 32.17, 43.32, 51.87, 55.45, 69.22, 72.34, 115.15, 116.01, 119.20, 124.20, 124.37, 128.20, 128.66, 128.69, 129.94, 138.33, 141.28, 141.54, 145.54, 152.75, 167.46, 172.62; m/z (ESI MS): calculated for $C_{31}H_{34}N_4O_4$ obtained 527.41 $(M + H)^+$.

2-(3-(Benzyloxy)-2-methyl-4-oxopyridin-1(4H)-yl)-N-(3-((6-chloro-1,2,3,4-tetrahydroacridin-9-yl)amino)-2-hydroxypropyl)acetamide (**21**):

Compound **21** was obtained as a yellow solid at room temperature in 70% isolated yield from the reaction of **13** with **5** by following the general procedure; m.p. 134-136 °C; ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 1.80 (br.s, 4H, H-2 & H-3), 2.00 (s, 3H, CH_3), 2.71 (br.s, 2H, H-1), 2.89 (br.s, 2H, H-4), 3.18 (br.s, 2H, H-3′), 3.32-3.42 (m, 2H, H-1′), 3.70 (br.s, 1H, H-2′), 4.61 (s, 2H, NCH_2), 4.99 (s, 2H, OCH_2Ph), 5.31-5.33 (m, 1H, NH (D_2O exchanged)), 5.36-5.38 (br.s, 1H, OH (D_2O exchanged)), 6.14 (d, 2H, *J* = 10.4 Hz, H-5″), 7.30-7.43 (m, 6H, Ph & H-7), 7.51 (d, 1H, *J* = 7.5 Hz, H-8), 7.73 (s, 1H, H-5), 8.11-8.14 (m, 1H, H-6″), 8.32-8.34 (m, 1H, NH (D_2O exchanged); ^{13}C NMR (400 MHz, DMSO-d6), δ (ppm): 12.47, 22.74, 23.00, 25.03, 31.13, 43.38, 52.18, 55.48, 69.40, 72.34, 116.02, 116.90, 119.10, 123.99, 125.94, 127.25, 128.18, 128.64, 128.69, 132.94, 138.33, 141.25, 141.51, 145.55, 148.10, 151.07, 159.88, 167.40, 172.63; m/z (ESI MS): calculated for $C_{31}H_{33}ClN_4O_4$ obtained 561.37 $(M + H)^+$.

B.) Characterization of remaining final compounds (23-29 i.e. TACHHP-9, 10,12,13,14,15,16):

General synthesis procedure described in article (**section 2.):**

2-(3-Hydroxy-2-methyl-4-oxopyridin-1(4H)-yl)-N-(3-((1,2,3,4-tetrahydroacridin-9-yl)amino)propyl)acetamide (**23**):

Compound **23** was obtained as light yellow solid in 93% yield through the hydrogenolysis of **15** by following general procedure; m.p. 170-172 °C; ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 1.83 (br.s, 4H, H-2 & H-3), 1.92 (br.s, 2H, H-2′), 2.10 (s, 3H, CH_3), 2.69 (br.s, 2H, H-1), 3.03 (br.s, 2H, H-4), 3.21 (br.s, 2H, H-3′), 3.91 (br.s, 2H, H-1′), 4.68 (s, 2H, NCH_2), 6.11 (d, 2H, *J* = 10.9 Hz, H-5″), 7.49-7.51 (m, 1H, H-7), 7.57 (t, 1H, *J* = 7.5 Hz, H-6), 7.84-7.86 (m, 2H, H-5 & NH (D_2O exchanged)), 8.00 (d, 1H, *J* = 7.1 Hz, H-8), 8.45 (1H, *J* = 7.5 Hz, H-6″), 8.72 (s, 1H, NH (D_2O exchanged)), 14.05 (s, 1H, OH (D_2O exchanged)); ^{13}C NMR (400 MHz, DMSO-d6), δ (ppm): 11,95, 20.75, 21.94, 24.52, 28.46, 30.30, 36.42, 45.17, 55.58, 110.73, 111.83, 116.13, 119.73, 125.47, 125.54, 129.67, 132.96, 138.44, 139.52, 145.48, 151.24, 156.12, 167.28, 169.68; m/z (ESI MS): calculated for $C_{24}H_{28}N_4O_3$ obtained 421.40 $(M + H)^+$.

2-(3-hydroxy-2-methyl-4-oxopyridin-1(4H)-yl)-N-(4-((1,2,3,4-tetrahydroacridin-9-yl)amino)butyl)acetamide (**24**):

Compound **24** was obtained as light yellow solid in 93% yield through the hydrogenolysis of **16** by following general procedure; m.p. 113-114 °C; ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 1.43-1.47 (m, 2H, H-2′), 1.55-1.56 (m, 2H, H-3′), 1.81 (brs, 4H, H-2 & H-3), 2.10 (s, 3H, CH_3), 2.72 (br.s, 2H, H-1), 2.91 (br.s, 2H, H-4), 3.08-3.10 (m, 2H, H-4′), 3.42 (br.s, 2H, H-1′), 4.60 (s, 2H, NCH_2), 5.45 (br.s, 1H, NH (D_2O exchanged)), 6.10 (d, 2H, *J* = 10.1 Hz, H-5″), 7.35 (t, 1H, *J* = 7.5 Hz, H-7), 7.47 (d, 1H, *J* = 7.1 Hz, H-5), 7.53 (t, 1H, *J* = 7.5 Hz, H-6), 7.72 (d, 1H, *J* = 8.0 Hz, H-8), 8.12 (1H, *J* = 10.9 Hz, H-6″), 8.23 (t, 1H, *J* = 4.0 Hz, NH (D_2O exchanged)); ^{13}C NMR (400, MHz, DMSO-d6), δ (ppm): 11,86, 22.89, 23.21, 25.58, 26.93, 28.52, 33.93., 48.05, 55.59, 110.70, 116.35, 120.71, 123.50, 123.74, 128.39, 128.66, 129.51, 139.54, 145.53, 147.26, 150.75, 158.34, 166.87, 169.78; m/z (ESI MS): calculated for $C_{25}H_{30}N_4O_3$ obtained 435.46 $(M + H)^+$.

***N*-(2-((6-chloro-1,2,3,4-tetrahydroacridin-9-yl)amino)ethyl)-2-(3-hydroxy-2-methyl-4-oxopyridin-1(4H)-yl)acetamide** (**25**):

Compound **25** was obtained as light yellow solid in 95% yield through the hydrogenolysis of **17** by following general procedure; m.p. 181-182 °C; ^{1}H NMR (400 MHz, MeOD-d4), δ (ppm): 1.92 (br.s, 4H, H-2 & H-3), 2.18 (s, 3H, CH_3), 2.67 (t, 2H, *J* = 4.0 Hz, H-1), 3.04 (t, 2H, *J* = 4.0 Hz, H-4), 3.74 (br.s, 2H, H-2′), 4.18-4.19 (m, 2H, H-1′) 4.81 (s, 2H, NCH_2), 6.38 (d, 2H, *J* = 10.5 Hz, H-5″), 7.54 (d, 2H, *J* = 7.1 Hz, H-7), 7.59 (t, 1H, *J* = 7.5 Hz, NH (D_2O exchanged)), 7.81 (d, 2H, *J* = 1.5 Hz, H-5), 7.88 (d, 1H, *J* = 7.1 Hz, H-8), 8.44 (d, 1H, *J* = 10.9 Hz, H-6″); ^{13}C NMR (400 MHz, DMSO-d6), δ (ppm): 11.80, 20.77, 21.92, 24.32, 28.48, 31.10, 47.85, 55.54, 110.73, 111.97, 116.09, 119.78, 125.52, 125.60, 129.60, 132.96, 138.54, 139.53, 145.45, 151.38, 156.30, 168.26, 169.69; m/z (ESI MS): calculated for $C_{23}H_{25}ClN_4O_3$ obtained 441.46 $(M + H)^+$.

***N*-(3-((6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)amino)propyl)-2-(3-hydroxy-2-methyl-4-oxopyridin-1(4H)-yl)acetamide** (**26**):

Compound **26** was obtained as light yellow solid in 92% yield through the hydrogenolysis of **18** by following general procedure; m.p. 162-163 °C; ^{1}H NMR (400 MHz, MeOD-d4), δ (ppm): 1.98-1.99 (m, 4H, H-2 & H-3), 2.08-2.11 (m, 2H, H-2′), 2.26 (s, 3H, CH_3), 2.76 (br.s, 2H, H-1), 3.04 (br.s, 2H, H-4), 3.45 (t, 2H, *J* = 8.0 Hz, H-3′), 4.03 (t, 2H, *J* = 8.0 Hz, H-1′), 4.79 (s, 2H, NCH_2), 6.40 (d, 2H, *J* = 8.0 Hz, H-5″), 7.53 (d, 1H, *J* = 8.0 Hz, H-7), 7.61 (t, 1H, *J* = 8.0 Hz, NH (D_2O exchanged)), 7.80 (d, 1H, *J* = 8.0 Hz, H-5), 7.88 (d, 1H, *J* = 8.0 Hz, H-8), 8.41 (d, 1H, *J* = 8.0 Hz, H-6″); ^{13}C NMR (400 MHz, DMSO-d6), δ (ppm): 11.96, 20.73, 21.93, 24.52, 28.41, 30.30, 35.42, 45.15, 55.61, 110.75, 111.76, 116.07, 119.64, 125.54, 129.82, 132.97, 138.35, 139.53, 145.45, 151.15, 156.12, 167.27, 169.60; m/z (ESI MS): calculated for $C_{24}H_{27}ClN_4O_3$ obtained 455.91 $(M + H)^+$.

***N*-(4-((6-chloro-1,2,3,4-tetrahydroacridin-9-yl)amino)butyl)-2-(3-hydroxy-2-methyl-4-oxopyridin-1(4H)-yl)acetamide** (**27**):

Compound **27** was obtained as light yellow solid in 96% yield through the hydrogenolysis of **19** by following general procedure; m.p. 148-149 °C; ^{1}H NMR (400 MHz, MeOD-d4), δ (ppm): 1.66-1.70 (m, 2H, H-2′), 1.86-1.88 (m, 2H, H-3′), 1.97 (br.s, 4H, H-2 & H-3), 2.27 (s, 3H, CH_3), 2.72 (br.s, 2H, H-1), 3.03 (br.s, 2H, H-4), 3.28-3.30 (m, 2H, H-4′), 4.01 (t, 2H, *J* = 7.0 Hz, H-1′), 4.78 (s, 2H, NCH_2), 6.39 (d, 2H, *J* = 10.5 Hz, H-5″), 7.55 (d, 1H, *J* = 7.5 Hz, H-7), 7.59 (t, 1H, *J* = 8.0 Hz, NH (D_2O

exchanged)), 7.78 (d, 1H, *J* = 7.0 Hz, H-5), 7.85 (d, 1H, *J* = 8.5 Hz, H-8), 8.40 (d, 1H, *J* = 10.9 Hz, H-6″); ^{13}C NMR (400 MHz, DMSO-d6), δ (ppm): 11.93, 20.78, 21.97, 24.55, 26.58, 27.84, 28.52, 38.76, 47.24, 55.66, 110.69, 111.73, 116.16, 119.82, 125.47, 129.73, 132.84, 138.54, 139.59, 145.47, 151.32, 155.95, 167.00, 169.67; m/z (ESI MS): calculated for $C_{25}H_{29}ClN_4O_3$ obtained 470.14 $(M + H)^+$.

2-(3-hydroxy-2-methyl-4-oxopyridin-1(4H)-yl)-N-(2-hydroxy-3-((1,2,3,4-tetrahydroacridin-9-yl)amino)propyl)acetamide (28):

Compound **28** was obtained as light yellow solid in 91% yield through the hydrogenolysis of **20** by following general procedure; m.p. 150-152 °C; ^{1}H NMR (400 MHz, DMSO-d6), δ (ppm): 1.82 (br.s, 4H, H-2 & H-3), 2.09 (s, 3H, CH_3), 2.72 (br.s, 2H, H-1), 2.94 (br.s, 2H, H-4), 3.23 (t, 2H, *J* = 7.0 Hz, H-3′), 3.47-3.49 (m, 1H, Ha-1′), 3.58-3.61 (m, 1H, Hb-1′), 3.79-380 (m, 1H, H-2′), 4.67 (s, 2H, NCH_2), 5.99 (br.s, 1H, NH (D_2O exchanged)), 6.10 (d, 2H, *J* = 10.1 Hz, H-5″), 7.41 (t, 1H, *J* = 7.5 Hz, H-7), 7.48 (d, 1H, *J* = 7.1 Hz, H-5), 7.65 (t, 1H, *J* = 7.0 Hz, H-6), 7.79 (d, 1H, *J* = 7.5 Hz, H-8), 8.21 (d, 1H, *J* = 10.5 Hz, H-6″), 8.47 (t, 1H, NH (D_2O exchanged)); ^{13}C NMR (400 MHz, DMSO-d6), δ (ppm): 11.93, 22.21, 22.74, 24.84, 31.09, 43.33, 51.89, 55.52, 69.25, 110.73, 115.18, 119.21, 124.21, 124.38, 125.80, 129.58, 129.94, 139.54, 144.43, 145.49, 152.74, 156.04, 167.58, 169.78; m/z (ESI MS): calculated for $C_{24}H_{28}N_4O_4$ obtained 437.40 $(M + H)^+$.

N-(3-((6-chloro-1,2,3,4-tetrahydroacridin-9-yl)amino)-2-hydroxypropyl)-2-(3-hydroxy-2-methyl-4-oxopyridin-1(4H)-yl)acetamide (**29**):

Compound **29** was obtained as light yellow solid in 91% yield through the hydrogenolysis of **21** by following general procedure; m.p. 161-162 °C; ^{1}H NMR (400, MHz, MeOD-d4), δ (ppm): 1.97-1.98 (br.s, 4H, H-2 & H-3), 2.27 (s, 3H, CH_3), 2.75 (br.s, 2H, H-1), 3.05 (br.s, 2H, H-4), 3.41-3.47 (br.s, 2H, H-3′), 3.91-3.95 (m, 1H, H-1′), 4.0-4.09 (m, 1H, H-2′), 4.80 (s, 2H, NCH_2), 6.37 (d, 2H, *J* = 10.0 Hz, H-5″), 7.50 (d, 1H, *J* = 7.5 Hz, H-7), 7.58 (t, 1H, NH (D_2O exchanged)), 7.78 (d, 1H, *J* = 7.7 Hz, H-5), 7.85 (d, 1H, *J* = 8.5 Hz, H-8), 8.43 (d, 1H, *J* = 10.5 Hz, H-6″); ^{13}C NMR (400, MHz, DMSO-d6), δ (ppm): 12.00, 20.84, 21.94, 24.37, 28.61, 43.19, 51.25, 55.56, 68.86, 110.75, 112.13, 116.34, 119.96, 125.40, 125.53, 129.72, 132.83, 138.70, 139.55, 145.46, 151.48, 156.35, 167.61, 169.73; m/z (ESI MS): calculated for $C_{24}H_{27}ClN_4O_4$ obtained 471.84 $(M + H)^+$.