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Triazole substituted metal-free, metallo-phthalocyanines and their water soluble derivatives as potential cholinesterases inhibitors: Design, synthesis and *in vitro* inhibition study

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ABSTRACT

In this study, 1,2,3-triazole substituted metal-free and metallo phthalocyanines (**4**, **5**, **6**) and their water soluble derivatives (**4a**, **5a**, **6a**) were designed, synthesized for the first time and tested *in vitro* on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes. Most phthalocyanines exhibited good inhibitory activities on these enzymes. Among the six phthalocyanines and starting compounds, **4a** showed the most interesting profile as a submicromolar selective inhibitor of AChE (IC_{50} = 0.040 μM) and **5a** showed the most effective inhibitor of BChE (IC_{50} = 0.1198 μM).

1. Introduction

The cause of Alzheimer's disease (AD) is a neurodegenerative disease related to aging with multifactorial etiopathology known as oxidative stress, environmental conditions, genetic and endogenous factors, mitochondrial abnormalities, or neuroinflammation. The effects of this disease, especially in developed countries, are increasing with the increasing number of elderly people [1]. Regardless of the obvious need for an effective treatment for AD, medications today can stabilize some of the symptoms of early-mid-stage forms of AD for a while. Although many strategies have been foresighted in recent years, increased cholinergic neurotransmission with the inhibition of AChE enzyme is still one of the most valid treatment options for AD. The cholinesterase inhibitors (ChEIs) temporarily replenish the natural levels of neurotransmitter acetylcholine (ACh), compensating for the intense degeneration of cholinergic neurons and the cholinergic degradation that causes cholinergic conduction loss in some parts of the brain [2,3]. To date, AChE inhibition has been basically associated with the reduction of AD symptoms, but AChE plays a role in the pathogenesis of AD by affecting Aβ accumulation, as shown in both *in vitro* [4] and *in vivo* [5] studies. Recent research has shown that selective AChE/BChE inhibitors can lead to a novel pathway for anticholinesterase inhibitors (AChEI) in the treatment of AD by reducing the Aβ-induced inflammatory process [6–8]. In addition, new research results showed that AChE and BChE inhibitors may be useful in the treatment of AD. Studies in a healthy human brain showed that the BChE enzyme plays a secondary role in the hydrolysis of ACh, and that its role is dominated by AD progression in an AD brain as the level of AChE enzyme decreases and the BChE level increases [9]. Therefore AChE and BChE inhibitors are currently used for AD due to increasing the neurotransmitters [10].

Both metall-free and metallophthalocyanines (Pcs), which have very good thermal and chemical stability are important class of tetrapyrrole compounds [11,12]. Due to they have a much conjugated π-electron system, widespreadly have been used in distinct fields of technology such as sensor, electrochromism, catalysis, liquid non-linear optical devices, photochromic materials, and photodynamic therapy (PDT) [13–20]. Recently, research on phthalocyanine has focused especially on the biological and pharmaceutical applications. Phthalocyanines were investigated by our group various biological properties such as DNA binding modes and cleavage activities, topoisomerase inhibitor and carbonic anhydrase isozymes hCA I and II inhibitory, antioxidant activities and cytotoxicity effects [11,21,22].

We report herein, the synthesis and characterization of the novel soluble peripherally tetra-substituted metal-free (**4**) and metallo phthalocyanine (**5 and 6**) bearing 1,2,3-triazole group. Their cationic derivatives (**4a**, **5a** and **6a**) were also synthesized by quaternization of the nitrogen atom on the N, *N*-dimethylamino group with iodomethane. The target of this study is to synthesize novel phthalocyanine

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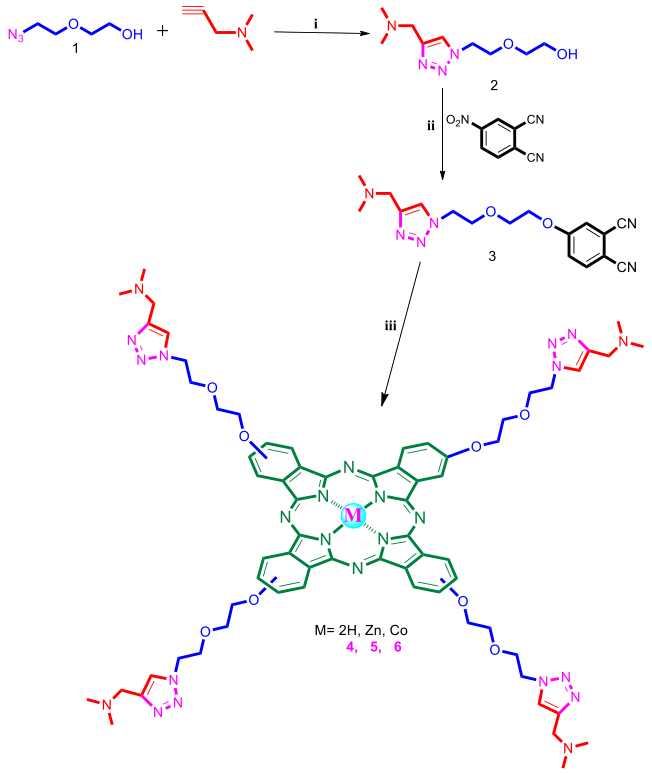

Fig. 1. The synthesis of the metal-free (**4**) and metallophthalocyanines (**5**,**6**).(i) $CuSO_4{\cdot}5H_2O$, Sodium ascorbate, *t*-BuOH/H_2O (ii) K_2CO_3, N_2, DMF. (ii) $ZnCl_2$, $CoCl_2$, *n*-pentanol, DBU, 160 °C.

compounds that inhibit AChE and BChE enzymes which are very important for the treatment of AD.

2. Results and discussion

2.1. Synthesis and characterization

All newly synthesized compounds are given in Figs. 1 and 2. In this study, 2- (2-azido-ethoxy) -ethanol (**1**) which is to be used as the beginning compound in the prepare of the phthalonitrile precursors was synthesized from the 2-(2-chloro-ethoxy)-ethanol according to the literature [23]. To synthesize the target phthalocyanines, our first initiative begined with the click reaction between N,N-dimethylprop-2-yn-1-amine and compound **1**. This reaction was carried out in the presence of $CuSO_4{\cdot}5H_2O$ (0.1 mmol) and sodium ascorbate (0.4 mmol) in BuOH:H_2O (10:10 mL) at room temperature to give 2-(2-(4-((dimethylamino)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethanol (**2**) in 88% yield. Thereafter, the phthalonitrile derivative (**3**), which would facilitate our transition to the phthalocyanines, was synthesized by the SNAr type substitution reaction of compound **2** with 4-nitrophthalonitrile in the presence of dry DMF and K_2CO_3 at 50 °C under $N_{2(g)}$.

The peripherally tetra-(2-(2-(4-((dimethylamino)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)-substituted metal-free, zinc(II) and cobalt (II) phthalocyanines (**4, 5, 6**) were synthesized by the cyclotetramerization of the phthalonitrile **3** in the presence of anhydrous Zn $(CH_3COO)_2$, $CoCl_2$ in *n*-pentanol (5 mL) and a few drops DBU under $N_{2(g)}$. Water soluble peripherally tetra-substituted metal-free, zinc(II) and cobalt(II) phthalocyanines (**4a, 5a, 6a**) were synthesized starting from phthalocyanines (**4, 5** and **6**) with an excessive of iodomethane as a quaternization reagent in chloroform at rt. The products were purified by chromatography using a mixture of methanol and dichloromethane or methanol and chloroform. The structures of all novel compounds were characterized by a combination of ^{1}H NMR, ^{13}C NMR, IR, UV–Vis, mass spectroscopic data.

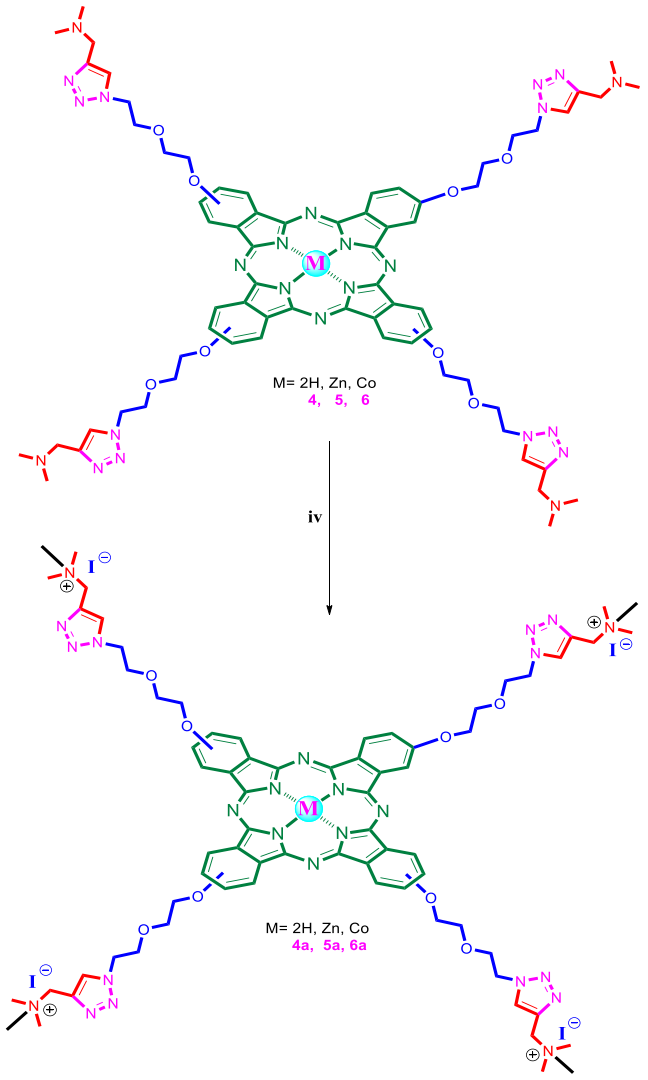

Fig. 2. The synthesis of water soluble phthalocyanines **4a, 5a, 6a**. (iv) CH_3-I, $CHCl_3$, room temperature.

In the FT-IR spectrum of 2-(2-(4-((dimethylamino)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethanol (**2**), the extinction of the vibration of the azide group present in the starting compounds and the N=N double bond peak of the triazole ring at 1590 cm^{-1} are supported by the structure of the synthesized compound for the IR spectrum.

Disappearance of peak at ~2100 cm^{-1} in FT-IR of 2-(2-azidoethoxy)ethanol (**1**) and in the ^{1}H NMR spectrum of compound **2** appearance of triazole ring proton signal at 7.96 ppm in both cases identified the success of this “click” reaction. A color change from yellow to dark green was also observed in the triazole-containing reaction solution. The CH_2–O protons arised as a triplet at 3.83 and 3.44 ppm respectively, the CH_2 protons appeared as a triplet at 4.50 and 3.68 ppm and the methyl protons appeared as a singlet at 2.10 ppm.

The ^{13}C NMR spectroscopic data of 2-(2-(4-((dimethylamino)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethanol (**2**), the signal of triazole ring olefinic carbons is observed at 141.2 and 125.1 ppm, respectively. The aliphatic carbons arised at 72.5, 69.0, 60.8, 52.9, 49.9 ppm, respectively. Also, the molecular ion peak of 2-(2-(4-((dimethylamino) methyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethanol (**2**) was found at *m/z* 215.1564 $[M+H]^+$. ^{1}H and ^{13}C NMR, Mass spectrum of compound **2** are given in supplementary data.

The structure of 4-(2-(2-(4-((dimethylamino)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)phthalonitrile (**3**) was clearly approved by the appearance of the −C≡N− band at 2230 in the FT-IR spectrum. The

^{1}H NMR spectrum of **3**, the aromatic protons appeared as a doublet, doublet and doublet at 8.00, 7.67 and 7.43 ppm, respectively. In the ^{13}C NMR spectrum the presence of nitrile carbon atoms was defined, with peaks at δ 115.8, 115.4 ppm respectively. The structure of target compound **3** were also elucidated by the appearance of [M + H] and [M + Na] ion peaks *m/z* at 341.1862 and 363.1693 respectively. After direct cyclotetramerization of phthalonitrile compound **3** into phthalocyanines **4–6** in DBU and *n*-pentanol, the sharp −C≡N vibration was no longer present in the FT-IR spectrum. The ^{1}H NMR spectrum of compound **4** was almost the same compound **3** except for some minor shifts. The ^{1}H NMR spectrum of **4** demonstrated aromatic protons at 7.90 ppm as broad signal, 7.66 and 7.08 ppm as multiplet signal. The appearance of aliphatic proton signals at 4.17, 3.64, 3.53, 1.30 (for $-CH_2-O$), 3.44, 2.94 (for $-CH_2-N$) as multiplet and 2.25 ($-CH_3$) ppm as singlet supported the formation of **4**. In the MALDI-TOF mass spectra of metal-free compound **4**, the molecular ion peak was watched at *m/z* 1363.885 $[M]^+$.

In the IR spectrums of both the metallophthalocyanine **5** and **6**, cyclotetramerization of **3** was endorsed by the disappearance of the sharp −C≡N− stretching vibration at 2230 cm^{-1}. The ^{1}H NMR and FT-IR spectra of zinc(II) and cobalt(II) phthalocyanines are very similar to that of the precursor metal-free phthalocyanine **4**. The broadening of ^{1}H NMR signals of compound **5** is probably due to the formation of aggregation due to high concentration NMR analysis. [24,25]. ^{1}H NMR analysis of the cobalt(II) phthalocyanine **6** were precluded because of its paramagnetic nature [26].

MALDI-TOF MS spectrum of tetra-substituted zinc(II) and cobalt(II) phthalocyanines (**5** and **6**) and water soluble derivatives (**4a, 5a, 6a**), the molecular ion peaks were watched at *m/z* = 1426.455 as $[M]^+$, 1420.291 as $[M]^+$, 355.837 $[M\text{-}4I]^{+4}$, 371.799 $[M\text{-}4I]^{+4}$, 370.730 $[M\text{-}4I]^{+4}$, respectively, approved the propose formula for these compounds. Mass spectrum of compound **5** and **6** are given in supplementary data. In the MALDI-TOF mass spectra of the molecular ion peak was watched at *m/z* 1363.885 $[M]^+$.

The electronic absorption spectra of phthalocyanines **4–6** in $CHCl_3$, water soluble phthalocyanines **4a–6a** in DMF at room temperature is shown in Fig. 3a and 3b. UV–Vis spectra of phthalocyanines **4–6** and their water soluble derivatives **4a-6a** exhibit intense single Q band absorption of $\pi \rightarrow \pi^*$ transitions at (703, 667), 683, 672, (703, 672), 679, 669 nm, respectively. B bands of phthalocyanines **4–6** and their water soluble derivatives **4a–6a** were observed in the UV region at 340, 355, 330, 341, 355, 329 nm, respectively.

Numerous findings have been found that cholinesterase inhibitors can be used to treat many neurodegenerative diseases such as myasthenia gravis, glaucoma, Lewy body dementia and Parkinson's disease, especially AD treatment [27–30]. In a study conducted by Tian et al., AChE inhibition of three series of tetrapyrrolic phthalocyanines carrying positive charges was tested [31]. Two porphyrin derivatives modified with piperidine salts were found to be the most potent AChE inhibitors. In this study, due to the strong inhibition of AChE and good water solubility similar to our study, it was concluded that these compounds could be used as potential candidates in the treatment of Alzheimer's disease [31]. In another study conducted in 2016 reported that zinc (II) phthalocyanines bearing some schiff base did not demonstrate AChE inhibitory effects [32]. Recently, the acetylcholinesterase/butyrylcholinesterase inhibitory effects of peripherally tetra substituted lead(II),nickel(II) and cobalt(II) phthalocyanines have been examined to determine the therapeutic potential [33].

In our earlier study, the inhibitor activity of some phthalocyanines on carbonic anhydrase I and II were investigated. These phthalocyanines shown good to moderate inhibitory activity against hCA I and II [11]. For this reason, it was decided to screen for these enzymes in molecules of similar structure. Compounds **2–6a** were evaluated for their inhibitory activity of these two cholinergic enzymes by means of the Ellman's colorimetric assay [34]. IC_{50} graphs of the interaction of all newly synthesized compounds (**2–6a**) are given in supplementary data. Rivastigmine and neostigmine (reference compounds) was used as cholinesterase inhibitors.

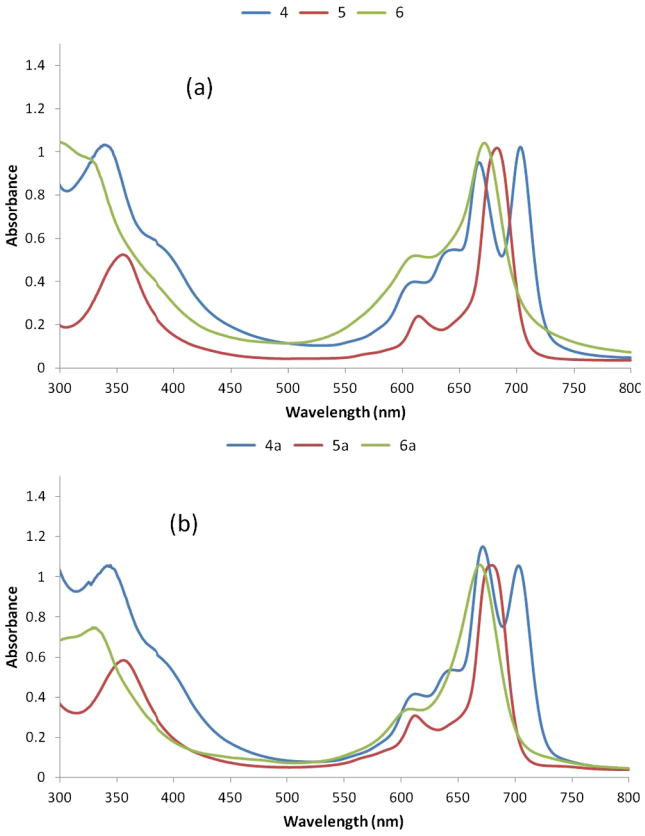

Fig. 3. (a) The UV–Vis spectrum of **4**, **5**, **6** in $CHCl_3$. Concentration = 1.00 × 10^{-5} mol × dm^{-3}. (b) The UV–Vis spectrum of water soluble **4a**, **5a**, **6a** in DMF. Concentration = 1.00 × 10^{-5} mol × dm^{-3}.

The concentration of inhibitors **2–6a** required to inhibit 50% of AChE and BChE activity was computed from different inhibitor concentrations and gived in Table 1.

A comparison of the IC_{50} values of **2–6a** indicated that their inhibition was mixed in nature, IC_{50} values of the inhibitors ranged from 0.040 to 9.762 μM for AChE and from 0.1198 to 0.8041 μM for BChE. According to these results compounds showed IC_{50} values moderate compared to the reference compounds neostigmine (IC_{50} AChE = 0.136 μM and IC_{50} BuChE = 0.084 μM) and rivastigmine (IC_{50} AChE = 0.060 μM and IC_{50} BChE = 0.014 μM) against both AChE and

Table 1
IC_{50} values of inhibitors **2-6a**, rivastigmine and neostigmine for AChE and BChE.

Tested compounds	AChE IC_{50} (μM)[a]	BuChE IC_{50} (μM)[b]	Selectivity index[c]
2	9.7620	0.7037	0.07
3	3.4480	0.8041	0.23
4	0.2237	0.2965	1.32
5	0.4642	0.1526	0.33
6	0.1509	0.1217	0.80
4a	0.0400	0.1718	4.29
5a	0.0828	0.1198	1.44
6a	0.2663	0.1426	0.53
Rivastigmine	0.0600	0.0140	0.23
Neostigmine	0.1360	0.0840	0.62

[a] IC_{50}: 50% inhibitory concentration (means ± SD of three experiments) of AChE.
[b] IC_{50}: 50% inhibitory concentration (means ± SD of three experiments) of BChE.
[c] Selectivity Index: IC_{50} of BChE/IC_{50} of AChE.

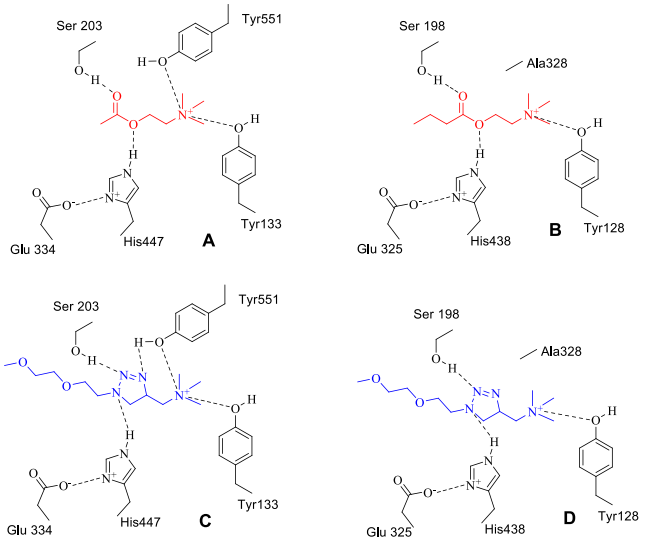

Fig. 4. (A) The binding of acetylcholine with the active region of the hAChE enzyme (PDB: 4ey4) [36]. (B) the binding pattern of butyrylcholine with the active site of the hBChE enzyme (PDB: 1p0i) [36]. (C) the estimated AChE binding form of **4b**, (D) the predicted BChE binding pattern of **4b**.

BChE. The strongest inhibition was observed with water soluble quaternized tetra substituted peripherally metal-free **4a** phthalocyanine (IC_{50} = 0.040 μM) against AChE but was 1,5 fold and 3.4 fold active compared to rivastigmine and neostigmine respectively. Water soluble quaternized zinc(II) (**5a)** (IC_{50} = 0.1198 μM) exhibited the strongest inhibition of BChE however **5a** was 8.5 fold and 1.4 fold less active compared to rivastigmine and neostigmine respectively. The IC_{50} values of all compounds followed the order 4a > 5a > 6 > 4 > 6a > 5 > 3 > 2 for AChE and 5a > 6 > 6a > 5 > 4a > 4 > 2 > 3 for BChE. They are quite similar in these small groups of N,N-dimethylamino in new compounds, considering the structure of standard drugs rivastigmine and neostigmine. These data suggested that these compounds indicated AChE and BChE inhibitory activity due to in the presence of the N,N-dimethylamino group in compounds. AChE has two active ends which are the catalytic active site (CAS) and the peripheral anionic site (PAS) [35–36]. The N,N-dimethylamino substituent and nitrogen atoms of the triazole group in both phthalocyanine precursor (**2–3**) and phthalocyanines (**4–6a**) structure could easily be predicted to be involved interacts with the catalytic and anionic site as observed in classical AChE and BChE inhibitors (Fig. 4).

3. Experimental

The used materials, equipments, cholinesterase inhibition experiments, AChE and BChE inhibition assay, were supplied as supplementary information. 2-(2-Azidoethoxy)-ethanol (**1**) was synthesized according to the literature [23].

3.1. Synthesis

3.1.1. 2-(2-(4-(Dimethylamino)methyl)-1H-1,2,3-triazol-1-yl)ethoxy) ethanol (2)

2-(2-Azidoethoxy)-ethanol (500 mg, 1 mmol), 3-dimethylamino-1-propyne (317 mg, 1 mmol) solved in *t*-BuOH:H_2O (10:10 mL). Copper sulfate pentahydrate (0.1 mmol) and sodium ascorbate (0.4 mmol) were added and the reaction was left under gentle stirring at room temperature. The reactions were monitored through TLC. Then the mixture were added H_2O (25 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried over anhydrous sodium sulfate concentrated in vacuo and purified by column chromatography using dichloromethane and methanol (97:3) to give *2-(2-(4-((dimethylamino) methyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethanol* **2**. Yield: 0.72 g (88%). IR (ATR) ν (cm^{-1}): 3368 (O—H), 2951–2875 (Aliph. C—H), 1656, 1590, 1466, 1347, 1230, 1123, 1058, 1015, 955.^{1}H NMR (400 MHz, $CDCl_3$), (δ:ppm): 7.96 (s, 1H), 4.50 (t, *J* = 4.0 Hz, 2H), 3.83 (t, *J* = 4.0 Hz, 2H), 3.68 (t, *J* = 4.0 Hz, 2H), 3.50 (br, 2H), 3.44 (t, *J* = 4.0 Hz, 2H), 2.10 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$), (δ:ppm): 141.78, 125.15, 72.59, 69.09, 60.87, 52.59, 49.90, 42.95. MS (ESI), (*m/z*) calcl. 214.14; found: $[M+H]^+$: 215.1564, $[M+Na]^+$: 237.1390.

3.1.2. 4-(2-(2-(4-((Dimethylamino)methyl)-1H-1,2,3-triazol-1-yl)ethoxy) ethoxy)phthalonitrile (3)

2-(2-(4-((Dimethylamino)methyl)-1H-1,2,3-triazol-1-yl)ethoxy) ethanol (**2**) (1,5 g, 7 mmol), 4-nitrophthalonitrile (1.21 g, 7 mmol) and K_2CO_3 (2.9 g, 21 mmol) in dry DMF (15 mL) were stirred at 60 °C for 4 days under a nitrogen atmosphere. Then, reaction mixture was poured into water and stirring at room temperature. After filtration under vacuum, the crude product was purified by column chromatography. Yield: 1.65 g (70%). IR (ATR) ν (cm^{-1}): 3078 (Ar-H), 2940–2823 (Aliph. C—H), 2230 (C≡N), 1708, 1596, 1562, 1491, 1457, 1427, 1319, 1293, 1255, 1223, 1171, 1129, 1097, 1088, 1045, 963, 838. ^{1}H NMR (400 MHz, $CDCl_3$), (δ:ppm): 8.00 (d, *J* = 8 Hz. 1H), 7.92 (s, 1H), 7.67 (d, *J* = 4 Hz. 1H), 7.43 (dd, *J* = 4 1H), 4.55 (t, *J* = 4.0 Hz, 2H), 4.29 (t, *J* = 4.0 Hz, 2H), 3.93 (t, *J* = 4.0 Hz, 2H), 3.80 (t, *J* = 4.0 Hz, 2H), 3.50 (br, 2H), 2.12 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$), (δ:ppm): 162.29, 141.75, 135.59, 124.96, 120.10, 120.02, 116.92, 115.86, 115.48, 106.81, 69.31, 68.76, 68.68, 54.62, 49.78, 43.11. MS (ESI), (*m/z*) calcl. 340.16; found: $[M+H]^+$: 341.1862, $[M+Na]^+$: 363.1693.

3.1.3. Synthesis of metal-free phthalocyanine (4)

4-(2-(2-(4-((Dimethylamino)methyl)-1H-1,2,3-triazol-1-yl)ethoxy) ethoxy)phthalonitrile **3** (0.15 g, 0.44 mmol) was dissolved in 5 mL of pentanol and was added 3 drop of 1.8-diazabicyclo[5.4.0] undec-7-ene (DBU) in mixture. The mixture was heated and stirred at 160 °C in a sealed glass tube for 24 h under N_2. After cooling to room temperature, the green crude product was precipitated with ethanol, filtered. Finally, pure metal-free phthalocyanine was obtained by column chromatography using basic aluminum oxide and $CHCl_3$:CH_3OH (100:3) as solvent system. Yield: 0.07 g (47%). mp: > 300 °C. IR (KBr Tablet), ν/cm^{-1}: 3291 (N—H), 3045 (Ar—H), 2919–2851 (Alif. C—H), 1608, 1455, 1343, 1323, 1278, 1221, 1115, 1095, 1012, 949, 849, 821, 744. ^{1}H NMR ($CDCl_3$), (δ:ppm): 7.90 (bs, 4H, Ar—H), 7.66 (m, 4H, Ar—H), 7.08 (m, 8H, Ar—H), 4.17 (m, 8H, CH_2—O), 3.64 (m, 8H, CH_2—O), 3.53 (m, 8H, CH_2—O), 3.44 (m, 8H, CH_2—N), 2.94 (m, 8H, CH_2—N), 2.25 (s, 24H, CH_3). ^{13}C NMR ($CDCl_3$), (δ:ppm): 166.24, 144.92, 137.32, 129.62, 124.02, 123.93, 117.88, 113.52, 110.45, 104.36, 69.89, 69.73, 67.77, 54.66, 48.84, 45.06. UV–Vis ($CHCl_3$), λ_{maks}(logε)nm : 703 (5.01), 667 (4.97), 641 (4.73), 608 (4.59), 340 (5.01). MALDI-TOF-MS *m/z*: 1363 $[M]^+$.

3.1.4. Synthesis of zinc (II) phthalocyanine (5)

4-(2-(2-(4-((Dimethylamino)methyl)-1H-1,2,3-triazol-1-yl)ethoxy) ethoxy)phthalonitrile **3** (0.15 g, 0.44 mmol), anhydrous $Zn(CH_3COO)_2$ (0.040 g, 0.22 mmol) and *n*-pentanol (5 mL) were placed in a standard schlenk tube in presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (3 drops) under N_2 gas atmosphere and was heated at 160 °C for 24 h. After cooling to room temperature the reaction mixture refluxed with ethanol to precipitate the product which was filtered off. The crude product was collected by filtration and washed with hot ethanol, diethyl ether and dried in vacuo. The obtained green solid product was purified with column chromatography on silica gel with chloroform:methanol (10:1) as eluents. Yield: 30 mg (19%). mp: > 300 °C. IR (ATR), ν/cm^{-1}: 3065 (Ar—H), 2919–2851 (Alif. C—H), 1606, 1487, 1453, 1394, 1367, 1335, 1279, 1222, 1116, 1090, 1049, 954, 849, 810, 745. ^{1}H NMR ($CDCl_3$), (δ:ppm): 8.76 (bs, 4H, Ar—H), 7.64–7.46 (m, 12H, Ar—H), 4.45 (bs, 16H, CH_2—O), 3.84 (m, 16H, CH_2-O,N), 2.39 (bs, 8H, CH_2-N), 1.27 (s, 24H, CH_3). ^{13}C NMR ($CDCl_3$), (δ:ppm): 160.38,

140.31, 135.31, 131.95, 131.92, 125.14, 123.86, 120.68, 118.47, 105.57, 69.64, 69.43, 67.75, 50.07, 44.81, 43.82. UV–Vis ($CHCl_3$), λ_{maks}(logε)nm : 683 (5.00), 614 (4.37), 355 (4.71). MALDI-TOF-MS *m/z*: 1426 $[M]^+$.

3.1.5. Synthesis of cobalt (II) phthalocyanine (6)

This compound was synthesized similarly to **5** from **3** by using anhydrous $CoCl_2$. The pure green solid product was obtained by column chromatography using basic aluminum oxide and $CHCl_3$: CH_3OH (10:2) as solvent system. Yield: 64 mg (36%). mp: > 300 °C. IR (ATR), ν/cm^{-1}: 3070 (Ar—H), 2921–2867 (Alif. C—H), 1608, 1520, 1455, 1408, 1337, 1279, 1227, 1119, 1093, 1062, 961, 849, 817, 753. UV–Vis ($CHCl_3$), λ_{maks}(logε)nm : 672 (5.01), 610 (4.71), 330 (4.97). MALDI-TOF-MS *m/z*: 1420 $[M]^+$.

3.1.6. Synthesis of water soluble metal-free phthalocyanine (4a)

Metal-free phthalocyanine **4** (40 mg, 0.029 mmol) was dissolved in $CHCl_3$ (4 mL) and stirred with 3 mL of iodomethane at room temperature for 1 day. The precipitate was filtered off, washed with chloroform, acetone and diethyl ether. The precipitate was dried in vacuo. Yield: 35 mg (83%), m.p. > 300 °C. IR (ATR) ν (cm^{-1}): 3008 (Ar-H), 2931–2870 (Aliph. C—H), 1607, 1473, 1343, 1322, 1281, 1232, 1114, 1096, 1051, 1016, 973, 949, 893, 827, 746. UV–Vis (DMF) λ_{max} nm (log ε): 703 (5.02), 672 (5.06), 642 (4.72), 611 (4.62), 341 (5.02). MALDI-TOF-MS *m/z* calc. 1931; found: 355 $[M\text{-}4I]^{+4}$.

3.1.7. Synthesis of water soluble zinc (II) phthalocyanine (5a)

Synthesized similarly to **4a** from **5**. Yield: 27 mg (52%). mp: > 300 °C. IR (ATR), ν/cm^{-1}: 3007 (Ar-H), 2928–2856 (Alif. C—H), 1715, 1605, 1484, 1395, 1336, 1282, 1229, 1115, 1092, 1051, 972, 954, 893, 829, 745. UV–Vis (DMF) λ_{max} nm (log ε): 679 (5.02), 612 (4.48), 355 (4.76). MALDI-TOF-MS *m/z* calc. 1994; found: 371 $[M\text{-}4I]^{+4}$.

3.1.8. Synthesis of water soluble cobalt (II) phthalocyanine (6a)

Synthesized similarly to **4a** from **6**. Yield: 32 mg (92%), m.p. > 300 °C. IR (ATR) ν (cm^{-1}): 3006, 2931–2871 (Aliph. C—H), 1716, 1607, 1522, 1471, 1409, 1338, 1279, 1231, 1118, 1094, 1061, 962, 918, 893, 822, 750. UV–Vis (DMF) λ_{max} nm (log ε): 669 (5.02), 607 (4.53), 329 (4.87). MALDI-TOF-MS *m/z* calc. 1988; found: 370 $[M\text{-}4I]^{+4}$.

4. Conclusion

To summarize, design, synthesis, characterization of novel peripherally tetra-substituted phthalocyanines and their water soluble cationic derivatives containing functional groups similar to choline group were reported for the first time and their AChE and BChE inhibitions were examined. Most of the synthesized compounds showed against both AChE and BChE inhibitory activity. Particularly, water soluble peripherally metal-free **4a** phthalocyanine (IC_{50} = 0.040 μM) showed very good inhibition against AChE while water soluble quaternized zinc (II) **(5a)** (IC_{50} = 0.1198 μM) observed the strongest inhibition against BChE. These results showed that newly synthesized phthalocyanines have potential for the treatment of AD.

Declaration of Competing Interest

The authors declare no competing interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.103100.

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