Synthesis and Biological Activity of New 2,3-dihydro-1*H*-cyclopenta[b]-quinoline Derivatives as Acetylcholinesterase Inhibitors

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Abstract: In this study we present synthesis and biological evaluation of derivatives of 4-fluorobenzoic acid and 2,3-dihydro-1*H*-cyclopenta[b]quinoline towards inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Synthesis of acquired molecules involved the reaction of condensation between the activated 4-fluorobenzoic acid and amino derivatives of 2,3-dihydro-1*H*-cyclopenta[b]quinoline. Biological testing towards the inhibition of cholinesterases was conducted according to the Ellman's spectrophotometric method. Compounds **4b** and **4e** were found to be less active in comparison with tacrine. However, compounds **4d**, **4g** and **4h** showed similar activity to tacrine. Compounds **4a**, **4c** and **4f** were more active towards inhibition of AChE than tacrine. Every synthesized compound displayed higher selectivity towards AChE and lower selectivity towards BChE in comparison with tacrine apart from compound **4b**. Compound **4b** was characterized by similar selectivity towards AChE and higher selectivity towards BChE than tacrine.

Keywords: Acetylcholinesterase inhibitors, Alzheimer's disease, Cyclopenta[b]quinoline, Ellman method, Fluorobenzoic acid, molecular docking.

INTRODUCTION

XXI st century medicine still faces numerous issues which at the current state of knowledge seem to be unsolvable. One of them is Alzheimer's disease (AD) which is characterized by a progressive neurodegeneration of the brain. Initially the symptoms (mainly memory deterioration) are almost imperceptible and frequently are considered as a common process connected with advanced age. But with time passing by the signs become more apparent. Typical AD symptoms include: progressive cognitive deterioration, neuropsychiatric and behavioral disturbances and impairment of everyday activities. [1,2] Pathologic lesions include cellular degeneration and neuronal loss which involve the cerebral cortex, initially affecting more temporal and cortical regions than the parietal and occipital cortices [3].

AD is associated with alterations of certain neurotransmitter systems in the central nervous system. The most significant changes concern a deficit of cholinergic transmission. The acetylcholine deficiency leads to the cholinergic treatment strategy whose aim is to increase the concentration of this neurotransmitter. Medicines based on this mechanism, acetylcholinesterase inhibitors (AChEIs), are best-developed and widely approved drugs for AD treatment. The first widely used AChEI, which has positive influence on cognitive function, was tacrine. Second generation AChEIs (donepezil, rivastigmine and galantamine) demonstrate greater efficacy in AD treatment. [4-6] Due to adverse side effects of these drugs, to date, the therapeutic strategies for the treatment of AD have been mainly focused on the restoration of cholinergic functionality. Furthermore, AChEIs do not only increase the level of ACh but also have other functions in pathogenesis and development of AD such as modulatory effects on cerebral blood flow, amyloid cascade, and tau phosphorylation of the inflammatory process [7]. Thus, novel compounds, such as Schiff bases of isatin [8], bisquaternary isoquinolinium derivatives [9], 7H-thiazolo[3,2-b]-1,2,4-triazin-7-one derivatives [10] that might increase the level of Ach, appear to be a promising strategy to be explored by scientists. Synthesis of the analogues of the approved drugs: donepezil, rivastigmine and galantamine is still of interest for many research groups [11,12]. The most promising novel compounds that belong to tacrine derivatives are presented in Fig. (**1**). [7]

Development of new therapies for AD is extremely important. Thus, in continuation of our research program [13], we present study concerning new derivatives of 2,3-dihydro-1*H*-cyclopenta[b]quinoline and fluorobenzoic acid. Synthesized compounds were obtained by introducing the side chain in position 9 into the structure of 2,3-dihydro-1*H*-cyclopenta[b]quinoline. After that we analyzed those molecules towards inhibition of AChE and BChE.

We introduced fluorobenzoic acid into the structure of synthesized compound because nowadays the number of drug candidates with one or more fluorine atoms is gradually

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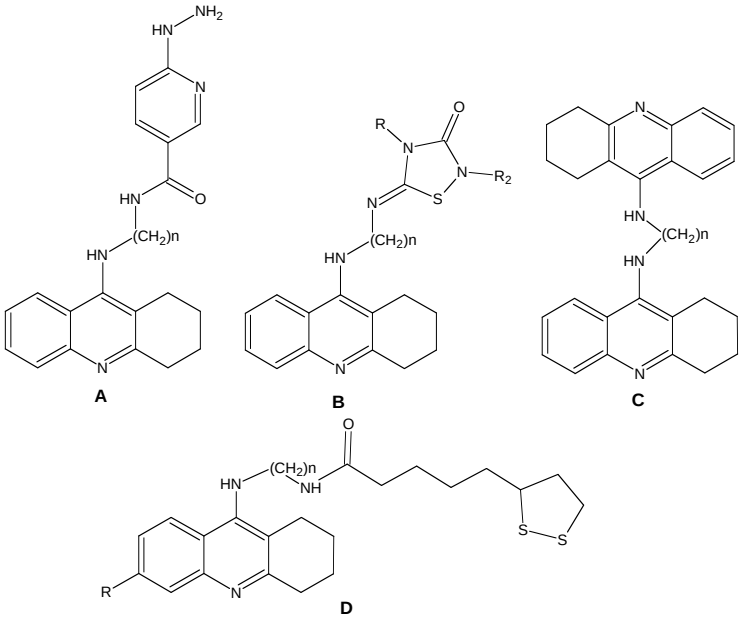


Fig. (1). Analogues of tacrine –AChE inhibitors: **A** 6-Hydrazino-N-[ω-(1,2,3,4-tetrahydroacridin-9-ylamino)alkilo]nicotinamide [8], **B** Tacrine-thiadiazolidinone Hybrids[11], **C** Bis-tacrines [12], **D** Tacrine and Lipoic Acid as a hybrid derivatives [13].

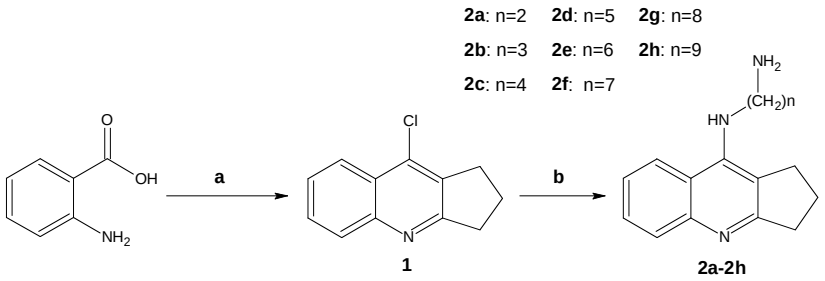
increasing and seems to become commonplace. Incorporation of fluorine atoms into drug candidate structure enables to obtain enhanced binding interactions, metabolic stability, changes in physical properties, and selective reactivity. [14]

Therefore, scientific teams search for highly selective and potent compounds with fluorine atoms in their structure as AChE inhibitors. Hu *et al*. synthesized a number of homodimeric tacrine congeners and estimated their effects on rat AChE and human BChE. They found that bis-(6-fluoro)-tacrines were more potent in inhibiting rat AChE than tacrine and the unsubstituted bis-tacrine. [15] Thus, we decided to synthesize a series of 2,3-dihydro-1*H*-cyclopenta[b]quinolone derivatives which were substituted by 4-fluorobenzoic acid.

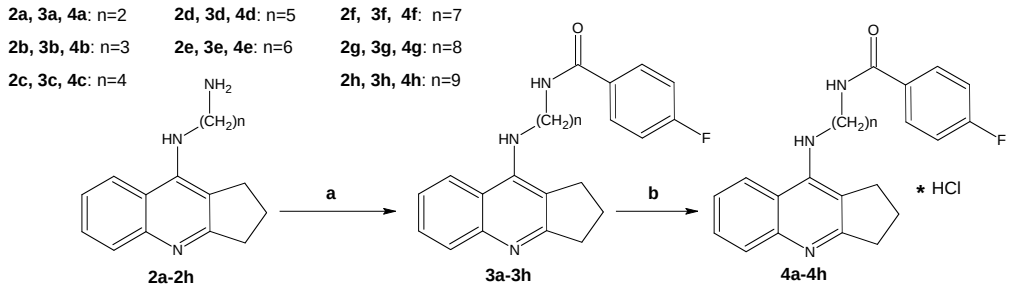
INVESTIGATIONS AND RESULTS

Synthesis

The synthesis of 9-chloro-2,3-dihydro-1*H*-cyclopenta[b-]quinoline were described in patent of Han-Zhoung Zhang [16]. This molecule was obtained by the cyclization of anthranilic acid with cyclopentanone in $POCl_3$. In the next step 1,ω-diamines were attached to the heterocycle, in similar reaction as presented in our previous paper [13]. Various 1,ω-diamines with different length of the carbon chain reacted with 9-chloro-2,3-dihydro-1*H*-cyclopenta[b]quinoline and generated compounds **(2a-2h)** (Scheme **1**) [17-22]. Afterwards we coupled 4-fluorobenzoic acid to compounds **(2a-2h)** in the presence of 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT). In this step the carboxylic group of 4-fluorobenzoic acid was activated by CDMT and *N*-methylmorpholine in tetrahydrofuran (THF). Subsequently, compounds **(2a-2h)** were added in small volume of THF to the solution of activated fluorobenzoic acid [23,24]. The mixture was mixed for 24 h. The resulting compounds **(3a-3h)** were then converted into hydrochlorides **(4a-4h)** by crystallization from HCl in ether (Scheme **2**). Barreiro *et al*. synthesized a series of derivatives of THA, where the A ring was replaced by a pyrazol ring with a phenyl substituent at different positions [25]. Unfortunately, all tested compounds appeared to be less active than tacrine. Also tacrine analogues such as pyrano[2,3-*b*]quinoline,



Scheme 1. Synthesis of compounds **1**, **2a** – **2h**. Reagents: **a:** cyclopentanone, $POCl_3$, reflux; **b:** 1,ω-diamine, phenol, NaI, reflux.



Scheme 2. Synthesis of compounds **3a – 3h** and **4a – 4h**. Reagents: **a:** 4-fluorobenzoic acid, CDMT, methylmorpholine, THF; **b:** HCl/ether.

Table 1. IC_{50} Values for Activities Towards Acetylcholinesterase and Butyrylcholinesterase

Compounds	AChE IC_{50}, [nM] ±SEM[a]	BChE IC_{50}, [nM] ±SEM[b]	Selectivity for AChE[c]	Selectivity for BChE[d]
4a	1.07 ±0.07	4.59 ±1.50	4.3	0.2
4b	10.80 ±0.50	4.70 ±0.30	0.4	2.3
4c	0.49 ±0.012	5.91 ±0.60	12.1	0.1
4d	5.83 ±0.25	14.00 ±1.40	2.4	0.4
4e	153.00 ±5.16	559.00 ±4.32	3.7	0.3
4f	2.77 ±0.19	717.00 ±7.07	258.8	0.004
4g	5.20 ±0.90	699.00 ±1.16	134.4	0.008
4h	5.48 ±0.20	744.00 ±9.89	135.8	0.007
tacrine	5.46 ±1.00	2.44 ±0.60	0.4	2.2

[a] Inhibitor concentration (means ± SEM of three experiments) for 50% inactivation of AChE
[b] Inhibitor concentration (means ± SEM of three experiments) for 50% inactivation of BChE
[c] Selectivity for AChE is defined as IC_{50}(BChE)/IC_{50}(AChE)
[d] Selectivity for BChE is defined as IC_{50}(AChE)/IC_{50}(BChE)

pyrano[3,2-*e*]pyridine and [1,8]naphthyridine synthesized by Marco and co-workers were less active in comparison with tacrine [26,27].

Within this study we describe the synthesis of 8 derivatives of 2,3-dihydro-1*H*-cyclopenta[b]quinoline which differed in the number of carbon atoms in chain linking 2,3-dihydro-1*H*-cyclopenta[b]quinoline with 4-fluorobenzoic acid.

Biological Activity

The inhibitory activity of the synthesized compounds towards cholinesterases was evaluated by spectrophotometric Ellman's method [28]. According to the procedure pioneered by Ellman, we acquired the following data concerning anticholinesterase activity of the newly synthesized compounds (Table **1**). Furthermore, values of the relative inhibitory effects towards acetylcholinesterase (ratio IC_{50} BChE/AChE) and towards butyrylcholinesterase (ratio IC_{50} AChE/BChE) are presented in Table **1**.

Every synthesized compound, apart from **4b** was characterized by higher selectivity towards AChE than tacrine. The most active compound **4c** was 55-fold more selective for AChE than tacrine. The most selective towards AChE was compound **4f**. Apart from **4b,** every synthesized compound was less selective towards BChE than tacrine.

Molecular Modeling Studies

Docking studies showed the binding mode of novel compounds. Each derivative presented similar conformation in the active center. The most interesting compound was **4c** due to its highest activity against acetylcholinesterase. GoldScore function for this compound was equaled 70.14 (AChE IC_{50} 0.49 nM) in comparison with 51.42 for tacrine (IC_{50} 5.46 nM) and 80.72 for bis-(7)-tacrine (IC_{50} 0.2 nM [15]). It occurred in extended conformation, interacting with catalytic and peripheral active site of AChE (Fig. **2**). The four carbon linker provided the best fit of the outermost moieties to both active sites. The cyclopentaquinoline fragment was engaged in formation of a characteristic sandwich due to π-π stacking with Trp84 and Phe330. Its protonated form created H-bond with His440 carbonyl group. The tether was organized in the middle of the gorge and it formed hydrophobic interactions with Tyr334. The linker amine and amide groups could create weak hydrogen bonds with carbonyl group of Ser81 and hydroxyl substituent in Tyr121, respectively. The benzamide phenyl ring

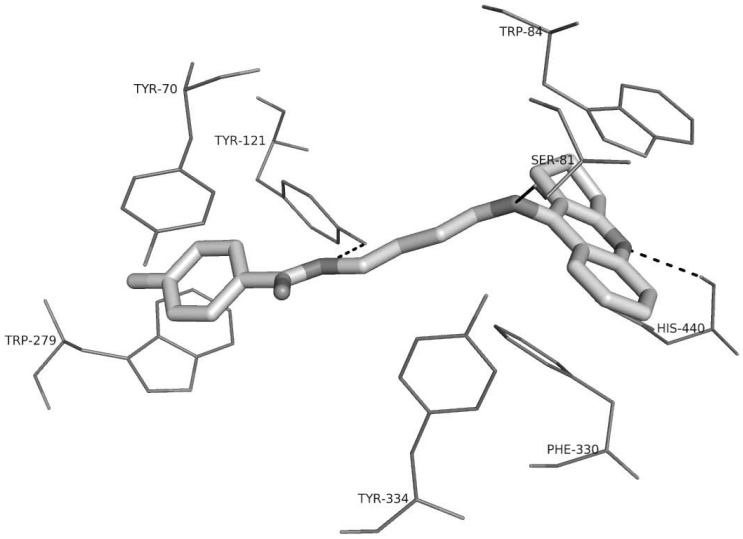


Fig. (2). Binding mode of compound **4c** with acetylcholinesterase.

interacted with Trp279 and Tyr70, forming the other sandwich. It was also engaged in hydrophobic interactions with Tyr121. Derivative **4c** appeared in bent conformation in the active gorge of butyrylcholinesterase (Fig. **3**). For this compound ChemScore function equaled 41.38 (BChE IC_{50} 5.91 nM) in comparison with 52.63 for tacrine (IC_{50} 2.44 nM). The cyclopentaquinoline moiety created π-π stacking with Trp82, was engaged in hydrophobic interaction with Trp430, Tyr440 and Met437 and could form weak H-bond with carbonyl group of His438. The tetramethylene linker was located near Tyr332. The fluorobenzamide moiety was placed in hydrophobic pocket which was built of Phe329, Leu286, Trp231, Val288 and Phe398. The compound **4c** was the most promising inhibitor among the whole series because of the highest potency against AChE and high activity against BChE. It fitted well into the active gorge of both cholinesterases, especially acetylcholinesterase.

EXPERIMENTAL SECTION

Chemistry

Anhydrous Na_2SO_4 was used to dry organic solutions during work-up and the removal of solvents was carried out under vacuum with a rotary evaporator. Progress of the reactions was monitored by TLC using 25 DC-Alufolien Kieselgel $60F_{254}$ (Merck), and detection was done by a UV Lamp (254 nm). Column chromatography was performed using silica gel 60 (200-400 mesh, Merck). IR spectra were recorded in KBr using a Mattson Infinity Series FT-IR

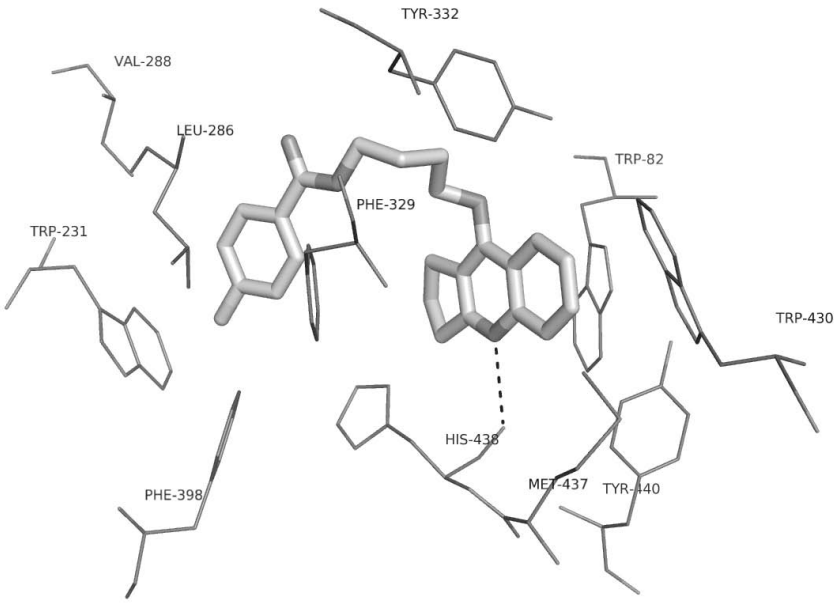


Fig. (3). Binding mode of compound **4c** with butyrylcholinesterase.

spectrophotometer. ^{1}H NMR spectra were recorded with a Varian Mercury 300 MHz spectrometer, using tetramethylsilane as an internal standard. Mass spectra were performed by the Centre of Molecular and Macromolecular Studies in Lodz (Polish Academy of Sciences).

9-chloro-2,3-dihydro-1H-cyclopenta[b]quinoline *(1)*

To a mixture of anthranilic acid (7.4 g, 53.9 mmol) and cyclopentanone (1.64 ml, 53.9 mmol) 30 ml of $POCl_3$ was carefully added in an ice bath. The mixture was heated under reflux for 2 h, then cooled at room temperature, and concentrated under reduced pressure to give slurry. The residue was diluted with ethyl acetate (50 ml), neutralized with aqueous K_2CO_3 (30 ml), and washed with brine (2 x 20 ml). The organic layer was dried over $MgSO_4$ and concentrated to dryness under reduced pressure to give a brown solid. Recrystallization from acetone gave the desired product **1** as yellow solid. Compound **1**: mp 85-87° C; yield 54%**;** IR (KBr) ν (cm^{-1}): 766.2 (Cl), 1607.2 (Ar), 2955.1 (CH2CH2CH2), 2920.7(CH2CH2CH2), 3417.8(ArN); ^{1}H NMR ($CDCl_3$) (δ ppm.): 8.1 (1H, d, *J* = 8.3 Hz, ArH), 7.9 (1H, d, *J* = 8.5 Hz, ArH), 7.6 (1H, t, *J* = 6.9 Hz, ArH), 7.4 (1H, t, *J* = 6.9 Hz, ArH), 3.0 (2H, d, *J* = 5.9 Hz, CH_2), 2.9 (2H, d, *J* = 5.0 Hz, CH_2), 1.8 (2H, t, *J* = 6.9 CH_2).

N'-(2,3-dihydro-1H-cyclopenta[b]quinolin-9-yl)ethane-1,2-diamine *(2a)*

A mixture of **1** (0.71g, 3.5 mmol), 1,2-diaminoethane (0.47 ml, 7 mmol), phenol (1.5g), and NaI (0.07g) was carefully heated at 180°C for 2 h and then cooled at room temperature. The mixture was diluted with ethyl acetate (50 ml) and made basic with 10% KOH solution (30 ml). The organic layer was washed with water (20 ml) and brine (2 x 20 ml), and then dried over $MgSO_4$ and concentrated under reduced pressure. The resulting residue was purified on silica gel chromatography (CH_2Cl_2:CH_3OH:NH_3=10:4.6:0.5) to afford **2a** as an oil. Compound **2a**: yield 64%; IR (KBr) ν (cm^{-1}): 1570.4 (NH2), 2856.4 (CH2CH2CH2), 2924.5(CH2CH2CH2), 2950.0 (CH2CH2CH2), 3355.7 (ArN); ^{1}H NMR ($CDCl_3$) (δ ppm.): 7.8 (1H, d, *J* = 8.3 Hz, ArH), 7.7 (1H, d, *J* = 8.3 Hz, ArH), 7.5 (1H, t, *J* = 6.9 Hz, ArH), 7.3 (1H, t, *J* = 8.1 Hz, ArH), 5.4 (1H, s, NH), 3.6 (2H, d, *J* = 5.4 Hz, $NHCH_2$), 3.1 (2H, t, *J* = 7.3 Hz CH_2NH_2), 2.8 - 3.0 (4H, m, CH_2), 2.6 (2H, p, *J* = 7.5, 7.5 Hz CH_2), 1.6 (2H, s, NH_2), MS (FAB) *m/z* (M+1) 228.1, 197.0, 185.0; MS-HR (FAB) Calc. for $C_{14}H_{17}N_3$: 227.1422 Found: 227.1420.

N'-(2,3-dihydro-1H-cyclopenta[b]quinolin-9-yl)propane-1,3-diamine *(2b)*

A mixture of **1** (0.71g, 3.5 mmol), 1,3-diaminopropane (0.58 ml, 7 mmol), phenol (1.5g), and NaI (0.07g) was combined as above to afford **2b** as an oil. Compound **2b**: yield 72%; IR (film) ν (cm^{-1}): 1568.8 (NH2), 2870.4 (CH2CH2CH2), 2951.9 (CH2CH2CH2), 3348.7 (ArN); ^{1}H NMR (CD_3OD) (δ ppm.): 8.0 (1H, d, *J* = 7.6 Hz, ArH), 7.7 (1H, d, *J* = 7.6 Hz, ArH), 7.6 (1H, t, *J* = 7.1 Hz, ArH), 7.4 (1H, t, *J* = 6.8 Hz, ArH), 3.7 (2H, t, *J* = 7.1 Hz, $NHCH_2$), 3.3 (2H, t, *J* = 7.1 CH_2NH_2), 3.0 (2H, t, *J* = 7.1 Hz, CH_2), 2.8 (2H, t, *J* = 7.1 Hz CH_2), 2.1 (2H, p, *J* = 7.6, 7.8 Hz CH_2), 1.9 (2H, p, *J* = 7.1, 7.1 Hz, CH_2); MS (FAB) *m/z* (M+1) 242.2, 197.0, 185.0; MS-HR (FAB) Calc. for $C_{15}H_{19}N_3$: 241.1579 Found: 241.1579.

N'-(2,3-dihydro-1H-cyclopenta[b]quinolin-9-yl)butane-1,4-diamine *(2c)*

A mixture of **1** (0.71g, 3.5 mmol), 1,6-diaminohexane (0.81g, 7 mmol), phenol (1.5g), and NaI (0.07g) was combined as above to afford **2c** as an oil. Compound **2c**: yield 52%; IR (film) ν (cm^{-1}): 1566.8 (NH2), 2865.0 (CH2CH2CH2), 2934.1(CH2CH2CH2), 3304.1 (ArN); ^{1}H NMR (CD_3OD) (δ ppm.): 8.0 (1H, d, *J* = 7.6 Hz, ArH), 7.7 (1H, d, *J* = 7.3 Hz, ArH), 7.5 (1H, t, *J* = 6.8 Hz, ArH), 7.4 (1H, t, *J* = 8.3 Hz, ArH), 3.6 (2H, t, *J* = 6.3 Hz, $NHCH_2$), 3.2 (2H, t, *J* = 7.1 CH_2NH_2), 2.9 (2H, t, *J* = 7.8 Hz, CH_2), 2.7 (2H, t, *J* = 7.1 Hz CH_2), 2.1 (2H, p, *J* = 7.6, 7.6 Hz CH_2), 1.6 -1.8 (4H, m, CH_2);MS (FAB) *m/z* (M+1) 256.2, 197.0, 185.0; MS-HR (FAB) Calc. for $C_{16}H_{21}N_3$: 255.1735 Found: 255.1733.

N'-(2,3-dihydro-1H-cyclopenta[b]quinolin-9-yl)penthane-1,5-diamine (2d)

A mixture of **1** (0.71g, 3.5 mmol), 1,5-diaminopentane (0.82 ml, 7 mmol), phenol (1.5g), and NaI (0.07g) was combined as above to afford **2d** as an oil. Compound **2d**: yield 75%; IR (film) ν (cm^{-1}): 1567.9 (NH2), 2856.5 (CH2CH2CH2), 2931.8(CH2CH2CH2), 3310.4 (ArN); ^{1}H NMR ($CDCl_3$) (δ ppm.): 7.8 (1H, d, *J* = 7.3 Hz, ArH), 7.6 (1H, d, *J* = 7.5 Hz, ArH), 7.5 (1H, t, *J* = 6.9 Hz, ArH), 7.3 (1H, t, *J* = 6.9 Hz, ArH), 4.6 (1H, s, NH), 3.5 (2H, m, $NHCH_2$), 3.1 (2H, t, *J* = 6.9 Hz, CH_2), 3.0 (2H, t, *J* = 7.7 Hz, CH_2NH_2), 2.6 (2H, t, *J* = 6.7 Hz, CH_2), 2.1 (2H, p, *J* = 3.9, 3.8 Hz, CH_2), 1.9 (2H, s, NH_2), 1.6 (2H, p, *J* = 7.3, 6.5 Hz, CH_2), 1.3 - 1.5 (4H, br, CH_2); MS (FAB) *m/z* (M+1) 270.3, 197.1, 185.0; MS-HR (FAB) Calc. for $C_{17}H_{23}N_3$: 269.1892 Found: 270.1971 (M+1).

N'-(2,3-dihydro-1H-cyclopenta[b]quinolin-9-yl)hexane-1,6-diamine (2e)

A mixture of **1** (0.71g, 3.5 mmol), 1,6-diaminohexane (0.82g, 7 mmol), phenol (1.5g), and NaI (0.07g) was combined as above to afford **2e** as an oil. Compound **2e**: yield 64%; IR (film) ν (cm^{-1}): 1567.5 (NH2), 2855.0 (CH2CH2CH2), 2928.4(CH2CH2CH2), 3350.4 (ArN); ^{1}H NMR (CD_3OD) (δ ppm.): 8.0 (1H, d, *J* = 8.3 Hz, ArH), 7.7 (1H, d, *J* = 8.3 Hz, ArH), 7.5 (1H, t, *J* = 7.8 Hz, ArH), 7.3 (1H, t, *J* = 7.8 Hz, ArH), 3.6 (2H, t, *J* = 7.1 Hz, $NHCH_2$), 3.2 (2H, t, *J* = 7.1 Hz, CH_2), 3.0 (4H, t, *J* = 7.8 Hz, CH_2NH_2), 2.6 (2H, t, *J* = 6.8 Hz, CH_2), 2.1 (2H, p, *J* = 7.6, 7.3 Hz, CH_2), 1.6 - 1.7 (2H, m, CH_2), 1.3 - 1.5 (6H, br, CH_2); MS (FAB) *m/z* (M+1) 284.3, 197.0, 185.0; MS-HR (FAB) Calc. for $C_{18}H_{25}N_3$: 283.2048 Found: 283.2043.

N'-(2,3-dihydro-1H-cyclopenta[b]quinolin-9-yl)heptane-1,7-diamine (2f)

A mixture of **1** (0.75g, 3.5 mmol), 1,7-diaminoheptane (0.92g, 7 mmol), phenol (1.5g), and NaI (0.07g) was combined as above to afford **2f** as an oil. Compound **2f**: yield 72%; IR (film) ν (cm^{-1}): 1567.9 (NH2), 2853.9 (CH2CH2CH2), 2927.9(CH2CH2CH2), 3294.4 (ArN); ; ^{1}H NMR ($CDCl_3$) (δ ppm.): 7.9 (1H, d, *J* = 7.5 Hz, ArH), 7.7 (1H, t, *J* = 8.3 Hz, ArH), 7.5 (1H, t, *J* = 7.1 Hz, ArH), 7.4 (1H, t, *J* = 6.9 Hz, ArH), 4.6 (1H, s, NH), 3.6 (2H, m,

$NHCH_2$), 3.3 (2H, t, *J* = 7.5 Hz, CH_2), 3.0 (2H, t, *J* = 7.7 Hz, CH_2NH_2), 2.6 (2H, t, *J* = 6.7 Hz, CH_2), 2.1 (2H, p, *J* = 7.7, 7.3 Hz, CH_2), 1.7 (2H, s, NH_2), 1.5 - 1.6 (2H, m, CH_2CH_2), 1.2 - 1.5 (8H, br, CH_2CH_2); MS (FAB) *m/z* (M+1) 298.3, 197.0, 185.0; MS-HR (FAB) Calc. for $C_{19}H_{27}N_3$: 297.2205 Found: 298.2291 (M+1).

N'-(2,3-dihydro-1H-cyclopenta[b]quinolin-9-yl)octane-1,8-diamine (2g)

A mixture of **1** (0.71g, 3.5 mmol), 1,8-diaminooctane (1.00g, 7 mmol), phenol (1.5g), and NaI (0.07g) was combined as above to afford **2g** as an oil. Compound **2g**: yield 68%; IR (film) ν (cm^{-1}): 1567.2 (NH2), 2853.2 (CH2CH2CH2), 2925.5(CH2CH2CH2), 3329.5 (ArN); ; ^1H NMR ($CDCl_3$) (δ ppm.): 7.8 (1H, d, *J* = 8.3 Hz, ArH), 7.5 (1H, t, *J* = 7.3 Hz, ArH), 7.3 (1H, t, *J* = 7.5 Hz, ArH), 4.5 (1H, s, NH), 3.5 (2H, m, $NHCH_2$), 3.1 (2H, t, *J* = 7.1 Hz, CH_2), 3.0 (2H, d, *J* = 7.7 Hz, CH_2NH_2), 2.6 (2H, t, *J* = 6.5 Hz, CH_2), 1.5 - 1.6 (4H, br, CH_2, NH_2), 1.1 - 1.5 (10H, br, CH_2); MS (FAB) *m/z* (M+1) 312.5, 197.1, 185.1; MS-HR (FAB) Calc. for $C_{20}H_{29}N_3$: 311.2361 Found: 312.2444 (M+1).

N'-(2,3-dihydro-1H-cyclopenta[b]quinolin-9-yl)nonane-1,9-diamine (2h)

A mixture of **1** (0.71g, 3.5 mmol), 1,9-diaminononane (1.10g, 7 mmol), phenol (1.5g), and NaI (0.07g) was combined as above to afford **2h** as an oil. Compound **2h**: yield 66%; IR (film) ν (cm^{-1}): 1568.0 (NH2), 2852.5 (CH2CH2CH2), 2925.7 (CH2CH2CH2), 3294.4 (ArN); ; ^1H NMR ($CDCl_3$) (δ ppm.): 7.8 (1H, d, *J* = 7.3 Hz, ArH), 7.6 (1H, d, *J* = 7.5 Hz, ArH), 7.5 (1H, t, *J* = 6.9 Hz, ArH), 7.2 (1H, t, *J* = 6.9 Hz, ArH), 4.6 (1H, s, NH), 3.5 (2H, m, $NHCH_2$), 3.1 (2H, t, *J* = 7.1 Hz CH_2), 3.0 (2H, t, *J* = 7.7 Hz, CH_2NH_2), 2.6 (2H, t, *J* = 6.9 Hz CH_2), 2.1 (2H, p, *J* = 7.7, 7.7 Hz CH_2), 1,7 (2H, s, NH_2), 1.5 - 1.6 (2H, m, CH_2CH_2), 1.1 - 1.3 (14H, br, CH_2CH_2); MS (FAB) *m/z* (M+1) 326.3, 185.0; MS-HR (FAB) Calc. for $C_{21}H_{31}N_3$: 325.2518 Found: 326.2609 (M+1).

N-[2-(2,3-dihydro-1H-cyclopenta[b]quinolin-9-ylamino)ethyl]-4-fluorobenzamide (3a)

To a stirred mixture of 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) (1.76g, 10.2 mmol), 4-fluorobenzoic acid (1.43 g, 10.2 mmol) in THF (8 ml), *N*-methylmorpholine (1.12 ml, 10.2 mmol) was added dropwise at such a rate as to keep temperature at -5°C to 0°C. Stirring has been continued at 0°C for 1- 4 h until all CDMT has been consumed. Then to the crude mixture, obtained as described above **2a** (2.32 g, 10.2 mmol) in THF (10 ml), at -5°C to 0°C, was added. Stirring has been continued at 0°C for 2 h, and then for 12 h at room temperature. Precipitate formed and was isolated by filtration. Recrystallization from ethyl acetate afforded the desired product **3a** as yellow solid. Compound **3a**: mp 220-222°C; yield 60%; IR (KBr) ν (cm^{-1}): 765.4 (F), 1587.4 (NH), 1633.4 (NHCO), 2846.0 (CH2CH2CH2), 2929.5 (CH2CH2CH2), 3264.4 (CON); ^1H NMR (CD_3OD) (δ ppm.): 8.3 (1H, d, *J* = 8.1 Hz, ArH), 7.8 – 7.9 (4H, m, ArH), 7.7 (1H, d, *J* = 7.1 Hz, ArH), 7.6 (1H, t, *J* = 7.1 Hz, ArH), 7.2 (1H, t, *J* = 8.8 Hz, ArH), 4.1 (2H, t, *J* = 5.6 CH_2NH_2), 3.7 (2H, t, *J* = 6.1 Hz, CH_2), 3.4 (2H, t, *J* = 7.1 Hz CH_2), 3.1 (2H, t, *J* = 8.1 Hz, CH_2), 2.3 (2H, p, *J* = 7.8, 7.8 Hz, CH_2); MS (FAB) *m/z* (M+1) 350.1, 185.0; MS-HR (FAB) Calc. for $C_{21}H_{20}FN_3O$: 349.1590 Found: 350.1669 (M+1).

N-[3-(2,3-dihydro-1H-cyclopenta[b]quinolin-9-ylamino)propyl]-4-fluorobenzamide (3b)

A mixture of 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) (1.76g, 10.2 mmol), 4-fluorobenzoic acid (1.43g, 10.2 mmol), *N*-methylmorpholine (1.12 ml, 10.2 mmol) and **2b** (2.60g, 10.2 mmol) in THF (10 ml) were combined as above to afford **3b** as yellow solid. Compound **3b**: mp 112-114°C; yield 58%; IR (KBr) ν (cm^{-1}): 765.0 (F), 1585.8 (NH), 1661.5 (NHCO), 2736.9 (CH2CH2CH2), 2786.8 (CH2CH2CH2), 3239.7 (CON); ^1H NMR (CD_3OD) (δ ppm.): 8.3 (1H, d, *J* = 8.5 Hz, ArH), 7.8 – 7.9 (4H, m, ArH), 7.7 (1H, d, *J* = 9.3 Hz, ArH), 7.6 (1H, t, *J* = 7.3 Hz, ArH), 7.2 (1H, t, *J* = 6.8 Hz, ArH), 3.9 (2H, t, *J* = 7.1 Hz, $NHCH_2$), 3.6 (2H, t, *J* = 7.1 CH_2NH_2), 3.4 (2H, t, *J* = 7.3 Hz, CH_2), 3.1 (2H, t, *J* = 8.1 Hz CH_2), 2.3 (2H, p, *J* = 7.8, 7.3 Hz, CH_2), 2.0 (2H, p, *J* = 6.1, 6.6 Hz, CH_2); MS (FAB) *m/z* (M+1) 364.2, 185.0; MS-HR (FAB) Calc. for $C_{22}H_{22}FN_3O$: 363.1747 Found: 364.1825 (M+1).

N-[4-(2,3-dihydro-1H-cyclopenta[b]quinolin-9-ylamino)buthyl]-4-fluorobenzamide (3c)

A mixture of 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) (1.76g, 10 mmol), 4-fluorobenzoic acid (1.43g, 10.2 mmol), *N*-methylmorpholine (1.12 ml, 10.2 mmol) and **2c** (3.03 g, 10.2 mmol) in THF (10 ml) were combined as above to afford **3c** as yellow solid. Compound **3c**: mp 176-178°C; yield 64%; IR (KBr) ν (cm^{-1}): 762.2 (F), 1561.1 (NH), 1634.7 (NHCO), 2871.8 (CH2CH2CH2), 2934.4 (CH2CH2CH2), 3297.1 (CON); ^1H NMR (CDOD) (δ ppm.): 8.3 (1H, d, *J* = 8.1 Hz, ArH), 8.2 (1H, d, *J* = 7.8 Hz, ArH), 7.7 - 7.9 (3H, m, ArH), 7.5 - 7.6 (2H, m, ArH), 7.1 (1H, t, *J* = 8.5 Hz, ArH), 3.7 - 4.1 (4H, m, CH_2), 3.2 - 3.4 (4H, m, CH_2), 2.2 (2H, p, *J* = 7.1, 7.3 Hz, CH_2), 1.8 - 1.9 (4H, m, CH_2) ;MS (FAB) *m/z* (M+1) 378.3, 185.0; MS-HR (FAB) Calc. for $C_{23}H_{24}FN_3O$: 377.1903 Found: 378.1982 (M+1).

N-[5-(2,3-dihydro-1H-cyclopenta[b]quinolin-9-ylamino)penthyl]-4-fluorobenzamide (3d)

A mixture of 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) (1.76g, 10 mmol), 4-fluorobenzoic acid (1.43g, 10.2 mmol), *N*-methylmorpholine (1.12 ml, 10.2 mmol) and **2d** (2.75g, 10.2 mmol) in THF (10 ml) were combined as above to afford **3d** as yellow solid. Compound **3a**: mp 100-103°C; yield 62%; IR (KBr) ν (cm^{-1}): 766.6 (F), 1584.1 (NH), 1632.5 (NHCO), 2862.8 (CH2CH2CH2), 2933.7 (CH2CH2CH2), 3261.5 (CON); ^1H NMR (CDOD) (δ ppm.): 8.3 (1H, d, *J* = 8.5 Hz, ArH), 7.6 - 7.8 (5H, m, ArH), 7.6 (1H, t, *J* = 1.5 Hz, ArH), 7.1 (1H, t, *J* = 6.6 Hz, ArH), 3.8 (2H, t, *J* = 7.6 Hz CH_2), 3.4 (2H, m, CH_2), 3.1 (2H, t, *J* = 7.8 Hz, CH_2NH_2), 2.9 (2H, m, CH_2), 2.3 (2H, p, *J* = 7.8, 7.6 Hz, CH_2), 1.8 (4H, m, CH_2), 1.7 (2H, m, CH_2), 1.5 (2H, m, CH_2);MS (FAB) *m/z* (M+1) 392.3, 185.0; MS-HR (FAB) Calc. for $C_{24}H_{26}FN_3O$: 391.2060 Found: 392.2128 (M+1).

N-[6-(2,3-dihydro-1H-cyclopenta[b]quinolin-9-ylamino)hexyl]-4-fluorobenzamide (3e)

A mixture of 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) (1.76g, 10.2 mmol), 4-fluorobenzoic acid (1.43g,

10.2 mmol), *N*-methylmorpholine (1.12 ml, 10.2 mmol) and **2e** (2.89 g, 10.2 mmol) in THF (10 ml) was combined as above to afford **3e** as yellow solid. Compound **3e**: mp 125-127°C; yield 38%; IR (KBr) ν (cm^{-1}): 764.9 (F), 1584.2 (NH), 1632.7 (NHCO), 2857.3 (CH2CH2CH2), 2932.7 (CH2CH2CH2), 3222.6 (CON); ^{1}H NMR (CD_3OD) (δ ppm.): 8.3 (1H, d, *J* = 8.1 Hz, ArH), 7.8 - 7.9 (3H, m, ArH), 7.7 (1H, d, *J* = 7.8 Hz, ArH), 7.6 (1H, t, *J* = 7.3 Hz, ArH), 7.2 (1H, t, *J* = 6.8 Hz, ArH), 3.8 (2H, t, *J* = 7.1 Hz CH_2), 3.7 (2H, t, *J* = 6.3 Hz CH_2), 3.4 (2H, t, *J* = 6.1 Hz, CH_2NH_2), 3.2 (2H, t, *J* = 7.8 Hz, CH_2), 2.3 (2H, p, *J* = 7.6, 7.6 Hz, CH_2), 1.8 (2H, m, CH_2), 1.7 (2H, m, CH_2), 1.4 - 1.5 (4H, m, CH_2); MS (FAB) *m/z* (M+1) 406.4, 183.1; MS-HR (FAB) Calc. for $C_{25}H_{28}FN_3O$: 405.2216 Found: 406.2293 (M+1).

N-[7-(2,3-dihydro-1H-cyclopenta[b]quinolin-9-ylamino)heptyl]-4-fluorobenzamide (3f).

A mixture of 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) (1.76g, 10.2 mmol), 4-fluorobenzoic acid (1.43g, 10.2 mmol), *N*-methylmorpholine (1.12 ml, 10.2 mmol) and **2f** (3.03 g, 10.2 mmol) in THF (10 ml) was combined as above to afford **3f** as yellow solid. Compound **3c**: mp 105-107°C; yield 40%; IR (KBr) ν (cm^{-1}):1565.2.4 (NH), 1632.4 (NHCO), 2847.5 (CH2CH2CH2), 2933.0 (CH2CH2CH2), 3259.7 (CON); ^{1}H NMR (CD_3OD) (δ ppm.): 8.3 (1H, d, *J* = 8.1 Hz, ArH), 7.8 - 7.9 (3H, m, ArH), 7.7 (1H, d, *J* = 7.8 Hz, ArH), 7.6 (1H, t, *J* = 7.3 Hz, ArH), 7.2 (1H, t, *J* = 6.8 Hz, ArH), 3.8 (2H, t, *J* = 7.3 Hz CH_2), 3.7 (2H, t, *J* = 6.8 Hz CH_2), 3.4 (2H, m, CH_2NH_2), 3.2 (2H, t, *J* = 7.8 Hz, CH_2), 2.3 (2H, p, *J* = 7.8, 7.8 Hz, CH_2), 1.8 (2H, m, CH_2), 1.6 (2H, m, CH_2), 1.4 - 1.5 (6H, m, CH_2); MS (FAB) *m/z* (M+1) 420.4, 183.0; MS-HR (FAB) Calc. for $C_{26}H_{30}FN_3O$: 419.2373 Found: 420.2446 (M+1).

N-[8-(2,3-dihydro-1H-cyclopenta[b]quinolin-9-ylamino)octyl]-4-fluorobenzamide (3g).

A mixture of 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) (1.76g, 10.2 mmol), 4-fluorobenzoic acid (1.43 g, 10.2 mmol), *N*-methylmorpholine (1.12 ml, 10.2 mmol) and **2g** (3.18 g, 10.2 mmol) in THF (10 ml) was combined as above to afford **3g** as yellow solid. Compound **3d**: mp 121-123°C; yield 35%; IR (KBr) ν (cm^{-1}): 764.9 (F), 1565.2 (NH), 1680.0 (NHCO), 2868.4 (CH2CH2CH2), 2928.9 (CH2CH2CH2), 3227.0 (CON); ^{1}H NMR (CD_3OD) (δ ppm.): 8.3 (1H, d, *J* = 8.1 Hz, ArH), 7.8 - 7.9 (3H, m, ArH), 7.7 (1H, d, *J* = 7.1 Hz, ArH), 7.6 (1H, t, *J* = 7.1 Hz, ArH), 7.2 (1H, t, *J* = 6.8 Hz, ArH), 3.9 (2H, m, CH_2), 3.7 (2H, t, *J* = 7.3 Hz CH_2), 3.4 (2H, m, CH_2NH_2), 3.2 (2H, t, *J* = 7.8 Hz, CH_2), 2.3 (2H, p, *J* = 7.8, 7.8 Hz, CH_2), 1.8 (2H, m, CH_2), 1.6 (2H, m, CH_2), 1.4 - 1.5 (8H, m, CH_2); MS (FAB) *m/z* (M+1) 434.4, 185.0; MS-HR (FAB) Calc. for $C_{27}H_{32}FN_3O$: 433.2529 Found: 434.2603 (M+1).

N-[9-(2,3-dihydro-1H-cyclopenta[b]quinolin-9-ylamino)nonyl]-4-fluorobenzamide (3h).

A mixture of 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) (1.76g, 10.2 mmol), 4-fluorobenzoic acid (1.43 g, 10.2 mmol), *N*-methylmorpholine (1.12 ml, 10.2 mmol) and **2h** (3.32 g, 10.2 mmol) in THF (10 ml) was combined as above to afford **3h** as yellow solid. Compound **3e**: mp 128-130°C; yield 36%; IR (KBr) ν (cm^{-1}): 749.4 (F), 1565.5 (NH), 1680.4 (NHCO), 2866.5 (CH2CH2CH2), 2926.9 (CH2CH2CH2), 3217.5 (CON); ^{1}H NMR (CD_3OD) (δ ppm.): 8.3 (1H, d, *J* = 8.5 Hz, ArH), 7.8 - 7.9 (3H, m, ArH), 7.7 (1H, d, *J* = 7.1 Hz, ArH), 7.6 (1H, t, *J* = 7.1 Hz, ArH), 7.2 (1H, t, *J* = 8.5 Hz, ArH), 3.9 (2H, m, CH_2), 3.8 (2H, t, *J* = 7.3 Hz CH_2), 3.4 (2H, t, *J* = 8.3 Hz, CH_2NH_2), 3.2 (2H, t, *J* = 7.6 Hz, CH_2), 2.3 (2H, p, *J* = 7.8, 7.6 Hz, CH_2), 1.8 (2H, m, CH_2), 1.6 (2H, m, CH_2), 1.3 - 1.4 (10H, m, CH_2); MS (FAB) *m/z* (M+1) 448.3, 185.0; MS-HR (FAB) Calc. for $C_{28}H_{34}FN_3O$: 447.26859 Found: 448.2763 (M+1).

N-[2-(2,3-dihydro-1H-cyclopenta[b]quinolin-9-ylamino)ethyl]-4-fluorobenzamide hydrochloride (4a).

A solution of HCl in ether has been prepared by bubbling HCl into ether (50 ml) at a moderate rate for 20 min. **3a** (0.20 g, 0.5183 mmol) was dissolved in ether (2 ml), HCl/ether (4 ml) was added, and the reaction mixture was stirred at room temperature. After 2 min, the solution became cloudy and precipitate formed. The precipitate was isolated by filtration and the solid was washed with ether and dried in vacuum. Compound **4a**: mp >280°C; yield 28%; IR (KBr) ν (cm^{-1}): 764.0 (F), 1563.6 (NH), 1634.3 (NHCO), 2851.0 (CH2CH2CH2), 2931.8 (CH2CH2CH2), 3263.6 (CON); ^{1}H NMR (DMSO) (δ ppm.): 14.0 (1H, s, HCl), 8.9 (1H, m, NH), 8.5 (1H, d, *J* = 8.3 Hz, ArH), 7.8 - 7.9 (5H, m, ArH), 7.6 (1H, t, *J* = 7.3 Hz, ArH), 7.3 (1H, t, *J* = 9.1 Hz, ArH), 3.9 (2H, m, CH_2), 3.6 (2H, m, CH_2), 3.5 (3H, m, NH, CH_2), 3.1 (2H, t, *J* = 8.1 Hz, CH_2), 2.1 (2H, m, CH_2).

N-[3-(2,3-dihydro-1H-cyclopenta[b]quinolin-9-ylamino)propyl]-4-fluorobenzamide hydrochloride (4b).

A **3b** (0.20 g, 0.4831 mmol) were combined as above to afford **4b** as yellow solid. Compound **4b**: mp >280°C; yield 24%; IR (KBr) ν (cm^{-1}): 751.3 (F), 1563.7 (NH), 1667.8 (NHCO), 2848.4 (CH2CH2CH2), 2932.0 (CH2CH2CH2), 3265.9 (CON); ^{1}H NMR (DMSO) (δ ppm.): 14.0 (1H, s, HCl), 8.8 (1H, m, NH), 8.5 (1H, d, *J* = 8.9 Hz, ArH), 7.8 - 7.9 (5H, m, ArH), 7.6 (1H, t, *J* = 6.5 Hz, ArH), 7.3 (1H, t, *J* = 8.9 Hz, ArH), 3.7 – 3.8 (2H, m, CH_2), 3.3 - 3.4 (3H, m, NH, CH_2), 3.2 (2H, t, *J* = 7.7 Hz, CH_2), 3.1 (2H, t, *J* = 7.9 Hz, CH_2), 2.1 (2H, m, CH_2), 1.9 (2H, m, CH_2).

N-[4-(2,3-dihydro-1H-cyclopenta[b]quinolin-9-ylamino)butyl]-4-fluorobenzamide hydrochloride (4c).

A **3c** (0.20 g, 0.4673 mmol) were combined as above to afford **4c** as brown solid. Compound **4c**: mp >280°C; yield 33%; IR (KBr) ν (cm^{-1}): 762.6 (F), 1560.8 (NH), 1632.4 (NHCO), 2851.2 (CH2CH2CH2), 2936.1 (CH2CH2CH2), 3308.8 (CON); ^{1}H NMR (DMSO) (δ ppm.): 13.9 (1H, s, HCl), 8.7 (1H, m, NH), 8.4 (1H, d, *J* = 8.7 Hz, ArH), 7.7 - 7.9 (4H, m, ArH), 7.5 -7.6 (2H, m, ArH), 7.2 - 7.3 (1H, m, ArH), 3.7 – 3.9 (2H, m, CH_2), 3.2 - 3.5 (3H, m, NH, CH_2), 3.0 (2H, t, *J* = 7.9 Hz, CH_2), 2.8 – 2.9 (2H, m, CH_2), 2.0 – 2.1 (2H, m, CH_2), 1.6 – 1.7 (4H, m, CH_2).

N-[5-(2,3-dihydro-1H-cyclopenta[b]quinolin-9-ylamino)penthyl]-4-fluorobenzamide hydrochloride (4d)

A **3d** (0.20 g, 0.5109 mmol) were combined as above to afford **4d** as brown solid. Compound **4d**: mp >300°C; yield 30%; IR (KBr) ν (cm^{-1}): 764.3 (F), 1568.7 (NH), 1632.2 (NHCO), 2737.4 (CH2CH2CH2), 2937.1 (CH2CH2CH2), 3243.0 (CON); ^{1}H NMR (DMSO) (δ ppm.): 14.2 (1H, s,

HCl), 8.8 (1H, m, NH), 8.5 (1H, d, *J* = 5.4 Hz, ArH), 7.7 - 7.9 (5H, m, ArH), 7.5 -7.6 (1H, m, ArH), 7.2 (1H, t, *J* = 8.7 Hz, ArH), 3.5 – 3.6 (5H, m, NH, CH_2), 3.3 (2H, t, *J* = 7.3, CH_2), 3.1 (2H, t, *J* = 7.9, CH_2), 2.1 (2H, p, *J* = 7.7, 7.3, CH_2), 1.7 (2H, m, CH_2), 1.6 (2H, p, *J* = 7.1, 6.7, CH_2), 1.4 (2H, m, CH_2).

N-[6-(2,3-dihydro-1H-cyclopenta[b]quinolin-9-ylamino)hexyl]-4-fluorobenzamide hydrochloride (4e).

A **3e** (0.20 g, 0.4932 mmol) was combined as above to afford **4e** as yellow solid. Compound **4e**: mp 240-242°C; yield 28%; IR (KBr) ν (cm^{-1}): 762.3.4 (F), 1566.7 (NH), 1633.6 (NHCO), 2854.6 (CH2CH2CH2), 2929.9 (CH2CH2CH2), 3196.1 (CON); ^{1}H NMR (DMSO) (δ ppm.): 14.0 (1H, s, HCl), 8.7 (1H, m, NH), 8.5 (1H, m, ArH), 7.8 - 7.9 (3H, m, ArH), 7.6 -7.7 (2H, m, ArH), 7.2 (1H, t, *J* = 8.9 Hz, ArH), 3.7 (4H, m, NH CH_2, CH_2), 3.2 -3.3 (3H, m, NH, CH_2), 3.1 (2H, t, *J* = 7.9, CH_2), 2.1 (2H, m, CH_2), 1.7 (2H, m, CH_2), 1.4 (2H, m, CH_2), 1.2 (4H, m, CH_2).

N-[7-(2,3-dihydro-1H-cyclopenta[b]quinolin-9-ylamino)heptyl]-4-fluorobenzamide hydrochloride (4f).

A **3f** (0.20 g, 0.4757 mmol) was combined as above to afford **4f** as yellow solid. Compound **4f**: mp 179-181°C; yield 25%; IR (KBr) ν (cm^{-1}): 764.0 (F), 1562.7 (NH), 1633.7 (NHCO), 2853.3 (CH2CH2CH2), 2928.0 (CH2CH2CH2), 3227.8 (CON); ^{1}H NMR (DMSO) (δ ppm.): 14.0 (1H, s, HCl), 8.7 (1H, m, NH), 8.5 (1H, d, *J* = 7.3 Hz, ArH), 7.8 - 7.9 (5H, m, ArH), 7.6 (1H, t, *J* = 6.8 Hz, ArH), 7.3 (1H, t, *J* = 8.9 Hz, ArH), 3.7 (2H, m, $NHCH_2$), 3.2 -3.3 (5H, m, NH, CH_2), 3.1 (2H, t, *J* = 7.9, CH_2), 2.1 (2H, m, CH_2), 1.6 (2H, m, CH_2), 1.5 (2H, m, CH_2), 1.2 (6H, m, CH_2).

N-[8-(2,3-dihydro-1H-cyclopenta[b]quinolin-9-ylamino)octyl]-4-fluorobenzamide hydrochloride (4g).

A **3g** (0.20 g, 0.4613 mmol) was combined as above to afford **4g** as brown solid. Compound **4g**: mp 174-176°C; yield 44%; IR (KBr) ν (cm^{-1}): 764.0 (F), 1562.4 (NH), 1632.8 (NHCO), 2855.1 (CH2CH2CH2), 2924.3 (CH2CH2CH2), 3218.1 (CON); ^{1}H NMR (DMSO) (δ ppm.): 14.0 (1H, s, HCl), 8.7 (1H, m, NH), 8.5 (1H, d, *J* = 8.1 Hz, ArH), 7.8 - 7.9 (5H, m, ArH), 7.6 (1H, t, *J* = 6.8 Hz, ArH), 7.3 (1H, t, *J* = 8.9 Hz, ArH), 3.7 (2H, m, $NHCH_2$), 3.2 -3.3 (5H, m, NH, CH_2), 3.1 (2H, m, CH_2), 2.1 (2H, m, CH_2), 1.6 (2H, m, CH_2), 1.5 (2H, m, CH_2), 1.2 -1.4 (8H, m, CH_2).

N-[9-(2,3-dihydro-1H-cyclopenta[b]quinolin-9-ylamino)nonyl]-4-fluorobenzamide hydrochloride (4h).

A **3h** (0.20 g, 0.4468 mmol) was combined as above to afford **4h** as brown solid. Compound **4h**: mp 118-120°C; yield 32%; IR (KBr) ν (cm^{-1}): 759.2 (F), 1588.0 (NH), 1644.0 (NHCO), 2849.9 (CH2CH2CH2), 2925.3 (CH2CH2CH2), 3226.9 (CON); ^{1}H NMR (DMSO) (δ ppm.): 13.9 (1H, s, HCl), 8.7 (1H, m, NH), 8.5 (1H, d, *J* = 7.5 Hz, ArH), 7.8 - 7.9 (5H, m, ArH), 7.6 (1H, t, *J* = 8.5 Hz, ArH), 7.3 (1H, t, *J* = 8.9 Hz, ArH), 3.7 (2H, m, $NHCH_2$), 3.2 -3.3 (5H, m, NH, CH_2), 3.1 (2H, t, *J* = 7.7 Hz, CH_2), 2.2 (2H, m, CH_2), 1.7 (2H, m, CH_2), 1.5 (2H, m, CH_2), 1.2 -1.4 (10H, m, CH_2).

Enzyme Studies

All new derivatives of 2,3-dihydro-1*H*-cyclopenta[b]quinoline were examined towards AChE and BChE inhibition. We used spectrophotometric Ellman's method with own modifications. All measurements were conducted in one and the same conditions. Determination of the inhibitory activity was carried in the environment of phosphate buffer (0.1M, pH 8.0) at 37° C. The content of every single sample was as follows: 5,5'-dithiobisnitrobenzoic acid (DTNB, 0.05 ml, 0.5M), AChE (5 units/ml), acetylthiocholine iodide (substrate) and analyzed, newly synthesized inhibitor. The volume of every probe was equal and it totaled 3 ml. The absorbance was measured after 1 minute incubation of all ingredients. The inhibition curves of every compound were obtained by usage of 7 concentrations of acetylthiocholine iodide. Every procedure was repeated three times. All measurements were conducted with the accompaniment of sample without inhibitor in order to obtain the 100% of AChE activity. The only alteration in estimation of the drugs inhibitory activity towards butyrylcholinesterase (BChE) was utilization of BChE (5 units/ml) instead of AChE. The drug concentration that contributes to the inhibition of 50% AChE or BChE activity (value of IC_{50}) was determined by non-linear and linear regression. All reagents: DTNB, enzymes (acetylcholinesterase from *Electrophorus electricus* (electric eel) and butyrylcholinesterase from equine serum) and acetylthiocholine iodide were purchased from Sigma-Aldrich.

Molecular Modeling

Structures of inhibitors were created by Corina on-line (Molecular Networks) and then Gasteiger-Marsili charges were assigned by Sybyl 8.0 (Tripos). Ligands were docked to both cholinesterases with application of GoldSuite 4.1 (CCDC). Before docking acetylcholinesterase from 2CKM and butyrylcholinesterase from 1P0I crystal complex were prepared. Protonation at Nε of histidine residues were assigned, the hydrogen atoms were added, ligand and water molecules were deleted. The binding site was defined as all amino acid residues within 10 Å from bis-(7)-tacrine for AChE and 20 Å from the glycerol molecule, present in the active gorge of BChE. A standard set of genetic algorithm with population size 100, number of operations 100000 and clustering with a tolerance of 1Å were applied to find 10 poses for each ligand, sorted by GoldScore (AChE) and ChemScore (BChE) function. The results were visualized by PyMOL 0.99rc6 (DeLano Scientific LLC).

DISCUSSION AND CONCLUSION

Enhancement of cholinergic function in the central nervous system in the course of AD is of extreme importance. This goal might be achieved by use of acetylcholinesterase inhibitors such as tacrine, the first AChEI drug approved by FDA for clinical use, rivastigmine, galantamine and donepezil. However, there is still a great need to search for new compounds with anticholinesterase activity. In this study we described synthesis of a series of tacrine-fluorobenzoic acid hybrids.

In our earlier work we also presented derivatives of fluorobenzoic acid but on the basis of the 1,2,3,4-tetrahydroacridin-9-ylamine. All novel compounds described in this study were characterized, in comparison with tacrine, by 4-fold higher inhibitory activity towards AChE. In case of BChE inhibition, slightly higher IC_{50} values were obtained for all constituents than for tacrine. These three synthesized compounds were differing from each other only in the length of the aliphatic chain. According to the results of the study, introduction of longer aliphatic chain contributed to the remarkable increase in compounds' inhibitory activity suggesting that it was good way to introduce such alteration [17].

In comparison with our previous study, the exchange of a six membered ring for a five membered ring significantly influenced the activity and interaction with active site. However, in the current study we did not record such coherent and straightforward results. We cannot observe that together with the length of aliphatic chain between fluorobenzoic acid and 2,3-dihydro-1*H*-cyclopenta[b]quinolone the activity of synthesized compounds is increased. Among obtained compounds the most active towards AChE were compounds **4c**, **4a** and **4f.** They were 11-, 5- and 2-fold more active than tacrine, respectively. The 11-fold more active than tacrine, compound **4c** is more selective towards AChE in comparison with tacrine. Compounds **4d**, **4g** and **4h** exhibited similar to tacrine value of IC_{50}.

Apart from AChE inhibitory activity, we also tested all compounds towards BChE inhibition. Obtained data are shown in Table **1**. All the synthesized molecules were characterized by lower BChE inhibitory activity than tacrine but compounds **4a**-**4e** had higher selectivity. Selectivity of compound **4c,** which is the most active towards AChE, is very interesting because in AD the level of BChE rises in comparison to AChE in cholinergic neurons [29]. Last clinical trials have demonstrated that patients treated with inhibitors of both enzymes shows attenuated loss of brain volume and cortical changes [30, 31].

Considering that AD is related with a significant reduction in AChE activity and this enzyme is found in both the cholinergic axons and the cholinoceptive neurons, AChE is an attractive target for the diagnosis of AD using both positron emission tomography (PET) and single photon emission computed tomography (SPECT) [32]. Radiolabeled AChE inhibitors have been developed and evaluated as means of visualizing the AChE density. However, some radioligands such as N-[^{11}C]methyltacrine and [^{11}C]physostigmine allow only nonspecific binding in the brain regions because of their low selectivity of AChE over BChE and mild binding properties to AChE [33,34]. Apart from ^{11}C-labeled radioligands there are other radioligands containing ^{18}F atom, such as 1-(4-[18F]fluorobenzyl)-4-[(5,6-dimethoxy-1-oxoindan-2-yl)methyl]piperidine (4-FDP) [35], 3-[1-(4-[^{18}F]fluorobenzyl)piperidin-4-yl]-1-(1-methyl-1H-indol-3-yl)propan-1-one, its 3-[^{18}F]fluoromethylbenzyl derivative [36]. Ryu *et al*. synthesized a novel radioligand 5,7-dihydro-3-[2-[1-(2-fluorobenzyl)-4-piperidinyl]ethyl]-6H-pyrrolo[3,2,f]-1,2-benzisoxazol-6-one (2-[^{18}F]fluoro-CP-118,954) which in *in vivo* distribution studies demonstrated a high level of radioligand accumulation both in the striatum and the olfactory tubercle which are AChE-rich regions [32].

On the basis of the current stage of knowledge the next step of our study will be labeling of compounds presented in this work with ^{18}F atoms and then evaluating them as radioligands for the *in vivo* mapping of AChE.

In summary, results of our synthesis and analysis suggest that obtained hybrids of tacrine and 4-fluorobenzoic acid may be considered to be a kind of novel anti-Alzheimer's drug candidates. This kind of new potential drugs could be used in the treatment of cholinergic deficit occurring in AD, inhibiting both enzymes AChE and BChE which could restore the acetylcholine level.

CONFLICT OF INTEREST

Declared none.

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