Received: 1 June 2018 | Revised: 7 July 2018 | Accepted: 11 July 2018
DOI: 10.1002/ardp.201800167

FULL PAPER

DPhG ARCH PHARM
Archiv der Pharmazie

Synthesis, characterization, and SAR of arylated indenoquinoline-based cholinesterase and carbonic anhydrase inhibitors

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We report the synthesis of bromoindenoquinolines (**15a–f**) by Friedlander reactions in low yields (13–50%) and the conversion of the corresponding phenyl-substituted indenoquinoline derivatives **16–21** in high yields (80–96%) by Suzuki coupling reactions. To explore the structure–activity relationship (SAR), their inhibition potentials to inhibit acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and human carbonic anhydrase cyctosolic (hCA I and II) enzymes were determined. Monophenyl (**16–18**) indenoquinolines significantly inhibited the AChE and BChE enzymes in ranges of IC_{50} 37–57 nM and 84–93 nM, respectively, compared with their starting materials **15a–c** and reference compounds (galanthamine and tacrine). On the other hand, these novel arylated indenoquinoline-based derivatives were effective inhibitors of the BChE, hCA I and II, BChE and AChE enzymes with K_i values in the range of 37 ± 2.04 to 88640 ± 1990 nM for AChE, 120.94 ± 37.06 to 1150.95 ± 304.48 nM for hCA I, 267.58 ± 98.05 to 1568.16 ± 438.67 nM for hCA II, and 84 ± 3.86 to 144120 ± 2910 nM for BChE. As a result, monophenyl indenoquinolines **16–18** may have promising anti-Alzheimer drug potential and 3,8-dibromoindenoquinoline amine (**15f**) can be novel hCA I and hCA II enzyme inhibitors.

KEYWORDS
acetylcholinesterase, bromoindenoquinolines, butyrylcholinesterase, carbonic anhydrase, enzyme inhibition, phenyl indenoquinolines, SAR

1 | INTRODUCTION

Alzheimer's disease (AD) is acknowledged as progressive multifarious neurodegenerative disorder[1] and considered as lack of cognitive abilities, i.e., thinking and judging, in the elder people.[1,2] AD is accompanied by an increase of acetylcholinesterase (AChE) activity that leads to run out of cholinergic neurotransmission in the brain areas related to memory and learning.[3] Amyloid beta-protein (A beta) plaques, the major pathological sign in the brain of AD patients, is a degradation product of cholinesterase (ChE) enzyme.[2] Currently, there are several approved drugs as AChE inhibitors (AChEIs) including donepezil, rivastigmine, and galanthamine for the treatment of AD.[4,5] These drugs increase acetylcholine (ACh) level in the central nervous system to enhance cholinergic neurotransmission.[6] The inhibition of both hydrolysis products of ACh, AChE and butyrylcholinesterase (BChE), is essential for successful treatment of AD because recent

studies in AD patients reported that while the AChE activity is significantly reduced in specific regions of the brain, BChE activity can increase.[3,7,8] Most preferred medication is discovery of either new cholinesterase inhibitors or *N*-methyl-D-aspartate receptor antagonists such as memantine.[9] Adverse effects of some synthetic AChEIs used for the treatment of AD such as tacrine and donepezil let us to find innocent and most potent derivatives of those drugs.[10] Therefore, it is very important to find new substances, more efficient and less expensive than the currently used drugs is urgently needed.

Carbonic anhydrase (CA, EC 4.2.1.1) isoforms catalyze a simple, yet decisive, reaction: the reversible hydration of water and carbon dioxide to proton and a bicarbonate anion.[11–13] This reaction, in the absence of CA cannot proceed with a perceptible rate under physiological positions. CAs are ubiquitous metalloenzymes comprising Cd(II)-, Zn(II)-, or Fe(II)-, available in eukaryotes and prokaryotes, and classified at least seven class (α-CA, β-CA, δ-CA, γ-CA, ζ-CA, η-CA, θ-CA).[14] CA isoforms play a momentous role in multitude physiological activities of eukaryotes such as CO_2 transport, respiration, photosynthesis between others and electrolyte secretion.[15] Additionally, to the defined role of CA inhibitors (CAIs) as antiglaucoma drugs and diuretics, it has newly appointed that they have potential as antiobesity, anticonvulsant, anti-infective drugs, and anticancer.[16]

The quinoline skeleton occurs in several natural compounds and pharmacologically active substances displaying a broad range of biological activity.[17–20] In addition to the medicinal uses, quinolines have been employed in the study of bioorganic and bioorganometallic processes. The Friedlander reaction is a well-known method for preparing quinolines,[21] it is still considered as one of the most useful methods for preparing quinolines and related bicyclic azaaromatic compounds. Indenoquinolines are quinoline derivatives bearing tetra-aromatic heterocycle and distinguished as profound chemical and biological agents due to diverse range of their biological activities such as 5-HT-receptor binding and anti-inflammatory activities.[22] They have also acted as antitumor agents,[23,24] steroid reductase inhibitors,[25] new potential topo I/II inhibitors,[26] antimalarials,[27] and also AChEIs.[2,28] Thus, the synthesis of these molecules has attracted considerable attention. However, there are restricted reports about synthesis of substituted indenoquinoline amine, displayed AChE inhibitory activity. Some reported studies showed that halogenated indenoquinoline analogues have better inhibitory activity compared with unsubstituted ones.[28] This study reported that especially fluoro substituted at C-2 position of benzene ring fused 1-*H* indene having more AChE inhibitory activity potential compared with methoxy, chloro-substituted indenoquinoline amine derivatives.[28]

Halogenated aromatic compounds, including quinoline,[29,30] tacrine,[31] anthracene,[32] indane,[33] and naphthalenes,[34,35] would enable the synthesis of diverse aromatic frameworks because the halogen, especially bromine, could enhance biological activity in many cases and could also be used for further functionalization in preparing other molecules.[31] For quinoline-based compounds, tacrine, indenoquinoline, etc., ring substitution by halogen is rather complex.[31,36] Particularly, direct bromination of polycyclic aromatics with *N*-function is difficult due to formation of *N*-bromine complex.[29]

Recently, we have reported the preparation of novel tricyclic tacrine derivatives, including five-, six-, and seven-membered hydrocycles,[31] through cyclodehydration reaction of several ketones and 2-amino-3,5-dibromobenzonitrile via Friedlander reaction furnished a series of dibrominated tacrines. Then bromotacrine analogues were converted to corresponding disubstituted tacrine analogues by metal catalyzed or metal halogen exchange. In continuation of our research on bioactive molecules, we designed and synthesized a new series of bromo- and phenyl-substituted indenoquinoline amine. Due to having the AChE, hCA I and hCA II inhibition potentials of the tricyclic structure of the 1,2,3,4-tetrahydroacridine nucleus,[37,38] we expanded to tetracyclic ones. Thus, this work was to explore the synthesis of novel bromoindenoquinoline amines via Friedlander reaction between 2-amino-3,5-dibromobenzonitrile (**7**) and 2-amino-5-bromobenzonitrile (**11**) and bromoindan-1-ones (**12** and **13**) in the presence of some Lewis acids. In this series, we synthesized phenyl-substituted indenoquinoline amine analogues by Suzuki coupling reactions. In addition, we focused on investigation of anti-Alzheimer activities of novel-synthesized tetracyclic polyfunctional indenoquinoline amine as potentials of AChE and BChE inhibitors. Although there is no association between carbonic anhydrase inhibition and Alzheimer, as another study, we determined their carbonic anhydrase isoforms, the cytosolic hCA I and II inhibition activities. Furthermore, to fill the gap in the structure–activity relationship (SAR) of substituted indenoquinoline amine derivatives, IC_{50} values for the inhibition of AChE, BChE, hCA I and hCA II were studied by means of SAR approach, in search for describing the dependence of AChE and BChE inhibitory activities on substituents of indenoquinoline amine cycles.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

In our previous papers,[31,36] one-pot synthesis was described for tricyclic bromotacrine derivatives (**1**–**6**) (Figure 1) using Friedlander reactions of several cyclic ketones and brominated 2-amino-3,5-dibromobenzonitrile (**12**) in the presence of some Lewis acids. Then, tacrine bromides (**2** and **3**) were converted corresponding disubstituted tacrine derivatives via metal-halogen and copper-assisted substitution reactions.[31] Furthermore, the evaluation of their anti-Alzheimer and anticarbonic anhydrase potentials indicated that methoxy (**10**), cyano (**7**), silyl (**6** and **9**), and thiomethyl (**8**) tacrine

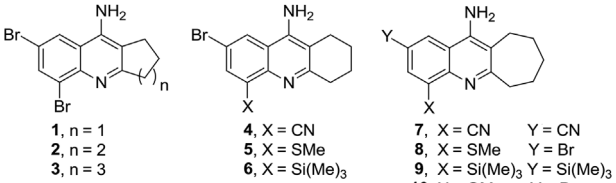


FIGURE 1 Tricyclic disubstituted tacrine derivatives (**1–10**)

derivatives had selective AChE and BChE inhibitory activities and showed significant inhibition against hCA I and hCA II enzymes.[37,38]

In view of these observations and in continuation of our current interest in the synthesis of disubstituted tacrines for biological evaluations,[38,39] the promising AChE and BChE inhibitory features of disubstituted tacrine derivatives have encouraged us to design and synthesize tetracyclic indenoquinoline amine bearing phenyl ring **16**–**21** for anti-Alzheimer properties as depicted in Figure 2. We have planned to investigate anti-Alzheimer and carbonic anhydrase activities of the novel-synthesized compounds **16**–**21** against AChE and BChE and the cytosolic hCA I and hCA II enzymes.

To achieve this goal, functional bromo tetracyclic indenoquinoline amine derivatives were obtained because heterocyclic aromatics are key structures in a large amount of pharmacological compounds.[19,20,40] Due to that the direct bromination leads to some problems in obtaining brominated *N* function aromatics.[29,31,36] We attempted Friedlander reactions between 2-aminobenzonitrile (**11**) or corresponding brominated 2-aminobenzonitriles (**12** and **13**) and bromoindan-1-ones (**14a–c**), instead of direct bromination of indenoquinoline nucleus. First, the reaction of 2-aminobenzonitrile (**11**) with Br_2 was examined in AcOH at 10–15°C. After the reaction between 2-aminobenzonitrile and 1 equivalent of Br_2, the ^{1}H NMR spectra of the mixture of products indicated 2-amino-3,5-dibromobenzonitrile (**12**) and 2-amino-5-bromobenzonitrile (**13**). Then the products were efficiently isolated with column chromatography. Compound **12** was furnished with treatment of 2 equivalents of Br_2 in 98% yield as the sole product (Scheme 1).

The cyclodehydration reaction under toluene reflux was further evaluated with several bromoindan-1-ones **14a–c** and 2-aminobenzonitrile (**11**) in the presence of $InCl_2$, Lewis acid as catalyst. Anthranilonitrile (2-aminobenzonitrile, **11**) and 4-bromo **14a**, 5-bromo **14b**, and 6-bromoindan-1-one (**14c**), instead of monocyclic ketones in our previous study,[31] furnished moderate yields of products **15a** (50%), **15b** (48%), and **15c** (44%), respectively (Scheme 2). Furthermore, 2-amino-5-bromobenzonitrile (**13**) and 2-amino-3,5-dibromobenzonitrile (**12**) with corresponding bromoindan-1-ones **14a–c** under the same reaction conditions furnished dibromo (**15d–f**) and tribromo (**15g–i**) indenoquinoline amines with low yields (35, 30, 23, 20, 13, and 15%, respectively) (Scheme 2).

Structures of bromoindenoquinoline amines (**15a–i**) were determined by ^{1}H NMR, ^{13}C NMR, FT-IR spectroscopy, and high resolution

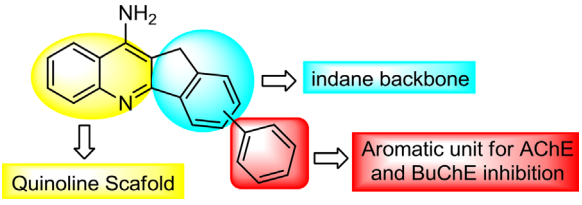
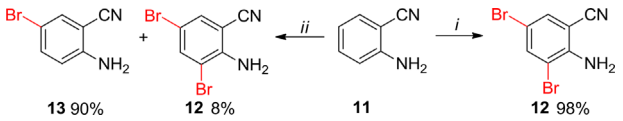


FIGURE 2 Design strategy for new indenoquinoline amines

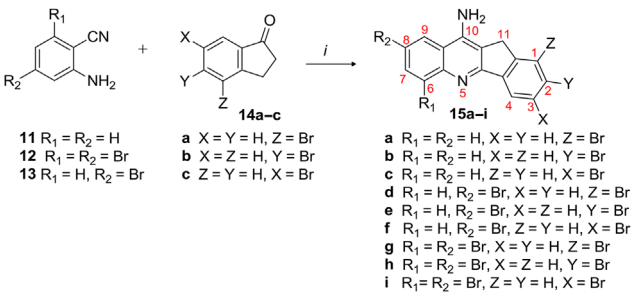


SCHEME 1 Bromination of 2-aminobenzonitrile **11** and synthesis of bromoaminobenzonitrile derivatives (**12** and **13**). Reagents and conditions; (*i*) Br_2 (2.2 equiv), AcOH, 10–15°C, 10 min; (*ii*) Br_2 (1.1 equiv), AcOH, 10–15°C, 10 min

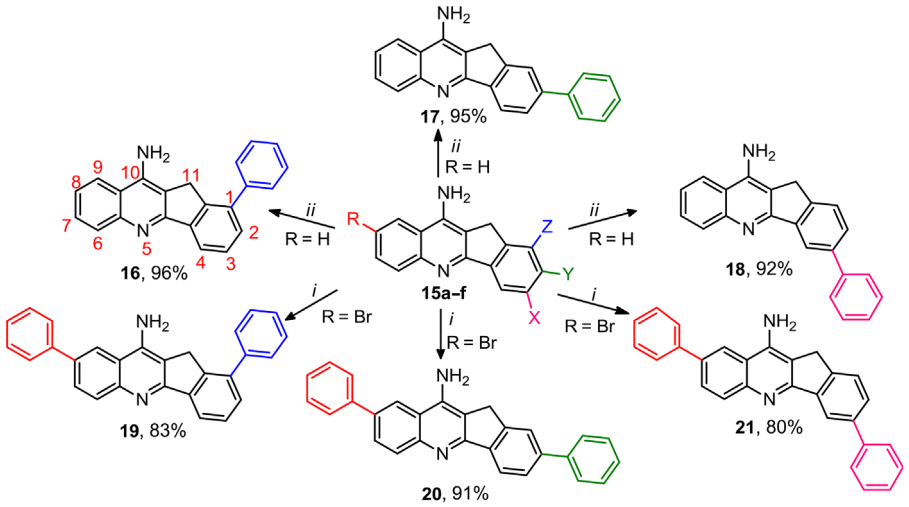
mass spectrometry. The ^{1}H NMR spectra of compounds **15a–i**, which consisted of aryl Hs, alkyl Hs (δ 3.79–3.99 ppm), and NH signals matched quite well with the suggested structures. For example, the ^{1}H NMR spectra of **15g–i** clearly show tribromo structures due to two aryl signals with *meta* coupling constants (^4J = 1.8 Hz). One aliphatic [3.84 (s), 3.99 (s), and 3.88 (s), respectively] and NH_2 shifts [8.35–8.68 (brs)] are recorded for each **15g–i**. In the ^{13}C NMR spectra of compounds **15g–i**, one carbon resonates in the sp^3 region and 15 carbon resonances in the sp^2 region are also in agreement with the proposed structures.

To demonstrate the value of brominated indenoquinoline amines **15a–f** as starting materials for numerous useful compounds, we investigated their Suzuki Miyaura cross-coupling reactions. These coupling reactions of monobromo **15a–c** and dibromoindenoquinoline amines **15d–f** with different equivalents of phenylboronic acid (1.1 and 2.2 equivalents) afforded 1-phenyl **16**, 2-phenyl **17**, 3-phenyl **18**, 1,8-diphenyl **19**, 2,8-diphenyl **20**, and 3,8-diphenyl **21** indenoquinoline amines in high yields (80–96%) (Scheme 3 and Table 1). The reactions were carried out with $Pd(PPh_3)_4$ (5 mol%) as the catalyst and aq Na_2CO_3 (3 M) as the base. 1,4-Dioxane was used as the solvent and the reactions were performed at 100°C for 4 h.

Structural characterization of the phenyl-substituted indenoquinoline amines **16**–**21** was further confirmed by elemental analysis and ^{1}H NMR, ^{13}C NMR, and FT-IR spectra. In the ^{1}H NMR of phenyl derivatives **16**–**21** provided new aromatic signals at δ_H 7.33–7.73 (m), 7.40–7.72 (m), 7.37–7.82 (m), 7.43–7.90 (m), 7.38–7.79 (m), 7.32–7.76 (m), respectively. In addition, ^{13}C NMR spectra of **16**–**21**, consisting of new sp^2 resonances supported the proposed structures.



SCHEME 2 Synthesis of mono- (**15a–c**), di- (**15d,e**), and tribromo (**15g,h**) indenoquinolines. Reagents and conditions; (*i*) toluene, $InCl_3$ (1.1 equiv), reflux, 24 h, then NaOH (2 M), reflux, 24 h



SCHEME 3 Synthesis of phenyl-substituted (**16–21**) indenoquinoline amines. Reagents and conditions; (*i*) $PhB(OH)_2$ (1.3 equiv), $Pd(PPh_3)_4$ (0.05 equiv), K_2CO_3 (3 M), dioxane, reflux, 4 h. (*ii*) $PhB(OH)_2$ (2.6 equiv), $Pd(PPh_3)_4$ (0.05 equiv), K_2CO_3 (3 M), dioxane, reflux, 4 h

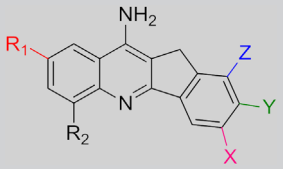
2.2 | Biological activity

2.2.1 | Anticholinesterase activity results

The inhibitory activity against AChE and BChE of novel-synthesized bromo- and phenyl-substituted indenoquinoline amine derivatives and of references compounds, tacrine (IC_{50} = 59 nM for AChE and IC_{50} = 23 nM for BChE, taken in our study) and galanthamine (IC_{50} = 1280 nM for AChE and IC_{50} = 19530 nM for BChE, obtained in our study) is reported in Table 1, expressed as IC_{50} values.

The monophenyl-substituted indenoquinoline amines **16–18** were found to be potent inhibitors of cholinesterases with IC_{50} in

TABLE 1 Structural data and AChE and BChE inhibitory activities (IC_{50}) of novel-substituted indenoquinoline amine derivatives



No.	R_1	R_2	X	Y	Z	Yield (%)	Mp (°C)	IC_{50} for AChE (nM)	r^2	IC_{50} for BChE (nM)	r^2
15a	H	H	H	H	Br	50	227–228	2610 ± 170	0.992	1340 ± 760	0.994
15b	H	H	H	Br	H	48	238–239	5700 ± 127	0.999	3890 ± 183	0.999
15c	H	H	Br	H	H	44	240–241	2170 ± 273	0.998	46550 ± 2670	0.999
15d	Br	H	H	H	Br	35	291–292	230 ± 15	0.999	2620 ± 390	0.999
15e	Br	H	H	Br	H	30	283–284	3730 ± 204	0.989	59790 ± 2140	0.999
15f	Br	H	Br	H	H	23	280–281	23420 ± 1620	0.992	3660 ± 259	0.994
15g	Br	Br	H	H	Br	20	297–298	5410 ± 1450	0.999	4790 ± 239	0.994
15h	Br	Br	H	Br	H	13	294–295	88640 ± 1990	0.999	38600 ± 2180	0.999
15i	Br	Br	Br	H	H	15	291–292	3530 ± 117	0.998	2420 ± 283	0.997
16	H	H	H	H	Ph	96	234–235	37 ± 2.04	0.999	93 ± 2.39	0.999
17	H	H	H	Ph	H	95	235–236	57 ± 2.39	0.987	87 ± 3.78	0.998
18	H	H	Ph	H	H	92	234–235	56 ± 2.46	0.998	84 ± 3.86	0.998
19	Ph	H	H	H	Ph	83	251–252	4870 ± 102	0.988	144120 ± 2910	0.998
20	Ph	H	H	Ph	H	91	253–254	1090 ± 175	0.999	13780 ± 253	0.989
Tacrine (reference compound A)								59 ± 1.47	0.999	23 ± 1.06	0.999
Galantamine (reference compound B)								1280 ± 106	0.997	19530 ± 1740	0.999

nanomolar concentration scale. The most active AChE inhibitor in the assayed series of substituted indenoquinoline amine analogues are dibromoindenoquinoline amine **15d** and monophenyl derivatives **16**–**18** with IC_{50} ranging between 37 and 230 nM. The 1,8-dibromo derivative **15d** significantly inhibited AChE enzyme at IC_{50} of 230 nM, while 3,8-dibromo **15f** (IC_{50} = 23420 nM) and 2,6,8-tribromoindenoquinolines **15h** (IC_{50} = 88640 nM) showed low inhibition against AChE enzyme compared with galanthamine (Table 1). Despite the high inhibition of **15d** against AChE enzyme, this compound moderately inhibited BChE enzyme (IC_{50} = 2620 nM). According to reported results, compound **15d** proved to be the most selective AChE inhibitor, compared with galanthamine and unsubstituted tacrine. Furthermore, the high AChE inhibition potentials of **16**–**18**, as close to IC_{50} value of tacrine, indicated that they could be novel promising AChE inhibitors (Table 1).

The BChE inhibition results showed that the most active compounds are also monophenyl indenoquinoline amines **16**–**18** with IC_{50} values of 93, 87 and 84 nM, respectively. Furthermore, most tested compounds showed good inhibition against BChE with IC_{50} ranging between 1340–13780 nM even better than standard compound galanthamine (IC_{50} = 19530 μM), except for compounds **15c** (IC_{50} = 46550 nM), **15e** (IC_{50} = 59790 nM), **15h** (IC_{50} = 38600 nM) and **19** (IC_{50} = 144120 nM).

2.2.2 | Anticarbonic anhydrases activity results

The substituted indenoquinoline amine derivatives reported here were determined as inhibitors of two physiologically relevant CA isoforms, the cytosolic hCA I and II by an esterase assay.[41] The hCA I and II isoenzymes were inhibited by the novel-synthesized bromo- and phenyl-substituted indenoquinoline amine derivatives, with K_i values ranging 120.94–1150.95 and 267.58–1568.16 nM, respectively. Acetazolamide (AZA), a clinically used sulfonamide CAI was used in the assays as a reference drug. As shown in Table 2, 13 compounds (**15a–h** and **16–20**) were found to have IC_{50} in the range of 138.63–1064.51 nM against hCA I. Also, these compounds have IC_{50} in the range of 233.41–1300.18 nM against hCA II. All these compounds displayed good activity against hCA I and hCA II as compared to AZA with IC_{50} values of 3745.94 and 3347.82, respectively (Table 2). Among all the above compounds, only compound **15f** displayed significant activity, i.e., IC_{50} 138.63 and 213.66 nM, as compared to AZA. Moreover, compounds **15a**, **15c–e**, and **18–20** were found to possess good activity against hCA I, i.e., IC_{50} 330.76 nM in agreement with AZA. Although all other remaining compounds were found to possess moderate activity, their inhibition activities more than as AZA against hCA I. Furthermore, all tested compounds showed inhibition activity against hCA II in the same manner as hCA I, but in higher concentrations, i.e., IC_{50} 543.18 nM (Table 2). This study clearly indicates that bromo- and phenyl-substituted indenoquinoline amine derivatives (**15a–h**, **16–20**) could be promising hCA I and hCA II inhibitor candidates.

2.2.3 | The SAR study for enzyme inhibition

Substituents were introduced in synthetically accessible positions of the aryl rings indenoquinoline amine as well as on primary amino group in order to investigate the SAR of the series. The structural characteristics of novel-substituted indenoquinoline amines and AChE and BChE inhibition data expressed as IC_{50} values are shown in Tables 1 and 2. Also hCA I and II inhibitory potentials' data expressed as IC_{50} and K_i values in nM concentrations are shown in Table 2.

TABLE 2 The hCA I and hCA II inhibitory activities (IC_{50} and K_i) of novel-substituted indenoquinoline amine derivatives

	IC_{50} (nM)				K_i (nM)	
Compound no.	hCA I	r^2	hCA II	r^2	hCA I	hCA II
15a	330.76	0.9869	612.18	0.9436	322.75 ± 52.58	682.22 ± 72.85
15b	693.06	0.9778	734.58	0.9579	543.18 ± 91.98	580.98 ± 132.22
15c	409.08	0.9861	620.41	0.9572	573.33 ± 196.12	495.68 ± 136.84
15d	403.61	0.9595	548.66	0.9735	377.08 ± 72.21	495.02 ± 94.08
15e	428.33	0.9661	560.67	0.9858	332.97 ± 47.37	714.07 ± 315.33
15f	138.63	0.9885	233.41	0.9621	120.94 ± 37.06	267.58 ± 98.05
15g	863.11	0.9884	1300.18	0.9149	587.03 ± 100.71	1568.16 ± 438.67
15h	1213.66	0.9854	579.43	0.9238	1150.95 ± 304.48	720.88 ± 210.97
16	1051.58	0.9800	797.46	0.9368	986.01 ± 254.22	762.88 ± 270.16
17	1064.51	0.9709	889.62	0.9139	1131.54 ± 277.61	1447.70 ± 522.32
18	402.22	0.9834	502.92	0.9574	341.88 ± 42.02	677.93 ± 244.07
19	421.02	0.9773	535.96	0.9663	526.27 ± 224.21	421.63 ± 87.32
20	415.77	0.9771	427.75	0.9642	408.06 ± 124.27	491.18 ± 171.03
AZA	3745.94	0.9653	3347.82	0.9899	3320.46 ± 716.02	2718.92 ± 531.18

It is evident that substituents at C-1, C-2, C-3, and C-8 for indenoquinoline amines present various inhibitory activity. When comparing the compounds containing the Br substituent with different positions and the numbers, it is observed that Br at both C-1 and C-8 (IC_{50} 230 nM) increased the inhibition of AChE activity for dibromo compounds (**15d–f**); however, this substituent at both C-3 and C-8 (IC_{50} 23420 nM) led to reduction in the activity. The only one bromine atom of monobromides **15a–c** at C-1, C-2, or C-3 positions did not increase the AChE and BChE inhibition activities, in particular, Br at C-3 in the indane backbone significantly reduced the BChE inhibition activity (IC_{50} 46550 nM). Furthermore, the presence of Br substituent at the positions of C-6, C-8, and anywhere in the benzene ring fused penta atomic cycle (C-1, C-2, or C-3) in tribromoindenoquinolines (**15g–i**) decreased the both AChE and BChE inhibition activities, i.e., IC_{50} values of **15h** 88640 nM for AChE and 38600 nM for BChE. The other interesting observation is that Br atom in the presence at the position of C-1, C-2, or C-3 for dibromoindenoquinoline is crucially important for cholinesterase inhibition activity. The bromine at C-1 in **15d** significantly inhibited AChE (IC_{50} 230 nM) and moderately inhibited BChE (IC_{50} 2620 nM), while Br at C-3 in **15f** and Br at C-2 in **15e** significantly reduced inhibitions of AChE (IC_{50} 23420 nM) and BChE (IC_{50} 59790 nM), respectively.

The introduction of phenyl substituent on compounds **16**–**18** led to incredibly increased inhibition activities against AChE and BChE, compared with their starting materials (**15a–c**). Also, these compounds (**16**–**18**) (average IC_{50} = 50 nM for AChE and 88 nM for BChE) displayed similar inhibition activities to tacrine. The significant increase on inhibition activity was observed in case of phenyl groups introduced at C-1, C-2, or C-3 positions of benzene ring fused penta atomic cycle in **16**–**18**. Actually, the phenyl substituent bounded at C-2 and C-8 in diphenyl indenoquinoline **20** slightly increased the inhibition activity against both ChE enzymes (IC_{50} = 1090 nM for AChE and 13780 nM for BChE), however, the phenyl groups at C-1 and C-8 in compound **19** significantly decreased the inhibition, especially against BChE (IC_{50} = 144120 nM), compared with starting compound **15d** (IC_{50} = 2620 nM).

When comparing hCA I and II inhibition activities of the indenoquinolines containing the Br and phenyl substituent with the same positions, it is observed that the presence of Br at both C-3 and C-8 indenoquinoline nucleus displayed high inhibition against hCA I (IC_{50} = 138.63 nM) and hCA II (IC_{50} = 233.41 nM), compared with the other derivatives and AZA. The evaluation of bromine in tribromides (**15g**,**h**) indicated that the bromine atom at C-1 in **15g** reduced the inhibition activity against hCA II (IC_{50} = 1300.18 nM) while Br at C-1 in **15h** led to show low inhibition against hCA I (IC_{50} = 1213.66 nM) enzyme. Furthermore, the phenyl groups did not show significant effect on inhibition activity. However, the presence of phenyl groups at only C-1 or C-2 in compounds **16** and **17** causes to have lower inhibition effect against hCA I (IC_{50} = 1051.58 and 1064.51 nM, respectively), compared with monobromoindenoquinoline analogues (**15a**,**b**) (IC_{50} = 330.76 and 639.06 nM, respectively).

These results indicated that phenyl substitutent at position of C-1/C-2/C-3 for indenoquinoline derivatives are more critical for AChE and BChE inhibitory activities than bromo-substituted ones. The position and kinds of substituents are important to anti-AChE and BChE activities.

3 | CONCLUSION

In this work, we have decribed a convenient route to synthesize bromoindenoquinoline amines (**15a–h**) by Friedlander reactions starting with 2-aminobenzonitrile (**11**) and its brominated derivatives **12** and **13**. In addition, we showed a simple synthetic methodology to obtain phenyl-substituted indenoquinolines by Suzuki cross-coupling reactions between bromoindenoquinolines **15a–f** and phenylboronic acid. Several bromo- and phenyl-substituted analogues were found to be potent and selective inhibitors of AChE, BChE, hCA I and hCA II in nanomolar concentration scale. Moreover, we attempted to determine the SAR of the inhibition effects of the introduction of the bromo and phenyl substituents in synthetically accessible positions of tetracyclic nucleus. In case of the presence of phenyl groups at only C-1, C-2, or C-3, the inhibition activities of arylated indenoquinolines against cholinesterase enzymes (AChE and BChE) were found the highest potency. On the contrary, the phenyl bound to positions of both C-1 and C-8 significantly reduced the inhibition. Furthermore, the inhibition effect of two Br atoms bound at both C-2 and C-8 increased, but three bromine atom at C-2, C-6, and C-8 led to significant decrease of the inhibition activity.

In conclusion, monophenyl-substituted indenoquinoline analogues have been potential of cholinesterase inhibitors. In addition, bromoindenoquinoline have potential to convert novel cholinesterase inhibitor by substitution or coupling reactions. This promising result can contribute to the investigation of a cure for Alzheimer or therapy in the future.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

Thin layer chromatography was carried out on Merck silica F_{254} 0.255 mm plates and spots were visualized by UV at 254 nm. Flash column chromatography was performed using Merck 60 (70–230 mesh) silica gel. Melting points were determined on a MPM-H1 capillary melting points apparatus. Solvents were concentrated at reduced pressure. IR spectra were recorded on a Shimadzu Prestige-21 (200 VCE) FT/IR instrument. Mass spectra were recorded on an Agilent 6530 Accurate-Mass spectrometer under electron-impact (EI) and chemical ionization conditions. Elemental analysis was recorded on an Elementar Vario MICRO Cube instrument. NMR spectra were recorded on a Varian Infinity Plus at 300 MHz and on an Agilent at 600 MHz for ^{1}H and at 75 MHz for ^{13}C NMR.

The NMR spectra and the InChI codes of the investigated compounds are provided as Supporting Information.

4.1.2 | Synthesis of 2-amino-3,5-dibromobenzonitrile (12)

Bromine (3.52 g, 0.022 mol) was added dropwise to a magnetically stirred, cooled (ice-water bath) solution of 2-aminobenzonitrile **11** (1.18 g, 10 mmol) in acetic acid (30 mL) at 15–20°C over 10 min. The reaction mixture was stirred at room temperature for 5 h, then added to ice-water (100 mL), and the white precipitate was collected by filtration. The precipitate was washed thoroughly with water and dried on Na_2SO_4 at room temperature to afford a white solid mass **12** (98%), mp = 155–156°C (Lit.,[41] 156°C); R_f (30% EtAcO/hexane) = 0.37. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.73 (d, ^4J = 2.3 Hz, 1H), 7.48 (d, ^4J = 2.3 Hz, 1H), 4.90 (brs, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 146.4, 139.6, 133.8, 115.8, 109.9, 108.4, 98.1 ppm. All data were identical to those reported in the literature.[40]

4.1.3 | Synthesis of 2-amino-5-bromobenzonitrile (13)

Bromine (1.63 g, 10.2 mmol) was added dropwise to a mechanically stirred, cooled (ice-water bath) solution of 2-aminobenzonitrile **11** (1.18 g, 10.0 mmol) in acetic acid (15 mL) at 15–20°C in 10 min. Reaction mixture was stirred at room temperature for 2 h, then added to ice-water (100 mL) and the white precipitate was collected by filtration. The precipitate was washed thoroughly with water and dried on Na_2SO_4 at room temperature to afford white solid. The TLC analysis revealed that the final product was a mixture of two products. The dibromide **12** and monobromide **13** were isolated using a silica gel column eluting with a mixture of hexane/chloroform (5:1). **12** (0.21 g) 8%, **13** (1.77 g) 90% were isolated in pure forms. White solid powder **13** (90%), mp = 94–95°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.58 (d, J = 2.4 Hz, 1H), 7.41 (dd, J = 9.0, 2.4 Hz, 1H), 6.74 (d, J = 9.0 Hz, 1H), 6.26 (brs, 2H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ 151.0, 136.7, 133.9, 117.3, 116.8, 105.2, 95.0 ppm. FT-IR (neat $\tilde{\nu}$ cm^{-1}): 3434, 3351, 2218, 1629, 1485, 1303, 1255.

4.1.4 | General method for the cyclodehydration reaction

Unsubstituted or brominated anthranilonitrile (1 mmol, 1 equivalent), bromoindanone (1.0 mmol, 1 equivalent), and sodium-dried toluene (15 mL) were placed in a three-necked round bottomed flask fitted with an overhead stirrer. $InCl_3$ as Lewis acid (1.1 mmol, 1.1 equivalent) was added. The reaction mixture was heated at 120–130°C for 24 h under stirring. On cooling at rt, the toluene was decanted to liberate the product and the remaining solids were treated with sodium hydroxide (2 M, 15 mL) and heated at reflux for 24 h. After cooling, the organic components were extracted with chloroform, the organic layers were combined and dried over Na_2SO_4. The solvent was evaporated *in vacuo* to give the desired product.

10-Amine-1-bromo-11-*H*-indeno[1,2-*b*]quinoline (15a)

Unsubstituted anthranilonitrile (**11**) (0.35 g, 3.3 mmol), 4-bromoindan-1-one (**14a**) (0.7 g, 3.3 mmol), and $InCl_3$ (0.8 g, 3.6 mmol) gave the title compound (**15a**) (0.51 g, 50%). Light yellow powder solid, mp 227–228°C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.80 (s, 2H, CH_2), 6.95 (brs, 2H, NH_2), 7.38–7.43 (m, 2H, ArH), 7.60 (t, ^3J = 8.4, ^3J = 6.9 Hz, 1H, ArH), 7.65 (d, ^3J = 8.1 Hz, 1H, ArH), 7.88 (d, ^3J = 8.4 Hz, 1H, ArH), 8.02 (d, ^3J = 7.5 Hz, 1H), 8.25 (t, ^3J = 8.1, ^3J = 8.4 Hz, 1H, ArH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.9, 149.7, 147.5, 144.8, 144.1, 132.0, 129.8, 129.3, 129.0, 123.7, 122.8, 120.6, 120.4, 118.4, 112.8, 34.4. FT-IR (neat $\tilde{\nu}$ cm^{-1}): 3473–3302 (NH_2), 3163 (Arom. C—H), 2970–2866 (Aliph. C—H), 1641, 1509 (C═N), 1439, 1385, 1125, 753–727 (C—Br). HRMS (ESI) *m/z*: calcd. for $C_{16}H_{12}N_2{}^{79}Br$ $[M+H]^+$: 311.0184. Found: 310.9884.

10-Amine-2-bromo-11-*H*-indeno[1,2-*b*]quinoline (15b)

Unsubstituted anthranilonitrile (**11**) (0.24 g, 2.2 mmol), 5-bromoindan-1-one (**14b**) (0.46 g, 2.2 mmol), and $InCl_3$ (0.52 g, 2.4 mmol) gave the title compound (**15b**) (0.30 g, 48%). Light yellow powder solid, mp 238–239°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.96 (s, 2H, CH_2), 7.57 (t, ^3J = 8.3 Hz, 1H, ArH), 7.66 (d, ^3J = 8.7 Hz, 1H, ArH), 7.85 (t, 1H, ^3J = 8.5 Hz, ArH), 7.88 (s, 1H, ArH), 7.96 (d, ^3J = 8.4 Hz, 1H, ArH), 8.21 (d, ^3J = 8.3 Hz, 1H, ArH), 8.45 (d, ^3J = 8.8 Hz, 1H, ArH), 8.60 (brs, 1H, NH), 8.87 (brs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 154.2, 151.5, 148.1, 138.8, 134.0, 133.7, 131.6, 129.8, 126.6, 126.1, 125.0, 124.3, 120.4, 116.4, 114.3, 33.9. FT-IR (neat $\tilde{\nu}$ cm^{-1}): 3330 (NH_2), 3066 (Arom. C—H), 2970 (Aliph. C—H), 1738, 1602, 1508 (C═N), 1428, 1384, 965, 822–671 (C—Br). HRMS (ESI) *m/z*: calcd. for $C_{16}H_{12}N_2{}^{79}Br$ $[M+H]^+$: 311.0184. Found: 310.9879.

10-Amine-3-bromo-11-*H*-indeno[1,2-*b*]quinoline (15c)

Unsubstituted anthranilonitrile (**11**) (0.35 g, 3.3 mmol), 6-bromoindan-1-one (**14c**) (0.7 g, 3.3 mmol), and $InCl_3$ (0.8 g, 3.6 mmol) gave the title compound (**15c**) (0.45 g, 44%). Light yellow powder solid, mp 240–241°C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 3.80 (s, 2H, CH_2), 6.88 (brs, 2H, NH_2), 7.39 (t, 1H, ^3J = 8.4, ^3J = 4.0 Hz, ArH), 7.60–7.61 (m, 3H, ArH), 7.88 (d, ^3J = 8.4 Hz, 1H, ArH), 8.09 (s, 1H, ArH), 8.25 (d, ^3J = 8.1 Hz, 1H, ArH). ^{13}C NMR (75 MHz, DMSO-d_6, ppm): δ 159.2, 149.4, 147.5, 144.3, 144.0, 131.7, 129.3, 129.0, 128.1, 123.9, 123.7, 122.8, 120.7, 118.4, 113.9, 32.4. FT-IR (neat $\tilde{\nu}$ cm^{-1}): 3298 (NH_2), 3075 (Arom. C—H), 2970–2906 (Aliph. C—H), 1738, 1644, 1562 (C═N), 1435, 1363, 883–743 (C—Br). HRMS ESI *m/z*: calcd. for $C_{16}H_{12}N_2{}^{79}Br$ $[M+H]^+$: 311.0184. Found: 310.9890.

10-Amine-1,8-dibromo-11-*H*-indeno[1,2-*b*]quinoline (15d)

2-Amino-5-bromobenzonitrile (**13**) (0.35 g, 1.78 mmol), 4-bromoindan-1-one (**14a**) (0.38 g, 1.78 mmol), and $InCl_3$ (0.51 g, 2.30 mmol) gave the title compound (**15d**) (0.24 g, 35%). Light yellow powder solid, mp 291–292°C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 3.79 (s, 2H, CH_2), 7.08 (brs, NH_2), 7.42 (t, ^3J = 7.5 Hz, 1H, ArH), 7.67–7.71 (m, 2H, ArH), 7.81 (d, ^3J = 9.0 Hz, 1H, ArH), 7.99 (d, ^3J = 7.5 Hz, 1H, ArH), 8.52 (d, ^4J = 2.0 Hz, 1H, ArH). ^{13}C NMR (75 MHz, DMSO-d_6, ppm): δ 160.7, 148.4, 147.2, 145.1, 143.8, 132.5, 132.3, 131.6, 130.2, 125.4, 121.0, 120.7, 120.0, 116.8, 113.8, 34.7. FT-IR (neat $\tilde{\nu}$ cm^{-1}) 3446 (NH_2), 3198–3027 (Arom. C—H), 2968–2841 (Aliph. C—H), 1735, 1636, 1575 (C═N), 1459, 832–754 (C—Br).

HRMS (ESI) *m/z*: calcd. for $C_{16}H_{11}N_2Br_2$ $[M+H]^+$: 390.9269. Found: 390.8980.

10-Amine-2,8-dibromo-11-*H*-indeno[1,2-*b*]quinoline (15e)

2-Amino-5-bromobenzonitrile (**13**) (0.35 g, 1.78 mmol), 5-bromoindan-1-one (**14b**) (0.38 g, 1.78 mmol), and $InCl_3$ (0.47 g, 2.13 mmol) gave the title compound (**15e**) (0.21 g, 30%). Light yellow powder solid, mp 283–284°C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 3.98 (s, 2H, CH_2), 7.80 (d, ^4J = 2.8, 1H, ArH), 8.0–8.1 (m, 3H, ArH), 8.27 (d, ^3J = 8.0 Hz, 1H, ArH), 8.78 (s, 1H, ArH), 8.85 (brs, —NH), 9.04 (brs, —NH). ^{13}C NMR (75 MHz, DMSO-d_6, ppm): δ 153.0, 151.5, 147.9, 137.6, 136.5, 133.3, 131.4, 129.6, 126.5, 126.1, 125.0, 122.3, 119.0, 117.6, 114.8, 33.8. FT-IR (neat $\tilde{\nu}$ cm^{-1}) 3403–3318 (NH_2), 3184 (Arom. C—H), 2923–2766 (Aliph. C—H), 1635, 1493, 1376, 1059, 862–741 (C—Br). HRMS (ESI) *m/z*: calcd. for $C_{16}H_{11}N_2Br_2$ $[M+H]^+$: 390.9269. Found: 390.8984.

10-Amine-3,8-dibromo-11-*H*-indeno[1,2-*b*]quinoline (15f)

2-Amino-5-bromobenzonitrile (**13**) (0.35 g, 1.78 mmol), 6-bromoindan-1-one (**14c**) (0.38 g, 1.78 mmol), and $InCl_3$ (0.51 g, 2.30 mmol) gave the title compound (**15f**) (0.16 g, 23%). Light brown powder solid, mp 280–281°C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm): δ 3.89 (s, 2H, CH_2), 7.70–7.84 (m, 2H, ArH), 7.92 (d, ^3J = 9.3 Hz, 1H, ArH), 8.03 (dd, ^3J = 9.0 Hz, ^4J = 2.1 Hz,1H, ArH), 8.48 (s, 1H, ArH), 8.72 (s, 1H, ArH), 8.89 (brs, NH_2) ppm. ^{13}C NMR (150 MHz, DMSO-d_6, ppm): δ 153.0, 151.0, 144.7, 137.5, 136.5, 136.1, 134.5, 128.4, 126.5, 126.0, 122.3, 121.0, 119.1, 117.5, 115.4, 33.6. FTIR (neat $\tilde{\nu}$ cm^{-1}) 3556–3426 (NH_2), 3198–3027 (Arom. C—H), 2967–2841 (Aliph. C—H), 1735, 1646, 1575 (C═N), 1459, 831–734 (C—Br). HRMS (ESI) *m/z*: calcd. for $C_{16}H_{11}N_2Br_2$ $[M+H]^+$: 390.9269. Found: 390.8980.

10-Amine-1,6,8-tribromo-11-*H*-indeno[1,2-*b*]quinoline (15g)

2-Amino-3,5-dibromobenzonitrile (**12**) (0.35 g, 1.27 mmol), 4-bromoindan-1-one (**14a**) (0.27 g, 1.27 mmol), and $InCl_3$ (0.51 g, 2.30 mmol) gave the title compound (**15g**) (0.12 g, 20%). Light yellow powder solid, mp 297–298°C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 3.84 (s, 2H, CH_2), 7.02 (s, 1H, ArH), 7.19 (s, 1H, ArH), 7.49 (t, 4.6 Hz, ^3J = 3.8, 1H, ArH), 7.78 (d, ^3J = 3.8 Hz, 1H, ArH), 8.32 (d, ^3J = 4.6 Hz, 1H, ArH), 8.35 (brs, NH), 8.68 (brs, NH). ^{13}C NMR (75 MHz, DMSO-d_6, ppm): δ 155.7, 151.6, 148.3, 142.3, 141.2, 136.5, 133.5, 129.3, 128.7, 126.6, 124.8, 122.5, 121.5, 121.4, 32.7. FT-IR (neat $\tilde{\nu}$ cm^{-1}) 3334–3225 (NH_2), 3102–3033 (Arom. C—H), 2970 (Aliph. C—H), 1656, 1574 (C═N), 1402, 1377, 855–764 (C—Br). HRMS (ESI) *m/z*: calcd. for $C_{16}H_{10}N_2Br$ $[M+H]^+$: 468.8374. Found: 469.1268.

10-Amine-2,6,8-tribromo-11-*H*-indeno[1,2-*b*]quinoline (15h)

2-Amino-3,5-dibromobenzonitrile (**12**) (0.83 g, 3 mmol), 5-bromoindan-1-one (**14b**) (0.63 g, 3 mmol), and $InCl_3$ (0.8 g, 3.6 mmol) gave the title compound (**15h**) (0.08 g, 13%). Light yellow powder solid, mp 294–295°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.99 (s, 2H, CH_2), 7.49 (d, ^3J = 4.0 Hz, 1H, ArH), 7.55 (d, ^3J = 3.9 Hz, 1H, ArH), 7.59–7.62 (m, 2H, ArH), 7.70 (s, 2H, ArH), 7.71 (s, 1H, ArH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 32.6, 125.0 (2C), 125.4, 126.1, 126.9, 128.7, 129.1, 129.2, 130.3, 131.0, 138.3, 139.6, 149.9, 153.7, 154.3. FT-IR (neat $\tilde{\nu}$ cm^{-1}) 3384 (NH_2), 3086, 2920–2849, 1673, 1585 (C═N), 1465, 1317, 1123, 866–615 (C—Br). HRMS (ESI) *m/z*: calcd. for $C_{16}H_{10}N_2Br$ $[M+H]^+$: 468.8374. Found: 469.1268.

10-Amine-3,6,8-tribromo-11-*H*-indeno[1,2-*b*]quinoline (15i)

2-Amino-3,5-dibromobenzonitrile (**12**) (0.35 g, 1.27 mmol), 6-bromoindan-1-one (**14c**) (0.27 g, 1.27 mmol), and $InCl_3$ (0.31 g, 1.39 mmol) gave the title compound (**15i**) (0.09 g, 15% crude). Light yellow powder solid, mp 291–292°C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 3.88 (s, 2H, CH_2), 7.67 (d, ^3J = 4.8 Hz, 1H), 7.74 (d, ^3J = 4.8 Hz, 1H), 8.30 (d, ^3J = 1.8 Hz, 1H), 8.28 (s, 1H), 8.42 (brs, 1H), 8.65 (brs, 1H), 8.73 (d, ^3J = 4.2 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6, ppm): δ 153.0, 151.0, 144.7, 137.5, 136.5, 136.1, 134.5, 128.4, 126.5, 126.0, 122.3, 121.0, 119.1, 117.5, 115.4, 33.6. FT-IR (neat $\tilde{\nu}$ cm^{-1}) 3312–3205 (NH_2), 3131–3078, 2970, 1739, 1573 (C═N), 1492, 1389, 1217, 1156, 875–734 (C—Br). HRMS (ESI) *m/z*: calcd. for $C_{16}H_{10}N_2Br$ $[M+H]^+$: 468.8374. Found: 468.8329.

4.1.5 | General procedures for the synthesis of arylated indenoquinolines

To a solution of indenoquinoline (1.0 equiv) in 1,4-dioxane, aq Na_2CO_3 (3.0 M) 15 mL was added and stirred for 10 min at room temperature under N_2, followed by the addition of $Pd(PPh_3)_4$ (0.05 equiv) and phenylboronic acid (1.3 equivalents for monobromides, 2.6 equivalents for dibromides). The mixture was refluxed for 4 h at 90°C. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was diluted with H_2O and extracted with $CHCl_3$ (3 × 20 mL). The combined org. layers were dried (Na_2SO_4), filtered, and the filtrate was concentrated *in vacuo*. The resulting residue was purified by column chromatography (silica gel, $CHCl_3$/MeOH).

10-Amine-1-phenyl-11-*H*-indeno[1,2-*b*]quinoline (16)

Starting with **15a** (300 mg, 0.96 mmol), $Pd(PPh_3)_4$ (75.0 mg, 0.048 mmol), dioxane (15 ml), aq. K_2CO_3 solution (15 mL), and $PhB(OH)_2$ (150 mg, 1.25 mmol) were isolated as a brownish–yellow crystalline solid 285 mg (96%), mp 234–235°C. ^{1}H NMR (300 MHz, $CDCl_3$): 3.69 (s, 2H, CH_2); 4.77 (brs, 2H, NH_2); 7.33–7.73 (m, 10H, ArH); 8.12 (d, ^3J = 7.8 Hz, 1H, ArH); 8.24 (d, ^3J = 6.6 Hz, 1H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$): 31.6, 114.8, 118.2, 120.4, 121.1, 124.2, 127.6, 128.2, 128.7, 128.8, 129.0, 129.6, 130.0, 131.9, 132.2, 132.4, 139.4, 140.7, 142.0, 145.0, 149.4, 161.5. FT-IR (neat $\tilde{\nu}$ cm^{-1}) 3389 (NH_2), 3059 Arom. C—H), 2921–2850 (Aliph. C—H), 1633, 1567 (C═N), 1432, 1387. Anal. calcd. for $C_{22}H_{16}N_2$: C, 85.69%, H, 5.23%, N, 9.08%. Found: C, 85.72%, H, 5.25%, N, 9.03%.

10-Amine-2-phenyl-11-*H*-indeno[1,2-*b*]quinoline (17)

Starting with **15b** (164 mg, 0.53 mmol), $Pd(PPh_3)_4$ (30.0 mg, 0.026 mmol), dioxane (15 ml), 3 M aq. K_2CO_3 solution (15 mL), and $PhB(OH)_2$ 84.0 mg, 0.68 mmol) were isolated as a brownish–yellow crystalline solid 155 mg (95%), mp 235–236°C. ^{1}H NMR (300 MHz, $CDCl_3$-DMSO-d_6): 3.93 (s, 2H, CH_2); 6.00 (brs, 2H, NH_2); 7.40–7.50 (m,

4H, ArH); 7.60–7.72 (m, 4H, ArH); 7.85 (s, 1H, ArH); 8.03 (d, ^3J = 8.5 Hz, 1H, ArH); 8.12 (d, ^3J = 8.4 Hz, 1H, ArH); 8.23 (d, ^3J = 8.0 Hz, 1H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$-DMSO-d_6): 32.0, 114.1, 118.1, 121.5, 121.7, 123.5, 124.0, 126.1, 127.1 (2C), 127.4, 128.6, 128.8 (2C), 129.0, 140.6, 140.9, 141.8, 145.1, 146.3, 149.2, 160.7. FT-IR (neat $\nu^{\sim}$ cm^{-1}) 3451–3392 (NH_2), 3058 Arom. C—H), 2922–2854 (Aliph. C—H), 1648, 1568 (C═N). Anal. calcd. for $C_{22}H_{16}N_2$: C, 85.69%, H, 5.23%, N, 9.08%. Found: C, 85.55%, H, 5.20%, N, 9.02%.

10-Amine-3-phenyl-11-*H*-indeno[1,2-*b*]quinoline (18)

Starting with **15c** (76.0 mg, 0.24 mmol), $Pd(PPh_3)_4$ (14.0 mg, 0.012 mmol), dioxane (15 mL), 3 M aq. K_2CO_3 solution (15 mL), and $PhB(OH)_2$ (39.0 mg, 0.32 mmol) were isolated as a brownish–yellow crystalline solid 68 mg (92%), mp 234–235°C. ^{1}H NMR (300 MHz, $CDCl_3$): 3.82 (s, 2H, CH_2); 4.76 (brs, 2H, NH_2); 7.37–7.50 (m, 4H, ArH); 7.70–7.82 (m, 6H, ArH); 8.16 (d, ^3J = 8.5, 1H, ArH); 8.49 (s, 1H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$): 31.5, 115.3, 118.3, 120.2, 120.5, 124.5, 125.8, 127.6, 128.7 (3C), 129.0, 129.1 (2C), 129.9, 132.3, 140.8, 141.2, 142.1, 143.2, 145.0, 161.6. FT-IR (neat $\nu^{\sim}$ cm^{-1}) 3803–3510 (NH_2), 3128 Arom. C—H), 2924 (Aliph. C—H), 1645, 1565 (C═N), 1506, 1433, 1363, 817, 758, 693. Anal. calcd. for $C_{22}H_{16}N_2$: C, 85.69%, H, 5.23%, N, 9.08%. Found: C, 85.65%, H, 5.22%, N, 9.15%.

10-Amine-1,8-diphenyl-11-*H*-indeno[1,2-*b*]quinoline (19)

Starting with **15d** (30.0 mg, 0.077 mmol), $PhB(OH)_2$ (24 mg, 0.2 mmol) and $Pd(PPh_3)_4$ (9.0 mg, 0.008 mmol) in dioxane (15 mL) and 3 M aq. K_2CO_3 solution (15 mL), **19** was isolated as a brownish–yellow crystalline solid (25 mg, 83%), mp 251–252°C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 3.93 (s, 2H, CH_2); 6.95 (brs, 2H, NH_2); 7.36–7.38 (t, J = 3.7, 1H, ArH); 7.43–7.58 (m, 7H, ArH); 7.68–7.70 (d, J = 3.8 Hz, 2H, ArH); 7.88–7.90 (d, J = 3.8 Hz, 2H, ArH); 7.96 (brs, 2H, ArH); 8.05–8.06 (d, J = 3.7, 1H, ArH); 8.57 (s, 1H, ArH). ^{13}C NMR (75 MHz, DMSO-d_6, ppm): δ 29.4, 114.0, 120.4, 120.6, 126.6, 127.3 (3C), 127.7, 127.9, 128.3, 129.0 (4C), 129.1 (4C), 129.3 (3C), 129.7, 139.3, 140.5, 142.5, 151.9, 162.6. FT-IR (neat $\nu^{\sim}$ cm^{-1}) 3456–3322 (NH_2), 3192, 3034 (Arom. C—H), 2921, 2770 (Aliph. C—H), 1639, 1558 (C═N), 1491, 1385. Anal. calcd. for $C_{28}H_{20}N_2$: C, 87.47%, H, 5.24%, N, 7.29%. Found: C, 87.55%, H, 5.20%, N, 7.32%.

10-Amine-2,8-diphenyl-11-*H*-indeno[1,2-*b*]quinoline (20)

Starting with **15e** (97.0 mg, 0.248 mmol), $PhB(OH)_2$ (79 mg, 0.65 mmol) and $Pd(PPh_3)_4$ (29.0 mg, 0.025 mmol) in dioxane (15 mL) and 3 M aq. K_2CO_3 solution (15 mL), **20** was isolated as a brownish–yellow crystalline solid (87 mg, 91%), mp 253–254°C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 3.93 (s, 2H, CH_2); 7.05 (brs, 2H, NH_2); 7.38–7.40 (m, 2H, ArH); 7.48–7.53 (m, 4H, ArH); 7.76–7.79 (m, 3H, ArH); 7.88–7.90 (d, J = 3.9, 2H, ArH); 7.96 (brs, 2H, ArH); 8.09–8.11 (d, J = 3.9, 1H, ArH); 8.59 (s, 1H, ArH). ^{13}C NMR (75 MHz, DMSO-d_6, ppm): δ 29.5, 114.2, 118.5, 120.4, 121.9, 124.3, 126.3, 127.3 (4C), 127.4 (4C), 127.7, 128.0, 129.3 (4C), 129.4 (4C), 140.3, 140.7, 145.8. FT-IR (neat $\nu^{\sim}$ cm^{-1}) 3346 (NH_2), 3088 (Arom. C—H), 2919–2848 (Aliph. C—H), 1634, 1567 (C═N), 1489, 1386. Anal. calcd. for $C_{28}H_{20}N_2$: C, 87.47%, H, 5.24%, N, 7.29%. Found: C, 87.40%, H, 5.22%, N, 7.34%.

10-Amine-3,8-diphenyl-11-*H*-indeno[1,2-*b*]quinoline (21)

Starting with **15f** (110 mg, 0.28 mmol), $PhB(OH)_2$ (90 mg, 0.74 mmol) and $Pd(PPh_3)_4$ (30 mg, 0.025 mmol) in dioxane (15 mL) and 3 M aq. K_2CO_3 solution (10 mL), **21** was isolated as a brownish–yellow crystalline solid (86 mg, 80%), mp 252–253°C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 3.83 (s, 2H, CH_2); 7.32–7.76 (m, 15H, ArH); 8.09–8.17 (m, 2H, ArH); 8.59 (s, 1H, ArH). ^{13}C NMR (75 MHz, DMSO-d_6, ppm): δ 29.6, 115.0, 116.0, 121.4, 123.9, 126.4 (3C), 126.8, 127.1 (3C), 127.3 (3C), 127.4, 128.2 (3C), 129.0 (3C), 129.1, 129.2, 129.7, 140.6, 143.1, 145.6. FT-IR (neat $\nu^{\sim}$ cm^{-1}) 3392 (NH_2), 3068 (Arom. C—H), 2922–2774 (Aliph. C—H), 1648, 1611, 1568 (C═N), 1433, 1389, 965, 747. Anal. calcd. for $C_{28}H_{20}N_2$: C, 87.47%, H, 5.24%, N, 7.29%. Found: C, 87.49%, H, 5.22%, N, 7.21%.

4.2 | Biology

4.2.1 | Anticholinesterase activity tests

Inhibitory activities of tested compounds against acetyl- and butyrylcholinesterase were measured by slightly modified spectrophotometric method developed by Ellman et al.[42] Acetylthiocholine iodide and butyrylthiocholine iodide were used as substrates of the reaction and DTNB method was used for the measurement of the anticholinesterase activity. One hundred thirty microliters of 100 mM sodium phosphate buffer (pH 8.0), test compound solutions and of solution of AChE or BChE were mixed and incubated for 15 min at 25°C, and 0.5 mM DTNB was added. The reaction was then initiated by the addition of acetylthiocholine iodide (0.71 mM) or butyrylthiocholine chloride (0.2 mM). The hydrolysis of these substrates was monitored spectrophotometrically by the formation of yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine iodide or butyrylthiocholine chloride, at a wavelength of 412 nm. Acetylcholinesterase enzyme obtained from *Electrophorus electricus* (Sigma–Aldrich, code: C3389) and butyrylcholinesterase enzyme obtained from *Equus caballus* (Sigma–Aldrich, code: C7512) were supplied from market. Tacrine and galanthamine were used as standard compound. Ethanol was used as a solvent to dissolve test compounds and the controls. The effect of ethanol excluded in the calculation.

Percentage of inhibition of AChE or BChE was determined by a comparison of the rates of reaction of samples relative to blank sample (ethanol in phosphate buffer pH 8) using the formula:

$$[(E - S)/E] \times 100$$

where E is the activity of enzyme without test sample and S is the activity of enzyme with test sample.

The anticholinesterase activity of 14 indenoquinoline amine derivatives was measured at different concentrations (from 10^{-8} to 200 μM) for calculation of the IC_{50} values. Each substance was tested three times in triplicate against AChE and BChE enzymes.

4.2.2 | Carbonic anhydrase I and II isoenzymes purification and inhibition studies

The purification of both cytosolic CA isoforms (CA I and II) of human blood cells was previously explained with a simple one-step procedure by a Sepharose-4B-L-tyrosine-sulphanilamide affinity chromatoghraphy.[43–45] The protein quantity in the column effluents were evaluated spectrophotometrically at 280 nm and previously described. For purity of both isoenzymes sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was applied. CA isoenzymes activities were obtained in conforming with the procedure of Verpoorte et al.[46] explained previously. The enhance in absorbance of the reaction medium was spectrophotometrically obtained at 348 nm. We utilized the esterase activity procedure for ascertaining the inhibition agents by the Lineweaver–Burk procedure.[47] CA activity (%) versus inhibitory concentration and $1/V$ versus 1/[S] graphs were drawn. The quantity of protein was determined at 595 nm according to Bradford's procedure.[48] Bovine serum albumin was used as standard protein as given previously in detail. For the designation of the inhibition efficacy of each novel indenoquinoline amine derivatives on both hCA isoenzymes, an activity (%)-[indenoquinoline derivatives] graph was drawn. The IC_{50} values were obtained from activity (%) versus compounds plots. For the calculation of K_i values, substituted indenoquinoline derivatives' concentrations were used.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES

[1] M. A. Ceschi, J. S. da Costa, J. P. B. Lopes, V. S. Câmara, L. F. Campo, A. C. A. Borges, C. A. S. Gonçalves, D. F. de Souza, E. L. Konrath, A. L. M. Karl, I. A. Guedes, L. E. Dardenne, *Eur. J. Med. Chem.* **2016**, *121*, 758.
[2] J. B. Standridge, *Clin. Ther.* **2004**, *26*, 615.
[3] M. Eghtedari, Y. Sarrafi, H. Nadri, M. Mahdavi, A. Moradi, F. H. Moghadam, S. Emami, L. Firoozpour, A. Asadipour, O. Sabzevari, A. Foroumadi, *Eur. J. Med. Chem.* **2017**, *128*, 237.
[4] H. M. Bryson, P. Benfield, *Drugs Aging* **1997**, *10*, 234.
[5] L. J. Scott, K. L. Goa, *Drugs* **2000**, *60*, 1095.
[6] M. Shanks, M. Kivipelto, R. Bullock, R. Lane, *Curr. Med. Res. Opin.* **2009**, *25*, 2439.
[7] M. A. Kamal, P. Klein, W. Luo, Y. Li, H. W. Holloway, D. Tweedie, N. H. Greig, *Neurochem. Res.* **2008**, *33*, 745.
[8] R. M. Lane, S. G. Potkin, A. Enz, *Int. J. Neuropsychopharmacol.* **2005**, *9*, 1.
[9] F. K. Hank, *ACS Med. Chem. Lett.* **2012**, *3*, 265.
[10] P. K. Mukherjee, V. Kumar, M. Mal, P. J. Houghton, *Phytomedicine* **2007**, *14*, 289.
[11] P. Taslimi, H. Akıncıoğlu, İ. Gulcin, *J. Biochem. Mol. Toxicol.* **2017**, *31*, e21973.
[12] M. Boztas, Y. Cetinkaya, M. Topal, I. Gülçin, A. Menzek, E. Sahin, C. T. Supuran, *J. Med. Chem.* **2014**, *58*(2), 640.
[13] A. Yıldırım, U. Atmaca, A. Keskin, M. Topal, M. Celik, İ. Gülçin, C. T. Supuran, *Bioorg. Med. Chem.* **2015**, *23*(10), 2598.
[14] U. M. Koçyiğit, Y. Budak, F. Eligüzel, P. Taslimi, D. Kılıç, İ. Gulçin, M. Ceylan, *Arch. Pharm.* **2017**, *350*, e1700198.
[15] P. Taslimi, C. Caglayan, İ. Gulcin, *J. Biochem. Mol. Toxicol.* **2017**, *31*, e21995.
[16] U. M. Koçyiğit, Y. Budak, M. B. Gürdere, F. Ertürk, B. Yencilek, P. Taslimi, İ. Gulçin, M. Ceylan, *Arch. Physiol. Biochem.* **2017**, *124*, 61.
[17] S. Ökten, O. Çakmak, R. Erenler, Ş. Tekin, Ö. Yüce, *Turk. J. Chem.* **2013**, *37*, 896.
[18] S. Ökten, Ö. Y. Şahin, Ş. Tekin, O. Çakmak, *J. Biotechnol.* **2014**, *185*, S106.
[19] S. Ökten, O. Çakmak, Ş. Tekin, *Turk. J. Clin. Lab.* **2017**, *8*, 152.
[20] S. Ökten, O. Çakmak, Ş. Tekin, T. K. Köprülü, *Lett. Drug Des. Dis.* **2017**, *14*, 1415.
[21] R. Zong, D. Wang, R. Hammitt, R. P. Thummel, *J. Org. Chem.* **2006**, *71*, 167.
[22] M. Anzini, A. Cappelli, S. Vomero, A. Cagnotto, M. Skorupska, *Med. Chem. Res.* **1993**, *3*, 249.
[23] M. Yamato, Y. Takeuchi, K. Hashigaki, Y. Ikeda, M. C. Chang, K. Takeuchi, M. Matsushima, T. Tsuruo, T. Tashiro, S. Tsukagoshi, Y. Yamashita, H. Nakano, *J. Med. Chem.* **1989**, *32*, 1295.
[24] L. W. Deady, J. Desneves, A. J. Kaye, G. J. Finlay, B. C. Baguley, W. A. Denny, *Bioorg. Med. Chem.* **2000**, *8*, 977.
[25] J. R. Brooks, C. Berman, M. Hichens, R. L. Primka, G. F. Reynolds, G. H. Rasmusson, *Proc. Soc. Exp. Biol. Med.* **1982**, *169*, 67.
[26] L. W. Deady, A. J. Kaye, G. J. Finlay, B. C. Baguley, W. A. Denny, *J. Med. Chem.* **1997**, *40*, 2040.
[27] B. Venugopalan, C. P. Bapat, E. P. Desouza, N. J. Desouza, *Indian J. Chem. B* **1992**, *31*, 35.
[28] A. Rampa, A. Bisi, F. Belluti, S. Gobbi, P. Valenti, V. Andrisano, V. Cavrini, A. Cavalli, M. Recanatini, *Bioorg. Med. Chem.* **2000**, *8*, 497.
[29] S. Ökten, O. Çakmak, *Tetrahedron Lett.* **2015**, *56*, 5337.
[30] O. Çakmak, S. Ökten, *Tetrahedron* **2017**, *73*, 5389.
[31] M. Ekiz, A. Tutar, S. Ökten, *Tetrahedron* **2016**, *72*, 5323.
[32] O. Çakmak, R. Erenler, A. Tutar, N. Çelik, *J. Org. Chem.* **2006**, *71*, 1795.
[33] A. Tutar, K. Berkil, R. R. Hark, M. Balcı, *Synth. Commun.* **2008**, *38*, 1333.
[34] R. Erenler, O. Çakmak, *J. Chem. Res.* **2004**, *2004*, 566.
[35] R. Erenler, I. Demirtaş, B. Buyukkidan, O. Çakmak, *J. Chem. Res.* **2006**, *2006*, 753.
[36] A. Şahin, O. Çakmak, I. Demirtas, S. Ökten, A. Tutar, *Tetrahedron* **2008**, *64*, 10068.
[37] İ. Çelik, M. Akkurt, M. Ekiz, S. Ökten, A. Tutar, C. C. Ersanlı, *IUCrData* **2017**, *2*, x171011.
[38] M. Ekiz, A. Tutar, S. Ökten, B. Bütün, G. Topcu, Third International Drug and Pharmacy Congress, April 26–29, **2017**, İstanbul, Turkey, Abstract no: 0176, p. 334.
[39] M. Ekiz, S. Ökten, Ü. M. Koçyiğit, A. Tutar, P. Talsimi, Fourth International Congress for Applied Biological Sciences, May 3–5, **2018**, Eskişehir, Turkey.
[40] A. P. Rajput, A. R. Kankhare, *Int. J. Pharm. Sci. Investig.* **2017**, *6*, 19.
[41] U. M. Kocyigit, P. Taslimi, F. Gurses, S. Soylu, S. Durna Dastan, İ. Gulcin, *J. Biol. Chem.* **2018**, *32*(3), e22031.
[42] G. L. Ellman, K. D. Courtney, V. Andres, R. M. Featherston, *Biochem. Pharmacol.* **1961**, *7*, 88.
[43] A. Scozzafava, M. Passaponti, C. T. Supuran, İ. Gülçin, *J. Enzyme Inhib. Med. Chem.* **2015**, *30*(4), 586.
[44] B. Arabaci, I. Gulcin, S. Alwasel, *Molecules* **2014**, *19*(7), 10103.
[45] A. Scozzafava, P. Kalın, C. T. Supuran, İ. Gülçin, S. H. Alwasel, *J. Enzyme Inhib. Med. Chem.* **2015**, *30*(6), 941.
[46] J. A. Verpoorte, S. Mehta, J. T. Edsall, *J. Biol. Chem.* **1967**, *242*, 4221.

[47] H. Lineweaver, D. Burk, *J. Am. Chem. Soc*. **1934**, *56*, 658.
[48] M. M. Bradford, *Anal. Biochem*. **1976**, *72*, 248.

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How to cite this article: Ekiz M, Tutar A, Ökten S, et al. Synthesis, characterization, and SAR of arylated indenoquinoline-based cholinesterase and carbonic anhydrase inhibitors. *Arch Pharm Chem Life Sci*. 2018;1–11. https://doi.org/10.1002/ardp.201800167