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FULL PAPER

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Synthesis and biological evaluation of phloroglucinol derivatives possessing α-glycosidase, acetylcholinesterase, butyrylcholinesterase, carbonic anhydrase inhibitory activity

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Abstract

A series of novel phloroglucinol derivatives were designed, synthesized, characterized spectroscopically and tested for their inhibitory activity against selected metabolic enzymes, including α-glycosidase, acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and human carbonic anhydrase I and II (hCA I and II). These compounds displayed nanomolar inhibition levels and showed K_i values of 1.14–3.92 nM against AChE, 0.24–1.64 nM against BChE, 6.73–51.10 nM against α-glycosidase, 1.80–5.10 nM against hCA I, and 1.14–5.45 nM against hCA II.

KEYWORDS
α-glycosidase, acetylcholinesterase, butyrylcholinesterase, carbonic anhydrase, phloroglucinol derivatives

1 | INTRODUCTION

In nature, phenolic compounds, that is, compounds containing an aromatic ring with one or more hydroxyl groups are typically found in esters or glycosides rather than in free structures (Figure 1). These compounds are some of the most studied groups of compounds in the world.[1] Phenolic compounds also have a very important place in everyday life and are commonly used as additives in drugs and food products. Many natural and synthetic phenolic compounds have been reported to be bioactive and possess anticancer, antimutagenic, antiviral, antibacterial, anti-inflammatory, and antioxidant activities.[2] Phloroglucinol (**1**) is a phenolic compound containing three hydroxyl groups attached to its aromatic ring.[3] Phloroglucinol itself is isolated from natural sources and is used in drugs, cosmetics, agrochemicals, cement, and paints.[4]

Phloroglucinol has been reported to have a protective effect against intracellular oxidative stress caused by H_2O_2 owing to its catalase activation properties and can protect against cell damage caused by oxidative stress due to gamma radiation.[5]

As cancer, diabetes, and Alzheimer's disease (AD) are some of the more prevalent diseases in the world today, there is a need for understanding the mechanism of these diseases and the development of novel active therapeutic agents. These diseases are correlated with carbonic anhydrases, α-glycosidase, acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and monoamine oxidases (MAOs) enzymes. These chronic and neurodegenerative diseases urgently require novel drug candidates. For this purpose, it is particularly important to combine experimental studies of synthesis of new compounds and determination of their kinetic, biochemical, pharmacological properties with structural and computational studies.[6–8]

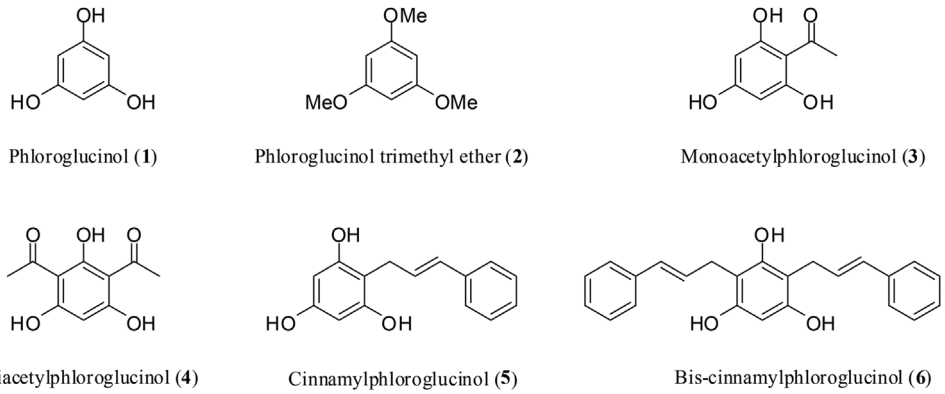


FIGURE 1 The structures of some phloroglucinol (**1**) derivatives

Carbonic anhydrases (CAs, E.C.4.2.1.1) are zinc-containing metalloenzymes that are efficient catalysts for the interconversion of carbon dioxide (CO_2) to bicarbonate (HCO_3^-).[9]

$$CO_2 + H_2O \overset{CA}{\Leftrightarrow} H_2CO_3 \Leftrightarrow H^+ + HCO_3^-$$

In addition to their biochemical functions, CA catalyzes the hydrolysis of esters.[10,11] CAs are commonly found in living organisms, and different isoenzymes can be seen according to various environmental conditions and biological requirements. To date, CAs are known to be encoded by seven different unrelated gene families (α-, β-, γ-, ε-, ζ-, n-, and θ-CAs). Currently, 16 α-CA isoenzymes have been identified.[12,13] All human CAs (hCAs) belong to the α-class. These include several cytosolic isoforms (hCA I, II, III, VII, and XIII), five membrane-bound isoforms (hCA IV, IX, XII, XIV, and XV), two mitochondrial isoforms (hCA VA and VB), and one secreted form (hCA VI). The hCAs are involved in various biological processes such as gluconeogenesis, lipogenesis, and ureagenesis.[14–16] The most widely available and studied isoenzymes are hCA I and II.[17] Numerous studies have investigated synthetic compounds associated with the inhibition of CA I. CA inhibitors (CAIs) are clinically used for the treatment of certain diseases such as glaucoma and cancer. Recently, CAIs have demonstrated the potential to act as antiobesity drugs, diuretics, and anti-infective drugs.[18] The hCA I isoenzyme is found in many tissues, occurs in high concentrations in blood and the gastrointestinal tract, and is involved in retinal and cerebral edema. The inhibition of hCA I may be a valuable tool for fighting retinal and cerebral edema.[19] The hCA II isoenzyme is a ubiquitous cytosolic isoform. Its role in glaucoma has been well characterized. Indeed, HCO_3^- production serves as a mechanism to transport sodium ions into the eye along with the influx of water, leading to an increase in intraocular pressure.[20]

The inhibition and activation mechanism of CAs are well known.[21,22] CAIs are clinically important compounds.[23,24] Many efforts have been made to develop CAIs for therapeutic cancer treatments and for lowering intraocular pressure. Most types of classical CAIs bind directly to the metal center within the enzyme active site. In contrast, activator molecules bind at the entrance of the active center cavity.[25–27] In non-insulin-dependent (type II) diabetes mellitus (T2DM), inadequate or inappropriate amounts of insulin are secreted, and peripheral cells become resistant to the action of insulin.[28] One T2DM therapy uses the inhibition of enzymes that can hydrolyze polysaccharide molecules and convert them into monosaccharide or simple sugar units.[29] This type of enzyme, such as α-glycosidase, acts by hydrolyzing disaccharide molecules to glucose. The hypoglycemic agents retard the digestion and assimilation of simple carbohydrates in the intestine through α-glycosidase inhibition. As a result, postprandial blood glucose levels in T2DM can be controlled.[30] Some α-glycosidase-inhibiting medicines that are used for T2DM treatment, such as acarbose, miglitol, and voglibose, bind to the enzyme active site, thereby limiting their catalytic activities, to reduce carbohydrate digestion and glucose uptake. However, these hypoglycemic agents elicit several undesirable effects.[30,31] Owing to the unwanted side effects of these hypoglycemic agents, attention has been directed toward antidiabetic nutraceuticals, natural derivatives, and other phytoconstituents.[32] It was reported that DM is characterized by hyperglycemia, which could result from insulin secretion deficiencies and/or the inability of pancreatic β-cells to produce insulin. Inhibition of α-glycosidase is important in the management of DM.[32]

AD, the most common form of dementia among the elderly, is a progressive neurodegenerative disease of the central nervous system.[33] The 2015 World Alzheimer Report estimates that there are 46.8 million people living with dementia, and this number is expected to increase rapidly with over 10 million new cases of dementia reported each year.[33] AChE EC.3.1.1.7 present in the postsynaptic membrane is responsible for the termination of neuronal signal transmission by hydrolyzing acetylcholine (ACh). BChE (EC 3.1.1.8) is produced in the liver and found primarily in blood plasma, the central nervous system, and the peripheral nervous system. ACh can normally be hydrolyzed by both AChE and BChE: AChE mostly for α-glycoproteins of neuronal origin and BChE mostly for α-glycoproteins of glial origin.[34,35] Under normal conditions, ACh is dominantly decomposed by AChE instead of BChE.[36,37] Although BChE is considered to have a minor role in regulating brain ACh levels, BChE levels have been reported to correlate with drug metabolism and detoxification. Additionally, BChE is closely associated with lipoprotein

metabolism and diseases such as obesity, hepatic adiposity, and cardiovascular disease.[38,39] It was also reported that BChE activity increases mainly in areas of the brain most affected by AD.[40,41] Abnormal acetylation of histone is involved in the pathology of AD. Therefore, AChE and BChE have been attractive targets for AD therapeutic drug discovery.[42,43] The overall structures of the two ChEs are very similar. AChE and BChE contain a catalytic active site, a deep gorge, and a peripheral anionic site. Almost 65% of the amino acid sequences in AChE and BChE are homological.[44,45]

In this study, we describe the synthesis of some novel phloroglucinol derivatives (**12a–d** and **13a–d**) and determine their inhibition profiles against selected metabolic enzymes, including AChE, BChE, α-glycosidase enzymes, and CA I and II isoenzymes, toward therapies for cancer, diabetes, and AD.

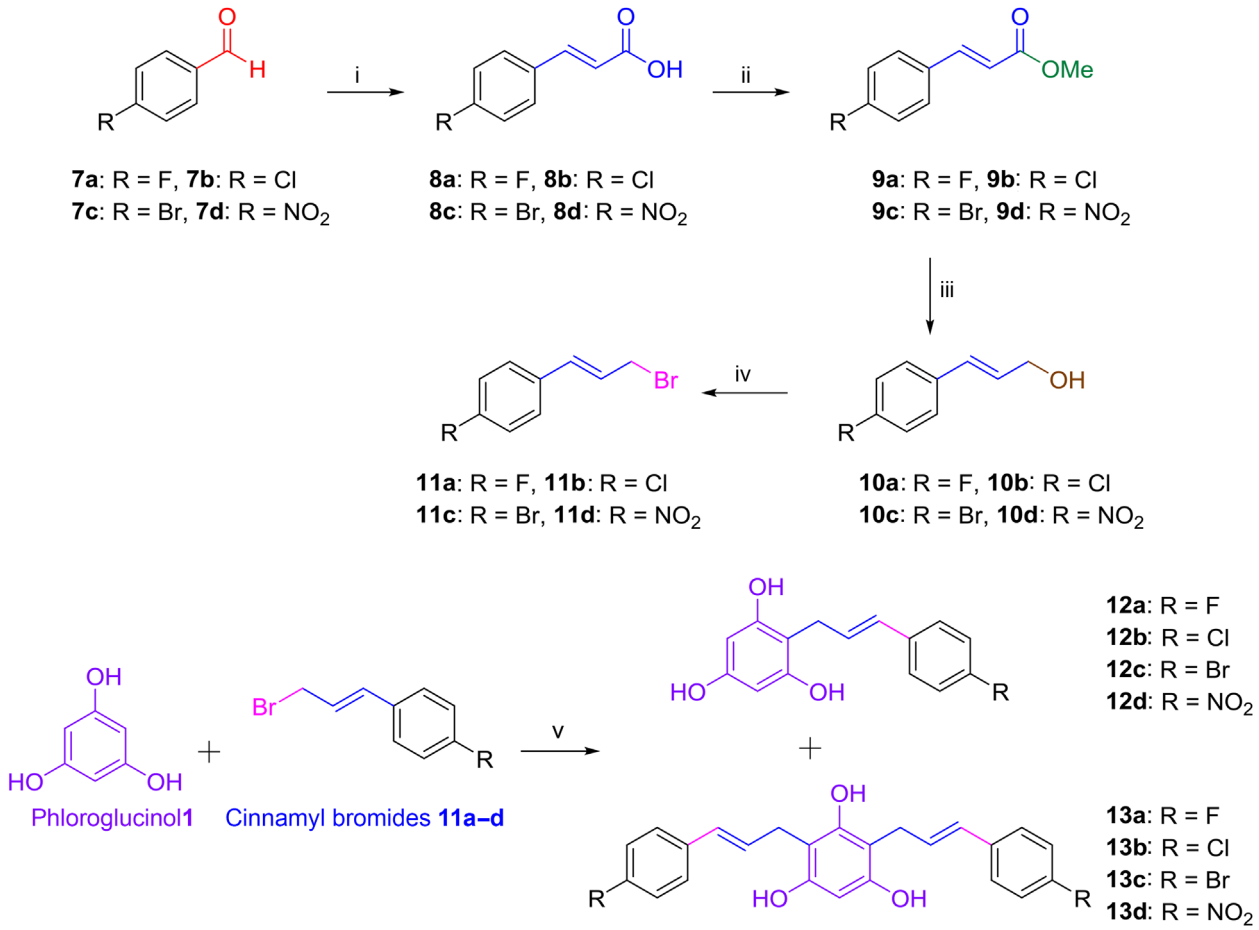
2 | RESULTS AND DISCUSSION

2.1 | Chemistry

The first ever synthesis of cinnamyl-substituted phloroglucinol derivatives **12a–d** and **13a–d** is summarized in Scheme 1. Our synthesis was based on a one-step reaction between phloroglucinol (**1**) and appropriate *p*-substituted cinnamylbromides **11a–d**. Compounds **11a–d** were synthesized in four steps starting from commercially available *p*-substituted benzaldehydes **7a–d**. The reactions between benzaldehydes **7a–d** and malonic acid in the presence of piperidine and pyridine resulted in cinnamic acids **8a–d** in 54–97% yields.[46] Acid-catalyzed esterification of **8a–d** with MeOH gave the ester derivatives **9a–d** in 80–96% yields.[47] Reduction of the cinnamic esters **9a–d** with diisobutylaluminum hydride gave cinnamyl alcohols **10a–d** in 80–98% yields.[48] The reaction of **10a–d** with PBr_3 gave cinnamyl bromides **11a–d** in 53–88% yields (Scheme 1).[48]

After successfully synthesizing cinnamyl bromides **11a–d**, we turned our attention to the alkylation reaction with phloroglucinol (**1**).[3] The base-catalyzed reaction of **1** with **11a**, **11b**, **11c**, or **11d** gave target compounds **12a–d** and **13a–d** in good yields (Scheme 1).

To the best of our knowledge, this is the first reported synthesis of compounds **12a–d** and **13a–d**. All the novel phloroglucinol derivatives were isolated as solids and characterized by ^{1}H NMR, ^{13}C NMR, IR, and elemental analysis (see the Supporting Information).



SCHEME 1 Synthesis of compounds **12a–d** and **13a–d**. Reagents and conditions: (i) Malonic acid, piperidine, pyridine, reflux, 2–3 h, 54–97%; (ii) *p*-TSA, MeOH, reflux, 20–24 h, 80–96%; (iii) DIBAL-H, DCM, −78°C, 1.5 h, 80–98%; (iv) PBr_3, Et_2O, 0°C, 1–2 h, 53–88%; (v) NaH, THF, rt, 17–23 h, 35–60%

2.2 | Biochemistry

Novel compounds are attractive in terms of permitting the design of new chemotypes acting as CAIs. The CA inhibitory effects of the novel phloroglucinol derivatives (**12a–d** and **13a–d**) were measured against hCA I, hCA II isoenzymes and AChE, BChE, and α-glycosidase enzymes. The results are summarized in Table 1 and compared with the K_i values of acetazolamide, tacrine, and acarbose as reference compounds.

As can be seen in Figure 2, acetazolamide (AZA) was used as standard for hCA I and II isoenzymes, acarbose (ACR) was used as standard for α-glycosidase (α-GLY) enzyme. On the other hand, tacrine (TAC) was used as standard for both AChE and BChE enzymes. In these standard molecules, ACR contains hydroxyl groups, but AZA and TAC molecules do not contain any hydroxyl group.

The K_i values of novel phloroglucinol derivatives were found in the range of 1.80 ± 0.25 to 5.10 ± 0.80 nM for hCA I. In comparison, the K_i value for the broad-spectrum CA inhibitor acetazolamide (AZA), an efficient hCA inhibitor, was 13.66 ± 1.91 nM. All the novel phloroglucinol derivatives demonstrated a lower K_i than AZA. Among the novel phloroglucinol derivatives, **13c**, which had three hydroxyl groups and two bromine groups, was the best hCA I inhibitor (K_i: 1.80 ± 0.25 nM). It is well known that compounds containing hydroxyl and halogen groups are effective CA inhibitors.[49]

The novel phloroglucinol derivatives synthesized in this study effectively inhibited hCA II with K_i in the low nanomolar range, between 1.14 ± 0.26 and 5.45 ± 1.64 nM. As with hCA I, **13c** demonstrated the best hCA II inhibition (K_i: 1.14 ± 0.26 nM) in this series. However, all novel phloroglucinol derivatives had better hCA II inhibition when compared with AZA (K_i of AZA: 10.01 ± 0.70 nM). AZA is a clinical CAI and is used to reverse metabolic alkalosis when fluid and potassium replacements are insufficient to correct blood alkalinity. AZA has also been shown to be effective at reducing the frequency of both vertigo attacks and severe headache.[50]

It was reported that some phenolic compounds including 2,6-dimethylphenol, 2,6-diisopropylphenol, 2,6-di-*t*-butylphenol, butylated hydroxytoluene, butylated hydroxyanisole, vanillin, guaiacol, di(2,6-dimethylphenol), di(2,6-diisopropylphenol), and di(2,6-di-*t*-butylphenol) demonstrated K_i values in the range of 37.5–274.5 μM for hCA I and of 0.29–113.5 μM against hCA II, respectively.[51] Also, Balaydın and coworkers reported that ellagic acid, gallic acid, ferulic acid, caffeic acid, quercetin, *p*-coumaric acid, *p*-hydroxybenzoic acid, and syringic acid showed K_i values in the range of 99–1061 μM for hCA I and of 105–758 μM against hCA II, respectively.[52] In some recent studies, it was shown that dimethoxybromophenol derivatives had exhibited excellent inhibitory effects like our new phloroglucinol derivatives, in the low nanomolar range, with K_i values in the range of 0.54–59 nM against hCA I and in the range of 0.97–12.14 nM against hCA II.[53]

The newly synthesized phloroglucinol derivatives were tested against α-glycosidase enzyme and displayed better activity than the standard inhibitor acarbose (K_i: 12.60 ± 7.80 nM). The phloroglucinol derivatives had K_i values in the range of 6.73–51.10 nM against α-glycosidase (Table 1). All the phloroglucinol derivatives

TABLE 1 The inhibition parameters (IC_{50} and K_i values) of some novel phloroglucinol derivatives (**12a–d** and **13a–d**) against some metabolic enzymes including α-glycosidase (α-GLY), acetylcholinesterase (AChE), butyrylcholinesterase (BChE) enzymes and carbonic anhydrase I and II isoenzymes

	IC_{50} (nM)								K_i (nM)						
Compounds	hCA I	r^2	hCA II	r^2	AChE	r^2	BChE	r^2	α-GLY	r^2	hCA I	hCA II	AChE	BChE	AChE/BChE	α-GLY
12a	4.95	0.9906	3.22	0.9925	4.81	0.9754	1.30	0.9879	41.34	0.9969	5.10 ± 0.80	1.77 ± 0.32	2.88 ± 0.40	0.44 ± 0.06	6.54	36.66 ± 6.31
12b	4.53	0.9802	2.04	0.9887	5.05	0.9762	1.42	0.9714	30.32	0.9967	4.35 ± 0.74	1.48 ± 0.18	3.92 ± 1.28	0.90 ± 0.26	4.35	23.12 ± 1.76
12c	4.62	0.9921	2.08	0.9770	3.72	0.9755	1.46	0.9712	33.14	0.9843	5.08 ± 0.97	1.86 ± 0.43	3.51 ± 0.15	0.62 ± 0.30	5.66	24.91 ± 3.33
12d	4.58	0.9661	2.87	0.9984	3.80	0.9922	2.01	0.9927	43.88	0.9901	3.17 ± 0.43	2.84 ± 1.02	2.25 ± 0.47	1.64 ± 0.34	1.37	25.15 ± 6.52
13a	3.26	0.9665	2.13	0.9820	2.21	0.9822	0.77	0.9611	34.21	0.9831	4.32 ± 1.13	1.93 ± 0.22	1.32 ± 0.14	0.34 ± 0.16	3.35	51.10 ± 15.34
13b	3.78	0.9949	6.02	0.9758	2.58	0.9812	0.54	0.9695	13.35	0.9867	3.28 ± 0.77	5.45 ± 1.64	2.71 ± 0.21	0.25 ± 0.11	10.84	11.13 ± 2.04
13c	2.22	0.9801	1.51	0.9877	2.03	0.9833	0.45	0.9787	36.62	0.9947	1.80 ± 0.25	1.14 ± 0.26	1.14 ± 0.14	0.24 ± 0.11	5.50	6.73 ± 2.33
13d	3.61	0.9574	1.48	0.9749	2.53	0.9965	1.40	0.9941	33.90	0.9868	3.22 ± 0.83	1.24 ± 0.26	1.27 ± 0.28	0.86 ± 0.25	1.47	22.02 ± 5.98
AZA	16.50	0.9897	10.82	0.9934	–	–	–	–	–	–	13.66 ± 1.91	10.01 ± 0.70	–	–	–	–
Tacrine	–	–	–	–	34.65	0.9942	26.65	0.9667	–	–	–	–	14.57 ± 2.02	11.03 ± 1.35	1.32	–
Acarbose	–	–	–	–	–	–	–	–	22.80	–	–	–	–	–	–	12.60 ± 7.80

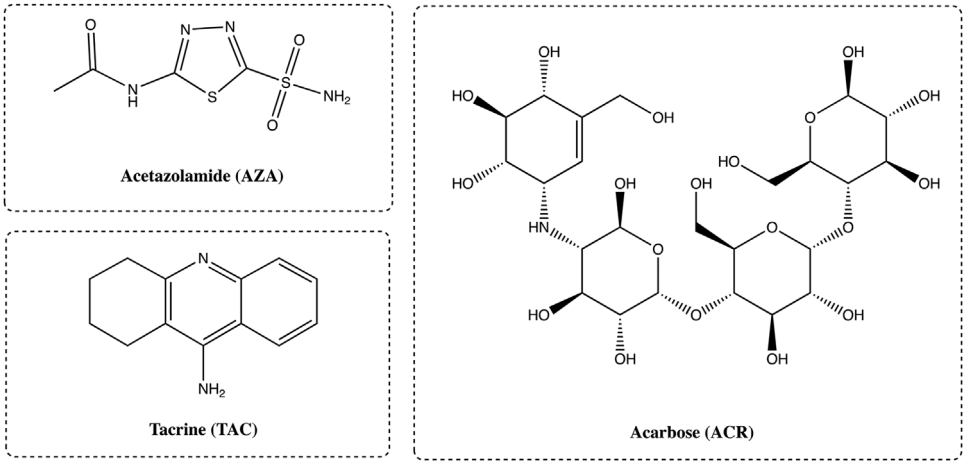


FIGURE 2 Standard molecules were used for enzymes inhibition. Acetazolamide (AZA) was used as standard for hCA I and II isoenzymes. Acarbose (ACR) was used as standard for α-glycosidase enzyme. Tacrine (TAC) was used as standard for both AChE and BChE enzymes

showed excellent inhibition potencies, and their K_i values can be ranked as follows: **13c** (6.73 ± 2.33 nM) < **13b** (11.13 ± 2.04 nM) < acarbose (12.60 ± 7.80 nM) < **13d** (22.02 ± 5.98 nM) < **12b** (23.12 ± 1.76 nM) < **12c** (24.91 ± 3.33 nM) < **12d** (25.15 ± 6.52 nM) < **13a** (51.10 ± 15.34 nM). The most powerful inhibition effect was found for **13c**. It was previously demonstrated that bromo-substituted derivatives showed better results than unsubstituted derivatives. The K_i values clearly show that the bromo-substituent is key and may be responsible for the enrichment of inhibition activity.[30] Thus, the results clearly show that the halogen substitutions enhanced α-glycosidase inhibition. Recently, Taslimi and Gulcin showed that some phenolic compounds including tetrakis, *p*-coumaric acid, caffeic acid phenethyl ester, resveratrol curcumin, olivetol, and rosmarinic acid had K_i values in the range of of 12.60–126.50 nM against α-glycosidase.[54] At the same manner, it was found that some phenolic compounds including synephrine from the leaves of diverse citrus trees and phenylephrine as vasoconstrictor confidants had K_i values of 61.87 and 135.06 nM against α-glycosidase, respectively.[55]

AChE was also highly inhibited by the novel phloroglucinol derivatives with K_i values in the low nanomolar range of 1.14 ± 0.14 to 3.92 ± 1.28 nM (Table 1). These results clearly indicate that novel synthesized phloroglucinol derivatives showed effective AChE inhibition properties. The most powerful AChE inhibition was observed with **13c** (K_i: 1.14 ± 0.14 nM). For comparison, tacrine, the first centrally acting cholinesterase inhibitor approved for the treatment of AD, demonstrated a K_i value of 14.57 ± 2.02 nM against cholinergic AChE. Tacrine has since been removed from the market owing to adverse effects, including hepatotoxicity in a significant percentage of patients.[56] Donepezil hydrochloride, which is used for the treatment of mild-to-moderate AD and various other memory impairments, was also shown to lower AChE inhibition activity (IC_{50}: 55.0 nM).[57]

All the novel phloroglucinol derivatives inhibited BChE with K_i values in the range of 0.24 ± 0.11 to 1.64 ± 0.34 nM. The K_i values of novel phloroglucinol derivatives for AChE and BChE were calculated from Lineweaver–Burk plots.[58] For comparison, tacrine had a K_i value of 11.03 ± 1.35 nM. In a recent study, a series of novel bromophenols were tested against some metabolic enzymes including AChE and BChE.[59] The bromophenols showed K_i values in a range of 4.60–38.13 nM against AChE and 7.36–29.38 nM against BChE. Similarly, it was reported that humic acid effectively inhibited AChE and BChE enzymes with K_i values of 0.208 and 0.112 nM, respectively.[60]

2.3 | Molecular docking

In order to explain how 4,6-bis-*p*-bromocinnamyl phloroglucinol (**13c**) conjugates with hCA I, hCA II, AChE, BChE, and α-glycosidase enzymes, the molecular docking analyses were carried out with Glide/XP docking protocols. Glide scores of the compound **13c** in hCA I, hCA II, AChE, BChE, and α-glycosidase enzymes are determined as −6.24, −4.61, −8.38, −7.83, and −6.18, respectively. Compared to the glide scores, AChE and BChE seem to be higher than hCA I, α-glucosidase, hCA II. These scores give information about whether the compound has affinity to these enzymes.

As result of the docking study, the best XP poses were obtained and are given in Figure 3. Ligand interaction diagrams were used to examine exhaustively the interaction of the ligand observed in these poses with the enzymes as two dimensions. By means of these diagrams, the interactions of the ligand with the key amino acids of the enzymes have been elaborated and also the type of these interactions were determined. The ligand interaction diagrams of compound **13c** at the hCA I (Figure 4a), hCA II (Figure 4b), AChE (Figure 4c), BChE (Figure 4d), and α-glycosidase (Figure 4e) proteins are presented as two-dimensional. His94 and His200 residues formed polar interactions with the compound **13c** in hCA I (Figure 4a), whereas Trp5, Tyr20, Pro21, Ile22, Phe91, Leu198, Pro201, Pro202, Tyr204, Leu141, Leu131, and Ala135 formed hydrophobic interactions. Also, the compound **13c** formed hydrogen bonds with the residues of Tyr20 and Tyr204, π–π stacking interaction with residue Tyr20.

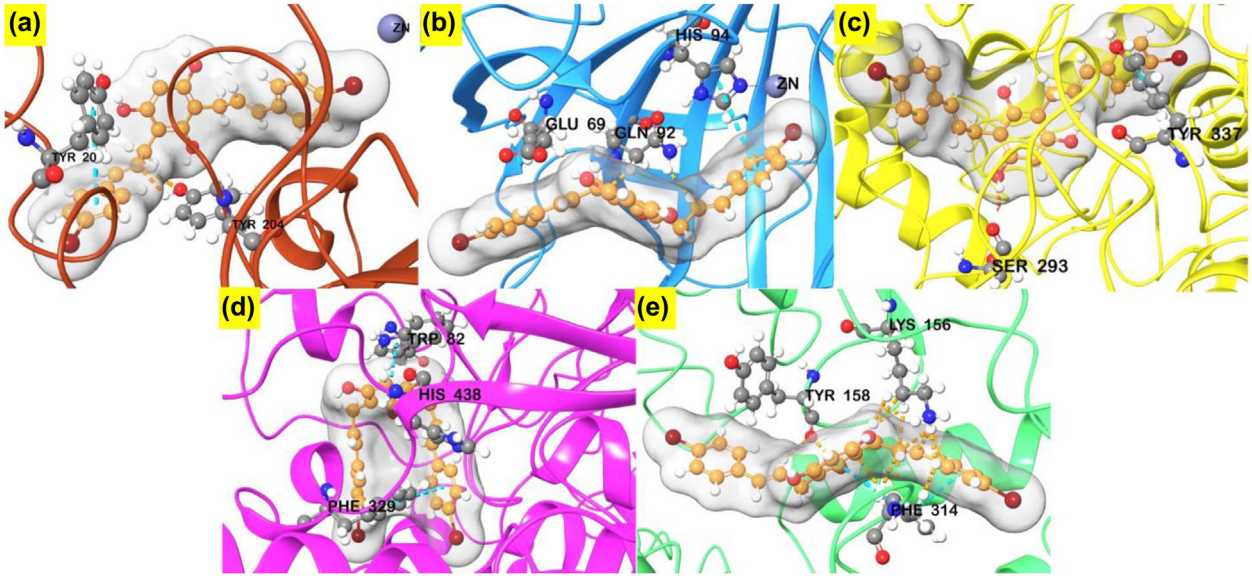


FIGURE 3 The docking poses of compound **13c** in ligand binding sites of hCA I (a), hCA II (b), AChE (c), BChE (d) and α-glycosidase (e). In the images, the key residues are represented in ball and stick model with grey carbon atoms, white hydrogen atoms, blue nitrogen atoms and red oxygen atoms. Zn atoms are represented as purple ball in hCA I and hCA II. Compound **13c** is represented in ball and stick model with orange carbon atoms and white hydrogen atoms, red oxygen atoms and dark red bromine atoms

Asn62, Asn67, His119, Thr199, Thr200, His94, and Gln92 were involved in polar interactions with the compound **13c** in hCA II (Figure 4b), while Leu57, Leu60, Val121, Phe70, Val143, Trp209, Leu198, Ile91, and Phe131 created hydrophobic interactions. Moreover, positive and negative charge interactions were found with Arg58 and Asp71, Asp72, Glu69 residues, respectively. The compound **13c** formed hydrogen bond with the residue Glu69 and π–π stacking interaction with residue His94.

The residues Thr75, Ser203, His 447, and Ser293 made polar interactions with compound **13c** in AChE (Figure 4c), whereas the residues Leu76, Tyr341, Tyr72, Tyr124, Phe338, Tyr337, Trp86, Phe297, Phe295, Val294, and Trp286 made hydrophobic interactions. Moreover, positive and negative charge interactions were found with Arg296 and Asp74, Glh202 residues, respectively. The compound **13c** formed hydrogen bond with the residue Ser293 and π–π stacking interaction with residue Tyr337.

The ligand **13c** in BChE (Figure 4d) formed polar interactions with Ser79, His438, and Ser198 whereas it formed hydrophobic interactions with Trp430, Trp82, Met437, Tyr440, Tyr332, Pro285, Phe329, Ala328, Leu286, Val288, Trp231, and Phe398. Additionally, negative charge interaction was found with Asp70. The compound **13c** formed hydrogen bond with the residue His438 and π–π stacking interaction with residues Trp82, Phe329, and Tyr332. The residues Asn415, Ser157, Ser236, Asn235, and Ser240 made polar interactions with compound **13c** in α-glycosidase (Figure 4e), while the residues Ala418, Ile419, Leu313, Phe314, Tyr158, Phe159, Tyr316, Phe303, Phe178, and Val216 made hydrophobic interactions. Moreover, positive and negative charge interactions were found with Lys156, Arg315, His423 and Glu429, Asp233, Asp352, Glh277, Glu411 residues, respectively. The compound **13c** formed hydrogen bond with the residue Lys156 and π–π stacking interactions with residue Phe314.

3 | CONCLUSION

In conclusion, we designed and synthesized new analogs of phloroglucinol (**12a–d** and **13a–d**) bearing halogen or nitro functionality at the *para* position of the phenyl ring. All the synthesized compounds were tested for their inhibitory effects against hCA I, hCA II, AChE, BChE, and α-glycosidase enzymes. All the compounds showed low nanomolar inhibition levels, especially analogs **13a–d** for hCA I, **13c** and **13d** for hCA II, **13a–d** for AChE, **13a–c** for BChE, and **13b** for α-glycosidase. The inhibition of α-glycosidase by **13b** displayed the highest activity of all the compounds under evaluation. Preliminary SAR analysis suggested that effective enzyme inhibition required the presence of a *para*-substituted phenyl and a bis-structure further increased the inhibition activity. Newly synthesized phloroglucinol derivatives **12a–d** and **13a–d** are an emerging class of potent α-glycosidase inhibitors. However, among the new phloroglucinol derivatives, phloroglucinol derivative **13c** had the most effective α-GLY, AChE, BChE, and both hCA isoenzymes profiles. These enzymes inhibitors have been reported as anticancer, antiglaucoma, and antiobesity agents, or for the treatment of several of neurological disturbances including AD, PD, altitude, and epilepsy diseases.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

Commercially available reagents and solvents were of analytical grade or were purified by standard procedures prior to use. Reactions were

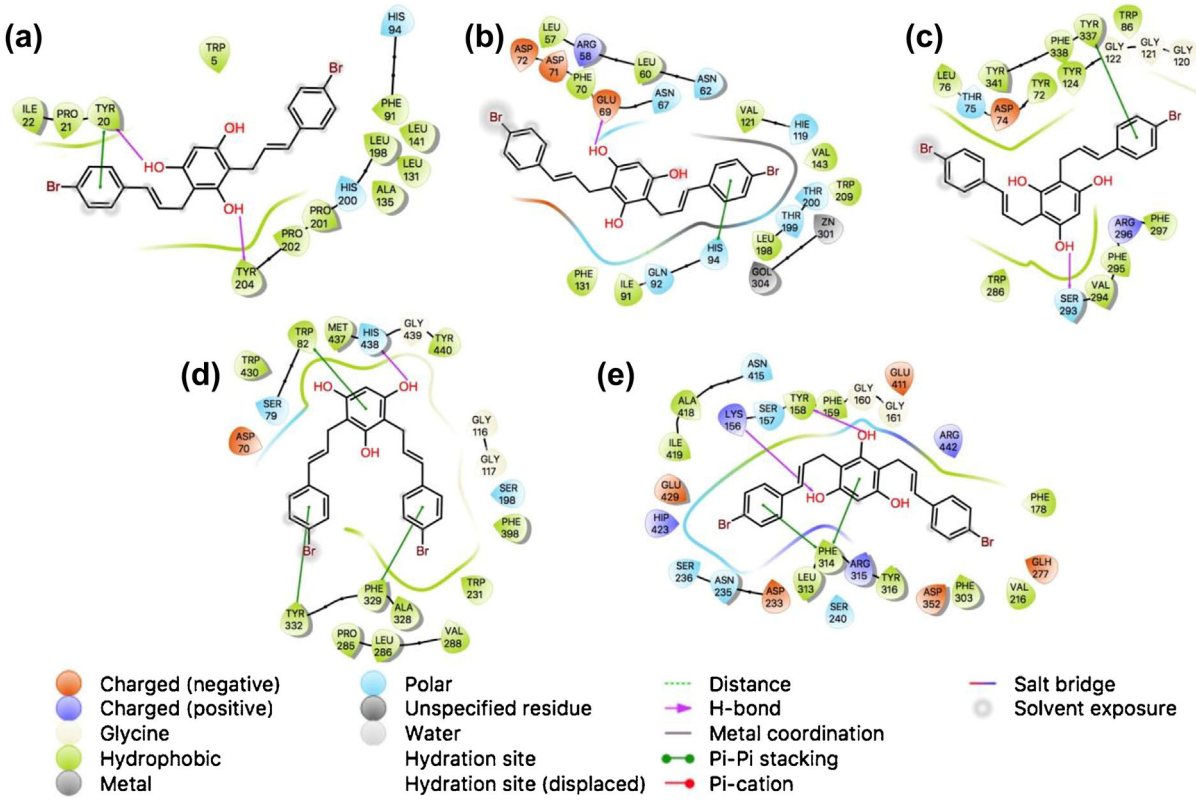


FIGURE 4 Ligand interaction diagrams of compound **13c** in hCA I (a), hCA II (b), AChE (c), BChE (d), and α-glycosidase (e). Purple arrows symbolize a hydrogen bond and two-point green line represents π–π stacking

monitored via thin layer chromatography (TLC). The ^{1}H NMR and ^{13}C NMR spectra were recorded on a 400 (100) MHz Varian spectrometer using $CDCl_3$ and acetone-d_6. Column chromatography was performed on silica gel 60 (70–230 mesh ASTM), and TLC was carried out on silica gel (254–366 mesh ASTM). Melting points were determined on a capillary melting apparatus (Buchi 530) and are uncorrected. Infrared (IR) spectra were obtained from solutions in 0.1-mm cells with a PerkinElmer spectrophotometer (Waltham, MA). Elemental analyses were performed on a Leco CHNS-932 apparatus.

The NMR spectra as well as the InChI codes of the investigated compounds, together with some biological activity data, are provided as Supporting Information.

4.1.2 | General procedure for the synthesis of *p*-substituted cinnamic acids

In a round bottom flask was added benzaldehyde derivatives **7a–d** (1 eq), malonic acid (2.2 eq), piperidine (0.1 eq), and pyridine (2.5 mL). The reaction mixture was stirred and heated to a gentle reflux for 2–3 h. The mixture was cooled to room temperature and slowly poured over ice-cold aq HCl (2 M, 30 mL). Then the mixture was extracted with EtAOc three times. The organic phase was dried with Na_2SO_4 and the solvent was evaporated under reduced pressure.

4-Fluorocinnamic acid (8a)

Yield: 97%; ^{1}H NMR (400 MHz, acetone): δ 10.84 (s, 1H), 7.87–7.74 (m, 2H), 7.69 (d, 1H, *J* = 16.0 Hz), 7.23 (t, 2H, *J* = 8.8 Hz), 6.51 (d, 1H, *J* = 16.0 Hz). The ^{1}H NMR spectrum is in agreement with reported data.[61]

4-Bromocinnamic acid (8c)

Yield: 90%; ^{1}H NMR (400 MHz, acetone): δ 10.86 (s, 1H), 7.71–7.57 (m, 5H), 6.59 (d, 1H, *J* = 16.0 Hz). The ^{1}H NMR spectrum is in agreement with reported data.[62]

4-Nitrocinnamic acid (8d)

Yield: 54%; ^{1}H NMR (400 MHz, DMSO): δ 13.15–12.30 (m, 1H), 8.25 (d, 2H, *J* = 7.8 Hz), 7.99 (d, 2H, *J* = 7.9 Hz), 7.71 (d, 1H, *J* = 16.2 Hz), 6.76 (d, 1H, *J* = 16.1 Hz). The ^{1}H NMR spectrum is in agreement with reported data.[62]

4.1.3 | General procedure for the synthesis of 4-substituted cinnamic acid methyl esters

Cinnamic acid derivative was dissolved in MeOH (equal volume) and added cat. *p*-TSA at room temperature. The reaction mixture was stirred and heated at reflux temperature for 20–24 h. The reaction mixture was cooled to room temperature. The solvent was evaporated under reduce pressure. The mixture was extracted with EtAOc and

washed with water three times. Then organic phase was dried with Na_2SO_4 and the solvent was evaporated under reduced pressure.

4-Fluorocinnamic acid methyl ester (9a)

Yield: 92%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66 (d, 1H, *J* = 16.0 Hz), 7.52 (dd, 2H, *J* = 8.6, 5.4 Hz), 7.08 (t, 2H, *J* = 8.6 Hz), 6.37 (d, 1H, *J* = 16.0 Hz), 3.81 (s, 3H). The ^{1}H NMR spectrum is in agreement with reported data.[63]

4-Chlorocinnamic acid methyl ester (9b)

Yield: 96%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, 1H, *J* = 16.0 Hz), 7.46 (d, 2H, *J* = 8.4 Hz), 7.36 (d, 2H, *J* = 8.4 Hz), 6.41 (d, 1H, *J* = 16.0 Hz), 3.81 (s, 3H). The ^{1}H NMR spectrum is in agreement with reported data.[64]

4-Bromocinnamic acid methyl ester (9c)

Yield: 85%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, 1H, *J* = 16.0 Hz), 7.52 (d, 2H, *J* = 8.5 Hz), 7.39 (d, 2H, *J* = 8.5 Hz), 6.43 (d, 1H, *J* = 16.0 Hz), 3.81 (s, 3H). The ^{1}H NMR spectrum is in agreement with reported data.[64]

4-Nitrocinnamic acid methyl ester (9d)

Yield: 80%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (d, 2H, *J* = 8.6 Hz), 7.73 (d, 1H, *J* = 16.1 Hz), 7.68 (d, 2H, *J* = 8.7 Hz), 6.57 (d, 1H, *J* = 16.1 Hz), 3.84 (s, 3H, *J* = 6.6 Hz). The ^{1}H NMR spectrum is in agreement with reported data.[64]

4.1.4 | General procedure for the synthesis of 4-substituted cinnamyl alcohols

To a stirred solution of the α,β-unsaturated ester (1 eq) in anhydrous DCM (0.2 M) at −78°C under N_2 was added DIBAL-H (1.0–1.2 in toluene or hexane, 2.2 eq) dropwise. The reaction was stirred for 1.5 h at −78°C, and quenched with 10% aq. NaOH (equal volume). The resultant mixture was allowed to warm to room temperature and stirred for 1 h. The layers were separated and the aqueous layer was extracted with DCM (equal volume). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to get the pure cinnamyl alcohols.

4-Fluorocinnamyl alcohol (10a)

Yield: 80%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43–7.31 (m, 2H), 7.01 (t, 2H, *J* = 8.7 Hz), 6.59 (d, 1H, *J* = 15.9 Hz), 6.29 (dt, 1H, *J* = 15.9, 5.7 Hz), 4.32 (d, 2H, *J* = 5.7 Hz). The ^{1}H NMR spectrum is in agreement with reported data.[63]

4-Chlorocinnamyl alcohol (10b)

Yield: 98%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35–7.22 (m, 4H), 6.58 (d, 1H, *J* = 15.9 Hz), 6.34 (dt, 1H, *J* = 15.9, 5.6 Hz), 4.33 (dd, 2H, *J* = 5.6, 1.4 Hz). The ^{1}H NMR spectrum is in agreement with reported data.[65]

4-Bromocinnamyl alcohol (10c)

Yield: 90%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, 2H, *J* = 8.4 Hz), 7.25 (d, 2H, *J* = 7.3 Hz), 6.57 (d, 1H, *J* = 16.0 Hz), 6.36 (dt, 1H, *J* = 16.0, 5.5 Hz), 4.33 (s, 2H). The ^{1}H NMR spectrum is in agreement with reported data.[66]

4-Nitrocinnamyl alcohol (10d)

Yield: 88%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, 2H, *J* = 8.6 Hz), 7.52 (d, 2H, *J* = 8.5 Hz), 6.72 (d, 1H, *J* = 16.1 Hz), 6.54 (dt, 1H, *J* = 15.8, 5.1 Hz), 4.41 (s, 2H). The ^{1}H NMR spectrum is in agreement with reported data.[66]

4.1.5 | General procedure for the synthesis of 4-substituted cinnamyl bromides

To a stirred solution of cinnamyl alcohol (1 eq) in anhydrous Et_2O (0.2 M) at 0°C under N_2 in dark was added PBr_3 (0.4 eq). The reaction was stirred until complete by TLC analysis, and poured into sat. aq. $NaHCO_3$ (equal volume). The layers were separated and the aqueous layer extracted with Et_2O (2× equal volume). The combined organic layers were washed with sat. aq. $Na_2S_2O_3$ and brine (1:1, equal volume), dried over Na_2SO_4, filtered and concentrated *in vacuo* to get the pure cinnamyl bromides.

4-Fluorocinnamyl bromide (11a)

Yield: 88%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (dd, 2H, *J* = 8.2, 5.6 Hz), 7.01 (t, 2H, *J* = 8.5 Hz), 6.59 (d, 1H, *J* = 15.6 Hz), 6.30 (dt, 1H, *J* = 15.6, 7.8 Hz), 4.14 (d, 2H, *J* = 7.7 Hz). The ^{1}H NMR spectrum is in agreement with reported data.[67]

4-Chlorocinnamyl bromide (11b)

Yield: 77%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.28 (m, 4H), 6.60 (d, 1H, *J* = 15.6 Hz), 6.37 (dt, 1H, *J* = 15.6, 7.8 Hz), 4.14 (d, 2H, *J* = 7.4 Hz). The ^{1}H NMR spectrum is in agreement with reported data.[68]

4-Bromocinnamyl bromide (11c)

Yield: 63%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, 2H, *J* = 8.4 Hz), 7.25 (d, 2H, *J* = 8.3 Hz), 6.58 (d, 1H, *J* = 15.6 Hz), 6.39 (dt, 1H, *J* = 15.5, 7.7 Hz), 4.14 (d, 2H, *J* = 7.7 Hz). The ^{1}H NMR spectrum is in agreement with reported data.[66]

4-Nitrocinnamyl bromide (11d)

Yield: 53%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, 2H, *J* = 7.9 Hz), 7.53 (d, 2H, *J* = 8.0 Hz), 6.71 (d, 1H, *J* = 15.7 Hz), 6.63–6.48 (m, 1H), 4.16 (d, 2H, *J* = 7.5 Hz). The ^{1}H NMR spectrum is in agreement with reported data.[66]

4.1.6 | General procedure for the synthesis of mono- and bis-*p*-substituted cinnamyl phloroglucinols

To a suspension of NaH (1.25 eq) in dry THF (equal volume), phloroglucinol (**1**) (1 eq) was added under N_2 atm. After 5 min, a solution of cinnamyl bromide (1.25 eq) in dry THF (equal volume) was added to the mixture. The reaction was stirred until complete by TLC analysis (17–23 h). The reaction was quenched in NH_4Cl (equal

volume) and then the mixture was neutralized with 2.0 M HCl until pH 1–2. The reaction mixture was extracted with EtAOc (3× equal volume). The organic phase was dried over Na_2SO_4 and solvent was evaporated under reduce pressure. The crude product was purified by silica gel chromatography.

4-Fluorocinnamyl phloroglucinol (12a)

The above procedure was followed with **11a** to yield **12a** as orange solid. Yield: 54%; mp: 123–124°C, R_f: 0.2 (5% MeOH/DCM), Anal. calcd. for $C_{15}H_{13}FO_3$: C, 69.22; H, 5.03; Found C, 69.43; H, 5.38, IR (neat cm^{-1}): 3373.30, 3284.22, 3038.93. ^{1}H NMR (400 MHz, acetone) δ 8.09 (bs, 2H), 7.97 (bs, 1H), 7.38–7.33 (m, 2H), 7.04–6.98 (m, 2H), 6.41–6.31 (m, 2H), 6.02 (s, 2H), 3.47 (d, 2H, J = 4.8 Hz); ^{13}C NMR (100 MHz, acetone) δ 161.8 (d, C-16, J_{CF} = 242 Hz), 156.9, 156.8, 134.9 (d, C-13, J_{CF} = 3.3 Hz), 129.9, 127.8, 127.6 (d, C-18, J_{CF} = 7.7 Hz), 115.38 (d, C-17, J_{CF} = 21.4 Hz), 104.6.

4-Chlorocinnamyl phloroglucinol (12b)

The above procedure was followed with **11b** to yield **12b** as yellow solid. Yield: 45%; mp: 134–135°C, R_f: 0.2 (5% MeOH/DCM), Anal. calcd. for $C_{15}H_{13}ClO_3$: C, 64.08; H, 4.68; Found C, 64.17; H, 4.75, IR (neat cm^{-1}): 3429.90, 3030.34, 2970.09. ^{1}H NMR (400 MHz, acetone) δ 8.03 (bs, 2H), 7.88 (bs, 1H), 7.36 (d, 2H, J = 8 Hz), 7.29 (d, 2H, J = 8 Hz), 6.47–6.40 (m, 2H), 6.01 (s, 2H), 3.46 (d, 2H, J = 2.7 Hz); ^{13}C NMR (100 MHz, acetone) δ 156.9, 156.9, 137.4, 131.7, 131.1, 128.6, 127.7, 127.5, 104.4, 94.8, 26.2.

4-Bromocinnamyl phloroglucinol (12c)

The above procedure was followed with **11c** to yield **12c** as brown solid. Yield: 60%; mp: 162–163°C. R_f: 0.2 (5% MeOH/DCM), Anal. calcd. for $C_{15}H_{13}BrO_3$: C, 55.36 H, 4.04; Found C, 55.41; H, 4.21, IR (neat cm^{-1}): 3431.08, 3351.37, 3028.96. ^{1}H NMR (400 MHz, acetone) δ 8.08 (bs, 2H), 7.94 (bs, 1H), 7.42 (d, 2H, J = 8.5 Hz), 7.28 (d, 2H, J = 8.5 Hz), 6.48–6.33 (m, 2H), 6.00 (s, 2H), 3.46 (d, 2H, J = 6.0 Hz); ^{13}C NMR (100 MHz, acetone) δ 156.9 (C2-C4), 137.7, 131.6, 131.2, 127.9, 127.8, 119.7, 104.4, 94.9, 26.8.

4-Nitrocinnamyl phloroglucinol (12d)

The above procedure was followed with **11d** to yield **12d** as yellow solid. Yield: 40%; mp: 194–195°C. R_f: 0.3 (5% MeOH/DCM), Anal. calcd. for $C_{15}H_{13}NO_5$: C, 62.32; H, 4.67; N, 4.78; Found C, 62.07; H, 4.60; N, 4.72, IR (neat cm^{-1}): 3336.98. ^{1}H NMR (400 MHz, acetone) δ 8.16–8.12 (m, 2H), 7.61–7.58 (m, 2H), 6.70 (dt, 1H, J = 15.7, 6.4 Hz), 6.53 (d, 1H, J = 15.9 Hz), 6.02 (s, 2H), 3.54 (d, 2H, J = 6.3 Hz), 3.32 (bs, 1H), 3.05 (bs, 2H); ^{13}C NMR (100 MHz, acetone) δ 157.1, 156.9, 145.3, 135.8, 127.3, 126.7, 123.9 (C11-C12), 103.8, 94.9, 26.5.

4,6-Bis-*p*-fluorocinnamyl phloroglucinol (13a)

The above procedure was followed with **11a** to yield **13a** as brown solid. Yield: 42%; mp: 115–116°C, R_f: 0.5 (5% MeOH/DCM), Anal. calcd. for $C_{24}H_{20}F_2O_3$: C, 72.25; H, 5.07; Found C, 71.96; H, 4.90, IR (neat cm^{-1}): 3292.44, 3032.42, 2968.21. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29–7.25 (m, 4H), 6.98–6.93 (m, 4H), 6.46 (d, 2H, J = 15.9 Hz), 6.25 (dt, 2H, J = 15.9, 6.2 Hz), 6.03 (s, 1H), 3.54 (d, 4H, J = 6.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.4 (d, C-16, J_{CF} = 242 Hz), 154.5, 153.6, 133.3 (d, C-13, J_{CF} = 3.2 Hz), 130.0, 127.9 (d, C-14, J_{CF} = 7.9 Hz), 127.8, 115.6 (d, C-17, J_{CF} = 21.4 Hz), 105.1, 96.4, 26.9.

4,6-Bis-*p*-chlorocinnamyl phloroglucinol (13b)

The above procedure was followed with **11b** to yield **13b** as yellow solid. Yield: 40%; mp: 85–86°C, R_f: 0.4 (5% MeOH/DCM), Anal. calcd. for $C_{24}H_{20}Cl_2O_3$: C, 63.27; H, 4.53; Found C, 63.22; H, 4.48, IR (neat cm^{-1}): 3373.59, 3028.26. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25–7.16 (m, 8H), 6.42 (d, 2H, J = 16 Hz), 6.30 (dt, 2H, J = 15.9, 6.1 Hz), 6.02 (s, 1H), 5.27 (bs, 1H), 3.53 (d, 4H, J = 6.0 Hz), 2.19 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.5, 153.5, 135.6, 133.1, 129.8, 128.8, 128.8, 127.6, 105.1, 96.4, 26.9.

4,6-Bis-*p*-bromocinnamyl phloroglucinol (13c)

The above procedure was followed with **11c** to yield **13c** as brown solid. Yield: 45%; mp: 104–105°C, R_f: 0.4 (5% MeOH/DCM), Anal. calcd. for $(C_{24}H_{20}Br_2O_3)_5(CH_2Cl_2)$: C, 54.51; H, 3.85; Found C, 54.42; H, 4.09, IR (neat cm^{-1}): 3535.53, 3426.71, 3026.82. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (d, 4H, J = 8.4 Hz), 7.19 (d, 4H, J = 8.4 Hz), 6.43 (d, 2H, J = 16.0 Hz), 6.33 (dt, 2H, J = 15.9, 5.9 Hz), 6.02 (s, 1H), 5.17 (bs, 1H), 4.86 (bs, 2H), 3.54 (d, 4H, J = 6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.4, 153.5, 136.1, 131.8, 129.9, 128.9, 127.9, 121.3, 105.0, 96.4, 26.9.

4,6-Bis-*p*-nitrocinnamyl phloroglucinol (13d)

The above procedure was followed with **11d** to yield **13d** as yellow solid. Yield: 55%, mp: 175–176°C, R_f: 0.4 (5% MeOH/DCM), Anal. calcd. for $C_{24}H_{20}N_2O_7$: C, 61.97; H, 5.13; N, 5.63; Found C, 62.02; H, 5.07; N, 6.08, IR (neat cm^{-1}): 3444.96, 2949.73, 2919.19. ^{1}H NMR (400 MHz, acetone) δ 8.2 (bs, 2H), 8.12 (d, 4H, J = 8.6 Hz), 7.57 (d, 4H, J = 8.6 Hz), 7.24 (bs, 1H), 6.71 (dt, 2H, J = 15.6, 6.2 Hz), 6.54 (d, 2H, J = 15.9 Hz), 6.21 (s, 1H), 3.63 (d, 4H, J = 5.9 Hz); ^{13}C NMR (100 MHz, acetone) δ 154.8, 154.62, 146.5, 145.1, 135.4, 127.5, 126.7, 123.9, 104.4, 95.4, 26.8.

4.2 | Biological studies

4.2.1 | hCA I and II isoenzymes purification assays

The hCA isoenzymes were purified by affinity chromatography on a Sepharose-4B-L-tyrosine-sulfanilamide matrix for selective retention of CAs.[69] The amount of eluted protein was quantitated spectrophotometrically at 280 nm.[70] Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE, 0.1% SDS) was used to determine the purities of both hCA isoenzymes. This biochemical method was established with 10 and 3% acrylamide for the running and stacking gels, respectively. A single band was observed for each hCA isoenzyme.[71] During the purification steps, the quantity of protein was measured spectrophotometrically at 595 nm according to the Bradford method.[72]

4.2.2 | CA I and II isoenzymes activities

CA activities were determined according to the method of Verpoorte et al.[73] as described previously.[74] For this purpose, *p*-NPA hydrolysis was assayed by measuring the change in absorbance at 348 nm over a period of 30 min at 25°C using a spectrophotometer (Beckman Coulter, Germany). The reaction mixture contained 400 µL Tris-HCl buffer (pH 7.4), 360 µL *p*-NPA (3.0 mM), 220 µL H_2O, and 20 µL purified hCA isoenzyme. A control measurement was obtained by preparing the same cuvette without enzyme. Bovine serum albumin was used as the standard protein.[75] Negative control includes all solutions for enzymatic activity determination except for CA isoenzymes. Acetazolamide (AZA) was used as a positive control for hCA I and II isoenzymes.

4.2.3 | AChE and BChE activities

AChE and BChE activities were measured according to Ellman's method.[76,77] Acetylcholinesterase (C2888-500UN; Sigma–Aldrich Chemie GmbH) was obtained from electric eel (*Electrophorus electricus*). Butyrylcholinesterase (C2490-1KU; Sigma–Aldrich Chemie GmbH) was obtained from equine serum. AChI/BChI and DTNB were used for the determination of AChE/BChE activities. In brief, 100 mL buffer (Tris-HCl, 1 M, pH 8.0) and 10 mL of sample solutions with different concentrations were dissolved in deionized water. Then, 50 mL AChE/BChE (5.3210^{-3} EU) solution was added, and the resulting solution was incubated for 10 min at 25°C. After incubation, a portion of DTNB (50 mL, 0.5 mM) was added. Finally, the reaction was started by the addition of 50 mL AChI/BChI (10/10 mM). The enzymatic hydrolysis of both substrates was determined spectrophotometrically at 412 nm from the formation of yellow 5-thio-2-nitrobenzoate anion.[78] Negative control contains all solutions for enzymatic activity determination except for AChE/BChE. Tacrine (TAC) was used as a positive control for both AChE and BChE enzymes.

4.2.4 | α-Glycosidase activity

α-Glycosidase enzyme activity was determined using *p*-nitrophenyl-D-glycopyranoside (*p*-NPG) as the substrate, according to the procedure of Tao et al.[79] α-Glycosidase enzyme (G5003-100UN; Sigma–Aldrich Chemie GmbH) was obtained from *Saccharomyces cerevisiae*. Samples (20 mg) were prepared by dissolving in 2 mL EtOH/H_2O. Several solutions in phosphate buffer (pH 7.4) were prepared. First, 75 µL phosphate buffer was mixed with 20 µL enzyme solution in phosphate buffer (0.15 U/mL, pH 7.4) and 5 µL sample. Then, the sample was preincubated at 35°C for 10 min. Adding *p*-NPG initiated the reaction. Also, 20 µL *p*-NPG in phosphate buffer (5 mM, pH 7.4) after preincubation was added and again incubated at 35°C. Negative control contains all solutions for enzymatic activity determination except for α-glycosidase enzyme. Acarbose (ACR) was used as a positive control for α-glycosidase enzyme.

4.2.5 | Enzyme inhibition assays

For determination of the 50% inhibition concentration (IC_{50}) and inhibition constant (K_i) of each novel phloroglucinol derivatives, three concentrations of each derivative were tested. The activities (%) were plotted against the concentrations in Lineweaver–Burk plots,[58] as previously described.[80,81] The IC_{50} and K_i values were calculated by curve fitting the data.

4.3 | *In silico* experiments

Taking into account the experimental data obtained, it was observed that 4,6-bis-*p*-bromocinnamyl-phloroglucinol (**13c**) had the best inhibitory property among the synthesized compounds. Based on this, the molecular docking analyses of this compound were carried out to determine the theoretical binding affinity to the enzymes. In addition to this, sites of interaction of the compound **13c** with human carbonic anhydrase I (hCA I), human carbonic anhydrase II (hCA II), human acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and α-glycosidase enzymes were also determined. Crystal structures in complex with inhibitor (PDB ID: 2FW4 for hCA I, 4PQ7 for hCA II, 4M0E for AChE, 5LKR for BChE, and 3A4A for α-glycosidase) were prepared using protein preparation wizard of Maestro.[70–76,82–88] Three-dimensional structure of compound **13c** was constructed using the LigPrep module of Maestro.[89] The details of these modules were described in the previous work.[90,91] Grids that define the receptor structures were prepared using the receptor grid generation panel, excluding of co-crystallized ligands. In addition, these co-crystallized ligands were selected as the docking site. Glide/XP was selected as docking protocol.[92]

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CONFLICTS OF INTEREST

The authors report no conflicts of interests.

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