Synthesis and Characterization of Novel Phthalimide-pyrano[3,2-*c*] chromene and Phthalimide-pyrano-2-one Hybrids

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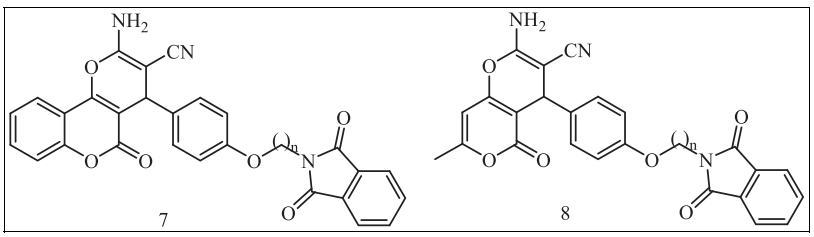

Coumarin skelton holds substantial promise for further exploration because of its immense pharmacological potential. In this pursuit, a series of phthalimide-chromen and phthalimide-pyran-2-one hybrids were synthesized in efficient yields *via* one-pot multicomponent reaction of aldehyde linked to phthalimide moiety, 4-hydroxy coumarin/4-hydroxy-6-methyl-2H-pyran-2-one, and malanonitrile by Knoevenagel reaction at room temperature in the presence of DABCO as catalyst. The compounds were characterized by ^{1}H NMR and ^{13}C NMR, MS, and FTIR. All the compounds consisting of phthalimide-chromen/pyrano-2-one moieties tethered by spacers of varying lengths were evaluated for their biological activity in Ellman's assay. Most of the compounds feebly inhibited Acetylcholinesterase Enzyme and were inactive toward Butyrylcholinesterase Enzyme.

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INTRODUCTION

Coumarins are plant-derived polyphenolic compounds belonging to the benzopyrone family. They exhibit a wide range of pharmacotherapuetic properties and represent one of the most versatile and applicable family in fluorescent probes. The diverse pharmacological properties make them useful as anti-cancer and anti-proliferative [1], anti-inflammatory, anti-HIV agents [2], neuro-protective [3,4], anti-oxidative, antibacterial [5], antifungal [6] anticoagulants and antiplatelet [7], vasorelaxants [8], and anti-Alzheimer agents [9].

Coumarins exhibit fascinating and unique physicochemical properties, and their applications in different area have been extensively studied. Coumarins have high applicability in fluorescent probes of heterogeneous environments, such as supramolecular host cavities, micelles, polymers, and solids [10]. Several coumarin derivatives have been designed as a new class of far-red emitting fluorgenic agents, such as cyanocoumarins and 7-hydroxycoumarin-hemicyanine hybrids [11]. Several other coumarin derivatives like 3-(2′-benzimidazolyl) coumarins and biscoumarins have been synthesized with specific properties and applications including layser dyes, bioactive compounds with different biological activities, additive in food and cosmetics, and fluorescent transthyretin folding sensors [11–15].

The physiochemical properties of coumarins devise the extent of their biological properties. Coumarin eliciting vast pharmacological effects has been widely explored for the development of ligands for combating multifaceted diseases like cancer, neurodegenerative, and metabolic syndromes [16]. It is well established that coumarin exerts its anti-Alzheimer activity by inhibiting acetylcholinestrase enzyme, which remains a main target in the symptomatic treatment of disease. Pertinently, four among the five FDA-approved drugs for Alzheimer's disease are cholinesterase inhibitors (Fig. 1) [17,18]. Coumarin is a well-known acetylcholinestrase peripheral anionic site binding moiety, and most of the reported scaffolds are based on linking coumarin with some catalytic binding site moiety tethered by an appropriate spacer [19,20]. In this regard, several coumarin-based scaffolds like emasulin [21] and AP 2238 [22] are under clinical trial (Fig. 2)

Multicomponent reactions (MCRs) occupy an important place in organic synthesis because of the formation of

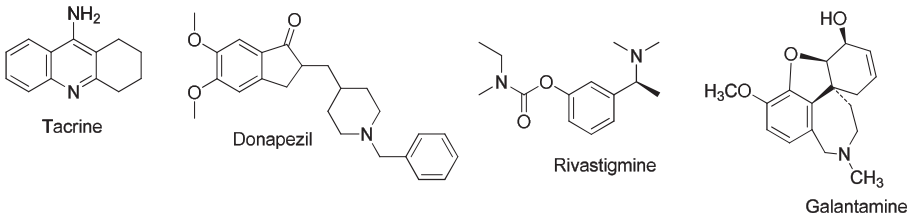


Figure 1. Structures of AChE inhibitors used in Alzheimer's disease. Tacrine was withdrawn because of its hepatotoxicity; however, it is used in research and as a reference inhibitor and design of new scaffolds.

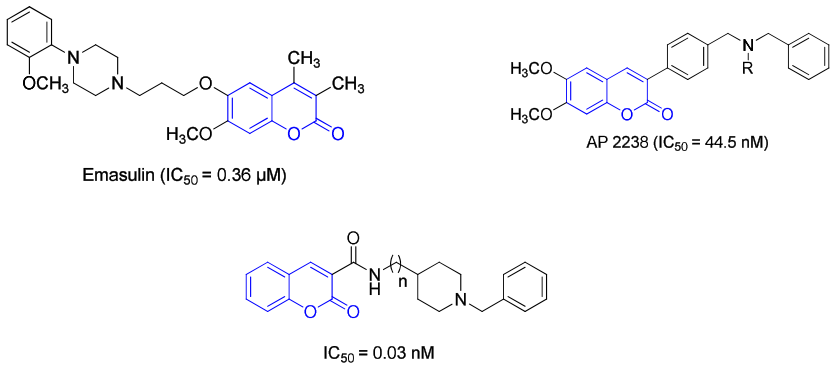


Figure 2. Related coumarin-based and phthalimide-based scaffolds as cholinesterase inhibitors. [Color figure can be viewed at wileyonlinelibrary.com]

carbon–carbon and carbon–heteroatom bond involving one pot, simple procedures, low cost, time and energy saving, and less environmental hazards. Pyrano[3,2-c] chromens being an imperative class of heterocycles have previously been synthesized from 4-hydroxy coumarin, malanonitrile, and aromatic aldehydes in the presence of different catalysts. In continuation of our interest in the development of coumarin-based scaffolds in the spotlight of previously mentioned facts and MCRs [4], we herein report the DABO-catalyzed Knoevenagel reaction followed by cyclization that produced novel phthalimide-pyrano[3,2-c] chromene and phthalimide-pyrano-2-one hybrids bearing different linker lengths (Fig. 3) with this aim that they could have some chemical and biological interest (Scheme 1).

RESULTS AND DISCUSSION

Chemistry. In continuation of our work based on the synthesis and evaluation of coumarin-based scaffolds [4,23,24], we synthesized phthalimide-pyrano[3,2-c] chromene and phthalimide-pyrano-2-one hybrids and evaluated them for their biological activity. The synthesis pathway for preparation of compounds **7** and **8** was illustrated in Scheme 1. The structure of the target compounds was confirmed by the spectral data. The IR showed the appearance of characteristic NH_2 band around 3193–3367 cm^{-1} and CN 2105–2295 cm^{-1}. ^1H NMR spectra showed a peak at a range of 4.1–4.4 ppm corresponding to the chiral proton (CH). Further evidence was gained from their mass spectra that displayed characteristic mass fragments and abundant peaks.

Phthalimide (**1**) (20 mmol) was dispersed in a solution of KOH in EtOH. The solution was refluxed for 7 h, reaction progress was monitored by TLC, and the solution was concentrated under vacuum. The obtained solid was washed with little amount of cold ethanol, dried to afford **2a**. In the next step, dibromomethane **2b** (9 mmol) was added to a solution of **2a** (3 mmol) in 6 mL of DMF and left to stir for 9 h at 85°C. The reaction mixture was poured in ice-cold water, and the precipitate formed was filtered, dried, and crystallized in ethanol to afford **3**. Compounds **3a**, **3b** (10 mmol), and aldehydes **4** (10 mmol) were mixed in dry acetone and left to stir under reflux temperature for 22–24 h (compounds **3a** (n = 3) and **3b** (n = 4) are commercially available). The reaction progress was monitored by TLC, and then the mixture was concentrated under vacuum. The obtained solid was washed with water, dried and crystallized in appropriate solvent to furnish pure compound **5**. Finally, the desired compounds **7** and **8** were prepared *via* one-pot MCR by stirring the intermediate (**5**) (2 mmol) with malanonitrile (**6**) (2 mmol), or with 4-hydroxy coumarin/6-methyl, 4-hydroxy-2-one-pyrone (2 mmol) in ethanol in the presence of catalytic amount of DABCO overnight at ambient temperature, respectively. The reaction progress was monitored by TLC, and the

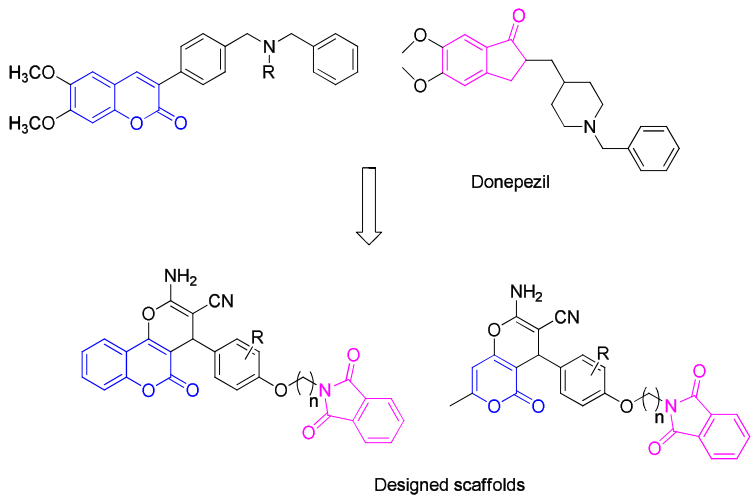
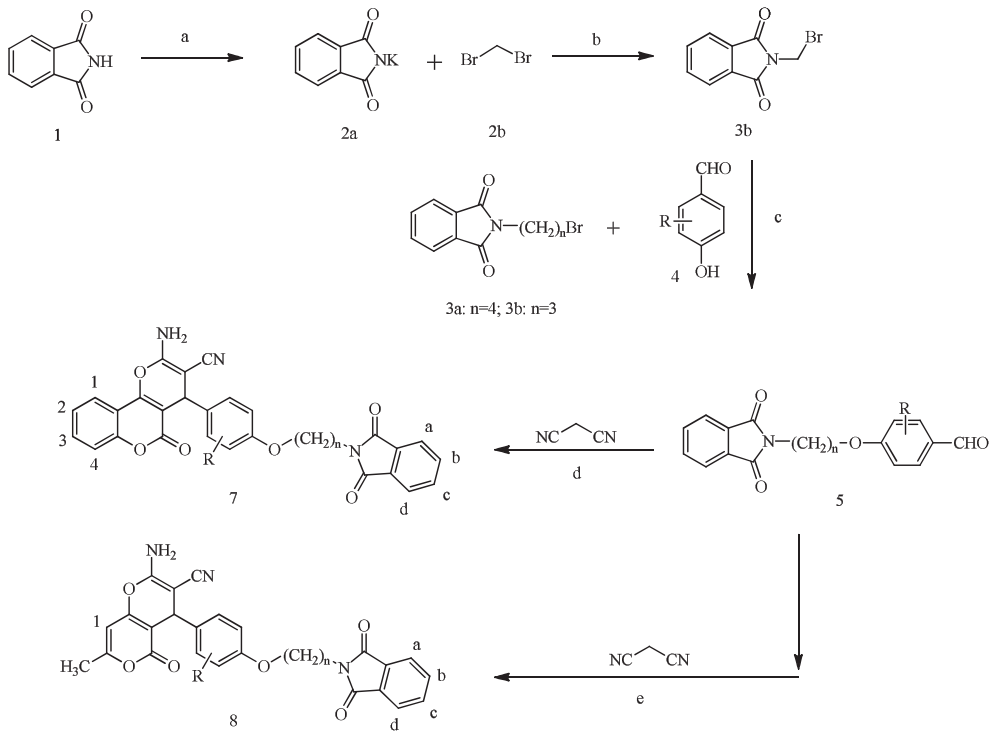


Figure 3. Design strategy of coumarin/pyranone- phthalimide hybrids. [Color figure can be viewed at wileyonlinelibrary.com]

Scheme 1. Reagents and conditions: (a) KOH, EtOH, reflux; (b) DMF, 85°C, 9 h; (c) K_2CO_3, acetone, reflux, 22–24 h; (d) 4-hydroxy coumarin, DABCO, EtOH, rt, 24 h; (e) 4-hydroxy-6-methyl-2H-pyran-2-one, DABCO, EtOH, rt.

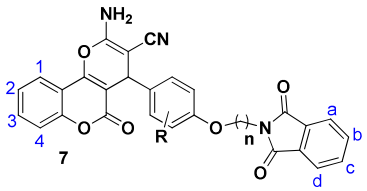


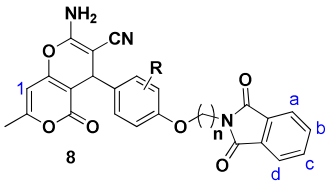
precipitate formed was filtered off and washed with EtOH to afford pure compound in 85–95% yields.

Biological assay. The activity against Acetylcholinesterase Enzyme (AChE) and Butyrylcholinesterase Enzyme (BuChE) was determined in spectrophotometric Elman's assay. Donepezil was used as reference drug. The enzyme inhibitory activity of all the compounds is summarized in Table 1. Interestingly, change substitution on the phenyl ring-linking phthalimide and coumarin and replacement of chromene moiety with pyran-2-one did not display any significant effect on the activity of compounds either on AChE or BuChE. The changing in spacer length changed the percentage of inhibition AChE at 70 μM significantly, as optimum distance between phthalimide and *O*-phenyl ring is provided with three methylene groups. However, these changes in spacer length for inhibition of BuChE did not display important role. The positive effects of spacer length in increasing

Table 1

AChE and BuChE inhibitory activity of compounds **7** and **8**.





Entry	Product 7/8	R	*n*	% of inhibition of AChE at 70 μM	% of inhibition of BuChE at 70 μM
1	**7a**	H	4	36	<10
2	**7b**	H	1	55	16
3	**7c**	H	3	69	18
4	**7d**	3,5-OCH_3	1	31	<10
5	**7e**	3-OCH_3	1	53	13
6	**7f**	3-OCH_3	3	67	19
7	**7g**	3-OCH_3	4	37	17
8	**8a**	H	4	31	<10
9	**8b**	H	1	45	16
10	**8c**	H	3	65	19
11	**8d**	3,5-OCH_3	1	32	<10
12	**8e**	3-OCH_3	1	42	14
13	**8f**	3-OCH_3	3	61	16
14	**8g**	3-OCH_3	4	30	<10
Donepezil				100	100

the activity for both series 7 and 8 and also for phenyl (**7a–c** and **8a–c**) or substituted-phenyl group (**7d–g** and **8d–g**) were observed. All synthesized compound did not inhibit BuChE significantly at concentration of 70 μM.

EXPERIMENTAL

General. All commercially available materials and reagents were purchased from Merck and Aldrich and were used without further purification. Thin-layer chromatography was performed using silica gel 250 micron and F254 plates. Melting points were obtained using Koftler hot stage apparatus. The IR spectra were recorded on Nicolet FT magna 550 spectrograph (KBr pellets). ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker spectrometer at 500 and 125 MHz, respectively. The chemical shifts and coupling constants (*J*) are expressed in parts per millennium (ppm) and hertz (Hz), respectively. Mass spectra were acquired on an Agilent Technology (HP) mass spectrometer operating at an ionization potential of 70 eV. Elemental analyses were carried out with a Perkin Elmer Model 240-C apparatus. Elemental analyses were within ±0.4% of theoretical values for C, H, and N.

General procedure for the synthesis of compounds 7a–g and 8a–g. The target compounds **7** and **8** were prepared as illustrated in Scheme 1. Phthalimide (**1**) (20 mmol) was dispersed in a solution of KOH in ethanol. The solution was refluxed for 7 h, and the reaction progress was monitored by TLC. The mixture was concentrated under reduced pressure, and the solid material was washed with little amount of cold ethanol, dried to afford **2a**. In the next step, dibromomethane (9 mmol) was added to a solution of **2a** (3 mmol) in 6 mL of DMF and left to stir for 9 h at 85°C. The reaction mixture was poured in ice-cold water, and the precipitate formed was filtered, dried, and crystalized in ethanol to afford **3b**. Compounds **3** (10 mmol) and aldehyde **4** (10 mmol) were mixed in dry acetone and left to stir under reflux condition for 24 h (compounds **3a**, *n* = 4, and **3c**, *n* = 3, are commercially available). The reaction progress was monitored by TLC, and after completion, the mixture was concentrated under reduced pressure. The obtained solid material was washed with water, dried, and crystalized in appropriate to furnish pure compound **5**. Finally, the desired compounds **7** and **8** were prepared *via* one-pot MCR by stirring the intermediate 5 (2 mmol) with malononitrile (**6**) (2 mmol) or with 4-hydroxy coumarin/6-methyl, 4-hydroxy-2-one-pyrone (2 mmol) in ethanol in the presence of catalytic amount of DABCO overnight in ambient temperature, respectively. The reaction progress was monitored by TLC, and precipitate was filtered off and washed with ethanol. The solid material was crystalized in ethanol to afford pure compound in 85–95% yield.

2-Amino-4-(4-(4-(1,3-dioxoisoindolin-2-yl)butoxy)phenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (7a). White solid; yield 92.5%; mp = 223–225°C; IR (KBr) cm^{-1}: 3425, 3327, 2195, 1777, 1723. ^{1}H NMR (500 MHz, DMSO-d6): 7.89–7.81 (m, 5H, $H_{a–d}$, and H_1),

7.70 (t, J = 7.7 Hz, 1H, H_3), 7.43–7.49 (m, 2H, H_2, and H_4), 7.33 (s, 2H, NH_2), 7.15 (d, J = 8.5 Hz, 2H, $H_{3,5}$-phenyl), 6.84 (d, J = 8.0 Hz, 2H, $H_{2,6}$-phenyl), 4.38 (s, 1H,CH), 3.94 (t, J = 6.0 Hz, 2H, CH_2), 3.62 (t, J = 6.5 Hz, 2H, CH_2), 1.71–1.73 (m, 4H, 2 × CH_2). ^{13}C NMR (125 MHz, DMSO-d6): 167.8, 159.3, 157.8, 157.6, 153.0, 152.0, 135.2, 134.2, 132.7, 131.5, 128.6, 124.5, 123.0, 122.3, 119.1, 116.4, 114.3, 112.9, 104.2, 66.8, 58.2, 37.1, 36.1, 26.1, 24.6. MS (m/z, %): [M^+; 533 (3%)], 160 (100), 202 (76), 130 (29), 77 (16). *Anal*. Calcd for $C_{31}H_{23}N_3O_6$: C, 69.79; H, 4.35; N, 7.88. Found: C, 69.43; H, 4.39; N, 7.65.

2-Amino-4-(4-((1,3-dioxoisoindolin-2-yl)methoxy)phenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (7b). White solid; yield 84%; mp = 242–244°C; IR (KBr) cm^{-1}: 3419, 3327, 2283, 1789, 1729. ^{1}H NMR (500 MHz, DMSO-d6): 7.89–7.95 (m, 5H, $H_{a–d}$, and H_1), 7.69 (t, J = 7.5 Hz, 1H, H_3), 7.43–7.49 (m, 2H, H_2, and H_4), 7.35 (s, 2H, NH_2), 7.23 (d, J = 8.0 Hz, 2H, $H_{3,5}$-phenyl), 7.06 (d, J = 8.0 Hz, 2H, $H_{2,6}$- phenyl), 5.55 (s, 2H, CH_2), 4.43 (s, 1H, CH). ^{13}C NMR (125 MHz, DMSO-*d6*): 166.8, 159.4, 157.9, 155.2, 153.1, 152.0, 136.7, 134.9, 132.7, 131.1, 128.8, 124.5, 123.7, 122.4, 119.1, 116.4, 115.4, 112.9, 104.0, 65.2, 58.1, 55.9, 36.1, 18.4. MS (m/z, %): [M^+; 491 (5%)], 120 (100), 162 (99), 92 (77). *Anal*. Calcd for $C_{28}H_{17}N_3O_6$: C, 68.43; H, 3.49; N, 8.55. Found: C, 68.63; H, 3.39; N, 8.65.

2-Amino-4-(4-(3-(1,3-dioxoisoindolin-2-yl)propoxy)phenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (7c). White solid; yield 83%; mp = 223–226°C; IR (KBr): cm^{-1} 3351, 3193, 2204, 1763, 1735. ^{1}H NMR (500 MHz, DMSO-*d6*): 7.79–7.83 (m, 5H, $H_{a–d}$, and H1), 7.70 (dt, J = 8.5, and 1.5 Hz, 1H, H_3), 7.43–7.49 (m, 2H, H_2, and H_4), 7.32 (s, 2H, NH_2), 7.13 (d, J = 8.5 Hz, 2H, $H_{3,5}$-phenyl), 6.75 (d, J = 8.5 Hz, 2H, $H_{2,6}$- phenyl), 4.37 (s, 1H, CH), 3.97 (t, J = 6.0 Hz, 2H, CH_2), 3.74 (t, J = 6.5 Hz, 2H, CH_2), 2.01–2.06 (m, 2H, CH_2). ^{13}C NMR (125 MHz, DMSO-d_6): 167.8, 159.5, 157.9, 157.5, 153.1, 152.1, 135.3, 134.2, 132.8, 131.7, 128.6, 124.6, 122.9, 122.4, 119.2, 116.5, 114.3, 112.9, 104.3, 65.5, 36.1, 34.9, 27.6. MS (m/z, %): [M^+; 519 (2%)], 160 (100), 188 (100), 130 (35), 265 (25), 104 (11), 77 (7). *Anal*. Calcd for $C_{30}H_{21}N_3O_6$: C, 69.36; H, 4.07; N, 8.09. Found: C, 69.53; H, 4.19; N, 7.88.

2-Amino-4-(4-((1,3-dioxoisoindolin-2-yl)methoxy)-3,5-dimethoxyphenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (7d). White solid; yield 85%; mp = 250–253°C; IR (KBr): cm^{-1} 3391, 3315, 2928, 2198, 1778, 1726. ^{1}H NMR (500 MHz, DMSO-*d6*): 7.89–7.95 (m, 5H, $H_{a–d}$, and H_1), 7.69 (t, J = 7.5 Hz, 1H, H_3), 7.44–7.48 (m, 2H, H_2, and H_4), 7.29 (s, 2H, NH_2), 6.45 (s, 2H, $H_{2,6}$-phenyl), 5.36 (s, 2H, CH_2), 4.42 (s, 1H,CH), 3.50 (s, 6H, 2 × OCH_3). ^{13}C NMR (125 MHz, DMSO-d_6): 166.8, 159.5, 158.1, 153.5, 153.0, 152.2, 140.0, 135.0, 133.6, 133.0, 131.2, 124.5, 123.3, 123.0, 119.0, 116.5, 113.0, 104.5, 103.8, 67.7, 57.7, 55.7, 37.1. MS (m/z, %): [M^+; 551 (1%)], 160 (100), 133 (14), 104 (17), 77 (22). *Anal*. Calcd for $C_{30}H_{21}N_3O_8$: C, 65.49; H, 3.84; N, 7.62. Found: C, 65.40; H, 4.03; N, 7.39.

2-Amino-4-(4-((1,3-dioxoisoindolin-2-yl)methoxy)-3-methoxyphenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (7e). White solid; yield 84%; mp = 261–262°C; IR (KBr): cm^{-1} 3373, 3202, 2944, 2195, 1777, 1731. ^{1}H NMR (500 MHz, DMSO-*d6*): 7.83–7.87 (m, 5H, $H_{a–d}$, and H_1), 7.70 (t, J = 8.0 Hz, 1H, H_3), 7.44–7.49 (m, 2H, H_2, and H_4), 7.33 (s, 2H, NH_2), 7.07 (d, J = 8.0 Hz, 1H, H_5-phenyl), 6.91 (s, 1H, H_2-phenyl), 6.75 (d, J = 8.0 Hz, 1H, H_6-phenyl), 5.49 (s, 2H, CH_2), 4.44 (s, 1H, CH), 3.68 (s, 3H, OCH_3). ^{13}C NMR (125 MHz, DMSO-d_6): 166.7, 159.4, 157.9, 153.2, 152.1, 149.8, 144.6, 138.5, 134.9, 132.7, 131.2, 124.5, 123.5, 122.4, 119.6, 119.1, 117.5, 116.4, 112.3, 103.8, 66.6, 57.9, 55.6, 36.5. MS (m/z, %): [M^+; 521 (1%)], 160 (100), 133 (8), 103 (11). *Anal*. Calcd for $C_{29}H_{19}N_3O_7$: C, 66.79; H, 3.67; N, 8.06. Found: C, 66.51; H, 3.70; N, 7.95.

2-Amino-4-(4-(3-(1,3-dioxoisoindolin-2-yl)propoxy)-3-methoxyphenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (7f). White solid; yield 88%; mp = 237–238°C; IR (KBr): cm^{-1} 3376, 3196, 2941, 2198, 1734, 1775.^{1}H NMR (500 MHz, DMSO-*d6*): 7.79–7.85 (m, 5H, $H_{a–d}$, and H_1), 7.70 (t, J = 7.5 Hz, 1H, H_3), 7.44–7.49 (m, 2H, H_2, and H_4), 7.29 (s, 2H, NH_2), 6.78–6.84 (m, 2H, H_5, and H_6-phenyl), 6.70 (d, J = 2.0 Hz, H_2-phenyl), 4.38 (s, 1H, CH), 3.96 (t, J = 6.0 Hz, 2H, CH_2), 3.75 (t, J = 6.5 Hz, 2H, CH_2), 3.55 (s, 3H, OCH_3), 2.04 (d, J = 6.5 Hz, 2H, CH_2). ^{13}C NMR (125 MHz, DMSO-d_6): 167.8, 159.5, 157.9, 153.1, 152.0, 148.7, 147.1, 135.9, 134.1, 132.7, 131.7, 124.5, 122.8, 122.4, 119.5, 119.1, 116.5, 113.3, 112.9, 111.9, 04.1, 66.5, 55.5, 36.4, 35.2, 27.7. MS (m/z, %): [M^+ 549; (1%)], 188 (100), 160 (60), 130 (15), 66 (10). *Anal*. Calcd for $C_{31}H_{23}N_3O_7$: C, 67.75; H, 4.22; N, 7.65. Found: C, 67.69; H, 4.29; N, 7.54.

2-Amino-4-(4-(4-(1,3-dioxoisoindolin-2-yl)butoxy)-3-methoxyphenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (7g). White solid; yield 81%; mp = 184–186°C; IR (KBr): cm^{-1} 3403, 3327, 2934, 2201, 1761, 1718. ^{1}H NMR (500 MHz, DMSO-*d6*): 7.81–7.86 (m, 5H, $H_{a–d}$, and H_1), 7.70 (t, J = 8.5 Hz, 1H, H_3), 7.44–7.50 (m, 2H, H_2, and H_4), 7.31 (s, 2H, NH_2), 6.83–6.86 (m, 2H, H_5, and H_6-phenyl), 6.70 (d, J = 1.5 Hz, H_2-phenyl), 4.3 (s, 1H, CH), 3.92 (t, J = 6.0 Hz, 2H, CH_2), 3.70 (s, 3H, OCH_3), 3.63 (t, J = 6.5 Hz, 2H, CH_2), 1.71–1.75 (m, 4H, 2× CH_2). ^{13}C NMR (125 MHz, DMSO-d_6): 167.9, 159.5, 157.8, 153.1, 152.1, 148.8, 147.3, 135.9, 134.3, 132.7, 131.6, 124.5, 122.9, 122.4, 119.6, 119.2, 116.5, 113.4, 112.9, 112.0, 104.1, 67.8, 55.6, 37.1, 36.4, 26.2, 24.7. MS (m/z, %): [M^+; 563 (1%)], 160 (100), 202 (48), 120 (16), 92 (9).

Anal. Calcd for $C_{32}H_{25}N_3O_7$: C, 68.20; H, 4.47; N, 7.46. Found: C, 67.97; H, 4.51; N, 7.61.

2-Amino-4-(4-(4-(1,3-dioxoisoindolin-2-yl)butoxy)phenyl)-7-methyl-5-oxo-4,5-dihydropyrano[4,3-b]pyran-3-carbonitrile (8a). White solid; yield 90%; mp = 215–216°C; IR (KBr): cm^{-1} 3406, 3327, 2195, 1720, 1765. ^1H NMR (500 MHz, DMSO-*d*6): 7.87–.81 (m, 4H, $H_{a–d}$), 7.10–7.05 (m, 4H, NH_2, and $H_{3,5}$-phenyl), 6.82 (d, 2H, *J* = 8.5 Hz, $H_{2,6}$-phenyl), 6.23 (s, 1H, H_1), 4.20 (s, 1H, CH), 3.95 (t, *J* = 6.0 Hz, 2H, CH2), 3.63 g (t, *J* = 6.5 Hz, 2H, CH_2), 2.21 (s, 3H, CH_3), 1.74–1.72 (m, 4H, 2 × CH_2). ^{13}C NMR (125 MHz, DMSO-d_6): 167.8, 162.5, 161.1, 157.9, 157.7, 157.5, 135.5, 134.2, 131.5, 128.4, 122.8, 119.2, 114.2, 100.9, 7.8, 66.8, 58.2, 37.1, 35.4, 26.1, 24.6, 19.2. MS (m/z, %): [M^+; 497 (6%)], 160 (100), 229 (24), 202 (22), 130 (9), 104 (11), 66 (13). *Anal*. Calcd for $C_{28}H_{23}N_3O_6$: C, 67.60; H, 4.66; N, 8.45. Found: C, 67.49; H, 4.75; N, 8.31.

2-Amino-4-(4-((1,3-dioxoisoindolin-2-yl)methoxy)phenyl)-7-methyl-5-oxo-4,4a,5,8a-tetrahydropyrano[4,3-b]pyran-3-carbonitrile (8b). White solid; yield 87%; mp = 249–251°C; IR (KBr): cm^{-1} 3400, 3199, 2201, 1720, 1775. ^1H NMR (500 MHz, DMSO-*d*6): 7.96–7.88 (m, 4H, $H_{a–d}$), 7.14 (d, *J* = 8.5 Hz, 2H, $H_{3,5}$-phenyl), 7.08 (s, 2H, NH_2), 7.04 (d, *J* = 8.5 Hz, 2H, $H_{2,6}$-phenyl), 6.23 (s, 1H, H_1), 5.55 (s, 2H, CH_2), 4.24 (s, 1H, CH), 2.21 (s, 3H, CH_3). ^{13}C NMR (125 MHz, DMSO-d_6): 167.8, 162.6, 161.2, 157.9, 157.8, 155.2, 137.0, 134.9, 131.1, 128.6, 123.6, 119.1, 115.3, 100.8, 97.8, 65.2, 35.4, 19.2. MS (m/z, %): [M^+; 455 (8%)], 160 (100), 66 (9), 229 (4), 104 (3). *Anal*. Calcd for $C_{25}H_{17}N_3O_6$: C, 65.93; H, 3.76; N, 9.23. Found: C, 65.81; H, 3.61; N, 9.32.

2-Amino-4-(4-(3-(1,3-dioxoisoindolin-2-yl)propoxy)phenyl)-7-methyl-5-oxo-4,4a,5,8a-tetrahydropyrano[4,3-b]pyran-3-carbonitrile (8c). White solid; yield 84%; mp = 225–227°C; IR (KBr): cm^{-1} 3403, 3324, 2198, 1718, 1779. ^1H NMR (500 MHz, DMSO-*d*6): 7.86–7.81 (m, 4H, $H_{a–d}$), 7.09 (s, 2H, NH_2), 7.05 (d, *J* = 9.0 Hz, $H_{3,5}$-phenyl), 6.75 (d, *J* = 9.0 Hz, 2H, $H_{2,6}$-phenyl), 6.24 (s, 1H, H_1), 4.19 (s, 1H, CH), 3.98 (t, *J* = 6.0 Hz, 2H, CH_2), 3.75 (t, *J* = 6.5 Hz, 2H, CH_2), 2.21 (s, 3H, CH_3), 2.05 (t, *J* = 6.5 Hz, 2H, CH_2). ^{13}C NMR (125 MHz, DMSO-d_6): 167.8, 162.6, 161.2, 157.9, 157.8, 157.4, 135.6, 134.2, 131.7, 128.4, 122.9, 119.2, 114.2, 97.8, 65.4, 58.2, 35.4, 34.9, 27.6, 19.2. MS (m/z, %): [M^+; 483 (4%)], 188 (100), 160 (86), 229 (3.5), 130 (6), 77 (3). *Anal*. Calcd for $C_{27}H_{21}N_3O_6$: C, 67.07; H, 4.38; N, 8.69. Found: C, 67.21; H, 4.57; N, 8.55.

2-Amino-4-(4-((1,3-dioxoisoindolin-2-yl)methoxy)-3,5-dimethoxyphenyl)-7-methyl-5-oxo-4,4a,5,8a-tetrahydropyrano[4,3-b]pyran-3-carbonitrile (8d). White solid; yield 83%; mp = 253–254°C; IR (KBr): cm^{-1} 3452, 3327, 2938, 2189, 1780, 1727.^1H NMR (500 MHz, DMSO-*d*6): 7.92–7.86 (m, 4H, $H_{a–d}$), 7.13 (s, 2H, NH_2), 6.37 (s, 2H, $H_{2,6}$-phenyl), 6.24 (s, 1H, H_1), 5.35 (s, 1H, CH), 4.24 (s, 1H, CH), 3.49 (s, 6H, 2 × OCH_3), 2.23 (s, 3H, CH_3).^{13}C NMR (125 MHz, DMSO-d_6): 166.7, 162.7, 161.2, 158.1, 152.7, 140.3, 134.7, 131.3, 123.3, 118.9, 104.3, 100.4, 97.8, 67.7, 55.6, 36.3, 19.2. MS (m/z, %): [M^+; 515 (1%)], 160 (100), 133 (6), 104 (6), 77 (7.5). *Anal*. Calcd for $C_{27}H_{21}N_3O_8$: C, 62.91; H, 4.11; N, 8.15. Found: C, 62.82; H, 3.96; N, 8.24.

2-Amino-4-(4-((1,3-dioxoisoindolin-2-yl)methoxy)-3-methoxyphenyl)-7-methyl-5-oxo-4,4a,5,8a-tetrahydropyrano[4,3-b]pyran-3-carbonitrile (8e). White solid; yield 82%; mp = 240–243°C; IR (KBr): cm^{-1} 3214, 3029, 2105, 1734, 1768.^1H NMR (500 MHz, DMSO-*d*6): 7.95–7.89 (m, 4H, $H_{a–d}$), 7.13 (s, 2H, NH_2), 7.05 (d, *J* = 8.5 Hz, 1H, H_5-phenyl), 6.82 (s, 1H, H_2-phenyl), 6.65 (d, *J* = 8.5 Hz, H_6-phenyl), 6.25 (s, 1H, H_1), 5.48 (s, 2H, CH_2), 4.25 (s, 1H, CH), 3.66 (s, 3H, OCH_3), 2.22 (s, 3H, CH_3). ^{13}C NMR (125 MHz, DMSO-d_6): 166.7, 162.7, 161.2, 160.2, 158.1, 157.9, 149.8, 144.5, 138.8, 134.9, 131.2, 123.5, 119.3, 119.1, 117.5, 112.2, 100.6, 97.8, 66.6, 55.5, 35.7, 19.2. MS (m/z, %): [M^+; 485 (2%)], 160 (100), 104 (18), 66 (31). *Anal*. Calcd for $C_{26}H_{19}N_3O_7$: C, 64.33; H, 3.95; N, 8.66. Found: C, 64.52; H, 3.96; N, 8.54.

2-Amino-4-(4-(3-(1,3-dioxoisoindolin-2-yl)propoxy)-3-methoxyphenyl)-7-methyl-5-oxo-4,4a,5,8a-tetrahydropyrano[4,3-b]pyran-3-carbonitrile (8f). White solid; yield 89%; mp = 210–213°C; IR (KBr): cm^{-1} 3391, 3193, 2204, 1723, 1776.^1H NMR (500 MHz, DMSO-*d*6): 7.86–7.80 (m, 4H, $H_{a–d}$), 7.09 (s, 2H, NH_2), 6.84 (d, *J* = 8.0 Hz, 1H, H_5-phenyl), 6.73 (s, 1H, H_2-phenyl), 6.64 (d, *J* = 8.0 Hz, 1H, H_6-phenyl), 6.24 (s, 1H, H_1), 4.21(s, 1H, CH), 3.97 (t, *J* = 6.5 Hz, 2H, CH_2), 3.76 (t, *J* = 6.5 Hz, 2H, CH_2), 3.55 (s, 3H, OCH_3), 2.22 (s, 3H, CH_3), 2.08–2.01 (m, 4H, 2× CH_2). ^{13}C NMR (125 MHz, DMSO-d_6): 167.8, 162.6, 161.2, 158.0, 157.8, 148.6, 147.0, 136.3, 134.1, 131.7, 122.8, 119.3, 119.2, 113.3, 111.8, 100.8, 97.8, 66.5, 58.1, 55.4, 35.7, 35.2, 27.7, 19.2. MS (m/z, %): [M^+; 513 (3%)], 188 (100), 160 (100), 130 (31), 66 (25). *Anal*. Calcd for $C_{28}H_{23}N_3O_7$: C, 65.49; H, 4.51; N, 8.18. Found: C, 65.29; H, 4.27; N, 8.29.

2-Amino-4-(4-(4-(1,3-dioxoisoindolin-2-yl)butoxy)-3-methoxyphenyl)-7-methyl-5-oxo-4,4a,5,8a-tetrahydropyrano[4,3-b]pyran-3-carbonitrile (8g). White solid; yield 87%; mp = 223–224°C; IR (KBr): cm^{-1} 3367, 3193, 2947, 2201, 1776, 1725. ^1H NMR (500 MHz, DMSO-*d*6): 7.85–7.82 (m, 4H, $H_{a–d}$), 7.06 (s, 2H, NH_2), 6.87 (d, *J* = 8.0 Hz, 1H, H_5-phenyl), 6.77 (s, 1H, H_2-phenyl), 6.65 (d, *J* = 8.0 Hz, 1H, H_6-phenyl), 6.23 (s, 1H, H_1), 4.22 (s, 1H, CH), 3.93 (t, *J* = 6.1 Hz, 2H, CH_2), 3.71 (s, 3H, OCH_3), 3.64 (t, *J* = 6.1 Hz, 2H, CH_2), 2.21 (s, 3H, CH_3), 1.75–1.72 (m, 4H, 2× CH_2). ^{13}C NMR (125 MHz, DMSO-d_6): 167.8, 162.6, 161.3, 158.0, 157.8, 148.7, 147.1, 136.2, 134.2, 131.5, 122.8, 119.4, 119.2, 113.4, 111.9, 100.8, 97.8, 67.8, 58.1, 55.6,

37.1, 35.6, 26.2, 24.7, 19.2. MS (m/z, %): [M^+; 527 (3%)], 160 (100), 202 (89), 130 (28), 102 (10), 77 (15). *Anal*. Calcd for $C_{29}H_{25}N_3O_7$: C, 66.03; H, 4.78; N, 7.97. Found: C, 66.12; H, 4.90; N, 7.97.

Cholinesterase inhibition assay. Electric eel (*Torpedo californica*), AChE (type VI-S), Butyrylcholinesterase Enzyme (BChE) (E.C.3.1.1.8, from equine serum), acetylthiocholine iodide, butyrylthiocholine iodide, 5,5-di thio bis[2-nitrobenzoic acid] (DTNB), and donepezil hydrochloride were purchased from Sigma–Aldrich (Steinheim, Germany). Compounds stock solutions were prepared by dissolving them in absolute ethanol and then diluted in the phosphate buffer (0.1 M, pH¼ 8) to obtain required concentrations. The AChE inhibitory activity of the synthesized compounds was determined by the Ellman's spectroscopic method [25], acetylthiocholine iodide as substrate in 24-well plates. The solutions consisted of 2 mL of phosphate buffer, 65 mL of DTNB, 20 mL of AChE, 35 mL of inhibitors solution, which were mixed and incubated for 15 min at 25°C. The reaction was then initiated by adding 20 mL of acetylthiocholine to each well. The formation of the thiolate dianion was monitored at 412 nm in a Synergy HTX multimode plate reader. Donepezil hydrochloride was used as the positive control. The BChE inhibition assay was similarly performed by using butyrylthiocholine iodide as a substrate.

CONCLUSION

In summary, a series of phthalimide-pyrano[3,2-c] chromene and phthalimide-pyran-2-one hybrids were synthesized by one-pot MCR *via* Knoevenagel condensation in efficient yields. The compounds were characterized by FTIR, ^{1}H NMR, ^{13}C NMR, and MS. Furthermore, the two series of compounds were evaluated against AChE/BuChE inhibitory activity using modified Ellman's method. The results showed that most of the compounds were feebly active toward AChE and were inactive toward BuChE. Change in spacer length did not elicit any significant effect on activity. However, linker length of three seemed better relative to one and four with >60% inhibition. Introduction of electron-donating group (OCH_3) on phenyl ring in series **7** compounds with linker length three led to a slight decline in activity. Similar structure activity relationship (SAR) was observed in compounds of series **8** with spacer of three CH_2 groups. In general, the introduction of an additional methoxy group mounted on inactivity.

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