Design, synthesis, docking study and biological evaluation of some novel tetrahydrochromeno [3',4':5,6]pyrano[2,3-b]quinolin-6(7H)-one derivatives against acetyl- and butyrylcholinesterase

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PII: S0223-5234(13)00495-9
DOI: 10.1016/j.ejmech.2013.07.045
Reference: EJMECH 6334

To appear in: European Journal of Medicinal Chemistry

Received Date: 23 April 2013
Revised Date: 23 June 2013
Accepted Date: 1 July 2013


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Graphical abstract


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A novel series of hybrid structures, composed of two known scaffolds including tetrahydraminoquinoline and coumarin were synthesized and evaluated for both acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory activities.
Research highlights

- In this study the synthesis of some tetrahydrochromeno[3’,4’:5,6]pyrano[2,3-b]quinolin-6(7H)-one derivatives is reported.
- We examine the anticholinesterase activity of the target compounds.
- It was revealed that all compounds exhibited high anticholinesterase activity.
- The docking study showed that all compounds were dual binding site inhibitors.
- According to the kinetic study the mixed-type of inhibition was revealed.
Design, synthesis, docking study and biological evaluation of some novel tetrahydrochromeno [3’,4’:5,6]pyranopyrano[2,3-b]quinolin-6(7H)-one derivatives against acetyl- and butyrylcholinesterase

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Abstract

Novel hybrid derivatives of two known scaffolds; tetrahydroaminoquinoline and coumarin were synthesized and evaluated for both acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activities. By means of an efficient nanocatalyst, the reaction time for the syntheses of the target compounds was reduced. Subsequently, Ellman’s modified method was used to evaluate the enzyme inhibitory activity of the synthesized structures. It was observed that most hybrid structures were moderate to potent inhibitors of AChE compared to Tacrine as the reference drug among which 7f with 4-fluorophenyl substituent was the most active compound (IC$_{50}$ = 5 nM).

Keywords: Alzheimer’s disease; Coumarin; Tacrine; Cholinesterase inhibitor; Docking study

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1. Introduction

Alzheimer’s disease (AD) is the most common form of dementia [1]. The principle pathological hallmarks of the disease are senile plaques that consist of amyloid β, and neurofibrillary tangles. The formation of these plaques in hippocampal cholinergic neurons results in neuronal cell death via apoptotic pathway [2]. In view of the fact that, most of the AD symptoms were related to loss of cholinergic function in basal forebrain, cholinergic hypothesis was developed [3]. Since Acetylcholinesterase (AChE) is responsible for the hydrolysis of acetylcholine (Ach) in the synaptic cleft, inhibition of this enzyme can increase the synaptic level of Ach and might lead to the improvement of cognition and some behavioral problems [4]. Moreover, the role of Butyrylcholinesterase (BuChE) in progression of AD has been proved. Furthermore, BuChE inhibitors could recover cholinergic activity through restoring the AChE/BuChE activity ratios as seen in healthy brain [5]. As a result, investigations have been focused on dual AChE/BuChE inhibitors [6]. Tacrine (Fig. 1) (THA, 9-amino-1,2,3,4-tetrahydroacridine), the first AChE inhibitor approved for treatment of AD, has shown selectivity towards BuChE instead of AChE [7]. In this context several Tacrine-like derivatives (such as compounds I [8], II [9], III [10] and IV [11]) have been synthesized in which tetrahydroaminoquinoline moiety present in tacrine was fused with different ring systems (Figure 1).

On the other hand, coumarin (2H-chromen-2-one) scaffold has recently gained interest for the study of its biological activities. Compounds tethering coumarin scaffold have shown wide range of biological activities such as anti-inflammatory, anti-tumor, hepatoprotective, anti-allergic, anti-HIV-1, antiviral, antifungal, antimicrobial, antiasthmatic, anti-oxidant, antinociceptive, anti-diabetic and antidepressant effects [12]. Moreover, synthetic coumarins have exhibited potent AChE inhibitory activity [13]. Further studies have been focused on modification of coumarin ring that led to development of new analogs of coumarin with superior activity (Figure 2) [14].

In continuation of the previous efforts in order to find more potent compounds [15], new series of 8-amino-tetrahydrochromeno[3’,4’:5,6]pyrano[2,3-b]quinolin-6(7H)-one derivatives were designed and their BuChE/AChE inhibitor activities were determined (Figure 3).

2. Results and discussion
2.1. Chemistry
The synthesis of a novel series of Tacrine analogues 7a–r in a rather convenient way is described in this work. As outlined in Scheme 1, these compounds could be easily prepared by the AlCl₃ promoted Friedländer reaction [16] between the corresponding known dihydropyrano[c]chromenes and cyclohexanone. Several methods have been reported for the synthesis of dihydropyrano[c]chromenes. Recently, the successful usage of magnetic nanocatalyst including (2-aminomethyl)phenol moiety on the surface of hydroxyapatite encapsulated maghemite (γ-Fe₂O₃) in aqueous medium for the synthesis of 2-amino-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitriles has been reported [17a]. In refluxing water and in the presence of magnetic nanocatalyst, the addition of 4-hydroxycoumarin to the stirring mixture of various aldehydes, 1a-r, and malonitrile 2 yielded 7-substituted 2-amino-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitriles 5a-r. This ‘one-pot’ reaction protocol proceeds through formation of alkylidene or arylidenedimalononitrile intermediate 3. Treatment of pyranochromene 5a-r, with cyclohexanone 6, in the presence of aluminum trichloride and in refluxing 1,2-dicholorethane as solvent, afforded the target molecules 7a-r. A notable feature of this procedure is the straightforward product isolation without formation of any side-products. All products were characterized using spectral data. For example, compound 7m was fully characterized by IR, ¹H and ¹³C NMR spectra and MS. The mass spectrum of 7m displayed a molecular ion signal at m/z 426 and an ion signal at m/z 319 indicating the loss of the 4-methoxyphenyl group. In the ¹H-NMR spectrum of compound 7m, in addition to the aromatic protons of coumarin ring and those assigned to the phenyl ring (δ= 8.01–6.78 ppm), a sharp singlet due to hydrogen in the pyrano moiety on 7 position (5.16 ppm) was observed. A broad singlet, exchangeable with D₂O, at 5.80 ppm was also assigned to amine group. In addition, the aliphatic protons of tetrahydroquinoline moiety were observed at δ= 2.64-1.62 ppm. The most important absorption band of 7m in the IR spectrum is detected at 3475 and 3374 cm⁻¹ and it is attributed to the amine group stretching frequency. Absorption bands at 1709 cm⁻¹ were associated to the carbonyl group. The ¹H decoupled ¹³C NMR spectrum of 7m showed 23 distinct signals. In this spectrum, the methine of the pyrano moiety on 7 position resonated at δ = 33.6 and the signal for the carbonyl was observed at δ = 157.9. In addition, six methines and eleven quaternary carbons, all in the aromatic region were in agreement with the structure.
2.2. Cholinesterase inhibition study

Compounds 7a-r were evaluated as AChE/BuChE inhibitors using colorimetric Ellman’s method [18,19]. Tacrine was used as the reference drug. The inhibitory activity of the target compounds was summarized in Table 1. It was demonstrated that 7f was the most active compound against eelAChE (IC$_{50}$ = 5 nM) and compound 7p was the most potent inhibitor of BuChE (IC$_{50}$ = 570 nM). It was also found that most of the synthesized structures were more selective against AChE. On the other hand, the compounds 7a, 7b, and 7e could be considered as dual inhibitors of both enzymes.

According to the table values, replacement of the aromatic substituents at position 7 by aliphatic methyl group (7a) led to an increase in the activity towards BuChE but the AChE inhibition potency was diminished. The resulting compound could be therefore considered as a dual inhibitor. Meanwhile, elongation of aliphatic side chain in case of compound 7b and 7c increased the activity due to the more lipophilic property of these substituents. Insertion of aromatic groups at position 7 with the ability to form π-π interaction in the hydrophobic cavity caused significant increase in activity. However, the substituent position on the aromatic ring could largely affect the activity of the target compounds. Substitution at positions 2 and 4 was therefore more tolerated while substitution at position 3 was not favorable as seen in compound 7e, 7h and 7k with the IC$_{50}$ values of 67, 288 and 141 nM, respectively. The order of activity in 4-substituted compounds was F>CH$_3$=OCH$_3$=H>Cl. The electron withdrawing substituents such as fluoro and chloro at position 2 and 4 increased the inhibitory activity probably through reinforcing the π-π interactions as seen in compounds 7f, 7g and 7i. On the other hand, electron donating groups such as methyl and methoxy induce their enhancing effects through lipophilic interaction with the active site as seen in 7l, 7m and 7p.

In order to verify the validity of our hypothesis on the efficacy of compounds with hybrid structures of tetrahydroaminoquinoline and coumarin, all synthesized intermediates 5a-r, with only one scaffold of coumarin were evaluated for their inhibitory activity. It was observed that the intermediates were significantly less potent than their corresponding target compounds. This finding has therefore confirmed our hypothesis for design of hybrid structure using the two aforementioned scaffolds.
To determine the kinetic type of AChE inhibition in case of the most active compound 7f, a kinetic study was carried out. The inhibition type was established through analysis of Lineweaver-Burk reciprocal plots (Figure 4). A mixed-type inhibition was concluded during the study. This finding proposed that the target compounds interact with enzyme in both peripheral anionic site (PAS) and central anionic site (CAS) and was in good agreement with docking data. $K_i$ value of 22.4 nM was obtained from the slopes of the double reciprocal plots versus compound 7f concentrations.

2.3. Docking studies

In order to find out the binding mode for the synthesized compounds, docking simulation studies were conducted using Autodock vina 1.1.1 [20]. To verify the predictive ability of vina scoring function for this target, a set of 107 AChE inhibitors together with 1097 decoys were retrieved from DUD database and docked with Autodock vina. The two common metrics of virtual ligand screening including enrichment factor (EF) and the area under the curve of ROC ($\text{AUC}_{\text{ROC}}$) were calculated for the docking results [21]. As depicted in (Figure 5) both values of $\text{EF}_{\text{max}}$ and $\text{AUC}_{\text{ROC}}$ were within reasonable range ($\text{EF}_{\text{max}}=5.63$ and $\text{AUC}_{\text{ROC}}=0.671$) verifying that the used parameters and scoring function are able to discriminate between active and inactive compound. Being satisfied with the predictive ability of the docking procedure, the target compounds were subjected to vina as described in the experimental section.

The binding mode of the most active compounds 7f as depicted in Figure 6 revealed a proper fitting of the compound in the gorge of eelAChE. The phenyl ring at position 7 is oriented towards the hydrophobic pocket of the binding cavity composed of Phe330, Tyr334 and Phe331. A $\pi$-$\pi$ stacking between the phenyl side chain of Tyr334 and the phenyl moiety in compound 7f takes role in stabilization of the ligand-receptor complex. It seems that the observed decrease in the activity of the compounds with aliphatic methyl group, 7a, might be due to lack of this $\pi$-$\pi$ interaction. On the other hand, in compound 7c the possible hydrophobic interaction of butyl group with the mentioned hydrophobic pocket might lead to an increase in the activity compared to 7a. Another $\pi$-$\pi$ stacking interaction between pyridine ring
and indole side chain of Trp279 is a common feature of all target compounds. This interaction is able to donate specific conformation to the compounds so that the lipophilic cyclohexane ring is fitted in the hydrophobic packet composed by Phe290, Leu282, Phe288, Ile287 and Ser286. Other notable interactions of the synthesized hybrid structures were as follow: a hydrogen bonding of coumarin carbonyl moiety with hydroxyl of Tyr121 and CH-π stacking between side chain of Gln74 and phenyl ring of coumarin scaffold.

3. Conclusions

New hybrid structures of tetrahydrochromeno[3′,4′:5,6]pyrano[2,3-b]quinolin-6(7H)-one were synthesized and evaluated for AChE and BuChE inhibitory activity. By using magnetic nanocatalyst in water medium, a very efficient method for the preparation of the intermediate compounds with short reaction times was obtained. Most of the synthesized compounds revealed significant AChE inhibitory activity among which 7f was the most active compound (IC\textsubscript{50} = 5 nM). Based on the docking studies it was suggested that the complex of ligand protein in the active site is stabilized through π-π stacking and hydrogen bond interactions. Finally, it was concluded that the hybrid of tetrahydroaminoquinoline and coumarin scaffold could be proposed as promising lead structure for further modifications.

4. Experimental

4.1. General

All commercially available reagents were used without further purification. TLC was conducted on silica gel 250 micron, F254 plates. Melting points were measured on a Kofler hot stage apparatus and are uncorrected. The IR spectra were taken using Nicolet FT-IR Magna 550 spectrographs (KBr discs). \textsuperscript{1}H NMR spectra were recorded on a Varian 400 or Brucker 500 MHz NMR instruments. The atoms numbering of the intermediate and target compounds used for \textsuperscript{1}H NMR data are depicted in Scheme 1. The chemical shifts (δ) and coupling constants (J) are expressed in parts per million and hertz, respectively. Mass spectra of the products were obtained with an HP (Agilent technologies) 5937 Mass Selective Detector. Elemental analyses were
carried out by a CHN-Rapid Heraeus elemental analyzer. The results of elemental analyses (C, H, N) were within ± 0.4% of the calculated values.

4.2. General procedure for the synthesis of 2-amino-4-alkyl or aryl-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitriles 5a-r

A stirring mixture of an appropriate aldehyde (1 mmol), malononitrile (1.2 mmol), magnetic catalytic system (1.5 mol %) and water (5 mL) were heated under refluxing conditions for a few minutes. To this stirred mixture, 4-hydroxycoumarin (1 mmol) was added. The reaction mixture was refluxed for 10 to 30 minutes. The progress of the reaction was monitored by TLC. After completion of the reaction, it was allowed to cool at room temperature and the reaction mixture was diluted with ethyl acetate and the catalyst was easily separated from the reaction mixture with an external magnet and washed twice with ethyl acetate. The combined organic layers were concentrated in vacuum and the resulting residue was purified by recrystallization from ethanol.

4.2.1. 2-amino-4-methyl-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (5a)

Yield 70%, white solid, mp 227-229 °C, IR $\nu_{max}$/cm$^{-1}$ (KBr): 3390 and 3314 (NH$_2$), 2192 (CN), 1708 (C=O). $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ 7.81 (d, 1H, $J = 7.5$ Hz, H$_{10}$), 7.70 (t, 1H, $J = 7.5$ Hz, H$_8$), 7.49-7.41 (m, 2H, H$_{7,9}$), 7.25 (s, 2H, NH$_2$), 4.34 (q, 1H, $J = 7.0$ Hz, H$_4$), 1.30 (d, 3H, CH$_3$, $J = 7.0$ Hz).

4.2.2. 2-amino-4-ethyl-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (5b)

Yield 88%, white solid, mp 239-241 °C, IR $\nu_{max}$/cm$^{-1}$ (KBr): 3395 and 3316 (NH$_2$), 2191 (CN), 1710 (C=O). $^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$ 7.82 (dd, 1H, $J = 1.4$ and 7.7 Hz, H$_{10}$), 7.71 (dt, 1H, $J = 1.4$ and 7.7 Hz, H$_8$), 7.50-7.44 (m, 2H, H$_{7,9}$), 7.32 (s, 2H, NH$_2$), 3.45 (t, 1H, $J = 7.0$ Hz, H$_4$), 1.83-1.56 (m, 2H, CH$_2$), 0.77 (t, 3H, $J = 7.5$ Hz, CH$_3$). Anal. Calcd for C$_{15}$H$_{12}$N$_2$O$_3$ C, 67.16; H, 4.51; N, 10.44. Found C, 67.35, H, 4.39, N, 10.73.

4.2.3. 2-amino-4-butyl-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (5c)

Yield 70%, white solid, mp 202-205 °C, IR $\nu_{max}$/cm$^{-1}$ (KBr): 3405 and 3291 (NH$_2$), 2192 (CN), 1723 (C=O). $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ 7.81 (d, 1H, $J = 7.5$ Hz,
H_{10}), 7.70 (t, 1H, J = 7.5 Hz, H_8), 7.49-7.44 (m, 2H, H_{7,9}), 7.31 (s, 2H, NH_2), 3.43 (t, 1H, J = 7.0 Hz, H_4), 1.74-1.56 (m, 2H, CH_2CH_2CH_2CH_3), 1.27-1.14 (m, 4H, CH_2CH_2CH_2CH_3), 0.83 (t, 3H, J = 7.0 Hz, CH_3CH_2CH_2CH_3). Anal. Calcd for C_{17}H_{16}N_2O_3: C, 68.91; H, 5.44; N, 9.45. Found: C, 69.15, H, 5.18, N, 9.73.

4.2.4. 2-amino-5-oxo-4-phenyl-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (5d)

Yield 78%, white solid, mp 256-258 °C, IR ν_{max}/cm^{-1} (KBr): 3378 and 3286 (NH_2), 2196 (CN), 1709 (C=O). ^1H NMR (DMSO-d_6, 500 MHz) δ 7.90 (d, 1H, J = 7.5 Hz, H_{10}), 7.70 (t, 1H, J = 7.5 Hz, H_8), 7.52-7.44 (m, 2H, H_{7,9}), 7.41 (s, 2H, NH_2), 7.35-7.23 (m, 5H, H_{phenyl}), 4.45 (s, 1H, H_4).

4.2.5. 2-amino-4-(3-fluorophenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (5e)

Yield 76%, pale yellow solid, mp 234-236 °C, IR ν_{max}/cm^{-1} (KBr): 3384 and 3315 (NH_2), 2199 (CN), 1701 (C=O). ^1H NMR (DMSO-d_6, 500 MHz) δ 7.91 (d, 1H, J = 7.5 Hz, H_{10}), 7.73 (t, 1H, J = 7.5 Hz, H_8), 7.54-7.44 (m, 4H, H_{7,9} and NH_2), 7.38-7.34 (m, 1H, H_{phenyl}), 7.14-7.07 (m, 3H, H_{phenyl}), 4.52 (s, 1H, H_4).

4.2.6. 2-amino-4-(4-fluorophenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (5f)

Yield 79%, yellow solid, mp 250-252 °C, IR ν_{max}/cm^{-1} (KBr): 3380 and 3293 (NH_2), 2192 (CN), 1715 (C=O). ^1H NMR (DMSO-d_6, 400 MHz) δ 7.90 (d, 1H, J = 7.5 Hz, H_{10}), 7.71 (t, 1H, J = 7.5 Hz, H_8), 7.52-7.45 (m, 2H, H_{7,9}), 7.43 (s, 2H, NH_2), 7.35-7.31 (m, 2H, H_{phenyl}), 7.16-7.11 (m, 2H, H_{phenyl}), 4.48 (s, 1H, H_4).

4.2.7. 2-amino-4-(2-chlorophenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (5g)

Yield 70%, pale yellow solid, mp 273-275 °C, IR ν_{max}/cm^{-1} (KBr): 3407 and 3282 (NH_2), 2200 (CN), 1718 (C=O). ^1H NMR (DMSO-d_6, 500 MHz) δ 7.92 (d, 1H, J = 7.5 Hz, H_{10}), 7.73 (t, 1H, J = 7.5 Hz, H_8), 7.51 (t, 1H, J = 7.5 Hz, H_9), 7.48 (d, 1H, J = 7.5 Hz, H_7), 7.44-7.37 (m, 3H, 1H_{phenyl} and NH_2), 7.33-7.24 (m, 3H, H_{phenyl}), 4.98 (s, 1H, H_4).
4.2.8. 2-amino-4-(3-chlorophenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (5h)

Yield 74%, yellow solid, mp 253-255 °C, IR $\nu_{\text{max}}$/cm$^{-1}$ (KBr): 3384 and 3323 (NH$_2$), 2204 (CN), 1697 (C=O). $^1$H NMR (DMSO-$d_6$, 500 MHz) δ 7.91 (d, 1H, $J = 7.5$ Hz, H$_{10}$), 7.73 (t, 1H, $J = 7.5$ Hz, H$_8$), 7.52-7.49 (m, 4H, H$_{7,9}$), 7.48-7.43 (m, 3H, 1H$_{\text{phenyl}}$ and NH$_2$), 7.39-7.33 (m, 3H, H$_{\text{phenyl}}$), 4.74 (s, 1H, H$_4$).

4.2.9. 2-amino-4-(4-chlorophenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (5i)

Yield 89%, white solid, mp 266-268 °C, IR $\nu_{\text{max}}$/cm$^{-1}$ (KBr): 3380 and 3312 (NH$_2$), 2192 (CN), 1713 (C=O). $^1$H NMR (DMSO-$d_6$, 500 MHz) δ 7.90 (d, 1H, $J = 7.5$ Hz, H$_{10}$), 7.72 (t, 1H, $J = 7.5$ Hz, H$_8$), 7.52-7.43 (m, 4H, H$_{7,9}$ and NH$_2$), 7.37 (d, 2H, $J = 8.5$ Hz, H$_{\text{phenyl}}$), 7.31 (d, 2H, $J = 8.5$ Hz, H$_{\text{phenyl}}$), 4.49 (s, 1H, H$_4$).

4.2.10. 2-amino-4-(2,4-dichlorophenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (5j)

Yield 78%, white solid, mp 244-246 °C, IR $\nu_{\text{max}}$/cm$^{-1}$ (KBr): 3459 and 3414 (NH$_2$), 2198 (CN), 1719 (C=O). $^1$H NMR (DMSO-$d_6$, 500 MHz) δ 7.91 (dd, 1H, $J = 7.7$ and 1.4 Hz, H$_{10}$), 7.74 (dt, 1H, $J = 1.4$ and 7.7 Hz, H$_8$), 7.59 (d, 1H, $J = 1.7$ Hz, H$_{\text{phenyl}}$), 7.53-7.48 (m, 4H, H$_{7,9}$ and NH$_2$), 7.41-7.34 (m, 2H, H$_{\text{phenyl}}$), 4.98 (s, 1H, H$_4$).

4.2.11. 2-amino-4-(3-nitrophenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (5k)

Yield 84%, white solid, mp 262-264 °C, IR $\nu_{\text{max}}$/cm$^{-1}$ (KBr): 3404 and 3322 (NH$_2$), 2202 (CN), 1703 (C=O), 1527 and 1345 (NO$_2$). $^1$H NMR (DMSO-$d_6$, 500 MHz) δ 8.14-8.10 (m, 2H, H$_{\text{phenyl}}$), 7.92 (d, 1H, $J = 7.5$ Hz, H$_{10}$), 7.80 (d, 1H, $J = 7.2$ Hz, H$_{\text{phenyl}}$), 7.72 (t, 1H, $J = 7.5$ Hz, H$_8$), 7.63 (t, 1H, $J = 7.2$ Hz, H$_{\text{phenyl}}$), 7.54 (s, 2H, NH$_2$), 7.50 (t, 1H, $J = 7.5$ Hz, H$_9$), 7.45 (d, 1H, $J = 7.5$ Hz, H$_7$), 4.72 (s, 1H, H$_4$).

4.2.12. 2-amino-5-oxo-4-p-tolyl-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (5l)

Yield 78%, white solid, mp 227-229 °C, IR $\nu_{\text{max}}$/cm$^{-1}$ (KBr): 3387 and 3312 (NH$_2$), 2192 (CN), 1713 (C=O). $^1$H NMR (DMSO-$d_6$, 400 MHz) δ 7.90 (d, 1H, $J = 7.5$ Hz,
H_{10}), 7.70 (t, 1H, J = 7.5 Hz, H_{8}), 7.52-7.43 (m, 2H, H_{7,9}), 7.37 (s, 2H, NH_{2}), 7.16-7.08 (m, 4H, H_{phenyl}), 4.40 (s, 1H, H_{4}), 2.28 (s, 3H, CH_{3}).

4.2.13. 2-amino-4-(4-methoxyphenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (5m)

Yield 78%, white solid, mp 240-242 °C, IR v_{max}/cm^{-1} (KBr): 3365 and 3280 (NH_{2}), 2202 (CN), 1707 (C=O). ^{1}H NMR (DMSO-\textit{d}_6, 500 MHz) δ 7.89 (d, 1H, J = 7.5 Hz, H_{10}), 7.69 (t, 1H, J = 7.5 Hz, H_{8}), 7.50-7.42 (m, 2H, H_{7,9}), 7.35 (s, 2H, NH_{2}), 7.16 (d, 2H, J = 7.2 Hz, H_{phenyl}), 6.85 (d, 2H, J = 7.2 Hz, H_{phenyl}), 4.39 (s, 1H, H_{4}), 3.72 (s, 3H, OCH_{3}).

4.2.14. 2-amino-4-(3,4-dimethoxyphenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (5n)

Yield 84%, white solid, mp 234-236 °C, IR v_{max}/cm^{-1} (KBr): 3370 and 3323 (NH_{2}), 2191 (CN), 1723 (C=O). ^{1}H NMR (DMSO-\textit{d}_6, 500 MHz) δ 7.90 (d, 1H, J = 7.5 Hz, H_{10}), 7.72 (dt, 1H, J = 1.4 and 8.2 Hz, H_{8}), 7.51-7.46 (m, 2H, H_{7,9}), 7.36 (s, 2H, NH_{2}), 6.88 (d, 1H, J = 8.3 Hz, H_{phenyl}), 6.51 (s, 1H, H_{phenyl}), 6.75 (d, 1H, J = 8.3 Hz, H_{phenyl}), 4.41 (s, 1H, H_{4}), 3.72 (s, 6H, 2xOCH_{3}). Anal. Calcd for C_{21}H_{16}N_{2}O_{5} C, 67.02; H, 4.28; N, 7.44. Found C, 66.83, H, 4.46, N, 7.64.

4.2.15. 2-amino-4-(3,4,5-trimethoxyphenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (5o)

Yield 82%, white solid, mp 218-220 °C, IR v_{max}/cm^{-1} (KBr): 3433 and 3303 (NH_{2}), 2192 (CN), 1723 (C=O). ^{1}H NMR (DMSO-\textit{d}_6, 500 MHz) δ 7.90 (dd, 1H, J = 1.4 and 8.2 Hz, H_{10}), 7.72 (dt, 1H, J = 1.4 and 8.2 Hz, H_{8}), 7.52-7.47 (m, 2H, H_{7,9}), 7.38 (s, 2H, NH_{2}), 6.53 (s, 2H, H_{phenyl}), 4.44 (s, 1H, H_{4}), 3.72 (s, 6H, 2xOCH_{3}), 3.63 (s, 3H, OCH_{3}). Anal. Calcd for C_{22}H_{18}N_{2}O_{6} C, 65.02; H, 4.46; N, 6.89. Found C, 65.31, H, 4.69, N, 6.63.

4.2.16. 2-amino-4-(benzo[d][1,3]dioxol-5-yl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (5p)

Yield 79%, white solid, mp 253-255 °C, IR v_{max}/cm^{-1} (KBr): 3399 and 3317 (NH_{2}), 2190 (CN), 1721 (C=O). ^{1}H NMR (DMSO-\textit{d}_6, 400 MHz) δ 7.89 (d, 1H, J = 7.5 Hz, H_{10}), 7.70 (t, 1H, J = 7.5 Hz, H_{8}), 7.52-7.41 (m, 2H, H_{7,9}), 7.37 (s, 2H, NH_{2}), 6.85-
6.79 (m, 2H, H\textsubscript{phenyl}), 6.73 (d, 1H, J = 7.2 Hz, H\textsubscript{phenyl}), 5.98 (s, 2H, OCH\textsubscript{2}O), 4.39 (s, 1H, H\textsubscript{a}). Anal. Calcd for C\textsubscript{20}H\textsubscript{12}N\textsubscript{2}O\textsubscript{5}: C, 66.67; H, 3.36; N, 7.77. Found: C, 66.89, H, 3.13, N, 8.02.

4.2.17. 2-amino-5-oxo-4-(pyridin-4-yl)-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (5q)

Yield 70%, white solid, mp 270-272 °C,\textsuperscript{17a} IR \( \nu_{\text{max}}/\text{cm}^{-1} \) (KBr): 3375 and 3283 (NH\textsubscript{2}), 2192 (CN), 1707 (C=O). \(^1\)H NMR (DMSO-\textit{d}_6, 500 MHz) \( \delta \): 8.51 (d, 2H, J = 4.5 Hz, H\textsubscript{pyridin}), 7.91 (d, 1H, J = 7.5 Hz, H\textsubscript{10}), 7.72 (t, 1H, J = 7.5 Hz, H\textsubscript{8}), 7.53 (s, 2H, NH\textsubscript{2}), 7.51-7.45 (m, 2H, H\textsubscript{7,9}), 7.33 (d, 2H, J = 4.5 Hz, H\textsubscript{pyridin}), 4.51 (s, 1H, H\textsubscript{a}).

4.2.18. 2-amino-5-oxo-4-(thiophen-2-yl)-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (5r)

Yield 75%, white solid, mp 256-258 °C,\textsuperscript{17a} IR \( \nu_{\text{max}}/\text{cm}^{-1} \) (KBr): 3370 and 3272 (NH\textsubscript{2}), 2200 (CN), 1709 (C=O). \(^1\)H NMR (DMSO-\textit{d}_6, 500 MHz) \( \delta \): 7.87 (d, 1H, J = 7.5 Hz, H\textsubscript{10}), 7.68 (t, 1H, J = 7.5 Hz, H\textsubscript{8}), 7.52 (s, 2H, NH\textsubscript{2}), 7.49-7.37 (m, 3H, 2H\textsubscript{7,9} and 1H\textsubscript{Thiophen}), 7.16 (d, 1H, J = 4.5 Hz, H\textsubscript{Thiophen}), 6.95 (t, 1H, J = 4.5 Hz, H\textsubscript{Thiophen}), 4.81 (s, 1H, H\textsubscript{a}).

4.3. General method for the Friedländer reaction

Aluminium chloride (1.5 equiv) was suspended in dry 1,2-dichloroethane (10 mL) at ambient temperature under argon atmosphere. The suspension was stirred for a few minutes. Then the corresponding pyranochromene (1 equiv) and cyclohexanone (1.5 equiv) were added. The reaction mixture was heated under reflux (24 h). After completion of the reaction (monitored by TLC), an aqueous solution of sodium hydroxide (10%) was added drop wise to the mixture until the aqueous solution became basic. After stirring for 30 min, the precipitate was filtered and washed with water. For further purification the solid was crystallized from ethanol or acetonitrile to give corresponding products.

4.3.1. 8-amino-7-methyl-9,10,11,12-tetrahydrochromeno[3',4':5,6]pyrano[2,3-b]quinolin-6(7H)-one (7a)
Yield 90%, white solid, mp 250-252 °C, IR \( \nu_{\text{max}}/\text{cm}^{-1} \) (KBr): 3425 and 3375 (NH$_2$), 1706 (C=O). $^1$H NMR (DMSO-$d_6$, 500 MHz) \( \delta \) 7.93 (d, 1H, \( J = 7.6 \) Hz, H$_1$), 7.77-7.74 (m, 3H, H$_3$ and NH$_2$), 7.55-7.52 (m, 2H, H$_{2,4}$), 4.16 (q, 1H, \( J = 6.2 \) Hz, H$_7$), 2.82-2.75 (m, 2H, H$_{12}$), 2.445-2.32 (m, 2H, H$_9$), 1.82-1.71 (m, 4H, 2H$_{10}$ and 2H$_{11}$), 1.33 (d, 3H, \( J = 6.2 \) Hz, CH$_3$). $^{13}$C NMR (DMSO-$d_6$, 125 MHz) \( \delta \) 160.0, 156.2, 153.5, 151.9, 148.7, 145.6, 133.0, 124.8, 122.1, 116.6, 113.7, 112.8, 106.4, 99.2, 26.8, 23.2, 22.3, 20.8, 20.5, 19.3. MS m/z (%) 334 (M$^+$, 7), 319 (100), 303 (9), 291 (13), 121 (18), 77 (13). Anal. Calcd for C$_{20}$H$_{18}$N$_2$O$_3$ C, 71.84, H, 5.43, N, 8.38. Found C, 72.06, H, 4.62, N, 8.46.

4.3.2. 8-AMINO-7-ETHYL-9,10,11,12-TETRAHYDROCHROMENO[3',4':5,6]PYRANO[2,3-b]QUINOLIN-6(7H)-ONE (7b)

Yield 93%, white solid, mp 260-262 °C, IR \( \nu_{\text{max}}/\text{cm}^{-1} \) (KBr): 3428 and 3385 (NH$_2$), 1706 (C=O). $^1$H NMR (CDCl$_3$, 500 MHz) \( \delta \) 8.17 (dd, 1H, \( J = 1.4, 7.5 \) Hz, H$_1$), 7.58 (dt, 1H, \( J = 1.4, 7.5 \) Hz, H$_3$), 7.39-7.33 (m, 2H, H$_{2,4}$), 4.40 (s, 2H, NH$_2$), 4.18 (t, 1H, \( J = 4.0 \) Hz, H$_7$), 2.84-2.82 (m, 2H, H$_{12}$), 2.45 (t, 2H, \( J = 6.1 \) Hz, H$_9$), 2.04-1.82 (m, 6H, 2H$_{10}$, 2H$_{11}$ and CH$_2$CH$_3$), 0.74 (t, 3H, \( J = 7.5 \) Hz, CH$_3$). $^{13}$C NMR (CDCl$_3$, 125 MHz) \( \delta \) 161.9, 157.9, 153.7, 152.6, 150.4, 140.4, 132.0, 124.2, 123.3, 116.5, 114.2, 114.1, 102.8, 97.8, 32.3, 30.3, 24.5, 23.1, 22.4, 22.2, 8.8. MS m/z (%) 348 (M$^+$, 2), 319 (100), 303 (10), 291 (13), 121 (10), 77 (5). Anal. Calcd for C$_{21}$H$_{20}$N$_2$O$_3$ C, 72.40; H, 5.79; N, 8.04. Found C, 72.66, H, 5.51, N, 7.81.

4.3.3. 8-AMINO-7-BUTYL-9,10,11,12-TETRAHYDROCHROMENO[3',4':5,6]PYRANO[2,3-b]QUINOLIN-6(7H)-ONE (7c)

Yield 90%, white solid, mp 242-244 °C, IR \( \nu_{\text{max}}/\text{cm}^{-1} \) (KBr): 3410 and 3355 (NH$_2$), 1709 (C=O). $^1$H NMR (DMSO-$d_6$, 500 MHz) \( \delta \) 7.93 (d, 1H, \( J = 7.3 \) Hz, H$_1$), 7.67 (t, 1H, \( J = 7.3 \) Hz, H$_3$), 7.47-7.42 (m, 2H, H$_{2,4}$), 6.04 (s, 2H, NH$_2$), 4.28-4.2 (m, 1H, H$_7$), 2.65-2.57 (m, 2H, H$_{12}$), 2.39-2.33 (m, 2H, H$_9$), 1.79-1.62 (m, 4H, 2H$_{10}$ and 2H$_{11}$), 1.11-1.06 (m, 2H, CH$_2$CH$_2$CH$_2$CH$_3$), 1.06-0.83 (m, 4H, CH$_2$CH$_2$CH$_2$CH$_3$), 0.68 (t, 3H, \( J = 6.7 \) Hz, CH$_2$CH$_2$CH$_2$CH$_3$). $^{13}$C NMR (DMSO-$d_6$, 125 MHz) \( \delta \) 160.6, 156.8 (2C), 154.1, 152.0, 151.5, 132.3, 124.5, 122.3, 116.4, 113.7, 113.6, 103.6, 96.7, 31.8, 31.2, 28.7, 26.1, 23.0, 22.3, 22.2, 22.0, 13.8. MS m/z (%) 376 (M$^+$, 38), 319 (100), 303 (40), 291 (77), 121 (31), 77 (19), 57 (27). Anal. Calcd for C$_{23}$H$_{24}$N$_2$O$_3$ C, 73.38, H, 6.43, N, 7.44. Found C, 73.57, H, 6.68, N, 7.35.
4.3.4. 8-amino-7-phenyl-9,10,11,12-tetrahydrochromeno[3′,4′:5,6]pyrano[2,3-b]quinolin-6(7H)-one (7d)

Yield 95%, white solid, mp >260 °C, IR $\nu_{\text{max}}$/cm$^{-1}$ (KBr): 3409 and 3359 (NH$_2$), 1710 (C=O). $^1$H NMR (DMSO-d$_6$, 500 MHz) $\delta$ 8.02 (d, 1H, $J = 7.5$ Hz, H$_1$), 7.67 (t, 1H, $J = 7.5$ Hz, H$_3$), 7.48-7.39 (m, 4H, H$_{2,4,2,4}$ and 2H$_{\text{phenyl}}$), 7.22 (t, 2H, $J = 7.2$ Hz, H$_{\text{phenyl}}$), 7.14 (t, 1H, $J = 7.2$ Hz, H$_{\text{phenyl}}$), 5.84 (s, 2H, NH$_2$), 5.23 (s, 1H, H$_7$), 2.64-2.62 (m, 2H, H$_{12}$), 2.39-2.17 (m, 2H, H$_9$), 1.85-1.65 (m, 4H, 2H$_{10}$ and 2H$_{11}$).

$^{13}$C NMR (DMSO-d$_6$, 125 MHz) $\delta$ 160.1, 155.2, 153.1, 152.6, 152.0, 151.5, 142.8, 132.5, 128.3, 128.0, 126.7, 124.6, 116.4, 113.9, 113.8, 104.8, 97.9, 34.4, 31.8, 23.0, 22.1, 21.9. MS m/z (%) 396 (M$^+$, 18), 319 (100), 121 (14), 92 (15), 77 (70), 51 (42). Anal. Calcd for C$_{25}$H$_{20}$N$_2$O$_3$ C, 75.74, H, 5.08, N, 7.07. Found C, 75.91, H, 4.83, N, 7.21.

4.3.5. 8-amino-7-(3-fluorophenyl)-9,10,11,12-tetrahydrochromeno[3′,4′:5,6]pyrano[2,3-b]quinolin-6(7H)-one (7e)

Yield 92%, white solid, mp >260 °C, IR $\nu_{\text{max}}$/cm$^{-1}$ (KBr): 3436 and 3363 (NH$_2$), 1708 (C=O). $^1$H NMR (DMSO-d$_6$, 500 MHz) $\delta$ 8.01 (d, 1H, $J = 7.5$ Hz, H$_1$), 7.68 (t, 1H, $J = 7.5$ Hz, H$_3$), 7.48-7.37 (m, 3H, 1H$_{\text{phenyl}}$ and 2H$_{2,4}$), 7.27-7.23 (m, 1H, H$_{\text{phenyl}}$), 7.11 (d, 1H, $J = 7.4$ Hz, H$_{\text{phenyl}}$), 6.98 (t, 1H, $J = 7.4$ Hz, H$_{\text{phenyl}}$), 5.93 (s, 2H, NH$_2$), 5.28 (s, 1H, H$_7$), 2.65-2.61 (m, 2H, H$_{12}$), 2.39-2.15 (m, 2H, H$_9$), 1.73-1.65 (m, 4H, 2H$_{10}$ and 2H$_{11}$).

$^{13}$C NMR (DMSO-d$_6$, 125 MHz) $\delta$ 162.6, 160.6, 160.1, 155.4, 153.0, 152.1, 151.6, 145.6, 132.6, 130.1, 124.6, 124.1, 122.7, 116.4, 115.7, 115.5, 114.0, 113.7, 113.6, 113.4, 104.6, 97.7, 34.2, 31.8, 23.0, 22.1, 21.9. MS m/z (%) 414 (M$^+$, 50), 319 (75), 162 (44), 121 (38), 109 (100), 95 (58), 77 (60), 62 (83). Anal. Calcd for C$_{25}$H$_{19}$FN$_2$O$_3$ C, 72.45, H, 4.62, N, 6.76. Found C, 72.36, H, 4.95, N, 6.91.

4.3.6. 8-amino-7-(4-fluorophenyl)-9,10,11,12-tetrahydrochromeno[3′,4′:5,6]pyrano[2,3-b]quinolin-6(7H)-one (7f)

Yield 92%, orange solid, mp >260 °C, IR $\nu_{\text{max}}$/cm$^{-1}$ (KBr): 3373 and 3230 (NH$_2$), 1708 (C=O). $^1$H NMR (DMSO-d$_6$, 500 MHz) $\delta$ 8.01 (d, 1H, $J = 7.6$ Hz, H$_1$), 7.68 (t, 1H, $J = 7.6$ Hz, H$_3$), 7.48-7.43 (m, 4H, H$_{2,4}$ and H$_{2,4}$), 7.07-7.04 (m, 2H, H$_{\text{phenyl}}$), 6.87 (s, 2H, NH$_2$), 5.26 (s, 1H, H$_7$), 2.62-2.62 (m, 2H, H$_{12}$), 2.39-2.15 (m, 2H, H$_9$), 1.79-1.70 (m, 2H, H$_{10}$ and 2H$_{11}$). $^{13}$C NMR (DMSO-d$_6$, 125 MHz) $\delta$ 161.8, 160.1, 159.9, 155.2, 153.0, 152.8, 152.0, 151.5, 139.8, 132.5, 130.2 (2C), 124.6, 122.6,
116.4, 114.8, 114.6, 114.0, 113.8, 104.6, 97.7, 33.6, 31.8, 23.0, 22.1, 21.9. MS m/z (%): 414 (M+, 70), 319 (100), 121 (18), 95 (15), 77 (10). Anal. Calcd for C25H19FN2O3 C, 72.45, H, 4.62, N, 6.76. Found C, 72.67, H, 4.85, N, 6.93.

4.3.7. 8-amino-7-(2-chlorophenyl)-9,10,11,12-tetrahydrochromeno[3′,4′:5,6]pyrano[2,3-b]quinolin-6(7H)-one (7g)

Yield 90%, white solid, mp >260 ºC, IR $\nu_{\text{max}}$/cm$^{-1}$ (KBr): 3489 and 3383 (NH$_2$), 1710 (C=O). $^1$H NMR (DMSO-d$_6$, 500 MHz) $\delta$ 8.02 (d, 1H, $J = 7.5$ Hz, H$_1$), 7.69 (t, 1H, $J = 7.1$ Hz, H$_3$), 7.57 (d, 1H, $J = 7.1$ Hz, H$_{\text{phenyl}}$), 7.27-7.22 (m, 2H, H$_{\text{phenyl}}$), 5.48 (s, 2H, NH$_2$), 5.37 (s, 1H, H$_7$), 2.65-2.60 (m, 2H, H$_{12}$), 2.39-2.15 (m, 2H, H$_9$), 1.78-1.65 (m, 4H, 2H$_{10}$ and 2H$_{11}$). $^{13}$C NMR (DMSO-d$_6$, 125 MHz) $\delta$ 159.8, 155.7, 153.0, 152.8, 152.1, 151.5, 138.7, 132.7 (2C), 132.3, 129.6, 128.8, 127.3, 124.6, 122.8, 116.4, 114.1, 113.5, 102.4, 96.6, 34.0, 31.7, 22.9, 22.1, 21.8. MS m/z (%) 432 (M$^+$+2, 19), 430 (M$^+$, 55), 395 (42), 319 (100), 175 (47), 121 (41), 111 (41), 77 (30). Anal. Calcd for C$_{25}$H$_{19}$FN$_2$O$_3$ C, 69.69, H, 4.44, N, 6.50. Found C, 69.58, H, 4.71, N, 6.39.

4.3.8. 8-amino-7-(3-chlorophenyl)-9,10,11,12-tetrahydrochromeno[3′,4′:5,6]pyrano[2,3-b]quinolin-6(7H)-one (7h)

Yield 92%, white solid, mp >260 ºC, IR $\nu_{\text{max}}$/cm$^{-1}$ (KBr): 3423 and 3375 (NH$_2$), 1710 (C=O). $^1$H NMR (DMSO-d$_6$, 500 MHz) $\delta$ 8.00 (d, 1H, $J = 6.4$ Hz, H$_1$), 7.68-7.62 (m, 2H, H$_3$ and H$_{\text{phenyl}}$), 7.45-7.39 (m, 2H, H$_{\text{phenyl}}$), 7.27-7.18 (m, 3H, H$_{\text{phenyl}}$), 5.93 (s, 2H, NH$_2$), 5.26 (s, 1H, H$_7$), 2.69-2.53 (m, 2H, H$_{12}$), 2.42-2.15 (m, 2H, H$_9$), 1.75-1.61 (m, 4H, 2H$_{10}$ and 2H$_{11}$). $^{13}$C NMR (DMSO-d$_6$, 125 MHz) $\delta$ 160.1, 155.5 (2C), 152.9, 152.1, 151.6, 145.1, 132.6, 132.4, 130.1, 128.5, 126.7 (2C), 124.6, 122.7, 116.4, 114.0, 113.7, 104.1, 97.2, 34.1, 31.8, 23.0, 22.1, 21.8. MS m/z (%) 432 (M$^+$+2, 1), 430 (M$^+$, 3), 319 (100), 121 (27), 111 (50), 92 (36), 77 (59), 62 (90), 55 (89). Anal. Calcd for C$_{25}$H$_{19}$ClN$_2$O$_3$ C, 69.69, H, 4.44, N, 6.50. Found C, 69.90, H, 4.70, N, 6.83.

4.3.9. 8-amino-7-(4-chlorophenyl)-9,10,11,12-tetrahydrochromeno[3′,4′:5,6]pyrano[2,3-b]quinolin-6(7H)-one (7i)

Yield 93%, white solid, mp 248-250 ºC, IR $\nu_{\text{max}}$/cm$^{-1}$ (KBr): 3497 and 3386 (NH$_2$), 1707 (C=O). $^1$H NMR (CDCl$_3$, 500 MHz) $\delta$ 8.23 (d, 1H, $J = 7.7$ Hz, H$_1$), 7.57 (t, 1H, $J = 7.7$ Hz, H$_3$), 7.37 (t, 1H, $J = 7.7$ Hz, H$_2$), 7.31 (d, 1H, $J = 7.7$ Hz, H$_4$), 7.28-7.25
(m, 4H, Hphenyl), 5.04 (s, 1H, H7), 4.17 (s, 2H, NH2), 2.89-2.86 (m, 2H, H12), 2.39-2.30 (m, 2H, H9), 1.89-1.87 (m, 4H, 2H10 and 2H11). 13C NMR (DMSO-d6, 125 MHz) δ 161.2, 155.7, 154.7, 153.6, 150.8, 139.9, 133.3, 132.2, 130.0, 128.9, 124.2, 123.5, 116.5, 114.7, 114.1, 103.8, 98.4, 35.9, 32.4, 22.9, 22.4, 22.1. MS m/z (%) 432 (M+2, 3), 430 (M+, 9), 350 (23), 319 (100), 283 (22), 249 (46), 239 (98), 121 (33), 92 (26). Anal. Calcd for C25H19ClN2O3 C, 69.69; H, 4.44; N, 6.50. Found C, 69.41, H, 4.63, N, 6.28.

4.3.10. 8-amino-7-(2,4-dichlorophenyl)-9,10,11,12-tetrahydrochromeno[3',4':5,6]pyrano[2,3-b]quinolin-6(7H)-one (7j)

Yield 93%, white solid, mp >300 ºC, IR vmax/cm−1 (KBr): 3485 and 3388 (NH2), 1718 (C=O). 1H NMR (CDCl3, 500 MHz) δ 8.25 (d, 1H, J= 7.8 Hz, H1), 7.59 (t, 1H, J= 7.8 Hz, H3), 7.41-7.36 (m, 2H, H2 and Hphenyl), 7.33 (d, 1H, J= 8.2 Hz, Hphenyl), 7.23 (d, 1H, J= 8.2 Hz, Hphenyl), 7.15 (d, 1H, J= 7.8 Hz, H4), 5.45 (s, 1H, H7), 4.49 (s, 2H, NH2), 2.85-2.80 (m, 2H, H12), 2.40-2.26 (m, 2H, H9), 1.86-1.83 (m, 4H, 2H10 and 2H11). 13C NMR (CDCl3, 125 MHz) δ 160.8, 156.3, 15.9, 153.2, 152.8, 151.0, 137.9, 133.8, 133.6, 132.5, 132.1, 131.0, 129.2, 128.2, 124.3, 123.6, 116.6, 114.4, 113.9, 98.0, 33.2, 32.3, 22.9, 22.3, 22.1. MS m/z (%) 468 (M+4, 2), 466 (M+2, 12), 464 (M+, 19), 429 (33), 396 (18), 319 (100), 291 (20), 264 (13), 121 (15), 77 (16). Anal. Calcd for C25H18Cl2N2O3 C, 64.53; H, 3.90; N, 6.02. Found C, 64.74, H, 4.21, N, 6.37.

4.3.11. 8-amino-7-(3-nitrophenyl)-9,10,11,12-tetrahydrochromeno[3',4':5,6]pyrano[2,3-b]quinolin-6(7H)-one (7k)

Yield 91%, white solid, mp >260 ºC, IR vmax/cm−1 (KBr): 3419 and 3363 (NH2), 1708 (C=O). 1H NMR (DMSO-d6, 500 MHz) δ 8.47 (s, 1H, Hphenyl), 8.05-8.01 (m, 2H, H1 and Hphenyl), 7.77-7.67 (m, 2H, H3 and Hphenyl), 7.54-7.42 (m, 3H, 2H2,4 and Hphenyl), 6.04 (s, 2H, NH2), 5.46 (s, 1H, H7), 2.66-2.61 (m, 2H, H12), 2.41-2.13 (m, 2H, H6), 1.79-1.62 (m, 4H, 2H10 and 2H11). 13C NMR (DMSO-d6, 125 MHz) δ 160.1, 155.6, 153.0, 152.9, 152.1, 151.8, 147.2, 144.7, 134.8, 132.7, 129.7, 124.6, 123.3, 122.7, 121.9, 116.5, 114.2, 113.7, 103.8, 96.9, 34.1, 31.7, 22.9, 22.0, 21.8. MS m/z (%) 441 (M+, 6), 401 (11), 319 (100), 212 (31), 177 (20), 159 (85), 120 (20), 89 (21), 77 (17). Anal. Calcd for C25H19N3O5 C, 68.02, H, 4.34, N, 9.52. Found C, 68.25, H, 4.13, N, 9.84.
4.3.12. 8-amino-7-(p-tolyl)-9,10,11,12-tetrahydrochromeno[3’,4’:5,6]pyrano[2,3-b]quinolin-6(7H)-one (7l)

Yield 95%, white solid, mp >260 ºC, IR \( \nu_{\text{max}} \)/cm\(^{-1}\) (KBr): 3474 and 3375 (NH\(_2\)), 1708 (C=O). \(^1\)H NMR (DMSO-\(d_6\), 500 MHz) \( \delta \) 8.01 (d, 1H, \( J = 6.9 \) Hz, H\(_1\)), 7.66 (t, 1H, \( J = 6.9 \) Hz, H\(_3\)), 7.46-7.40 (m, 2H, H\(_{2,4}\)), 7.27 (d, 2H, \( J = 6.5 \) Hz, H\(_{\text{phenyl}}\)), 7.02 (d, 2H, \( J = 6.5 \) Hz, H\(_{\text{phenyl}}\)), 5.80 (s, 2H, NH\(_2\)), 5.17 (s, 1H, H\(_7\)), 2.64-2.59 (m, 2H, H\(_{12}\)), 2.39-2.11 (m, 5H, 2H\(_9\) and CH\(_3\)), 1.77-1.62 (m, 4H, 2H\(_{10}\) and 2H\(_{11}\)). \(^{13}\)C NMR (DMSO-\(d_6\), 125 MHz) \( \delta \) 160.1, 155.0, 153.0, 152.6, 152.0, 151.5, 139.8, 135.8, 132.4, 128.6, 128.2, 124.6, 116.4, 113.9, 113.8, 104.9, 98.0, 34.0, 31.8, 23.0, 22.1, 21.9, 20.5. MS m/z (%) 410 (M\(^+\), 81), 319 (100), 121 (18), 91 (26), 65 (33). Anal. Calcd for C\(_{26}\)H\(_{22}\)N\(_2\)O\(_3\) C, 76.08, H, 5.40, N, 6.82. Found C, 76.29, H, 5.73, N, 7.03.

4.3.13. 8-amino-7-(4-methoxyphenyl)-9,10,11,12-tetrahydrochromeno[3’,4’:5,6]pyrano[2,3-b]quinolin-6(7H)-one (7m)

Yield 93%, white solid, mp >260 ºC, IR \( \nu_{\text{max}} \)/cm\(^{-1}\) (KBr): 3475 and 3374 (NH\(_2\)), 1709 (C=O). \(^1\)H NMR (DMSO-\(d_6\), 500 MHz) \( \delta \) 8.01 (d, 1H, \( J = 7.5 \) Hz, H\(_1\)), 7.68 (t, 1H, \( J = 7.5 \) Hz, H\(_3\)), 7.48-7.40 (m, 2H, H\(_{2,4}\)), 7.31 (d, 2H, \( J = 7.8 \) Hz, H\(_{\text{phenyl}}\)), 6.78 (d, 2H, \( J = 7.8 \) Hz, H\(_{\text{phenyl}}\)), 5.80 (s, 2H, NH\(_2\)), 5.16 (s, 1H, H\(_7\)), 3.66 (s, 3H, OCH\(_3\)), 2.64-2.62 (m, 2H, H\(_{12}\)), 2.37-2.20 (m, 2H, H\(_9\)), 1.75-1.62 (m, 2H, H\(_{10}\) and 2H\(_{11}\)). \(^{13}\)C NMR (DMSO-\(d_6\), 125 MHz) \( \delta \) 160.1, 157.9, 154.9, 153.0, 152.6, 152.0, 151.4, 134.8, 132.4, 129.3, 124.6, 116.4, 113.8 (2C), 113.4, 105.0, 98.1, 54.9, 33.6, 31.8, 23.0, 22.2, 21.9. MS m/z (%) 426 (M\(^+\), 40), 319 (33), 98 (30), 64 (98), 62 (100), 49 (70). Anal. Calcd for C\(_{26}\)H\(_{22}\)N\(_2\)O\(_4\) C, 73.23, H, 5.20, N, 6.57. Found C, 73.46, H, 5.51, N, 6.81.

4.3.14. 8-amino-7-(3,4-dimethoxyphenyl)-9,10,11,12-tetrahydrochromeno[3’,4’:5,6]pyrano[2,3-b]quinolin-6(7H)-one (7n)

Yield 91%, white solid, mp 171-173 ºC, IR \( \nu_{\text{max}} \)/cm\(^{-1}\) (KBr): 3471 and 3342 (NH\(_2\)), 1720 (C=O). \(^1\)H NMR (CDCl\(_3\), 500 MHz) \( \delta \) 8.22 (d, 1H, \( J = 7.5 \) Hz, H\(_1\)), 7.56 (t, 1H, \( J = 7.5 \) Hz, H\(_3\)), 7.35 (t, 1H, \( J = 7.7 \) Hz, H\(_2\)), 7.31 (d, 1H, \( J = 7.7 \) Hz, H\(_4\)), 7.02 (d, 1H, \( J = 1.7 \) Hz, H\(_{\text{phenyl}}\)), 6.96 (dd, 1H, \( J = 1.7 \), 8.3 Hz, H\(_{\text{phenyl}}\)), 6.78 (d, 1H, \( J = 8.3 \) Hz, H\(_{\text{phenyl}}\)), 5.02 (s, 1H, H\(_7\)), 4.22 (s, 2H, NH\(_2\)), 3.83 (s, 3H, OCH\(_3\)), 3.82 (s, 3H, OCH\(_3\)), 2.88-2.84 (m, 2H, H\(_{12}\)), 2.42-2.30 (m, 2H, H\(_9\)), 1.90-1.82 (m, 4H, 2H\(_{10}\) and 2H\(_{11}\)). \(^{13}\)C NMR (CDCl\(_3\), 125 MHz) \( \delta \) 159.9, 154.6, 152.8, 152.2, 151.8, 151.0, 145.9, 143.8.
136.1, 133.2, 124.2, 123.1, 122.3, 115.8, 114.1, 113.1, 109.8, 108.2, 105.4, 98.5, 95.9, 55.7, 34.5, 32.1, 23.3, 22.2, 22.0. MS m/z (%) 456 (M$^+$, 27), 319 (100), 291 (19), 137 (16), 121 (24), 92 (17), 77 (31). Anal. Calcd for C$_{27}$H$_{24}$N$_2$O$_5$ C, 71.04; H, 5.30; N, 6.14. Found C, 71.37, H, 5.17, N, 6.46.

4.3.15. **8-amino-7-(3,4,5-trimethoxyphenyl)-9,10,11,12-tetrahydrochromeno[3',4':5,6]pyrano[2,3-b]quinolin-6(7H)-one (7o)**

Yield 95%, white solid, mp 262-264 °C, IR $\nu_{\text{max}}$/cm$^{-1}$ (KBr): 3476 and 3334 (NH$_2$), 1726 (C=O). $^1$H NMR (CDCl$_3$, 500 MHz) $\delta$ 8.23 (d, 1H, $J$ = 7.5 Hz, H$_1$), 7.57 (t, 1H, $J$ = 7.5 Hz, H$_3$), 7.37 (t, 1H, $J$ = 7.5 Hz, H$_2$), 7.31 (d, 1H, $J$ = 7.5 Hz, H$_4$), 6.66 (s, 2H, H$_{\text{phenyl}}$), 5.02 (s, 1H, H$_7$), 4.31 (s, 2H, NH$_2$), 3.80 (s, 6H, 2×OCH$_3$), 3.79 (s, 3H, OCH$_3$), 2.90-2.85 (m, 2H, H$_{12}$), 2.43-2.29 (m, 2H, H$_9$), 1.92-1.86 (m, 4H, 2H$_{10}$ and 2H$_{11}$). $^{13}$C NMR (CDCl$_3$, 125 MHz) $\delta$ 161.3, 153.4 (3C), 152.6, 137.4 (2C), 136.8, 132.3, 124.3, 116.5, 114.6, 114.1, 105.9 (2C), 104.0, 98.8, 60.7, 56.2, 36.8, 29.6, 22.8, 22.2, 22.1. MS m/z (%) 486 (M$^+$, 7), 319 (100), 291 (5), 167 (3), 121 (18), 97 (35), 85 (42), 71 (64), 57 (98). Anal. Calcd for C$_{28}$H$_{26}$N$_2$O$_6$ C, 69.12; H, 5.39; N, 5.76. Found C, 69.38, H, 5.57, N, 5.54.

4.3.16. **8-amino-7-(benzo[d][1,3]dioxol-5-yl)-9,10,11,12-tetrahydrochromeno[3',4':5,6]pyrano[2,3-b]quinolin-6(7H)-one (7p)**

Yield 94%, white solid, mp >260 °C, IR $\nu_{\text{max}}$/cm$^{-1}$ (KBr): 3432 and 3355 (NH$_2$), 1720 (C=O). $^1$H NMR (DMSO-d$_6$, 500 MHz) $\delta$ 8.01 (d, 1H, $J$ = 7.5 Hz, H$_1$), 7.68 (t, 1H, $J$ = 7.5 Hz, H$_3$), 7.48-7.41 (m, 2H, H$_2,4$), 7.03 (s, 1H, H$_{\text{phenyl}}$), 6.78-6.75 (m, 2H, H$_{\text{phenyl}}$), 5.92 (s, 2H, OCH$_2$O), 5.85 (s, 2H, NH$_2$), 5.15 (s, 1H, H$_7$), 2.65-2.60 (m, 2H, H$_{12}$), 2.40-2.18 (m, 2H, H$_9$), 1.79-1.63 (m, 4H, 2H$_{10}$ and 2H$_{11}$). $^{13}$C NMR (DMSO-d$_6$, 125 MHz) $\delta$ 160.1, 155.0, 153.0, 152.6, 152.0, 151.5, 146.7, 145.9, 136.7, 132.5, 124.6, 122.6, 121.4, 116.4, 113.9, 113.8, 109.1, 107.9, 104.8, 100.8, 97.9, 34.0, 31.8, 23.0, 22.1, 21.9. MS m/z (%) 440 (M$^+$, 7), 319 (100), 291 (5), 167 (3), 121 (18), 97 (35), 85 (42), 71 (64), 57 (98). Anal. Calcd for C$_{26}$H$_{20}$N$_2$O$_5$ C, 69.12; H, 5.39; N, 5.76. Found C, 69.38, H, 5.57, N, 5.54.

4.3.17. **8-amino-7-(pyridin-4-yl)-9,10,11,12-tetrahydrochromeno[3',4':5,6]pyrano[2,3-b]quinolin-6(7H)-one (7q)**

Yield 94%, white solid, mp >260 °C, IR $\nu_{\text{max}}$/cm$^{-1}$ (KBr): 3432 and 3355 (NH$_2$), 1720 (C=O). $^1$H NMR (DMSO-d$_6$, 500 MHz) $\delta$ 8.42 (d, 2H, $J$ = 5.1 Hz, H$_{\text{pyridine}}$), 8.03 (d,
1H, J = 7.5 Hz, H\textsubscript{1}), 7.70 (t, 1H, J = 7.5 Hz, H\textsubscript{3}), 7.50-7.40 (m, 4H, H\textsubscript{2,4} and 2H\textsubscript{pyridine}), 5.94 (s, 2H, NH\textsubscript{2}), 5.32 (s, 1H, H\textsubscript{7}), 2.68-2.54 (m, 2H, H\textsubscript{12}), 2.61-2.18 (m, 2H, H\textsubscript{9}), 1.77-1.64 (m, 4H, 2H\textsubscript{10} and 2H\textsubscript{11}). \textsuperscript{13}C NMR (DMSO-\textsubscript{d6}, 125 MHz) δ 160.1, 155.9 (2C), 153.1, 152.1, 151.7, 151.0, 149.4, 132.7, 124.7, 123.5, 122.7, 116.5, 114.1, 113.7, 103.4, 96.5, 33.9, 31.8, 23.0, 22.1, 21.8. MS m/z (%) 397 (M\textsuperscript{+}, 73), 319 (100), 121 (35), 79 (40). Anal. Calcd for C\textsubscript{24}H\textsubscript{19}N\textsubscript{3}O\textsubscript{3}C, 72.53, H, 4.82, N, 10.57. Found C, 72.29, H, 5.14, N, 10.78.

4.3.18. 8-amino-7-(thiophen-2-yl)-9,10,11,12-tetrahydrochromeno[3',4':5,6]pyrano[2,3-b]quinolin-6(7H)-one (7r)

Yield 90%, white solid, mp >260 ºC, IR \(\nu_{\text{max}}\)/cm\(^{-1}\) (KBr): 3413 and 3367 (NH\textsubscript{2}), 1710 (C=O). \(^1\)H NMR (DMSO-\textsubscript{d6}, 500 MHz) δ 8.00 (d, 1H, J = 7.6 Hz, H\textsubscript{1}), 7.70 (t, 1H, J = 7.6 Hz, H\textsubscript{3}), 7.49-7.45 (m, 2H, H\textsubscript{2,4}), 7.26 (d, 1H, J = 4.7 Hz, H\textsubscript{thiophen}), 7.15 (d, 1H, J = 2.2 Hz, H\textsubscript{thiophen}), 6.87 (dd, 1H, J = 4.7 and 2.2 Hz, H\textsubscript{thiophen}), 6.07 (s, 2H, NH\textsubscript{2}), 5.65 (s, 1H, H\textsubscript{7}), 2.71-2.60 (m, 2H, H\textsubscript{12}), 2.42-2.23 (m, 2H, H\textsubscript{9}), 1.69-1.75 (m, 4H, 2H\textsubscript{10} and 2H\textsubscript{11}). \textsuperscript{13}C NMR (DMSO-\textsubscript{d6}, 125 MHz) δ 160.1, 155.3, 152.8, 152.0, 151.8, 146.5, 132.7, 126.4, 125.8, 124.7(2C), 122.6, 116.5, 113.9, 113.7, 104.4, 97.5, 31.9, 29.7, 23.0, 22.1, 21.9. MS m/z (%) 402 (M\textsuperscript{+}, 80), 319 (100), 121 (50), 84 (43), 77 (41). Anal. Calcd for C\textsubscript{23}H\textsubscript{18}N\textsubscript{2}O\textsubscript{3}S C, 68.64, H, 4.51, N, 6.96. Found C, 68.85, H, 4.74, N, 7.18.

4.4. Determination of anticholinesterase activity

The inhibitory potency of target compounds on AChE and BuChE was determined using colorimetric Ellman’s method [18,19]. Acetylcholinesterase (AChE, E.C. 3.1.1.7, Type V-S, lyophilized powder, from electric eel, 1000 unit), butyrylcholinesterase (BuChE, E.C. 3.1.1.8, from equine serum), butyrylthiocholine iodide (BTC), were obtained from Sigma–Aldrich. 5, 5′-Dithiobis-(2-nitrobenzoic acid) (DTNB), potassium dihydrogen phosphate, dipotassium hydrogen phosphate, potassium hydroxide, sodium hydrogen carbonate, and acetylthiocholine iodide were purchased from Fluka. In brief, 50 µl of five different concentration of the test compounds was added to the mixture of 3 ml phosphate buffer 0.1M (pH=8.0), 100 µl of DTNB solution (0.1M), and 50 µl of AChE (2.5 IU/ml). After 10 minutes incubation at 25ºC, 10 µl solution of acetylthiocholine iodide (0.15M) as substrate was added. The change of absorbance was measured at 412 nm for 6 minutes. The
IC$_{50}$ values were determined graphically from inhibition linear curves (log inhibitor concentration Vs percent of inhibition). UV-2100 Rayleigh Double Beam Spectrophotometer was used for spectrophotometric measurements. The same method was used for BuChE inhibition assay.

4.5. Kinetic study

To obtain estimates of the inhibition model and inhibition constant $K_i$, reciprocal plots of $1/V$ versus $1/[S]$ were constructed at different concentrations of the substrate acetylthiocholine (0.14-0.69 mM) by using Ellman’s method. For this purpose, experiments were performed similar to enzyme inhibition assay. The rate of enzymatic reaction was measured with different inhibitor concentrations and without inhibitor for proposed substrate concentrations. For each experiment, reaction was initiated by adding acetylthiocholine as substrate. Progress curves were monitored at 412 nm over 2 min. Then, double reciprocal plots ($1/v$ vs. $1/[s]$) were constructed using the slopes of progress curves to obtain the type of inhibition. Slopes of the reciprocal plots were then plotted against the concentration of inhibitor, to evaluate $K_i$ data. Data analysis was performed with Microsoft Excel 2003. All rate measurements were performed in triplicate.

4.6. Docking studies

The pdb structure of 3I6Z (Torpedo californica acetylcholinesterase) was downloaded from the Brookhaven protein database [22] in complex with the inhibitor $N$-sacharinoheptyl-galantamine. Afterwards, the co-crystallized ligand and the water molecules were removed from the protein. The structures of the target compounds were drawn using MarvineSketch 5.8.3, 2012, ChemAxon (http://www.chemaxon.com) and converted to 3D by means of Openbabel 2.3.1 [23]. Autodock Tools 1.5.4 [24] was finally used to prepare the proper pdbqt format of the protein and ligands with default parameters. For docking purpose Autodock vina 1.1.1 was taken. The exhaustiveness was set to 10 and other parameters were remained unchanged. The box size was set to the dimensions of 50, 50, 50, to cover all binding sites occurred in the active site of the enzyme. The center of grid box was
set to $x=6.231$, $y = 67.871$, $z = 58.888$ and the lowest energy conformations were selected for analyzing the interactions between the AChE and inhibitor. PosViewWeb 1.97.035 [25] and Chimera 1.6 [26] were used for visual representation of the ligand protein complex. Calculation and plot of ROC and EF metrics were done using our application implemented in vb.net.

**Acknowledgments**

This work was financially supported by Grants from the Research Council of Tehran University of Medical Sciences, Iran National Elite Foundation (INEF) and Iran National Science Foundation (INSF). Molecular graphics and analyses were performed with the UCSF Chimera package. Chimera is developed by the 25 Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco.
References


Figure captions:

**Figure 1.** Tacrine and Tacrine-like derivatives as dual AChE/BuChE inhibitors.

**Figure 2.** Some potent anticholinesterase structures bearing coumarin moiety.

**Figure 3.** Design strategy of the target compounds 7a–r by juxtaposition of coumarin and tetrahydroaminoquinoline.

**Figure 4.** Steady-state inhibition of AChE by compound 7f. (left) Lineweaver–Burk plot of reciprocal of initial velocities versus reciprocal of acetylthiocholine iodide concentrations (0.14-0.69 mM) in the absence and presence of 7f at 2 nM, 5 nM and 10 nM; (right) secondary plots of the Lineweaver-Burk plot, slope versus various concentrations of 7f.

**Figure 5.** (left) ROC plot of virtual screening step, (right) enrichment factor plot in logarithmic scale.

**Figure 6.** (left) residues involved in the interactions with compound 7f, (right) 2D representation of binding mode of 7f in the gorge of TcAChE.
Table 1. Inhibition of AChE from *electrophorus electricus* (*eel*AChE) and horse serum butyrylcholinesterase (*eq*BuChE) by compounds 7a-r.

![Chemical structure](https://example.com/structure.png)

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<sup>a</sup> Selectivity for AChE  
<sup>b</sup> No activity (IC<sub>50</sub> > 30 µM)
Figure 1

Figure 2
Figure 3

Figure 4
Figure 5

Figure 6
Scheme 1. Synthetic pathway of the target compounds 7a-r.
Supplementary material

Design, synthesis, docking study and biological evaluation of some novel 
tetrahydrochromeno [3’,4’:5,6]pyrano[2,3-b]quinolin-6(7H)-one derivatives against acetyl- 
and butyrylcholinesterase 

Mehdi Khoobi, Masoumeh Alipour, Alireza Moradi, Amirhossein Sakhteman, Hamid Nadri, 
Seyyede Faeze Razavi, Mehdi Ghandi, Alireza Foroumadi, Abbas Shafiee*

$^1$H and $^{13}$C spectra for selected compounds:

8-amino-7-methyl-9,10,11,12-tetrahydrochromeno[3’,4’:5,6]pyrano[2,3-b]quinolin-6(7H)-one (7a)

8-amino-7-ethyl-9,10,11,12-tetrahydrochromeno[3’,4’:5,6]pyrano[2,3-b]quinolin-6(7H)-one (7b)

8-amino-7-butyl-9,10,11,12-tetrahydrochromeno[3’,4’5,6]pyrano[2,3-b]quinolin-6(7H)-one (7c)

8-amino-7-phenyl-9,10,11,12-tetrahydrochromeno[3’,4’5,6]pyrano[2,3-b]quinolin-6(7H)-one (7d)

8-amino-7-(4-fluorophenyl)-9,10,11,12-tetrahydrochromeno[3’,4’5,6]pyrano[2,3-b]quinolin-6(7H)-one (7f)

8-amino-7-(4-chlorophenyl)-9,10,11,12-tetrahydrochromeno[3’,4’:5,6]pyrano[2,3-b]quinolin-6(7H)-one (7i)

8-amino-7-(2,4-dichlorophenyl)-9,10,11,12-tetrahydrochromeno[3’,4’:5,6]pyrano[2,3-b]quinolin-6(7H)-one (7j)

8-amino-7-(3-nitrophenyl)-9,10,11,12-tetrahydrochromeno[3’,4’5,6]pyrano[2,3-b]quinolin-6(7H)-one (7k)

8-amino-7-(3,4-dimethoxyphenyl)-9,10,11,12-tetrahydrochromeno[3’,4’:5,6]pyrano[2,3-b]quinolin-6(7H)-one (7n)

8-amino-7-(3,4,5-trimethoxyphenyl)-9,10,11,12-tetrahydrochromeno[3’,4’:5,6]pyrano[2,3-b]quinolin-6(7H)-one (7o)
8-amino-7-(benzo[d][1,3]dioxol-5-yl)-9,10,11,12-tetrahydrochromeno[3',4':5,6]pyrano[2,3-b]quinolin-6(7H)-one (7p)

8-amino-7-(benzo[d][1,3]dioxol-5-yl)-9,10,11,12-tetrahydrochromeno[3',4'5,6]pyrano[2,3-b]quinolin-6(7H)-one (7p)

8-amino-7-(thiophen-2-yl)-9,10,11,12-tetrahydrochromeno[3',4'5,6]pyrano[2,3-b]quinolin-6(7H)-one (7r)