Regular Article

Synthesis and Biological Evaluation of New Tacrine Analogues under Microwave Irradiation

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Efficient routes to various kinds of heterocycles incorporating the *p*-halophenyl moiety have been synthesized. Different pyrrole derivatives have been synthesized, as well, by Thorpe–Ziegler cyclization. Therefore, we synthesized different analogues of tacrine by Friedländer reaction of *o*-amino nitriles (pyrazolo, furano and pyrrolo) with different cycloalkanones. The use of microwave irradiation leads to shorter production times and high product conversion. These synthesized compounds were biologically evaluated by Ellman's test on acetylcholinesterase inhibition.

Key words tacrine; Friedländer reaction; enaminone; Alzheimer's disease; acetylcholinesterase; microwave irradiation

Alzheimer's disease (AD) is recognized as one of the most common diseases to affect the elderly, and its effect on this set of population is serious. It is characterized by neuronal loss, synaptic damage, a deficit in neurotransmitter acetylcholine and vascular plaques. Unfortunately, the occurrence of AD is now rising exponentially in both genders as the general population ages. Until recently an exact scientific description of the cause of this disease had not been defined. And despite an impressive amount of development in understanding the molecular mechanisms behind AD, an effective therapeutic drug is still not obtainable. The inhibition of acetylcholinesterase (AChE) to increase the acetylcholine (ACh) level in synapses is the most recent method for the treatment of AD.[1–3)]

At this time there are four AChE inhibitor (AChEI) drugs accepted by the U.S. Food and Drug Administration (FDA) for the treatment of AD: tacrine, rivastigmine, donepezil and galantamine (Fig. 1). Tacrine, the first AChEI generally presented in therapy, under the trade name Cognex® since 1993 has low selectivity for AChE leading to side effects, it is now infrequently used due to its hepatotoxicity (in *ca.* 50% of patients), which does not happen with the other three drugs.[4,5)] Nevertheless, studies using tacrine analogues have continued in the search for more powerful, safer tacrine derivatives.[6)]

Attempts to modify the structure of tacrine have been achieved either by changing the ring size, increasing the number of rings, or by introducing heteroatoms.[7–16)] One study found that tacrine is more active against AChE than other compounds that contain four rings. But these compounds have shown potential activity against β-amyloid protein aggregation in the brain and they are more selective.[7)] Five membered ring tacrine analogues have been identified for a long period.[8)]

Recently, Kirsch and colleagues synthesized velnacrine and tacrine anlogues that contain thiazole, thiophene, selenophene or 4-azaisoindole heterocycles.[9–12)] New analogues of tacrine have been developed by replacing the primary amino group with the azetidine moiety.[13)] Analogues of tacrine that contain heterocyclic rings as pyridine, oxazole and pyran have been developed by a Spanish–Portuguese group and the inhibitory effects of these on butyryl cholinesterase and AChE have been reported.[14)] Barreiro *et al.* synthesized tacrine analogues containing pyrazolonaphthyridine or pyrazolopyridine systems and concluded that these compounds were the good inhibitors of AChE.[15)] It has also been reported that the attendance of halogen atoms in tacrine derivatives advances the activity.[16)]

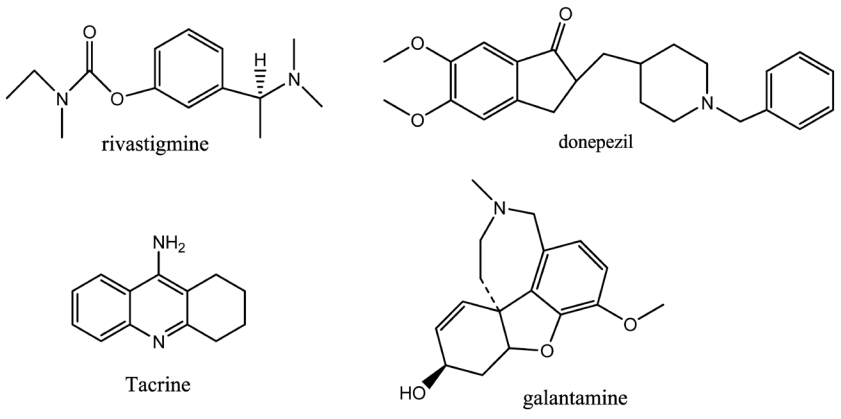

Fig. 1. Structures of the Four Acetylcholinesterase Inhibitors (AChEIs)

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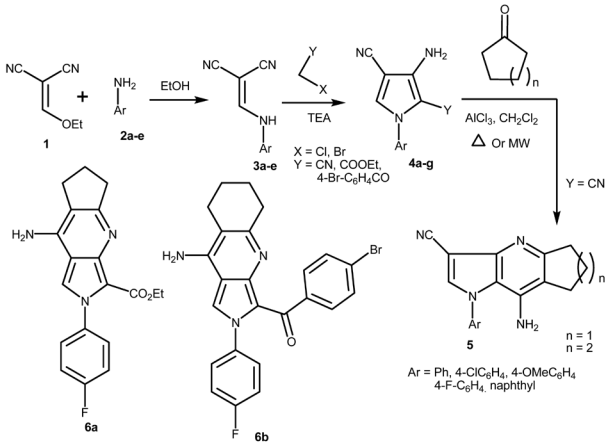
Here, the synthesis and biological evaluation of fused five-membered aromatic ring analogues of tacrine containing halogens such as the pyrrolotacrines, pyrazolotacrines and furanotacrines heterocyclic ring systems are reported (Charts 1–3). We designed new tacrine analogues from heterocyclic system containing an enaminonitrile moiety by reaction of a cyclic ketone with an *ortho*-aminonitrile in the attendance of a Lewis acid, according to the Friedländer reaction.

Results and Discussion

Chemistry As an extension of our interest on the chemistry of *β*,*β*-enaminonitriles, we report here the results aimed at exploring the possible utility of 3-anilino-2-cyanoacrylonitrile in the synthesis of heterocyclic compounds. We converted the *β*,*β*-enaminonitriles **3** which were prepared by recognized procedures[17] into the corresponding 3-aminopyrrole derivatives **4** through reaction with chloroacetonitrile in presence of a base, a Thorpe–Ziegler cyclization[18–22] (Chart 1).

The new pyrrolotacrines **5** and **6** were synthesized, as illustrated in Chart 1, from the readily available N_1-aryl-3-aminopyrrolocarbonitrile derivatives **4** according to Friedländer reaction (FR). Under thermal conditions the reaction time was 8–10 h, which was reduced to about 30 min, under microwave irradiation and compound **5** was obtained in good yields (75–87%); see Table 1. Using cyclopentanone or cyclohexanone, we obtain the expected target molecules in good yield. However, when Y was an ester or ketone group **(4f**, **g)** regioisomers **6** were formed. The cyclization happened wholly in the direction of the nitrile in position 4 of the pyrrole, so regioisomers **6** were formed. The structures of these novel compounds were evaluated by mass spectrometry, and by ^{1}H- and ^{13}C-NMR spectroscopy. For example in compound **6a** the ^{1}H-NMR spectrum showed the presence of a *quartet* signal at 4.22 ppm and a *triplet* signal at 1.14 ppm of the ester group, as well as the nonappearance of a CN absorption band in the IR spectrum. The structures of the synthesized compounds are in covenant with their spectroscopic and analytical data (see Experimental).



	Ar	Y		Ar	n
4a	C_6H_5	CN	**5a**	C_6H_5	1
4b	4-ClC_6H_4	CN	**5b**	C_6H_5	2
4c	4-$OMeC_6H_4$	CN	**5c**	4-ClC_6H_4	1
4d	4-FC_6H_4	CN	**5d**	4-$OMeC_6H_4$	1
4e	Naphthyl	CN	**5e**	4-$OMeC_6H_4$	2
4f	4-FC_6H_4	CO_2Et	**5f**	4-FC_6H_4	2
4g	4-FC_6H_4	4-BrC_6H_4CO	**5g**	Naphthyl	1

Chart 1. Syntheses of Compounds **4a**–**g**, **5a**–**g**, **6a** and **b**

Pyrazolo[3,4-*b*]pyridines are significant compounds due to their structural relationship to azaindoles and their biological activity. A number of these are potentially biologically active compounds as new inhibitors of xantine oxidase.[23,24] Due to their variety of activities, we have been extended our work on *ortho*-aminocyanopyrazoles[24,25] and their derivatives as inhibitors of xanthine oxidase,[26] For this purpose we started from the key intermediates (N_1-substituted-5-amino-4-cyanopyrazoles) **8a**–**d**[17] (Chart 2). The introduction of a CF_3 group provided compounds with increased lipophilicity and activity when compared to their non-fluorinated analogues. The trifluoromethyl substituted compounds, in particular, have been reported to possess such biological activity as herbicidal, fungicidal, analgesic, antipyretic, and inhibitors of platelet aggregation.[27]

The 5-amino-1-substituted pyrazole-4-carbonitriles **8** were used as starting materials as they contain a cyano group adjacent to an amino group which is necessary to synthesize the condensed rings of the fused heterocycles *via* reactions of heterocyclic enaminonitrile derivatives *via* FR under thermal and microwave heating to produce the pyarazolotacrine derivatives

Table 1. Friedländer Cyclization Reaction under Classical Heating and Microwave Irradiation

Compounds	Classical heating		MW irradiation	
	Time (h)	Yield %	Time (min)	Yield %[a]
5a	9	67	2×15	85
5b	9	60	2×15	80
5c	7	64	2×12	78
5d	7	66	2×12	86
5e	10	55	2×16	72
5f	10	48	2×16	78
5g	8	64	2×15	79
6a	8	62	2×15	76
6b	8	62	2×15	87
9a	9	44	2×16	62
9b	9	48	2×16	69
9c	10	30	2×16	45
9d	10	42	2×15	66
9e	9	50	2×15	65
14	10	52	2×15	70

a) Yield of pure compounds.

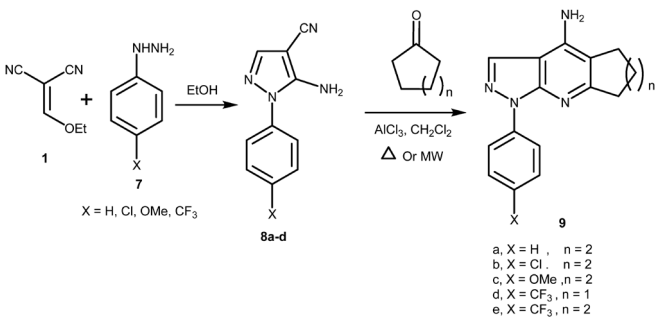


Chart 2. Synthesis of Compounds **8a**–**d** and **9a**–**e**

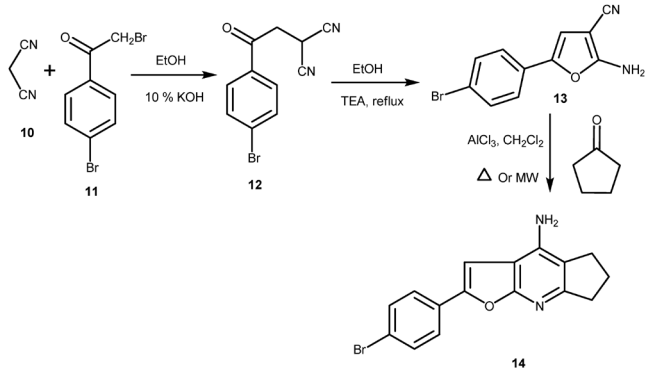


Chart 3. Synthesis of Compound **14**

9a–**e**. The structures of these compounds were established and determined by their spectroscopic and analytical data.

Reaction of 4-bromophenacyl bromide **11** with malononitrile **10** in ethanol and KOH yielded phenacyl malononitrile derivative **12**, which underwent cyclization into furan derivative **13** under basic conditions[28] (Chart 3).

The synthesis of the new furanotacrine **14** was achieved as shown in Chart 3, starting from previously synthesized 2-amino-5-arylfuran-3-carbonitriles **13** according to FR under thermal and microwave heating.

Biology The inhibitory activities of the synthesized compounds against AChE were studied in comparison with the reference compound donepezil using the method of Ellman *et al.*[29] The results are recorded in Tables 2 and 3.

Cholinesterase is a family of enzymes that catalyzes the hydrolysis of the neurotransmitter acetylcholine (ACh) into choline and acetic acid, a reaction necessary to allow a cholinergic neuron to return to its resting state after activation. AChE used in the present study (E.C. 3.1.1.7) is found in motor neurons of the mammalian brain; the majority of AChE occurs in a tetrameric, G4 form, wihile much smaller amounts are present in a monomeric G1 (4S) form. According to our data, all compounds inhibited AChE, with donepezil used as reference compound.

From these results, and for the pyrrolotacrines **5** and **6a**, **b** we conclude that: (a) all synthesized tacrine compounds have the potent to moderate activity; (b) compound **5b** is the most potent inhibitor; (c) the substiution of a fused cyclohexane ring with fused cyclopentane ring [compare **5a** with **5b**] improved the anti-AChE activity of the tacrine derivatives; (d) regarding the type of substituent at C-4 in the aromatic ring in compounds that bear the same cycloalkane fused ring system the inhibitory potency followed this order: either H≫4-OMe>4-F [compare **5b** with **e** and **f** (cyclohexane)] or H>naphthyl>4-Cl [compare **5a** with **c** and **g** (cyclopentane); (e) introducing a methoxy group at *para* position of the phenyl led us to the less active **5e** (around 37% less active than **5b**). Some authors have reported that the presence of halogen atoms in tacrine derivatives, especially the chlorine atom, improved anti-AChE activity, but this was not observed in our study.[16] Compounds **6a** and **b** with a different annelations between the pyridine and pyrrolo rings, when compared with all the others presented lower activity than **5b** (**6a** was lower by 32% and **6b** by 27%). Once again, the five membered saturated ring doesn't ameliorate the anti-AChE activity.

From the data in Table 2 and for the pyrazolotacrines **9a**, **b** and **e** regarding the type of substituent at the C-4 in the aromatic ring and the effect of the size of the fused cycloalkane ring, the same structure–activity relationships (SARs) could also be determined. The most potent inhibitor was compound **9a** while **9e** showed lower activity.

The furanotacrine analogue **14** showed reactivity of 85% compared with the donepezil reference compound. Overall, compounds **5b** (98%) and **9a** (96%) demonstrated the most potent inhibition of AChE compared with the donepezil reference compound.

Table 2. Anti-Alzheimer's Activity % (Anticholinesterase Inhibitory Activity) of Different Concentrations of Organic Compounds Compared with Donepezil

Compounds	Concentration			Donepezil
	25 μmol/mL	50 μmol/mL	100 μmol/mL	100 μmol/mL
5a	28.47	33.40	58.35	66.67
5b	36.53	44.69	63.35	64.54
5c	26.24	30.25	41.46	53.81
5e	25.48	32.40	40.10	56.44
5f	28.00	31.00	41.10	61.56
5g	33.00	35.12	49.34	59.50
6a	29.87	33.69	41.60	62.74
6b	27.56	29.89	46.00	56.40
9a	37.36	44.60	51.30	53.40
9b	31.89	38.90	50.50	57.56
9e	28.36	31.54	37.40	58.40
14	30.34	33.00	42.00	49.00

Data represented as the mean of three replicates in each group. Statistical analysis was carried out using an SPSS computer program, (one way ANOVA), coupled with a co-state computer program, where the unshared letter is significant at $p \leq 0.01$.

Table 3. IC_{50} Results of the Different Organic Compounds Compared with Donepezil on AChE *in Vitro*

Compounds	IC_{50} (nM)
5a	6.32
5b	6.87
5c	4.50
5e	4.35
5f	4.45
5g	5.35
6a	4.51
6b	4.99
9a	5.56
9b	5.48
9e	4.06
14	4.55
Donepezil	7.23

IC_{50}: The dose of the compounds that inhibit 50% of the AChE.

Conclusion

Three heterocyclic compounds, pyrrole, pyrazole and furan derivatives were prepared as starting materials for the synthesis of tacrin analogues, according to typical FR under thermal and microwave heating. The inhibition of AChE by the synthesized tacrine analogues was evaluated. Compounds **5b** (98%) and **9a** (96%) were the most potent for the inhibition of AChE compared with the donepezil itself.

Experimental

Materials and Methods Melting points were measured in open capillaries (uncorrected) on a Gallenkamp melting point apparatus. IR spectra were verified using a Shimadzu FT IR 8101 PC spectrometer using KBr technique. A Varian Mercury VXR-300 NMR spectrometer was used for recording NMR spectra. ^{13}C-NMR (75.46 MHz) and ^{1}H-NMR (300 MHz)) were verified in deuterated chloroform ($CDCl_3$) or dimethyl sulfoxide (DMSO-d_6). Tetramethylsilane (TMS) was used as an internal reference. Whenever possible the complete assignment of ^{1}H and ^{13}C in the NMR spectra was carried out by heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC) double resonance experiments. Electron impact (EI) mass spectra were recorded on a Shimadzu GCMS-QP-2010 plus mass spectrometer at 70 eV. Microwave irradiation was performed using a *CEM MARS* oven. Elemental analyses were carried out at the Micro-Analytical Centre of Cairo University, Giza, Egypt and recorded on an Elementar-Vario EL automatic analyzer. Biological evaluations were carried out at the Animal Reproduction Research Institute (ARRI) at the, Ministry of Agriculture, Dokki, Cairo, Egypt. Compounds **3** were prepared as in the literature.[18,20]

General Procedure for the Preparation of 2-((Arylamino)methylene) Malononitrile Derivatives 3a–e To a solution of the aromatic amine derivatives **2a**–**e** (0.1 mol) in ethanol (100 mL) ethoxymethylene malononitrile (EMMN) **1** (0.1 mol) was added slowly (only half the total quantity); when the solution began to boil the other half was added and stirred for 30 min. On cooling the precipitate formed was filtered off and recrystallized from ethanol. Compounds **3a**–**c** were prepared according to the literature.[19,21]

2-((4-Fluorophenylamino)methylene)malononitrile **3d**

Yellow solid in 84% yield, mp 250–252°C (EtOH-DMF). ^{1}H-NMR (DMSO-d_6) δ: 6.91–6.94 (d, 2H, Ar-H, J=0, 9.0 Hz), 7.30–7.33 (d, 2H, Ar-H, J=9.0 Hz), 8.37 (s, 1H), 11.02 (brs, 1H, NH). IR (KBr): 3301 (NH), 2216 (CN) cm^{-1}. MS (EI)=187 (84%). *Anal.* Calcd for $C_{10}H_6FN_3$ (187.17): C, 64.17; H, 3.23; N, 22.45. Found: C, 64.35; H, 3.48; N, 22.40.

2-((Naphthalen-1-ylamino)methylene)malononitrile **3e**

Pink solid in 84%yield, mp 123–125°C (EtOH). ^{1}H-NMR (DMSO-d_6) δ: 7.10–7.18 (m, 1H, Ar-H), 7.50–7.61 (m, 3H, Ar-H), 7.85 (s, 1H), 8.05–8.12 (m, 3H, Ar-H). IR (KBr): 3425 (NH), 2216 (CN) cm^{-1}. MS (EI)=219 (100%). *Anal.* Calcd for $C_{14}H_9N_3$ (219.24): C, 76.70; H, 4.14; N, 19.17. Found: C, 76.85; H, 4.25; N, 19.36.

General Procedure for the Preparation of 3-Aminopyrrole Derivatives 4a–g To a solution of the intermediate **3a**–**e** (0.01 mol) the α-halo compound (chloroacetonitrile, 4-bromophenacylbromide and ethyl bromoacetate) (0.01 mol) and triethylamine (TEA, 5 mL) were added, with cooling. The reaction mixture was then refluxed for 15–30 min, cooled 30 mL of water was added. The precipitate formed was filtered off, washed several times with water and recrystallized from a proper solvent.[22]

3-Amino-1-phenyl-1*H*-pyrrole-2,4-dicarbonitrile **4a**

White solid, in 88% yield, mp 204–206°C (EtOH) (lit.[21], 187–188°C). ^{1}H-NMR ($CDCl_3$) δ: 7.22 (s, 1H, H-C(5)), 4.29 (s, 2H, NH_2), 7.47–7.56 (m, 3H, Ar-H), 7.39–7.42 (m, 2H, Ar-H). IR (KBr): 2205, 2227 (CN), 3456, 3360 (NH_2) cm^{-1}. MS (EI)=208 (64%). *Anal.* Calcd for $C_{12}H_8N_4$ (208.22): C, 69.22; H, 3.87; N, 26.91. Found: C, 69.12; H, 4.07; N, 26.81.

3-Amino-1-(4-chlorophenyl)-1*H*-pyrrole-2,4-dicarbonitrile **4b**

Yellowish white solid, in 85% yield, mp 243–245°C (EtOH). ^{1}H-NMR ($CDCl_3$) δ: 7.19 (s, 1H, H-C(5)), 4.30 (s, 2H, NH_2), 7.51 (d, 2H, J=9.0 Hz, H-C(3′), H-C(5′)), 7.35 (d, 2H, J=9.0 Hz, H-C(2′), H-C(6′)). IR (KBr): 2232, 2200 (CN), 3468, 3363 (NH_2) cm^{-1}. MS (EI)=242 (77%). *Anal.* Calcd for $C_{12}H_7ClN_4$ (242.66): C, 59.39; H, 2.91; N, 23.09. Found: C, 59.33; H, 2.90; N, 23.11.

3-Amino-1-(4-methoxyphenyl)-1*H*-pyrrole-2,4-dicarbonitrile **4c**

Beige solid, in 91% yield, mp 186–188°C (EtOH). ^{1}H-NMR (DMSO-d_6) δ: 6.11 (s, 2H, NH_2), 3.80 (s, 3H, OCH_3), 7.84 (s, 1H, H-5), 7.07 (d, 2H, J=8.8 Hz, Ar-H, 3′, 5′), 7.41 (d, 2H, J=8.8 Hz, Ar-H, 2′, 6′); ^{13}C-NMR (DMSO-d_6) δ: 83.23 (C-4), 88.18 (C-2), 55.56 ($O\underline{C}H_3$), 113.38 (CN), 114.71 (C-3′,5′), 114.23 (CN), 125.44 (C-2′,6′), 130.12 (C-1′), 132.52 (C-5), 148.32 (C-3), 159.21 (C-4′). IR (KBr): ν=2227, 2205 (CN), 3437, 3349 (NH_2) cm^{-1}.

3-Amino-1-(4-fluorophenyl)-1*H*-pyrrole-2,4-dicarbonitrile **4d**

Yellowish white solid, in 82% yield, mp 186–188°C (EtOH). ^{1}H-NMR ($CDCl_3$) δ: 6.15 (s, 2H, NH_2), 7.32 (s, 1H, H-C(5)), 7.25–7.28 (d, 2H, Ar-H, J=9.0 Hz), 7.85–7.88 (d, 2H, Ar-H, J=9.0 Hz). IR (KBr): 3445, 3348 (NH_2), 2222 (CN) cm^{-1}. MS (EI)=226 (80%). *Anal.* Calcd for $C_{12}H_7FN_4$ (226.21): C, 63.71; H, 3.12; N, 24.77. Found: C, 63.60; H, 3.25; N, 24.66.

3-Amino-1-(naphthalen-1-yl)-1*H*-pyrrole-2,4-dicarbonitrile **4e**

Pink solid in 63% yield, mp 206–208°C (EtOH). ^{1}H-NMR (DMSO-d_6) δ: 6.20 (brs, 2H, NH_2), 7.30–7.40 (m, 1H, Ar-H), 7.62–7.68 (m, 3H, Ar-H), 7.97 (s, 1H, H-5), 8.10–8.18 (m, 3H, Ar-H). IR (KBr): 3410–3337 (NH_2), 2221 (CN) cm^{-1}. MS (EI)=258 (100%). *Anal.* Calcd for $C_{16}H_{10}N_4$ (258.28): C, 74.40; H, 3.90; N, 21.69. Found: C, 74.66; H, 3.85; N, 21.60.

Ethyl 3-Amino-4-cyano-1-(4-fluorophenyl)-1*H*-pyrrole-2-carboxylate **4f**

White solid in 74% yield, mp 128–130°C (EtOH). ^{1}H-NMR ($CDCl_3$) δ: 1.05 (t, 3H, J=7.2 Hz, CH_3), 4.11 (q, 2H, J=7.2 Hz, CH_2), 6.14 (s, 2H, NH_2), 7.38–7. 41 (d, 2H, Ar-H, J=9.0 Hz), 7.55–7.58 (d, 2H, Ar-H, J=9.0 Hz), 7.92 (s, 1H, H-5). IR (KBr): 3455, 3340 (NH_2), 2224 (CN), 1656 (CO) cm^{-1}. MS (EI)=273 (67%). *Anal.* Calcd for $C_{14}H_{12}FN_3O_2$ (273.26): C, 61.53; H, 4.43; N, 15.38. Found: C, 61.64; H, 4.31; N, 15.50.

4-Amino-5-(4-bromobenzoyl)-1-(4-fluorophenyl)-1*H*-pyrrole-3-carbonitrile **4g**

Pale yellow solid, in 80% yield, mp 220–222°C (EtOH). ^{1}H-NMR (DMSO-d_6) δ: 6.58 (s, 2H, NH_2), 7.41–7.44 (d, 2H, J=9 Hz, Ar-H), 7.57–7.60 (d, 2H, J=9 Hz, Ar-H), 7.65–7.68 (d, 2H, J=9 Hz, Ar-H), 8.05 (s, 1H, H-5), 7.92–7.95 (d, 2H, J=9 Hz, Ar-H); IR (KBr): 1678 (CO), 3423, 3318 (NH_2), 2220 (CN) cm^{-1}. MS (EI)=384 (15%), 383 (M^+, ^{79}Br, 95), 385 (M^+, ^{81}Br, 87); *Anal.* Calcd for $C_{18}H_{11}BrFN_3O$ (384.20): C, 56.27; H, 2.89; N, 10.94. Found: C, 56.45; H, 3.04; N, 11.15.

General Procedure to Prepare Analogues of Tacrine (*via* Friedländer Reaction) 5a–g, 6a and b

Thermal Method

A mixture of cyclopentanone or cyclohexanone (3.1 mmol), 2-substituted-3-aminopyrrole-4-carbonitrile **4** (0.3 mmol), and anhydrous $AlCl_3$ (3.1 mmol) in distilled 1,2-dichloroethane

(15 mL), was refluxed for 8–10 h controlled by TLC, then cooled to room temperature. After cooling a mixture of tetrahydrofurane and water (1 : 1, 20 mL) was added, then NaOH (aq. solution 10%) drop wise until the solution was basic. After stirring for 30 min, the mixture was extracted with dichloromethane (3×20 mL). The joint extracts were washed by saline (20 mL) and dehydrated over $MgSO_4$, then filtered, and evaporated to obtain a solid followed by purification by preparative layer chromatography (PLC) (CH_2Cl_2–MeOH, 9 : 1) or recrystallization from EtOH.

Microwave Irradiation Method

In a round bottomed flask of 100 mL equipped with a condenser we added a solution of 2-substituted-3-aminopyrrole-4-carbonitrile **4** (1 mmol) with either cyclopentanone or cyclohexanone (1.4 mmol) in 30 mL of distilled 1,2-dichloroethane. Anhydrous $AlCl_3$ (4 mmol) was then added, and the mixture was refluxed for 30 and 32 min, respectively, under microwave irradiation (at a constant power of 400 W). The mixture was then cooled to room temperature. After cooling a mixture of tetrahydrofurane and water (1 : 1, 20 mL) was added, followed by the drop wise addition of NaOH (aq. solution 10%) until the solution was basic. After this, the mixture was stirred for 30 min, and extracted with dichloromethane (3×20 mL). The combined extracts were washed with saline (20 mL), dried over $MgSO_4$, filtered, and the solvent evaporated to obtain a solid, which was identical in all respects with that obtained from the thermal method (mp, TLC, NMR).

8-Amino-1-phenyl-1,5,6,7-tetrahydrocyclopenta[*e*]pyrrolo-[3,2-*b*]pyridine-3-carbonitrile **5a**

Yellow solid, in 85% yeild, mp 242–244°C. ^{1}H-NMR (DMSO-d_6) δ: 2.71 (t, 2H, J=7.7 Hz, H-C(7)), 2.16–2.26 (m, 2H, H-C(6)), 4.85 (s, 2H, NH_2), 2.91 (t, 2H, J=7.8 Hz, H-C(5)), 7.55–7.66 (m, 5H, Ar-H), 8.28 (s, 1H, H-C(2)). ^{13}C-NMR (DMSO-d_6) δ: 22.82 (C(6)), 27.30 (C(7)), 86.75 (C(3)), 34.18 (C(5)), 115.46 (CN), 116.15 (C(8a)), 119.23 (C(3a)), 124.54 (C(4′)), 126.52 (C(2′), C(6′)), C(5′)), 129.97 (C(7a)), 129.61 (C(3′), 138.21 (C(1′)), 137.17 (C(2)), 145.76 (C(8)), 162.19 (C(4a)). IR (KBr): 2224 (CN), 3465, 3360 (NH_2) cm^{-1}. MS (EI)=274 (63%). *Anal.* Calcd for $C_{17}H_{14}N_4$ (274.32): C, 74.43; H, 5.14; N, 20.42. Found: C, 74.39; H, 5.10; N, 20.45.

9-Amino-1-phenyl-5,6,7,8-tetrahydro-1*H*-pyrrolo[3,2-*b*]-quinoline-3-carbonitrile **5b**

Yellowish white solid, in 80% yield, mp 223–224°C. ^{1}H-NMR (DMSO-d_6) δ: 2.40–2.52 (m, 2H, H-C(8)), 1.70–1.84 (m, 4H, H-C(6), H-C(7)), 2.74–2.86 (m, 2H, H-C(5)), 7.53–7.65 (m, 5H, Ar-H), 4.74 (s, 2H, NH_2), 8.29 (s, 1H, H-C(2)). ^{13}C-NMR (DMSO-d_6) δ: 115.75 (CN), 23.28 (C(8)), 22.61 (C(7)), 22.40 (C(6)), 33.06 (C(5)), 86.52 (C(3)), 110.56 (C(9a)), 118.48 (C(3a)), 124.49 (C(4′)), 129.64 (C(3′), 126.62 (C(2′), C(6′), C(5′)), 138.28 (C(2)), 138.69 (C(1′)), 143.49 (C(8a)), 145.93 (C(9)), 153.41 (C(4a)). IR (KBr): 2219 (CN), 3467, 3356 (NH_2) cm^{-1}. MS (EI)=288 (49%). *Anal.* Calcd for $C_{18}H_{16}N_4$ (288.35): C, 74.98; H, 5.59; N, 19.43. Found: C, 74.95; H, 5.56; N, 19.40.

8-Amino-1-(4-chlorophenyl)-1,5,6,7-tetrahydrocyclopenta[*e*]-pyrrolo[3,2-*b*]pyridine-3-carbonitrile **5c**

Yellow solid, in 78% yield, mp 304–306°C. ^{1}H-NMR (DMSO-d_6) δ: 2.14–2.24 (m, 2H, H-6), 2.81 (t, 2H, J=7.7 Hz, H-7), 3.11 (t, 2H, J=7.7 Hz, H-5), 6.75 (brs, 2H, NH_2), 7.59 (d, 2H, J=9.0 Hz, H-2′,6′), 7.71 (d, 2H, J=9.0 Hz, H-3′,5′), 8.64 (s, 1H, H-2). IR (KBr): 2226 (CN), 3446, 3325 (NH_2) cm^{-1}. MS (EI)=(^{35}Cl) 309 (88%), (^{37}Cl) 311 (30%). *Anal.* Calcd for $C_{17}H_{13}ClN_4$ (308.76): C, 66.13; H, 4.24; N, 18.15. Found: C, 66.10; H, 4.19; N, 17.96.

8-Amino-1-(4-methoxyphenyl)-1,5,6,7-tetrahydro-cyclopenta[*e*]pyrrolo[3,2-*b*]pyridine-3-carbonitrile **5d**

Yellow solid, in 86% yield, mp 220–222°C. ^{1}H-NMR ($CDCl_3$) δ: 2.74 (t, 2H, J=7.8 Hz, H-7), 2.15–2.23 (m, 2H, H-6), 3.01 (t, 2H, J=7.8 Hz, H-5), 3.90 (s, 3H, OCH_3), 3.78 (s, 2H, NH_2), 7.07 (d, 2H, J=9.2 Hz, H-3′,5′), 7.55 (s, 1H, H-2), 7.38 (d, 2H, J=9.2 Hz, H-2′,6′). ^{13}C-NMR ($CDCl_3$) δ: 114.70 (CN), 55.71 (OCH_3), 23.26 (C-6), 26.97 (C-7), 34.50 (C-5), 88.30 (C-3), 114.82 (C-3′,5′), 116.26 (C-7a), 117.29 (C-8a), 125.05 (C-3a), 128.05 (C-2′,6′), 131.14 (C-1′), 136.15 (C-2), 145.93 (C-8), 160.32 (C-4′), 163.49 (C-4a). IR (KBr): ν=2218 (CN), 3393, 3299 (NH_2) cm^{-1}. MS (EI)=304 (71%). *Anal.* Calcd for $C_{18}H_{16}N_4O$ (304.35): C, 71.04; H, 5.30; N, 18.41. Found: C, 71.09; H, 5.25; N, 18.30.

9-Amino-1-(4-methoxyphenyl)-5,6,7,8-tetrahydro-1*H*-pyrrolo[3,2-*b*]quinoline-3-carbonitrile **5e**

Yellow solid, in 72% yield, mp 213–215°C. ^{1}H-NMR (DMSO-d_6) δ: 6.34 (brs, 2H, NH_2), 7.53 (s, 1H, H-2), 1.85–1.88 (m, 4H, H-6,7), 2.43–2.47 (m, 2H, H-8), 2.98–3.02 (m, 2H, H-5), 7.03 (d, 2H, J=8.8 Hz, H-3′,5′), 7.38 (d, 2H, J=8.8 Hz, H-2′,6′). ^{13}C-NMR (DMSO-d_6) δ: 22.69 (C-6), 22.80 (C-7), 23.19 (C-8), 33.48 (C-5), 87.88 (C-3), 110.97 (C-9a), 114.78 (C-3′,5′), 114.84 (CN), 116.80 (C-3a), 128.08 (C-2′,6′), 131.02 (C-1′), 136.76 (C-2), 137.81 (C-7a), 143.53 (C-9), 154.70 (C-4a), 160.27 (C-4′). IR (KBr): 2223 (CN), 3485, 3360 (NH_2) cm^{-1}. MS (EI)=318 (68%). *Anal.* Calcd for $C_{19}H_{18}N_4O$ (318.37): C, 71.68; H, 5.70; N, 17.60. Found: C, 71.62; H, 5.79; N, 17.41.

9-Amino-1-(4-fluorophenyl)-5,6,7,8-tetrahydro-1*H*-pyrrolo[3,2-*b*]quinoline-3-carbonitrile **5f**

Pale yellow solid, in 78% yield, mp. 252–254°C. ^{1}H-NMR ($CDCl_3$) δ: 3.86 (s, 2H, NH_2), 1.85–1.92 (m, 4H, H-6,7), 2.49 (m, 2H, H-8), 3.03 (m, 2H, H-5), 7.42 (d, 2H, J=9.0 Hz, H-2′,6′), 7.57 (d, 2H, J=9.0 Hz, H-3′,5′), 7.54 (s, 1H, H-2). ^{13}C-NMR ($CDCl_3$) δ: 114.42 (CN), 23.29 (C-6), 22.70 (C-7), 26.81 (C-8), 33.59 (C-5), 89.41 (C-3), 111.45 (C-8a), 116.39 (C-9a), 127.79 (C-2′,6′), 130.02 (C-3′,5′), 135.49 (C-4′), 136.46 (C-3a), 136.92 (C-2), 137.49 (C-1′), 143.93 (C-9), 155.19 (C-4a). IR (KBr): 2229 (CN) 3412, 3335 (NH_2) cm^{-1}. MS (EI)=306 (58%). *Anal.* Calcd for $C_{18}H_{15}FN_4$ (306.34): C, 70.56; H, 4.95; N, 18.28. Found: C, 70.81; H, 4.84; N, 18.35.

4-Amino-1-(naphthalen-1-yl)-1,5,6,7-tetrahydrocyclopenta-[*e*]pyrrolo[2,3-*b*]pyridine-3-carbonitrile **5g**

Dark pink solid, in 79% yield. mp 289–292°C. ^{1}H-NMR (DMSO-d_6) δ: 2.14–2.25 (m, 2H, H-6), 2.73 (t, 2H, J=7.7 Hz, H-7), 2.99 (t, 2H, J=7.8 Hz, H-5), 4.88 (s, 2H, NH_2), 7.49–7.54 (m, 4H, Ar-H), 8.25 (s, 1H, H-2). ^{13}C-NMR (DMSO-d_6) δ: 22.42 (C-6), 27.98 (C-7), 34.59 (C-5), 86.45 (C-3), 116.436 (CN), 117.95 (C-8a), 120.83 (C-3a), 123.54, 124.27, 125.15, 126.17, 126.99, 127.58, 128.65, 129.23, 133.15 (Ar-C), 130.84 (C-7a), 135.14 (C-2), 136.48 (C-1′), 149.66 (C(8)), 162.19 (C(4a)). IR (KBr): 3458, 3365 (NH_2), 2219 (CN) cm^{-1}. MS (EI)=324 (88%). *Anal.* Calcd for $C_{21}H_{16}N_4$ (324.36): C, 77.75; H, 4.98; N, 17.26. Found: C, 77.89; H, 5.12; N, 17.35.

Ethyl 8-Amino-2-(4-fluorophenyl)-2,5,6,7-tetrahydrocyclo-penta[*e*]pyrrolo[3,4-*b*]pyridine-3-carboxylate **6a**

Yellow solid, in 76% yield, mp 208–210°C. ^{1}H-NMR ($CDCl_3$) δ: 1.14 (t, 3H, J=7.5 Hz, CH_3), 2.04–2.14 (m, 2H,

H-6), 2.78 (t, 2H, *J*=8.0 Hz, H-7), 3.03 (t, 2H, *J*=8.1 Hz, H-5), 4.21 (q, 2H, *J*=7.5 Hz, CH_2), 6.31 (s, 2H, NH_2), 7.12 (d, 2H, *J*=9.0 Hz, H-3′,5′), 7.40 (d, 2H, *J*=9.0 Hz, H-2′,6′), 7.83 (s, 1H, H-1). ^{13}C-NMR ($CDCl_3$) δ: 14.25 (CH_3), 22.71 (C-6), 26.94 (C-7), 34.35 (C-5), 60.12 (CH_2), 109.59 (C-8a), 110.04 (C-3a), 111.48 (C-7a), 113.61 (C-3′,5′), 124.47 (C-1), 125.15 (C-2′,6′), 133.39 (C-1′), 138.39 (C-3), 145.94 (C-8), 159.44 (C-4′), 161.48 (CO), 165.68 (C-4a). IR (KBr): 3477, 3339 (NH_2), 1712 (CO) cm^{-1}. MS (EI)=351 (81%). *Anal.* Calcd for $C_{19}H_{18}FN_3O_2$ (351.40): C, 67.24; H, 5.35; N, 12.38. Found: C, 67.41; H, 5.45; N, 12.29.

(9-Amino-2-(4-fluorophenyl)-5,6,7,8-tetrahydro-2*H*-pyrrolo[3,4-*b*]quinolin-3-yl)(4-bromophenyl)methanone **6b**

Pale yellow solid, in 87% yield, mp 263–265°C. ^{1}H-NMR (DMSO-d_6) δ: 1.77–1.82 (m, 4H, H-6,7), 2.44 (m, 2H, H-8), 2.81 (m, 2H, H-5), 6.42 (s, 2H, NH_2), 7.31–7.35 (d, 2H, Ar-H, H-3′,5′), 7.41–7.46 (d, 2H, Ar-H, H-2″,6″), 7.52–7.53 (d, 2H, Ar-H, H-2′,6′), 7.63–7.66 (d, 2H, Ar-H, H-3″,5″), 7.85 (s, 1H, H-1). ^{13}C-NMR (DMSO-d_6) δ: 22.67 (C-7), 22.91 (C-6), 26.17 (C-8), 34.59 (C-5), 104.63 (C-8a), 112.97 (C-9a), 119.47 (C-3), 122.37 (C-1), 125.78 (C-2′,6′), 128.53 (C-3′,5′), 129.30 (C-4″), 130.25 (C-3″,5″), 131.30 (C-2″,6″), 131.42 (C-3a), 141.66 (C-1′), 145.81 (C-9), 157.41 (C-4a), 159.92 (C-4′), 175.56 (CO). IR (KBr): 3458, 3342 (NH_2), 1657 (CO) cm^{-1}. MS (EI)=463 (M^+, ^{79}Br, 64%), 465 (M^+, ^{81}Br, 61%). *Anal.* Calcd for $C_{24}H_{19}BrFN_3O$ (464.33): C, 62.08; H, 4.12; N, 9.05. Found: C, 62.15; H, 4.22; N, 9.27.

5-Amino-4-cyano-1-substituted Pyrazoles 8a–d Were Previously Prepared by Us[22] Preparation of Pyrazolotacrine 9a–e

1-Phenyl-5,6,7,8-tetrahydro-1*H*-pyrazolo[3,4-*b*]quinolin-4-amine **9a**

A yellowish white solid, in 62% yield, mp 195–197°C. δ_H (DMSO-d_6): 6.68 (brs, 2H, NH_2), 8.40–8.29 (m, 2H, H-2′,6′), 7.47 (t, 2H, *J*=7.2 Hz, H-3′,5′), 8.33 (s, 3H, H-3), 7.21 (t, 1H, *J*=7.5 Hz, H-4′), 2.85–2.74 (m, 2H, H-8), 2.52–2.40 (m, 2H, H-5), 1.85–1.71 (m, 4H, H-7, H-6); δ_C (DMSO-d_6): 22.72 and 22.53 (C-7, C-6), 22.92 (C-5), 33.78 (C-8), 105.05 (C-3a), 106.83 (C-4a), 119.45 (C-2′,6′), 124.53 (C-4′), 128.84 (C-3′,5′), 132.94 (C-3), 140.28 (C-1′), 146.99 (C-9a), 150.07 (C-4), 157.05 (C-8a). IR (KBr): 3415, 3330 (NH_2) cm^{-1}. MS (EI)=264(44%). *Anal.* Calcd for $C_{16}H_{16}N_4$: C, 72.70; H, 6.10; N, 21.20. Found: C, 72.97; H, 6.09; N, 20.93.

1-(4-Chlorophenyl)-5,6,7,8-tetrahydro-1*H*-pyrazolo[3,4-*b*]-quinolin-4-amine **9b**

Yellowish white solid, in 69% yield, mp 151–152°C; δ_H (DMSO-d_6): 6.72 (brs, 2H, NH_2), 7.52 (d, 2H, *J*=7.0 Hz, H-3′,5′),8.41 (d, 2H, *J*=7.0 Hz, H-2′,6′), 8.34 (s, 1H, H-3), 2.52–2.42 (m, 2H, H-5), 2.85–2.74 (m, 2H, H-8), 1.86–1.70 (m, 4H, H-7, H-6). δ_C (DMSO-d_6): 150.09 (C-4), 157.15 (C-8a), 147.11 (C-9a), 133.45 (C-3), 139.12 (C-1′), 128.79 (C-3′,5′), 128.34 (C-4′), 120.61 (C-2′,6′), 105.05 (C-3a), 107.08 (C-4a), 33.73 (C-8), 22.89 (C-5), 22.65 and 22.45 (C-7, C-6). MS (EI)=297 (44%), 300 (M^+, ^{37}Cl, 23), 299 (19%), 298 (M^+, ^{35}Cl, 100%). *Anal.* Calcd for $C_{16}H_{15}N_4Cl$: C, 64.32; H, 5.06; N, 18.75. Found: C, 63.89; H, 4.97; N, 18.54.

1-(4-Methoxyphenyl)-5,6,7,8-tetrahydro-1*H*-pyrazolo[3,4-*b*]-quinolin-4-amine **9c**

Beige solid, in 45% yield, mp 107–108°C. δ_H (400 MHz, $CDCl_3$): 4.61 (brs, 2H, NH_2), 8.12 (d, 2H, *J*=7.0 Hz, H-2′,6′), 7.97 (s, 1H, H-3), 7.01 (d, 2H, *J*=6.8 Hz, H-3′,5′), 2.98 (t, 2H, *J*=6.0 Hz, H-8), 2.54 (t, 2H, *J*=6.0 Hz, H-5), 2.0–1.80 (m, 4H, H-7, H-6); δ_C ($CDCl_3$): 55.51 (OMe), 158.51 (C-8a), 157.40 (C-4′), 149.76 (C-4), 144.85 (C-9a), 133.46 (C-1′), 129.78 (C-3), 122.72 (C-2′,6′), 114.12 (C-3′,5′), 105.23 (C-3a), 107.40 (C-4a), 34.16 (C-8), 22.93 (C-5), 22.88 and 22.87 (C-7, C-6); MS (EI)=294 (93%). *Anal.* Calcd for $C_{17}H_{18}N_4O$; (294.15): C, 68.37; H, 6.20; N, 18.54. Found: C, 68.38; H, 6.26; N, 18.58.

1-(4-Trifluoromethylphenyl)-1,5,6,7-tetrahydrocyclopenta[*e*]-pyrazolo[3,4-*b*]pyridin-4-amine **9d**

A brown solid, in 66% yield, mp 266–268°C. δ_H (DMSO-d_6): 6.84 (brs, 2H, NH_2), 8.37 (s, 1H, H-C3), 8.62 (d, 2H, *J*=8.7 Hz, H-2′,6′), 7.85 (d, 2H, *J*=8.4 Hz, H-3′,5′), 2.90 (t, 2H, *J*=7.5 Hz, H-7), 2.07 (m, 2H, H-6), 2.73 (t, 2H, *J*=7.5 Hz, H-5); δ_C (DMSO-d_6): 166.33 (C-7a), 152.89 (C-8a), 145.01 (C-4), 143.21 (C-1′), 134.41 (C-3), 126.18 (C-3′,5′), 124.50 (q, ^2J=31.8 Hz, C-4′), 122.58, 126.18, 129.89 (the other peak of the q is hidden under C2′, C-6′, ^1J=274.5 Hz, CF_3), 119.19 (C-2′,6′), 112.05 (C-4a), 105.82 (C-3a), 34.57 (C-7), 26.70 (C-5), 22.52 (C-6); MS (EI)=318 (55)%. *Anal.* Cald for $C_{16}H_{13}F_3N_4$. (318.11): C, 59.65; H, 4.20; N, 17.17. Found: C, 59.42; H, 4.54; N, 16.98.

1-(4-Trifluoromethylphenyl)-5,6,7,8-tetrahydro-1*H*-pyrazolo[3,4-*b*]quinolin-4-amine **9e**

Light brown solid, in 56% yield, mp 115–118°C; δ_H (DMSO-d_6): 6.78 (brs, 2H, NH_2), 8.65 (d, 2H, *J*=8.4 Hz, H-2′,6′), 8.40 (s, 3H, H-3), 7.85 (d, 2H, *J*=8.7 Hz, H-3′,5′), 2.52–2.42 (m, 2H, H-5), 2.88–2.78 (m, 2H, H-8), 1.86–1.74 (m, 4H, H-7, H-6); δ_C (DMSO-d_6): 157.35 (C-8a), 150.55 (C-4), 147.28 (C-9a), 143.30 (C1′), 134.45 (C-3), 126.21 (C-3′,5′), 122.61, 126.17, 129.80 (the other peak of the q is hidden under C-2′, C-6′, ^1J=272.9 Hz, CF_3), 124.32 (q, ^2J=31.9 Hz, C-4′), 118.92 (C-2′,6′), 107.59 (C-4a), 105.21 (C-3a), 33.75 (C-8), 22.91 (C-6 or C-7), 22.63 (C-5), 22.43 (C-6 or C-7). MS (EI)=332 (45%). *Anal.* Calcd for $C_{17}H_{15}F_3N_4$: C, 61.44; H, 4.51; N 16.86. Found C, 61.02; H, 5.21; N, 16.57.

Preparation of 2-(2-(4-Bromophenyl)-2-oxoethyl)malononitrile 12 To a solution of 4-bromophenacyl bromide **11** (0.01 mol) the malononitrile (0.01 mol) and KOH 10% (5 mL) were added with cooling. The reaction mixture was stirred for 30 min, after cooling ice-water (50 mL) was added, the solid product was filtered off washed thoroughly with cold water and crystallized from ethanol. Pale white solid, in 80% yield, mp 116–118°C (EtOH). ^{1}H-NMR ($CDCl_3$) δ: 3.61–3.65 (d, 2H,CH_2), 4.10–4.14 (t, 1H, CH), 7.61–7.64 (d, 2H, Ar-H, *J*=9.0 Hz), 8.10–8.13 (d, 2H, Ar-H, *J*=9.0 Hz). IR (KBr): 2216, 2224 (CN), 1630 (CO) cm^{-1}. MS (EI)=263 (80%). *Anal.* Calcd for $C_{11}H_7BrN_2O$ (263.09): C, 50.21; H, 2.68; N, 10.65. Found: C, 50.32; H, 2.89; N,10.70.

To a Preparation of 2-Amino-5-(4-bromophenyl)furan-3-carbonitrile 13 The intermediate **12** (0.01 mol) in ethanol (40 mL) and TEA (2 mL) was added. The reaction mixture was refluxed for 2 h. After cooling overnight, the solid product was filtered off, washed thoroughly with cold ethanol–water and crystallized from ethanol. Brown solid, in 67% yield, mp 148–150°C (EtOH). ^{1}H-NMR ($CDCl_3$) δ: 5.15 (brs, 2H, NH_2), 7.31–7.34 (d, 2H, Ar-H, *J*=9.0 Hz), 7.45 (s, 1H, H-4), 8.01–8.04 (d, 2H, Ar-H, *J*=9.0 Hz). IR (KBr): 3340–3180 (NH_2), 2210 (CN) cm^{-1}. MS (EI)=263 (65%). *Anal.* Calcd for $C_{11}H_7BrN_2O$ (263.09): C, 50.22; H, 2.68; N, 10.65. Found: C, 50.29; H, 2.62; N, 10.72.

2-(4-Bromophenyl)-6,7-dihydro-5*H*-cyclopenta[*e*]furo-

[2,3-*b*]pyridin-4-amine 14 Light brown solid, in 70% yield, mp 283–285°C. ^{1}H-NMR (DMSO-d_6) δ: 2.05–2.14 (m, 2H, H-6), 2.75 (t, 2H, J=8.1 Hz, H-7), 3.05 (t, 2H, J=8.0 Hz, H-5), 6.14 (br s, 2H, NH_2), 6.95 (s, 1H, H-3), 7.62–7.65 (d, 2H, J=9.2 Hz, H-3′,5′), 8.14–8.17 (d, 2H, J=9.2 Hz, H-2′,6′). IR (KBr): 3465, 3358 (NH_2) cm^{-1}. MS (EI)=328 (M^+, ^{79}Br, 91%), 330 (M^+, ^{81}Br, 85%). *Anal.* Calcd for $C_{16}H_{13}BrN_2O$ (329.19): C, 58.38; H, 3.98; Br, 24.27; N, 8.51. Found: C, 58.52; H, 4.14; Br, 24.35; N, 8.64.

Anti-Alzheimer's Activity of Various Extracts of *Taonia* (*T.*) *atomaria* Using a Cholinesterase Inhibitory Assay The inhibition of cholinesterase various extracts of *T. atomaria* was assessed by a colorimetric method performed in flat-bottom 96-well microtitre plates. A typical study involved the use of 5 μL of AChE solution, at a final assay concentration of 0.08 U/mL; 200 μL of 0.1 M phosphate buffer pH 8; 5 μL of 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) at a final concentration of 0.5 mM prepared in 0.1 M phosphate buffer pH 7 containing 0.12 M of sodium bicarbonate; and 5 μL of the test extract. The reactants were mixed and pre-incubated for 15 min at 30°C. The reaction was initiated by adding 5 μL of acetylthiocholine iodide (ATCI) at a final concentration of 0.5 mM. As a control the inhibitor solution was replaced with buffer. The change in absorbance at 412 nm was measured on a spectrophotometer (Ellman *et al.*).[29] Donepezil was used as a standard. Statistical analysis was carried out using an SPSS computer program, (one way ANOVA), coupled with a co-state computer program, where the unshared letter is significant at $p \leq 0.01$.

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Conflict of Interest The authors declare no conflict of interest.

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