

Article

Synthesis and Biological Evaluation of New Pyrrolotacrines for the Treatment of Alzheimer's Disease

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Abstract: An efficient routes to pyrroles by the reaction of versatile enamionitriles and aniline derivatives. Different pyrrole derivatives have been synthesized by Thorpe-Ziegler cyclization followed by its transformation into pyrrolotacrine analogues by Friedländer-type of reaction by classical heating and by microwave irradiation. The synthesized tacrine analogues were evaluated for their ability to inhibit acetylcholinesterase, using Ellman's test, are reported.

Keywords: Enaminones, Tacrine, Friedländer reaction, Alzheimer disease, Acetylcholinesterase

1. Introduction

Alzheimer disease (AD) is a speedily growing clinical and public health problem, mostly due to the increasing numbers of elderly. The disease is characterized by neuronal loss, synaptic damage, vascular plaques and a deficit in neurotransmitter acetylcholine that leads to a progressive impairment in memory cognitive functions and behavioral disturbances. Unfortunately, the incidence of AD is now rising in both sexes and more over exponentially with age. Until now, the perfect scientific description of the origin of this neurodegenerative disease is not clearly defined. Despite the impressive amount of progress in understanding the molecular mechanisms behind AD, an effective treatment is still not

available. In order to increase the acetylcholine (ACh) level in the synapse, the inhibition of acetylcholinesterase (AChE) represents the currently employed approach for the treatment of AD (Watkins et al, 1994; Valenti et al, 1997; Goedert, and Spillantini, 2006).

Up till now, four AChE inhibitor (AChEI) drugs have been approved by the U.S. Food and Drug Administration (FDA) for the treatment of AD: tacrine, donepezil, rivastigmine, and galantamine, although none of them proved to be effective against the progression of AD (Zemek et al, 2014) (Fig. 1). Tacrine, the first AChEI introduced in therapy, sold under the name of (Cognex®) since 1993 but the poor selectivity of this drug for AChE resulted in side effects, is now rarely used because of its hepatotoxicity (in ~50% of patients), which does not occur with the other three (Chaki et al, 1995). In spite of this, studies with tacrine analogues have continued (Fernndez-Bachiller et al, 2012) in the search for more potent and safer tacrine derivatives.

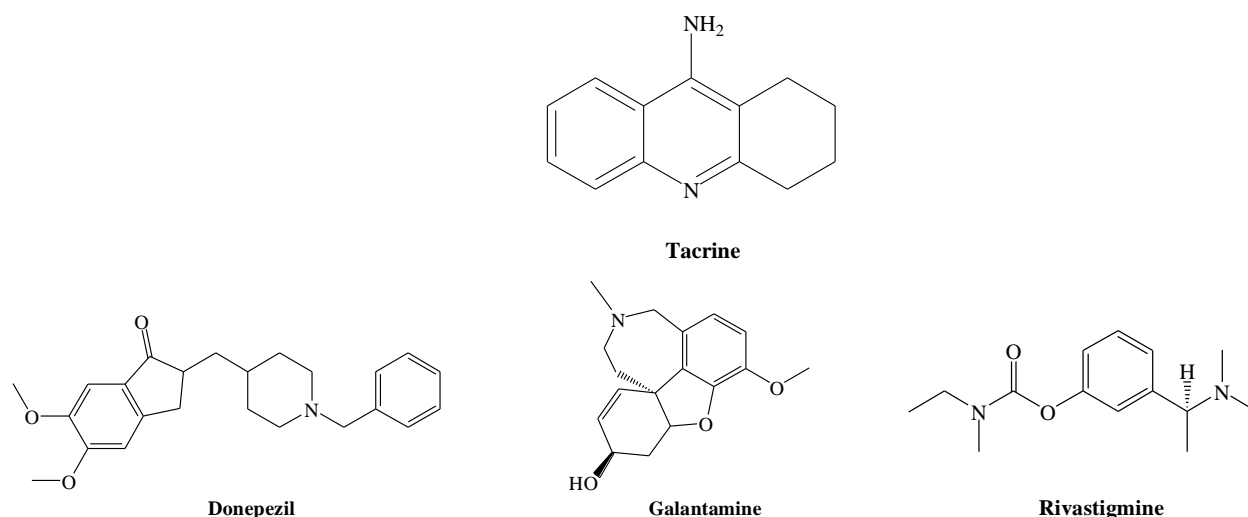


Fig. 1: Structures of AChEIs

Modifications on the tacrine structure have been performed, either by increasing the number of rings or changing their size or introducing heteroatoms (Martinez-Grau, and Marco, 1997; Marco-Contelles et al, 2006; Thomae et al, 2007; Bekolo, and Kirsch, 2007; Thomae et al, 2008; Michalson et al, 1999; Marco et al, 2004; Barreiro et al, 2003; Recanatini et al, 2000). Spanish group has been found that compounds containing four rings were less active than tacrine against AChE, however they were more selective and have shown potential activity against β -amyloid protein accumulation in the brain (Martinez-Grau, and Marco, 1997).

Tacrine analogues containing a furan ring have been known for some time [8] and recently, Kirsch *et al.* synthesized analogues of tacrine and velnacrine containing thiophene, thiazole, 4-azaisoindole, or selenophene heterocycles (Thomae et al, 2007; Bekolo, and Kirsch, 2007; Thomae et al, 2008). An analogue of tacrine was also described, in which the primary amino group was replaced by the azetidene

moiety (Michalson et al, 1999). A Spanish-Portuguese group has described the synthesis of tacrine analogues containing heterocyclic rings, such as pyridine, pyran and oxazole, and their inhibitory effects on AChE and butyrylcholinesterase (Marco et al, 2004). Other families of tacrine analogues, containing pyrazolopyridine or pyrazolonaphthyridine systems as isosteres of the quinoline ring of tacrine, has been described by Barreiro *et al* (Barreiro et al, 2003). They concluded that these compounds were, in their class, the most potent inhibitors of AChE. Some authors report that, the presence of halogen atoms in tacrine derivatives improves the activity anti-AChE (Recanatini et al, 2000).

We report here the synthesis and biological evaluation of pyrrolotacrine analogues containing halogens, (Scheme 1). We design to synthesis new pyrrolotacrine analogues, from heterocyclic system containing enamionitrile moiety, by reaction of an *ortho*-aminonitrile with a cyclic ketone in the presence of a Lewis acid according to Friedländer Reaction (Rodrigues et al, 2008).

2. Materials and Methods

2.1. General Part

All melting points were measured on a Gallenkamp melting point apparatus. The infrared spectra were recorded in potassium bromide discs on a Pye Unicam SP 3–300 and Shimadzu FT IR 8101 PC infrared spectrophotometers. The NMR spectra were recorded on a Varian Mercury VXR-300 NMR spectrometer (^1H NMR (300 MHz) and ^{13}C NMR (75.46 MHz)) were run in deuterated chloroform (CDCl_3) or dimethyl sulfoxide ($\text{DMSO}-d_6$), using TMS as an internal reference, and results are expressed as β -values. Double resonance, HMQC and HMBC experiments were carried out for complete assignment of ^1H and ^{13}C in the NMR spectra, whenever possible. Mass spectra were recorded on a Shimadzu GCMS-QP-2010 plus mass spectrometer at 70 eV. A *CEM MARS* oven was used for *Friedländer's* reaction under microwave irradiation. Elemental analyses were carried out at the Micro-analytical Centre of Cairo University, Giza, Egypt and recorded on Elementar-Vario EL automatic analyzer. Biological evaluation were carried out at the Animal Reproduction Research Institute (ARRI), Ministry of Agriculture, Dokki, Cairo Egypt. Compounds **3** were prepared according to the literature (Alshareef et al, 2017; Gewald et al, 1992).

General procedure for 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) the ethoxymethylene malononitrile (EMMN) **1** (0.1 mol) was added slowly (half the quantity), when the solution start to boil added the other half of the EMMN and stir for 30 min. leave to cool, the solid product was filtered off, washed thoroughly with ethanol. Compound 3a-c was prepared according to the literature (Alshareef et al, 2017; Gewald et al, 1992).

2-[(4-Fluorophenylamino)methylene]malononitrile (3d):

Yield 84%; yellow solid; m.p. 250-252°C (EtOH-DMF). IR (KBr): 3301 (NH), 2216 (CN). ¹H-NMR (DMSO-d₆): 6.91-6.94 (d, 2H, Ar-H, J = 9.0 Hz), 7.30-7.33 (d, 2H, Ar-H, J = 9.0 Hz), 8.37 (s, 1H), 11.02 (b, s, 1H, NH). MS (EI) = 187 (84%). Anal. calc. for C₁₀H₆FN₃ (187.17): C 64.17, H 3.23, N 22.45; found: C 64.35, H 3.48, N 22.40.

2-[(4-nitrophenylamino)methylene]malononitrile (3e):

Yield 89%; yellow solid; m.p. 285-287°C (EtOH-DMF). IR (KBr): 3204 (NH), 2206 (CN). ¹H-NMR (DMSO-d₆): 6.57-6.60 (d, 2H, Ar-H, J = 9.0 Hz), 8.22-8.25 (d, 2H, Ar-H, J = 9.0 Hz), 8.67 (s, 1H), 11.46 (b, s, 1H, NH). MS (EI) = 214 (64%). Anal. calc. for C₁₀H₆N₄O₂ (214.18): C, 56.08; H, 2.82; N, 26.16; found: C 56.24, H 2.69, N 25.94.

General procedure for 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) (0.011 mol) and Et₃N (4 ml) were added with external cooling. The reaction mixture was refluxed for 15-30 minutes, after cooling, water (50 mL) was added, and the solid product was filtered off, washed thoroughly with cold water and crystallized from ethanol (Salaheldin et al, 2008).

3-Amino-1-phenyl-1H-pyrrole-2,4-dicarbonitrile (4a).

Yield 88%; white solid; m.p. 204-206 °C (EtOH) ([21]: 187-188 °C). IR (KBr): 3456, 3360 (NH₂), 2205, 2227 (CN). ¹H-NMR (CDCl₃): 4.29 (s, 2H, NH₂), 7.22 (s, 1H, H-C(5)), 7.39-7.42 (m, 2H, Ar-H), 7.47-7.56 (m, 3H, Ar-H). Anal. calc. for C₁₂H₈N₄ (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)-1H-pyrrole-2,4-dicarbonitrile (4b).

Yield 84%; yellowish white solid; m.p. 243-245 °C (EtOH). IR (KBr): 3468, 3363 (NH₂), 2232, 2200 (CN). ¹H-NMR (CDCl₃): 4.30 (s, 2H, NH₂), 7.19 (s, 1H, H-C(5)), 7.35 (d, 2H, J = 9.0, H-C(2')), H-C(6')), 7.51 (d, 2H, J = 9.0 Hz, H-C(3')), H-C(5')). Anal. calc. for C₁₂H₇ClN₄ (242.66): C 59.39, H 2.91, N 23.09; found: C 59.32, H 2.88, N 23.10.

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

Yield 91%; beige solid, m.p. 186-188 °C (EtOH) [Lit. (Salaheldin et al, 2008) m.p. 187-188 °C]; IR (KBr): ν = 3437, 3349 (NH₂), 2227, 2205 (CN) cm⁻¹; ¹H NMR (DMSO-d₆): δ = 3.80 (s, 3H, OCH₃), 6.11 (s, 2H, NH₂), 7.07 (d, 2H, J = 8.8 Hz, Ar-H, 3', 5'), 7.41 (d, 2H, J = 8.8 Hz, Ar-H, 2', 6'), 7.84 (s, 1H, H-5); ¹³C NMR (DMSO-d₆): δ = 55.56 (OCH₃), 83.23 (C-4), 88.18 (C-2), 113.38 (CN), 114.23 (CN),

114.71 (C-3',5'), 125.44 (C-2',6'), 130.12 (C-1'), 132.52 (C-5), 148.32 (C-3), 159.21 (C-4').

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

Yield 82%; yellowish white solid; m.p. 186-188 °C (EtOH) IR (KBr): 3445, 3348 (NH₂), 2222 (CN) cm⁻¹; ¹H-NMR (CDCl₃): 6.15 (s, 2H, NH₂), 7.25-7.28 (d, 2H, Ar-H, J = 9.0 Hz), 7.32 (s, 1H, H-C(5)), 7.85-7.88 (d, 2H, Ar-H, J = 9.0 Hz). MS (EI) = 226 (80%). Anal. calc. for C₁₂H₇FN₄ (226.21): C 63.71, H 3.12, N 24.77; found: C 63.60, H 3.25, N 24.66.

Ethyl 3-amino-4-cyano-1-(4-fluorophenyl)-1H-pyrrole-2-carboxylate (4e)

Yield 74%; white solid; m.p. 128-130 °C (EtOH). IR (KBr): 3455, 3340 (NH₂), 2224 (CN), 1656 (CO) cm⁻¹. ¹H-NMR (CDCl₃): 1.05 (t, 3H, J = 7.2, CH₃); 4.11 (q, 2H, J = 7.2, CH₂); 6.14 (s, 2H, NH₂); 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). MS (EI) = 273 (67%); Anal. calc. for C₁₄H₁₂FN₃O₂ (273.26): C, 61.53; H, 4.43; N, 15.38; found: C 61.64, H 4.31, N 15.50.

Ethyl 3-amino-4-cyano-1-(4-nitrophenyl)-1H-pyrrole-2-carboxylate (4f)

Yield 82%; pale yellow solid; m.p. 185-187 °C (EtOH). IR (KBr): 3446, 3347 (NH₂), 2220 (CN), 1668 (CO), 1618, 1557 (NO₂). ¹H-NMR (CDCl₃): 1.38 (t, 3H, J = 7.2, CH₃); 4.17 (q, 2H, J = 7.2, CH₂); 5.14 (s, 2H, NH₂); 7.12 (s, 1H, H-5); 7.43-7.46 (d, 2H, Ar-H, J = 9.0 Hz), 8.30-8.33 (d, 2H, Ar-H, J = 9.0 Hz). MS (EI) = 300 (77%); Anal. calc. for C₁₄H₁₂N₄O₄ (300.27): C, 56.00; H, 4.03; N, 18.66; found: C 56.14, H 4.21, N 18.50.

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

Yield 80%; pale yellow solid, m.p. 220-222 °C (EtOH); IR (KBr): 3423, 3318 (NH₂), 2220 (CN), 1678 (CO) cm⁻¹; ¹H NMR (DMSO-d₆): δ = 6.58 (s, 2H, NH₂), 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), 7.92-7.95 (d, 2H, J = 9 Hz, Ar-H), 8.05 (s, 1H, H-5); MS (EI) = 384 (15), 383 (M⁺, 79Br, 95), 385 (M⁺, 81Br, 87); Anal. calc. for C₁₈H₁₁BrFN₃O (384.20): C, 56.27; H, 2.89; N, 10.94; found: C 56.45, H 3.04, N 11.15.

2.2. Friedländer Reaction

General procedure for the preparation of tacrine analogues 5a-h, 6a and 6b.

a) *By thermal heating.* A mixture of 2-substituted-3-aminopyrrole-4-carbonitrile (**4**) (0.3 mmol), cyclohexanone or cyclopentanone (3.1 mmol) and AlCl₃ (anhyd. 3.1 mmol) in distilled 1,2-dichloroethane (20 ml), was heated to reflux for 7-10 h (TLC control). After cooling to r.t., a mixture of THF/H₂O (1:1, 25 ml) was added, and then an aq. soln. of NaOH (10%) was added dropwise until basic. After stirring for 30 min, the mixture was extracted with CH₂Cl₂ (3 x 20 ml) and

the combined extracts were washed with brine (20 ml) and dried (MgSO₄), filtered, and the solvent was evaporated to give a solid, which was purified by PLC (CH₂Cl₂/MeOH, 9:1) or crystallized from EtOH.

b) *Under Microwave irradiation.* In a round bottom flask of 100 ml equipped with a condenser, cyclohexanone or cyclopentanone (1.4 mmol) was added to a soln. of 2-substituted-3-aminopyrrole-4-carbonitrile **4** (1 mmol) in 40 ml of distilled 1,2-dichloroethane. AlCl₃ (4 mmol) was added and the mixture was heated at reflux during 30 and 32 min under microwave irradiation (at a constant power of 400 W). After cooling to r.t., a mixture of THF/H₂O (1:1, 25 ml) was added, and then an aq. soln. of NaOH (10%) was added dropwise until basic. After stirring for 30 min, the mixture was extracted with CH₂Cl₂ (3 x 20 ml) and the combined extracts were washed with brine (20 mL) and dried (MgSO₄), filtered, and the solvent was evaporated to give a solid, which was identical in all respects with that obtained from the above reaction (TLC, m.p., NMR).

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

Yield 85%; yellow solid; m.p. 242-244 °C. IR (KBr): 3465, 3360 (NH₂), 2224 (CN). ¹H-NMR (DMSO-d₆): 2.16-2.26 (*m*, 2H, H-C(6)), 2.71 (*t*, 2H, *J* = 7.7, H-C(7)); 2.91 (*t*, 2H, *J* = 7.8, H-C(5)); 4.85 (*s*, 2H, NH₂); 7.55-7.66 (*m*, 5H, Ar-H); 8.28 (*s*, 1H, H-C(2)). ¹³C-NMR NMR (DMSO-d₆): 22.82 (C(6)); 27.30 (C(7)); 34.18 (C(5)); 86.75 (C(3)); 115.46 (CN); 116.15 (C(8a)); 119.23 (C(3a)); 124.54 (C(4`)); 126.52 (C(2`); C(6`)); 129.61 (C(3`), C(5`)); 129.97 (C(7a)); 137.17 (C(2)); 138.21 (C(1`)); 145.76 (C(8)); 162.19 (C(4a)). MS (EI) = 244 (63). Anal. calc. for C₁₇H₁₄N₄ (274.32): C 74.43, H 5.14, N 20.42; found: C 74.34, H 4.96, N 20.54.

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

Yield 79%; yellow solid; m.p. 222-224°C. IR (KBr): 3467, 3356 (NH₂), 2219 (CN). ¹H-NMR NMR (DMSO-d₆): 1.70-1.84 (*m*, 4H, H-C(6),H-C(7)); 2.40-2.52 (*m*, 2H, H-C(8)); 2.74-2.86 (*m*, 2H, H-C(5)); 4.74 (*s*, 2H, NH₂); 7.53-7.65 (*m*, 5H, Ar-H); 8.29 (*s*, 1H, H-C(2)). ¹³C-NMR NMR (DMSO-d₆): 22.40 (C(6)); 22.61 (C(7)); 23.28 (C(8)); 33.06 (C(5)); 86.52 (C(3)); 110.56 (C(9a)); 115.75 (CN); 118.48 (C(3a)); 124.49 (C(4`)); 126.62 (C(2`), C(6`)); 129.64 (C(3`), C(5`)); 138.28 (C(2)); 138.69 (C(1`)); 143.49 (C(8a)); 145.93 (C(9)); 153.41 (C(4a)). MS (EI) = 288 (49). Anal. calc. for C₁₈H₁₆N₄ (288.35): C 74.98, H 5.59, N 19.43; found: C 74.86, H 5.38, N 19.25.

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

Yield 78%; yellow solid, m.p. 304-306 °C; IR (KBr): 3446, 3325 (NH₂), 2226 (CN) cm⁻¹; ¹H NMR (DMSO-d₆): 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, NH₂), 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). MS (EI) = (³⁵Cl) 309 (88), (³⁷Cl) 311 (30). Anal. Calcd. for C₁₇H₁₃ClN₄ (308.76): C, 66.13; H, 4.24; N,

18.15. Found: C, 66.09; H, 4.20; N, 17.95.

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

Yield 62%; pale yellow solid, m.p. 260-262 °C; IR (KBr): 3412, 3335 (NH₂), 2229 (CN) cm⁻¹; ¹H NMR (CDCl₃): δ = 1.85-1.92 (m, 4H, H-6,7), 2.49 (m, 2H, H-8), 3.03 (m, 2H, H-5), 3.86 (s, 2H, NH₂), 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). ¹³C NMR (CDCl₃): δ = 22.70 (C-7), 23.29 (C-6), 26.81 (C-8), 33.59 (C-5), 89.41 (C-3), 111.45 (C-8a), 114.42 (CN), 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). MS (EI) = (³⁵Cl) 323. (65), (³⁷Cl) 325 (25). Anal. Calcd. for C₁₈H₁₅ClN₄ (322.79): C, 66.98; H, 4.68; N, 17.36. Found: C, 66.94; H, 4.72; N, 17.12.

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

Yield 86%; yellow solid, m.p. 221-222 °C; IR (KBr): 3393, 3299 (NH₂), 2218 (CN) cm⁻¹; ¹H NMR (CDCl₃): δ = 2.15-2.23 (m, 2H, H-6), 2.74 (t, 2H, *J* = 7.8 Hz, H-7), 3.01 (t, 2H, *J* = 7.8 Hz, H-5), 3.78 (s, 2H, NH₂), 3.90 (s, 3H, OCH₃), 7.07 (d, 2H, *J* = 9.2 Hz, H-3',5'), 7.38 (d, 2H, *J* = 9.2 Hz, H-2',6'), 7.55 (s, 1H, H-2). ¹³C NMR (CDCl₃): δ = 23.26 (C-6), 26.97 (C-7), 34.50 (C-5), 55.71 (OCH₃), 88.30 (C-3), 114.70 (CN), 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). MS (EI) = 304 (71). Anal. Calcd. for C₁₈H₁₆N₄O (304.35): C, 71.04; H, 5.30; N, 18.41. Found: C, 71.15; H, 4.94; N, 18.20.

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

Yield 72%; yellow solid; m.p. 214-215 °C; IR (KBr): 3485, 3360(NH₂), 2223 (CN) cm⁻¹; ¹H NMR (DMSO-d₆): δ = 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), 6.34 (brs, 2H, NH₂), 7.03 (d, 2H, *J* = 8.8 Hz, H-3',5'), 7.38 (d, 2H, *J* = 8.8 Hz, H-2',6'), 7.53 (s, 1H, H-2). ¹³C NMR (DMSO-d₆): 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'). MS (EI) = 318 (68). Anal. Calcd. for C₁₉H₁₈N₄O (318.37): C, 71.68; H, 5.70; N, 17.60. Found: C, 71.62; H, 5.79; N, 17.41.

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

Yield 81%; yellowish white solid; m.p. 288-290 °C (EtOH-DMF) IR (KBr): 3434, 3342 (NH₂), 2220 (CN) cm⁻¹; ¹H NMR (DMSO-d₆): δ = 2.02-2.07 (m, 2H, H-6), 2.69 (t, 2H, *J* = 7.8 Hz, H-7), 2.89 (t, 2H, *J* = 7.8 Hz, H-5), 6.14 (s, 2H, NH₂), , 7.40-7.43 (d, 2H, *J* = 8.7 Hz, H-2',6'), 7.57-7.59 (d, 2H, *J* = 8.7 Hz, H-3',5'), 7.90 (s, 1H, H-2),. MS (EI) = 292 (40%). Anal. calc. for C₁₇H₁₃FN₄ (292.11): C, 69.85; H, 4.48; N, 19.17; found: C 69.76, H 4.29, N 19.28.

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

Yield 78%; pale yellow solid, m.p. 252-254 °C; IR (KBr): 3412, 3335 (NH₂), 2229 (CN) cm⁻¹; ¹H NMR (CDCl₃): δ = 1.85-1.92 (m, 4H, H-6,7), 2.49 (m, 2H, H-8), 3.03 (m, 2H, H-5), 3.96 (s, 2H, NH₂), 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). ¹³C NMR (CDCl₃): δ = 22.70 (C-7), 23.29 (C-6), 26.81 (C-8), 33.59 (C-5), 89.41 (C-3), 111.45 (C-8a), 114.42 (CN), 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). MS (EI) = 306 (58). Anal. Calcd. for C₁₈H₁₅FN₄ (306.34): C, 70.57; H, 4.94; N, 18.29. Found: C, 70.81; H, 4.84; N, 18.35.

Ethyl 9-amino-2-(4-nitrophenyl)-5,6,7,8-tetrahydro-2H-pyrrolo[3,4-b]quinoline-3-carboxylate (6a)

Yield 75%; yellow solid, m.p. 210-212 °C; IR (KBr): 3475, 3332 (NH₂), 1714 (CO) cm⁻¹; ¹H NMR (CDCl₃): δ = 1.14 (t, 3H, *J* = 7.5 Hz, CH₃), 1.77-1.82 (m, 4H, H-6,7), 2.44 (m, 2H, H-8), 2.81 (m, 2H, H-5), 4.21 (q, 2H, *J* = 7.5 Hz, CH₂), 6.31 (s, 2H, NH₂), 7.43-7.46 (d, 2H, Ar-H, *J* = 9.0 Hz, H-2',6'), 8.30-8.33 (d, 2H, Ar-H, *J* = 9.0 Hz, H-3',5'), 7.83 (s, 1H, H-1). ¹³C NMR (CDCl₃): δ = 14.25 (CH₃), 22.67 (C-7), 22.91 (C-6), 26.17 (C-8), 34.59 (C-5), 60.12 (CH₂), 104.63 (C-8a), 110.04 (C-3a), 112.97 (C-9a), 122.37 (C-1), 122.78 (C-2',6'), 125.53 (C-3',5'), 138.39 (C-3), 143.66 (C-1'), 145.81 (C-4'), 158.92 (C-9), 161.48 (CO), 163.68 (C-4a). MS (EI) = 380 (77). Anal. Calcd. for C₂₀H₂₀N₄O₄ (380.40): C, 63.15; H, 5.30; N, 14.73;. Found: C, 63.25; H, 5.45; N, 14.59.

[8-Amino-2-(4-fluorophenyl)-2,5,6,7-tetrahydrocyclopenta[e]pyrrolo[3,4-b]pyridin-3-yl](4-bromophenyl)methanone (6b)

Yield 87%; pale yellow solid; m.p. 263-265 °C; IR (KBr): 3458, 3342 (NH₂), 1657 (CO) cm⁻¹; ¹H NMR (DMSO-d₆): δ = 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), 6.42 (s, 2H, NH₂), 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). ¹³C NMR (DMSO-d₆): δ = 22.71 (C-6), 26.94 (C-7), 34.35 (C-5), 112.97 (C-8a), 122.37 (C-3a), 125.78 (C-2',6'), 128.53 (C-3',5'), 129.30 (C-4"), 130.25 (C-3",5"), 131.30 (C-2",6"), (131.92 (C-1), 141.66 (C-1'), 155.81 (C-8), 157.41 (C-4a), 159.92 (C-4'), 175.56 (CO).

MS (EI) = 449 (M⁺, 79Br, 64), 451 (M⁺, 81Br, 61). Anal. Calcd. for C₂₃H₁₇BrFN₃O (450.30): C, 61.35; H, 3.81; N, 9.33;. Found: C, 61.15; H, 3.92; N, 9.27.

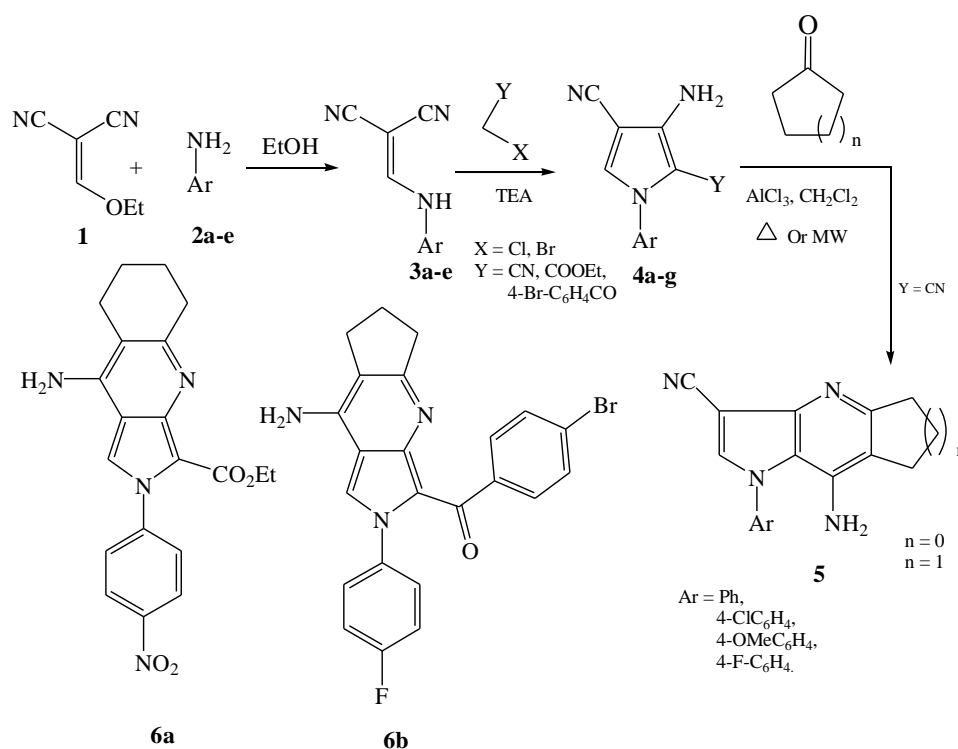
2.3. Inhibition of AChE Using Ellman's Method

To assess the inhibitory activity of the compounds towards acetylcholinesterase (AChE) we followed the Ellman's method using a solution of AChE and ATCI as a substrate. The cuvette was filled with 0.1 M phosphate buffer pH 7.4, 10 μL of Ellman's reagent (0.15 mM final concentration of DNTB in

0.1 M phosphate buffer pH 7.4) and 6 μ L of the compound solution in DMSO. After homogenization, 7 mL of a solution of AChE (final concentration 0.037 U/mL in 0.1 M phosphate buffer pH 7.4) were added and the enzymatic reaction was initiated by addition of 10 μ L of ACTI solution (final concentration 2 mM). The final assay volume was 1000 μ L. The cuvette was shaken for 5 s and the increase of absorbance at 412 nm was monitored at 37°C for 3 min. For controls, test compounds solutions were replaced by the corresponding volume of DMSO and buffer.

3. Results and Discussion

In continuance of our concentration in the chemistry of β , β -enaminonitriles, the outcomes aimed at discovering the potential utility of 3-anilino-2-cyanoacrylonitrile in the synthesis of heterocycles, are reported here. The β , β -enaminonitriles **3** which were synthesized by known methods [Mingos, and Baghurst, 1991) are converted into the corresponding 3-aminopyrrole derivatives **4** by reaction with chloroacetonitrile, ethylbromoacetate and 4-bromophenacyl bromide under basic conditions, a Thorpe-Ziegler cyclization (Scheme 1) [Alshareef et al, 2017; Mitteilung, and Wolfbeis 1981, Gewald et al, 1992, Salaheldin et al, 2007, 2008; Campos et al, 2007).



| | Ar | Y | | Ar | n |
|-----------|---|--------------------------------------|-----------|------------------------------------|---|
| 4a | C ₆ H ₅ | CN | 5a | C ₆ H ₅ | 0 |
| 4b | 4-ClC ₆ H ₄ | CN | 5b | C ₆ H ₅ | 1 |
| 4c | 4-OMeC ₆ H ₄ | CN | 5c | 4-ClC ₆ H ₄ | 0 |
| 4d | 4-FC ₆ H ₄ | CN | 5d | 4-ClC ₆ H ₄ | 1 |
| 4e | 4-FC ₆ H ₄ | CO ₂ Et | 5e | 4-OMeC ₆ H ₄ | 0 |
| 4f | 4-NO ₂ C ₆ H ₄ | CO ₂ Et | 5f | 4-OMeC ₆ H ₄ | 1 |
| 4g | 4-FC ₆ H ₄ | 4-BrC ₆ H ₄ CO | 5g | 4-FC ₆ H ₄ | 0 |
| | | | 5h | 4-FC ₆ H ₄ | 1 |

Scheme 1

The synthesis of the new pyrrolotacrines **5**, **6** has been achieved, as shown in Scheme 1, starting from the readily available, *N*₁-aryl-3-aminopyrrolocarbonitrile derivatives **4**, according to typical Friedländer reaction (FR) protocols. Under the thermal conditions for 7-10 h and microwave irradiation, the time of reaction was reduced from 7-10 h to about 30 min, using cyclopentanone or cyclohexanone, we isolated the expected target molecules in good yields (70-90%); only the regioisomers **6** are formed when Y was an ester or ketone group (**4f** and **4g**), the cyclization occurred exclusively towards the nitrile in position 4 of the pyrrole, thus forming regioisomers **6**. The structures of the new compounds were determined by mass spectrometry, ¹H- and ¹³C-NMR spectroscopy. For example, the ¹H-NMR spectrum of compound **6a** showed the presence of a *triplet* at 1.14 ppm and a *quartet* at 4.22 ppm for the ester function and no absorption band for the CN group in the IR spectrum. The structures of these compounds are in very good agreement with their analytical and spectroscopic data (see Experimental part).

3.1. Biology

The inhibitory activities of the synthesized compounds against AChE were studied by determining the rate of hydrolysis of acetylthiocholine (ATCI) in comparison with the reference compound tacrine using the method of (Ellman *et al* 1961). The results are listed in table 1 as IC₅₀ values (Concentration of compound that produces 50% AChE activity inhibition).

From these data, and for the pyrrolotacrines **5a-h** and **6a,b** we conclude that: (a) these compounds are from potent to moderate, in the micromolar range. (b) the most potent inhibitor was *9-Amino-1-phenyl-5,6,7,8-tetrahydro-1H-pyrrolo[3,2-b]quinoline-3-carbonitrile* (**5b**) (IC₅₀ = 0.20 μM). (c) the substitution of a fused cyclohexane ring, instead of a fused cyclopentane ring [compare **5a,c,e,g** with **5b,d,f,h**] didn't improved the anti-AchE activity of the tacrine derivatives. (d) for the compounds bearing the same cycloalkane fused ring system, and regarding the type of the substituent at C4 in the aromatic ring, the inhibitory potency followed the order: H >> 4-Cl > 4-F > 4-OMe [compare **5b** with **5d**, **5f** and **5h** (cyclohexane)]; or H > 4-OMe > 4-Cl > 4-F [compare **5a** with **5c**, **5e** and **5g** (cyclopentane)]. (e)

The introduction of a methoxy group on *para* position of the phenyl led us to the less active compounds (**5e**, $IC_{50} = 5.3 \mu\text{M}$ and **5f**, $IC_{50} = 45.3 \mu\text{M}$, around 25 to 216-fold respectively less active than tacrine). Some authors report that, the presence of halogen atoms in tacrine derivatives, especially the chlorine atom, improves the activity anti-AChE but this is not observed in our case [16]. Compounds **6a** and **6b** with a different annelation between the pyridine and pyrrolo rings when compared with all the others, presented lower activity than **tacrine** (**6a** ($IC_{50} = 35.0 \mu\text{M}$), and **6b** ($IC_{50} = 3.1 \mu\text{M}$). Once again the five membered saturated ring doesn't ameliorated the activity anti-AchE.

Overall, compounds **5b** ($IC_{50} = 0.20 \mu\text{M}$), this derivative displayed potency for the inhibition of AchE in the same order of magnitude to the tacrine as a reference compound.

Table 1: IC_{50} values for the AChE inhibition

| Compound | $IC_{50} (\mu\text{M}) \pm SD^a$ |
|-----------|----------------------------------|
| Tacrine | 0.21 ± 0.01 |
| 5a | 0.96 ± 0.2 |
| 5b | 0.20 ± 0.04 |
| 5c | 7.5 ± 0.3 |
| 5d | 6.6 ± 0.2 |
| 5e | 5.3 ± 0.3 |
| 5f | 45.3 ± 0.8 |
| 5g | 9.2 ± 0.2 |
| 5h | 7.3 ± 0.3 |
| 6a | 35.0 ± 5.2 |
| 6b | 3.1 ± 0.5 |

^aData is mean of at least five different experiments in triplicate.

4. Conclusion

The 3-amino-4-cyanopyrrole derivatives reacted with cyclopentanone and cyclohexanone to afford the corresponding pyrrolotacrine analogues through Friedländer reaction under classical heating and microwave irradiation. The use of microwave irradiation leads to high product conversion and shorter times. The synthesized tacrine analogues were evaluated for their ability to inhibit AChE and compound **5b** was found the most active one (similar to tacrine itself).

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