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Short Note

3-((2-(4-Chloro-5-ethoxy-2-nitrophenoxy)acetamido)methyl) phenyl-dimethylcarbamate

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Abstract: In this study, we report the synthesis of 3-((2-(4-chloro-5-ethoxy-2-nitrophenoxy)acetamido) methyl)phenyl-dimethylcarbamate, designed on the basis of the structures of the commercial acetylcholinesterase inhibitor drug rivastigmine and a substituted aryloxyacetic acid, aiming at a multi-target approach to the therapy of Alzheimer's disease. The hybrid was obtained thanks to a synthesized intermediate by-product. The compound was fully characterized by using ^{1}H and ^{13}C NMR, FT-IR and HRMS.

Keywords: 3-((2-(4-chloro-5-ethoxy-2-nitrophenoxy)acetamido)methyl)phenyl-dimethylcarbamate; Alzheimer's disease; by-product; S_NAr

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder. It is characterized by an age-related progressive loss of cognitive functions, and available drugs are only syntomatic [1]. The origin of AD is still unknown; however, several classical targets, such as cholinesterases (ChEs), inhibition of Aβ aggregation, antioxidant activity and chelation [2], as well as innovative targets, like the endocannabinoid-hydrolyzing enzyme fatty acid amide hydrolase (FAAH) and monoamino oxidases (MAOs), have been studied [3]. Considering the multifactorial profile of AD, a multi-target direct ligands (MTDLs) have been designed in the last few years [3,4].

Recently, we designed and synthesized different series of hybrids containing an aryloxy portion condensed via an amide bond with fragments mimicking anti-AD drugs donepezil [4] or rivastigmine [3] and in vitro tested them as inhibitors of various enzymes. Compounds bearing a halogen and a nitro group in the *ortho* position in aryloxy moiety exhibited an excellent activity against ChEs [3,4].

In this work, we present a compound that was designed with the aim of having a rivastigmine-like portion condensed with an aryloxy ring containing a nitro group and both fluorine and chlorine atoms. However, in its synthesis, we obtained a by-product final compound, which was due to a nucleophilic aromatic substitution (SNAr) during an intermediate synthetic step (Figure 1) [5,6].

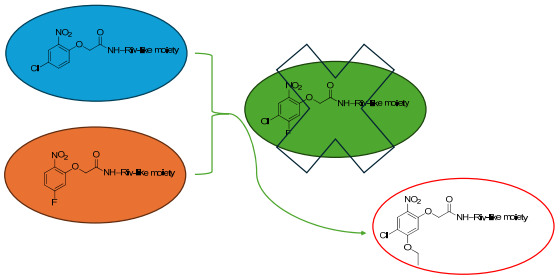

Figure 1. Design of new multi-target anti-AD hybrids.

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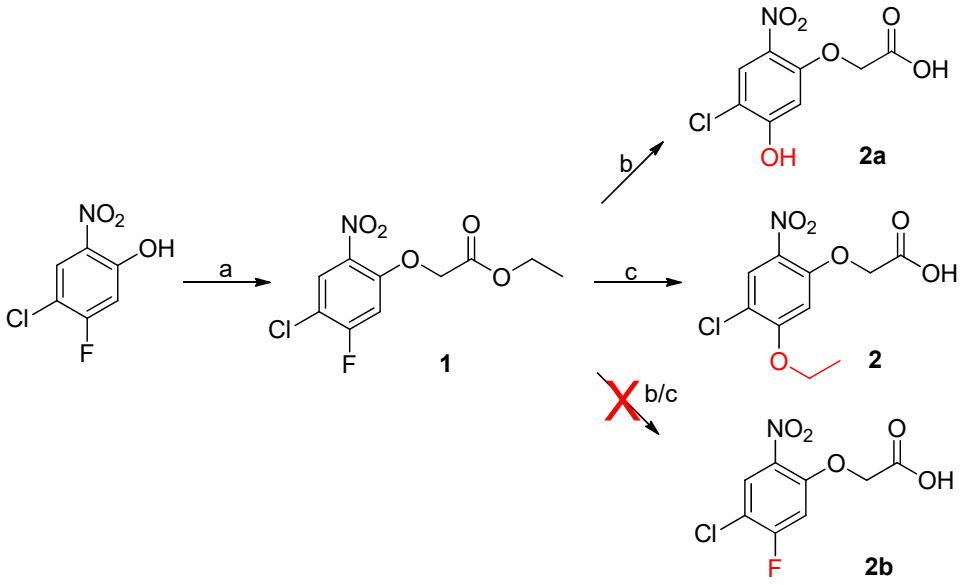
2. Results and Discussion

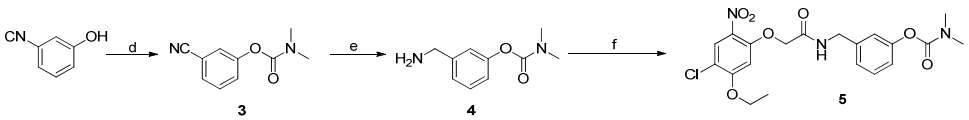
Scheme 1 represents the synthesis of intermediate **1**. After the nucleophilic substitution of commercial 4-chloro-5-fluoro-2-nitrophenol on ethyl bromoacetate, using sodium ethoxide as a base [4], the ethyl ester **1** thus obtained was hydrolyzed under basic conditions, using NaOH 1N in ethanol 96% at reflux [7]. Instead of the desired acid **2b**, we obtained the acid **2** because of an SNAr reaction.



Scheme 1. Synthesis of by-product intermediate **2**. (a) Na, abs EtOH, ethyl bromoacetate, reflux, 23 h; (b) 1.5 N NaOH, THF, RT, 20 h; (c) 1 N NaOH, 96% EtOH, reflux, 4h.

The substitution of fluorine with the ethoxy group in 2 was promoted by the presence, in the aryloxy ring, of both a nitro group in the *para* position and a chlorine atom in the *ortho* position of fluorine. Meanwhile, when the hydrolysis was performed in classical condition THF/NaOH 1.5N (1:1), a hydroxy group directly replaced fluorine through an SNAr mechanism, again (compound **2a**, Scheme 1). On the other hand, when the chlorine is not present, the SNAr does not happen, and we were able to obtain the title compound and use it for the synthesis of the correct hybrid with 2-nitro,5-fluorine-substitution [3,4].

Scheme 2 reported the synthesis of final compound **5**. The preparation of rivastigmine-like intermediate **4** involves a reaction between commercial 3-cyanophenol and dimethyl carbamoyl chloride in the presence of triethylamine (TEA) [8], obtaining **3**. The catalytic hydrogenation of the cyano group using Pd/C 10% *w*/*w* as a catalyst [8] gave primary amine **4**. The condensation of carboxylic acid **2** and the primary amine 4 in the presence of propylphosphonic anhydride (T3P) and 4-methylmorpholine (NMM) as condensing agents [9] gave final compound **5**.



Scheme 2. Synthesis of final compound 5. (d) dimethyl carbamoyl chloride, TEA, reflux 7 h RT overnight; (e) Pd/C 10% *w*/*w*, MeOH, H_2 4 atm, RT, 4 h; (f) **2**, T3P, NMM, anhydrous CH_2Cl_2, N_2, RT, 72 h.

3. Material and Methods

Reagents were purchased from common suppliers and used without additional purification. Reactions were monitored via TLC. Column chromatography was performed by using Geduran silica gel 60 (63–200 µm) as a stationary phase. Hydrogenation reactions were performed using a FID Tower Plus Hydrogen Gas Generator by PerkinElmer

(Waltham, MA, USA). Mass spectra were recorded on an HP MS 6890-5973 MDS spectrometer, electron impact 70 eV, which was equipped with an HP ChemStation (Santa Clara, CA, USA). High-resolution mass spectrometry (HRMS) was performed using a Bruker microTOF QII mass spectrometer equipped with an electrospray ion source (ESI) (Billerica, MA, USA). ^{1}H-NMR and ^{13}C-NMR spectra were recorded using the appropriate deuterated solvent on a Varian Mercury 300 or 500 NMR Spectrometer (Palo Alto, CA, USA). FT-IR was recorded by using the sample as it was through a Jasco FT/IR-4200 instrument (Cremella, LC, Italy). The purity of the compounds was estimated as >95%. All data are available in the Supplementary Material File (Figures S1–S4).

Preparation of ethyl 2-(4-chloro-5-fluoro-2-nitrophenoxy)acetate (**1**)

A solution of sodium ethoxide, prepared dissolving Na (2.5 mmol, 1 eq) in absolute ethanol (15 mL), was prepared; then, commercial 4-chloro-5-fluoro-2-nitrophenolphenol (2.5 mmol, 1 eq) was added, and the mixture was stirred for 30 min at room temperature. Subsequently, ethyl bromoacetate (2.5 mmol, 1 eq), dissolved in absolute ethanol (5 mL), was added dropwise, and the reaction mixture was refluxed for 23 h. The solvent was removed in vacuo, and the crude was treated with diethyl ether and washed with 0.5 N NaOH (three times) and brine, dried over anhydrous Na_2SO_4, filtered and concentrated to dryness, obtaining the title compound as a yellow solid. Yield 71%. ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.30 (t, *J* = 7.1 Hz, 3H, OCH_2CH_3), 4.28 (q, *J* = 7.1 Hz, 2H, OCH_2CH_3), 4.76 (s, 2H, OCH_2CO), 6.78–6.84 (m, 1H aromatic), 8.04–8.08 (m, 1H aromatic). GC-MS *m*/*z* (%): 278 (3) $[M+2]^+$, 277 (7) $[M]^+$, 205 (33), 203 (100), 160 (16), 158 (39).

Preparation of 2-(4-chloro-5-ethoxy-2-nitrophenoxy)acetic acid (**2**)

First, **1** N NaOH (1.77 mmol, 2 eq) was added to a solution of **1** (0.88 mmol, 1 eq) dissolved in ethanol 96° (8 mL), and the reaction mixture was refluxed for 4 h. Then, the organic solvent was removed in vacuo, and the aqueous residue was washed with diethyl ether and acidified with 2 N HCl. The acid aqueous phase was extracted with diethyl ether (three times). The organic portions were collected and washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to dryness, affording a brown solid. Analysis revealed that compound **2** was obtained instead of the desired compound **2b** (2-(4-chloro-5-fluoro-2-nitrophenoxy)acetic acid). Yield 95%. ^{1}H-NMR (500 MHz, DMSO-d6) δ (ppm): 1.35 (t, *J* = 7.0 Hz, 3H, $PhOCH_2CH_3$), 4.22 (q, *J* = 7.0 Hz, 2H, $PhOCH_2CH_3$), 4.98 (s, 2H, OCH_2CO), 6.91 (s, 1H aromatic), 8.06 (s, 1H aromatic). ESI-MS *m*/*z*: (IP: negative) 276 $[M+2-H]^-$, 274 $[M-H]^-$. HRMS ($C_{10}H_9ClNO_6^-$): calculated 274.0124 found 274.0154.

Preparation of 2-(4-chloro-5-hydroxy-2-nitrophenoxy)acetic acid (**2a**)

First, **1** (1.64 mmol, 1 eq) was dissolved in THF (11 mL); then, 1.5 N NaOH (16.4 mmol, 10 eq) was added, and the reaction mixture was stirred for 20 h at room temperature. Then, the organic solvent was removed under reduced pressure, and the aqueous residue was acidified with 6 N HCl and extracted with diethyl ether (three times). The organic portions were collected and washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to dryness, affording a brown solid. Analysis revealed that compound **2a** was obtained instead of the desired compound **2b** (2-(4-chloro-5-fluoro-2-nitrophenoxy)acetic acid). Yield 97%. ^{1}H-NMR (500 MHz, DMSO-d6) δ (ppm): 4.82 (s, 2H, OCH_2CO), 6.62 (s, 1H aromatic), 8.03 (s, 1H aromatic). ESI-MS *m*/*z*: (IP: negative) 246 $[M+2-H]^-$, 248 $[M-H]^-$. HRMS ($C_8H_5ClNO_6^-$): calculated 245.9811 found 245.9849.

Preparation of 3-cyanophenyl dimethylcarbamate (**3**)

Commercial dimethyl carbamoyl chloride (3.7 mmol, 1.02 eq) and 3-cyanophenol (3.63 mmol, 1 eq) were added to triethylamine (2.5 mL). The mixture was refluxed for 7 h and stirred at room temperature overnight. Then, the mixture was diluted with CH_2Cl_2 and the organic phase was washed with 1N NaOH (three times) and dried over anhydrous Na_2SO_4, filtered and concentrated to dryness. The obtained crude was purified by a chromatography column (eluent 100% CH_2Cl_2) to give the title compound as a yellow

oil. Yield 84%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.02 (s, 3H, CH_3), 3.08 (s, 3H, CH_3), 7.35–7.52 (m, 4H aromatics). GC-MS *m*/*z* (%): 190 (4) $[M]^+$, 90 (3), 72 (100).

Preparation of 3-(aminomethyl)phenyl dimethyl carbamate (**4**)

First, **3** (3.045mmol, 1 eq) was dissolved in MeOH (25 mL) and hydrogenated at a pressure of 4 atm in the presence of 10% Pd/C (4.40 mmol, 1.46 eq) for 4 h at room temperature. The catalyst was filtered off, the solvent was removed in vacuo, and the resulting oil was purified with a column chromatography (eluent EtOAc/MeOH 95:5) to obtain the title compound as a yellow oil. Yield 30%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 3.01 (s, 3H, CH_3), 3.09 (s, 3H, CH_3), 3.85 (s, 2H, CH_2NH_2), 6.91–7.03 (m, 1H aromatic), 7.08–7.16 (m, 2H aromatics), 7.25–7.34 (m, 1H aromatic). GC-MS *m*/*z* (%): 194 (4) $[M]^+$; 106 (26); 72 (100).

Preparation of 3-((2-(4-chloro-5-ethoxy-2-nitrophenoxy)acetamido)methyl)phenyl-dimethylcarbamate (**5**)

Acid **2** (0.8 mmol, 1 eq) was dissolved in anhydrous CH_2Cl_2 (8 mL), which was followed by NMM (2.04 mmol, 2.5 eq) and T_3P (1.22 mmol, 1.5 eq). After 30 min, amine 2, dissolved in anhydrous CH_2Cl_2 (6 mL), was added dropwise. The reaction mixture was stirred for 72 h at room temperature; then, it was diluted with CH_2Cl_2 and washed with water (three times). The collected aqueous portion was extracted three times with CH_2Cl_2; then, all the organic phases were collected and washed with 1N NaOH (three times), dried over anhydrous Na_2SO_4, filtered and concentrated to dryness. The obtained crude was purified by a chromatography column (eluent 100% CH_2Cl_2) to give the title compound as a yellow solid. Yield 19%. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.51 (t, *J* = 6.8 Hz, 3H, $PhOCH_2CH_3$), 2.98 (s, 3H, CH_3), 3.08 (s, 3H, CH_3), 4.15 (q, *J* = 6.8 Hz, 2H, $PhOCH_2CH_3$), 4.53 (d, *J* = 5.7 Hz, 2H, $NHCH_2CH$), 4.64 (s, 2H, OCH_2CO), 6.47 (s, 1H aromatic), 6.99–7.18 (m, 3H aromatics), 7.28–7.35 (m, 1H aromatic), 7.64–7.75 (m, NH), 8.16 (s, 1H aromatic). ^{13}C NMR (125.8 MHz, $CDCl_3$) δ (ppm): 14.3, 36.4, 36.7, 42.8, 65.9, 68.2, 98.6, 115.7, 121.1, 124.4, 128.3, 129.5, 131.2, 138.9, 151.8, 152.1, 154.7, 160.0, 166.3. FT-IR (cm^{-1}): 734, 754, 1038, 1116, 1171, 1212, 1239, 1272, 1335, 1387, 1417, 1438, 1488, 1501, 1530, 1578, 1604, 1680, 1717, 2937, 3054, 3413. HRMS ($C_{20}H_{22}ClN_3O_7$+Na^+): calculated 474.1038 found 474.1038.

Supplementary Materials: Figure S1: ^{1}H-NMR spectrum of compound 5; Figure S2: ^{13}C-NMR spectrum of compound 5; Figure S3: FT-IR spectrum of compound 5; Figure S4: HR-MS of compound 5.

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