Supporting Information

Synthesis and in vitro evaluation of donepezil-based reactivators and analogues for nerve agent-inhibited human acetylcholinesterase

Julien Renou, José Dias, Guillaume Mercey, Tristan Verdelet, Catherine Rousseau, Anne-Julie Gastellier, Mélanie Arboléas, Mélanie Touvrey-Loiodice, Rachid Baati, Ludovic Jean*, Florian Nachon, Pierre-Yves Renard*

Table of contents

1. Biological assays .................................................................................................................2
2. $^1$H and $^{13}$C NMR Spectra and HPLC .............................................................................7
1. Biological assays

Time-dependent reactivation of VX-inhibited hAChE and GB-inhibited hAChE by 100 µM of 1, for the determination of $k_{\text{obs}}$ (See Table 1 and experimental section).

Time-dependent reactivation of VX, paraoxon (POX), tabun (GA)-inhibited hAChE and VX-inhibited hBChE by 100 µM of 2, for the determination of $k_{\text{obs}}$ (See Table 1 and experimental section).
Time-dependent reactivation of VX-, paraoxon (POX-), tabun (GA)-inhibited hAChE by 50 µM of 3, for the determination of $k_{obs}$ (See Table 1 and experimental section).

Time-dependent reactivation of VX-, paraoxon or POX-, tabun by 100 µM, of GA-inhibited hAChE by 50 µM of 4, and VX-inhibited hBChE by 100 µM of 4, for the determination of $k_{obs}$ (See Table 1 and experimental section).
Concentration-dependent reactivation of VX- and GB-inhibited hAChE by compound 1 for the determination of $k_r$, $K_D$ and $k_{r2}$ (See Table 1 and experimental section)

![Graph showing reactivation of VX- and GB-inhibited hAChE](image)

Concentration-dependent reactivation of VX-inhibited hBChE, VX-inhibited hAChE and paraoxon-inhibited hAChE by compound 2 for the determination of $k_r$, $K_D$ and $k_{r2}$ (See Table 1 and experimental section)

![Graph showing reactivation of VX-inhibited hBChE, VX-inhibited hAChE and paraoxon-inhibited hAChE](image)
Concentration-dependent reactivation of VX- and paraoxon-inhibited hAChE by compound 3 for the determination of $k_r$, $K_D$ and $k_{r2}$ (See Table 1 and experimental section)

![Graph of k_{obs} vs [3] (μM)](image)

Concentration-dependent reactivation of VX-inhibited hAChE by compound 4 for the determination of $k_{r2}$ (See Table 1 and experimental section)

![Graph of k_{obs} vs [4] (μM)](image)
IC$_{50}$ of compound 1 for hAChE

![Graph showing IC$_{50}$ for compound 1]

\[ IC_{50} = 1.0 \pm 0.1 \, \mu M \]
2. $^1$H and $^{13}$C NMR Spectra and HPLC
Under these non-optimized HPLC conditions, the basic compound 1 is partially protonated and the purity (97.5%) was determined by the sum of the two peak areas of the protonated form ($t_R = 26.08$ min) and of the unprotonated form ($t_R = 22.48$ min). The UV absorption spectra were completely similar.
Under these non-optimized HPLC conditions, the basic compound 3 is partially protonated and the purity (95.1%) was determined by the sum of the two peak areas of the protonated form ($t_R = 23.07$ min) and of the unprotonated form ($t_R = 22.48$ min). The UV absorption spectra were completely similar.
Under these non-optimized HPLC conditions, the basic compound 4 is partially protonated and the purity (98.2 %) was determined by the sum of the two peak areas of the protonated form ($t_R = 24.76$ min) and of the unprotonated form ($t_R = 23.62$ min). The UV absorption spectra were completely similar.