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RESEARCH ARTICLE

Novel phenyl(thio)ureas bearing (thio)oxothiazoline group as potential BACE-1 inhibitors: synthesis and biological evaluation

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Abstract

We report the synthesis and the β-site amyloid precursor protein cleaving enzyme-1 inhibitory properties of novel phenyl(thio)ureas bearing 2-(thio)oxothiazoline derivatives. A library of analogues was prepared according to specific synthetic schemes and the inhibitory activity was monitored using a fluorescence resonance energy transfer assay. Several analogues show potent inhibitory activities ranging between 1 and 0.01 μM and the activity is related to the NH acidity of the (thio)urea motif. Our results illustrate once again the close relationship between molecular recognition, complexation of the active site in enzymatic system, and organocatalysis utilizing explicit hydrogen bonding.

Keywords: BACE-1, N-aryl-thiazoline-2-(thi)ones, ureas, thioureas, Alzheimer disease

Introduction

A recent critical review outlined "the close relationship and mutual interplay between molecular recognition, active site considerations in enzyme catalysis involving anions, and organocatalysis utilizing explicit hydrogen bonding[1]". Aryl(thio)ureas presenting two N-H groups are well recognized in the very active field of non-covalent organocatalysis for their ability to bind ketones, aldehydes and nitro groups through hydrogen bonding[2–8]. In addition, they are outstanding neutral compounds for the selective complexation of various anions[9–14] and they found application in the (enantio) selective extraction of carboxylate anions[15–18]. We have shown that some ureas and thioureas derived from 3-(2-aminophenyl)-4-methylthiazole-2(3H)-thione **1a** enantioselectively bind to carboxylate anion derived from amino-acids[19] and act as organocatalysts for addition to aldehydes[20]. In an extension of our interest in the binding capabilities of aryl(thi)oureas through hydrogen bonding, we hypothesized that aspartyl proteases are prone to be selectively complexed by the aryl(thio)urea scaffold. The inhibition of BACE-1 (β-site amyloid precursor protein (APP) cleaving enzyme), a key enzyme in the production of α, β peptides involved in the formation of extracellular senile plaques in Alzheimer's disease[21–23], was chosen to monitor the binding capability (if any) of aryl(thio)ureas to that important enzyme. The search of BACE-1 inhibitors is a very active field of research[24–31]. Arylthioureas have been recently described in several patents as potentially active scaffolds for neurodegenerative diseases including Alzheimer[32,33]. Moreover, (thio)oxothiazolphenyl heterocycles represent a privileged moiety very often encountered in bioactive molecules utilized for the treatment of several pathologies including neurodegenerative diseases[34,35]. We report herein the synthesis of focused library of heterocyclic aryl(thio)ureas. They were screened as BACE-1 inhibitors by the mean of a fluorescence resonance energy transfer (FRET) assay, which

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uses purified baculovirus-expressed (BACE-1) and a specific substrate (Rh-EVNLDAEFK-Quencher) based on the Swedish mutation of the APP. This peptidic substrate becomes highly fluorescent upon enzymatic cleavage.

Methods

Chemistry general methods

^{1}H NMR spectra were recorded at 500, 400, 300 or 200 MHz and ^{13}C NMR spectra at 125, 100, 75 or 50 MHz on Bruker Avance DRX-500 or 400, DPX-300 or 200 instruments, respectively. Chemical shifts are reported in ppm with the signal for residual solvent as internal standard. *J* values are reported in Hz. Elemental analyses were within 0.4% of theoretical values. High resolution mass spectra were performed on Q-STAR Elite spectrometer. Melting points were measured using a Büchi Melting Point B-545 apparatus or a Kofler hot stage apparatus and are not corrected. Filtrations through silica gel were performed with silica gel 60 (230–400 mesh). TLCs were carried out on Merck 60F254 silica plates.

Thiazoline thiones and ones

3-(2-Aminophenyl)-4-methylthiazole-2(3H)-thione (1a)

Twenty-eight grams of 1,2-phenylenediamine (258 mmol) was suspended in CS_2 (600 mL), then NEt_3 (2 eq, 72 mL) was added dropwise and the mixture stirred at r.t. for 3 h. The resulting dithiocarbamate salt (73 g, yellow powder, 99%) was filtered off, washed with Et_2O and used without further purification. Seventy-three grams of dithiocarbamate salt (393 mmol) were suspended in CH_3CN (600 mL), then chloroacetone (1 eq, 21 mL) was added dropwise and the mixture stirred at r.t. for 12 h. The solvent was then removed and HCl 37% (69 mL) was added and the mixture vigorously stirred for 15 min. Water (400 mL) was added and the mixture extracted with 3 × 400 mL of CH_2Cl_2. The organic layer was then washed with 3 × 500 mL of water, dried over $MgSO_4$ and evaporated under reduced pressure. Thiazoline **1a** was then recovered by recrystallization from EtOH: yield 70% (41 g, pale yellow crystals); product description is given in Roussel et al.[36] and Bellec et al.[37].

3-(2-Aminophenyl)-4,5-dimethylthiazole-2(3H)-thione (1b)

Seven grams of 1,2-phenylenediamine (64.6 mmol) were suspended in CS_2 (150 mL), then NEt_3 (2 eq, 18 mL) was added dropwise and the mixture stirred at r.t. for 3 h. The resulting dithiocarbamate salt (18 g, yellow powder, 99%) was filtered off, washed with Et_2O and used without further purification. Eighteen grams of dithiocarbamate salt (93 mmol) were suspended in CH_3CN (150 mL), then 3-chlorobutan-2-one (1 eq, 6.4 mL) was added dropwise and the mixture stirred at r.t. for 24 h. The solvent was then removed and H_2SO_4 (8 mL) was added and the mixture vigorously stirred for 15 min. Water (100 mL) was added and the mixture extracted with 3 × 100 mL of CH_2Cl_2. The organic layer was then washed with 3 × 200 mL of water, dried over $MgSO_4$ and evaporated under reduced pressure. Thiazoline **1b** was then recovered by recrystallization from EtOH: yield 73% (11 g, pale yellow crystals); product description is given in Roussel et al.[36] and Bellec et al.[37].

3-(2-Aminophenyl)-4-methylthiazol-2(3H)-one (1c)

In 50 mL of acetone solution, 2.5 g of **1a** (11.2 mmol) were solubilized, then iodomethane (10 eq, 70 mL) was added and the solution stirred at r.t. After 24 h, the solvent and the excess of reagent were evaporated and the resulting thiazolium salt recovered quantitatively (4.1 g). Then, a solution of sodium methoxide (4 eq) in CH_3OH was prepared, 4.1 g (11.24 mmol) of thiazolium salt were added and the solution stirred at r.t. After 48 h, the solvent was evaporated, water (200 mL) was added and the mixture extracted 3 × 100 mL of CH_2Cl_2. The organic layer was then dried over $MgSO_4$ and evaporated under reduced pressure. The **1c** was finally recovered by recrystallization from EtOH: yield 74% (1.73 g, pale yellow crystals); for product description see ref. Vanthuyne et al.[38].

3-[2-Amino-5-(trifluoromethyl)phenyl]-4-methyl-1,3-thiazole-2(3H)-thione (1d)

Three grams of 1,2-diamino-4-trifluoromethylbenzene (17.03 mmol) were suspended in CS_2 (40 mL), then NEt_3 (2 eq, 5 mL) was added dropwise and the mixture stirred at r.t. for 3 h. The resulting dithiocarbamate salt (5.98 g, yellow powder, 99%) was filtered off, washed with Et_2O and used without further purification. In 50 mL of CH_3CN solution, 5.98 g of dithiocarbamate salt (16.92 mmol) were suspended, then chloroacetone (1 eq, 1.36 mL) was added dropwise and the mixture stirred at r.t. for 12 h. The solvent was then removed and HCl 37% (4 mL) was added and the mixture vigorously stirred for 15 min. Water (200 mL) was added and the mixture extracted with 3 × 200 mL of CH_2Cl_2. The organic layer was then washed with 3 × 400 mL of water, dried over $MgSO_4$ and evaporated under reduced pressure. Thiazoline **1d** was then recovered by recrystallization from EtOH: yield 68% (3.32 g, pale yellow crystals); mp 188°C, R_f = 0.61 (CH_2Cl_2/AcOEt 9:1), ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.95 (d, J = 1.2 Hz, 3H, CH_3), 4.04 (br s, 2H, NH_2), 6.39 (q, J = 1.2 Hz, 1H, *H5*), 6.91–6.99 (m, 1H, *Ar*), 7.27–7.31 (m, 1H, *Ar*), 7.47–7.58 (m, 1H, *Ar*). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 15.53, 107.00, 117.18, 121.31 (q, J = 34 Hz, 1C), 122.69, 123.96 (q, J = 271 Hz, 1C), 126.41 (q, J = 4 Hz, 1C), 127.98 (q, J = 4 Hz, 1C), 132.60, 146.90, 172.15. High resolution mass spectrometry (HRMS) *m/z* $C_{11}H_9N_2F_3S_2$ $[M+H]^+$ calcd: 291.0231, found: 291.0231. The structure of 3-[2-amino-5-(trifluoromethyl)phenyl]-4-methyl-1,3-thiazole-2-(3*H*)-thione was fully established by X-ray single crystal analysis.

3-(2-Amino-4-fluorophenyl)-4-methyl-1,3-thiazole-2(3H)-thione (1e)

Ten grams (79 mmol) of 1,2-diamino-4-fluorobenzene were suspended in CS_2 (500 mL), then NEt_3 (2 eq, 22 mL) was added dropwise and the mixture stirred at r.t. for 2 h.

The resulting dithiocarbamate salt was then filtered off, washed with Et_2O and used without further purifications (24 g, yellow powder, 99%). Twenty-four grams (79 mmol) of dithiocarbamate salt were suspended in CH_3CN (500 mL), then chloroacetone (1.1 eq, 7.14 mL) was added dropwise and the mixture stirred at r.t. for 12 h. The solvent was then removed and HCl 37% (21 mL) was added under vigorous stirring. After 15 min, water (150 mL) was added and the mixture extracted 3 × 150 mL of CH_2Cl_2. The organic layer was washed 3 × 400 mL of water, dried with $MgSO_4$ and evaporated under reduced pressure. Thiazoline **1e** was then recovered by recrystallization from CH_2Cl_2: yield 68% (12.1 g, yellow crystals); mp = 138°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.95 (d, J = 1.2 Hz, 3H, CH_3), 3.80 (br s, 2H, NH_2), 6.37 (q, J = 1.2 Hz, 1H, *H5*), 6.57–6.99 (m, 3H, *Ar*). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 15.44, 103.9 (d, J = 26 Hz, 1C), 106.4 (d, J = 24 Hz, 1C), 106.6, 119.3 (d, J = 3 Hz, 1C), 130.0 (d, J = 11 Hz, 1C), 144.6 (d, J = 12 Hz, 1C), 163.8 (d, J = 248 Hz, 1C), 189.2, HRMS m/z $C_{10}H_{10}N_2FS_2$ $[M+H]^+$ calcd: 241.0264, found: 241.0262. The structure of 3-(2-amino-4-fluorophenyl)-4-methyl-1,3-thiazole-2(3*H*)-thione was fully established by X-ray single crystal analysis.

Isothiocyanates

3-(2-Isothiocyanatophenyl)-4-methylthiazole-2(3H)-thione (2a)

To a suspension of thiazoline **1a** (1 g, 4.5 mmol) in CS_2 (40 mL), NEt_3 was added dropwise (10 eq, 6.3 mL) and the mixture stirred first 1 h under reflux, then 5 h at room temperature. The resulting dithiocarbamate salt (1.79 g, 99%) was filtered off, washed with anhydrous Et_2O and used without further purification. Then, to a suspension of the dithiocarbamate salt (916 mg, 2.29 mmol) in CH_3CN (30 mL), DiCyclohexylCarbodiimide (DCC) (1.05 eq, 475 mg) was added and the mixture stirred at room temperature. After 24 h, the formed Dicyclohexylurea (DCU) was filtered off and the pure isothiocyanate was recovered by column chromatography (CH_2Cl_2): yield 74% referring to dithiocarbamate salt (448 mg, white powder); for product description see ref. Vanthuyne et al.[38].

3-(2-Isothiocyanatophenyl)-4,5-dimethylthiazole-2(3H)-thione (2b)

To a suspension of thiazoline **1b** (1.09 g, 4.61 mmol) in CS_2 (40 mL), NEt_3 was added dropwise (10 eq, 6.5 mL) and the mixture stirred first 1 h under reflux, then 4 h at room temperature. The resulting dithiocarbamate salt (1.51 g, 79%) was filtered off, washed with anhydrous Et_2O and used without further purification. Then, to a suspension of the dithiocarbamate salt (1.51 g, 3.65 mmol) in CH_3CN (40 mL), DCC (1.05 eq, 790 mg) was added and the mixture stirred at room temperature. After 24 h, the formed DCU was filtered off and the pure isothiocyanate was recovered by column chromatography (CH_2Cl_2): yield 92% referring to the dithiocarbamate salt (931 mg, pale yellow powder); mp 140°C (Bellec et al. [37] 138°C); R_f = 0.57 (CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.85 (s, 3H, CH_3), 2.22 (s, 3H, CH_3), 7.28–7.55 (m, 4H, *Ar*); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 11.9, 13.0, 118.6, 126.2, 128.2, 130.0, 130.9, 131.0, 134.3, 135.2, 141.7, 188.6. NMR data are in agreement with literature[37].

3-(2-Isothiocyanatophenyl)-4-methylthiazole-2(3H)-one (2c)

To a suspension of thiazoline **1c** (600 mg, 2.91 mmol) in CS_2 (25 mL), NEt_3 was added dropwise (10 eq, 4.0 mL) and the mixture stirred first 1 h under reflux, then 5 h at room temperature. The resulting dithiocarbamate salt (880 mg, 79%) was filtered off, washed with anhydrous Et_2O and used without further purification. Then, to a suspension of the dithiocarbamate salt (880 mg, 2.29 mmol) in CH_3CN (25 mL), DCC (1.05 eq, 493 mg) was added and the mixture stirred at room temperature. After 24 h, the formed DCU was filtered off and the pure isothiocyanate was recovered by column chromatography (CH_2Cl_2): yield 80% referring to dithiocarbamate salt (455 mg, pale yellow powder); for product description see ref. Vanthuyne et al.[38].

General procedure for the synthesis of thioureas and ureas

To a solution of isothiocyanate (238 mg, 0.9 mmol) in CH_3CN (5 mL), the corresponding aniline was added (1.05 eq) and the solution stirred for 24 h at room temperature. Then, the solvent was partially evaporated and the resulting thiourea recovered by filtration. Modifications to the general procedure are given when needed.

1-(2-(4-Methyl-2-thioxothiazol-3(2*H*)-yl)phenyl)-3-phenylthiourea **(3a),** 1-(2-(4-methyl-2-thioxothiazol-3(2*H*)-yl)phenyl)-3-phenylthiourea **(3b),** 1-(2-(4-methyl-2-thioxothiazol-3(2*H*)-yl)phenyl)-3-(pyridin-3-yl)thiourea **(3c),** 1-(2-hydroxyphenyl)-3-(2-(4-methyl-2-thioxothiazol-3(2*H*)-yl)phenyl)thiourea **(3d),** 1-(3,5-dimethylphenyl)-3-(2-(4-methyl-2-thioxothiazol-3(2*H*)-yl)phenyl)thiourea **(3e),** 1-(2-methoxyphenyl)-3-(2-(4-methyl-2-thioxothiazol-3(2*H*)-yl)phenyl)thiourea **(3f),** 1-(2-(4-methyl-2-thioxothiazol-3(2*H*)-yl)phenyl)-3-(3-(trifluoromethyl)-phenyl)thiourea **(3g),** 1-(3,5-bis(trifluoromethyl)phenyl)-3-(2-(4-methyl-2-thioxothiazol-3(2*H*)-yl)phenyl)thiourea **(3h),** 1-(2-(4-methyl-2-thioxothiazol-3(2*H*)-yl)phenyl)-3-phenylurea **(4a)** were available from a previous study[19]. 1-(3,5-Bis(trifluoromethyl)phenyl)-3-(2-(4-methyl-2-thioxothiazol-3(2*H*)-yl)phenyl)urea **(4c),** 1-(2-(4-methyl-2-thioxothiazol-3(2*H*)-yl)phenyl)-3-(4-nitrophenyl)urea **(4d),** 1-(2-(4-methyl-2-thioxothiazol-3(2*H*)-yl)phenyl)-3-*p*-tolylurea **(4e)** were available from a previous study[39].

1-(2,4-Dichlorophenyl)-3-(2-(4-methyl-2-thioxothiazol-3(2H)-yl)phenyl)thiourea (3i)

According to the general procedure, the reaction of 2,4-dichlorobenzenamine with isothiocyanate **2a** afforded thiourea **3i**: yield 62% (238 mg, white solid); mp 180°C; ^{1}H NMR (300 MHz, dimethyl sulfoxide (DMSO) d_6): δ_H 1.99 (d, J = 1.0 Hz, 3H, CH_3), 6.88 (q, J = 1.0 Hz, 1H, *H5*), 7.34–7.81 (m, 7H, *Ar*), 8.84 (s, 1H, N*H*), 9.95 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO d_6): δ_C 15.42, 107.50, 127.32, 127.67, 128.90, 128.93, 129.66, 130.58, 130.88, 130.91,

131.13, 133.12, 135.04, 135.92, 140.66, 181.88, 188.54; HRMS: *m/z* [M+H]$^+$ calcd. for $C_{17}H_{14}N_3S_3Cl_2^+$: 425.9721, found: 425.9717.

1-(2-(4-methyl-2-thioxothiazol-3(2H)-yl)phenyl)-3-(pyrimidin-2-yl)thiourea (3j)

According to the general procedure, the reaction of 2-aminopyrimidine with isothiocyanate **2a** afforded thiourea **3j**; in this case, CH_3CN was evaporated and thiourea **3j** recovered by column chromatography (CH_2Cl_2/AcOEt 9/1): yield 42% (136 mg, white solid); mp 242°C; R_f = 0.49 (CH_2Cl_2/AcOEt 9/1); ^{1}H NMR (300 MHz, DMSO d_6): δ_H 1.95 (d, *J* = 1.1 Hz, 3H, C*H*$_3$), 6.78 (q, *J* = 1.1 Hz, 1H, *H5*), 7.22 (t, *J* = 4.9 Hz, 1H, *Ar*), 7.38–7.54 (m, 2H, *Ar*), 7.55–7.65 (m, 1H, *Ar*), 8.01–8.09 (m, 1H, *Ar*), 8.60 (d, *J* = 4.9 Hz, 2H, *Ar*), 11.26 (s, 1H, N*H*), 13.03 (s, 1H, N*H*). ^{13}C NMR (75 MHz, DMSO d_6): δ_C 15.27, 107.63, 116.24, 127.61, 128.69, 129.03, 129.60, 132.12, 136.09, 140.13, 157.12, 158.01 (2C), 179.47, 189.19; HRMS: *m/z* [M+H]$^+$ calcd. for $C_{15}H_{14}N_5S_3^+$: 360.0405, found: 360.0411.

4-(3-(2-(4-Methyl-2-thioxothiazol-3(2H)-yl)phenyl)thioureido) benzoic acid (3k)

A different procedure was used: a solution of isothiocyanate **2a** (238 mg, 0.9 mmol) in CH_3CN (3 mL) and a Na_2CO_3 saturated aqueous solution containing 4-aminobenzoic acid (1.1 eq) were mixed together and then the mixture put under vigorous stirring at room temperature. After 24 h, the suspension was acidified with HCl 37% and the thiourea **3k** recovered by filtration: yield 84% (304 mg, white solid); mp 146°C; ^{1}H NMR (300 MHz, DMSO d_6): δ_H 2.00 (d, *J* = 1.0 Hz, 3H, C*H*$_3$), 6.87 (q, *J* = 1.0 Hz, 1H, *H5*), 7.33–7.63 (m, 3H, *Ar*), 7.68–7.79 (m, 3H, *Ar*), 7.84–7.93 (m, 2H, *Ar*), 8.79 (s, 1H, N*H*), 10.57 (s, 1H, N*H*), 12.74 (br s, 1H, COO*H*). ^{13}C NMR (75 MHz, DMSO d_6): δ_C 15.43, 107.60, 121.38 (2C), 126.04, 127.73, 128.94, 129.65, 129.97 (2C), 130.85, 133.27, 135.90, 140.57, 143.24, 166.85, 180.10, 188.61; HRMS: *m/z* [M+H]$^+$ calcd. for $C_{18}H_{16}N_3O_2S_3^+$: 402.0399, found: 402.0398.

1-(4-Fluorophenyl)-3-(2-(4-methyl-2-thioxothiazol-3(2H)-yl) phenyl)thiourea (3l)

According to the general procedure, the reaction of 4-fluorobenzenamine with isothiocyanate **2a** afforded thiourea **3l**: yield 86% (291 mg, white solid); mp 179°C; ^{1}H NMR (300 MHz, DMSO d_6): δ_H 1.99 (d, *J* = 1.0 Hz, 3H, C*H*$_3$), 6.87 (q, *J* = 1.0 Hz, 1H, *H5*), 7.13–7.23 (m, 2H, *Ar*), 7.32–7.60 (m, 5H, *Ar*), 7.72–7.80 (m, 1H, *Ar*), 8.44 (s, 1H, N*H*), 10.21 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO d_6): δ_C 15.39, 107.55, 115.27 (d, *J* = 22 Hz, 2C), 125.70 (d, *J* = 8 Hz, 2C), 127.46, 128.71, 129.56, 130.63, 133.02, 134.96 (d, *J* = 3 Hz, 1C), 136.03, 140.56, 159.28 (d, *J* = 242 Hz, 1C), 180.56, 188.46; HRMS: *m/z* [M+H]$^+$ calcd. for $C_{17}H_{15}FN_3S_3^+$: 376.0406, found: 376.0404.

1-(2-(4,5-Dimethyl-2-thioxothiazol-3(2H)-yl)phenyl)-3-(3-(trifluoromethyl)phenyl)thiourea (3m)

According to the general procedure, the reaction of 3,5-bis(trifluoromethyl)benzenamine with isothiocyanate **2b** (203 mg, 0.73 mmol) afforded thiourea **3m**: yield 66% (210 mg, white solid); mp 191°C; ^{1}H NMR (300 MHz, DMSO d_6): δ_H 1.91 (s, 3H, C*H*$_3$), 2.18 (s, 3H, C*H*$_3$), 7.13–7.23 (m, 2H, *Ar*), 7.31–7.62 (m, 5H, *Ar*), 7.72–7.80 (m, 1H, *Ar*), 7.70–7.80 (m, 1H, *Ar*), 8.11 (br s, 1H, *Ar*), 8.76 (s, 1H, N*H*), 10.51 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO d_6): δ_C 11.39, 13.23, 117.43, 118.94 (q, *J* = 4 Hz, 1C), 120.83 (q, *J* = 4 Hz, 1C), 124.01 (q, *J* = 272 Hz, 1C), 126.38, 127.66, 128.89, 129.03 (q, *J* = 32 Hz, 1C), 129.64, 129.72, 130.55, 133.77, 135.73, 135.85, 139.98, 180.41, 186.09; HRMS: *m/z* [M+H]$^+$ calcd. for $C_{19}H_{17}N_3F_3S_3^+$: 440.0531, found: 440.0532.

1-(3,5-Bis(trifluoromethyl)phenyl)-3-(2-(4,5-dimethyl-2-thioxothiazol-3(2H)-yl)phenyl)-thiourea (3n)

According to the general procedure, the reaction of 4-fluorobenzenamine with isothiocyanate **2b** (207 mg, 0.74 mmol) afforded thiourea **3n**; in this case, the solvent was evaporated and **3n** recovered by column chromatography (CH_2Cl_2): yield 78% (295 mg, pale yellow solid); mp 170°C, R_f = 0.34 (CH_2Cl_2), ^{1}H NMR (300 MHz, DMSO d_6): δ_H 1.90 (s, 3H, C*H*$_3$), 2.16 (s, 3H, C*H*$_3$), 7.33–7.41 (m, 1H, *Ar*), 7.43–7.63 (m, 2H, *Ar*), 7.70–7.77 (m, 1H, *Ar*), 7.81 (br s, 1H, *Ar*), 8.33 (br s, 2H, *Ar*), 9.03 (s, 1H, N*H*), 10.73 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO d_6): δ_C 11.36, 13.21, 117.03 (m, 1C) 117.46, 122.42 (q, *J* = 3 Hz, 2C), 123.19 (q, *J* = 273 Hz, 2C), 127.91, 129.10, 129.82, 130.12 (q, *J* = 33 Hz, 2C), 130.33, 133.71, 135.51, 135.67, 141.38, 180.38, 186.10; HRMS: *m/z* [M+H]$^+$ calcd. for $C_{20}H_{16}N_3F_6S_3^+$: 508.0405, found: 508.0409.

1-(2-(4-Methyl-2-oxothiazol-3(2H)-yl)phenyl)-3-phenylthiourea (3o)

To a solution of isothiocyanate **2c** (200 mg, 0.8 mmol) in CH_3CN (5 mL), aniline was added (1.05 eq) and the solution stirred for 24 h at room temperature. Then, the solvent was partially evaporated and the resulting thiourea recovered by filtration: yield 80% (220 mg, white solid); mp 183°C; ^{1}H NMR (200 MHz, DMSO d_6): δ_H 2.00 (d, *J* = 1.0 Hz, 3H, C*H*$_3$), 6.28 (q, *J* = 1.0 Hz, 1H, *H5*), 7.06–7.21 (m, 1H, *Ar*), 7.24–7.60 (m, 7H, *Ar*), 7.63–7.76 (m, 1H, *Ar*), 8.87 (s, 1H, N*H*), 10.06 (s, 1H, N*H*); ^{13}C NMR (50 MHz, DMSO d_6): δ_C 15.21, 96.45, 123.11 (2C), 124.67, 127.13, 128.56 (2C), 129.18, 129.26, 130.12, 131.28, 133.02, 136.79, 139.00, 171.43, 180.27; HRMS: *m/z* [M+H]$^+$ calcd. for $C_{17}H_{16}N_3OS_2^+$: 342.0729, found: 342.0728.

1-(2-(4-Methyl-2-thioxothiazol-3(2H)-yl)phenyl)-3-(3-(trifluoromethyl)phenyl)urea (4b)

To a solution of thiazoline **1a** (1 g, 4.5 mmol) in CH_3CN (10 mL), 3-(trifluoromethyl)phenyl isocyanate (1.05 eq) was added and the solution stirred at room temperature. After 24 h, the solvent was evaporated and urea **4b** recovered by column chromatography (CH_2Cl_2/AcOEt 9.5/0.5): yield 85% (1.57 g, white solid); mp 151°C, R_f = 0.32 (CH_2Cl_2/AcOEt 9.5/0.5), ^{1}H NMR (300 MHz, DMSO d_6): δ_H 1.85 (d, *J* = 1.1 Hz, 3H, C*H*$_3$), 6.93 (q, *J* = 1.1 Hz, 1H, *H5*), 7.17–7.35 (m, 3H, *Ar*), 7.42–7.59 (m, 3H, *Ar*), 7.77 (s, 1H, N*H*), 7.98 (br s, 1H, *Ar*), 8.06–8.14 (m, 1H, *Ar*), 9.52 (s, 1H, N*H*). ^{13}C NMR (75 MHz,

DMSO d_6): δ_C 14.94, 107.86, 114.04 (q, J=4 Hz, 1C), 118.29 (d, J=4 Hz, 1C), 121.63, 123.19, 124.12 (q, J=272 Hz, 1C), 124.17, 128.01, 129.01, 129.56 (d, J=31 Hz, 1C), 130.01 (2C), 135.42, 139.97, 140.18, 152.29, 189.13; HRMS: m/z $[M+H]^+$ calcd. for $C_{18}H_{15}N_3OF_3S_2^+$: 410.0603, found: 410.0611.

1-(3,5-Bis(trifluoromethyl)phenyl)-3-(2-(4-methyl-2-thioxothiazol-3(2H)-yl)-4-(trifluoro-methyl)phenyl)urea (4f)

To a solution of thiazoline **2d** (100 mg, 0.34 mmol) in CH_3CN (10 mL), 3,5-bis(trifluoromethyl)phenyl isocyanate (1.05 eq) was added and the solution stirred at room temperature. After 24 h, the stirring was stopped and **4f** recovered by crystallization: yield 70% (131 mg, colourless crystals); mp 268°C, ^{1}H NMR (300 MHz, DMSO d_6): δ_H 1.88 (d, J=1.0 Hz, 3H, CH_3), 6.99 (q, J=1.0 Hz, 1H, *H5*), 7.69 (br s, 1H, *Ar*), 7.74–7.79 (m, 1H, *Ar*), 7.84–7.91 (m, 1H, *Ar*), 8.07 (br s, 2H, *Ar*), 8.19 (s, 1H, N*H*), 8.43–8.51 (m, 1H, *Ar*), 9.96 (s, 1H, N*H*). ^{13}C NMR (75 MHz, DMSO d_6): δ_C 14.92, 108.42, 115.19 (sept, J=4 Hz, 1C), 118.22 (d, J=3 Hz, 2C), 122.22, 123.22 (q, J=272 Hz, 2C), 123.72 (q, J=271 Hz, 1C), 123.98 (q, J=33 Hz, 1C), 127.07 (q, J=4 Hz, 1C), 127.12, 127.31 (q, J=4 Hz, 1C), 130.86 (q, J=33 Hz, 2C), 139.29, 139.76, 140.92, 151.87, 189.63; HRMS: m/z $[M+H]^+$ calcd. for $C_{20}H_{13}N_3OF_9S_2^+$: 546.0350, found: 546.0349.

1-(3,5-Bis(trifluoromethyl)phenyl)-3-(5-fluoro-2-(4-methyl-2-thioxothiazol-3(2H)-yl)-phenyl)urea (4g)

To a solution of thiazoline **1e** (300 mg, 1.25 mmol) in CH_3CN (10 mL), 3,5-bis(trifluoromethyl)phenyl isocyanate (1.05 eq) was added and the solution stirred at room temperature. After 24 h, the stirring was stopped and **4g** recovered by crystallization: yield 72% (445 mg, colourless crystals); mp 250°C, ^{1}H NMR (300 MHz, DMSO d_6): δ_H 1.87 (d, J=1.0 Hz, 3H, CH_3), 6.96 (q, J=1.0 Hz, 1H, *H5*), 7.05–7.15 (m, 1H, *Ar*), 7.25–7.36 (m, 1H, *Ar*), 7.68 (br s, 1H, *Ar*), 7.98–8.11 (m, 4H, *Ar* and N*H*), 9.92 (s, 1H, N*H*). ^{13}C NMR (75 MHz, DMSO d_6): δ_C 14.97, 108.00, 108.74 (d, J=28 Hz, 1C), 110.82 (d, J=23 Hz, 1C), 115.04 (sept, J=4 Hz, 1C), 118.10 (d, J=3 Hz, 2C), 123.22 (q, J=273 Hz, 2C), 123.40 (d, J=3 Hz, 1C), 130.83 (q, J=33 Hz, 2C), 131.22 (d, J=10 Hz, 1C), 137.10 (d, J=13 Hz, 1C), 140.05, 141.00, 151.97, 162.16 (d, J=244 Hz, 1C), 189.60; HRMS: m/z $[M+H]^+$ calcd. for $C_{19}H_{13}N_3OF_7S_2^+$: 496.0383, found: 496.0382.

1-(2-(4-Methyl-2-oxothiazol-3(2H)-yl)phenyl)-3-phenylurea (4h)

To a solution of thiazoline **1c** (185 mg, 0.90 mmol) in CH_3CN (5 mL), phenyl isocyanate (1.05 eq) was added and the solution stirred at room temperature. After 24 h, the solvent was evaporated and replaced with CH_2Cl_2 (10 mL); then the solution was washed with HCl 5% (3 × 10 mL), and the organic phase evaporated to dryness: yield 76% (221 mg, white solid); mp 146°C, ^{1}H NMR (300 MHz, DMSO d_6): δ_H 1.78 (d, J=1.2 Hz, 3H, CH_3), 6.36 (q, J=1.2 Hz, 1H, *H5*), 6.95–7.50 (m, 8H, *Ar*), 7.79 (s, 1H, 1N*H*), 8.14 (m, 1H, *Ar*), 9.14 (s, 1H, N*H*). ^{13}C NMR (75 MHz, DMSO d_6): δ_C 14.78, 96.98, 118.17 (2C), 122.05, 122.14, 123.23, 125.40, 128.85 (2C), 129.52, 129.72, 132.57, 136.83, 139.37, 152.26, 171.55; Anal. calcd. for $C_{17}H_{15}N_3O_2S$ (325.39): C, 62.75; H, 4.65; N, 12.91; S, 9.85%. Found: C, 62.76; H, 4.87; N, 13.09; S, 9.79%.

1-(2-(4-Methyl-2-oxothiazol-3(2H)-yl)phenyl)-3-(3-(trifluoromethyl)phenyl)urea (4i)

To a solution of thiazoline **1c** (236 mg, 1.17 mmol) in CH_3CN (5 mL), 3-(trifluoromethyl)phenyl isocyanate (1.05 eq) was added and the solution stirred at room temperature for 24 h. Then, the solvent was partially evaporated and the resulting urea recovered by filtration: yield 79% (355 mg, white solid); mp 234°C, ^{1}H NMR (300 MHz, DMSO d_6): δ_H 1.78 (d, J=1.2 Hz, 3H, CH_3), 6.36 (q, J=1.2 Hz, 1H, *H5*), 7.12–7.37 (m, 3H, *Ar*), 7.40–7.58 (m, 3H, *Ar*), 7.94–8.16 (m, 3H, *Ar* and N*H*), 9.48 (s, 1H, N*H*). ^{13}C NMR (75 MHz, DMSO d_6): δ_C 14.79, 97.02, 114.09 (q, J=4 Hz, 1C), 118.29 (d, J=4 Hz, 1C), 121.69, 122.45, 123.68, 124.14 (q, J=272 Hz, 1C), 125.73, 129.55, 129.57 (d, J=31 Hz, 1C), 129.76, 130.01, 132.54, 136.40, 140.21, 152.27, 171.52; Anal. calcd. for $C_{18}H_{14}F_3N_3O_2S$ (393.39): C, 54.96; H, 3.59; N, 10.68; S, 8.15%. Found: C, 55.03; H, 3.55; N, 10.78; S, 8.13%.

1-(3,5-Bis(trifluoromethyl)phenyl)-3-(2-(4-methyl-2-oxothiazol-3(2H)-yl)phenyl)urea (4j)

To a solution of thiazoline **1c** (236 mg, 1.17 mmol) in CH_3CN (5 mL), 3,5-bis(trifluoromethyl)phenyl isocyanate (1.05 eq) was added and the solution stirred at room temperature for 24 h. Then, the solvent was partially evaporated and the resulting urea recovered by filtration: yield 75% (396 mg, white solid); mp 267°C, ^{1}H NMR (300 MHz, DMSO d_6): δ_H 1.79 (d, J=1.2 Hz, 3H, CH_3), 6.36 (q, J=1.0 Hz, 1H, *H5*), 7.17–7.30 (m, 2H, *Ar*), 7.42–7.53 (m, 1H, *Ar*), 7.65 (br s, 1H, *Ar*), 7.93–8.20 (m, 4H, *Ar* and N*H*), 9.81 (s, 1H, N*H*). ^{13}C NMR (75 MHz, DMSO d_6): δ_C 14.81, 97.04, 114.62 (sept, J=4 Hz, 1C), 117.86 (d, J=3 Hz, 2C), 122.75, 123.24 (q, J=272 Hz, 2C), 124.12, 126.09, 129.60, 129.80, 130.78 (q, J=33 Hz, 2C), 132.54, 136.02, 141.41, 152.22, 171.51; Anal. calcd. for $C_{19}H_{13}F_6N_3O_2S$ (461.29): C, 49.46; H, 2.84; N, 9.11; S, 6.95 %. Found: C, 49.73; H, 2.78; N, 9.23; S, 7.11%.

1,3-Bis(3,5-bis(trifluoromethyl)phenyl)urea (4k)

To a solution of 3,5-bis(trifluoromethyl)benzenamine (200 mg, 0.87 mmol) in CH_3CN (5 mL), 3,5-bis(trifluoromethyl)phenyl isocyanate (1.05 eq) was added and the solution stirred at room temperature for 1 h. Then, the solvent was partially evaporated and the resulting urea recovered by filtration: yield 70% (296 mg, white solid); mp 244°C ([40] 240–242°C), ^{1}H NMR (300 MHz, DMSO d_6): δ_H 7.16 (br s, 2H), 7.60 (br s, 2H), 7.94 (br s, 4H). NMR data are in agreement with literature[40].

1-(3,5-Bis(trifluoromethyl)phenyl)-3-(3,5-dimethylphenyl) urea (4l)

To a solution of 3,5-dimethylbenzenamine (200 mg, 1.65 mmol) in CH_3CN (5 mL), 3,5-bis(trifluoromethyl) phenyl isocyanate (1.05 eq) was added and the solution

stirred at room temperature for 1 h. Then, the solvent was partially evaporated and the resulting urea recovered by filtration: yield 76% (472 mg, white solid); mp 235°C, ^{1}H NMR (300 MHz, DMSO d_6): δ_H 2.24 (s, 6H, 2CH_3), 6.66 (br s, 1H, *Ar*), 7.10 (br s, 2H, *Ar*), 7.62 (br s, 1H, *Ar*), 8.12 (br s, 2H, *Ar*), 8.78 (s, 1H, N*H*), 9.32 (s, 1H, N*H*). ^{13}C NMR (75 MHz, DMSO d_6): δ_C 21.07 (2C), 114.25 (sept, J = 4 Hz, 1C), 116.58 (2C), 117.88 (d, J = 4 Hz, 2C), 123.32 (q, J = 272 Hz, 2C), 124.13, 130.69 (q, J = 33 Hz, 2C), 137.81 (2C), 138.82, 141.91, 152.36; HRMS: m/z $[M+H]^+$ calcd. for $C_{17}H_{15}N_2OF_6^+$: 377.1083, found: 377.1084.

Biology

BACE-1 enzymatic assay

These experiments have been performed using BACE-1 (β-secretase) FRET Kit assay, from PanVera Corporation (Madison, WI, USA), according to the described protocol and using a multiwell spectrofluorometer instrument capable of 530–545 nm excitation and 570–590 nm emission wavelengths (Wallac Victor$^{2-8}$ 1420, Perkin Elmer, Turku, Finland). The procedure is as follow: the substrate and enzyme are diluted according to the described protocol into the provided assay buffer (50 mM Tris, pH 7.5, 10% glycerol). Each inhibitor is diluted into DMSO at the desired concentration. The substrate (Rh-EVNLDAEFK-Quencher; 10 µL of the main solution) and each inhibitor (1 µL of the corresponding solution) are introduced in the 96-well flat bottom black polystyrene plate (Corning, NY, USA). The resulting mixtures are gently mixed and 10 µL of the enzyme solution are then added to each well to start the reaction. The reaction mixtures are incubated at 25°C for 90 min and the spectrofluorescence is monitored at 530–545 nm (excitation wavelength) and 570–590 nm (emission wavelength). The kinetic assays are performed in sixplicate for each inhibitor, using BACE-1 inhibitor (H-Lys-Thr-Glu-Glu-Ile-Ser-Glu-Val-Asn-(3*S*,4*S*)-Stat-Val-Ala-Glu-Phe-OH, IC_{50} = 30 nM; Calbiochem, Beeston, UK) as reference (negative test, no clivage), the provided BACE-1 Product Standard (Rh-EVNL) as positive test (100% clivage) and a control test using only the enzyme and the substrate in the same conditions to allow a 15% clivage of the substrate after 90 minutes.

Molecular docking

BACE-1 protein coordinates were obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank, code 1SGZ and modified with Discovery Studio 2.1 (Accelrys, San Diego, USA). Chains were separated and considered individually, water molecules were removed, incomplete residues were modified with the "Clean Protein" command from the Protein Reports and Utilities module. Finally, hydrogen atoms were added, charges were calculated with CHARMm forcefield and the resulting structures were saved in the mol2 format. The obtained receptor was submitted to a quick minimization protocol to ensure correct angles and atom distances (500 steps Steepest Descent algorithm, 0.01 gradient, then 2000 steps Conjugate Gradient algorithm, 0.01 gradient). All ligands used in the study were prepared in the mol2 format with DS 2.1, and were considered as fully flexible during the docking process.

The docking software used in this study was CDocker (J Comp Chem 2003, 24, 1549–1562) as implemented in Discovery Studio 2.1. Briefly, the grid based algorithm used a simulated annealing process to account for the flexibility of the ligand into a rigid receptor, the obtained complex energy was considered to evaluate the affinity of the docked ligand. The ligands were docked into the active site using a 10 Å sphere centered on a point situated at equal distance between Asp 228 and Tyr 71 Cα. Pymol software was used to prepare Figure 1 (DeLano, W.L. The PyMOL Molecular Graphics System. (2008) DeLano Scientific LLC, Palo Alto, CA, USA. http://www.pymol.org).

Results

A library of thioureas **3a–o** was prepared according to Scheme 1. N-(2-aminophenyl)-thiazoline-2-thiones **1a,b** and N-(2-aminophenyl)-thiazolin-2-one **1c** were transformed into the corresponding isothiocyanates **2a–c** by the CS_2, DCC route under very mild conditions. These isothiocyanates are stable, they can be chromatographied using alcohol-free mobile phases and stored in the fridge for a long period of time. Intermediates **2a–c** afforded a platform from which the thioureas **3a–o** were easily prepared in good yield by reaction with the corresponding aniline, aminopyridine or aminopyrimidine at room temperature in CH_3CN.

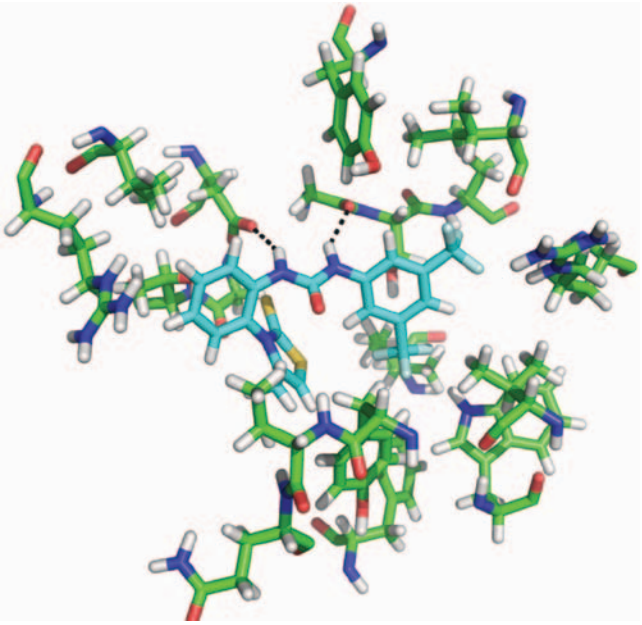
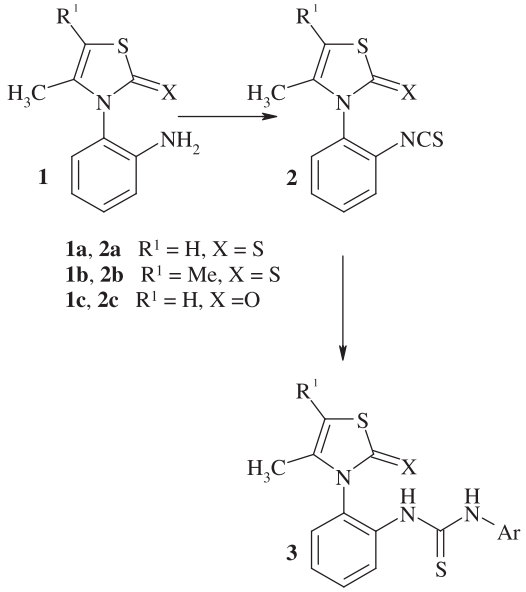
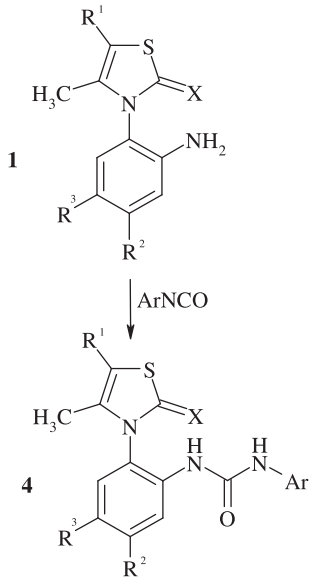


Figure 1. Molecular docking for **4c** with BACE-1.



Scheme 1. Synthesis of thioureas 3.



Scheme 2. Synthesis of ureas 4.

In contrast to isothiocyanates **2**, the isocyanates of N-(2-aminophenyl)-thiazoline-2-(thi)ones 1 were not stable. Another route was used to prepare ureas **4a–j** in good yields. N-(2-aminophenyl)-thiazoline-2-thiones **1a,b** and N-(2-aminophenyl)-thiazolin-2-ones **1c–e** were reacted with commercially available arylisocyanates under very mild conditions (Scheme 2).

All the compounds are displayed at the same time in Schemes 1 and 2 for convenience. Of course the library was elaborated step by step and the logic behind the design was strongly connected with the arriving inhibition results.

Enzymatic assays were conducted as reported in the experimental part. The percent inhibition values were determined at least six times. Selected values are summarized in Table 1.

Results in Table 1 show that the inhibition range covered by the library goes from no inhibition at 30 μM (**3k** and **4e**) to 30% inhibition at 100 nM (**4c**). Taking **3a** which produced 30% inhibition at 3 μM as a reference, the introduction of electron donating groups on the N-aryl group drastically decreased the binding. For instance, compounds **3d**, **3e** and **3f** in which the aryl group is equipped with OH, di-Me or MeO groups are inactive at 3 μM. On the other hand, a ten-fold increase in the binding (230 nM) is observed for **3g** and **3h** which present one or two CF_3 group on the aryl. Interestingly, compounds **3i**, **3k** and **3l** which present electron attracting groups weaker than CF_3 are inactive. Compounds **3b**, **3c** and **3j** (Scheme 1) in which the aryl group was replaced by a pyridine or a pyrimidine as electro-deficient groups were found inactive at 30 μM. Comparison of **3g** vs **3m** and **3h** vs **3n** indicates that the introduction of a methyl group in position 5 of the thiazoline ring significantly decreases the binding. The smaller binding for **3o** vs **3a** is probably reflecting a detrimental intramolecular hydrogen bond between a N-H of the thiourea and the carbonyl group of the heterocycle in **3o**.

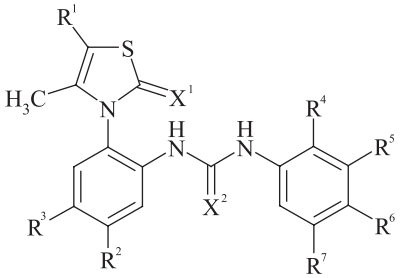
Compound **4c** gave the best binding of the whole library: 30% inhibition at 100 nM. The binding difference with thiourea **3h** and urea **4c** deserves a brief comment. It is well documented that thioureas are more acidic than the corresponding ureas and one should expect a better binding through H-bonding for the thioureas. We have previously shown that the thioureas of the **3** series adopted a Z,E conformation while the urea analogue adopted a Z,Z conformation[19]. The preorganization in the urea **4** was accounting for a better binding to carboxylate anion than the thiourea **3** which required a costly conformational change for the same binding. We believe that the same consideration is operating for the better binding observed in **4c**. Similar observations were recently reported for the binding abilities of urea and thiourea in 1,3-disubstituted thia[4]calixarenes and corresponding monofunctional receptors for their anion recognition properties[41].

The two atropisomers of **4c** showed no significant difference in binding and they gave binding values very close the one obtained for the racemate.

Figure 1 reported molecular docking studies with analogue **4c** showed that Asp 228 from BACE-1 interacts with the first NH donor of the urea while the second NH interacts with Gly 34. A close proximity was observed between the ligand CF_3 and residues Trp 76 and Arg 128.

The discussion of the data reported in Table 1 led to a coherent view of the important feature to achieve a good binding in series **3** and **4**. Ureas are better than thioureas and the east phenyl group should be equipped with two CF_3 in 3,5 positions. Based on these remarks, two very simple ureas **4k** and **4l** were prepared and assayed as BACE-1 inhibitors. 1,3-Bis(3,5-Bis(trifluoromethyl)phenyl)urea, **4k** is well known in

Table 1. BACE-1 inhibition according to a FRET assay.



Cpd	X1	X2	R1	R2	R3	R4	R5	R6	R7	% Inh (μM)*
3a	S	S	H	H	H	H	H	H	H	30 (3)
3d	S	S	H	H	H	OH	H	H	H	Inac (3)
3e	S	S	H	H	H	H	CH_3	H	CH_3	Inac (3)
3f	S	S	H	H	H	OCH_3	H	H	H	Inac (3)
3g	S	S	H	H	H	H	CF_3	H	H	35 (0.23)
3h	S	S	H	H	H	H	CF_3	H	CF_3	35 (0.23)
3i	S	S	H	H	H	Cl	H	Cl	H	Inac (3)
3k	S	S	H	H	H	H	H	CO_2H	H	Inac (30)
3l	S	S	H	H	H	H	H	F	H	Inac (3)
3m	S	S	CH_3	H	H	H	CF_3	H	H	40 (3)
3n	S	S	CH_3	H	H	H	CF_3	H	CF_3	40 (1)
3o	O	S	H	H	H	H	H	H	H	Inac (10)
4a	S	O	H	H	H	H	H	H	H	Inac (3)
4b	S	O	H	H	H	H	CF_3	H	H	Inac (3)
4c	S	O	H	H	H	H	CF_3	H	CF_3	30 (0.1)
4d	S	O	H	H	H	H	H	NO_2	H	Inac (3)
4e	S	O	H	H	H	H	H	CH_3	H	Inac (30)
4f	S	O	H	H	CF_3	H	CF_3	H	CF_3	Inac (3)
4g	S	O	H	F	H	H	CF_3	H	CF_3	Inac (3)
4h	O	O	H	H	H	H	H	H	H	30 (3)
4i	O	O	H	H	H	H	CF_3	H	H	50 (10)
4j	O	O	H	H	H	H	CF_3	H	CF_3	30 (1)

*Standard deviation on the inhibition percentages are ± 5% of indicated value.

the field of organocatalysis[42–50]. The two N-H are highly acidic and equivalent in **4k**. 1-(3,5-Bis(trifluoromethyl)-phenyl)-3-(3,5-dimethylphenyl)urea **4l** is a new compound in which the two N-H groups should present marked difference in acidity.

Urea **4k** produced 15% inhibition at 10 nM level, while urea **4l** gave 80% inhibition at 100 nM level. For the most active BACE-1 inhibitors (Table 2) **3h**, **4c**, **4k** and **4l**, curves plotting drug concentrations versus inhibition percentage were established allowing determination of the IC_{50} values (values which correspond to the drug concentration leading to 50% enzyme inhibition). The most potent BACE-1 inhibitor is analogue **4k** (IC_{50} value ranging between 0.01 and 0.1 μM), while IC_{50} values for analogues **4c** and **4l** were respectively 0.1 ± 0.03 μM and 0.08 ± 0.03 μM; in contrast IC_{50} value for analogue **3h** ranged between 0.1 and 1 μM.

In summary our study revealed a new field of application of urea and thiourea derivatives as BACE-1 inhibitors[51]. Unfortunately cell assays showed that further structure optimization is required to address the toxicity issues revealed in compounds **3** and **4** series.

Table 2. Comparison of % of inhibition for 3h, 4c, 4k and 4l at various concentrations.

Entry	10 μM	1 μM	0.1 μM	0.01 μM
3h	100%	80%	25 ± 5% (%)	Inactive
4c	100%	100%	50 ± 5%	Inactive
4k	100%	100%	100	15 ± 5%
4l	100%	100%	80 ± 5%	Inactive

Declaration of interest

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