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Substituted 2-aminothiazoles are exceptional inhibitors of neuronal degeneration in tau-driven models of Alzheimer's disease

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ABSTRACT

A novel series of 2-aminothiazoles with strong protection in an Alzheimer's disease (AD) model comprising tau-induced neuronal toxicity is disclosed. These derivatives can be synthesized in one-pot and a small SAR of the substitution within these series afforded several compounds that counteracted tau-induced cell toxicity at nanomolar concentrations. These congeners therefore have strong potential as possible treatment for Alzheimer's disease and other related tauopathies.

1. Introduction

In view of the continuously rising life expectancy of mankind, increasing numbers of patients with neurodegenerative diseases are expected particularly in the western world. This has triggered major research both at universities and in the pharmaceutical industry, with efforts centered on delineating the underlying disease mechanisms as well as on the development of new therapeutic treatments.

Generation of proteinaceous aggregates is a predominant pathological process in many common neurodegenerative diseases. In AD, accumulation of β-amyloid plaques (Aβ) (Suh and Checler, 2002) and neurofibrillary tangles comprising tau (Ballatore et al., 2007) are major pathological hallmarks. Although the underlying mechanisms of brain degeneration and cognitive impairment have not been uncovered, it is nevertheless clear that the formation of these aggregates is a noxious event responsible for neuronal demise and ultimately death. Most therapeutic efforts have concentrated so far on modulation of Aβ levels. Different strategies hereto are available, with among others the use of anti-aggregation molecules (Aisen et al., 2006), inhibitors of the processing of the amyloid precursor protein leading to reduced Aβ formation [either with β-secretase inhibitors (Citron, 2004; Dominguez et al., 2001) or γ-secretase modulators (Garofalo, 2008; Serneels et al., 2009; Weggen et al., 2001)] or aiming at increased Aβ clearance using antibodies (Gilman et al., 2005). However, where a large reduction in Aβ levels clearly can be attained, there seems to be a lack of significant clinical effects, which has led some researchers to put the amyloid cascade hypothesis into question (van Marum, 2009). Others however argue the scientific basis for the hypothesis to remain quite compelling (Aisen, 2009).

Another self-aggregating protein involved in neurodegenerative disorders is tau (Buee et al., 2000). Tau is an intracellular protein with the ability to bind and consequently stabilize and define microtubule structure and function. Apart from its physiological function tau also plays a direct role in numerous neurodegenerative disorders collectively known as "tauopathies" with the most notable examples AD and Pick's disease. Tauopathies are characterized by insoluble aggregates or polymers of tau which are formed by self-polymerization of tau monomers. An important aspect of tau-misfolding and subsequent aggregation is its inherent cytotoxicity reducing cellular integrity or even triggering cell death. In case of neurodegenerative diseases loss of affected neurons leads to cognitive and/or motoric dysfunctioning. A direct role of tau in disease onset has been established unequivocally by the elucidation of familial mutations in tau which appear to be responsible for a very early and sometimes aggressive form of tauopathy (Lee et al., 2001). Such mutations lead to changes in the amino acid

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sequence of tau (e.g. P301L or R406W) promoting toxic aggregation and thereby provoke loss of cellular integrity.

Treatments aimed to suppress cytotoxic tau pathology are presently not available. Thus there is a medical need for designing new drugs for therapeutic treatments that target the underlying molecular mechanism of tau related pathologies and toxicity such as in AD. The present manuscript will highlight a short SAR study on a series of 2-aminothiazoles, which proved to be endowed with a remarkable potency in counteracting tau-induced cytotoxicity in a human neuronal cell line model. In light of some recent publications wherein analogous but different structures based on the same 2-aminothiazole scaffold were described as interesting therapeutic leads for prion diseases (Gallardo-Godoy et al., 2011; Ghaemmaghami et al., 2010) we felt it appropriate to communicate our preliminary results on inhibition of tau-induced neuronal toxicity.

2. Materials and methods

2.1. General

NMR spectra were recorded on a Bruker AM-300 spectrometer (^{1}H 300.12 MHz, ^{13}C 75.47 MHz). All NH protons were assigned by exchange with D_2O. In case of AA'BB' systems determination of *J* is based on the assumption of an AB quartet (Becker, 1969). Mass spectrometry was carried out on a Finnigan LC Duo spectrometer (FAB^+, FAB^-). The reactions were monitored by thin-layer chromatography (TLC) analysis using silica gel plates (Kieselgel 60 F_{254}, E. Merck). Compounds were visualized by UV irradiation. Column chromatography was performed on Silica Gel 60 M (0.040–0.063 mm, E. Merck). Nomenclature of the obtained compounds follows the rules of IUPAC and was checked with Autonome (ACD-Chem-Sketch).

2.2. Syntheses of precursor ketones from nitriles – general procedure

In a 2-neck flask equipped with a reflux condenser and a dropping funnel, a suspension of magnesium (2.83 g, 0.12 mol) and alkyl bromide (0.12 mol) in dry ether were stirred under nitrogen atmosphere. After addition of a crystal of iodine the reaction started. After the reaction was completed a solution of the nitrile (0.1 mol) in dry ether (15 ml) was added drop wise. Following heating under reflux for 6 h the mixture was quenched with 6 N H_2SO_4 ice (100 ml). To complete the hydrolysis of the ketimine, the mixture was warmed and afterwards extracted with ether. After removing the solvent, the resulting ketones were used without further purification.

Example: Isobutylphenylketone (Evans and Gordon, 1938): Yield: quant; ^{1}H NMR ($CDCl_3$): 0.97, 1.00 (6H, 2s, 2xCH_3), 2.29 (1H, m, CH), 2.80 (2H, d, J = 6.6 Hz, CH_2), 7.43–7.63 (3H, m, 3,4,5H Ar), 7.93 (2H (d), 2,6H Ar).

2.3. Syntheses of α-haloketones (**4**) – general procedure

To an ice-cooled solution of the corresponding ketone (0.04 mol) in diethyl ether, bromine (0.04 mol, 2 ml) was added dropwise. The almost colorless solution was stirred at room temperature for another 20 min. After addition of aqueous $NaHCO_3$-solution (100 ml) the organic layer was separated and dried over Na_2SO_4. Following removal of the solvent, the haloketones could be used without further purification.

Example: α-Bromoisobutylphenylketone (Boswell et al., 1996) (**4b**): Yield: quant; ^{1}H NMR ($CDCl_3$): 1.05, 1.23 (6H, 2d, J = 6.6 Hz, 2xCH_3), 2.47 (1H, m, CH), 5.01 (1H, d, J = 8.4 Hz, CHC═O), 7.41–7.66 (5H, m, Ar).

2.4. General procedure for the preparation of thiazoles (**3**)

A solution of the halocarbonyl compound **4** (3 mmol) and potassium thiocyanate (0.44 g, 4.5 mmol) in methanol (10 ml) was stirred at ambient temperature for 30 min. Subsequently the respective amine **5** was added drop wise (solution in 5 ml MeOH) and the reaction mixture was heated under reflux overnight. After reducing the volatiles to approximate 1 ml and cooling to ambient temperature the precipitate was filtered off and washed with water. Recrystallization from either ethanol/water (4:1) or acetone/water (5:1) afforded the crystalline products **3**. The purity of the products was verified by TLC (ethyl acetate/petrol ether 1:3). In case the product did not crystallize from the reaction mixture, the volatiles were removed. The obtained solid was treated with water (5 ml) to remove inorganic impurities and extracted with ethyl acetate. After drying the organic layer over Na_2SO_4 and removal of the solvent the desired products either crystallized or could be obtained by flash-column chromatography (silica, *n*-hexane/ethyl acetate 3:1) as a powder. Alternatively this modified Hantzsch synthesis can be carried out under microwave assisted conditions, heating the mixture under reflux at 100 W/80 °C for 60 min. Workup was carried out as described above.

2.4.1. 5-Methyl-N,4-diphenylthiazol-2-amine (**3a**)

Yield 85%; ^{1}H NMR (DMSO-d_6): δ 2.43 (3H, s, 5-CH_3), 6.89 (1H, t, J = 7.3 Hz, 4-H NPh), 7.26–7.36 (3H, m, 2,4,6-H 4-Ph), 7.46 (2H, (t), J ∼ 7.5 Hz, 3,5-H 4-Ph), 7.64–7.68 (4H, m, 2,3,5,6H NPh), 10.06 (1H, s, br, ex, NH); ^{13}C NMR (DMSO-d_6): δ 117.3, 145.3, 159.4 (5, 4, 2C thiazole); R^1: 127.5, 128.4, 128.7, 135.1 (4C, 2,6C, 3,5C, 1C); R^2: 12.4; R^3: 117.3, 120.8, 129.4, 141.1 (2,6C, 3,5C, 4C, 1C); MS *m/z* 267 $[M+H]^+$.

2.4.2. 5-Isopropyl-N,4-diphenylthiazol-2-amine (**3b**)

Yield 87%; ^{1}H NMR (DMSO-d_6): δ 1.26, 1.27 (6H, d, J = 6.6 Hz, 2xCH_3), 2.50 (1H, m, 5-CH), 6.91 (1H, t, J = 7.3 Hz, 4-H NPh), 7.28 (2H, (t), J ∼ 7.6 Hz, 3,5H 4-Ph), 7.35 (1H, t, J = 7.3 Hz, 4-H 4Ph), 7.49 (2H, (t), J ∼ 7.4 Hz, 3,5-H NPh), 7.58 (2H, d, J = 8.1 Hz, 2,6-H 4-Ph), 7.64 (2H, d, J = 8.1 Hz, 2,6-H NPh), 10.08 (1H, s, br, ex, NH); ^{13}C NMR (DMSO-d_6): δ 117.2, 144.5, 160.1 (5, 4, 2C thiazole); R^1: 127.7, 128.7, 129.4, 136.0 (4, 2,6, 3,5, 1C); R^2: 26.0 (2xCH_3), 27.4 (CH); R^3: 117.2, 121.3, 131.0, 141.8 (2,6, 3,5, 4, 1C); MS *m/z* 295 $[M+H]^+$.

2.4.3. 4-(4-Fluorophenyl)-5-methyl-N-phenylthiazol-2-amine (**3c**)

Yield 82%; ^{1}H NMR (DMSO-d_6): δ 2.40 (3H, s, 5-CH_3), 6.91 (1H, t, J = 7.3 Hz, 4-H NPh), 7.24–7.31 (4H, m, 2,6-H 4-Ar, 2,6H NPh), 7.64–7.72 (4H, m, 3,5-H 4-Ar, 3,5-H NPh), 10.10 (1H, s, br, ex, NH); ^{13}C NMR (DMSO-d_6): δ 116.7, 144.6, 159.7 (5, 4, 2C thiazole); R^1: 115.5, 115.8; 130.3, 130.4; 132.1, 132.15; 160.0, 163.2 (3,5, 2,6, 1, 4); R^2: 12.4; R^3: 117.1, 121.4, 130.3, 141.7 (2,6, 4, 3,5, 1C); MS *m/z* 285 $[M+H]^+$.

2.4.4. 4-(4-Chlorophenyl)-5-methyl-N-phenylthiazol-2-amine (**3d**)

Yield 86%; ^{1}H NMR (DMSO-d_6): δ 2.46 (3H, s, 5-CH_3), 6.92(1H, t, J = 7.3 Hz, 4-H NPh), 7.30 (2H, (t), J ∼ 7.3 Hz, 3,5-H NPh), 7.49 (2H, d, J = 8.1 Hz, 2,6-H NPh), 7.62, 7.65; 7.68, 7.71 (4H, AA′BB′, J ∼ 8.5 Hz, 4-Ar), 10.07 (1H, s, br, ex, NH); ^{13}C NMR (DMSO-d_6): δ 117.6, 144.5, 159.8 (5, 4, 2C thiazole); R^1: 128.8, 130.1, 132.1, 134.4, (3,5, 2,6, 1, 4); R^2: 12.4; R^3: 117.1, 121.5, 129.4, 141.7, (2,6, 4, 3,5, 1C); MS *m/z* 301 $[M+H]^+$.

2.4.5. 4-(4-Chlorophenyl)-5-methyl-N-(naphtalen-1-yl)thiazol-2-amine (**3e**)

Yield 82%; ^{1}H NMR (DMSO-d_6): δ 2.42 (3H, s, 5-CH_3), 7.49–7.70, 7.92, 8.22–8.31 (11H, m, H Ar), 9.98 (1H, s, br, ex, NH); ^{13}C NMR (DMSO-d_6): δ 118.1, 144.3, 161.8 (5, 4, 2C thiazole); R^1: 128.8,

130.1, 134.4, 134.5 (3,5, 2,6, 1, 4C); R^2: 12.5; R^3: 116.4, 122.6, 123.3, 126.0, 126.2, 126.5, 126.5, 128.7, 132.0, 132.2 (2, 4, 8, 7, 8a, 6, 3, 5, 4a, 1C); MS *m/z* 351 $[M+H]^+$.

*2.4.6. 4-(2,4-Dichlorophenyl)-5-methyl-N-(naphtalen-1-yl)thiazol-2-amine (**3f**)*

Yield 88%; ^{1}H NMR (DMSO-d_6): δ 2.15 (3H, s, 5-CH_3), 7.41–7.55 (6H, m, H-Ar), 7.60 (1H, s, 3-H 4Ar), 7.71, 7.89, 8.32 (3H, NAr), 10.01 (1H, s, br, ex, NH); ^{13}C NMR (DMSO-d_6): δ 199.9, 142.9, 162.2 (5, 4, 2C thiazole); R^1: 127.7, 129.6, 133.7, 134.3, 134.4, 137.1 (5, 6, 3, 2, 4, 1C); R^2: 11.9; R^3: 116.1, 122.6, 123.1, 125.9, 126.0, 126.5, 126.6, 128.7, 133.6, 133.8 (2, 4, 8, 7, 8a, 6, 3, 5, 4a, 1C); MS *m/z* 385 $[M+H]^+$.

*2.4.7. 4-(4-Bromophenyl)-N-(3-chlorophenyl)-5-methylthiazol-2-amine (**3g**)*

Yield 73%; ^{1}H NMR (DMSO-d_6): δ 2.27 (3H, s, 5-CH_3), 6.97 (1H, d, *J* = 7.5 Hz, 6-H NAr), 7.31 (1H, t, *J* = 7.9 Hz, 5-H NAr), 7.64–7.99 (6H, m, H-Ar), 10.35 (1H, s, br, ex, NH); ^{13}C NMR (DMSO-d_6): δ 118.3, 144.5, 160.7 (5, 4, 2C thiazole); R^1: 119.7, 130.4, 131.6, 132.7 (4, 3,5, 2,6, 1C); R^2: 12.4; R^3: 116.7, 119.7, 129.4, 131.0, 131.2, 132.6 (6, 2, 4, 5, 3,1C); MS *m/z* 379 $[M+H]^+$.

*2.4.8. 4-(4-Chlorophenyl)-N-(3-chlorophenyl)-5-methylthiazol-2-amine (**3h**)*

Yield 72%; ^{1}H NMR (DMSO-d_6): δ 2.43, (3H, s, 5-CH_3), 6.94 (1H, d, *J* = 7.5 Hz, 6-H NAr), 7.31 (1H, t, *J* = 7.95 Hz, 5-H NAr), 7.50, 7.53, 7.67, 7.70 (4H, AA′BB′, *J* = 8.2 Hz, 4-Ar), 7.87 (1H, s, 2-H NAr), 8.07 (1H, d, *J* = 8.7 Hz, 4-H NAr), 10.04 (1H, s, br, ex, NH); ^{13}C NMR (DMSO-d_6): δ 118.5, 143.0, 159.3 (5, 4, 2C thiazole); R^1: 128.9, 130.1, 133.7, 134.3, (3,5, 2,6, 1, 4C), R^2: 12.3, R^3: 116.4, 120.9, 129.5, 131.0, 131.3, 132.2 (6, 2, 4, 5, 3, 1C); MS *m/z* 335 $[M+H]^+$.

*2.4.9. N^1-(4-(3-Bromophenyl)-5-methylthiazol-2-yl)benzene-1,4-diamine (**3i**)*

Yield 76%; ^{1}H NMR (DMSO-d_6): δ 2.49 (3H, s, 5-CH_3), 4.20 (2H, s, br, ex, NH_2), 7.40 (1H, (t) *J* ∼ 7.8 Hz, 5-H 4Ar), 7.51 (1H, d, *J* = 7.9 Hz, 6-H 4Ar), 7.58 (4H, m, NAr), 7.65 (1H, d, *J* = 7.7 Hz, 4-H 4Ar), 7.80 (1H, s, 2-H 4Ar), 10.03 (1H, s, br, ex, NH); ^{13}C NMR (DMSO-d_6): δ 122.1, 137.9, 160.2 (5, 4, 2C thiazole), R^1: 120.1, 127.3, 130.2, 130.8, 131.0, 135.8 (3, 6, 4, 2, 5, 1C); R^2: 12.4; R^3: 117.7, 118.2, 135.8, 143.0 (3,5, 2,6, 1, 4C); MS *m/z* 360 $[M+H]^+$.

*2.4.10. N^1-(4-(4-Chlorophenyl)-5-methylthiazol-2-yl)benzene-1,4-diamine (**3j**)*

Yield 72%; ^{1}H NMR (DMSO-d_6): δ 2.38 (3H, s, 5-CH_3), 4.13 (2H, s, br, ex, NH_2), 7.47, 7.65 (4H, AA′BB′, *J* = 7 Hz 4Ar), 7.57 (4H, m, NAr), 10.02 (1H, s, br, ex, NH); ^{13}C NMR (DMSO-d_6): δ 118.3, 144.0, 159.9 (5, 4, 2C thiazole), R^1: 128.6, 129.8, 131.6, 134.2 (3,5, 2,6, 1, 4C); R^2: 12.0; R^3: 117.8, 117.9, 135.6, 144.0 (3,5, 2,6, 1, 4C); MS *m/z* 316 $[M+H]^+$.

*2.4.11. N^1-(4-(4-Bromophenyl)-5-methylthiazol-2-yl)benzene-1,4-diamine (**3k**)*

Yield 69%; ^{1}H NMR (DMSO-d_6): δ 2.40 (3H, s, 5-CH_3), 7.57 (2H, s, br, ex, NH_2), 7.63 (4H, m, H Ar), 9.97 (1H, s, br, ex, NH); ^{13}C NMR (DMSO-d_6): δ 120.5, 144.5, 160.3 (5, 4, 2C thiazole), R^1: 126.3, 130.4, 131.7, 134.4 (4, 3,5, 2,6, 1C); R^2: 12.5; R^3: 116.9, 118.2, 135.8, 143.0 (3,5, 2,6, 1, 4C); MS *m/z* 360 $[M+H]^+$.

*2.4.12. N^1-(4-(2,4-Dichlorophenyl)-5-methylthiazol-2-yl)benzene-1,4-diamine (**3l**)*

Yield 74%; ^{1}H NMR (DMSO-d_6): δ 22.09 (3H, s, 5-CH_3), 4.85 (2H, s, br, ex, NH_2), 6.53, 7.19 (4H, AA′BB′, *J* = 8.5 Hz, NAr), 7.46 (2H, (s), 5,6H 4-Ar), 7.70 (1H, s, 3-H 4-Ar), 9.49 (1H, s, br, ex, NH); ^{13}C NMR (DMSO-d_6): δ 117.5, 144.3, 162.3 (5, 4, 2C thiazole); R^1: 127.7, 129.5, 133.6, 133.7, 133.9, 134.2 (5, 6,3, 2, 4, 1C); R^2: 11.9; R^3: 114.8, 120.3, 131.4, 143.0 (3,5, 2,6, 1, 4C); MS *m/z* 350 $[M+H]^+$.

*2.4.13. N-(3,5-Dimethylphenyl)-4,5-dimethylthiazol-2-amine (**3m**)*

Yield 63%; ^{1}H NMR (DMSO-d_6): δ 2.24, 2.27 (2x3H, 2xs, 4-CH_3, 5-CH_3), 6.73 (1H, s, 4-H NAr), 6.98 (2H, s, 2,6-H, NAr), 9.00 (1H, s, br, ex, NH); ^{13}C NMR (DMSO-d_6): δ 113.6, 142.3, 162.5 (5, 4, 2C thiazole); R^1: 14.5; R^2: 11.0; R^3: 21.4 (2xCH_3), 116.2, 124.4, 139.0, 141.0 (4, 3,5, 2,6, 1C); MS *m/z* 233 $[M+H]^+$.

*2.4.14. N-(3,5-Dimethylphenyl)-5-methyl-4-phenylthiazol-2-amine (**3n**)*

Yield 86%; ^{1}H NMR (DMSO-d_6): δ 2.24 (6H, s, 2xCH_3), 2.42 (5-CH_3), 6.58 (1H, s, 4-H NAr), 7.24 (2H, s, 2,6H NAr), 7.33 (1H, t, *J* = 7.3 Hz, 4-H 4-Ph), 7.46 (2H, (t), *J* ∼ 7.4 Hz, 3,5-H 4-Ph), 7.66 (2H, d, *J* = 7.5 Hz, 2,6-H, 4-Ph), 9.89 (1H, s, br, ex, NH); ^{13}C NMR (DMSO-d_6): δ 116.6, 145.7, 159.8 (5, 4, 2C thiazole); R^1: 127.5, 128.4, 128.8, 135.7 (4, 2,6, 3,5, 1C); R^2: 12.4; R^3: 21.7 (2xCH_3), 115.0, 123.1, 138.3, 141.7 (4, 3,5, 2,6, 1C); MS *m/z* 295 $[M+H]^+$.

*2.4.15. N-(3,5-Dimethylphenyl)-5-isopropyl-4-phenylthiazol-2-amine (**3o**)*

Yield 89%; ^{1}H NMR (DMSO-d_6): δ 1.25, 1.27 (6H, d, *J* = 6.6 Hz, 2xCH_3), 2.22 (6H, s, 2xCH_3Ar), 2.50 (1H, m, 5-CH), 6.57 (s, 4-H NAr), 7.22 (2H, s, 2,6-H, NAr), 7.34 (1H, t, *J* = 7.3 Hz, 4-H 4Ph), 7.46 (2H, (t), *J* ∼ 7.5 Hz, 2,6-H 4Ph), 7.55 (2H, d, *J* = 7.5 Hz, 2,6-H 4Ph), 9.22 (1H, s, br, ex, NH); ^{13}C NMR (DMSO-d_6): δ 118.0, 144.3, 160.2 (5, 4, 2C thiazole); R^1: 127.7, 128.7, 128.8, 136.1 (4, 2,6, 3,5, 1C); R^2: 26.02 (2xCH_3), 27.4 (CH); R^3: 21.7 (2xCH_3), 115.1, 123.2, 138.3, 141.7 (4, 3,5, 2,6, 1C); MS *m/z* 323 $[M+H]^+$.

*2.4.16. 4-(3-Bromophenyl)-N-(3,5-dimethylphenyl)-5-methylthiazol-2-amine (**3p**)*

Yield 82%; ^{1}H NMR (DMSO-d_6): δ 2.24 (6H, s, 2xCH_3Ar), 2.43 (3H, s, 5-CH_3), 6.59 (1H, s, 4-H NAr), 7.24 (2H, s, 2,6-H NAr), 7.44 (1H, (t), *J* ∼ 7.7 Hz, 5-H 4Ar), 7.52 (1H, d, *J* = 7.7 Hz, 4-H 4Ar), 7.68 (1H, d, *J* = 7.5 Hz, 6-H 4Ar), 7.86 (1H, s, 2-H 4Ar), 9.97 (1H, s, br, ex, NH); ^{13}C NMR (DMSO-d_6): δ 118.1, 143.7, 159.9 (5, 4, 2C thiazole); R^1: 122.2, 127.0, 130.2, 130.9, 131.0, 137.9 (3, 6, 4, 5, 2, 1C); R^2: 12.4; R^3: 21.7 (2xCH_3), 115.1, 123.3, 138.4, 141.6 (4, 3,5, 2,6, 1C); MS *m/z* 373 $[M+H]^+$.

*2.4.17. 4-(4-Chlorophenyl)-N-(3,5-dimethylphenyl)-5-methylthiazol-2-amine (**3q**)*

Yield 87%; ^{1}H NMR (DMSO-d_6): δ 2.23 (6H, s, 2 CH_3Ar), 2.41 (3H, s, 5-CH_3), 6.57 (1H, s, 4-H NAr), 7.23 (2H, s, 2,6-H NAr), 7.49, 7.51; 7.67, 7.69 (4H, AA′BB′, *J* = 7.5 Hz, 4-Ar), 9.94 (1H, s, br, ex, NH); ^{13}C NMR (DMSO-d_6): δ 117.3, 144.4, 159.9 (5, 4, 2C thiazole); R^1: 128.8, 130.0, 132.0, 134.5 (4, 3,5, 2,6, 1C); R^2: 12.4; R^3: 21.7 (2xCH_3), 115.0, 123.2, 138.3, 141.6 (4, 3,5, 2,6, 1C); MS *m/z* 329 $[M+H]^+$.

*2.4.18. 4-(4-Bromophenyl)-N-(3,5-dimethylphenyl)-5-methylthiazol-2-amine (**3r**)*

Yield 81%; ^{1}H NMR (DMSO-d_6): δ 2.23 (6H, s, 2xCH_3Ar), 2.41 (3H, s, 5-CH_3), 6.57 (1H, s, 4-H, NAr), 7.23 (2H, s, 2,6-H NAr), 7.62 (4H, AA′BB′, 4-Ar), 9.96 (1H, s, br, ex, NH); ^{13}C NMR (DMSO-d_6): δ 117.4, 144.6, 159.9 (5, 4, 2C thiazole); R^1: 120.6, 130.3, 131.7, 134.8, (4, 3,5, 2,6, 1C); R^2: 12.4; R^3: 21.7 (2xCH_3), 115.0, 123.2, 138.3, 141.6 (4, 3,5, 2,6, 1C); MS *m/z* 373 $[M+H]^+$.

*2.4.19. 4-(4-Fluorophenyl)-N-(3,5-dimethylphenyl)-5-methylthiazol-2-amine (**3s**)*

Yield 74%; ^{1}H NMR (DMSO-d_6): δ 2.23 (6H, s, 2xCH_3Ar), 2.40 (3H, s, 5-CH_3), 6.57 (1H, s, 4-H NAr), 7.23 (2H, s, 2,6-H NAr), 7.27, 7.69 (4H, AA‘BB‘4-Ar), 9.94 (1H, s, br, ex, NH); ^{13}C NMR (DMSO-d_6): δ 116.4, 144.6, 159.8 (5, 4, 2C thiazole), R^1: 115.5, 115.8;

130.3, 130.4; 132.2; 160.2, 163.3 (3,5, 2,6, 1, 4C); R^2: 12.4; R^3: 21.7 (2xCH_3), 115.0, 123.2, 138.3, 141.7 (4, 3,5, 2,6, 1C); MS *m/z* 313 $[M+H]^+$.

*2.4.20. 4-(2,4-Dichlorophenyl)-N-(3,5-dimethylphenyl)-5-methylthiazol-2-amine (**3t**)*

Yield 82%; ^{1}H NMR (DMSO-d_6): δ 2.15 (3H, s, 5-CH_3), 2.20 (6H, s, 2xCH_3Ar), 6.56 (1H, s, 4-H NAr), 7.17 (2H, s, 2,6-H NAr), 7.49 (2H, 5,6-H 4-Ar), 7.73 (1H, s, 3-H 4Ar), 9.92 (1H, s, br, ex, NH); ^{13}C NMR (DMSO-d_6): δ 119.3, 143.0, 160.4 (5, 4, 2C thiazole); R^1: 127.7, 129.7, 133.5, 133.6, 133.8, 134.3 (5, 6, 3, 2, 4, 1C); R^2: 11.9; R^3: 21.7 (2xCH_3), 115.0, 123.2, 138.3, 141.5 (4, 3,5, 2,6, 1C); MS *m/z* 363 $[M+H]^+$.

*2.4.21. N-Benzyl-5-methyl-4-phenylthiazol-2-amine (**3u**)*

Yield 65%; ^{1}H NMR (DMSO-d_6): δ 2.33 (3H, s, 5-CH_3), 4.43 (2H, d, *J* = 5.9 Hz, CH_2Bn), 7.22–7.561 (10H, m, 4-Ph, Bn), 7.85 (1H, t, ex, *J* = 5.9 Hz, NH); ^{13}C NMR (DMSO-d_6): δ 114.9, 145.5, 164.9 (5, 4, 2C thiazole); R^1: 127.3, 128.6, 128.7, 136.0 (4, 2,6, 3,5, 1C); R^2: 12.6; R^3: 47.9 (CH_2), 127.2, 127.9, 128.4, 139.9 (4, 2,6, 3,5, 1C); MS *m/z* 281 $[M+H]^+$.

*2.4.22. 4-(3-Bromophenyl)-5-methyl-N-(4-methylpyridin-2-yl)thiazol-2-amine (**3v**)*

Yield 48%; ^{1}H NMR (DMSO-d_6): δ 2.50 (3H, s, 5-CH_3), 2.56 (3H, s, Ar-CH_3), 7.43–7.85 (8H, m, aromatic-H), 10.2 (1H, s, br, ex, NH); ^{13}C NMR (DMSO-d_6): δ 118.0, 145.5, 158.8 (5, 4, 2C thiazole); R^1: 131.0, 132.0, 132.1, 140.8 (4, 3,5, 2,6, 1C); R^2: 12.6; R^3: 22.7 (CH_3), 118.0, 122.1, 132.1, 148.0, 148.4 (6, 4, 5, 1, 3C); MS *m/z* 360 $[M+H]^+$.

2.5. Use of tau expressing cells as a model of neuronal degradation

Sub-cloning the cDNA of human TAU-P301L into mammalian expression vector pcDNA3.1 resulted in plasmid pcDNA3.1-TAU-P301L. Plasmids pcDNA3.1 and pcDNA3.1-TAU-P301L were transfected to human neuroblastoma cells (BE-M17; ATCC No. CRL-2267) and independent clonal lines with the plasmids stably integrated into the genome were selected. These resulted in cell lines named M17-3.1 and M17-TAU-P301L (transfected with pcDNA3.1 and pcDNA3.1-TAU-P301L, respectively). Expression of the TAU-P301L genes in the cell lines was confirmed by Western analysis.

Tau induced cytotoxicity was determined as follows: from appropriate precultures of M17-3.1 and M17-TAU-P301L, cells were differentiated for 7 days with 2.5 μM retinoic acid in the presence of the tested compounds. Hereto, stock solutions of the test compounds were prepared in DMSO and the appropriate amount was added to the respective wells. The same amount of DMSO was used throughout all experiments. LDH activity was determined using Promega Cytotox 96 non-radioactive cytotoxicity assay, (Cat. G1780) according the supplier's instructions. The potency of the compounds under study for protection versus neuronal insult, could be determined by evaluating these substances at different concentrations ranging from non-effective (thus at a relatively low concentration) to an effective concentration for their ability to reduce LDH activity of retinoic acid incubated M17-TAU-P301L cells. These measurements were used to calculate the respective EC_{50} values.

3. Results and discussion

3.1. Synthesis of the 2-aminothiazole congeners

The cytoprotective effect of a series of *N*-aminoimidazole (NAIM) compounds (highlighted by compounds **1** and **2**, Fig. 1) has been documented before (Stevens et al., 2007), and was followed-up by an in-house study uncovering the 2-aminothiazole scaffold **3** as a new lead for tau-induced cytotoxicity. This general scaffold has three variables, the thiazole 4- and 5-substituents R^1 and R^2, and the amine moiety R^3, with overall geometry resembling the NAIM core.

The thiazoles were assembled according to a slightly modified version of the Hantzsch thiazole synthesis (Hantzsch, 1890; Hantzsch and Weber, 1887; Schantl and Lagoja, 1998). Herein, the condensation of one eq of α-halo ketones **4** with potassium thiocyanate (1.2 eq) and an (hetero)aromatic or aliphatic amine derivative **5** in refluxing methanol yielded the corresponding *N*-aminothiazole derivatives **3a–v** in good to excellent yield. By use of microwave assisted synthesis the reaction time could significantly be decreased as worked out for some analogs (1.5 h compared to 10 h), without affecting the yield. The reaction is suitable for aromatic, heteroaromatic or aliphatic substituted halocarbonyl aldehydes and ketones, and aromatic, heteroaromatic or aliphatic amines. A possible synthetic pathway is lined out in Scheme 1. The required (halo)ketones if commercially not available, could be obtained from the respective nitriles *via* Grignard reaction followed by α-bromination.

3.2. Cytoprotective activity in a neuronal cell line model of tau-induced toxicity

Specific clinical mutations predispose tau for toxic aggregation. To recapitulate this process a tau expression plasmid was constructed by sub-cloning the cDNA of human TAU-P301L (encoding for tau with proline 301 substituted by a leucine residue) into a mammalian expression vector, which subsequently was transfected to human neuroblastoma cells. The expression of TAU-P301L in M17-TAU-P301L cells was found to confer increased toxicity relative to control cells not over-expressing wild type tau (data not shown). In degenerated or dead cells intracellular LDH leaks out into the extracellular environment due to a loss of plasma-membrane integrity. This principle was used to determine tau related cytotoxicity by quantifying the level of leaked LDH into the growth medium. The M17-TAU-P301L cell line therefore made it possible to assess the ability of novel compounds (when brought in contact with the cells) to counteract tau cytotoxicity since such compounds are expected to inhibit tau-instigated LDH leakage. In this way the neuroprotective potential of the aminothiazoles as described above was evaluated (Table 1).

As can be seen in Table 1, several compounds with nanomolar activity were uncovered. Although the number of compounds is still too small for complete SAR conclusions, some interesting trends can already be deduced. The starting compound **3a** with unsubstituted phenyl rings already inhibited the tau-induced toxicity at 53 ng/ml or approximately 180 nM concentrations. In search of the key variables, substitution of the aromatic R^1-phenyl for a methyl moiety reduced the potency 5-fold (compare **3n** with **3m**). On the other hand, introduction of the larger isopropyl moiety at the 5-position while keeping the 4-phenyl ring, afforded conflicting results. The 10-fold reduction in potency for **3b** versus **3a** is in sharp contrast with the results for *N*-3,5-dimethylphenyl substituted aminothiazoles, with **3o** displaying the strongest inhibitory activity within this series with a 5-fold reduction compared to **3n**.

In general, para-halogenation of the phenyl ring R^1 increased the potency (**3c and d** versus **3a**, and **3q–s** versus **3n**), but meta-bromination likewise does (compare **3p** with **3n** and notice the analogous activity for **3i**, **3j** and **3k**). Introducing a *p*-methoxy-phenyl substituent at R^1 however annihilated the activity, regardless the nature of R^2 and R^3 (not shown). Considerable improvement of activity is noticed with the more bulky α-naphtyl at R^3 (**3g and h**) and even more pronounced with a *p*-amino-phenyl (**3i–l**)

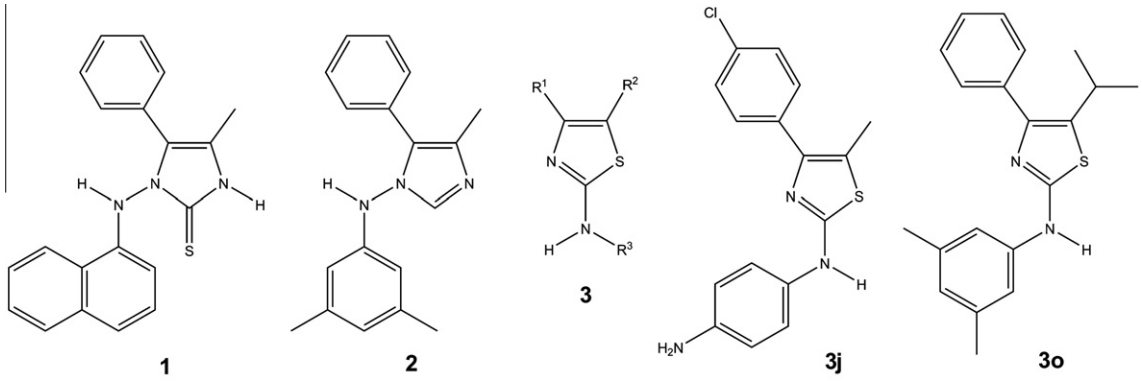
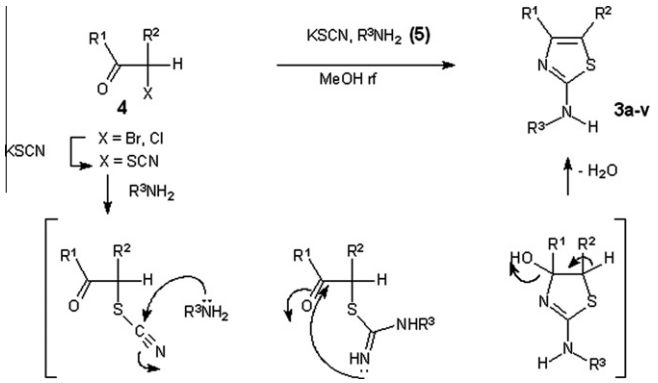


Fig. 1. Chemical structures of the cytoprotective *N*-aminoimidazole lead compounds **1** and **2** and general new thiazole scaffold **3a**–**v** under study with **3j** and **3o** as prototype examples.

or a 3,5-dimethylphenyl (**3m–t**) moiety, culminating as mentioned in **3o** with an IC_{50} of 4 ng/ml or 14 nM. However, the lipophilicity as calculated average for different programs is further increased, and might hamper future *in vivo* applications. Nevertheless, while all compounds are endowed with a high log *P* value, this does not seem to prevent the *in vitro* activity in this cellular system. Lipophilicity by itself proved not to be a prime determinant for the inhibitory properties within this series as can be deduced from Table 1. The strong lipophilicity is reflected as well in the high R*f*-values for the system n-hexane: ethyl acetate 3:1, which for all compounds are found in the range of 0.86–0.88.

Furthermore, although the orientation of the meta-methyl is preserved in **3v**, the introduction of the more polar pyridine ring at R^3 almost annihilated the inhibitory potency in comparison with **3p**. Likewise introducing an extra methylene spacer using an *N*-benzyl moiety as in **3u** afforded a considerable loss in activity.



Scheme 1. Plausible reaction mechanism for synthesis of the aminothiazole scaffold.

Table 1
Substitution pattern, calculated log *P* data and inhibitory activity of the aminothiazoles **3**.

Compound	R^1	R^2	R^3	log *P*[a]	IC_{50}[b]
3a	Ph	Me	Ph	4.75	53
3b	Ph	*i*-Pr	Ph	5.55	582
3c	4-F-Ph	Me	Ph	4.92	46
3d	4-Cl-Ph	Me	Ph	5.36	32
3e	4-Cl-Ph	Me	α-Naphtyl	6.46	7
3f	2,4-DiCl-Ph	Me	α-Naphtyl	7.05	51
3g	4-Br-Ph	Me	3-Cl-Ph	6.09	1750
3h	4-Cl-Ph	Me	3-Cl-Ph	5.97	220
3i	3-Br-Ph	Me	4-NH_2-Ph	4.59	9
3j	4-Cl-Ph	Me	4-NH_2-Ph	4.45	7
3k	4-Br-Ph	Me	4-NH_2-Ph	4.59	8
3l	2,4-DiCl-Ph	Me	4-NH_2-Ph	5.05	11
3m	Me	Me	3,5-DiMe-Ph	4.14	90
3n	Ph	Me	3,5-DiMe-Ph	5.52	20
3o	Ph	*i*-Pr	3,5-DiMe-Ph	6.30	4
3p	3-Br-Ph	Me	3,5-DiMe-Ph	6.26	9
3q	4-Cl-Ph	Me	3,5-DiMe-Ph	6.13	6
3r	4-Br-Ph	Me	3,5-DiMe-Ph	6.25	12
3s	4-F-Ph	Me	3,5-DiMe-Ph	5.68	16
3t	2,4-DiCl-Ph	Me	3,5-DiMe-Ph	6.72	10
3u	Ph	Me	Bn	4.61	300
3v	3-Br-Ph	Me	4-Me-2-pyridinyl	5.24	1250

[a] The average log *P* values are given as calculated by a variety of programs on the ALOGPS 2.1 program website (VCCLAB, 2005).
[b] In ng/ml.

3.3. Comparison with related aminothiazole scaffolds for modulation of proteinopathies

Generation of proteinaceous aggregates either by misprocessing or misfolding cause serious cellular toxicity leading to neurodegenerative diseases. In our study we focused on counteracting the cytotoxicity as provoked by formation of tau neurofibrillary tangles, one of the hallmarks of AD, using compounds based on a 2-aminothiazole scaffold. Remarkably, some very recent communications by Prusiner and Renslo likewise report on the use of 2-aminothiazoles as novel lead compounds with antiprion properties (Gallardo-Godoy et al., 2011; Ghaemmaghami et al., 2010). Either formation of the misfolded endogenous prion protein into a β-sheet-rich form (PrP^{Sc}) leading to oligomeric deposits or their clearance seem to be influenced by the 2-aminothiazole scaffold, leading to lowered toxicity. The structures for the most active compound within their series (**6a**) and for the one evaluated for bioavailability in mice (**6b**) are shown in Fig. 2.

While prion diseases and AD both can be classified belonging to proteinopathies, the latter is very different from the former. Both proteins PrP^{Sc} and tau eventually end up as β-sheet fibrils, which are structurally similar, but it has not been proven that full blown aggregates are the primary toxic conformation for the tau related toxicity. Hence, where for antiprion properties one primarily screens for reduction in the PrP^{Sc} load, here the specific cytotoxicity elicited by tau aggregation is determined by evaluating the plasma membrane integrity.

As already described by Ghaemmaghami et al., the aminothiazole scaffold exhibits very diverse pharmacology and can be found in many therapeutic classes with at first glance only slight structural changes. Clearly also some differences can be found between our set of compounds and the one of the University of California (UCal). Structurally, the most obvious one is that UCal's most active

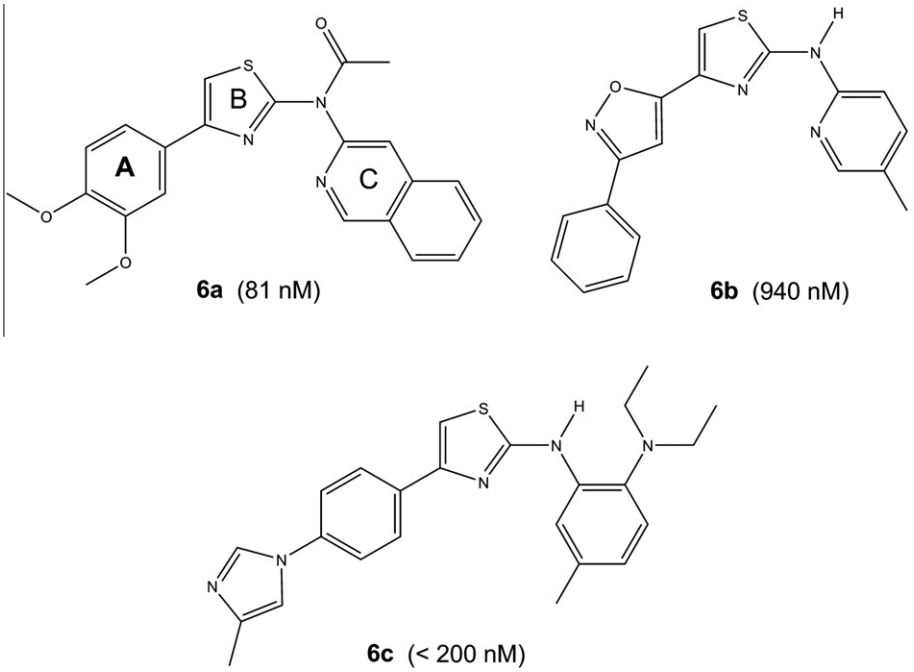


Fig. 2. Resembling known compounds: representative structures for the antiprion aminothiazole derivatives (**6a** and **b**) with annotation of the aromatic ring system A, B and C, and for the anti-β-amyloid compounds (**6c**) with their reported activity in nM.

derivative (**6a**) has an acyl moiety on the amino group, whereas all the derivatives we tested have a secondary amine. Further, while all compounds from UCal lack a substituent on position 5 of the thiazole ring (excluding the tricyclic derivatives), all our congeners carry a 5-methyl or 5-isopropyl moiety. Where coplanar arrangement of the aromatic A- and B-ring (respectively the 4-aryl and the thiazole ring, as shown in **6a**) is believed to be important for antiprion activity as shown in the UCal papers, one can argue therefore that analogs comprising a 5-alkyl substituent prohibiting this coplanarity, will be hampered in their antiprion activity. Yet, most active congeners in the 5-H series display low micromolar or submicromolar activity, with only the most active compound having a potency of 81 nM. In contrast, within the 5-alkyl series several congeners were uncovered being endowed with sub-50 nM activity in reducing tau instigated toxicity. It is therefore plausible that the mechanism or target by which the compounds act will be different.

This is further illustrated with the lack of activity in our series when introducing a pyridine ring on the amine, where all antiprion compounds carry either a pyridine or quinoline ring as the C-moiety. In addition, where ortho substitution on the A ring is not tolerated for antiprion activity, we do not observe this intolerance, with a strong anti-tau toxicity found for compounds **3l** and **3t** (sub-50 nM), and to a lower extent for **3f**, all having a 2,4-dichlorophenyl moiety at the 4-position of the thiazole scaffold. Some patent reports filed by Neurogenetic Pharmaceuticals likewise claim reduction in β-amyloid levels using a 2-aminothiazole scaffold as the central part of a series of compounds comprising several aromatic rings as exemplified by structure **6c** (Neurogenetic Pharmaceuticals, 2004, 2010). Most of the hundreds of analogs reported herein likewise are devoid of a 5-alkyl substituent, and show in general only micromolar activities. These structures therefore have more resemblance to the UCal derivatives. Overall, we can conclude that we have uncovered a new scaffold based on the aminothiazole ring system, providing strong inhibition of neuronal toxicity induced by tau self-polymerization via a new as yet unidentified mechanism.

4. Conclusion

A cellular assay was developed for evaluation of tau-instigated neuronal toxicity by sub-cloning and transfecting the cDNA of a clinically relevant mutated tau into a human neuroblastoma cell-line. The lead compound 5-methyl-*N*,4-diphenylthiazol-2-amine (**3a**) was found to counteract tau-induced cell toxicity at submicromolar concentration. Hence, a straightforward one-pot modification of the Hantzsch thiazole synthetic scheme was used to obtain a 2-aminothiazole scaffold with 3 variables. Several congeners were found which strongly counteracted tau-induced cell toxicity culminating in **3o** with an IC_{50} of 14 nM. A clear structural distinction on several points was made with some resembling 2-aminothiazole derivatives endowed with antiprion properties. The here uncovered thiazole derivatives therefore seem to have strong potential as possible treatment for Alzheimer's disease and other related tauopathies.

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