Research article

MRC

Received: 26 March 2014 Revised: 11 May 2014 Accepted: 15 May 2014 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/mrc.4088

A comparative study between *para*-aminophenyl and *ortho*-aminophenyl benzothiazoles using NMR and DFT calculations

G. K. Pierens,* T. K. Venkatachalam and D. Reutens

***Ortho*-substituted and *para*-substituted aminophenyl benzothiazoles were synthesised and characterised using NMR spectroscopy. A comparison of the proton chemical shift values reveals significant differences in the observed chemical shift values for the NH protons indicating the presence of a hydrogen bond in all *ortho*-substituted compounds as compared to the *para* compounds. The presence of intramolecular hydrogen bond in the *ortho* amino substituted aminophenyl benzothiazole forces the molecule to be planar which may be an additional advantage in developing these compounds as Alzheimer's imaging agent because the binding to amyloid fibrils prefers planar compounds. The splitting pattern of the methylene proton next to the amino group also showed significant coupling to the amino proton consistent with the notion of the existence of slow exchange and hydrogen bond in the *ortho*-substituted compounds. This is further verified by density functional theory calculations which yielded a near planar low energy conformer for all the *o*-aminophenyl benzothiazoles and displayed a hydrogen bond from the amine proton to the nitrogen of the thiazole ring. A detailed analysis of the ^{1}H, ^{13}C and ^{15}N NMR chemical shifts and density functional theory calculated structures of the compounds are described. Copyright © 2014 John Wiley & Sons, Ltd.**

Keywords: aminophenyl benzothiazoles; hydrogen bond; ^{1}H NMR; ^{13}C NMR; ^{15}N NMR; DFT calculations

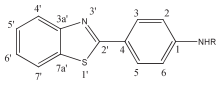
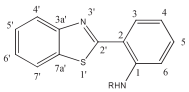
Introduction

Aminophenyl benzothiazoles are a unique class of compounds possessing extraordinary biological activity. In recent years, attention has been focused on the development of novel derivatives of aminophenyl benzothiazoles to utilise them as biologically useful compounds.[1,2] Stevens *et al*.[3–5] have extensively studied these compounds for their anticancer properties. The cytotoxicity profiles of polyhydroxylated 2-phenyl benzothiazoles against human tumour cell lines compared well with Genistein and the benzothiazoles acted as tyrosine kinase inhibitors similar to Genistein and other known flavones. Several aminophenyl benzothiazole derivatives have been reported.[6] Interestingly, introduction of groups at the 3′ position of the phenyl moiety enhanced the potency of the compounds and derivatives with methyl, chloro, bromo and iodo groups in that position showed unprecedented activity against several tumour cell lines. In light of the biological activity of benzothiazole derivatives, other applications have been explored including possible application as a diagnostic agent in Alzheimer's disease. The progressive neuronal loss associated with this disease is believed to occur because of the formation of senile plaques and neurofibrillary tangles within the brain. Several studies have implicated a short peptide containing 39–43 amino acids as the main constituent of amyloid plaques.[7,8] These are produced by the proteolytic cleavage of a precursor protein by secretases[9] to form insoluble fibrils that deposit in the brain. Todd *et al*.[10] has reported a comprehensive review on the development and application of various compounds for amyloid imaging. Aminophenyl benzothiazoles have been highlighted as ideal candidates for brain imaging. The [^{11}C]-6-Me-benzothiazole has been prepared by methylation of 4-(6-methyl-2-benzothiazolyl) aniline using [^{11}C] methyl iodide.[11] Additional modification by removing the 6-methyl group in the structure gave [^{11}C] BTA ((*N*-methyl-[^{11}C])-2-(4′-(methylaminophenyl)-benzothiazole)) which showed improved uptake and washout characteristics in normal mice. It also showed vivo specificity towards amyloid fibrils in the brain of transgenic mouse models of Alzheimer's disease and human AD brain homogenates.[11,12] The 6-hydroxy substituted amino methyl benzothiazole derivative is also known as Pittsburgh Compound B (PIB)[13–17] and has received Federal Drug Administration approval as an imaging contrast agent for Alzheimer's disease. Although the 4-aminophenyl benzothiazoles have been studied extensively, the synthesis and characterisation of the *o*-aminophenyl benzothiazole derivatives have not been extensively described and is the subject of this paper. The compounds we studied were 2-(4′-aminophenyl)benzothiazole (4′-(benzo[d]thiazole-2yl)aniline) (**1**) and 2-(2′-aminophenyl) benzothiazole (2′-(benzo[d]thiazole-2yl)aniline) (**8**) analogues (Table 1). The compounds throughout this manuscript will be referred to as aminophenyl benzothiazoles. We include analysis of the structure using density functional theory (DFT) calculations. The aim for studying the structural feature of

* *Correspondence to: G. K. Pierens, Centre for Advanced Imaging, The University of Queensland, St. Lucia, Brisbane 4072, Australia.*
E-mail: g.pierens@uq.edu.au

Centre for Advanced Imaging, The University of Queensland, St.Lucia, Brisbane 4072, Australia

Table 1. Structure numbering scheme and IUPAC names for the synthesised compounds

Compound number (IUPAC name)	R group	Compound number (IUPAC name)
		
1 (4-(benzo[d]thiazole-2′-yl)aniline)	−H	**8** (2-(benzo[d]thiazole-2′-yl)aniline)
2 (4-(benzo[d]thiazole-2′-yl)-*N*-methylaniline)	$-CH_3$	**9** (2-(benzo[d]thiazole-2′-yl)-*N*-methylaniline)
3 (4-(benzo[d]thiazole-2′-yl)-*N*-ethylaniline)	$-CH_2CH_3$	**10** (2-(benzo[d]thiazole-2′-yl)-*N*-ethylaniline)
4 (4-(benzo[d]thiazole-2′-yl)-*N*-propylaniline)	$-CH_2CH_2CH_3$	**11** (2-(benzo[d]thiazole-2′-yl)-*N*-propylaniline)
5 (4-(benzo[d]thiazole-2′-yl)-*N*-butylaniline)	$-CH_2CH_2CH_2CH_3$	**12** (2-(benzo[d]thiazole-2′-yl)-*N*-butylaniline)
6 (4-(benzo[d]thiazole-2′-yl)-*N*-isopropylaniline)	$-CH(CH_3)_2$	**13** (2-(benzo[d]thiazole-2′-yl)-*N*-isopropylaniline)
7 (4-(benzo[d]thiazole-2′-yl)-*N*-(but-2″-en-1″-yl)aniline)	$-CH_2CH=CHCH_3$	**14** (2-(benzo[d]thiazole-2′-yl)-*N*-(but-2″-en-1″-yl)aniline)

ortho-aminophenyl substituted benzothiazole is to investigate whether the hydrogen bond formation helps to further stabilise the molecule due to planarity. This aspect is important in terms of utility of these compounds as Alzheimer's imaging agents, because all the known imaging agents (thioflavin T derivatives) are planar which aids the binding of these molecules to amyloid fibrils.[18–20] Recently, Petric *et al.*[21] reported the binding affinities of two planar molecules of dicyanovinyl naphthalenes for neuroimaging of amyloid and came to the conclusion that the most planar analogues showed the highest binding affinities towards amyloid while the least planar analogue showed lowest binding affinity.

Experimental

All the chemicals were obtained from Sigma Aldrich and were used without further purification. NMR spectra were recorded in deuterated chloroform, and the chemical shifts for protons are reported relative to the residual chloroform signal at 7.25 ppm. The NMR data were acquired on a Bruker 900 MHz NMR spectrometer equipped with a cryoprobe. The proton spectra were acquired with a sweep width of 12 ppm centred at 5 ppm. The carbon spectra were acquired with a sweep width of 200 ppm centred at 105 ppm. The residual chloroform peak at 77 ppm was used as a ^{13}C chemical shift reference. The COSY experiments were acquired with a sweep width of 12 ppm using a 90° pulse of 9 μs with 128 increments, respectively. The ^{13}C HSQC spectra were acquired with sweep widths of 12 and 200 ppm for proton and carbon, respectively, and the carbon centred at 100 ppm. Additionally, HMBC spectral data were also acquired to establish the structures of the compounds (^{13}C sweep width of 200 ppm). The ^{15}N spectra were acquired using a sweep width of 400 ppm and urea was used as external standard at 73.4 ppm.

The synthesis of the *o*-substituted aminophenyl benzothiazole was accomplished by following the synthetic scheme in Fig. 1.

In brief, *o*-amino thiophenol was condensed with *o*-aminobenzoic acid in polyphosphoric acid at 220 °C to furnish the *o*-aminophenyl benzothiazole. This was further alkylated using alkyl bromide/iodide in the presence of potassium carbonate and acetonitrile to yield the desired products. A detailed description of synthesis and isolation of the products will be reported elsewhere.[22] The *para*-substituted aminophenyl benzothiazoles were prepared in a similar fashion using

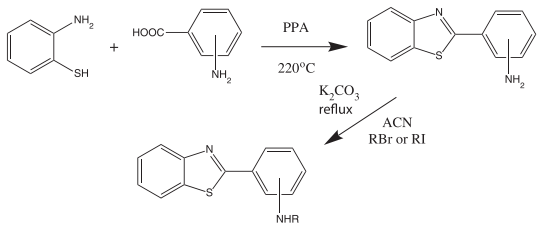


Figure 1. Scheme illustrating the synthesis of *p*-aminophenyl and *o*-aminophenyl alkyl substituted compounds.

4-aminobenzoic acid instead of *o*-aminobenzoic acid. Several mono alkylated amino derivatives were prepared, and their melting points (°C) are (1):156–157;(2):166–167; (3):127–128; (5):119–120; (6):137–138; (7):126–127; (8):128–130; (9):11–112; (10):61–62; (11):81–82; (12):71–72; (13):93–94; (14):128–130.

Molecular Modelling

Monte Carlo conformational searching was performed using Macromodel V10.1 (Schrodinger, LLC, New York).[23] Torsional sampling (MCMM) was performed with 1000 steps per rotatable bond. Each step was minimised with the OPLS-2005 force field using the TNCG method with maximum iterations of 50 000 and energy convergence threshold of 0.02. All other parameters were left as the default values. All low energy conformations (<5 kcal/mol from global minimum) were further optimised using DFT calculations in Jaguar V8.1 (Schrödinger, LLC, New York) [24] using B3LYP/6-311++G(d,p) with chloroform solvent).

Results and Discussion

Tables 2 and 3 show the ^{1}H NMR chemical shifts observed for the *para*-substituted and *ortho*-substituted aminophenyl benzothiazoles in deuterated chloroform at room temperature, respectively. Compound **1** which is the parent *para* amino substituted derivative showed a broad singlet at 4.0 ppm corresponding to the free amino group in the structure. The NMR spectrum further showed four doublets and two triplets associated with the aromatic protons. Compound **2** showed the methyl resonance at 2.90 ppm as a singlet and the NH protons appeared at 4.12 ppm as a broad singlet and all other proton signals were consistent

Table 2. ^{1}H NMR chemical shifts of *p*-aminophenyl benzothiazoles (**1–7**), in $CDCl_3$

Proton	1[28,29]	2	3	4	5	6	7
2	6.72 (d, 1H, *J* = 8.5)	6.64 (d, 1H, *J* = 8.6)	6,64 (d, 1H, *J* = 7.4)	6,63 (d, 1H, *J* = 8.6)	6.63 (d, 1H, *J* = 8.6)	6.61 (d, 1H, *J* = 8.4)	6.64 (d, 1H, *J* = 8.5)
3	7.88 (d, 1H, *J* = 8.3)	7.92 (d, 1H, *J* = 8.6)	7.91 (d, 1H, *J* = 8.6)	7.90 (d, 1H, *J* = 8.6)	7.90 (d, 1H, *J* = 8.6)	7.89 (d, 1H, *J* = 8.4)	7.90 (d, 1H, *J* = 8.5)
5	7.88 (d, 1H, *J* = 8.3)	7.92 (d, 1H, *J* = 8.6)	7.91 (d, 1H, *J* = 8.6)	7.90 (d, 1H, *J* = 8.6)	7.90 (d, 1H, *J* = 8.6)	7.89 (d, 1H, *J* = 8.4)	7.90 (d, 1H, *J* = 8.5)
6	6.72 (d, 1H, *J* = 8.5)	6.64 (d, 1H, *J* = 8.6)	6.64 (d, 1H, *J* = 7.4)	6.63 (d, 1H, *J* = 8.6)	6.63 (d, 1H, *J* = 8.6)	6.61 (d, 1H, *J* = 8.4)	6.64 (d, 1H, *J* = 8.5)
4′	7.99 (d, 1H, *J* = 8.2)	7.98 (d, 1H, *J* = 8.1)	7.98 (d, 1H, *J* = 8.2)	7.96 (d, 1H, *J* = 8.0)	7.97 (d, 1H, *J* = 8.1)	7.97 (d, 1H, *J* = 8.0)	7.97 (d, 1H, *J* = 8.4)
5′	7.43 (t, 1H, *J* = 7.75)	7.43 (t, 1H, *J* = 7.5)	7.42 (t, 1H, *J* = 7.98)	7.42 (t, 1H, *J* = 7.5)	7.42 (t, 1H, *J* = 7.5)	7.42 (t, 1H, *J* = 7.6)	7.42 (t, 1H, *J* = 7.5)
6′	7.31 (t, 1H, *J* = 7.55)	7.30 (t, 1H, *J* = 7.4)	7.30 (t, 1H, *J* = 7.6)	7.29 (t, 1H, *J* = 7.6)	7.29 (t, 1H, *J* = 7.5)	7.29 (t, 1H, *J* = 7.4)	7.30 (t, 1H, *J* = 7.5)
7′	7.84 (d, 1H, *J* = 7.7)	7.83 (d, 1H, *J* = 7.8)	7.82 (d, 1H, *J* = 7.6)	7.83 (d, 1H, *J* = 7.9)	7.83 (d, 1H, *J* = 7.7)	7.83 (d, 1H, *J* = 7.9)	7.83 (d, 1H, *J* = 7.8)
1″	4.0 (bs, 2H)	4.12 (bs, 1H)	4.0 (bs, 1H)	4.04 (bs, 1H)	4.04 (bs, 1H)	3.90 (bs, 1H)	4.10 (bs, 1H)
2″	—	2.90 (s, 3H)	3.23 (q, 2H, *J* = 7.0)	3.16 (t, 2H, *J* = 7.2)	3.18 (t, 2H, *J* = 7.3)	3.71 (m, 1H)	3.75 (d, 2H, *J* = 5.7)
3″	—	—	1.29 (t, 3H, *J* = 7.0)	1.67 (m, 2H)	1.63 (m, 2H)	1.24 (d, 3H, *J* = 6.4)	5.57 (m, 1H)
4″	—	—	—	1.02 (t, 3H, *J* = 7.5)	1.44 (m, 2H)	1.24 (d, 3H, *J* = 6.4)	5.73 (m, 1H)
5″	—	—	—	—	0.97 (t, 3H, *J* = 7.2)	—	1.71 (d, 3H, *J* = 6.4)

Table 3. ^{1}H NMR chemical shifts of *o*-aminophenyl benzothiazoles (**8–14**) in $CDCl_3$

Proton	8[29]	9	10	11	12	13	14
3	7.70 (d, 1H, *J* = 8.0)	7.73 (d, 1H, *J* = 7.7)	7.74 (d, 1H, *J* = 7.8)	7.72 (d, 1H, *J* = 8.3)	7.72 (d, 1H, *J* = 7.8)	7.72 (dd, 1H, *J* = 7.8,1.4)	7.74 (d, 1H, *J* = 7.6)
4	6.74 (t, 1H, *J* = 7.1)	6.69 (t, 1H, *J* = 7.4)	6.69 (t, 1H, *J* = 7.0)	6.66 (t, 1H, *J* = 7.0)	6.67 (t, 1H, *J* = 7.4)	6.65 (t, 1H, *J* = 7.5)	6.69 (t, 1H, *J* = 6.4)
6	6.78 (d, 1H, *J* = 8.1)	6.77 (d, 1H, *J* = 8.5)	6.79 (d, 1H, *J* = 8.4)	6.78 (d, 1H, *J* = 8.3)	6.78 (d, 1H, *J* = 8.2)	6.79 (d, 1H, *J* = 8.3)	6.78 (d, 1H, *J* = 4.3)
5	7.22 (t, 1H, *J* = 7.1)	7.34 (t, 1H, *J* = 7.7)	7.32 (t, 1H, *J* = 7.8)	7.30 (t, 1H, *J* = 7.6)	7.31 (t, 1H, *J* = 7.8)	7.29 (t, 1H, *J* = 7.7)	7.30 (t, 1H, *J* = 7.6)
4′	7.97 (d, 1H, *J* = 8.1)	7.96 (d, 1H, *J* = 8.0)	7.96 (d, 1H, *J* = 8.0)	7.93 (d, 1H, *J* = 8.3)	7.93 (d, 1H, *J* = 8.0)	7.93 (d, 1H, *J* = 7.8)	7.95 (d, 1H, *J* = 8.0)
5′	7.45 (t, 1H, *J* = 7.1)	7.44 (t, 1H, *J* = 7.7)	7.45 (t, 1H, *J* = 7.6)	7.43 (t, 1H, *J* = 7.6)	7.44 (t, 1H, *J* = 7.4)	7.43 (t, 1H, *J* = 7.5)	7.44 (t, 1H, *J* = 7.4)
6′	7.35 (t, 1H, *J* = 7.7)	7.34 (t, 1H, *J* = 7.5)	7.34 (t, 1H, *J* = 7.4)	7.33 (t, 1H, *J* = 7.5)	7.34 (t, 1H, *J* = 7.6)	7.33 (t, 1H, *J* = 7.5)	7.34 (t, 1H, *J* = 7.4)
7′	7.87 (d, 1H, *J* = 7.7)	7.85 (d, 1H, *J* = 7.7)	7.86 (d, 1H, *J* = 7.8)	7.85 (d, 1H, *J* = 7.8)	7.86 (d, 1H, *J* = 7.8)	7.85 (d, 1H, *J* = 7.8)	7.85 (d, 1H, *J* = 7.6)
1″	6.40 (bs, 2H)	8.79 (bs, 1H)	8.86 (bs, 1H)	8.96 (bs, 1H)	8.96 (bs, 1H)	8.94 (bs, 1H)	9.1 (bs, 1H)
2″	—	3.04 (d, 3H)	3.38 (m, 2H, *J* = 7.0)	3.29 (q, 2H, *J* = 6.9)	3.32 (m, 2H)	3.82 (m, 1H)	3.93 (d, 2H, *J* = 4.3)
3″	—	—	1.46 (t, 3H, *J* = 7.2)	1.82 (m, 2H)	1.79 (m, 2H)	1.36 (d, 3H, *J* = 6.3)	5.68 (m, 1H)
4″	—	—	—	1.12 (t, 3H, *J* = 7.5)	1.54 (m, 2H)	1.36 (d, 3H, *J* = 6.3)	5.82 (m, 1H)
5″	—	—	—	—	1.03 (t, 3H, *J* = 7.5)	—	1.78 (d, 3H, *J* = 6.4)

with the structure. The comparison of the ^{1}H NMR chemical shifts of all the *p*-aminophenyl substituted derivatives showed that the aromatic signals were almost identical. This is expected because there is no significant change in the main structural frame of the compounds. As expected, the methyl signal from compound **2** appeared as a singlet and the methylene signal of other compounds in the series showed the expected splitting patterns due to coupling to the adjacent protonated carbons (Fig. 2).

The ^{1}H NMR chemical shifts for the *o*-aminophenyl substituted benzothiazole (compound **8**) showed the NH_2 proton as a broad singlet at 6.4 ppm. Comparing this NH_2 proton with compound **1** described in the previous paragraph, it is evident that the *o*-aminophenyl substituted compound showed a downfield shift of nearly 2.40 ppm compared to *p*-aminophenyl substituted compound **1**, indicating that this proton is in slow exchange and is possibly involved in a hydrogen bond with the thiazole moiety. There are two positions in the thiazole ring that contain heterocyclic atoms such as nitrogen or sulfur which could be involved in the hydrogen bond. On the basis of electronegativity differences among them, the most preferred is likely to be the nitrogen atom of the thiazole ring. The hydrogen bonded structure should yield a stable six membered ring.

The formation of such intramolecular hydrogen bond has been proposed previously by Dey *et al.*[25] in dealing with the solvatochromism of aminophenyl benzothiazole derivatives. It was proposed that the lone pair of electrons associated with the nitrogen group is being forced to be parallel with the π-cloud, thereby altering the resonance characteristics. The formation of such an intramolecular hydrogen bond is expected to shift the resonance downfield, and this was confirmed experimentally. Comparing the other chemical shift values between the aromatic proton resonances, we found that there was only a marginal change in the chemical shift values between the *o*-aminophenyl and the *p*-aminophenyl benzothiazole derivatives.

Comparison of the chemical shift values between the *N*-methyl substituted derivatives (compounds **2** and **9**) showed small changes in the methyl proton's chemical shift value from 2.90 to 3.04 ppm, respectively. However, there was a significant change in the splitting pattern which changed from a singlet to a doublet (Fig. 2). The doublet arises in compound **9** due to the coupling with the amino proton. The amino proton showed a significant change from 4.12 to 8.79 ppm and from a very broad resonance to a broad peak with some structure resembling a quartet. This splitting pattern is expected because this proton is coupled to the methyl proton and further confirms that the amino proton is involved in a hydrogen bond with the proton in slow exchange. All other aromatic proton signals in the benzothiazole fragment were unchanged.

The other substituted aminophenyl compounds showed a similar chemical shift change for the amino group from 3.90–4.12 to 8.86–9.10 ppm for the *para*-substituted and *ortho*-substituted compounds, respectively. All other aromatic proton signals in the benzothiazole fragment are very similar. All the *para*-compounds showed the expected splitting pattern, but the *ortho*-substituted derivatives showed the extra splitting in the methylene multiplet adjacent to the amino group. Again, this is due to the coupling to the amino proton which is in slow exchange due to being involved in a hydrogen bond. Examples of the observed splitting pattern for the methylene proton for the *para*-substituted and *ortho*-substituted compounds are shown in Fig. 2. The coupling constant to the amino proton in the methyl, ethyl, propyl and butyl substituted compounds is 4.8 Hz; however, it increases to 6.2 Hz in the isopropyl-substituted compound. The amino peak in the *ortho*-substituted compounds showed splitting associated with the coupling to the adjacent protons in the alkyl group (Fig. 3)

We then examined the carbon NMR chemical shifts of the compounds in chloroform solution (Tables 4 and 5). Most of the carbon chemical shift values were very similar with no significant change in the chemical shift values with different alkyl groups. Additionally, there was no significant change in the chemical shift values of the benzothiazole carbons for the *para*-substituted and *ortho*-substituted compounds. In summary, introduction of various alkyl groups in the amine functionality of the compounds had no significant effect on the carbon chemical shift values.

The effect of various alkyl groups on ^{15}N chemical shift values were examined (Table 6). Chemical shifts were determined by acquiring a ^{15}N HMBC experiment. The ^{15}N chemical shift for the thiazole ring nitrogen appeared at approximately 290 ppm in all compounds and was usually much less intense than for the amine nitrogen. This was expected as changes in the

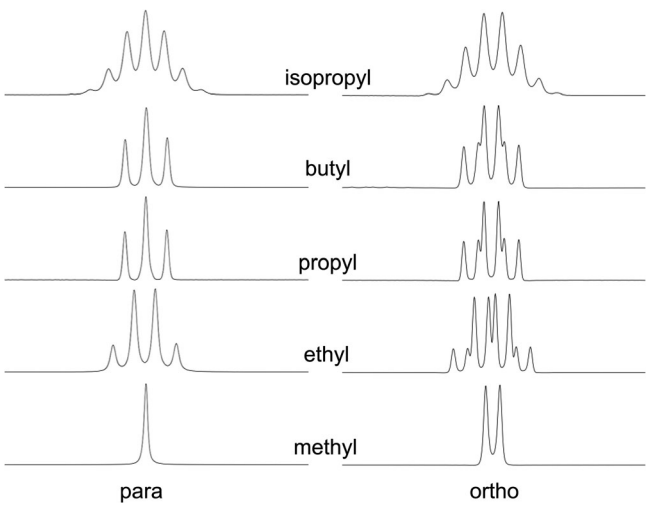


Figure 2. Expansion of the methylene multiplet adjacent to the amino group for the *para*-substituted and *ortho*-substituted compounds (the spectra are referenced so that all the peaks are aligned and slight resolution enhancement has been applied before FT).

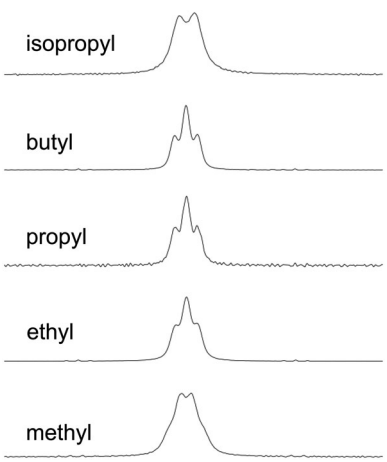


Figure 3. Expansion of the amino multiplet of the ortho compounds showing the splitting pattern (the spectra are referenced so that all the peaks are aligned and slight resolution enhancement has been applied before FT).

Table 4. ^{13}C NMR chemical shifts for *p*-aminophenyl benzothiazoles (**1–7**) in $CDCl_3$

Carbon	1[28,29]	2	3	4	5	6	7
1	149.2	151.6	150.7	150.8	150.8	149.8	150.6
2	114.7	112.0	112.3	112.2	112.2	112.6	112.5
3	129.6	129.1	129.2	129.1	129.1	129.2	129.1
4	123.8	122.4	122.4	122.4	122.8	122.2	122.8
5	129.6	129.1	129.2	129.1	129.1	129.2	129.1
6	114.7	112.0	112.3	112.2	112.2	112.6	112.5
2′	168.5	168.4	168.8	168.8	168.8	168.7	168.9
3a′	154.2	154.2	154.1	154.4	154.3	154.3	154.5
4′	122.4	122.3	122.3	122.3	122.3	122.3	122.3
5′	126.0	126.0	126.0	126.0	126.0	125.9	126.0
6′	124.4	124.2	124.3	124.2	124.2	124.2	124.3
7′	121.4	121.3	121.4	121.3	121.3	121.3	121.4
7a′	134.5	134.4	134.4	134.5	134.5	134.5	134.6
2″	—	30.3	38.1	45.3	43.2	44.0	45.5
3″	—	—	14.7	29.7	31.5	22.8	127.1
4″	—	—	—	11.6	20.2	22.8	128.6
5″	—	—	—	—	13.9	—	17.8

Table 5. ^{13}C NMR chemical shifts for *o*-aminophenyl benzothiazoles (**8–14**) in $CDCl_3$

carbon	8[29]	9	10	11	12	13	14
1	146.1	148.0	147.5	147.7	147.7	146.1	147.4
2	115.3	114.8	114.5	114.5	114.5	114.4	114.0
3	130.3	130.6	130.7	130.7	130.7	130.9	130.6
4	116.9	115.0	114.9	114.8	114.8	114.6	115.2
5	131.5	132.1	132.1	132.1	132.1	131.9	132.0
6	116.8	110.9	111.3	111.3	111.3	111.8	111.8
2′	169.2	169.6	169.6	169.6	169.6	169.5	169.5
3a′	153.7	153.6	153.6	153.6	153.6	153.6	153.5
4′	122.4	122.2	122.2	122.2	122.2	122.2	122.3
5′	126.0	125.9	125.9	125.9	125.9	125.9	126.0
6′	124.8	124.7	124.7	124.7	124.7	124.7	125.0
7′	121.2	121.1	121.1	121.1	121.1	121.0	121.1
7a′	137.2	133.0	133.1	133.1	133.2	133.1	133.2
2″	—	30.4	37.8	44.8	42.7	43.6	44.8
3″	—	—	14.6	22.4	31.3	22.9	127.6
4″	—	—	—	11.8	20.5	22.9	127.2
5″	—	—	—	—	14.0	—	17.8

Table 6. ^{15}N NMR chemical shifts for *p* and *o*-aminophenyl benzothiazoles in $CDCl_3$

Compound	*Para*-substituted			*Ortho*-substituted		
	#	NH–R	ring N	#	NH–R	ring N
NH_2	**1**	58.9	293.3	**8**	65.2	295.3
$NHCH_3$	**2**	57.6	289.6	**9**	61.7	291.7
$NHCH_2CH_3$	**3**	76.3	*	**10**	79.8	293.3
$NHCH_2CH_2CH_3$	**4**	73.2	*	**11**	77.2	291.5
$NHCH_2CH_2CH_2CH_3$	**5**	73.5	290.6	**12**	77.7	292.0
$CH(CH_3)_2$	**6**	90.8	291.3	**13**	95.0	291.2
$CH_2CH=CHCH_3$ (trans)	**7**	72.5	291.5	**14**	74.9	292.6

* Not observable using the similar experimental conditions.

structural characteristics of the benzothiazole moiety did not affect proton or carbon chemical shifts, as discussed previously. The ^{15}N chemical shift of the amino group in the molecule was significantly affected by the alkyl substituent. For example, in the case of *para*-aminophenyl benzothiazole derivatives, compound **1** showed a chemical shift value of 58.9 ppm for the

amino group. Increasing length of the alkyl side chain caused a deshielding of the NH chemical shifts. The chemical shift value was 57.6 ppm for the methyl-substituted derivative and ethyl, propyl and butyl and crotyl substitution showed a significant shift in the chemical shift values (73–76 ppm). The isopropyl-substituted derivative showed the highest chemical shift value of 90.8 ppm perhaps owing to the dimethyl group in the structure of the compound.

The ^{15}N chemical shift values of the alkyl substituted *o*-aminophenyl benzothiazole revealed the following trend; compound **8** showed a chemical shift value of 65.2 ppm as compared to 58.9 ppm for the *para* amino substituted compound **1**. The other compounds in the *ortho* series showed similar trend in chemical shift values (Table 6) to that observed for the *p*-aminophenyl analogues. The isopropyl-substituted compound again showed the highest chemical shift value of 95 ppm akin to that obtained for the *para*-substituted compound 6 (90.0 ppm). Duthaler *et al.*[26] have reported the effects of *N*-alkyl substituents upon the ^{15}N chemical shifts of the aminic nitrogen. The increments on the chemical shift values satisfactorily apply to the effects observed in the present study. The chemical shift values did not follow a systematic trend as larger alkyl groups were introduced into the structure of aminophenyl benzothiazole compounds[27–29]. *Ortho*-substituted derivatives had higher chemical shift values than the corresponding *para*-substituted derivatives.

We then proceeded to investigate the structures of the compounds using molecular modelling. Firstly, the conformational space was investigated by performing a Monte Carlo conformational search using the MacroModel software (Schrodinger, LLC),[23] and then, each conformation was further optimised by Jaguar (Schrodinger, LLC)[24] with the inclusion of chloroform solvent. The final structures were compared to eliminate any duplicate structures. After the macromodel conformational search, all the *para*-substituted derivatives showed the two aromatic ring being in a planar arrangement and the N–H bond being in-plane to the phenyl ring. On the other hand, the *ortho*-substituted derivatives had a non-planar arrangement of the aromatic rings from 40° to 80° and none of the compounds showed a hydrogen bond to the nitrogen or the sulfur. From the DFT calculations, the *p*-aminophenyl benzothiazole (compound **1**) showed one planar conformer due to the symmetry of the molecule. The angle between the thiazole and phenyl ring was 1.3°, and the amine had a planar arrangement with respect to the phenyl ring due to delocalisation of the nitrogen lone pair.

The *o*-aminophenyl benzothiazole derivative (compound **8**) resulted in two conformers. The lowest energy conformer had an angle between the benzothiazole and the phenyl ring of 1.6°, which was significantly different to that predicted by the molecular mechanics calculation. The N–H bond was almost coplanar with the phenyl ring at 8.9°. The conformation resulted in a hydrogen bond between the NH proton and the nitrogen atoms of the thiazole ring, in agreement with proton NMR results. The second conformer was found to have an angle between the benzothiazole ring and the phenyl ring of 34.1°, and the N–H bond was 16.8° out of the plane of the phenyl ring. The Boltzmann population distribution of these two conformers was calculated to be 99.92 : 0.08 indicating that the hydrogen bonded conformer was almost exclusively preferred.

For compound **9** (NHMe), conformational searching and further optimization with Jaguar yielded similar results. The *p*-methylamino compound **2** had two conformers due to rotation around the C–N bond. The benzothiazole to phenyl ring angle of 1.3° was very close to a planar configuration. The NH bond was out of plane by only 0.4°. The Boltzmann population distribution between these two conformers was almost 50 : 50. The *o*-methylamino substituted compound **9** had five conformers, the lowest of which was near planar with an angle between the benzothiazole and phenyl rings of 0.5° and an N–H bond that was only 0.1° out of plane with the phenyl ring. This conformer also resulted in a hydrogen bond between the NH proton and the thiazole nitrogen. The other four conformers where not in a planar arrangement and did not form the hydrogen bond. From the energies, the Boltzmann population distribution of the lowest energy conformer *versus* the four other conformations was 99.98 : 0.02. The results are in keeping with observed NMR chemical shift values for the amino proton which provide evidence of hydrogen bonding. Figure 4 shows the DFT optimised structures of the *o*-methylaminophenyl benzothiazole **9**. For the *p*-ethylamino, compound **3** resulted in four conformations due to rotamers within the ethyl chain. The lowest energy conformation represented 42.6% of the Boltzmann population. The lowest three energy conformation had a near planar aromatic substructure ($<1.1^0$) and the N–H bond of the amino group was planar with the phenyl ring in the top two structures.

The *o*-ethylamino compound **10** resulted in 16 conformers, of which five were hydrogen bonded to the nitrogen atom in the thiazole ring. These five conformers comprised 99.98% of the Boltzmann population, the lowest energy conformer contributing 41.3% of the room temperature population. The latter was a planar structure with the N–H bond being out of plane by only 0.5°. The other four structures had benzothiazole to phenyl ring angles of 0.3°–2.3° with the N–H bond being out of plane by 2.8°–3.7°.

The *p*-propylamino compound **4** resulted in 10 conformers due to the rotation of the propyl side chain. Of the rotamers, five showed the N–H bond parallel to the phenyl ring while the remainder showed the N–H bond antiparallel (i.e. 180° in the opposite direction) due to rotation about the C–N bond.

Optimization of the structure of the *o*-propylamino compound **11** using Jaguar resulted in 28 conformations. The six lowest energy conformers showed a hydrogen bond between the NH group and the nitrogen atom of the thiazole. These conformers contributed 99.97% of the Boltzmann population. In the lowest energy conformers, the benzothiazole ring and the phenyl ring were out of plane slightly by 0.7°. The N–H bond for the amino group was 0.5° out of plane with the phenyl ring. This resulted in a planar conformation due to the formation of the hydrogen bond and is consistent with the NMR data.

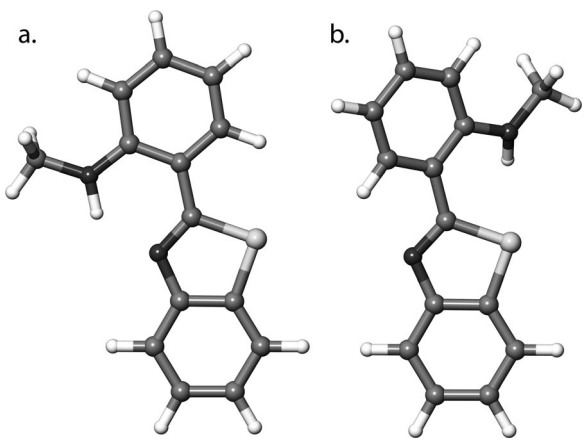


Figure 4. Density functional theory optimised structures of (*o*-methylamino phenyl) benzothiazole (**9**). a. Lowest energy planar structure and b. higher energy non-planar structure.

Conclusions

In summary, we have prepared several *ortho*-aminophenyl and *para*-aminophenyl benzothiazole derivatives and characterised them using ^{1}H, ^{13}C and ^{15}N NMR spectroscopy. The data revealed that there were only marginal changes in the aromatic proton chemical shift in the *para* and *ortho* series of compounds. However, the NH proton chemical shifts varied significantly between the *para* and *ortho* compounds due to the formation of a hydrogen bond between the NH group and the nitrogen in thiazole ring. Additionally, we observed significant changes in the splitting patterns for the CH/CH_2 groups in the structure of the *o*-aminophenyl benzothiazoles. This is in contrast to that obtained for the *p*-aminophenyl benzothiazole derivatives and it is rationalised due to the slow exchange of hydrogen due to the hydrogen bond. The presence of intramolecular hydrogen bond in *o*-aminophenyl benzothiazole derivatives may aid the binding of these compounds towards amyloid fibrils due to their resulting planar conformation. DFT calculations provided further evidence regarding the number of conformers for each of the molecules. When the hydrogen bond was present, the energy was reduced in conformers which comprised >99% of the Boltzmann population. The conformational search of *para*-substituted compounds did not show the presence of any hydrogen bonds. DFT calculations were consistent with the observed NMR chemical shifts.

References

[1] Y. Zhang, M. Chakraborty, C. G. Cerda-Smith, R. N. Bratton, N. E. Maurer, E. M. Senser, M. Novak. *J. Org. Chem.* **2013**, *78*, 6992.
[2] M. Chakraborty, K. J. Jin, S. A. Glover, M. Novak. *J. Org. Chem.* **2010**, *75*, 5296.
[3] M. F. G. Stevens, C. J. McCall, P. Lelievald, P. Alexander, A. Richter, D. E. Davies. *J. Med. Chem.* **1994**, *37*, 1689.
[4] M. F. G. Stevens, D. F. Shi, A. Castro. *J. Chem. Soc. Perkin Trans.* **1996**, *1*, 83.
[5] D. F. Shi, T. D. Bradshaw, S. Wrigley, C. J. McCall, P. Lelieveld, I. Fichtner, M. F. G. Stevens. *J. Med. Chem.* **1996**, *39*, 3375.
[6] R. Wang, D. Sweeney, S. E. Gandy, S. S. Sisodia. *J. Biol. Chem.* **1996**, *271*, 31894.
[7] S. F. Lichtenthaler, N. Ida, G. Multhaup, C. L. Masters, K. Beyreuther. *Biochemistry* **1997**, *36*, 15396.
[8] J. D. Harper, C. M. Lieber, P. T. Landsbury. *Chem. Biol.* **1997**, *119*, 4.
[9] J. D. Harper, P. T. Landsbury Jr.. *Ann. Rev. Biochem.* **1997**, *66*, 385.
[10] T. J. Eckroat, A. S. Mayhoub, S. G. Tsodikova. *Beilstein J. Org. Chem.* **2013**, *9*, –1012.
[11] W. E. Klunk, Y. Wang, G. Huang, M. L. Debnath, D. P. Holt, C. A. Mathis. *Life Sci.* **2001**, *69*, 1471.
[12] W. E. Klunk, Y. Wang, G. Huang, M. L. Debnath, D. P. Holt, L. Shao, R. L. Hamilton, M. D. Ikonomovic, S. T. DeKosky, C. A. Mathis. *J. Neuro Sci.* **2003**, *23*, 2086.
[13] C. A. Mathis, Y. Wang, D. P. Holt, G. Huang, M. L. Debnath, W. E. Klunk. *J. Med. Chem.* **2003**, *46*, 2740.
[14] C. Solbach, M. Uebele, G. Reischl, H.-J. Machulla. *J. Appl. Radiat. Isot.* **2005**, *62*, 591.
[15] W. E. Klunk, H. Engler, A. Nordberg, Y. Wang, G. Blomqvist, D. P. Holt, M. Bergström, I. Savitcheva, G. Huang, S. Estrada, B. Ausén, M. L. Debnath, J. Barletta, J. C. Price, J. Sandell, B. J. Lopresti, A. Wall, P. Koivisto, G. Antoni, C. A. Mathis, B. Långström. *Ann. Neurol.* **2004**, *55*, 306.
[16] M. M. Svedberg, H. Hall, E. H. Lindahl, S. Estrada, Z. Z. Guan, A. Nordberg, B. Långström. *Neurochem. Int.* **2009**, *54*, 347.
[17] K. Serdons, T. Verduyckt, D. Vanderghinste, P. Borghgraef, J. Cleynhens, F. Van Leuven, H. Kung, G. Bormans, A. Verbruggen. *Eur. J. Med. Chem.* **2009**, *44*, 1415.
[18] A. E. Johnson, F. Jeppsson, J. Sandell, D. Wensbo, J. A. M. Neelissen, A. Juréus, P. Ström, H. Norman, L. Farde, S. P. S. Svensson. *J. Neurochem.* **2009**, *108*, 1177.
[19] C. H. Lin, R. Bhatia, R. Lal. *FSAEB* **2001**, *15*, 2433.
[20] E. Gazit. *FASEB* **2002**, *16*, 77.
[21] A. Petric, S. A. Johnson, H. V. Pham, Y. Li, S. Ceh, A. Golobic, E. D. Agdeppa, G. Timbol, J. Liu, G. Keum, N. Satyamoorthy, V. Kepe, K. N. Hauk, J. R. Barrio. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 16492.
[22] T. K. Venkatachalam, D. Stimson, R. Bhalla, G. K. Pierens, D. Reutens, (manuscript under review by J. Labelled Compd. Rad.)
[23] Macro Model, version 10.1, Schrödinger, LLC, New York, NY, **2013**.
[24] Jaguar, version 8.1, Schrödinger, LLC, New York, NY, **2013**.
[25] J. K. Dey, S. K. Dogra. *Bull. Chim. Soc. Jpn.* **1991**, *64*, 3142.
[26] R. O. Duthaler, J. D. Roberts. *J. Am. Chem. Soc.* **1978**, *100*, 3889.
[27] C. H. Cheung, D. S. Surry, S. L. Buchwald. *Org. Lett.* **2013**, *15*, 3734.
[28] Y. Zhang, L. Y. Chen, W. X. Yin, J. Yin, S. B. Zhang, C. L. Liu. *Dalton Trans.* **2011**, *40*, 4830.
[29] N. Park, Y. Heo, M. R. Kumar, Y. Kim, K. H. Song, S. Lee. *Eur. J. Org. Chem.* **2012**, *10*, 1984.