Chapter 3

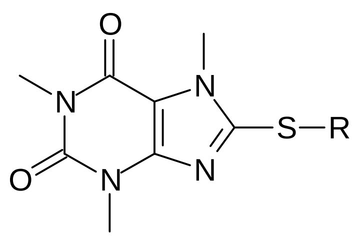
Synthesis

3.1 Introduction

The compounds that were prepared and evaluated as human MAO-A and MAO-B inhibitors in this study are shown in tables 3.1 and 3.2. As mentioned in the introduction, substitution of the caffeine at position 8 of the ring yields structures that are potent MAO-B inhibitors. A particularly potent MAO-B inhibitor is 8-[(phenylethyl)sulfanyl]caffeine (**2a**) with an IC_{50} value of 0.223 µM (Booysen *et al*., 2011). In the present study, this compound was used as lead for the design of novel MAO-B inhibitors. As stated in the introduction the following compounds will be synthesized in this study:

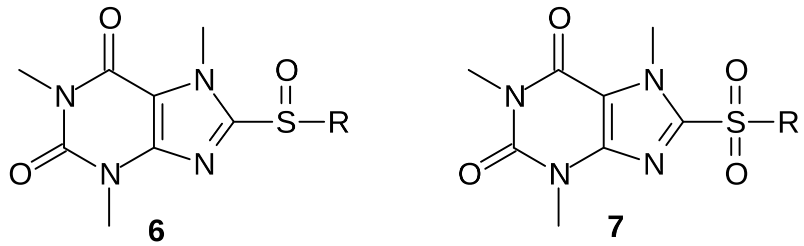
1. 8-[(phenylethyl)sulfanyl]caffeines (**3a**–**e**),
2. 8-[(phenylpropyl)sulfanyl]caffeines (**4a**–**c**),
3. 8-(benzylsulfanyl)caffeines (**5a**–**b**),
4. 8-sulfinylcaffeines (**6a**–**b**)
5. and 8-sulfonylcaffeine (**7**)

Table 3.1. Series 1 - 8-sulfanylcaffeine derivatives that will be synthesized in this study.



Compound	R-Group	Compound	R-Group
3a	**$–(CH_2)_2-(3-Cl-C_6H_4)$**	**4a**	**$–(CH_2)_3-C_6H_5$**
3b	**$–(CH_2)_2-(3-Br-C_6H_4)$**	**4b**	**$–(CH_2)_3-(3-Cl-C_6H_4)$**
3c	**$–(CH_2)_2-(3-CF_3-C_6H_4)$**	**4c**	**$–(CH_2)_3-(4-Cl-C_6H_4)$**
3d	**$–(CH_2)_2-(3-CH_3-C_6H_4)$**	**5a**	**$–CH_2-(3-Cl-C_6H_4)$**
3e	**$–(CH_2)_2-(3-OCH_3-C_6H_4)$**	**5b**	**$–CH_2-(3-Br-C_6H_4)$**

Table 3.2. Series 2 - 8-sulfinyl- and 8-sulfonylcaffeine derivatives selected for this study.



6 7

Compound	R-Group	Compound	R-Group
6a	**$–CH_2-C_6H_5$**	**7**	**$–(CH_2)_2-C_6H_5$**
6b	**$–CH_2-(4-F-C_6H_4)$**		

3.2 General synthetic approaches

The target 8-sulfanylcaffeine analogues, compounds **3a**–**e**, **4a**–**c** and **5a**–**b** will be synthesized according to the literature procedure as shown in figure 3.1 (Long, 1947). For this purpose, 8-chlorocaffeine (**8**) will be reacted with an appropriate mercaptan (**9**) in the presence of NaOH, with 50% aqueous ethanol serving as solvent. This will yield the target 8-sulfanylcaffeine analogues.

8-Chlorocaffeine, in turn, will be synthesized by reacting chlorine with caffeine (**1**) in chloroform (Fischer & Reese, 1883). This procedure yields 8-chlorocaffeine in good yield (typically >70%) and high purity (figure 3.2).

In certain instances, the mercaptan starting materials that will be required for the synthesis of the 8-sulfanylcaffeine analogues are not commercially available and will thus be synthesized according to the literature procedure (Manoury *et al*., 1986). For this purpose an appropriate alkylbromide will be reacted with thiourea (**10**) in ethanol (figure 3.3). The resulting thiouronium salt (**11**) will be hydrolyzed in the presence of NaOH to yield the target mercaptan (**9**).

The 8-sulfinylcaffeine analogues, **6a**–**b**, and the 8-sulfonylcaffeine, **7**, will be synthesized by reacting the 8-sulfanylcaffeines with H_2O_2 in the presence of glacial acetic acid and acetic anhydride (figure 3.4) (Long, 1947).

The structures of the target compounds will be characterized with MS and NMR while the purities of the target compounds will be estimated via HPLC analysis.

Series 1

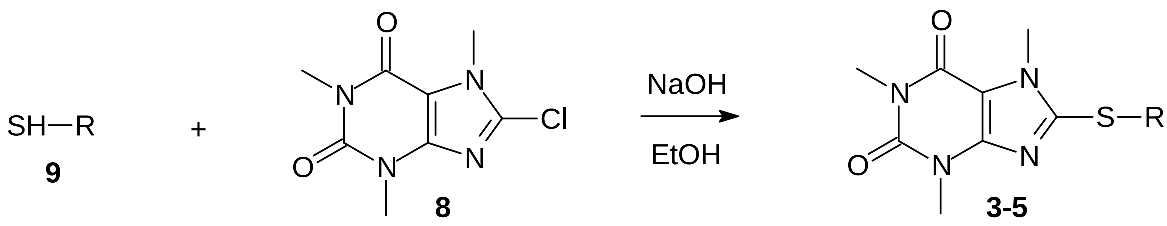


Figure 3.1. Synthetic route to the 8-sulfanylcaffeine analogues, compounds **3a**–**e**, **4a**–**c** and **5a**–**b**.

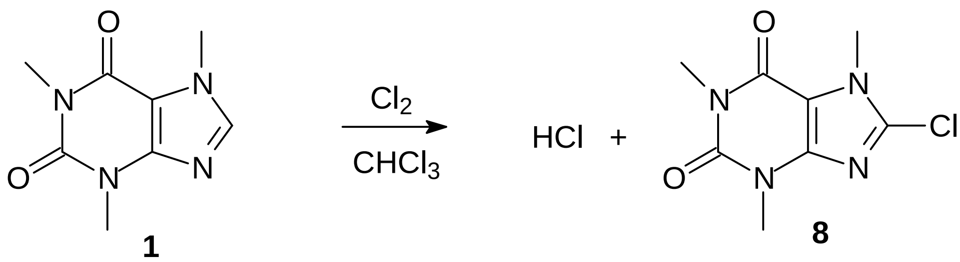


Figure 3.2. Synthetic route to 8-chlorocaffeine.

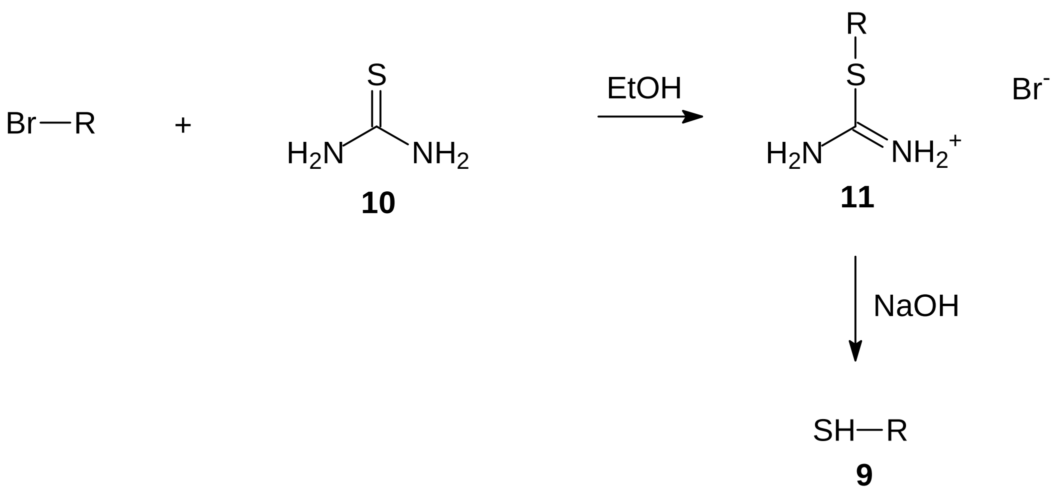


Figure 3.3. Synthetic route to the mercaptan starting materials.

Series 2

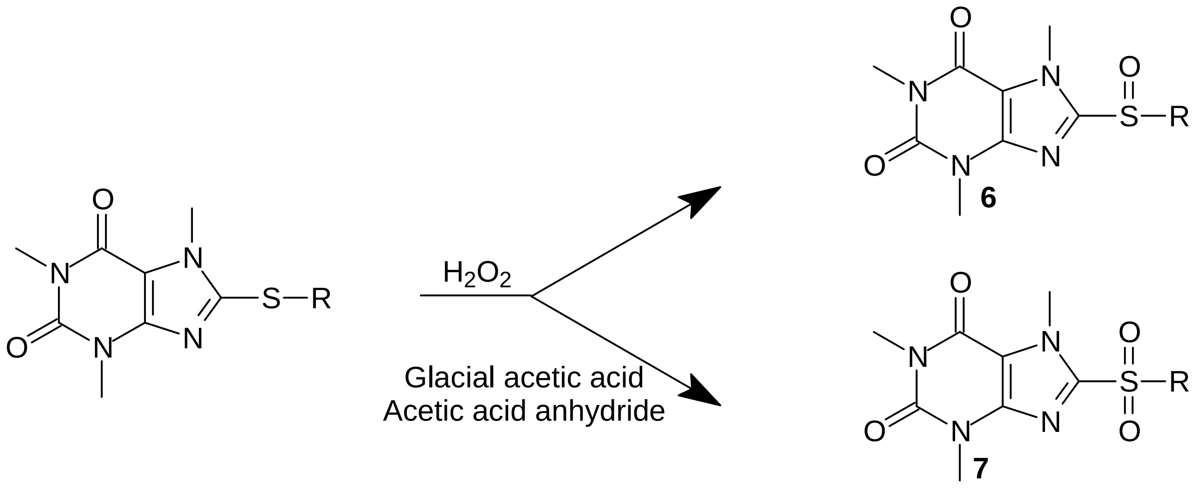


Figure 3.4. Synthetic route to the 8-sulfinylcaffeine analogues, **6a**–**b**, and 8-sulfonylcaffeine, **7**.

3.3 Materials and instrumentation

Thin layer chromatography (TLC):

TLC was employed during each synthesis to determine whether the reactions were complete. TLC was carried out using silica gel sheets (Merck) with UV_{254} fluorescent indicator. The mobile phase constisted of a mixture of 60% ethyl acetate and 30% n-hexane. All compounds were dissolved in ethyl acetate for TLC analysis. The TLC sheets were observed using a UV-lamp at a wavelength of 254 nm. In some cases an iodine chamber was also used to visualize the TLC sheets.

Melting points:

All melting points were determined using a Buchi B-545 apparatus and are uncorrected.

Mass spectra (MS):

High resolution mass spectra (HRMS) were obtained on a DFS high resolution magnetic sector mass spectrometer (Thermo Electron Corporation) in electron ionization (EI) mode.

Nuclear magnetic resonance (NMR):

Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Bruker Avance III 600 spectrometer at frequencies of 600 MHz and 150 MHz, respectively. NMR measurements of compounds **3a**–**e**, **4a**–**c** and **5a**–**b** were conducted in $CDCl_3$ while the spectra of compounds **6a**–**b** and **7** were were recorded in DMSO-*d6*. Chemical shifts are reported in parts per million (δ) downfield from the signal of tetramethylsilane added to the deuterated solvent. Spin multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet), qn (quintet) or m (multiplet). The coupling constants (J) are expressed in Hertz (Hz).

HPLC analysis:

The purities of the synthesized compounds were estimated via HPLC analyses. For this purpose an Agilent 1100 HPLC system equipped with a quaternary pump and an Agilent 1100 series diode array detector were employed. HPLC grade acetonitrile (Merck) and Milli-Q water (Millipore) were used for the chromatography. A Venusil XBP C18 column (4.60 × 150 mm, 5 µm) was used and the mobile phase consisted initially of 30% acetonitrile and 70% Milli-Q water at a flow rate of 1 ml/min. At the start of each HPLC run a solvent gradient program was initiated by linearly increasing the composition of the acetonitrile in the mobile phase to 85% acetonitrile over a period of 5 min. Each HPLC run lasted 15 min and a time period of 5 min was allowed for equilibration between runs. A volume of 20 µl of solutions of the test compounds (1 mM) in acetonitrile was injected into the HPLC system and the eluent was monitored at a wavelength of 254 nm.

3.4 Detailed synthetic procedures

3.4.1 The synthesis of 8-chlorocaffeine

8-Chlorocaffeine is not commercially available, and thus was synthesized in the laboratory. As can be seen in figure 3.2, caffeine (**1**) reacts with chlorine gas in chloroform to yield 8-chlorocaffeine (**8**).

Chlorine gas was prepared from the reaction between $KMnO_4$ and HCl (Vogel *et al*., 1989):

- 0.367 g of $KMnO_4$, for the production of 0.412 g Cl_2, was placed in a round-bottom flask. This flask was attached to a pressure equalizing funnel, containing 20.17 ml of 32% HCl. The funnel was secured by an elastic band. Caution was taken when working with chlorine as it is toxic and an irritant.

- The HCl was slowly added dropwise onto the $KMnO_4$ crystals, while the flask was regularly shaken.
- When about half the acid had been added, the rate of formation of chlorine gas receded. At this point the flask was warmed to 50 ˚C.
- After all the acid had been added, the mixture was boiled gently to complete the formation of chlorine.

The chlorine gas formed was passed through a Drechsel bottle, containing water, to remove any HCl from the chlorine while a second Drechsel bottle containing H_2SO_4 was used to dry the gas. A third Drechsel bottle was used as a safety trap between the reaction vessel and the dry chlorine generating source.

The chlorine gas so prepared was passed through a second round-bottomed flask to produce 8-chlorocaffeine (Fischer & Reese, 1883):

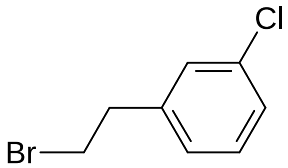
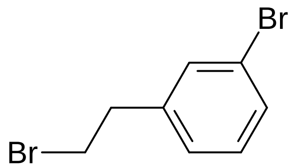
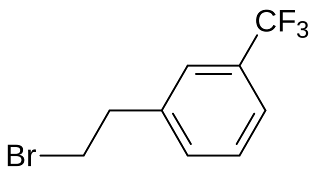
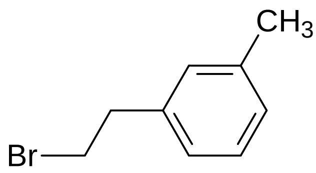
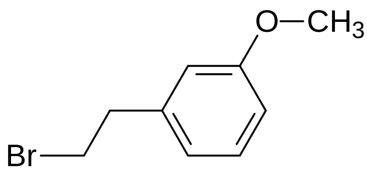
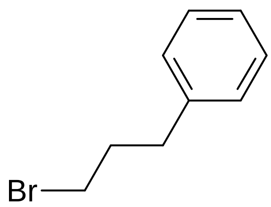
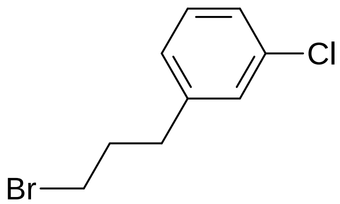
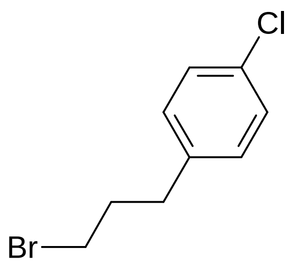
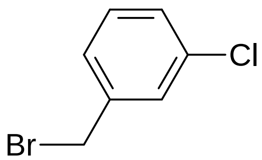
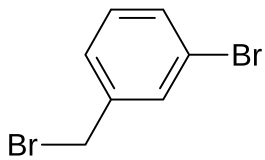
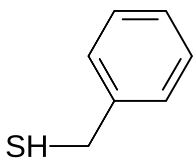
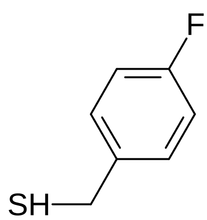
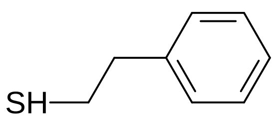
- 2.5 g of caffeine was heated in 20 ml of chloroform under reflux until all the caffeine was dissolved.
- The prepared chlorine gas was subsequently bubbled through the solution.
- Solid material precipitated from the solution but later dissolved with continuation of the reaction.
- The chloroform was then removed via distillation, using a rotary evaporator and the crystals were freed from the residual chloroform by heating it with a small amount of water.

3.4.2 The synthesis of the mercaptan derivatives

As mentioned, several mercaptans were required for the synstesis of the 8-sulfanylcaffeine analogues. Since many of these mercaptans were not commercially available, they were synthesized. The synthetic routes to these mercaptans are shown in figure 3.3. As shown, a commercially available alkylbromide was reacted with thiourea (**10**) in the presence of ethanol to yield the thiouronium salt (**11**) as intermediate. Hydrolysis of the thiouronium in the presence of NaOH yielded the target mercaptans (**9**).

- 13.5 ml of ethanol was added to 7 mmol of the alkylbromide to produce a solution. Thiourea (7 mmol) was subsequently added to the reaction.
- The reaction mixture was heated under reflux at 100 ˚C for 120 min to form a colourless solution.
- The mixture was cooled at room temperature for approximately 60 min.
- The ethanol was then removed under reduced pressure to obtain crystals. The crystals were freed from the residual ethanol by heating it with a small amount of water.
- A solution of NaOH (0.420 g) in water (8.75 ml) was added, and the resulting mixture was refluxed at 120 ˚C for 120 min.
- An aqueous solution of H_2SO_4 (1 ml) in water (5 ml) was prepared and subsequently added drop wise to the reaction.
- The reaction was extracted to 30 ml of diethylether and the ether phase was washed twice with 20 ml of water.
- The ether phase was subsequently dried over 2 g of anhydrous $MgSO_4$.
- The ether was removed under reduced pressure and the resulting crystals were freed from the residual diethylether by heating it with a small amount of water.

Table 3.3. The structures of the alkylbromides that were required as starting materials. The structures of the commercially available mercaptans are also shown.

Alkylbromides and commercially available mercaptans	
	
	
	
	
	
	
	

3.4.3 The synthesis of 8-sulfanylcaffeine analogues 3a–e, 4a–c and 5a–b

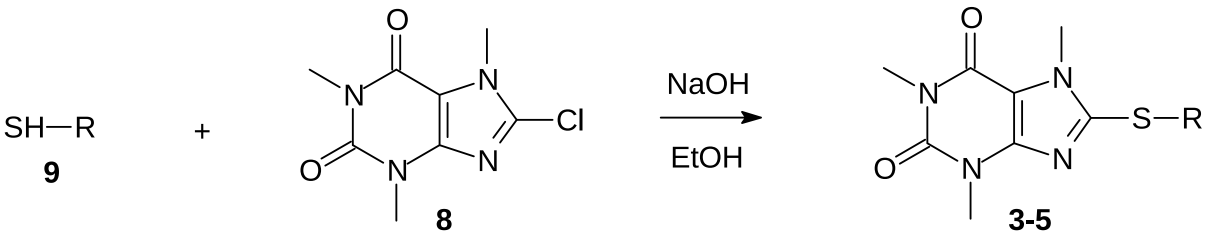
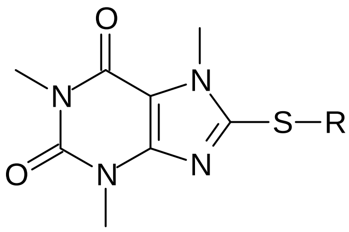


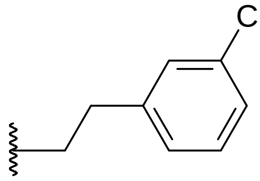
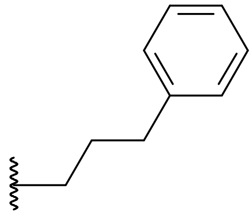
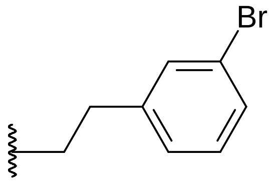
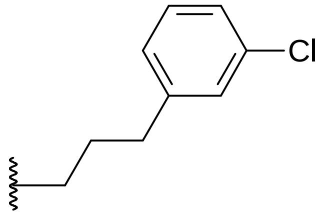
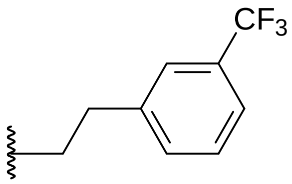
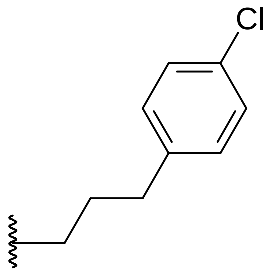
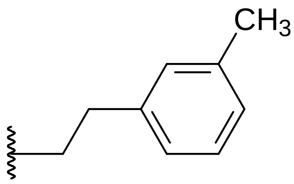
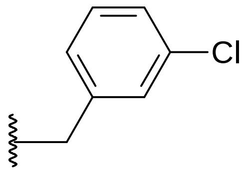
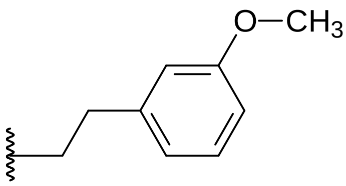
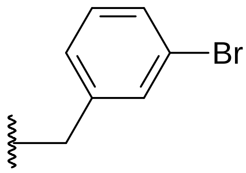
Figure 3.5. The synthesis of 8-sulfanylcaffeine analogues **3a–e, 4a–c** and **5a–b**.

As shown in figure 3.5, 8-chlorocaffeine (**8**) was reacted with the appropriate mercaptan (**9**) to yield an 8-sulfanylcaffeine derivative (**3–5**). This reaction was conducted in ethanol in the presence of sodium hydroxide.

- NaOH (4 mmol) was dissolved in water (4 ml) at room temperature after which ethanol (4 ml) was added.
- The reaction mixture was cooled in an ice bath and the appropriate mercaptan (4 mmol) was added. The mercaptan presented as insoluble droplets at the bottom of the reaction vessel.
- 8-Chlorocaffeine (4 mmol) was rapidly added in a single portion.
- The reaction mixture was heated under reflux at 130 ˚C for 60 min.
- The mixture was cooled in an ice bath and the resulting white precipitate was collected by filtration.
- The precipitate was washed with 60 ml ethanol.
- The powder was recrystallized from 60 ml of boiling ethanol, and the crystals were collected by filtration and washed with 60 ml ethanol.

Table 3.4. The structures of 8-sulfanylcaffeine analogues **3a**–**e, 4a**–**c** and **5a**–**b**.



	R-Group		R-Group
3a		**4a**	
3b		**4b**	
3c		**4c**	
3d		**5a**	
3e		**5b**	

3.4.4 The synthesis of 8-sulfinylcaffeine analogues, 6a–b and the 8-sulfonylcaffeine, 7

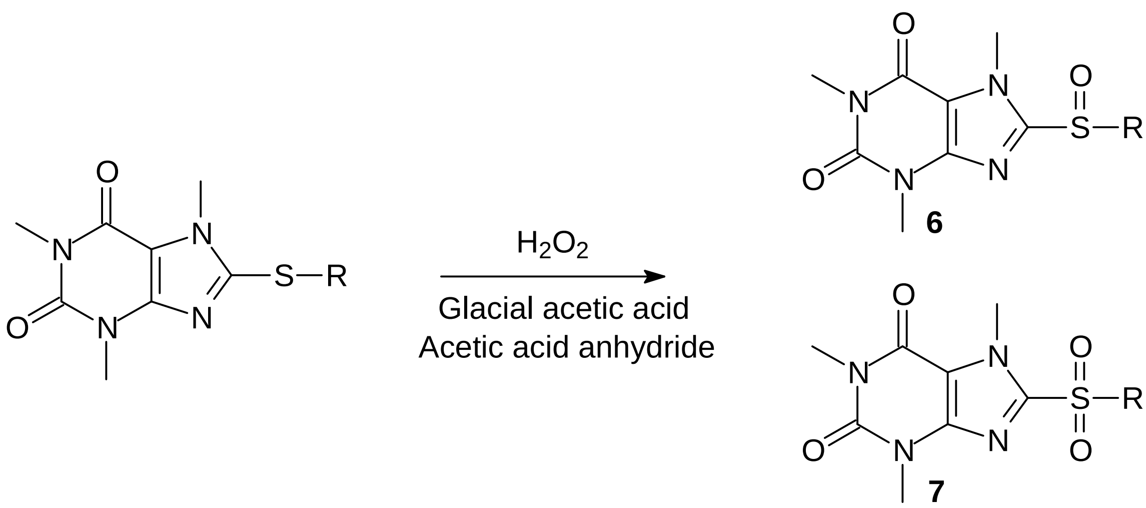
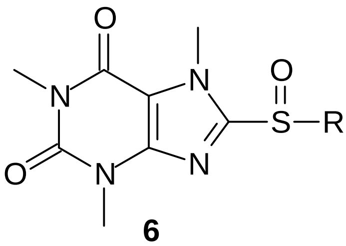


Figure 3.6. The synthesis of 8-sulfinylcaffeine analogues, **6a**–**b**, and the 8-sulfonylcaffeine, **7**.

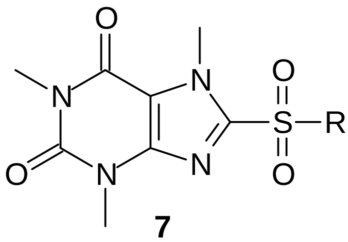
As shown in figure 3.6, the 8-sulfinylcaffeine analogues, **6a**–**b**, and the 8-sulfonylcaffeine, **7**, were synthesized by reacting an appropriate 8-sulfanylcaffeine analogue with H_2O_2 in glacial acetic acid and acetic anhydride.

- The 8-sulfanylcaffeine analogue (3 mmol) was dissolved with glacial acetic acid (6 ml) and acetic anhydride (3 ml) in an Erlenmeyer flask.
- 30% H_2O_2 (3 ml) was subsequently carefully added to the reaction.
- The temperature of the reaction mixture was increased to 60 ˚C. Care was taken to keep the reaction temperature below 80 ˚C.
- After 2 hours the clear reaction solution was diluted with two volumes of cold water.
- The solution was subsequently evaporated to dryness at reduced pressure. Care was taken not to apply extensive heat as the residue may contain acetyl peroxide.
- The solid residue was then recrystallized from boiling ethyl acetate to yield 8-sulfinylcaffeine analogues, **6a**–**b**, and the 8-sulfonylcaffeine, **7**.

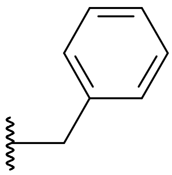
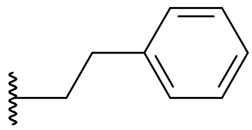
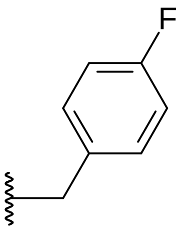
Table 3.5. The structures of 8-sulfinylcaffeine analogues, **6a**–**b**, and the 8-sulfonylcaffeine, **7**.



6



7

	R-Group		R-Group
6a		**7**	
6b			

3.5 Results

3.5.1 Melting points, yields and purities

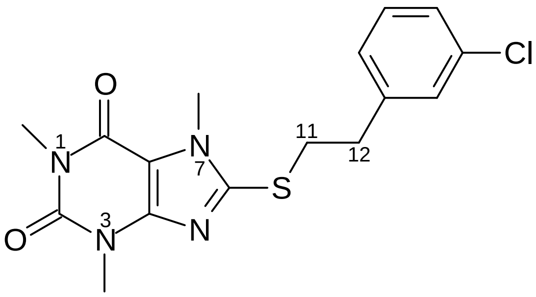
The yields obtained for the synthesis of the target 8-sulfanylcaffeine analogues are given in table 3.6. As shown, the yields ranged from 6.4% to 56.5%. The poor yields of some compounds are due to incomplete recrystallization. Although some of the yields were poor, the compounds were of high purity as indicated by HPLC analysis. For the HPLC analyses, strong eluting conditions were employed (up to 85% acetonitrile) to ensure that most compounds present in the samples elute. At the low wavelength selected (254 nm), most organic compounds should be detected. The chromatograms obtained for each compound are provided in the addendum. All chromatograms showed that the 8-sulfanylcaffeine analogues are of a high degree of purity. Where additional peaks occurred, the percentage purities were calculated. These calculations were based on the integrated surface areas of the analyte and impurity peaks. As shown in table 3.6, with the exception of **7** (85%), the purities of the 8-sulfanylcaffeine analogues are estimated to be 94–99%. These purities are acceptable.

Table 3.6. Melting points, yields and purities obtained after successful synthesis.

Compound	Yields (%)	Melting points (˚C)	Purities (%)
3a	6.4	125.5-126.1	97
3b	32.2	122.6-124.3	99
3c	18.2	130.4-132.7	99
3d	28.0	110.5-112.0	99
3e	40.7	126.6-127.5	97
4a	21.4	76.4-78.3	99
4b	24.1	87.7-89.3	98
4c	26.7	95.5-98.0	94
5a	50.7	156.5-158.1	99
5b	45.8	143.9-145.7	99
6a	42.1	165.6-169.7	99
6b	56.5	190.4-191.9	98
7	27.0	185.7-187.5	85

3.5.2 The physical data for the 8-sulfanylcaffeine analogues 3a–e, 4a–c and 5a–b

8-{[2-(3-Chlorophenyl)ethyl]sulfanyl}caffeine (3a)

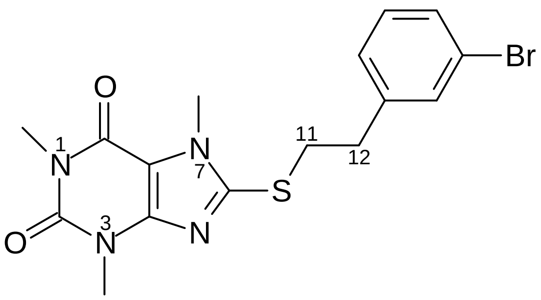


The title compound was prepared from 2-(3-chlorophenyl)ethanethiol in a yield of 6.40%: mp 125.5–126.1 ℃ (ethanol). **^{1}H NMR** (Bruker Avance III 600, $CDCl_3$) δ 3.02 (t, 2H, J = 7.2 Hz), 3.36 (s, 3H), 3.47 (t, 2H, J = 7.2 Hz), 3.55 (s, 3H), 3.78 (s, 3H), 7.08 (d, 1H, J = 7.5 Hz), 7.17 (m, 1H) 7.20 (m, 2H); **^{13}C NMR** (Bruker Avance III 600, $CDCl_3$) δ 27.8, 29.7, 32.1, 33.5, 35.8, 108.6, 126.8, 126.9, 128.7, 129.8, 134.3, 141.2, 148.4, 150.5, 151.5, 154.5; EI-HRMS m/z: calcd for $C_{16}H_{17}ClN_4O_2S$, 364.0761, found 364.0757; Purity (HPLC): 97%.

<u>^{1}H NMR</u>

- The three methyl groups at N1, N3 and N7 respectively correspond to the singlets at 3.36, 3.55 and 3.78 ppm. The signals integrate for 3 protons each.
- The methylene groups at C11 and C12, respectively, correspond to the triplets at 3.02 and 3.47 ppm. The signals integrate for 2 protons each.
- Aromatic protons on the phenyl ring correspond with the doublet at 7.08 and the multiplets at 7.17 and 7.20 ppm respectively. The signals integrate for 1 proton for the doublet and 1 proton for first multiplet and 2 protons for second multiplet.

8-{[2-(3-Bromophenyl)ethyl]sulfanyl}caffeine (3b)

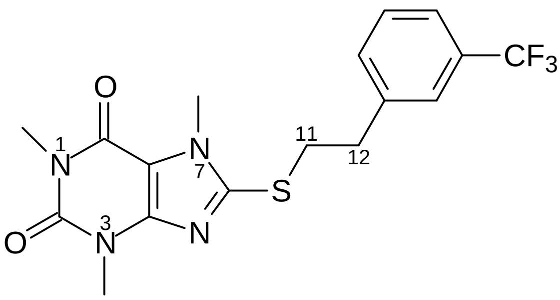


The title compound was prepared from 2-(3-bromophenyl)ethanethiol in a yield of 32.15%: mp 122.6–124.3 ℃ (ethanol). **^{1}H NMR** (Bruker Avance III 600, $CDCl_3$) δ 3.01 (t, 2H, J = 7.2 Hz), 3.36 (s, 3H), 3.47 (t, 2H, J = 7.2 Hz), 3.55 (s, 3H), 3.77 (s, 3H), 7.13 (m, 2H), 7.32 (m, 1H), 7.35 (m, 1H) ; **^{13}C NMR** (Bruker Avance III 600, $CDCl_3$) δ 27.8, 29.7, 32.1, 33.5, 35.8, 108.6, 122.5, 127.3, 129.8, 130.0, 131.6, 141.5, 148.4, 150.5, 151.5, 154.5 ; EI-HRMS m/z: calcd for $C_{16}H_{17}BrN_4O_2S$, 408.0256, found 408.0251; Purity (HPLC): 99%.

<u>^{1}H NMR</u>

- The three methyl groups at N1, N3 and N7, respectively, correspond to the singlets at 3.36, 3.55 and 3.77 ppm. The signals integrate for 3 protons each.
- The methylene groups at C11 and C12, respectively, correspond to the triplets at 3.01 and 3.47 ppm. The signals integrate for 2 protons each.
- Aromatic protons on the phenyl ring correspond with the multiplets at 7.13, 7.32 and 7.35 ppm, respectively. The signals integrate for 2 protons for the first multiplet and 1 proton each for the second and third multiplets.

8-{[2-(3-(Trifluoromethyl)phenyl)ethyl]sulfanyl}caffeine (3c)

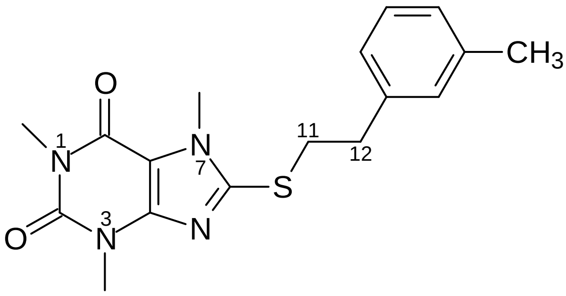


The title compound was prepared from 2-[3-(trifluoromethyl)phenyl]ethanethiol in a yield of 18.22%: mp 130.4–132.7 ℃ (ethanol). **^{1}H NMR** (Bruker Avance III 600, $CDCl_3$) δ 3.12 (t, 2H, J = 7.5 Hz), 3.37 (s, 3H), 3.50 (t, 2H, J = 7.5 Hz), 3.55 (s, 3H), 3.78 (s, 3H), 7.44 (m, 4H); **^{13}C NMR** (Bruker Avance III 600, $CDCl_3$) δ 27.8, 29.7, 32.1, 33.4, 35.9, 108.6, 123.6 (q), 125.3 (q), 129.0, 130.9 (q) 132.0, 140.2, 148.4, 150.4, 151.5, 154.5; EI-HRMS m/z: calcd for $C_{17}H_{17}F_3N_4O_2S$, 398.1024, found 398.1031; Purity (HPLC): 99%.

^{1}H NMR

- The three methyl groups at N1, N3 and N7, respectively, correspond to the singlets at 3.37, 3.55 and 3.78 ppm. The signals integrate for 3 protons each.
- The methylene groups at C11 and C12, respectively, correspond to the triplets at 3.12 and 3.50 ppm. The signals integrate for 2 protons each.
- Aromatic protons on the phenyl ring correspond with the multiplet at 7.44 ppm. The signal integrates for 4 protons.

8-{[2-(3-Methylphenyl)ethyl]sulfanyl}caffeine (3d)

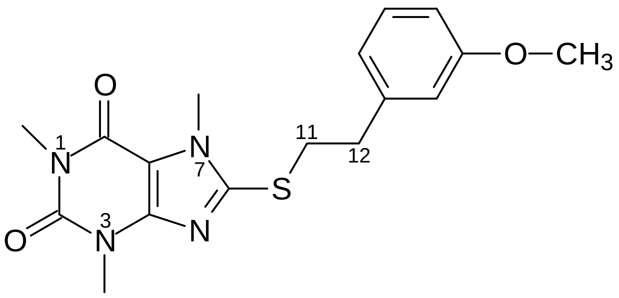


The title compound was prepared from 2-(3-methylphenyl)ethanethiol in a yield of 28.01%: mp 110.5–112.0 ℃ (ethanol). **^{1}H NMR** (Bruker Avance III 600, $CDCl_3$) δ 2.31 (s, 3H), 3.00 (t, 2H, J = 7.5 Hz), 3.37 (s, 3H), 3.48 (t, 2H, J = 7.5 Hz), 3.56 (s, 3H), 3.79 (s, 3H), 7.02 (m, 3H), 7.17 (t, 1H, J = 7.5 Hz) ; **^{13}C NMR** (Bruker Avance III 600, $CDCl_3$) δ 21.3, 27.8, 29.7, 32.1, 33.9, 36.0, 108.5, 125.5, 127.4, 128.4, 129.3, 138.1, 139.3, 148.5, 150.9, 151.5, 154.5; EI-HRMS m/z: calcd for $C_{17}H_{20}N_4O_2S$, 344.1307, found 344.1259; Purity (HPLC): 99%

^{1}H NMR

- The three methyl groups at N1, N3 and N7, respectively, correspond to the singlets at 3.37, 3.56 and 3.79 ppm. The signals integrate for 3 protons each.
- The methylene groups at C11 and C12, respectively, correspond to the triplets at 3.00 and 3.48 ppm. The signals integrate for 2 protons each.
- The methyl group on the phenyl ring corresponds to the singlet at 2.31 ppm. The signal integrates for 3 protons.
- Aromatic protons on the phenyl ring correspond with the signals at 7.02 and 7.17 ppm, respectively. The signals integrate for 3 protons for the first multiplet and 1 proton for the triplet.

8-{[2-(3-Methoxyphenyl)ethyl]sulfanyl}caffeine (3e)

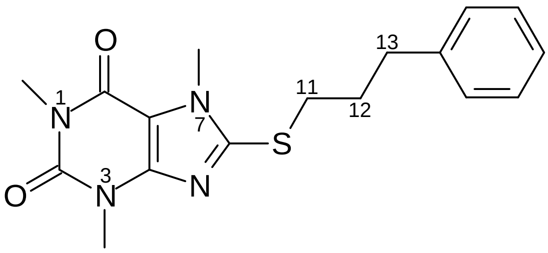


The title compound was prepared from 2-(3-methoxyphenyl)ethanethiol in a yield of 40.69%: mp 126.6–127.5 ℃ (ethanol). **^{1}H NMR** (Bruker Avance III 600, $CDCl_3$) δ 3.01 (t, 2H, J = 7.5 Hz), 3.36 (s, 3H), 3.48 (t, 2H, J = 7.5 Hz), 3.55 (s, 3H), 3.76 (s, 3H,), 3.78 (s, 3H), 6.76 (m, 3H), 7.20 (m, 1H) ; **^{13}C NMR** (Bruker Avance III 600, $CDCl_3$) δ 27.8, 29.7, 32.1, 33.8, 36.1, 55.1, 108.5, 111.6, 114.6, 120.9, 129.5, 140.9, 148.5, 150.8, 151.5, 154.5, 159.7; EI-HRMS m/z: calcd for $C_{17}H_{20}N_4O_3S$, 360.1256, found 360.1255; Purity (HPLC): 97%

^{1}H NMR

- The four methyl groups at N1, N3, N7 and C17, respectively, correspond to the singlets at 3.36, 3.55, 3.76 and 3.78 ppm. The signals integrate for 3 protons each.
- The methylene groups at C11 and C12, respectively, correspond to the triplets at 3.01 and 3.48 ppm. The signals integrate for 2 protons each.
- Aromatic protons on the phenyl ring correspond with the multiplets at 6.76 and 7.20 ppm, respectively. The signals integrate for 3 protons for the first multiplet and 1 proton for the second multiplet.

8-[(3-Phenylpropyl)sulfanyl]caffeine (4a)

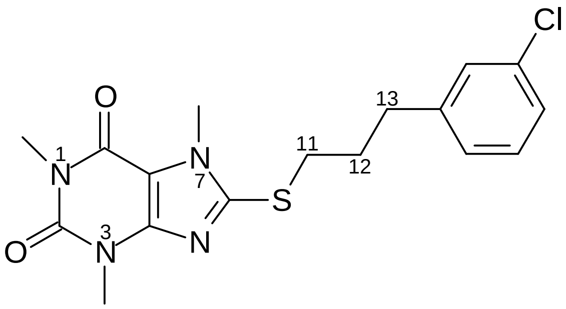


The title compound was prepared from 3-phenylpropane-1-thiol in a yield of 21.39%: mp 76.4–78.3 ℃ (ethanol). **^{1}H NMR** (Bruker Avance III 600, $CDCl_3$) δ 2.07 (qn, 2H, J = 7.5 Hz), 2.75 (t, 2H, J = 7.5 Hz), 3.24 (t, 2H, J = 7.5 Hz), 3.36 (s, 3H), 3.50 (s, 3H), 3.81 (s, 3H), 7.17 (m, 3H), 7.26 (m, 2H) ; **^{13}C NMR** (Bruker Avance III 600, $CDCl_3$) δ 27.8, 29.6, 31.1, 32.0, 32.1, 34.5, 108.5, 126.1, 128.4, 128.4, 140.7, 148.4, 151.0, 151.5, 154.5; EI-HRMS m/z: calcd for $C_{17}H_{20}N_4O_2S$, 344.1307, found 344.1308; Purity (HPLC): 99%

^{1}H NMR

- The three methyl groups at N1, N3 and N7, respectively, correspond to the singlets at 3.36, 3.50 and 3.81 ppm. The signals integrate for 3 protons each.
- The methylene groups at C11, C12 and C13, respectively, correspond to the quintet at 2.07 and the triplets at 2.75 and 3.24 ppm. The signals integrate for 2 protons each.
- Aromatic protons on the phenyl ring correspond with the multiplets at 7.17 and 7.26 ppm, respectively. The signals integrate for 3 protons for the first multiplet and 2 protons for the second multiplet.

8-{[3-(3-Chlorophenyl)propyl]sulfanyl}caffeine (4b)

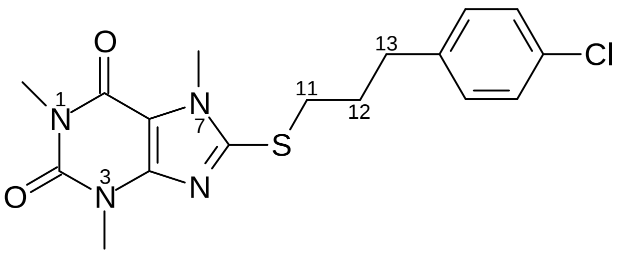


The title compound was prepared from 3-(3-chlorophenyl)propane-1-thiol in a yield of 24.10%: mp 87.7–89.3 ℃ (ethanol). **^{1}H NMR** (Bruker Avance III 600, $CDCl_3$) δ 2.07 (qn, 2H, J = 7.5 Hz), 2.73 (t, 2H, J = 7.5 Hz), 3.23 (t, 2H, J = 7.5 Hz), 3.36 (s, 3H), 3.50 (s, 3H), 3.81 (s, 3H), 7.04 (d, 1H, J = 7.5 Hz), 7.15 (m, 2H), 7.19 (m, 1H); **^{13}C NMR** (Bruker Avance III 600, $CDCl_3$) δ 27.8, 29.7, 30.8, 31.7, 32.1, 34.2, 108.5, 126.3, 126.6, 128.5, 129.7, 134.2, 142.7, 148.4, 150.8, 151.5, 154.5; EI-HRMS m/z: calcd for $C_{17}H_{19}ClN_4O_2S$, 378.0917, found 378.0902; Purity (HPLC): 98%.

^{1}H NMR

- The three methyl groups at N1, N3 and N7, respectively, correspond to the singlets at 3.36, 3.50 and 3.81 ppm. The signals integrate for 3 protons each.
- The methylene groups at C11, C12 and C13, respectively, correspond to the quintet at 2.07 and the triplets at 2.73 and 3.23 ppm. The signals integrate for 2 protons each.
- Aromatic protons on the phenyl ring correspond with the doublet at 7.04 and the multiplets at 7.15 and 7.19 ppm respectively. The signals integrate for 1 proton for the doublet and second multiplet, and 2 protons for first multiplet.

8-{[3-(4-Chlorophenyl)propyl]sulfanyl}caffeine (4c)

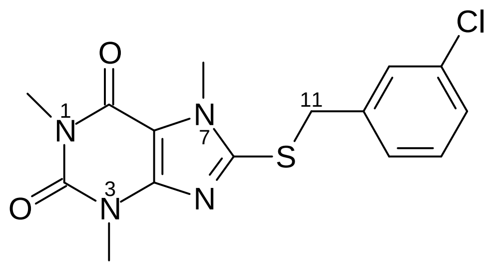


The title compound was prepared from 3-(4-chlorophenyl)propane-1-thiol in a yield of 26.70%: mp 95.5–98.0 ℃ (ethanol). **^{1}H NMR** (Bruker Avance III 600, $CDCl_3$) δ 2.04 (qn, 2H, J = 7.5 Hz), 2.72 (t, 2H, J = 7.5 Hz), 3.22 (t, 2H, J = 7.5 Hz), 3.36 (s, 3H), 3.50 (s, 3H), 3.81 (s, 3H), 7.09 (d, 2H, J = 8.7 Hz), 7.22 (d, 2H, J = 8.3 Hz); **^{13}C NMR** (Bruker Avance III 600, $CDCl_3$) δ 27.8, 29.6, 31.0, 31.7, 32.1, 33.8, 108.5, 128.5, 129.7, 131.9, 139.1, 148.4, 150.8, 151.5, 154.5; EI-HRMS m/z: calcd for $C_{17}H_{19}ClN_4O_2S$, 378.0917, found 378.0903; Purity (HPLC): 94%.

<u>^{1}H NMR</u>

- The three methyl groups at N1, N3 and N7, respectively, correspond to the singlets at 3.36, 3.50 and 3.81 ppm. The signals integrate for 3 protons each.
- The methylene groups at C11, C12 and C13, respectively, correspond to the quintet at 2.04 and the triplets at 2.72 and 3.22 ppm. The signals integrate for 2 protons each.
- Aromatic protons on the phenyl ring correspond with the doublets at 7.09 and 7.22 ppm, respectively. The signals integrate for 2 protons each.

8-[(3-Chlorobenzyl)sulfanyl]caffeine (5a)

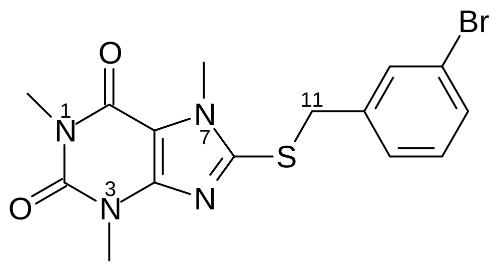


The title compound was prepared from (3-chlorophenyl)methanethiol in a yield of 50.67%: mp 156.5–158.1 ℃ (ethanol). **^{1}H NMR** (Bruker Avance III 600, $CDCl_3$) δ 3.35 (s, 3H), 3.56 (s, 3H), 3.73 (s, 3H), 4.39 (s, 2H), 7.23 (m, 3H), 7.38 (s, 1H); **^{13}C NMR** (Bruker Avance III 600, $CDCl_3$) δ 27.8, 29.7, 32.2, 36.3, 108.8, 127.1, 128.0, 129.3, 129.9, 134.3, 138.7, 148.3, 149.6, 151.4, 154.5; EI-HRMS m/z: calcd for $C_{15}H_{15}ClN_4O_2S$, 350.0604, found 350.0600; Purity (HPLC): 99%.

^{1}H NMR

- The three methyl groups at N1, N3 and N7, respectively, correspond to the singlets at 3.35, 3.56 and 3.73 ppm. The signals integrate for 3 protons each.
- The methyl group at C11 corresponds to the singlet at 4.39 ppm. The signal integrates for 2 protons.
- Aromatic protons on the phenyl ring correspond with the multiplet at 7.23 and the singlet at 7.38 ppm, respectively. The signals integrate for 3 protons for the multiplet and 1 proton for the singlet.

8-[(3-Bromobenzyl)sulfanyl]caffeine (5b)



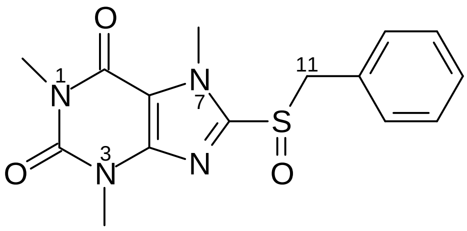
The title compound was prepared from (3-bromophenyl)methanethiol in a yield of 45.75%: mp 143.9–145.7 ℃ (ethanol). **[1]H NMR** (Bruker Avance III 600, $CDCl_3$) δ 3.35 (s, 3H), 3.56 (s, 3H), 3.73 (s, 3H), 4.38 (s, 2H), 7.14 (t, 1H, J = 7.9 Hz), 7.26 (d, 1H, J = 7.5 Hz), 7.37 (d, 1H, J = 7.9 Hz), 7.54 (s, 1H) ; **[13]C NMR** (Bruker Avance III 600, $CDCl_3$) δ 27.8, 29.7, 32.2, 36.3, 108.8, 122.4, 127.5, 130.1, 130.9, 132.2, 139.0, 148.3, 149.5, 151.4, 154.5; EI-HRMS m/z: calcd for $C_{15}H_{15}BrN_4O_2S$, 394.0099, found 394.0113; Purity (HPLC): 99%.

^{1}H NMR

- The three methyl groups at N1, N3 and N7, respectively, correspond to the singlets at 3.35, 3.56 and 3.73 ppm. The signals integrate for 3 protons each.
- The methyl group at C11 corresponds to the singlet at 4.38 ppm. The signal integrates for 2 protons.
- Aromatic protons on the phenyl ring correspond with the triplet at 7.14, the doublets at 7.26 and 7.37 and the singlet at 7.54 ppm, respectively. The signals integrate for 1 proton for each.

3.5.3 The physical data for the 8-sulfinylcaffeine analogues 6a–b, and the 8-sulfonylcaffeine, 7

8-(Benzylsulfinyl)caffeine (6a)

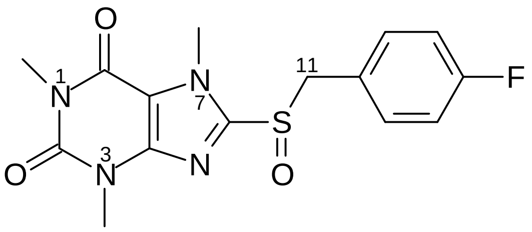


The title compound was prepared from 8-(benzylsulfanyl)caffeine in a yield of 42.12%: mp 165.6–169.7 ℃ (ethyl acetate). **^{1}H NMR** (Bruker Avance III 600, DMSO-*d6*) δ 3.20 (s, 3H), 3.44 (s, 3H), 3.58 (s, 3H), 4.61 (q, 2H, J = 12.4), 7.16 (m, 2H), 7.31 (m, 3H) ; **^{13}C NMR** (Bruker Avance III 600, DMSO-*d6*) δ 27.7, 29.6, 32.2, 59.3, 108.8, 128.6, 128.6, 129.2, 130.5, 146.9, 149.4, 150.8, 154.4; EI-HRMS m/z: calcd for $C_{15}H_{16}N_4O_3S$, 332.0943, found 332.0932; Purity (HPLC): 99%.

<u>^{1}H NMR</u>

- The three methyl groups at N1, N3 and N7, respectively, correspond to the singlets at 3.20, 3.44 and 3.58 ppm. The signals integrate for 3 protons each.
- The methylene group at C11 corresponds to the quartet at 4.61 ppm. The signal integrates for 2 protons.
- Aromatic protons on the phenyl ring correspond with multiplets at 7.16 and 7.31 ppm, respectively. The signals integrate for 2 protons for the first multiplet and 3 protons for the second multiplet.

8-{[(4-Fluorophenyl)methyl]sulfinyl}caffeine (6b)

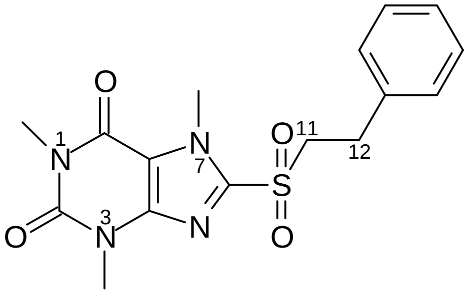


The title compound was prepared from 8-[(4-trifluorobenzyl)sulfanyl]caffeine in a yield of 56.49%: mp 190.4–191.9 ℃ (ethyl acetate). **^{1}H NMR** (Bruker Avance III 600, DMSO-*d6*) δ 3.20 (s, 3H), 3.43 (s, 3H), 3.67 (s, 3H), 4.62 (s, 2H), 7.15 (m, 2H), 7.22 (m, 2H) ; **^{13}C NMR** (Bruker Avance III 600, DMSO-*d6*) δ 27.7, 29.6, 32.3, 58.2, 109.0, 115.5, 115.6, 125.5, 125.5, 132.7, 132.7, 146.9, 149.2, 150.8, 154.5, 161.5, 163.1; EI-HRMS m/z: calcd for $C_{15}H_{15}FN_4O_3S$, 350.0849, found 350.0833; Purity (HPLC): 98%.

^{1}H NMR

- The three methyl groups at N1, N3 and N7, respectively, correspond to the singlets at 3.20, 3.43 and 3.67 ppm. The signals integrate for 3 protons each.
- The methylene group at C11 corresponds to the singlet at 4.62 ppm. The signal integrates for 2 protons.
- Aromatic protons on the phenyl ring correspond with multiplets at 7.15 and 7.22 ppm, respectively. The signals integrate for 2 protons each.

8-[(2-Phenylethyl)sulfonyl]caffeine (7)



The title compound was prepared from 8-[2-(phenylethyl)sulfanyl]caffeine in a yield of 27.06%: mp 185.7–187.5 ℃ (ethyl acetate). **^{1}H NMR** (Bruker Avance III 600, DMSO-*d6*) δ 3.04 (t, 2H, J = 7.5), 3.23 (s, 3H), 3.38 (s, 3H), 3.96 (t, 2H, J = 7.5), 4.14 (s, 3H), 7.09 (m, 1H), 7.20 (m, 4H) ; **^{13}C NMR** (Bruker Avance III 600, DMSO-*d6*) δ 27.9, 27.9, 29.6, 34.0, 55.4, 109.5, 126.3, 128.2, 128.3, 128.4, 137.0, 143.6, 145.7, 150.7, 154.9; EI-HRMS m/z: calcd for $C_{16}H_{18}N_4O_4S$, 362.1049, found 346.1100; Purity (HPLC): 85%.

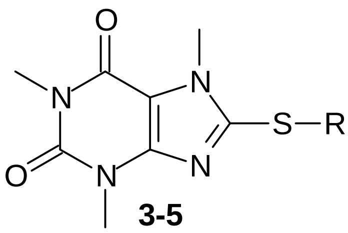
<u>^{1}H NMR</u>

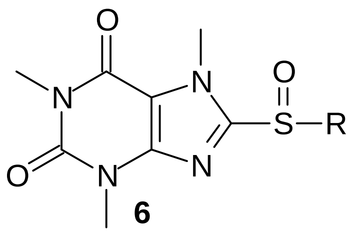
- The three methyl groups at N1, N3 and N7, respectively, correspond to the singlets at 3.23, 3.38 and 4.14 ppm. The signals integrate for 3 protons each.
- The methylene groups at C11 and C12, respectively, correspond to the triplets at 3.04 and 3.96 ppm. The signals integrate for 2 protons each.
- Aromatic protons on the phenyl ring correspond with multiplets at 7.09 and 7.20 ppm respectively. The signals integrate for 1 proton for the first multiplet and 4 protons for the second multiplet.

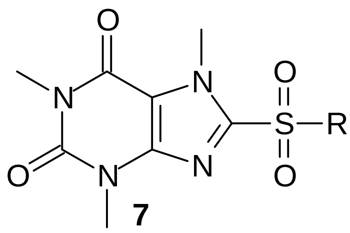
3.5.4 Interpretation of mass spectra

To further provide evidence that the structures of the 8-sulfanylcaffeine analogues are correct, their exact masses were recorded. As shown in table 3.7, the high resolution masses that were obtained for each of the 8-sulfanylcaffeine analogues closely correspond (<5 ppm) to that of the calculated values. This is further confirmation of the structures of these compounds.

Table 3.7. Correlation of the calculated exact masses with the experimentally obtained masses of the 8-sulfanylcaffeine analogues.


3-5


6


7

			Mass spectrometry		
Compound	**-R**	**Formula**	**Calcd.**	**Found**	**ppm**
3a	$-(CH_2)_2-(3-Cl-C_6H_4)$	$C_{16}H_{17}ClN_4O_2S$	364.0761	364.0757	0.48
3b	$-(CH_2)_2-(3-Br-C_6H_4)$	$C_{16}H_{17}BrN_4O_2S$	408.0256	408.0251	0.28
3c	$-(CH_2)_2-(3-CF_3-C_6H_4)$	$C_{17}H_{17}F_3N_4O_2S$	398.1024	398.1031	2.97
3d	$-(CH_2)_2-(3-CH_3-C_6H_4)$	$C_{17}H_{20}N_4O_2S$	344.1307	344.1259	0.75
3e	$-(CH_2)_2-(3-OCH_3-C_6H_4)$	$C_{17}H_{20}N_4O_3S$	360.1256	360.1255	1.18
4a	$-(CH_2)_3-C_6H_5$	$C_{17}H_{20}N_4O_2S$	344.1307	344.1308	1.79
4b	$-(CH_2)_3-(3-Cl-C_6H_4)$	$C_{17}H_{19}ClN_4O_2S$	378.0917	378.0902	-2.56
4c	$-(CH_2)_3-(4-Cl-C_6H_4)$	$C_{17}H_{19}ClN_4O_2S$	378.0917	378.0903	-2.25
5a	$-CH_2-(3-Cl-C_6H_4)$	$C_{15}H_{15}ClN_4O_2S$	350.0604	350.0600	0.21
5b	$-CH_2-(3-Br-C_6H_4)$	$C_{15}H_{15}BrN_4O_2S$	394.0099	394.0113	4.95
6a	$-CH_2-C_6H_5$	$C_{15}H_{16}N_4O_3S$	332.0943	332.0932	-1.78
6b	$-CH_2-(4-F-C_6H_4)$	$C_{15}H_{15}FN_4O_3S$	350.0849	350.0833	-2.96
7	$-(CH_2)_2-C_6H_5$	$C_{16}H_{18}N_4O_4S$	362.1049	346.1100	1.65

ppm = (found – calcd.)/calcd. x 1 000 000. In general a ppm difference smaller than 5 is considered to be in good agreement

3.6 Conclusion

This chapter described the successful synthesis of the following compounds:

1. 8-[(phenylethyl)sulfanyl]caffeines (**3a**–**e**),
2. 8-[(phenylpropyl)sulfanyl]caffeines (**4a**–**c**),
3. 8-(benzylsulfanyl)caffeines (**5a**–**b**),
4. 8-sulfinylcaffeines (**6a**–**b**)
5. and 8-sulfonylcaffeine (**7**)

The structures of the compounds were confirmed by NMR and MS and the purities were estimated by HPLC analysis. Both the ^{1}H NMR and ^{13}C NMR spectra corresponded with the proposed structures. In addition, the expected exact masses corresponded to the theoretical exact masses for each compound. The results of the HPLC analysis show that the compounds are of good purity. With the exception of the 8-sulfonylcaffeine analogue **7** (85%), the purities of the compounds ranged from 94–99%. In the next chapter, the MAO inhibitory properties of the synthesized compounds will be examined.