Chapter 6

First article

Novel sulfanylphthalimide analogues as highly potent inhibitors of monoamine oxidase B

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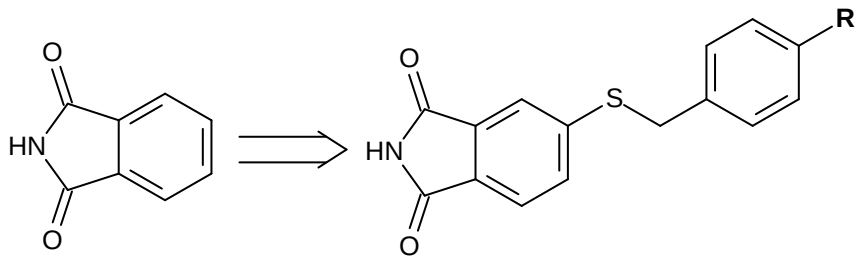
6.1 Graphical abstract



IC_{50} (MAO-B) = 134 µM

MAO-B	
R	**IC_{50} (µM)**
H	0.0045
Cl	0.0056
Br	0.0074
F	0.0068

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6.2 Author's contributions

Novel sulfanylphthalimide analogues as highly potent inhibitors of monoamine oxidase B

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The above mentioned article is part of the doctoral thesis of M.M. van der Walt. As required, the table below indicates the contribution of each author to this research article. Also, the written declaration for consent by each author is provided. This article was submitted for publication in Bioorganic & Medicinal Chemistry Letters and the guidelines to authors are also given.

Author contributions:	**Author name:**	**Description of contribution**
research design:	J.P. Petzer	Concept of the study was provided.
performed research:		
a) Synthetic work	M.M. van der Walt	Performed all the syntheses.
b) Characterization via NMR and MS	SASOL centre for chemistry, NWU	Carried out by the SASOL Centre for Chemistry, North-West University. NMR spectra was recorded by André Joubert and MS was recorded by Johan Jordaan.
c) Compound purity determination by HPLC	M.M. van der Walt	The degree of purity of each compound was determined by HPLC analysis.
d) IC_{50} value determination	M.M. van der Walt	The IC_{50} values for the inhibition of MAO-A and MAO-B were measured.
e) Recovery of enzyme activity after dilution study	M.M. van der Walt A. Petzer	The recovery of enzyme activity after dilution was performed for compound **8a**.
contributed new reagents/ analytic tools:		
a) Synthesis of compounds	G. Terre'Blanche	Providing the facilities, instrumentation and relevant reagents for performing the synthetic work.
b) IC_{50} value determinations and the recovery of enzyme activity	A. Petzer	Providing the facilities, instrumentation and relevant

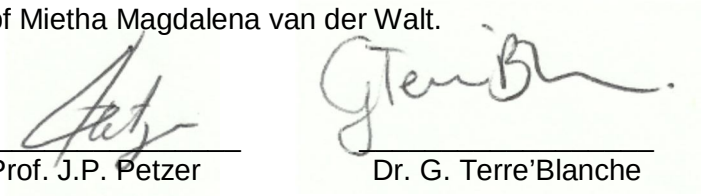
after dilution study		reagents for performing the IC_{50} value determintation and the recovery of enzyme activity after dilution study
analyzed data:		
a) Characterization by NMR and MS	M.M. van der Walt J.P. Petzer	The analyses of the NMR and MS data were performed by M.M. van der Walt, with critical feedback by J.P. Petzer.
b) Compound purity measurement determination	M.M. van der Walt J.P. Petzer	The analyses of the compound purity data were performed by M.M. van der Walt, with critical feedback by J.P. Petzer.
c) IC_{50} value measurements	M.M. van der Walt A. Petzer J.P. Petzer	Calculations of the IC_{50} values were performed by M.M. van der Walt, with critical feedback by J.P. Petzer and A. Petzer.
d) Recovery of enzyme activity after dilution study	M.M. van der Walt A. Petzer J.P. Petzer	The analyses and interpretation of the data was performed by M.M. van der Walt with critical feedback by J.P. Petzer and A. Petzer.
manuscript:		
a) Writing of manuscript	M.M. van der Walt J.P. Petzer	Contribution was equally for the mentioned authors.
b) Comments, suggestions and proof reading	G. Terre'Blanche A. Petzer	Contribution was equally for the mentioned authors

6.3 Declaration and consent of each co-author

Novel sulfanylphthalimide analogues as highly potent inhibitors of monoamine oxidase B

The following is a declaration by the co-authors that confirms their individual roles in the above mentioned article, as described in the table of author's contributions. The authors hereby give consent that this article may form part of this doctoral thesis.

I declare that I approve the inclusion of above mentioned article in this thesis, that my role in this study is as indicated in the author's contributions table and is representative of my actual contribution. Herewith, I grant consent that this article may be published as part of the doctoral thesis of Mietha Magdalena van der Walt.



Prof. J.P. Petzer	Dr. G. Terre'Blanche	Dr. A. Petzer

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6.6 Published article

Novel sulfanylphthalimide analogues as highly potent inhibitors of monoamine oxidase B

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Abstract—Monoamine oxidase (MAO) plays an essential role in the catabolism of neurotransmitter amines. The two isoforms of this enzyme, MAO-A and –B, are considered to be drug targets for the therapy of depression and neurodegenerative diseases, respectively. Based on a recent report that the phthalimide moiety may be a useful scaffold for the design of potent MAO-B inhibitors, the present study examines a series of 5-sulfanylphthalimide analogues as potential inhibitors of both human MAO isoforms. The results document that 5-sulfanylphthalimides are highly potent and selective MAO-B inhibitors with all of the examined compounds possessing IC_{50} values in the nanomolar range. The most potent inhibitor, 5-(benzylsulfanyl)phthalimide, exhibits an IC_{50} value of 0.0045 µM for the inhibition of MAO-B with a 427–fold selectivity for MAO-B compared to MAO-A. We conclude that 5-sulfanylphthalimides represent an interesting class of MAO-B inhibitors and may serve as lead compounds for the design of antiparkinsonian therapy.

Keywords: isatin; phthalimide; sulfanylphthalimide; monoamine oxidase; MAO; inhibition.

Monoamine oxidase A and B (MAO-A and –B) are flavine adenine dinucleotide (FAD) containing enzymes, bound to the outer membrane of mitochondria.[1,2] These enzymes catalyze the oxidative deamination of neurotransmitter and dietary amines thereby terminating their physiological actions.[3] Although MAO-A and –B share 70% sequence identity, they exhibit different substrate and inhibitor specificities.[4] MAO-A metabolizes serotonin, adrenaline and noradrenaline and inhibitors of this enzyme are in use for the treatment of clinical depression and anxiety.[5] MAO-B preferentially metabolizes the dietary amine, 2-phenylethylamine, and may therefore act as a metabolic brain barrier, limiting the entry of this false neurotransmitter into the central nervous system.[6,7] Since MAO-B also catabolizes dopamine in the brain, inhibitors of this enzyme are used in the treatment of Parkinson's disease.[5,7,8] In Parkinson's disease, MAO-B inhibitors conserve the depleted supply of central dopamine and enhance dopamine levels following administration of levodopa, the metabolic precursor of dopamine.[9] For these reasons, MAO-B inhibitors are frequently combined with levodopa in Parkinson's disease therapy. It should be noted that MAO-A also metabolizes dopamine in the primate brain, and MAO-A inhibitors may consequently also elevate dopamine levels in the central nervous system.[9] In addition, MAO-A inhibitors may be employed to treat non-motor symptoms of Parkinson's disease such as depression and anxiety.[3,10] MAO-A inhibitors in conjunction with levodopa should, however, be used with caution since this may lead to a severe hypertensive response.[11]

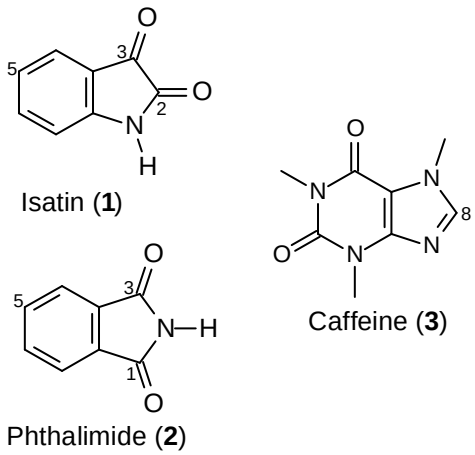


Figure 1. The structures of isatin (**1**), phthalimide (**2**) and caffeine (**3**).

A wide variety of heterocyclic moieties have been employed in the design of MAO inhibitors. Among these are isatin (**1**), an endogenous small molecule inhibitor of MAO-A and –B, and caffeine (**3**) (Fig. 1).[12–14] Phthalimide (**2**), an isomer of isatin, has also recently been reported to be a potentially useful scaffold for the design of MAO-B selective inhibitors.[15] Although phthalimide is a weak MAO inhibitor, substitution on C5 yields structures endowed with highly potent and selective MAO-B inhibitory activities. In contrast, N-substitution yields structures that are essentially devoid of MAO inhibitory properties.[15] The MAO-B inhibitory properties of isatin

and caffeine may similarly be enhanced by substitution on the C5 and C6 positions of isatin and the C8 position of caffeine. In this regard, the benzyloxy substituent has been shown to be particularly favorable, and benzyloxy substitution of isatin, caffeine and phthalimide yields compounds **4**–**6** which are several orders of magnitude more potent MAO-B inhibitors than the parent compounds (Fig. 2).[13–15]In all instances, halogen substitution on the benzyloxy ring further improves inhibition potency. Modeling studies have shown that productive interactions of the benzyoxy side chain with the MAO-B entrance cavity may be responsible for this behavior. Interestingly, the benzylsulfanyl side chain appears to exhibit similar properties to that of the benzyloxy moiety, since a series of 8-(benzylsulfanyl)caffeine (**7**) analogues has recently been shown to exhibit similar MAO-B inhibition potencies to those of the 8-benzyloxycaffeine (**5**) analogues.[16] Based on these analyses, the present study examines the possibility that benzylsulfanyl substitution on C5 of phthalimide (to yield **8a**) would also lead to highly potent MAO-B inhibition. For this purpose, the effect that substitution (Cl, Br, F and OCH_3) on the benzylsulfanyl ring has on MAO inhibition will be explored. In addition, this study also determines the effect on MAO inhibition by the phenylsulfanyl, (2-phenylethyl)sulfanyl, cyclohexylsulfanyl and (3-methylbutyl)sulfanyl substituents. This study therefore aims to discover new highly potent MAO-B inhibitors and to contribute to the structure-activity relationships (SARs) of MAO inhibition by phthalimide derived compounds.

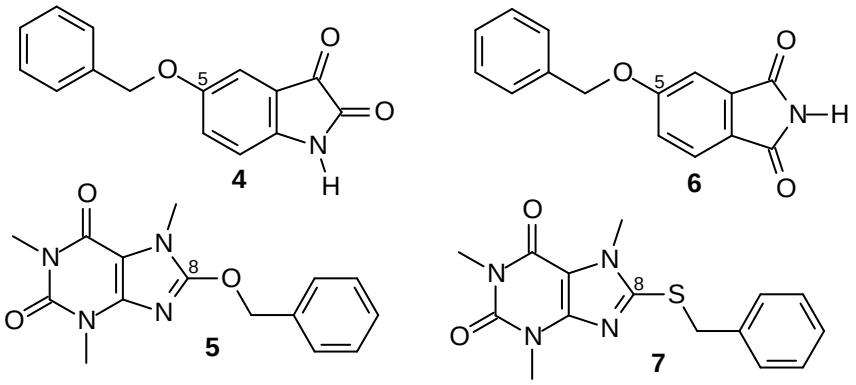
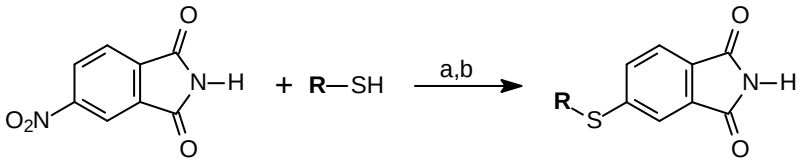


Figure 2. The structures of 5-benzyloxyisatin (**4**), 8-benzyloxycaffeine (**5**), 5-benzyloxyphthalimide (**6**) and 8-(benzylsulfanyl)caffeine (**7**).

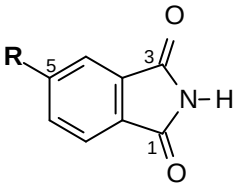
The 5-sulfanylphthalimides (**8a**–**k**) were conveniently synthesized according to a previously described protocol (Scheme 1).[17] The appropriate thiol reagents were reacted with 5-nitrophthalimide in the presence of K_2CO_3 to yield the target compounds in low to good yields (4–76%). The 5-sulfanylphthalimides were purified via crystallization from an appropriate solvent. In each instance, the structures and purities of the target compounds were verified by ^{1}H NMR, ^{13}C NMR, mass spectrometry and HPLC analysis as cited in the supplementary material. The presence of two ^{13}C NMR signals at 167.6–168.8 ppm, which corresponds to the

carbonyl carbons at C1 and C3, and a ^{1}H NMR signal at 8.12–8.20 ppm ($CDCl_3$) or 11.28–11.36 ppm (DMSO-*d6*), which corresponds to the phthalimide NH proton, confirmed the presence of the phthalimide ring (Table 1).



Scheme 1. Synthetic pathway to 5-sulfanylphthalimide analogues. Reagents and conditions: (**a**) K_2CO_3, acetone, reflux, 24 h; (**b**) HCl (6 N).

Table 1. The ^{1}H NMR and ^{13}C NMR chemical shifts for the NH proton and carbonyl C1 and C3 of phthalimide analogues **8a**–**k**.



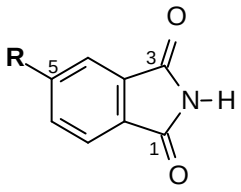
	R	NH	C1/C3	
8a	–S–(CH_2)–C_6H_5	11.28[a]	168.8[a]	168.8[a]
8b	– S–(CH_2)–(4-Cl-C_6H_4)	11.29[a]	168.8[a]	168.8[a]
8c	– S–(CH_2)–(4-Br-C_6H_4)	11.29[a]	168.7[a]	168.8[a]
8d	– S–(CH_2)–(4-F-C_6H_4)	11.29[a]	168.8[a]	168.8[a]
8e	–S–(CH_2)–(4-OCH_3-C_6H_4)	11.28[a]	168.8[a]	168.8[a]
8f	–S–C_6H_5	8.20[b]	167.7[b]	167.8[b]
8g	–S–(4-Cl-C_6H_4)	11.35[a]	168.5[a]	168.6[a]
8h	– S–(4-Br-C_6H_4)	11.36[a]	168.5[a]	168.6[a]
8i	–S–(CH_2)$_2$–C_6H_5	11.28[a]	168.8[a]	168.8[a]
8j	–S–C_6H_{11}	11.30[a]	167.6[b]	167.8[b]
8k	–S–(CH_2)$_2$–CH(CH_3)$_2$	8.12[b]	167.9[b]	168.0[b]

[a] NMR experiments conducted in DMSO-*d6*.

[b] NMR experiments conducted in $CDCl_3$.

To examine the MAO inhibitory properties of the 5-sulfanylphthalimides, recombinant human MAO-A and –B were employed.[18] The enzyme catalytic activities in the absence and presence of the test inhibitors were determined by fluorometrically measuring the MAO-catalyzed formation of 4-hydroxyquinoline from the mixed MAO–A/B substrate, kynuramine.[14,18] This approach was suitable for evaluating the MAO inhibitory properties of all the 5-sulfanylphthalimides. The inhibition potencies of the test compounds were calculated from the sigmoidal dose–response curves and are expressed as the corresponding IC_{50} values. These IC_{50} values as well as the selectivity index (SI) values [SI = IC_{50}(MAO–A)/(IC_{50}(MAO–B)] are given in table 2. The results document that all of the 5-sulfanylphthalimides are potent inhibitors of human MAO-B with IC_{50} values ranging from 0.0045–0.986 µM. In accordance with expectation (see introduction), benzylsulfanyl substitution of phthalimide to yield **8a**, resulted in highly potent MAO-B inhibition. An IC_{50} value of 0.0045 µM was recorded for **8a**. In fact **8a** proved to be the most potent MAO-B inhibitor among the compounds of the present series. Compared to its C5 benzyloxy substituted phthalimide homologue, compound **6** (IC_{50} = 0.043 µM), compound **8a** is approximately eightfold more potent as a MAO-B inhibitor.[15] Compound **8a** also proved to be a more potent MAO-B inhibitor than 5-benzyloxyisatin (**4**) (IC_{50} = 0.103 µM),[13] 8-benzyloxycaffeine (**5**) (IC_{50} = 1.77 µM)[14] and 8-(benzylsulfanyl)caffeine (**7**) (IC_{50} = 1.86 µM).[16] Substitution on the benzylsulfanyl ring with Cl, Br, F and OCH_3, to yield **8b**–**e**, also resulted in highly potent inhibition, with these homologues exhibiting IC_{50} values of 0.0056–0.020 µM. It is interesting to note that particularly the halogen substituted homologues **8b**–**d** are weaker MAO-B inhibitors than compound **8a**. This is in contrast to the results obtained with 8-benzyloxycaffeine (**5**), 5-benzyloxyphthalimide (**6**) and 8-(benzylsulfanyl)caffeine (**7**), where particularly bromine substitution on the phenyl ring leads to an enhancement in MAO-B inhibitory potency.[14–16] From these results it may therefore be concluded that 5-(benzylsulfanyl)phthalimides are highly potent MAO-B inhibitors and superior to the lead structures, compounds **4**–**7**, of this study.

Table 2. The IC_{50} values for the inhibition of recombinant human MAO-A and –B by 5-sulfanylphthalimides **8a**–**k**.



		IC_{50} (μM)[a]		
	R	MAO-A	MAO-B	SI[b]
8a	–S–(CH_2)–C_6H_5	1.92 ± 0.172	0.0045 ± 0.0004	427
8b	– S–(CH_2)–(4-Cl-C_6H_4)	0.506 ± 0.032	0.0056 ± 0.0003	90
8c	– S–(CH_2)–(4-Br-C_6H_4)	0.273 ± 0.041	0.0074 ± 0.0029	37
8d	– S–(CH_2)–(4-F-C_6H_4)	0.958 ± 0.003	0.0068 ± 0.0009	141
8e	–S–(CH_2)–(4-OCH_3-C_6H_4)	1.63 ± 0.023	0.020 ± 0.0042	82
8f	–S–C_6H_5	8.03 ± 0.622	0.986 ± 0.026	8.1
8g	–S–(4-Cl-C_6H_4)	1.68 ± 0.343	0.457 ± 0.093	3.7
8h	– S–(4-Br-C_6H_4)	1.01 ± 0.053	0.364 ± 0.050	2.8
8i	–S–$(CH_2)_2$–C_6H_5	2.27 ± 0.136	0.030 ± 0.0094	76
8j	–S–C_6H_{11}	1.03 ± 0.062	0.179 ± 0.0082	5.8
8k	–S–$(CH_2)_2$–$CH(CH_3)_2$	0.380 ± 0.042	0.015 ± 0.0033	26

[a] All values are expressed as the mean ± SD of triplicate determinations.

[b] The selectivity index is the selectivity for the MAO-B isoform and is given as the ratio of [IC_{50}(MAO-A)]/[IC_{50}(MAO-B)].

Although still considered as a potent MAO-B inhibitor, the phenylsulfanyl homologue **8f** (IC_{50} = 0.986 μM) was the weakest inhibitor of the present series. In this case, however, halogen substitution in the phenyl ring to yield compounds **8g** (IC_{50} = 0.457 μM) and **8h** (IC_{50} = 0.364 μM), resulted in enhanced MAO-B inhibition. The (2-phenylethyl)sulfanyl substituted homologue **8i** was also found to be a potent MAO-B inhibitor with an IC_{50} value of 0.030 μM, approximately sixfold weaker than **8a**. Since both **8f** and **8i** are weaker MAO-B inhibitor than **8a**, it may be concluded that the benzylsulfanyl side chain is particularly suitable for enhancing the MAO-B inhibitory potency of phthalimide, and that neither a reduction in side chain length (to yield **8f**), nor an increase in chain length (to yield **8i**) would further increase inhibitory activity. The general suitability of 5-sulfanylphthalimides for MAO-B inhibition was further demonstrated with the

finding that the cyclohexylsulfanyl (**8j**) and (3-methylbutyl)sulfanyl (**8k**) substituted homologues are also potent MAO-B inhibitors with IC_{50} values of 0.179 µM and 0.015 µM, respectively.

The 5-sulfanylphthalimides were found to also act as inhibitors of MAO-A. Four homologues, **8b**–**d** and **8k**, exhibited IC_{50} values in the nanomolar range, with **8c** being the most potent MAO-A inhibitor with an IC_{50} value of 0.273 µM. As evident from the SI values, all of the 5-sulfanylphthalimides were, however, selective inhibitors of the MAO-B isoform. The most potent MAO-B inhibitor of the series, **8a**, is also the most selective inhibitor with an SI value of 427. This compound may therefore be considered the most suitable homologue of the series where MAO-B selectivity is desired. As already noted, MAO-A inhibition may lead to adverse effects when combined with certain antiparkinsonian therapies. In contrast, **8c** may be considered as a potent MAO inhibitor with comparatively low isoform selectivity.

Since it is reported that the benzyloxyphthalimide class of MAO inhibitors interacts reversibly with MAO, the current study examined if this property is also shared by sulfanylphthalimides.[15]For this purpose, the reversibility of MAO-B inhibition by the most potent sulfanylphthalimide MAO-B inhibitor, compound **8a**, was investigated by measuring the degree of enzyme recovery after dilution of the enzyme-inhibitor complex. MAO-B was preincubated with **8a** at concentrations of 10 × IC_{50} and 100 × IC_{50} for 30 min and then diluted to 0.1 × IC_{50} and 1 × IC_{50}, respectively.[19] The results show that after dilution of the enzyme-inhibitor complexes to concentrations of **8a** equal to 0.1 × IC_{50} and 1 × IC_{50}, the MAO-B catalytic activities are recovered to levels of approximately 72% and 28%, respectively, of the control value (Fig. 3). This behavior is consistent with a reversible interaction of **8a** with MAO-B. In contrast, incubation of MAO-B with the irreversible inhibitor (R)-deprenyl (10 × IC_{50}), the MAO-B activities were not recovered (2.1% of control). Interestingly, after dilution of the **8a**–MAO-B complex to 0.1 × IC_{50} and 1 × IC_{50}, the enzyme activities are not recovered to 90% and 50%, respectively, as expected. This result suggests that, for the inhibition of MAO-B, **8a** may possess a quasi-reversible or tight-binding component.

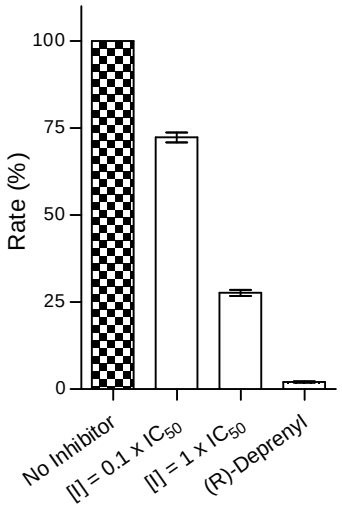


Figure 3. The reversibility of the inhibition of MAO-B by **8a**. The enzyme was preincubated with **8a** at 10 × IC_{50} and 100 × IC_{50} for 30 min and then diluted to 0.1 × IC_{50} and 1 × IC_{50}, respectively. For comparison, (R)-deprenyl, at 10 × IC_{50} was similarly incubated with MAO-B and diluted to 0.1 × IC_{50}. The residual enzyme activities were subsequently measured.

In conclusion, the present study shows that 5-sulfanylphthalimides are potent and selective inhibitors of MAO-B. In this regard, the benzylsulfanyl side chain is particularly suitable for enhancing the MAO-B inhibitory potency of phthalimide. It is noteworthy that compound **8a** (IC_{50} = 0.0045 μM) is approximately 30,000-fold more potent than phthalimide (**2**) (IC_{50} = 134 μM).[15]This illustrates the importance of the C5 side chain for MAO-B inhibitory activity. Based on their MAO-B inhibition potencies and appropriate selectivity profiles, this study concludes that 5-sulfanylphthalimides are suitable lead compounds for the development of antiparkinsonian drugs. From a design point of view it is noteworthy that a wide variety of C5 substituents yield 5-sulfanylphthalimides with potent MAO-B inhibitory actions. This suggests that structural modifications made to the C5 side chain in order to improve the properties of the compound are less likely to reduce MAO-B inhibition potency.

Acknowledgements

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6.7 Supplementary material

Novel sulfanylphthalimide analogues as highly potent inhibitors of monoamine oxidase B

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6.7.1 Experimental procedures

6.7.1.1 Chemicals and instrumentation

Unless otherwise noted, all the reagents were obtained from Sigma-Aldrich and were used without further purification. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Bruker Avance III 600 spectrometer in $CDCl_3$ or DMSO-*d6*. The chemical shifts are reported in parts per million (δ) relative to the signal of tetramethylsilane. Spin multiplicities are abbreviated as follows: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet) or m (multiplet). High resolution mass spectra (HRMS) were recorded with a Bruker micrOTOF-Q II mass spectrometer in atmospheric-pressure chemical ionization (APCI) mode. Melting points (mp) were measured with a Buchi M-545 melting point apparatus and are uncorrected. A Varian Cary Eclipse fluorescence spectrophotometer was used for fluorescence spectrophotometry. For the enzymology, kynuramine.2HBr and microsomes from insect cells containing recombinant human MAO-A and –B (5 mg protein/mL) were obtained from Sigma-Aldrich.

6.7.1.2 Synthesis of the 5-sulfanylphthalimide analogues (**8a–k**)

A mixture of the appropriate thiol (6 mmol), 4-nitrophthalimide (4 mmol) and K_2CO_3 (10 mmol) in 20 mL acetone was heated under reflux for 24 h. The reaction was cooled to room temperature and diluted with 150 mL water. The mixture was subsequently acidified to pH = 2 with 6 N HCl. The resulting precipitate was collected by filtration and dried at 50 ºC.[1]

5-(Benzylsulfanyl)phthalimide (8a)

The title compound (yellow crystals) was prepared from 4-nitrophthalimide and benzyl mercaptan in a yield of 65%: mp 177.5–177.6 ºC (ethanol); ^{1}H NMR (Bruker Avance III 600, DMSO-*d6*) δ 4.43 (s, 2H), 7.23–7.26 (m, 1H), 7.30–7.33 (m, 2H), 7.42 (d, 2H, J = 7.5 Hz), 7.67 (s, 3H), 11.28 (s, 1H); ^{13}C NMR (Bruker Avance III 600, DMSO-*d6*) δ 35.4, 120.2, 123.2, 127.4,

128.6, 128.9, 129.0, 131.5, 133.5, 136.3, 145.5, 168.8, 168.8; APCI-HRMS *m/z*: calcd for $C_{15}H_{12}NO_2S$ (MH^+), 270.0589, found 270.0568; Purity (HPLC): 100%.

5-[(4-Chlorobenzyl)sulfanyl]phthalimide (8b)

The title compound (white powder) was prepared from 4-nitrophthalimide and 4-chlorobenzyl mercaptan in a yield of 9%: mp 228.0–228.14 ºC (ethanol); ^{1}H NMR (Bruker Avance III 600, DMSO-*d6*) δ 4.45 (s, 2H), 7.37 (d, 2H, J = 8.7 Hz), 7.44 (d, 2H, J = 8.7 Hz), 7.67 (m, 3H), 11.29 (s, 1H); ^{13}C NMR (Bruker Avance III 600, DMSO-*d6*) δ 34.6, 120.4, 123.3, 128.5, 129.2, 130.7, 131.8, 131.9, 133.5, 135.7, 145.0, 168.8, 168.8; APCI-HRMS *m/z*: calcd for $C_{15}H_{11}ClNO_2S$ (MH^+), 304.0199, found 304.0174; Purity (HPLC): 99%.

5-[(4-Bromobenzyl)sulfanyl]phthalimide (8c)

The title compound (light yellow powder) was prepared from 4-nitrophthalimide and 4-bromobenzyl mercaptan in a yield of 7%: mp 255.7–255.9 ºC (ethanol); ^{1}H NMR (Bruker Avance III 600, DMSO-*d6*) δ 4.43 (s, 2H), 7.38 (d, 2H, J = 8.3 Hz), 7.51 (d, 2H, J = 8.3 Hz), 7.68 (m, 3H), 11.29 (s, 1H); ^{13}C NMR (Bruker Avance III 600, DMSO-*d6*) δ 34.6, 120.4, 120.5, 123.3, 129.2, 131.0, 131.4, 131.8, 133.5, 136.1, 145.0, 168.7, 168.8; APCI-HRMS *m/z*: calcd for $C_{15}H_{11}BrNO_2S$ (MH^+), 347.9694, found 347.9662; Purity (HPLC): 100%.

5-[(4-Fluorobenzyl)sulfanylphthalimide (8d)

The title compound (dark yellow powder) was prepared from 4-nitrophthalimide and 4-fluorobenzyl mercaptan in a yield of 70%: mp 179.8–180.2 ºC (ethanol); ^{1}H NMR (Bruker Avance III 600, DMSO-*d6*) δ 4.43 (s, 2H), 7.13 (t, 2H, J = 8.7 Hz), 7.44–7.46 (m, 2H), 7.67 (s, 3H), 11.29 (s, 1H); ^{13}C NMR (Bruker Avance III 600, DMSO-*d6*) δ 34.6, 115.3, 115.4, 120.4, 123.3, 129.1, 130.8, 130.9, 131.7, 132.6, 133.5, 145.2, 160.6, 162.2, 168.8, 168.8; APCI-HRMS *m/z*: calcd for $C_{15}H_{11}FNO_2S$ (MH^+), 288.0495, found288.0484; Purity (HPLC): 96%.

5-[(4-Methoxybenzyl)sulfanyl]phthalimide (8e)

The title compound was prepared from 4-nitrophthalimide and 4-methoxybenzyl mercaptan in a yield of 63%: mp 172.7–172.8 ºC (ethanol); ^{1}H NMR (Bruker Avance III 600, DMSO-*d6*) δ 3.70 (s, 3H), 4.37 (s, 2H), 6.87 (d, 2H, J = 8.7 Hz), 7.33 (d, 2H, J = 8.7 Hz), 7.66 (s, 3H), 11.28 (s, 1H); ^{13}C NMR (Bruker Avance III 600, DMSO-*d6*) δ 35.0, 55.0, 114.0, 120.2, 123.2, 127.9, 128.9, 130.1, 131.5, 133.5, 145.8, 158.5, 168.8, 168.8; APCI-HRMS *m/z*: calcd for $C_{16}H_{14}NO_3S$ (MH^+), 300.0694, found 300.0675; Purity (HPLC): 100%.

5-(Phenylsulfanyl)phthalimide (8f)

The title compound (yellow crystals) was prepared from 4-nitrophthalimide and thiophenol in a yield of 4%: mp 166.6–168.0 ºC (ethanol); ^{1}H NMR (Bruker Avance III 600, $CDCl_3$) δ 7.43–7.44 (m, 4H), 7.47 (m, 1H), 7.51–7.52 (m, 2H), 7.66 (d, 1H, J = 7.9 Hz), 8.20 (s, 1H); ^{13}C NMR (Bruker Avance III 600, $CDCl_3$) δ 121.5, 123.8, 129.1, 129.7, 130.1, 130.4, 131.9, 133.5, 134.6, 148.3, 167.7, 167.8; APCI-HRMS *m/*z: calcd for $C_{14}H_{10}NO_2S$ (MH^+), 256.0432, found 256.0404; Purity (HPLC): 100%.

5-[(4-Chlorophenyl)sulfanyl]phthalimide (8g)

The title compound (white powder) was prepared from 4-nitrophthalimide and 4-chlorothiophenol in a yield of 76%: mp 198.8–200.4 ºC (ethanol); ^{1}H NMR (Bruker Avance III 600, DMSO-*d6*) δ 7.40 (s, 1H), 7.54–7.56 (m, 5H), 7.73 (d, 1H, J = 7.9 Hz), 11.35 (s, 1H); ^{13}C NMR (Bruker Avance III 600, DMSO-*d6*) δ 121.0, 123.9, 129.9, 130.1, 130.2, 132.6, 133.8, 134.5, 135.5, 144.8, 168.5, 168.6.; APCI-HRMS *m/*z: calcd for $C_{14}H_9ClNO_2S$ (MH^+), 290.0043, found 290.0014; Purity (HPLC): 100%.

5-[(4-Bromophenyl)sulfanyl]phthalimide (8h)

The title compound (white powder) was prepared from 4-nitrophthalimide and 4-bromothiophenol in a yield of 46%: mp 203.2–203.3 ºC (ethanol); ^{1}H NMR (Bruker Avance III 600, DMSO-*d6*) δ 7.41 (d, 1H, J = 1.5 Hz), 7.48 (d, 2H, J = 8.7 Hz), 7.55 (dd, 1H, J = 1.5, 7.9 Hz), 7.69 (d, 2H, J = 8.7 Hz), 7.73 (d, 1H, J = 7.9 Hz), 11.36 (s, 1H);^{13}C NMR (Bruker Avance III 600, DMSO-*d6*) δ 121.2, 123.1, 123.9, 130.1, 130.5, 132.7, 133.1, 133.8, 135.6, 144.6, 168.5, 168.6; APCI-HRMS *m/*z: calcd for $C_{14}H_9BrNO_2S$ (MH^+), 333.9537, found 333.9538; Purity (HPLC): 99%.

5-[(2-Phenylethyl)sulfanyl]phthalimide (8i)

The title compound (dark yellow needles) was prepared from 4-nitrophthalimide and 2-phenylethyl mercaptan in a yield of 75%: mp 139.0–139.5 ºC (ethanol); ^{1}H NMR (Bruker Avance III 600, DMSO-*d6*) δ 2.92 (t, 2H, J = 7.6 Hz), 3.40 (t, 2H, J = 7.2 Hz), 7.19–7.21 (m, 1H), 7.26–7.30 (m, 4H), 7.63–7.69 (m, 3H), 11.28 (s, 1H); ^{13}C NMR (Bruker Avance III 600, DMSO-*d6*) δ 32.3, 34.0, 120.0, 123.3, 126.4, 128.4, 128.6, 128.8, 131.4, 133.6, 139.6, 145.6, 168.8, 168.8; APCI-HRMS *m/*z: calcd for $C_{16}H_{14}NO_2S$ (MH^+), 284.0745, found 284.0720; Purity (HPLC): 100%.

5-(Cyclohexylsulfanyl)phthalimide (8j)

The title compound (light yellow powder) was prepared from 4-nitrophthalimide and cyclohexanethiol in a yield of 5%: mp 180.7–180.9 ºC (ethanol); ^{1}H NMR (Bruker Avance III 600, DMSO-*d6*) δ 1.22–1.43 (m, 6H), 1.58–1.60 (m, 1H), 1.69–1.72 (m, 2H), 1.94–1.97 (m, 2H), 3.59–3.63 (m, 1H), 7.67–7.71 (m, 2H), 11.30 (s, 1H); ^{13}C NMR (Bruker Avance III 600, $CDCl_3$) δ 25.6, 25.8, 32.9, 45.1, 122.4, 123.7, 128.8, 133.3, 133.5, 146.5, 167.6, 167.8; APCI-HRMS *m/z*: calcd for $C_{14}H_{16}NO_2S$ (MH^+), 262.0902, found 262.0882; Purity (HPLC): 96%.

5-[(3-Methylbutyl)sulfanyl]phthalimide (8k)

The title compound (light yellow powder) was prepared from 4-nitrophthalimide and 3-methyl-1-butanethiol in a yield of 19%: mp 64-115 ºC (ethanol); ^{1}H NMR (Bruker Avance III 600, $CDCl_3$) δ 0.94 (d, 6H, J = 6.8 Hz), 1.56–1.60 (q, 2H, J = 7.2 Hz), 1.72–1.76 (m, 1H), 3.01–3.03 (m, 2H), 7.51 (dd, 1H, J = 1.5, 7.9 Hz), 7.63 (d, 1H, J = 1.5 Hz), 7.69 (d, 1H, J = 7.9 Hz), 8.12 (s, 1H); ^{13}C NMR (Bruker Avance III 600, $CDCl_3$) δ 22.2, 27.5, 30.2, 37.2, 120.4, 123.6 128.3, 131.5, 133.4, 147.9, 167.9, 168.0; APCI-HRMS *m/z*: calcd for $C_{13}H_{16}NO_2S$ (MH^+), 250.0902, found 250.0884; Purity (HPLC): 100%.

6.7.1.3 IC_{50} value determination

Recombinant human MAO-A and MAO-B containing microsomes (5 mg protein/mL) were pre-aliquoted and stored at –70 ºC. All incubations were carried out in potassium phosphate buffer (K_2HPO_4/KH_2PO_4 100 mM, made isotonic with KCl, pH 7.4) to a final volume of 500 µL. The reactions contained various concentrations of the test inhibitor (0-100 µM) and kynuramine, a mixed MAO-A/B substrate. The final concentrations of kynuramine in the reactions were 45 µM and 30 µM for MAO-A and MAO-B, respectively. The stock solutions of all the test inhibitors were prepared in DMSO and added to the reactions to yield a final volume of 4% (v/v) DMSO. The reactions were initiated with the addition of MAO-A or MAO-B (0.0075 mg protein/mL) and allowed to incubate for 20 min at 37 ºC. The reactions were subsequently terminated with the addition of 400 µL NaOH (2 N) and 1000 µL water, and the concentrations of the MAO generated 4-hydroxyquinoline were measured by fluorescence spectrophotometry (λ_{em} = 310; λ_{ex} = 400 nm).[2,3] By employing a linear calibration curve (4-hydroxyquinoline: 0.047–1.56 µM), the enzyme catalytic rates were calculated and fitted to the one site competition model incorporated into the Prism software package (GraphPad). The IC_{50} values were determined in triplicate and are expressed as mean ± standard deviation (SD).

6.7.1.4 Recovery of enzyme activity after dilution

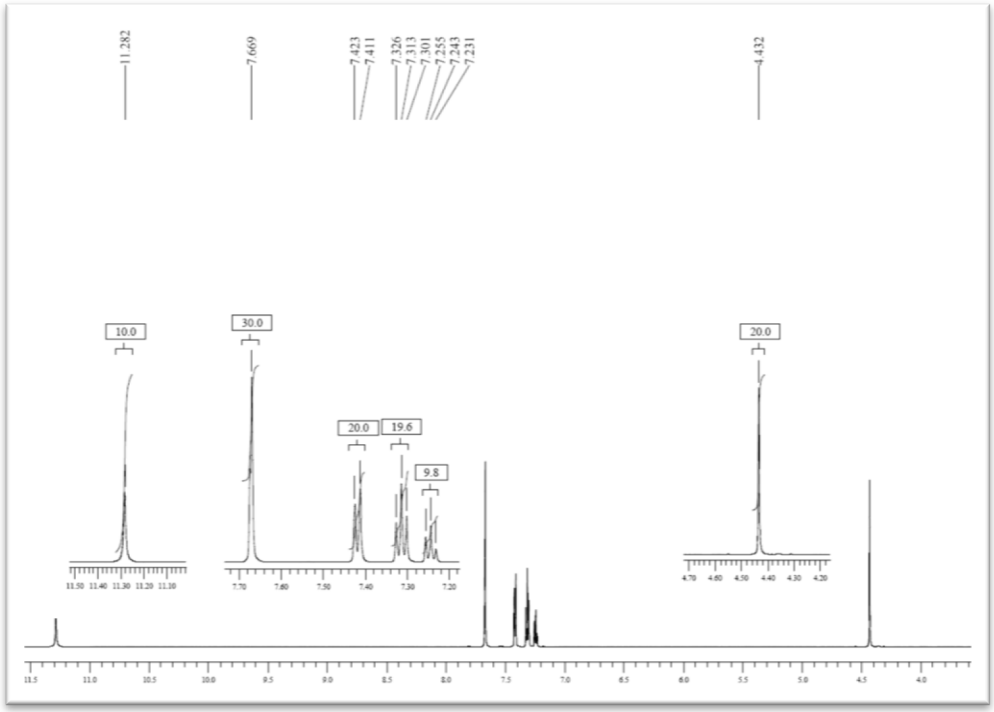
Compound **8a** [IC_{50}(MAO-B) = 0.0045 μM], at concentrations equal to 0 × IC_{50}, 10 × IC_{50} and 100 × IC_{50}, was preincubated with recombinant human MAO-B (0.75 mg/ml) for 30 min at 37 ºC. The reaction solvent was K_2HPO_4/KH_2PO_4 (pH 7.4, 100 mM, made isotonic with KCl). DMSO at a final concentration of 4% was added as co-solvent to all preincubations. The reactions were subsequently diluted 100-fold with the addition of a solution of kynuramine to yield final concentrations of **8a** equal to 0 × IC_{50}, 0.1 × IC_{50} and 1 × IC_{50}, and a final concentration of kynuramine equal to 30 μM. The final concentration of MAO-B was 0.0075 mg/mL. The reactions were incubated for a further 20 min at 37 °C, terminated and the residual rates of 4-hydroxyquinoline formation were measured as described above for the IC_{50} value determinations.[4] As control experiments, (R)-deprenyl (IC_{50} = 0.079 μM) was similarly preincubated with recombinant human MAO-A and –B (0.75 mg/ml) at concentrations equal to 10 × IC_{50} and diluted 100-fold with the addition of kynuramine to yield final concentrations of (R)-deprenyl equal to 0.1 × IC_{50}.[4]

6.7.2 References

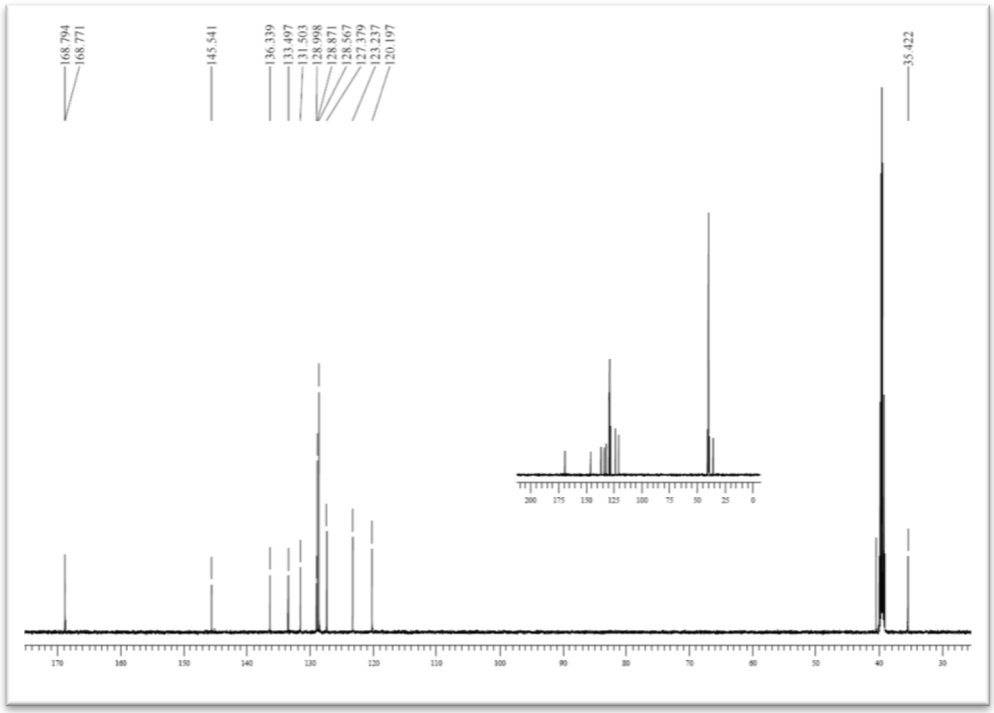
1. Arend, M. P.; Flippin, L. A.; Guenzler-Pukall, V.; Ho, W. –B.; Turtle, E. D.; Du, X. U. S. Patent 20040254215, **2004**.
2. Strydom, B.; Malan, S. F.; Castagnoli, N., Jr.; Bergh, J. J.; Petzer, J. P. *Bioorg. Med. Chem.* **2010**, *18,* 1018.
3. Novaroli, L.; Reist, M.; Favre, E.; Carotti, A.; Catto, M.; Carrupt, P. A. *Bioorg. Med. Chem.* **2005**, *13,* 6212.
4. Petzer, A.; Harvey, B. H.; Wegener, G.; Petzer, J. P. *Toxicol. Appl. Pharm*. **2012**, *258*, 403.

6.7.3. NMR spectra of the 5-sulfanylphthalimide analogues (**8a–k**)

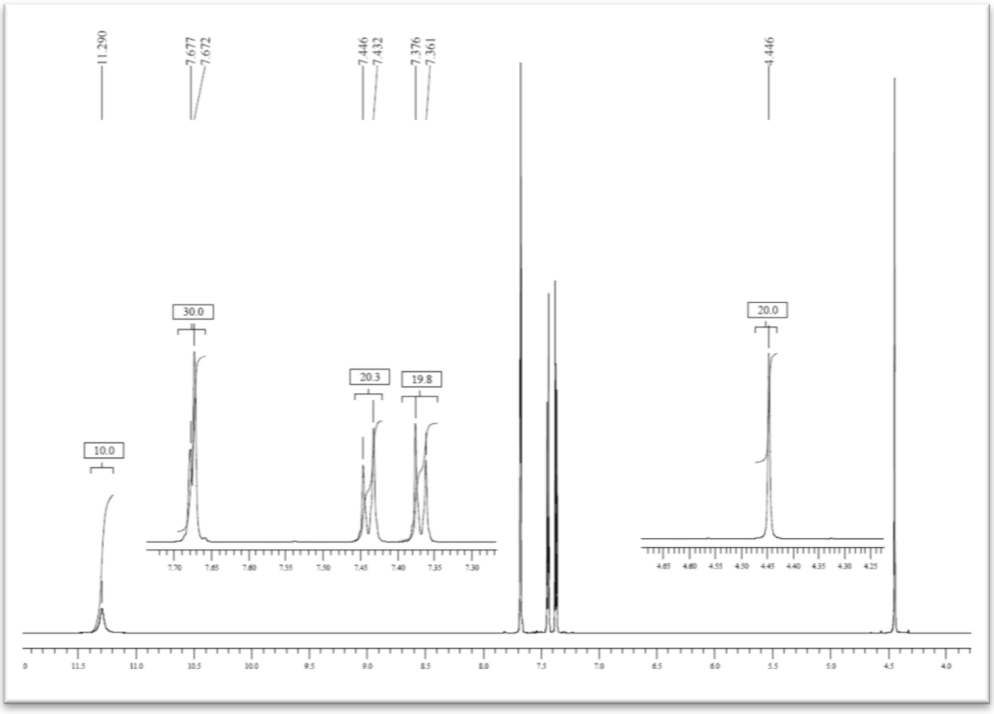
^{1}H NMR (DMSO-*d6*): **5-(Benzylsulfanyl)phthalimide** (**8a**)



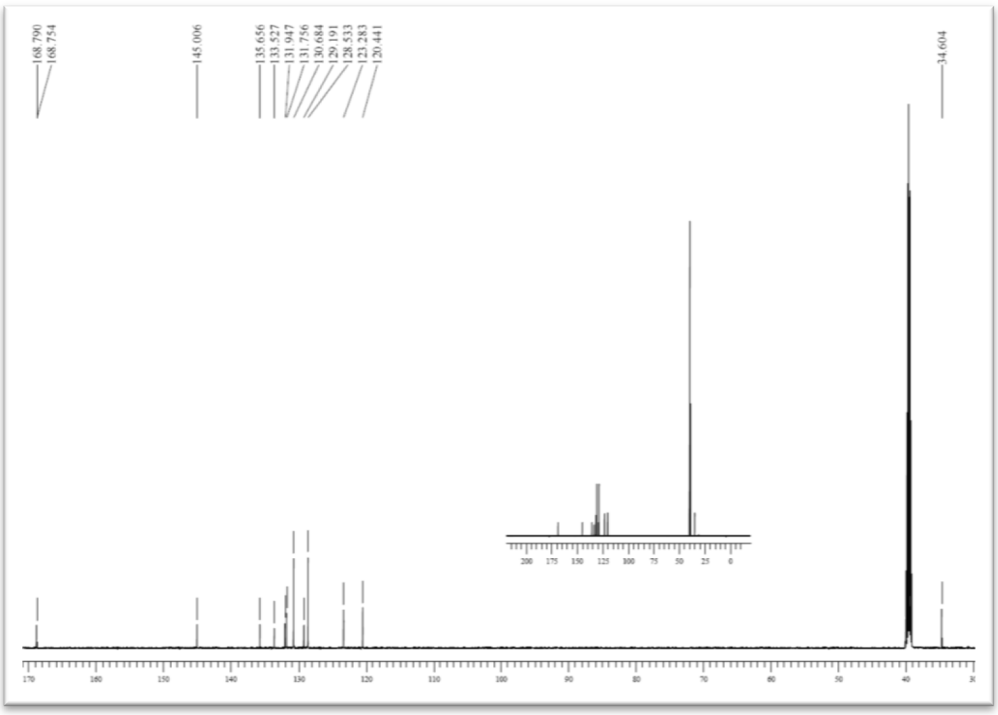
^{13}C NMR (DMSO-*d6*): **5-(Benzylsulfanyl)phthalimide** (**8a**)



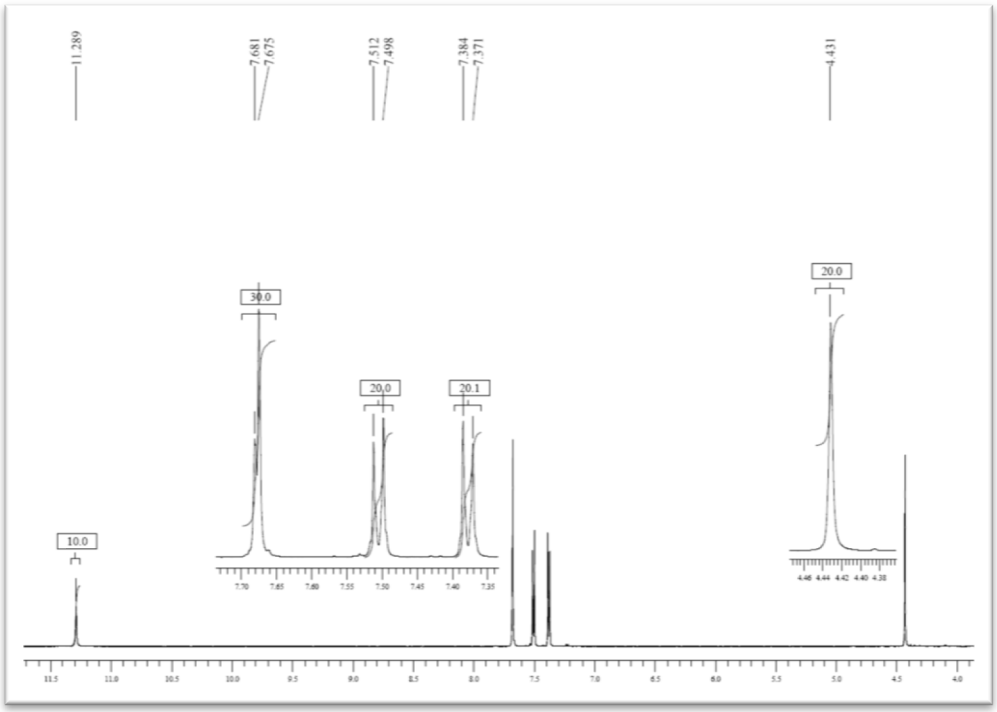
^{1}H NMR (DMSO-*d6*): **5-[(4-Chlorobenzyl)sulfanyl]phthalimide** (**8b**)



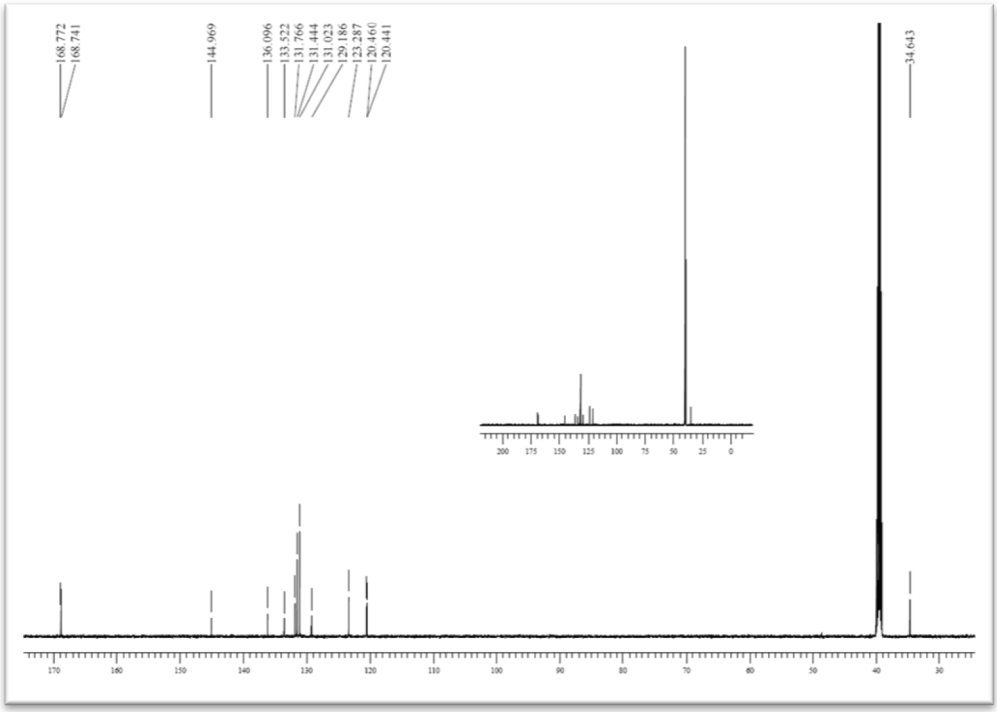
^{13}C NMR (DMSO-*d6*): **5-[(4-Chlorobenzyl)sulfanyl]phthalimide** (**8b**)



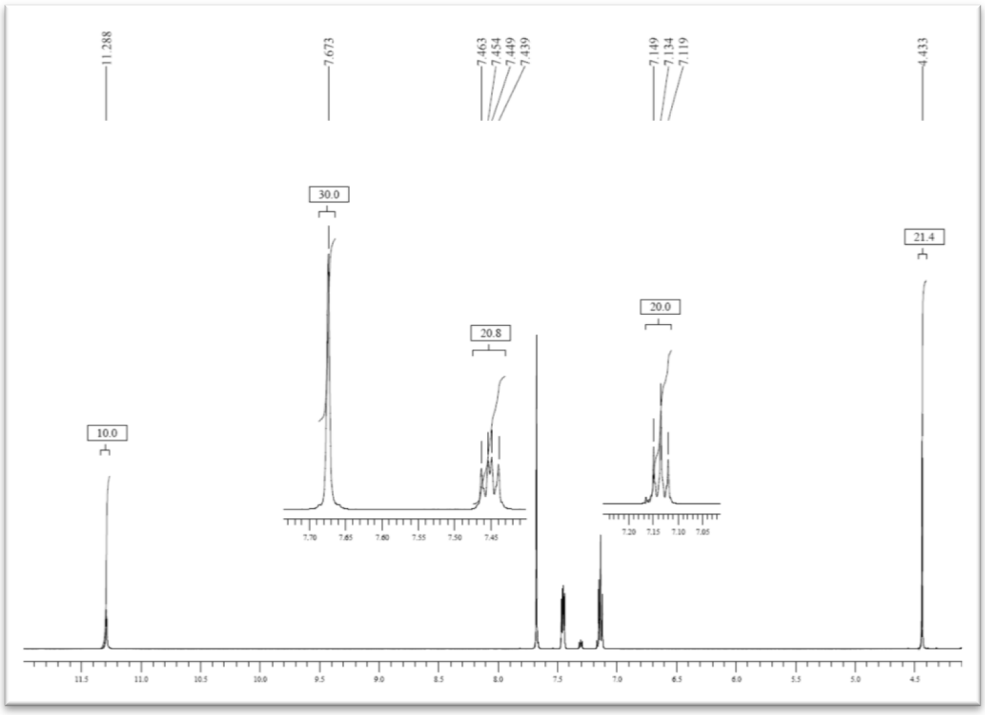
^{1}H NMR (DMSO-*d6*): **5-[(4-Bromobenzyl)sulfanyl]phthalimide** (**8c**)



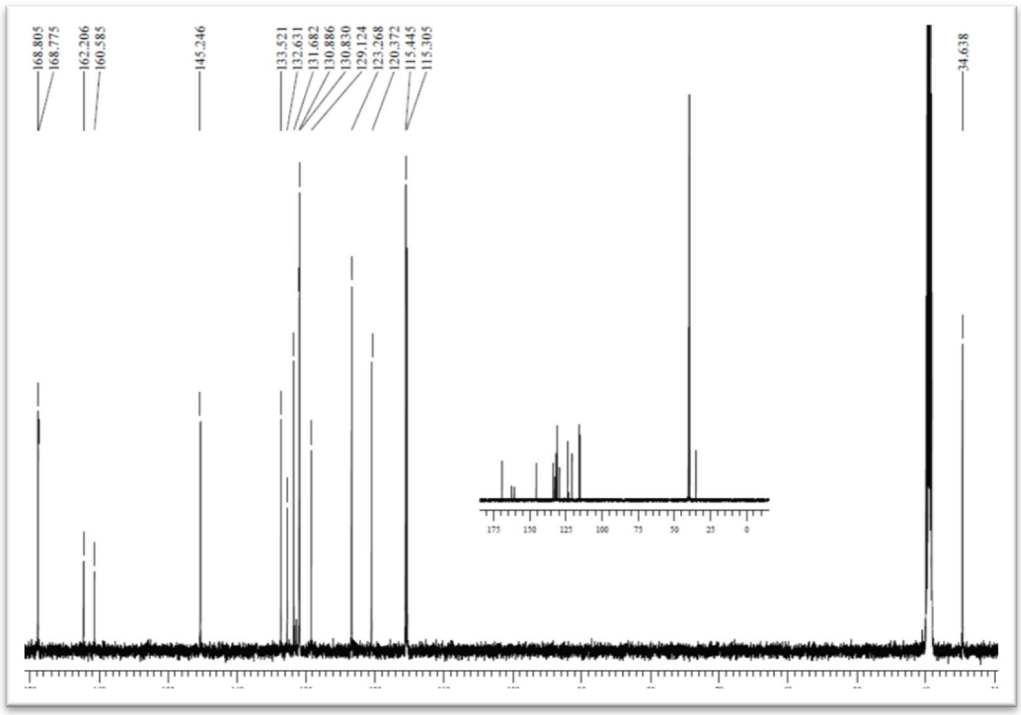
^{13}C NMR (DMSO-*d6*): **5-[(4-Bromobenzyl)sulfanyl]phthalimide** (**8c**)



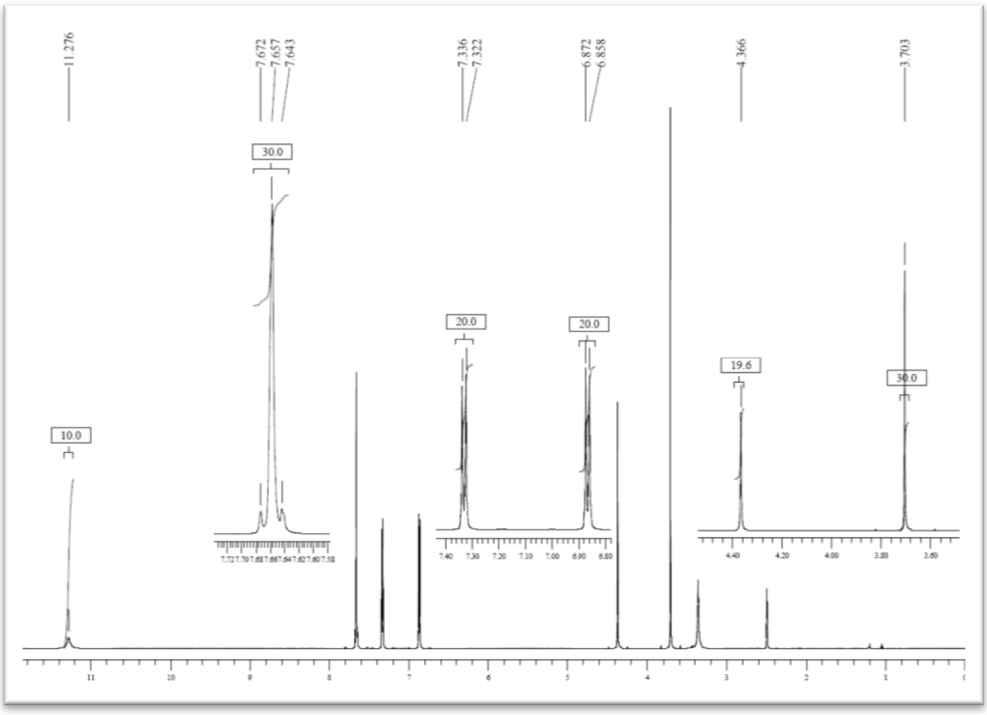
^{1}H NMR (DMSO-*d6*): **5-[(4-Fluorobenzyl)sulfanylphthalimide** (**8d**)



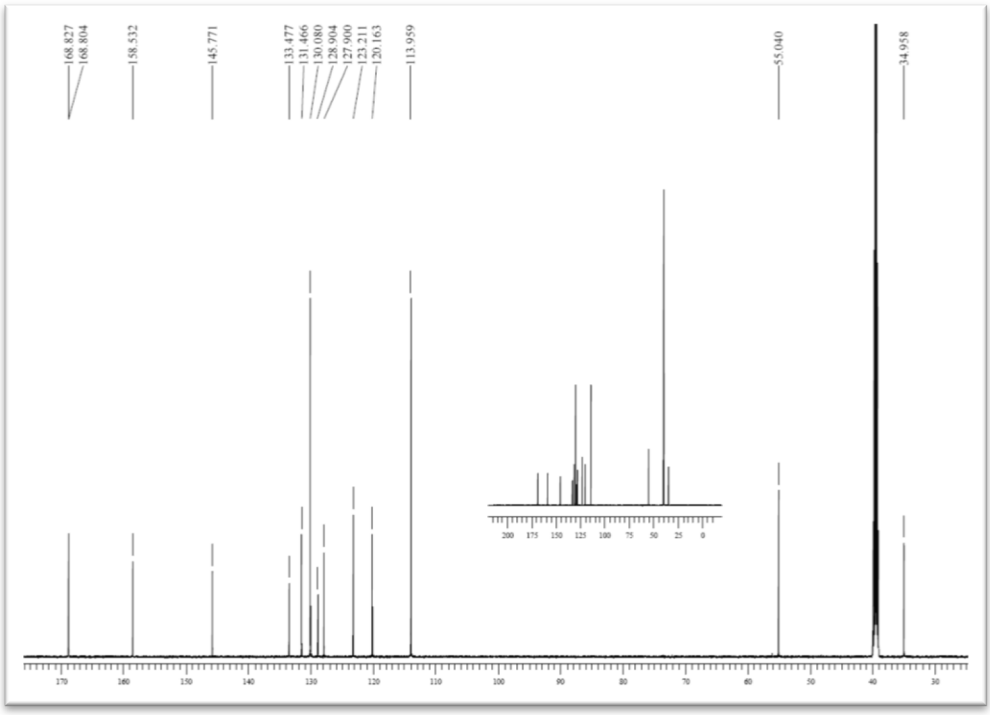
^{13}C NMR (DMSO-*d6*): **5-[(4-Fluorobenzyl)sulfanylphthalimide** (**8d**)



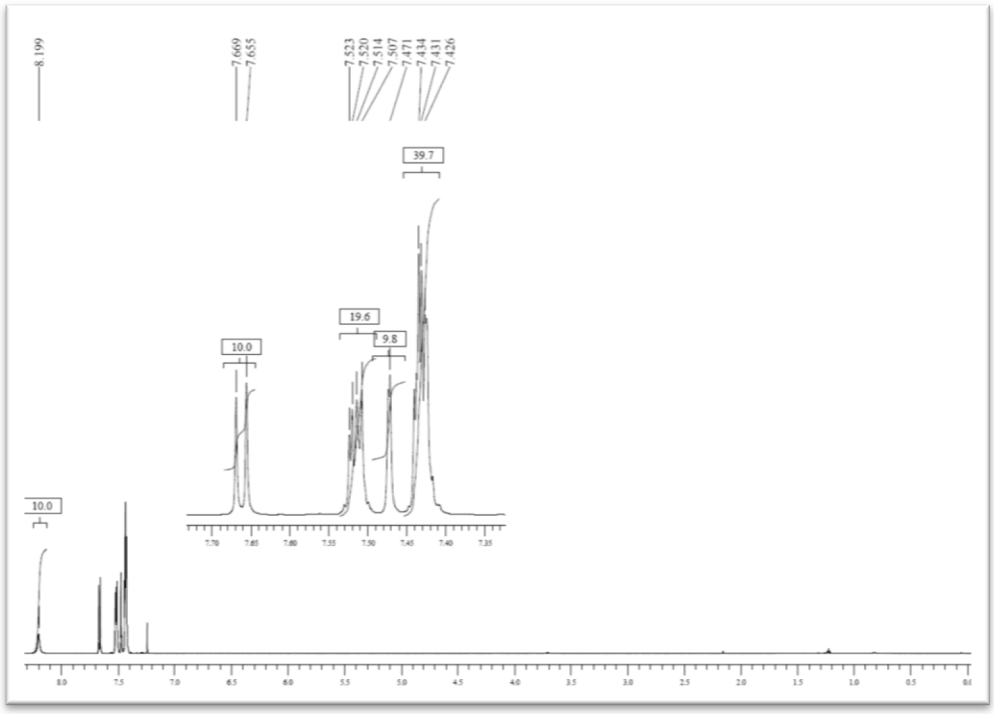
^{1}H NMR (DMSO-*d6*): **5-[(4-Methoxybenzyl)sulfanyl]phthalimide** (**8e**)



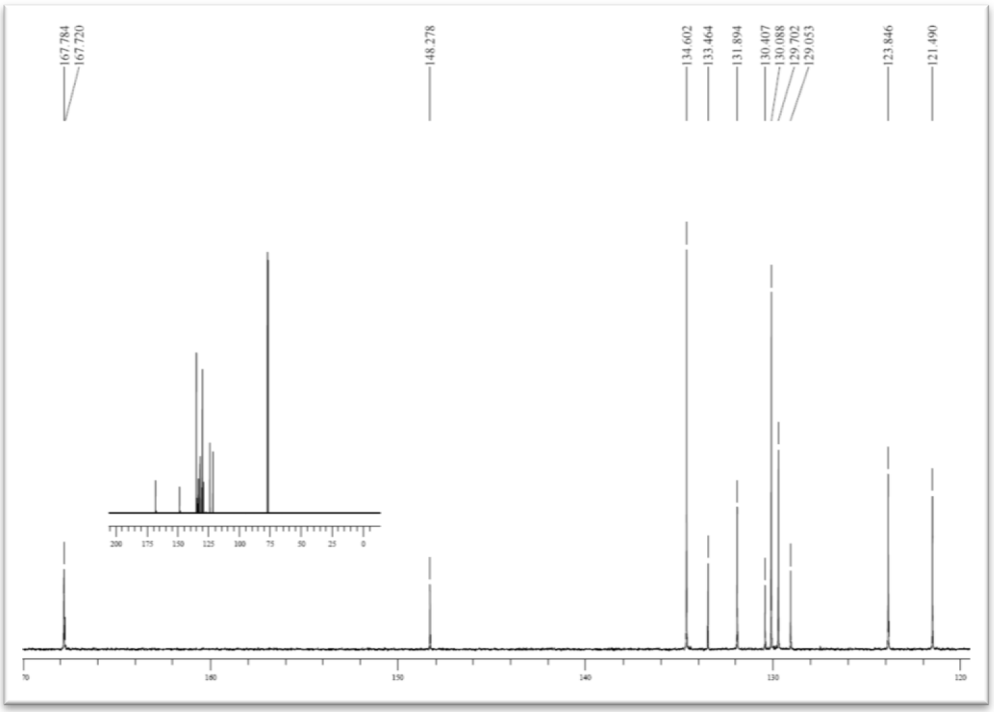
^{13}C NMR (DMSO-*d6*): **5-[(4-Methoxybenzyl)sulfanyl]phthalimide** (**8e**)



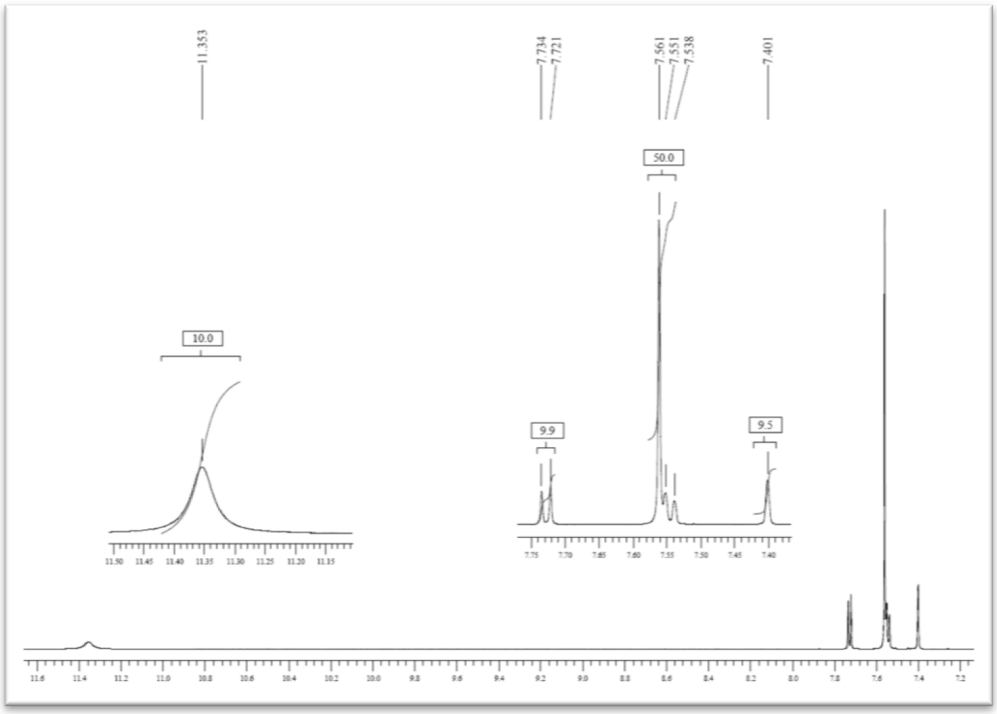
^{1}H NMR ($CDCl_3$): **5-(Phenylsulfanyl)phthalimide** (**8f**)



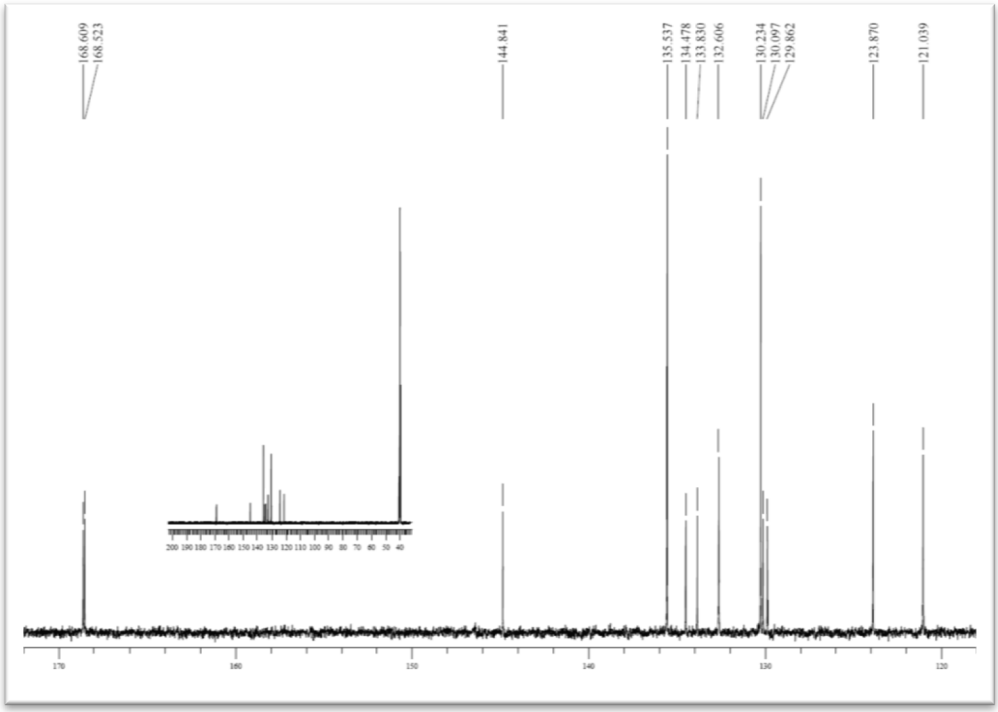
^{13}C NMR ($CDCl_3$): **5-(Phenylsulfanyl)phthalimide** (**8f**)



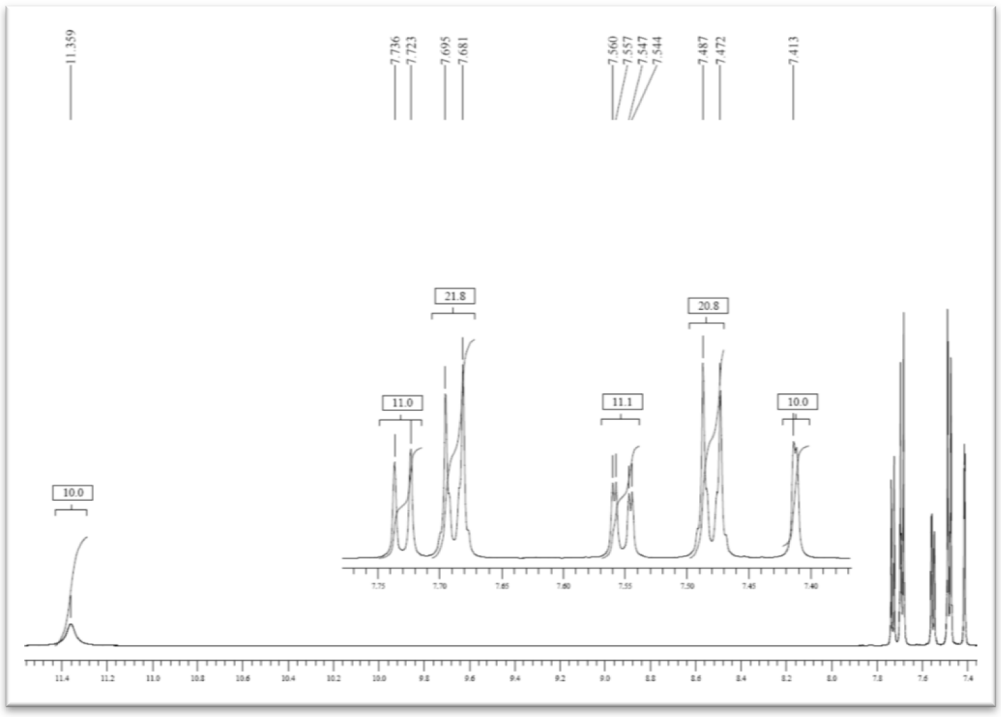
^{1}H NMR (DMSO-*d6*): **5-[(4-Chlorophenyl)sulfanyl]phthalimide** (**8g**)



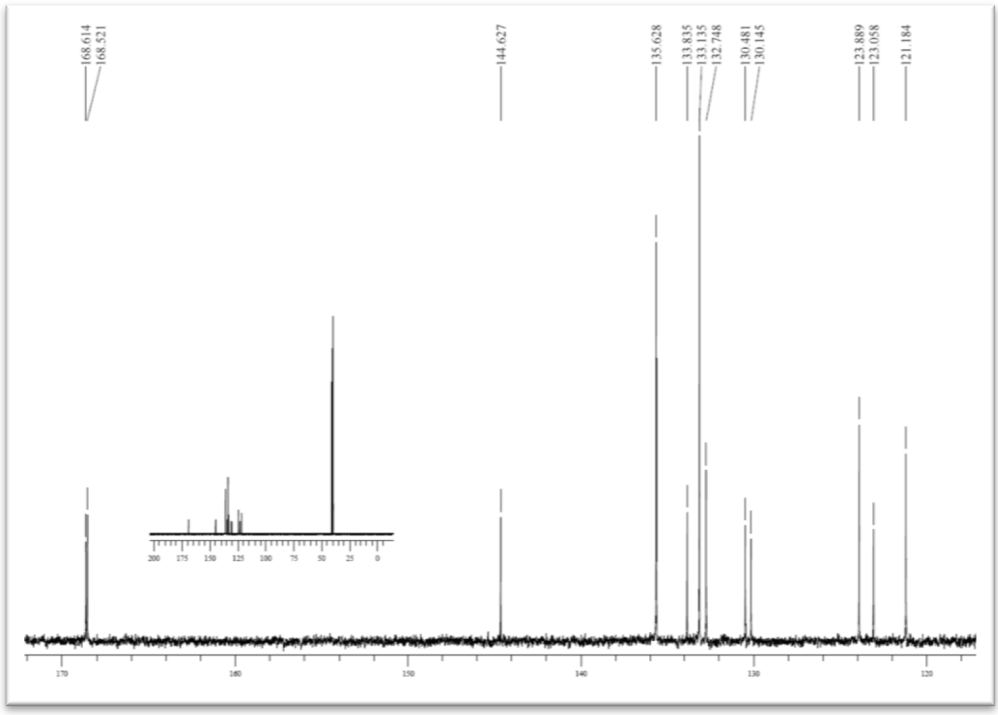
^{13}C NMR (DMSO-*d6*): **5-[(4-Chlorophenyl)sulfanyl]phthalimide** (**8g**)



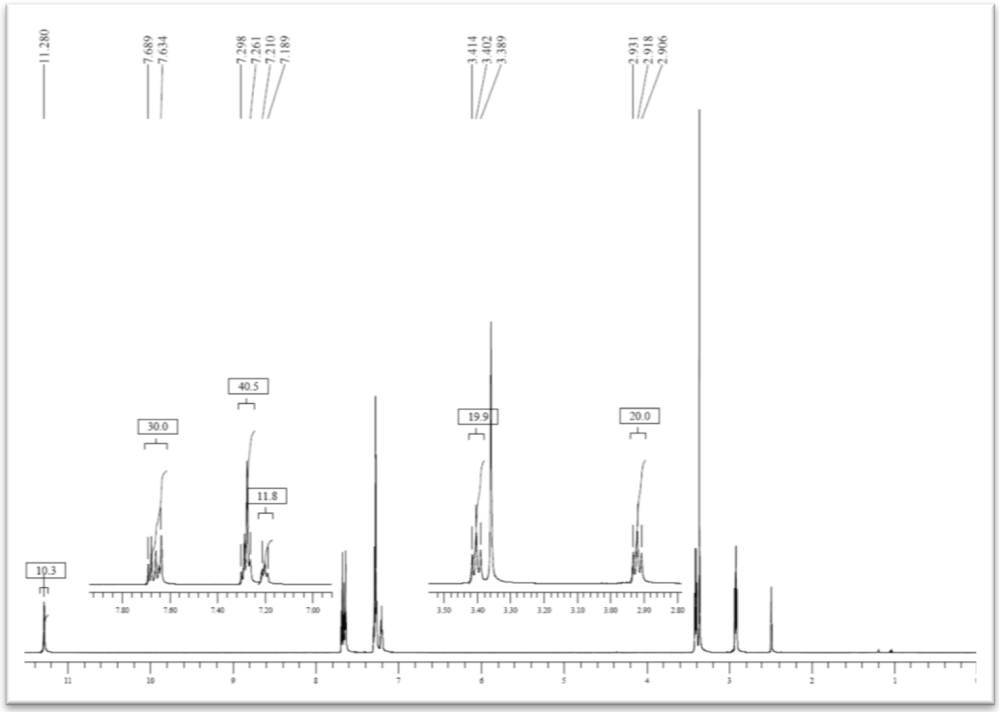
^{1}H NMR (DMSO-*d6*): **5-[(4-Bromophenyl)sulfanyl]phthalimide** (**8h**)



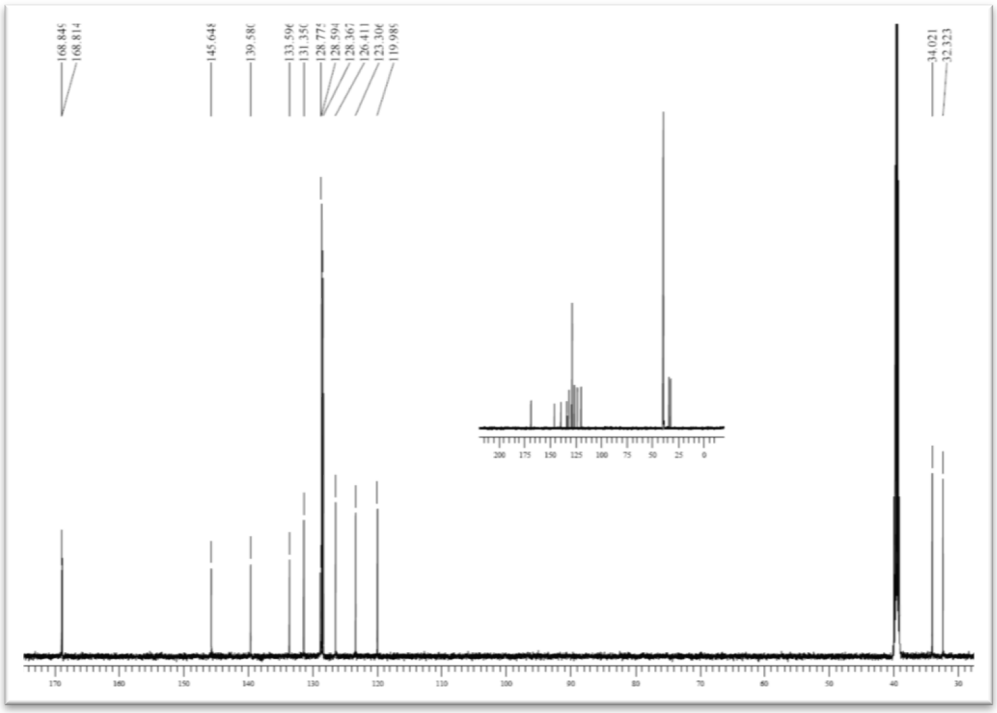
^{13}C NMR (DMSO-*d6*): **5-[(4-Bromophenyl)sulfanyl]phthalimide** (**8h**)



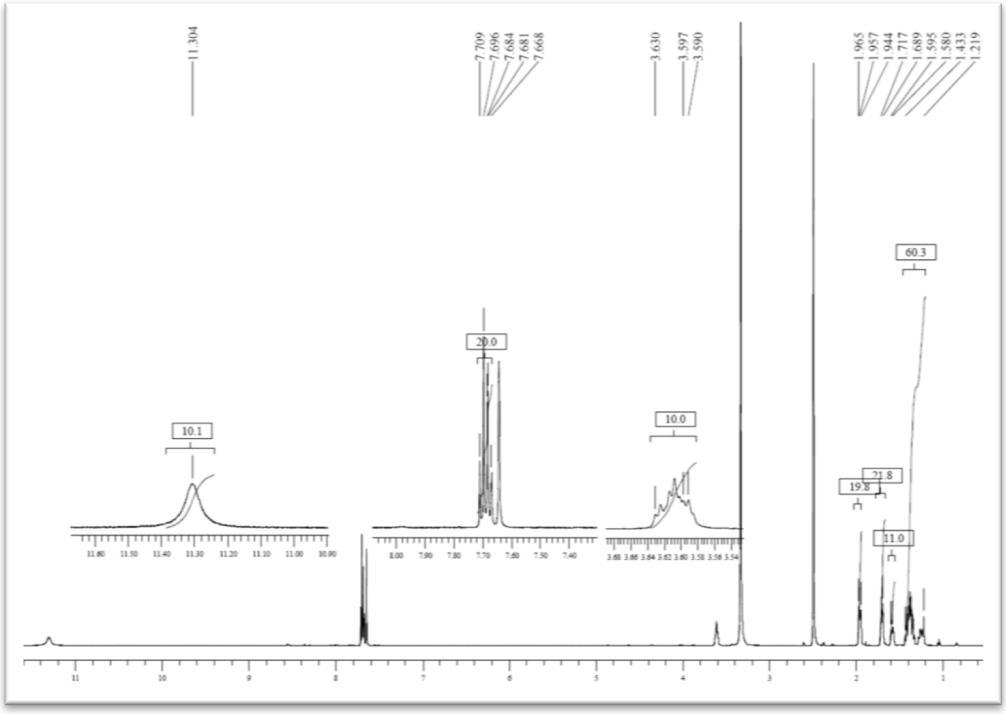
^{1}H NMR (DMSO-*d6*): **5-[(2-Phenylethyl)sulfanyl]phthalimide** (**8i**)



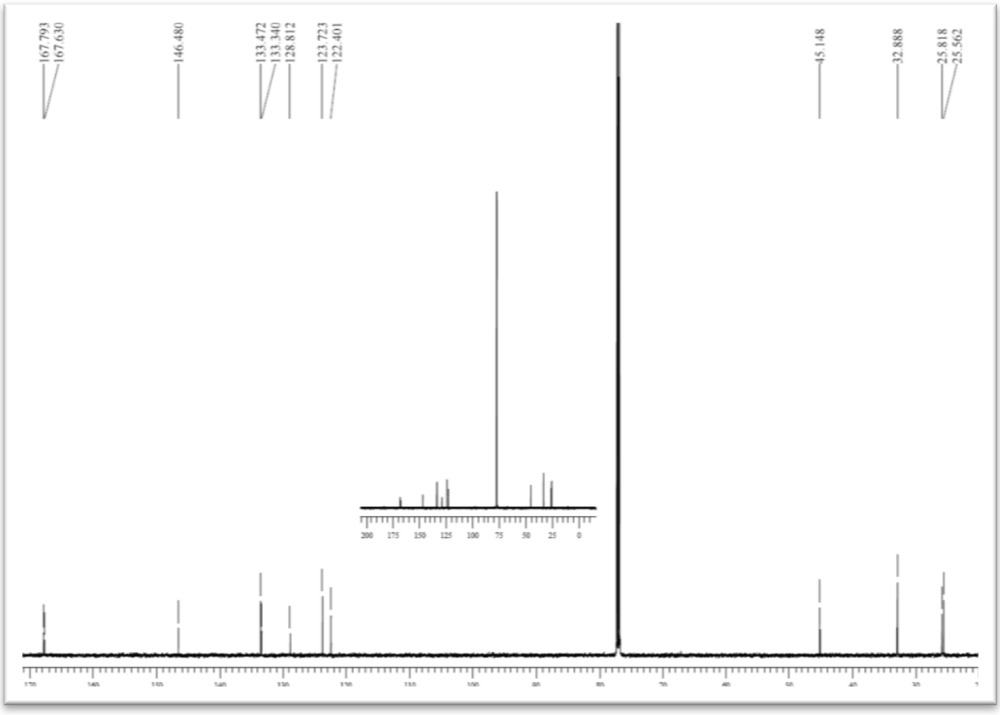
^{13}C NMR (DMSO-*d6*): **5-[(2-Phenylethyl)sulfanyl]phthalimide** (**8i**)



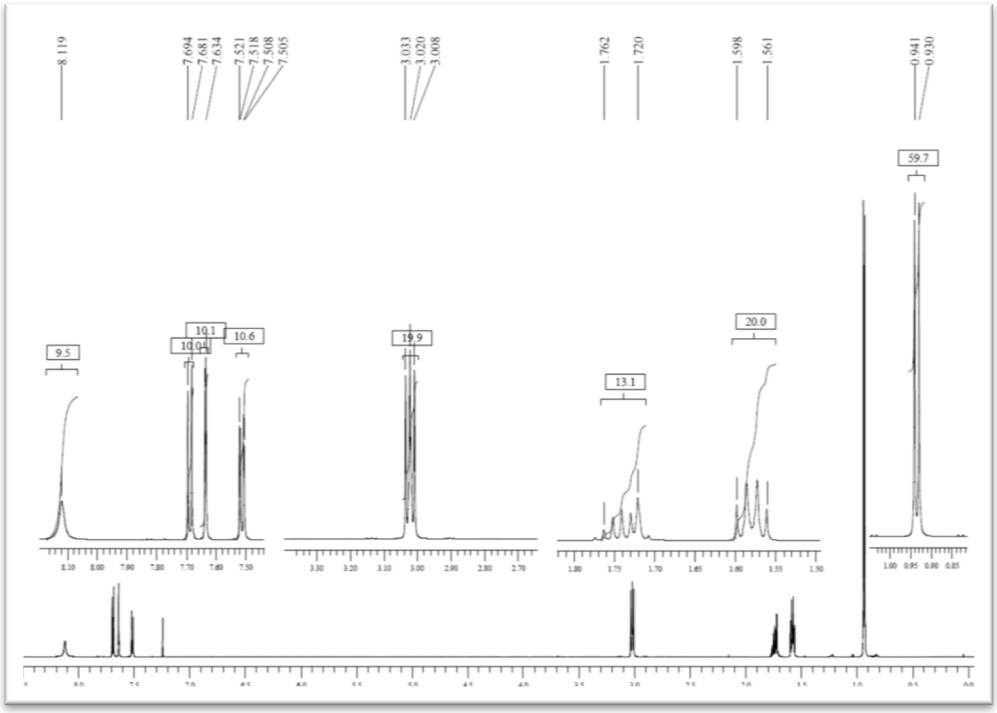
^{1}H NMR (DMSO-*d6*): **5-(Cyclohexylsulfanyl)phthalimide** (**8j**)



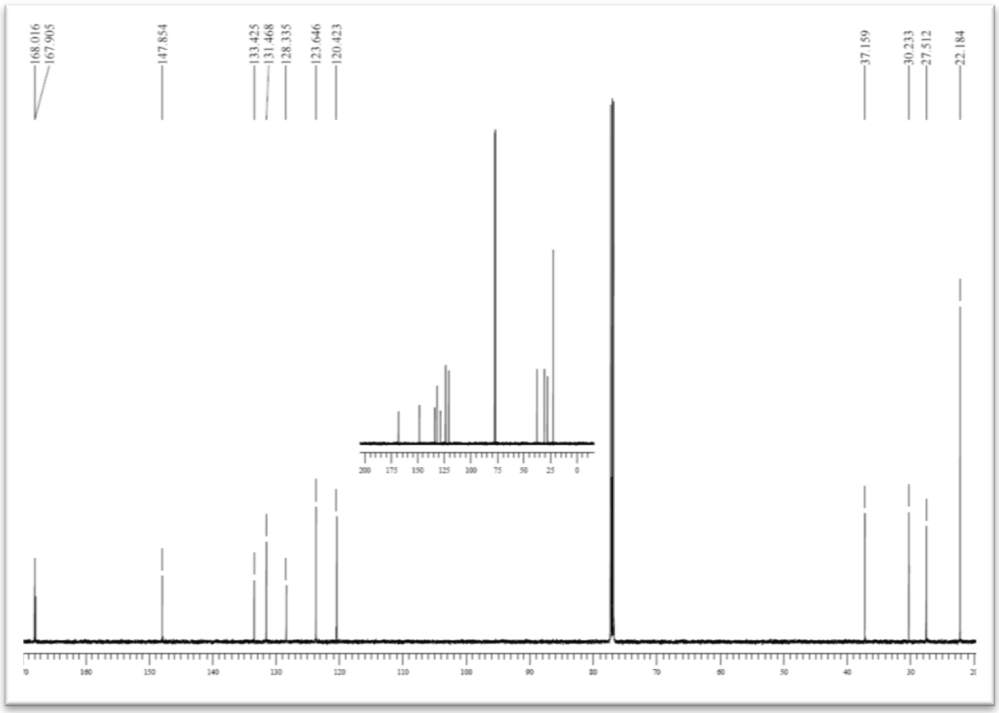
^{13}C NMR ($CDCl_3$): **5-(Cyclohexylsulfanyl)phthalimide** (**8j**)



^{1}H NMR ($CDCl_3$): **5-[(3-Methylbutyl)sulfanyl]phthalimide** (**8k**)



^{13}C NMR ($CDCl_3$): **5-[(3-Methylbutyl)sulfanyl]phthalimide** (**8k**)

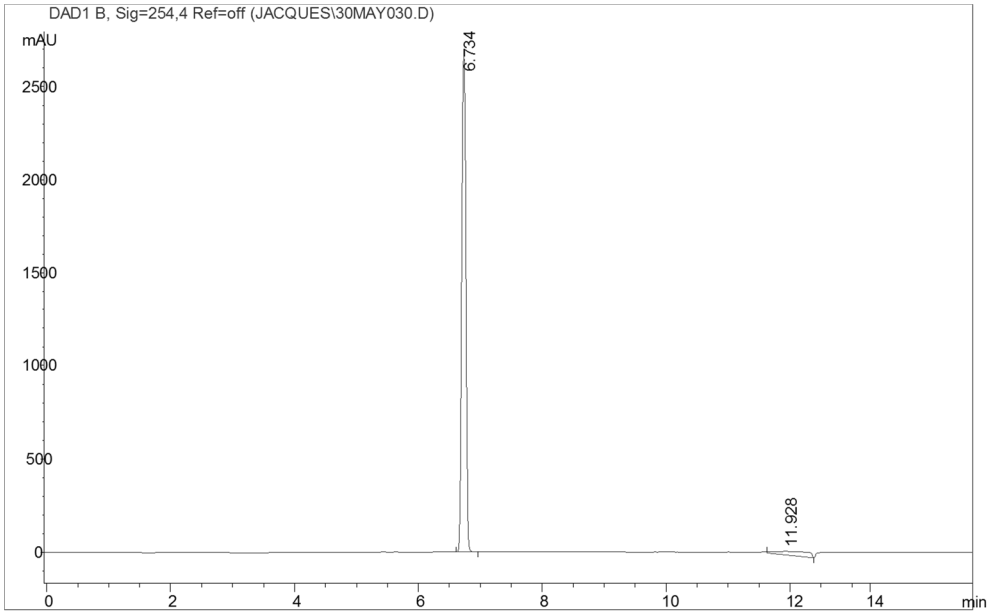


6.7.4 HPLC traces of the 5-sulfanylphthalimide analogues (**8a–k**)

The degree of purity for each of the synthesized 5-sulfanylphthalimide analogues (**8a-k**) were estimated with HPLC analyses, which were carried out with an Agilent 1100 HPLC system equipped with a quaternary pump and an Agilent 1100 series diode array detector. Milli-Q water (Millipore) and HPLC grade acetonitrile (Merck) 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% MilliQ 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 in acetonitrile (1 mM) was injected into the HPLC system and the eluent was monitored at wavelengths of 254 nm.

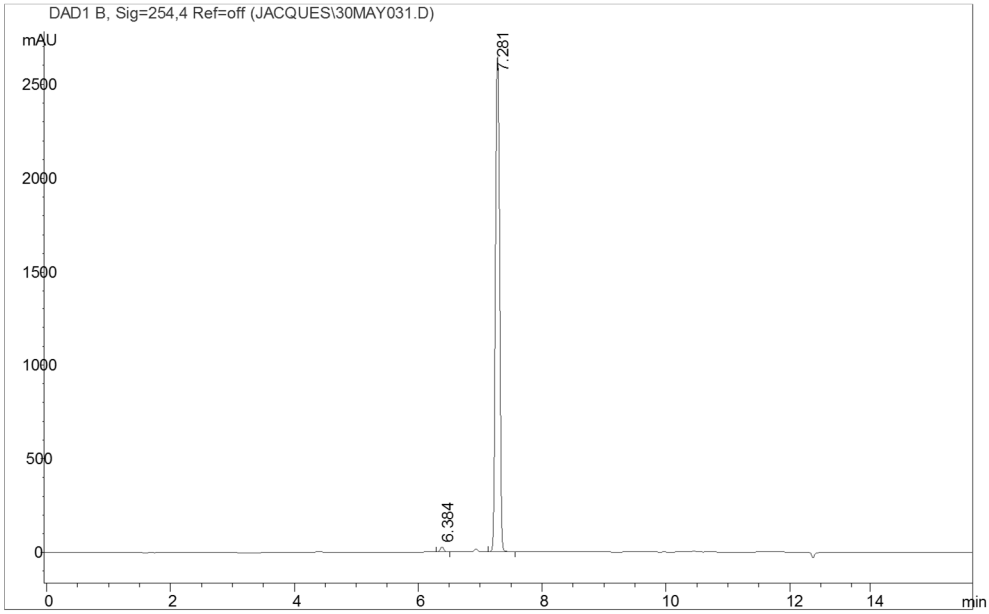
5-(Benzylsulfanyl)phthalimide (8a)

Purity (HPLC): 100%.



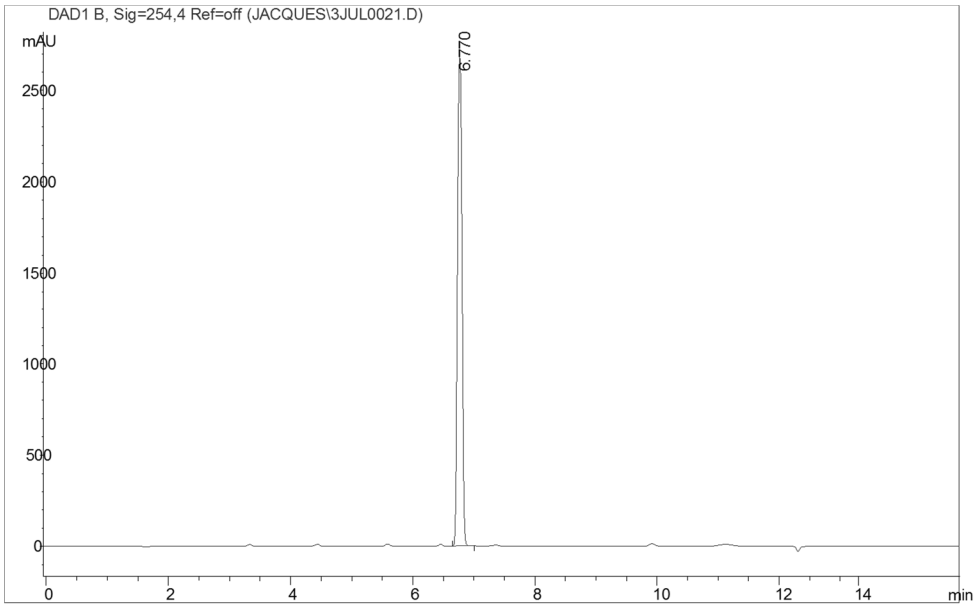
5-[(4-Chlorobenzyl)sulfanyl]phthalimide (8b)

Purity (HPLC): 99%



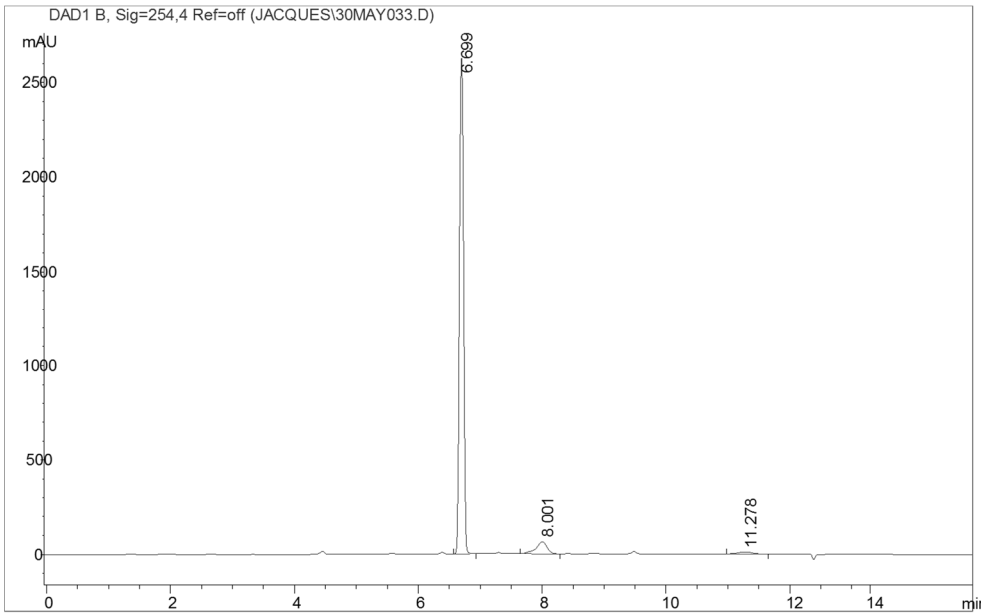
5-[(4-Bromobenzyl)sulfanyl]phthalimide (8c)

Purity (HPLC): 100%



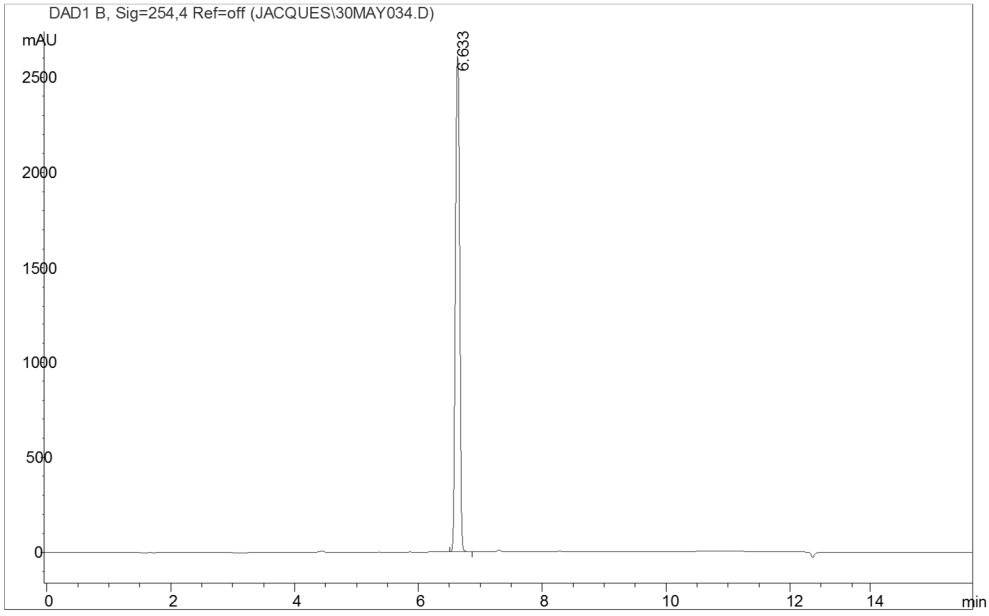
5-[(4-Fluorobenzyl)sulfanyl]phthalimide (8d)

Purity (HPLC): 96%



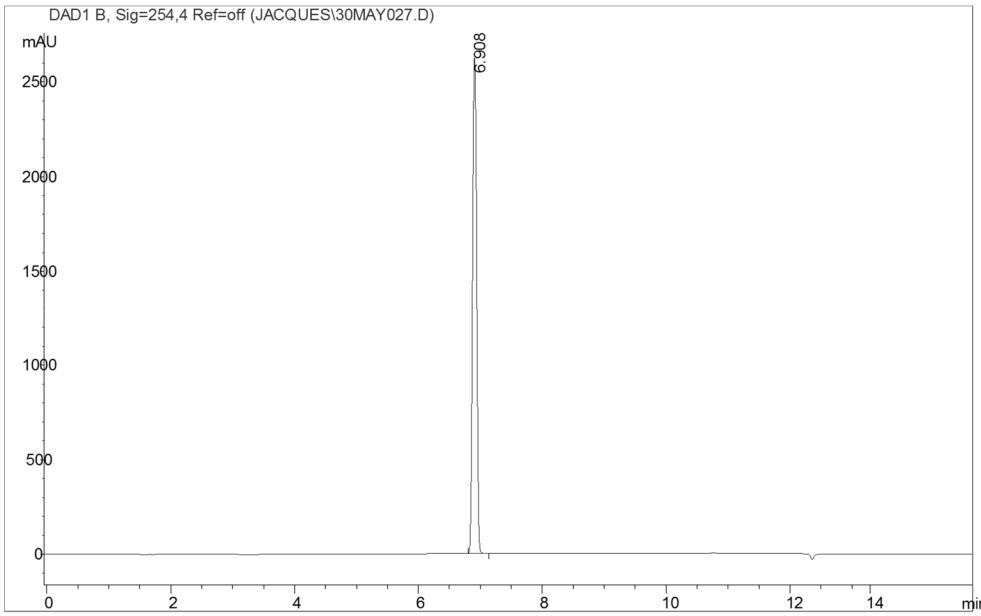
5-[(4-Methoxybenzyl)sulfanyl]phthalimide (8e)

Purity (HPLC): 100%



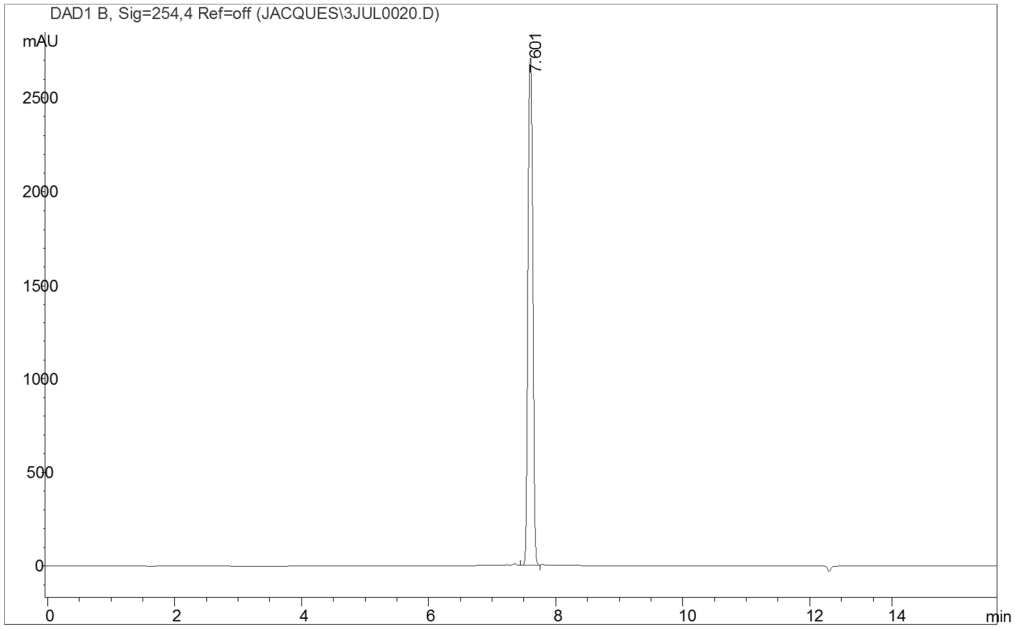
5-(Phenylsulfanyl)phthalimide (8f)

Purity (HPLC): 100%



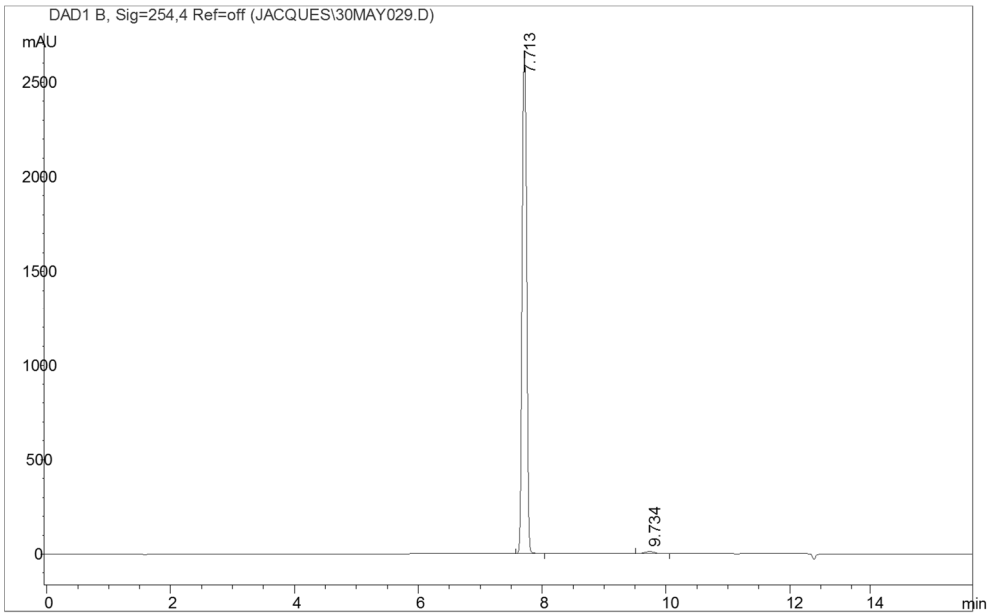
5-[(4-Chlorophenyl)sulfanyl]phthalimide (8g)

Purity (HPLC): 100%



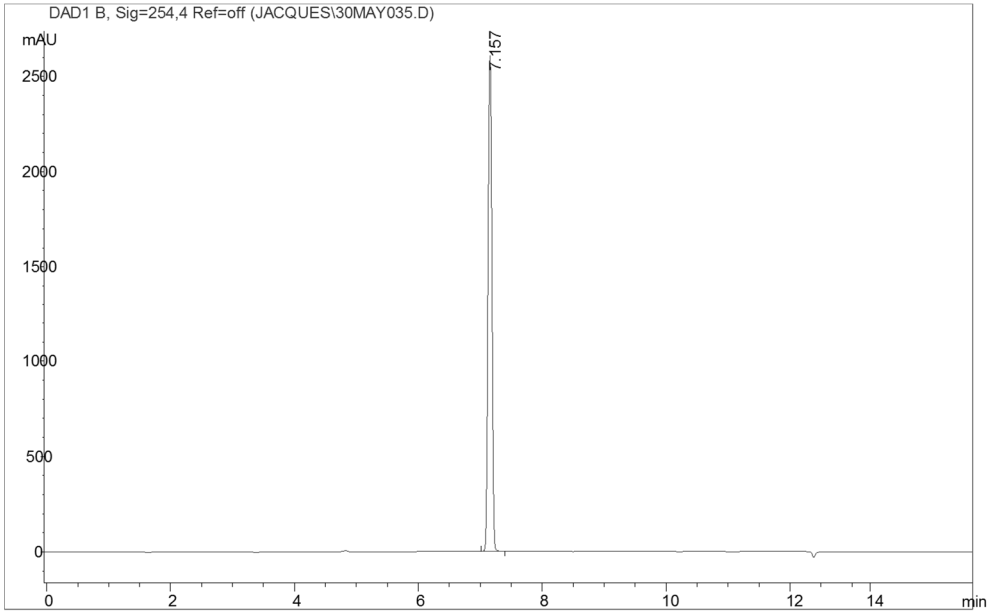
5-[(4-Bromophenyl)sulfanyl]phthalimide (8h)

Purity (HPLC): 99%



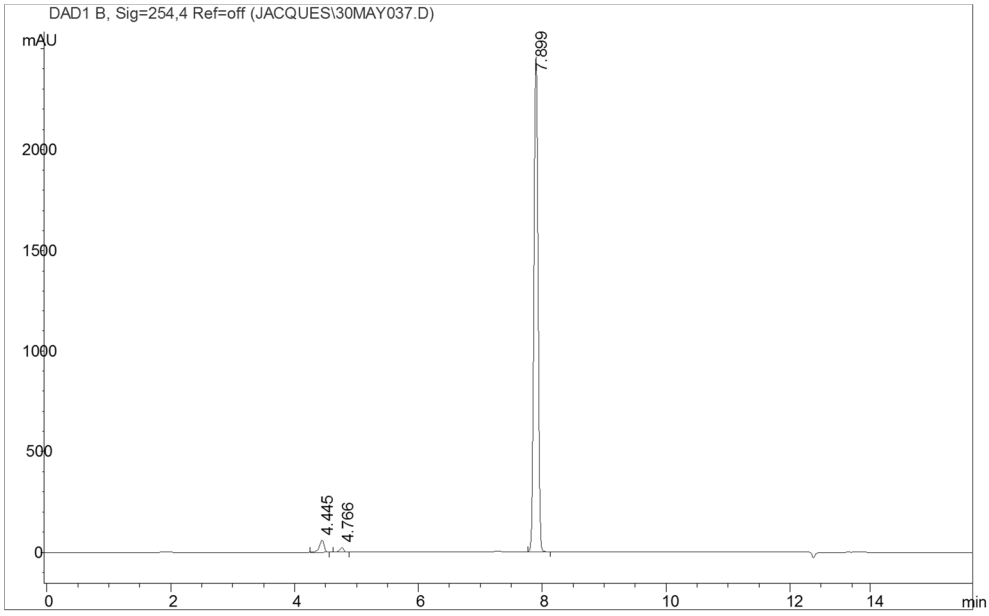
5-[(2-Phenylethyl)sulfanyl]phthalimide (8i)

Purity (HPLC): 100%



5-(Cyclohexylsulfanyl)phthalimide(8j)

Purity (HPLC): 96%



5-[(3-Methylbutyl)sulfanyl]phthalimide (8k)

Purity (HPLC): 100%