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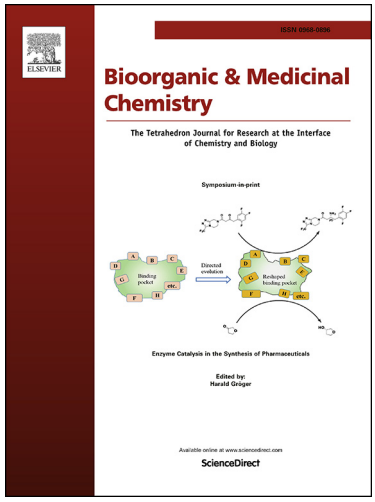

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Synthesis and evaluation of 2-substituted 4(3*H*)-quinazolinone thioether derivatives as monoamine oxidase inhibitors

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Abstract

In the present study, a series of fourteen 2-mercapto-4(3*H*)-quinazolinone derivatives was synthesised and evaluated as potential inhibitors of the human monoamine oxidase (MAO) enzymes. Quinazolinone is the oxidised form of quinazoline, and although this class has not yet been extensively explored as MAO inhibitors, it has been shown to possess a wide variety of biological activities. Among the quinazolinone derivatives investigated, seven compounds (IC_{50} < 1 μM) proved to be potent and specific MAO-B inhibitors, with the most potent inhibitor, 2-[(3-iodobenzyl)thio]quinazolin-4(3*H*)-one, exhibiting an IC_{50} value of 0.142 μM. Further investigation showed that this inhibitor is a reversible and competitive inhibitor of MAO-B with a K_i value of 0.068 μM. None of the test compounds were MAO-A inhibitors. Analysis of the structure-activity relationships (SARs) for MAO-B inhibition shows that substitution on the C2 position of quinazolinone with a benzylthio moiety bearing a Cl, Br or I on the *meta* position yields the most potent inhibitors of the series. In contrast, substitution with the unsubstituted benzylthio moiety (IC_{50} = 3.03 μM) resulted in significantly weaker inhibition activity towards MAO-B. This study suggests that quinazolinones are promising leads for the development of selective MAO-B inhibitors which may be used for the treatment of neurodegenerative disorders such as Parkinson's disease.

Keywords: monoamine oxidase, MAO, inhibition, reversible, quinazolinone, Parkinson's disease

1. Introduction

The human monoamine oxidases (MAOs) are mitochondrial bound flavin-dependent enzymes that catalyse the oxidative deamination of neurotransmitters such as serotonin, dopamine, epinephrine and norepinephrine.[1] The MAO enzymes exist as two isoforms, MAO-A and MAO-B, which are approximately 70% identical on the amino acid sequence level.[2,3] In spite of this, the MAOs differ in substrate and inhibitor specificities, and tissue distribution. For example, MAO-B is specifically and irreversibly inhibited by selegiline and rasagiline, and preferentially metabolises benzylamine and phenylethylamine.[1] On the other hand, the MAO-A isoform has specificity for bulkier endogenous amines such as serotonin, and is irreversibly inhibited by clorgyline.[1] Dopamine, epinephrine, norepinephrine, tyramine and tryptamine are considered substrates for both forms of the enzyme.[1] The MAO enzymes are found in most tissues including the brain, gut, liver, placenta, platelets and lymphocytes.[4] MAO-B is exclusively expressed in platelets and is the main form in human liver tissue,[5] while MAO-A is the main form in human placental and gut tissues.[6,7] In the brain, the two MAOs are not evenly distributed with MAO-B being the main form in the basal ganglia.[8] The main function of MAO is to metabolise and terminate the actions of neurotransmitter amines. In the peripheral tissues, the MAOs also function as metabolic barriers, with MAO-A regulating the entry of biogenic amines such as tyramine into the systemic circulation,[9,10] while MAO-B in the microvessels of the blood-brain barrier limits the entry of certain trace amines such as phenylethylamine into the central nervous system.[11,12]

An imbalance of the concentrations of monoamine neurotransmitters in the brain is responsible for many of the clinical symptoms of various neurological disorders including depression and Parkinson's disease. In this respect, MAO-A metabolises serotonin in the brain, and specific MAO-A inhibitors are thus used clinically for the treatment of depression and anxiety disorder.[13] MAO-B on the other hand is a key dopamine metabolising enzyme in the brain and MAO-B specific inhibitors are used for the treatment of Parkinson's disease.[14] In Parkinson's disease, MAO-B inhibitors are often combined with L-dopa in an attempt to enhance the therapeutic efficacy of L-dopa.[8] MAO-B inhibitors may also protect against neurodegeneration in Parkinson's disease by reducing levels of hydrogen peroxide and aldehyde

compounds, potentially harmful by-products of MAO-B catalysis.[8,15] Since brain MAO-B activity increases with age, the involvement of hydrogen peroxide and aldehyde derivatives in neurodegeneration in Parkinson's disease may be particularly relevant.[16]

Although MAO-A also metabolises dopamine in the brain,[8] MAO-A inhibitors are not used in Parkinson's disease due to concerns over potentially serious elevations in blood-pressure when combined with antiparkinsonian drugs such as L-dopa.[17] In fact drugs that inhibit MAO-A, particularly irreversible inhibitors, are used with caution in the clinic because of their ability to cause a potentially lethal hypertensive crisis when combined with tyramine-containing food.[9,10,18] This is known as the "cheese reaction" and is the result of the inhibition of intestinal MAO-A, which normally inactivates tyramine, an indirectly acting sympathomimetic amine, present in certain food such as cheese. When MAO-A is inhibited, tyramine reaches the systemic circulation leading to the release of norepinephrine from the peripheral adrenergic neurons and a rapid blood-pressure increase. MAO-B specific inhibitors and reversible MAO-A inhibitors are not associated with the cheese reaction.[18]

Numerous scaffolds have been explored as leads for the design of potent MAO inhibitors. Among these is the quinazolinone moiety. It has been demonstrated that quinazolinone derivatives containing the hydrazine and pyrazoline moieties (e.g. **1** and **2**) are highly potent MAO inhibitors (Fig. 1).[19] Other 4(3*H*)-quinazolinones that have been reported as MAO inhibitors include structures **3**–**5** as representative examples.[20–24] Based on these reports, substitution on C2 of the 4(3*H*)-quinazolinone was considered in the present study. The benzyl, pyridinylmethyl and substituted benzyl moieties were linked, via a thiol ether, to 4(3*H*)-quinazolinone to yield **6a**–**k** (Fig. 2). For the purpose of this study, benzylthio substitution was selected since it is similar to the benzyloxy substituent which is present in numerous experimental MAO-B inhibitors as well as in safinamide. For cavity-spanning MAO-B inhibitors, the benzyloxy moiety interacts with the entrance cavity via Van der Waals interactions, thus enhancing binding affinity. Since halogen substitution was shown to increase MAO inhibition potency in previous studies, the 4(3*H*)-quinazolinone thioether derivatives were substituted with halogens on the *meta* and *para* positions of the benzyl ring. For comparison with benzyloxy substitution, the 2-oxo-

2-phenylethyl (**6l**), 3-phenylpropyl (**6m**) and 2-phenoxyethyl (**6n**) substituents were also explored in this study. Based on the previous studies, these 4(3*H*)-quinazolinone derivatives may act as potential MAO inhibitors. Although the scaffold has been described, substitution via the thioether linkage at C2 has only been described by one previous report. The present study is the first comprehensive investigation of the MAO inhibition properties of 4(3H)-quinazolinone thioether derivatives, and the first report of high potency inhibition by members of this class.

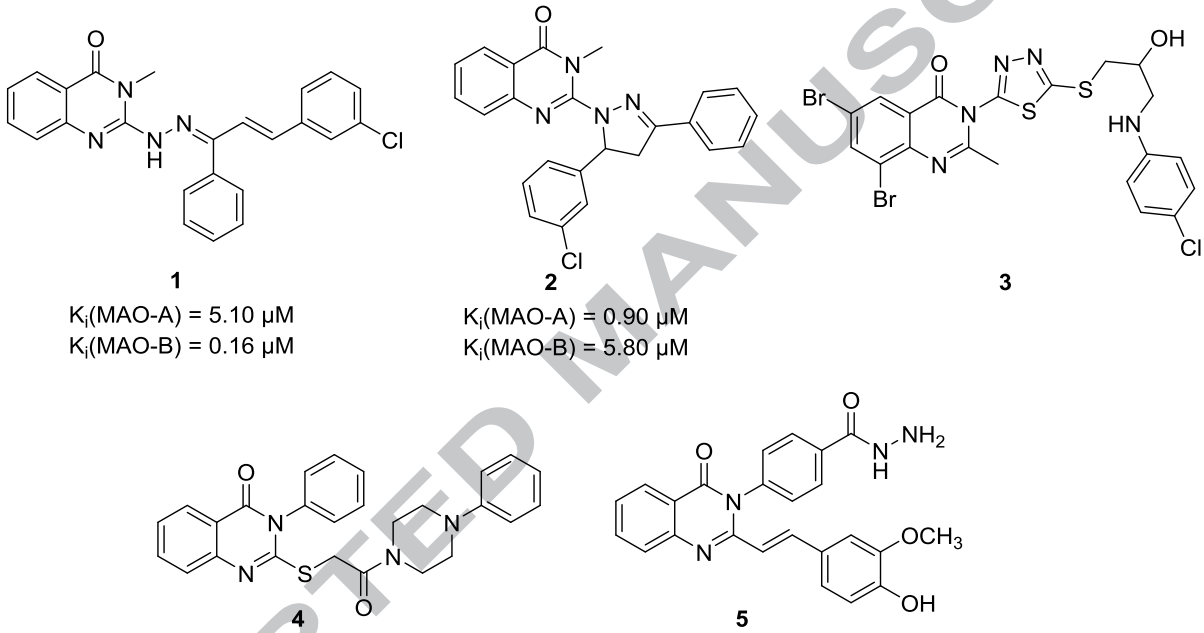


Fig. 1. The structures of quinazolinone derivatives **1**–**5**.

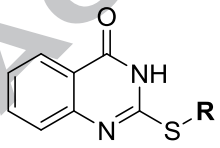


Fig. 2. The general structure of the 4(3*H*)-quinazolinone thioether derivatives **6a**–**n**.

2. Results and discussion

2.1. Chemistry

The 4(3*H*)-quinazolinone thioether derivatives (**6a**–**n**) were synthesised in poor to excellent yields (5-95%) by employing the nucleophilic substitution reaction shown in Fig. 3. Commercially available 2-mercapto-4(3*H*)-quinazolinone (**7**) and an appropriate arylalkyl bromide or chloride were suspended in ethanol (10 mL) in the presence of K_2CO_3 or NaOH, and stirred for 2 h. With the exception of **6n**, the target products were precipitated with the addition of ice-cold water, collected by filtration and dried. Compounds **6b** and **6n** were isolated by extraction to ethyl acetate. The crude products obtained were purified by recrystallisation from ethyl acetate, and the structures and purities of the target compounds were verified by ^{1}H NMR, ^{13}C NMR, mass spectrometry and HPLC analyses as cited in the experimental section. Thin-layer chromatography shows that the low yields obtained for certain reactions are a result of incomplete reactions. In addition, for more polar compounds, solvent-solvent extraction resulted in loss of the product, thus reducing the yield.

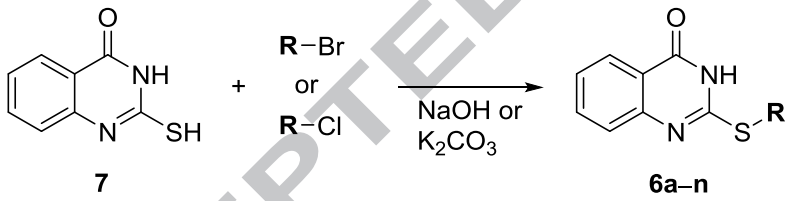


Fig. 3. Route for the synthesis of 4(3*H*)-quinazolinone thioether derivatives **6a**–**n**.

2.2. IC_{50} values for the inhibition of MAO

Recombinant human MAO-A and MAO-B were employed to evaluate the MAO inhibitory properties of the newly synthesised compounds.[25] Kynuramine served as enzyme substrate for both MAO-A and MAO-B. Kynuramine is oxidised to yield 4-hydroxyquinoline as ultimate product, a metabolite that fluoresces (λ_{ex} = 310 nm; λ_{em} = 400 nm) in alkaline media and can thus be readily measured by fluorescence spectrophotometry.[26] By measuring the fluorescence of 4-hydroxyquinoline in the presence and absence of the test inhibitors (10 and 100 µM), it was established that the 4(3*H*)-quinazolinone thioether derivatives do not fluoresce under the specific assay conditions, and thus do

not affect with the fluorescence measurements. A typical enzyme reaction contained the substrate (50 μM) and the test inhibitors (at 0.003–100 μM), and were initiated with addition of enzymes. As negative control, enzyme reactions were also carried out in absence of inhibitor. The reactions were incubated at 37 °C for 20 min and terminated by the addition of sodium hydroxide (2 N). The rate of formation of 4-hydroxyquinoline in each incubation was calculated from the fluorescence measurements and these data were fitted to a sigmoidal curve of rate versus the logarithm of inhibitor concentration (Fig. 4). The IC_{50} values for the inhibition of MAO were estimated from these plots. For comparison, the known reversible MAO-A inhibitor, toloxatone (IC_{50} = 3.92 μM), and the MAO-B inhibitor, lazabemide (IC_{50} = 0.091 μM), were included as positive controls and were evaluated under identical conditions.[27]

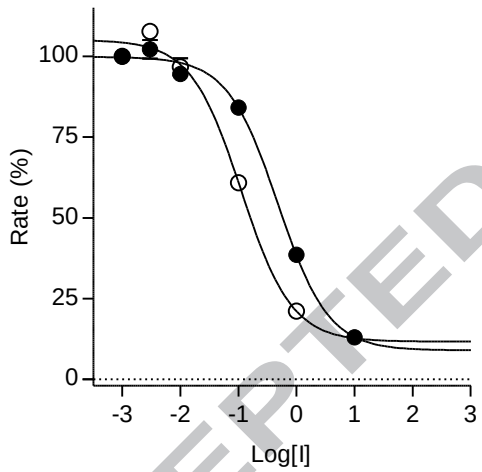


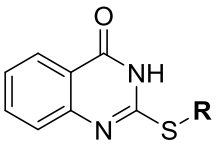
Fig. 4. Sigmoidal curve of rate versus the logarithm of inhibitor concentration for the inhibition of MAO-B by **6c** (IC_{50} = 0.566 μM; filled circles) and **6k** (IC_{50} = 0.142 μM; open circles).

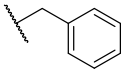
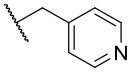
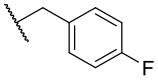
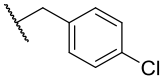
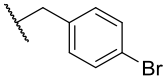
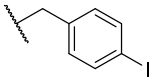
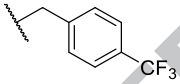
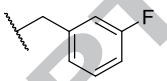
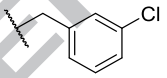
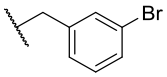
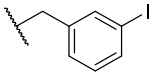
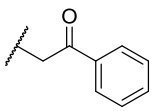
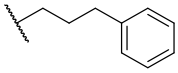
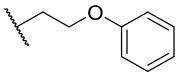
The IC_{50} values for the inhibition of MAO by the 2-substituted 4(3*H*)-quinazolinone thioether derivatives are reported in table 1. The results show that 2-substituted 4(3*H*)-quinazolinones are indeed MAO inhibitors, with IC_{50} ranging from 0.142 to 31.8 μM for the inhibition of the MAO-B isoform. The results further demonstrate that the majority of the test inhibitors (12 of 14) are selective for MAO-B, with no compounds displaying inhibition towards the MAO-A isoform. With the exception of **6c** and **6h**, those derivatives bearing a halogen on the *meta*-position of the benzyl ring (**6i, 6j** and **6k**) are more

potent than the corresponding *para*-substituted derivatives. Compound **6k**, the *meta*-iodobenzyl derivative, is the most potent inhibitor among the evaluated compounds (IC_{50} = 0.142 μM). However, its *para*-iodo substituted isomer, **6f**, is devoid of inhibition activity for MAO-B. Since the *meta* and the *para* isomers **6f** and **6k** can be considered as having similar lipophilicities and very similar electronic properties, the loss of activity of the *para*-iodo compound could likely be attributed to steric effects. In this respect, steric bulk at the *meta* position seems to be beneficial while being detrimental at the *para* position. This is supported by the observation that for both the chlorine and bromine substituted compounds, *meta* substitution yields more potent MAO-B inhibition than *para* substitution, presumably due to the steric bulk of the halogens. The weaker inhibition potency observed for **6h** (*meta*-F) compared to **6c** (*para*-F) further supports the notion that low steric bulk on the *meta* position reduces inhibition potency while low steric bulk *para* is beneficial for inhibition.

It is interesting to note that substitution on the benzyl ring with both halogens (F, Cl, Br, I) and alkyl (CF_3) substituents enhances MAO-B inhibitory potency relative to the derivative bearing an unsubstituted benzyl ring (**6a**). In fact, with the exception of **6f**, all halogen-substituted derivatives act as more potent MAO-B inhibitors than **6a**. Also of note is that the 3-phenylpropyl (**6m**) and 2-phenoxyethyl (**6n**) substituted compounds exhibited similar MAO-B inhibition potencies compared to **6a**, which may be attributed to the absence of substituents (e.g. halogens) on the side chain phenyl rings. The pyridine substituted compound, **6b**, did not inhibit either MAO isoforms, which suggests that non-polar, lipophilic phenyl-containing substituents are more appropriate for MAO-B inhibition compared to the pyridine ring. The 2-oxo-2-phenylethyl substituted derivative **6l** also displayed relatively poor MAO-B inhibition, which shows that introduction of an oxo group to the side chain linker reduces MAO-B inhibition. These results demonstrate the appropriate substitution and substitution pattern of 4(3*H*)-quinazolinones via thioether linkage for the design of MAO-B inhibitors.

Table 1. The IC_{50} values for the inhibition of MAO-A and MAO-B by 4(3*H*)-quinazolinone thioether derivatives.



		IC_{50} (μM)[a]		SI[b]	cLogP[c]
		MAO-A	MAO-B		
6a		NI[d]	3.03 ± 0.131	>33	3.52 ± 0.64
6b		NI[d]	NI[d]	–	2.03 ± 0.64
6c		NI[d]	0.566 ± 0.024	>177	3.57 ± 0.66
6d		NI[d]	0.482 ± 0.036	>207	4.12 ± 0.64
6e		NI[d]	0.779 ± 0.188	>128	4.29 ± 0.66
6f		NI[d]	NI[d]	–	4.55 ± 0.66
6g		NI[d]	0.700 ± 0.005	>143	4.09 ± 0.65
6h		NI[d]	2.50 ± 0.112	>40	3.57 ± 0.66
6i		NI[d]	0.230 ± 0.020	>435	4.12 ± 0.64
6j		NI[d]	0.208 ± 0.015	>481	4.29 ± 0.66
6k		NI[d]	0.142 ± 0.011	>704	4.55 ± 0.66
6l		NI[d]	31.8 ± 0.707	>3.1	3.22 ± 0.65
6m		NI[d]	5.99 ± 1.60	>17	4.37 ± 0.63
6n		NI[d]	1.24 ± 0.053	>81	3.66 ± 0.64

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

[b] Selectivity index (SI) = IC_{50}(MAO-A)/IC_{50}(MAO-B).

[c] Calculated with ACD/ChemSketch 1.2 (Advanced Chemistry Development).

[d] No inhibition observed at a maximal tested concentration of 100 µM.

2.3. Reversibility and mode of MAO-B inhibition

To examine the reversibility of MAO inhibition by the 4(3*H*)-quinazolinone thioether derivatives, the recovery of enzymatic activity after dialysis of enzyme-inhibitor mixtures was measured.[25] MAO-B and a selected test inhibitor, compound **6k**, at a concentration equal to 4 × IC_{50} were pre-incubated for 15 min and subsequently dialysed for 24 h. After dialysis, the incubations were diluted twofold with the addition of kynuramine and the residual enzyme activity was measured. As negative control, MAO-B was similarly dialysed in the absence of the inhibitor, and as positive control in the presence of the irreversible inhibitor selegiline. For reversible inhibition, enzymatic activity is expected to recover to the level of the negative control value (100%) following dialysis. In contrast, for irreversible inhibition, enzyme activity is not expected to recover after dialysis. In this experiment, MAO-B activity was recovered to 108% of the control value after dialysis of incubations containing MAO-B and **6k**. Enzyme activity of undialysed mixtures of MAO-B and **6k** are 46% of the negative control value, while only 3% activity was recovered after dialysis of incubations containing MAO-B and selegiline. These results are given in figure 5, and it may thus be concluded that **6k** is a reversible inhibitor of MAO-B.

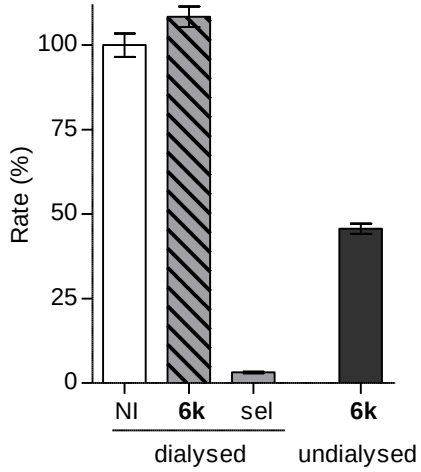


Fig. 5. Reversibility of MAO-B inhibition by **6k**. MAO-B was pre-incubated in the presence of **6k** (at 4 × IC_{50}) for 15 min, dialysed for 24 h and the residual enzyme activity was measured (**6k** – dialysed). Similarly, MAO-B was pre-incubated and dialysed in the absence of the inhibitor (NI – dialysed) and in the presence of the irreversible MAO-B inhibitor, selegiline (sel – dialysed). The residual activity of undialysed mixtures of MAO-B and **6k** (**6k** – undialysed) was also measured for comparison.

To investigate the mode of MAO-B inhibition by **6k**, a set of Lineweaver-Burke plots (Fig. 6) was constructed. The set comprised of 6 plots which were constructed by measuring enzyme activities in the absence and presence of different inhibitor concentrations (0 µM, $^1/_4$ × IC_{50}, ½ × IC_{50}, ¾ × IC_{50}, 1 × IC_{50}, $1^1/_4$ × IC_{50}). Eight different substrate (kynuramine) concentrations ranging from 15 to 250 µM were used for each plot. The results show that the plots are linear and intersect on the y-axis. This indicates that **6k** inhibits MAO-B competitively. For MAO-B inhibition, the K_i value was estimated by plotting the slopes of the Lineweaver-Burke plots versus the inhibitor concentration, with the K_i equal to 0.068 µM.

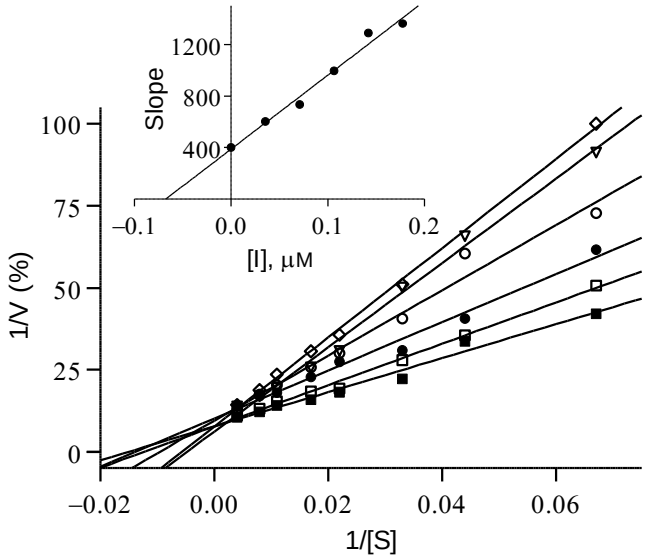


Fig. 6. Lineweaver-Burke plots of human MAO-B catalytic activities in the absence (filled squares) and presence of various concentrations of **6k** (K_i = 0.068 μM).

3. Conclusion

The present study shows that, with the appropriate substitution, C2-substituted 4(3*H*)-quinazolinone thioether derivatives are potent and specific inhibitors of human MAO-B, with compound **6k** being the most potent inhibitor (IC_{50} = 0.142 μM). This inhibition potency is similar in range to that recorded for lazabemide (IC_{50} = 0.091 μM).[27] An analysis of the SARs for MAO-B inhibition shows that substitution with a benzyl moiety bearing a halogen (Cl, Br, I) on the *meta* position is particularly favourable for MAO-B inhibition.[28] In contrast, pyridine substitution (e.g. **6b**) leads to loss of MAO-B inhibition activity, which suggests that an increase of polarity of the C2 substituent is unfavourable for MAO-B inhibition. Of note is the finding that none of the derivatives inhibited MAO-A up to a maximal tested concentration of 100 μM, and thus would not possess the liability for the cheese reaction. Based on their good selectivity profile for MAO-B, this series of 4(3*H*)-quinazolinone thioether derivatives represents promising leads for future development of novel specific MAO-B inhibitors, which have potential in the treatment of Parkinson's disease. In this regard, the synthetic accessibility of this class could facilitate their further development as clinically useful agents.

4. Experimental section

4.1. Chemicals and instrumentation

Unless otherwise noted, all starting materials and 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 at frequencies of 600 MHz and 151 MHz, respectively, with DMSO-*d*6 serving as NMR solvent (Merck). Chemical shifts are reported in parts per million (δ) and spin multiplicities are given as s (singlet), d (doublet), dd (doublet of doublets), ddd (doublet of doublet of doublets), t (triplet) and m (multiplet). The processing and analyses of the NMR data were carried out with MestreNova. High resolution mass spectra (HRMS) were recorded on a Bruker micrOTOF-Q II mass spectrometer in atmospheric-pressure chemical ionisation (APCI) mode. Chemical purities were determined by HPLC (Agilent 1200 system) with a Venusil XBP C18 column (4.60 × 150 mm, 5 μm) and a mobile phase of 30% acetonitrile and 70% MilliQ water at a flow rate of 1 mL/min. At the start of each injection, a solvent gradient program was initiated by linearly increasing the percentage acetonitrile to 85% over a period of 5 min. Each run lasted 15 min, 20 μL of the test compounds in acetonitrile (1 mM) was injected into the HPLC system and the eluent was monitored at wavelengths of 210 and 254 nm. The melting points (mp) were determined with a Buchi B-545 melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was carried out using silica gel 60 F254 precoated aluminium sheets (0.25 mm, Merck). Microsomes from insect cells containing recombinant human MAO-A and MAO-B (5 mg protein/mL), and kynuramine dihydrobromide were obtained from Sigma-Aldrich. Fluorescence spectrophotometry was carried out with a Varian Cary Eclipse fluorescence spectrophotometer.

4.2. General procedure for synthesis of 4(3H)-quinazolinone derivatives

A mixture of commercially available 2-mercapto-4(3*H*)-quinazolinone (**7**, 1.68 mmol), ethanol (10 mL) and an appropriately substituted arylalkyl halide (1.68 mmol) was stirred in the presence of K_2CO_3 (3.36 mmol) or NaOH (3.36 mmol) for 2 h at room temperature. The reaction products were precipitated with the addition of ice-cold water (15 mL), collected by filtration and dried. For derivatives **6b** and **6n**, the

reaction was extracted to ethyl acetate (60 mL), dried over anhydrous magnesium sulfate and the solvent was removed *in vacuo*. The target products were purified by recrystallisation from appropriate solvents. To monitor the progress of the reactions, silica gel TLC was performed with a mobile phase that consisted of petroleum ether and ethyl acetate (3:1).

*4.2.1. 2-(Benzylthio)quinazolin-4(3H)-one (**6a**)*

The title compound was prepared in a yield of 93%: mp 212.4–213.1 °C (ethanol), (lit. 214–216).[29] ^{1}H NMR (600 MHz, DMSO-*d6*) δ 12.59 (s, 1H), 8.04 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.78 (ddd, *J* = 8.5, 7.3, 1.6 Hz, 1H), 7.61 (d, *J* = 7.9 Hz, 1H), 7.51 – 7.46 (m, 2H), 7.46 – 7.40 (m, 1H), 7.33 (dd, *J* = 10.3, 4.7 Hz, 2H), 7.28 – 7.22 (m, 1H), 4.50 (s, 2H). ^{13}C NMR (151 MHz, DMSO-*d6*) δ 161.83, 155.91, 148.76, 137.87, 135.10, 129.67, 128.92, 127.75, 126.53, 126.38, 126.14, 120.49, 34.01. APCI-HRMS m/z: calcd for $C_{15}H_{13}N_2OS$, 269.0743, found 269.0755. Purity (HPLC): 99.2%.

*4.2.2. 2-[(Pyridin-4-ylmethyl)thio]quinazolin-4(3H)-one (**6b**)*

The title compound was prepared in a yield of 5%: mp 336.6–336.7 °C. ^{1}H NMR (600 MHz, DMSO-*d6*) δ 12.65 (s, 1H), 8.49 (d, *J* = 5.9 Hz, 2H), 8.01 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.78 – 7.72 (m, 1H), 7.57 (d, *J* = 8.0 Hz, 1H), 7.51 – 7.47 (m, 2H), 7.44 – 7.38 (m, 1H), 4.47 (s, 2H). ^{13}C NMR (151 MHz, DMSO-*d6*) δ 161.37, 154.84, 149.60, 148.16, 147.02, 134.61, 126.05, 125.89, 125.75, 124.19, 120.01, 32.13. APCI-HRMS m/z: calcd for $C_{14}H_{12}N_3OS$ (MH^+), 270.0696, found 270.0705. Purity (HPLC): 97.1%.

*4.2.3. 2-[(4-Fluorobenzyl)thio]quinazolin-4(3H)-one (**6c**)*

The title compound was prepared in a yield of 67%: mp 200.4–202.5 °C (ethanol), (lit. 217–219).[29] ^{1}H NMR (600 MHz, DMSO-*d6*) δ 12.56 (s, 1H), 8.04 – 7.98 (m, 1H), 7.78 – 7.70 (m, 1H), 7.57 (d, *J* = 8.1 Hz, 1H), 7.51 (dd, *J* = 8.4, 5.7 Hz, 2H), 7.43 – 7.35 (m, 1H), 7.12 (t, *J* = 8.8 Hz, 2H), 4.46 (s, 2H). ^{13}C NMR (151 MHz, DMSO-*d6*) δ 161.73, 161.35 (d, *J* = 243.4 Hz), 155.83, 148.41, 134.48, 133.96 (d, *J* = 3.1 Hz), 131.20 (d, *J* = 8.2 Hz), 126.07, 125.84, 125.53, 120.08, 115.18 (d, *J* = 21.4 Hz), 32.69. APCI-HRMS m/z: calcd for $C_{15}H_{12}FN_2OS$ (MH^+), 287.0649, found 287.0660. Purity (HPLC): 99.1%.

*4.2.4. 2-[(4-Chlorobenzyl)thio]quinazoline-4(3H)-one (**6d**)*

The title compound was prepared in a yield of 90%: mp 228.7–232.3 °C.[30] ^{1}H NMR (600 MHz, DMSO-*d6*) δ 12.58 (s, 1H), 8.04 – 7.98 (m, 1H), 7.79 – 7.72 (m, 1H), 7.59 (d, *J* = 8.1 Hz, 1H), 7.50 (d, *J* = 8.4 Hz, 2H), 7.41 (dd, *J* = 11.1, 4.0 Hz, 1H), 7.35 (d, *J* = 8.4 Hz, 2H), 4.46 (s, 2H). ^{13}C NMR (151 MHz, DMSO-*d6*) δ 161.28, 155.08, 148.28, 136.81, 134.69, 131.86, 131.09, 128.36, 126.07, 125.78, 120.00, 105.03, 32.69. APCI-HRMS m/z: calcd for $C_{15}H_{12}ClN_2OS$ (MH^+), 303.0353, found 303.0384. Purity (HPLC): 96.5%.

*4.2.5. 2-[(4-Bromobenzyl)thio]quinazolin-4(3H)-one (**6e**)*

The title compound was prepared in a yield of 95%: mp 242.6–245.0 °C. ^{1}H NMR (600 MHz, DMSO-*d*6) δ 12.60 (s, 1H), 8.04 – 7.98 (m, 1H), 7.75 (s, 1H), 7.57 (d, *J* = 8.1 Hz, 1H), 7.49 (d, *J* = 8.4 Hz, 2H), 7.45 – 7.37 (m, 3H), 4.44 (s, 2H). ^{13}C NMR (151 MHz, DMSO-*d*6) δ 161.54, 155.51, 148.31, 137.34, 134.59, 131.44, 131.28, 126.07, 125.89, 125.66, 120.35, 120.04, 32.75. APCI-HRMS m/z: calcd for $C_{15}H_{12}BrN_2OS$ ($M+2H^+$), 346.9848, found 348.9869. Purity (HPLC): 100.0%.

*4.2.6. 2-[(4-Iodobenzyl)thio]quinazolin-4(3H)-one (**6f**)*

The title compound was prepared in a yield of 93%: mp 251.3–261.3 °C. ^{1}H NMR (600 MHz, DMSO-*d*6) δ 12.59 (s, 1H), 8.01 (d, *J* = 7.8 Hz, 1H), 7.76 (s, 1H), 7.65 (d, *J* = 8.3 Hz, 2H), 7.58 (d, *J* = 8.1 Hz, 1H), 7.41 (s, 1H), 7.29 (d, *J* = 8.2 Hz, 2H), 4.42 (s, 2H). ^{13}C NMR (151 MHz, DMSO-*d*6) δ 161.18, 154.96, 148.28, 137.56, 137.15, 134.72, 131.57, 126.05, 125.80, 121.95, 120.01, 93.27, 32.91. APCI-HRMS m/z: calcd for $C_{15}H_{12}IN_2OS$ (MH^+), 394.9698, found 394.9710. Purity (HPLC): 96.2%.

*4.2.7. 2-[(4-Trifluoromethylbenzyl)thio]quinazolin-4(3H)-one (**6g**)*

The title compound was prepared in a yield of 82%: mp 217.3–219.7 °C.[31] ^{1}H NMR (600 MHz, DMSO-*d*6) δ 12.61 (s, 1H), 8.03 – 7.97 (m, 1H), 7.77 – 7.69 (m, 3H), 7.66 (d, *J* = 8.2 Hz, 2H), 7.57 (d, *J* = 8.1 Hz, 1H), 7.39 (s, 1H), 4.54 (s, 2H). ^{13}C NMR (151 MHz, DMSO-*d*6) δ 161.87, 155.76, 148.42, 143.08, 134.43, 130.00, 127.72 (q, *J* = 31.8 Hz), 126.07, 125.82, 125.52, 125.22 (q, *J* = 3.6 Hz), 123.36, 120.11, 32.82. APCI-HRMS m/z: calcd for $C_{16}H_{12}F_3N_2SO$ (MH^+), 337.0617, found 337.0626. Purity (HPLC): 98.1%.

*4.2.8. 2-[(3-Fluorobenzyl)thio]quinazolin-4(3H)-one (**6h**)*

The title compound was prepared in a yield of 63%: mp 193.7–266.1 °C (ethanol). ^{1}H NMR (600 MHz, DMSO-*d*6) δ 12.60 (s, 1H), 8.01 (dd, *J* = 7.9, 0.9 Hz, 1H), 7.76 (s, 1H), 7.58 (d, *J* = 8.0 Hz, 1H), 7.44 – 7.38 (m, 1H), 7.33 (dd, *J* = 12.2, 6.9 Hz, 3H), 7.06 (s, 1H), 4.48 (s, 2H). ^{13}C NMR (151 MHz, DMSO-*d*6) δ 161.96 (d, *J* = 243.5 Hz), 161.51, 155.39, 148.28, 140.66, 134.62, 130.33 (d, *J* = 8.4 Hz), 126.09, 125.85, 125.68, 125.34 (d, *J* = 2.6 Hz), 120.05, 115.95 (d, *J* = 21.7 Hz), 114.09, 32.86. APCI-HRMS m/z: calcd for $C_{15}H_{12}FN_2OS$ (MH^+), 287.0649, found 287.0650. Purity (HPLC): 98.2%.

*4.2.9. 2-[(3-Chlorobenzyl)thio]quinazolin-4(3H)-one (**6i**)*

The title compound was prepared in a yield of 86%: mp 209.6–212.7 °C. ^{1}H NMR (600 MHz, DMSO-*d*6) δ 12.60 (s, 1H), 8.02 (dd, *J* = 7.9, 1.1 Hz, 1H), 7.79 – 7.73 (m, 1H), 7.61 – 7.54 (m, 2H), 7.48 – 7.37 (m, 2H), 7.36 – 7.25 (m, 2H), 4.47 (s, 2H). ^{13}C NMR (151 MHz, DMSO-*d*6) δ 161.36, 155.17, 148.22, 140.39, 134.69, 132.82, 130.26, 129.11, 127.96, 127.20, 126.09, 125.84, 125.76, 120.03, 32.77. APCI-HRMS m/z: calcd for $C_{15}H_{12}ClN_2OS$ (MH^+), 303.0353, found 303.0367. Purity (HPLC): 97.8%.

*4.2.10. 2-[(3-Bromobenzyl)thio]quinazoline-4(3H)-one (**6j**)*

The title compound was prepared in a yield of 79%: mp 218.9–219.9 °C. ^{1}H NMR (600 MHz, DMSO-*d*6) δ 12.58 (s, 1H), 8.01 (dd, *J* = 7.9, 0.9 Hz, 1H), 7.76 (s, 1H), 7.71 (s, 1H), 7.58 (d, *J* = 8.1 Hz, 1H), 7.49 (d, *J* = 7.7 Hz, 1H), 7.42 (dd, *J* = 5.4, 4.1 Hz, 2H), 7.27 (d, *J* = 7.8 Hz, 1H), 4.46 (s, 2H). ^{13}C NMR (151 MHz, DMSO-*d*6) δ 161.46, 155.25, 148.26, 140.72, 134.72, 132.05, 130.59, 130.10, 128.38, 126.13, 125.86, 125.79, 121.45, 120.05, 32.75. APCI-HRMS m/z: calcd for $C_{15}H_{12}BrN_2OS$ ($M+2H^+$), 346.9848, found 348.9825. Purity (HPLC): 98.2%.

*4.2.11. 2-[(3-Iodobenzyl)thio]quinazolin-4(3H)-one (**6k**)*

The title compound was prepared in a yield of 87%: mp 228.5–230.9 °C. ^{1}H NMR (600 MHz, DMSO-*d*6) δ 12.59 (s, 1H), 8.02 (dd, *J* = 7.9, 1.0 Hz, 1H), 7.90 (s, 1H), 7.77 (s, 1H), 7.62 – 7.55 (m, 2H), 7.51 (d, *J* = 7.8 Hz, 1H), 7.45 – 7.39 (m, 1H), 7.11 (t, *J* = 7.8 Hz, 1H), 4.42 (s, 2H). ^{13}C NMR (151 MHz, DMSO-*d*6) δ 161.23, 140.47, 137.93, 135.89, 134.72, 130.56, 128.72, 127.26, 126.09, 125.81, 120.01, 118.40, 94.61, 77.42, 32.62. APCI-HRMS m/z: calcd for $C_{15}H_{12}IN_2OS$ (MH^+), 394.9709, found 394.9704. Purity (HPLC): 98.8%.

*4.2.12. 2-[(2-Oxo-2-phenylethyl)thio]quinazolin-4(3H)-one (**6l**)*

The title compound was prepared in a yield of 6%: mp 263.7–265.9 °C (ethanol), (lit. 196–197).[32] ^{1}H NMR (600 MHz, DMSO-*d*6) δ 12.71 (s, 1H), 8.09 (d, *J* = 7.7 Hz, 2H), 7.99 (d, *J* = 7.8 Hz, 1H), 7.72 – 7.61 (m, 2H), 7.58 (t, *J* = 7.7 Hz, 2H), 7.36 (t, *J* = 7.5 Hz, 1H), 7.11 (d, *J* = 8.2 Hz, 1H), 4.84 (s, 2H). ^{13}C NMR (151 MHz, DMSO-*d*6) δ 193.76, 161.15, 155.02, 148.05, 136.22, 134.56, 133.50, 128.79, 128.32, 126.03, 125.66, 124.61, 119.79, 37.50. APCI-HRMS m/z: calcd for $C_{16}H_{13}N_2O_2S$ (MH^+), 297.0692, found 297.0723. Purity (HPLC): 99.2%.

*4.2.13. 2-[(3-Phenylpropyl)thio]quinazolin-4(3H)-one (**6m**)*

The title compound was prepared in a yield of 39%: mp 51.0–51.1 °C. ^{1}H NMR (600 MHz, DMSO-*d*6) δ 12.54 (s, 1H), 8.00 (d, *J* = 7.9 Hz, 1H), 7.72 (dd, *J* = 11.2, 4.1 Hz, 1H), 7.43 (d, *J* = 8.1 Hz, 1H), 7.38 (t, *J* = 7.5 Hz, 1H), 7.28 (t, *J* = 7.5 Hz, 2H), 7.24 – 7.13 (m, 3H), 3.18 (t, *J* = 7.3 Hz, 2H), 2.71 (t, *J* = 7.5 Hz, 2H), 2.05 – 1.92 (m, 2H). ^{13}C NMR (151 MHz, DMSO-*d*6) δ 161.67, 156.29, 148.50, 141.14, 134.44, 128.40, 128.36, 126.04, 125.92, 125.78, 125.41, 120.02, 34.19, 30.50, 29.12. APCI-HRMS m/z: calcd for $C_{17}H_{17}N_2OS$ (MH^+), 297.1056, found 297.1080. Purity (HPLC): 98.4%.

*4.2.14. 2-[(Phenoxyethyl)thio]quinazolin-4(3H)-one (**6n**)*

The title compound was prepared in a yield of 32%: mp 176.0–178.7 °C (ethyl acetate). ^{1}H NMR (600 MHz, DMSO-*d*6) δ 12.64 (s, 1H), 8.03 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.79 – 7.73 (m, 1H), 7.52 (d, *J* = 8.1 Hz, 1H), 7.44 – 7.38 (m, 1H), 7.32 – 7.26 (m, 2H), 7.01 (d, *J* = 7.9 Hz, 2H), 6.94 (t, *J* = 7.3 Hz, 1H), 4.27 (t, *J* = 6.6 Hz, 2H), 3.60 (t, *J* = 6.6 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-*d*6) δ 161.23, 158.03,

153.27, 134.67, 129.56, 126.07, 125.87, 125.72, 120.85, 120.02, 114.52, 65.87, 30.69, 28.56. APCI-HRMS m/z: calcd for $C_{16}H_{15}N_2O_2S$ (MH^+), 299.0849, found, 299.0884. Purity (HPLC): 96.3%.

4.3. Protocol for the measurement of IC_{50} values

The IC_{50} values for inhibition of MAO-A and MAO-B were measured according to the literature method.[25] The recombinant human MAOs served as enzyme sources in this study and the enzyme reactions were carried out in white 96-well microtiter plates (Eppendorf) in potassium phosphate buffer (100 mM, pH 7.4, made isotonic with KCl). The final volume of the reactions was 200 μL and contained kynuramine (50 μM) and the test inhibitors at concentrations of 0.003–100 μM. Stock solutions of the inhibitors were prepared in DMSO and added to the reactions to yield a final DMSO concentration of 4%. The reactions were initiated with the addition of MAO-A (0.0075 mg protein/mL) or MAO-B (0.015 mg protein/mL), incubated for 20 min at 37 °C in a convection oven, and terminated with 80 μL NaOH (2 N). At endpoint, 4-hydroxyquinoline generated by the oxidation of kynuramine was measured by fluorescence spectrophotometry (λ_{ex} = 310 nm; λ_{em} = 400 nm) and quantitated with a linear calibration curve constructed with authentic 4-hydroxyquinoline (0.047–1.56 μM). The inhibition data were fitted to the one site competition model incorporated into the Prism 5 software package (GraphPad) and the IC_{50} values were determined from the resulting sigmoidal plots (rate versus the logarithm of inhibitor concentration). IC_{50} values were determined in triplicate and are expressed as mean ± standard deviation (SD).

4.4. Dialysis of enzyme-inhibitor mixtures

The reversibility of MAO-B inhibition by a selected 4(3*H*)-quinazolinone thioether derivative (**6k**) was examined by dialysis using Slide-A-Lyzer® dialysis cassettes (Thermo Scientific) with a molecular weight cut-off of 10 000 and sample volume capacity of 0.3–5 mL.[25] A mixture of MAO-B (0.03 mg/ml) and **6k**, at a concentration equal to fourfold the IC_{50}, was prepared in potassium phosphate buffer (100 mM, pH 7.4, containing 5% sucrose) to a final volume of 0.8 mL and placed in the dialysis cassettes. DMSO (4%) was added to the mixtures as co-solvent. These mixtures were pre-incubated for 15 min at 37 °C and subsequently dialysed at 4 °C in 80 mL dialysis buffer (100 mM potassium phosphate, pH

7.4, containing 5% sucrose). The dialysis buffer was replaced at 3 h and 7 h after the start of dialysis. As controls, MAO-B was similarly pre-incubated and dialysed in the presence of the irreversible MAO-B inhibitor, selegiline (IC_{50} = 0.079 μM), as well as in the absence of the inhibitor.[33] After 24 h of dialysis, the dialysed samples (250 μL) were diluted twofold with the addition of kynuramine to yield a final inhibitor concentration of 2 × IC_{50} and a final kynuramine concentration of 50 μM. These enzyme reactions were incubated for 20 min at 37 °C, after which they were terminated with addition of 400 μL NaOH (2 N) and 1000 μL of water. The residual MAO activities were measured by fluorescence spectrophotometry as described for the IC_{50} determinations. For comparison, undialysed mixtures of MAO-B and **6k** were maintained at 4 °C for 24 h and thereafter diluted and assayed as above. All reactions were carried out in triplicate, and the residual enzyme catalytic rates were expressed as mean ± SD.

4.5. Construction of Lineweaver-Burk plots and K_i determination

The mode of MAO-B inhibition by **6k** was investigated by constructing a set of six Lineweaver-Burk plots.[25] The first plot was constructed in the absence of inhibitor, while the remaining plots were constructed in the presence of five different inhibitor concentrations ($^1/_4$ x IC_{50}, ½ x IC_{50}, ¾ x IC_{50}, 1 x IC_{50}, $1^1/_4$ x IC_{50}). The substrate, kynuramine, was used at concentrations ranging from 15-250 μM, while the final concentration of MAO-B was 0.015 mg/mL. All enzyme reactions and catalytic activity measurements were carried out as described above for the IC_{50} determinations. Linear regression analysis was performed using the Prism version 5.0 software package. The K_i value was estimated from a plot of the slopes of the Lineweaver-Burke plots versus inhibitor concentration where the x-axis intercept equals $–K_i$.

Conflict of interest

The authors declare no conflict of interest.

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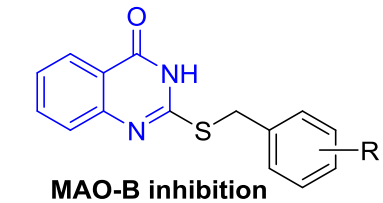
Graphical abstract



MAO-B inhibition

6c: R = 4-F IC_{50} = 0.566 μM
6k: R = 3-I IC_{50} = 0.142 μM