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Monoamine oxidase inhibitory activities of heterocyclic chalcones

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ABSTRACT

Studies have shown that natural and synthetic chalcones (1,3-diphenyl-2-propen-1-ones) possess monoamine oxidase (MAO) inhibition activities. Of particular importance to the present study is a report that a series of furanochalcones acts as MAO-B selective inhibitors. Since the effect of heterocyclic substitution, other than furan (and more recently thiophene, piperidine and quinoline) on the MAO inhibitory properties of the chalcone scaffold remains unexplored, the aim of this study was to synthesise and evaluate further heterocyclic chalcone analogues as inhibitors of the human MAOs. For this purpose, heterocyclic chalcone analogues that incorporate pyrrole, 5-methylthiophene, 5-chlorothiophene and 6-methoxypyridine substitution were examined. Seven of the nine synthesised compounds exhibited IC_{50} values <1 μM for the inhibition of MAO-B, with all compounds exhibiting higher affinities for MAO-B compared to the MAO-A isoform. The most potent MAO-B inhibitor (**4h**) displays an IC_{50} value of 0.067 μM while the most potent MAO-A inhibitor (**4e**) exhibits an IC_{50} value of 3.81 μM. It was further established that selected heterocyclic chalcones are reversible and competitive MAO inhibitors. **4h**, however, may exhibit tight-binding to MAO-B, a property linked to its thiophene moiety. We conclude that high potency chalcones such as **4h** represent suitable leads for the development of MAO-B inhibitors for the treatment of Parkinson's disease and possibly other neurodegenerative disorders.

Neuropsychiatric and neurodegenerative disorders are often closely linked to reduced levels of monoamine neurotransmitters in the central nervous system. For example, reduced levels of serotonin, and to a lesser extent norepinephrine,[1,2] have been implicated in depressive illness, while depletion of central dopamine is responsible for the characteristic motor symptoms of Parkinson's disease.[3] Replacement therapy with metabolic precursors or agonist drugs, and inhibition of neurotransmitter reuptake are approaches frequently used to restore synaptic neurotransmission. Blocking the metabolic degradation of the affected monoamines and thereby increasing their levels in the central nervous system represent another useful approach. The flavin adenine dinucleotide (FAD)-containing enzyme monoamine oxidase (MAO) is a key metabolising enzyme of monoamine neurotransmitters in peripheral tissues and in the central nervous system.[4] MAO consists of two isoforms of which the MAO-A form catalyses the degradation of serotonin, as well as epinephrine and norepinephrine.[5] MAO-A inhibitors are thus an established class of antidepressant drugs. Inhibitors of the MAO-B isoform, in turn, are used in the treatment of Parkinson's disease where these drugs block the MAO-B-catalysed metabolism of dopamine.[5,6] MAO-B inhibitors are often combined with L-Dopa, the direct metabolic precursor of dopamine. This combination further enhances central dopamine levels and allows for the effective L-Dopa dose to be reduced.[7–9]

MAO inhibitors may also reduce potentially injurious by-products of MAO-catalysed reactions. These are hydrogen peroxide, which is generated from the re-oxidation of the FAD cofactor by molecular oxygen, and aldehyde species, which form when the imine product of most amine substrates are hydrolysed. Since these by-products may contribute to the neurodegenerative processes in Parkinson's disease, MAO inhibitors may be neuroprotective by reducing their central concentrations.[4,10] In this respect, MAO-B inhibitors may be of particular relevance to Parkinson's disease since MAO-B activity increases with age while MAO-A activity remains unchanged.[11] MAO-A inhibitors may have a similar role in cardiovascular pathophysiology. Experimental evidence suggests that MAO-A is a major source of hydrogen peroxide in the heart, which may be responsible for increased oxidative stress and age-related impairment of cardiac function.[12] The by-products of MAO-catalysis may target mitochondrial function and in this way affect the function and viability of the myocardium.[13]

Based on these considerations, MAO inhibitors are of importance in medicine and numerous research groups are involved in the discovery of novel MAO inhibitors of both natural and synthetic origin. Chalcones (1,3-diphenyl-2-propen-1-ones) (**1**) is

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a class of compounds that have been shown to inhibit the MAOs (Fig. 1).[14–20] Chalcones are intermediates in the biosynthesis of flavonoids and are reported to possess a wide range of biological activities.[21,22] Since chalcones are relatively facile to prepare in the laboratory, they often serve as reagents and intermediates in organic chemistry. In fact, chalcones have been used in the synthesis of pyrazoline derivatives as part of an effort to discover novel MAO inhibitors.[23–30] Literature reports that, with the appropriate substitution, chalcones themselves are, however, high potency MAO-B inhibitors. For example, synthetic chalcone **2** inhibits human MAO-B with an IC_{50} value of 0.0044 μM.[14] As exemplified by **2** for which no inhibition of MAO-A is observed (up to 50 μM), chalcones are in general selective MAO-B inhibitors. A related study has recently investigated the human MAO inhibition properties of a series of furanochalcones in an attempt, for the first time, to determine the effect of heterocyclic substitution on inhibition potency.[31] The resulting furanochalcones also proved to be potent MAO-B-selective inhibitors with the most active compound (**3a**) exhibiting an IC_{50} value of 0.174 μM (Table 1). Since the effect of heterocyclic substitution other than furan, and more recently thiophene, piperidine and quinoline,[32,33] on the MAO inhibitory properties of chalcones remains unexplored, the present study examines a series of heterocyclic chalcone analogues that incorporates pyrrole, 5-methylthiophene, 5-chlorothiophene and 6-methoxypyridine substitution (Table 2). This study is part of a systematic investigation of the effect of heterocyclic substitution of chalcones on MAO inhibition.

A further point of interest is that, in the reported study, two representative substituted furanochalcones were found to inhibit MAO-B reversibly.[31] Reversibility of MAO inhibition is an important consideration since the irreversible inhibition of MAO-A in the periphery is associated with potentially fatal changes in blood-pressure when these drugs are taken with certain foods.[34,35] For this reason MAO-A inhibitors are used with caution in the clinic and dietary restrictions are imposed. Reversible MAO-A inhibitors are less likely to cause blood-pressure changes and are considered to possess better safety profiles in this regard.[36,37] Since MAO-B inhibition is not associated with serious adverse effects, selective (reversible or irreversible) MAO-B inhibitors are considered safer drugs.[38,39] It should, however, be kept in mind that long-lasting enzyme inhibition by irreversible MAO-B inhibitors may in theory lead to immunogenicity, and de novo synthesis of the MAO protein is required for activity to recover.

The heterocyclic chalcone analogues, **4a**–**h**, were synthesised by the Claisen–Schmidt condensation between an aromatic aldehyde and heteroaromatic methyl ketone in yields of 11–60% (Scheme 1).[40] For the synthesis of **4i**, a substituted acetophenone was reacted with a heteroaromatic aldehyde (yield 1.8%). For all reactions, sodium hydroxide served as base. The structures and purities of the chalcone analogues were verified by ^{1}H NMR, ^{13}C NMR, mass spectrometry and HPLC analysis as cited in Supplementary material. The most characteristic signals observed in the ^{1}H NMR spectra, were those of the double bond protons, that were present as two doublets with coupling constants of 15.1–15.8 Hz, indicating the *trans* geometry. In the ^{13}C NMR spectra, the most downfield signal, at 177.5–190.8 ppm, signified the presence of the carbonyl group. For all compounds, NMR (chemical shifts, integration, multiplicities and coupling constants) and mass spectra corresponded with the proposed structures. Purities, as determined by HPLC were acceptable (94–100%).

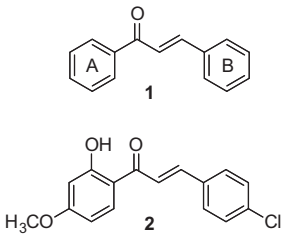
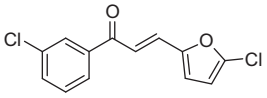
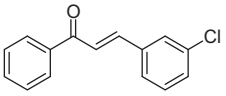
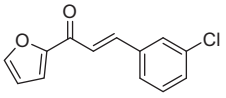
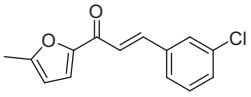
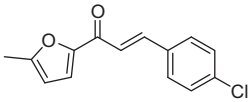
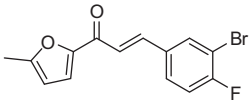
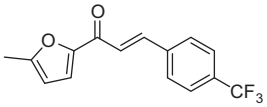


Figure 1. The structures of chalcones discussed in the text.

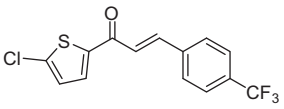
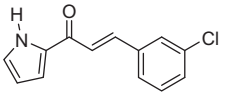
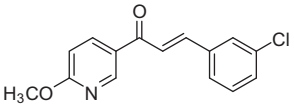
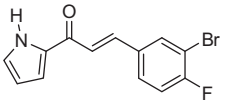
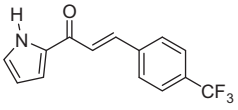
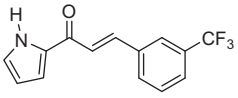
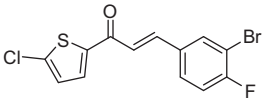
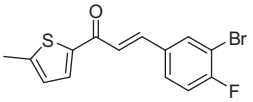
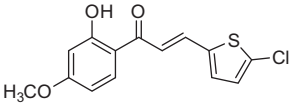
Table 1
The reported IC_{50} values for the inhibition of human MAO-B by selected chalcone analogues[31]

		MAO-B IC_{50} (μM)
3a		0.174
3b		0.529
3c		2.10
3d		0.490
3e		0.616
3f		0.200
3g		0.275

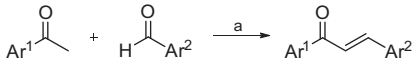
To determine the inhibitory activities of the heterocyclic chalcone analogues, the recombinant human MAO-A and MAO-B enzymes were used according to a previously published protocol.[41,42] For both MAO isoforms, kynuramine served as enzyme substrate. Kynuramine is metabolised by the MAOs to yield 4-hydroxyquinoline, a metabolite which fluoresces in alkaline media. MAO catalytic rates were thus determined by measuring the formation of 4-hydroxyquinoline with fluorescence spectrophotometry. By carrying out these experiments in the presence of various concentrations of the test inhibitors (0.003–100 μM), sigmoidal plots of catalytic rate versus logarithm of inhibitor concentration were constructed. From these plots IC_{50} values were calculated. Examples of sigmoidal plots for the inhibition of MAO-A and MAO-B are given in Figure 2.

The IC_{50} values for the inhibition of the human MAOs are given in Table 2. From the results it is clear that all the heterocyclic chalcone analogues possess higher affinities for the MAO-B isoform with selectivity index (SI) values of >4.6. The most selective MAO-B inhibitor, compound **4h** (SI = 240), also is the most potent MAO-B inhibitor of the current study with an IC_{50} value of 0.067 μM. The inhibition potency of this compound is in the same range as that of the reference MAO-B inhibitor, lazabemide (IC_{50} = 0.091 μM).[43] With the exception of **4b** and **4f**, all chalcones display IC_{50} <1 μM for the inhibition of MAO-B. The most potent MAO-A inhibitor is compound **4e** (IC_{50} = 3.81 μM). Although not considered highly potent, this value is similar to that of toloxatone,

Table 2
The IC_{50} values for the inhibition of human MAO-A and MAO-B by heterocyclic chalcone analogues, **4a–i**, and reference inhibitors

		IC_{50}[a] (μM)		SI[b]
		MAO-A	MAO-B	
4a		7.56 ± 1.36	0.133 ± 0.048	57
4b		143 ± 24.1	3.27 ± 0.419	44
4c		15.3 ± 6.16	0.330 ± 0.053	46
4d		8.98 ± 1.52	0.803 ± 0.037	11
4e		3.81 ± 0.387	0.830 ± 0.100	4.6
4f		267 ± 80.5	1.40 ± 0.140	191
4g		10.5 ± 1.20	0.116 ± 0.030	91
4h		16.1 ± 2.14	0.067 ± 0.016	240
4i		9.99 ± 2.64	0.185 ± 0.050	54
Toloxatone		3.92[c]	–	
Lazabemide		–	0.091[c]	

[a] All values are expressed as the mean ± standard deviation (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).
[c] Value obtained from Ref. 43.



Scheme 1. The general synthetic route for heterocyclic chalcone analogues, **4a–i**. Reaction conditions: (a) ketone (1 equiv), aldehyde (1 equiv), NaOH (40%; 0.5 equiv), EtOH, rt.

a MAO-A inhibitor in clinical use, which is reported to inhibit MAO-A with an IC_{50} value of 3.92 μM.[43]

From the inhibition data, the following structure–activity relationships (SARs) and comparisons may be made: (1) by comparing the MAO-A and MAO-B inhibition potencies of the pyrrole derivatives, it is clear that trifluoromethyl substitution on the *para* position of the phenyl ring (**4e**) is preferable over substitution in the *meta* position (**4f**) for both isoforms. In fact the *para* substituted compound is the most potent MAO-A inhibitor of the current series. (2) A comparison of the MAO-B inhibition potencies of the 5-chlorothiophene derivatives, **4a** (IC_{50} = 0.133 μM) and **4g** (IC_{50} = 0.116 μM), indicate that a 3-bromo-4-fluorophenyl and 4-trifluoromethylphenyl substituents yields similar MAO-B inhibition potencies. (3) Previously reported compound **2**, the most active analogue among a series of 1,3-diphenylprop-2-en-1-ones, was resynthesised for comparative purposes.[14] In the present

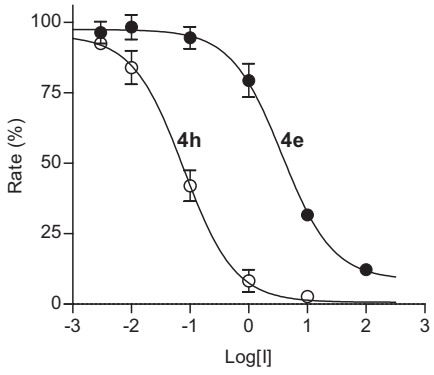


Figure 2. The sigmoidal plots for the inhibition of MAO-A by **4e** (filled circles) and MAO-B by **4h** (open circles).

study this compound exhibits an IC_{50} value of 0.093 ± 0.022 μM for the inhibition of human MAO-B, which is slightly less potent than the most active analogue, **4h** (IC_{50} = 0.067 μM). This indicates that heterocyclic substitution of the chalcone scaffold (with a methylthiophene ring in particular) is a viable design strategy. It should be noted that the difference in activities of these compounds are within experimental error, and their activities may thus be viewed as similar. The difference in the IC_{50} values of **2** in the present and the reported study (IC_{50} = 0.0044 μM) may be due to the use of different substrates and experimental conditions. (4) Comparison of the MAO-B inhibition potencies of 3-bromo-4-fluorophenyl derivatives **4h** (IC_{50} = 0.067 μM) and **4g** (IC_{50} = 0.116 μM) reveals that an electron donating methyl substituent in the thiophene ring (**4h**) results in slightly improved MAO-B inhibition compared to substitution with an electron withdrawing chlorine substituent (**4g**). (5) When the MAO-B inhibition potencies of the 3-bromo-4-fluorophenyl derivatives **4g** (IC_{50} = 0.116 μM) and **4d** (IC_{50} = 0.803 μM), and the MAO-B inhibition potencies of the 4-trifluoromethylphenyl derivatives **4a** (IC_{50} = 0.133 μM) and **4e** (IC_{50} = 0.830 μM) are compared, the results indicate that 5-chlorothiophene substitution is preferable over pyrrole substitution. Similarly, when the MAO-B inhibition potencies of the 3-chlorophenyl derivatives, **4c** (IC_{50} = 0.330 μM) and **4b** (IC_{50} = 3.27 μM) are compared, it is evident that 6-methoxypyridine substitution is also preferable over pyrrole substitution.

As mentioned above, in a previous study a series of furanochalcones has been shown to inhibit the MAOs.[31] The inhibition potencies of the heterocyclic chalcones of the current study compounds may thus be compared to the results obtained with the furanochalcones (Table 1). This direct comparison is possible, since these derivatives were evaluated under the same experimental conditions. In order to identify the heterocyclic substituent that confers the most potent MAO-B inhibition activity, the potencies of the following derivatives were compared: (1) the 3-chlorophenyl derivatives **3b–d** (IC_{50} = 0.529, 2.10, 0.490 μM, respectively), **4b** (IC_{50} = 3.27 μM) and **4c** (IC_{50} = 0.330 μM). An analysis of the MAO-B inhibition activities of these compounds indicate that the effect of the heteroaromatic/aromatic substituent on activity, in decreasing order is: 6-methoxypyridine > 5-methylfuran > phenyl > furan > pyrrole. It should be noted that the activities of **3b**, **3d** and **4c** are similar in range. (2) The 3-bromo-4-fluorophenyl derivatives, **3f** (IC_{50} = 0.200 μM), **4d** (IC_{50} = 0.803 μM), **4g** (IC_{50} = 0.116 μM) and **4h** (IC_{50} = 0.067 μM). An analysis of the MAO-B inhibition activities indicates that the effect of the heteroaromatic substituent for these compounds, on activity, in decreasing order is: 5-methylthiophene > 5-chlorothiophene > 5-methylfuran > pyrrole. It should be noted that the activities of **3f** and **4g** are, however, similar. (3) The 4-trifluoromethylphenyl derivatives, **3g** (IC_{50} = 0.275 μM), **4a** (IC_{50} = 0.133 μM) and **4e** (IC_{50} = 0.830 μM). An analysis of the MAO-B inhibition activities indicate that the effect of the heteroaromatic substituent for these compounds, on activity, in decreasing order is: 5-chlorothiophene > 5-methylfuran > pyrrole. These results show that substitution with a 5-methylthiophene group is an improvement on furan or methylfuran substitution, and results in optimal MAO-B inhibition activity, while pyrrole substitution is associated with decreased MAO-B inhibition activity. This is exemplified by the finding that **4h** is 2.6-fold more potent as a MAO-B inhibitor than the most potent furanochalcone (**3a**) (IC_{50} = 0.174 μM) investigated by Robinson et al.,[31] and has similar activity to **2**, previously investigated by Chimenti and co-workers.[14] It should be kept in mind that this is a preliminary study and future work will include the expansion of this series, to investigate the effect of further variations in the substitution of the phenyl ring in particular, and also to investigate the effect of heteroaromatic substitution other than those considered in this study.

Since reversibility of MAO inhibition is frequently a consideration in inhibitor design and development, the reversibility of MAO inhibition by the most potent MAO-A and MAO-B inhibitors, **4e**, and **4h**, respectively, were examined. This study also investigated the reversibility of MAO-B inhibition by compound **4d**. To examine the reversibility of inhibition, the recoveries of enzyme activity after dilution of enzyme–inhibitor mixtures were evaluated. MAO-A and MAO-B were preincubated (for 30 min) with the test compounds at concentrations of 10 × IC_{50} and 100 × IC_{50} for the inhibition of the respective enzymes. These mixtures were subsequently diluted 100-fold to yield inhibitor concentrations of 0.1 × IC_{50} and 1 × IC_{50}, and the residual MAO activities were measured. For reversible inhibition, enzyme activity is expected to recover to 90% after dilution of the enzyme–inhibitor mixtures to an inhibitor concentration of 0.1 × IC_{50}, while enzyme activity is expected to recover to 50% after dilution to 1 × IC_{50}. As positive controls, the irreversible MAO-A and MAO-B inhibitors, pargyline and (*R*)-deprenyl, respectively, were also evaluated. For irreversible inhibition, enzyme activity is not expected to recover after dilution of enzyme–inhibitor mixtures.

The results show that the pyrrole derivative, **4e**, behaves as a reversible inhibitor of both MAO-A and MAO-B (Fig. 3). After dilution, of mixtures containing MAO and **4e** to 0.1 × IC_{50} and 1 × IC_{50}, the MAO-A activity is recovered to levels 90% and 67%, respectively, of the negative control value (experiment conducted in absence of inhibitor). After dilution, MAO-B activity is recovered to levels of 85% and 32%, respectively, of the negative control value. In contrast, after similar treatment of MAO-A and MAO-B with the irreversible inhibitors pargyline and (*R*)-deprenyl, respectively, the MAO activities are not recovered as dilution to concentrations of 0.1 × IC_{50} resulted in the recovery of only 1.2% and 3.4% enzyme activity. These results were as expected, since a reversible mode of binding to MAO-B was also illustrated for a related series of furanochalcones.[31]

Unexpected results were, however, obtained when the reversibility of binding of the most potent MAO-B inhibitor, **4h**, was examined. As shown in Figure 4, after dilution of mixtures containing MAO-B and **4h** to concentrations equal to 0.1 × IC_{50} and 1 × IC_{50}, the MAO-B activities are recovered to levels of only 26% and 5%, respectively, of the control value. This behaviour is not fully consistent with a reversible interaction of **4h** with MAO-B. As mentioned above, for reversible inhibition, after dilution of enzyme–inhibitor mixtures to inhibitor concentrations of 0.1 × IC_{50} and 1 × IC_{50}, enzyme activity is expected to recover to

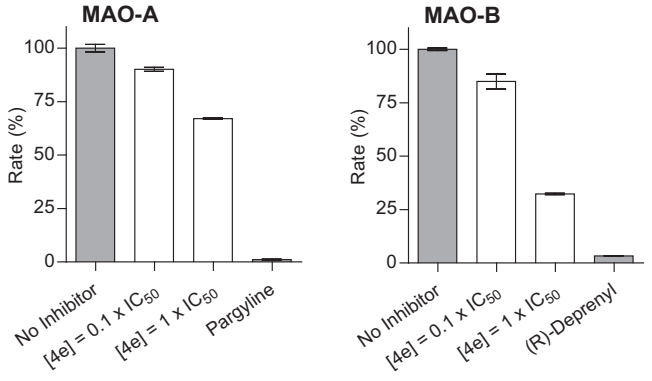


Figure 3. Dilution reverses MAO-A and MAO-B inhibition by **4e**. The MAOs and **4e** were incubated at inhibitor concentrations of 10 × IC_{50} and 100 × IC_{50} for 30 min and subsequently diluted 100-fold to 0.1 × IC_{50} and 1 × IC_{50}. For comparison, MAO-A and MAO-B were similarly preincubated with pargyline and (*R*)-deprenyl, respectively, at concentrations equal to 10 × IC_{50} and diluted to 0.1 × IC_{50}. The residual enzyme activities after dilution were measured and are shown.

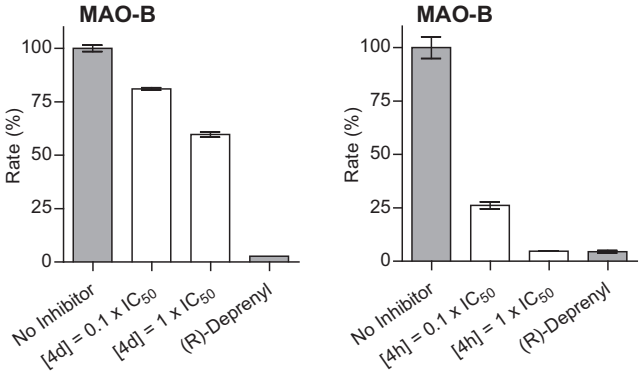


Figure 4. Dilution reverses MAO-B inhibition by **4d** but not inhibition by **4h**. MAO-B and the test inhibitors were incubated at inhibitor concentrations of 10 × IC_{50} and 100 × IC_{50} for 30 min and subsequently diluted 100-fold to 0.1 × IC_{50} and 1 × IC_{50}. For comparison, MAO-B was similarly preincubated with (*R*)-deprenyl at a concentration equal to 10 × IC_{50} and diluted to 0.1 × IC_{50}. The residual enzyme activities after dilution were measured and are shown.

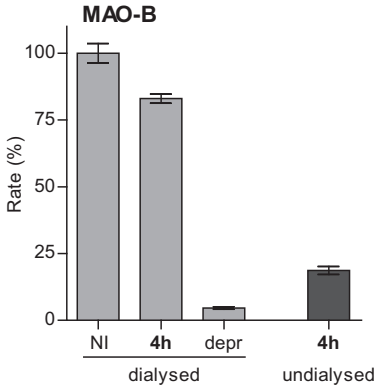


Figure 5. Dialysis reverses MAO-B inhibition by **4h**. MAO-B and **4h** (at a concentration of 4 × IC_{50}) were incubated for 15 min, dialysed for 24 h and the residual enzyme activity was measured (**4h** dialysed). Similar dialysis of MAO-B in the absence (NI dialysed) and presence of the irreversible inhibitor, (*R*)-deprenyl (depr dialysed) were also carried out. The residual MAO-B activity of undialysed mixtures of MAO-B with **4h** was also recorded (**4h** undialysed).

90% and 50%, respectively. A possible explanation for this finding is that **4h** may exhibit tight-binding to the MAO-B enzyme, and the inhibition caused by this compound is not readily reversed by dilution. This possibility is further explored below where the reversibility of MAO-B inhibition by **4h** is examined by dialysis. To investigate whether potential tight-binding of **4h** was due to the presence of the phenyl or thiophene moieties, the reversibility of MAO-B inhibition of **4d** was also examined. These results, given in Figure 4, show that after dilution of mixtures containing MAO-B and **4d** to inhibitor concentrations equal to 0.1 × IC_{50} and 1 × IC_{50}, the MAO-B activities are recovered to levels of 81% and 60%, respectively, of the control value. This behaviour is consistent with a reversible interaction of **4d** with MAO-B. From these results it could thus be derived that the tight-binding observed for **4h** is due to the presence of the thiophene moiety, as the pyrrole derivative **4d**, with a similar phenyl substituent, binds reversibly to MAO-B. Potential tight-binding of inhibitors to the MAOs has been previously reported for a variety of different classes of compounds.[44–47]

Since the inhibition of MAO-B by compound **4h** may not to be readily reversible and **4h** therefore may exhibit a degree of tight-binding to the MAO-B active site, the reversibility of MAO-B inhibition by **4h** was further examined by dialysis. For this purpose MAO-B and **4h**, at a concentration of 4 × IC_{50}, were preincubated for a period of 15 min and subsequently dialysed for 24 h. The residual enzyme activity was measured and compared to similar dialysis experiments performed in the absence of inhibitor and presence of the irreversible inhibitor, (*R*)-deprenyl. For reversible inhibition, the enzyme activity is expected to recover to 100% after dialysis. The results, given in Figure 5, show that inhibition of MAO-B by **4h** is almost completely reversed after 24 h of dialysis, with the MAO-B activity recovering to a level of 83% of the control value (activity after dialysis in absence of inhibitor). In contrast, MAO-B activity in undialysed mixtures of the enzyme and **4h** is only 18% of the control value. This behaviour is consistent with reversible inhibition of MAO-B by **4h**. As expected, enzyme activity is not recovered after dialysis of mixtures containing MAO-B and (*R*)-deprenyl, with activity at only 5% of the control value.

To further investigate MAO-B inhibition by **4h**, the present study examines the mode (e.g., competitive) of inhibition. For this purpose Lineweaver–Burk plots were constructed. Six plots were constructed employing the following inhibitor concentrations: 0 μM, ¼ × IC_{50}, ½ × IC_{50}, ¾ × IC_{50}, 1 × IC_{50} and 1¼ × IC_{50}. The results show that the Lineweaver–Burk plots are linear and

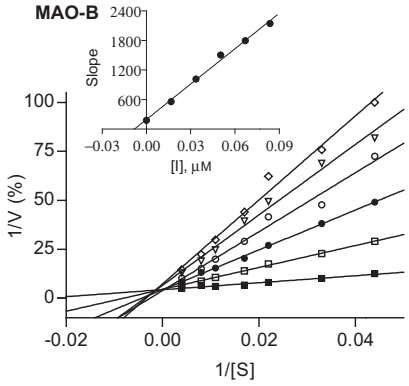


Figure 6. Lineweaver–Burk plots of human MAO-B activities in the absence (filled squares) and presence of various concentrations of **4h** [IC_{50}(MAO-B) = 0.067 μM]. The inhibitor concentrations used were: ¼ × IC_{50}, ½ × IC_{50}, ¾ × IC_{50}, 1 × IC_{50} and 1¼ × IC_{50} and the inset is a graph of the slopes of the Lineweaver–Burk plots versus inhibitor concentration.

Table 3
The percentage viable HeLa cells remaining after treatment with **4d**, **4e** and **4h**, compared to untreated cells (100%)

	% viable cells[a]	
	1 μM	10 μM
4d	99.4 ± 3.4	98.0 ± 8.6
4e	77.5 ± 11.6*	4.66 ± 6.9*
4h	104 ± 4.7	95.7 ± 4.0

* p <0.05 compared to untreated cells.
[a] Values are given as mean ± SD of triplicate determinations.

intersect on the *y*-axis (Fig. 6). This suggests that **4h** is a competitive inhibitor of human MAO-B. This is in agreement with the finding that this compound is a reversible MAO-B inhibitor. Global (shared) fitting of the inhibition data directly to the Michaelis–Menten equation yields a K_i value of 0.0064 ± 0.0004 μM (r^2 = 0.999) for the inhibition of MAO-B.

To obtain an early assessment of the 'drug-like' properties of some the heterocyclic chalcones, the toxicity of selected inhibitors, **4d**, **4e** and **4h**, towards cultured cells was determined. For this

purpose, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay was employed.[48,49] Cytotoxicity was evaluated by exposing HeLa cells for 24 h to concentrations of 1 and 10 μM of the test compounds. To determine if statistical differences exist between mean values of the drug-treated and drug-naive cells, the viability data were analysed by One-way ANOVA followed by Post-hoc Dunnett's analysis. As shown in Table 3, the most potent MAO-B inhibitor of the present series, **4h**, was non-toxic [$F(2,6) = 2.17$; $p < 0.20$] at 1 and 10 μM, with 104% and 95.7% viable cells, respectively, remaining. Compound **4e**, the most potent MAO-A inhibitor, however, exhibited significant toxicity [$F(2,6) = 86.1$; $p < 0.000038$] at 10 μM, with only 4.66% viable cells remaining ($p = 0.000038$). As expected, at a lower concentration of 1 μM, a smaller degree of toxicity (77.5% viable cells remaining) was observed ($p = 0.04$). This compound exhibits an estimated half-maximal cytotoxic concentration (CC_{50}) of 28.2 ± 10.8 μM. Compared to **4h**, compound **4e** is thus toxic to cultured HeLa cells. To determine whether the pyrrole or phenyl substituents were responsible for the observed toxicity of **4e**, the cytotoxicity of another pyrrole derivative, **4d**, was investigated. Interestingly, the results show that **4d** is nontoxic at 1 μM and 10 μM, with 99.4% and 98.0% viable cells, respectively, remaining [$F(2,6) = 0.08$; $p < 0.92$]. Since **4d** is nontoxic, it may be concluded that the presence of the pyrrole substituent is not responsible for the higher degree of cytotoxicity of **4e**, but possibly the trifluoromethyl substituted phenyl ring. Further investigation in this regard is required, specifically to determine the mode of cytotoxicity and to investigate the possibility that **4e**, but not **4h** and **4d**, is metabolised to reactive intermediates or toxic products.

In conclusion, this study discovers a number of potent new MAO-B inhibitors among the heterocyclic chalcone class of compounds. This is exemplified by compound **4h**, which is a highly potent chalcone analogue. **4h** is more potent as a MAO-B inhibitor than the most potent furanochalcone (**3a**) investigated by Robinson et al.,[31] and has similar activity to **2**, previously investigated by Chimenti and co-workers.[14] An analysis of the SARs for MAO-B inhibition shows that substitution with a 5-methylthiophene group is an improvement on furan or methylfuran substitution reported previously.[31] Pyrrole substitution on the other hand is associated with decreased MAO-B inhibition activity. Experimental results further show that selected heterocyclic chalcones are reversible MAO inhibitors, with **4h** possibly exhibiting tight-binding to MAO-B, a property likely linked to its thiophene moiety. The finding that heterocyclic chalcones are reversible MAO inhibitors are of note, particularly since irreversible inhibition of the MAO-A isoform is associated with potentially fatal adverse reactions. Also noteworthy is that some chalcones (such as **4e**) may be toxic to cultured cells. This should alert scientists to possible toxicity issues and that cytotoxicity studies should be included in property characterisations of this class of compounds. Interestingly, the toxicity of **4e** is more likely due to the trifluoromethyl substituted phenyl ring than the heterocyclic moiety.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.09.049.

References and notes

1. Baumeister, A. A.; Hawkins, M. F.; Uzelac, S. M. *J. Hist. Neurosci.* **2003**, *12*, 207.
2. Lanni, C.; Govoni, S.; Lucchelli, A.; Boselli, C. *Cell. Mol. Life Sci.* **2009**, *66*, 2985.
3. Olanow, C. W.; Stern, M. B.; Sethi, K. *Neurology* **2009**, *72*, S1.
4. Youdim, M. B.; Bakhle, Y. S. *Br. J. Pharmacol.* **2006**, *147*, S287.
5. Youdim, M. B.; Edmondson, D.; Tipton, K. F. *Nat. Rev. Neurosci.* **2006**, *7*, 295.
6. Finberg, J. P. *Pharmacol. Ther.* **2014**, *143*, 133.
7. Shoulson, I.; Oakes, D.; Fahn, S.; Lang, A.; Langston, J. W.; LeWitt, P.; Olanow, C. W.; Penney, J. B.; Tanner, C.; Kieburtz, K.; Rudolph, A.Parkinson Study Group *Ann. Neurol.* **2002**, *51*, 604.
8. Fernandez, H. H.; Chen, J. J. *Pharmacotherapy* **2007**, *27*, 174S.
9. Finberg, J. P.; Wang, J.; Bankiewicz, K.; Harvey-White, J.; Kopin, I. J.; Goldstein, D. S. *J. Neural Transm. Suppl.* **1998**, *52*, 279.
10. Edmondson, D. E. *Curr. Pharm. Des.* **2014**, *20*, 155.
11. Fowler, J. S.; Volkow, N. D.; Wang, G. J.; Logan, J.; Pappas, N.; Shea, C.; MacGregor, R. *Neurobiol. Aging* **1997**, *18*, 431.
12. Maurel, A.; Hernandez, C.; Kunduzova, O.; Bompart, G.; Cambon, C.; Parini, A.; Francés, B. *Am. J. Physiol. Heart Circ. Physiol.* **2003**, *284*, H1460.
13. Kaludercic, N.; Mialet-Perez, J.; Paolocci, N.; Parini, A.; Di Lisa, F. *J. Mol. Cell. Cardiol.* **2014**, *73*, 34.
14. Chimenti, F.; Fioravanti, R.; Bolasco, A.; Chimenti, P.; Secci, D.; Rossi, F.; Yáñez, M.; Orallo, F.; Ortuso, F.; Alcaro, S. *J. Med. Chem.* **2009**, *52*, 2818.
15. Tanaka, S.; Kuwai, Y.; Tabata, M. *Planta Med.* **1987**, *53*, 5.
16. Haraguchi, H.; Tanaka, Y.; Kabbash, A.; Fujioka, T.; Ishizu, T.; Yagi, A. *Phytochemistry* **2004**, *65*, 2255.
17. Pan, X.; Kong, L. D.; Zhang, Y.; Cheng, C. H.; Tan, R. X. *Acta Pharmacol. Sin.* **2000**, *21*, 949.
18. Gao, G. Y.; Li, D. J.; Keungm, W. M. *J. Med. Chem.* **2001**, *44*, 3320.
19. Morales-Camilo, N.; Salas, C. O.; Sanhueza, C.; Espinosa-Bustos, C.; Sepúlveda-Boza, S.; Reyes-Parada, M.; Gonzalez-Nilo, F.; Caroli-Rezende, M.; Fierro, A. *Chem. Biol. Drug Des.* **2015**, *85*, 685.
20. Mathew, B.; Mathew, G. E.; Uçar, G.; Baysal, I.; Suresh, J.; Vilapurathu, J. K.; Prakasan, A.; Suresh, J. K.; Thomas, A. *Bioorg. Chem.* **2015**, *62*, 22.
21. Go, M. L.; Wu, X.; Liu, X. L. *Curr. Med. Chem.* **2005**, *12*, 481.
22. Dimmock, J. R.; Elias, D. W.; Beazely, M. A.; Kandepu, N. M. *Curr. Med. Chem.* **1999**, *6*, 1125.
23. Chimenti, F.; Fioravanti, R.; Bolasco, A.; Chimenti, P.; Secci, D.; Rossi, F.; Yáñez, M.; Orallo, F.; Ortuso, F.; Alcaro, S.; Cirilli, R.; Ferretti, R.; Sanna, M. L. *Bioorg. Med. Chem.* **2010**, *18*, 1273.
24. Chimenti, F.; Carradori, S.; Secci, D.; Bolasco, A.; Bizzarri, B.; Chimenti, P.; Granese, A.; Yáñez, M.; Orallo, F. *Eur. J. Med. Chem.* **2010**, *45*, 800.
25. Chimenti, F.; Fioravanti, R.; Bolasco, A.; Manna, F.; Chimenti, P.; Secci, D.; Befani, O.; Turini, P.; Ortuso, F.; Alcaro, S. *J. Med. Chem.* **2007**, *50*, 425.
26. Chimenti, F.; Maccioni, E.; Secci, D.; Bolasco, A.; Chimenti, P.; Granese, A.; Befani, O.; Turini, P.; Alcaro, S.; Ortuso, F.; Cirilli, R.; La Torre, F.; Cardia, M. C.; Distinto, S. *J. Med. Chem.* **2005**, *48*, 7113.
27. Chimenti, F.; Bolasco, A.; Manna, F.; Secci, D.; Chimenti, P.; Granese, A.; Befanim, O.; Turini, P.; Alcaro, S.; Ortuso, F. *Chem. Biol. Drug Des.* **2006**, *67*, 206.
28. Gökhan-Kelekçi, N.; Koyunoğlu, S.; Yabanoğlu, S.; Yelekçi, K.; Ozgen, O.; Uçar, G.; Erol, K.; Kendi, E.; Yeşilada, A. *Bioorg. Med. Chem.* **2009**, *17*, 675.
29. Secci, D.; Carradori, S.; Bolasco, A.; Bizzarri, B.; D'Ascenzio, M.; Maccioni, E. *Curr. Top. Med. Chem.* **2012**, *12*, 2240.
30. Mathew, B.; Suresh, J.; Anbazhagan, S.; Mathew, G. E. *Cent. Nerv. Syst. Agents Med. Chem.* **2013**, *13*, 195.
31. Robinson, S. J.; Petzer, J. P.; Petzer, A.; Bergh, J. J.; Lourens, A. C. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 4985.
32. Zaib, S.; Farooq Rizvi, S. U.; Aslam, S.; Ahmad, M.; Al-Rashida, M.; Iqbal, J. *Med. Chem.* **2015**, *11*, 497.
33. Zaib, S.; Rizvi, S. U.; Aslam, S.; Ahmad, M.; Ali Abid, S. M.; Al-Rashida, M.; Iqbal, J. *Med. Chem.* **2015**, *11*, 580.
34. Da Prada, M.; Zürcher, G.; Wüthrich, I.; Haefely, W. E. *J. Neural Transm. Suppl.* **1988**, *26*, 31.
35. Flockhart, D. A. *J. Clin. Psychiatry* **2012**, *73*, 17.
36. Bonnet, U. *CNS Drug Rev.* **2003**, *9*, 97.
37. Provost, J. C.; Funck-Brentano, C.; Rovei, V.; D'Estanque, J.; Ego, D.; Jaillon, P. *Clin. Pharmacol. Ther.* **1992**, *52*, 384.
38. Pae, C. U.; Bodkin, J. A.; Portland, K. B.; Thase, M. E.; Patkar, A. A. *J. Clin. Psychiatry* **2012**, *73*, 661.
39. Finberg, J. P.; Gillman, K. *Int. Rev. Neurobiol.* **2011**, *100*, 169.
40. Cocconcelli, G.; Diodato, E.; Caricasole, A.; Gaviraghi, G.; Genesio, E.; Ghiron, C.; Magnoni, L.; Pecchioli, E.; Plazzi, P. V.; Terstappen, G. C. *Bioorg. Med. Chem.* **2008**, *16*, 2043.
41. Novaroli, L.; Reist, M.; Favre, E.; Carotti, A.; Catto, M.; Carrupt, P. A. *Bioorg. Med. Chem.* **2005**, *13*, 6212.
42. Mostert, S.; Petzer, A.; Petzer, J. P. *ChemMedChem* **2015**, *10*, 862.
43. Petzer, A.; Pienaar, A.; Petzer, J. P. *Drug Res. (Stuttg)* **2013**, *63*, 462.

44. Gaspar, A.; Silva, T.; Yáñez, M.; Vina, D.; Orallo, F.; Ortuso, F.; Uriarte, E.; Alcaro, S.; Borges, F. *J. Med. Chem.* **2011**, *54*, 5165.
45. Gaspar, A.; Reis, J.; Fonseca, A.; Milhazes, N.; Viña, D.; Uriarte, E.; Borges, F. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 707.
46. Krueger, M. J.; Mazouz, F.; Ramsay, R. R.; Milcent, R.; Singer, T. P. *Biochem. Biophys. Res. Commun.* **1995**, *206*, 556.
47. Mazouz, F.; Gueddari, S.; Burstein, C.; Mansuy, D.; Milcent, R. *J. Med. Chem.* **1993**, *36*, 1157.
48. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.
49. Delport, A.; Harvey, B. H.; Petzer, A.; Petzer, J. P. *Life Sci.* **2014**, *117*, 56.

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