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ORIGINAL ARTICLE

The monoamine oxidase inhibition properties of C6- and N1-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives

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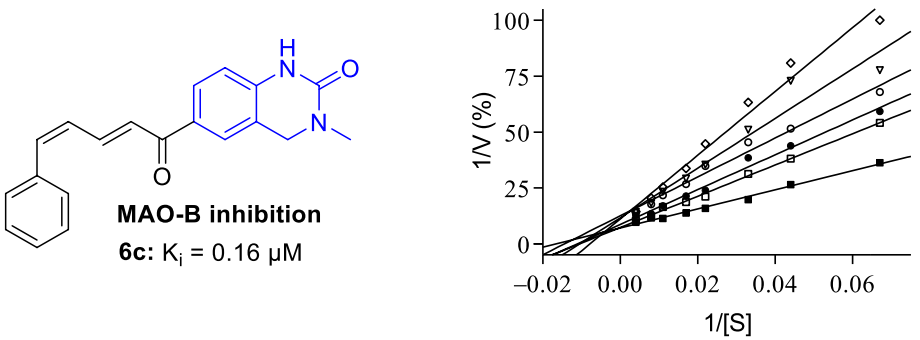
Abstract

Quinazolinone compounds are of interest in medicinal chemistry since they display a wide range of biological properties. In the present study, a series of C6- and N1-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives were synthesised and evaluated as inhibitors of recombinant human monoamine oxidase (MAO). Some of these quinazolinones are structurally related to a series of 3,4-dihydro-2(1*H*)-quinolinone derivatives, which have previously been reported to act as specific inhibitors of MAO-B. The results document that, among 37 compounds synthesised, seven displayed IC_{50} values <1 µM for the inhibition of MAO-B. The most potent MAO-A inhibitor exhibits an IC_{50} value of 7.43 µM while the most potent MAO-B inhibitor possesses an IC_{50} value of 0.269 µM. Good-potency MAO inhibition was only observed among C6-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives with N1-substitution yielding comparatively low-potency inhibition. MAO-B-specific inhibitors such as some of the quinazolinone compounds investigated here may act as leads for the design of therapies for neurodegenerative disorders such as Parkinson's disease.

Graphical abstract



Keywords Monoamine oxidase · MAO · Inhibition · Reversible · Quinazolinone · Neurodegenerative disorders

Introduction

The monoamine oxidase (MAO) enzymes are flavin adenine dinucleotide (FAD)-containing enzymes that catalyse the oxidation of neurotransmitter and dietary amines in both the peripheral and central tissues [1]. Two isoforms of MAO are expressed, MAO-A and MAO-B, which are products of distinct genes [2]. Although MAO-A and MAO-B share a high degree of sequence and structural similarity,

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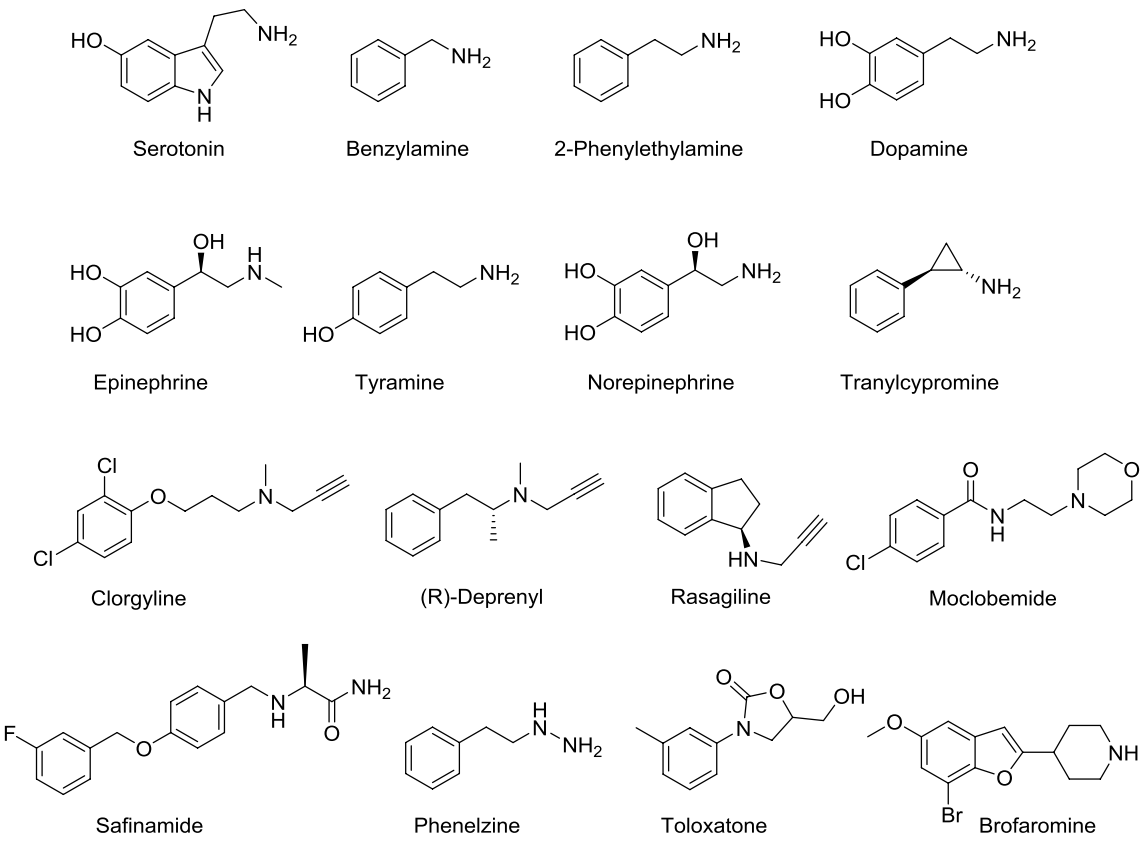


Fig. 1 Structures of selected MAO substrates and inhibitors

they exhibit different substrate and inhibitor specificities [1, 3, 4]. MAO-A metabolises serotonin and is specifically inhibited by the propargylamine compound clorgyline, while MAO-B metabolises the arylalkylamines, benzylamine and 2-phenethylamine and is specifically inhibited by (*R*)-deprenyl (Fig. 1). Certain substrates such as dopamine, epinephrine, tyramine and norepinephrine are oxidised by both MAO isoforms, while a number of inhibitors (e.g. tranylcypromine) also do not display isoform specificity [1].

Because of their involvement in the catabolism of neurotransmitter amines, MAO-A and MAO-B are considered targets for the treatment of neurological disorders [1, 5]. In this respect, MAO-A inhibitors are used in the treatment of depression and anxiety, while MAO-B inhibitors are often combined with L-dopa in the treatment of Parkinson's disease [1, 6]. In Parkinson's disease, MAO-B inhibitors block the MAO-B-catalysed metabolism of dopamine in the brain and thus enhance dopaminergic neurotransmission. In combination with L-dopa, MAO-B inhibitors may enhance dopamine levels derived from the conversion of L-dopa to dopamine and may thus allow for a reduction in the dose of L-dopa required for a therapeutic effect [7, 8]. MAO-B inhibitors may also protect against neuronal injury and neurodegeneration in Parkinson's disease by reducing oxidative damage mediated by hydrogen peroxide, a by-product of MAO catalysis [5]. Currently, two irreversible acting propargylamine compounds, (*R*)-deprenyl and rasagiline, are clinically used for the management of Parkinson's disease, while a reversible inhibitor, safinamide, has recently been approved [1, 9]. MAO-A inhibitors that are used in the clinic for the treatment of depression include tranylcypromine, phenelzine, moclobemide, toloxatone and brofaromine [1]. It should be cautioned that reversibility of MAO-A inhibition is an important consideration since irreversible inhibitors such as tranylcypromine and phenelzine may cause a severe hypertensive crisis when combined with food that contains the sympathomimetic amine, tyramine [10, 11]. With the irreversible inhibition of MAO-A in the gastrointestinal tract and vascular endothelium, dietary tyramine gains access to the circulatory system which leads to the release of norepinephrine from the sympathetic neurons and subsequently a hypertensive reaction [5, 12]. In some instances, this hypertensive reaction can be fatal. This led to the development of reversible MAO-A inhibitors such as moclobemide and brofaromine, which possess a lower liability for increases in blood pressure [13, 14]. MAO-B-specific inhibitors also do not cause tyramine-induced hypertension since MAO-B is absent from the gut tissues [5].

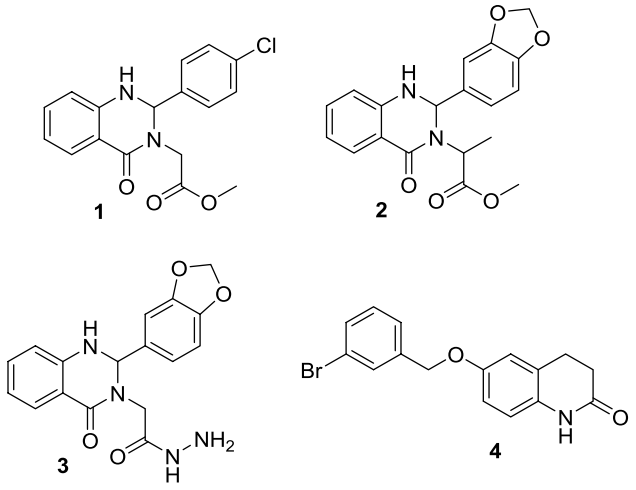


Fig. 2 Structures of 4(3*H*)-quinazolinone (**1**–**3**) and 3,4-dihydro-2(1*H*)-quinolinone (**4**) derivatives

Because of their biochemical and clinical significance, the discovery and development of MAO inhibitors are being actively pursued [15]. Quinazolinone is a heterocyclic nitrogen containing system composed of fused phenyl and pyrimidinone (pyrimidone) rings. Over the years, natural and synthetic quinazolinones have attracted attention since they display a variety of biological activities [16–19] including MAO inhibition [20–24]. Other biological activities of quinazolinones include antimicrobial, anticonvulsant, anticancer, antimalarial, antihypertensive, anti-inflammatory, antidiabetic, antitumor, anticholinesterase, dihydrofolate reductase inhibition, as well as the inhibition of cellular phosphorylation and kinases [25]. As mentioned, the MAO inhibitory properties of quinazolinone derivatives have also been reported [20–22]. For example, Khattab and co-workers synthesised a series of 4(3*H*)-quinazolinone derivatives and evaluated them as potential inhibitors of MAO-A and MAO-B. Compounds **1** (IC_{50} = 3.6 nM), **2** (IC_{50} = 2.8 nM) and **3** (IC_{50} = 2.1 nM) were found to be most active inhibitors and displayed MAO-A inhibition that was comparable to the reference inhibitor, clorgyline (Fig. 2) [22]. Quinazolinones that have been studied as MAO inhibitors are from the 4(3*H*)-quinazolinone class of compounds, while 2(1*H*)-quinazolinones have not yet been investigated. Based on promising properties of 4(3*H*)-quinazolinones, we envisage that isomeric 2(1*H*)-quinazolinone derivatives may also act as MAO inhibitors. In support of this, structurally related compounds such as 3,4-dihydro-2(1*H*)-quinolinone derivatives are known to potently and specifically inhibit MAO-B. For example, among a series of 3,4-dihydro-2(1*H*)-quinolinone derivatives substituted on the C6 and C7 positions, the most active compound (**4**) exhibits an IC_{50} value of 0.086 μM [26, 27]. Based on these findings, the present study synthesises series of C6- and N1-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives. In total, 26 derivatives with C6-substitution (**5a**–**m**; **6a**–**m**) and 11 derivatives with N1-substitution (**7a**–**e**; **8a**–**f**) were synthesised. The present study is the first investigation of the MAO inhibition properties of 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives.

Results and discussion

Chemistry

In the current study, two series of 3-methyl-3,4-dihydroquinazolin-2(1*H*)-ones were synthesised. The first series consists of C6-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives **5a**–**m** and **6a**–**m** for which benzoyl/acyl and cinnamoyl C6-substitutions were considered, respectively. The C6-substituted benzoyl/acyl derivatives (**5**) were synthesised by Friedel–Crafts acylation (Fig. 3). To a solution of aluminium trichloride and 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one in carbon disulphide, the appropriate acyl chloride or bromide was added. The reaction mixture was heated under reflux (50 °C) for 24 h, and on completion, the reaction was terminated by the addition of a mixture of ice and water. The product was collected by filtration, dried in a convection oven and recrystallised from ethanol. Attempts to synthesise the C6-substituted cinnamoyl derivatives by direct acylation failed, and with the exception of **6b**, the target cinnamoyl derivatives **6** were thus synthesised from 6-acetyl-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (**5l**). Derivative **6b** was synthesised by employing **5m** as starting material. To a solution of **5l** or **5m** in a mixture of hydrochloric acid (32%) and methanol, an appropriate benzaldehyde (or cinnamaldehyde for **6c**) was added. The reaction was heated under reflux for 24–48 h, and on completion, the product was isolated and recrystallised as described above.

The target 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives **5** and **6** were obtained in yields of 13–100%. The structures were determined by ^{1}H NMR, ^{13}C NMR and mass spectrometry, while purities were estimated by HPLC as cited in Experimental section. The successful synthesis of the cinnamoyl derivatives **6** was verified by the presence of the ^{1}H NMR signals of the vinylic protons of the substituent.

The second series, the N1-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives **7a**–**e** and **8a**–**f**, was synthesised according to the pathway shown in Fig. 4. 3,4-Dihydro-3-methyl-2(1*H*)-quinazolinone was dissolved in N,N-dimethylformamide (DMF) and treated with sodium hydride at 0 °C. The reaction was stirred for 20 min, and an appropriate alkyl bromide or alkyl chloride was subsequently added. The reaction was stirred for a further 1 h at 0 °C, and after completion was partitioned

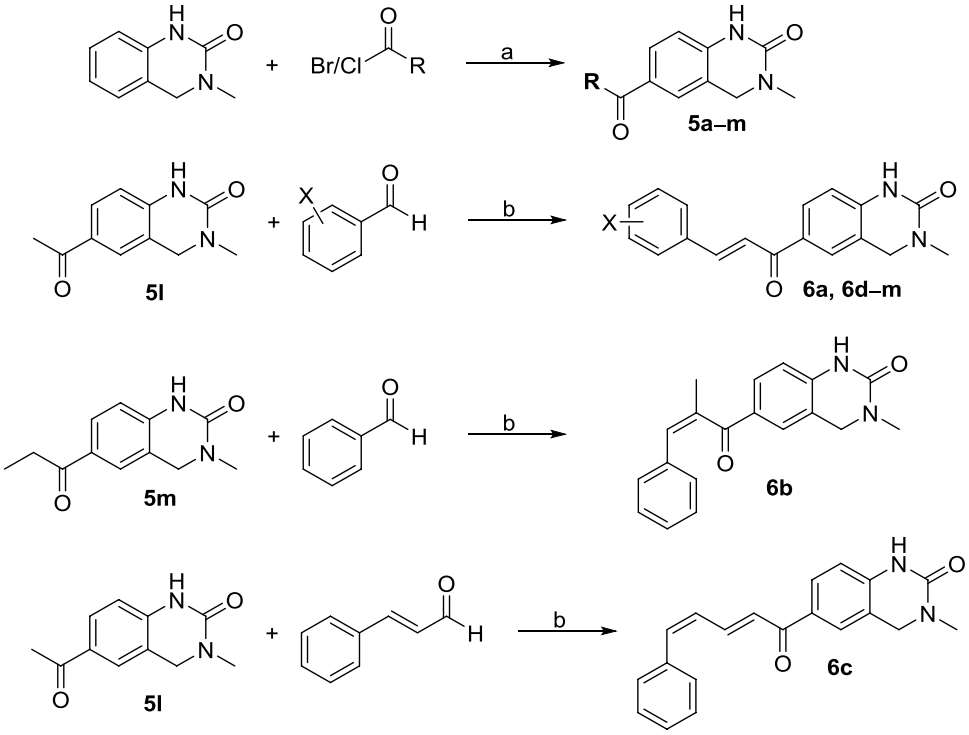


Fig. 3 Synthesis of C6-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives **5** and **6**. Key **a** $AlCl_3$, CS_2, reflux, 24 h; **b** HCl (32%)/methanol, reflux, 24–48 h

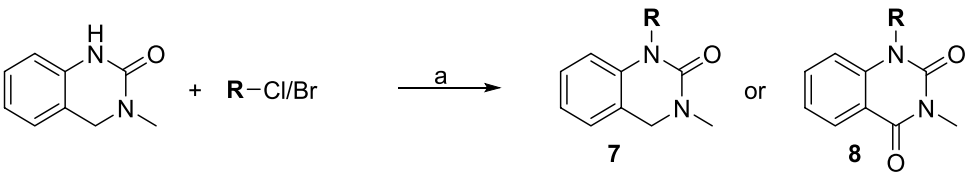


Fig. 4 Synthesis of N1-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives **7** and **8**. Key **a** NaH, DMF, 0 °C

between water and ethyl acetate. The organic phase was dried over $MgSO_4$ and then concentrated *in vacuo*, and the resulting residue was recrystallised from ethanol, ethyl acetate or a mixture of petroleum ether and ethyl acetate/ethanol. Compounds **7b**, **7c**, **7e**, **8a** and **8c** were purified by column chromatography. The 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives were obtained in yields of 10–67%. The structures were verified, as cited in Experimental section, by ^{1}H NMR, ^{13}C NMR and mass spectrometry, and purity was estimated by HPLC. Interestingly, this procedure yielded both the 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives **7a**–**e** and the 3-methyl-3,4-dihydroquinazolin-2(1*H*,3*H*)-diones, compounds **8a**–**f**. For **7a**–**e**, ^{1}H NMR spectra exhibit a singlet (2H) at approximately 4.5 ppm for the methylene protons at position 4, which was absent for compound **8a**–**f**. Furthermore, in the ^{13}C NMR spectra of **8a**–**f**, an additional carbonyl carbon signal at approximately 162 ppm was observed.

IC_{50} values for the inhibition of MAO

The IC_{50} values for the inhibition of MAO-A and MAO-B were determined by employing the human recombinant enzymes [28, 29]. Kynuramine, a nonspecific MAO substrate, was used to measure the catalytic rates of both enzyme isoforms. Kynuramine is non-fluorescent, and after MAO-catalysed oxidative deamination yields 4-hydroxyquinoline, a metabolite which fluoresces in alkaline media. The rate of kynuramine oxidation was thus determined by measuring the production of 4-hydroxyquinoline by fluorescence spectrophotometry ($\lambda_{ex} = 310$, $\lambda_{em} = 400$) at the endpoint of the enzyme reaction. The enzyme reactions contained substrate (50 μM) and test inhibitor (0.003–100 μM) in potassium phosphate buffer (pH 7.4, 100 μM). After the reactions were initiated with the addition of the enzyme, the reactions were incubated for 20 min at 37 °C and terminated with the addition of sodium hydroxide (2 N). Control reactions were conducted in the absence of inhibitor. IC_{50} values were estimated by constructing sigmoidal plots of enzyme catalytic rate versus the logarithm of inhibitor concentration. Examples of such plots are shown in Fig. 5.

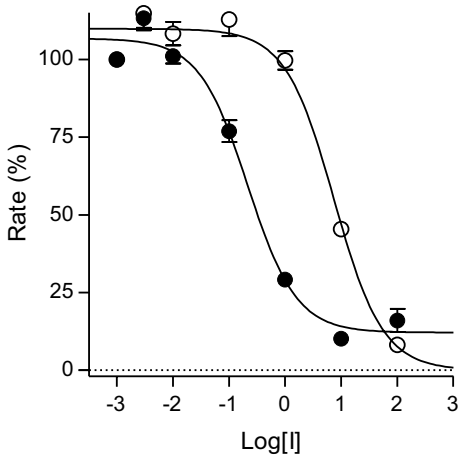
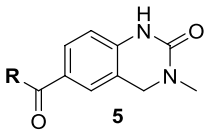
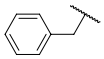
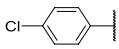
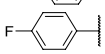
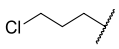


Fig. 5 Sigmoidal plots for the inhibition of MAO-A and MAO-B by **6b** (open circles) and **6c** (filled circles), respectively

The IC_{50} values for the inhibition of the human MAOs by 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives are given in Tables 1, 2, 3 and 4. From the results, it is evident that the 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives **5**–**8** are weak MAO-A inhibitors with $IC_{50} > 7.43$ µM. Only three compounds (e.g. **6b**, **6g** and **6h**) possess $IC_{50} < 15$ µM for the inhibition of MAO-A. Furthermore, with the exception of **5c**, **5j** and **7a–d,** none of the compounds evaluated are specific for the MAO-A isoform. These compounds are, however, very weak inhibitors of MAO-A. While no clear trend exists for the inhibition of MAO-A, it is noteworthy that none of the 3-methyl-3,4-dihydroquinazolin-2(1*H*,3*H*)-diones (**8a**–**f**) exhibited inhibition, even at a maximal tested concentration of 100 µM. Furthermore, the three most potent MAO-A inhibitors, **6b**, **6g** and **6h,** are all cinnamoyl-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives.

Table 1 IC_{50} values for the inhibition of recombinant human MAO-A and MAO-B by 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives **5**



	R	IC_{50} (µM)[a]		SI[b]
		MAO A	MAO B	
5a		51.7 ± 3.70	30.8 ± 0.638	1.7
5b		50.9 ± 14.3	36.8 ± 1.334	1.4
5c		86.7 ± 4.62	No inh[c]	–
5d		24.3 ± 2.84	2.22 ± 0.409	10.9
5e		54.9 ± 27.2	27.2 ± 3.104	2.0
5f		71.6 ± 12.1	20.4 ± 1.410	3.5
5g		50.0 ± 4.774	9.66 ± 0.897	5.2
5h		No inh[c]	No inh[c]	–
5i		No inh[c]	No inh[c]	–
5j		86.2 ± 16.2	No inh[c]	–
5k		No inh[c]	No inh[c]	–
5l		No inh[c]	No inh[c]	–
5m		No inh[c]	No inh[c]	–
Toloxatone		3.92[d]	–	–
Lazabemide		–	0.091[d]	–

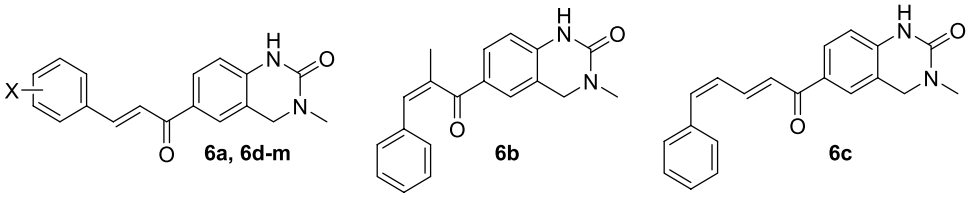
[a]All values are expressed as the mean ± standard deviation (SD) of triplicate determinations

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

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

[d]Value obtained from literature [34]

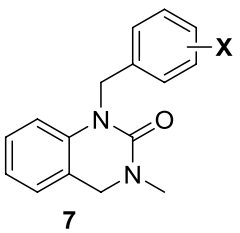
Table 2 IC_{50} values for the inhibition of recombinant human MAO-A and MAO-B by 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives **6**



	X	IC_{50} (μM)[a]		SI[b]
		MAO A	MAO B	
6a	H	No inh[c]	1.06 ± 0.066	–
6b	–	7.43 ± 0.178	2.54 ± 0.103	2.9
6c	–	24.7 ± 2.36	0.269 ± 0.071	91.8
6d	4-OCH_3	No inh[c]	3.72 ± 0.044	–
6e	4-OH	24.6 ± 3.80	3.16 ± 0.460	7.8
6f	4-Cl	78.1 ± 9.16	0.607 ± 0.034	128.7
6g	4-CN	8.86 ± 0.532	1.23 ± 0.289	7.2
6h	4-CF_3	11.1 ± 1.63	0.406 ± 0.024	27.3
6i	3-Cl	44.2 ± 7.10	0.350 ± 0.030	126.3
6j	2-Cl	52.6 ± 2.93	0.416 ± 0.029	126.4
6k	4-F	No inh[c]	0.446 ± 0.082	–
6l	4-Br	21.8 ± 0.566	1.01 ± 0.101	21.6
6m	3-F	No inh[c]	0.513 ± 0.031	–

See Table 1 for footnotes

Table 3 IC_{50} values for the inhibition of recombinant human MAO-A and MAO-B by 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives **7**



7

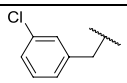
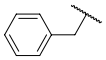
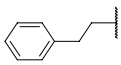
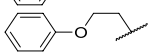
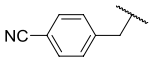
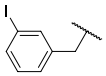
	X	IC_{50} (μM)[a]		SI[b]
		MAO A	MAO B	
7a	4-Cl	86.7 ± 4.97	No inh[c]	–
7b	3-Br	50.2 ± 7.22	No inh[c]	–
7c	4-Br	47.9 ± 3.17	No inh[c]	–
7d	4-I	55.8 ± 5.12	No inh[c]	–
7e	4-CF_3	No inh[c]	No inh[c]	–

See Table 1 for footnotes

In general, the 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives are more potent MAO-B inhibitors. Among the 37 compounds synthesised, seven displayed $IC_{50} < 1$ μM for the inhibition of MAO-B. The most potent MAO-B inhibitor (**6c**) possesses an IC_{50} value of 0.269 μM. Good-potency MAO inhibition was only observed among C6-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives, specifically among the cinnamoyl-substituted compounds **6**, which all display IC_{50} values < 3.72 μM. Among the benzoyl/acyl-substituted derivatives **5**, only two compounds (**5d** and **5g**) possess $IC_{50} < 10$ μM of the inhibition of MAO-B. With N1-substitution, no MAO-B inhibition was observed, even at a maximal tested concentration of 100 μM.

The following structure–activity relationships (SARs) for MAO inhibition were observed. Substitution with chlorine in the *para* and fluorine in *meta* positions of the benzoyl moiety led to more potent MAO-B inhibitors (**5d** and **5g**) among the 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives **5**. The position of substituent is important since the *para* (**5e**) and *ortho* (**5f**) fluorine-substituted derivatives are lower potency MAO-B inhibitors. Replacement of the benzoyl phenyl ring (**5a**) with the cyclohexyl (**5c**) led to the loss of MAO-B inhibition activity, suggesting that the aromatic ring is essential for activity. This is supported by

Table 4 IC_{50} values for the inhibition of recombinant human MAO-A and MAO-B by 3-methyl-3,4-dihydroquinazolin-2,4(1*H*,3*H*)-diones derivatives **8**

	R	IC_{50} (μM)[a]		
		MAO A	MAO B	SI[b]
8a		No inh[c]	No inh[c]	–
8b		No inh[c]	No inh[c]	–
8c		No inh[c]	No inh[c]	–
8d		No inh[c]	No inh[c]	–
8e		No inh[c]	No inh[c]	–
8f		No inh[c]	No inh[c]	–

See Table 1 for footnotes

the observation that the compounds bearing non-aromatic alkyl or alkyl halide substituents, **5i–m,** are also devoid of MAO-B inhibition activity. Compounds substituted with heteroaromatic groups, such as furanyl, (**5h**), also do not inhibit MAO-B which indicates that heteroaromatic groups are less appropriate than the phenyl. Interestingly, phenyl- and benzyl-containing compounds (**5a**) (IC_{50} = 51.7 μM) and **5b** (IC_{50} = 50.9 μM) possess similar MAO-A inhibition potencies, while a reduction and loss of MAO-A inhibition were observed when the phenyl group was replaced with the cyclohexyl (**5c**) and furanyl (**5h**) groups, respectively.

In contrast to the benzoyl-substituted derivative **5a** (IC_{50} = 30.8 μM), cinnamoyl substitution to yield **6a** (IC_{50} = 1.06 μM) resulted in more potent MAO-B inhibition. Compound **6c** (IC_{50} = 0.269 μM), in turn, is approximately fourfold more potent than compound **6a**, which shows that extension of conjugation (by replacing the styryl group with a phenylbutadienyl) further enhances MAO-B inhibition activity. Recently, it has been reported that compounds containing α, β, γ, δ-diunsaturated ketones, such as cinnamylidene acetophenones, display antioxidant and anti-inflammatory activities [30]. Compound **6c** may thus be a candidate compound that displays multiple biological activities relevant to the treatment of Parkinson's disease including MAO-B inhibition, antioxidant and anti-inflammatory activities. MAO inhibitors with antioxidant and anti-inflammatory properties would be beneficial in the treatment of Parkinson's disease, since inflammation and oxidative stress have been linked to the pathogenesis of Parkinson's disease [31]. Substitution with halogens and alkyl groups (e.g. **6f–m**) on the cinnamoyl phenyl ring leads to improved MAO-B inhibition activity compared to substitution with a polar hydroxyl group (e.g. **6e**). Compound **6a** is devoid MAO-A inhibition activity; however, extension of conjugation (e.g. **6c**) results in a compound with MAO-A inhibition, although weak. Substitution on the cinnamoyl phenyl ring of **6a** with the *para* nitrile (**6g**) and trifluoromethyl (**6h**) groups yields the second and third most potent MAO-A inhibitors of the study. Substitution with a smaller halogen (fluorine) on the cinnamoyl phenyl ring produced compounds which lacked MAO-A inhibition activity (**6k** and **6m**), although substitution with bromine and chlorine produced active MAO-A inhibitors (**6f, 6i, 6j** and **6l**). Interestingly, compound **6e**, the hydroxy-substituted compound, was active as a MAO-A inhibitor (IC_{50} = 24.6 μM), although the methoxy-substituted homologue (**6d**) was found to be inactive. For compounds **6**, in general, the electron-withdrawing halogen substituents yielded more potent MAO-B inhibition compared to electron-releasing substituents (OCH_3 and OH).

Although the N1-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives (**7a–d**) are weak MAO-A inhibitors, they exhibited specificity for the MAO-A isoform. In contrast, the 3-methyl-3,4-dihydroquinazolin-2,4(1*H*,3*H*)-diones (**8a–f**) were found to be inactive against both MAO isoforms. For the 3-methyl-3,4-dihydroquinazolin-2,4(1*H*,3*H*)-diones, a number of structural modifications were explored; however, no MAO inhibition was observed. It may thus be concluded that 3-methyl-3,4-dihydroquinazolin-2,4(1*H*,3*H*)-diones are not suitable for the design of MAO inhibitors. Among the N-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives, substitution with halogens at the appropriate position on the benzyl

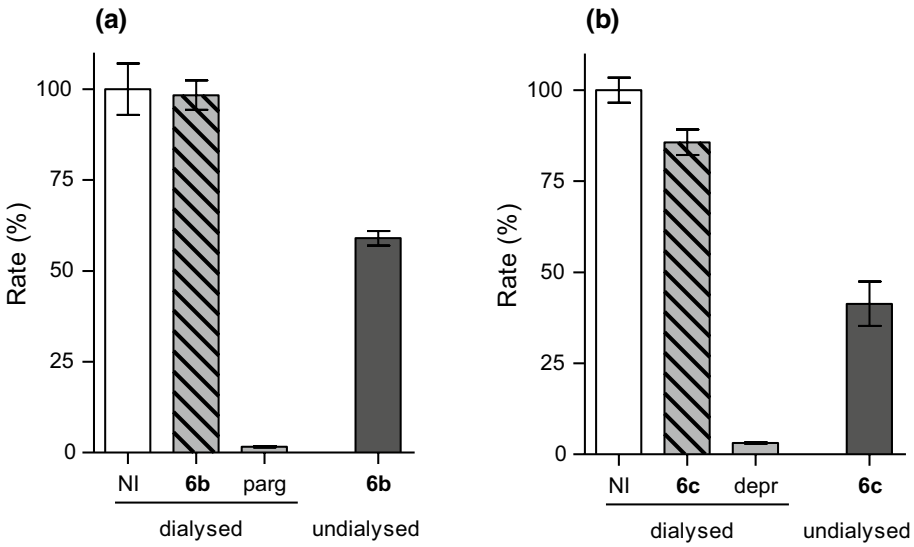


Fig. 6 Reversibility of inhibition of MAO-A and MAO-B by compounds **6b** and **6c**, respectively. MAO-A was pre-incubated in the absence of inhibitor and presence of **6b** and pargyline (**a**), and MAO-B was pre-incubated in the absence of inhibitor and presence of **6c** and (*R*)-deprenyl (**b**). After dialysis, the residual enzyme activities were measured. For comparison, the activities of undialysed mixtures of the MAOs and the test inhibitors were also measured

moiety produced compounds with MAO-A inhibition activity (**7a**–**d**), with compound **7c** displaying the highest inhibition potency (IC_{50} = 47.9 μM). Interestingly, the MAO-A inhibition potencies of the bromine-substituted compounds (**7b** and **7c**) were within experimental error identical, which shows that the position (*para* versus *meta*) is not an important consideration.

Reversibility of MAO inhibition

As mentioned, reversibility of MAO inhibition, especially of the MAO-A isoform, is an important consideration since irreversible MAO-A inhibitors may lead to a potentially fatal hypertensive crisis when combined with certain food [11]. Furthermore, compared to reversible inhibition, irreversible inhibitors also may have the disadvantage of slow and variable rates of enzyme recovery after drug withdrawal [32, 33]. For these reasons, we have set out to investigate the reversibility of MAO inhibition by selecting the 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives. Compounds **6b** and **6c** were selected to investigate the reversibility of MAO-A and MAO-B inhibition, respectively. Since these compounds do not possess functional groups [e.g. propargylamine, hydrazine, cyclopropylamine, haloallylamine or N-(2-aminoethyl)carboxamide] that are associated with irreversible MAO inhibition, it is expected that **6b** and **6c** would act as reversible inhibitors. Dialysis was used to investigate the reversibility of inhibition. Compounds **6b** and **6c**, at concentrations of 4 × IC_{50}, were combined with the MAO enzyme and pre-incubated for 20 min followed by dialysis for 24 h. Following dialysis, the reactions were diluted twofold with the addition of kynuramine to yield an inhibitor concentration of 2 × IC_{50}. The residual enzyme activities were measured and are presented graphically (Fig. 6). Similar dialysis experiments in the presence of the irreversible inhibitors, pargyline and (*R*)-deprenyl, served as positive controls, while dialysis of the enzymes in the absence of inhibitor served as negative control. For comparison, the MAO activities of undialysed mixtures of the MAOs and the test inhibitors were also measured.

For the negative control (absence of the inhibitor), the residual activity was set to 100%. As shown by the results, **6b** and **6c** are reversible MAO inhibitors since dialysis restores MAO-A and MAO-B activity to 98% and 86%, respectively. Inhibition, however, persists in undialysed mixtures of MAO-A and MAO-B with these test inhibitors, with the residual activity at 59% and 41%, respectively. Dialysis of the mixtures containing the MAOs and the irreversible inhibitors, pargyline and (*R*)-deprenyl, does not restore activity with the residual activities at 1.6% and 3.1%, respectively.

Mode of inhibition

Lineweaver–Burk plots were constructed to investigate the modes of MAO-A and MAO-B inhibition by **6b** and **6c**, respectively. MAO activity were measured at eight different kynuramine concentrations (15–250 μM), in the absence and presence of five different concentrations of the inhibitors. As shown in Fig. 7, the Lineweaver–Burk plots for the inhibition of both MAO-A and MAO-B are linear and have common y-intercepts. This is typical for competitive inhibition, and from these data, it may be concluded that **6b** and **6c** are competitive inhibitors of the MAO isozymes, respectively. This further supports the finding of the dialysis studies that the inhibition is reversible. From replots of the slopes of the Lineweaver–Burk plots versus inhibitor concentration, K_i values of 6.9 and 0.16 μM for the inhibition of MAO-A and MAO-B by **6b** and **6c**, respectively, are estimated.

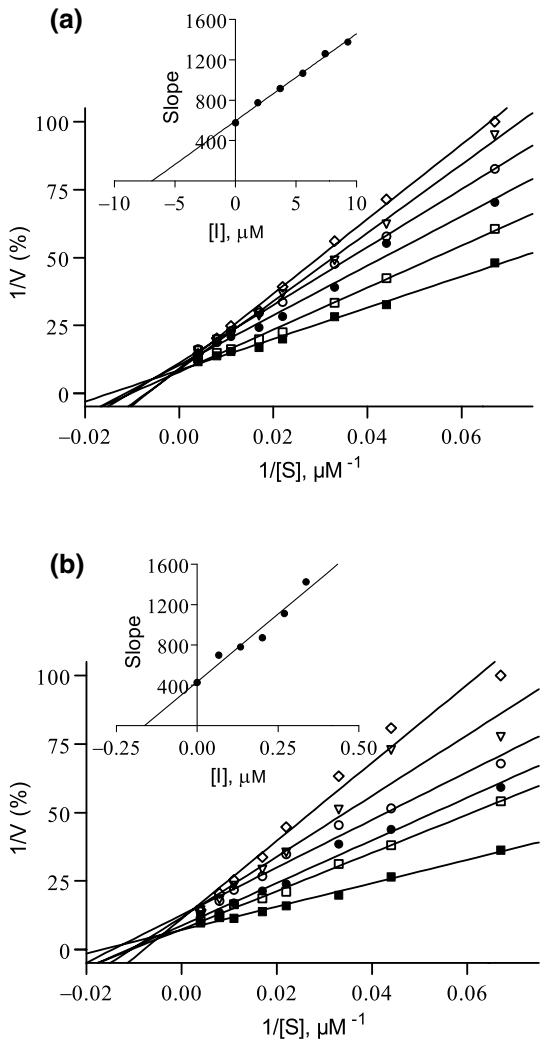


Fig. 7 Lineweaver–Burk plots of human MAO-A and MAO-B catalytic activities in the absence (filled squares) and presence of various concentrations of **6b** (**a**) and **6c** (**b**), respectively. For MAO-A, the concentrations of **6b** were 1.86 μM (open squares), 3.72 μM (filled circles), 5.58 μM (open circles), 7.43 μM (triangles) and 9.29 μM (diamonds). For the studies with MAO-B, the concentrations of **6c** were 0.067 μM (open squares), 0.134 μM (filed circles), 0.202 μM (open circles), 0.269 μM (triangles) and 0.336 μM (diamonds)

Conclusions

The present study shows that a number of compounds among a series of C6-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives act as reversible and specific MAO-B inhibitors. Such compounds are thus suitable leads for the development of selective MAO-B inhibitors that may find application in neurodegenerative disorders such as Parkinson's disease. The N1-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives (**7**) proved to be MAO-A-specific inhibitors, although the inhibition potencies were weak. An interesting SAR for MAO-B inhibition by the C6-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives is the increase in potency when the conjugation is extended by replacing the cinnamyl group with a cinnamylidene group. In this respect, the cinnamylidene derivative **6c** is the most potent MAO-B inhibitor of the series. Some derivatives also exhibited MAO-A inhibition, with **6b** in particular displaying an IC_{50} value of 7.43 μM. This potency is comparable to reference inhibitors such as toloxatone, which is reported to inhibit MAO-A with an IC_{50} value of 3.92 μM [34]. Since dopamine is metabolised by both MAO isoforms, nonspecific MAO inhibitors such as **6b** may be considered useful for the therapy of Parkinson's disease. The present study also shows that the 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one class of compounds are reversible inhibitors of both MAO-A and MAO-B. This is significant since reversible MAO inhibitors are less likely to cause tyramine-induced hypertension than irreversible MAO inhibitors. Based on these findings, it may be concluded that C6-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives are promising MAO-B inhibitors for the treatment of Parkinson's disease, while nonspecific MAO inhibitors may also find application in the treatment of depression, which often is a comorbid condition of Parkinson's disease.

Experimental section

Chemicals and instrumentation

Unless otherwise specified, all chemicals were obtained from Sigma-Aldrich and were used without further purification. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded in deuterated dimethyl sulfoxide (DMSO-*d*6) or deuterochloroform ($CDCl_3$) with a Bruker Avance III 600 spectrometer at frequencies of 600 MHz and 151 MHz, respectively. MestReNova was used to process and analyse NMR data. ^{1}H NMR data are reported by providing the chemical shift (δ), the integration and multiplicity of the signals. The chemical shifts (*δ*) are given in parts per million (ppm) and the coupling constants (*J*) in Hz. Spin multiplicities are given as singlet (s), doublet (d), doublet of doublets (dd), doublet of doublet of doublets (ddd), triplet (t), triplet of doublet (td) and multiplet (m). High-resolution mass spectra (HRMS) were recorded on a Bruker micrOTOF-QII mass spectrometer in atmospheric-pressure chemical ionisation (APCI) mode. Melting points (mp) were determined with a Buchi B-545 melting point apparatus and are uncorrected. Thin-layer chromatography (TLC) was carried out to monitor the progress of reactions. Silica gel 60 (Merck) F_{254} sheets were used for TLC, and the developed sheets were visualised under a UV254 light or by staining with iodine vapour. A Varian Cary Eclipse instrument was employed for fluorescence spectrophotometry. For the enzymology,

microsomes from insect cells containing recombinant human MAO-A and MAO-B (5 mg protein/mL) and kynuramine dihydrobromide were obtained from Sigma-Aldrich.

General procedure for synthesis of 5a–m

Friedel–Crafts acylation was employed to synthesise the C6-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives. Aluminium trichloride (4.62 mmol), 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (3.08 mmol) and the appropriate acyl chloride or bromide (3.08 mmol) were dissolved in carbon disulphide (6 mL). The reaction was heated under reflux (50 °C) for 24 h. The progress of the reaction was monitored by silica gel TLC with ethyl acetate as mobile phase. On completion of the reaction, 30 mL of a mixture of ice and water was added. The precipitate that formed was collected by filtration and dried in a convection oven overnight. The crude product was purified by recrystallisation from a suitable solvent (ethanol).

6-Benzoyl-3-methyl-3,4-dihydroquinazolin-2(1H)-one (5a) The title compound was prepared in a yield of 39%: mp 235.6–258.4 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.69 (s, 1H), 7.66 (d, J = 7.6 Hz, 2H), 7.63 (t, J = 7.4 Hz, 1H), 7.57 (d, J = 8.3 Hz, 1H), 7.54–7.51 (m, 3H), 6.87 (d, J = 8.3 Hz, 1H), 4.46 (s, 2H), 2.85 (d, J = 11.5 Hz, 3H). ^{13}C NMR (151 MHz, DMSO) δ 194.18, 153.08, 142.20, 137.78, 132.02, 130.72, 129.47, 129.25, 128.44, 127.93, 117.72, 112.94, 49.41, 33.88; APCI-HRMS *m/z* calcd for $C_{16}H_{14}N_2O_2$ (MH^+), 267.1128, found 267.1124. Purity (HPLC): 96%.

3-Methyl-6-(2-phenylacetyl)-3,4-dihydroquinazolin-2(1H)-one (5b) The title compound was prepared in a yield of 53%: mp 259.4–259.5 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.66 (s, 1H), 7.88 (dd, J = 8.4, 1.6 Hz, 1H), 7.82 (s, 1H), 7.30 (t, J = 7.5 Hz, 2H), 7.26 (d, J = 7.1 Hz, 2H), 7.23 (d, J = 7.2 Hz, 1H), 6.85 (d, J = 8.4 Hz, 1H), 4.47 (s, 2H), 4.27 (s, 2H), 2.87 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 196.25, 153.49, 142.81, 135.94, 130.07, 129.83, 129.75, 128.77, 126.99, 126.87, 118.18, 113.48, 49.96, 44.71, 34.37; APCI-HRMS *m/z* calcd for $C_{17}H_{17}N_2O_2$ (MH^+), 281.1285, found 281.1285. Purity (HPLC): 92.8%.

6-(Cyclohexanecarbonyl)-3-methyl-3,4-dihydroquinazolin-2(1H)-one (5c) The title compound was prepared in a yield of 39%: mp 207.3–242.2 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.62 (s, 1H), 7.78 (dd, J = 8.4, 1.6 Hz, 1H), 7.73 (s, 1H), 6.84 (d, J = 8.4 Hz, 1H), 4.47 (s, 2H), 3.29 (dd, J = 12.6, 9.7 Hz, 1H), 2.87 (s, 3H), 1.80–1.70 (m, 4H), 1.67 (d, J = 12.8 Hz, 1H), 1.36 (ddd, J = 23.4, 17.3, 10.8 Hz, 4H), 1.18 (d, J = 12.6 Hz, 1H). ^{13}C NMR (151 MHz, DMSO) δ 201.74, 153.56, 142.56, 129.28, 129.22, 126.69, 118.23, 113.54, 49.99, 44.41, 34.37, 29.70, 26.10, 25.69; APCI-HRMS *m/z* calcd $C_{16}H_{21}N_2O_2$ (MH^+), 273.1597, found 273.1583. Purity (HPLC): 98.8%.

6-(4-Chlorobenzoyl)-3-methyl-3,4-dihydroquinazolin-2(1H)-one (5d) The title compound was prepared in a yield of 69%: mp 236.3–242.1 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.71 (s, 1H), 7.71–7.64 (m, 2H), 7.62–7.52 (m, 4H), 6.87 (d, J = 8.3 Hz, 1H), 4.46 (s, 2H), 2.86 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 193.04, 153.04, 142.39, 136.87, 136.47, 131.14, 130.73, 129.13, 128.56, 127.97, 117.78, 112.99, 49.39, 33.88; APCI-HRMS *m/z* calcd for $C_{16}H_{14}ClN_2O_2$ (MH^+), 301.0738, found 301.0756. Purity (HPLC): 99.4%.

6-(4-Fluorobenzoyl)-3-methyl-3,4-dihydroquinazolin-2(1H)-one (5e) The title compound was prepared in a yield of 64%: mp 233.6–236.2 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.69 (s, 1H), 7.81–7.68 (m, 2H), 7.58–7.51 (m, 2H), 7.40–7.28 (m, 2H), 6.87 (d, J = 8.3 Hz, 1H), 4.46 (s, 2H), 2.85 (d, J = 12.4 Hz, 3H). ^{13}C NMR (151 MHz, DMSO) δ 192.85, 165.12, 163.46, 153.08, 142.24, 134.30, 132.18, 132.11, 130.68, 129.38, 127.91, 117.76, 115.57, 115.42, 112.96, 49.41, 33.89; APCI-HRMS *m/z* calcd for $C_{16}H_{14}FN_2O_2$ (MH^+), 285.1033, found 285.1059. Purity (HPLC): 98.9%.

6-(2-Fluorobenzoyl)-3-methyl-3,4-dihydroquinazolin-2(1H)-one (5f) The title compound was prepared in a yield of 52%: mp 268.3–268.5 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.74 (s, 1H), 7.62 (d, J = 8.3 Hz, 1H), 7.58–7.51 (m, 2H), 7.49 (dd, J = 7.4, 1.7 Hz, 1H), 7.38–7.31 (m, 2H), 6.86 (d, J = 8.4 Hz, 1H), 4.45 (s, 2H), 2.84 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 190.81, 159.72, 158.08, 152.93, 143.11, 132.94, 132.89, 130.55, 130.06, 130.04, 129.50, 127.57, 127.05, 126.95, 124.74, 124.72, 117.95, 116.23, 116.09, 113.19, 49.30, 33.86; APCI-HRMS *m/z* calcd for $C_{16}H_{14}FN_2O_2$ (MH^+), 285.1033, found 285.1021. Purity (HPLC): 99.2%.

6-(3-Fluorobenzoyl)-3-methyl-3,4-dihydroquinazolin-2(1H)-one (5g) The title compound was prepared in a yield of 81%: mp 243.9–354.9 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.72 (s, 1H), 7.58 (ddd, J = 13.7, 8.0, 4.6 Hz, 3H), 7.52–7.42 (m, 3H), 6.88 (d, J = 8.3 Hz, 1H), 4.47 (s, 2H), 2.86 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 192.78, 162.62, 160.99, 153.01, 142.52, 140.12, 140.08, 130.83, 130.67, 130.62, 128.92, 128.03, 125.41, 118.91, 118.77, 117.82, 115.74, 115.59, 113.03, 49.37, 33.87; APCI-HRMS *m/z* calcd for $C_{16}H_{14}FN_2O_2$ (MH^+), 285.1033, found 285.1017. Purity (HPLC): 99.0%.

6-(Furan-2-carbonyl)-3-methyl-3,4-dihydroquinazolin-2(1H)-one (5h) The title compound was prepared in

a yield of 100%: mp 244.8–248.2 ^{1}H NMR (600 MHz, DMSO) δ 9.72 (s, 1H), 8.09 (dd, *J* = 1.7, 0.7 Hz, 1H), 7.79 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.74 (s, 1H), 7.39 (d, *J* = 3.5 Hz, 1H), 6.92 (d, *J* = 8.4 Hz, 1H), 6.78 (dd, *J* = 3.6, 1.7 Hz, 1H), 4.51 (s, 2H), 2.88 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 180.24, 153.54, 151.98, 148.40, 142.68, 130.29, 129.84, 127.68, 120.74, 118.25, 113.57, 112.99, 49.95, 34.39; APCI-HRMS *m/z* calcd for $C_{14}H_{13}N_2O_3$ (MH^+), 257.0920, found 257.0902. Purity (HPLC): 99.3%.

6-(2-Chloroacetyl)-3-methyl-3,4-dihydroquinazolin-2(1H)-one (5i) The title compound was prepared in a yield of 81%: mp 249.8–252.1 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.72 (s, 1H), 7.80 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.76 (s, 1H), 6.85 (d, *J* = 8.4 Hz, 1H), 5.07 (s, 2H), 4.47 (s, 2H), 2.88 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 190.11, 153.39, 143.35, 129.70, 127.66, 126.97, 118.27, 113.53, 49.87, 47.60, 34.37; APCI-HRMS *m/z* calcd for $C_{11}H_{12}ClN_2O_2$ (MH^+), 239.0581, found 239.0584. Purity (HPLC): 99.4%.

6-(3-Chloropropanoyl)-3-methyl-3,4-dihydroquinazolin-2(1H)-one (5j) The title compound was prepared in a yield of 79%: mp 171.7–171.8 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.68 (s, 1H), 7.80 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.76 (s, 1H), 6.84 (d, *J* = 8.4 Hz, 1H), 4.47 (s, 2H), 3.91 (t, *J* = 6.3 Hz, 2H), 3.44 (t, *J* = 6.3 Hz, 2H), 2.88 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 195.36, 153.47, 142.99, 129.84, 129.26, 126.65, 118.15, 113.50, 49.93, 34.38; APCI-HRMS *m/z* calcd for $C_{12}H_{14}ClN_2O_2$ (MH^+), 253.0738, found 253.0736. Purity (HPLC): 96.3%.

6-(4-Chlorobutanoyl)-3-methyl-3,4-dihydroquinazolin-2(1H)-one (5k) The title compound was prepared in a yield of 57%: mp 180.8–238.3 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.65 (s, 1H), 7.79 (dd, *J* = 8.4, 1.8 Hz, 1H), 7.74 (s, 1H), 6.84 (d, *J* = 8.4 Hz, 1H), 4.47 (s, 2H), 3.70 (t, *J* = 6.7 Hz, 2H), 3.08 (t, *J* = 7.1 Hz, 2H), 2.87 (s, 3H), 2.08–2.02 (m, 2H). ^{13}C NMR (151 MHz, DMSO) δ 197.46, 153.52, 142.76, 130.03, 129.06, 126.47, 118.11, 113.47, 49.95, 45.44, 35.13, 34.37, 27.47; APCI-HRMS *m/z* calcd for $C_{13}H_{16}ClN_2O_2$ (MH^+), 267.0894, found 267.0896. Purity (HPLC): 94.5%.

6-Acetyl-3-methyl-3,4-dihydroquinazolin-2(1H)-one (5l) The title compound was prepared in a yield of 76%: mp 213.3–224.8 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.65 (s, 1H), 7.77 (dd, *J* = 8.3, 1.9 Hz, 1H), 7.72 (s, 1H), 6.83 (d, *J* = 8.3 Hz, 1H), 4.47 (s, 2H), 2.87 (s, 3H), 2.48 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 196.47, 153.53, 142.69, 130.48, 129.39, 126.74, 118.03, 113.40, 49.95, 34.37, 26.77; APCI-HRMS *m/z* calcd for $C_{11}H_{13}N_2O_2$ (MH^+), 205.0971, found 205.0973. Purity (HPLC): 98.9%.

3-Methyl-6-propionyl-3,4-dihydroquinazolin-2(1H)-one (5m) The title compound was prepared in a yield of 80%: mp 301.0–312.6 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.63 (s, 1H), 7.78 (d, *J* = 8.4 Hz, 1H), 7.73 (s, 1H), 6.83 (d, *J* = 8.4 Hz, 1H), 4.47 (s, 2H), 2.94 (q, *J* = 7.2 Hz, 2H), 2.87 (s, 3H), 1.06 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, DMSO) δ 199.03, 153.57, 142.54, 130.16, 128.99, 126.37, 118.05, 113.42, 49.98, 34.37, 31.17, 8.83; APCI-HRMS *m/z* calcd for $C_{12}H_{15}N_2O_2$ (MH^+), 219.1128, found 219.1137. Purity (HPLC): 99.1%.

General procedure for preparation of compounds 6a–m

6-Acetyl-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (**5l**) for the synthesis of **6a** and **6c**–**m** or 3-methyl-6-propionyl-3,4-dihydroquinazolin-2(1*H*)-one (**5m**) (1.375 mmol) for the synthesis of **6b** and the appropriate benzaldehyde (or cinnamaldehyde for **6c**) (1.375 mmol) were dissolved in a mixture of hydrochloric acid (32%; 5.5 mL) and methanol (3.67 mL). The reaction was heated under reflux for 24 to 48 h, and the progress was monitored by using silica gel TLC with ethyl acetate as the mobile phase. Upon completion, 30 mL of a mixture of ice and water was added to the reaction. The residue that formed was collected by filtration and dried in a convection oven overnight. The crude product was purified by recrystallisation from ethanol.

6-Cinnamoyl-3-methyl-3,4-dihydroquinazolin-2(1H)-one (6a) The title compound was prepared in a yield of 40%: mp 249.9–250.0 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.70 (s, 1H), 8.04–7.97 (m, 2H), 7.95–7.85 (m, 3H), 7.71 (d, *J* = 15.6 Hz, 1H), 7.57–7.44 (m, 3H), 6.89 (d, *J* = 8.4 Hz, 1H), 4.52 (s, 2H), 2.90 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 187.45, 153.49, 143.50, 142.85, 135.32, 131.06, 130.91, 129.80, 129.38, 129.22, 127.28, 122.42, 118.28, 113.61, 50.03, 34.42; APCI-HRMS *m/z* calcd for $C_{18}H_{17}N_2O_2$ (MH^+), 293.1284, found 293.1281. Purity (HPLC): 91.9%.

(Z)-3-Methyl-6-(2-methyl-3-phenylacryloyl)-3,4-dihydroquinazolin-2(1H)-one (6b) The title compound was prepared in a yield of 17%: mp 122.8–122.9 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.64 (s, 1H), 7.61 (dd, *J* = 8.3, 1.8 Hz, 1H), 7.56 (s, 1H), 7.51 (d, *J* = 7.4 Hz, 2H), 7.45 (t, *J* = 7.7 Hz, 2H), 7.38 (d, *J* = 7.3 Hz, 1H), 7.05 (s, 1H), 6.87 (d, *J* = 8.3 Hz, 1H), 4.49 (s, 2H), 2.87 (s, 3H), 2.15 (d, *J* = 1.3 Hz, 3H). ^{13}C NMR (151 MHz, DMSO) δ 197.38, 153.64, 142.16, 139.32, 136.65, 135.95, 130.74, 130.55, 130.12, 128.97, 128.92, 128.04, 118.09, 113.32, 49.96, 34.36, 15.34; APCI-HRMS *m/z* calcd for $C_{19}H_{19}N_2O_2$ (MH^+), 307.1441, found 307.1436. Purity (HPLC): 91.8%.

3-Methyl-6-((2E,4Z)-5-phenylpenta-2,4-dienoyl)-3,4-dihydroquinazolin-2(1H)-one (6c) The title compound was prepared in a yield of 68%: mp 243.7–247.2 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.70 (s, 1H), 7.87 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.83 (s, 1H), 7.60 (d, *J* = 7.3 Hz, 2H), 7.55–7.44 (m, 1H), 7.44–7.30 (m, 4H), 7.27–7.18 (m, 2H), 6.88 (d, *J* = 8.4 Hz, 1H), 4.51 (s, 2H), 2.89 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 187.48, 153.49, 143.92, 142.72, 141.64, 136.56, 131.14, 129.62, 129.43, 127.79, 127.68, 126.88, 125.86, 118.33, 113.62, 50.00, 34.40; APCI-HRMS *m/z* calcd for $C_{20}H_{19}N_2O_2$ (MH^+), 319.1441, found 319.1449. Purity (HPLC): 99.6%.

(E)-6-(3-(4-Methoxyphenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1H)-one (6d) The title compound was prepared in a yield of 47%: mp 220.3–331.9 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.70 (s, 1H), 8.03 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.98 (s, 1H), 7.91 (d, *J* = 15.6 Hz, 1H), 7.68 (d, *J* = 15.5 Hz, 1H), 7.48–7.40 (m, 2H), 7.37 (t, *J* = 7.9 Hz, 1H), 7.02 (dd, *J* = 8.1, 2.4 Hz, 1H), 6.90 (d, *J* = 8.4 Hz, 1H), 4.52 (s, 2H), 3.83 (s, 3H), 2.90 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 187.46, 160.12, 153.48, 143.50, 142.86, 136.72, 131.05, 130.39, 129.86, 127.26, 122.67, 121.94, 118.28, 116.84, 113.93, 113.59, 55.78, 50.04, 34.41; APCI-HRMS *m/z* calcd for $C_{19}H_{19}N_2O_3$ (MH^+), 323.1390, found 323.1389. Purity (HPLC): 96.4%.

(E)-6-(3-(4-Hydroxyphenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1H)-one (6e) The title compound was prepared in a yield of 16%: mp 290.9–294.8 °C. ^{1}H NMR (600 MHz, DMSO) δ 10.07 (s, 1H), 9.67 (s, 1H), 8.00–7.92 (m, 2H), 7.71 (dd, *J* = 12.0, 10.4 Hz, 3H), 7.64 (d, *J* = 15.4 Hz, 1H), 6.88 (d, *J* = 8.3 Hz, 1H), 6.84 (d, *J* = 8.6 Hz, 2H), 4.51 (s, 2H), 2.89 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 187.33, 160.41, 153.53, 143.95, 142.53, 131.44, 131.28, 129.54, 127.04, 126.42, 118.83, 118.19, 116.26, 113.54, 56.51, 50.06, 34.41, 19.06; APCI-HRMS *m/z* calcd for $C_{18}H_{17}N_2O_3$ (MH^+), 309.1233, found 308.1145. Purity (HPLC): 98.9%.

(E)-6-(3-(4-Chlorophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1H)-one (6f) The title compound was prepared in a yield of 42%: mp 244.3–256.6 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.70 (s, 1H), 8.02 (dd, *J* = 8.4, 1.8 Hz, 1H), 7.98 (s, 1H), 7.92 (t, *J* = 12.1 Hz, 3H), 7.69 (d, *J* = 15.6 Hz, 1H), 7.53 (d, *J* = 8.5 Hz, 2H), 6.89 (d, *J* = 8.4 Hz, 1H), 4.52 (s, 2H), 2.88 (d, *J* = 13.3 Hz, 3H). ^{13}C NMR (151 MHz, DMSO) δ 187.32, 153.46, 142.91, 142.02, 135.35, 134.31, 130.96, 130.91, 129.86, 129.41, 127.30, 123.16, 118.29, 113.60, 50.02, 34.41; APCI-HRMS *m/z* calcd for $C_{18}H_{16}ClN_2O_2$ (MH^+), 327.0894, found 327.0864. Purity (HPLC): 75.4%.

(E)-4-(3-(3-Methyl-2-oxo-1,2,3,4-tetrahydroquinazolin-6-yl)-3-oxoprop-1-en-1-yl)benzonitrile (6g) The title compound was prepared in a yield of 13%: mp 220.9–224.8 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.72 (s, 1H), 8.10–8.06 (m, 2H), 8.05–8.03 (m, 1H), 8.03–8.01 (m, 1H), 8.00–7.92 (m, 3H), 7.73 (d, *J* = 15.6 Hz, 1H), 6.89 (d, *J* = 8.4 Hz, 1H), 4.52 (s, 2H), 2.90 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 187.25, 153.42, 143.11, 141.28, 139.92, 133.19, 130.76, 130.02, 129.95, 129.78, 127.44, 125.74, 118.34, 113.64, 112.60, 50.01, 34.42; APCI-HRMS *m/z* calcd for $C_{19}H_{16}N_3O_2$ (MH^+), 318.1237, found 318.1250. Purity (HPLC): 76.5%.

(E)-3-Methyl-6-(3-(4-(trifluoromethyl)phenyl)acryloyl)-3,4-dihydroquinazolin-2(1H)-one (6h) The title compound was prepared in a yield of 26%: mp 216.9–252.0 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.72 (s, 1H), 8.09 (d, *J* = 8.2 Hz, 2H), 8.07–8.02 (m, 2H), 8.00 (s, 1H), 7.81 (d, *J* = 8.3 Hz, 2H), 7.75 (d, *J* = 15.6 Hz, 1H), 6.90 (d, *J* = 8.4 Hz, 1H), 4.52 (s, 2H), 2.90 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 187.30, 153.43, 143.06, 141.49, 139.37, 130.80, 130.45, 130.24, 129.98, 129.76, 127.39, 126.16, 126.14, 125.46, 125.12, 123.66, 118.33, 113.63, 50.01, 34.41; APCI-HRMS *m/z* calcd for $C_{19}H_{16}F_3N_2O_2$ (MH^+), 361.1158, found 361.1158. Purity (HPLC): 78.7%.

(E)-6-(3-(3-Chlorophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1H)-one (6i) The title compound was prepared in a yield of 49%: mp 257.7–257.9 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.70 (s, 1H), 8.08–8.02 (m, 2H), 7.99 (d, *J* = 15.4 Hz, 2H), 7.80 (d, *J* = 6.9 Hz, 1H), 7.68 (d, *J* = 15.6 Hz, 1H), 7.49 (t, *J* = 4.8 Hz, 2H), 6.89 (d, *J* = 8.4 Hz, 1H), 4.52 (s, 2H), 2.90 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 187.30, 153.45, 142.98, 141.78, 137.60, 134.27, 131.15, 130.90, 130.43, 129.95, 128.27, 128.24, 127.37, 123.96, 118.29, 113.61, 50.04, 34.42; APCI-HRMS *m/z* calcd for $C_{18}H_{16}ClN_2O_2$ (MH^+), 327.0894, found 327.0884. Purity (HPLC): 93.2%.

(E)-6-(3-(2-Chlorophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1H)-one (6j) The title compound was prepared in a yield of 56%: mp 257.6–265.1 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.72 (s, 1H), 8.19 (d, *J* = 6.6 Hz, 1H), 7.9–8.0 (m), 7.58 (d, *J* = 6.9 Hz, 1H), 7.47 (s, 2H), 6.90 (d, *J* = 8.2 Hz, 1H), 4.52 (s, 2H), 2.90 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 187.20, 153.44, 143.08, 138.06, 134.72, 132.94, 132.31, 130.77, 130.52, 129.98, 128.95, 128.14, 127.41, 125.22, 118.35, 113.65, 50.01, 34.42; APCI-HRMS *m/z* calcd for $C_{18}H_{16}ClN_2O_2$ (MH^+), 327.0894, found 327.0900. Purity (HPLC): 92.3%.

(E)-6-(3-(4-Fluorophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1H)-one (6k) The title compound was prepared in a yield of 27%: mp 241.5–253.3 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.69 (s, 1H), 8.01 (dd, *J* = 8.4, 1.9 Hz,

1H), 7.99–7.93 (m, 3H), 7.87 (d, $J = 15.6$ Hz, 1H), 7.71 (d, $J = 15.6$ Hz, 1H), 7.31 (t, $J = 8.8$ Hz, 2H), 6.89 (d, $J = 8.4$ Hz, 1H), 4.52 (s, 2H), 2.90 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 187.37, 164.60, 162.95, 153.47, 142.84, 142.26, 132.02, 132.00, 131.56, 131.51, 131.04, 129.81, 127.25, 122.33, 118.27, 116.46, 116.31, 113.59, 50.04, 34.41; APCI-HRMS *m/z* calcd for $C_{18}H_{16}FN_2O_2$ (MH^+), 311.1190, found 310.1112. Purity (HPLC): 95.4%.

(E)-6-(3-(4-Bromophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1H)-one (6l) The title compound was prepared in a yield of 15%: mp 260.3–265.0 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.70 (s, 1H), 8.01 (dd, $J = 8.4$, 1.8 Hz, 1H), 7.98 (s, 1H), 7.94 (d, $J = 15.6$ Hz, 1H), 7.84 (d, $J = 8.5$ Hz, 2H), 7.66 (d, $J = 8.3$ Hz, 3H), 6.89 (d, $J = 8.4$ Hz, 1H), 4.52 (s, 2H), 2.90 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 187.33, 153.46, 142.92, 142.12, 134.63, 132.33, 131.13, 130.96, 129.87, 127.31, 124.23, 123.21, 118.29, 113.61, 50.03, 34.42; APCI-HRMS *m/z* calcd for $C_{18}H_{16}BrN_2O_2$ (MH^+), 371.0389, found 372.0323. Purity (HPLC): 96.8%.

(E)-6-(3-(3-Fluorophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1H)-one (6m) The title compound was prepared in a yield of 23%: mp 317.3–336.2 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.70 (s, 1H), 8.03 (dd, $J = 8.4$, 1.9 Hz, 1H), 8.00–7.95 (m, 2H), 7.83 (d, $J = 10.1$ Hz, 1H), 7.72–7.65 (m, 2H), 7.50 (dd, $J = 7.9$, 1.7 Hz, 1H), 7.28 (d, $J = 2.4$ Hz, 1H), 6.89 (d, $J = 8.4$ Hz, 1H), 4.52 (s, 2H), 2.90 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 187.33, 163.79, 162.18, 153.45, 142.96, 142.02, 137.95, 137.89, 131.33, 131.28, 130.90, 129.91, 127.36, 125.93, 123.88, 118.28, 117.61, 117.47, 115.04, 114.89, 113.62, 50.03, 34.41; APCI-HRMS *m/z* calcd for $C_{18}H_{16}FN_2O_2$ (MH^+), 311.1190, found 311.1200. Purity (HPLC): 84.6%.

General synthesis of 7a–e and 8a–f

Commercially available 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (1.85 mmol) and sodium hydride (3.7 mmol) were dissolved in anhydrous DMF. The reaction was stirred for 20 min at 0 °C. The appropriate alkyl bromide or alkyl chloride (1.85 mmol) was added to the mixture, and stirring was continued for 1 h at 0 °C. The reaction progress was monitored by TLC with petroleum ether and ethyl acetate (2:1) as the mobile phase. Upon completion, 30 mL of a mixture of ice and water was added and the mixture was extracted to 40 mL ethyl acetate. The organic phase was dried over $MgSO_4$, filtered and evaporated *in vacuo*. In most instances, the residue was purified by recrystallisation from ethanol, ethyl acetate or from a mixture of petroleum ether and ethyl acetate/ethanol (1:1). Compounds **7b**, **7c**, **7e**, **8a** and **8c** were purified by silica gel column chromatography with a mixture of petroleum ether and ethyl acetate (2:1) serving as mobile phase.

1-(4-Chlorobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1H)-one (7a) The title compound was prepared in a yield of 26%: mp 118.9–123.9 °C (ethanol). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.28–7.24 (m, 2H), 7.19 (d, $J = 8.3$ Hz, 2H), 7.13–7.03 (m, 2H), 6.94 (t, $J = 7.4$ Hz, 1H), 6.63 (d, $J = 8.2$ Hz, 1H), 5.08 (s, 2H), 4.46 (s, 2H), 3.09 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 155.04, 138.40, 136.38, 132.55, 128.79, 128.18, 127.79, 125.57, 121.95, 119.56, 113.69, 50.47, 46.22, 35.83; APCI-HRMS *m/z* calcd for $C_{16}H_{16}ClN_2O$ (MH^+), 287.0945, found 301.0731. Purity (HPLC): 97.4%.

1-(3-Bromobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1H)-one (7b) The title compound was prepared in a yield of 67%: mp 96.8–100.1 °C (petroleum ether: ethyl acetate). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41 (d, $J = 8.4$ Hz, 2H), 7.13 (d, $J = 8.3$ Hz, 2H), 7.09 (d, $J = 8.0$ Hz, 1H), 7.06 (d, $J = 7.4$ Hz, 1H), 6.94 (t, $J = 7.4$ Hz, 1H), 6.62 (d, $J = 8.2$ Hz, 1H), 5.06 (s, 2H), 4.46 (s, 2H), 3.09 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 155.03, 138.39, 136.93, 131.73, 128.19, 128.16, 125.58, 121.96, 120.62, 119.56, 113.68, 50.48, 46.27, 35.83; APCI-HRMS *m/z* calcd for $C_{16}H_{16}BrN_2O$ (MH^+), 331.0440, found 331.0407. Purity (HPLC): 98.4%.

1-(4-Bromobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1H)-one (7c) The title compound was prepared in a yield of 57%: mp 095.8–098.6 °C (petroleum ether: ethyl acetate). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.44–7.39 (m, 2H), 7.16–7.03 (m, 4H), 6.94 (td, $J = 7.4$, 0.6 Hz, 1H), 6.62 (d, $J = 8.2$ Hz, 1H), 5.06 (s, 2H), 4.46 (s, 2H), 3.09 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 155.02, 138.39, 136.93, 131.73, 128.19, 128.16, 125.58, 121.96, 120.62, 119.56, 113.68, 50.47, 46.27, 35.83; APCI-HRMS *m/z* calcd for $C_{16}H_{16}BrN_2O$ (MH^+), 331.0440, found 331.0440. Purity (HPLC): 99.2%.

1-(4-Iodobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1H)-one (7d) The title compound was prepared in a yield of 11%: mp 106.8–109.1 °C (petroleum ether: ethyl acetate). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.61 (d, $J = 8.3$ Hz, 2H), 7.12–7.03 (m, 2H), 7.01 (d, $J = 8.2$ Hz, 2H), 6.95 (d, $J = 7.4$ Hz, 1H), 6.62 (d, $J = 8.2$ Hz, 1H), 5.06 (s, 2H), 4.46 (s, 2H), 3.09 (s, 3H), 1.65 (s, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 155.02, 138.39, 137.68, 137.65, 128.44, 128.20, 125.58, 121.96, 119.56, 113.68, 92.07, 50.47, 46.35, 35.84; APCI-HRMS *m/z* calcd for $C_{16}H_{16}IN_2O$ (MH^+), 379.0301, found 379.0278. Purity (HPLC): 95.4%.

3-Methyl-1-(4-(triflouromethyl)benzyl)-3,4-dihydroquinazolin-2(1H)-one (7e) The title compound was prepared in a yield of 25%: mp 086.4–088.9 °C (petroleum ether: ethyl acetate). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.53 (s, 1H),

7.51–7.45 (m, 1H), 7.41 (d, J = 6.4 Hz, 2H), 7.13–7.05 (m, 2H), 6.95 (td, J = 7.4, 0.7 Hz, 1H), 6.61 (d, J = 8.2 Hz, 1H), 5.17 (s, 2H), 4.48 (s, 2H), 3.10 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 154.99, 139.01, 138.36, 129.67, 129.18, 128.27, 125.67, 123.89, 123.86, 123.21, 123.19, 123.17, 122.07, 119.58, 113.56, 50.48, 46.52, 35.86; APCI-HRMS *m/z* calcd for $C_{17}H_{16}F_3N_2O$ (MH^+), 321.1209, found 321.1240. Purity (HPLC): 99.1%.

1-(3-Chlorobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1H,3H)-dione (8a) The title compound was prepared in a yield of 15%: mp 127.6–130.0 °C (petroleum ether: ethyl acetate). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.26 (dd, J = 7.9, 1.4 Hz, 1H), 7.57 (ddd, J = 8.6, 7.3, 1.6 Hz, 1H), 7.29–7.22 (m, 4H), 7.14 (d, J = 6.8 Hz, 1H), 7.06 (d, J = 8.4 Hz, 1H), 5.36 (s, 2H), 3.56 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 161.89, 151.55, 139.61, 137.85, 135.13, 134.98, 130.30, 129.13, 128.01, 126.60, 124.63, 123.30, 115.69, 114.09, 46.93, 28.73; APCI-HRMS *m/z* calcd for $C_{16}H_{14}ClN_2O_2$ (MH^+), 301.0738, found 301.0738. Purity (HPLC): 99.8%.

1-Benzyl-3-methyl-3,4-dihydroquinazolin-2(1H,3H)-dione (8b) The title compound was prepared in a yield of 11%: mp 155.2–156.1 °C (ethanol: petroleum ether). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.25 (dd, J = 7.9, 1.6 Hz, 1H), 7.54 (ddd, J = 8.7, 7.3, 1.6 Hz, 1H), 7.34 (t, J = 7.4 Hz, 2H), 7.26 (dd, J = 7.6, 3.3 Hz, 3H), 7.24–7.21 (m, 1H), 7.12 (d, J = 8.4 Hz, 1H), 5.39 (s, 2H), 3.56 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 162.01, 151.62, 139.86, 135.71, 135.00, 128.99, 127.68, 126.46, 123.08, 115.65, 114.37, 47.40, 28.70; APCI-HRMS *m/z* calcd for $C_{16}H_{15}N_2O_2$ (MH^+), 267.1128, found 267.1118. Purity (HPLC): 100%.

3-Methyl-1-phenethyl-3,4-dihydroquinazolin-2(1H,3H)-dione (8c) The title compound was prepared in a yield of 25%: mp 143.8–145.3 °C (petroleum ether: ethyl acetate). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.26 (dd, J = 7.9, 1.3 Hz, 1H), 7.71–7.64 (m, 1H), 7.36–7.29 (m, 4H), 7.27–7.22 (m, 3H), 4.37–4.31 (m, 2H), 3.50 (s, 3H), 3.06–2.99 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 161.96, 150.90, 139.55, 137.74, 135.08, 129.24, 128.82, 128.81, 126.91, 122.90, 115.63, 113.32, 45.18, 33.61, 28.42; APCI-HRMS *m/z* calcd for $C_{17}H_{17}N_2O_2$ (MH^+), 281.1284, found 281.1262. Purity (HPLC): 99.6%.

3-Methyl-1-(2-phenoxyethyl)-3,4-dihydroquinazolin-2(1H,3H)-dione (8d) The title compound was prepared in a yield of 10%: mp 148.8–165.3 °C (ethanol). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.23 (dd, J = 7.9, 1.5 Hz, 1H), 7.70–7.65 (m, 1H), 7.52 (d, J = 8.5 Hz, 1H), 7.30–7.22 (m, 3H), 6.94 (t, J = 7.3 Hz, 1H), 6.83 (d, J = 8.2 Hz, 2H), 4.55 (t, J = 5.8 Hz, 2H), 4.34 (t, J = 5.8 Hz, 2H), 3.49 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 161.95, 158.14, 151.25, 140.32, 134.83, 129.55, 128.87, 123.08, 121.29, 115.53, 114.42, 114.32, 65.19, 43.45, 28.43; APCI-HRMS *m/z* calcd for $C_{17}H_{17}N_2O_3$ (MH^+), 297.1233, found 297.1229. Purity (HPLC): 94.1%.

4-((3-Methyl-2,4-dioxo-3,4-dihydroquinazolin-2(1H)-yl)methyl)benzonitrile (8e) The title compound was prepared in a yield of 30%: mp 194.5–281.9 °C (petroleum ether: ethanol). ^{1}H NMR (600 MHz, DMSO) δ 8.09 (dd, J = 7.8, 1.1 Hz, 1H), 7.81 (d, J = 8.2 Hz, 2H), 7.68–7.62 (m, 1H), 7.52 (d, J = 8.2 Hz, 2H), 7.28 (t, J = 7.5 Hz, 1H), 7.21 (d, J = 8.5 Hz, 1H), 5.47 (s, 2H), 3.37 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 161.80, 151.49, 142.78, 139.82, 135.63, 133.03, 128.49, 127.98, 123.46, 119.20, 115.72, 115.12, 110.54, 46.65, 28.79; APCI-HRMS *m/z* calcd for $C_{17}H_{14}N_3O_2$ (MH^+), 292.1080, found 292.1059. Purity (HPLC): 97.0%.

1-(3-Iodobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1H,3H)-dione (8f) The title compound was prepared in a yield of 34%: mp 131.4–134.0 °C (ethyl acetate). ^{1}H NMR (600 MHz, DMSO) δ 8.08 (dd, J = 7.8, 1.5 Hz, 1H), 7.76 (s, 1H), 7.70–7.61 (m, 2H), 7.33–7.24 (m, 3H), 7.12 (t, J = 7.8 Hz, 1H), 5.35 (s, 2H), 3.37 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 161.80, 151.49, 139.91, 139.55, 136.54, 135.60, 135.55, 131.25, 128.42, 126.34, 123.38, 115.68, 115.22, 95.67, 46.17, 28.80; APCI-HRMS *m/z* calcd for $C_{16}H_{14}IN_2O_2$ (MH^+), 393.0094, found 393.0085. Purity (HPLC): 96.3%.

The determination of IC_{50} values for MAO inhibition

The IC_{50} values were measured according to the literature procedure [28]. Recombinant human MAO-A and MAO-B were used as enzyme sources, and kynuramine served as enzyme–substrate. The enzyme reactions were carried out to a volume of 200 µL in white polypropylene 96-well microtiter plates. The reactions consisted of substrate (50 µM) and the test inhibitors (0.003–100 µM) dissolved in potassium phosphate buffer (pH 7.4, 100 mM). The reactions were initiated with the addition of MAO-A (0.0075 mg/mL) or MAO-B (0.015 mg/mL) and were incubated for 20 min at 37 °C. At endpoint, the reactions were terminated with the addition of 80 µL NaOH (2 N) and the fluorescence was measured (λ_{ex} = 310, λ_{em} = 400). Quantitative estimations of 4-hydroxyquinoline were made by constructing a linear calibration curve (0.047–1.50 µM). From these data, the enzyme catalytic rates were calculated and the IC_{50} values were estimated by constructing sigmoidal plots of rate versus the logarithm of inhibitor concentration. For this purpose, the data were fitted to the one-site competition model incorporated into the Prism 5 software package. All IC_{50}

values were measured in triplicate and are reported as the mean ± standard deviation (SD).

Determination of reversibility of inhibition by dialysis

Reversibility of inhibition was determined by dialysis. Dialysis was carried out with Slide-A-Lyzer dialysis cassettes with a molecular weight cut-off of 10,000 and a sample volume of 0.5–3 mL. For this purpose, the literature procedure was followed [28]. MAO-A or MAO-B (0.03 mg/mL) and the test inhibitors (at concentrations of $4 \times IC_{50}$) were dissolved in potassium phosphate buffer (100 mM, pH 7.4) containing 5% sucrose and pre-incubated for 20 min at 37 °C. Stock solutions of the inhibitors were prepared in DMSO and added to yield a final concentration of 4% DMSO. The samples were subsequently dialysed for 24 h at 4 °C with the dialysis buffer (potassium phosphate buffer, 100 mM, pH 7.4, containing 5% sucrose) being replaced with fresh buffer at 3 h and 7 h after dialysis was started. As positive controls, MAO-A and MAO-B were pre-incubated and dialysed in the presence of the irreversible inhibitors, pargyline [IC_{50}(MAO-A) = 13 µM] and (*R*)-deprenyl [IC_{50}(MAO-B) = 0.079 µM], respectively [35, 36]. The concentrations of the irreversible inhibitors were $4 \times IC_{50}$. Dialysis of the enzymes was also carried out in the absence of inhibitor and served as negative control. After 24 h, the dialysis samples were diluted twofold with the addition kynuramine to yield an inhibitor concentration of $2 \times IC_{50}$ and a kynuramine concentration of 50 µM. These reactions were incubated for 20 min at 37 °C, terminated with the addition of NaOH (2 N), and the fluorescence of the samples was measured as described above. The residual enzyme activities were calculated and expressed as mean ± SD of triplicate determinations. For comparison, undialysed mixtures of the test inhibitors and the MAOs were maintained at 4 °C for the same time period (24 h), and the residual enzyme catalytic rates were measured.

Construction of Lineweaver–Burk plots

The modes of MAO-A and MAO-B inhibition by **6b** and **6c**, respectively, were investigated by constructing sets of Lineweaver–Burk plots. For each inhibitor, plots were constructed in the absence of inhibitor and in the presence of five different inhibitor concentrations ($^1/_4 \times IC_{50}$, ½ × IC_{50}, ¾ × IC_{50}, 1 × IC_{50}, $1^1/_4 \times IC_{50}$). The substrate, kynuramine, was used at concentrations ranging from 15–250 µM, while the final concentration of MAO-A and MAO-B was 0.015 mg protein/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 replot of the slopes of the Lineweaver–Burke plots versus inhibitor concentration (*x*-axis intercept equals $-K_i$).

Electronic supplementary material

^{1}H NMR, ^{13}C NMR, mass spectra and HPLC traces for the synthesised compounds.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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