Bioorganic & Medicinal Chemistry Letters xxx (xxxx) xxxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Evaluation of nitrocatechol chalcone and pyrazoline derivatives as inhibitors of catechol-O-methyltransferase and monoamine oxidase

Rialette Hitge, Sharissa Smit, Anél Petzer, Jacobus P. Petzer*

Pharmaceutical Chemistry, School of Pharmacy and Centre of Excellence for Pharmaceutical Sciences, North-West University, Potchefstroom 2520, South Africa

ARTICLE INFO

Keywords:
Monoamine oxidase
Catechol-O-methyltransferase
Chalcone
Pyrazoline
Multi-target-directed
Parkinson's disease

ABSTRACT

Literature reports that chalcones inhibit the monoamine oxidase (MAO) enzymes, mostly with specificity for the MAO-B isoform, while nitrocatechol compounds are established inhibitors of catechol–O-methyltransferase (COMT). Based on this, nitrocatechol derivatives of chalcone have been proposed to represent dual-target-directed compounds that may inhibit both MAO-B and COMT. Both these enzymes play key roles in the metabolism of dopamine and levodopa, and inhibitors are thus relevant to the treatment of Parkinson's disease. The present study expands on the discovery of dual MAO-B/COMT inhibitors by synthesising additional nitrocatechol derivatives of chalcones which include heterocyclic derivatives, and converting them to the corresponding pyrazoline derivatives. The newly synthesised chalcone and pyrazoline compounds were evaluated as inhibitors of human MAO and rat COMT, and the inhibition potencies were expressed as IC_{50} values. A pyrazoline derivative, compound **8b**, was the most potent COMT inhibitor with an IC_{50} value of 0.048 μM. This is more potent than the reference COMT inhibitor, entacapone, which has an IC_{50} value of 0.23 μM. The results indicated that the pyrazoline derivatives (IC_{50} = 0.048–0.21 μM) are more potent COMT inhibitors than the chalcones (IC_{50} = 0.14–0.29 μM). Unfortunately, the chalcone and pyrazoline derivatives were weak MAO inhibitors with IC_{50} values > 41.4 μM. This study concludes that the nitrocatechol derivatives investigated here are promising COMT inhibitors, while not being suitable as MAO inhibitors. Using molecular docking, potential binding modes and interactions of selected inhibitors with COMT are proposed.

Monoamine oxidase (MAO) metabolises biogenic amines in the central and peripheral tissues, and thus plays an important role in the inactivation of neurotransmitters.[1–3] Two isoenzymes of MAO have been identified, MAO-A and MAO-B, and inhibitors of these enzymes have found application in the treatment of neuropsychiatric and neurodegenerative disorders. In this regard, MAO-A degrades serotonin and noradrenaline in the central nervous system (CNS), and MAO-A inhibitors have been useful for the treatment of psychiatric illnesses such as depression.[1,4,5] In the CNS, MAO-B is a major metabolic enzyme for dopamine and β–phenylethylamine, and MAO-B inhibitors have thus been used for the treatment of neurodegenerative disorders, particularly Parkinson's disease.[2] The rationale for the use of specific MAO-B inhibitors in Parkinson's disease is based on the inhibition of dopamine metabolism which results in the enhancement of striatal dopaminergic activity.[6] MAO-B inhibitors are often co-administered with levodopa in an attempt to further enhance dopamine levels following levodopa treatment.[7,8] A more theoretical rationale for the use of MAO-B inhibitors in patients with Parkinson's disease originates from the observation that hydrogen peroxide is produced as by-product of MAO catalysis. Since hydrogen peroxide may lead to oxidative damage, it has been postulated that hydrogen peroxide produced by MAO-B in the brain may contribute to neurodegeneration in Parkinson's disease. MAO-B inhibitors have thus been advocated as potential neuroprotective agents.[2]

The enzyme, aromatic L-amino acid decarboxylase (AADC), decarboxylates levodopa in the gastrointestinal tract and liver to yield dopamine, and subsequently only a fraction a levodopa dose reaches the systemic circulation.[9] The combination of levodopa with an AADC inhibitor such as carbidopa or benserazide, increases the fraction of levodopa that reaches the systemic circulation, thus improving the bioavailability of levodopa to the brain.[10,11] Dopamine as well as levodopa is also metabolised by catechol–O–methyltransferase (COMT) in the central and peripheral tissues. In the periphery, COMT inhibitors such as tolcapone and entacapone reduce the O–methylation of levodopa, and thus enhance the fraction of levodopa that is available for uptake into the brain.[12–15] Central inhibitors of COMT (e.g. tolcapone) may also reduce levodopa and dopamine metabolism in the brain, which further may improve the therapeutic efficacy of levodopa.[16]

* Corresponding author.
E-mail addresses: 12264954@nwu.ac.za (A. Petzer), jacques.petzer@nwu.ac.za (J.P. Petzer).

https://doi.org/10.1016/j.bmcl.2020.127188
Received 14 February 2020; Received in revised form 8 April 2020; Accepted 9 April 2020

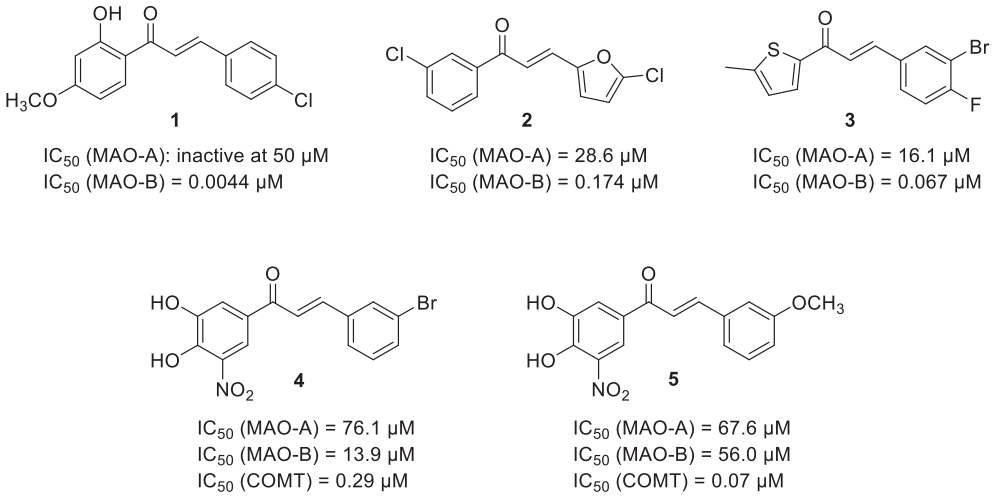


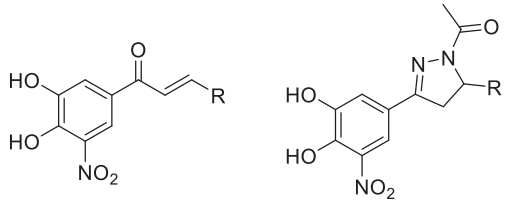
Fig. 1. The structures of chalcone compounds discussed in the text.

COMT inhibitors are usually administered in conjunction with a combination of carbidopa/levodopa. The inhibition of levodopa metabolism by AADC and COMT inhibitors allows for a reduction in the levodopa dose, which greatly reduces the potential of levodopa-associated adverse effects.[17,18] Levodopa continues to be the gold standard drug for the symptomatic treatment of Parkinson's disease, and because of this much interest in the development of inhibitors of the COMT enzyme exists.[16,19]

Based on the roles of MAO-B and COMT in the metabolism of dopamine and levodopa, dual inhibitors of these enzymes may synergise to enhance dopaminergic neurotransmission and provide an improved therapeutic outcome in Parkinson's disease. Chalcones are known to inhibit the MAOs, mostly with specificity for the MAO-B isoform.[20] For example, chalcone **1** is a high potency MAO-B inhibitor with an IC_{50} value 0.0044 μM, while no inhibition of MAO–A is observed at concentrations of 50 μM (Fig. 1).[20] Heterocyclic chalcone derivatives such as **2** and **3** are also known to inhibit MAO-B. These compounds inhibit MAO-B with IC_{50} values of 0.174 μM and 0.067 μM, respectively.[21,22] The nitrocatechol moiety, in turn, is present in the clinically used COMT inhibitors such as tolcapone and entacapone, and is considered privileged for the inhibition of COMT. Based on this, it has been proposed that nitrocatechol derivatives of chalcone may exhibit dual inhibition of COMT and MAO-B.[23] A recent study thus showed that nitrocatechol derivatives of chalcone are high potency inhibitors of COMT, although only moderate MAO-B inhibition was observed. For example, chalcone **4** is a potent COMT inhibitor (IC_{50} = 0.29 μM) while also acting as a moderately potent MAO-B inhibitor (IC_{50} = 13.9 μM).[23] Chalcone **5** was the most potent COMT inhibitor reported in the literature study (IC_{50} = 0.07 μM).[23]

To expand on the structure–activity relationships (SARs) for dual MAO/COMT inhibition, this study investigated additional nitrocatechol derivatives. This study included three nitrocatechol derivatives of chalcone (**6a–c**) bearing polar functional groups on ring B since these are expected to increase inhibition of MAO-B (Table 1). In particular, the nitrile functional group was considered as polar substituent since benzo- and phthalonitriles are well-known to potently inhibit MAO-B.[24] Hydroxy substitution also is known to enhance MAO-B inhibition.[25] This effect of polar substitution on MAO B inhibition is due to the interaction of the polar groups with the polar region of the MAO-B active site in proximity to the FAD. For the first time, heterocyclic chalcone derivatives (**7a–f**) that incorporate the nitrocatechol moiety will be investigated as potential dual MAO/COMT inhibitors. As mentioned above, heterocyclic chalcone derivatives have been reported to act as good potency MAO-B inhibitors. This study will also convert some of the chalcone compounds to the corresponding pyrazoline derivatives

Table 1
The IC_{50} values for the inhibition of human MAO-A and MAO-B, and the IC_{50} values for the inhibition of rat liver COMT by the chalcone and pyrazoline compounds and reference inhibitors.



	R	IC_{50} (μM) ± SD		IC_{50} (μM) ± SD
		MAO-A	MAO-B	COMT
6a	3-CN-C_6H_4-	58.9 ± 2.45	60.6 ± 5.17	0.24 ± 0.07
6b	4-CN-C_6H_4-	45.3 ± 2.52	56.6 ± 7.36	0.18 ± 0.06
6c	3-OH-C_6H_4-	43.9 ± 4.69	55.8 ± 5.00	0.14 ± 0.04
7a	2-thienyl	49.9 ± 4.53	62.9 ± 7.58	0.19 ± 0.05
7b	3-thienyl	41.4 ± 3.09	42.1 ± 4.14	0.23 ± 0.05
7c	2-thiazolyl	69.6 ± 5.27	59.2 ± 9.75	0.24 ± 0.03
7d	3-furyl	52.9 ± 2.34	71.4 ± 2.99	0.29 ± 0.03
7e	2-furyl	60.5 ± 2.99	68.2 ± 12.49	0.23 ± 0.03
7f	1-methyl-1*H*-pyrazol-4-yl	51.5 ± 4.89	66.2 ± 3.57	0.29 ± 0.03
8a	3-CN-C_6H_4-	70.6 ± 7.47	55.4 ± 3.21	0.079 ± 0.009
8b	4-CN-C_6H_4-	76. 6 ± 7.43	83.2 ± 14.5	0.048 ± 0.03
8c	3-OH-C_6H_4-	87.0 ± 2.98	85.2 ± 7.48	0.075 ± 0.03
9a	2-thiazolyl	84.6 ± 4.90	68.2 ± 12.64	0.16 ± 0.05
9b	3-furyl	90.9 ± 3.22	126 ± 3.06	0.18 ± 0.05
9c	2-furyl	98.0 ± 6.62	77.4 ± 17.16	0.18 ± 0.05
9d	2-thienyl	86.3 ± 11.26	69.2 ± 2.82	0.21 ± 0.05
Entacapone		–	–	0.23 ± 0.05
Curcumin		5.02 ± 0.45	2.56 ± 0.21	–

(**8a–c**; **9a–d**) since this class of compounds have been reported to be good potency MAO-B inhibitors.[26,27] The possibility that pyrazolines may inhibit COMT has not been investigated before.

The nitrocatechol derivatives of chalcone (**6a–c**; **7a–f**) were synthesised according to the reaction scheme given in Fig. 2. The synthetic route consisted of three steps. Firstly, nitration of 4-hydroxy-3-methoxyacetophenone (apocynin; **10**) was carried out with nitric acid in the

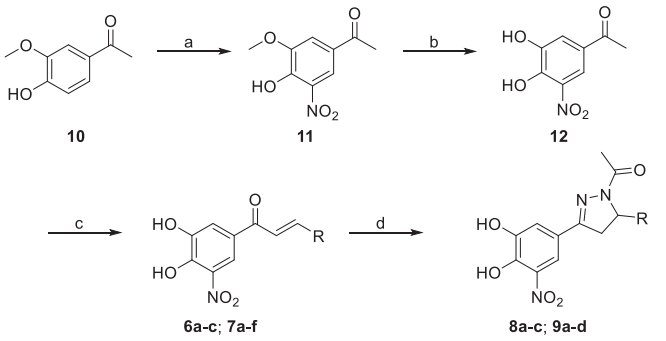


Fig. 2. The synthetic route for the synthesis of nitrocatechol derivatives of chalcone and the pyrazoline derivatives. Reagents and conditions: (a) HNO_3, acetic acid, rt; (b) $AlCl_3$, pyridine, ethyl acetate, 77 °C; (c) ethanol, KOH, RCHO, rt; (d) acetic acid, hydrazine hydrate, 120 °C.

presence of acetic acid to yield the nitro derivative **11** (5–nitroapocynin). In the second step, demethylation of **11** was carried out with $AlCl_3$/pyridine to yield the 5-acetyl-3-nitrocatechol **12.**[23,28] The target chalcones (**6a–c**; **7a–f**) were obtained via the Claisen-Schmidt condensation reaction between **12** and an appropriately substituted aldehyde in ethanol. Potassium hydroxide served as the base.[29] The nitrocatechol derivatives of chalcone were purified by crystallisation from an appropriate solvent (yields: 12–35%). The pyrazoline derivatives (**8a–c**; **9a–d**) were synthesised by reacting the corresponding chalcones with hydrazine hydrate in the presence of acetic acid.[26,30] The yields for these reactions ranged from 38 to 96%. The chalcone and pyrazoline derivatives were characterised by NMR and MS, while the purity was assessed by HPLC (see supplementary material).

The MAO inhibitory properties of the nitrocatechol derivatives of chalcone and the pyrazoline derivatives were investigated with the recombinant human MAO enzymes. The MAO activity measurements were carried out with kynuramine as substrate, which is converted by MAO to yield the fluorescent compound, 4-hydroxyquinoline.[31,32] After measuring the catalytic activities of MAO-A and MAO-B in the absence and presence (0.003–100 μM) of the test inhibitors, IC_{50} values were estimated in triplicate and are reported here as the means ± standard deviation (SD). These IC_{50} values are given in Table 1 and show that the nitrocatechol derivatives of chalcone (**6a–c**; **7a–f**) and the pyrazoline derivatives (**8a–c**; **9a–d**) are relatively weak inhibitors of MAO-A and MAO-B, with IC_{50} values > 41.4 μM. These values are significantly higher than those of the reference inhibitor, curcumin, which inhibit MAO-A and MAO-B with IC_{50} values of 5.02 μM and 2.56 μM, respectively. It is well-known that benzo- and phthalonitriles inhibits MAO-B potently, and hydroxy substitution is also known to enhance MAO-B inhibition of chalcones.[24,25] Since chalcones are in general good potency inhibitors of MAO-B, particularly compounds containing polar groups on the A-ring (e.g. OH), it may be concluded that nitro substitution on the A-ring greatly diminishes MAO-B inhibition.[20] It is interesting to note that the pyrazoline derivatives are in general weaker MAO inhibitors than the corresponding chalcones. The only exception is MAO-B inhibition by **8a** versus **6a**.

To determine whether the synthesised compounds are inhibitors of COMT, soluble fractions obtained from homogenates of rat liver tissue were used as enzyme source.[23,33,34] Ethical approval for the collection and use of animal tissue was obtained from the Research Ethics Committee of the North-West University (ethics approval numbers: NWU-00561-19-S5 and NWU-00562-19-S5). Esculetin (6,7–dihydroxycoumarin) served as substrate and is methylated by COMT to yield scopoletin, a reaction that may be monitored by fluorescence spectrophotometry.[35] COMT catalytic activity was thus measured in the absence and presence (0.003–80 μM) of the test inhibitors, and IC_{50} values were estimated in triplicate and are given as the means ± SD (Fig. 3). The IC_{50} values that were recorded are given in table 1, and show that

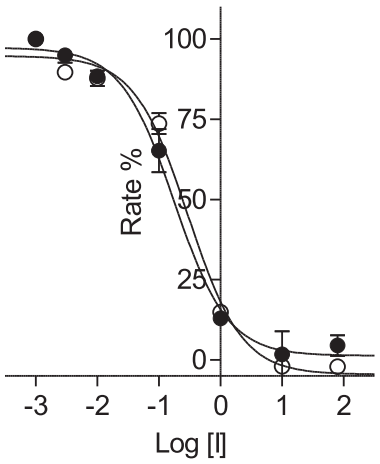


Fig. 3. Sigmoidal plots for the inhibition of COMT by **7f** (open circles) and **9c** (filled circles).

the nitrocatechol derivatives of chalcone (**6a–c**; **7a–f**) and the pyrazoline derivatives (**8a–c**; **9a–d**) are good potency inhibitors of COMT with IC_{50} values < 0.29 μM. The pyrazoline derivatives are more potent inhibitors than the chalcones, with **8b** being the most potent inhibitor with an IC_{50} value of 0.048 μM. COMT inhibitors containing the 3-nitrocatechol moiety that have been developed and introduced into the market include entacapone (IC_{50} = 0.23 μM) that display similar potency under these experimental conditions. Among the heterocyclic derivatives, pyrazoline **9a** was found to be the most potent inhibitor with an IC_{50} value of 0.16 μM.

It is noteworthy that all nitrocatechol derivatives of chalcone and the pyrazoline derivatives are good potency COMT inhibitors. This shows that the B-ring does not have a significant effect on COMT inhibition. The B-ring can thus be explored to improve MAO-B inhibition as it is unlikely to affect COMT inhibition to a large extent. Nitrocatechol derivatives of chalcone and the corresponding pyrazoline derivatives may serve as leads for the future design of potent COMT inhibitors. This study is the first report of COMT inhibition by pyrazoline compounds and thus proposes the further development of these nitrocatechol derivatives as potentially clinically useful COMT inhibitors. It should be kept in mind that the pyrazoline derivatives are chiral and represent the racemates of two enantiomers. It is not clear if both enantiomers contribute equally to COMT inhibition, but future studies should separate the enantiomers of a representative inhibitor (e.g. **8b**) to determine the stereochemistry of the eutomer and distomer.

As mentioned, nitrocatechol derivatives of chalcone have previously been investigated as COMT inhibitors.[23] In accordance with the findings of this study, the pyrazoline compounds are significantly more potent COMT inhibitors than the chalcones investigated previously. The most potent pyrazoline compound (**8b**, IC_{50} = 0.048 μM) is approximately 1.46-fold more potent that the most potent chalcone (**5**, IC_{50} = 0.07 μM) reported in literature.[23]

To obtain insight into potential binding orientations and interactions of pyrazoline compound **8b** with the COMT active site, molecular docking was carried out. For comparison, the corresponding chalcone, **6b**, was also investigated. For this study, rat COMT complexed with 3,5-dinitrocatechol was used as protein model (PDB code: 1VID).[36] Preparation of the protein models and ligands were carried out according to the previously reported protocol with Discovery Studio, while docking was done with AutoDock Vina.[31,37] The accuracy of the docking procedure was assessed by redocking the co-crystallized ligand into the COMT active site, which yielded a root mean square deviation (RMSD) of 0.684 Å between the docked orientation and the position of the co-crystallised ligand (Fig. 4). In the crystal structure, the Mg^{2+} co-factor is octahedrally coordinated to the side chains of Asp141 and Asp169, Asn170, the two hydroxy groups of the catechol substrate, and

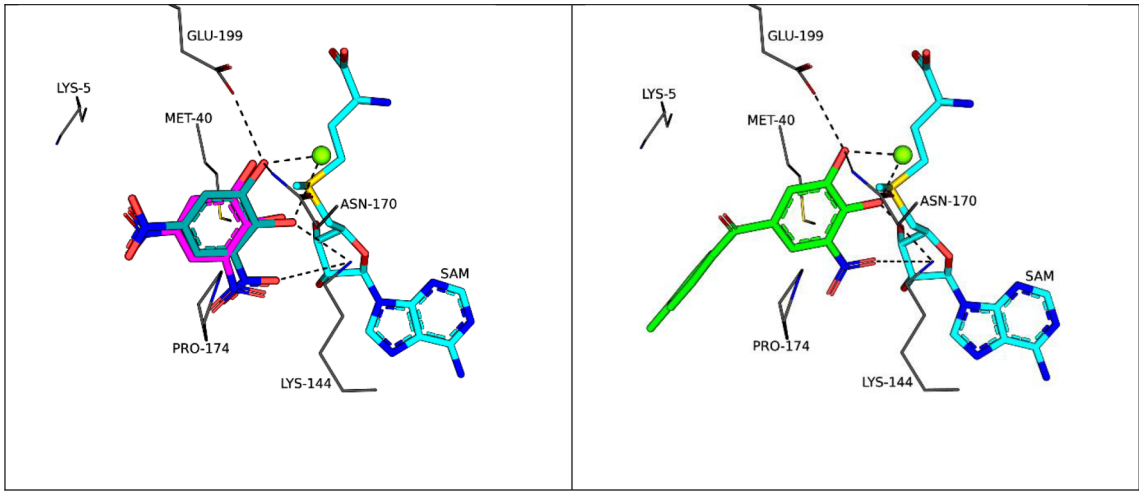


Fig. 4. The co-crystallised (teal) and docked (magenta) orientations of 3,5-dinitrocatechol in the active site of rat COMT (PDB code: 1VID). The orientation of tolcapone (green) in the active site of rat COMT (PDB code: 3S68).

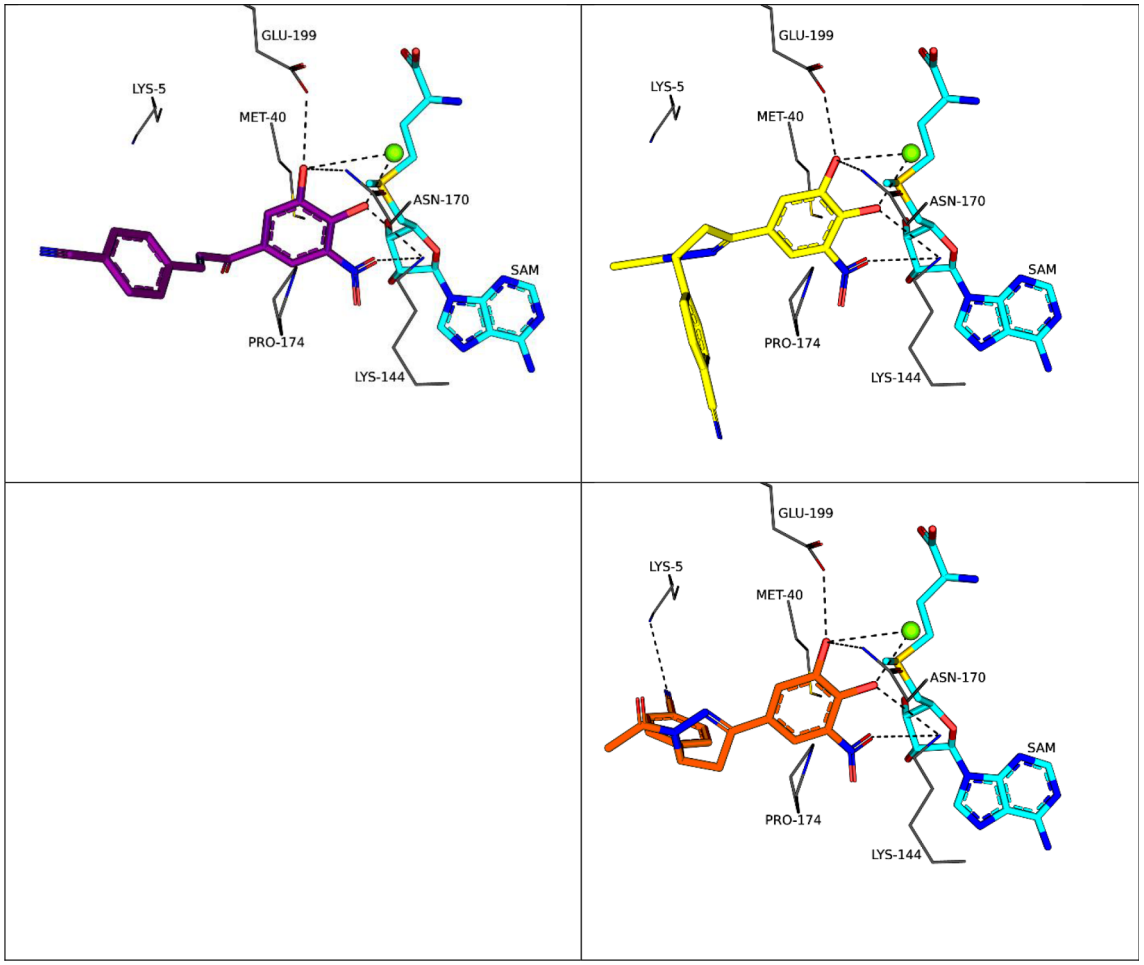


Fig. 5. The docked orientations of **6b** (purple) and the R- (yellow) and S-enantiomers (orange) of **8b** in the active site of rat COMT.

a water molecule.[36,38] The 1-hydroxy group of 3,5-dinitrocatechol is hydrogen bonded to Glu199 and Asn170, and the 2-hydroxy and 3-nitro groups are hydrogen bonded to Lys144. It may be concluded that the formation of a hydrogen bond network is crucial for the binding of nitrocatechol compounds to COMT.

Both enantiomers of **8b** as well as **6b** were docked into COMT, and among the solutions generated only those where the catechol hydroxyl groups of the initrocatechol moiety undergoes similar coordination with the Mg^{2+} as 3,5-dinitrocatechol above, are presented here as these are most probable. The crystal structure of tolcapone in complex with rat COMT also exhibit this orientation and interaction pattern.[39] As expected, chalcone **6b** adopts a similar binding orientation to 3,5-dinitrocatechol and forms a similar interaction network (Fig. 5). In this respect, the catechol OH groups coordinates with the magnesium ion,

while hydrogen bonding occurs with Lys144, Asn170 and Glu199. The B-ring of the chalcone projects out of the active site and undergoes very limited interactions, mainly a pi-alkyl interaction with Pro174, while the nitrocatechol ring forms a pi-alkyl interaction with Met40.

The results of the docking study further show that 3,5-dinitrocatechol of **8b** is correctly placed for coordination with the Mg^{2+} and the two enantiomers bind similar with respect to the 3,5-dinitrocatechol moieties. The two enantiomers exhibit very similar binding orientations and interactions. The catechol OH groups coordinates with the magnesium ion, while hydrogen bonding occurs with Lys144, Asn170 and Glu199. The nitrocatechol rings of both enantiomers form pi-alkyl interactions with Pro174 and Met40, while the pyrazoline rings form pi-alkyl interactions with Pro174. Interestingly, for the S-enantiomer, a hydrogen bond exists between the nitrile and Lys5. Compared to chalcone **6b**, the side chain of the S-enantiomer of **8b** undergoes more extensive interactions which may explain the better inhibition potencies observed for the pyrazolines. However, the AutoDock Vina binding affinity values (kcal/mol) for **6b** and **8b** are very similar, which underscores the observation that the polar interactions of the nitrocatechol moieties of these compounds are virtually identical.

In this study, series of novel nitrocatechol derivatives of chalcone and pyrazoline derivatives were investigated with the aim of discovering compounds that exhibit dual inhibition of MAO-B and COMT. The results show that the study compounds are weak MAO inhibitors. In contrast to their MAO inhibition potencies, the nitrocatechol chalcone and pyrazoline derivatives are potent inhibitors of COMT with IC_{50} values < 0.29 μM. The pyrazoline derivatives were found to be more potent inhibitors than the chalcones. Chalcones contain the α,β-usaturated carbonyl system which may be considered to be a Michael acceptor. Such compounds may act as electrophiles and could therefore be a liability from a medicinal chemistry point of view.[40,41] In this regard, the pyrazolines are more desirable for the future development of COMT inhibitors, and they are expected to be less reactive than the corresponding chalcone compounds. A further point of interest is that all nitrocatechol derivatives of chalcone and pyrazoline derivatives are good potency COMT inhibitors, which may be attributed to the 3-nitrocatechol moiety (A-ring). The B-ring therefore does not affect COMT inhibition to a large degree, and thus represents a site for future structural modification to improve MAO-B inhibition potency. Even in the absence of good potency MAO-B inhibition, nitrocatechol-containing pyrazoline derivatives may represent useful leads for the future design of COMT inhibitors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was financially supported by the National Research Foundation of South Africa [Grant specific unique reference numbers (UID) 85642 and 96180]. The Grantholders acknowledge that opinions, findings and conclusions or recommendations expressed in any publication generated by the NRF supported research are that of the authors, and that the NRF accepts no liability whatsoever in this regard.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127188.

References

1. Youdim MB, Edmondson D, Tipton KF. The therapeutic potential of monoamine oxidase inhibitors. *Nat Rev Neurosci.* 2006;7:295–309. https://doi.org/10.1038/nrn1883.
2. Youdim MB, Bakhle YS. Monoamine oxidase: isoforms and inhibitors in Parkinson's disease and depressive illness. *Br J Pharmacol.* 2006;147:S287–S296. https://doi.org/10.1038/sj.bjp.0706464.
3. Tipton KF. 90 years of monoamine oxidase: some progress and some confusion. *J Neural Transm (Vienna).* 2018;125:1519–1551. https://doi.org/10.1007/s00702-018-1881-5.
4. Lum CT, Stahl SM. Opportunities for reversible inhibitors of monoamine oxidase-A (RIMAs) in the treatment of depression. *CNS Spectr.* 2012;17:107–120.
5. Naoi M, Maruyama W, Shamoto-Nagai M. Type A monoamine oxidase and serotonin are coordinately involved in depressive disorders: from neurotransmitter imbalance to impaired neurogenesis. *J Neural Transm (Vienna).* 2018;125:53–66. https://doi.org/10.1007/s00702-017-1709-8.
6. Fernandez HH, Chen JJ. Monoamine oxidase-B inhibition in the treatment of Parkinson's disease. *Pharmacotherapy.* 2007;27:174s–185s. https://doi.org/10.1592/phco.27.12part2.174S.
7. Birkmayer W, Knoll J, Riederer P, Youdim MB. (-)-Deprenyl leads to prolongation of L-dopa efficacy in Parkinson's disease. *Mod Probl Pharmacopsychiatry.* 1983;19:170–176.
8. Riederer P, Muller T. Monoamine oxidase-B inhibitors in the treatment of Parkinson's disease: clinical-pharmacological aspects. *J Neural Transm.* 2018;125:1751–1757. https://doi.org/10.1007/s00702-018-1876-2.
9. Seeberger LC, Hauser RA. Carbidopa levodopa enteral suspension. *Expert Opin Pharmacother.* 2015;16:2807–2817. https://doi.org/10.1517/14656566.2015.1111336.
10. Freitas ME, Ruiz-Lopez M, Fox SH. Novel Levodopa formulations for Parkinson's disease. *CNS Drugs.* 2016;30:1079–1095. https://doi.org/10.1007/s40263-016-0386-8.
11. Poewe W, Antonini A. Novel formulations and modes of delivery of levodopa. *Mov Disord.* 2015;30:114–120. https://doi.org/10.1002/mds.26078.
12. Learmonth DA, Palma PN, Vieira-Coelho MA, Soares-da-Silva P. Synthesis, biological evaluation, and molecular modeling studies of a novel, peripherally selective inhibitor of catechol-O-methyltransferase. *J Med Chem.* 2004;47:6207–6217. https://doi.org/10.1021/jm040848o.
13. Nissinen E, Linden IB, Schultz E, Pohto P. Biochemical and pharmacological properties of a peripherally acting catechol-O-methyltransferase inhibitor entacapone. *Naunyn Schmiedebergs Arch Pharmacol.* 1992;346:262–266.
14. Salamon A, Zadori D, Szpisjak L, Klivenyi P, Vecsei L. Opicapone for the treatment of Parkinson's disease: an update. *Expert Opin Pharmacother.* 2019;20:2201–2207. https://doi.org/10.1080/14656566.2019.1681971.
15. Akhtar MJ, Yar MS, Grover G, Nath R. Neurological and psychiatric management using COMT inhibitors: a review. *Bioorg Chem.* 2020;94:103418https://doi.org/10.1016/j.bioorg.2019.103418.
16. Silva T, Mohamed T, Shakeri A, et al. Development of blood-brain barrier permeable nitrocatechol-based catechol O-methyltransferase inhibitors with reduced potential for hepatotoxicity. *J Med Chem.* 2016;59:7584–7597. https://doi.org/10.1021/acs.jmedchem.6b00666.
17. Guldberg HC, Marsden CA. Catechol-O-methyl transferase: pharmacological aspects and physiological role. *Pharmacol Rev.* 1975;27:135–206.
18. Mannisto PT, Kaakkola S. Catechol-O-methyltransferase (COMT): biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. *Pharmacol Rev.* 1999;51:593–628.
19. Learmonth DA, Vieira-Coelho MA, Benes J, Alves PC, Borges N, Freitas AP. da-Silva PS. Synthesis of 1-(3,4-dihydroxy-5-nitrophenyl)-2-phenyl-ethanone and derivatives as potent and long-acting peripheral inhibitors of catechol-O-methyltransferase. *J Med Chem.* 2002;45:685–695.
20. Chimenti F, Fioravanti R, Bolasco A, et al. Chalcones: a valid scaffold for monoamine oxidases inhibitors. *J Med Chem.* 2009;52:2818–2824. https://doi.org/10.1021/jm801590u.
21. Robinson SJ, Petzer JP, Petzer A, Bergh JJ, Lourens AC. Selected furanochalcones as inhibitors of monoamine oxidase. *Bioorg Med Chem Lett.* 2013;23:4985–4989. https://doi.org/10.1016/j.bmcl.2013.06.050.
22. Minders C, Petzer JP, Petzer A, Lourens AC. Monoamine oxidase inhibitory activities of heterocyclic chalcones. *Bioorg Med Chem Lett.* 2015;25:5270–5276. https://doi.org/10.1016/j.bmcl.2015.09.049.
23. Engelbrecht I, Petzer JP, Petzer A. Nitrocatechol derivatives of chalcone as inhibitors of monoamine oxidase and catechol-O-methyltransferase. *Cent Nerv Syst Agents Med Chem.* 2018;18:115–127. https://doi.org/10.2174/1871524918666180426125714.
24. Manley-King CI, Bergh JJ, Petzer JP. Monoamine oxidase inhibition by C4-substituted phthalonitriles. *Bioorg Chem.* 2012;40:114–124. https://doi.org/10.1016/j.bioorg.2011.10.003.
25. Legoabe L, Kruger J, Petzer A, Bergh JJ, Petzer JP. Monoamine oxidase inhibition by selected anilide derivatives. *Eur J Med Chem.* 2011;46:5162–5174. https://doi.org/10.1016/j.ejmech.2011.08.036.
26. Chimenti F, Bolasco A, Manna F, et al. Synthesis and selective inhibitory activity of 1-acetyl-3,5-diphenyl-4,5-dihydro-(1H)-pyrazole derivatives against monoamine oxidase. *J Med Chem.* 2004;47:2071–2074. https://doi.org/10.1021/jm031042b.
27. Manna F, Chimenti F, Bolasco A, et al. Inhibition of amine oxidases activity by 1-acetyl-3,5-diphenyl-4,5-dihydro-(1H)-pyrazole derivatives. Bioorg Med Chem Lett. 2002;12(24):3629–3633. https://doi.org/Pii S0960-894x(02)00699-6.
28. Kiss LE, Ferreira HS, Torrao L, et al. Discovery of a long-acting, peripherally selective

inhibitor of catechol-O-methyltransferase. *J Med Chem.* 2010;53:3396–3411. https://doi.org/10.1021/jm1001524.
29. Klinke P, Gibian H. Über chalkone. *Eur J Inorg Chem.* 1961;94:26–38.
30. Manna F, Chimenti F, Fioravanti R, et al. Synthesis of some pyrazole derivatives and preliminary investigation of their affinity binding to P-glycoprotein. *Bioorg Med Chem Lett.* 2005;15:4632–4635. https://doi.org/10.1016/j.bmcl.2005.05.067.
31. Mostert S, Petzer A, Petzer JP. Indanones as high-potency reversible inhibitors of monoamine oxidase. *ChemMedChem.* 2015;10:862–873. https://doi.org/10.1002/cmdc.201500059.
32. Novaroli L, Reist M, Favre E, Carotti A, Catto M, Carrupt PA. Human recombinant monoamine oxidase B as reliable and efficient enzyme source for inhibitor screening. *Bioorg Med Chem.* 2005;13:6212–6217. https://doi.org/10.1016/j.bmc.2005.06.043.
33. Hirano Y, Tsunoda M, Funatsu T, Imai K. Rapid assay for catechol-O-methyltransferase activity by high-performance liquid chromatography-fluorescence detection. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2005;819:41–46. https://doi.org/10.1016/j.jchromb.2005.01.019.
34. Zhu BT, Wang P, Nagai M, Wen Y, Bai HW. Inhibition of human catechol-O-methyltransferase (COMT)-mediated O-methylation of catechol estrogens by major polyphenolic components present in coffee. *J Steroid Biochem Mol Biol.* 2009;113:65–74. https://doi.org/10.1016/j.jsbmb.2008.11.011.
35. Kurkela M, Siiskonen A, Finel M, Tammela P, Taskinen J, Vuorela P. Microplate screening assay to identify inhibitors of human catechol-O-methyltransferase. *Anal Biochem.* 2004;331:198–200. https://doi.org/10.1016/j.ab.2004.04.026.
36. Vidgren J, Svensson LA, Liljas A. Crystal structure of catechol O-methyltransferase. *Nature.* 1994;368:354–358. https://doi.org/10.1038/368354a0.
37. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem.* 2010;31:455–461. https://doi.org/10.1002/jcc.21334.
38. Rutherford K, Le Trong I, Stenkamp RE, Person VW. Crystal structures of human 108V and 108M catechol O-methyltransferase. *J Mol Biol.* 2008;380:120–130. https://doi.org/10.1016/j.jmb.2008.04.040.
39. Ellermann M, Lerner C, Burgy G, et al. Catechol-O-methyltransferase in complex with substituted 3'-deoxyribose bisubstrate inhibitors. *Acta Crystallogr D Biol Crystallogr.* 2012;68:253–260. https://doi.org/10.1107/S0907444912001138.
40. Zhuang CL, Zhang W, Sheng CQ, Zhang WN, Xing CG, Miao ZY. Chalcone: a privileged structure in medicinal chemistry. *Chem Rev.* 2017;117:7762–7810. https://doi.org/10.1021/acs.chemrev.7b00020.
41. Srinivasan B, Johnson TE, Lad R, Xing CG. Structure-activity relationship studies of chalcone leading to 3-hydroxy-4,3 ',4 ',5 '-tetramethoxychalcone and its analogues as potent nuclear factor kappa B inhibitors and their anticancer activities. *J Med Chem.* 2009;52:7228–7235. https://doi.org/10.1021/jm901278z.