Bioorganic & Medicinal Chemistry Letters 18 (2008) 6362–6368

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Pyrazoline-based mycobactin analogues as MAO-inhibitors

Venkatesan Jayaprakash [a,*], Barij N. Sinha [a], Gulberk Ucar [b,†], Ayse Ercan [b]

[a] Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, Ranchi 835215, Jharkhand, India
[b] Department of Biochemistry, Faculty of Pharmacy, Hacettepe University, 06100 Sıhhıye, Ankara, Turkey

ARTICLE INFO

Article history:
Received 1 July 2008
Revised 23 September 2008
Accepted 20 October 2008
Available online 1 November 2008

Keywords:
Mycobaction analogs
Aryl carbo-thioamide pyrazolines
MAO inhibitors
Docking

ABSTRACT

3,5-Diaryl carbothioamide pyrazolines designed as mycobactin analogs (mycobacterial siderophore) were reported to be potent antitubercular agents under iron limiting condition in our earlier study. Clinical complications of newly introduced antibiotic Linezolid, due its MAO inhibitory activity, prompted us to evaluate our compounds for their MAO-inhibitory activity against rat liver MAO-A and MAO-B as pyrazolines were reported to be antidepressants and MAO inhibitors. The present study carried out with this pilot library of 32 compounds will provide us with necessary information for designing antitubercular molecules with reduced MAO-inhibitory activity and also help us in identifying a selective MAO-B inhibitor which has potential clinical utility in neurodegenerative disorders. Thirty-two compounds analyzed has shown spectrum of activity from selective to nonselective against two isoforms of rat liver MAO-A and MAO-B and also as competitive, reversible to non-competitive, irreversible. It is also interesting to note that anti-tubercular compound **11, 14** and **16** were also found to be selective inhibitors of rat liver MAO-B. Docking studies with human MAO shows that compound **11** interacts with the catalytic site of both the isoforms, suggesting compound **11** as non-selective inhibitor of human MAO isoforms.

Our group has reported 32 new 3,5-diaryl carbothioamide pyrazolines having structural similarities to siderophores of *Mycobacterium tuberculosis* (Mycobactin) and *Yersinia pestis* (Yersinibactin) in search of novel antimicrobials against them targeting critical pathways to iron scarcity adaptation.[1] Dual activity of drugs containing hydrazine derived hetero-cycles, which are available for clinical use (antibacterial with MAO inhibitory activity and MAO inhibitors with antibacterial activity) were reviewed and reported by many research groups.[2,3] Dual activity targeting different biochemical pathway of a pathogen generally provides a potent antibacterial agent. On the other hand, antibacterial drug acting on host's biochemical pathway will lead to clinical complications during long term therapy. Linezolid, an oxazolidinone antibiotic, has a major safety concern due to its reversible non-selective human MAO inhibitory activity.[4]

The above mentioned considerations prompted us to evaluate our compounds for their MAO inhibitory activity, since pyrazolines were reported to have anti-depressant[5–12] and MAO inhibitory activity.[13–22] This will provide us with necessary information for designing anti-tubercular molecules with reduced MAO-inhibitory activity and also help us in identifying a selective MAO-B inhibitor which has potential clinical utility in neurodegenerative disorders. Since results obtained using rat MAO cannot be extrapolated to human MAO, docking studies were also performed with human MAO using Autodock4.

Very few pyrazolines were reported with hydroxylphenyl substitution in 3rd and 5th position with antibacterial[23–29], antitubercular[30–32] and MAO inhibitory activity.[13] The presence of 2-hydroxyphenyl substitution in the 3rd position of pyrazoline imparts metal chelating property[33] and 4-hydroxyphenyl substitution in the 3rd and 5th position of pyrazoline imparts antioxidant property to the molecule.[34] Considering the effectiveness of multifunctional selective MAO-B inhibitor with iron chelating and anti-oxidant property in the treatment of neurodegenerative disorder, we strongly believe a molecule from our pilot library of 32 compounds with selective MAO-B inhibitory activity will be of great interest towards development of effective Neuroprotective agents.

3,5-Diaryl-1-carbothioamide-pyrazoline derivatives (1–32) were synthesized from 2′-hydroxy chalcone derivatives as per Scheme 1 as reported earlier.[1] Hydroxy chalcones were prepared through Claisen–Schmidt Condensation. Pyrazoline derivatives were then obtained by condensing 2′-hydroxy chalcones with hydrazine hydrate (80%). The final compounds **1–31** were obtained by the reaction of pyrazoline derivatives with phenyl/substituted phenyl isothiocyanates and Compound **32** was obtained by the reaction of chalcone with thiosemicarbazide in alkaline medium. All the intermediates were characterized by

* Corresponding author. Tel.: +91 651 2275843.
E-mail addresses: venkatesanj@bitmesra.ac.in (V. Jayaprakash), gulberk@hacettepe.edu.tr (G. Ucar).
† Tel.: +90 312 3052553; fax: +90 312 3114777.

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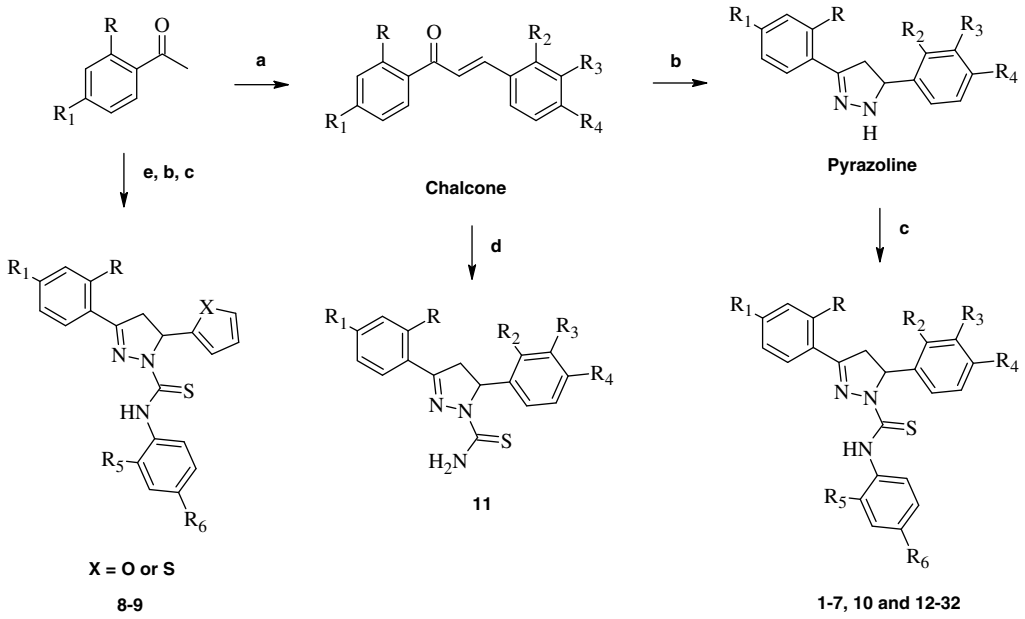
doi:10.1016/j.bmcl.2008.10.084



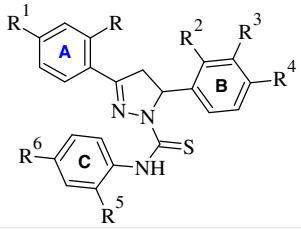
Scheme 1. *Synthesis of compounds **1–32**. Reagents and conditions: (a) i—R_2, R_3, R_4-C_6H_2–CHO, aq NaOH (60%), stirring at rt 4–48 h, ii—ice cold HCl (6 N), pH adjusted to 2; (b) NH_2NH_2/H_2O (80%) excess, C_2H_5OH, reflux 3–6 h; (c) R_5, R_6-C_6H_3-NCS, C_2H_5OH or CH_3OH, reflux 15–30 min.; (d) i—$NH_2NHC(S)NH_2$, NaOH excess, CH_3OH, reflux 8 h, ii—ice cold HCl (3 N), pH adjusted between 2 and 4; (e) thiophene-2-carboxaldehyde or furfuraldehyde followed by step (b) and (c). *Adopted and modified from Ref. 1.

IR spectroscopic analysis and elemental analysis for CHNS. In the elemental analysis, the observed values were within ±0.4% of the calculated values. Final compounds were characterized by ^{1}H NMR and FAB-MS. Structure of compounds synthesized was presented in Table 1.

MAO was purified from the rat liver and total MAO activity was measured spectrophotometrically according to the method of Holt.[35] Assay mixture contained a chromogenic solution consisted of vanillinic acid, 4-aminoantipyrin and peroxidase type II in potassium phosphate buffer, pH 7.6. Assay mixture was pre-incubated with substrate *p*-tyramine before addition of enzyme. The reaction was initiated by the addition of homogenate and increase in absorbance was monitored at 498 nm at 37 °C for 60 min. Molar absorption coefficient of 4654 M^{-1} cm^{-1} was used to calculate the initial velocity of the reaction. Results were expressed as nmol h^{-1} mg^{-1}. For selective measurement of MAO-A and MAO-B activities, homogenates were incubated with the substrate p-tyramine following the inhibition of one of the MAO isoform with selective inhibitors. After inhibition of homogenates with selective inhibitors, total MAO activity was determined by method of Holt. Newly synthesized compounds were dissolved in DMSO and used in the concentration range of 1–1000 μM. Inhibitors were then incubated with purified MAO at 37° C for 0–60 min prior to adding to the assay mixture. Reversibility of the inhibition of MAO by these compounds was assessed by dilution. Kinetic data for interaction of the enzyme with this compound was determined using Microsoft Excel package program. IC_{50} values were determined from plots of residual activity percentage, calculated in relation to a sample of the enzyme treated under the same conditions without inhibitor, versus inhibitor concentration [I].

All of the newly synthesized compounds (**1–32**) inhibited the total MAO activity of rat liver homogenates. The IC_{50} (μM) and K_i (μM) values of these compounds were presented in Table 2. and Table 3, respectively. Lineweaver-Burk plot for the inhibition of rat liver MAO-A by compound **7** was presented in Figure 1. It is interesting to note the activity profile of the library molecules, eight compounds were found to be non-selective (**25–32**), seven were found to be selective for MAO-B (**11–17**) and seventeen

Table 1



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Compound	R	R^1	R^2	R^3	R^4	R^5	R^6
1	–OH	–H	–H	–H	–H	–H	–H
2	–OH	–H	–Cl	–H	–H	–H	–H
3	–OH	–H	–H	–H	–Cl	–H	–H
4	–OH	–H	–OH	–H	–H	–H	–H
5	–OH	–H	–H	–H	–OH	–H	–H
6	–OH	–H	–OMe	–H	–H	–H	–H
7	–OH	–H	–H	–H	–OMe	–H	–H
8	–OH	–H	2-Thiophenyl			–H	–H
9	–OH	–H	2-Furfuryl			–H	–H
10	–H	–OH	–H	–H	–OH	–H	–H
11	–OH	–H	–H	–H	–OH	–H–	
12	–H	–H	–OH	–H	–H	–H	–H
13	–H	–H	–H	–H	–OH	–H	–H
14	–OH	–H	–OH	–H	–H	–H	–OMe
15	–OH	–H	–H	–H	–OH	–H	–OMe
16	–OH	–H	–OH	–H	–H	–H	–Me
17	–OH	–H	–H	–H	–OH	–H	–Me
18	–OH	–H	–H	–OMe	–OH	–H	–H
19	–OH	–H	–H	–OMe	–OH	–H	–OMe
20	–OH	–H	–H	–OMe	–OH	–H	–Me
21	–OH	–OH	–H	–OMe	–OH	–H	–H
22	–OH	–OH	–H	–OMe	–OH	–H	–OMe
23	–OH	–OH	–H	–H	–OH	–H	–H
24	–OH	–OH	–H	–H	–OH	–H	–OMe
25	–OH	–H	–H	–H	–OH	–OMe	–H
26	–OH	–H	–OH	–H	–H	–OMe	–H
27	–OH	–H	–H	–OMe	–OH	–OMe	–H
28	–OH	–OH	–H	–H	–OH	–OMe	–H
29	–OH	–OH	–H	–OMe	–OH	–OMe	–H
30	–OH	–H	–H	–H	–OH	–Me	–H
31	–OH	–H	–OH	–H	–H	–Me	–H
32	–OH	–H	–H	–OMe	–OH	–Me	–H

* Adopted and modified from Ref. 1.

Table 2
IC_{50} values corresponding to the inhibition of rat liver MAO isoforms by the newly synthesized 3,5-diaryl-1-carbothioamide-pyrazoline derivatives (**1–32**).*

Compound	IC_{50} for MAO-A** (μM)		IC_{50} for MAO-B** (μM)		MAO inhibitory Selectivity
	Preincubation 0	Preincubation 60 min	Preincubation 0	Preincubation 60 min	
1	60.35 ± 4.55	49.16 ± 3.50	480.32 ± 19.56	439.20 ± 25.11	Selective for MAO-A
2	49.10 ± 3.00	20.05 ± 3.56	470.34 ± 23.20	455.23 ± 27.10	Selective for MAO-A
3	60.16 ± 5.33	23.18 ± 1.58	450.12 ± 35.79	400.30 ± 29.25	Selective for MAO-A
4	80.22 ± 5.55	58.10 ± 3.63	475.06 ± 45.10	420.34 ± 34.20	Selective for MAO-A
5	80.10 ± 6.50	67.22 ± 5.80	498.12 ± 25.55	400.05 ± 23.35	Selective for MAO-A
6	87.60 ± 5.66	74.20 ± 6.76	431.0 ± 12.05	419.30 ± 26.76	Selective for MAO-A
7	39.20 ± 2.30	2.84 ± 0.19	489.00 ± 47.90	415.20 ± 40.85	Selective for MAO-A
8	31.20 ± 2.35	5.56 ± 0.45	481.56 ± 30.56	385.75 ± 22.10	Selective for MAO-A
9	74.12 ± 5.37	33.41 ± 2.85	450.12 ± 29.90	440.30 ± 28.60	Selective for MAO-A
10	82.16 ± 7.23	32.10 ± 3.00	490.22 ± 36.42	486.28 ± 30.12	Selective for MAO-A
11	479.18 ± 28.56	466.20 ± 30.12	45.13 ± 3.20	19.45 ± 1.02	Selective for MAO-B
12	456.75 ± 29.36	450.80 ± 30.40	77.20 ± 5.80	35.55 ± 3.10	Selective for MAO-B
13	490.36 ± 25.33	475.56 ± 30.12	89.60 ± 5.55	48.60 ± 3.80	Selective for MAO-B
14	472.20 ± 29.90	470.50 ± 40.21	75.50 ± 6.31	40.78 ± 3.66	Selective for MAO-B
15	405.45 ± 20.30	400.56 ± 29.60	70.88 ± 6.30	41.10 ± 3.50	Selective for MAO-B
16	480.30 ± 35.22	400.20 ± 31.10	77.96 ± 6.40	37.70 ± 3.05	Selective for MAO-B
17	500.01 ± 38.80	475.50 ± 30.56	82.00 ± 6.02	46.12 ± 3.70	Selective for MAO-B
18	77.70 ± 5.23	40.07 ± 3.56	455.45 ± 29.93	450.60 ± 30.27	Selective for MAO-A
19	65.54 ± 5.80	48.90 ± 4.02	481.00 ± 30.54	477.22 ± 31.50	Selective for MAO-A
20	88.60 ± 7.40	50.64 ± 4.30	490.45 ± 30.70	481.12 ± 29.99	Selective for MAO-A
21	90.12 ± 7.60	65.60 ± 5.13	499.50 ± 32.20	490.23 ± 30.30	Selective for MAO-A
22	84.45 ± 6.27	53.36 ± 4.40	488.20 ± 33.41	475.50 ± 29.58	Selective for MAO-A
23	91.20 ± 6.82	58.60 ± 4.20	476.35 ± 30.25	470.38 ± 33.90	Selective for MAO-A
24	96.50 ± 8.66	69.55 ± 5.74	480.22 ± 30.40	455.80 ± 28.89	Selective for MAO-A
25	203.36 ± 15.50	150.60 ± 10.88	200.55 ± 17.30	144.47 ± 10.85	Non-selective
26	190.21 ± 14.20	144.48 ± 9.30	219.60 ± 18.44	170.52 ± 12.75	Non-selective
27	240.79 ± 18.86	170.46 ± 13.60	286.32 ± 20.24	201.30 ± 16.65	Non-selective
28	181.77 ± 15.41	147.80 ± 10.66	225.33 ± 18.63	170.55 ± 13.36	Non-selective
29	255.60 ± 19.47	180.23 ± 14.20	201.10 ± 19.60	170.22 ± 16.32	Non-selective
30	240.20 ± 17.43	188.51 ± 13.34	210.18 ± 18.75	180.27 ± 13.92	Non-selective
31	200.05 ± 13.35	170.26 ± 12.74	223.88 ± 20.35	180.44 ± 12.36	Non-selective
32	240.22 ± 19.73	200.56 ± 13.30	244.57 ± 19.44	197.56 ± 14.88	Non-selective
Pargyline***	**432.26 ± 18.60**	**390.20 ± 17.23**	**5.05 ± 0.95**	**3.85 ± 0.23**	Selective for MAO-B
Clorgyline***	**6.16 ± 034**	**2.05 ± 0.19**	**410.15 ± 16.78**	**400.90 ± 15.54**	Selective for MAO-A
Selegiline***	**516.10 ± 19.25**	**499.01 ± 18.20**	**7.56 ± 0.35**	**2.01 ± 0.15**	Selective for MAO-B
Moclobemide***	**5.58 ± 0.28**	**3.90 ± 0.19**	**496.22 ± 30.15**	**480.12 ± 21.05**	Selective for MAO-A

* The IC_{50} values were determined from the kinetic experiments in which *p*-tyramine was used at 500 μM to measure MAO-A and 2.5 mM to measure MAO-B. Pargyline or clorgyline were added at 0.50 μM to determine the isoenzymes A and B.

** Each value represents the mean ± SEM of three independent experiments.

*** IC_{50} values corresponding to the inhibition of MAO isoforms by clorgyline and moclobemide as selective MAO-A inhibitors, and pargyline and selegiline as selective MAO-B inhibitors were also determined to assess the inhibitory potencies of the novel compounds.

were found to be selective for MAO-A (**1–10** and **18–24**). All the seventeen MAO-A selective inhibitors were found to be competitive and reversible. Compound **7** and **8** (IC_{50}: 2.84 ± 0.19 and 5.56 ± 0.45 μM, respectively, Table 2.) were the potent MAO-A inhibitors in this series, inhibitory activity of compound **7** was found to be better than moclobemide (IC_{50}: 3.90 ± 0.19 μM) and equivalent to clorgyline (IC_{50}: 2.05 ± 0.19 μM), respectively (Table 2.). Whereas only two (**11** and **14**) out of seven MAO-B selective inhibitors were found to be competitive and reversible, while other five (**12–13** and **15–17**) were noncompetitive and irreversible. Compound **11** (IC_{50}: 19.45 ± 1.02 μM, Table 2.) was the potent MAO-B inhibitor in this series and was found to be 5 and 7 times less potent than pargyline (IC_{50}: 3.85 ± 0.23 μM) and selegiline (IC_{50}: 2.01 ± 0.15 μM), respectively (Table 2.).

Structure–activity relationship within this small library reveals that *ortho*-methyl or *ortho*-methoxy substitution in ring C abolishes selectivity towards isoforms irrespective of substitution in ring A and B. *ortho*-hydroxy substitution in ring A has no role in selectivity towards isoforms, but ortho, *para*-dihydroxy substitution favors selectivity towards MAO-A that to when ring C was unsubstituted. In ring B, *ortho* or *para* mono-substitution with hydroxy, methoxy or chloro groups favors selectivity towards MAO-A when ring C is unsubstituted. Substitution in *ortho* position of ring B with chloro or hydroxy group was found to better than substitution in *para* position, but not significant. Whereas substitution with methoxy group, the difference was significant, *para*-methoxy substitution was found to be potent. When ring B was replaced with thiophene or furan ring, selectivity towards MAO-A was retained and thiophene substitution was found to be potent, but not better than *para*-methoxy phenyl substitution. *Ortho*-methoxy, *para*-hydroxy di-substitution in ring B also favors selectivity towards MAO-A, once again when ring C is unsubstituted. Selectivity towards MAO-B was favored when ring C was absent. In the presence of ring C, *para*-methyl or *para*-methoxy substitution favors selectivity towards MAO-B. It is interesting to note that compounds **11** and **14** were the only two which are competitive and reversible in this series while other five were non-competitive and irreversible. Except compound **11**, difference in substitution in compounds **12–17** shows no great difference in activity.

Docking studies were performed with human MAO isoforms using Autodock4. Selective inhibitors **7**, **8**, **9**, **11**, **14**, **16** along with Linezolid and crystallographic models 2BXR for human MAO-A and 2BYB for human MAO-B were considered for docking study. Marvin Sketch (Chemaxon), Protein Preparation Wizard (Schrodinger Inc.) and Open Babel were used for ligand and protein preparation. Top scoring molecules from the largest cluster were considered for interaction studies. Estimated free energy of binding (kcal/mol) was presented in Table 4. and detailed method of computational approach adopted is presented in Supplementary materials.

Table 3
Experimental kinetic parameters obtained by the inhibition of rat liver MAO isoforms by 3,5-diaryl–1-carbothioamide-pyrazoline derivatives (**1–32**).

Compound	Experimental $K_i$values (μM)	Selectivity, inhibition type and reversibility of the inhibition
1	40.22 ± 3.00	Selective for MAO-A, competitive, reversible
2	18.60 ± 1.56	Selective for MAO-A, competitive, reversible
3	20.14 ± 1.78	Selective for MAO-A, competitive, reversible
4	48.45 ± 3.08	Selective for MAO-A, competitive, reversible
5	50.45 ± 45.12	Selective for MAO-A, competitive, reversible
6	63.55 ± 5.40	Selective for MAO-A, competitive, reversible
7	2.50 ± 0.15	Selective for MAO-A, competitive, reversible
8	6.13 ± 0.48	Selective for MAO-A, competitive, reversible
9	27.53 ± 2.52	Selective for MAO-A, competitive, reversible
10	30.55 ± 3.90	Selective for MAO-A, competitive, reversible
11	15.20 ± 1.22	Selective for MAO-B, competitive, reversible
12	34.70 ± 2.86	Selective for MAO-B, noncompetitive, irreversible
13	40.64 ± 3.99	Selective for MAO-B, noncompetitive, irreversible
14	36.21 ± 3.05	Selective for MAO-B, competitive, reversible
15	39.18 ± 3.45	Selective for MAO-B, noncompetitive, irreversible
16	35.00 ± 2.85	Selective for MAO-B, noncompetitive, irreversible
17	42.89 ± 3.49	Selective for MAO-B, noncompetitive, irreversible
18	62.74 ± 5.55	Selective for MAO-A, competitive, reversible
19	60.54 ± 5.70	Selective for MAO-A, competitive, reversible
20	75.12 ± 5.90	Selective for MAO-A, competitive, reversible
21	79.19 ± 6.05	Selective for MAO-A, competitive, reversible
22	71.14 ± 5.82	Selective for MAO-A, competitive, reversible
23	80.00 ± 7.25	Selective for MAO-A, competitive, reversible
24	85.45 ± 7.23	Selective for MAO-A, competitive, reversible
Pargyline	**3.25 ± 0.21**	Selective for MAO-B, competitive, reversible
Clorgyline	**1.99 ± 0.10**	Selective for MAO-A, competitive, reversible
Selegiline	**1.90 ± 0.08**	Selective for MAO-B, competitive, reversible
Moclobemide	**2.25 ± 0.15**	Selective for MAO-A, competitive, reversible

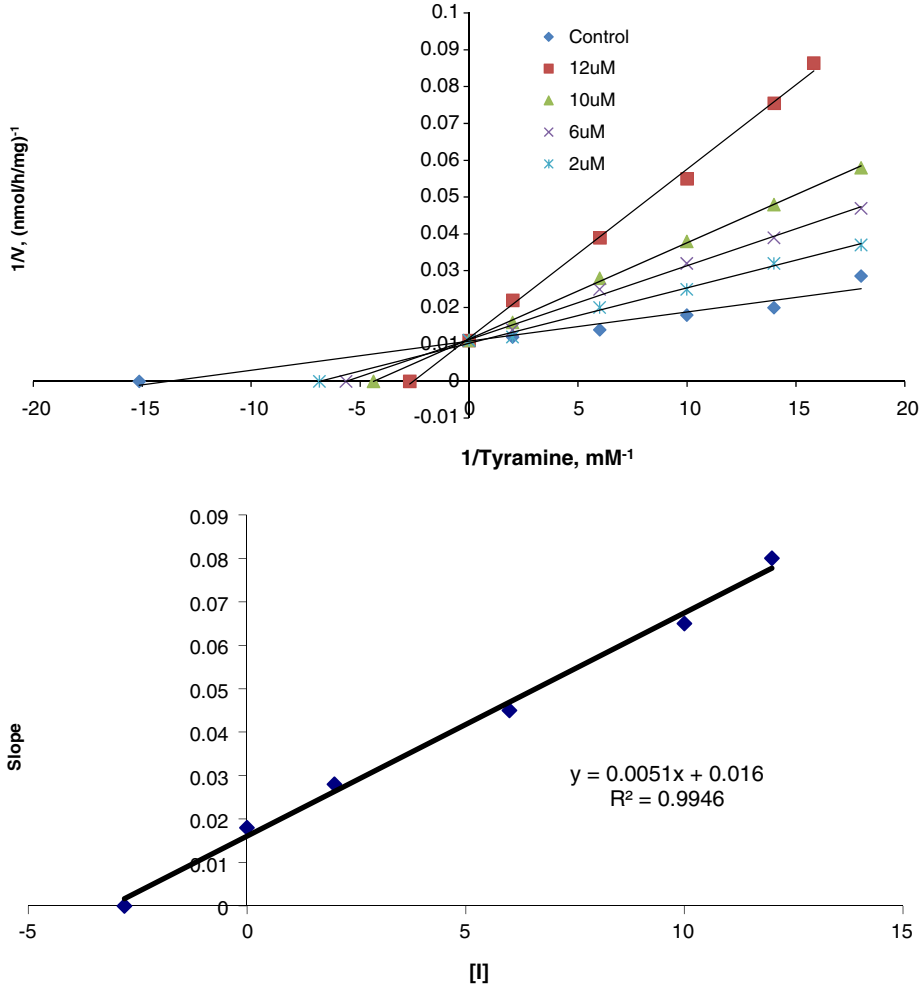

Figure 1. Lineweaver–Burk plot for the inhibition of rat liver MAO-A by the compound **7** with 60 min of preincubation at 37 °C. *p*-Tyramine was used as substrate in the concentration range of 10–100 μM. Replot of Y-intercepts vs I and apparent K_i value are shown in the second graph.

Table 4
Docking scores of compounds **7**–**9**, **11**, **14**, **16** and Linezolid.

Compound	Estimated free energy of binding	
	hMAO-A (2BXR)	hMAO-B (2BYB)
7	−9.07	−5.42
8	−9.33	−7.83
9	−9.31	−8.43
11	−8.27	−8.69
14	−9.40	−4.36
16	−8.97	−4.76
Linezolid	−9.07	−5.42

Estimated binding free energy for Linezolid with human MAO-A (2BXR) was found to be higher than with human MAO-B (2BYB), as expected. Interestingly the compound **11** was found to interact with the catalytic site of both isomers with appreciably equal but slightly better estimated binding free energy towards MAO-B. Complex of compound **11** with MAO-A, hydrogen bonding interactions were found between: amino hydrogen of compound **11** with side chain carbonyl group of ASN181, hydroxyl hydrogen in ring A with amino hydrogen of SER209. Both the aromatic ring A and B were away from aromatic cage formed by FAD, TYR407 and TYR444 (pocket 1). Ring A was positioned in hydrophobic pocket delimited by GLY71, GLN74, ARG206, ILE207, PHE208, GLU216 and TRP441 (pocket 2) whereas ring B was positioned in hydrophobic pocket delimited by ILE180, ILE335, LEU337, MET350 and PHE352 (pocket 3). Sulphur in thicarbamoyl group in compound **11** was found to position itself towards the aromatic cage formed by FAD, TYR407 and TYR444. Interactions of compound **11** with MAO-A were shown in Figure 2a. Estimated binding free energy of compounds **7**, **8**, **9**, **14** and **16** were all found to be lower than compound **11** with single hydrogen bond interaction of hydroxyl oxygen of ring A with amino nitrogen of SER209. In compounds **7**, **8** and **9** ring A was positioned in pocket 3, ring B in pocket 1 and ring C in pocket 2, where as in compounds **24** and **34** ring A in pocket 2, ring B in pocket 3 and ring C in pocket 1.

Complex of compound **11** with MAO-B, hydrogen bonding interactions were found between: amino hydrogen of compound **11** with hydroxyl oxygen of TYR398 (this also allows the positioning of ring B of compound **11** in aromatic cage formed by FAD, TYR398 and TYR435) and hydroxyl hydrogen of ring A with hydroxyl oxygen of TYR326. Interactions of compound **11** with MAO-B were shown in Figure 2b. Comparatively narrow active site cavity favors the positioning of ring B in aromatic cage of MAO-B. Hydroxyl functional group in *para* position of ring B further favors hydrophobic interaction of its phenyl ring with phenyl rings of TYR398 and TYR435. In compounds **7**, **8** and **9** both ring B and C were positioned in aromatic cage, also ring B methoxy oxygen of compound **7** and furan oxygen of compound **9** were found to have hydrogen bonding interaction with CYS172. In compounds **24** and **34** ring

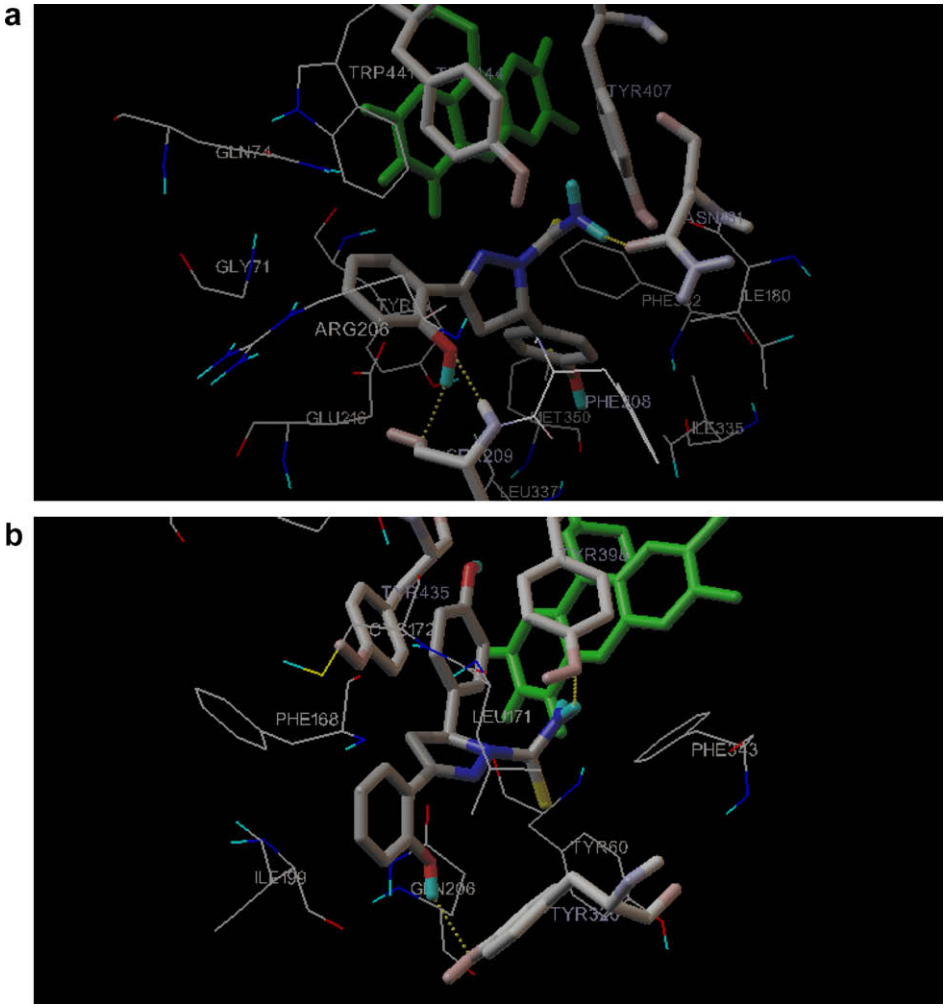

Figure 2. (a) Interaction of compound **11** with human MAO-A (2BXR), (b) Interaction of compound **11** with human MAO-B (2BYB). FAD shown in green color and hydrogen bonds in yellow dotted lines.

Table 5
MAO-B inhibitory and antitubercular activity of compounds **11**–**17**.

Compound[a]	IC50 (μM) Preincubation 60 min[a]	MAO inhibitory selectivity[a]	Compound [b]	IC_{50}[b] (μM)		$IC_{50GAST-D-Fe}/IC_{50GAST-D}$[b]
				GAST-D	GAST-D-Fe	
11	19.45 ± 1.02	Selective for MAO-B	**32**	8 ± 1	125 ± 0	16
12	35.55 ± 3.10	Selective for MAO-B	**30**	24 ± 6	42 ± 4	2
13	48.60 ± 3.80	Selective for MAO-B	**31**	500 ± 0	500 ± 0	1
14	40.78 ± 3.66	Selective for MAO-B	**10**	28 ± 5	167 ± 42	6
15	41.10 ± 3.50	Selective for MAO-B	**15**	>500	>500	nd
16	37.70 ± 3.05	Selective for MAO-B	**12**	27 ± 3	208 ± 42	8
17	46.12 ± 3.70	Selective for MAO-B	**17**	417 ± 83	>500	>1

[a] Table 2 in this article.
[b] Reproduced from Ref. 1.

A was positioned in aromatic cage, which is favored by the hydrogen bonding interaction between hydroxyl oxygen of ring B and hydroxyl hydrogen of TYR398.

Two unsubstituted aromatic rings were found to be conveniently accommodated in aromatic cage of MAO-B (compound **8** and **9**), while a ring with substitution drastically reduces estimated binding free energy (compound **7**). When all the three rings were substituted only one ring was accommodated in aromatic cage of MAO-B, which forces other two ring to be accommodated in the narrow cavity leading to poor estimated binding free energy. In other words these factors make them selective towards MAO-A. Only compounds **8**, **9** and **11** were found to be non-selective towards human MAO-A and B with good estimated binding free energy on both the isoforms, while all other compounds studied were found to be selective towards human MAO-A. Observations from docking studies comply with the statement of De Colibus et al. that "results from investigation of one mammalian form of MAO cannot be unambiguously extrapolated to other mammalian forms.[36]

Compound **11** has higher estimated binding free energy than Linezolid on both the isoforms of human MAO. The compound **11** was a novel antitubercular lead in spite of its very low potency compared with standard, Rifampicin, used in our study. Novelty of the compound **11** compared with antitubercular pyrazolines reported earlier[30–32] is its increased potency in iron limiting conditions compared with iron fed condition. This suggests its inhibitory action on mycobacterial iron metabolism and will greatly help in identifying a new potential target, which is needed in addressing global threats due to multi-drug resistant and emerging extensively drug resistant Tuberculosis. Further design and development of this class of molecules as antitubercular agents should consider its potential human MAO inhibitory activity as a major problem to be addressed.

It is interesting to note that the potent antitubercular molecules in this series were found to be selective inhibitors of rat MAO-B (Table 5.). Once again one cannot unambiguously extrapolate it to mycobacterial MAO inhibition for its antitubercular activity. At the same time under iron limiting condition expression of oxidation enzymes required for metabolism of carbohydrates in mycobacterium tuberculosis grown in culture medium and oxidation enzymes required for metabolism of fatty acid in mycobacterium tuberculosis grown in macrophages were reported to be elevated.[37–39] Possibility of these enzymes as a target for this class of molecules should also be investigated, since this may provide us a new potential target for antitubercular drug design.

Acknowledgment

We are grateful to the Sophisticated Analytical Instrument Facility (CDRI, Lucknow, India) for providing spectral data.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.10.084.

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