

Accepted Manuscript

Title: Design, synthesis, molecular docking and MAO inhibitory activity of 2-(arylmethylidene)-2,3-dihydro-1-benzofuran-3-one derivatives

Authors: Vishnu Nayak Badavath, Chandrani Nath, Narayana Murthy Ganta, Gulberk Ucar, Barij Nayan Sinha, Venkatesan Jayaprakash

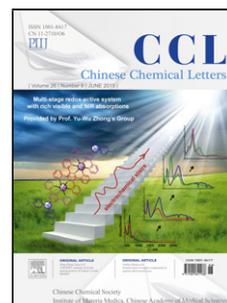
PII: S1001-8417(17)30065-7
DOI: <http://dx.doi.org/doi:10.1016/j.ccllet.2017.02.009>
Reference: CCLET 3982

To appear in: *Chinese Chemical Letters*

Received date: 2-11-2016
Revised date: 26-12-2016
Accepted date: 17-2-2017

Please cite this article as: Vishnu Nayak Badavath, Chandrani Nath, Narayana Murthy Ganta, Gulberk Ucar, Barij Nayan Sinha, Venkatesan Jayaprakash, Design, synthesis, molecular docking and MAO inhibitory activity of 2-(arylmethylidene)-2,3-dihydro-1-benzofuran-3-one derivatives, *Chinese Chemical Letters* <http://dx.doi.org/10.1016/j.ccllet.2017.02.009>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



Original article

Design, synthesis, molecular docking and MAO inhibitory activity of 2-(arylmethylidene)-2,3-dihydro-1-benzofuran-3-one derivatives

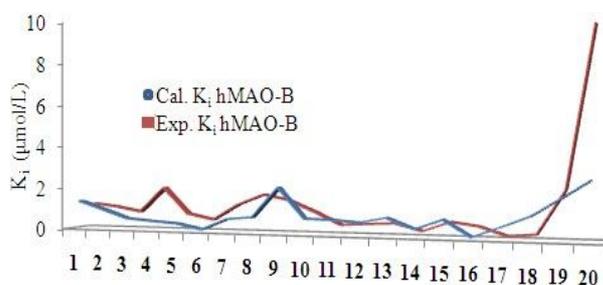
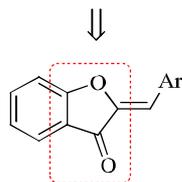
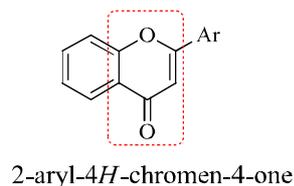
Vishnu Nayak Badavath^{1,*}, Chandrani Nath¹, Narayana Murthy Ganta¹, Gulberk Ucar^{2,*}, Barij Nayan Sinha¹, Venkatesan Jayaprakash^{1,□}

¹Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology, Mesra, Ranchi, Jharkhand 835 215, India ²Department of Biochemistry, Faculty of Pharmacy, Hacettepe University, Sıhhiye 06100, Ankara, Turkey

□ Corresponding authors.

E-mail addresses: venkatesanj@bitmesra.ac.in (V. Jayaprakash), gulberk@hacettepe.edu.tr (G. Ucar), vishnu.niper@gmail.com (Vishnu N. Badavath)

Graphical abstract



Twenty synthetic aurones were tested for their hMAO-isoform inhibitory activity. Seventeen compounds with less bulkier Ar-group were found to be potent, selective and reversible inhibitors of hMAO-B.

ABSTRACT

A series of 2-(arylmethylidene)-2,3-dihydro-1-benzofuran-3-one derivatives (aurones, **1-20**) were synthesized and screened for their inhibitory activity against hMAO. Seventeen compounds (**1-5**, **717**, **19**) were found to be selective towards hMAO-B, while two were non-selective (**6** and **20**) and one (**18**) selective towards hMAO-A. Compound **17** ($K_i=0.10\pm0.01$ $\mu\text{mol/L}$) was found to be equally potent and selective towards hMAO-B, when compared with the standard drug Selegiline ($K_i=0.12\pm0.01$ $\mu\text{mol/L}$). Nature and position of substitution in aryl ring at 2nd position of benzofuranone influences hMAO-B inhibitory potency, while their structural bulkiness influences selectivity between hMAO-A and hMAO-B. Molecular docking simulation was also carried out to understand the interaction of inhibitor with the enzyme at molecular level, and we found the docking results were in good agreement with the experimental values. Comparison of the activity profile of the aurones with their corresponding flavones reported earlier by our group revealed that there exists no difference in potency as well as selectivity.

Keywords: 2-(Arylmethylidene)-2,3-dihydro-1-benzofuran-3-one derivatives; hMAO Inhibitors; Molecular docking simulation; Synthesis.

1. Introduction

Monoamine oxidase (MAO) inhibitors introduced into clinical practice during 1960's were abandoned due to adverse effects, Such as hepatotoxicity, and "cheese effect", which was characterized by hypertensive crisis [1,2]. These adverse effects are due to non-selective inhibition of MAO-isoforms. Development of newer inhibitors that inhibits MAO selectively and reversibly is thus a rational approach in overcoming the adverse effects reported with earlier MAO-inhibitors. Aurones, 2benzylidenebenzofuran-3-(2*H*)-ones, are a subclass of flavonoids, recently research group synthesized a series of aurones and evaluated there *in vitro* rMAO-B (rat MAO) inhibitory activity in the micromolar range [3]. Earlier few researchers synthesized flavones, thioflavones, and flavanones and evaluated as potential inhibitors of monoamine oxidase isoforms (hMAO-A and -B, human MAO) [4]. Our group has also reported a series of flavones having inhibitory potential on hMAO isoforms [5]. The objective of the presented work is to evaluate the inhibitory activity of analogues aurones against MAO isoforms. The results were compared and molecular docking simulation studies were also performed. In the presented work evaluate their respective aurones are reported. Design strategy for designing MAO inhibitors is shown in Fig. 1.

2. Results and discussion

2.1. Chemistry:

All the compounds were synthesized according to the reactions outlined in Scheme 1. Chalcones intermediates (**a-t**) were prepared through Claisen-Schmidt condensation of appropriate aromatic/heteroaromaticaldehydes with 2-hydroxyacetophenone in 60% aqueous ethanolic sodium hydroxide solution. The final products (**1-20**) were obtained by refluxing the chalcones (**a-t**), with mercuric acetate in pyridine at 110 °C and characterized by ¹H NMR, ¹³C NMR and Mass spectroscopy. ¹H NMR of all the compounds, the olefinic proton (=CH-Ar) appeared as a singlet between δ 6.87-7.25, the hydroxyl proton of compounds **7-9** appeared as singlet between δ 10.20-12.37, the methoxy protons of compounds **10-14** appeared as singlet between δ 3.50-3.90, while methyl protons of compound **15** appeared as a singlet at δ 2.3. ¹³C-NMR characterization has been carried out for group of compounds (**6-8**, **16-17** and **20**) representing the enumerated library of twenty compounds (**1-20**). Compounds displayed carbonyl carbon (C-3) peak and (C-8) peak between δ 183.19-184.34 and δ 165.2-166.19 respectively. For compound **18**, the carbonyl carbon (C-3) peak appeared at δ 169.66, while and (C-8) peak at δ 95.67. For compounds **7** and **8**, the carbon having phenolic group exhibited the peak at δ 157.48 and δ 158.10, respectively. Mass spectra of all the compounds displayed M⁺ as molecular ion peak, except **2** (M+1)⁺.

2.2. Biochemistry

All the synthesized compounds **1-20** were screened for their hMAO inhibitory activity [6,7] using recombinant human MAO isoforms. hMAO inhibitory activity was determined by measuring the production of H₂O₂ from *p*-tyramine, the common substrate for both hMAO-A and hMAO-B, using the Amplex®-Red MAO assay kit. The compounds and reference inhibitors did not react directly with the Amplex®-Red reagent indicating that they do not interfere with the measurements. The compounds were also confirmed, as they did not interact with resorufin by treating the maximum concentration of compounds with various concentrations of resorufin in order to detect if the fluorescence signal is the same with or without our compounds in the medium. No significant quenching of resorufin was observed. The experimental data concerning to the inhibition of hMAO-A and hMAO-B inhibition by the novel compounds **1-20** were presented in Table 1, together with their selectivity indices. The selectivity index was expressed as $SI=K_i(\text{MAO-A})/K_i(\text{MAO-B})$ for selectivity towards hMAO-B. Protein was determined according to the method of Bradford method [8] using bovine serum albumin as the standard.

Compounds, **1-5**, **7-17** and **19** were found to inhibit hMAO-B selectively and reversibly, while, compound **18** having bulkier aldehyde portion (anthracen-9-yl) was found to be competitive, reversible and selective inhibitor of hMAO-A isoforms with $K_i=0.11\pm 0.01$ $\mu\text{mol/L}$, while compound **6** and **20** which carrying 4-NO₂, and thiophen-2-yl were found to be nonselective towards the MAO-isoforms. The most potent hMAO-B inhibitor in this series was compound **17** ($K_i=0.10\pm 0.01$ $\mu\text{mol/L}$), which was carrying naphth-2-yl group at aldehyde position. It was also found to have potency and selectivity almost equal to selegiline, a well-known selective MAO-B inhibitor ($K_i=0.12\pm 0.01$ $\mu\text{mol/L}$), Compound **14** which was carrying 3,4-diOMe functional group exhibited the best selectivity (SI=26.14) towards hMAO-B. The activity profiles of this series of compounds were then compared

with their flavone counterparts reported earlier by our group [5]. In spite of structural variation *viz:* (i) fused furan in place of pyran and (ii) exocyclic double bond in place of endocyclic double bond, this series displayed similar activity profile as that of their flavone counterparts in potency as well as selectivity towards MAO isoforms.

A comprehensive SAR study revealed the following: (i) Moving from phenyl (**1**) to 9-anthracenyl (**18**) substitution through 1-naphthyl (**16**) – potency increases but selectivity reversed from hMAO-B (**1,16**) to hMAO-A (**18**). Interestingly, 2-naphthyl derivative (**17**) was found to have potency equivalent to **18** with improved selectivity towards hMAO-B than **1**. (ii) Electron withdrawing functional groups at *o*- or *p*- positions did not exhibit significant improvement in either the potency or the selectivity towards hMAO-B for chloro (**2** and **3**), bromo (**5**) and cyano (**4**) substitutions in comparison with compound **1**. The only exception is with compound having nitro group at *p*- position (**6**), which displayed improved potency with loss of selectivity in comparison with compound **1**. (iii) Electron pumping functional groups: (a) Hydroxyl group at *o*-, *m*- and *p*- (**7-9**) did not displayed significant improvement in either potency or selectivity. (b) Replacing hydroxyl group with methoxy (**11-13**) improved the potency as well as selectivity towards hMAO-B to 3-4 fold & 7-9 fold, respectively for *o*- and *m*- substituted derivatives (**11** and **12**). *p*- Substituted derivative (**13**) displayed improved potency with no improvement in selectivity towards hMAO-B. A similar effect is observed with the methyl substitution at *p*- position (**15**) too. (c), While **13** was displaying increase in potency, the selectivity was found to be improved by the additional methoxy group at *m*-position (**14**). Almost 18- fold improvement was observed and this compound was found to have the best selectivity within the series. (iv) Replacing the phenyl ring of **1** with 2-pyridyl (**19**) or 2-thiophenyl (**20**) was found to decrease the potency as well as selectivity. Compound **19** was found to be non-selective similar to compound **6**. This clearly establishes that deactivated ring (*p*-nitrophenyl of **6** and 2-pyridyl of **19**) makes the molecule to lose its selectivity towards isoforms in comparison with compound **1**.

2.3. Molecular docking simulation

Molecular docking simulation was carried to understand the effect of structural features that determines the potency and selectivity at molecular level. The approach has been adopted by other groups as well as our group in our earlier communications [5, 9-13]. The molecular docking simulation was done using AutoDock-4.2 by adopting the protocol reported earlier by our group. Estimated K_i values obtained were in good agreement with the experimental K_i values (Fig. 2).

2.3.1 Orientation of compounds (1-20) in active site of hMAO-B

Active site of hMAO-B is hydrophobic in nature and is designated to have an aromatic cage lined by Flavin adenine dinucleotide (FAD), TYR435, and TYR398. The narrow space (hydrophobic tunnel) between aromatic cage and the active site entrance is lined with hydrophobic amino acid residues (LEU171, TYR326, PHE168, ILE198, and ILE199). Volume is relatively small when compared with the active site of hMAO. All the synthesized compounds (except **16,18**) oriented in a similar fashion as that of the potent hMAO-B inhibitor **17**, the benzofuran-3(2*H*)-one portion oriented towards aromatic cage and the benzylidene portion was placed in the hydrophobic tunnel. The orientation is quite similar to the one reported by Morales-Camilo *et al.*, [3]. Compounds **16** and **18** exhibited reversed orientation with reference to compound **17**. *i.e.*, now the benzofuran-3(2*H*)-one portion was placed in the tunnel orienting the benzylidene portion in the aromatic cage. This unusual reversal due to ortho-fusion at 2,3-position may be the reason why these molecules are less selective towards hMAOB/selective towards hMAO-A. The interactions of the molecules are purely hydrophobic and they exhibited π - π stacking interaction with components of aromatic cage. Fig.3. displays the interaction of compound **17** with TYR435 (12.03Å) and FAD (30.35 Å).

2.3.2 Orientation of compounds (1-20) in active site of hMAO-A

Active site of hMAO-A is hydrophobic in nature as that of hMAO-B. Volume of the pocket is larger with three sub-pockets namely (i) pocket 1: aromatic cage lined by FAD, Tyr407 & Tyr444; (ii) pocket 2: lined by Ile180, Ile335, Leu337, Met350 & Phe352 and (iii) pocket 3: lined by Gly74, Arg206, Ile207, Phe208, Glu216 and Trp441. This could be able to accommodate larger molecules. Compounds **1, 2, 7-9, 16, 19** and **20**, the benzylidene portion orient towards pocket 1, while the benzofuran-3(2*H*)-one portion orient towards pocket 3 and left the pocket 2 unoccupied. In case of compounds **3, 10-12** and **14**, the benzofuran-3(2*H*)-one portion orient towards pocket 1 placing the benzylidene portion at the center leaving pocket 2 and pocket 3 unoccupied. While in compounds **4-6, 13** and **15**, the benzylidene portion was found to completely fill the pocket 3 which keeps the benzofuran-3(2*H*)-one portion to accommodate in pocket 1 leaving pocket 2 unoccupied. Compound **17** places the entire molecule in the axis that connects aromatic cage with the entrance, leaving pockets 2 and 3 unoccupied. Incomplete occupation of the sub-pockets seems to be the reason behind the poor interaction of ligand with the protein leading to reduced potency and selectivity for the compounds in this series. Surprisingly the most potent hMAO-A inhibitor of this series, compound **18** that did not occupy any pockets but it resided at the entrance lined by THR201, VAL210, TYR121, TRP128, PHE208, TYR124, GLY110 and PHE173. The bulkier anthracene ring found to close the entrance cavity displaying hydrophobic interaction with the hydrophobic residues at the entrance and projecting the rest of the molecules into the entrance cavity. This resembles the cork closing the wine-bottle.

3. Conclusion

A series of twenty 2-(arylmethylidene)-2,3-dihydro-1-benzofuran-3-one derivatives were synthesized and screened for their potential to inhibit hMAO isoforms selectively. With the limited variations at single site, benzylidene portion the activity profile was recorded and a concise SAR was derived. In summary, weak to moderate electron pumping as well as electron withdrawing groups favor selectivity towards hMAO-B, while strong deactivators make them non-selective towards isoforms. Mono-substitution of methoxy group at *o*- and *m*- position increases potency while bi-substitution lead to the improved potency as well as selectivity. The same may be extended to flavones reported in our earlier communication⁵.

4. Experimental

4.1 Materials and methods

Chemicals and solvents were of reagent grade and purchased from Sigma-Aldrich. Melting points were determined using OPTIMELT (SRS, Sweden) an automated melting point system and are uncorrected melting point. ¹H NMR spectra were recorded on Varian 400MHz and (ECX-500 (JEOL) 300 MHz, DMSO-*d*₆ and CDCl₃ as a solvent. In ¹H NMR spectra the coupling constants (*J*) are expressed in hertz (Hz). Chemical shifts (δ) of NMR are reported in parts per million (ppm) units relative to the solvent. ¹³C-NMR 100 MHz, in DMSO-*d*₆ and CDCl₃ as a solvent. Mass spectra were recorded in WATERS-QToF Premier-HAB213 and ESI-MS technique. hMAO-A and hMAO-B (both recombinant, expressed in baculovirus-infected BTI insect cells), Selegiline, Moclobemide, Resorufin, DMSO, and other chemicals were purchased from Sigma-Aldrich (Munich, Germany). The Amplex[®]-Red MAO assay kit containing benzylamine, *p*-tyramine, Clorgyline, Pargyline, and horseradish peroxidase was purchased from Cell Technology Inc., Mountain View, CA, USA. Molecular modelling studies were carried using AutoDock-4.2 on RHEL-5.0 Operating system installed on Dell Precision workstation with Intel core-2 quad processor and 8 GB RAM.

4.2. Chemistry

4.2.1 General procedure for synthesis of Chalcones (*a-t*)

To a solution of 2-hydroxy acetophenone (0.01 mol/L) and appropriate benzaldehyde (0.01 mol/L) in ethanol (10 mL), was added 60% aq. sodium hydroxide solution at <10 °C with continuous stirring for a period of 30-45 min. The reaction mixture was then left at room temperature for about 48 h with occasional shaking. After 48 h, the reaction mixture was poured into icecold water, and was then adjusted to pH 2 using 6 mol/L HCl. The yellow precipitate obtained was filtered, washed with water and dried. The obtained crude was purified by column chromatography and then recrystallized from ethanol to give yellow product [14-16].

4.2.2 General procedure for synthesis of compounds (*1-20*)

To a solution of mercuric acetate (0.1 mol/L) in pyridine (10 mL) was added chalcone (0.1 mol/L) at room temperature and the mixture was refluxed at 110°C for 1 h. After completion, the reaction mixture was poured into ice cold water and acidified with hydrochloric acid (10% aqueous solution). The precipitated solid was extracted with DCM, the extracts were dried on sodium sulfate and the solvent was evaporated to give a solid which was further purified by column chromatography [17].

4.3. Biochemistry

4.3.1. Determination of hMAO-A and -B activities

The activities of hMAO-A and hMAO-B were determined using *p*-tyramine as common substrate and calculated as 177.00±9.55 pmol/mg/min (n=3) and 140.90±11.70 pmol/mg/min (n=3), respectively. The interactions of the synthesized compounds with hMAO isoforms were determined by a fluorimetric method described and modified previously activity [6,7]. The production of H₂O₂ catalyzed by MAO isoforms was detected using Amplex[®]-Red reagent, a non-fluorescent, highly sensitive, and stable probe that reacts with H₂O₂ in the presence of horseradish peroxidase to produce the fluorescent product resorufin. The reaction was started by adding (final concentrations) 200 μmol/L Amplex Red reagent, 1 U/mL horseradish peroxidase, and *p*-tyramine (concentration range 0.1-1mmol/L). Control experiments were carried out by replacing the synthesized compound and reference inhibitors. The possible capacity of compounds to modify the fluorescence generated in the reaction mixture due to nonenzymatic inhibition was determined by adding these compounds to solutions containing only the Amplex Red reagent in a sodium phosphate buffer.

4.3.2. Kinetic experiments

Synthesized compounds were dissolved in DMSO, with a maximum concentration of 1%, and used in the concentration range of 1-100 μmol/L. Kinetic data for interaction of the enzyme isoforms with the compounds were determined using the Microsoft

Excel package program. The specificity index was expressed as $SI=K_i(MAO-A)/K_i(MAO-B)$. The protein was determined according to the Bradford method [8], in which bovine serum albumin was used as standard.

4.3.3. Reversibility experiments

Reversibility of the MAO inhibition with synthesized compounds were evaluated by a centrifugation-ultrafiltration method [18]. In brief, adequate amounts of the recombinant hMAO-A or B were incubated together with a single concentration of the synthesized compounds or the reference inhibitors in a sodium phosphate buffer (0.05 mol/L, pH 7.4) for 1 h at 37 °C. After this incubation period, an aliquot was stored at 4 °C and used for the measurement of MAO-A and -B activity. The remaining incubated sample was placed in an Ultrafree-0.5 centrifugal tube with a 30 kDa biomax membrane and centrifuged at 9000×g for 20 min at 4 °C. The enzyme retained in the 30 kDa membrane was resuspended in a sodium phosphate buffer at 4 °C and centrifuged again two successive times. After the third centrifugation, the enzyme retained in the membrane was resuspended in sodium phosphate buffer (300 mL) and an aliquot of this suspension was used for MAO-A and -B activity determination. Control experiments were performed simultaneously (to define 100% MAO activity) by replacing the test drugs with appropriate dilutions of the vehicles. The corresponding values of percent (%) MAO isoform inhibition was separately calculated for samples with and without repeated washing.

4.4. Molecular docking simulation

In order to understand the interaction at molecular level, compounds (**1-20**) were docked with X-ray crystal structure of hMAO-A (PDB: 2BXR) and hMAO-B (PDB: 2BYB) using AutoDock 4.2 following the protocol reported in our earlier communications [5, 9-13]. For docking, grid parameter file (.gpf) and docking parameter files (.dpf) were written using MGLTools-1.5.6. Receptor grids were generated using 60×60×60 grid points in xyz with grid spacing of 0.375 Å. Grid box was centered on N5 atom of FAD. Map types were generated using autogrid-4.2. Docking was carried out with following parameters: number of runs: 50, population size: 150, number of evaluations: 2,500,000 and number of generations: 27,000, using autodock 4.2. Analysis of docking results was done using MGLTools-1.5.6. Top scoring molecule in the largest cluster was analyzed for its interaction with the protein.

Acknowledgement

First author acknowledges to Birla Institute of Technology for providing financial support as a prestigious Institute fellowship, also acknowledge to Central Instrumentation Facility and NMR Facility at BIT, Mesra for Spectral Characterization of the compounds.

References

- [1] Knapp, D. Robert, Cheese and monoamine oxidase inhibitors Headache, The Journal of Head and Face Pain 3 (1964) 157-158.
- [2] B. Blackwell, Hypertensive crisis due to monoamine-oxidase inhibitors, The Lancet 282 (1963) 849-851.
- [3] N. Morales-Camilo, C. O. Salas, C. Sanhueza, et al., Synthesis, Biological Evaluation, and Molecular Simulation of Chalcones and Aurones as Selective MAO_B Inhibitors, Chem. Biol. Drug Des. 85 (2015) 685–695.
- [4] F. Chimenti, R. Fioravanti, A. Bolasco, et al, A new series of flavones, thioflavones, and flavanones as selective monoamine oxidase-B inhibitors, Bioorganic Med. Chem. 18 (2010) 1273–1279.
- [5] V. N. Badavath, S. Ciftci-Yabanoglu, S. Bhakat, et al, Monoamine oxidase inhibitory activity of 2-aryl-4H-chromen-4-ones, Bioorg. Chem. 58 (2015) 72–80.
- [6] F. Chimenti, E. Maccioni, D. Secci, et al., Synthesis, stereochemical identification, and selective inhibitory activity against human monoamine oxidase-B of 2-methylcyclohexylidene-(4-arylthiazol-2-yl) hydrazones, J. Med. Chem. 51 (2008) 4874–4880.
- [7] M. Yanez, N. Fraiz, E. Cano, F. Orallo, Inhibitory effects of cis- and trans-resveratrol on noradrenaline and 5-hydroxytryptamine uptake and on monoamine oxidase activity, Biochem. Biophys. Res. Commun. 344 (2006) 688–95.
- [8] Bradford, M. Marion, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72.1-2 (1976) 248-254.
- [9] V. N. Badavath, S. Ciftci-Yabanoglu, S. S. Jadav, M. Jagrat, B. N. Sinha., G. Ucar, V. Jayaprakash, Monoamine oxidase inhibitory activity of 3, 5-biaryl-4, 5-dihydro-1H-pyrazole-1-carboxylate derivatives, Eur. J. Med. Chem. 69 (2013) 762-767.
- [10] V. N. Badavath, B. Ipek, G. Ucar, B. N. Sinha, V. Jayaprakash, Monoamine oxidase inhibitory activity of novel Pyrazoline analogues: Curcumin based design and synthesis, ACS Med. Chem. Lett. 7 (2016) 56-61.
- [11] V. N. Badavath, G. Ucar, B. N. Sinha, S. K. Mondal, V. Jayaprakash, Monoamine Oxidase Inhibitory Activity of Novel Pyrazoline Analogues: Curcumin Based Design and Synthesis-II, Chemistry Select. 1 (2016) 1-7.
- [12] V. N. Badavath, B. Ipek, G. Ucar, S. K. Mondal, B. N. Sinha, V. Jayaprakash, MAO inhibitory activity of Ferulic Acid Amides: Curcumin based design and synthesis, Arch Pharm. 348 (2015) 1-11.

- [13] V. N. Badavath, B. N. Sinha, V. Jayaprakash, Design, in-silico docking and predictive ADME properties of novel Pyrazoline derivatives with selective human MAO inhibitory activity, *Int. j. pharm. pharm. sci.* 7 (2015) 277-282.
- [14] V. N. Badavath, S.S. Jadav, B. Pastorino, et al., Synthesis and antiviral activity of 2-Phenyl-4H-chromen-4-one derivatives against Chikungunya virus, *Lett Drug DesDiscov.* 13 (2016) 1-6
- [15] T. Narender, K. Venkateswarlu, V. N. Badavath, S. Sarkar, A new chemical access for 3'-acetyl-4'-hydroxychalcones using $\text{BF}_3\text{-Et}_2\text{O}$ via a regioselective Claisen-Schmidt condensation and its application in the synthesis of chalcone hybrids, *Tetrahedron Lett.* 52 (2011) 5794-5798.
- [16] S. S. Jadav, S. Kaptein, T. Ajay kumar, et al., Design, synthesis, optimization and antiviral activity of a class of hybrid dengue virus E protein inhibitors. *Bioorg. Med. Chem. Lett.* 25. (2015) 1747-1752.
- [17] S. Venkateswarlu, G. K. Panchagnula, A. L. Gottumukkala, G. V. Subbaraju, Synthesis, structural revision, and biological activities of 4'-chloroaurone, a metabolite of marine brown alga *Spatoglossum variabile*, *Tetrahedron* 63 (2007) 6909–6914.
- [18] F. Chimenti, S. Carradori, D. Secci, et al., Synthesis and inhibitory activity against human monoamine oxidase of *N*1-thiocarbamoyl-3, 5-di (hetero) aryl-4, 5-dihydro-(1H)-pyrazole derivatives, *Eur. J. Med. Chem.* 45 (2010) 800-804.

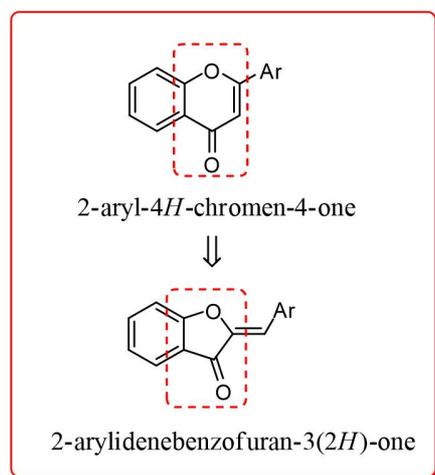


Fig. 1. Design strategy adopted for designing MAO inhibitors (**1-20**)

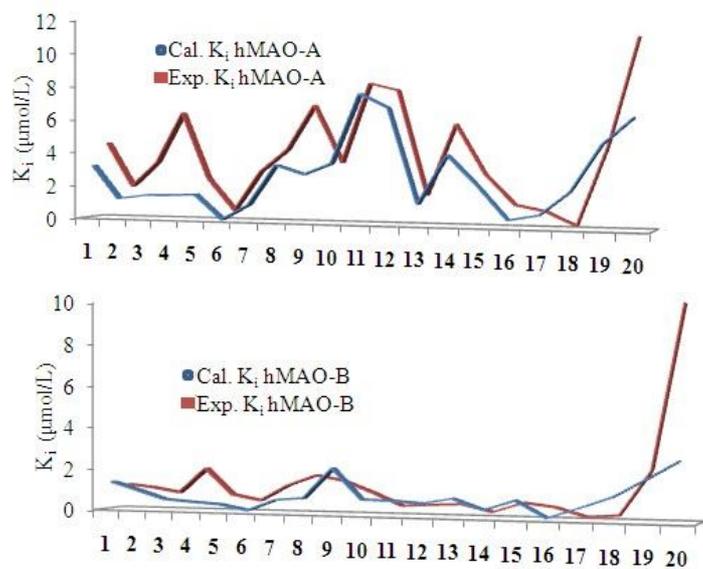


Fig. 2. Experimental and calculated K_i values corresponding to the inhibition of hMAO-isoforms with aurone derivatives

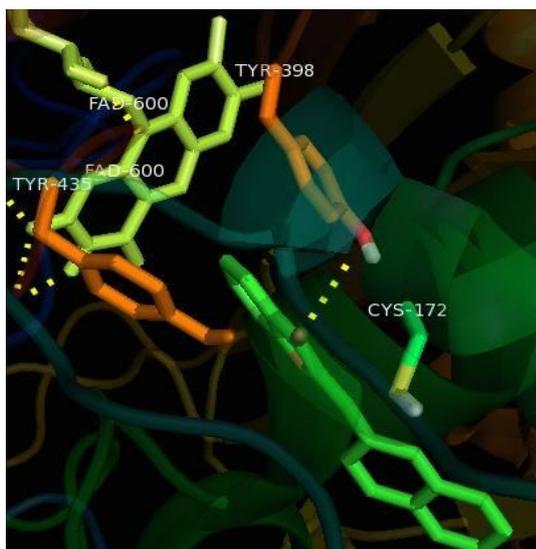
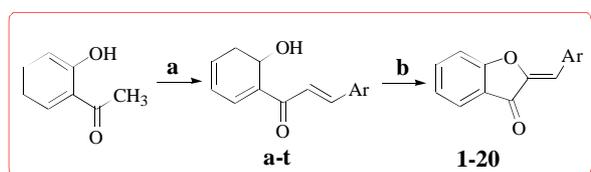


Fig. 3. Interaction of compound **17** with hMAO-B (PDB: 2BYB) active site



Ar
 a, **1**= C₆H₅ b, **2**= 2-Cl-C₆H₄ c, **3**= 4-Cl-C₆H₄ d, **4**= 4-CN-C₆H₄ e, **5**= 4-Br-C₆H₄ f, **6**= 4-NO₂-C₆H₄ g, **7**= 2-OH-C₆H₄ h, **8**= 3-OH-C₆H₄ i, **9**= 4-OH-C₆H₄ j, **10**= 3-OMe, 4-OH-C₆H₃

Ar
 k, **11**= 2-OMe-C₆H₄ l, **12**= 3-OMe-C₆H₄ m, **13**= 4-OMe-C₆H₄ n, **14**= 3, 4-OMe-C₆H₃ o, **15**= 4-Me-C₆H₄ p, **16**= naphth-1-yl q, **17**= naphth-2-yl r, **18**= anthracen-9-yl s, **19**= pyridin-2-yl t, **20**= thiophen-2-yl

Scheme 1. Reagents and conditions: a) Ar-CHO, aq. NaOH (60%), EtOH, 48 h, r.t.; b) Hg(OAc)₂, pyridine, 110 °C, 1 hr.

Table 1. Human monoamine oxidase inhibitory activity of compounds (1-20)

Compound	Cal. K_i value ($\mu\text{mol/L}$)		Exp. K_i value ($\mu\text{mol/L}$)**		MAO-B SI [†]	MAO-A SI [†]	Inhibition type	Reversibility	MAO selectivity
	MAO-A (2BXR)	MAO-B (2BYB)	MAO-A	MAO-B					
1	3.20	1.35	4.55±0.21	1.16±0.09	3.92	0.25	Competitive	Reversible	MAO-B
2	1.22	0.95	1.90±0.01	1.02±0.009	1.86	0.53	Competitive	Reversible	MAO-B
3	1.44	0.55	3.39±0.18	0.80±0.05	4.24	0.24	Competitive	Reversible	MAO-B
4	1.47	0.45	6.41±0.40	2.00±0.14	3.21	0.31	Competitive	Reversible	MAO-B
5	1.54	0.37	2.43±0.18	0.75±0.05	3.24	0.30	Competitive	Reversible	MAO-B
6	0.10	0.12	0.55±0.03	0.50±0.04	1.10	0.90	Competitive	Reversible	Non selective
7	1.02	0.64	2.90±0.15	1.25±0.09	2.32	0.43	Competitive	Reversible	MAO-B
8	3.35	0.75	4.22±0.31	1.75±0.12	2.41	0.41	Competitive	Reversible	MAO-B
9	2.83	2.16	6.85±0.51	1.50±0.13	4.57	0.21	Competitive	Reversible	MAO-B
10	3.48	0.74	3.48±0.025	0.98±0.09	3.55	0.28	Competitive	Reversible	MAO-B
11	7.47	0.72	8.10±0.55	0.40±0.02	20.25	0.05	Competitive	Reversible	MAO-B
12	6.66	0.63	7.69±0.53	0.48±0.02	16.02	0.06	Competitive	Reversible	MAO-B
13	1.24	0.88	1.66±0.11	0.54±0.03	3.07	0.33	Competitive	Reversible	MAO-B
14	4.03	0.4	5.75±0.38	0.22±0.01	26.14	0.04	Competitive	Reversible	MAO-B
15	2.35	0.86	2.90±0.16	0.67±0.04	4.33	0.23	Competitive	Reversible	MAO-B
16	0.46	0.12	1.20±0.10	0.50±0.03	2.40	0.41	Competitive	Reversible	MAO-B
17	0.76	0.59	0.88±0.06	0.10±0.01	8.80	0.11	Competitive	Reversible	MAO-B
18	2.08	1.09	0.11±0.01	0.20±0.01	0.55	1.81	Competitive	Reversible	MAO-A
19	4.66	1.86	4.50±0.36	2.20±0.18	2.05	0.48	Competitive	Reversible	MAO-B
20	6.12	2.68	10.55±1.22	9.48±0.76	1.11	0.89	Competitive	Reversible	Non selective
Selegiline	-	-	7.82±0.06	0.12±0.01	65.16	0.01	Competitive	Irreversible	MAO-B
Moclobemide	-	-	0.02±0.001	1.44±0.11	0.014	72	Competitive	Reversible	MAO-A

[†]Selectivity index. It was calculated as $K_i(\text{MAO-A})/K_i(\text{MAO-B})$ for MAO-B and $K_i(\text{MAO-B})/K_i(\text{MAO-A})$ for MAO-A.

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