Pyrazoline carboxylates as selective MAO-B inhibitors: Synthesis and Biological screening


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Abstract: A series of carboxylates of pyrazolines (3a-3b) were synthesized from ethyl 2-benzylidene-3-oxobutanato esters using hydrazine hydrate and ethanol as solvent. All the compounds (3a-3b) screened for their MAO inhibitory activity using rat brain MAO. All the compounds were found to be selective, reversible and competitive inhibitors of MAO-B except compound 3d and 3h which was found to be non-selective. These compounds were found to be active against MAO-B at concentration close to 1 μM (3e = 0.99 μM, 3a = 0.92 μM and 3b = 1.11μM). Compound 3a was potent amongst these while 3b was more selective towards MAO-B with selectivity index 2.88.

Keywords: Pyrazoline; Rat liver enzyme; MAO isoform; Selectivity; Inhibitors

1. Introduction

Monoamine oxidases (MAOs) are flavoenzymes bound to the outer mitochondrial membrane and are responsible for the oxidative deamination of neurotransmitters, dietary amines and trace of amines.1,11 The scientist Mary Bernheim reported existence of MAO enzyme first time from the various animal livers.2-3 Existence of two isoforms have been identified MAO-A and MAO-B based on their amino acid sequences4, substrate preference and inhibitor selectivity by Youdim et al.5, 6 MAO-A has a higher affinity for noradrenaline and serotonin, where as phenyl ethylamine and benzylamine were preferentially deaminated by MAO-B, this leads to the rapid degradation of these molecules and ensure that the proper functioning of synaptic neurotransmission, regulation of emotional behaviours and other brain functions.8 The lack of selectivity towards specific isoforms (MAO-A and MAO-B) created problems during the clinical use of several MAO inhibitors due their side effects. 9 In the current perspectives, enormous MAO inhibitors with selectivity towards either MAO-A or MAO-B were developed and employed for curing of the psychic disorders like anxiety, depression, Parkinson’s and Alzheimer’s diseases respectively.10-12 Pyrazolines are a class of compounds that are extensively studied for their inhibitory activity and a small review of the same is presented in the following paragraph.13-15

Pyrazolines are a class of heterocyclic compounds, important five membered nitrogen containing heterocycle and have been reported for their anti-inflammatory,16 antimicrobial,17 antitubercular,18 antiviral,19 cerebroprotective,20 analgesic,21 anticancer,22 anti tubercular and anticonvulsant etc.23,24 Pyrazolines are now becoming a popular pharmacophore for developing the new drug entities against the MAO and as anti depressant agents.15 Pyrazolines are having the capability of binding to MAO enzyme and its subtypes reversible or irreversible, they could be considered a valid pharmacophore for design, synthesis of selective monoamine oxidase (MAO) inhibitor.25 Our research team has reported a divergent class of 3,5 diaryl pyrazolines which consists of carbothiamides along with their monoamine oxidase inhibitory activity in rat MAO (either A or B).[Figure 1]26-29 The ethyl and phenyl carbamates of pyrazoline against human MAO A and B isoforms became the promising lead molecules also been reported recently.30 Stability and possibility for carrying various substitutions on pyrazolines led us to develop further newer agents against monoamine oxidase enzyme (MAO-A & MAO-B).25 The current work explains the effect of carboxylate group in the 4th position of pyrazoline and without an aryl group at 3rd position.

2. Results and Discussions

In the present context we have synthesized a few different pyrazoline analogues i.e, ethyl 3-methyl-5-phenyl-4,5-dihydro-1H-pyrazole-4-carboxylates (3a-3b) and screened for their inhibitory properties against both isoforms of MAO, as a part of continuous effort to develop the suitable lead molecules. Interestingly all the carboxylates of pyrazolines were found to be inhibiting the MAO-B isofrm by competitive, reversible and selectively except the compound 3f which was found to inhibit the MAO-A isofrm as others.

2.1. Chemistry

The Pyrazolines-4-carboxylate derivatives were synthesized through reactions outlined in Scheme 1.
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The reaction completion was monitored by using TLC plate (Merck) with iodine chamber/UV chamber. The crude products were recrystallized from suitable solvent and are characterized by FT-IR (Intermediates and final). Compound (3a-3h) have been confirmed by the by the difference in the melting point from that of the reactant.

The melting point of intermediate 1a m.p: 68 °C (Lit. 69-70 °C), 1b m.p: 140 °C (Lit. 142 °C), 1c m.p: 48 °C (Lit. 46-48 °C), 1d m.p: 88 °C (Lit. 87 °C), 1e m.p: 50 °C (Lit. 59.5-61.5 °C). All the final compounds (3a-3h) have been characterized using 1H-NMR and FAB-MS spectra.

The 1H-NMR spectra of compound (3a-3h) shows methyl protons present at N3 position of the pyrazoline ring between δ: 1.29-1.97 ppm and appeared as singlet. The -OH proton present at 5th position of the pyrazoline ring between δ: 4.47-4.50 ppm and appeared as doublet. The -NH proton appeared between δ: 6.01-8.94 ppm. For compound (3c and 3e) 2-CH2 of phenyl ring and 4-CH2 of phenyl ring on 5th position of pyrazoline ring δ: 3.63-3.83 ppm appeared as singlet respectively. For compound (3d and 3f) 2-CH of phenyl ring and 4-CH2 of phenyl ring on 5th position of pyrazoline ring at δ: 2.31-2.37 ppm appeared as singlet respectively. FAB-MS spectra of all the final compounds displayed the characteristic molecular ion peak (M+) along with (M+1)- as base peak and another [M+2]- as isotopic peak.

The Compound 3b and Compound 3g have displayed the characteristic [M+2]- isotopic peak for the presence of chloride.

2.2. Biochemistry

MAO enzyme was purified from rat liver according the procedure reported by Holt.36 The compounds (3a-3h) was screened for their inhibitory activity on MAO isoforms according to the established procedure. The biochemistry results revealed that newer carboxylates of pyrazolines (3e, 3c, 3b, 3a, 3f and 3g-3b) were found to be competitive, reversible and selective towards the MAO-B isoform except compound 3d which was non-selective towards the MAO isoform. The compounds 3a and 3e were having highest potency (IC50 = 0.92μM and 0.99μM) among the series with significant selectivity towards MAO-B, having the nitro group and methoxy group substitution at 2nd and 4th position on aromatic ring respectively. Compound 3b with chloro group substitution on 2nd position of aromatic ring exerted slight less potency and higher selectivity (IC50 = 1.11μM) than 3a and 3e. While other compounds 3g & 3f, 3h & 3c were exhibited the similar potency (IC50 = 1.0-2.0 μM) and lesser selectivity than 3b, 3h and 3e; which were having the chloro & methyl, nitro & methoxy groups substitution at 4th and 2nd position on aromatic ring respectively. The only one compound 3d was found to be having the competitive and reversible inhibitors of MAO-A isoform with weak selectivity having the methyl group at 2nd position on aromatic ring. However, the present activity data may relates with the electronegativity (EN) effect of compounds; the decline in MAO activity with decreasing EN effect was observed in ortho substituted compounds (3a-3d). The effect of EN was observed on selectivity index values of compounds 3a-3d, but the only variation was the chloro group (3b) is slightly better than nitro group (3a) were noticed. Whereas the effect of EN on the para substituted compounds were also studied and given in Figure 2. All the experimental results were presented in Table 1.

3. Experimental

Materials and Methods: Melting points were determined using automated melting point system Optimelt (Stanford research systems) by capillary method. Infrared (IR) spectra were taken on a Fourier Transform Infrared Spectrophotometer IR-Prestige 21 (Shimatu Corporation, Japan) from 4000-400 cm-1 using KBr discs. 1H-NMR spectra were recorded at 400 MHz in DMSO-d6 using a Bruker Avance 400 instrument (Bruker Instruments Inc., USA). Chemical shifts were measured at d units (ppm) relative to tetramethylsilane.

Figure 1. Pyrazolines as MAO inhibitors reported by our group

Knoevenagel condensation of appropriately substituted aromatic aldehyde with ethylacetoacetate in the presence of piperidine and glacial acetic acid provided benzylidene derivatives (2a-2h). Benzylidene derivatives (2a-2h) upon reflux with hydrazine hydrate (99%) in ethanol for about 5-6 h provided the final pyrazoline carboxylate derivatives (3a-3h).

Scheme 1. Reagents and conditions: a) R-CaH2-CHO, ethylacetoacetate, piperidine, AcOH, rt, 4-5 h; b) NH2NH2H2O 99%, EtOH, reflux, 5-6 h.
Table 1. Ki values corresponding to the inhibition of rat liver MAO isozymes by compound (3a-3h)

<table>
<thead>
<tr>
<th>Compd No.</th>
<th>R</th>
<th>R1</th>
<th>Ki-Value for (μM)</th>
<th>SI**</th>
<th>Inhibition Type</th>
<th>Reversibility</th>
<th>MAO-Selectivity</th>
</tr>
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<tbody>
<tr>
<td>3a</td>
<td>2-NO₂</td>
<td>H</td>
<td>2.45</td>
<td>0.92</td>
<td>2.66</td>
<td>Competitive</td>
<td>Reversible</td>
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<tr>
<td>3b</td>
<td>2-Cl</td>
<td>H</td>
<td>3.20</td>
<td>1.11</td>
<td>2.88</td>
<td>Competitive</td>
<td>Reversible</td>
</tr>
<tr>
<td>3c</td>
<td>2-OCH₃</td>
<td>H</td>
<td>2.22</td>
<td>1.41</td>
<td>1.53</td>
<td>Competitive</td>
<td>Reversible</td>
</tr>
<tr>
<td>3d</td>
<td>2-CH₃</td>
<td>H</td>
<td>2.16</td>
<td>2.20</td>
<td>0.98</td>
<td>Competitive</td>
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</tr>
<tr>
<td>3e</td>
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<td>4-OCH₃</td>
<td>2.03</td>
<td>0.99</td>
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<tr>
<td>3f</td>
<td>H</td>
<td>4-CH₃</td>
<td>2.09</td>
<td>1.27</td>
<td>1.64</td>
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<td>Reversible</td>
</tr>
<tr>
<td>3g</td>
<td>H</td>
<td>4-Cl</td>
<td>2.61</td>
<td>1.38</td>
<td>1.89</td>
<td>Competitive</td>
<td>Reversible</td>
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<tr>
<td>3h</td>
<td>H</td>
<td>4-NO₂</td>
<td>1.90</td>
<td>1.44</td>
<td>1.31</td>
<td>Competitive</td>
<td>Reversible</td>
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<tr>
<td>SEL</td>
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<td></td>
<td>105.66</td>
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<td>53.90</td>
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<tr>
<td>MOC</td>
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<td>0.0055</td>
<td>1.08</td>
<td>0.004</td>
<td>Competitive</td>
<td>Reversible</td>
</tr>
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</table>

SEL-selegeline, MOC-moclobemide. *Values were determined from the kinetic experiments in which p-tyramine (substrate) was used at 500 μM to measure MAO-A and 2.5 mM to measure MAO-B. Pargyline or clorgyline were added at 20 μM to determine the isozymes A and B. Newly synthesized compounds and the known inhibitors were preincubated with the homogenates for 60 min at 37 °C. Each value represents the mean ± SEM of three independent experiments. **Selectivity index was calculated as Ki (MAO-A)/Ki(MA0-B).

(TMS). Fast-atom bombardment (FAB) mass spectra were recorded on a Jeol SX 102/DA-6000 mass spectrometer (Jeol Ltd Akishima, Tokyo, Japan) using argon/xenon (6 kV, 10 mA) as FAB gas, m-nitrobenzyl alcohol as matrix, and 10 kV as accelerating voltage at room temperature. All chemicals were purchased from Merck, Spectrochem or CDH, India. Solvents were of reagent grade and were purified and dried by standard procedure. Reactions were monitored by thin-layer chromatography on silica gel plates in either iodine or UV chambers. Final compounds were characterized by 1H NMR and FAB mass spectrometry (MS).

Figure 2. The Ki values and selectivity of compounds (3a-3d) against MAO-B

3.1. Chemistry

3.1.1. General procedure for synthesis of (Z)-ethyl 2-benzylidene-3-oxobutanoate (2a-2h)

The equimolar quantities of substituted aromatic benzaldehyde (1a-1h, 0.01M) and ethylacetoacetate (0.01M) were taken in RBF. To this 2.96 mL of piperidine and 3.36mL of glacial acetic acid were added. The resulting mixture was stirred for 4-5h in an inert atmosphere at room temperature. The completion of reaction was monitored by thin layer chromatography using 20% ethyl acetate: hexane as mobile phase. After the completion of reaction, the reaction mixture was concentrated under vacuum and without further spectral characterization the resulting crude mixture was utilized for the next step.

3.1.2. General procedure for synthesis of ethyl 3-methyl-5-phenyl-4,5-dihydro-1H-pyrazole-4-carboxylate (3a-3h)

The intermediate (2a-2h) 0.01M and 0.015 M (excess) of hydrazine hydrate 99% was taken in RBF. To this 20 mL ethanol was added and mixture was refluxed for 5-6 h. The completion of reaction was monitored by thin layer chromatography using 20% ethyl acetate: hexane as mobile phase. After the reaction completion, it was kept overnight for the crystallization process. The obtained solid product was filter under vacuum and dried.

**Ethyl 3-methyl-5-(2-nitrophenyl)-4,5-dihydro-1H-pyrazole-4-carboxylate (3a) Yield: 46%; Rf: 0.33 (n-Hex: EtOAc, 8:2); mp: 167-168 °C; IR (KBr, cm⁻¹) Vmax: 3213, 2934, 2433, 1589, 1336 cm⁻¹; 'H NMR (400 MHz, DMSO-d₆): δ (ppm) 1.28 (t, 3H, J = 5.6Hz, -CH₃(CH₂)₃), 1.90 (s, 3H, Pyr-CH₃), 2.90 (d, 1H, J = 2.0Hz, Pyr-C(H)₂), 4.26-4.17 (q, 2H, J = 2.4Hz, -CH₂-CH₃), 4.49 (d, 1H, J = 1.2Hz, Pyr-C5-H), 7.01 (d, 1H, -NH), 7.72-7.66 (m, 2H, ArH), 8.06 (d, 1H, ArH), 8.16 (m, 1H, ArH); FAB-MS: 278.1 (M + 1)⁺

**Ethyl 5-(2-chlorophenyl)-3-methyl-4,5-dihydro-1H-pyrazole-4-carboxylate (3b) Yield: 54%; Rf: 0.78 (n-Hex: EtOAc, 8:2); mp: 145-147 °C; IR (KBr, cm⁻¹) Vmax: 3009, 2484, 1934, 1610, 1446, 1273, 1043 cm⁻¹; 'H NMR (400 MHz, DMSO-d₆): δ (ppm) 2.46 (s, 5H, -CH₂-CH₃), 3.27 (s, 1H, Pyr-CH₃), 7.47 (t, 2H, J = 0.8Hz, Pyr-C₄ & C₅-H), 7.51 - 7.59 (m, 3H, J = 2.0Hz, ArH), 8.12 (d, 2H, J = 1.6Hz, ArH), 8.94 (s, 1H, Pyr-NH); FAB-MS: 267.7 (M + 2)⁺

**Ethyl 5-(2-methoxphenyl)-3-methyl-4,5-dihydro-1H-pyrazole-4-carboxylate (3c) Yield: 59%; Rf: 0.70 (n-Hex: EtOAc, 8:2); mp: 218-220°C; IR (KBr, cm⁻¹) Vmax: 3061, 2961, 2843, 2033, 1606, 1486, 1031 cm⁻¹; 'H NMR (400 MHz, DMSO-d₆): δ (ppm) 1.29 (t, 3H, J = 1.8Hz, -CH₂-CH₃), 1.96 (s, 3H, Pyr-CH₃), 2.96 (d, 1H, J = 2.0Hz, Pyr-C(H)₂), 3.85 (s, 3H, Ar-CH₃), 4.15 (t, 2H, J = 3.2Hz, -CH₂-CH₃), 4.50 (d, 1H, J = 1.6Hz, Pyr-C₅-H), 6.85-6.97 (t, 1H, J = 3.6Hz, ArH), 7.01 (d, 2H, J = 0.8Hz, Pyr-NH merged with ArH), 7.15 (d, 2H, J = 2.0Hz, ArH); FAB-MS: 261.0 (M - 1)⁺

**Ethyl 3-methyl-5-o-tolyl-4,5-dihydro-1H-pyrazole-4-carboxylate (3d) Yield: 74%; Rf: 0.85 (n-Hex: EtOAc, 8:2); mp: 203-205 °C; IR (KBr, cm⁻¹) Vmax: 3001, 1923, 1610, 1460, 1219, 1039 cm⁻¹; 'H NMR (400 MHz, DMSO-
Badavath inhibitors, versus inhibitor concentration. Activity percentage, calculated in relation to a sample of intera 7.6). Assay mixture was preperoxidise type II in potassium phosphate buffer (pH 7.6). Inhibitors were then incubated with purified MAO at 37 °C for 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 these compounds were determined using Microsoft Excel package program. IC50 values were determined from plots of residual activity percentage, calculated in relation to a sample of the enzyme treated under the same conditions without inhibitors, versus inhibitor [1] concentration.

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References