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# Pyrazoline-based Mycobactin Analogues as Dual Inhibitors of MAO/Cholinesterase

## [MAO ve Kolinesteraz Dual İnhibitörü Pirazolin-Bazlı Mikobaktin Analogları]

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**ABSTRACT**

**Introduction:** We earlier reported on anti-mycobacterial and monoamine oxidase (MAO) inhibitory activity of 3, 5-diaryl-2-pyrazoline 1-carbothioamides with structural similarities to Mycobactin. The same thirty two compounds have been evaluated for Acetylcholinesterase (AChE) inhibitory activity in a search for dual inhibitors against MAO-B/AChE which may have potential therapeutic utility in Alzheimer Disease (AD).

**Materials and Methods:** Each compound was evaluated for its ability to inhibit hAChE and hBuChE using Ellman's spectrophotometric method.

**Results:** All the compounds inhibited AChE and BuChE at nM to low μM concentration. Compounds **c1-c7** were highly selective towards AChE, **c3**, **c5-7** being mixed-type reversible and the rest being non-competitive irreversible. Compounds **c12-17** were highly selective towards BuChE, **c12**, **c14-17** being mixed-type reversible and **c13** being noncompetitive irreversible. Of the selective inhibitors of rat liver MAO-B c11-17 reported earlier, **c11** was selective for AChE while **c12-17** were selective for BuChE.

**Keywords**: mycobactin analogues, aryl carbothioamide pyrazolines, MAO inhibitors.

**ÖZET**

**Giriş:** Kimyasal yapı olarak Mikobaktine benzeyen 3, 5-diaril-2-pirazolin 1-karbotiyoamid türevlerinin anti-mikobakteriyel ve monoamin oksidaz (MAO) inhibitör aktiviteleri grubumuzca daha önce yayınlanmıştır. Aynı grup bileşiklerin, MAO-B/Asetilkolinesteraz (AChE) inhibisyonu açısından dual etkili inhibitör arayışı çerçevesinde AChE inhibitör aktiviteleri bu çalışmada incelenmiştir. Bu şekilde dual etkili bir inhibitör Alzheimer Hastalığının tedavisi için yol gösterici olacaktır.

**Materyal ve Metod:** Her bileşiğin hAChE ve hBuChE enzimlerini inhibe edebilme yeteneği Ellman'nın spektrofotometrik yöntemi kullanılarak değerlendirilmiştir.

**Sonuçlar**: Test edilen otuz iki bileşiğin tamamı nM-Mm konsantrasyonlarında AChE ve BuChE enzimlerini inhibe etmiştir. Bunlardan AChE enzimine karşı selektif olan 7'sinden **(c1-7)** 4'ü **(c3, 5-7)** karışık tipte tersinir inhibisyona, geri kalanları ise yarışmasız tipte tersinmez inhibisyona yol açmıştır. BuChE enzimine karşı selektif olan 6'sından **(c12-17)** 5'i (**c12**, **c14-17**) karışık tipte tersinir inhibisyona, diğeri **(c13)** ise yarışmasız tipte tersinmez inhibisyona yol açmıştır. Daha önceden sıçan karaciğer kaynaklı MAO-B enziminin selektif inhibitörü olduğu bildirilen 7 bileşikten potent olanının (**c11**) AChE enzimine karşı geri kalan 6'sının (**c12-17**) ise BuChE enzimine karşı selektif olduğu saptanmıştır.

**Anahtar Kelimeler:** mikobaktin analogları, aril karbotiyoamid pirazolinler, MAO inhibitörleri.

## Introduction

Alzheimer's disease (AD) is the most common neurodegenerative dementia in elderly people, is characterized by the presence of senile plaques and neurofibrillary tangles in the brain and is associated with cognitive dysfunction and progressive deterioration of memory and learning processes as a result of cholinergic deficit [1,2]. Severe loss of basal forebrain cholinergic cells particularly in neocortex, hippocampus, and amygdala has been suggested to lead to diminished level of the transmitter acetylcholine (ACh) [3]. Inhibitors of AChE and BuChE that raise ACh level in brain have been successful in treatment of certain symptoms of AD [4,5].

Drugs which are selective against isoforms of a particular biological target but nonselective against two or more targets are very useful in multifactorial disorders such as the neurodegenerative diseases (Alzheimer and Parkinson) [6]. Design strategy and advantages of dual inhibitors in neurodegenerative disorders have been extensively reviewed by Andrea Cavalli et al [7]. Ladostigil, which has pharmacophoric features of both rivastigmine (AChEI) and rasagline (MAO-BI) (Figure 1), is in phase II clinical trials for the treatment of dementia with PD-like symptoms and depression [8]. Similarly dual inhibitory activity of 3, 5-diaryl-2-pyrazoline-1-thiocarbmoyl derivatives against MAO/AChE has been reported [9]. They presumed the presence of (i) hydrazine pharmacophore of isocarbaxazid in ring nitrogen of pyrazoline and (ii) carbamate pharmacophore of rivastigmine in thiocarbamoyl group at 1N position of pyrazoline. [9].

We here report the acetylcholinesterase inhibitory activity of the thirty two 3, 5-diaryl-2-pyrazoline-1-thiocarbamoyl derivatives which were synthesized as analogues of Mycobactin [10] and which have been previously reported as potent MAO inhibitors [11].

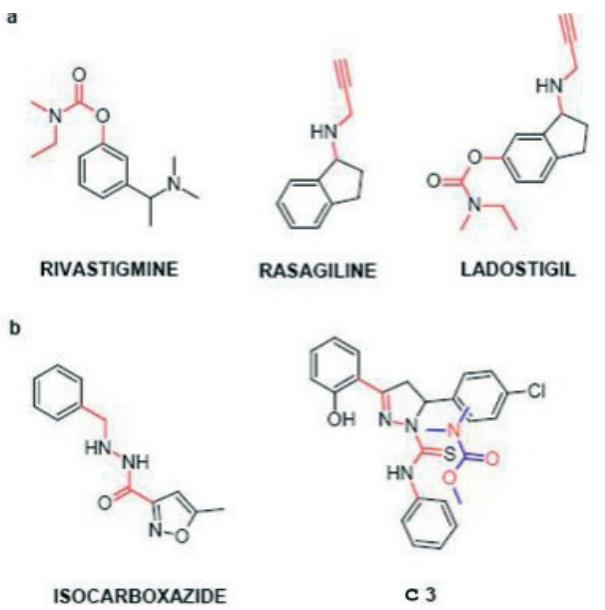



**Figure 1. (a)** Structure of Ladostigil, a dual inhibitor of MAO/AChE. **(b)** Pharmacophoric features of isocarboxazide and rivastigmine in compound **3** (carbamate superimposed on thiocarbamate).

## Materials and methods

### *Materials*

3, 5-diaryl-2-pyrazoline-1-thiocarbamoyl derivatives (1-32) were synthesized as reported earlier [10]. Human AChE (recombinant, expressed in HEK 293 cells, lyophilized powder containing phosphate buffer salt, 2000 U/mg protein) was purchased from Sigma-Aldrich (Germany). One unit is defined as amount of enzyme that hydrolyzes 1.0 µmole of acetylcholine to choline and acetate per minute at pH 8.0 at $37^0$C. Activity of AChE from electric eel on acetylcholine is 30-100 times that on butyrylcholine. BuChE from equine serum (lyophilized powder, highly purified containing buffer salts, 900 U/mg protein) was purchased from Sigma-Aldrich (Germany). One unit was defined as amount of enzyme that hydrolyzes 1.0 µmole of butyrylcholine to choline and butyrate per minute at pH 8.0 at $37^0$C. Activity on butyrylcholine was 2.5 times that on acetylcholine. All other chemicals were purchased from Sigma–Aldrich or Merck (Germany).

## Methods

Each freshly synthesized compound was dissolved in dimethyl sulfoxide (DMSO) and used in the range of 0.10-20,000 nM for inhibition of human AChE and equine serum BuChE using Ellman's spectrophotometric method [12]. AChE and BuChE activities were assayed at 25 °C, in 50 mM 3-(N-Morpholino)-propanesulfonic acid (MOPS) buffer (pH 8.0) containing 0.05–0.50 mM butyrylcholine or acetylcholine, 0.125 mM 5, 5'-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent or DTNB) and 0.1–20,000 nM of inhibitor. Reaction was initiated by addition of enzyme (0.02 U/mL) and the increase in *A*412 was measured, using a Shimadzu 1701 PC spectrophotometer equipped with a Peltier unit. The DMSO content of the assay mixture, 2% v/v, had no effect on enzyme activity. Initial rates were calculated considering $\varepsilon$TNB (412 nm) = 14.2 $mM^{-1}$ $cm^{-1}$ [13]. Each assay was repeated at least three times. Lineweaver-Burk plots were generated for each compound in the absence of inhibitor and at least at three different concentrations of inhibitor. Slopes of the lines were replotted against inhibition constant (Ki). Reversibility of inhibition was assessed by dialysis performed over 24h at 25°C relative to a 100 mM MOPS buffer (pH 8.0) capable of restoring 98-100% of the activity.

## Results and Discussion

All the compounds were active against both AChE and BuChE at nM to low µM concentration. $IC_{50}$ values in nM with Selectivity Index ($SI_{AChE}$ and $SI_{BuChE}$) and $K_i$ va-

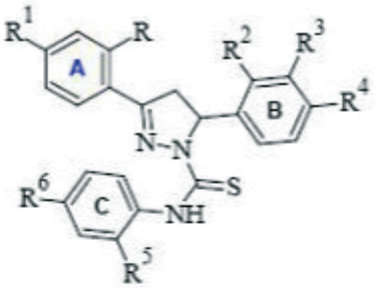
**Table 1.** Structure of 3, 5-diaryl-1-carbothioamide-pyrazoline derivatives (**c1-32**)[a]

[a]adopted from reference [11]

[a]Each value represents the mean±SEM of three independent ASSAYS .

[b]Selectivity Index for Acetylcholinesterase (BuChE/AChE)

[c]Selectivity Index for Butylcholinesterase (AChE/BuChE)




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

lues in nM with inhibition type and reversibility are presented in **Table 2** and **Table 3,** respectively. Compounds **c1-11** and **c18-24** were selective towards AChE, of which **c3**, **c5-7**, **c18** were mixed-type reversible and **c1-2**, **c4**, **c8-17** were noncompetitive irreversible. Compounds **c12-17** and **c25-30** were selective against BuChE, of which **c12**, **c14-17** and **c25-28** were mixed-type reversible and **c13**, **c29-30** were non-competitive irreversible.

Compounds c**1-7** were potent AChE inhibitors at concentration 12.93±0.20 nM (**3**) to 41.56±1.30 nM (**1**) with $SI_{AChE}$ between 263 and 96. Compound c**14** inhibited BuChE with $SI_{BuChE}$ of 391.69. Compound c**3** (Ki=12.93±0.20 nM) was a potent AChE inhibitor. Structure activity relationship for AChE inhibitory will be discussed with reference to c**3**. Hydroxy substitution in ortho position of ring A, electron rich substitution (chloro > methoxy >hydroxyl, in order of decreasing activity) in para position of ring B and presence of unsubstituted phenyl ring C were optimal for activity. Replacing phenyl ring B with thiophene (**c8**) or furan ring (c9)

**Table 2.** $IC_{50}$ values for inhibition of recombinant hAChE and equine BuChE enzymes by 3, 5-diaryl-1-carbothioamide-pyrazoline derivatives (**c1-32**)

| Code | $IC_{50}$ for AChE[a] (nM) | $IC_{50}$ for BuChE[a] (nM) | SI[b] | SI[c] | Selectivity |
|---|---|---|---|---|---|
| c 1 | 81.00±1.54 | 8653.33±136.14 | 106.83 | 0.0094 | Selective for AChE |
| c 2 | 72.33±2.08 | 8066.67±351.19 | 111.53 | 0.009 | Selective for AChE |
| c 3 | 23.47±1.17 | 6730.00±204.21 | 286.75 | 0.0035 | Selective for AChE |
| c 4 | 58.39±3.04 | 5433.33±472.58 | 93.05 | 0.0107 | Selective for AChE |
| c 5 | 39.29±0.72 | 5138.33±156.07 | 130.78 | 0.0076 | Selective for AChE |
| c 6 | 33.66±0.51 | 6195.67±137.79 | 184.07 | 0.0054 | Selective for AChE |
| c 7 | 30.64±1.00 | 4292.00±215.34 | 140.08 | 0.0071 | Selective for AChE |
| c 8 | 168.58±7.81 | 3877.33±682.47 | 23.00 | 0.0435 | Selective for AChE |
| c 9 | 192.85±12.14 | 4676.67±120.14 | 24.25 | 0.0412 | Selective for AChE |
| c 10 | 305.27±18.04 | 5430.00±242.69 | 17.79 | 0.0562 | Selective for AChE |
| c 11 | 483.20±6.79 | 3216.67±202.07 | 6.66 | 0.1502 | Selective for AChE |
| c 12 | 3836.67±261.60 | 11.58±0.43 | 0.0030 | 331.32 | Selective for BuChE |
| c 13 | 3780.00±80.00 | 21.39±1.36 | 0.0056 | 176.72 | Selective for BuChE |
| c 14 | 3230.00±286.88 | 6.31±0.34 | 0.0020 | 511.89 | Selective for BuChE |
| c 15 | 3300.00±274.04 | 9.85±0.68 | 0.0030 | 335.03 | Selective for BuChE |
| c 16 | 3393.33±460.58 | 6.45±0.37 | 0.0019 | 526.10 | Selective for BuChE |
| c 17 | 4362.00±329.17 | 16.91±0.17 | 0.0039 | 257.95 | Selective for BuChE |
| c 18 | 411.92±19.19 | 5033.33±152.75 | 12.22 | 0.0818 | Selective for AChE |
| c 19 | 474.34±17.33 | 6043.33±128.97 | 12.74 | 0.0785 | Selective for AChE |
| c 20 | 490.11±19.89 | 9140.00±163.71 | 18.65 | 0.0536 | Selective for AChE |
| c 21 | 988.63±19.93 | 9010.00±101.49 | 9.11 | 0.1097 | Selective for AChE |
| c 22 | 1110.05±105.29 | 9530.00±442.38 | 8.59 | 0.1165 | Selective for AChE |
| c 23 | 1240.3±55.67 | 10796.67±262.74 | 8.70 | 0.1149 | Selective for AChE |
| c 24 | 1500.00±147.99 | 16200.00±984.89 | 10.80 | 0.0926 | Selective for AChE |
| c 25 | 13123.33±395.77 | 403.24±3.59 | 0.0307 | 32.54 | Selective for BuChE |
| c 26 | 13050.00±180.28 | 447.18±14.66 | 0.0343 | 29.18 | Selective for BuChE |
| c 27 | 11300.00±754.98 | 480.70±4.26 | 0.0425 | 23.51 | Selective for BuChE |
| c 28 | 13353.33±455.01 | 605.07±10.43 | 0.0453 | 22.07 | Selective for BuChE |
| c 29 | 13386.67±500.53 | 585.28±30.32 | 0.0437 | 22.87 | Selective for BuChE |
| c 30 | 14326.67±857.55 | 1326.67±68.07 | 0.0926 | 10.80 | Selective for BuChE |
| c 31 | 44200.00±1178.98 | 22500±2291.29 | 0.5090 | 1.96 | Non-selective |
| c 32 | 45766.67±3262.41 | 24988.33±1426.40 | 0.5460 | 1.83 | Non-selective |

**Table 3.** Kinetic parameters for inhibition of recombinant hAChE and equine BuChE enzymes by 3, 5-diaryl-1-carbothioamide-pyrazoline derivatives (**c1-32**).

[a]Each value represents the mean±SEM of three independent assays.

| Code | $K_i$ (nM) for AChE[a] | Inhibition type and reversibility | $K_i$ (nM) for BuChE[a] | Inhibition type and reversibility |
|---|---|---|---|---|
| C 1 | 41.56±1.30 | Non-competitive Irreversible | 4003.33±15.28 | Non-competitive Irreversible |
| C 2 | 34.93±0.70 | Non-competitive Irreversible | 3963.33±55.08 | Non-competitive Irreversible |
| C 3 | 12.93±0.20 | Mix-type Reversible | 3411.33±177.37 | Non-competitive Irreversible |
| C 4 | 29.46±0.50 | Non-competitive Irreversible | 2716.67±236.29 | Non-competitive Irreversible |
| C 5 | 19.61±1.00 | Mix-type Reversible | 2683.33±76.38 | Non-competitive Irreversible |
| C 6 | 17.69±0.20 | Mix-type Reversible | 3130.00±75.50 | Non-competitive Irreversible |
| C 7 | 15.61±0.70 | Mix-type Reversible | 2706.67±120.97 | Non-competitive Irreversible |
| C 8 | 101.77±9.90 | Non-competitive Irreversible | 1466.67±83.27 | Non-competitive Irreversible |
| C 9 | 125.80±3.50 | Non-competitive Irreversible | 1535.00±45.00 | Non-competitive Irreversible |
| C 10 | 146.50±8.70 | Non-competitive Irreversible | 1602.67±21.94 | Non-competitive Irreversible |
| C 11 | 208.87±3.40 | Non-competitive Irreversible | 1636.67±15.28 | Non-competitive Irreversible |
| C 12 | 1146.67±55.10 | Non-competitive Irreversible | 5.55±0.20 | Mix-type Reversible |
| C 13 | 995.00±13.20 | Non-competitive Irreversible | 10.16±0.29 | Non-competitive Irreversible |
| C 14 | 880.00±98.50 | Non-competitive Irreversible | 2.25±0.14 | Mix-type Reversible |
| C 15 | 1250.00±50.00 | Non-competitive Irreversible | 8.79±0.30 | Mix-type Reversible |
| C 16 | 970.00±63.80 | Non-competitive Irreversible | 3.49±0.13 | Mix-type Reversible |
| C 17 | 1550.00±51.10 | Non-competitive Irreversible | 8.93±0.34 | Mix-type Reversible |
| C 18 | 153.77±10.20 | Mix-type Reversible | 2580.00±80.00 | Non-competitive Irreversible |
| C 19 | 270.43±10.40 | Non-competitive Irreversible | 2773.33±179.54 | Non-competitive Irreversible |
| C 20 | 335.43±14.90 | Non-competitive Irreversible | 3865.00±58.95 | Non-competitive Irreversible |
| C 21 | 528.83±22.60 | Non-competitive Irreversible | 3953.33±55.08 | Non-competitive Irreversible |
| C 22 | 641.87±23.80 | Non-competitive Irreversible | 4340.00±151.00 | Non-competitive Irreversible |
| C 23 | 857.67±19.90 | Non-competitive Irreversible | 6483.33±596.52 | Non-competitive Irreversible |
| C 24 | 858.00±50.30 | Non-competitive Irreversible | 7800.00±871.78 | Non-competitive Irreversible |
| C 25 | 5820.00±230.70 | Non-competitive Irreversible | 196.83±15.11 | Mix-type Reversible |
| C 26 | 5174.67±203.60 | Non-competitive Irreversible | 215.57±13.15 | Mix-type Reversible |
| C 27 | 4906.67±90.20 | Non-competitive Irreversible | 286.57±5.72 | Mix-type Reversible |
| C 28 | 5633.33±208.20 | Non-competitive Irreversible | 323.87±11.57 | Mix-type Reversible |
| C 29 | 5983.33±76.40 | Non-competitive Irreversible | 372.53±11.10 | Non-competitive Irreversible |
| C 30 | 5953.33±147.40 | Non-competitive Irreversible | 681.07±28.25 | Non-competitive Irreversible |
| C 31 | 17200.00±1249 | Non-competitive Irreversible | 11733.33±1501.1 | Non-competitive Irreversible |
| C 32 | 18800.00±264.0 | Non-competitive Irreversible | 13933.33±960.90 | Non-competitive Irreversible |

reduced potency to 8 and 10 fold respectively. In ring C, ortho (**c14-17**) or para (**c25-26**, **c29-30**) substitution reduced potency drastically between 68-119 and 400-462 fold, respectively. Disubstitution in ring A or B or both with unsubstituted or monosubstitution in C decreased potency between 12-1453 fold.

Compounds c**12-17** were potent BuChE inhibitors at concentration between 2.25±0.14 nM (**c14**) and 10.16±0.29 nM (**c13**) with $SI_{BuChE}$ between 391.69 and 97.90. **c31 and c32** inhibited nonselectively and were active at µM concentration. SAR for BuChE inhibitory activity will be discussed with reference to c**14** ($K_i$=2.25±0.14 nM). Hydroxyl substitution in ortho position of ring A and ring B and a methoxy or methyl substitution in para position (methoxy or methyl) of ring C were optimal for activity. When ring C is unsubstituted (**c1-10**) or absent (**c11**) potency decreased between 651-1779 fold. Methoxy or methyl substitution in ortho position of ring C (**c25-26**, **c29-30**) decreased the potency 87-303 fold. Disubstitution in ring A or B or both with no substitution or monosubstitution in C decreased potency 127-6192 fold.

Needed structural features for selectivity towards AChE are ortho-hydroxy functional group in ring A with electron rich functional group in either para or ortho position of ring B and an unsubstituted ring C and for selectivity towards BuChE are a substitution in ring C preferably at para position or at ortho position.

We previously reported [11] **c1-10** and **c18-24** to be selective inhibitors for rat liver MAO-A, **c11-17** to be selective inhibitors for rat liver MAO-B and **c25-32** to be nonselective and inhibit both MAO-A and MAO-B (**Table 4**).

**Table 4.** Anticholinesterase and monoamine oxidase inhibitory activity of compounds 1-7 and 11-17.

[a]Each value represents the mean±SEM of three independent assays.

[b]adopted from reference [11]

[c]Values are in µM against erythrocyte AchE from [4]

[d]Values are in µM against plasma BuChE from [4]

ovasc Res. 52: 181–198.

| Code | $IC_{50}$ for AChE[a] (nM) | $IC_{50}$ for BuChE[a] (nM) | $IC_{50}$ for MAO-A[a,b] (µM) | $IC_{50}$ for MAO-B[a,b] (µM) | Dual inhibitors proposed |
|---|---|---|---|---|---|
| c 1 | 81.00±1.54 | | 49.16±3.50 | | |
| c 2 | 72.33±2.08 | | 20.05±3.56 | | |
| **c 3** | **23.47±1.17** | | **23.18±1.58** | | **AChE/MAO-A type** |
| c 4 | 58.39±3.04 | | 58.10±3.63 | | |
| c 5 | 39.29±0.72 | | 67.22±5.80 | | |
| c 6 | 33.66±0.51 | | 74.20±6.76 | | |
| **c 7** | **30.64±1.00** | | **2.84±0.19** | | **AChE/MAO-A type** |
| **c 11** | **483.20±6.79** | | | **19.45±1.02** | **AChE/MAO-B type** |
| c 12 | | 11.58±0.43 | | 35.55±3.10 | |
| c 13 | | 21.39±1.36 | | 48.60±3.80 | |
| **c 14** | | **6.31±0.34** | | **40.78±3.66** | **BuChE/MAO-B type** |
| c 15 | | 9.85±0.68 | | 41.10±3.50 | |
| c 16 | | 6.45±0.37 | | 37.70±3.05 | |
| c 17 | | 16.91±0.17 | | 46.12±3.70 | |
| Phenothiazine | | 30.16±3.14 | | | |
| Ethopropazine | | 15.34±1.99 | | | |
| Rivastigmine | 12.23±1.34[c] | | | | |
| Donepezil | 2.45±0.39[c] | | | | |
| Pargyline | | | | 3.85±0.23[d] | |
| Selegiline | | | | 2.01±0.15[d] | |
| Clorgyline | | | 2.05±0.19 | | |
| Moclobemide | | | 3.90±0.19 | | |

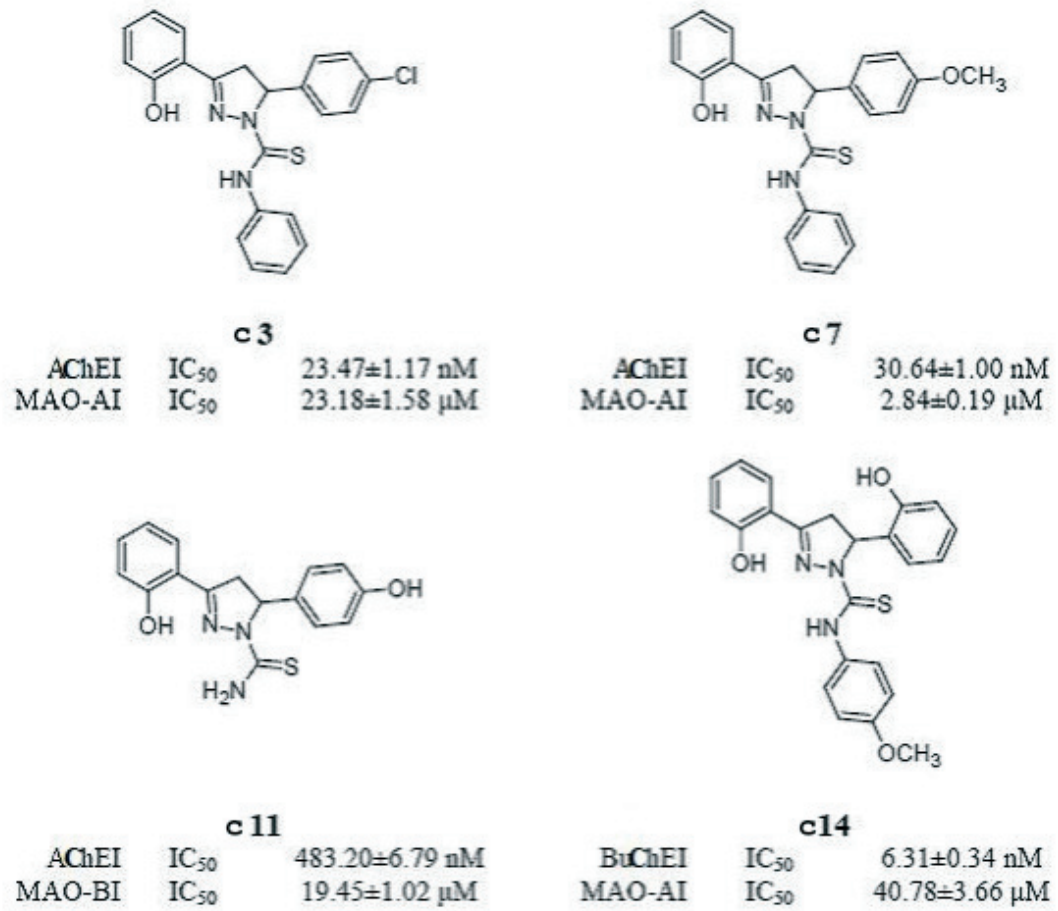



**Figure 2.** Structures of proposed dual inhibitors and their $IC_{50}$ values.

Our present study revealed that c**1-7** had dual inhibitory activity against MAO-A and AChE, c**12-17** had dual inhibitory activity against MAO-B and BuChE, and c**11** had dual inhibitory activity against MAO-B and AChE (**Table 4**) In order to have a dual activity molecule should display balanced activity profile against both targets [6]. No compound displayed such balanced activity profile against MAO/AChE. However c**3**, c**7**, c**11** and c**14** could serve as potential leads for the design and development of dual inhibitors of MAO-A/AChE, MAO-B/AChE and MAO-B/BuChE, respectively (**Figure 2**).

## Conclusion

All compounds tested were potent inhibitors of cholinesterase but very much less of monoamine oxidase. Work on other modifications c**3**, c **11** and c**14** that could provide a dual inhibitor with balanced activity profile is currently in progress.

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