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Synthesis of 3-benzyl-2-substituted quinoxalines as novel monoamine oxidase A inhibitors

Seham Y. Hassan,[a] Sherine N. Khattab,[a] Adnan A. Bekhit[b,*] and Adel Amer[a,*,†]

[a]*Department of Chemistry, Faculty of Science, University of Alexandria, Alexandria 21521, Egypt*
[b]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Alexandria, Alexandria 21521, Egypt*

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Abstract—A new series of 3-benzyl-2-substituted quinoxalines have been synthesized by means of microwave enhancement of nucleophilic substitution reaction involving the corresponding 2-chloroquinoxaline analogs and substituted amines or hydrazine. The synthesized compounds were evaluated for their monoamine oxidase A and B inhibitory activity by determination of their IC_{50}. All the newly synthesized compounds showed more selective inhibitory activity toward MAO-A than MAO-B. In addition, the acute toxicity of the synthesized compounds was determined. This work may be a fruitful matrix of the synthesis of a new series of novel MAO-A inhibitors with good safety margins.

Human monoamine oxidases A and B (MAO-A and B) are the most intensively investigated flavin-dependent amine oxidases. This is due to their roles in the metabolism of neurotransmitters such as serotonin and dopamine.[2,3] MAO-A and MAO-B are separate gene products of ~70% sequence identity with both isoforms containing 8 α-*S*-cysteinyl-FAD coenzymes as the sole redox cofactors and retaining different but partly overlapping substrate and inhibitor specificities. Due to the key role played by monoamine oxidases (MAOs) in the metabolism of neurotransmitters, MAO inhibitors (MAOIs) represent a useful tool for the treatment of several neurological diseases.[4] MAOs are implicated in a large number of neurological disorders such as Parkinson's disease and depression, and have been important targets for drug, therapy over the past 40 years.[2] Among selective MAOIs, MAO-A inhibitors are used as antidepressant and antianxiety drugs and are claimed to protect neuronal cells against apoptosis.[5] There are many different structures of MAOIs due to the fact that the active sites of the MAO-A are still unknown today which limits the design of potent selective MAOIs.[6,7] The three-dimensional structures of both MAO-A and MAO-B are therefore of interest. However, both enzymes are bound to the outer mitochondrial membrane through a C-terminal polypeptide segment and this feature has made structural investigation by X-ray crystallography more difficult. With the recent high resolution crystal structure of human MAO-B[8] and a lower resolution structure of rat MAO-A,[9] new insights into the molecular basis of MAO inhibition are now available. MAOIs can be classed into three categories: (1) irreversible inhibitors, (2) 'quasi-irreversible' inhibitors, and (3) reversible inhibitors. Those belonging to the irreversible class include the acetylenic inhibitors and the arylalkyl hydrazines.[8] These mechanism-based inhibitors form covalent adducts with the flavin. Stable N (5) flavocyanine adducts (acetylenic inhibitors) and C (4a) adducts (proposed) are formed on hydrazine inhibition. Inhibition by acetylenic inhibitors occurs in a single turnover event, while phenethylhydrazine inhibition requires ~15 turnovers/inhibition event. Attempts to determine the structure of hydrazine-inactivated MAO-B have been unsuccessful due to the lability of the adduct in the X-ray beam. The 'quasi-irreversible' inhibitors are so classified since denaturation of the inhibited enzyme results in their dissociation and include phenylcyclopropylamine ('tranylcypromine') and the *N*-(2-aminoethyl)benzamides (which belong to the 'lazabemide' class of MAO-B inhibitors). It was worthy to design a hybride structure of reversible and irreversible inhibitors to study the effect of such molecular variation on MAO inhibitor activity.

Keywords: MAO-A inhibitors; 3-Benzyl-2-substituted quinoxalines.
* Corresponding authors. Tel.: +2 012 2219463; fax: +1 781 8465563 (A.A); e-mail: ShKh2@link.net
† See Ref. 1.

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As part of our medicinal chemistry program aimed at the search for novel quinoxaline-based bioactive molecules,[10,11] we report herein our attempt to design a new lead compound by combining the different possible active sites of two previously known MAO-A inhibitors (namely, moclobemide and pargyline) in a quinoxaline model as shown in Figure 1.

Attempt to synthesize the 3-benzyl-2-(2-morpholin-4-yl-ethyl)amino-quinoxaline **4a** from the reaction of 3-benzyl-2-chloroquinoxaline with the 2-morpholin-4-ylethylamine in *n*-butanol for 48 h reflux gave a mixture of products, while the separation of the target molecule was troublesome. The reaction was repeated in the presence of ammonium chloride,[12] affording also a mixture of undesired products. However, we were able to obtain our aimed product in very high yield by effecting the reaction using microwave radiation of the reactants in isopropanol in a Pyrex-glass open vessel. The reaction mixture was irradiated in a domestic microwave oven for 15 min. Similarly, compounds **4b–4h** were prepared.[13,14] Their structures were established by IR, ^{1}H NMR, and elemental analyses.[14]

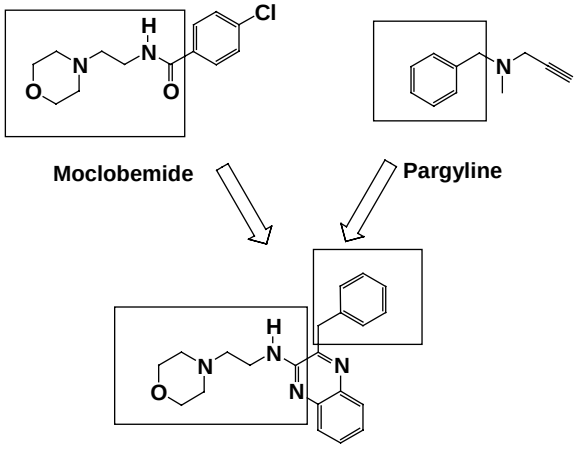


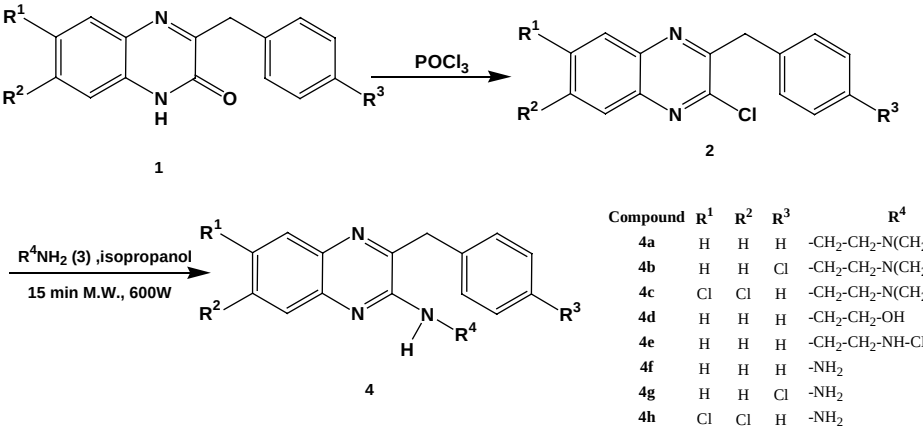
Figure 1. Planned modification and newly designed MAO-A inhibitor.

For the synthesis of desired compounds, Scheme 1 was adopted.

The final compounds **4a–4h** were evaluated for their MAO-A inhibitory activity in vitro by the method described by Undenfriend et al.[15] using serotonin (5HT) as substrate. The method depends on the determination of MAO-A activity of rat liver mitochondria.[16] All compounds under test were used at a final concentration of 1×10^{-4} M. The results were expressed as percentage inhibition of the activity of MAO-A (Table 1).

Furthermore, the synthesized compounds **4a–4h** were tested to determine their activity toward MAO-A and MAO-B selectivity in the presence of the specific substrate, serotonin or benzylamine, respectively. Bovine brain mitochondria were isolated according to Basford.[17] The activity of MAO-A and MAO-B was determined by the fluorimetric method, according to Matsumoto et al.[18] The mitochondrial fractions were preincubated at 38 °C for 30 min before adding the specific inhibitor, L-depreny (l0.5 μM), to determine the MAO-A activity and clorgyline (10.5 μM) to determine the MAO-B activity. The incubation mixture contained (0.1 ml, 0.25 M) phosphate buffer, pH 7.4, mitochondrial suspension (6 mg/1 ml), the specific substrate for MAO-A or MAO-B (0.1 mM), and test compounds at four different concentrations ranging from 5 μM to 0.1 mM dissolved in propylene glycol. The mixture was incubated in a shaking water bath at 37 °C for 60 min. The reaction was quenched by adding perchloric acid. The samples were centrifuged at 10000*g* for 5 min and the supernatant was completed to 2.7 ml of 1 N NaOH and measured on a Perkin-Elmer Lf 45 Spectrofluorimeter. Protein concentration was determined according to the reported method.[19] The MAO-A and MAO-B results were expressed as IC_{50} (Table 1). The selectivity index is also given in (Table 1).

The results revealed that all the test compounds **4a–4h** showed MAO-A inhibitory activity higher than that of MAO-B. Compounds **4a**, **4b**, and **4g** are the most selective compounds as MAO-A inhibitors.



Compound	R^1	R^2	R^3	R^4
4a	H	H	H	$-CH_2-CH_2-N(CH_2-CH_2)_2O$
4b	H	H	Cl	$-CH_2-CH_2-N(CH_2-CH_2)_2O$
4c	Cl	Cl	H	$-CH_2-CH_2-N(CH_2-CH_2)_2O$
4d	H	H	H	$-CH_2-CH_2-OH$
4e	H	H	H	$-CH_2-CH_2-NH-CH_3$
4f	H	H	H	$-NH_2$
4g	H	H	Cl	$-NH_2$
4h	Cl	Cl	H	$-NH_2$

Scheme 1.

Table 1. Effect of some quinoxaline derivatives on the activity of MAO-A and MAO-B of rat liver mitochondria

Compound	% MAO-A inhibition	MAO-A IC_{50}	MAO-B IC_{50}	Selectivity index (SI)[a]
4a	46.13 ± 1.28	$1.3 \times 10^{-9} \pm 0.03$	$8.4 \times 10^{-4} \pm 0.08$	**646153**
4b	50.52 ± 1.46	$1.7 \times 10^{-9} \pm 0.04$	$3.6 \times 10^{-4} \pm 0.16$	**211764**
4c	39.99 ± 1.67	$7.3 \times 10^{-8} \pm 0.12$	$9.6 \times 10^{-4} \pm 0.12$	13150
4d	45.97 ± 1.54	$3.7 \times 10^{-9} \pm 0.05$	$8.6 \times 10^{-5} \pm 0.24$	23243
4e	40.92 ± 2.74	$9.2 \times 10^{-8} \pm 0.04$	$7.9 \times 10^{-4} \pm 0.24$	8977
4f	54.09 + 1.36	$8.8 \times 10^{-9} \pm 0.06$	$8.4 \times 10^{-4} \pm 0.32$	95454
4g	49.48 ± 1.84	$2.1 \times 10^{-9} \pm 0.08$	$5.7 \times 10^{-4} \pm 0.42$	**271428**
4h	41.99 ± 3.12	$7.6 \times 10^{-9} \pm 0.06$	$7.4 \times 10^{-5} \pm 0.36$	9736

The results are expressed as means ± SEM. Data were analyzed by one-way analysis of variance. Student's *t* test for unpaired observations was used. *P* value = <0.001 and was significant. The number of experiments was 6.

[a] SI = MAO-B IC_{50}/MAO-A IC_{50}.

The test compounds **4a**–**4h** were further evaluated for their oral acute toxicity in male mice using a literature method.[20,21] The results indicated that test compounds proved to be non-toxic and well tolerated by the experimental animals up to 250 mg/kg, although no mortality was recorded at 500 mg/kg. Moreover, these compounds were tested for their toxicity through the parenteral route.[22] The results revealed that all the test compounds were non-toxic up to 125 mg/kg. We could conclude that the synthesis and biochemical evaluation of the new series of compound **4** led to the design of a novel class of MAO-A inhibitors with a good safety margin.

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13. General procedure for the preparation of 2-benzyl-3-chloroquinoxaline derivatives 2: a mixture of 3-benzyl-1*H*-quinoxalin-2-one derivatives **1** (10 mmol) and phosphoryl chloride (30 ml) was refluxed for 2 h, cooled, poured over crashed ice, neutralized with saturated sodium bicarbonate, filtered, and washed with water. The crude product was recrystallized from ethanol. Compound **2a**: This compound was obtained as pink crystals, 2.35 g (92.27%) yield, mp 85 °C (Lit 84–85 °C).[23] Compound **2b**: This compound was obtained as pale pink crystals, 1.96 g (67.7%) yield, mp 120 °C, ^{1}H NMR ($CDCl_3$): δ 4.46 (s, 2H, CH_2), 7.27 (m, 4H, H-2′, H-3′, H-5′, H-6′Ph), 7.76 (dt, 2H, H-6Q, H-7Q), 7.99, 8.07 (2m, 2H, H-5Q, H-8Q). Anal. Calcd for $C_{15}H_{10}Cl_2N_2$: C, 62.31; H, 3.49; N, 9.69. Found: C, 62.57; H, 3.36; N, 9.51. Compound **2c**: This compound was obtained as pale pink crystals, 2.25 g (69.5%) yield, mp 122 °C; ^{1}H NMR ($CDCl_3$): δ 4.47 (s, 2H, CH_2), 7.26 (br s, 5H, Ph), 8.09, 8.21 (2s, 2H, H-5Q, H-8Q). Anal. Calcd for $C_{15}H_9Cl_3N_2$: C, 55.67; H, 2.80; N, 8.66. Found: C, 55.83; H, 2.67; N, 8.53.
14. General procedure for the preparation of 3-benzyl-2-substituted aminoquinoxalines 4: (1 mmol) of 2-benzyl-3-chloroquinoxaline derivatives **2** was dissolved in 5 ml isopropyl alcohol in a Pyrex-glass open vessel. (2 mmol) of the primary amine **3** was added to the reaction mixture. The reaction mixture was irradiated in a domestic microwave oven for 15 min. After removal of the solvent, the product was separated by gradiant column chromatography using hexane and ethyl acetate as eluents.
Compound **4a**: This compound was obtained as yellow crystals, 0.259 g (74.3%) yield, mp 86 °C; IR (KBr): 3393 (NH) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.34 (t, 4H, 2 CH_2-N) 2.49 (t, 2H, CH_2-N), 3.53 (q, 2H, CH_2), 3.59 (t, 4H, CH_2-O), 4.27 (s, 2H, CH_2), 5.49 (br s, 1H, NH), 7.27 (m, 5H, Ph), 7.38, 7.54 (2dt, 2H, H-6Q, H-7Q), 7.69, 7.90 (2d, 2H, H-5Q, H-8Q). Anal. Calcd for $C_{21}H_{24}N_4O$: C, 72.39; H, 6.94; N, 16.08. Found: C, 72.61; H, 6.74; N, 15.89.
Compound **4b**: This compound was obtained as yellow crystals, 0.266 g (69.47%) yield, mp 77 °C; IR (KBr): 3400 (NH) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.37 (br s, 4H, 2 CH_2-N), 2.51 (t, 2H, CH_2-N), 3.53 (q, 2H, CH_2), 3.62 (br s, 4H, CH_2-O), 4.22 (s, 2H, CH_2), 5.43 (br s, 1H, NH), 7.20, 7.26 (2d, 4H, Ph), 7.39, 7.55 (2t, 2H, H-6Q, H-7Q), 7.69, 7.88 (2d, 2H, H-5Q, H-8Q). Anal. Calcd for $C_{21}H_{23}ClN_4O$: C, 65.87; H, 6.05; N, 14.63. Found: C, 66.01; H, 5.87; N, 14.39.
Compound **4c**: This compound was obtained as yellow crystals, 0.325 g (77.88%) yield, mp 140 °C; IR (KBr): 3386 (NH) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.36 (br s, 4H, 2 CH_2-N) 2.50 (t, 2H, CH_2-N), 3.47 (q, 2H, CH_2), 3.61 (br s, 4H, CH_2-O), 4.22 (s, 2H, CH_2), 5.68 (br s, 1H, NH), 7.27 (m, 5H, Ph), 7.77, 7.97 (2s, 2H, H-5Q, H-8Q). Anal. Calcd for $C_{21}H_{22}Cl_2N_4O$: C, 60.44; H, 5.31; N, 13.43. Found: C, 60.22; H, 5.57; N, 13.68.
Compound **4d**: This compound was obtained as yellow crystals, 0.196 g (70.2%) yield, mp 73–74 °C; IR (KBr): 3393 (NH), 3280 (br s, OH) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.98 (br s, 1H, OH), 3.58 (q, 2H, CH_2), 3.72 (t, 2H,

CH_2-O), 4.27 (s, 2H, CH_2), 5.17 (br s, 1H, NH), 7.28 (m, 5H, Ph), 7.40, 7.55 (2dt, 2H, H-6Q, H-7Q), 7.65, 7.91 (2d, 2H, H-5Q, H-8Q). Anal. Calcd for $C_{17}H_{17}N_3O$: C, 73.10; H, 6.13; N, 15.04. Found: C, 73.25; H, 5.93; N, 14.86.

Compound **4e**: This compound was obtained as yellow crystals, 0.252 g (86.19%) yield, mp 65 °C; IR (KBr): 3400, 3382 (NH) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.95 (br s, 1H, NH, D_2O exchangable), 2.26 (s, 3H, CH_3), 2.68 (t, 2H, CH_2), 3.51 (q, 2H, CH_2), 4.22 (s, 2H, CH_2), 5.39 (br s, H, NH, D_2O exchangable), 7.27 (m, 5H, Ph), 7.36, 7.51 (2dt, 2H, H-6Q, H-7Q), 7.67, 7.88 (2d, 2H, H-5Q, H-8Q). Anal. Calcd for $C_{18}H_{20}N_4$: C, 73.94; H, 6.89; N, 19.16. Found: C, 74.13; H, 6.59; N, 18.94.

Compound **4f**: This compound was obtained as red crystals, 0.23 g (92.0%) yield, mp 154 °C (Lit 155-157 °C).[23]

Compound **4g**: This compound was obtained as red crystals, 0.202 g (71.1%) yield, mp 135 °C; IR (KBr): 3400 (NH) cm^{-1}; ^1H NMR ($CDCl_3$): 4.18 (s, 2H, CH_2), 6.84 (m, 2H, NH_2), 7.15–7.36 (m, 4H, Ph), 7.55–7.63 (m, 2H, H-6Q, H-7Q), 7.74, 7.84 (2d, 2H, H-5Q, H-8Q), 8.61 (br s, 1H, NH). Anal. Calcd for $C_{15}H_{13}ClN_4$: C, 63.27; H, 4.60; N, 19.68. Found: C, 63.01; H, 4.82; N, 19.94.

Compound **4h**: This compound was obtained as yellow crystals, 0. 256 g (80.23%) yield, mp 225 °C; IR (KBr): 3410 (NH) cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.26 (s, 2H, CH_2), 4.86 (br s, 2H, NH_2), 7.28 (m, 5H, Ph), 7.73, 8.03 (2s, 2H, H-5Q, H-8Q), 8.69 (br s, 1H, NH). Anal. Calcd for $C_{15}H_{12}Cl_2N_4$: C, 56.44; H, 3.79; N, 17.55. Found: C, 56,69; H, 3.57; N, 17.28.

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