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Original article

# Synthesis of new series of quinoxaline based MAO-inhibitors and docking studies

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ABSTRACT

A series of 2-benzyl-3-(2-arylidenehydrazinyl)quinoxalines **3**, 4-benzyl-1-aryl-[1,2,4]triazolo[4,3-a]quinoxalines **4** and phenyl(1-aryl-[1,2,4]triazolo[4,3-a]quinoxalin-4-yl)methanones **5** analogues were synthesized and investigated for their monoamine oxidase (MAO) inhibitory property. The inhibition profile was found to be competitive for compounds **3k**, **3m**, **5f** and **5n** with MAO-A selectivity. Observation of the docked positions of these compounds revealed interactions with many residues previously reported to have an effect on the inhibition of the enzyme. The structural features of the new compounds have been determined from the microanalytical, IR, $^{1}H$, $^{13}C$ NMR spectral studies and X-ray crystalography.



## 1. Introduction

Monoamine oxidase A and B (MAO-A and -B) are flavin adenine dinucleotide (FAD) containing enzymes, which are localized in the outer mitochondrial membranes of neuronal, glial, and other cells [1,2] particularly abundant in the liver and brain [3]. These FAD dependent enzymes catalyze the oxidative deamination of a range of endogenous and exogenous monoamines [4]. The two mammalian isoforms are characterized by their different sensitivities to inhibitors and their different specificities to substrates. Thus, MAO-A is selectively inhibited by clorgyline and metabolizes serotonin preferentially, whereas MAO-B is inhibited by L-deprenyl and prefers benzylamine and phenylethylamine as substrates. Selective MAO-A inhibitors are used clinically as antidepressants and anxiolytics, while MAO-B inhibitors are used for reduction of the progression of Parkinson's disease and of symptoms associated with Alzheimer's disease. Earlier MAO-inhibitors introduced into clinical practice for the treatment of depression were abandoned due to adverse side-effect, such as 'cheese effect' characterized by hypertensive crises [5]. For this reason, research has been directed at the synthesis of new potential agents with clinical practice. Among these targeted compounds are heterocyclic hydrazines and hydrazides. Their prototype, *N′*-propan-2-ylpyridine-4-carbohydrazide, was the first modern antidepressant and was introduced into the market under the trade names of ipronid, iprozid, marsilid, propilniazida and rivivol. The discovery of this class of drugs has led to a considerable increase in their preparation as potential therapeutic agents for the treatment of central nervous system (CNS) depression.

In the course of our ongoing studies aimed at the synthesis of heterocyclic compounds of potential pharmaceutical relevance [6–10], we were interested in novel derivatives bearing a 2-benzylquinoxalinyl core. Among these compounds is 2-benzyl-3-hydrazinylquinoxaline **1** which was found to possess antimicrobial, antifungal, MAO inhibition and CNS activity [11,12]. This compound caused a significant and dose-dependent increase in pentobarbitone-sleeping time in mice when it was given intraperitoneally and orally [11]. The promising bioactive diversity of compound **1** urge us to synthesize and biologically evaluate a series of its Schiff bases and novel structural variants of [1,2,4]triazolo[4,3-a]quinoxalines derivatives on the MAO inhibitory activity.

## 2. Results and discussion

### 2.1. Chemistry

The synthesis of compounds **3**–**6** followed the general pathway elicited in Scheme 1. Treatment of **1** with carbonyl compounds

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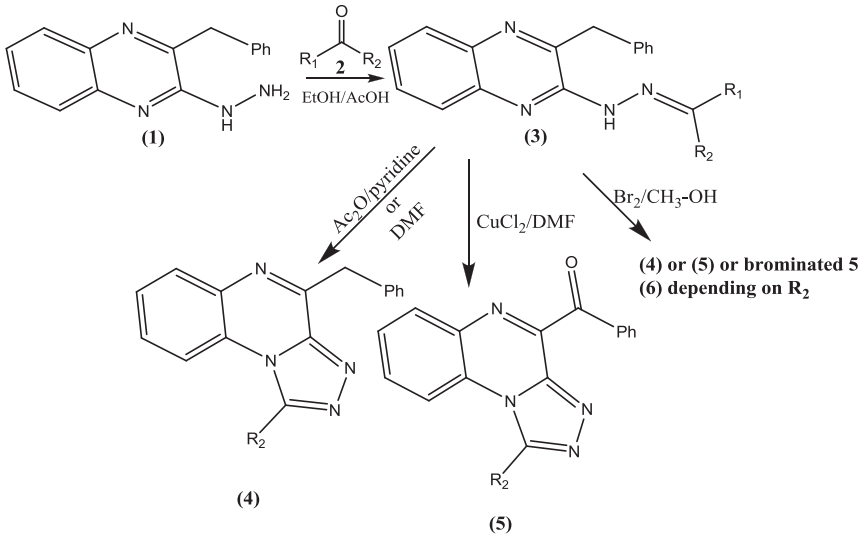


| Compound No. | $R_1$ | $R_2$ | Compound No. | $R_1$ | $R_2$ |
|---|---|---|---|---|---|
| **3a** | H | 2-$OCH_3$-$C_6H_4$ | **3l** | $CH_3$ | 4-$NO_2$-$C_6H_4$ |
| **3b** | H | 3,4-$(OCH_3)_2$-$C_6H_3$ | **3m** | H | 4-HO-$C_6H_4$ |
| **3c** | H | 3,4-$(OCH_2O)$-$C_6H_3$ | **3n** | H | 4-$N(CH_3)_2$-$C_6H_4$ |
| **3d** | $CH_3$ | 3,4,5-$(OCH_3)_3$-$C_6H_2$ | **3o** | $CH_3$ | 2-$C_5H_4N$ |
| **3e** | $CH_3$ | 4-$OCH_3$-$C_6H_4$ | **3p** | $CH_3$ | 2-$C_4H_3S$ |
| **3f** | H | $C_6H_5$ | **3q** | $CH_3$ | 4-$CH_3$-$C_6H_4$ |
| **3g** | H | 4-Cl-$C_6H_4$ | **3r** | $CH_3$ | 4-$NH_2$-$C_6H_4$ |
| **3h** | H | 2-Cl-$C_6H_4$ | **3s** | H | 4-$NO_2$-$C_6H_4$ |
| **3i** | H | 4-$OCH_3$-$C_6H_4$ | **3t** | H | 3-Br-$C_6H_4$ |
| **3j** | $CH_3$ | $C_6H_5$ | **3u** | H | 3-$CH_3$-(2-$C_4H_2S$) |
| **3k** | $CH_3$ | 4-HO-$C_6H_4$ | **3v** | H | H |

* *Same $R_1$ and $R_2$ are used for compounds 4, 5 and 6 with the same notation of 3*

**Scheme 1.**

(namely, substituted benzaldehydes, acetophenones or formaldehyde) **2** afforded the corresponding Schiff bases **3** in good yield. The structures of compounds **3** were found in agreement with the assigned molecular structure confirmed by their consistent IR and NMR spectra.

Attempt annelation of compounds **3** *via* pyrolysis in aprotic polar solvent like dimethyl-formamide led to the formation of their corresponding 1-aryl-4-benzyl-[1,2,4]triazolo[4,3-a]quinoxalines **4**. Same products were obtained when compounds **3** were exposed to acylation with acetic anhydride in pyridine. The structures of **4** were based on their NMR and elemental analysis.

On the other hand, using copper (II) chloride in dimethyl-formamide as a promoter for the annelation reaction of type **3** compounds *via* double oxidation processes afforded compounds **5** in excellent yields. Their structures were confirmed by scrutiny of their $^1$H NMR, $^{13}$C NMR, IR spectral data and elemental analyses. Compound **5v** showed unusual downfield shift of H-1 at $\delta$ 10.41 ppm and its structure was secured by X-ray crystallography (Table 1 for crystal's data). Perspective view of the molecule with atom numbering and hydrogen bonding pattern are shown in Figs. 1 and 2, respectively. Selected bond distances and angles are given in Table 2 and hydrogen bonds are shown in Table 3.

Furthermore, our attempt to cyclize **3** with bromine in methanol showed that the reaction product depends on the nature of $R_2$ which could be due to the solubility of the intermediate in each case. Thus, compound **3f** ($R_2 = C_6H_5$) afforded **4f** which precipitated out within 90 min stirring while in case of **3s** ($R_2 = 4\text{-}NO_2\text{–}C_6H_4$) and **3v** ($R_2 = H$) the oxidized compounds **5s** and **5v** were obtained. Compounds **3h** ($R_2 = 2\text{-}Cl\text{–}C_6H_4$) and **3n** ($R_2 = 4\text{-}Me_2N\text{–}C_6H_4$) furnished the brominated products **6h** and **6n** (Fig. 3), respectively. The sites of bromination were determined and secured by NMR analyses.

### 2.2. Biological activity

The newly synthesized compounds **3**–**6** were evaluated for their MAO-A inhibitory activity *in vitro* by the method described by Undenfriend et al. [13] by determining the MAO-A activity of rat liver mitochondria [14] (Table 4). Furthermore, the synthesized compounds **3**–**6** were tested to determine their activity toward MAO-A and MAO-B selectivity in the presence of their specific substrate serotonin or benzylamine, respectively [15]. Bovine brain mitochondria were isolated according to Basford [15]. The compounds **3**–**6** were tested to determine their activity toward MAO-A and MAO-B according to the methods of Matsumoto et al. [16] and Bradford [17]. The MAO-A and MAO-B results are expressed as $IC_{50}$ (Table 4). The selectivity index is also given in Table 4. The results revealed that compounds **3k**, **3m**, **5f** and **5n** showed MAO-A

**Table 1**
Crystal data and structure refinement for **5v**.

| | |
|---|---|
| Empirical formula | $C_{16}H_{10}N_4O$ |
| Formula weight | 274.28 |
| Temperature | 153(2) K |
| Wavelength | 0.71073 Å |
| Crystal system | Orthorhombic |
| Space group | $Pna2_1$ |
| Unit cell dimensions | $a = 11.1840(8)$ Å $a = 90°$<br>$b = 15.1398(11)$ Å $b = 90°$<br>$c = 7.3035(5)$ Å $g = 90°$ |
| Volume | 1236.65(15) Å$^3$ |
| Z | 4 |
| Density (calculated) | 1.473 Mg/m$^3$ |
| Absorption coefficient | 0.097 mm$^{-1}$ |
| $F(000)$ | 568 |
| Crystal size | 0.71 × 0.18 × 0.14 mm$^3$ |
| Theta range for data collection | 2.26–33.07°. |
| Index ranges | $-15 \le h \le 17$, $-22 \le k \le 21$, $-11 \le l \le 10$ |
| Reflections collected | 16,065 |
| Independent reflections | 2401 [$R$(int) = 0.0313] |
| Completeness to theta = 32.00° | 99.9% |
| Absorption correction | Multi-scan |
| Max. and min. transmission | 0.9865 and 0.9341 |
| Refinement method | Full-matrix least-squares on $F^2$ |
| Data/restraints/parameters | 2401/1/200 |
| Goodness-of-fit on $F^2$ | 1.001 |
| Final $R$ indices [$I > 2$sigma(I)] | $R1 = 0.0385$, w$R2 = 0.0980$ |
| $R$ indices (all data) | $R1 = 0.0424$, w$R2 = 0.1013$ |
| Absolute structure parameter | Not determined |
| Largest diff. peak and hole | 0.284 and −0.303 e Å$^{-3}$ |

inhibition comparable to that of Clorgyline. In addition, compounds **3k** and **5n** possessed selectivity inhibition index toward MAO-A and MAO-B comparable to those found with Clorgyline.

### 2.2.1. Modeling studies

Molecular modeling study and conformational alignment studies of the newly synthesized compounds **3**–**6** were performed in order

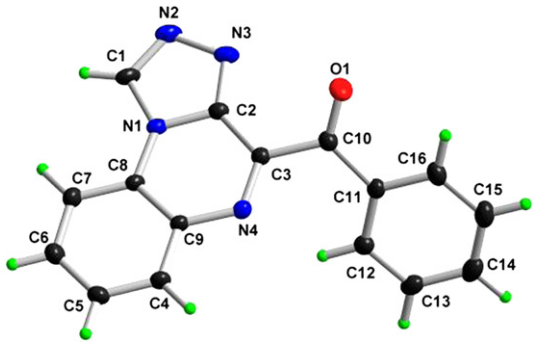


**Fig. 1.** Numbering scheme with atomic displacement ellipsoids drawn at 50% probability level of compound **5v**.

to rationalize the obtained biological results. Structure–affinity relationships (SAR) and the hypothetical binding motif of these compounds using human MAO-A crystal structure (PDB ID: 2BXR) were carried as described earlier [6,18,19]. Compounds **3k**, **3m**, **5f** and **5n** are the most active in this study, possessed good hydrogen bonding interaction beside hydrophobic interactions with amino acid residues of the active site of MAO-A enzyme. Docking of compound **3k** into the MAO-A active site revealed that several molecular interactions were considered to be responsible for the observed affinity. For instance, hydrophobic interaction was observed with PHE208 and three hydrogen bonds to SER209 and PRO72 residues (Fig. 4). Docking of compound **3m** showed a hydrophobic interaction with PHE208 and three hydrogen bonds to SER209, TYR 69 and GLN74 residues (Fig. 5). Docking of compound **5f** showed five hydrophobic interactions with ILE335, LEU337, PHE208, TYR444 and GLU216 and one hydrogen bond to SER209 (Fig. 6). On the other hand, docking of compound **5n** into the MAO-A active site showed that the molecular interactions responsible for the observed affinity were: (i) three hydrogen bond interactions with the

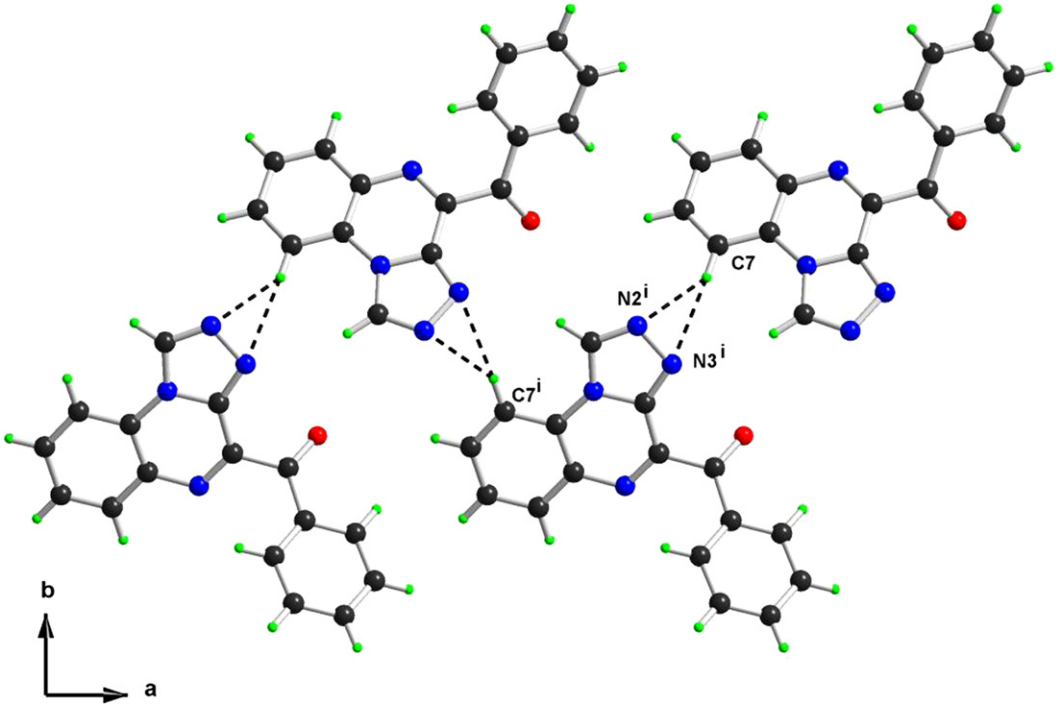


**Fig. 2.** Hydrogen bonding pattern of compound **5v**.

**Table 2**
Bond lengths [Å] and angles [°] for **5v**.

| | | | |
|---|---|---|---|
| $O_1$–$C_{10}$ | 1.2197(17) | $C_6$–$C_7$ | 1.384(2) |
| $N_1$–$C_1$ | 1.3654(17) | $C_6$–$H_6$ | 0.9500 |
| $N_1$–$C_2$ | 1.3787(16) | $C_7$–$C_8$ | 1.3952(18) |
| $N_1$–$C_8$ | 1.3941(16) | $C_7$–$H_7$ | 0.9500 |
| $N_2$–$C_1$ | 1.3134(18) | $C_8$–$C_9$ | 1.4015(16) |
| $N_2$–$N_3$ | 1.3861(18) | $C_{10}$–$C_{11}$ | 1.4926(18) |
| $N_3$–$C_2$ | 1.3224(16) | $C_{11}$–$C_{12}$ | 1.3983(19) |
| $N_4$–$C_3$ | 1.3058(16) | $C_{11}$–$C_{16}$ | 1.4005(18) |
| $N_4$–$C_9$ | 1.3936(15) | $C_{12}$–$C_{13}$ | 1.3928(19) |
| $C_1$–$H_1$ | 0.9500 | $C_{12}$–$H_{12}$ | 0.9500 |
| $C_2$–$C_3$ | 1.4455(17) | $C_{13}$–$C_{14}$ | 1.392(2) |
| $C_3$–$C_{10}$ | 1.5114(18) | $C_{13}$–H13 | 0.9500 |
| $C_4$–$C_5$ | 1.3829(19) | $C_{14}$–$C_{15}$ | 1.391(2) |
| $C_4$–$C_9$ | 1.4033(16) | $C_{14}$–$H_{14}$ | 0.9500 |
| $C_4$–$H_4$ | 0.9500 | $C_{15}$–$C_{16}$ | 1.387(2) |
| $C_5$–$C_6$ | 1.400(2) | $C_{15}$–$H_{15}$ | 0.9500 |
| $C_5$–$H_5$ | 0.9500 | $C_{16}$–$H_{16}$ | 0.9500 |
| $C_1$–$N_1$–$C_2$ | 104.98(11) | $C_5$–$C_4$–$C_9$ | 120.07(12) |
| $C_1$–$N_1$–$C_8$ | 132.33(12) | $C_9$–$C_4$–$H_4$ | 120.0 |
| $C_2$–$N_1$–$C_8$ | 122.64(11) | $C_8$–$C_7$–$H_7$ | 120.7 |
| $C_1$–$N_2$–$N_3$ | 108.62(12) | $C_6$–$C_7$–$H_7$ | 120.7 |
| $C_2$–$N_3$–$N_2$ | 106.30(11) | $N_1$–$C_8$–$C_7$ | 122.61(11) |
| $C_3$–$N_4$–$C_9$ | 119.21(11) | $N_1$–$C_8$–$C_9$ | 115.98(11) |
| $N_2$–$C_1$–$N_1$ | 109.75(13) | $C_7$–$C_8$–$C_9$ | 121.40(11) |
| $N_2$–$C_1$–$H_1$– | 125.1 | $N_4$–$C_9$–$C_8$ | 122.91(11) |
| $N_1$–$C_1$–$H_1$ | 125.1 | C11–C10–C3 | 120.01(11) |
| $N_3$–$C_2$–$N_1$ | 110.34(12) | $C_{12}$–$C_{11}$–$C_{16}$ | 119.30(13) |
| $N_3$–$C_2$–$C_3$ | 132.51(12) | $C_{12}$–$C_{11}$–$C_{10}$ | 123.59(12) |
| $N_1$–$C_2$–$C_3$ | 117.14(11) | $C_{16}$–$C_{11}$–$C_{10}$ | 117.04(12) |
| $N_4$–$C_3$–C2 | 122.09(11) | $C_{13}$–$C_{12}$–$C_{11}$ | 119.90(13) |
| $N_4$–$C_3$–$C_{10}$ | 116.92(11) | $C_{13}$–$C_{12}$–$H_{12}$ | 120.1 |
| $C_2$–$C_3$–$C_{10}$ | 120.71(11) | $C_{11}$–$C_{12}$–$H_{12}$ | 120.1 |
| $C_4$–$C_5$–$C_6$ | 120.07(13) | $C_{12}$–$C_{13}$–$C_{14}$ | 120.37(14) |
| $C_4$–$C_5$–$H_5$ | 120.0 | $C_{12}$–$C_{13}$–$H_{13}$ | 119.8 |
| $C_6$–$C_5$–$H_5$ | 120.0 | $C_{14}$–$C_{13}$–$H_{13}$ | 119.8 |
| $C_7$–$C_6$–$C_5$ | 121.03(13) | $C_{15}$–$C_{14}$–$C_{13}$ | 119.90(14) |
| $C_7$–$C_6$–$H_6$ | 119.5 | $C_{15}$–$C_{14}$–$H_{14}$ | 120.0 |
| $C_5$–$C_6$–$H_6$ | 119.5 | $C_{13}$–$C_{14}$–$H_{14}$ | 120.0 |
| $C_8$–$C_7$–$C_6$ | 118.57(12) | $C_{14}$–$C_{15}$–$C_{16}$ | 119.97(14) |
| $N_4$–$C_9$–$C_4$ | 118.22(11) | $C_{14}$–$C_{15}$–$H_{15}$ | 120.0 |
| $C_8$–$C_9$–$C_4$ | 118.85(11) | $C_{16}$–$C_{15}$–$H_{15}$ | 120.0 |
| $O_1$–$C_{10}$–$C_{11}$ | 122.25(12) | $C_{15}$–$C_{16}$–$C_{11}$ | 120.52(14) |
| $O_1$–$C_{10}$–$C_3$ | 117.71(12) | $C_{15}$–$C_{16}$–$H_{16}$ | 119.7 |
| $C_5$–$C_4$–$H_4$ | 120.0 | $C_{11}$–$C_{16}$–$H_{16}$ | 119.7 |

following residue: SER209, GLN74 and TYR444. (ii) Hydrophobic interactions with PHE208 (Fig. 7). As a result, the inhibitory profile of these compounds could be competitive. The hydrogen bonding calculation and scoring through docking in active site of MAO-A enzyme support the obtained experimental enzyme inhibitory activities for these compounds. Compound **3k** has three hydrogen bondings with good scoring and it is the most active and selective compound against MAO-A enzyme (Tables 4 and 5).

### 2.2.2. Acute toxicity

The test compounds **3k**, **3m**, **5f**, **5n** were further evaluated for their oral acute toxicity in male mice using a literature method [20–22]. The results indicated that test compounds proved to be non-toxic and well tolerated by the experimental animals up to

**Table 3**
Hydrogen bonds for **5v** [Å and°].

| D–H···A | d(D–H) | d(H···A) | d(D···A) | <(DHA) |
|---|---|---|---|---|
| C7–H7···N2$^{i}$ | 0.95 | 2.43 | 3.2607(19) | 146.0 |
| C7–H7···N3$^{i}$ | 0.95 | 2.55 | 3.4978(18) | 176.8 |
| C12–H12···N4 | 0.95 | 2.43 | 2.9751(17) | 116.3 |

Symmetry transformations used to generate equivalent atoms: (i): $x-1/2, -y+1/2, z$.

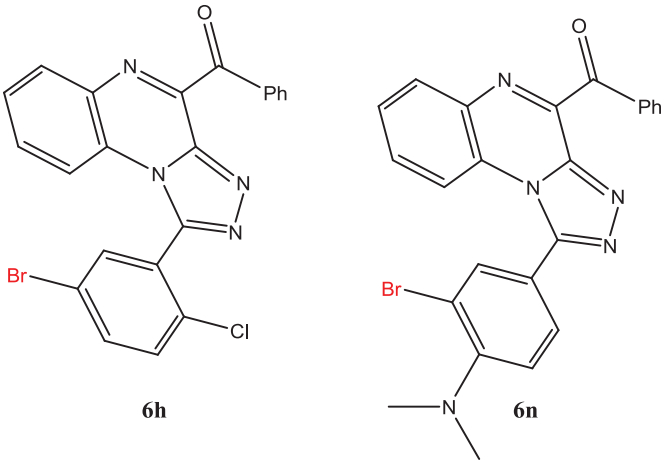


**Fig. 3.** The brominated products **6h** and **6n**.

200 mg/kg, although no mortality was recorded at 300 mg/kg. Moreover, these compounds were tested for their parenteral toxicity and the results revealed that all the test compounds were non-toxic up to 100 mg/kg. We could conclude that the synthesis and biochemical evaluation of the newly synthesized compounds led to the design of a novel class of MAO-A inhibitors with good safety margins.

**Table 4**
Effect of some quinoxaline derivatives on the activity of MAO-A and MAO-B.

| Compound | MAO-A $IC_{50}$ (M) | MAO-B $IC_{50}$ (M) | Selectivity inhibition index (SI)[a] |
|---|---|---|---|
| **3a** | $8.3 \times 10^{-8} \pm 0.12$ | $7.9 \times 10^{-4} \pm 0.4$ | $0.951 \times 10^{4}$ |
| **3b** | $5.6 \times 10^{-8} \pm 0.14$ | $4.6 \times 10^{-4} \pm 0.32$ | $0.821 \times 10^{4}$ |
| **3c** | $6.3 \times 10^{-9} \pm 0.22$ | $8.7 \times 10^{-5} \pm 0.52$ | $1.380 \times 10^{4}$ |
| **3d** | $2.9 \times 10^{-8} \pm 0.13$ | $2.6 \times 10^{-4} \pm 0.34$ | $0.896 \times 10^{4}$ |
| **3e** | $8.9 \times 10^{-8} \pm$o.18 | $2.8 \times 10^{-4} \pm 0.42$ | $0.314 \times 10^{4}$ |
| **3f** | $8.1 \times 10^{-8} \pm 0.17$ | $3.2 \times 10^{-4} \pm 0.28$ | $0.395 \times 10^{4}$ |
| **3g** | $7.6 \times 10^{-8} \pm 0.24$ | $8.4 \times 10^{-5} \pm 0.2$ | $1.105 \times 10^{3}$ |
| **3h** | $5.8 \times 10^{-9} \pm 0.12$ | $8.7 \times 10^{-5} \pm 0.12$ | $1.500 \times 10^{4}$ |
| **3i** | $6.3 \times 10^{-8} \pm 0.27$ | $7.9 \times 10^{-5} \pm 0.36$ | $1.111 \times X10^{3}$ |
| **3j** | $7.9 \times 10^{-8} \pm 0.09$ | $9.8 \times 10^{-4} \pm 0.45$ | $1.240 \times 10^{4}$ |
| **3k** | $2.8 \times 10^{-9} \pm 0.13$ | $8.4 \times 10^{-3} \pm 0.14$ | $3.000 \times 10^{6}$ |
| **3l** | $6.8 \times 10^{-7} \pm 0.54$ | $8.9 \times 10^{-5} \pm 0.49$ | $1.308 \times 10^{3}$ |
| **3m** | $3.4 \times 10^{-9} \pm 0.17$ | $7.9 \times 10^{-3} \pm 0.18$ | $2.323 \times 10^{6}$ |
| **3n** | $8.3 \times 10^{-8} \pm 0.81$ | $8.8 \times 10^{-4} \pm 0.34$ | $1.060 \times 10^{4}$ |
| **3o** | $9.1 \times 10^{-8} \pm 0.14$ | $9.8 \times 10^{-4} \pm 0.36$ | $1.076 \times 10^{4}$ |
| **3p** | $6.4 \times 10^{-8} \pm 0.21$ | $8.5 \times 10^{-4} \pm 0.15$ | $1.328 \times X10^{4}$ |
| **3q** | $8.6 \times 10^{-8} \pm 0.19$ | $7.4 \times 10^{-4} \pm 0.27$ | $0.860 \times 10^{4}$ |
| **3r** | $9.8 \times 10^{-9} \pm 0.53$ | $8.6 \times 10^{-4} \pm 0.26$ | $0.877 \times 10^{5}$ |
| **3s** | $8.8 \times 10^{-9} \pm 0.18$ | $9.6 \times 10^{-4} \pm 0.42$ | $1.090 \times 10^{4}$ |
| **3t** | $9.8 \times 10^{-9} \pm 0.44$ | $4.8 \times 10^{-5} \pm 0.08$ | $0.489 \times 10^{5}$ |
| **3u** | $7.4 \times 10^{-8} \pm 0.17$ | $8.6 \times 10^{-4} \pm 0.25$ | $1.162 \times 10^{4}$ |
| **3v** | $8.6 \times 10^{-8} \pm 0.34$ | $9.5 \times 10^{-4} \pm 0.54$ | $1.04 \times 10^{4}$ |
| **4f** | $8.2 \times 10^{-8} \pm 0.18$ | $9.8 \times 10^{-4} \pm 0.42$ | $1.195 \times 10^{4}$ |
| **4h** | $6.1 \times 10^{-9} \pm 0.26$ | $7.2 \times 10^{-5} \pm 0.46$ | $1.180 \times 10^{4}$ |
| **5b** | $6.8 \times 10^{-9} \pm 0.11$ | $8.6 \times 10^{-5} \pm 0.47$ | $1.264 \times 10^{4}$ |
| **5f** | $2.7 \times 10^{-9} \pm 0.11$ | $2.4 \times 10^{-4} \pm 0.26$ | $0.888 \times 10^{5}$ |
| **5h** | $8.8 \times 10^{-8} \pm 0.37$ | $3.8 \times 10^{-4} \pm 0.15$ | $0.431 \times 10^{4}$ |
| **5i** | $6.2 \times 10^{-9} \pm 0.48$ | $8.5 \times 10^{-5} \pm 0.37$ | $1.370 \times 10^{4}$ |
| **5n** | $2.7 \times 10^{-9} \pm 0.02$ | $7.3 \times 10^{-3} \pm 0.05$ | $2.703 \times 10^{6}$ |
| **6n** | $9.6 \times 10^{-9} \pm 0.18$ | $7.3 \times 10^{-5} \pm 0.34$ | $0.812 \times 10^{5}$ |
| **5s** | $2.2 \times 10^{-8} \pm 0.08$ | $2.3 \times 10^{-45} \pm 0.06$ | $1.045 \times 10^{4}$ |
| **5v** | $7.5 \times 10^{-7} \pm 0.04$ | $9.4 \times 10^{-4} \pm 0.18$ | $1.253 \times 10^{3}$ |
| **4v** | $8.7 \times 10^{-8} \pm 0.14$ | $9.5 \times 10^{-5} \pm 0.11$ | $1.091 \times 10^{3}$ |
| Clorgyline | $3.1 \times 10^{-9} \pm 0.13$ | $9.4 \times 10^{-3} \pm 0.16$ | $3.032 \times 10^{6}$ |

The results were expressed as mean ± S.E.M. Number of experiments was 6.
[a] SI_MAO-B $IC_{50}$/MAO-A $IC_{50}$.

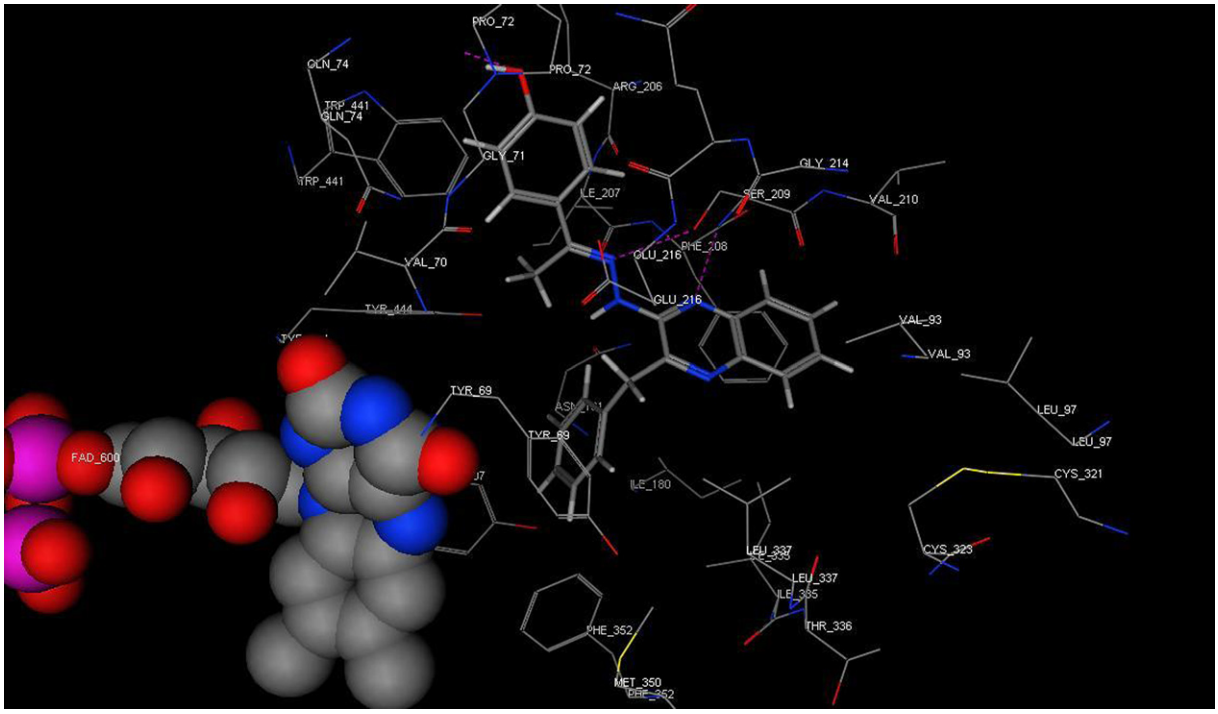


**Fig. 4.** 3D view from a molecular modelling study, of minimum-energy structure of the complex of **3k** (stick) docked in the active site of MAO-A (PDB ID: 2BXR). Dashed lines depict hydrogen bond interactions. Viewed using Molecular Operating Environment (MOE) module.

## 3. Experimental protocols

### 3.1. Chemistry

Melting points were determined with a Mel-Temp apparatus and are uncorrected. Magnetic resonance spectra ($^{1}$H NMR and $^{13}$C NMR spectra) were recorded using a JEOL 500 MHz spectrometer with the chemical shift values reported in $\delta$ units (part per million). Infrared data were obtained using a Perkin–Elmer 1600 series Fourier transform instrument as KBr pellets. The compounds were named using Chem. Draw Ultra version 12, Cambridge soft Corporation. Elemental analyses were performed on Perkin–Elmer 2400

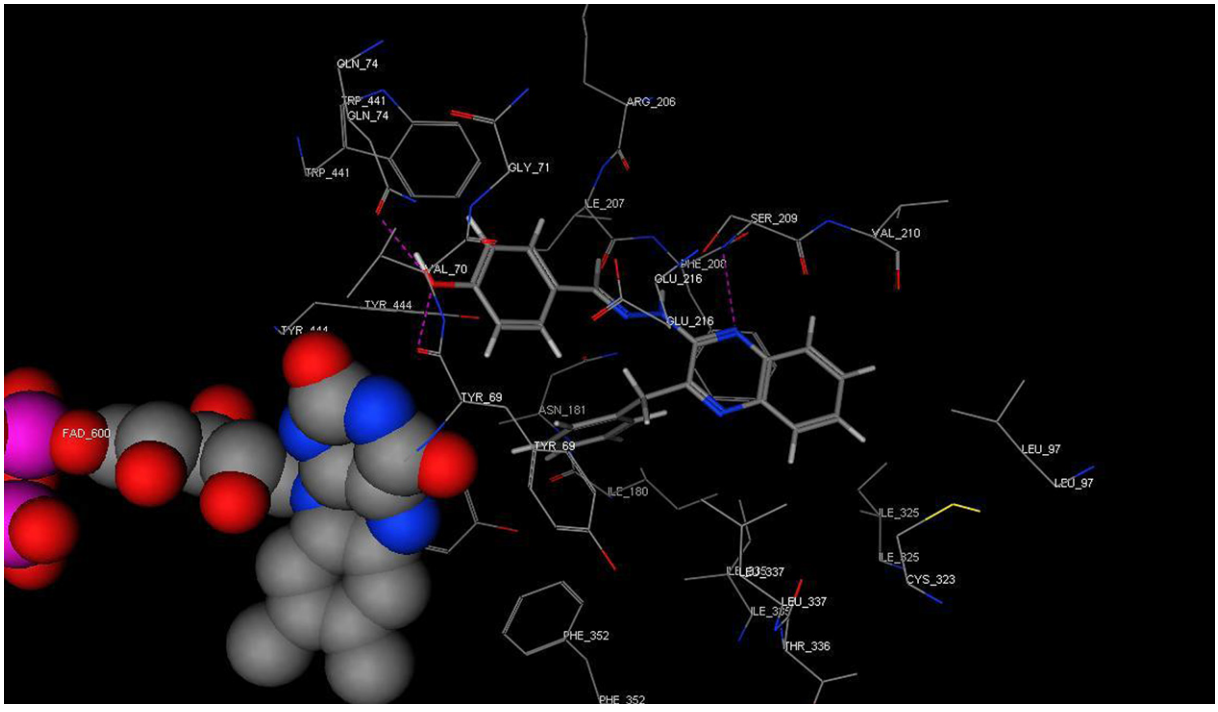


**Fig. 5.** 3D view from a molecular modelling study, of minimum-energy structure of the complex of **3m** (stick) docked in the active site of MAO-A (PDB ID: 2BXR). Dashed lines depict hydrogen bond interactions. Viewed using Molecular Operating Environment (MOE) module.

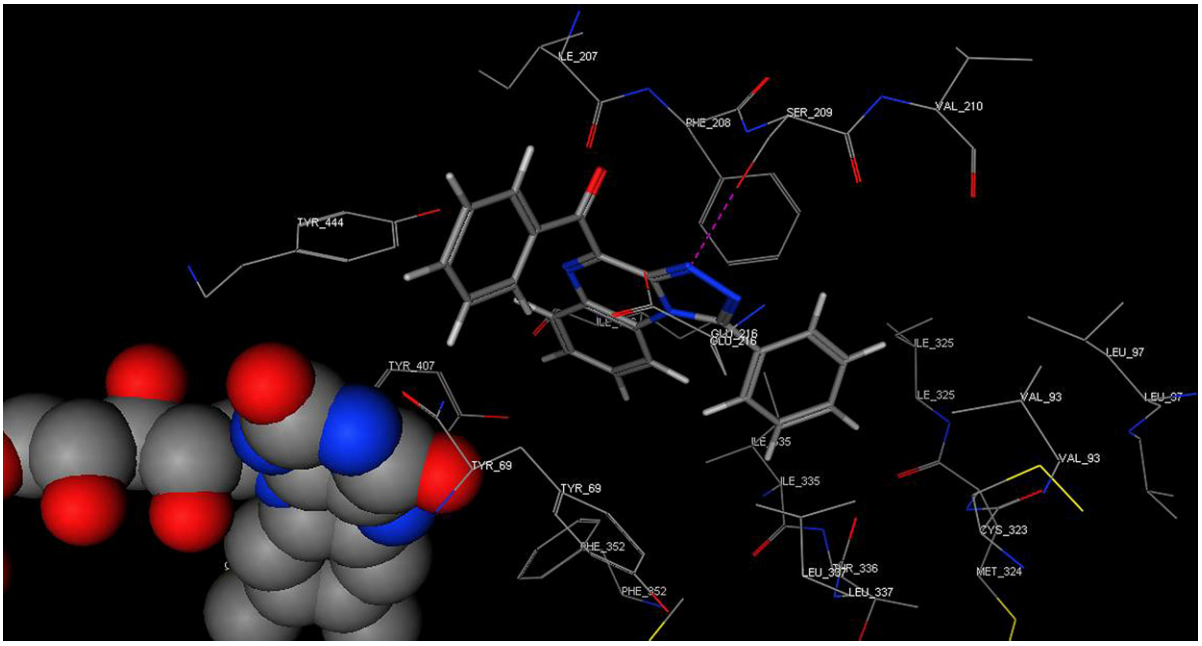


**Fig. 6.** 3D view from a molecular modelling study, of minimum-energy structure of the complex of **5f** (stick) docked in the active site of MAO-A (PDB ID: 2BXR). Dashed lines depict hydrogen bond interactions. Viewed using Molecular Operating Environment (MOE) module.

elemental analyzer, and the obtained values were within ±0.3% of the theoretical values. Follow up of the reactions and checking the purity of the compounds were made by TLC on silica gel-protected aluminum sheets (Type 60 GF254, Merck) and the spots were detected by exposure to UV-lamp at $\lambda$ 254 nm for few seconds.

*3.1.1. General procedure for the preparation of compound **(3)***

A solution of 3-benzyl-2-hydrazinylquinoxaline **1** (0.25 g, 1 mmol) in ethanol (10 mL) was added to substituted aldehydes or ketones (1 mmol) which were dissolved in ethanol (10 mL), and glacial acetic acid (2 drops) and the reaction mixture was then refluxed with for 3 h. The product was separated out on cooling, filtered off, recrystallized from ethanol and dried.

*3.1.1.1. 3-Benzyl-2-(2-(2-methoxybenzylidene)hydrazinyl)quinoxaline (**3a**).* This compound was obtained as yellow crystals (ethyl alcohol), 0.32 g (86.96%) yield, mp (°C) 130–131; IR (KBr): 3251 (NH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 3.91 (s, 3H, $CH_3$), 4.28 (s, 2H, $CH_2$),

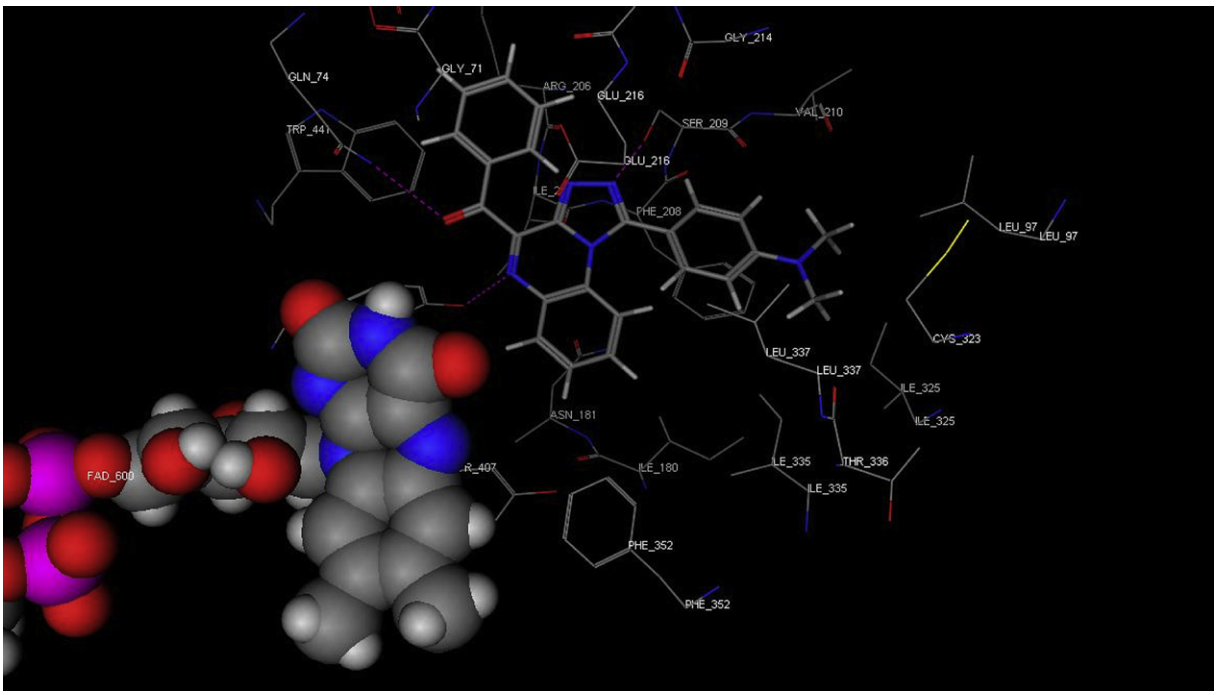


**Fig. 7.** 3D view from a molecular modelling study, of minimum-energy structure of the complex of **5n** (stick) docked in the active site of MAO-A (PDB ID: 2BXR). Dashed lines depict hydrogen bond interactions. Viewed using Molecular Operating Environment (MOE) module.

**Table 5**
Calculated docking hydrogen bonding results for compounds **3k**, **3m**, **5f** and **5n**.

| Compound | Residue | Type | Score (%) | Distance |
|---|---|---|---|---|
| **3k** | PRO72 | H-don | 46.3 | 1.07 |
| | SER209 | H-acc | 78.0 | 2.73 |
| | SER209 | H-acc | 26.4 | 2.59 |
| **3m** | TYR69 | H-don | 51.3 | 2.92 |
| | GLN74 | H-don | 68.6 | 1.67 |
| | SER209 | H-acc | 12.9 | 2.82 |
| **5f** | SER209 | H-acc | 13.5 | 2.46 |
| **5n** | GLN74 | H-acc | 22.9 | 3.04 |
| | SER209 | H-acc | 20.9 | 1.81 |
| | TYR444 | H-acc | 21.4 | 2.83 |

6.95 (t, 2H, Ar–H, $J = 9.2$ Hz), 7.01, 7.11, 7.22 (3 t, 3H, Ar–H, $J = 6.9$ Hz), 7.28–7.31 (m, 3H, Ar–H), 7.39 (t, 1H, Ar–H, $J = 7.7$ Hz), 7.54 (d, 2H, Ar–H, $J = 6.9$ Hz), 7.63 (d, 1H, Ar–H, $J = 7.7$ Hz), 8.07 (d, 1H, Ar–H, $J = 7.7$ Hz), 9.02 (s, 1H, C–H), 9.39 (s, 1H, NH, $D_2O$ exchangeable). *Anal.* Calcd for $C_{23}H_{20}N_4O$: C, 74.98; H, 5.47; N, 15.21. Found: C, 75.22; H, 5.25; N, 14.98.

*3.1.1.2. 3-Benzyl-2-(2-(3,4-dimethoxybenzylidene)hydrazinyl)quinoxaline (**3b**).* This compound was obtained as yellow crystals (ethyl alcohol), 0.373 g (93.72%) yield, mp (°C) 95–96; IR (KBr): 3253 (NH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 3.93 (s, 6H, 2 $CH_3$), 4.26 (s, 2H, $CH_2$), 6.90 (d, 1H, Ar–H, $J = 8.4$ Hz), 6.94 (d, 1H, Ar–H, $J = 9.2$ Hz), 7.10 (t, 1H, Ar–H, $J = 8.4$ Hz), 7.20 (t, 1H, Ar–H, $J = 7.7$ Hz), 7.27–7.31 (m, 4H, Ar–H), 7.43 (d, 1H, Ar–H, $J = 2.3$ Hz), 7.51 (d, 2H, Ar–H, $J = 7.7$ Hz), 7.63 (d, 1H, Ar–H, $J = 6.9$ Hz), 8.54 (s, 1H, C–H), 9.45 (s, 1H, NH, $D_2O$ exchangeable). *Anal.* Calcd for $C_{24}H_{22}N_4O_2$: C, 72.34; H, 5.57; N, 14.06. Found: C, 72.08; H, 5.41; N, 14.33.

*3.1.1.3. 2-(2-(Benzo[d][1,3]dioxol-5-ylmethylene)hydrazinyl)-3-benzylquinoxaline (**3c**).* This compound was obtained as yellow crystals (ethyl alcohol), 0.35 g (91.62%) yield, mp (°C) 179–180; IR (KBr): 3249 (NH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 4.25 (s, 2H, $CH_2$), 6.03 (s, 2H, $CH_2$), 6.86, 6.98 (2d, 2H, Ar–H, $J = 8.4$ Hz), 7.11 (t, 1H, Ar–H, $J = 6.9$ Hz), 7.17–7.22 (m, 2H, Ar–H), 7.27–7.31 (m, 3H, Ar–H), 7.45 (s, 1H, Ar–H), 7.51 (d, 2H, Ar–H, $J = 6.9$ Hz), 7.63 (d, 1H, Ar–H, $J = 6.9$ Hz), 8.49 (s, 1H, C–H), 9.31 (s, 1H, NH, $D_2O$ exchangeable). *Anal.* Calcd for $C_{23}H_{18}N_4O_2$: C, 72.24; H, 4.74; N, 14.65. Found: C, 71.97; H, 4.55; N, 14.81.

*3.1.1.4. 3-Benzyl-2-(2-(1-(3,4,5-trimethoxyphenyl)ethylidene)hydrazinyl)quinoxaline (**3d**).* This compound was obtained as yellow crystals (ethyl alcohol), 0.419 g (94.69%) yield, mp (°C) 120–121; IR (KBr): 3370 (NH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 2.48 (s, 3H, $CH_3$), 3.90 (s, 3H, $CH_3$), 3.93 (s, 6H, 2 $CH_3$), 4.28 (s, 2H, $CH_2$), 6.90 (d, 1H, Ar–H, $J = 7.7$ Hz), 7.06 (d, 2H, Ar–H, $J = 1.6$ Hz), 7.11, 7.21 (2 t, 2H, Ar–H, $J = 7.7$ Hz), 7.27–7.32 (m, 3H, Ar–H), 7.51 (d, 2H, Ar–H, $J = 7.6$ Hz), 7.66 (d, 1H, Ar–H, $J = 8.4$ Hz), 9.08 (s, 1H, NH, $D_2O$ exchangeable). *Anal.* Calcd for $C_{26}H_{26}N_4O_3$: C, 70.57; H, 5.92; N, 12.66. Found: C, 70.87; H, 6.19; N, 12.40.

*3.1.1.5. 3-Benzyl-2-(2-(1-(4-methoxyphenyl)ethylidene)hydrazinyl)quinoxaline (**3e**).* This compound was obtained as yellow crystals (ethyl alcohol), 0.375 g (98.06%) yield, mp (°C) 129–130; IR (KBr): 3377 (NH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 2.50 (s, 3H, $CH_3$), 3.86 (s, 3H, $CH_3$), 4.28 (s, 2H, $CH_2$), 6.93–6.96 (m, 3H, Ar–H), 7.10 (t, 1H, Ar–H, $J = 7.7$ Hz), 7.20 (t, 1H, Ar–H, $J = 6.9$ Hz), 7.29 (t, 3H, Ar–H, $J = 7.7$ Hz), 7.52 (d, 2H, Ar–H, $J = 6.9$ Hz), 7.64 (dd,1H, Ar–H, $J = 7.7$, $J = 1.6$ Hz), 7.84 (d, 2H, Ar–H, $J = 8.4$ Hz), 9.17 (s, 1H, NH, $D_2O$ exchangeable). *Anal.* Calcd for $C_{24}H_{22}N_4O$: C, 75.37; H, 5.80; N, 14.65. Found: C, 75.61; H, 6.08; N, 14.50.

*3.1.1.6. 3-Benzyl-2-(2-benzylidenehydrazinyl)quinoxaline (**3f**).* This compound was obtained as yellow needles (ethyl alcohol), 0.31 g (91.72%) yield, mp (°C) 145–146; IR (KBr): 3261 (NH) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 4.15 (s, 2H, $CH_2$), 7.09 (t, 1H, Ar–H, $J = 6.9$ Hz), 7.16 (t, 1H, Ar–H, $J = 7.7$ Hz), 7.26 (t, 2H, Ar–H, $J = 7.7$ Hz), 7.33–7.46 (m, 6H, Ar–H), 7.53 (t, 2H, Ar–H, $J = 7.6$ Hz),8.01 (d, 2H, Ar–H, $J = 6.9$ Hz), 8.55 (s, 1H, C–H), 11.00 (s, 1H, NH, $D_2O$ exchangeable). $^{13}C$ nmr (DMSO-$d_6$): $\delta$ 39.50, 115.56, 122.77, 126.82, 127.21, 128.56, 128.79, 128.87, 129.13, 129.76, 129.98, 130.81, 132.32, 133.10, 135.59, 138.43, 146.13, 156.60, 159.60. *Anal.* Calcd for $C_{22}H_{18}N_4$: C, 78.08; H, 5.36; N, 16.56. Found: C, 78.32; H, 5.09; N, 16.38.

*3.1.1.7. 3-Benzyl-2-(2-(4-chlorobenzylidene)hydrazinyl)quinoxaline (**3g**).* This compound was obtained as reddish brown crystals (ethyl alcohol), 0.26 g (69.90%) yield, mp (°C) 159–160; IR (KBr): 3253 (NH) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 4.15 (s, 2H, $CH_2$), 7.10–7.16 (m, 1H, Ar–H), 7.17–7.25 (m, 4H, Ar–H), 7.26 (t, 1H, Ar–H, $J = 6.9$ Hz), 7.53 (d, 4H, Ar–H, $J = 8.4$ Hz), 7.69 (m, 1H, Ar–H), 7.90 (d, 1H, Ar–H, $J = 8.4$ Hz), 8.03 (d, 1H, Ar–H, $J = 7.7$ Hz), 8.54 (s, 1H, CH), 11.06 (s, 1H, NH, $D_2O$ exchangeable). $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 39.50, 126.87, 128.42, 128.59, 128.80, 129.23, 129.29, 129.49, 129.73, 130.03, 130.21, 130.45, 131.69, 132.23, 133.16, 134.56, 135.25, 138.31, 146.30. *Anal.* Calcd for $C_{22}H_{17}ClN_4$: C, 70.87; H, 4.60; N, 15.03. Found: C, 71.05; H, 4.36; N, 14.82.

*3.1.1.8. 3-Benzyl-2-(2-(2-chlorobenzylidene)hydrazinyl)quinoxaline (**3h**).* This compound was obtained as red crystals (ethyl alcohol), 0.32 g (86.02%) yield, mp (°C) 160–161; IR (KBr): 3250 (NH) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 4.17 (s, 2H, $CH_2$), 7.12 (t, 1H, Ar–H, $J = 6.9$ Hz), 7.16 (t, 1H, Ar–H, $J = 7.7$ Hz), 7.26 (t, 3H, Ar–H, $J = 9.2$ Hz), 7.36–7.38 (m, 3H, Ar–H), 7.43–7.45 (m, 2H, Ar–H), 7.50–7.52 (m, 1H, Ar–H), 7.55 (d, 1H, Ar–H, $J = 6.9$ Hz), 8.50 (m, 1H, Ar–H), 8.79 (s, 1H, CH), 11.18 (s, 1H, NH, $D_2O$ exchangeable). *Anal.* Calcd for $C_{22}H_{17}ClN_4$: C, 70.87; H, 4.60; N, 15.03. Found: C, 70.65; H, 4.42; N, 14.77.

*3.1.1.9. 3-Benzyl-2-(2-(4-methoxybenzylidene)hydrazinyl)quinoxaline (**3i**).* This compound was obtained as yellow crystals (ethyl alcohol), 0.32 g (86.96%) yield, mp (°C) 170–171; IR (KBr): 3251 (NH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 3.86 (s, 3H, $CH_3$), 4.26 (s, 2H, $CH_2$), 6.95–6.97 (m, 3H, Ar–H), 7.10 (t, 1H, Ar–H, $J = 8.4$ Hz), 7.21 (t, 1H, Ar–H, $J = 6.9$ Hz), 7.27–7.31 (m, 3H, Ar–H), 7.53 (d, 2H, Ar–H, $J = 7.7$ Hz), 7.63 (d, 1H, Ar–H, $J = 6.9$ Hz), 7.78 (d, 2H, Ar–H, $J = 9.2$ Hz), 8.55 (s, 1H, C–H), 9.35 (s, 1H, NH, $D_2O$ exchangeable). *Anal.* Calcd for $C_{23}H_{20}N_4O$: C, 74.98; H, 5.47; N, 15.21. Found: C, 75.22; H, 5.24; N, 14.99.

*3.1.1.10. 3-Benzyl-2-(2-(1-phenylethylidene)hydrazinyl)quinoxaline (**3j**).* This compound was obtained as yellow crystals (ethyl alcohol), 0.34 g (96.60%) yield, mp (°C)- 140–141; IR (KBr): 3251 (NH) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 2.41 (s, 3H, $CH_3$), 4.16 (s, 2H, $CH_2$), 7.07 (t, 1H, Ar–H, $J = 7.7$ Hz), 7.17 (t, 1H, Ar–H, $J = 7.7$ Hz), 7.26 (t, 2H, Ar–H, $J = 7.7$ Hz), 7.31 (t, 1H, Ar–H, $J = 6.9$ Hz), 7.37–7.42 (m, 5H, Ar–H), 7.52 (d, 2H, Ar–H, $J = 7.7$ Hz), 8.03 (dd, 1H, Ar–H, $J = 7.7$, $J = 1.5$ Hz), 10.68 (s, 1H, NH, $D_2O$ exchangeable). $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 15.23, 39.50, 115.66, 122.59, 126.81, 127.57, 128.49, 128.71, 128.78, 129.60, 129.82, 129.98, 132.50, 133.03, 138.51, 138.96, 144.18, 160.17, 161.15. *Anal.* Calcd for $C_{23}H_{20}N_4$: C, 78.38; H, 5.72; N, 15.90. Found: C, 78.63; H, 5.98; N, 16.11.

*3.1.1.11. 3-Benzyl-2-(2-(1-(4-hydroxyphenyl)ethylidene)hydrazinyl)quinoxaline (**3k**).* This compound was obtained as yellow powder (ethyl alcohol), 0.31 g (84.24%) yield, mp 185–186 °C; IR (KBr): 3240 (NH) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 2.36 (s, 3H, $CH_3$), 4.14 (s, 2H, $CH_2$), 6.78 (d, 2H, Ar–H, $J = 8.4$ Hz), 7.04 (t, 1H, Ar–H, $J = 6.9$ Hz), 7.16 (t, 1H, Ar–H, $J = 7.7$ Hz), 7.23–7.30 (m, 3H, Ar–H), 7.37 (d, 2H,

Ar–H, $J = 7.7$ Hz), 7.49 (t, 2H, Ar–H), 7.91 (d, 2H, Ar–H, $J = 8.4$ Hz), 9.85 (br.s, 1H, OH, $D_2O$ exchangeable), 10.53 (s, 1H, NH, $D_2O$ exchangeable). *Anal.* Calcd for $C_{23}H_{20}N_4O$: C, 74.98; H, 5.47; N, 15.21. Found: C, 75.24; H, 5.72; N, 15.55.

*3.1.1.12. 3-Benzyl-2-(2-(1-(4-nitrophenyl)ethylidene)hydrazinyl)quinoxaline (**3l**).* This compound was obtained as yellow powder (ethyl alcohol), 0.36 g (90.68%) yield, mp (°C)- 135–136; IR (KBr): 3250 (NH) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 2.40 (s, 3H, $CH_3$), 4.19 (s, 2H, $CH_2$), 7.11 (t, 1H, Ar–H, $J = 7.7$ Hz), 7.17 (t, 1H, Ar–H, $J = 7.7$ Hz), 7.27 (t, 2H, Ar–H, $J = 6.9$ Hz), 7.33–7.39 (m, 3H, Ar–H), 7.52 (d, 1H, Ar–H, $J = 8.4$ Hz), 7.56 (d, 1H, Ar–H, $J = 7.7$ Hz), 7.72 (t, 1H, Ar–H, $J = 7.7$ Hz), 8.24 (d, 1H, Ar–H, $J = 6.9$ Hz), 8.51 (d, 1H, Ar–H, $J = 7.7$ Hz), 8.72 (s, 1H, Ar–H), 10.91 (s, 1H, NH, $D_2O$ exchangeable). $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 15.22, 31.26, 115.81, 121.84, 122.90, 124.32, 126.84, 128.60, 128.80, 129.60, 129.98, 130.25, 132.26, 133.17, 133.84, 138.44, 140.63, 144.59, 148.60, 159.10, 159.91. *Anal.* Calcd for $C_{23}H_{19}N_5O_2$: C, 69.51; H, 4.82; N, 17.62. Found: C, 69.79; H, 5.11; N, 17.45.

*3.1.1.13. 3-Benzyl-2-(2-(4-hydroxybenzylidene)hydrazinyl)quinoxaline (**3m**).* This compound was obtained as yellow powder (ethyl alcohol), 0.33 g (93.22%) yield, mp (°C) 210–211; IR (KBr): 3249 (NH) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 4.12 (s, 2H, $CH_2$), 6.82 (d, 2H, Ar–H, $J = 8.4$ Hz), 7.05 (t, 1H, Ar–H, $J = 6.9$ Hz), 7.16 (t, 1H, Ar–H, $J = 7.7$ Hz), 7.25 (t, 2H, Ar–H, $J = 7.7$ Hz), 7.31 (t, 1H, Ar–H, $J = 7.7$ Hz), 7.38 (d, 2H, Ar–H, $J = 6.9$ Hz), 7.48–7.52 (m, 2H, Ar–H), 7.84 (d, 2H, Ar–H, $J = 8.4$ Hz), 8.42 (s, 1H, CH), 9.95 (br.s, 1H, OH, $D_2O$ exchangeable), 10.81 (s, 1H, NH, $D_2O$ exchangeable). $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 39.5, 115.33, 116.03, 122.47, 126.70, 126.79, 128.46, 128.77, 128.95, 129.73, 129.84, 130.79, 132.56, 132.97, 138.49, 145.53, 156.69, 159.79, 160.30. *Anal.* Calcd for $C_{22}H_{18}N_4O$: C, 74.56; H, 5.12; N, 15.81. Found: C, 74.34; H, 4.89; N, 16.10.

*3.1.1.14. 3-Benzyl-2-(2-(4-dimethylaminobenzylidene)hydrazinyl) quinoxaline (**3n**).* This compound was obtained as red crystals (ethyl alcohol), 0.31 g (81.36%) yield, mp (°C) 170–171; IR (KBr): 3254 (NH) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 2.95 (s, 6H, $2CH_3$), 4.10 (s, 2H, $CH_2$), 6.71 (d, 2H, Ar–H, $J = 9.2$ Hz), 7.03 (t, 1H, Ar–H, $J = 8.4$ Hz), 7.15 (t, 1H, Ar–H, $J = 7.6$ Hz), 7.23–7.30 (m, 3H, Ar–H), 7.37 (d, 2H, Ar–H, $J = 7.7$ Hz), 7.45 (d, 2H, Ar–H, $J = 7.7$ Hz), 7.79 (d, 2H, Ar–H, $J = 9.2$ Hz), 8.37 (s, 1H, CH), 10.72 (s, 1H, NH, $D_2O$ exchangeable). *Anal.* Calcd for $C_{24}H_{23}N_5$: C, 75.56; H, 6.08; N, 18.36. Found: C, 75.84; H, 6.35; N, 18.07.

*3.1.1.15. 3-Benzyl-2-(2-(1-(pyridin-2-yl)ethylidene)hydrazinyl)quinoxaline (**3o**).* This compound was obtained as orange crystals (ethyl alcohol), 0.32 g (90.65%) yield, mp (°C) 140–141; IR (KBr): 3253 (NH)$cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 2.42 (s, 3H, $CH_3$), 4.19 (s, 2H, $CH_2$), 7.11 (t, 1H, Ar–H, $J = 7.7$ Hz), 7.16 (t, 1H, Ar–H, $J = 6.9$ Hz), 7.26 (t, 2H, Ar–H, $J = 7.7$ Hz), 7.33–7.40 (m, 4H, Ar–H), 7.52–7.57 (m, 2H, Ar–H), 7.85 (dt, 1H, Ar–H, $J = 7.7$, $J = 1.6$ Hz), 8.54 (d, 1H, Ar–H, $J = 8.4$ Hz), 8.60 (d, 1H, Ar–H, $J = 4.6$ Hz), 10.83 (s, 1H, NH, $D_2O$ exchangeable). $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 14.11, 39.50, 115.75, 122.15, 122.86, 124.62, 126.83, 128.60, 128.78, 129.65, 129.95, 132.29, 133.17, 138.43, 144.49, 149.14, 156.25, 160.02, 162.24. *Anal.* Calcd for $C_{22}H_{19}N_5$: C, 74.77; H, 5.42; N, 19.82. Found: C, 75.06; H, 5.21; N, 20.10.

*3.1.1.16. 3-Benzyl-2-(2-(1-(thiophen-2-yl)ethylidene)hydrazinyl)quinoxaline (**3p**).* This compound was obtained as yellow powder (ethyl alcohol), 0.32 g (89.39%) yield, mp (°C) 145–146; IR (KBr): 3248 (NH) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 2.39 (s, 3H, $CH_3$), 4.14 (s, 2H, $CH_2$), 7.06–7.17 (m, 3H, Ar–H), 7.22–7.27 (m, 2H, Ar–H), 7.31 (t, 1H, Ar–H, $J = 7.7$ Hz), 7.34–7.40 (m, 2H, Ar–H), 7.48–7.56 (m, 3H, Ar–H), 7.61 (d, 1H, Ar–H, $J = 5.4$ Hz), 10.44 (s, 1H, NH, $D_2O$ exchangeable). $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 15.92, 39.50, 115.74, 122.65, 126.80, 128.20, 128.46, 128.76, 129.45, 129.61, 129.72, 129.80, 132.39, 133.09, 133.77, 138.49, 143.52, 144.60, 152.22, 156.94, 160.03. *Anal.* Calcd for $C_{21}H_{18}N_4S$: C, 70.36; H, 5.06; N, 15.63. Found: C, 70.25; H, 5.32; N, 15.91.

*3.1.1.17. 3-Benzyl-2-(2-(1-(p-tolyl)ethylidene)hydrazinyl)quinoxaline (**3q**).* This compound was obtained as orange crystals (ethyl alcohol), 0.33 g (90.16%) yield, mp (°C)- 130–131; IR (KBr): 3249 (NH) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 2.32 (s, 3H, $CH_3$), 2.39 (s, 3H, $CH_3$), 4.16 (s, 2H, $CH_2$), 7.06 (t, 1H, Ar–H, $J = 7.7$ Hz), 7.16 (t, 1H, Ar–H, $J = 6.9$ Hz), 7.21–7.27 (m, 4H, Ar–H), 7.31 (t, 1H, Ar–H, $J = 6.9$ Hz), 7.38 (d, 2H, Ar–H, $J = 7.7$ Hz), 7.52 (d, 2H, Ar–H, $J = 7.7$ Hz), 7.95 (d, 2H, Ar–H, $J = 7.7$ Hz), 10.65 (s, 1H, NH, $D_2O$ exchangeable). $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 15.13, 21.51, 39.50, 115.63, 122.51, 126.78, 127.55, 128.48, 128.76, 129.30, 129.59, 129.76, 132.57, 133.03, 136.24, 138.55, 139.62, 144.06, 160.24, 161.11. *Anal.* Calcd for $C_{24}H_{22}N_4$: C, 78.66; H, 6.05; N, 15.29. Found: C, 78.49; H, 5.88; N, 15.52.

*3.1.1.18. 3-Benzyl-2-(2-(1-(4-aminophenyl)ethylidene)hydrazinyl) quinoxaline (**3r**).* This compound was obtained as orange powder (ethyl alcohol), 0.32 g (87.19%) yield, mp (°C)- 152–153; IR (KBr): 3261 (NH) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 2.33 (s, 1H, $CH_3$), 4.12 (s, 2H, $CH_2$), 5.52 (s, 2H, $NH_2$, $D_2O$ exchangeable), 6.55 (d, 2H, Ar–H, $J = 8.4$ Hz), 7.02 (t, 1H, Ar–H, $J = 7.7$ Hz), 7.16 (t, 1H, Ar–H, $J = 6.9$ Hz), 7.23–7.28 (m, 3H, Ar–H), 7.37 (d, 2H, Ar–H, $J = 7.7$ Hz), 7.47 (d, 2H, Ar–H, $J = 8.4$ Hz), 7.79 (d, 2H, Ar–H, $J = 8.4$ Hz), 10.43 (s, 1H, NH, $D_2O$ exchangeable). $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 14.70, 39.50, 113.53, 115.37, 122.16, 126.22, 126.76, 128.34, 128.75, 129.03, 129.59, 132.88, 132.96, 138.64, 143.41, 151.05, 160.55, 161.49. *Anal.* Calcd for $C_{23}H_{21}N_5$: C, 75.18; H, 5.76; N, 19.06. Found: C, 74.90; H, 5.96; N, 18.82.

*3.1.1.19. 3-Benzyl-2-(2-(4-nitrobenzylidene)hydrazinyl)quinoxaline (**3s**).* This compound was obtained as purple crystals (ethyl alcohol), 0.34 g (88.77%) yield, mp (°C) 140–141; IR (KBr): 3255 (NH) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 4.18 (s, 2H, $CH_2$), 7.17 (t, 2H, Ar–H, $J = 7.7$ Hz), 7.26 (t, 3H, Ar–H, $J = 7.7$ Hz), 7.40 (m, 2H, Ar–H), 7.57 (d, 2H, Ar–H, $J = 7.7$ Hz), 8.28 (d, 3H, Ar–H, $J = 6.9$ Hz), 8.67 (s, 1H, CH), 11.30 (s, 1H, NH, $D_2O$ exchangeable). $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 39.50, 115.83, 123.31, 124.35, 126.86, 128.68, 128.82, 129.55, 129.76, 130.24, 131.94, 133.42, 138.32, 141.92, 146.90, 148.37, 154.23, 159.33. *Anal.* Calcd for $C_{22}H_{17}N_5O_2$: C, 68.92; H, 4.47; N, 18.27. Found: C, 69.11; H, 4.36; N, 18.02.

*3.1.1.20. 3-Benzyl-2-(2-(3-bromobenzylidene)hydrazinyl)quinoxaline (**3t**).* This compound was obtained as yellow powder (ethyl alcohol), 0.38 g (91.13%) yield, mp (°C) 145–146; IR (KBr): 3253 (NH) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 4.15 (s, 2H, $CH_2$), 7.11 (t, 1H, Ar–H, $J = 7.6$ Hz), 7.17 (t, 1H, Ar–H, $J = 6.9$ Hz), 7.26 (t, 2H, Ar–H, $J = 7.7$ Hz), 7.34–7.42 (m, 4H, Ar–H), 7.54 (t, 2H, Ar–H, $J = 7.6$ Hz), 7.59 (d, 1H, Ar–H, $J = 7.7$ Hz), 7.92 (d, 1H, Ar–H, $J = 7.7$ Hz), 8.31 (s, 1H, Ar–H), 8.52 (s, 1H, CH), 11.11 (s, 1H, NH, $D_2O$ exchangeable). $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 39.50, 115.70, 122.76, 122.96, 126.83, 128.26, 128.60, 128.79, 128.97, 129.77, 130.06, 130.50, 131.27, 132.16, 133.19, 133.25, 138.05, 138.37, 143.14, 146.44, 155.01, 159.49. *Anal.* Calcd for $C_{22}H_{17}BrN_4$: C, 63.32; H, 4.11; N, 13.43. Found: C, 63.55; H, 4.32; N, 13.21.

*3.1.1.21. 3-Benzyl-2-(2-((3-methylthiophen-2-yl)methylene)hydrazinyl)quinoxaline (**3u**).* This compound was obtained as yellow powder (ethyl alcohol), 0.33 g (92.18%) yield, mp (°C) 147–148; IR (KBr): 3250 (NH) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 2.36 (s, 3H, $CH_3$), 4.13 (s, 2H, $CH_2$), 6.96–6.99 (m, 1H, Ar–H), 7.05–7.10 (m, 1H, Ar–H), 7.16 (t, 1H, Ar–H, $J = 6.9$ Hz), 7.22–7.27 (m, 2H, Ar–H), 7.30–7.34 (m, 1H,

Ar–H), 7.37–7.41 (m, 2H, Ar–H), 7.47–7.54 (m, 2H, Ar–H), 7.6 (d, 1H, Ar–H, $J = 4.6$ Hz), 8.69 (s, 1H, CH), 10.76 (s, 1H, NH, $D_2O$ exchangeable). $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 14.31, 39.50, 115.73, 122.69, 126.79, 128.44, 128.78, 128.98, 129.41, 129.45, 129.77, 129.84, 131.54, 138.48, 138.60, 141.41, 141.99, 144.22, 145.14, 149.82, 159.63. *Anal.* Calcd for $C_{21}H_{18}N_4S$: C, 70.36; H, 5.06; N, 15.63. Found: C, 70.11; H, 5.34; N, 15.89.

*3.1.2. Procedure for the preparation of 2-benzyl-3-(2-methylenehydrazinyl) quinoxaline (**3v**)*

*3.1.2.1. Method A.* A solution of 3-benzyl-2-hydrazinylquinoxaline **1** (0.25 g, 1 mmol) in ethanol (10 mL) was added to paraformaldehyde (2 mmol) which was dissolved in ethanol (10 mL),and glacial acetic acid (2 drops) and the reaction mixture was then refluxed with for 3 hours. The product was separated out on cooling, filtered off, recrystallized from ethanol, and dried. The product was obtained as beige needles (ethyl alcohol), 0.21 g (80%) yield, mp (°C) 205; IR (KBr): 3210 (NH) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 4.54 (s, 2H, $CH_2$), 7.18 (t, 1H, Ar–H, $J = 7.7$ Hz), 7.27 (t, 2H, Ar–H, $J = 7.6$ Hz), 7.43 (d, 2H, Ar–H, $J = 7.7$ Hz), 7.74 (t, 2H, Ar–H, $J = 7.7$ Hz), 8.03 (d, 1H, Ar–H, $J = 7.6$ Hz), 8.36 (d, 1H, Ar–H, $J = 7.6$ Hz), 9.99 (s, 1H, NH, $D_2O$ exchangeable). *Anal.* Calcd for $C_{16}H_{14}N_4$: C, 73.26; H, 5.38; N, 21.36. Found: C, 73.01; H, 5.12; N, 21.18.

*3.1.2.2. Method B.* A solution of 3-benzyl-2-hydrazinylquinoxaline **1** (0.25 g, 1 mmol) in ethanol (10 mL) was added to ethylorthoformate (1 mmol) which was dissolved in ethanol (10 mL), and glacial acetic acid (2 drops) and the reaction mixture was then refluxed with for 3 hours. The product was separated out on cooling, filtered off, recrystallized from ethanol, and dried. The product was obtained as beige needles (ethyl alcohol), 0.24 g (91.49%) yield.

*3.1.3. General procedure for the preparation of compound (**4**)*

*3.1.3.1. Method A.* Compound **3** (1 mmol) was stirred in a mixture of pyridine (3 mL)/acetic anhydride (1 mL) at room temperature for 3 h followed by 1 h reflux. The reaction mixture was poured onto ice water with stirring and the solid that precipitated was collected by filtration, washed with water, recrystallized from ethanol, filtered and dried.

*3.1.3.2. Method B.* Compound **3** (1 mmol) was dissolved in DMF (5 mL). The solution was refluxed for 5 h. The reaction mixture was poured onto ice water and the solid that precipitated was collected by filtration, washed with water, recrystallized from ethanol, filtered and dried.

*3.1.3.3. 4-Benzyl-1-phenyl-[1,2,4]triazolo[4,3-a]quinoxaline (**4f**).* This compound was obtained as colorless crystals (ethyl alcohol), 0.26 g (77.31%) yield, mp (°C) 219–220; $^1H$ NMR (DMSO-$d_6$): $\delta$ 4.58 (s, 2H, $CH_2$), 7.20 (t, 1H, Ar–H, $J = 6.9$ Hz), 7.29 (m, 3H, Ar–H), 7.42 (t, 1H, Ar–H, $J = 8.4$ Hz), 7.49 (d, 2H, Ar–H, $J = 6.9$ Hz), 7.57 (t, 1H, Ar–H, $J = 7.7$ Hz), 7.64 (m, 2H, Ar–H), 7.68 (d, 1H, Ar–H, $J = 7.7$ Hz), 7.75 (d, 2H, Ar–H, $J = 6.9$ Hz), 8.03 (d, 1H, Ar–H, $J = 7.7$ Hz). *Anal.* Calcd for $C_{22}H_{16}N_4$: C, 78.55; H, 4.79; N, 16.66. Found: C, 78.28; H, 5.04; N, 16.87.

*3.1.3.4. 4-Benzyl-1-(2-chlorophenyl)-[1,2,4]triazolo[4,3-a]quinoxaline (**4h**).* This compound was obtained as white powder (ethyl alcohol), 0.29 g (78.38%) yield, mp (°C) 194–195; $^1H$ NMR (DMSO-$d_6$): $\delta$ 4.62 (2d, 2H, $CH_2$, $J = 13.8$ Hz), 7.06 (d, 1H, Ar–H, $J = 8.4$ Hz), 7.21 (t, 1H, Ar–H, $J = 6.9$ Hz), 7.31 (t, 2H, Ar–H, $J = 6.9$ Hz), 7.47–7.51 (m, 3H, Ar–H), 7.60–7.67 (m, 2H, Ar–H), 7.78–7.82 (m, 3H, Ar–H), 8.06 (d, 1H, Ar–H, $J = 7.7$ Hz). *Anal.* Calcd for $C_{22}H_{15}ClN_4$: C, 71.25; H, 4.08; N, 15.11. Found: C, 71.43; H, 4.35; N, 14.90.

*3.1.3.5. 4-Benzyl-1-(4-methoxyphenyl)-[1,2,4]triazolo[4,3-a]quinoxaline (**4i**).* This compound was obtained as white powder (ethyl alcohol), 0.27 g (73.77%) yield, mp (°C) 145–146; $^1H$ NMR (DMSO-$d_6$): $\delta$ 4.06 (2d, 2H, $CH_2$, $J = 14.55$ Hz), 6.61 (d, 1H, Ar–H, $J = 7.7$ Hz), 6.86 (d, 2H, Ar–H, $J = 9.2$ Hz), 7.00 (t, 1H, Ar–H, $J = 6.9$ Hz), 7.17 (t, 1H, Ar–H, $J = 6.9$ Hz), 7.23 (t, 1H, Ar–H, $J = 7.7$ Hz), 7.33 (t, 3H, Ar–H, $J = 9.2$ Hz), 7.37–7.42 (m, 3H, Ar–H), 7.49 (d, 1H, Ar–H, $J = 6.9$ Hz). *Anal.* Calcd for $C_{23}H_{18}N_4O$: C, 75.39; H, 4.95; N, 15.29. Found: C, 75.12; H, 4.76; N, 15.57.

*3.1.3.6. 4-Benzyl-[1,2,4]triazolo[4,3-a]quinoxaline (**4v**).* This compound was obtained as white powder (ethyl alcohol), 0.23 g (88.46%) yield, mp (°C) >320; $^1H$ NMR (DMSO-$d_6$): $\delta$ 4.41 (m, 2H, $CH_2$), 7.57 (t, 2H, Ar–H, $J = 7.7$ Hz), 7.65 (t, 2H, Ar–H, $J = 7.7$ Hz), 7.90 (t, 1H, Ar–H, $J = 7.7$ Hz), 8.05 (d, 2H, Ar–H, $J = 7.7$ Hz), 8.13 (d, 1H, Ar–H, $J = 8.4$ Hz), 8.55 (d, 1H, Ar–H, $J = 8.4$ Hz), 10.29 (s, 1H, Ar–H). *Anal.* Calcd for $C_{16}H_{12}N_4$: C, 73.83; H, 4.65; N, 21.52. Found: C, 74.05; H, 4.88; N, 21.26.

*3.1.4. General procedure for the preparation of compound (**5**)*

Compound **3** (1 mmol) was dissolved in absolute DMF (5 mL) at 50 °C and a warm solution of $CuCl_2$ (2 mmol, 0.27 g) was added. The reaction mixture was stirred at 50 °C for 30 min and then heated at 100 °C for 1 h under nitrogen. After the reaction mixture had been cooled to ambient temperature, a solution of water (100 mL), concentrated ammonia (50 mL), and NaCl (5 g) was added and the mixture was stirred at 40 °C for 20 min in the presence of air. The precipitated solid was filtered off, washed with water and recrystallized from ethanol, filtered and dried.

*3.1.4.1. 4-Benzoyl-1-(3,4-dimethoxyphenyl)-[1,2,4]triazolo[4,3-a]quinoxaline (**5b**).* This compound was obtained as colorless crystals (ethyl alcohol), 0.35 g (88.35%) yield, mp (°C) 256–257; IR (KBr): 1686 (C=O), 1665 (C = N) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 3.74, 3.88 (2s, 6H, 2 $CH_3$), 7.24 (d, 1H, Ar–H, $J = 8.4$ Hz), 7.32–7.35 (m, 2H, Ar–H), 7.55–7.69 (m, 5H, Ar–H), 7.78 (t, 1H, Ar–H, $J = 7.7$ Hz),8.07 (d, 2H, Ar–H, $J = 7.7$ Hz), 8.12 (d, 1H, Ar–H, $J = 6.9$ Hz). *Anal.* Calcd for $C_{24}H_{18}N_4O_3$: C, 70.23; H, 4.42; N, 13.65. Found: C, 70.56; H, 4.69; N, 13.92.

*3.1.4.2. 4-Benzoyl-1-phenyl-[1,2,4]triazolo[4,3-a]quinoxaline (**5f**).* This compound was obtained as white crystals (ethyl alcohol), 0.29 g (82.86%) yield, mp (°C) 220–221; IR (KBr): 1690 (C=O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 7.43 (d, 1H, Ar–H, $J = 8.4$ Hz), 7.60 (t, 3H, Ar–H, $J = 7.7$ Hz), 7.65–7.73 (m, 4H, Ar–H), 7.78 (t, 3H, Ar–H, $J = 8.4$ Hz), 8.08 (d, 3H, Ar–H, $J = 6.9$ Hz), 8.13 (d, 1H, Ar–H, $J = 8.4$ Hz). $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 116.28, 127.09, 128.47, 128.55, 129.63, 129.83, 130.54, 131.01, 131.35, 131.73, 134.94, 135.66, 135.73, 143.41, 149.77, 149.88, 190.80. *Anal.* Calcd for $C_{22}H_{14}N_4O$: C, 75.42; H, 4.03; N, 15.99. Found: C, 75.66; H, 3.87; N, 16.13.

*3.1.4.3. 4-Benzoyl-1-(2-chlorophenyl)-[1,2,4]triazolo[4,3-a]quinoxaline (**5h**).* This compound was obtained as colorless crystals (ethyl alcohol), 0.31 g (83.78%) yield, mp (°C) 154–155; IR (KBr): 1692 (C=O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 7.19 (d, 1H, Ar–H, $J = 8.4$ Hz), 7.61 (t, 2H, Ar–H, $J = 6.9$ Hz), 7.64–7.73 (m, 3H, Ar–H), 7.77–7.85 (m, 4H, Ar–H), 8.11 (d, 2H, Ar–H, $J = 8.4$ Hz), 8.17 (dd, 1H, Ar–H, $J = 8.4$, 1.6 Hz). *Anal.* Calcd for $C_{22}H_{13}ClN_4O$: C, 68.67; H, 3.41; N, 14.56. Found: C, 68.89; H, 3.70; N, 14.42.

*3.1.4.4. 4-Benzoyl-1-(4-methoxyphenyl)-[1,2,4]triazolo[4,3-a]quinoxaline (**5i**).* This compound was obtained as coorless crystals (ethyl alcohol), 0.29 g (86.96%) yield, mp (°C) 182–183; IR (KBr): 1685 (C=O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 3.89 (s, 3H, $CH_3$), 7.23 (d, 2H, Ar–H, $J = 8.4$ Hz), 7.53 (d, 1H, Ar–H, $J = 8.4$ Hz), 7.58–7.68 (m,

4H, Ar–H), 7.71 (d, 2H, Ar–H, $J = 8.4$ Hz), 7.77 (t, 1H, Ar–H, $J = 7.7$ Hz), 8.07 (d, 2H, Ar–H, $J = 8.4$ Hz), 8.12 (d, 1H, Ar–H, $J = 8.4$ Hz). *Anal.* Calcd for $C_{23}H_{16}N_4O_2$: C, 72.62; H, 4.24; N, 14.73. Found: C, 72.91; H, 4.47; N, 14.88.

*3.1.4.5. 4-Benzoyl-1-(4-(dimethylamino)phenyl)-[1,2,4]triazolo[4,3-a]quinoxaline (**5n**).* This compound was obtained as pale yellow powder (ethyl alcohol), 0.33 g (861.98%) yield, mp (°C) 216–217; IR (KBr): 1686 (C=O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 3.03 (s, 6H, $2CH_3$), 6.74 (d, 1H, Ar–H, $J = 8.4$ Hz), 6.92 (d, 1H, Ar–H, $J = 8.4$ Hz), 7.45 (d, 1H, Ar–H, $J = 8.4$ Hz), 7.55–7.67 (m, 5H, Ar–H), 7.72 (t, 1H, Ar–H, $J = 8.4$ Hz), 7.77 (t, 1H, Ar–H, $J = 7.7$ Hz), 8.08 (m, 3H, Ar–H). *Anal.* Calcd for $C_{24}H_{19}N_5O$: C, 73.27; H, 4.87; N, 17.80. Found: C, 72.99; H, 5.07; N, 17.55.

*3.1.4.6. 4-Benzoyl- [1,2,4]triazolo[4,3-a]quinoxaline (**5v**).* This compound was obtained as colorless needles (ethyl alcohol), 0.22 g (80.29%) yield, mp (°C) 243–244; IR (KBr): 1685 (C=O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 7.57 (t, 2H, Ar–H, $J = 7.7$ Hz), 7.75 (t, 2H, Ar–H, $J = 8.4$ Hz), 7.92 (t, 1H, Ar–H, $J = 7.7$ Hz), 8.05 (d, 2H, Ar–H, $J = 8.4$ Hz), 8.13 (d, 1H, Ar–H, $J = 8.4$ Hz), 8.50 (d, 1H, Ar–H, $J = 8.4$ Hz), 10.24 (s, 1H, Ar–H). $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 117.40, 126.00, 128.64, 129.52, 130.90, 131.07, 131.85, 134.89, 135.01, 135.47, 137.90, 149.03, 190.71. *Anal.* Calcd for $C_{16}H_{10}N_4O$: C, 70.06; H, 3.67; N, 20.43. Found: C, 70.23; H, 3.54; N, 20.67.

*3.1.5. General procedure for the bromination of compound (**3**)*

*3.1.5.1. Method A.* Compound **3** (1 mmol) was dissolved in methanol (5 mL). Bromine (0.5 mL) in methanol (10 mL) was added dropwise for 30 min. The reaction mixture was stirred at room temperature overnight. The solvent was evaporated under vacuum and the crude solid obtained was dried and recrystallized from ethanol, filtered and dried.

*3.1.5.2. 4-Benzoyl-1-(5-bromo-2-chlorophenyl)-[1,2,4]triazolo[4,3-a]quinoxaline (**6h**).* This compound was obtained as colorless crystals (ethyl alcohol), 0.38 g (81.95%) yield, mp (°C) 195–196; IR (KBr): 1691 (C=O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 7.19 (s, 1H, Ar–H), 7.61 (t, 2H, Ar–H, $J = 6.9$ Hz), 7.73 (t, 1H, Ar–H, $J = 7.7$ Hz), 7.77–7.91 (m, 5H, Ar–H), 8.11 (dd, 1H, Ar–H, $J = 8.4$, 3.8 Hz). *Anal.* Calcd for $C_{22}H_{12}BrClN_4O$: C, 56.98; H, 2.61; N, 12.08. Found: C, 57.25; H, 2.80; N, 11.86.

*3.1.5.3. 4-Benzoyl-1-(4-nitrophenyl)-[1,2,4]triazolo[4,3-a]quinoxaline (**5s**).* This compound was obtained as pale yellow crystals (ethyl alcohol), 0.32 g (81.01%) yield, mp (°C) 124–125; IR (KBr): 1687 (C=O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): $\delta$ 7.18–7.21 (m, 1H, Ar–H), 7.32–7.42 (m, 4H, Ar–H), 7.53 (t, 1H, Ar–H, $J = 8.4$ Hz), 7.61–7.68 (m, 2H, Ar–H), 7.84 (d, 2H, Ar–H, $J = 7.7$ Hz), 8.08 (d, 2H, Ar–H, $J = 8.4$ Hz), 8.14 (d, 1H, Ar–H, $J = 7.7$ Hz). *Anal.* Calcd for $C_{22}H_{13}N_5O_3$: C, 66.83; H, 3.31; N, 17.71. Found: C, 67.13; H, 3.08; N, 17.45.

*3.1.5.4. 4-Benzoyl-[1,2,4]triazolo[4,3-a]quinoxaline (**5v**).* This compound was obtained as colorless needles (ethyl alcohol), 0.25 g (91.24%) yield. Melting point and spectral data are identical to that of **5v**.

*3.1.5.5. Method B.* One millimole of compound **3** was dissolved in 5 mL methanol, 0.5 mL Bromine in 10 mL methanol was added dropwise for 30 min. The reaction mixture was stirred at room temperature for 1 h. The formed precipitate was filtered off, dried and recrystallized from ethanol, filtered and dried.

*3.1.5.6. 4-Benzyl-1-phenyl-[1,2,4]triazolo[4,3-a]quinoxaline (**4f**).* This compound was obtained as colorless cystals (ethyl alcohol), 0.29 g (86.31%) yield. Melting point and spectral data are identical to that of **4f**.

*3.1.5.7. 4-Benzoyl-1-(3-bromo-4-(N,N-dimethylaminophenyl))-[1,2,4]triazolo[4,3-a]quinoxaline (**6n**).* This compound was obtained as colorless crystals (ethyl alcohol), 0.35 g (76.42%) yield, mp (°C) 224–225; $^1H$ NMR (DMSO-$d_6$): $\delta$ 4.07 (s, 2H, $CH_2$), 7.20 (t, 1H, Ar–H, $J = 6.9$ Hz), 7.29 (t, 2H, Ar–H, $J = 7.6$ Hz), 7.34 (d, 1H, Ar–H, $J = 8.4$ Hz), 7.47 (m, 4H, Ar–H), 7.59 (t, 1H, Ar–H, $J = 6.9$ Hz), 7.69 (d, 1H, Ar–H, $J = 7.7$ Hz), 7.96 (s, 1H, Ar–H), 8.03 (d, 1H, Ar–H, $J = 7.7$ Hz). *Anal.* Calcd. for $C_{24}H_{20}BrN_5$: C, 62.89; H, 4.40; N, 15.28. Found: C, 63.14; H, 4.31; N, 15.02.

*3.2. Biology*

The newly synthesized compounds **3**–**6** 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 [15]. The activities of MAO-A and MAO-B were determined using a fluorimetric method described by Matsumoto et al. [16]. The mitochondrial fractions were incubated at 38 °C for 30 min with the substrate following the inhibition of one of the MAO isoforms with the specific inhibitor, L-deprenyl (l0.5 mM) to determine MAO-A activity and clorgyline (10.5 mM) to determine MAO-B activity. The incubation mixture contained 0.1 ml phosphate buffer (0.25 M, pH 7.4), mitochondrial suspension (6 mg/ml), the specific substrate for MAO-A or MAO-B (0.1 mM) and test compounds at five different concentrations ranging from 0.5 nM to 0.1 M (0, 0.5 nM, 5 nM, 5 μM, 5 mM and 100 mM) were 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 10,000*g* for 5 min and the supernatant was completed to 2.7 ml using 1 N NaOH and measured with a Perkin–Elmer Lf 45 Spectrofluorimeter. The values were from 6 independent samples that were measured in duplicate. The average value of the duplicate measurements was used for the statistical analysis. Protein concentration was determined according to a previously reported method [17]. The MAO-A and MAO-B results are expressed as $IC_{50}$ (Table 4). Propylene glycol was used as negative control and did not show any effect on the enzyme activity.

The test compounds were further evaluated for their oral acute toxicity in male mice (20 g each obtained from Medical Research Institute, Alexandria University) according to previously reported methods [18,19]. The animals were divided into groups of six mice each. The compounds were suspended in 1% gum acacia and given orally in doses of 1, 10, 100, 200, 250, 300 mg/kg. The mortality percentage in each group was recorded after 24 h. Additionally the test compounds were investigated for their parenteral acute toxicity in groups of mice of six animals each. The compounds or their vehicle, propylene glycol (control), were given by intraperitoneal injection in doses of 10, 25, 50, 75, 100 mg/kg. The percentage survival was followed up to 7 days [20].

*3.2.1. Modeling studies*

Computer-assisted simulated docking experiments were carried out under MMFF94X in human MAO-A crystal structure (PDB ID: 2BXR). Docking simulation study of the synthesized compounds **3**–**6** using MOE dock [18] with the following protocol.

(1) Enzyme structures were checked for missing atoms, bonds and contacts.
(2) Hydrogen atoms were added to enzyme structure. Water molecules and bound ligands were manually deleted.

(3) The ligand molecules were constructed using the builder module and were energy minimized.
(4) The active site was generated using the MOE-Alpha Site Finder.
(5) Dummy atoms were created from the obtained alpha spheres.
(6) Ligands were docked within the MAO-A active site using the MOEDock with simulated annealing used as the search protocol and MMFF94X molecular mechanics forcefield for 8000 iterations.
(7) The lowest energy conformation was selected and subjected to an energy minimization using MMFF94X force field.

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