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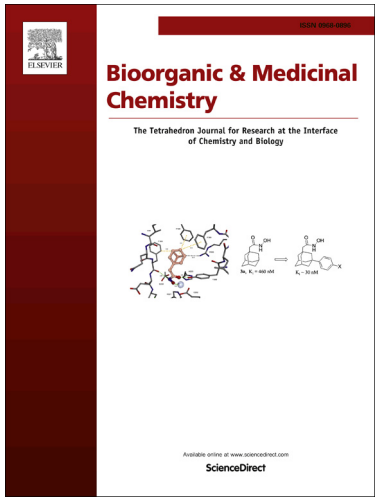

Synthesis and Evaluation of Quinazoline Amino Acid Derivatives as Mono Amine Oxidase (MAO) Inhibitors

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Graphical Abstract

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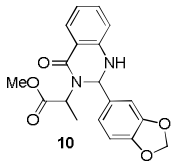
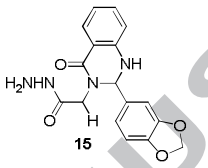
Compounds **7**, **10**, and 1**5** showed MAO-A inhibition activity comparable to that of the standard clorgyline. In addition, compound **15** showed the highest selectivity within the test compounds as MAO-A inhibitors. The docking of compound **15** into the MAO-A active site showed hydrogen bond interaction with GLU 216, SER 209, and TYR 444.



10

MAO-A IC50 (M) SI

$2.8\times10^{-9} \pm 0.11$ **30714**



15

MAO-A IC50 (M) SI

$2.1\times10^{-9} \pm 0.11$ **39524**

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ABSTRACT

A series of quinazolinone amino acid ester and quinazolinone amino acid hydrazides were prepared under microwave irradiation as well as conventional condition. The microwave irradiation afforded the product in less reaction time, higher yield and purity. The structures of the synthesized compounds were confirmed by IR, NMR, and elemental analysis. The new synthesized compounds were studied for their monoamine oxidase inhibitory activity. They showed more selective inhibitory activity toward MAO-A than MAO-B. Compounds **7**, **10**, and **15** showed MAO-A inhibition activity (IC_{50} = 3.6×10^{-9}, 2.8×10^{-9}, 2.1×10^{-9} M respectively) comparable to that of the standard clorgyline (IC_{50} = 2.9×10^{-9} M). 2-(2-(benzo[*d*][1,3]dioxol-5-yl)-4-oxo-1,2-dihydroquinazolin-3(4*H*)-yl)acetohydrazide **15** showed selective MAO-A inhibition activity (SI = 39524) superior to that of the standard clorgyline (SI = 33793). The acute toxicity of the synthesized compounds was determined. In addition, computer-assisted simulated docking experiments were performed to rationalize the biological activity.

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] particularly abundant in the liver and brain.[2] These FAD dependent enzymes are responsible for regulation and metabolism of major monoamine neurotransmitters such as serotonin (5-OH tryptamine), nor-adrenaline and dopamine. It is also involved in the biodegradation of exogenic amines such as benzylamine, tyramine, MPTT, MPP+ and a Parkinsonian producing neurotoxin.[1] It catalyzes the oxidative deamination of a range of endogenous and exogenous monoamines.[3] The two mammalian isoforms are encoded by two different genes[4] and distinguished by different substrate specificities and sensitivities to the selective inhibitors.[5] 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 'cheese effect' characterized by hypertensive crises.[6]

In spite of considerable progress in understanding of the interactions of the two enzyme forms with their preferred substrates and inhibitors, no general rules are yet available for the rational design of potent and selective inhibitors of MAO. This is partly due to the fact that the mechanism of interaction of several new drugs with MAOs has not been fully characterized. Therefore, the discovery of several selective MAO inhibitors has relied on serendipity. 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[7]. 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.

Recently we have demonstrated a series of 3-benzyl-2-substituted quinoxalines as selective MAO-A inhibitors bearing substituted amino or hydrazino functionalities at position 2 [8] and novel structural variants of [1,2,4]triazolo[4,3-*a*]quinoxalines derivatives.[9] In addition, substituted pyridazine-1-yl acetic acid derivatives [10] and α-ketoamino acid ester[11] were established as selective monoamine oxidase-A inhibitors.

The promising bioactive diversity of the quinazoline moiety[12-18] urged us to synthesize and biologically evaluate a series of novel quinazoline-based molecules on the MAO inhibitory activity.

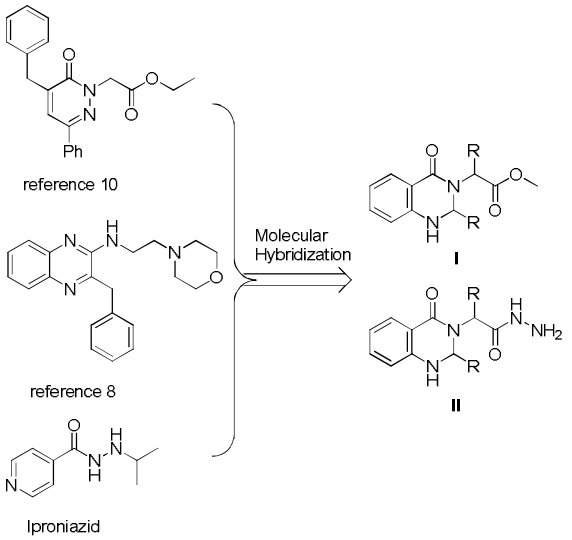


Figure 1. Design of the target compounds

The rational design of these compounds was based on hybrid structure of known inhibitors. The aim of the present study was tailoring MAO-A inhibitors considering some factors responsible for selectivity against the A isoform[19] which are i) the presence of electron-rich aromatic moieties (e.g. Bazinaprine[20], quinazoline), ii) the presence of hydrazido functionality (e.g. Iproniazid[21]), iii) the presence of ethoxycarbonyl methylene group side chain of aromatic system (e.g. pyridazine-1-yl acetic acid derivatives[10] and eugenol analog[22]) changing the ester group by amido was also considered, and iv) the presence of amino acid moiety,[10,11] Figure 1.

2. Results and Discussion

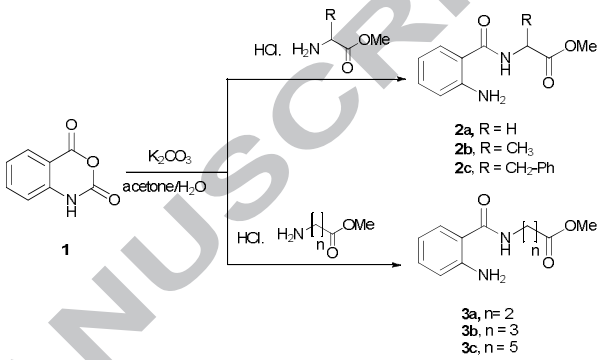
2.1. Chemistry

A series of novel quinazoline-based molecules were synthesized and biologically evaluated for their MAO inhibitory activity.

Preparation of methyl (2-aminobenzamido) ester derivatives ***2a-2c, 3a-3c***

Lately, Doveston *et al*[23] reported the synthesis of substituted aminobenzamido acid esters from the reaction of isatoic anhydride with amino acid esters. The reaction was carried out in ethyl acetate and toluene in the presence of triethylamine as base.[23] Robin *et al* reported recently the synthesis in pyridine in the presence of DMAP as base.[24] Earlier, the synthesis of substituted aminobenzamido acids was performed using free amino acids in the presence of triethylamine and acetic acid.[25]

Herein, we used greener reaction conditions for the synthesis of the substituted aminobenzamido acid esters . The reaction of isatoic anhydride **1** with glycine methyl ester hydrochloride, L-alanine methyl ester hydrochloride, L-phenylalanine methyl ester hydrochloride, β-alanine methyl ester hydrochloride, γ-aminobutanoic methyl ester hydrochloride, and 6-aminohexanoic methyl ester hydrochloride in acetone/water, in presence of potassium carbonate, by stirring 24 hours at room temperature gave smoothly the derivatives **2a-2c**, **3a-3c** in high yield, Scheme 1. The structure of the ester derivatives **2a-2c**, **3a-3b** was determined by spectroscopic methods (IR, ^{1}H and ^{13}C NMR) and by elemental analysis. Compound **3c** was obtained as oily brown syrup and was used subsequently in the following reaction.

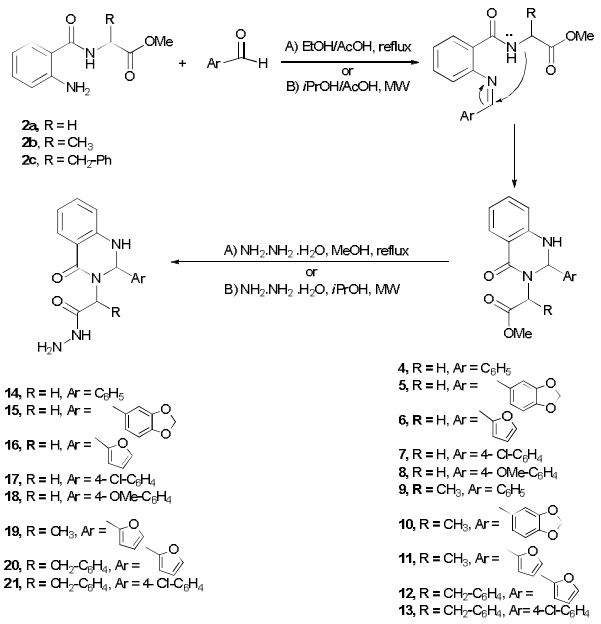


Scheme 1. Preparation of methyl (2-aminobenzamido) ester derivatives **2a-2c, 3a-3c**

Reactions of methyl (2-aminobenzamido) ester derivatives ***2a-2c*** *with aldehydes*

The reaction of methyl (2-aminobenzamido) ester **2a-2c** with different aldehydes in ethanol in the presence of 2 drops of glacial acetic, by conventional method by heating under reflux for 8 hrs or by using microwave irradiation (LG, 1000W) for 10 min, gives the corresponding Schiff's bases, which subsequently cyclized to give the substituted quinazolinones **4-13** (Scheme 2). Using microwave irradiation gave the desired products in shorter time, good yield, and higher purity. The structure of the quinazolinone derivatives **4-13** were found in agreement with the assigned molecular structure and were confirmed by IR, ^{1}H NMR, ^{13}C NMR spectroscopy and elemental analysis.

According to the obtained ^{1}H-NMR spectra, it was found that methyl 2-(4-oxo-2-phenyl-1,2-dihydroquinazolin-3(4*H*)-yl)propanoate **9** and methyl 2-(2-(benzo[*d*][1,3]dioxol-5-yl)-1,2-dihydro-4-oxoquinazolin-3(4*H*)-yl)propanoate **10** are present in two isomers. As a prototype, the ^{1}H-NMR spectrum of compound **9**, reveals that there are two isomers in ratio 67 %: 23 %, where they differ in the chemical shift of the alanine CH_3 group, as well as the chemical shift of the methyl ester group. The diastereomeric ratio of compound **9** was almost the same in both conventional or microwave synthesis. Two doublet peaks were observed equivalent to three protons at δ 1.14 and 1.23 ppm corresponding to the alanine methyl group. In addition, two singlet peaks were also observed equivalent to three protons at δ 3.45 and 3.53 ppm corresponding to the methyl ester group.



Scheme 2. Preparation of quinazolinone amino acid esters **4-13** and quinazolinone amino acid hydrazides **14-21**.

Since the formation of the cyclic compound **9** involves the formation of a new chiral center on the original chiral starting material **2b**, one should expect the formation of two diastereomeric products (Figure 2). Therefore, it is considered worthwhile to model the compounds using molecular mechanics MM2 calculations. In addition, quantum chemical calculations were carried out with the GAUSSIAN 98 suite of programs. Geometry optimizations were carried out using the DFT level (B3LYP/6-31G**) of theory to assess the relative stability of the diastereomeric species. Calculated relative energies of **9** isomers (R,S) and (S,S) is -1030.956657 au and -1030.7032970 au respectively. Computed energies indicate the stability of the (R,S) isomer over the (S,S) one by 0.25336 au (158.9868 kcal/mol), Figure 3.

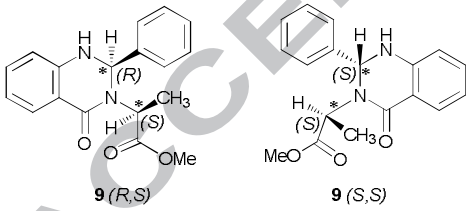


Figure 2. The expected diastereomeric isomers (R,S) and (S,S) of methyl 2-(4-oxo-2-phenyl-1,2-dihydroquinazolin-3(4*H*)-yl)propanoate **9**.

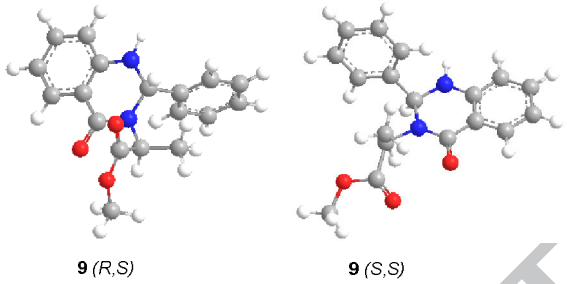


Figure 3. The expected 3D structure of diastereomeric isomers (*R,S*) and (*S,S*) of methyl 2-(4-oxo-2-phenyl-1,2-dihydroquinazolin-3(4*H*)-yl)propanoate **9**

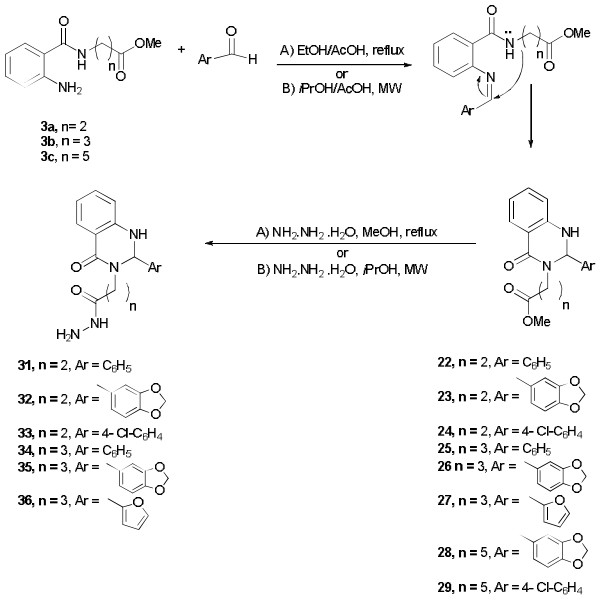
The ^{13}C-NMR of compounds **4, 5, 8, 10** showed a carbon signal in the sp3 region at 72.18, 72.04, 71.94, and 72.87 ppm respectively, confirming the cyclization of the quinazolinone derivatives.

3. Reactions of methyl(2-aminobenzamido) ester derivatives **3a-3c** with aldehydes

In order to study the effect of the linker's length on the bio-activity of the target molecules, we undertook the synthesis of methyl(2-aminobenzamido) ester derivatives **3a-3c.** The reaction of **3a-3c** with different aldehydes in ethanol in the presence of 2 drops of glacial acetic acid, by conventional method or by using microwave irradiation (LG, 1000W), gives the corresponding Schiff's bases, which subsequently cyclize to give substituted quinazolinones **22-29** (Scheme 3). Using microwave irradiation gave the desired products in shorter time, good yield, and higher purity.

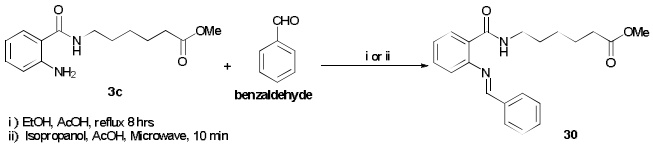
The structure of the cyclized quinazolinone derivatives **22-29** were found in agreement with the assigned molecular structure confirmed by their IR, NMR spectroscopy and elemental analysis.

The ^{13}C NMR spectrum of compound **28** in DMSO-d_6 showed 21 resolved carbon signals, seven in the sp3 region, 12 in the aromatic region and the carbonyl signals at δ 162.21 and 173.34 ppm. The ^{13}C-NMR of compound **28** showed a carbon signal in the sp3 region at 69.81 ppm, confirming the cyclization of the quinazolinone derivatives.



Scheme 3. Preparation of quinazolinone amino acid esters **22-29** and quinazolinone amino acid hydrazides **31-36**

The reaction of methyl 6-(2-aminobenzamido)hexanoate **3c** with benzaldehyde, afforded methyl 6-(2-(benzylideneamino)benzamido)hexanoate **30** (Scheme 4). Isolation of the Schiff's base in this reaction confirmed the proposed mechanism in Scheme 2 and 3, in which the quinazolinone ring is formed through the formation of Schiff's base as a first step followed by intramolecular cyclization to give the desired products.



Scheme 4. Preparation of methyl 6-(2-(benzylideneamino)benzamido) hexanoate **30**

The structure of compound **30** was found in agreement with the assigned molecular structure confirmed by IR, ^{1}H-NMR spectroscopy and elemental analysis. The ^{1}H-NMR spectrum of compound **30** in DMSO-d_6 showed a multiplet at δ 7.90-7.91 ppm, corresponding to three aromatic protons and the aldehydic proton, while the peak which is commonly observed in all the prepared quinazolinone derivatives **4-13** and **22-29** at the range 5.71-6.06 ppm, corresponding to the sp^{3}CH proton, was not observed.

4. Hydrazinolysis of quinazolinone amino acid esters **4-13** and **22-29**

Reaction of quinazolinone amino acid esters **4-13** and **22-29** with hydrazine hydrate using conventional heating about 8 hours or by using microwave irradiation (LG, 1000W) for 10 min yielded the corresponding hydrazides **14-21** and **31-36** (Scheme 2 and 3). Using microwave irradiation gave the desired products in shorter time, good yield, and higher purity. The structure of the hydrazides was found in agreement with the assigned molecular structure confirmed by their IR, NMR spectroscopy and elemental analysis.

2.2. Biological Activity

The newly synthesized compounds **2a-2c, 3a-3c, 4-36** were tested to determine their activity toward MAO-A and MAO-B and selectivity in the presence of the specific substrate, serotonin or benzylamine respectively. Bovine brain mitochondria were isolated according to Basford.[26] Compounds **2a-2c, 3a-3c, 4-36** were tested to determine their activity toward MAO-A and MAO-B according to methods of Matsumoto *et al* and Bradford.[27,28] MAO-A and MAO-B inhibitory results were expressed as IC_{50}, Table 1. The selectivity index was also given in Table 1.

Table 1. Effect of some quinazolinone derivatives on the MAO-A and MAO-B activity.

Compound	MAO-A IC_{50} (M)	MAO-B IC_{50} (M)	Selectivity inhibition index (SI)[a]
2a	$5.2\times10^{-8}\pm0.14$	$8.5\times10^{-4}\pm0.12$	16346
2b	$6.3\times10^{-8}\pm0.18$	$4.1\times10^{-4}\pm0.22$	6507
2c	$3.8\times10^{-8}\pm0.21$	$6.8\times10^{-4}\pm0.28$	17895
3a	$6.2\times10^{-8}\pm0.44$	$5.6\times10^{-4}\pm0.12$	9032
3b	$7.1\times10^{-8}\pm0.22$	$4.6\times10^{-4}\pm0.11$	6479
3c	$5.6\times10^{-8}\pm0.32$	$4.4\times10^{-4}\pm0.18$	7857
4	$9.3\times10^{-8}\pm0.28$	$7.2\times10^{-4}\pm0.26$	7742
5	$6.1\times10^{-8}\pm0.26$	$7.4\times10^{-4}\pm0.14$	12131
6	$4.4\times10^{-8}\pm0.13$	$3.8\times10^{-4}\pm0.16$	8636
7	$3.6\times10^{-9}\pm0.22$	$8.8\times10^{-5}\pm0.14$	**24444**
8	$4.8\times10^{-8}\pm0.28$	$5.6\times10^{-4}\pm0.16$	11666
9	$3.9\times10^{-8}\pm0.12$	$4.6\times10^{-4}\pm0.16$	11795
10	$2.8\times10^{-9}\pm0.11$	$8.6\times10^{-5}\pm0.14$	**30714**
11	$8.2\times10^{-9}\pm0.16$	$6.1\times10^{-5}\pm0.22$	7439
12	$7.3\times10^{-8}\pm0.12$	$3.4\times10^{-4}\pm0.28$	4658
13	$4.2\times10^{-8}\pm0.38$	$5.4\times10^{-4}\pm0.22$	12857
14	$3.8\times10^{-8}\pm0.12$	$3.1\times10^{-4}\pm0.18$	8158
15	$2.1\times10^{-9}\pm0.11$	$8.3\times10^{-5}\pm0.18$	**39524**
16	$3.6\times10^{-8}\pm0.22$	$5.6\times10^{-4}\pm0.16$	15555
17	$4.8\times10^{-8}\pm0.26$	$9.4\times10^{-4}\pm0.18$	19583
18	$8.2\times10^{-8}\pm0.32$	$8.9\times10^{-4}\pm0.28$	10854
19	$3.2\times10^{-8}\pm0.38$	$3.1\times10^{-4}\pm0.11$	9688
20	$2.8\times10^{-8}\pm0.24$	$2.2\times10^{-4}\pm0.26$	7857
21	$1.8\times10^{-8}\pm0.14$	$1.2\times10^{-4}\pm0.26$	6666
22	$9.2\times10^{-8}\pm0.21$	$8.6\times10^{-4}\pm0.32$	9348
23	$8.4\times10^{-8}\pm0.16$	$1.7\times10^{-4}\pm0.13$	2024
24	$9.8\times10^{-8}\pm0.44$	$7.2\times10^{-4}\pm0.22$	7347
25	$6.3\times10^{-8}\pm0.11$	$5.4\times10^{-4}\pm0.18$	8571
26	$8.8\times10^{-8}\pm0.52$	$6.6\times10^{-4}\pm0.42$	7500
27	$9.6\times10^{-8}\pm0.32$	$7.8\times10^{-4}\pm0.18$	8125
28	$8.4\times10^{-8}\pm0.14$	$8.2\times10^{-4}\pm0.28$	9762
29	$4.4\times10^{-8}\pm0.22$	$2.9\times10^{-4}\pm0.16$	6591
30	$6.4\times10^{-8}\pm0.28$	$5.4\times10^{-4}\pm0.22$	8438
31	$1.6\times10^{-8}\pm0.18$	$2.1\times10^{-4}\pm0.22$	13125
32	$3.2\times10^{-8}\pm0.16$	$3.8\times10^{-4}\pm0.12$	11875
33	$5.1\times10^{-8}\pm0.26$	$4.2\times10^{-4}\pm0.28$	8235
34	$4.7\times10^{-8}\pm0.22$	$3.7\times10^{-4}\pm0.16$	7872
35	$8.2\times10^{-8}\pm0.14$	$4.2\times10^{-4}\pm0.11$	5122
36	$3.6\times10^{-8}\pm0.18$	$2.7\times10^{-4}\pm0.24$	7500
Clorgyline	$2.9\times10^{-9}\pm0.12$	$9.8\times10^{-5}\pm0.16$	**33793**

The results were expressed as mean ± S.E.M. Data were analyzed by one-way 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 results revealed that all test compounds showed higher inhibitory activity toward MAO-A than MAO-B. An overall review of the results showed that the inhibition profile was competitive for compounds **7**, **10** and **15**, which showed inhibitory activity towards MAO-A similar to that of the standard drug clorgyline (IC_{50} = 2.9×10^{-9} M), Table 1. Compounds **10** and **15** were the most selective compounds as MAO-A inhibitors. Compound **15** showed selective MAO-A inhibition activity (SI = 39524) superior to that of the standard clorgyline (SI = 33793).

Among all tested compounds, the glycine- and alanine-containing compounds **7, 10, 15** showed high inhibitory activity towards MAO-A. The increase in the linker's length did not improve the bio-activity, where compounds **22-29** and **31-36** attributed the lowest activity. This indicates that the shorter the linker between the hydrophobic head and the terminal function groups the better the inhibitor activity. Which can probably orient the compound to form hydrogen bonding with the backbone of the MAO-A enzyme.

An additional factor which could be responsible for the

improvement of inhibitory activity towards MAO-A, observed in compounds **10** and **15**, could be the presence of piperonal moiety, which could form hydrogen bonding with the backbone of the MAO A enzyme.

In an attempt to understand the reason for the observed MAO-A inhibitory activity of **10** and **15** a molecular modeling study, as well as conformational alignment studies have been done.

Molecular docking studies further helps in understanding the various interactions between the ligands and enzyme active sites in detail (Table 2).

MOE (Molecular Operating Environment),[29,30] docking studies of the inhibitors were performed using human MAO-A crystal structure (PDB ID: 2BXR). The docking of compounds **10** and **15** into the MAO-A active site revealed that several hydrogen bonds and hydrophobic interactions are considered to be responsible for the observed affinity (Table 2, Figure 4, Figure 5). The docking results of the test compounds **10** and **15** showed that they bound to the MAO-A active site with position and orientation better than the others (Figure 4, Figure 5), where:

i) Compound **10** showed four hydrogen bond interactions with the following residues: ILE 207, GLN 74, SER 209, and TYR 444 (Figure 4).
ii) Compound **15** showed three hydrogen bond interactions with the following residues: GLU 216, SER 209, and TYR 444 (Figure 5)

Thus these observations provide a good explanation for the potent inhibitory activity of compounds **10** and **15**.

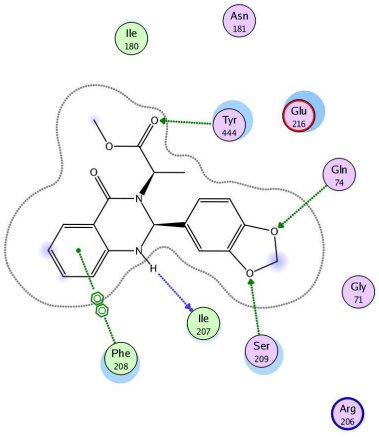


Figure 4. 2D binding modes of compound **10** docked in the active site of MAO-A (PDB ID: 2BXR). Dashed lines depict hydrogen bond interactions. Viewed using Molecular Operating Environment (MOE) module.

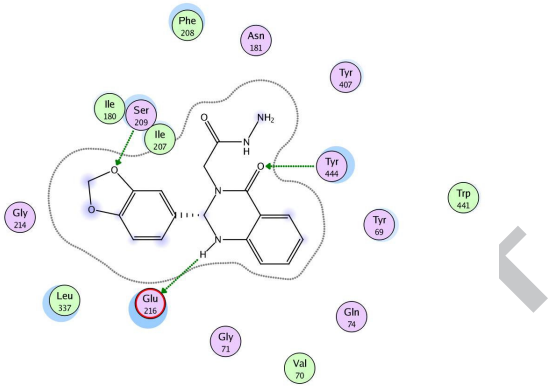


Figure 5. 2D binding modes of compound **15** docked in the active site of MAO-A (PDB ID: 2BXR). Dashed lines depict hydrogen bond interactions. Viewed using Molecular Operating Environment (MOE) module.

Acute toxicity

The newly synthesized compounds were further evaluated for their oral and parenteral acute toxicity in male mice using a literature method.[31-33] Sign recorded during acute toxicity studies: increased motor activity, anesthesia, tremors, arching and rolling, clonic convulsions, ptosis, tonic extension, lacrimation, straub reaction, exophthalmos, pilo-erection, salivation, muscle spasm, opisthotonus, writhing, hyperesthesia, loss of righting reflex, depression, ataxia, stimulation, analgesia, sedation, blanching, hypnosis, cyanosis or death. The results indicated that test compounds proved to be orally non-toxic and well tolerated by the experimental animals up to 300 mg/kg and were also non-toxic up to 125 mg /kg through the parenteral route.[33] On the other hand, clorgyline showed loss of righting reflex of the animals at the same dose levels, two hours after oral or half an hour after parenteral administration. This sign continued for 20 to 30 minutes without any mortality of the animals.

Table 2. Calculated docking hydrogen bonding results for compounds **10**, **15**

Compd	Residue	Type	Score	Distance
10	ILE207	H-don	1.7%	3.20
	GLN74	H-acc	5.4%	3.64
	SER209	H-acc	**95.8%**	2.75
	TYR444	H-acc	19.1%	3.00
15	GLU216	H-don	2.2%	2.56
	SER209	H-acc	**98.9%**	2.40
	TYR444	H-acc	25.9%	2.86

3. Conclusions

We could conclude that the synthesis and biochemical evaluation of the newly synthesized quinazolinone amino acid esters and quinazolinone amino acid hydrazides led to the design of a novel class of MAO-A inhibitors with good safety margin. Compounds **7**, **10**, and **15** showed MAO-A inhibition activity (IC_{50} = 3.6×10^{-9}, 2.8×10^{-9}, 2.1×10^{-9} M respectively) comparable to that of the standard clorgyline (IC_{50} = 2.9×10^{-9} M). 2-(2-(benzo[*d*][1,3]dioxol-5-yl)-4-oxo-1,2-dihydroquinazolin-3(4*H*)-yl)acetohydrazide **15** showed selective MAO-A

inhibition activity (SI = 39524) superior to that of the standard clorgyline (SI = 33793). The acute toxicity results indicated that the test compounds proved to be non-toxic. The docking results of compounds **10** and **15** showed that they bound to the MAO-A active site through hydrogen bonding and hydrophobic interactions and provide a good explanation for their potent inhibitory activity. It is worth mentioning that such kind of compounds would represent a fruitful matrix for the development of new class of selective MAO-A inhibitors that could deserve further investigation and derivatization.

4. Experimental protocol

4.1 Chemistry

General: The solvents used were of HPLC reagent grade. Melting points were determined with a Mel-Temp apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer 1600 series Fourier transform instrument as KBr pellets. Nuclear Magnetic resonance spectra (^{1}H NMR and ^{13}C NMR spectra) were recorded on 500 MHz JEOL spectrometer at room temperature. Chemical shifts are reported in parts per million (ppm) and are referenced relative to residual solvent (e.g. $CHCl_3$ at δ H 7.26 ppm for $CDCl_3$, DMSO at δ H 2.50 ppm for DMSO-d_6). Spin multiplicities are represented by the following signals: singlet (s), broad singlet (br s), doublet (d), broad doublet (br d), doublet of doublets (dd), triplet (t), doublet of triplets (dt), and multiplet (m). Elemental analyses were performed on Perkin-Elmer 2400 elemental analyzer, and the values found were within ±0.3% of the theoretical values. Follow-up of the reactions and checks of the purity of the compounds was done by TLC on silica gel coated aluminium sheets (Type 60 GF254, Merck) and the spots were detected by exposure to UV-lamp at λ 254 nm for a few seconds. The compounds were named using Chem. Draw Ultra version 11, Cambridge soft Corporation.

4.1.1 General procedure for the preparation of methyl (2-aminobenzamido) ester derivatives

To a solution of amino acid methyl ester hydrochloride (3 mmol) in water (15 mL), (0.23 g, 6 mmol) of K_2CO_3 was slowly added. The mixture was stirred for 30 min and then (0.49g, 3mmol) isatoic anhydride **1** dissolved in 3mL acetone was added dropwise. The reaction mixture was kept at r.t. under stirring for 24 hrs. The reaction mixture was concentrated under reduced pressure. The product filtered off without further purification.

Methyl 2-(2-aminobenzamido)acetate ***2a*** [25, 34, 35]

Compound **2a** was obtained as colorless needles 0.49 g (78.5%) yield; mp 73-74ºC; IR (KBr): 3468, 3355 (NH+ NH_2), 1737 (C=O, ester), 1638 (C=O, amide) cm^{-1}; ^{1}H-NMR (500 MHz: DMSO-d_6):δ 3.62 (s, 3H, CH_3), 3.91 (d, 2H, *J* = 6.1 Hz, CH_2), 6.44 (s, 2H, NH_2, D_2O exchangeable), 6.49 (t, 1H, *J* = 7.6 Hz, Ar-H), 6.67 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.13 (t, 1H, *J* = 8.4 Hz, Ar-H), 7.50 (d, 1H, *J* = 7.6 Hz, Ar-H), 8.62-8.64 (m, 1H, NH, D_2O exchangeable). ^{13}C-NMR (125 MHz: DMSO-d_6): δ 41.45, 52.21, 113.93, 115.08, 117.02, 128.63, 132.62, 150.45, 169.78, 171.17. Anal. Calcd. for $C_{10}H_{12}N_2O_3$: C, 57.68; H, 5.81; N, 13.45. Found: C, 57.52; H, 5.64; N, 13.69.

Methyl 2-(2-aminobenzamido)propanoate ***2b*** [25]

Compound **2b** was obtained as colorless crystals 0.59 g (88.5%) yield; mp 57-56ºC; IR (KBr): 1341, 3356 (NH+NH_2), 1747 (C=O, ester), 1648 (C=O, amide) cm^{-1}; ^{1}H-NMR (500 MHz: DMSO-d_6):δ 1.34 (d, 3H, CH_3, *J* = 6.8 Hz, CH_3), 3.60 (s, 3H, OCH_3), 4.35-4.41 (m, H, CH), 6.37 (s, 2H, NH_2, D_2O exchangeable), 6.84 (t, 1H, *J* = 6.9 Hz, Ar-H), 6.65 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.11 (t, 1H, *J* = 8.4 Hz, Ar-H), 7.54 (d, 1H, *J* = 6.8 Hz, Ar-H), 8.47 (d, 1H, *J* = 6.8 Hz, NH, D_2O exchangeable). ^{13}C-NMR (125 MHz: DMSO-d_6): δ 17.20, 40.30, 40.50, 114.16, 114.96, 116.85, 129.02, 132.54, 150.33, 169.41, 173.96. Anal. Calcd. for $C_{11}H_{14}N_2O_3$: C, 59.45; H, 6.35; N, 12.60. Found: C, 59.60; H, 6.53; N, 12.47.

Methyl 2-(2-aminobenzamido)-3-phenylpropanoate ***2c*** [25]

Compound **2c** was obtained as white crystals 0.75 g (83.8%) yield; mp 104-105ºC; IR (KBr): 3479 (NH), 3373, 3307 (NH_2), 1723 (C=O, ester), 1634 (C=O, amide) cm^{-1}; ^{1}H-NMR (500 MHz: DMSO-d_6): δ 3.05-3.11 (m, 2H, CH_2), 3.60 (s, 3H, CH_3), 4.56-4.57 (m, 1H, CH), 6.29 (s,2H, NH_2, D_2O exchangeable), 6.47 (t, 1H, *J* = 7.6 Hz, Ar-H), 6.63 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.09-7.23 (m, 2H, Ar-H), 7.24-7.27 (m, 4H, Ar-H), 7.44 (d, 1H, *J*= 7.6 Hz, Ar-H), 8.51 (d, 1H, *J* = 7.6 Hz, NH, D_2O exchangeable). ^{13}C-NMR (125 MHz: DMSO-d_6): δ 39.88, 52.45, 54.49, 114.16, 115.08, 116.87, 127.02, 128.79, 128.87, 129.57, 132.62, 138.32, 150.23, 169.55, 172.98. Anal. Calcd. for $C_{17}H_{18}N_2O_3$: C, 68.44; H, 6.08; N, 9.39. Found: C, 68.59; H, 6.24; N, 9.57.

Methyl 3-(2-aminobenzamido)propanoate ***3a*** [35]

Compound **3a** was obtained as colorless crystals 0.53 g (79.5%) yield; mp 67-68ºC; IR (KBr): 3444, 3415, 3332 (NH+NH_2), 1716 (C=O, ester), 1645 (C=O, amide) cm^{-1}; ^{1}H-NMR (500 MHz: DMSO-d_6): δ 2.53 (t, 2H, *J* = 7.0 Hz, CH_2), 3.42 (s, 2H, CH_2), 3.57 (s, 3H, OCH_3), 6.33 (s, 2H, NH_2, D_2O exchangeable), 6.46 (t, 1H, *J* =7.6 Hz, Ar-H), 6.64 (d, 1H, *J* = 7.6 Hz, Ar-H), 7.09 (t, 1H, *J* = 7.6 Hz, Ar-H), 7.39 (d, 1H, *J* = 7.6 Hz, Ar-H), 8.23 (t, 1H, NH, D_2O exchangeable). Anal. Calcd. for $C_{14}H_{20}N_2O_3$: C, 63.62; H, 7.63; N, 10.60. Found: C, 63.79; H, 7.80; N, 10.87.

Methyl 4-(2-aminobenzamido)butanoate ***3b***

Compound **3b** was obtained colorless crystals 0.61 g (86%) yield; mp 80-82ºC; IR (KBr): 3455, 3345, 3272 (NH+NH_2), 1725 (C=O, ester), 1618 (C=O, amide) cm^{-1}; ^{1}H-NMR (500 MHz: DMSO-d_6): δ 1.74-1.79 (m, 2H, CH_2), 2.36 (t, 2H, *J* = 9.2 Hz, CH_2), 3.20-3.23 (m, 2H, CH_2), 3.78 (s, 3H, OCH_3), 6.48-6.50 (m, 2H, NH_2, D_2O exchangeable), 6.67-6.69 (m, 1H, Ar-H), 7.11-7.12 (m, 1H, Ar-H), 7.45-7.47 (m, 1H, Ar-H), 8.04-8.22 (m, 1H, Ar-H), 12.21 (s, 1H, NH, D_2O exchangeable). Anal. Calcd.for $C_{12}H_{16}N_2O_3$: C, 61.00; H, 6.83; N, 11.86. Found: C, 61.27; H, 6.91; N, 11.70.

4.1.2 General procedure for the preparation quinazolinone ester derivatives 4-13

Method A: *Conventional Procedure*: To the solution of methyl (2-aminobenzamido) ester derivatives **2a-2c** (1 mmol) in ethanol (15 mL), 2 drops acetic acid was added. The appropriate aldehydes were added at room temperature. The reaction mixture was heated under reflux for about 8 hours. The solvent was removed under vacuum and the crude product was recrystallized from ethanol, filtered and washed with ethanol to afford the pure product.

Method B: *Microwave-Irradiation:* Employing a domestic

microwave (LG, 1000W); the initial step was conducted in a Teflon vessel. Methyl (2-aminobenzamido) ester derivatives **2a-2c** (1 mmol) was mixed with the appropriate aldehydes in small amount of isopropyl alcohol in the Teflon closed vessel. Two drops of acetic acid were added to the reaction mixture. The reaction mixture was irradiated in the domestic microwave oven for 10 minutes. The corresponding crude products were filtered, dried, and recrystallized from ethanol.

*Methyl 2-(1,2-dihydro-4-oxo-2-phenylquinazolin-3(4H)-yl)acetate **4***

Compound **4** was obtained as white crystals. **Method A**: 0.23 g (77.6%) yield; **Method B**: 0.27 g (91%) yield; mp 162-163ºC; IR (KBr): 3312 (NH), 1735 (C=O, ester), 1637 (C=O, amide) cm^{-1}; ^{1}H-NMR (500 MHz: DMSO-d_6): δ 3.36 (d, 1H, *J* = 16.8 Hz, α-CH), 3.55 (s, 3H, OCH_3), 4.35 (d, 1H, *J* = 16.8 Hz, α-CH), 5.89 (d, 1H, *J* = 1.6 Hz, sp^3 C-H), 6.65-6.67 (m, 2H, Ar-H), 7.21-7,24 (m, 1H, Ar-H), 7.33-7.36 (m, 6H, 5 Ar-H + (NH, D_2O exchangeable)), 7.60 (d, 1H, *J* =7.6 Hz, Ar-H). ^{13}C-NMR (125 MHz: DMSO- d_6): δ 40.17, 40.51, 72.18, 114.48, 114.88, 117.85, 127.45, 128.14, 129.23, 129.53, 134.24, 140.20, 147.71, 163.45, 169.74. Anal. Calcd .for $C_{17}H_{16}N_2O_3$: C, 68.91; H, 5.44; N, 9.45. Found: C, 68.77; H, 5.26; N, 9.70.

*Methyl 2-(2-(benzo[d][1,3]dioxol-5-yl)-1,2-dihydro-4-oxoquinazolin-3(4H)-yl)acetate **5***

Compound **5** was obtained as white crystals. **Method A:** 0.25 g (73.5%) yield; **Method B:** 0.31 g (91%) yield; mp 210-211ºC; IR (KBr): 3455 (NH), 1752 (C=O, ester), 1635 (C=O, amide) cm^{-1}; ^{1}H-NMR (500 MHz: DMSO-d_6): δ 3.54 (d, 1H, *J* =16.8 Hz, α-CH), 3.56 (s, 3H, OMe), 4.32 (d. 1H, *J* =16.8 Hz, α-CH), 5.81 (d, 1H, *J* = 1.5 Hz, sp^3C-H), 5.98 (s, 2H, CH_2), 6.65-6.68 (m, 2H, Ar-H), 6.79-6.81 (m, 1H, A-H), 6.86 (d, 1H, *J* = 7.6 Hz, Ar-H), 6.92 (brs, 1H, Ar-H), 7.21-7.24 (m, 2H, 1Ar-H+ (NH, D_2O exchangeable)), 7.59 (d, 1H, *J* = 6.9 Hz, Ar-H). ^{13}C-NMR (125 MHz: DMSO-d_6): δ 40.00, 40.34, 72.04, 101.83, 107.55, 108.58, 114.42, 114.87, 117.88, 121.34, 128.12, 133.91, 134.23, 147.72, 148.10, 148.34, 163.47, 169.79. Anal. Calcd. for $C_{18}H_{16}N_2O_5$: C, 63.52; H, 4.74; N, 8.23.Found: C, 63.57; H, 4.71; N, 8.20.

*Methyl 2-(2-(furan-2-yl)-1,2-dihydro-4-oxoquinazolin-3(4H)-yl)acetate **6***

Compound **6** was obtained as pale brown crystals. **Method A:** 0.21 g (73.4%) yield; **Method B:** 0.27 g (94.3%) yield, mp 93-94 ºC; IR (KBr): 3443 (NH), 1730 (C=O, ester), 1642 (C=O, amide) cm^{-1}. ^{1}H-NMR (500 MHz: DMSO-d_6): δ 3.58 (s, 3H, OMe), 3.81 (d, 1H, *J* = 16.8 Hz, α-CH), 4.42 (d. 1H, *J* = 16.8 Hz, α-CH), 5.95 (d, 1H, *J* = 2.3 Hz, sp^3 C-H), 6.26 (d, 1H, *J* = 3.8 Hz, Ar-H), 6.33-6.34 (m, 1H, Ar-H), 6.65-6.71 (m, 2H, Ar-H), 7.22-7.26 (m, 1H, Ar-H), 7.42-7.43 (m, 1H, Ar-H), 7.57-7.58 (m, 2H, 1Ar-H+ (NH, D_2O exchangeable)). Anal. Calcd. for $C_{15}H_{14}N_2O_4$: C, 62.93; H, 4.93; N, 9.79. Found: C, 62.78; H, 4.80; N, 9.55.

*Methyl 2-(1,2-dihydro-2-(4-chlorophenyl)-4-oxoquinazolin-3(4H)-yl) **7***

Compound **7** was obtained as colorless crystals. **Method A:** 0.26 g (78%) yield; **Method B:** 0.30 g (89.9%) yield; mp 140-142ºC; IR (KBr): 3311 (NH), 1745 (C=O, ester), 1636 (C=O, amide) cm^{-1}. ^{1}H-NMR (500 MHz: DMSO-d_6):δ 3.39 (s, 3H, OCH_3), 3.61 (d, 1H, *J* = 17.2 Hz, α-CH), 4.32 (d, 1H, *J* = 17.2 Hz, α-CH), 5.91 (s, 1H, sp^3C-H), 6.64-6.69 (m, 2H, Ar-H), 7.22 (t, 1H, *J* = 7.6 Hz, Ar-H), 7.83-7.42 (m, 5H, 4Ar-H+ (NH, D_2O exchangeable)), 7.60 (d, 1H, *J* = 7.6 Hz, Ar-H). Anal. Calcd. for $C_{17}H_{15}ClN_2O_3$: C, 61.73; H, 4.57; N, 8.47. Found: C, 61.50; H, 4.72; N, 8.23.

*Methyl 2-(1,2-dihydro-2-(4-methoxyphenyl)-4-oxoquinazolin-3(4H)-yl)acetate **8***

Compound **8** was obtained as white crystals. **Method A:** 0.26 g (79.7%) yield; **Method B:** 0.31 g (95%) yield; mp 150-151ºC; IR (KBr): 3308 (NH), 1730 (C=O, ester), 1636 (C=O, amide) cm^{-1}. ^{1}H-NMR (500 MHz: DMSO-d_6): δ 3.49 (d, 1H, *J* = 16.8 Hz, α-CH), 3.55 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 4.30 (d, 1H, *J* = 16.8 Hz, α-CH), 5.84 (s, 1H, sp^3 C-H), 6.66-6.67 (m, 2H, Ar-H), 6.91 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.21-7.31 (m, 4H, 3Ar-H+ (NH D_2O exchangeable)), 7.60 (d, 1H, *J* =7.8 Hz, Ar-H). ^{13}C-NMR (125 MHz: DMSO-d_6): δ 40.03, 40.36, 40.52, 71.94, 114.53, 114.89, 117.83, 128.16, 129.01, 131.83, 134.19, 147.95, 160.32, 163.63, 169.81. Anal. Calcd. for.$C_{18}H_{18}N_2O_4$: C, 66.25; H, 5.56; N, 8.58. Found: C, 66.29; H, 5.53; N, 8.55.

*Methyl 2-(4-oxo-2-phenyl-1,2-dihydroquinazolin-3(4H)-yl)propanoate **9***

Compound **9** was obtained as white crystals. **Method A:** 0.27 g (87%) yield, **Method B:** 0.29 g (93.4%) yield; mp 159-160 ºC; IR (KBr): 3353 (NH), 1739 (C=O, ester), 1623 (C=O, amide) cm^{-1}. ^{1}H-NMR (500 Hz: DMSO-d_6): (Isomer I:67 %):δ 1.14 (d, 3H, *J* = 6.9 Hz, CH_3), 3.45 (s, 3H, OCH_3), 4.05 (q, 1H, *J* = 6.9 Hz, CH), 5.89 (d, 1H, *J* = 1.6 Hz, sp^3C-H), 6.59-6.67 (m, 4H, Ar-H), 7.19-7.24 (m, 2H, Ar-H + (NH, D_2O exchangeable)), 7.33-7.35 (m, 2H, Ar-H), 7.39-7.41 (m, 1H, Ar-H), 7.58 (d, 1H, *J* = 6.1 Hz, Ar-H). (Isomer II: 23 %):δ 1.23 (d, 3H, *J* = 6.9 Hz, CH_3), 3.53 (s, 3H, OCH_3), 4.05 (q, 1H, *J*= 6.9 Hz, CH), 5.89 (s, 1H, sp^3 C-H), 6.59-6.67 (m, 4H, Ar-H), 7.19-7.24 (m, 2H, Ar-H + (NH, D_2O exchangeable)), 7.33-7.35 (m, 2H, Ar-H), 7.39-7.41 (m, 1H, Ar-H), 7.58 (d, 1H, *J* = 6.1 Hz, Ar-H). Anal. Calcd. for $C_{18}H_{18}N_2O_5$: C, 69.66; H, 5.85; N, 9.03. Found: C, 69.80; H, 6.07; N, 9.28.

*Methyl 2-(2-(benzo[d][1,3]dioxol-5-yl)-1,2-dihydro-4-oxoquinazolin-3(4H)-yl)propanoate **10***

Compound **10** was obtained as white crystals. **Method A:** 0.29 g (81.8%) yield; **Method B:** 0.33 g (93%) yield; mp 165-166 ºC; IR (KBr): 3347 (NH), 1727 (C=O, ester), 1624 (C=O, amide)cm^{-1}; ^{1}H-NMR (500 MHz: DMSO-d_6): δ (isomer I: 82%): δ 1.17 (d, 3H, *J* = 6.8 Hz, CH_3), 3.53 (s, 3H, OCH_3), 4 .01 (q, 1H, *J* = 6.9 Hz, CH), 5.82 (s, 1H, sp^3 C-H), 5.98 (d, 2H, *J* = 9.1 Hz, CH_2), 6.60-6.66 (m, 2H, Ar-H), 6.83-6.96 (m, 3H, Ar-H), 7.17-7.22 (m, 2H, Ar-H+ (NH, D_2O exchangeable)), 7.56 (d, 1H, *J* = 6.85 Hz, Ar-H). (Isomer II: 18%): δ 1.24 (d, 3H, *J* = 6.9 Hz, CH_3), 3.57 (s, 3H, OCH_3), 4.23 (q, 1H, *J* = 6.9 Hz, CH), 5.82 (s, 1H, sp^3 C-H), 5.98 (d, 2H, *J* = 9.1Hz, CH_2), 6.60-6.66 (m, 2H, Ar-H), 6.83-6.96 (m, 3H, Ar-H), 7.17-7.22 (m, 2H, Ar-H+ (NH D_2O exchangeable)), 7.56 (d, 1H, *J* = 6.9 Hz, Ar-H). ^{13}C-NMR (125 MHz: DMSO-d_6): δ 15.56, 52.30, 54.64, 72.87, 101.82, 107.33, 108.61, 114.61, 117.75, 121.26, 127.99, 134.19, 135.17, 147.23, 147.94, 148.29, 162.61, 171.74. Anal. Calcd. for $C_{19}H_{18}N_2O_5$: C, 64.40; H, 5.12; N, 7.91. Found: C, 64.65; H, 5.30; N, 7.78.

*Methyl 2-(2-(furan-2-yl)-4-oxo-1,2-dihydroquinazolin-3(4H)-yl)propanoate **11***

Compound **11** was obtained as pale yellow crystals. **Method A:** 0.21 g (70%) yield; **Method B:** 0.28 g (93%) yield; mp 183-184ºC; IR (KBr): 3338 (NH), 1737 (C=O, ester), 1634 (C=O, amide) cm^{-1}; ^{1}H-NMR (500 MHz: DMSO-d_6): δ 1.19 (d, 3H, J = 6.9Hz, CH_3), 3.47 (s, 3H, OCH_3), 4.34 (q, 1H, J = 6.9 Hz, CH), 5.95 (d, 1H, J = 3.1 Hz, sp^3 C-H), 6.27 (d, 1H, J = 3.1 Hz, Ar-H), 6.33-6.34 (m, 1H, Ar-H), 6.65-6.68 (m, 2H, Ar-H), 7.22 (t, 1H, J = 6.8 Hz, Ar-H), 7.31 (s, 1H, NH, D_2O exchangeable), 7.53-7.56 (m, 2H, Ar-H). Anal. Calcd. for $C_{16}H_{16}N_2O_4$: C, 63.99; H, 5.37; N, 9.33. Found: C, 63.73; H, 5.59; N, 9.55.

Methyl 2-(2-(furan-2-yl)-4-oxo-1,2-dihydroquinazolin-3(4H)-yl)-3-phenylpropanoate ***12***

Compound **12** was obtained as pale brown crystals. **Method A:** 0.31 g (82.4%) yield; **Method B:** 0.35 g (93%) yield; mp 175-176; ºC. IR (KBr): 3298 (NH), 1729 (C=O, ester), 1634 (C=O, amide) cm^{-1}. ^{1}H-NMR (500 MHz: DMSO-d_6): δ 3.34 (s, 3H, OCH_3), 3.45 (s, 2H, CH_2), 4.99 (t, 1H, J =7.6 Hz, CH), 6.06 (d, 1H, J = 2.3 Hz, sp^3 C-H), 6.17 (d, 1H, J = 3.1 Hz, Ar-H), 6.28-6.30 (m, 2H, Ar-H), 6.64-6.67 (m, 2H, Ar-H), 7.17-7.22 (m, 5H, Ar-H), 7.30 (brs, 1H, NH, D_2O exchangeable), 7.49 (d, 1H, J = 6.1 Hz, Ar-H), 7.56 (t, 1H, J =8.4 Hz, Ar-H). Anal. Calcd. for $C_{22}H_{20}N_2O_4$: C, 70.20; H, 5.36; N, 7.44. Found: C, 70.47; H, 5.53; N, 7.69.

Methyl 2-(2-(4-chlorophenyl)-1,2-dihydro-4-oxoquinazolin-3(4H)-yl)-3-phenylpropanoate ***13***

Compound **13** was obtained as white crystals. **Method A:** 0.32 g (76%) yield; **Method B:** 0.39 g (92.7%) yield; mp148-149 ºC; IR (KBr): 3450 (NH), 1741 (C=O, ester), 1686 (C=O, amide) cm^{-1}; ^{1}H-NMR (500 MHz: DMSO-d_6): δ 3.54 (s, 3H, OCH_3), 3.59 (s, 2H, CH_2), 4.90-4.93 (m, 1H, CH), 6.28 (brs, 1H, sp^3 C-H), 6.46 (t, 1H, J = 7.6 Hz, Ar-H), 6.62 (d, 1H, J = 7.6 Hz, Ar-H), 7.00 (t, 1H, J = 8.6 Hz, Ar-H), 7.06- 7.11 (m, 1H, Ar-H), 7.24 (t, 2H, J = 8.3 Hz, Ar-H), 7.48 (d, 1H, J = 8.6 Hz, Ar-H), 7.54 (d, 2H, J = 8.6 Hz, Ar-H), 7.72 (d, 1H, J = 8.6 Hz, Ar-H), 7.95 (d, 1H, J = 7.6 Hz, Ar-H), 8.54 (d, 2H, J = 8.6 Hz, Ar-H), 9.42 (s, 1H, NH, D_2O exchangeable). Anal. Calcd. for $C_{24}H_{21}ClN_2O_3$: C, 68.49; H, 5.03; N, 6.66. Found: C, 68.23; H, 5.26; N, 6.49.

4.1.3 General procedure for the preparation of quinazolinone amino acid esters 22-30

Method A: *Conventional Procedure*: To the solution of methyl(2-aminobenzamido) ester derivatives **3a-3c** (1 mmol) in ethanol (15 mL), 2 drops acetic acid was added. The appropriate aldehydes were added at room temperature. The reaction mixture was heated under reflux for about 8 hours. The solvent was removed under vacuum and the crude product was recrystallized from ethanol, filtered and washed with ethanol to afford the pure product.

Method B: *Microwave-Irradiation:* Employing a domestic microwave (LG, 1000W); the initial step was conducted in a Teflon vessel. Methyl(2-aminobenzamido) ester derivatives **3a-3c** (1 mmol) was mixed with the appropriate aldehydes in small amount of isopropyl alcohol in the Teflon closed vessel. Two drops of acetic acid were added to the reaction mixture. The reaction mixture was irradiated in the domestic microwave oven for 10 minutes. The corresponding crude products were filtered, dried, and recrystallized from ethanol.

Methyl 3-(1,2-dihydro-4-oxo-2-phenylquinazolin-3(4H)-yl)propanoate ***22***

Compound **22** was obtained as white crystals. **Method A:** 0.25 g (80.5%) yield; **Method B:** 0.30 g (96.7%) yield; mp 125-126 ºC; IR (KBr): 3301 (NH), 1733 (C=O, ester), 1632 (C=O, amide) cm^{-1}. ^{1}H-NMR (500 MHz: DMSO-d_6): δ 2.44-2.49 (m, 1H, CH), 2.63-2.69 (m, 1H, CH), 3.02-3.07 (m, 1H, CH), 3.53 (s, 3H, OCH_3), 3.91-3.94 (m, 1H, CH), 5.89 (d, 1H, J = 1.5 Hz, sp^3 C-H), 6.56-6.63 (m, 2H, Ar-H), 7.14 (t, 1H, J = 6.9 Hz, Ar-H), 7.28-7.35 (m, 6H, 5Ar-H + (NH, D_2O exchangeable)), 7.59 (d, 2H, J =7.6 Hz, Ar-H). Anal. Calcd. for $C_{18}H_{18}N_2O_3$: C, 69.66; H, 5.85; N, 9.03. Found: C, 69.90; H, 5.61; N, 8.73.

Methyl 3-(2-(benzo[d][1,3]dioxol-5-yl)-1,2-dihydro-4-oxoquinazolin-3(4H)-yl)propanoate ***23***

Compound **23** was obtained as white crystals. **Method A:** 0.29 g (81.8%) yield; **Method B:** 0.34 g (95.9%) yield; mp 178-179 ºC; IR (KBr): 3304 (NH), 1733 (C=O, ester), 1632 (C=O, amide) cm^{-1}. ^{1}H-NMR (500 MHz: DMSO-d_6): δ 2.46-2.49 (m, 1H, CH), 2.61-2.68 (m, 1H,CH), 3.02-3.08 (m, 1H, CH), 3.54 (s, 3H, OCH_3), 3.88-3.91 (m, 1H, CH), 5.81 (brs, 1H, sp^3 C-H), 5.95 (d, 2H, J = 2.3 Hz, CH_2), 6.57-6.63 (m, 2H, Ar-H), 6.73 (d, 1H, J = 7.6 Hz, Ar-H), 6.83 (d, 2H, J = 7.6 Hz, Ar-H), 7.17 (t, 1H, J = 7.6 Hz, Ar-H), 7.24 (s, 1H, NH, D_2O exchangeable), 7.59 (d, 1H, J =7.6 Hz, Ar-H). Anal. Calcd. for $C_{19}H_{18}N_2O_5$: C, 64.40; H, 5.12; N, 7.91.Found: C, 64.65; H, 5.35; N, 7.78.

Methyl 3-(2-(4-chlorophenyl)-1,2-dihydro-4-oxoquinazolin-3(4H)-yl)propanoate ***24***

Compound **24** was obtained as colorless crystals. **Method A:** 0.27 g (78.3%) yield; **Method B:** 0.33 g (95.7%) yield; mp 147-148 ºC; IR (KBr): 3305 (NH), 1731 (C=O, ester), 1631 (C=O, amide) cm^{-1}. ^{1}H-NMR (500 MHz: DMSO-d_6): δ 2.47-2.51 (m, 1H, CH), 2.63-2.69 (m, 1H, CH), 3.03-3.09 (m, 1H, CH), 3.53 (s, 3H, OCH_3), 3.93-3.96 (m, 1H, CH), 5.92 (d, 1H, J = 2.3 Hz, sp^3 C-H), 6.57 (d, 1H, J = 8.4 Hz, Ar-H), 6.63 (t, 1H, J = 7.6 Hz, Ar-H), 7.16 (td, 1H, ^3J = 8.4 Hz, ^4J = 1.6 Hz, Ar-H), 7.29 (d, 2H, J = 8.4 Hz, Ar-H), 7.38 (s, 1H, NH, D_2O exchangeable), 7.37-7.40 (m, 2H, Ar-H), 7.59 (d, 1H, J = 7.6 Hz, Ar-H). Anal. Calcd. for $C_{18}H_{17}ClN_2O_3$: C, 62.70; H, 4.97; N, 8.12. Found: C, 62.96; H, 4.74; N, 8.39.

Methyl 4-(4-oxo-2-phenyl-1,2-dihydroquinazolin-3(4H)-yl)butanoate ***25***

Compound **25**was obtained as colorless crystals. **Method A:** 0.26 g (80%) yield; **Method B:** 0.32 g (95.5%) yield; mp 148-149 ºC; IR (KBr): 3303 (NH), 1742 (C=O, ester), 1627 (C=O, amide) cm^{-1}; ^{1}H-NMR (500 MHz: DMSO-d_6): δ 1.69-1.80 (m, 2H, CH_2), 2.26-2.30 (m, 2H, CH_2), 2.72-2.75 (m, 1H, CH), 3.52 (s, 3H, OCH_3), 3.86-3.91 (m, 1H, CH), 5.80 (d, 1H, J = 2.3 Hz, sp^3 C-H), 6.59-6.63 (m, 2H, Ar-H), 7.15 (t, 1H, J = 7.6 Hz, Ar-H), 7.29-7.36 (m, 6H, 5Ar-H + (NH, D_2O exchangeable)), 7.61 (d, 1H, J = 6.8 Hz, Ar-H). Anal. Calcd. for $C_{19}H_{20}N_2O_3$: C, 70.35; H, 6.21; N, 8.64. Found: C, 70.59; H, 6.48; N, 8.92.

Methyl 4-(2-(benzo[d][1,3]dioxol-5-yl)-4-oxo-1,2-dihydroquinazolin-3(4H)-yl)butanoate ***26***

Compound **26** was obtained as white crystals. **Method A:** 0.30 g (81.4%) yield; **Method B:** 0.35 g (95%) yield; mp 140-141ºC; IR (KBr): 3299 (NH), 1743 (C=O, ester), 1630 (C=O,

amide) cm^{-1}; ^{1}H-NMR (500 MHz: DMSO-d_6): δ 1.68-1.79 (m, 2H, CH_2), 2.26-2.29 (m, 2H, CH_2), 2.71-2.74 (m, 1H, CH), 3.52 (s, 3H, OCH_3), 3.82-3.86 (m, 1H, CH), 5.71 (d, 1H, *J* = 2.3 Hz, sp^3 C-H), 5.94 (d, 2H, *J* = 1.6 Hz, CH_2), 6.59-6.63 (m, 2H, Ar-H), 6.71 (d, 1H, *J* = 9.9 Hz, Ar-H), 6.81-6.83 (m, 2H, Ar-H), 7.17 (t, 1H, *J* = 8.8 Hz, Ar-H), 7.26 (s, 1H, NH, D_2O exchangeable), 7.59 (d, 1H, *J* = 6.9 Hz, Ar-H). Anal. Calcd. for $C_{20}H_{20}N_2O_5$: C, 65.21; H, 5.47; N, 7.60. Found: C, 65.49; H, 5.62; N, 7.86.

Methyl 4-(2-(furan-2-yl)-4-oxo-1,2-dihydroquinazolin-3(4H)-yl)butanoate ***27***

Compound **27** was obtained as pale brown crystals. **Method A:** 0.26 g (82.7%) yield; **Method B:** 0.29 g (92.3%) yield; mp 109-110 ºC; IR (KBr): 3432 (NH), 1729 (C=O, ester), 1648 (C=O, amide) cm^{-1}; ^{1}H-NMR (500 MHz: DMSO-d_6): δ 1.71-1.80 (m, 2H, CH_2), 2.27-2.29 (m, 2H, CH_2), 2.90-2.93 (m, 1H, CH), 3.53 (s, 3H, OCH_3), 3.82-3.87 (m, 1H, CH), 5.84 (d, 1H, *J* = 3.1 Hz, sp^3 C-H), 6.15 (d, 1H, *J* = 6.1 Hz, Ar-H), 6.31-6.32 (m, 1H, Ar-H), 6.63-6.68 (m, 2H, Ar-H), 7.19 (t, 1H, *J* = 6.9 Hz, Ar-H), 7.34 (s, 1H, D_2O exchangeable), 7.53 (brs, 1H, Ar-H), 7.58 (d, 1H, *J* =6.9 Hz, Ar-H). Anal. Calcd. for $C_{17}H_{18}N_2O_5$: C, 64.96; H, 5.77; N, 8.91. Found: C, 65.23; H, 5.92; N, 8.67.

Methyl 6-(2-(benzo[d][1,3]dioxol-5-yl)-1,2-dihydro-4-oxoquinazolin-3(4H)-yl)hexanoate ***28***

Compound **28** was obtained as white crystals. **Method A:** 0.32 g (80.7%) yield, **Method B:** 0.37 g (93.3%) yield, mp 116-117 ºC; IR (KBr): 3289 (NH), 1738 (C=O, ester), 1630 (C=O, amide) cm^{-1}; ^{1}H-NMR (500 MHz: DMSO-d_6): δ 1.19-1.21 (m, 2H, CH_2), 1.45-1.48 (m, 2H, CH_2), 2.23 (t, 2H, *J* = 7.6 Hz, CH_2), 2.66-2.89 (m, 2H, CH_2), 3.52 (s, 3H, OCH_3), 3.78-3.81 (m, 2H, CH_2), 5.72 (d, 1H, *J* = 5.3 Hz, sp^3 C-H), 5.95 (d, 2H, *J* = 2.3 Hz, CH_2), 6.58-6.63 (m, 2H, Ar-H), 6.73 (d, 1H, *J* = 6.1 Hz, Ar-H), 6.82 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.16 (t, 1H, *J* = 6.8 Hz, Ar-H), 7.21 (1H, NH, D_2O exchangeable), 7.59 (d, 1H, *J* = 6.1 Hz, Ar-H). ^{13}C-.NMR (125 MHz: DMSO-d_6): δ 24.11, 25.75, 27.04, 38.87, 44.06, 51.21, 69.81, 101.20, 106.46, 107.97, 114.26, 114.90, 117.14, 119.59, 127.363, 135.15, 146.22, 147.31, 147.47, 162.21, 173.36. Anal. Calcd. for $C_{22}H_{24}N_2O_4$: C, 69.46; H, 6.36; N, 7.36. Found: C, 69.72; H, 6.51; N, 7.12.

Methyl 6-(2-(4-chlorophenyl)-4-oxo-1,2-dihydroquinazolin-3(4H)-yl)hexanoate ***29***

Compound **29** was obtained as white crystals. **Method A:** 0.28 g (72.4%) yield, **Method B:** 0.36 g (93%) yield; mp 84-85 ºC; IR (KBr):3297 (NH), 1734 (C=O, ester), 1680 (C=O, amide) cm^{-1}; ^{1}H-NMR (500 MHz: DMSO-d_6): δ 1.19-1.22 (m, 2H, CH_2), 1.45-1.49 (m, 3H, CH_2 +CH), 2.23 (t, 1H, *J* = 7.6 Hz, CH_2), 2.42-2.47 (m, 2H, CH_2), 2.69-2.72 (m, 1H, CH), 3.53 (s, 3H, OCH_3), 3.79-3.84 (m, 1H, CH), 5.83 (d, 1H, *J* = 1.5 Hz, sp^3 C-H), 6.63 (t, 1H, *J* = 7.6 Hz, 1H, Ar-H), 7.16 (t, 1H, *J* = 8.4 Hz, Ar-H), 7.30 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.33 (s, 1H, NH, D_2O exchangeable), 7.37 (d, 1H, *J* = 8.6 Hz, Ar-H), 7.54 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.90 (d, 2H, *J* = 8.4 Hz, Ar-H). Anal. Calcd. for $C_{21}H_{23}ClN_2O_3$: C, 65.20; H, 5.99; N, 7.24. Found: C, 65.58; H, 5.73; N, 7.50.

Methyl 6-(2-(benzylideneamino)benzamido)hexanoate ***30***

Compound **30** was obtained as white crystals. **Method A:** 0.294 g (83.4%) yield, **Method B:** 0.34 g (96.5%) yield, mp 89-90 ºC. IR (KBr): 3441 (NH), 1700 (C=O, ester), 1684 (C=O, amide) cm^{-1}. ^{1}H-NMR (500 MHz: DMSO-d_6): δ 0.99-1.03 (m, 2H, CH_2),1.26-1.29 (m, 2H, CH_2), 1.45-1.47 (m, 2H, CH_2), 2.12 (t, 2H, *J* = 7.6Hz ,CH_2), 3.52 (s, 3H, OCH_3), 3.82(t, 2H, *J*= 7.6 Hz, CH_2), 7.45-7.48 (m, 4H, Ar-H), 7.52 (t, 1H, *J* = 7.7 Hz, 1H, NH, D_2O exchangeable), 7.57-7.59 (m, 2H, Ar-H), 7.90-7.91 (m, 4H, 3Ar-H+ CH). Anal. Calcd. for $C_{21}H_{24}N_2O_3$: C, 71.57; H, 6.86; N, 7.95. Found: C, 71.80; H, 6.99; N, 7.70.

4.1.4 General procedure for the preparation of quinazolinone amino acid hydrazides 14-21, 31-35

Method A: To the solution of (1 mmol) methyl 2-(1,2-dihydro-4-oxo-2-arylquinazolin-3(4*H*)-yl)acetate **4–8,** methyl 2-(4-oxo-2-aryl-1,2-dihydroquinazolin-3(4*H*)-yl)propanoate **10-11,** methyl 2-(2-aryl-4-oxo-1,2-dihydroquinazolin-3(4*H*)-yl)-3-phenylpropanoate **12-13,** methyl 3-(1,2-dihydro-4-oxo-2-arylquinazolin-3(4*H*)-yl)propanoate **22-24**, ormethyl 4-(aryl-4-oxo-1,2-dihydroquinazolin-1,2-dihydroquinazolin-3(4*H*)-yl)butanoate **25-27** in 10 mL methanol, 2 mL hydrazine hydrate was added. The reaction mixture was refluxed for 8 hrs. The solvent was removed under vacuum and the crude oily residue was recrystallized from ethanol/ether, filtered and washed with ether to afford the pure product.

Method B: *Microwave-Irradiation:* Employing a domestic microwave (LG, 1000W); the initial step was conducted in a Teflon vessel. Methyl 2-(1,2-dihydro-4-oxo-2-arylquinazolin-3(4*H*)-yl)acetate **4–8,** methyl 2-(4-oxo-2-aryl-1,2-dihydroquinazolin-3(4*H*)-yl)propanoate **10-11,** methyl 2-(2-aryl-4-oxo-1,2-dihydroquinazolin-3(4*H*)-yl)-3-phenylpropanoate **12-13,** methyl 3-(1,2-dihydro-4-oxo-2-arylquinazolin-3(4*H*)-yl)propanoate **22-24**, ormethyl 4-(aryl-4-oxo-1,2-dihydroquinazolin-1,2-dihydroquinazolin-3(4*H*)-yl)butanoate **25-27** (1 mmol) was mixed with 2 mL hydrazine hydrate in small amount of isopropyl alcohol in the Teflon closed vessel. The reaction mixture was irradiated in the domestic microwave oven for 10 minutes. The corresponding crude products were filtered, dried, and recrystallized from ethanol.

2-(4-oxo-2-phenyl-1,2-dihydroquinazolin-3(4H)-yl)acetohydrazide ***14***

Compound **14** was obtained as colorless crystals. **Method A:** 0.24 g (80.1%) yield; **Method B:** 0.275g (92.8%) yield, mp 162-163ºC; IR (KBr): 3449-3300 (NH+NH_2), 1743 (C=O, amide), 1658 (C=O, amide) cm^{-1}; ^{1}H-NMR (500 MHz: DMSO-d_6): δ 3.10 (d, 1H, *J* = 16.1 Hz, α-CH), 4.14 (s, 2H, NH_2, D_2O exchangeable), 4.40 (d, H, *J* = 16.1 Hz, α-CH), 5.88 (s, 1H, sp^3 C-H), 6.62-6.66 (m, 2H, Ar-H), 7.19-7.21 (m, 1H, Ar-H), 7.24 (s, 1H, NH, D_2O exchangeable), 7.30-7.39 (m, 5H, Ar-H), 7.61 (d, 1H, *J* = 7.6 Hz, Ar-H), 8.97 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (125 MHz: DMSO- d_6): δ 40.32, 72.07, 114.73, 117.66, 127.34, 128.23, 129.41, 134.00, 140.42, 147.45, 163.41, 167.77. Anal. Calcd. for $C_{16}H_{16}N_4O_2$: C, 64.85; H, 5.44; N, 18.91. Found: C, 64.69; H, 5.62; N, 18.79.

2-(2-(benzo[d][1,3]dioxol-5-yl)-4-oxo-1,2-dihydroquinazolin-3(4H)-yl)acetohydrazide ***15***

Compound **15** was obtained as colorless crystals. **Method A:** 0.27 g (79.3%) yield; **Method B:** 0.31 g (91%) yield; mp 200-201ºC; IR (KBr): 3325-3338 (NH+NH_2), 1679(C=O, amide), 1661 (C=O, amide) cm^{-1}; ^{1}H-NMR (500 MHz: DMSO-d_6): δ 3.13 (d, 1H, *J* = 16.1 Hz, α-CH), 4.14 (s, 2H, NH_2, D_2O

exchangeable), 4.35 (d, 1H, *J* = 16.1Hz, α-CH), 5.80 (s, 1H, sp^3 C-H), 5.98 (s, 2H, CH_2), 6.64 (t, 2H, *J* = 5.3 Hz, Ar-H), 6.67-6.85 (m, 1H, Ar-H), 6.87-6.93 (m, 2H, Ar-H),7.19 (s, 1H, NH, D_2O exchangeable), 7.22-7.25 (m, 1H, Ar-H), 7.59 (d, 1H, *J* =7.6 Hz, Ar-H), 8.96 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (125 MHz: DMSO- d_6): δ 40.18, 71.96, 101.80, 107.51, 108.58, 114.74, 117.69, 121.19, 128.10, 133.99, 134.18, 147.49, 148.08, 148.23, 163.44, 167.82. Anal. Calcd. for $C_{17}H_{16}N_4O_4$: C, 59.99; H, 4.74; N, 16.46. Found: C, 59.73; H, 4.57; N, 16.29.

2-(2-(furan-2-yl)-4-oxo-1,2-dihydroquinazolin-3(4H)-yl)acetohydrazide ***16***

Compound **16** was obtained as pale brown crystals. **Method A:** 0.22 g (76.8%) yield, **Method B:** 0.26 g (92.9%) yield, mp 65-66ºC. IR (KBr): 3728, 3284 (NH+NH_2), 1678 (C=O, amide), 1637 (C=O, amide) cm^{-1}. ^{1}H-NMR (500 MHz: DMSO-d_6): δ 4.14 (s, 2H, NH_2, D_2O exchangeable), 4.61 (d, 1H, *J* = 16.8 Hz, α-CH), 4.89 (d, 1H, *J* = 16.8 Hz, α-CH), 5.91 (brs, 1H, sp^3 C-H), 6.35-6.69 (m, 1H, Ar-H), 6.66-6.69 (m, 2H, Ar-H), 7.23-7.38 (m, 2H, Ar-H + (1NH, D_2O exchangeable)), 7.49-7.68 (m, 3H, Ar-H), 11.04 (s, 1H, NH, D_2O exchangeable). Anal. Calcd. for $C_{14}H_{14}N_4O_3$: C, 58.73; H, 4.93; N, 19.57. Found: C, 58.99; H, 4.70; N, 19.32.

2-(2-(4-chlorophenyl)-1,2-dihydro-4-oxoquinazolin-3(4H)-yl)acetohydrazide ***17***

Compound **17** was obtained as colorless crystals. **Method A:** 0.29 g (87.7%) yield; **Method B:** 0.31 g (93.7%) yield; mp 158-159 ºC. IR (KBr): 3338, 3229 (NH_2), 3042 (NH), 1700 (C=O, amide), 1657 (C=O, amide) cm^{-1}. ^{1}H-NMR (500 MHz: DMSO-d_6): δ 3.61 (d, 1H, *J* = 17.5 Hz, α-CH), 4.02 (s, 2H, NH_2, D_2O exchangeable), 4.33 (d, 1H, *J* = 17.5 Hz, α-CH), 5.96 (s, 1H, sp^3.C-H), 6.65-6.67 (m, 2H, Ar-H), 7.23-7.37 (m, 1H, Ar-H), 7.41-7.43 (m, 5H, 4 Ar-H + (1NH, D_2O exchangeable)), 7.56-7.62 (m, 1H, Ar-H), 9.63 (s, 1H, NH, D_2O exchangeable). Anal. Calcd. for $C_{16}H_{15}ClN_4O_2$: C, 58.10; H, 4.57; N, 16.94. Found: C, 58.34; H, 4.82; N, 17.18.

2-(2-(4-methoxyphenyl)-4-oxo-1,2-dihydroquinazolin-3(4H)-yl)acetohydrazide ***18***

Compound **18** was obtained as yellow crystals. **Method A:** 0.26 g (79.7%) yield; **Method B:** 0.30 g (92%) yield; mp 187-188ºC; IR (KBr): 3437, 3296 (NH+NH_2), 1672 (C=O, amide), 1633 (C=O, amide) cm^{-1}. ^{1}H-NMR (500 MHz: DMSO-d_6): δ 3.07 (d, 1H, *J* = 16.1 Hz, α-CH), 3.70 (s, 3H, OCH_3), 4.13 (s, 2H, NH_2, D_2O exchangeable), 4.35 (d, 1H, *J* = 16.1 Hz, α-CH), 5.83 (s, 1H, sp^3 C-H), 6.64 (t, 2H, *J* = 8.4 Hz, Ar-H), 6.92 (d, 2H, *J* = 9.1 Hz, Ar-H), 7.12 (s, 1H, NH, D_2O exchangeable), 7.20 (t, 1H, *J* = 6.9 Hz, Ar-H), 7.28 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.59 (d, 1H, *J* = 8.4 Hz, Ar-H), 8.59 (s, 1H, NH, D_2O exchangeable). Anal. Calcd. for $C_{17}H_{18}N_4O_3$: C, 62.57; H, 5.56; N, 17.17. Found: C, 62.33; H, 5.31; N, 17.43.

2-(2-(furan-2-yl)-1,2-dihydro-4-oxoquinazolin-3(4H)-yl)propanehydrazide ***19***

Compound **19** was obtained as white crystals. **Method A:** 0.23 g (76.6%) yield; **Method B:** 0.28 g (93%) yield; mp 173-174ºC; IR (KBr): 3423, 3344 (NH_2), 3223 (NH), 1737 (C=O, amide), 1634 (C=O, amide) cm^{-1}; ^{1}H-NMR (500 MHz: DMSO-d_6): δ 1.20 (d, 3H, *J* = 6.8 Hz, CH_3), 4.14 (s, 2H, NH_2, D_2O exchangeable), 4.33-4.35 (m, 1H, CH), 5.95 (s, 1H, sp^3 C-H), 6.27-6.33 (m, 2H, Ar-H), 6.65-6.68 (m, 2H, Ar-H), 7.21 (t, 1H, *J* = 5.3 Hz, Ar-H), 7.24 (s, 1H, NH, D_2O exchangeable), 7.52-7.57 (m, 2H, Ar-H), 9.63 (s, 1H, NH, D_2O exchangeable). Anal. Calcd. for $C_{15}H_{16}N_4O$: C, 59.99; H, 5.37; N, 18.66. Found: C, 60.25; H, 5.52; N, 18.91.

2-(2-(furan-2-yl)-1,2-dihydro-4-oxoquinazolin-3(4H)-yl)-3-phenylpropanehydrazide ***20***

Compound **20** was obtained as grey powder. **Method A:** 0.32 g (85%); yield; **Method B:** 0.35 g (93%); yield; mp 145-146 ºC.IR (KBr): 3465, 3313 (NH + NH_2), 1660 (C=O, amide), 1623 (C=O, amide) cm^{-1}. ^{1}H-NMR (500 MHz: DMSO-d_6): δ 2.98 (d, 2H, *J* = 7.6 Hz, CH_2), 4.25 (brs, 2H, NH_2, D_2O exchangeable), 4.57 (q, 1H, *J*=7.6Hz, α- CH), 5.92 (brs, 1H, sp^3 C-H), 6.22 (s, 2H, Ar-H), 6.45 (t, 1H, *J* = 7.6 Hz, Ar-H), 6.63 (d, 1H, *J* = 7.6 Hz, Ar-H), 7.07 (t, 1H, *J* = 7.6 Hz, Ar-H), 7.13 (t, 1H, *J* = 6.9 Hz, Ar-H) ,7.23-7.30 (m, 5H, 4Ar-H + (NH, D_2O exchangeable), 7.45 (d, 1H, *J* =7.6Hz, Ar-H), 8.18 (d, 1H, *J* =8.4Hz, Ar-H), 9.24 (s, 1H, NH, D_2O exchangeable). Anal. Calcd. for $C_{21}H_{20}N_4O_3$: C, 67.01; H, 5.36; N, 14.88. Found: C, 67.28; H, 5.51; N, 14.63.

2-(2-(4-Chlorophenyl)-1,2-dihydro-4-oxoquinazolin-3(4H)-yl)-3-phenylpropanehydrazide ***21***

Compound **21** was obtained as white crystals. **Method A:** 0.31 g (73%) yield. **Method B:** 0.39 g (91.8%) yield; mp 85-86ºC; IR (KBr): 3476 (NH), 3372, 3307 (NH_2), 1723 (C=O, amide), 1633 (C=O, amide) cm^{-1}. ^{1}H-NMR (500 MHz: DMSO-d_6): δ 3.07-3.09 (m, 2H, CH_2), 3.59 (s, 2H, NH_2, D_2O exchangeable), 4.55-4.57 (m, 1H, CH), 6.28 (s, 1H, sp^3C-H), 6.46 (t, 1H, *J* = 7.6 Hz, Ar-H), 6.62 (d, 1H, *J* = 7.6 Hz, Ar-H), 7.09 (t, 2H, *J* = 6.8 Hz, Ar-H), 7.15-7.17 (m, 1H, NH, D_2O exchangeable), 7.20-7.25 (m, 5H, Ar-H), 7.43 (d, 1H, *J* = 6.1 Hz, Ar-H), 7.56 (d, 2H, *J* = 6.8 Hz, Ar-H), 7.87 (d, 1H, *J* = 8.4 Hz, Ar-H), 8.65 (s, 1H, NH, D_2O exchangeable). Anal. Calcd. for $C_{23}H_{21}ClN_4O_2$: C, 65.63; H, 5.03; N, 13.31. Found: C, 65.89; H, 4.87; N, 13.17.

3-(1,2-Dihydro-4-oxo-2-phenylquinazolin-3(4H)-yl)propanehydrazide ***31***

Compound **31** was obtained as colorless crystals. **Method A:** 0.26 g (83.8%) yield; **Method B:** 0.30 g (96.7%) yield; mp 126-127 ºC; IR (KBr): 3445, 3302 (NH + NH_2), 1733 (C=O, amide), 1631 (C=O, amide) cm^{-1}. ^{1}H-NMR (500 MHz: DMSO-d_6): δ 2.44-2.46 (m, 1H, CH), 2.62-2.66 (m, 1H, CH), 3.03-3.08 (m, 1H, CH), 3.53 (s, 2H, NH_2, D_2O exchangeable), 3.90-3.93 (m, 1H, CH), 5.88 (s, 1H, sp^3 C-H), 6.56-6.63 (m, 2H, Ar-H), 7.16 (t, 1H, *J* = 6.9 Hz, Ar-H), 7.28-7.35 (m, 6H, 5Ar-H + 1NH, D_2O exchangeable), 7.59 (d, 1H, *J* = 8.4 Hz, Ar-H), 8.97 (s, 1H, NH, D_2O exchangeable). Anal .Calcd. for $C_{17}H_{18}N_4O_2$: C, 65.79; H, 5.85; N, 18.05. Found: C, 65.53; H, 5.69; N, 18.28.

3-(2-(Benzo[d][1,3]dioxol-5-yl)-1,2-dihydro-4-oxoquinazolin-3(4H)-yl)propanehydrazide ***32***

Compound **32** was obtained as colorless crystals. **Method A:** 0.28 g (79%) yield; **Method B:** 0.33 g (93%) yield; mp 155-156ºC; IR (KBr): 3728, 3304 (NH + NH_2), 1732 (C=O, amide), 1631 (C=O, amide) cm^{-1}. ^{1}H-NMR (500 MHz: DMSO-d_6): δ 2.46-2.49 (m, 1H, CH), 2.61-2.66 (m, 1H, CH), 3.03-3.06 (m, 1H, CH), 3.54 (s, 2H, NH_2, D_2O exchangeable), 3.88-3.91 (m, 1H, CH), 5.81 (brs, 1H, sp^3 C-H), 5.96 (brs, 2H, CH_2), 6.57-6.63 (m, 2H, Ar-H), 6.72 (d, 1H, *J* = 7.6 Hz, Ar-H), 6.82-6.85 (m, 2H, Ar-H), 7.15-7.17 (m, 1H, Ar-H), 7.25 (s,

1H, NH, D_2O exchangeable), 7.59 (d, 1H, *J* = 6.9 Hz, Ar-H), 9.24 (s, 1H, NH, D_2O exchangeable). Anal. Calcd. for $C_{18}H_{18}N_4O_4$: C, 61.01; H, 5.12; N, 15.81. Found: C, 61.29; H, 5.38; N, 15.67.

3-(2-(4-Chlorophenyl)-1,2-dihydro-4-oxoquinazolin-3(4H)-yl)propanehydrazide ***33***

Compound **33** was obtained as colorless crystals. **Method A:** 0.25 g (72.5%) yield, **Method B:** 0.33 g (95.7%) yield; mp 165-166 ºC; IR (KBr): 3442, 3308 (NH + NH_2), 1731 (C=O, amide), 1630 (C=O, amide) cm^{-1}. ^{1}H-NMR (500 MHz: DMSO-d_6): δ 2.49-2.50 (m, 1H, CH), 2.63-2.69 (m, 1H, CH), 3.03-3.07 (m, 1H, CH), 3.54 (s, 2H, NH_2, D_2O exchangeable), 3.91-3.96 (m, 1H, CH), 5.92 (brs, 1H, sp^3 C-H), 6.57 (d, 1H, *J* = 6.9 Hz, Ar-H), 6.63 (t, 1H, *J* = 6.9 Hz, Ar-H), 7.17 (t, 1H, *J* = 6.9 Hz, Ar-H), 7.29 (d, 2H, *J* = 9.1 Hz, Ar-H), 7.35-7.40 (m, 3H, 2Ar-H + (NH, D_2O exchangeable)), 7.59 (d, 1H, *J* = 6.9 Hz, Ar-H), 9.63 (s, 1H, NH, D_2O exchangeable). Anal. Calcd. for $C_{17}H_{17}ClN_4O_2$: C, 59.22; H, 4.97; N, 16.25. Found: C, 59.47; H, 4.83; N, 16.01.

4-(1,2-Dihydro-4-oxo-2-phenylquinazolin-3(4H)-yl)butanehydrazide ***34***

Compound **34** was obtained as colorless crystals. **Method A:** 0.23 g (70.9%) yield; **Method B:** 0.31 g (95.5%) yield; mp 130-131ºC; IR (KBr): 3435, 3303 (NH + NH_2), 1724 (C=O, amide), 1626 (C=O, amide) cm^{-1}; ^{1}H-NMR (500 MHz: DMSO-d_6): δ 1.69-1.79 (m, 2H, CH_2), 2.27-2.29 (m, 2H, CH_2), 2.72-2.75 (m, 1H, CH), 3.52 (s, 2H, NH_2, D_2O exchangeable), 3.85-3.89 (m, 1H, CH), 5.79 (d, 1H, *J* = 2.3 Hz, sp^3 C-H), 6.58-6.63 (m, 2H, Ar-H), 7.26 (t, 1H, *J* = 7.6 Hz, Ar-H), 7.29-7.34 (m, 6H, 5Ar-H + (1NH, D_2O exchangeable)), 7.61 (d, 1H, *J* = 6.8 Hz, Ar-H), 9.08 (s, 1NH, D_2O exchangeable). Anal. Calcd. for $C_{18}H_{20}N_4O_2$: C, 66.65; H, 6.21; N, 17.27. Found: C, 66.89; H, 6.08; N, 17.53.

4-(2-(Benzo[d][1,3]dioxol-5-yl)-1,2-dihydro-4-oxoquinazolin-3(4H)-yl)butanehydrazide ***35***

Compound **35** was obtained as white crystals. **Method A:** 0.30 g (81%) yield; **Method B:** 0.34 g (92.3%) yield; mp 64-65 ºC. IR (KBr): 3338, 3227 (NH_2), 3042 (NH), 1700 (C=O, amide), 1630 (C=O, amide) cm^{-1}; ^{1}H-NMR (500 MHz: DMSO-d_6): δ 1.70-1.79 (m, 2H, CH_2), 2.26-2.29 (m, 2H, CH_2), 2.71-2.74 (m, 1H, CH), 3.52 (s, 2H, NH_2, D_2O exchangeable), 3.82-3.86 (m, 1H, CH), 5.72 (brs, 1H, sp^3 C-H), 5.95 (brs, 2H, CH_2), 6.59-6.64 (m, 2H, Ar-H), 6.72 (d, 1H, *J* = 9.2 Hz, Ar-H), 6.82-6.83 (m, 2H, Ar-H), 7.15 (t, 1H, *J* = 6.8 Hz, Ar-H), 7.24 (s, 1H, NH, D_2O exchangeable), 7.59 (d, 1H, *J* =7.6 Hz, Ar-H), 9.63 (s, 1H, NH, D_2O exchangeable). Anal. Calcd. for $C_{19}H_{20}N_4O_4$: C, 61.95; H, 5.47; N, 15.21. Found: C, 61.77; H, 5.61; N, 15.46.

4-(2-(Furan-2-yl)-1,2-dihydro-4-oxoquinazolin-3(4H)-yl)butanehydrazide ***36***

Compound **36** was obtained as brown crystals. **Method A:** 0.23 g (73.1%) yield; **Method B:** 0.29 g (92.3%) yield; mp 80-81ºC; IR (KBr): 3435, 3306 (NH+NH_2), 1729 (C=O, amide), 1630 (C=O, amide) cm^{-1}; ^{1}H-NMR (500 MHz: DMSO-d_6): δ 1.72-1.79 (m, 1H, CH), 1.92-1.95 (m, 1H, CH), 2.34-2.36 (m, 1H, CH), 2.89-2.94 (m, 2H, CH_2), 3.82-3.85 (m, 2H, NH_2, D_2O exchangeable), 3.97-3.98 (m, 1H, CH), 5.82 (s, 1H, sp^3 C-H), 6.15-6.16 (m, 1H, Ar-H), 6.29-6.30 (m, 1H, Ar-H), 6.65-6.68 (m, 2H, Ar-H), 7.20 (t, 1H, *J* = 7.6 Hz, Ar-H), 7.32 (s, 1H, NH, D_2O exchangeable), 7.79 (t, 1H, *J* = 7.6 Hz, Ar-H), 8.12 (d, 1H, *J* =7.6 Hz, Ar-H). Anal. Calcd. for: $C_{16}H_{18}N_4O_3$: C, 61.13; H, 5.77; N, 17.82. Found: C, 61.39; H, 5.90; N, 18.10.

4.2 Biology

The newly synthesized compounds **2a-2c, 3a-3c, 4-36** 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.[26] The activity of MAO-A and MAO-B was determined by fluorimetric method, according to Matsumoto et al.[27] The mitochondrial fractions were pre-incubated at 38°C for 30 min before adding the specific inhibitor, L-deprenyl (10.5 µM) to determine MAO-A activity and Clorgyline (10.5 µM) to determine 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 0.5 nM 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 using 1N NaOH and measured on Perkin-Elmer Lf 45 Spectrofluorimeter. The IC_{50}value was calculated and analyzed using the four-parameter logistic function in SigmaPlot software (SigmaPlot 12.3, Systat Software. Inc., Richmond, CA, USA). Protein concentration was determined according to a previously reported method.[28] The MAO-A and MAO-B results are expressed as IC_{50} (Table 1). Propylene glycol was used as negative control and did not show any effect on the enzyme activity.

Oral acute toxicity of the test compounds was studied using male mice (20 g each, Medical Research Institute, Alexandria University) according to previously reported methods.[31-33] The animals were divided into groups of six mice each. The compounds were given orally, suspended in 1% gum acacia, in doses of 50, 150, 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[33] in groups of mice of six animals each. The compounds or their vehicle, propylene glycol (control), were given by intraperitoneal injection in doses of 25, 50, 75, 100, 125 mg/kg. The percentage survival was followed up to 7 days.

4.3 Modeling studies[29,30]

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 **10** and **15** was estimated as follows:

1) Enzyme structures were checked for missing atoms, bonds, and contacts.

2) Hydrogen atoms were added to the 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 MOE-Dock with simulated annealing used as the search protocol and MMFF94X molecular mechanics force field for 8000 iterations.

7) The lowest energy conformation was selected and subjected to an energy minimization using MMFF94X force field.

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Supplementary data

Supplementary data associated with this article can be found in the online version,

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