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1,3,4-Oxadiazole-3(2*H*)-carboxamide derivatives as potential novel class of monoamine oxidase (MAO) inhibitors: Synthesis, evaluation, and role of urea moiety

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ABSTRACT

A new series of 1,3,4-oxadiazole-3(2*H*)-carboxamide derivatives have been synthesized by direct heterocyclization reaction of substituted benzoylisocyanate with various aroylhydrazones as novel monoamine oxidase inhibitors (MAOIs). The target molecules have been identified on the basis of satisfactory analytical and spectra (IR, ^{1}H NMR, ^{13}C NMR, and HR-MS) data. The newly synthesized compounds were evaluated for their MAO inhibitory activity by kynuramine fluorimetric assay method. The preliminary results showed that most of the compounds have moderate inhibitory activities toward MAO at the concentration of 10^{-5}–10^{-3} M. This work may provide a novel class of lead compounds with potential MAO inhibitions for further optimization.

1. Introduction

Monoamine oxidase (MAO) is a ubiquitous membrane-bound, flavin-containing enzyme, which is particularly abundant in the liver and brain.[1] MAO is located intracellularly in the mitochondrial outer membranes of neuronal, glial, and other cells and catalyzes the oxidative deamination of monoamine neurotransmitters both from endogenous and exogenous sources to the corresponding aldehydes with consumption of oxygen and production of hydrogen peroxide, thereby affecting the concentrations of neurotransmitter amines as well as many xenobiotic ones.[2–4]

In mammals, two distinct isoforms of MAO are present in most tissues, namely, MAO-A and MAO-B, which were defined in 1968, based on their differential substrate and inhibitor specificity,[5–7] tissue and cell distribution,[8] and gene expression[9,10] characteristics. MAO-A preferentially deaminates aromatic monoamines such as the neurotransmitters serotonin, noradrenaline, and adrenaline, while MAO-B mainly oxidizes β-phenylethylamines and benzylamines. Both isoforms act on dopamine and tryptamine.[1]

In the central nervous system and peripheral tissues, MAO catalyzes the metabolic transformation of amine neurotransmitters; whereas in the liver, MAO functions as a detoxicating enzyme against foreign amine compounds that enter the body.[11,12] It is well recognized that MAO plays an important role in metabolism in that it has been linked to a variety of adverse health conditions including alcoholism, smoking, aggressive behavior, certain neurodegenerative disorders, and even to the growth inhibition and progression of cancer.[13,14] Due to the important function played by MAO in the metabolism of neurotransmitters, MAO inhibitors represent a useful tool for the treatment of several neurological diseases.[15,16] Recognition of the importance of monoamine oxidases as targets for drug intervention for treatment of a variety of conditions has produced an enormous interest in development of inhibitors of these enzymes during these years.

The first generation of MAO inhibitors, originally synthesized in the 1950s, were used as antidepressants due to their inhibitory effect on the metabolism of monoamine neurotransmitters. The next discovery was made by Johnston,[17] who showed that the MAO enzyme exists in two forms: A and B. This led to the synthesis of a new generation of MAO inhibitors exhibiting greater selectivity toward the individual isoforms. Following an initial experience with non-selective, irreversible MAO inhibitors in the treatment of depression associated with severe side effects,[18] it can be stated that today the selective MAO-A inhibitors are currently used for treating neurological disorders such as anxiety and depression, while selective inhibitors of the B isoform are administered alone or together with L-DOPA for the treatment of Parkinson's syndrome and Alzheimer's disease.[19–23]

During the past decades, extensive studies focused on the search for MAO inhibitors have led to the discovery of various structural classes of potent MAO inhibitors, including hydrazide,

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amide, thiazole, imidazole, oxazolidinone, oxadiazolone, diacylurea derivatives, etc.[24–37] In spite of considerable progress in understanding the interactions of the two enzyme forms with their corresponding substrates, no general rules are yet available for the rational design of potent and selective inhibitors of MAO. There are many different structures of MAO inhibitors partly due to the fact that the active sites of the MAO are ambiguous, which limits the design of potent selective MAO inhibitors. Figure 1 shows the chemical structures of some representative MAO inhibitors used in research or clinical practice.[24–37]

The aim of the present study was the identification of novel potent MAO inhibitors that could serve as potential lead molecules for drug discovery. Among above depicted representative structures of MAO inhibitors, many heterocycle nitrogen-containing derivatives, including thiadiazole, oxadiazole, thiazole, etc., behave as potential MAO inhibitors, and a common structural feature of substrates and inhibitors is an amino or imino group that is assumed to play an essential role in orientation and complex formation at the active site of the enzyme,[38] however, little attention has been paid to the synthesis of 1,3,4-oxadiazoline derivatives bearing urea moiety. Considering that aroylurea derivatives as dipeptide mimics tend to contain multi-structure in a molecule, the biological activity of these compounds may be improved by the promotion of these combinations with the cell's microstructure and the accumulation of various biological activities resulting from the incorporation of different heterocyclic and non-heterocyclic changes on biological activity of the compounds. Furthermore, the introduction of urea moiety, known to be an important partial structure of many bioactive compounds,[39–48] may be stabilized within the active site of the enzyme through several hydrogen bonds.[49]

As part of our medicinal chemistry and agrochemistry program aimed at the search for novel heterocycle oxa(thia)diazole-based bioactive molecules,[50–55] we report herein our effort to design and discover a new lead compound by combining the different possible active units in an oxadiazoline model as shown in Figure 2, in order to find novel class of lead compounds with potential MAO inhibitions. We utilized 1,3,4-oxadiazoline scaffold as key prototype structural unit and planed for the attachment of an urea functionality to the heterocycle, and speculated that we might be able to improve on the properties of compounds by extending the active system. Thus to test this hypothesis, we prepared compounds 1,3,4-oxadiazole-3(2*H*)-carboxamide derivatives **3** (Scheme 1) and evaluated their MAO inhibitory properties.

The synthetic route is shown in Scheme 1, and the structures of all newly synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR, IR, EIMS, and high-resolution mass spectra (HR-MS) data. Furthermore, for a few comparative activity measurements of compounds without urea moiety, some 1,3,4-oxadiazoline derivatives **4** as shown in Scheme 1 were also synthesized. The newly synthesized compounds were evaluated for their MAO inhibitory activity by kynuramine fluorimetric assay method. The preliminary results showed that all the urea-containing compounds have moderate inhibitory activities toward MAO at the concentration of 10^{-4}–10^{-3} M, however, the activities of compounds without aroylurea moiety were almost lost. The present work demonstrated that assembling the biological active unit of oxadiazoles and ureas might be able to result in a novel class of lead compounds with potential MAO inhibitions for further optimization of MAO inhibitors with good activity.

2. Results and discussion

2.1. Synthesis of substituted 1,3,4-oxadiazole derivatives

Although various methods have been reported for the synthesis of oxadiazoline derivatives,[56–60] these methods require high reaction temperature, long reaction time, and expensive reagent. Reddy

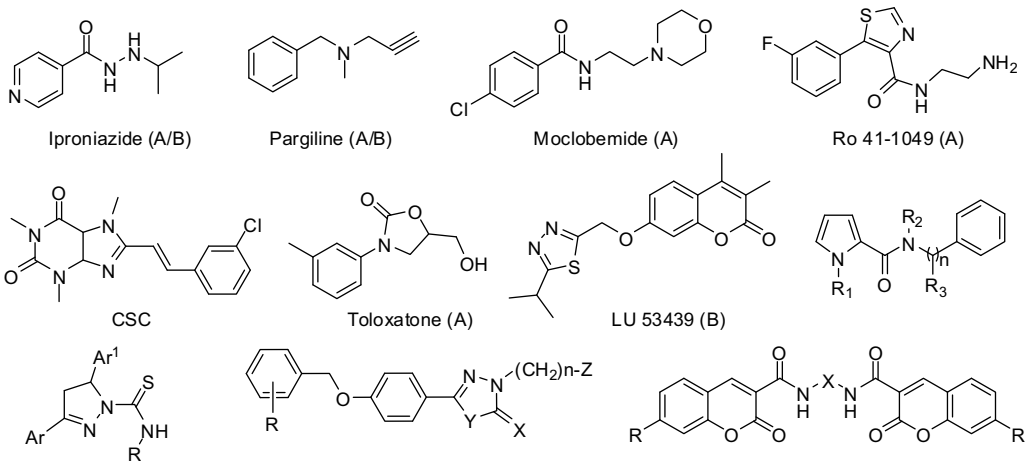


Figure 1. Some representative structures of MAO inhibitors used in research or clinical practice.

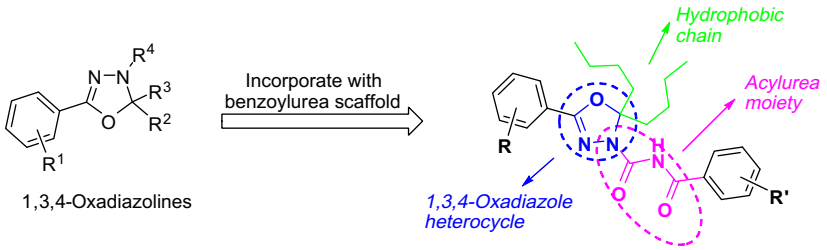
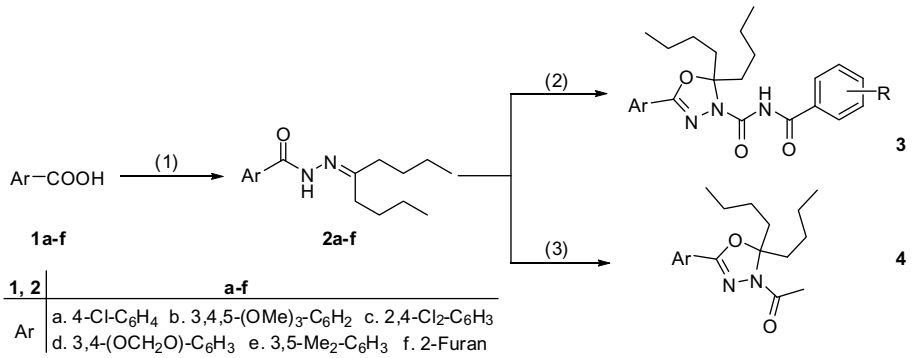


Figure 2. Design strategy of 1,3,4-oxadiazoline derivatives containing urea moiety.



Scheme 1. Reagents and conditions: (1) a—EtOH, concd H_2SO_4; b—$NH_2NH_2{\cdot}H_2O$, reflux for 8 h; c—nonan-5-one, EtOH, reflux for 6–8 h; (2) aroylisocyanate, toluene, reflux for 0.5–1.5 h; (3) Ac_2O, reflux for 1–2 h.

et al.[58] have synthesized some novel 1,3,4-oxadiazol-2-yl-4(3*H*)-quinazolinones by polyphosphoric acid-catalyzed cyclization method (reflux at 120–130 °C for 3–4 h and the yields are 51–67%). Hall et al.[59] reported that the hydrazides were converted to 1,3,4-oxadiazoles derivatives under drastic reaction conditions (in neat phosphorus oxychloride under microwave conditions at 100 °C). Ali et al.[60] obtained some novel oxadiazolines containing sugar moiety under reflux temperature with acetic anhydride, and the yields are 68–71%. However, in the present-described experimental conditions the heterocyclization reaction reached completion with a relative simple operation giving, high yield (64–80%) at shorter time (0.5–1.5 h) compared to the reported cyclization methods for 1,3,4-oxadiazole ring. The synthetic route of the designed 1,3,4-oxadiazoline derivatives 2,2-dibutyl-5-(substituted-phenyl)-*N*-(substituted-benzoyl)-1,3,4-oxadiazole-3(2*H*)-carboxamides **3a–k** is outlined in Scheme 1.

The various synthesized benzoates were treated with hydrazine hydrate in EtOH to afford the hydrazides. The following condensation reaction between hydrazides and nonan-5-one led to the important substrates, substituted benzoylhydrazone **2**. Another key intermediate, substituted benzoylisocyanates, was easily obtained by three steps under the conditions of experimental. The subsequent reaction of substituted benzoylhydrazone **2** with various substituted benzoylisocyanates were heating at about 75–80 °C in toluene for about 0.5–1.5 h afforded the target compounds characterized as 2,2-dibutyl-5-(substituted-phenyl)-*N*-(substituted-benzoyl)-1,3,4-oxadiazole-3(2*H*)-carboxamides **3a–k** in excellent yields of 64–80%. The heterocyclization reaction is most probably initiated by a nucleophilic attack of the imino hydrazone nitrogen to the carbonyl carbon of the isocyanate leading to an intermediate, with subsequent proton transfer and cyclization of the dipolar intermediate leading to the formation of target compounds. The possible mechanism of cyclization reaction to afford target compounds is shown in Scheme 2. In a conventional method, aroylhydrazone **2** undergoes convenient heterocyclization reaction in the presence of acetic anhydride to afford the 3-acetyl-5-aryl-2,3(2*H*)-1,3,4-oxadiazoles **4a–c**.

In the above-described experimental conditions the heterocyclization reaction reached completion giving a very high yield at shorter time compared to the reported cyclization methods for 1,3,4-oxadiazole ring derivatives.

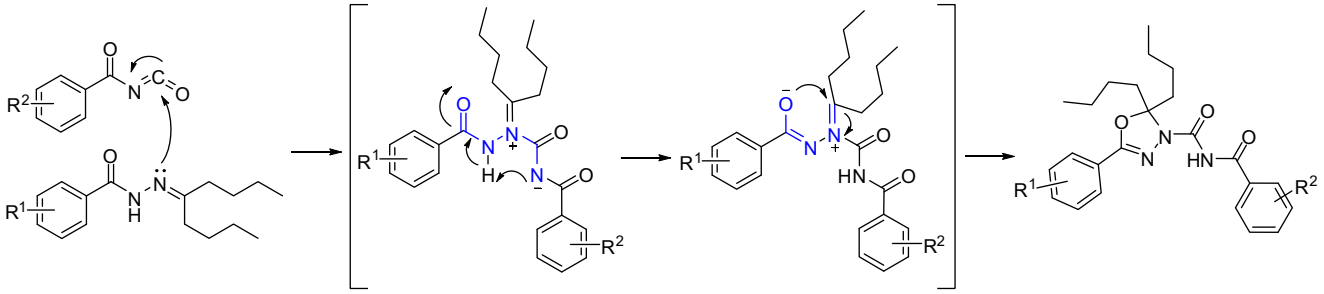
2.2. Spectroscopy

The structures of all the newly synthesized compounds were characterized as 2,2-dibutyl-5-(substituted-phenyl)-*N*-(substituted-benzoyl)-1,3,4-oxadiazole-3(2*H*)-carboxamide **3** and 2,2-dibutyl-3-acetyl-5-aryl-2,3(2*H*)-1,3,4-oxadiazole **4** on the basis of satisfactory analytical and spectral data including ^{1}H NMR, ^{13}C NMR, IR, EIMS, and HR-EIMS.

The IR spectra of the 1,3,4-oxadiazole-3(2*H*)-carboxamide derivatives **3a–k** show characteristic absorption bands at 3380–3230, 1750–1660, and 1635–1610 cm^{-1}, which have been assigned to N–H bond, amido carbonyl, and imino groups, respectively. The ^{1}H NMR spectra of 1,3,4-oxadiazole-3(2*H*)-carboxamides **3a–k** show signals in the range 10.50–11.25 ppm, assigned to the NH group proton, 6.80–8.50 ppm for the protons of the aromatic ring, and at 0.95–3.20 ppm for the aliphatic chain protons. Due to the instability of the oxadiazolines-benzoylurea derivatives, the molecular ion peak in their mass spectra was of low intensity. The appearance of peaks for $[M–ArCONHCO]^+$ shows that the amide C–N bond undergoes fission readily.

2.3. MAO inhibition activity

The in vitro inhibition activities against MAO of all synthesized compounds were investigated by kynuramine fluorimetric assay method.[61,62] Enzymatic assays revealed that all test compounds **3a–k** were weak to moderate MAO inhibitors at low micromolar concentrations. On the contrary, compounds **4a–c** almost lost



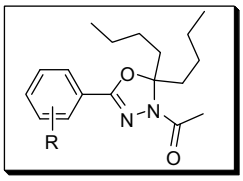
Scheme 2. The possible mechanism of cyclcondensation reaction with substituted benzoylisocyanate to afford 1,3,4-oxadiazolines.

inhibitory activities at the same concentration level, except **4c** with weak activity. The inhibition activity results of compounds **3a–k** and **4a–c** against MAO are shown in Tables 1 and 2, respectively.

From Table 1, which shows the structures and the MAO inhibition data, it can be seen that most of the 1,3,4-oxadiazole-3(2*H*)-carboxamide derivatives **3a–k** show obvious activity against MAO with values in the range of 6.03–41.02% at the low concentration of 100 μM. Compounds **3a** and **3b** have weaker activities at 100 μM and almost lost activity at the lower concentration of 8 μM. Compound **3d** was the most potent of the series, with values which reached 41.02% and 8.37% at 100 μM and 10 μM, respectively. Moreover, compounds 1,3,4-oxadiazole-3(2*H*)-carboxamide 3a–**k** and 3-acetyl-5-aryl-2,3(2*H*)-1,3,4-oxadiazoles **4a–c** show a striking contrast, compounds **4a–c** without urea moiety almost lost inhibitory activities at the same concentration level, except **4c** with weak activity (Table 2), which indicates that the acylurea moiety may be stabilized within the active site of the enzyme through hydrogen bonds. Additionally, we can find from Table 1 that the inhibition activities of the target compounds **3a–k** were influenced by the substituents on the phenyl ring gave the moderate activity. When the substituent R is 2,6-difluoro substituent, the aromatic ring (Ar) bearing piperonyl moiety (**3d**) is more favorable for inhibition activities. Furthermore, when Ar has the same substituents such as compounds **3d** and **3h**, **3e** and **3g**, the compounds (**3d** and **3e**) with strong electron-withdrawing group (2,6-difluor) increase the potency. Among all the active compounds, the higher inhibition activity in this series was obtained with the piperonyl derivatives **3d** and **3h**, which is similar to the conclusion given in the literature.[11]

The comparative semi-logarithmic plot of the inhibitory activity of target compounds **3a–k** and **4a–c** against rat brain mitochondrial MAO is given in Figure 3, which further testified compound **3d** was the most potential compound of the series of 1,3,4-oxadiazole-3(2*H*)-carboxamide derivatives. On the basis of these results, we can state that most of the assayed compounds **3a–k** possessed potential monoamine oxidase inhibitory activity. Futhermore, 1,3,4-oxadiazole-3(2*H*)-carboxamide derivatives, particularly with piperonyl derivatives, may be novel lead compounds with MAO inhibitory activity.

Table 2
In vitro inhibition activity of 1,3,4-oxadiazoline derivatives **4a–c** on rat brain mitochondria by kynuramine fluorimetric assay



Entry	Compound	Substituents (R)	Inhibitions against MAO (%)		
			0.008 (mM)	0.08 (mM)	0.8 (mM)
1	**4a**	3,4,5-$(MeO)_3$	0.00	0.00	0.00
2	**4b**	3,4-OCH_2O	1.21	0.00	0.00
3	**4c**	3,5-Me_2	2.89	17.69	42.81

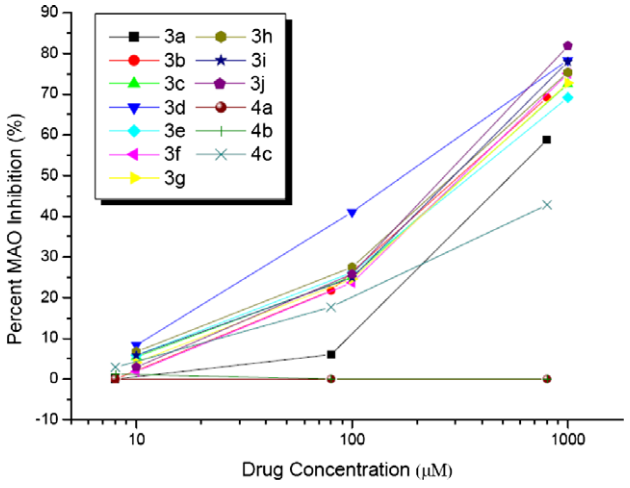
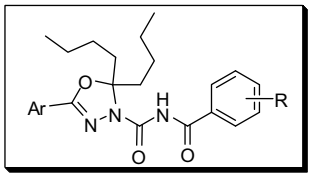


Figure 3. Semi-logarithmic plot of the inhibitory activity of target compounds **3c–k** against rat brain mitochondrial MAO at concentration of 1×10^{-5}–10^{-3} M and of compounds **3a–b**, **4a–c** at 0.8×10^{-5}–10^{-3} M, respectively.

Table 1
In vitro inhibition activity of 1,3,4-oxadiazole-3(2*H*)-carboxamide derivatives **3a–k** on rat brain mitochondria by kynuramine fluorimetric assay



Entry	Compound	Substituents		Inhibitions against MAO (%)		
		Ar	R	0.01 (mM)	0.1 (mM)	1 (mM)
1	**3a**	*p*-Cl-C_6H_4	2,6-2F	0.00[a]	6.03	58.79
2	**3b**	3,4,5-$(MeO)_3$-C_6H_2	2,6-2F	0.00[b]	21.72	69.18
3	**3c**	2,4-Cl_2-C_6H_3	2,6-2F	5.43	25.14	72.48
4	**3d**	3,4-OCH_2O-C_6H_3	2,6-2F	8.37	41.02	78.39
5	**3e**	3,5-Me_2-C_6H_3	2,6-2F	5.85	26.24	69.17
6	**3f**	2-Furan[c]	2,6-2F	1.84	23.68	75.07
7	**3g**	3,5-Me_2-C_6H_3	*o*-Cl	4.12	24.59	72.78
8	**3h**	3,4-OCH_2O-C_6H_3	*o*-Cl	6.73	27.49	75.37
9	**3i**	2,4-Cl_2-C_6H_3	*o*-Cl	5.86	24.90	77.96
10	**3j**	2,4-Cl_2-C_6H_3	2,3,5-F_3-4-MeO	2.92	25.85	81.84
11	**3k**	3,4,5-$(MeO)_3$-C_6H_2	2,3,5-F_3-4-MeO	ND[d]	ND	ND

[a,b] These two compounds were tested at the concentrations of 0.008, 0.08, and 0.8 mM, respectively.
[c] This aromatic ring is furan.
[d] ND, not determined.

3. Conclusion

In summary, we have described the molecular design, synthesis, and MAO inhibitory activities of a series of novel 1,3,4-oxadiazole-3(2*H*)-carboxamide derivatives and developed a practical and efficient procedure for the synthesis of 1,3,4-oxadiazole-3(2*H*)-carboxamide derivatives through the direct heterocyclization reaction of substituted benzoylhydrazone with various benzoylisocyanates in toluene for 0.5–1.5 h, according to the above-described experimental conditions, the cyclization reaction reached completion giving a very high yield at shorter time compared to the reported cyclization methods for 1,3,4-oxadiazole ring derivatives. The preliminary bioassay results indicated that some of 1,3,4-oxadiazole-3(2*H*)-carboxamide derivatives exhibited moderate MAO inhibition activity at the concentration of 10^{-4}–10^{-3} M. The present work demonstrated that assembling the biological active unit of oxadiazole and urea could result in new lead compounds with high lipophilicity and potent MAO inhibition activity. To our best knowledge, this is the first report about the syntheses and MAO inhibition activity of 1,3,4-oxadiazoles derivatives bearing urea moiety. Further structural optimization and structure–inhibition activity relationships about the designed 1,3,4-oxadiazole-3(2*H*)-carboxamide derivatives are well under way.

4. Experimental

4.1. Instrumentation and chemicals

All melting points (mp) were obtained using a Büchi Melting Point B540, and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Brucker AM-400 (400 MHz) spectrometer with $CDCl_3$ as the solvent and TMS as the internal standard. Chemical shifts are reported in δ (parts per million) values. Coupling constants ^nJ are reported in Hz. High-resolution electron impact mass spectra (HR-EIMS) were recorded under electron impact (70 eV) condition using a MicroMass GCT CA 055 instrument. Infrared (IR) spectra were measured on a Nicolet FT-IR-20SX instrument using a potassium bromide (KBr) disk, scanning from 400 to 4000 cm^{-1}. Analytical thin-layer chromatography (TLC) was carried out on precoated plates, and spots were visualized with ultraviolet (UV) light. All chemicals or reagents were purchased from standard commercial suppliers and treated with standard methods before use. Solvents were dried in a routine way and redistilled.

4.2. General synthetic procedure for substituted aroylhydrazones 2a–f

Substituted aroylhydrazones **2** were prepared via esterification, hydrazination and condensation three step reactions. Following are the brief descriptions: (1) Synthesis of aroylhydrazides. These were prepared by the usual esterification of appropriate benzoic acid (0.1 mol) with alcohol (200 mL) and concentrated sulfuric acid (10 drops) followed by condensation of the resulting ethyl benzoates (0.1 mol) with hydrazine hydrate (0.5 mol). The product was collected by filtration, washed with water and was recrystallined from ethanol. (2) Synthesis of aroylhydrazones **2**. Equal amounts of appropriate aroylhydrazide (0.01 mol) and nonan-5-one (1.42 g, 0.01 mol) were refluxed in absolute alcohol (15 mL) for several hours, which was detected by TLC. Then the solution was concentrated, and the condensation product aroylhydrazone separated out on cooling and was recrystallized from ethanol. Their physico-chemical properties and the spectra data are as follows:

4.2.1. 4-Chloro-*N*′-(nonan-5-ylidene)benzohydrazide (2a)

This compound was obtained as white powder following the above mentioned, yield 81%, mp 158.4–159.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.70 (s, 1H, N–H), 7.70 (d, J = 8.8 Hz, 2H, Ph-H), 7.44 (d, J = 8.4 Hz, 2H, Ph-H), 2.27–2.41 (m, 4H, N=C(CH_2)$_2$), 1.32–1.56 (m, 8H, CH_2), 0.93–0.99 (m, 6H, CH_3); MS: m/z = 294 (M^+), 237, 210, 168, 139, 111; EI-HRMS: calcd for $C_{16}H_{23}ClN_2O$ (M^+), 294.1499; found, 294.1500.

4.2.2. 3,4,5-Trimethoxy-*N*′-(nonan-5-ylidene)benzohydrazide (2b)

This compound was obtained as white powder following the above mentioned, yield 84%, mp 123.4–124.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.67 (s, 1H, N–H), 6.99 (s, 2H, Ph-H), 3.91 (s, 6H, OCH_3), 3.90 (s, 3H, OCH_3), 2.24–2.41 (m, 4H, N=C(CH_2)$_2$), 1.38–1.58 (m, 8H, CH_2), 0.98 (t, J = 7.2 Hz, 3H, CH_3), 0.93 (t, J = 7.2 Hz, 3H, CH_3); MS: m/z = 350 (M^+), 293, 209, 195, 167; EI-HRMS: calcd for $C_{19}H_{30}N_2O_4$ (M^+), 350.2206; found, 350.2207.

4.2.3. 2,4-Dichloro-*N*′-(nonan-5-ylidene)benzohydrazide (2c)

This compound was obtained as white powder following the above mentioned, yield 78%, mp 105.2–106.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.04 (s, 1H, N–H), 7.46 (d, ^4J = 2 Hz, 1H, Ph-H), 7.37–7.42 (m, 2H, Ph-H), 2.42 (t, J = 7.8 Hz, 2H, N=C–CH_2), 2.27 (t, J = 8 Hz, 2H, N=C–CH_2), 1.36–1.62 (m, 8H, CH_2), 0.93–0.98 (m, 6H, CH_3); MS: m/z = 328 (M^+), 271, 244, 229, 202, 173, 145, 109, 85, 73, 55; EI-HRMS: calcd for $C_{16}H_{22}Cl_2N_2O$ (M^+), 328.1109; found, 328.1100.

4.2.4. *N*′-(Nonan-5-ylidene)benzo[*d*][1,3]dioxole-5-carbohydrazide (2d)

This compound was obtained as white solid following the above mentioned, yield 80%, mp 143.4–144.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.64 (s, 1H, N–H), 7.28–7.33 (m, 2H, Ph-H), 6.86 (d, J = 8.4 Hz, 1H, Ph-H), 6.04 (s, 2H, OCH_2O), 2.26–2.38 (m, 4H, N=C–(CH_2)$_2$), 1.51–1.59 (m, 4H, CH_2), 1.35–1.45 (m, 4H, CH_2), 0.98 (t, J = 7.2 Hz, 3H, CH_3), 0.94 (t, J = 7.4 Hz, 3H, CH_3); MS: m/z = 304 (M^+), 247, 149, 121, 96; EI-HRMS: calcd for $C_{17}H_{24}N_2O_3$ (M^+), 304.1787; found, 304.1788.

4.2.5. 3,5-Dimethyl-*N*′-(nonan-5-ylidene)benzohydrazide (2e)

This compound was obtained as white solid following the above mentioned, yield 75%, mp 87.3–88.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.73 (s, 1H, N–H), 7.39 (s, 2H, Ph-H), 7.16 (s, 1H, Ph-H), 2.21–2.41 (m, 10H, N=C–(CH_2)$_2$ and Ph-CH_3), 1.36–1.58 (m, 8H, CH_2), 0.98 (t, J = 7.2 Hz, 3H, CH_3), 0.94 (t, J = 7.4 Hz, 3H, CH_3); MS: m/z = 288 (M^+), 259, 246, 231, 204, 189, 162, 133, 105, 91, 77; EI-HRMS: calcd for $C_{18}H_{28}N_2O$ (M^+), 288.2202; found, 288.2203.

4.2.6. *N*′-(Nonan-5-ylidene)furan-2-carbohydrazide (2f)

This compound was obtained as white crystal following the above mentioned, yield 78%, mp 46.3–48.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.09 (s, 1H, N–H), 7.48 (s, 1H, Furan-H), 7.29 (s, 1H, Furan-H), 6.55 (t, J = 1.6 Hz, 1H, Furan-H), 2.30–2.42 (m, 4H, N=C(CH_2)$_2$), 1.35–1.57 (m, 8H, CH_2), 0.99 (t, J = 7 Hz, 3H, CH_3), 0.93 (t, J = 7.2 Hz, 3H, CH_3); MS: m/z = 250 (M^+), 193, 166, 151, 137, 95, 81, 55; EI-HRMS: calcd for $C_{14}H_{22}N_2O_2$ (M^+), 250.1681; found, 250.1682.

4.3. General synthetic procedure for substituted benzoylisocyanate

These key intermediates were prepared by the usual method used for isocyanate. Substituted benzamides (0.01 mol), which were obtained by the usual chlorination of appropriate benzoic

acid (0.01 mol) with $SOCl_2$ (8–10 mL) and followed by ammonolysis of the resulting acyl chloride (0.01 mol) with concentrated ammonia liquor (15 mL, 28%), and 15 mL of toluene were placed in a dried round-bottomed flask containing a magnetic stirrer bar under nitrogen, and to this 10 mL of oxalyl chloride was added dropwise for 20 min at ice-bath. After addition, the resulting clear solution was heated at about 75 °C for 6–8 h, and then the excessive oxalyl chloride was removed under reduced pressure to give a clear solution of substituted benzoyl isocyanate, which was used for the next step reaction without further purification.

4.4. General synthetic procedure for 1,3,4-oxadiazole-3(2*H*)-carboxamide derivatives 3a–k

A solution of the appropriately substituted benzoyl isocyanate (2 mmol) in dry toluene or 1,2-dichloroethane (5 mL) was added dropwise to a stirred solution of the aroylhydrazone **2** (1.5 mmol) in dry toluene (20 mL) under reflux for over a period of 20 min. Then the reaction mixture was stirred at 75–85 °C for 0.5–1.5 h, which was monitored by TLC until the disappearance of aroylhydrazone **2**. The mixture was cooled to ambient temperature and washed with a brine solution. The organic layer was separated, dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using petroleum ether (60–90 °C)/ethyl acetate (v/v) as eluent, slowly increasing the polarity to give the target compounds **3a–k**. Their physico-chemical properties and the spectra data are as follows:

4.4.1. 2,2-Dibutyl-5-(4-chlorophenyl)-*N*-(2,6-difluorobenzoyl)-1,3,4-oxadiazole-3(2*H*)-carboxamide (3a)

This compound was obtained as white solid following the above mentioned, yield 66%, mp 126.5–128.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.11 (s, 1H, N–H), 7.77 (d, J = 8.4 Hz, 2H, Ph-H), 7.45 (d, J = 8.4 Hz, 2H, Ph-H), 7.40 (q, 1H, Ph-H), 6.97 (t, J = 8 Hz, 2H, Ph-H), 2.29–2.40 (m, 2H, CH_2), 1.92–1.99 (m, 2H, CH_2), 1.30–1.34 (m, 8H, CH_2), 0.88 (t, J = 7.2 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 160.80, 158.36, 154.15, 145.82, 138.13, 132.04, 129.14, 128.19, 122.45, 111.75, 111.50, 105.62, 36.29, 24.48, 22.26, 13.89; IR(KBr): ν = 3355 (N–H), 1714, 1695 (C=O), 1621 (C=N) cm^{-1}; ESI-HRMS: calcd for $C_{24}H_{26}ClF_2N_3O_3$ ([M+Na]$^+$), 500.1528; found, 500.1517.

4.4.2. 2,2-Dibutyl-*N*-(2,6-difluorobenzoyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole-3(*2H*)-carboxamide (3b)

This compound was obtained as white solid following the above mentioned, yield 78%, mp 141.2–142.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.15 (s, 1H, N–H), 7.38–7.45 (m, 1H, Ph-H), 7.06 (s, 2H, Ph-H), 6.97 (t, J = 8 Hz, 2H, Ph-H), 3.96 (s, 6H, OCH_3), 3.92 (s, 3H, OCH_3), 2.29–2.37 (m, 2H, CH_2), 1.90–1.98 (m, 2H, CH_2), 1.27–1.35 (m, 8H, CH_2), 0.89 (t, J = 7 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 161.09, 160.72, 154.89, 153.46, 145.77, 141.40, 131.94, 118.97, 111.66, 111.42, 105.32, 104.18, 60.98, 56.42, 36.29, 24.44, 22.26, 13.90; IR(KBr): ν = 3370 (N–H), 1710, 1688 (C=O), 1621 (C=N) cm^{-1}; ESI-HRMS: calcd for $C_{27}H_{33}F_2N_3O_6$ ([M+Na]$^+$), 556.2235; found, 556.2263.

4.4.3. 2,2-Dibutyl-5-(2,4-dichlorophenyl)-*N*-(2,6-difluorobenzoyl)-1,3,4-oxadiazole-3(2*H*)-carboxamide (3c)

This compound was obtained as white solid following the above mentioned, yield 74%, mp 97.2–98.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.10 (s, 1H, N–H), 7.74 (d, J = 8.4 Hz, 1H, Ph-H), 7.55 (s, 1H, Ph-H), 7.40–7.45 (m, 1H, Ph-H), 7.35–7.37 (m, 1H, Ph-H), 6.97 (t, J = 8.2 Hz, 2H, Ph-H), 2.29–2.37 (m, 2H, CH_2), 1.93–2.01 (m, 2H, CH_2), 1.27–1.40 (m, 8H, CH_2), 0.89 (t, J = 6.8 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 160.92, 160.38, 158.37, 152.34, 145.95, 137.90, 134.23, 132.12, 131.22, 127.34, 121.52, 113.85, 111.67, 105.16, 36.25, 24.53, 22.26, 13.90; IR(KBr): ν = 3259 (N–H), 1703, 1677 (C=O), 1618 (C=N) cm^{-1}; MS: m/z = 511, 328, 299, 286, 271, 244, 201, 172, 141, 113; EI-HRMS: calcd for $C_{24}H_{25}Cl_2F_2N_3O_3$ (M$^+$), 511.1241; found, 511.1241.

4.4.4. 5-(Benzo[*d*][1,3]dioxol-5-yl)-2,2-dibutyl-*N*-(2,6-difluorobenzoyl)-1,3,4-oxadiazole-3(2*H*)-carboxamide (3d)

This compound was obtained as white solid following the above mentioned, yield 76%, mp 159.5–160.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.11 (s, 1H, N–H), 7.37–7.45 (m, 2H, Ph-H), 7.27 (s, 1H, Ph-H), 6.97 (t, J = 8.2 Hz, 2H, Ph-H), 6.88 (d, J = 8 Hz, 1H, Ph-H), 6.06 (s, 2H, OCH_2O), 2.28–2.35 (m, 2H, CH_2), 1.90–1.98 (m, 2H, CH_2), 1.27–1.36 (m, 8H, CH_2), 0.88 (t, J = 7 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 160.83, 158.34, 154.74, 150.82, 148.06, 145.75, 131.90, 122.15, 117.68, 114.04, 111.61, 108.52, 106,92, 105.08, 101.83, 36.27, 24.48, 22.28, 13.91; IR(KBr): ν = 3237 (N–H), 1699, 1680 (C=O), 1624 (C=N) cm^{-1}; MS: m/z = 487, 304, 262, 247, 220, 205, 149, 121; EI-HRMS: calcd for $C_{25}H_{27}F_2N_3O_5$ (M$^+$), 487.1919; found, 487.1919.

4.4.5. 2,2-Dibutyl-*N*-(2,6-difluorobenzoyl)-5-(3,5-dimethylphenyl)-1,3,4-oxadiazole-3(2*H*)-carboxamide (3e)

This compound was obtained as white solid following the above mentioned, yield 68%, mp 141.2–142.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.15 (s, 1H, N–H), 7.46 (s, 2H, Ph-H), 7.37–7.43 (m, 1H, Ph-H), 7.17 (s, 1H, Ph-H), 6.97 (t, J = 8 Hz, 2H, Ph-H), 2.39 (s, 6H, Ph-CH_3), 2.39 (s, 6H, Ph-CH_3), 2.29–2.36 (m, 2H, CH_2), 1.91–1.98 (m, 2H, CH_2), 1.27–1.36 (m, 8H, CH_2), 0.88 (t, J = 7 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 160.84, 155.26, 145.82, 138.55, 133.62, 131.92, 131.82, 124.64, 123.73, 111.69, 111.45, 104.97, 36.33, 24.45, 22.29, 21.17, 13.91; IR(KBr): ν = 3237 (N–H), 1699, 1677 (C=O), 1625 (C=N) cm^{-1}; MS: m/z = 471, 288, 246, 231, 204, 189, 141, 133, 105, 79; EI-HRMS: calcd for $C_{26}H_{31}F_2N_3O_3$ (M$^+$), 471.2333; found, 471.2333.

4.4.6. 2,2-Dibutyl-*N*-(2,6-difluorobenzoyl)-5-(furan-2-yl)-1,3,4-oxadiazole-3(2*H*)-carboxamide (3f)

This compound was obtained as pale yellow solid following the above mentioned, yield 70%, mp 124.5–126.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.08 (s, 1H, N–H), 7.61–7.62 (m, 1H, Ar-H), 7.37–7.44 (m, 1H, Ar-H), 6.93–6.99 (m, 3H, Ar-H), 6.57 (q, 1H, Ar-H), 2.28–2.36 (m, 2H, CH_2), 1.91–1.98 (m, 2H, CH_2), 1.29–1.38 (m, 8H, CH_2), 0.89 (t, J = 6.8 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 160.86, 160.80, 158.35, 158.29, 147.97, 145.88, 139.42, 132.11, 132.01, 114.80, 111.95, 111.74, 111.49, 105.56, 36.27, 24.43, 22.26, 13.89; IR(KBr): ν = 3370 (N–H), 1710, 1688 (C=O), 1621 (C=N) cm^{-1}; MS: m/z = 433, 324, 297, 250, 221, 208, 193, 166, 141, 113, 95; EI-HRMS: calcd for $C_{22}H_{25}F_2N_3O_4$ (M$^+$), 433.1813; found, 433.1813.

4.4.7. 2,2-Dibutyl-*N*-(2-chlorobenzoyl)-5-(3,5-dimethylphenyl)-1,3,4-oxadiazole-3(2*H*)-carboxamide (3g)

This compound was obtained as white solid following the above mentioned, yield 75%, mp 108.5–110.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.33 (s, 1H, N–H), 7.62 (d, J = 6.8 Hz, 1H, Ph-H), 7.45 (s, 2H, Ph-H), 7.43 (dd, ^3J = 6 Hz, ^4J = 8 Hz, 2H, Ph-H), 7.36–7.40 (m, 1H, Ph-H), 7.16 (s, 1H, Ph-H), 2.39 (s, 6H, Ph-CH_3), 2.31–2.37 (m, 2H, CH_2), 1.91–1.98 (m, 2H, CH_2), 1.30–1.36 (m, 8H, CH_2), 0.88 (t, J = 7 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 155.06, 145.93, 138.52, 135.15, 133.52, 131.50, 130.53, 129.78, 129.57, 126.97, 124.59, 123.85, 104.95, 36.40, 24.54, 22.33, 21.16, 13.97; IR(KBr): ν = 3244 (N–H), 1699, 1680 (C=O), 1630 (C=N) cm^{-1}; MS: m/z = 469, 288, 246, 231, 204, 189, 139, 133, 105, 79, 57; EI-HRMS: calcd for $C_{26}H_{32}ClN_3O_3$ (M$^+$), 469.2132; found, 469.2130.

4.4.8. 5-(Benzo[*d*][1,3]dioxol-5-yl)-2,2-dibutyl-*N*-(2-chlorobenzoyl)-1,3,4-oxadiazole-3(2*H*)-carboxamide (3h)

This compound was obtained as white solid following the above mentioned, yield 76%, mp 119.4–121.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.32 (s, 1H, N–H), 7.64 (d, J = 6.8 Hz, 1H, Ph-H), 7.42–7.44 (m, 2H, Ph-H), 7.36–7.40 (m, 2H, Ph-H), 7.26 (d, ^4J = 1.6 Hz, 1H, Ph-H), 6.88 (d, J = 8 Hz, 1H, Ph-H), 6.05 (s, 2H, OCH_2O), 2.32–2.40 (m, 2H, CH_2), 1.92–1.99 (m, 2H, CH_2), 1.27–1.38 (m, 8H, CH_2), 0.88 (t, J = 7 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 154.54, 150.74, 148.04, 145.86, 135.05, 131.59, 130.49, 129.84, 129.75, 127.03, 122.08, 117.79, 108.52, 106.88, 105.08, 101.81, 36.35, 24.57, 22.33, 13.99; IR(KBr): ν = 3259 (N–H), 1703, 1665 (C=O), 1628 (C=N) cm^{-1}; MS: m/z = 485, 304, 262, 247, 220, 205, 149, 138, 121, 111, 91, 65; EI-HRMS: calcd for $C_{25}H_{28}ClN_3O_5$ (M^+), 485.1717; found, 485.1717.

4.4.9. 2,2-Dibutyl-*N*-(2-chlorobenzoyl)-5-(2,4-dichlorophenyl)-1,3,4-oxadiazole-3(2*H*)-carboxamide (3i)

This compound was obtained as white solid following the above mentioned, yield 64%, mp 104.3–105.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.36 (s, 1H, N–H), 7.74 (d, J = 8.8 Hz, 1H, Ph-H), 7.66 (d, J = 7.2 Hz, 1H, Ph-H), 7.54 (d, J = 6 Hz, 1H, Ph-H), 7.41–7.44 (m, 2H, Ph-H), 7.35–7.40 (m, 2H, Ph-H), 2.34–2.42 (m, 2H, CH_2), 1.96–2.05 (m, 2H, CH_2), 1.25–1.41 (m, 8H, CH_2), 0.89 (t, J = 7 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.79, 152.10, 146.04, 137.79, 134.76, 134.14, 131.78, 131.30, 131.08, 130.47, 129.99, 129.91, 127.34, 127.08, 121.62, 105.08, 36.33, 24.62, 22.31, 13.97; IR(KBr): ν = 3362 (N–H), 1710, 1691 (C=O), 1625 (C=N) cm^{-1}; MS: m/z = 509, 328, 286, 271, 244, 180, 139, 111, 75; EI-HRMS: calcd for $C_{24}H_{26}Cl_3N_3O_3$ (M^+), 509.1040; found, 509.1025.

4.4.10. 2,2-Dibutyl-5-(2,4-dichlorophenyl)-*N*-(2,3,5-trifluoro-4-methoxybenzoyl)-1,3,4-oxadiazole-3(2*H*)-carboxamide (3j)

This compound was obtained as white solid following the above mentioned, yield 75%, mp 88.1–89.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.89 (s, 1H, N–H), 7.77 (d, J = 8.4 Hz, 1H, Ph-H), 7.62–7.68 (m, 1H, Ph-H), 7.56 (d, ^4J = 2 Hz, 3H, Ar-H), 7.38 (dd, ^4J = 2 Hz, ^3J = 8.4 Hz, 1H, Ph-H), 4.08 (s, 3H, OCH_3), 2.38–2.47 (m, 2H, CH_2), 1.99–2.05 (m, 2H, CH_2), 1.31–1.41 (m, 8H, CH_2), 0.89 (t, J = 7 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.11, 159.34, 158.34, 151.88, 146.48, 145.89, 137.81, 134.10, 131.37, 130.99, 127.39, 121.45, 112.22, 112.03, 104.89, 60.37, 36.39, 24.62, 22.33, 13.95; IR(KBr): ν = 3377 (N–H), 1743, 1684 (C=O), 1620 (C=N) cm^{-1}; MS: m/z = 559, 362, 286, 271, 244, 231, 189, 172, 144, 113; EI-HRMS: calcd for $C_{25}H_{26}Cl_2F_3N_3O_4$ (M^+), 559.1252; found, 559.1252.

4.4.11. 2,2-Dibutyl-*N*-(2,3,5-trifluoro-4-methoxybenzoyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole-3(2*H*)-carboxamide (3k)

This compound was obtained as white solid following the above mentioned, yield 63%, mp 113.6–116.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.83 (s, 1H, N–H), 7.58 (q, ^3J = 16 Hz, ^4J = 8.8 Hz, 1H, Ph-H), 7.08 (s, 2H, Ph-H), 4.06 (s, 3H, OCH_3), 3.96 (s, 6H, OCH_3), 3.92 (s, 3H, OCH_3), 2.33–2.46 (m, 2H, CH_2), 1.94–2.07 (m, 2H, CH_2), 1.30–1.39 (m, 8H, CH_2), 0.89 (t, J = 6.4 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 159.65, 154.72, 153.44, 145.78, 141.37, 111.99, 111.77, 105.60, 104.10, 60.99, 56.21, 36.48, 29.68, 24.54, 22.33, 13.96; IR(KBr): ν = 3332 (N–H), 1747, 1691 (C=O), 1632 (C=N) cm^{-1}; MS: m/z = 581, 393, 350, 293, 251, 231, 195, 189, 167, 152; EI-HRMS: calcd for $C_{28}H_{34}F_3N_3O_7$ (M^+), 581.2349; found, 581.2350.

4.5. General synthetic procedure for 3-acetyl-5-aryl-2,3(2*H*)-1,3,4-oxadiazoles derivatives 4a–c

Appropriate aroylhydrazone **2** (1 mmol) was refluxed in acetic anhydride (5 mL) for 1–2 h, which was detected by TLC. The mixture was cooled, poured into crushed ice, and allowed to stand at room temperature overnight. The mixture was extracted with ethyl acetate (3× 15 mL). The organic layer was separated, dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using petroleum ether (60–90 °C)/ethyl acetate (v/v) as eluent, slowly increasing the polarity to give the target compounds **4a–c**. Their physico-chemical properties and the spectra data are as follows:

4.5.1. 2,2-Dibutyl-3-acetyl-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazoline (4a)

This compound was obtained as white powder following the above mentioned, yield 86%, mp 128.6–129.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.06 (s, 2H, Ph-H), 3.94 (s, 6H, OCH_3), 3.90 (s, 3H, OCH_3), 2.41–2.49 (m, 2H, CH_2), 2.35 (s, 3H, $COCH_3$), 1.92–1.99 (m, 2H, CH_2), 1.26–1.33 (m, 8H, CH_2), 0.89 (t, J = 6.8 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 154.49, 153.35, 140.91, 120.01, 105.20, 103.97, 60.94, 56.31, 36.14, 24.63, 22.37, 22.26, 13.99; IR(KBr): ν = 1651 (C=O), 1587 (C=N) cm^{-1}; MS: m/z = 392 (M^+), 335, 293, 251, 195, 167, 152, 137, 122; EI-HRMS: calcd for $C_{21}H_{32}N_2O_5$ (M^+), 392.2311; found, 392.2311.

4.5.2. 2,2-Dibutyl-3-acetyl-5-(benzo[*d*][1,3]dioxol-5-yl)-1,3,4-oxadiazoline (4b)

This compound was obtained as yellow liquid following the above mentioned, yield 74%, ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.38 (dd, ^3J = 8.4 Hz, ^4J = 1.4 Hz, 1H, Ph-H), 7.29 (d, ^4J = 1.4 Hz, 1H, Ph-H), 6.86 (d, J = 8.4 Hz, 1H, Ph-H), 6.04 (s, 2H, OCH_2O), 2.38–2.45 (m, 2H, CH_2), 2.32 (s, 3H, $COCH_3$), 1.90–2.02 (m, 2H, CH_2), 1.23–1.33 (m, 8H, CH_2), 0.88 (t, J = 6.8 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.70, 154.40, 150.27, 147.91, 121.68, 118.69, 108.37, 106.84, 104.94, 101.65, 36.09, 24.65, 22.39, 22.22, 13.99; IR(KBr): ν = 1658 (C=O), 1595 (C=N) cm^{-1}; MS: m/z = 346 (M^+), 289, 247, 149, 141, 114, 85, 57; EI-HRMS: calcd for $C_{19}H_{26}N_2O_4$ (M^+), 346.1893; found, 346.1893.

4.5.3. 2,2-Dibutyl-3-acetyl-5-(3,5-dimethylphenyl)-1,3,4-oxadiazoline (4c)

This compound was obtained as white solid following the above mentioned, yield 72%, mp 50.2–51.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.46 (s, 2H, Ph-H), 7.13 (s, 1H, Ph-H), 2.41–2.47 (m, 2H, CH_2), 2.38 (s, 6H, Ph-CH_3), 2.35 (s, 3H, $COCH_3$), 1.92–1.98 (m, 2H, CH_2), 1.29–1.34 (m, 8H, CH_2), 0.88 (t, J = 6.8 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.82, 154.94, 138.34, 133.02, 124.65, 124.39, 104.78, 36.16, 24.63, 22.40, 22.25, 21.18, 13.99; IR(KBr): ν = 1654 (C=O), 1595 (C=N) cm^{-1}; MS: m/z = 330 (M^+), 273, 231, 189, 133, 105, 85, 57; EI-HRMS: calcd for $C_{20}H_{30}N_2O_2$ (M^+), 330.2307; found, 330.2307.

4.6. MAO inhibitory activity determination

The prepared compounds were submitted to the Chinese National Center for Drug Screening for in vitro MAO inhibitory activity assays. MAO inhibitory activity of rat brain mitochondrial was determined by a fluorimetric procedure using kynuramine as a substrate according to the reported method[61,62] with some modifications. Fluorescence was detected using a Perkin-Elmer Victor II spectrofluorimeter. Test compounds were dissolved in DMSO/H_2O and different inhibitor concentrations (10^{-5}–10^{-3} mol L^{-1})

were used. A control was run for each test and MAO inhibiting activity of samples was expressed as % of the control.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.07.026.

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