European Journal of Medicinal Chemistry 44 (2009) 2113–2121

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Novel 4*H*-1,3,4-oxadiazin-5(6*H*)-ones with hydrophobic and long alkyl chains: Design, synthesis, and bioactive diversity on inhibition of monoamine oxidase, chitin biosynthesis and tumor cell

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ARTICLE INFO

Article history:
Received 25 September 2008
Received in revised form
13 October 2008
Accepted 17 October 2008
Available online 1 November 2008

Keywords:
1,3,4-Oxadiazin-5(6*H*)-ones
Heterocycle
Bioactive diversity

ABSTRACT

A new series of nitrogen-containing heterocycles 4*H*-1,3,4-oxadiazin-5(6*H*)-ones derivatives with hydrophobic and long chains were designed and synthesized by direct cyclization reaction of *N*′-alkylation substituted aroylhydrazines with chloroacetyl chloride. The preliminary assays showed that some of the compounds displayed moderate to good inhibitory activities toward monoamine oxidase (MAO) at the concentration of 10^{-5}–10^{-3} M, and antitumor activities against human lung cancer A-549 and human prostate cancer PC-3 cell lines at μM level, which might provide new scaffold for anticancer agents. Furthermore, compounds **5i** and **5m** exhibited significant inhibitory activity on chitin biosynthesis, which might represent a novel class of highly potential inhibitors of chitin synthesis.

1. Introduction

Azaheterocyclic compounds are of synthetic interest and display extensive biological activities, which constitute an important class of natural and unnatural products and are extremely versatile building blocks for the manufacture of bioactive compounds in pharmaceutical drug design and agrochemical industry [1–6]. Thus, developing new nitrogen-containing heterocycles derivatives as pharmaceuticals is still an important area of interest in the life science. The interest in six-membered heterocycles with two adjacent nitrogen atoms stems from the occurrence of saturated and partially saturated pyridazine in biologically active compounds [7,8]. Many pyridazine derivatives are used in various applications as pharmaceuticals and agrochemicals, especially those with oxadiazines heterocycles have a diversity of biological effects such as cardiovascular, antibacterial, antimicrobial, plant-growth regulating, miticidal and nematocidal, acricidal, and insecticidal activities and monoamine oxidase (MAO) inhibition [9–19]. The promising bioactive diversity of this class of heteroaryl compounds urge us to synthesize and biologically evaluate a series of novel structural variants of 4*H*-1,3,4-oxidiazine-5(6*H*)-ones derivatives and their related intermediates.

In 2000, Gellman and co-workers [20,21] at University of Wisconsin developed novel rigid amphiphiles for membrane protein manipulation. It is known that membrane proteins represent a large proportion of the proteins produced by living organisms and perform many crucial functions including transport, catalysis, photosynthesis, respiration and signal transduction. Their research results showed that the hydrophobic and long substituents in the molecules **A** and **B** (Fig. 1) were very important moieties for the binding with proteins. Whereafter, the researchers at FMC Corporation [22,23] developed a High-Throughput Screening (HTS) assay against the insect ecdysone receptor trying to identify new, unique scaffolds at a highly researched target site. From their results, many simple and unique scaffolds are of great interest such as isoxazolidine-3,5-dione derivative **C** and *N*′,*N*′-dibutyl-*N*-phenylthiourea **D** (Fig. 1). Both of them possess two hydrophobic and long alkyl chains, which might play a key role in regulating the octanol/water partition coefficient, improving chemical behavior and enhancing activities of the molecules. Recently, during the course of drug discovery, the researchers at Abbott Laboratories [24] have synthesized a series of 4,4-dialkyl-1-hydroxy-3-oxo-3,4-dihydronaphthalene-3-yl benzothiadiazine derivatives **E** (Fig. 1), and evaluated their activities as Hepatitis C NS5B polymerase inhibitors. The results indicated that the representative *gem*-dibutyl series of

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doi:10.1016/j.ejmech.2008.10.015

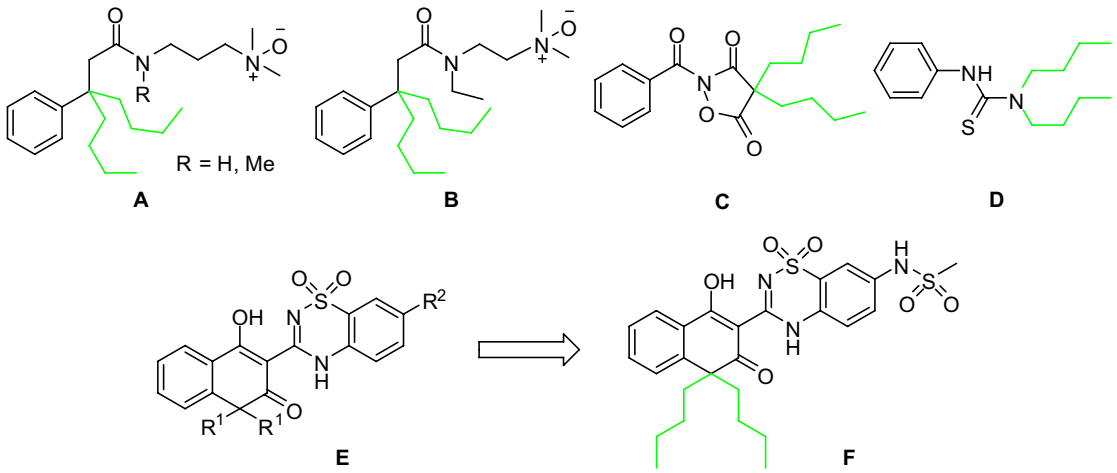


Fig. 1. Representative structures containing hydrophobic alkyl chains.

compounds **F** showed the best overall *in vitro* activity and improved pharmacokinetic behavior, these compounds with hydrophobic and long alkyl chains also displayed interesting bioactivity.

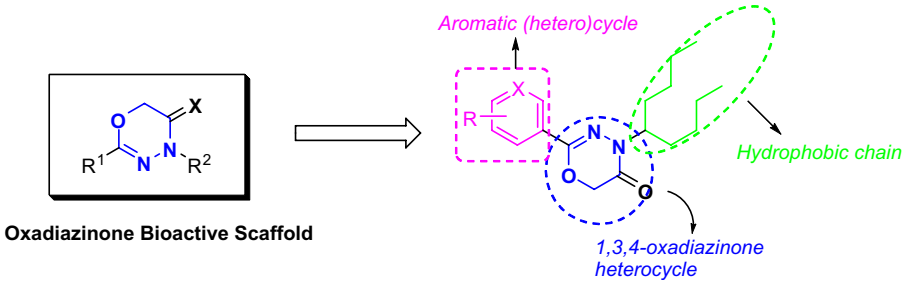
Inspired by these reports, we developed an idea to introduce hydrophobic and long alkyl chains into the important bioactive nitrogen-containing oxadiazinone heterocycles. Considering that the incorporation of hydrophobic and long alkyl chains into the heterocyclic skeleton may provide better interaction with the cell's microstructure and the active site of the enzymes, which lead to the promotion of the biological activity of the compounds and extend activity profiles, we utilized 1,3,4-oxadiazinone scaffold as prototype molecule and speculated that it might be able to improve on the properties of compounds by extending the active system. Therefore, a series of novel 4*H*-1,3,4-oxadiazin-5(6*H*)-one derivatives were designed and synthesized as shown in Scheme 1, and their antitumor activities, MAO inhibition activities and chitin synthesis inhibition were also evaluated. The availability of a reliable diversity measurement makes possible a comparison of data bases, so the aim of the present study is to evaluate the bioactive diversity and get an insight into the effect of the conjugates of 4*H*-1,3,4-oxadiazine-5(6*H*)-ones with hydrophobic and long alkyl chains on the biological activities.

2. Results and discussion

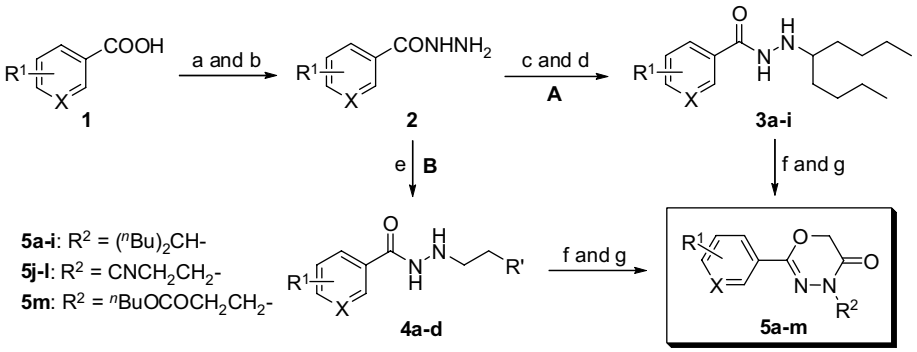
2.1. Synthesis of substituted 1,3,4-oxadiazin-5(6H)-one derivatives

The synthesis and reaction conditions of the novel 4*H*-1,3,4-oxadiazin-5(6*H*)-one derivatives containing *n*-butyl chains **5a**–**i** are outlined in Scheme 2. Furthermore, in order to broaden the scope of such kind of compounds, the easy derivatives **5j**–**m** were obtained conveniently in a different way by replacing the linear alkyl chains at 4-position of heterocycles with the different substituents such as 2-$CNCH_2CH_2$ and *n*-$BuOCOCH_2CH_2$ groups (Method B).

The various synthesized benzoates were treated with hydrazine hydrate in ethanol to afford the corresponding hydrazides **2**. It is well known that alkylation of a hydrazide usually occurs at different positions depending on different reaction conditions. Generally, in neutral medium, the terminal *N*′-atom is alkylated first, nevertheless in alkaline medium, the position of alkylation is distinctly determined by the nature of the solvent, namely, aprotic solvents such as benzene etc. are helpful to *N*-substitution, and in protic solvents like ethanol, *N*′-substitution plays a dominant role [25,26]. The condensation reaction (Method A in Scheme 2) between hydrazides **2** and nonan-5-one followed by a selective reduction of C=N double bond which was efficiently performed with a suspension of $NaBH_4$ in THF led exclusively to the key intermediates *N*′-alkylation products **3a**–**i**. The other four important alkylation products **4a**–**d** were obtained under mild reaction conditions by classical Michael addition of appropriate hydrazides **2** to α,β-unsaturated systems such as acrylonitrile and *n*-butyl acrylate (Method B in Scheme 2). In the above-described experimental conditions, all the alkylation reactions reached completion with a very high yield. The following cyclizations of the intermediates *N*′-(nonan-5-yl)aroylhydrazides **3a**–**i**, *N*′-(2-cyanoehtyl)aroylhydrazides and *N*′-(butoxycarbonylethyl)hydrazides **4a**–**d** were brought about with chloroacetyl chloride in $CHCl_3$ and following in the presence of potassium carbonate for about 0.5–2 h to afford the target compounds 5,6-dihydro-4*H*-1,3,4-oxadiazin-5-ones derivatives **5a**–**m** in excellent yields.



Scheme 1. Design strategy of new 1,3,4-oxadiazinone derivatives.



Scheme 2. Reagents and conditions: a. EtOH, Conc. H_2SO_4; b. 5 equiv $NH_2NH_2 \cdot H_2O$, reflux for 8 h; c. 1.1 equiv nonan-5-one, EtOH, reflux for 6–8 h; d. 2 equiv $NaBH_4$, THF, r.t. to 40 °C for 15 h; e. EtOH, acrylonitrile or *n*-butyl acrylate, at 60 °C for 30–48 h; f. 1.2 equiv $ClCH_2COCl$, $CHCl_3$, reflux for 1 h; g. 5 equiv K_2CO_3, EtOH, reflux for 0.5–2 h.

2.2. Spectroscopy

Structures of target compounds **5a**–**m** were confirmed by their ^1H and ^{13}C NMR spectra and high-resolution electron impact mass spectra (HR-EIMS). Their ^1H NMR spectra showed distinctive signals of methylene between oxygen and carbonyl in 1,3,4-oxadiazin-5(6*H*)-one ring, which presented a singlet at about 4.68–4.79 ppm. The multiplets at 4.57–4.70 ppm in the ^1H NMR spectra of compounds **5a**–**i** were assigned to the methine proton next to N atom in heterocycles as shown in the representative spectra (Fig. 2**)**. For compounds **5a**–**i**, the signals that appeared in their ^1H NMR spectra in the ranges 0.88–0.89 ppm, 1.19–1.39 ppm, 1.48–1.59 ppm and 1.72–1.92 ppm were attributed to the aliphatic protons of the dibutyl attached to the heterocycles. In particular, the two methylenes linked to the methine presents distinctly different signals at 1.48–1.59 ppm and 1.72–1.92 ppm, respectively, which may be related to the phenomena of magnetically nonequivalent protons due to the obstruction or restriction of the C–C bond free rotation by the long aliphatic chains. The ^{13}C NMR spectra of compounds **5a**–**m** indicated signals at 158.79–161.41 ppm as well as at 147.37–148.88 ppm, corresponding to carbonyl carbon and C=N carbon in the oxadiazinone heterocycle, respectively.

2.3. Biological activity evaluation

2.3.1. MAO inhibitory activity

The prepared compounds were submitted to the Chinese National Center for Drug Screening for *in vitro* MAO inhibitory activity and cytotoxicity assays. The *in vitro* inhibition activities against MAO of the synthesized compounds and the related intermediates were investigated by kynuramine fluorimetric assay method [27,28]. Enzymatic assays revealed that some of the tested compounds were weak to moderate MAO inhibitors at low concentrations (0.08 mmol/L). The inhibition activity of some active compound **5** and the intermediates **3** and **4** against MAO are shown in Tables 1 and 2, respectively.

As shown in Table 1, compounds **5b**, **5d**, **5g**, **5j** take on moderate inhibitory activity against MAO at the concentration of 0.08 mmol/L. Particularly, the compound **5b** bearing trimethoxyphenyl moiety exhibits better activity and the IC_{50} value is up to 0.41 mmol/L, which further indicates that trimethoxyphenyl moiety is an important active unit due to its naturally derived characterization [29–32]. For comparison, the intermediates **3** and **4** have been tested for the inhibition activity. On the basis of the results in Table 2, the intermediate **4a** exhibits better inhibitory activity against MAO compared with the other intermediates **3b**–**i**, the IC_{50} is up to 0.24 mmol/L. Furthermore, from Tables 1 and 2, it can be figured that the cyclization products **5b**, **5d**, **5g** have better activities than the corresponding intermediates **3b**, **3d**, **3g**, respectively, which demonstrates that the 4*H*-1,3,4-oxadiazin-5(6*H*)-one derivatives containing hydrophobic and long chains may be developed as potential lead compounds for optimization of novel MAO inhibitors that are useful not only in the treatment of neurodegenerative diseases (MAOI-B), but also for affective disorders (MAOI-A).

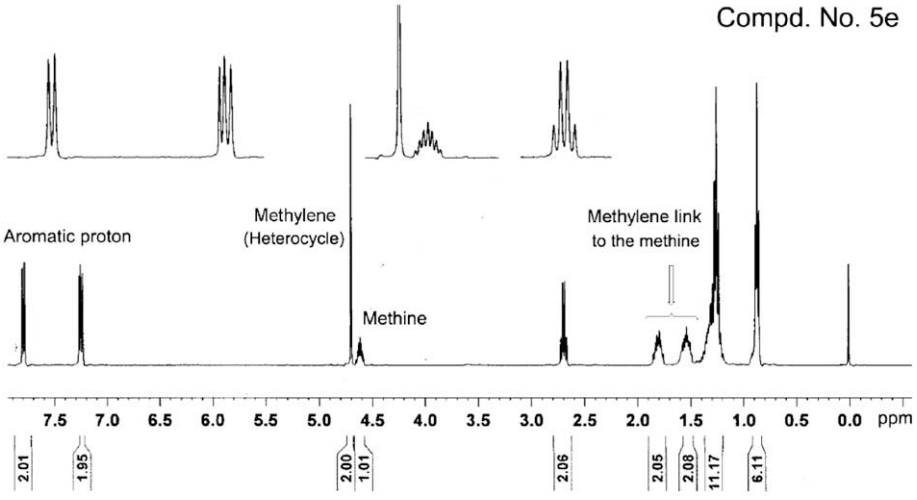
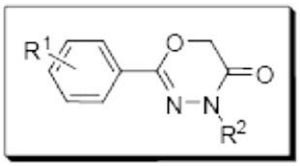


Fig. 2. Representative ^1H NMR spectral analysis of compound **5e**.

Table 1
In vitro inhibition activity of some active 4*H*-1,3,4-oxadiazin-5(6*H*)-one derivatives **5** against MAO by kynuramine fluorimetric assay.



Entry	Compound no.	Substituents		Inhibitions against MAO (%) (mmol/L)			
		R^1	R^2	0.008	0.08	0.8	IC_{50}[a]
1	**5b**	3,4,5-Trimethoxy	Nonan-5-yl	14.04	23.54	61.36	0.41
2	**5d**	3,4-Tetramethine	Nonan-5-yl	12.84	20.32	54.59	0.60
3	**5g**	*p*-Chloro	Nonan-5-yl	11.15	28.18	58.79	0.42
4	**5j**	*p*-Ethyl	2-Cyanoethyl	9.73	25.49	59.68	0.43

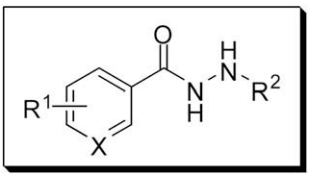
[a] IC_{50} – 50% inhibition concentration.

2.3.2. Antitumor activity evaluation

The 4*H*-1,3,4-oxadiazin-5(6*H*)-one heterocyclic derivatives **5** were further evaluated for their *in vitro* cytotoxicity effects against human lung cancer cell A-549 and human prostate cancer cell PC-3 using sulforhodamine B (SRB) dye-staining assay method [33]. Some of the results are summarized in Table 3. The IC_{50} value represents the drug concentration (μM) requires to inhibit cell growth by 50%.

In general, some of the new derivatives showed medium cytotoxic activity against A-549 and PC-3 cell lines. Among them, 4*H*-1,3,4-oxadiazin-5(6*H*)-one containing trimethoxyphenyl moiety **5b** represents most potential growth inhibitory activity against A-549 and PC-3 cell lines with IC_{50} values of 9.91 and 14.42 μM, respectively. Also, the cytotoxicities of compound **5d** exhibited selectivity for a special human prostate cancer cell type, the inhibition against PC-3 cell was obviously higher than A-549 cell. The results of preliminary antitumor assay indicated the heterocyclic molecule containing trimethoxyphenyl group might be an active scaffold, which further confirmed the compound **5b** should be a potential lead molecule for discovery of *N*-heterocyclic derivatives as potential drugs.

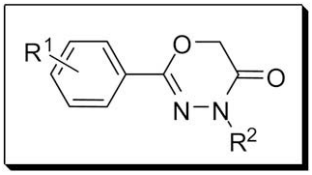
Table 2
In vitro inhibition activity of some active intermediates **3** and **4** against MAO by kynuramine fluorimetric assay.



Entry	Compound no.	Substituents			Inhibitions against MAO (%) (mmol/L)			
		X	R^1	R^2	0.01	0.1	1	IC_{50}[a]
1	**3b**	C	3,4,5-Trimethoxy	Nonan-5-yl	4.67	8.16	35.53	2.30
2	**3c**	C	3,4-Dioxomethene	Nonan-5-yl	0.00	13.77	62.82	–[b]
3	**3d**	C	3,4-Tetramethine	Nonan-5-yl	0.00	8.47	33.15	–
4	**3f**	C	*o*-Chloro	Nonan-5-yl	6.40	5.13	14.21	–
5	**3g**	C	*p*-Chloro	Nonan-5-yl	7.52	2.71	28.56	2.61
6	**3h**	C	2,4-Dichloro	Nonan-5-yl	9.33	3.52	10.51	–
7	**3i**	N	*o*-Ethoxy	Nonan-5-yl	0.00	6.09	21.70	–
8	**4a**	C	*p*-Ethyl	2-Cyanoethyl	2.80	36.98	71.18	0.24

[a] IC_{50} – 50% Inhibition concentration.
[b] –: Not determination.

Table 3
Cytotoxicities of some target compounds **5** against cell lines of A-549 and PC-3.



Entry	Compound no.	Substituents		Growth-inhibitory properties IC_{50}[a] (μ mol/L)	
		R^1	R^2	A-549[b]	PC-3[c]
1	**5b**	3,4,5-Trimethoxy	Nonan-5-yl	9.91	14.42
2	**5d**	3,4-Tetramethine	Nonan-5-yl	>100	42.45
3	**5g**	*p*-Chloro	Nonan-5-yl	24.79	ND[d]
4	**5j**	*p*-Ethyl	2-Cyanoethyl	>100	>100

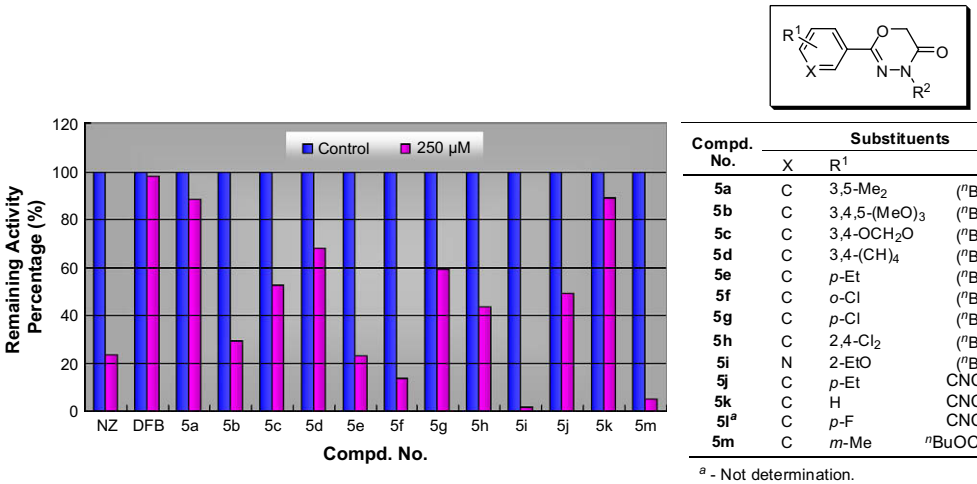
[a] IC_{50} – 50% Inhibition concentration.
[b] Human lung cancer cell line.
[c] Human prostate cancer cell line.
[d] ND – Not determination.

2.3.3. Chitin synthesis inhibitory activity

The chitin synthesis inhibition activities of the synthesized compounds were estimated using yeast *Saccharomyces cerevisiae* cell extracts by a non-radioactive chitin synthase assay according to a modified procedure described by Lecuro et al. [34]. Fig. 3 indicates the enzymatic activities in the presence of compounds **5a**–**m**.

The inhibitory activities against chitin synthesis were confirmed by control with nikkomycin Z (**NZ**) and diflubenzuron (**DFB**) to compare the potency of our compounds **5a**–**m**. As shown in Fig. 3, all the synthesized 4*H*-1,3,4-oxadiazin-5(6*H*)-one derivatives **5a**–**m** displayed moderate to good inhibition activities on chitin synthesis at 250 μM concentration, especially compounds **5i** and **5m** exhibited significant inhibitory effect on enzyme activity compared with **NZ** up to 98% and 95% at 250 μM, respectively. However, compound **DFB** produced almost no inhibition of chitin synthesis in this system. Compound **5i** bearing alkoxy-substituted pyridine ring exhibited obvious chitin synthesis inhibition, which might be due to the hydrophobic and long alkyl chains, e.g. dibutyl side chains increasing the lipophilicity of molecule, and the ether group in the structure possesses hydrophilicity as well, the double effects resulted in a better lipid–water partition coefficients of molecule **5i**. Additionally, compound **5m** containing *n*-$BuOCOCH_2CH_2$ moiety also showed a strong inhibition effect on chitin synthesis, however, the presence of cyanoethyl group attached to oxadiazinone heterocycle (**5j** and **5k**) decreased the inhibitory activity relative to the other analogues. Comparison of compounds **5e** and **5j**, the aromatic rings at C-2 of oxadiazinone scaffold are the same, but the different alkyl-substituents attached to the nitrogen atom of oxadiazinone heterocycle lead to the obviously different inhibition activity, which further reveals that the hydrophobic and long alkyl chains are superior to the cyanoethyl substituent.

In addition, we introduced various electron-withdrawing groups (such as chlorine atoms) and electron-donating substituents (Me, MeO, Et, etc.) into the aromatic ring for exploring the influence of structural changes on activity. As described in Fig. 3, the different substituents at the periphery of the molecules **5a**–**i** led to the obviously different inhibition activities. Among all the compounds containing electron-withdrawing groups, the compound containing *o*-chloro-substituent **5f** was the comparably highest potent, which led to 86% inhibition. Nevertheless, position changes of the chloro-substituent within the same ring (compounds **5f**, **5g** and **5h**) dramatically changed the inhibition activity, suggesting that the presence of a group in the *ortho*-position of benzene ring attached to oxadiazinone heterocycle



Compd. No.	Substituents		
	X	R^1	R^2
5a	C	3,5-Me_2	$(^nBu)_2CH$-
5b	C	3,4,5-$(MeO)_3$	$(^nBu)_2CH$-
5c	C	3,4-OCH_2O	$(^nBu)_2CH$-
5d	C	3,4-$(CH)_4$	$(^nBu)_2CH$-
5e	C	*p*-Et	$(^nBu)_2CH$-
5f	C	*o*-Cl	$(^nBu)_2CH$-
5g	C	*p*-Cl	$(^nBu)_2CH$-
5h	C	2,4-Cl_2	$(^nBu)_2CH$-
5i	N	2-EtO	$(^nBu)_2CH$-
5j	C	*p*-Et	$CNCH_2CH_2$-
5k	C	H	$CNCH_2CH_2$-
5l[a]	C	*p*-F	$CNCH_2CH_2$-
5m	C	*m*-Me	$^nBuOCOCH_2CH_2$-

[a] - Not determination.

Fig. 3. The structures and relative activity relationships of 4*H*-1,3,4-oxadiazin-5(6*H*)-one derivatives **5a**–**m**, and the chitin synthase activities were assayed in a total membrane fraction from *Saccharomyces cerevisiae*.

could introduce important steric and electronic effects. On the other hand, *para*-ethyl group bound to benzene rings in compound **5e** was better for inhibitory activity than the other alkyl- or alkoxy-substituted analogues. The drastic decrease in potency observed for compounds **5b**, **5c**, and **5d** can be explained by the reducing hydrophilicity resulting from the decreasing of ether group attached to the benzene ring. In comparison with the structures and activities of compounds **5b**, **5c** and **5d**, we can find the presence of trimethoxyphenyl moiety (**5b**) in the molecules is more favorable for inhibition activities, which may have a better lipid–water partition coefficient.

Furthermore, since compounds **5i** and **5m** displayed significant inhibition against chitin synthesis compared with **NZ**, five serial dilutions of them were further tested for enzyme activity. As indicated in Fig. 4, the inhibitory effects on chitin synthesis of target compounds showed obvious concentration-dependent manner.

3. Conclusion

In summary, the design, synthetic approach, analytical, spectroscopic and biological data of 2-substituted-aryl-5,6-dihydro-4-alkyl-4*H*-1,3,4-oxadiazin-5-one derivatives with hydrophobic and long alkyl chains (**5a**–**m**) have been presented here. Some of the tested compounds exhibited moderate to good inhibition activities against MAO at the dosage of 0.08 mmol L^{-1}, and several compounds showed good antitumor activities against A-549 and PC-3 cell lines. Meanwhile, compounds **5i** and **5m** exhibited better inhibitory activity on chitin synthesis at 250 μM level. The present work demonstrated that the integration of 1,3,4-oxadiazin-5-ones ring with hydrophobic and long alkyl chains showed an important biological effect and exhibited broad activity profiles, further modification of these structures might result in new compounds with high lipophilicity and variously potent activities. The preliminary structure–activity relationship established the importance of the alkoxy-substituted pyridine ring and highly lipophilic moieties.

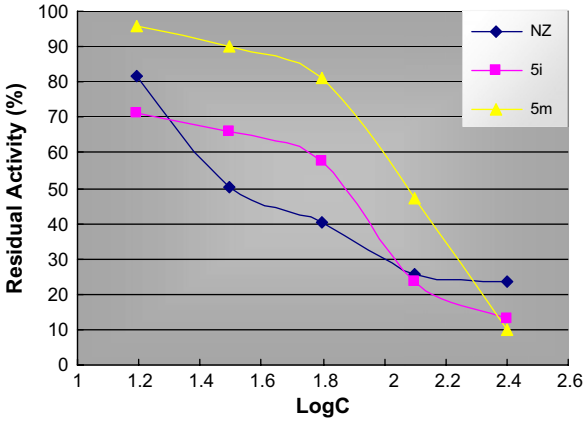


Fig. 4. Semi-logarithmic plot of the inhibitory activity of compounds **NZ**, **5i** and **5m** against chitin synthesis at concentrations of 15.63, 31.25, 62.5, 125 and 250 μM, expressed as residual activity of the enzyme.

4. Experimental section

4.1. Instrumentation and chemicals

All melting points (m.p.) were obtained using a Büchi Melting Point B540, and are uncorrected. ^1H 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 mass (HR-EIMS) spectra were recorded under electron impact (70 eV) condition using a MicroMass GCT CA 055 instrument. Analytical thin-layer chromatography (TLC) was carried out on precoated plates (silica gel 60 F254), and spots were visualized with ultraviolet (UV) light. All chemicals or reagents were purchased from standard commercial supplies. Anhydrous $CHCl_3$ and THF were prepared by standard methods. All other solvents and reagents were analytical reagent and used directly without purification, except for chloroacetyl chloride, which were distilled before use.

4.2. General synthetic procedure for N′-(nonan-5-yl)-aroylhydrazides *3a–i*

Synthesis of the intermediates aroylhydrazones: the equal amounts of appropriate acylhydrazide (0.01 mol), nonan-5-one (1.42 g, 0.01 mol) were refluxed in absolute ethanol (15 mL) for several hours, which was monitored by TLC. Then the solution was concentrated, and the condensation product hydrazone separated out on cooling and was recrystallized from aqueous ethanol.

The reduction of intermediates aroylhydrazones: sodium borohydride (0.76 g, 0.02 mol) was suspended in anhydrous THF (30 mL) under nitrogen, the above step product hydrazone (0.01 mol) was added batch to the stirred suspension solution under ice bath. The mixture was stirred at low temperature for about 0.5 h. After this, the mixture was allowed to react at 35–40 °C for about 10–15 h. The excess sodium borohydride was decomposed with 1 N hydrochloric acid under ice bath, the mixture was extracted with dichloromethane. The organic layer was washed with water and then brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo* to obtain crude products. The residue was purified by silica gel column chromatography to give the pure hydrazide, the eluent was petroleum ether/AcOEt (v/v, 6:1 → 4:1). Their physico-chemical properties and the spectra data are as follows.

*4.2.1. 3,5-Dimethyl-*N′*-(nonan-5-yl)benzohydrazide (**3a**)*

This compound was obtained as pale yellow liquid following the above method, yield 82%; ^{1}H NMR ($CDCl_3$): $\delta = 7.36$ (s, 2H, Ph-H), 7.15 (s, 1H, Ph-H), 4.63 (s, 1H, NH), 2.89–2.94 (m, 1H, CH), 2.37 (s, 6H, Ph-CH_3), 1.25–1.54 (m, 12H, CH_2), 0.92 (t, $J = 6.8$ Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 167.59$, 138.39, 133.36, 132.92, 124.82, 124.58, 60.38, 32.25, 27.86, 22.99, 21.22, 14.05; MS: $m/z = 290$ (M^+), 233, 190, 150, 133, 105.

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

This compound was obtained as white powder following the above method, yield 88%, m.p. 60.2–61.9 °C; ^{1}H NMR ($CDCl_3$): $\delta = 6.98$ (s, 2H, Ph-H), 3.89 (s, 6H, OCH_3), 3.88 (s, 3H, OCH_3), 2.89–2.91 (m, 1H, CH), 1.32–1.45 (m, 12H, CH_2), 0.91 (t, $J = 6$ Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 167.01$, 153.31, 141.19, 128.33, 104.25, 60.90, 60.39, 56.31, 32.38, 27.88, 22.99, 14.05; MS: $m/z = 352$ (M^+), 295, 211, 195.

4.2.3. N′*-(Nonan-5-yl)benzo[*d*][1,3]dioxole-5-carbohydrazide (**3c**)*

This compound was obtained as white solid following the above method, yield 78%, m.p. 35.4–36.2 °C; ^{1}H NMR ($CDCl_3$): $\delta = 7.23$–7.29 (m, 1H, Ph-H), 6.77–6.88 (m, 2H, Ph-H), 6.03 (s, 2H, OCH_2O), 5.95 (s, 1H, N–H), 4.59 (s, 1H, N–H), 2.85–2.90 (m, 1H, CH), 1.26–1.51 (m, 12H, CH_2), 0.92 (t, $J = 7$ Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 166.69$, 150.57, 148.04, 127.11, 121.47, 120.46, 108.15, 101.71, 60.32, 32.33, 27.83, 23.00, 14.04; MS: $m/z = 306$ (M^+), 249, 165, 149, 121, 84, 65.

4.2.4. N′*-(Nonan-5-yl)-2-naphthohydrazide (**3d**)*

This compound was obtained as white solid following the above method, yield 84%, m.p. 58.4–60.8 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.29$ (s, 1H, Ar-H), 7.79–7.93 (m, 4H, Ar-H), 7.53–7.60 (m, 2H, Ar-H), 2.94–2.99 (m, 1H, CH), 1.35–1.57 (m, 12H, CH_2), 0.93 (t, $J = 7$ Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 167.33$, 134.86, 132.62, 130.22, 128.92, 128.62, 127.78, 127.44, 126.85, 123.24, 60.43, 32.39, 27.88, 23.02, 14.07.

*4.2.5. 4-Ethyl-*N′*-(nonan-5-yl)benzohydrazide (**3e**)*

This compound was obtained as yellow liquid following the above method, yield 81%; ^{1}H NMR ($CDCl_3$): $\delta = 7.68$ (d, $J = 8.4$ Hz, 2H, Ar-H), 7.27 (d, $J = 8.4$ Hz, 2H, Ar-H), 4.66 (s, 1H, NH), 2.89–2.94 (m, 1H, CH), 2.70 (q, 2H, Ar-$\underline{CH_2}$), 1.35–1.46 (m, 12H, CH_2), 1.26 (t, $J = 7.6$ Hz, 6H, Ar-$CH_2\underline{CH_3}$), 0.92 (t, $J = 7$ Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 167.25$, 148.45, 130.39, 128.14, 126.92, 60.35, 32.33, 28.79, 27.85, 23.00, 15.26, 14.03; MS: $m/z = 290$ (M^+), 233, 133, 105, 79, 57.

*4.2.6. 2-Chloro-*N′*-(nonan-5-yl)benzohydrazide (**3f**)*

This compound was obtained as white crystal following the above method, yield 82%, m.p. 36.2–37.5 °C; ^{1}H NMR ($CDCl_3$): $\delta = 7.35$–7.72 (m, 4H, Ph-H), 3.07–3.13 (m, 1H, CH), 1.49–1.63 (m, 4H, CH_2), 1.39–1.45 (m, 8H, CH_2), 0.93 (t, $J = 7$ Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 165.76$, 133.23, 131.64, 131.07, 130.31, 130.29, 127.09, 60.55, 31.96, 27.74, 22.94, 13.99; MS: $m/z = 296$ (M^+), 239, 139, 111.

*4.2.7. 4-Chloro-*N′*-(nonan-5-yl)benzohydrazide (**3g**)*

This compound was obtained as white solid following the above method, yield 86%, m.p. 99.8–102.2 °C; ^{1}H NMR ($CDCl_3$): $\delta = 7.42$–7.82 (q, 4H, Ar-H), 4.69 (s, 1H, NH), 3.09–3.12 (m, 1H, CH), 1.34–1.59 (m, 12H, CH_2), 0.92 (t, $J = 7$ Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 166.10$, 138.83, 129.75, 129.27, 129.03, 128.84, 126.93, 61.74, 31.04, 27.55, 22.77, 13.91; MS: $m/z = 296$ (M^+), 239, 156, 139, 111.

*4.2.8. 2,4-Dichloro-*N′*-(nonan-5-yl)benzohydrazide (**3h**)*

This compound was obtained as white solid following the above method, yield 84%, m.p. 92.6–93.4 °C; ^{1}H NMR ($CDCl_3$): $\delta = 7.43$–7.62 (m, 2H, Ph-H), 7.31–7.35 (m, 1H, Ph-H), 2.92–2.97 (m, 1H, CH), 1.25–1.47 (m, 12H, CH_2), 0.92 (t, $J = 6.8$ Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 164.75$, 137.18, 131.77, 131.42, 130.14, 127.59, 60.33, 32.17, 27.75, 22.98, 14.04; MS: $m/z = 330$ (M^+), 273, 190, 173, 142, 111, 84.

*4.2.9. 2-Ethoxy-*N′*-(nonan-5-yl)nicotinohydrazide (**3i**)*

This compound was obtained as white powder following the above method, yield 75%, m.p. 55.6–56.2 °C; ^{1}H NMR ($CDCl_3$): $\delta = 9.31$ (s, 1H, N–H), 8.51 (dd, $^3J = 7.6$ Hz, $^4J = 1.2$ Hz, 1H, Py-H), 8.26 (dd, $^3J = 7.6$ Hz, $^4J = 1.2$ Hz, 1H, Py-H), 7.06 (dd, $^3J = 5$ Hz, $^4J = 2.4$ Hz, 1H, Py-H), 4.57 (q, $J = 7$ Hz, 2H, O$\underline{CH_2}CH_3$), 2.87–2.91 (m, 1H, CH), 1.26–1.51 (m, 15H, aliphatic CH_2 and $OCH_2\underline{CH_3}$), 0.93 (t, $J = 7$ Hz, 6H, aliphatic CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 162.93$, 160.27, 149.60, 141.23, 117.65, 114.55, 62.97, 60.45, 32.35, 27.86, 23.02, 14.64, 14.03; MS: $m/z = 307$ (M^+), 250, 167, 150, 122, 94.

*4.3. General synthetic procedure for aroylhydrazides **4a–d***

A mixture of appropriate acylhydrazide (10 mmol) and acrylonitrile or *n*-butyl acrylate (12.5 mmol) in EtOH (20 mL) was refluxed for 30–48 h, which was monitored by TLC. The solvent was removed *in vacuo*. The resulting crop was chromatographed, eluting with petroleum ether/AcOEt (v/v, 4:1 → 3:1). Their physico-chemical properties and the spectra data are as follows.

4.3.1. N′*-(2-Cyanoethyl)-4-ethylbenzohydrazide (**4a**)*

This compound was obtained as white powder following the above method, yield 81%, m.p. 103.8–104.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.14$ (bs, 1H, N–H), 7.71 (d, $J = 8$ Hz, 2H, Ph-H), 7.26 (d, $J = 8$ Hz, 2H, Ph-H), 4.93 (bs, 1H, N–H), 3.24 (t, $J = 6.2$ Hz, 2H, CH_2CN), 2.70 (q, $J = 7.2$ Hz, 2H, CH_2-Ph), 2.59 (t, $J = 6.4$ Hz, 2H, N–CH_2), 1.24 (t, $J = 7.8$ Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 168.25$, 149.02, 129.50, 128.24, 127.14, 118.89, 47.46, 28.82, 17.46, 15.26; MS: $m/z = 217$ (M^+), 177, 164, 149, 133, 105, 79.

4.3.2. N′*-(2-Cyanoethyl)-benzohydrazide (**4b**)*

This compound was obtained as white powder following the above method, yield 76%, m.p. 120.4–121.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.80$ (s, 1H, N–H), 7.45–7.78 (m, 5H, Ph-H), 4.25 (bs, 1H, N–H), 3.28 (t, $J = 6.4$ Hz, 2H, CH_2CN), 2.61 (t, $J = 6.4$ Hz, 2H, N–CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 156.17$, 132.32, 132.11, 128.84, 126.98, 118.68, 47.51, 17.56.

4.3.3. N′*-(2-Cyanoethyl)-4-fluorobenzohydrazide (**4c**)*

This compound was obtained as white solid following the above method, yield 70%, m.p. 93.4–95.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.91$ (bs, 1H, N–H), 7.81 (q, $J = 8.8$ Hz, 2H, Ph-H), 7.14 (t,

$J = 8.6$ Hz, 2H, Ph-H), 5.06 (bs, 1H, N–H), 3.26 (t, $J = 6.2$ Hz, 2H, CH_2CN), 2.61 (t, $J = 6.4$ Hz, 2H, N–CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 167.27$, 132.32, 129.49, 129.40, 128.83, 126.98, 116.07, 115.85, 47.44, 17.62; MS: $m/z = 207$ (M^+), 167, 154, 123, 95.

4.3.4. N′-(Butoxycarbonylethyl)-3-methylbenzohydrazide (**4d**)

This compound was obtained as pale yellow liquid following the above method, yield 83%; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.78$ (s, 1H, N–H), 7.59 (s, 1H, Ph-H), 7.32–7.54 (m, 3H, Ph-H), 4.11 (t, $J = 6.6$ Hz, 2H, OCH_2), 3.26 (t, $J = 6.4$ Hz, 2H, $NHCH_2$), 2.57 (t, $J = 6.4$ Hz, 2H, CH_2CO), 2.40 (s, 3H, Ph-CH_3), 1.58–1.65 (m, 2H, $COOCH_2\underline{CH_2}$), 1.33–1.43 (m, 2H, $CH_3\underline{CH_2}$), 0.93 (t, $J = 7.4$ Hz, 3H, $\underline{CH_3}CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 172.54$, 167.54, 138.60, 132.65, 128.56, 127.63, 123.78, 64.61, 47.61, 33.53, 30.60, 21.32, 19.11, 13.68; MS: $m/z = 278$ (M^+), 205, 163, 144, 91, 77, 65.

4.4. General synthetic procedure for 4H-1,3,4-oxadiazin-5(6H)-one derivatives **5a–m**

To a solution of N′-alkylaroylhydrazides (2 mmol) in anhydrous $CHCl_3$ (25 mL) was added chloroacetyl chloride (0.23 g, 2 mmol) dropwise at room temperature, and the stirred mixture was refluxed for 30 min. After cooling and evaporation of the solvent, the resulting crop was taken up into ethanol (20 mL) and charged with an excess of anhydrous K_2CO_3 (1.38 g, 10 mmol). The mixture was stirred to reflux for 0.5–2 h and then filtered and evaporated *in vacuo*. On completion of the reaction, as ascertained by TLC analysis, the residue was chromatographed with petroleum ether/AcOEt (v/v, 5:1 → 3:1) and recrystallized from EtOH to give substituted 1,3,4-oxadiazin-5(6*H*)-one derivatives.

4.4.1. 2-(3,5-Dimethylphenyl)-4-(nonan-5-yl)-4H-1,3,4-oxadiazin-5(6H)-one (**5a**)

This compound was obtained as yellow oil following the above method, yield 76%; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.49$ (s, 2H, Ph-H), 7.09 (s, 1H, Ph-H), 4.69 (s, 2H, OCH_2), 4.59–4.66 (m, 1H, CH), 2.37 (s, 6H, Ph-$\underline{CH_3}$), 1.78–1.87 (m, 2H, CH_2), 1.51–1.59 (m, 2H, CH_2), 1.19–1.37 (m, 8H, CH_2), 0.88 (t, $J = 6.8$ Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 159.12$, 148.81, 137.99, 132.45, 129.87, 129.25, 124.83, 124.32, 64.80, 54.70, 32.29, 28.38, 22.51, 21.27, 14.02; MS: $m/z = 330$ (M^+), 273, 217, 204, 190, 159, 133, 105; EI-HRMS: calcd for $C_{20}H_{30}N_2O_2$ (M^+), 330.2307; found, 330.2307.

4.4.2. 2-(3,4,5-Trimethoxyphenyl)-4-(nonan-5-yl)-4H-1,3,4-oxadiazin-5(6H)-one (**5b**)

This compound was obtained as white solid following the above method, yield 81%, m.p. 79.2–80.8 °C; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.13$ (s, 2H, Ph-H), 4.71 (s, 2H, OCH_2), 4.59–4.66 (m, 1H, CH), 3.91 (s, 6H, OCH_3), 3.89 (s, 3H, OCH_3), 1.75–1.85 (m, 2H, CH_2), 1.51–1.59 (m, 2H, CH_2), 1.23–1.35 (m, 8H, CH_2), 0.88 (t, $J = 6.8$ Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 158.96$, 153.11, 148.23, 140.53, 125.25, 103.94, 64.89, 60.94, 56.20, 54.73, 32.33, 28.33, 22.51, 14.00; MS: $m/z = 392$ (M^+), 335, 279, 266, 252, 195, 111; EI-HRMS: calcd for $C_{21}H_{32}N_2O_5$ (M^+), 392.2311; found, 392.2311.

4.4.3. 2-(Benzo[d][1,3]dioxol-5-yl)-4-(nonan-5-yl)-4H-1,3,4-oxadiazin-5(6H)-one (**5c**)

This compound was obtained as yellow oil following the above method, yield 72%; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.40$ (dd, $^3J = 8.4$ Hz, $^4J = 1.6$ Hz, 1H, Ph-H), 7.36 (d, $^4J = 1.6$ Hz, 1H, Ph-H), 6.83 (d, $J = 8.0$ Hz, 1H, Ph-H), 6.03 (s, 2H, OCH_2O), 4.68 (s, 2H, OCH_2), 4.57–4.64 (m, 1H, CH), 1.74–1.83 (m, 2H, CH_2), 1.49–1.57 (m, 2H, CH_2), 1.19–1.38 (m, 8H, CH_2), 0.88 (t, $J = 7$ Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 159.00$, 149.86, 148.33, 147.79, 124.05, 121.36, 108.00, 106.86, 101.54, 64.86, 54.65, 32.27, 28.34, 22.51, 14.01; MS: $m/z = 346$ (M^+), 289, 233, 220, 206, 175, 149, 121; EI-HRMS: calcd for $C_{19}H_{26}N_2O_4$ (M^+), 346.1893; found, 346.1893.

4.4.4. 2-(Naphthalen-2-yl)-4-(nonan-5-yl)-4H-1,3,4-oxadiazin-5(6H)-one (**5d**)

This compound was obtained as white solid following the above method, yield 78%, m.p. 70.6–72.3 °C; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 8.32$ (s, 1H, Ar-H), 8.01 (d, $J = 8.8$ Hz, 1H, Ar-H), 7.92 (d, $J = 8$ Hz, 1H, Ar-H), 7.86 (d, $J = 8.4$ Hz, 2H, Ar-H), 7.55 (m, 2H, Ar-H), 4.79 (s, 2H, OCH_2), 4.63–4.70 (m, 1H, CH), 1.82–1.92 (m, 2H, CH_2), 1.57–1.63 (m, 2H, CH_2), 1.26–1.38 (m, 8H, CH_2), 0.89 (t, $J = 6.6$ Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 159.11$, 148.50, 134.35, 132.75, 128.79, 128.10, 127.75, 127.37, 126.63, 126.61, 123.43, 64.92, 54.81, 32.33, 28.39, 22.52, 14.02; MS: $m/z = 352$ (M^+), 295, 239, 226, 212, 181, 155, 127; EI-HRMS: calcd for $C_{22}H_{28}N_2O_2$ (M^+), 352.2151; found, 352.2150.

4.4.5. 2-(4-Ethylphenyl)-4-(nonan-5-yl)-4H-1,3,4-oxadiazin-5(6H)-one (**5e**)

This compound was obtained as yellow oil following the above method, yield 76%; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.80$ (d, $J = 8$ Hz, 2H, Ph-H), 7.25 (d, $J = 8$ Hz, 2H, Ph-H), 4.70 (s, 2H, OCH_2), 4.58–4.66 (m, 1H, CH), 2.70 (q, 2H, Ph-CH_2), 1.76–1.86 (m, 2H, CH_2), 1.50–1.57 (m, 2H, CH_2), 1.22–1.36 (m, 11H, CH_2 and Ph-$CH_2\underline{CH_3}$), 0.88 (t, $J = 6.8$ Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 159.07$, 148.67, 147.40, 127.90, 127.48, 126.64, 64.81, 54.66, 32.29, 28.83, 28.35, 22.52, 15.40, 14.01; MS: $m/z = 330$ (M^+), 273, 217, 204, 190, 159, 133; EI-HRMS: calcd for $C_{20}H_{30}N_2O_2$ (M^+), 330.2307; found, 330.2307.

4.4.6. 2-(2-Chlorophenyl)-4-(nonan-5-yl)-4H-1,3,4-oxadiazin-5(6H)-one (**5f**)

This compound was obtained as yellow oil following the above method, yield 65%; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.60$ (d, $J = 7.6$ Hz, 1H, Ph-H), 7.47 (d, $J = 8$ Hz, 1H, Ph-H), 7.39 (t, $J = 7.6$ Hz, 1H, Ph-H), 7.32 (t, $J = 7.6$ Hz, 1H, Ph-H), 4.73 (s, 2H, OCH_2), 4.59–4.66 (m, 1H, CH), 1.74–1.83 (m, 2H, CH_2), 1.49–1.57 (m, 2H, CH_2), 1.23–1.39 (m, 8H, CH_2), 0.89 (t, $J = 6.8$ Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 159.08$, 148.47, 133.45, 131.49, 130.98, 130.72, 129.62, 126.66, 64.95, 54.75, 32.26, 28.36, 22.51, 14.03; MS: $m/z = 336$ (M^+), 279, 223, 139, 111; EI-HRMS: calcd for $C_{18}H_{25}ClN_2O_2$ (M^+), 336.1605; found, 336.1605.

4.4.7. 2-(4-Chlorophenyl)-4-(nonan-5-yl)-4H-1,3,4-oxadiazin-5(6H)-one (**5g**)

This compound was obtained as white solid following the above method, yield 76%, m.p. 45.1–46.0 °C; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.81$ (d, $J = 8.4$ Hz, 2H, Ph-H), 7.39 (d, $J = 8.4$ Hz, 2H, Ph-H), 4.72 (s, 2H, OCH_2), 4.59–4.66 (m, 1H, CH), 1.74–1.83 (m, 2H, CH_2), 1.51–1.56 (m, 2H, CH_2), 1.23–1.32 (m, 8H, CH_2), 0.88 (t, $J = 7$ Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 158.79$, 147.48, 136.84, 128.63, 128.53, 127.81, 64.83, 54.77, 32.27, 28.33, 22.49, 13.99; MS: $m/z = 336$ (M^+), 279, 223, 139, 111; EI-HRMS: calcd for $C_{18}H_{25}ClN_2O_2$ (M^+), 336.1605; found, 336.1605.

4.4.8. 2-(2,4-Dichlorophenyl)-4-(nonan-5-yl)-4H-1,3,4-oxadiazin-5(6H)-one (**5h**)

This compound was obtained as yellow oil following the above method, yield 74%; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.56$ (d, $J = 8.4$ Hz, 1H, Ph-H), 7.49 (d, $J = 2$ Hz, 1H, Ph-H), 7.31 (dd, $^3J = 8.4$ Hz, $^4J = 2$ Hz, 1H, Ph-H), 4.73 (s, 2H, OCH_2), 4.59–4.66 (m, 1H, CH), 1.72–1.81 (m, 2H, CH_2), 1.48–1.56 (m, 2H, CH_2), 1.21–1.37 (m, 8H, CH_2), 0.89 (t, $J = 7.2$ Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 158.88$, 147.37, 136.96, 134.21, 131.67, 130.71, 127.99, 127.07, 64.96, 54.82, 32.27, 28.34, 22.49, 14.02; MS: $m/z = 370$ (M^+), 313, 257, 245, 230, 199, 173, 145; EI-HRMS: calcd for $C_{18}H_{24}Cl_2N_2O_2$ (M^+), 370.1215; found, 370.1215.

4.4.9. 2-(2-Ethoxylpyridin-3-yl)-4-(nonan-5-yl)-4H-1,3,4-oxadiazin-5(6H)-one (**5i**)

This compound was obtained as yellow oil following the above method, yield 68%; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.23$ (dd, $^3J = 4.8$ Hz, $^4J = 2$ Hz, 1H, Py-H), 7.86 (dd, $^3J = 7.6$ Hz, $^4J = 2$ Hz, 1H, Py-H), 6.93 (q, $^3J = 4.8$ Hz, $^4J = 3$ Hz, 1H, Py-H), 4.68 (s, 2H, OCH_2CO), 4.58–4.65 (m, 1H, CH), 4.45 (q, $J = 7.2$ Hz, 2H, $O\underline{CH_2}CH_3$), 1.74–1.84 (m, 2H, CH_2), 1.49–1.56 (m, 2H, CH_2), 1.43 (t, $J = 7.2$ Hz, 3H, $OCH_2\underline{CH_3}$), 1.23–1.38 (m, 8H, CH_2), 0.89 (t, $J = 7$ Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 161.41, 159.30, 148.88, 147.91, 139.10, 116.07, 114.06, 64.95, 62.41, 54.68, 32.21, 28.38, 22.51, 14.66, 14.04$; MS: $m/z = 347$ (M^+), 290, 234, 221, 207, 176, 150, 121, 93; EI-HRMS: calcd for $C_{19}H_{29}N_3O_3$ (M^+), 347.2209; found, 347.2209.

4.4.10. 2-(4-Ethylphenyl)-4-(cyanoethyl)-4H-1,3,4-oxadiazin-5(6H)-one (**5j**)

This compound was obtained as white solid following the above method, yield 88%, m.p. 107.2–109.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.78$ (d, $J = 8$ Hz, 2H, Ph-H), 7.25 (d, $J = 8$ Hz, 2H, Ph-H), 4.75 (s, 2H, OCH_2CO), 4.10 (t, $J = 6.8$ Hz, 2H, NCH_2), 2.82 (t, $J = 6.8$ Hz, 2H, CH_2CN), 2.70 (q, 2H, Ph-CH_2), 1.26 (t, $J = 7.6$ Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 159.12, 149.66, 148.05, 128.04, 126.76, 126.46, 117.21, 64.83, 42.85, 28.84, 16.34, 15.29$; MS: $m/z = 257$ (M^+), 217, 159, 132, 116, 103, 77; EI-HRMS: calcd for $C_{14}H_{15}N_3O_2$ (M^+), 257.1164; found, 257.1164.

4.4.11. 2-Phenyl-4-(cyanoethyl)-4H-1,3,4-oxadiazin-5(6H)-one (**5k**)

This compound was obtained as white solid following the above method, yield 82%, m.p. 108.4–109.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.88$ (d, $J = 8$ Hz, 2H, Ph-H), 7.49–7.41 (m, 3H, Ph-H), 4.78 (s, 2H, OCH_2CO), 4.12 (t, $J = 6.8$ Hz, 2H, NCH_2), 2.83 (t, $J = 6.8$ Hz, 2H, CH_2CN); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 159.04, 149.34, 131.31, 129.04, 128.50, 126.67, 117.21, 64.85, 42.89, 16.38$; MS: $m/z = 229$ (M^+), 189, 131, 104, 77, 51; EI-HRMS: calcd for $C_{12}H_{11}N_3O_2$ (M^+), 229.0851; found, 229.0850.

4.4.12. 2-(4-Fluorophenyl)-4-(cyanoethyl)-4H-1,3,4-oxadiazin-5(6H)-one (**5l**)

This compound was obtained as white solid following the above method, yield 70%, m.p. 74.2–75.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.86$–7.89 (m, 2H, Ph-H), 7.09–7.13 (m, 2H, Ph-H), 4.77 (s, 2H, OCH_2CO), 4.10 (t, $J = 6.8$ Hz, 2H, NCH_2), 2.81 (t, $J = 6.8$ Hz, 2H, CH_2CN); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 165.88, 158.87, 148.52, 128.95, 125.25, 117.22, 115.68, 64.88, 42.90, 16.42$; MS: $m/z = 247$ (M^+), 207, 149, 122, 95, 75, 57; EI-HRMS: calcd for $C_{12}H_{10}FN_3O_2$ (M^+), 247.0757; found, 247.0755.

4.4.13. 2-(3-Methylphenyl)-4-(butoxycarbonylethyl)-4H-1,3,4-oxadiazin-5(6H)-one (**5m**)

This compound was obtained as yellow liquid following the above method, yield 76%; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.64$–7.67 (m, 2H, Ph-H), 7.26–7.32 (m, 2H, Ph-H), 4.71 (s, 2H, OCH_2CO), 4.09–4.14 (m, 4H, NCH_2 and $COOCH_2$), 2.77 (t, $J = 7.2$ Hz, 2H, CH_2CO), 2.40 (s, 3H, Ph-CH_3), 1.58–1.65 (m, 2H, $COOCH_2\underline{CH_2}$), 1.32–1.42 (m, 2H, $CH_3\underline{CH_2}$), 0.92 (t, $J = 7.4$ Hz, 3H, $\underline{CH_3}CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 171.32, 158.77, 148.86, 138.13, 131.79, 129.34, 128.30, 127.09, 123.74, 64.91, 64.64, 43.25, 32.48, 30.59, 21.36, 19.10, 13.66$; MS: $m/z = 318$ (M^+), 245, 203, 176, 160, 145, 118, 105, 91, 77, 65; EI-HRMS: calcd for $C_{17}H_{22}N_2O_4$ (M^+), 318.1580; found, 318.1580.

4.5. Biology assay

The prepared compounds were submitted to the Chinese National Center for Drug Screening for *in vitro* MAO inhibitory activity and cytotoxicity assays. MAO inhibitory activity of rat brain mitochondrial was determined by a fluorimetric procedure using kynuramine as a substrate according to the reported method [27,28] with some modifications. The *in vitro* cytotoxicity assays was evaluated against human lung cancer A-549 and prostate cancer PC-3 cell lines. Growth inhibitory effect on the cell lines (A-549 and PC-3) was measured by the sulforhodamine B (SRB) dye-staining assay [33]. The chitin synthesis activity from yeast *S. cerevisiae* was estimated using a non-radioactive chitin synthase assay according to a modified procedure described by Lecuro et al. [34].

Acknowledgements

This work was financially supported by the National Key Project for Basic Research (2003CB114400), National 863 High-Tech Research Award (2006AA10A201) and the National Natural Science Foundation of China (20536010) are highly appreciated. The authors also thank for the partial support from Shanghai Leading Academic Discipline Project (B507) and Shanghai Foundation of Science and Technology (073919107).

Appendix. Suplementary information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejmech.2008.10.015.

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