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Original article

Diacylhydrazine derivatives as novel potential chitin biosynthesis inhibitors: Design, synthesis, and structure–activity relationship

Shaoyong Ke [a], Xuhong Qian [a,*], Fengyi Liu [b], Ni Wang [b], Feng Fan [a], Zhong Li [a], Qing Yang [b,*]

[a] Shanghai Key Laboratory of Chemical Biology, Institute of Pesticides and Pharmaceuticals, School of Pharmacy, East China University of Science and Technology, Shanghai 200237, China
[b] Department of Bioscience and Biotechnology, Dalian University of Technology, Dalian 116012, China

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ABSTRACT

A series of diacylhydrazine derivatives containing hydrophobic alkyl chains have been designed and synthesized. The target molecules have been identified on the basis of analytical spectral (IR, ^{1}H NMR, ^{13}C NMR, and HRMS) data. All synthesized compounds have been screened for their potential inhibition *in vitro* against chitin synthesis using yeast cell extracts. The preliminary assays indicate that some of the compounds display moderate to good inhibitory activity. Structure–activity relationship (SAR) is also discussed based on the experimental data, and the further analysis of the quantitative structure–activity relationship (QSAR) indicates that the electronic parameter is the main factor to affect inhibition activities.

1. Introduction

Chitin is a linear polysaccharide joined by β-(1,4)-linked *N*-acetylglucosamine (GlcNAc) units (Fig. 1). It is one of the main structural components of cell walls of fungi and plays a crucial role in the determination of cell morphology [1]. Accordingly, the biosynthesis of chitin is essential for fungal growth and reproduction. Since chitin is absent in higher plants and vertebrate species [2], its biosynthetic pathway becomes an attractive target for the design of antifungal drugs and agrochemicals [3–5]. The biosynthesis of chitin involves a key enzyme, that is, chitin synthase, an integral membrane enzyme that catalyzes the transfer of *N*-acetylglucosamine from uridine diphosphate–*N*-acetylglucosamine (UDP–GlcNAc) to a nascent chitin chain (Fig. 1) [6,7]. However, it is difficult to separate and purify the membrane-bound chitin synthase, which leads to the structures of the active site are uncertainty [8–10]. Nowadays, novel potential inhibitors targeting chitin biosynthesis are very limited, but chitin synthesis inhibition still remains an important target site for the control of agricultural pests. The research strategy for the design of novel chitin synthesis inhibitors will be propitious to the R&D of pharmaceutical and agrochemical industry, and makes an active promote operation for the basic research on chemical biology [11].

It is well known that the insect growth regulators (IGRs) constitute effective tools for controlling fungus gnats, shore flies, and foliar feeding pests. The primary mode of action is by inhibiting chitin biosynthesis and interfering with formation and deposition of chitin occurring in immature stages of many pests [12]. Because of their unique mechanisms of action, simple structures, low toxicity to vertebrates, and environmental benign characters, IGRs have attracted considerable attention for decades. A variety of diacylhydrazine derivatives have been designed and synthesized with the purpose of optimizing new compounds with high insecticidal activity. RH-5849, RH-5992 (Tebufenozide), RH-0345 (Halofenozide), RH-2485 (Methoxyfenozide), ANS-118 and JS-118 (Fig. 2) were developed as successful high potent IGRs based on ecdysone receptor (EcR) [13–20].

The diacylhydrazine derivatives are a promising class of chemically and mechanistically novel insect control agents that were serendipitously discovered at Rohm and Haas Company [21]. RH-5849, for example, has been considered a prototype molecule, which was subjected to different chemical modifications to optimize their activities [22–30]. Further works on the SARs of the diacylhydrazines, both during and after the discovery of the commercialized compounds, have been extensively developed [31]. Nakagawa and his co-workers [32] have presented the extended 3D QSAR analysis of diacylhydrazine derivatives recently, which has

* Corresponding authors.
E-mail addresses: xhqian@ecust.edu.cn (X. Qian), qingyang@dlut.edu.cn (Q. Yang).

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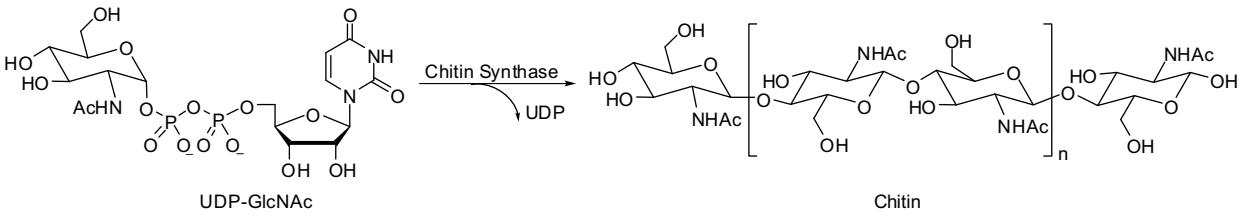


Fig. 1. Biosynthetic process of chitin.

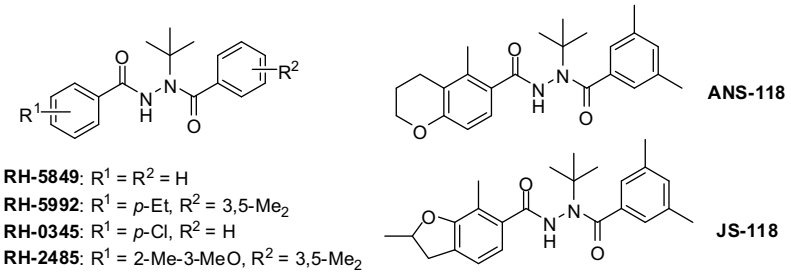
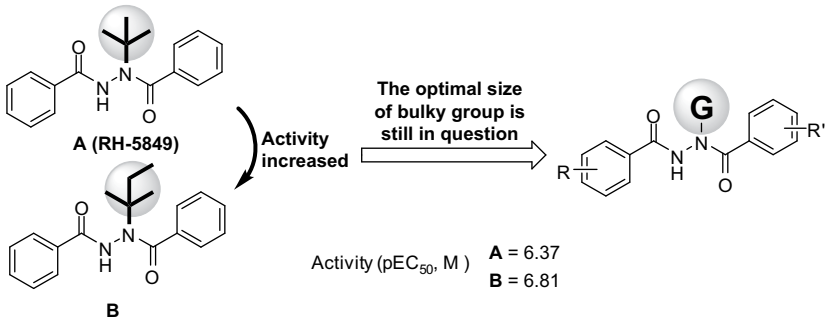
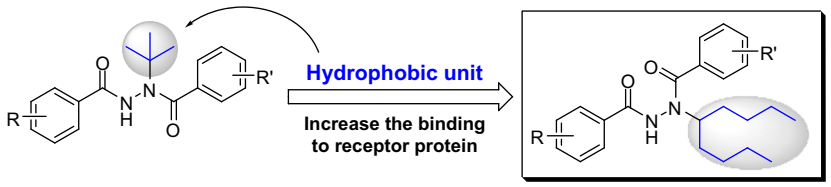


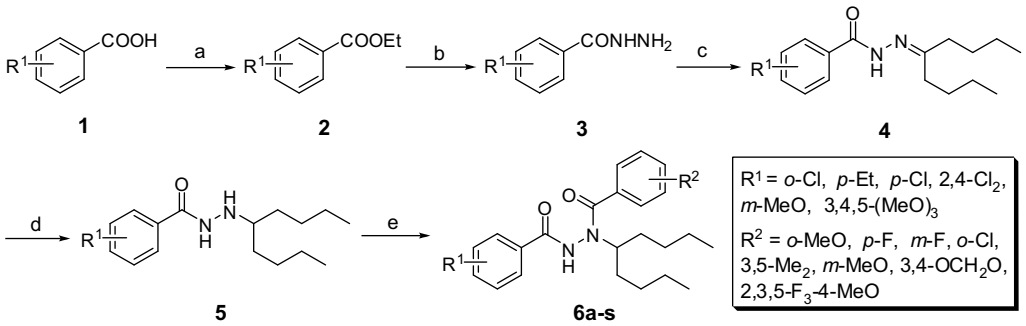
Fig. 2. Structures of some commercial diacylhydrazine derivatives.



Scheme 1. The research results of Nakagawa's group.



Scheme 2. Design strategy of novel diacylhydrazine derivatives containing hydrophobic alkyl chains.



Scheme 3. 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. 1.1 equiv. ArCOCl, 1.5 equiv. NEt_3, 0.05 equiv. 4-DMAP, CH_2Cl_2.

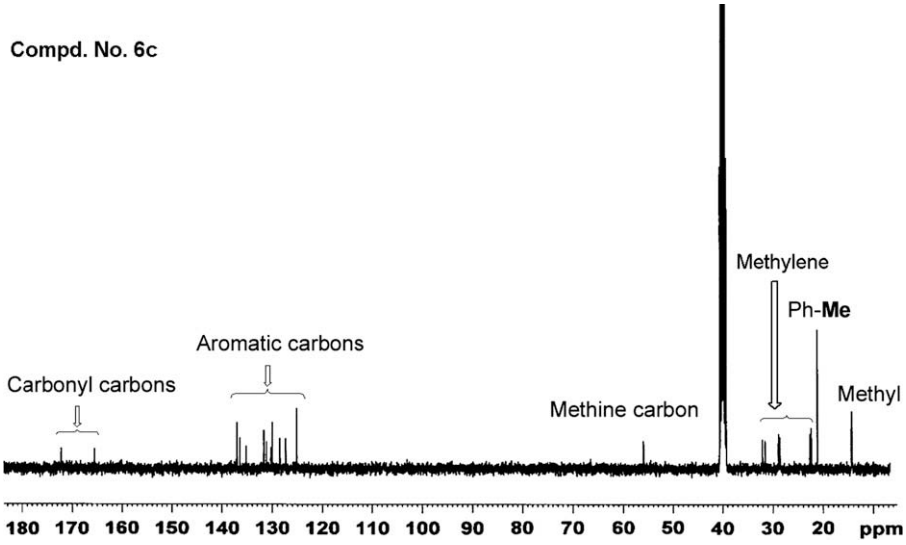
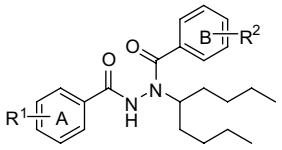
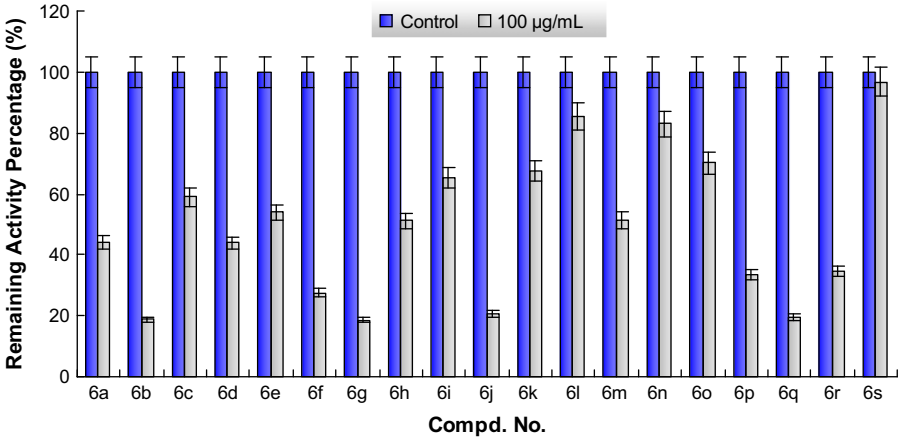


Fig. 3. Representative ^{13}C NMR spectral analysis of compound **6c**.

established that N-substituted sterically bulky groups (e.g. *tert*-butyl) are vital for activity. However, the optimal size of the bulky group is still unknown (Scheme 1).

Although many diacylhydrazine derivatives have been reported as insect growth regulators, few studies have been done concerning evaluation of this kind of compounds for their *in vitro* inhibition of chitin synthesis. Therefore, we decided to introduce a bulky hydrophobic substituent into *N*,*N*′-diacylhydrazines instead of the *tert*-butyl attached to the *N*′ atom. To further optimize active compounds, a series of novel *N*′-(nonan-5-yl)-*N*,*N*′-diacylhydrazines were designed and synthesized as depicted in Scheme 2, and we speculated that these compounds may regulate the chitin biosynthesis as above-described diacylhydrazines' insect growth regulator. Based on the identification of novel structures that can be potentially useful in the design of new and potent chitin synthesis inhibitors as potential agrochemical agents and motivated by the



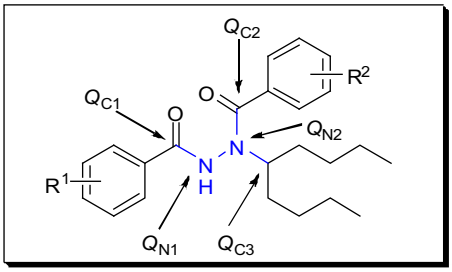


Structure of compounds 6a-s: **6a** R^1 = *o*-Cl, R^2 = H; **6b** R^1 = *o*-Cl, R^2 = *o*-MeO; **6c** R^1 = *o*-Cl, R^2 = 3,5-Me_2; **6d** R^1 = *o*-Cl, R^2 = *p*-F; **6e** R^1 = *o*-Cl, R^2 = *m*-F; **6f** R^1 = *p*-Cl, R^2 = 3,5-Me_2; **6g** R^1 = *p*-Cl, R^2 = *o*-MeO; **6h** R^1 = *p*-Et, R^2 = *o*-Cl; **6i** R^1 = *p*-Et, R^2 = *3,5-Me*$_2$; **6j** R^1 = *p*-Et, R^2 = *o*-MeO; **6k** R^1 = *p*-Et, R^2 = 3,4-OCH_2O; **6l** R^1 = *p*-Et, R^2 = $ClCH_2$[a]; **6m** R^1 = *p*-Et, R^2 = 2,3,5-F_3-4-MeO; **6n** R^1 = *m*-MeO, R^2 = H; **6o** R^1 = 2,4-Cl_2, R^2 = 3,5-Me_2; **6p** R^1 = 3,4,5-$(MeO)_3$, R^2 = *o*-Cl; **6q** R^1 = 3,4,5-$(MeO)_3$, R^2 = 3,5-Me_2; **6r** R^1 = 3,4,5-$(MeO)_3$, R^2 = 2,3,5-F_3-4-MeO; **6s** R^1 = 3,5-Me_2, R^2 = *p*-Et.

[a] The aromatic ring B was replaced with chloromethyl.

Fig. 4. The effect on chitin synthesis activities by different diacylhydrazine derivatives using yeast cell extracts.

Table 1
Molecular parameters of diacylhydrazine derivatives **6a–s**.



Compd.	Descriptor variables										
	E_T (a.u.)	E_{HOMO} (eV)	E_{LUMO} (eV)	Dipole (DM)	Q_{N1}	Q_{N2}	Q_{C1}	Q_{C2}	Q_{C3}	log *P*	*V* (Å^3)
6a	−110.0411	−9.7307	−0.1890	1.484	−0.255	−0.220	0.344	0.343	0.008	4.19	1175.91
6b	−120.9722	−9.0326	−0.1135	4.804	−0.226	−0.192	0.331	0.102	−0.032	3.19	1265.05
6c	−117.2324	−9.5108	−0.2869	1.427	−0.227	−0.272	0.330	0.369	0.017	4.49	1276.08
6d	−120.8765	−9.1448	−0.4625	4.583	−0.267	−0.173	0.329	0.056	0.059	3.59	1219.13
6e	−120.9121	−9.6739	−0.2917	1.820	−0.238	−0.250	0.336	0.356	0.016	3.59	1187.85
6f	−117.2325	−9.4514	−0.3121	0.827	−0.251	−0.246	0.339	0.355	0.006	4.49	1291.55
6g	−121.0161	−9.2350	−0.3131	1.303	−0.252	−0.237	0.341	0.368	0.009	3.19	1260.43
6h	−117.2289	−9.6114	−0.2023	0.754	−0.232	−0.257	0.331	0.367	0.007	4.74	1274.19
6i	−116.1147	−9.3614	−0.0273	1.162	−0.251	−0.239	0.339	0.354	−0.000	5.27	1337.96
6j	−119.8994	−9.5616	−0.2768	2.142	−0.263	−0.211	0.348	0.326	0.030	3.97	1314.61
6k	−126.6409	−9.1290	−0.2174	0.940	−0.255	−0.236	0.341	0.355	0.013	3.16	1324.04
6l	−101.8508	−9.7189	−0.2240	3.778	−0.200	−0.247	0.308	0.279	0.039	4.40	1159.59
6m	−152.5038	−9.7447	−0.8518	2.176	−0.254	−0.234	0.338	0.356	0.012	2.16	1354.47
6n	−112.7155	−9.2617	−0.1536	1.797	−0.229	−0.259	0.330	0.366	0.002	3.42	1222.70
6o	−125.5372	−9.5317	−0.5122	3.833	−0.211	−0.226	0.309	0.316	0.030	4.27	1340.75
6p	−142.9569	−9.1470	−0.1975	1.250	−0.207	−0.273	0.317	0.376	0.004	1.21	1365.00
6q	−141.8359	−8.8631	−0.5033	5.966	−0.256	−0.199	0.326	0.323	−0.029	1.74	1435.58
6r	−178.2299	−9.0675	−0.9227	2.365	−0.251	−0.241	0.338	0.357	0.018	−1.37	1450.11
6s	−116.0515	−8.7587	−0.4117	1.615	−0.225	−0.231	0.296	0.330	0.007	5.27	1261.02

aforementioned findings [33,34], we report herein the synthesis of novel *N*,*N*′-diacylhydrazine derivatives, and their inhibitory activities against chitin synthesis of *Saccharomyces cerevisiae*. Furthermore, the semiempirical quantum chemical method and molecular modeling are also used to study the structure–activity relationships (SARs) of the synthesized compounds.

2. Results and discussion

2.1. Synthesis of substituted diacylhydrazine derivatives

The synthetic route of the designed diacylhydrazine derivatives **6a–s** is outlined in Scheme 3. The various synthesized benzoates **2** were treated with hydrazine hydrate in ethanol to afford the hydrazides **3**. The key synthetic steps involved a condensation reaction between hydrazides **3** and nonan-5-one followed by a selective reduction reaction of C=N double bond which was efficiently performed with a suspension of $NaBH_4$ in anhydrous THF. This scheme led exclusively to the key intermediates *N*′-alkylation products **5**. The following reaction of *N*′-(nonan-5-yl)hydrazides **5** with various substituted benzoyl chlorides under NEt_3 and catalytic amount of DMAP in CH_2Cl_2 for about 3–6 h afforded the target compounds diacylhydrazine derivatives **6a–s** in excellent yields of 64–80%.

2.2. Spectroscopy

The structures of synthesized compounds **6a–s** were confirmed by their IR, ^{1}H and ^{13}C NMR spectra and high-resolution electron impact mass spectra (HR-EIMS). The IR spectra of the diacylhydrazine derivatives **6a–s** exhibited distinctive characteristic absorptions bands at about 3200, and 1710–1620 cm^{-1}, which were assigned to the N–H bond, amido carbonyl, respectively. Additionally, the IR spectra of the compounds showed prominent peaks corresponding to the two carbonyl groups on each backbone. A higher frequency vibration appears at ca. 1680–1710 cm^{-1} in addition to a more intense peak in the range 1610–1650 cm^{-1}.

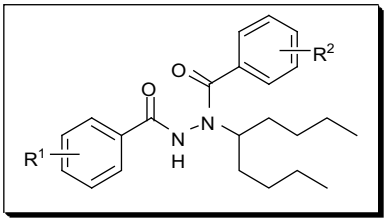
Their ^{1}H NMR spectra of compounds **6a–s** showed signals in the range 10–11 ppm assigned to the N–H group proton, the broad peaks that appeared at about 4.5 ppm in the ^{1}H NMR spectra of compounds diacylhydrazines were assigned to the methine proton attached to N atom. For compounds **6a–s**, the multiplet signals that appeared in their ^{1}H NMR spectra in the range 0.80–1.70 ppm were attributed to the aliphatic protons of the dibutyl chains. The ^{13}C NMR spectra of target compounds **6a–s** presented obvious four groups carbon signals at lower frequency 10–40 ppm, corresponding to the carbons of two aliphatic chains in the molecules. The carbons in the same chemical environment appeared in different signal peaks, which mainly due to the spatial configuration limitation of molecules. The representative ^{13}C NMR spectral analysis of target compound **6c** is shown in Fig. 3.

Due to the instability of the diacylhydrazine derivatives, the molecular ion peak in their mass spectra was of low intensity. The appearance of peaks for $[M\text{-}^nBu_2CH]^+$ shows that the C–N bond undergoes fission readily.

2.3. Biological activity

All the synthesized compounds were evaluated for their inhibition of chitin synthesis. The chitin biosynthesis inhibition activity assay was performed using yeast *S. cerevisiae* cell extracts, which were performed according to a modified procedure recently described by Lecuro et al. [35]. Fig. 4 showed the relative enzymatic activities in the presence of compounds **6a–s**.

Table 2
QSAR model test (where Obsvd. is experimental activity; Calcd. is predicted activity, and RES. is the residue of Calcd.).



Entry	Compd.	Substituents		Inhibition activity (*D*)		
		R^1	R^2	Obsvd.[a]	Calcd.[b]	RES.[c]
1	**6a**	*o*-Cl	H	−2.314	−2.356007	0.04200754
2	**6b**	*o*-Cl	*o*-MeO	−2.004	−2.240082	0.23608194
3	**6c**	*o*-Cl	3,5-diMe	−2.79	−2.799278	0.00927787
4	**6d**	*o*-Cl	*p*-F	−2.516	−2.35485	−0.1611497
5	**6e**	*o*-Cl	*m*-F	−2.691	−2.579079	−0.1119205
6	**6f**	*p*-Cl	3,5-diMe	−2.221	−2.550919	0.32991919
7	**6g**	*p*-Cl	*o*-MeO	−2.004	−2.462757	0.4587566
8	**6h**	*p*-Et	*o*-Cl	−2.649	−2.804733	0.15573287
9	**6i**	*p*-Et	3,5-diMe	−2.894	−2.534205	−0.3597951
10	**6j**	*p*-Et	*o*-MeO	−2.052	−2.170699	0.11869912
11	**6k**	*p*-Et	3,4-OCH_2O	−2.969	−2.483181	−0.4858195
12	**6l**	*p*-Et	$ClCH_2$–[d]	−3.317	−3.182347	−0.134653
13	**6m**	*p*-Et	2,3,5-F_3-4-MeO	−2.697	−2.480665	−0.2163351
14	**6n**	*m*-OMe	H	−3.286	−2.769355	−0.5166451
15	**6o**	2,4-Cl_2	3,5-diMe	−3.033	−3.140482	0.10748195
16	**6p**	3,4,5-$(MeO)_3$	*o*-Cl	−2.402	−3.218907	0.81690741
17	**6q**	3,4,5-$(MeO)_3$	3,5-diMe	−2.083	−2.082979	−2.05E-05
18	**6r**	3,4,5-$(MeO)_3$	2,3,5-F_3-4-MeO	−2.464	−2.459136	−0.0048639
19	**6s**	3,5-diMe	*p*-Et	−4.135	−3.851338	−0.2836621

[a] Inhibition activity at 100 μg/mL.
[b] Calculated activity by Eq. (1).
[c] RES is the residue of Calcd.
[d] The aromatic ring was replaced with chloromethyl.

As shown in Fig. 4, all the synthesized diacylhydrazine derivatives **6a**–**s** displayed moderate to good inhibition activities on chitin synthesis at 100 μg/mL concentration, especially compounds **6b**, **6g**, **6j** and **6q** exhibited higher inhibitory effect on enzymatic activity. In addition, we introduced various electron-withdrawing groups (such as halogen 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. 4, the different substituents at the periphery of the molecules **6a**–**s** can lead to the obviously different inhibition activities. Among all the compounds that containing electron-withdrawing groups (such as *ortho*-chloro) on the A-ring, it is obvious that compound **6b** containing *ortho*-methoxy group on the B-ring boasts the comparably highest potential activity, which leads to 81% inhibition. On the other hand, as to those compounds **6h**–**m** with electron-donating groups such as *para*-ethyl group bound to A-ring, compound **6j**, which contains methoxy group at the *ortho*-position of the B-ring, is the comparably most potent, which leads to 79% inhibition. Nevertheless, positional changes of the chloro-substituent within the A-ring (compounds **6a**–**g**) dramatically affect the inhibition activity, suggesting that the presence of a group in the *para*-position of aromatic ring (**6f** and **6g**) could introduce important electronic effects. Furthermore, we can find the presence of trimethoxyphenyl moiety (**6p**, **6q** and **6r**) in the molecules is more favorable for inhibition activities than the other alkyl- or alkoxy-substituted analogues, which may have a better lipid–water partition coefficient.

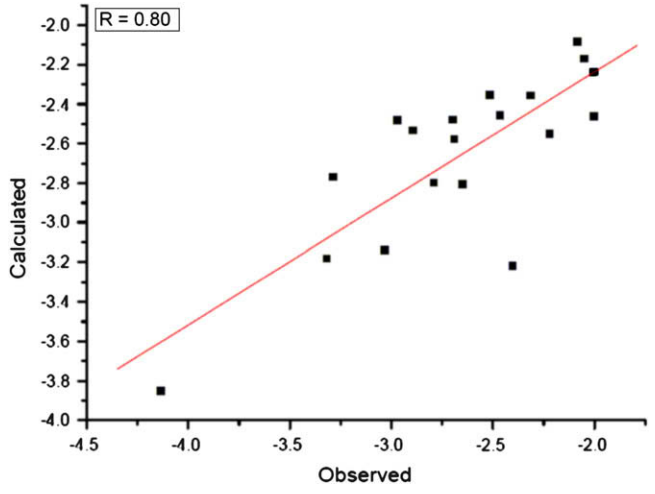


Fig. 5. Correlation between observed and calculated activities of compounds using regression model.

2.4. Quantitative structure–activity relationship analyses

To further explore the influential factors for the bioactivities of these compounds, a quantitative structure–activity relationship (QSAR) analysis was performed. Compounds **6a**–**s** showed different inhibitory activities against chitin synthesis. Results of the multiple regression analysis between structural parameters and their inhibitory activity are given below along with the statistical values. Of the combination of the parameters described in Table 1 as independent variables and the inhibitory activity data (*D*) (Table 2) as dependent parameters, Eq. (1) gave the best correlation.

$$D = -13.20077 + 0.02508(\text{Dipole})^2 + 31.36487 Q_{C3}$$

$$n = 19, r^2 = 0.64017, q^2 = 0.50250, \text{RMSE} = 0.31709 \qquad (1)$$

In this equation, n is the number of compounds, r^2 is the correlation coefficient, q^2 is the cross-validated squared correlation coefficient, RMSE is the root mean squared error.

Eq. (1) explains that the inhibitory activity against chitin synthesis of the tested compounds mainly depends on the molecular electronic properties (Dipole and Q_{C3}). It was found that the effects of these parameters on the activity descended in the order $Q_{C3} >$ Dipole. The coefficients of indicator parameters (Dipole and Q_{C3}) in Eq. (1) are positive. This indicates that the higher the value of Q_{C3} was, the better the activity against chitin synthesis was. The higher the value of dipole moment was, the higher the activity was. Unfortunately, from a statistic point of view, because of too many related variable parameters and less number of compounds, this limited the further QSAR analysis. However, our QSAR analysis revealed the essential structural requirements. The dipole moments of the molecules together with the net charge on C3 atom of compounds are strongly correlated with the activity, which might be used to predict the activity and provide insight into the designing novel chitin synthesis inhibitors. All descriptor data are listed in Table 1 and the inhibition activity values calculated by Eq. (1) are listed in Table 2.

The correlation between the calculated and experimental activity values of compounds **6a**–**s** is shown in Table 2 and Fig. 5. The calculated activities for **6a**–**s** by Eq. (1) were in good agreement with the observed activity. The equation showed small errors between the predicted activities and experimental activities according to the RES values.

3. Conclusions

In summary, we have described the molecular design, synthesis, and chitin synthesis inhibitory activities of a series of diacylhydrazine derivatives bearing nonan-5-yl moiety

replacement of *tert*-butyl group. The chitin synthesis assays indicate that all synthesized compounds exhibit moderate to good inhibition activity on chitin biosynthesis, especially, compounds **6b** and **6g** have significant activities toward chitin synthesis at low concentration (100 μg/mL), which may be developed as novel lead compounds and represent a novel class of highly potential inhibitors targeting chitin synthesis. Significant relations existed between the parameters including dipole moments, as well as net charge on C3 atom of molecules and the inhibitory activity against chitin synthesis. These correlations imply that electronic effects may play an important role in binding to the receptor. The understanding of quantitative structure–activity relationship against chitin synthesis may be advantageous for further structure optimization and thereby provides some insight into the rational design of new inhibitors of chitin synthesis.

4. Experimental section

4.1. Instrumentation and chemicals

All melting points (m.p.) were obtained with 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 ^{n}J are reported in Hz. High-resolution mass spectra (HRMS) 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 (silica gel 60 F254), and spots were visualized with ultraviolet (UV) light. All chemicals or reagents were purchased from standard commercial supplies and treated with standard methods before use. Solvents were dried in a routine way and redistilled.

4.2. General synthetic procedure for substituted acylhydrazines **5a–g**

The key synthetic intermediates **5a–g** were prepared following the literature methods [34] and their spectral and analytical data were also same as given in the literature except for the compound **5f**.

4.2.1. 3-Methoxy-N′-(nonan-5-yl)benzohydrazide (**5f**)

This compound was obtained following the above method as light yellow viscous liquid, yield 72%, ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.04–7.35 (m, 4H, Ph–H), 4.68 (s, 1H, NH), 3.85 (s, 3H, OCH_3), 2.92 (d, 1H, CH), 1.32–1.46 (m, 12H, CH_2), 0.92 (t, J = 7 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.08, 159.89, 134.37, 129.67, 118.57, 117.98, 112.26, 60.41, 55.42, 32.28, 27.84, 22.98, 14.03; MS: m/z = 292 (M^+), 235, 135, 107, 92, 77.

4.3. General synthetic procedure for diacylhydrazine derivatives **6a–s**

A mixture of intermediates acylhydrazines **5** (1 mmol) and triethylamine (1.5 mmol) and 4-dimethylaminopyridine (DMAP, 0.05 mmol) in anhydrous dichloromethane (10 mL) was stirred in an ice bath, and substituted benzoyl chloride (1.1 mmol) in dichloromethane (5 mL) was added. After stirring for 3 h at room temperature and another about 2–5 h at 35–40 °C, the reaction mixture was poured into water and extracted with dichloromethane. The combined organic layer was washed extensively with aqueous sodium bicarbonate, brine and water, and then dried over anhydrous sodium sulfate. The solvent was evaporated, and the residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate as eluent to afford the target compounds **6a–s**. Their physico-chemical properties and the spectral data are as follows.

4.3.1. N′-Benzoyl-2-chloro-N′-(nonan-5-yl)benzohydrazide (**6a**)

This compound was obtained following the above method as white solid, yield 72%, m.p. 113.4–114.7 °C; IR (KBr): ν = 3244 (N–H), 1703, 1617 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.61 (s, 1H, N–H), 7.23–7.50 (m, 8H, Ar–H), 6.39 (d, J = 7.6 Hz, 1H, Ar–H), 4.54 (br, 1H, CH), 1.18–1.80 (m, 12H, CH_2), 0.87 (t, J = 7 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 172.21, 165.50, 136.79, 135.01, 131.74, 130.07, 130.03, 129.92, 128.62, 128.04, 127.38, 127.34, 56.07, 32.11, 31.57, 28.94, 28.67, 22.73, 22.41, 14.45, 14.33; MS: m/z = 400 (M^+), 343, 274, 246, 203, 139, 105, 77; EI-HRMS: calcd for $C_{23}H_{29}ClN_2O_2$ (M^+), 400.1918; found, 400.1920.

4.3.2. 2-Chloro-N′-(2-methoxybenzoyl)-N′-(nonan-5-yl) benzohydrazide (**6b**)

This compound was obtained following the above method as white solid, yield 78%, m.p. 109.7–110.4 °C; IR (KBr): ν = 3185 (N–H), 1695, 1617 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.25 (s, 1H, N–H), 6.92–7.42 (m, 7H, Ar–H), 6.12 (d, J = 6.8 Hz, 1H, Ar–H), 4.55 (br, 1H, CH), 3.74 (s, 3H, OCH_3), 1.15–1.72 (m, 12H, CH_2), 0.88 (t, J = 7 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 170.43, 165.98, 135.58, 131.35, 130.47, 130.21, 130.03, 129.78, 128.43, 128.22, 127.12, 120.16, 55.93, 55.75, 32.44, 31.57, 28.97, 28.31, 22.75, 22.45, 14.60, 14.36; MS: m/z = 430 (M^+), 373, 304, 276, 191, 135; EI-HRMS: calcd for $C_{24}H_{31}ClN_2O_3$ (M^+), 430.2023; found, 430.2022.

4.3.3. N′-(2-Chlorobenzoyl)-3,5-dimethyl-N-(nonan-5-yl) benzohydrazide (**6c**)

This compound was obtained following the above method as white solid, yield 68%, m.p. 141.7–142.5 °C; IR (KBr): ν = 3200 (N–H), 1691, 1621 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.55 (s, 1H, N–H), 7.08–7.46 (m, 6H, Ar–H), 6.44 (d, J = 7.6 Hz, 1H, Ar–H), 4.53 (br, 1H, CH), 2.27 (s, 6H, Ph–CH_3), 1.27–1.56 (m, 12H, CH_2), 0.87 (t, J = 7 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 172.19, 165.52, 137.05, 136.49, 135.23, 131.69, 131.20, 130.27, 130.04, 128.53, 127.36, 125.19, 56.00, 32.14, 31.60, 28.93, 28.64, 22.73, 22.42, 21.21, 14.45, 14.34; MS: m/z = 428 (M^+), 371, 280, 237, 195, 133, 105, 79, 55; EI-HRMS: calcd for $C_{25}H_{33}ClN_2O_2$ (M^+), 428.2231; found, 428.2231.

4.3.4. 2-Chloro-N′-(4-fluorobenzoyl)-N′-(nonan-5-yl) benzohydrazide (**6d**)

This compound was obtained following the above method as white crystal, yield 82%, m.p. 145.2–145.8 °C; IR (KBr): ν = 3207 (N–H), 1703, 1691 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.65 (s, 1H, N–H), 7.21–7.54 (m, 7H, Ar–H), 6.61 (d, J = 7.6 Hz, 1H, Ar–H), 4.52 (br, 1H, CH), 1.39–1.67 (m, 12H, CH_2), 0.88 (t, J = 7 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 171.23, 165.56, 164.37, 161.92, 134.87, 133.07, 131.85, 130.34, 130.14, 130.03, 129.94, 128.74, 127.50, 115.15, 114.93, 56.30, 32.04, 31.54, 28.92, 28.66, 22.71, 22.40, 14.41, 14.31; MS: m/z = 418 (M^+), 361, 280, 237, 195, 139, 111; EI-HRMS: calcd for $C_{23}H_{28}ClFN_2O_2$ (M^+), 418.1823; found, 418.1822.

4.3.5. 2-Chloro-N′-(3-fluorobenzoyl)-N′-(nonan-5-yl) benzohydrazide (**6e**)

This compound was obtained following the above method as white solid, yield 76%, m.p. 125.3–126.8 °C; IR (KBr): ν = 3222

(N–H), 1703, 1617 (C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 10.68$ (s, 1H, N–H), 7.22–7.49 (m, 7H, Ar–H), 6.57 (d, $J = 7.2$ Hz, 1H, Ar–H), 4.52 (br, 1H, CH), 1.27–1.61 (m, 12H, CH_2), 0.87 (t, $J = 7$ Hz, 6H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 170.76$, 165.66, 165.56, 162.88, 160.46, 138.77, 138.70, 134.76, 134.70, 131.97, 131.34, 130.47, 130.39, 130.28, 130.18, 128.64, 127.52, 123.71, 117.03, 116.82, 114.42, 114.19, 56.33, 32.03, 31.51, 28.90, 28.63, 22.71, 22.40, 14.44, 14.33; MS: m/z = 418 (M^+), 361, 292, 221, 139, 123, 111, 95, 57; EI-HRMS: calcd for $C_{23}H_{28}ClFN_2O_2$ (M^+), 418.1823; found, 418.1823.

4.3.6. N′-*(4-Chlorobenzoyl)-3,5-dimethyl*-N-*(nonan-5-yl) benzohydrazide (**6f**)*

This compound was obtained following the above method as white solid, yield 71%, m.p. 136.2–137.7 °C; IR (KBr): $\nu = 3259$ (N–H), 1684, 1628 (C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 10.45$ (s, 1H, N–H), 6.95–7.91 (m, 7H, Ar–H), 4.53 (br, 1H, CH), 2.20 (s, 6H, Ph–CH_3), 1.28–1.62 (m, 12H, CH_2), 0.84 (t, $J = 6.8$ Hz, 6H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 172.26$, 165.82, 136.96, 136.85, 136.41, 132.03, 131.97, 131.24, 130.25, 129.58, 128.89, 125.04, 56.11, 31.90, 31.73, 28.97, 28.81, 22.71, 22.50, 21.16, 14.48, 14.33; MS: m/z = 428 (M^+), 371, 280, 237, 195, 133, 105, 79, 55; EI-HRMS: calcd for $C_{25}H_{33}ClN_2O_2$ (M^+), 428.2231; found, 428.2231.

4.3.7. N′-*(4-Chlorobenzoyl)-2-methoxy*-N-*(nonan-5-yl) benzohydrazide (**6g**)*

This compound was obtained following the above method as white solid, yield 73%, m.p. 132.2–134.4 °C; IR (KBr): $\nu = 3303$ (N–H), 1688, 1628 (C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 10.06$ (s, 1H, N–H), 6.79–7.94 (m, 8H, Ar–H), 4.55 (br, 1H, CH), 3.78 (s, 3H, OCH_3), 1.14–1.61 (m, 12H, CH_2), 0.85 (t, $J = 7.2$ Hz, 6H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 170.48$, 166.42, 137.09, 136.65, 132.25, 132.19, 131.99, 131.09, 130.50, 130.24, 129.62, 128.90, 128.64, 128.06, 56.00, 55.78, 32.22, 31.60, 29.00, 28.46, 22.73, 22.48, 14.63, 14.38; MS: m/z = 430 (M^+), 373, 304, 276, 233, 191, 135, 111, 92, 77; EI-HRMS: calcd for $C_{24}H_{31}ClN_2O_3$ (M^+), 430.2023; found, 430.2023.

4.3.8. 2-Chloro-N′-*(4-ethylbenzoyl)*-N-*(nonan-5-yl) benzohydrazide (**6h**)*

This compound was obtained following the above method as white solid, yield 68%, m.p. 92.3–93.7 °C; IR (KBr): $\nu = 3281$ (N–H), 1691, 1632 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.70$ (s, 1H, N–H), 7.53 (d, $J = 7.2$ Hz, 1H, Ph–H), 7.16–7.31 (m, 7H, Ph–H), 4.72–4.77 (m, 1H, CH), 2.65 (q, 2H, Ph–CH_2), 1.20–1.63 (m, 12H, CH_2), 1.21 (t, $J = 7.4$ Hz, 3H, Ph–$CH_2\underline{CH_3}$), 0.95 (t, $J = 7$ Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 170.24$, 166.27, 148.97, 136.12, 130.25, 129.63, 129.58, 128.37, 128.25, 127.07, 126.68, 56.94, 28.86, 28.75, 22.65, 15.14, 14.06; MS: m/z = 428 (M^+), 371, 280, 237, 133, 105, 90, 55; EI-HRMS: calcd for $C_{25}H_{33}ClN_2O_2$ (M^+), 428.2231; found, 428.2231.

4.3.9. N′-*(4-Ethylbenzoyl)-3,5-dimethyl*-N-*(nonan-5-yl) benzohydrazide (**6i**)*

This compound was obtained following the above method as white solid, yield 79%, m.p. 98.4–99.5 °C; IR (KBr): $\nu = 3288$ (N–H), 1684, 1628 (C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 10.30$ (s, 1H, N–H), 6.94–7.82 (m, 7H, Ar–H), 4.53 (br, 1H, CH), 2.59 (q, 2H, Ph–CH_2), 2.20 (s, 6H, Ph–CH_3), 1.27–1.63 (m, 12H, CH_2), 1.13 (t, $J = 7.6$ Hz, 3H, Ph–$CH_2\underline{CH_3}$), 0.89 (t, $J = 6.8$ Hz, 3H, CH_3), 0.84 (t, $J = 7.0$ Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 172.37$, 166.79, 148.25, 136.74, 136.60, 131.15, 130.86, 128.44, 128.07, 127.80, 125.12, 56.11, 31.91, 31.74, 28.99, 28.81, 28.47, 22.74, 22.52, 21.17, 15.78, 14.48, 14.34; MS: m/z = 422 (M^+), 365, 296, 274, 231, 189, 133, 105, 79, 57; EI-HRMS: calcd for $C_{27}H_{38}N_2O_2$ (M^+), 422.2933; found, 422.2933.

4.3.10. N′-*(4-Ethylbenzoyl)-2-methoxy*-N-*(nonan-5-yl) benzohydrazide (**6j**)*

This compound was obtained following the above method as white solid, yield 75%, m.p. 83.5–84.9 °C; IR (KBr): $\nu = 3303$ (N–H), 1684, 1632 (C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 9.89$ (s, 1H, N–H), 6.78–7.86 (m, 8H, Ar–H), 4.56 (br, 1H, CH), 3.79 (s, 3H, OCH_3), 2.56 (q, 2H, Ph–CH_2), 1.18–1.63 (m, 12H, CH_2), 1.11 (t, $J = 7.6$ Hz, 3H, Ph–$CH_2\underline{CH_3}$), 0.88 (t, $J = 9.0$ Hz, 6H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 170.57$, 167.23, 155.55, 147.94, 131.09, 130.40, 128.44, 128.09, 127.86, 126.84, 126.03, 120.68, 120.03, 111.76, 60.98, 55.79, 32.26, 31.61, 29.04, 28.83, 28.45, 22.63, 22.25, 15.80, 14.63, 14.43; MS: m/z = 424 (M^+), 367, 298, 276, 233, 191, 135, 105, 92, 77; EI-HRMS: calcd for $C_{26}H_{36}N_2O_3$ (M^+), 424.2726; found, 424.2726.

4.3.11. N′-*(4-Ethylbenzoyl)*-N-*(nonan-5-yl)benzo[*d*][1,3]dioxole-5-carbohydrazide (**6k**)*

This compound was obtained following the above method as white solid, yield 58%, m.p. 134.8–136.2 °C; IR (KBr): $\nu = 3192$ (N–H), 1691, 1669 (C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 10.34$ (s, 1H, N–H), 6.83–7.50 (m, 7H, Ar–H), 5.98 (s, 2H, OCH_2O), 4.49 (br, 1H, CH), 2.61 (q, 2H, Ph–CH_2), 1.26–1.62 (m, 12H, CH_2), 1.15 (t, $J = 7.6$ Hz, 3H, Ph–$CH_2\underline{CH_3}$), 0.85 (t, $J = 7.2$ Hz, 6H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 171.33$, 166.51, 148.56, 148.41, 146.67, 130.63, 130.25, 128.19, 127.91, 122.23, 108.19, 107.83, 101.70, 56.35, 31.79, 29.02, 28.83, 28.50, 22.72, 22.52, 15.78, 14.48, 14.34; MS: m/z = 438 (M^+), 381, 355, 281, 231, 207, 149, 133, 121, 105; EI-HRMS: calcd for $C_{26}H_{34}N_2O_4$ (M^+), 438.2519; found, 438.2520.

4.3.12. N′-*(2-chloroacetyl)-4-ethyl*-N′-*(nonan-5-yl)benzohydrazide (**6l**)*

This compound was obtained following the above method as white solid, yield 82%, m.p. 152.1–152.9 °C; IR (KBr): $\nu = 3244$ (N–H), 1688, 1643 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.75$ (d, $J = 8$ Hz, 2H, Ar–H), 7.69 (s, 1H, N–H), 7.35 (d, $J = 8$ Hz, 2H, Ar–H), 4.63 (br, 1H, CH), 4.08 (q, 2H, $COCH_2$), 2.75 (q, 2H, Ph–CH_2), 1.33–1.60 (m, 12H, CH_2), 1.28 (t, $J = 7.6$ Hz, 3H, Ph–$CH_2\underline{CH_3}$), 0.91 (t, $J = 6.4$ Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 168.81$, 166.52, 149.91, 129.01, 128.63, 127.34, 56.98, 41.98, 32.16, 31.41, 28.89, 28.64, 28.45, 22.63, 22.47, 15.23, 13.97; MS: m/z = 330 ($[M-Cl]^+$), 273, 217, 204, 190, 159, 133, 105; ESI-HRMS: calcd for $C_{20}H_{31}ClN_2O_2$ ($[M+H]^+$), 367.2152; found, 367.2151.

4.3.13. N′-*(4-Ethylbenzoyl)-2,3,5-trifluoro-4-methoxy*-N-*(nonan-5-yl)benzohydrazide (**6m**)*

This compound was obtained following the above method as white solid, yield 67%, m.p. 117.0–118.4 °C; IR (KBr): $\nu = 3274$ (N–H), 1684, 1636 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.67$ (s, 1H, N–H), 7.43 (d, $J = 8.4$ Hz, 2H, Ph–H), 7.24 (d, $J = 8$ Hz, 2H, Ph–H), 7.12–7.18 (m, 1H, Ph–H), 4.70–4.74 (m, 1H, CH), 3.91 (s, 3H, OCH_3), 2.68 (q, 2H, Ph–CH_2), 1.21–1.63 (m, 12H, CH_2), 1.23 (t, $J = 7.6$ Hz, 3H, Ph–$CH_2\underline{CH_3}$), 0.94 (t, $J = 7$ Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 166.67$, 165.89, 149.57, 129.03, 128.49, 126.78, 111.16, 110.91, 62.09, 57.35, 32.31, 31.90, 31.09, 28.81, 22.50, 15.20, 14.00; MS: m/z = 478 (M^+), 421, 330, 287, 245, 214, 189, 133, 105, 79; EI-HRMS: calcd for $C_{26}H_{33}F_3N_2O_3$ (M^+), 478.2443; found, 478.2443.

4.3.14. N′-*Benzoyl-3-methoxy*-N′-*(nonan-5-yl)benzohydrazide (**6n**)*

This compound was obtained following the above method as white solid, yield 64%, m.p. 89.4–90.2 °C; IR (KBr): $\nu = 3281$ (N–H), 1684, 1613 (C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 10.39$ (s, 1H, N–H), 6.88–7.95 (m, 9H, Ar–H), 4.53 (br, 1H, CH), 3.72 (s, 3H, OCH_3), 1.14–1.64 (m, 12H, CH_2), 0.87 (t, $J = 9.6$ Hz, 6H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 167.66$, 159.38, 136.79, 134.58, 133.32, 131.13, 129.99, 129.69, 129.01, 127.88, 127.26, 119.82, 117.52,

113.11, 56.17, 55.73, 31.87, 31.71, 28.97, 28.81, 22.72, 22.50, 14.47, 14.34; MS: m/z = 396 (M^+), 339, 276, 191, 135, 105, 92, 77; EI-HRMS: calcd for $C_{24}H_{32}N_2O_3$ (M^+), 396.2413; found, 396.2413.

4.3.15. 2,4-Dichloro-N′-(3,5-dimethylbenzoyl)-N′-(nonan-5-yl) benzohydrazide (**6o**)

This compound was obtained following the above method as white solid, yield 63%, m.p. 120.5–121.8 °C; IR (KBr): ν = 3185 (N–H), 1680, 1625 (C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 10.58 (s, 1H, N–H), 7.06–7.66 (m, 5H, Ar–H), 6.46 (d, J = 8.4 Hz, 1H, Ar–H), 4.52 (br, 1H, CH), 2.27 (s, 6H, Ar–CH_3), 1.14–1.60 (m, 12H, CH_2), 0.86 (t, J = 6 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 172.14, 165.42, 137.12, 136.39, 135.58, 131.55, 131.27, 129.83, 129.67, 127.72, 125.12, 55.98, 32.13, 31.58, 28.90, 28.63, 22.71, 22.40, 21.21, 14.44, 14.33; MS: m/z = 464 (M^+), 405, 336, 274, 231, 207, 189, 173, 133, 105; EI-HRMS: calcd for $C_{25}H_{32}Cl_2N_2O_2$ (M^+), 462.1841; found, 462.1841.

4.3.16. N′-(2-Chlorobenzoyl)-3,4,5-trimethoxy-N′-(nonan-5-yl) benzohydrazide (**6p**)

This compound was obtained following the above method as white solid, yield 77%, m.p. 74.3–76.8 °C; IR (KBr): ν = 3244 (N–H), 1688, 1632 (C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 10.23 (s, 1H, N–H), 7.27–7.51 (m, 4H, Ph–H), 6.54 (s, 2H, Ph–H), 4.55 (br, 1H, CH), 3.72 (s, 6H, OCH_3), 3.63 (s, 3H, OCH_3), 1.49–1.66 (m, 4H, CH_2), 1.18–1.35 (m, 8H, CH_2), 0.88 (t, J = 7 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 169.54, 166.54, 153.06, 152.83, 140.61, 130.76, 128.59, 128.35, 126.87, 123.78, 105.97, 105.33, 60.49, 56.52, 56.43, 32.12, 31.57, 29.07, 28.75, 22.71, 22.44, 14.45, 14.25; MS: m/z = 490 (M^+), 433, 364, 346, 331, 277, 224, 211, 195, 167, 152, 125, 81; EI-HRMS: calcd for $C_{26}H_{35}ClN_2O_5$ (M^+), 490.2235; found, 490.2234.

4.3.17. N′-(3,5-Dimethylbenzoyl)-3,4,5-trimethoxy-N′-(nona n-5-yl)benzohydrazide (**6q**)

This compound was obtained following the above method as white solid, yield 74%, m.p. 131.2–132.7 °C; IR (KBr): ν = 3281 (N–H), 1680, 1628 (C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 10.23 (s, 1H, N–H), 6.74–7.10 (m, 5H, Ar–H), 4.53 (br, 1H, CH), 3.75 (s, 6H, OCH_3), 3.65 (s, 3H, OCH_3), 2.22 (s, 6H, Ph–CH_3), 1.10–1.61 (m, 12H, CH_2), 0.88 (t, J = 6.6 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 172.40, 166.58, 153.00, 140.84, 136.92, 136.73, 131.18, 128.63, 124.93, 105.36, 60.54, 56.53, 56.20, 31.86, 29.08, 28.84, 22.71, 22.51, 21.21, 14.41; MS: m/z = 484 (M^+), 427, 340, 325, 211, 195, 133, 105, 79, 55; EI-HRMS: calcd for $C_{28}H_{40}N_2O_5$ (M^+), 484.2937; found, 484.2938.

4.3.18. 2,3,5-Trifluoro-4-methoxy-N-(nonan-5-yl)-N′-(3,4,5-trimethoxybenzoyl)benzohydrazide (**6r**)

This compound was obtained following the above method as white solid, yield 71%, m.p. 91.6–93.8 °C; IR (KBr): ν = 3288 (N–H), 1695, 1636 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.61 (s, 1H, N–H), 7.11–7.18 (m, 1H, Ph–H), 6.67 (s, 2H, Ph–H), 4.70 (br, 1H, CH), 3.96 (s, 3H, OCH_3), 3.87 (s, 9H, OCH_3), 1.32–1.70 (m, 12H, CH_2), 0.95 (t, J = 7 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.47, 165.86, 153.55, 141.91, 127.02, 111.18, 110.98, 104.09, 62.19, 60.91, 57.40, 56.34, 32.35, 28.57, 22.45, 14.03; MS: m/z = 540 (M^+), 483, 414, 383, 274, 195, 189, 152; EI-HRMS: calcd for $C_{27}H_{35}F_3N_2O_6$ (M^+), 540.2447; found, 540.2447.

4.3.19. N′-(4-Ethylbenzoyl)-3,5-dimethyl-N′-(nonan-5-yl) benzohydrazide (**6s**)

This compound was obtained following the above method as white solid, yield 80%, m.p. 167.2–168.0 °C; IR (KBr): ν = 3251 (N–H), 1684, 1628 (C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 10.34 (s, 1H, N–H), 7.41 (d, J = 8 Hz, 2H, Ph–H), 7.14 (d, J = 7.6 Hz, 2H, Ph–H), 7.10 (s, 1H, Ph–H), 7.02 (s, 2H, Ph–H), 4.52 (br, 1H, CH), 2.55 (q, 2H, Ph–CH_2), 2.23 (s, 6H, Ph–CH_3), 1.27–1.63 (m, 12H, CH_2), 1.10 (t, J = 7.6 Hz, 3H, Ph–CH_2CH_3), 0.89 (t, J = 7.2 Hz, 3H, CH_3), 0.84 (t, J = 7.2 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 172.25, 167.06, 137.86, 134.19, 133.43, 133.20, 127.52, 127.21, 125.34, 56.03, 31.87, 31.68, 29.02, 28.84, 28.41, 22.76, 22.50, 21.18, 15.89, 14.51, 14.40; MS: m/z = 422 (M^+), 365, 296, 274, 250, 189, 155, 133, 105, 91, 79; EI-HRMS: calcd for $C_{27}H_{38}N_2O_2$ (M^+), 422.2933; found, 422.2933.

4.4. Biology assay

For the chitin synthesis inhibition studies, enzymatic assays were performed using yeast cell extracts, which were performed according to a modified procedure recently described by Lecuro et al. [35]. The yeast *S. cerevisiae* were cultured overnight with shaking in YPD (1% yeast extract, 2% peptone, 2% glucose) at 30 °C, and then the cells were harvested by centrifugation at 1500 × *g* for 10 min at 4 °C. Cells were disrupted by grinding method with glass beads under liquid nitrogen condition. The inhibition activity was expressed in terms of *D* by the formula [36].

$$D = \log[a/(100 - a)] - \log M_w$$

where *a* indicates the percentage of inhibition and M_w is the molecular weight of the tested compounds.

4.5. Descriptor variables

The molecular total energy (E_T), the energy of the highest occupied molecular orbital (E_{HOMO}), the energy of the lowest unoccupied molecular orbital (E_{LUMO}), the net charges on N and C atoms (Q_N, and Q_C), the volume *V*, the dipole moment (Dipole), and the molecular hydrophobic parameter (log *P*) were calculated with Hyperchem Software (2002 edition) of Hypercube, Inc. Before all parameters of a compound were calculated, its spatial molecular conformation was also optimized with Hyperchem to acquire its most relaxed conformation. All descriptor data are listed in Table 1.

4.6. QSAR analyses

The QSAR modeling was operated on a Silicon Graphics workstation running on the IRIX 6.5 operating system which had a Cerius2 (version 4.8) software package of Accelrys [37,38]. QSAR equation was acquired according to different combinations of various descriptors such as E_T, E_{HOMO}, E_{LUMO}, Q_N, Q_C, *V*, Dipole and log *P* as independent parameters, and the inhibitory activity data (*D*) as dependent parameters. The data matrix was analyzed with the partial least squares (PLS) method. The quality of each regression model was evaluated using a squared correlation coefficient (r^2), cross-validation squared correlation coefficient (q^2), and root mean squared error (RMSE). The cross-validation squared correlation coefficient is a measure of the predictive ability of the equation.

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