Full Paper

Design and Synthesis of Novel Pyrazino[2,1-*a*]isoquinoline Derivatives with Potent Antifungal Activity

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A series of novel pyrazino[2,1-*a*]isoquinoline compounds were designed, synthesized, and their antifungal activities *in vitro* were evaluated. The results showed that all of the title compounds exhibited antifungal activities. Most of them exhibited stronger antifungal activities than the lead compounds; compound **7c** is more potent than fluconazole against two of the three tested fungal strains. The studies presented here provide a new structural type for the development of novel antifungal agents.

Keywords: Antifungal / Lanosterol 14α-demethylase / Pyrazino[2,1-*a*]isoquinoline / Synthesis

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Introduction

During the past decades, the incidence of systemic fungal infections has increased dramatically and became a major cause of morbidity and mortality in immune compromised individuals such as patients undergoing anticancer chemotherapy or organ transplants and patients with AIDS [1–4]. Although the arsenal of antifungal drugs has expanded, currently available antifungal drugs do not meet the increasing requirements of managing infection in the complex patient population. Therefore, the development of new antifungal drugs is an ongoing necessity in clinical therapy.

Lanosterol 14α-demethylase (CYP51) is one of the key enzymes of sterol biosynthesis in fungi and also a prime target for the development of antifungal drugs. Azole antifungals, such as fluconazole [5] and voriconazole [6] (Fig. 1), are the CYP51 inhibitors widely used in the clinical antifungal therapy, which exert antifungal activity through inhibiting the lanosterol 14α-demethylase (CYP51) of fungi by a mechanism in which the heterocyclic nitrogen atom [1] (N-3 of imidazole and N-4 of 1,2,4-triazole) binds to the sixth coordination position of the heme iron atom of the prophyrin in the substrate-binding site of the enzyme [7–9]. Unfortunately, the broad use of azoles has led to development of severe resistance, which significantly reduced their efficacy [10, 11]. Thus, the search for new non-azole CYP51 inhibitors is meaningful. In recent years, we focused on the search of non-azole CYP51 inhibitors. In our previous studies [12–13], we reported the design of non-azole lead molecules with a tetrahydroisoquinoline scaffold, based on the constructed three-dimensional model of *Candida albicans*

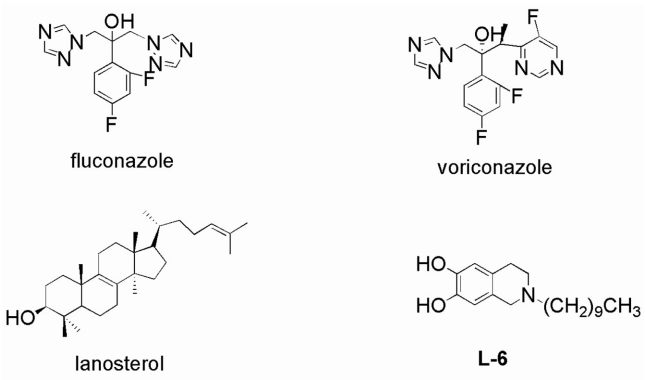

Figure 1. Some CYP51 inhibitors: Azole antifungals, lanosterol, and compound **L-6**.

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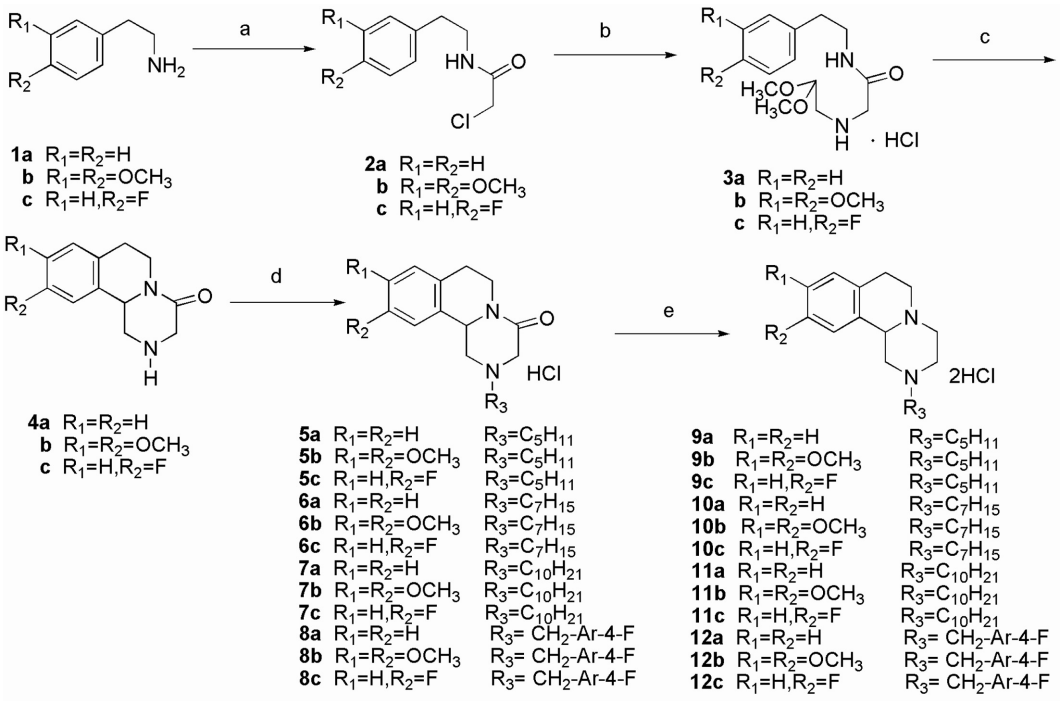
Abbreviation: Lanosterol 14α-demethylase from *Candida albicans* (CA-CYP51)



Reagents: a) Chloroacetyl chloride, $NaHCO_3$, CH_2Cl_2, rt; b) 2,2-dimethoxyethanamine toluene, reflux; c) H_2SO_4, CH_2Cl_2; d) RX, K_2CO_3/KI; e) $LiAlH_4$, THF.

Scheme 1. Synthetic routes for pyrazino[2,1-*a*]isoquinoline derivatives **5a–8c** and **9a–12c**.

CYP51 (CA-CYP51). The binding study showed that the tetrahydroisoquinoline scaffold was located on the substrate-binding site of fungal CYP51, and the affinity of the lead molecules for CYP51 was mainly attributed to their non-bonding interaction with the residues of the apoprotein, no binding with the heme, which was different from that of azole antifungals. **L-6** is the most potent compound discovered (Fig. 1). Considering lanosterol – the natural substrate for CYP51 with a four-ring skeleton in its structure – a possible increase in affinity for CYP51 and antifungal activities of the target compounds is expected by expanding the tetrahydroisoquinoline scaffold of the lead molecules. Therefore, novel pyrazino[2,1-*a*]isoquinoline derivatives were designed (Fig. 2), and their antifungal activities *in vitro* were evaluated.

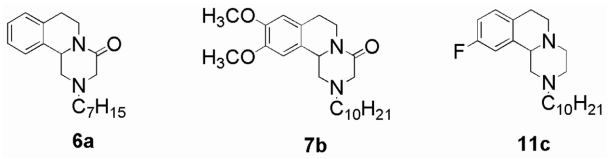


Figure 2. Novel pyrazino[2,1-*a*]isoquinoline derivatives.

Results and discussion

Chemistry

The synthetic routes of compounds **5a–8c** and **9a–12c** are reported in Scheme 1. Treatment of chloroacetyl chloride with several arylethylamines afforded compounds **2a–c**. The aminoalkylation reaction of the readily available 2,2-dimethoxyethanamine with **2a–c** gave the amine derivatives **3a–c**, which were isolated as hydrochloride salts without using column chromatography. Treatment with concentrated H_2SO_4 provided the key intermediates **4a–c** [14, 15]. Intermediates **4a–c** were refluxed with different halides to provide the target compounds **5a–8c**. Reduction of **5a–8c** with $LiAlH_4$ in THF yields the target compounds **9a–12c**. The structures of the synthesized compounds were characterized by elemental analysis and spectral data (IR, MS, and ^{1}H-NMR). The spectral analyses were consistent with the assigned structures, and spectral data are listed in the experimental section.

Binding mode of the pyrazino[2,1-*a*]isoquinoline

The binding mode of the compounds was validated by flexible molecular docking (Affinity module within InsightII 2000 software package [16]). The active site of CYP51 had been constructed in the previous studies [11, 12]. We found that the regions in the active site of CYP51 for non-covalent ligand binding can be divided into four subsites, S1–S4, next to the site coordinating with the heme. The S1 subsite is a hydrophilic hydrogen-bonding region; the S2 subsite is a hydrophobic region; the S3 sub-

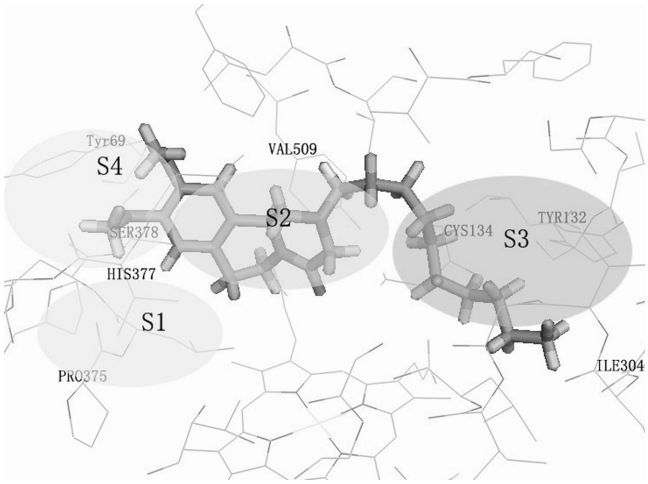


Figure 3. Docking conformation of compounds **7b** in the active site of CA-CYP51.

site is a narrow hydrophobic cleft formed by the residues in the helix B′-meander1 loop and the N terminus of helix I, and the S4 subsite is another hydrogen-bonding region in the active site. Figure 3 shows the docking conformation of compounds **7b** in the active site of CA-CYP51. The pyrazino[2,1-*a*]isoquinoline ring interacts with the S2 subsite. The methoxy group in the benzene ring forms H-bonding interactions with the residues Tyr69 and Ser378 in the S4 subsite. The long lipophilic side chain was oriented into the S3 pocket. No interaction was found between the compound and the heme. This suggests that the affinity of pyrazino[2,1-*a*]isoquinoline derivatives to the potential receptor CYP51 might be mainly attributed to their non-bonding interaction with the apoprotein part of the active site.

In-vitro antifungal activities

All compounds were evaluated for their antifungal activities *in vitro* by the standard broth microdilution method of the NCCLS [17]. The tested fungi species included three pathogenic fungi: dermatomycoses (*Trichophyton rubrum*) and systemic mycoses (*Candida albicans* and *Cryptococcus neoformans*). The antifungal activities of all compounds are listed in Table 1; fluconazole and **L-6** were used as controls.

All of the title compounds exhibit potent antifungal activities against three tested fungal strains. Compounds **6c**, **7a–c**, **10c**, and **11a–c** exhibit a higher antifungal activity than **L-6** against the test organisms. Compounds **6a**, **7a–c**, **9a**, **10a**, **10c**, and **11b–c** showed a stronger antifungal activity against *T. rubrum* than that of control drug fluconazole. The antifungal activities of compounds **5a–c** and **12a–c** are very low. That is probably because the N-substituted alkyl or the aromatic side chains (**5a–c** or **12a–c**, respectively) are too short to interact firmly with the hydrophobic S3 region. The S4 subsite is a hydrogen-bond donor and acceptor region, which interacts with the functional groups on the phenyl group of the isoquinoline ring. The compounds with a fluoro or methoxy group at positions 9 or 10 in the aromatic ring gave better results than those of none substituents in the aromatic ring. Compounds **7a–c** were the most potent compounds against all tested strains and exhibited much stronger activities than the compounds with other alkyl group, which suggests that the appropriate length of the substituents on the amino group of target derivatives is important for antifungal activities.

Table 1. *In-vitro* antifungal activity of compounds **5a–12c**.

Compound	MIC (μg/mL)		
	C. alb.[§]	C. neo.[&]	T. rub.[#]
5a	>64	16	>64
5b	>64	16	64
5c	64	8	32
6a	32	16	32
6b	32	16	16
6c	32	8	16
7a	32	4	16
7b	16	8	8
7c	8	4	8
8a	>64	64	64
8b	>64	32	64
8c	>64	64	32
9a	64	4	8
9b	64	>64	64
9c	64	32	>64
10a	32	16	16
10b	32	8	32
10c	32	16	16
11a	32	8	16
11b	16	4	8
11c	8	4	8
12a	>64	>64	64
12b	>64	64	32
12c	>64	64	32
L-6	64	16	32
Fluconazole	2	4	32

§ *C. alb.*: *Candida albicans*; & *C. neo.*: *Cryptococcus neoformans*; # *T. rub.*: *Trichophyton rubrum*.

Conclusion

In conclusion, a series of novel pyrazino[2,1-*a*]isoquinoline compounds were prepared and evaluated for antifungal activity *in vitro*. The results showed that most of the target compounds exhibited more potent antifungal activities than the lead compound **L-6**, in which com-

pound **7c** is more potent than fluconazole against two of the three testing fungal strains. The affinity of the molecules for CYP51 was mainly attributed to their non-bonding interaction with the residues of the apoprotein – without binding with the heme – which was different from that of azole antifungals. The studies presented here may provide a new structural type for the development of novel antifungal agents.

Experimental

Chemistry

Melting points were determined on an electrically heated RK-Z melting point apparatus (Analytical instrument factory in Tianjin, China) and are uncorrected. Mass spectra (MS) were measured on a Micromass QTof-Micro LC-MS instrument (Waters-Micromass). ^{1}H-NMR spectra were recorded at 300 MHz on a Bruker AC-300P spectrometer (Bruker Bioscience, USA) with Me_4Si as the internal standard. Chemical shifts are given in ppm (δ), and the spectral data are consistent with the assigned structures. Elemental analyses were performed with a MOD-1106 instrument (Carl Erba, Italy). Commercial solvents and reagents were of reagent grade.

*General procedure of preparation of N-2-(substituted phenyl)ethyl chloroacetamide **2a–c***

Chloroacetamides **2a–c** were obtained by a slight modification of the known procedure [11] as follows: To a mixture of substituted phenethylamine (0.1 mol) and $NaHCO_3$ (0.1 mol) in CH_2Cl_2 (200 mL) chloroacetyl chloride was added dropwise (0.12 mol) at 0°C. After stirring at 10°C for 2 h, the reaction mixture was quenched by the slow addition of water at 0°C. The organic layer was separated and washed with 10% aqueous HCl solution and brine. After evaporation of the solvent, the resulting solid was recrystallized with EtOH to afford **2a** as a light-colored needle (yield 96.6%); m.p.: 61.7 to 64.9°C (lit. m.p.: 67°C); **2b** (81.9%): m.p.: 93.6 to 95.2°C (lit. m.p.: 96°C); **2c** (79.3%): m.p.: 113.4 to 115.7°C.

*General procedure of the preparation of N-2-(substituted phenyl)ethyl-2-[(2,2-dimethoxyethyl)amino]acetamide hydrochloride **3a–c***

To a solution of **2a–c** (0.08 mol) in toluene (80 mL) 2,2-dimethoxyethanamine (0.167 mol) was added, and the mixture was heated to reflux. After cooling of the mixture, the resulting solid was recovered by filtration. The filtrate was washed with water, dried, and concentrated to give **3a–c**. This product was diluted with CH_2Cl_2 (50 mL) and treated with HCl gas at −30°C. The resulting solid was filtered and dried to afford **3a** hydrochloride in 73.8% yield as a white solid; m.p.: 151.5°C (lit. m.p.: 152 to 152.5°C); **3b** (77.7%): m.p.: 96.2 to 99.5°C (lit. m.p.: 98 to 99°C); **3c** (70.3%): m.p.: 166 to 167.5°C.

*General procedure of the preparation of key intermediates **4a–c***

Compound **3a–c** hydrochloride (12.1 mmol) was added in small portions to a solution of conc. H_2SO_4 (4 mL) at 5°C. After stirring for 3.5 h, the reaction mixture was poured into ice-water and adjusted to pH = 12 with 20% aqueous NaOH solution with cooling. The solution was extracted with CH_2Cl_2. The CH_2Cl_2 solution was washed with brine, dried, and concentrated to afford **4a** as a white solid. Yield 81.4%; m.p.: 116.8 to 118.4°C (lit. m.p.: 118 to 119°C); **4b** (77.6%): m.p.: 137.1 to 137.8°C (lit. m.p.: 136 to 137°C); **4c** (93%): m.p.: 116.7 to 119.8°C.

*General procedure of the preparation of **5a–8c***

To a refluxing mixture of intermediate **4** (5 mmol) and K_2CO_3 (5 mmol) in acetone, 6 mmol of bromo-compounds were added. After the mixture was heated to reflux for 6 h, the reaction mixture was treated with water and extracted with Et_2O three times. The Et_2O solution was dried and concentrated. The residue was treated with 1 M HCl and recrystallized with EtOH and Et_2O to afford the target compounds **5a–8c**.

*2-Pentyl-2,3,6,7-tetrahydro-1H-pyrazino[2,1-a] isoquinolin-4(11bH)-one **5a***

White solid; yield: 85.3%; m.p.: 251.2°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.92–0.95 (t, *J* = 6 Hz, 3H, CH_3), 1.37–1.45 (m, 4H, CH_2), 1.66–1.85 (m, 2H, CH_2), 2.84–3.22 (t, 4H, CH_2), 3.24–3.50 (t, 2H, CH_2), 4.02–4.31 (t, 2H, CH_2), 4.32–4.41, 4.51–4.78 (dd, 2H, CH_2), 5.82–5.91 (t, 1H, CH), 7.18–7.20 (m, 1H, Ar-H), 7.28–7.35 (t, *J* = 9 Hz, 3H, Ar-H); MS (ESI) *m/z*: 273.4 [M + H]. Anal. calcd. for $C_{17}H_{24}N_2O \cdot HCl$: C, 66.11; H, 8.16; N, 9.07. Found: C, 66.10; H, 8.04; N, 9.13.

*2-Pentyl-2,3,6,7-tetrahydro-9,10-dimethoxy-1H-pyrazino[2,1-a]isoquinolin-4(11bH)-one **5b***

White solid; yield: 88.5%; m.p.: 250.1°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.91–0.95 (t, *J* = 6 Hz, 3H, CH_3), 1.37–1.45 (m, 4H, CH_2), 1.81–2.03 (m, 2H, CH_2), 2.64–2.78 (t, 2H, CH_2), 3.07–3.44 (t, 4H, CH_2), 3.85 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 4.14–4.32 (m, 2H, CH_2), 4.78 (m, 1H, CH), 5.64–5.74 (dd, *J* = 9.3 Hz, 1H, Ar-H), 6.61 (s, 1H, Ar-H), 6.76 (s, 1H, Ar-H); MS (ESI) *m/z*: 333.4 [M + H]. Anal. calcd. for $C_{19}H_{28}N_2O_3 \cdot HCl$: C, 61.86; H, 7.92; N, 7.59. Found: C, 61.79; H, 8.01; N, 7.63.

*2-Pentyl-2,3,6,7-tetrahydro-10-fluoro-1H-pyrazino[2,1-a] isoquinolin-4(11bH)-one **5c***

Yellow solid; yield: 79.2%; m.p.: 260.3°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.94–1.00 (t, *J* = 6.5 Hz, 3H, CH_3), 1.36–1.91 (m, 6H, CH_2), 2.81–2.94 (t, 2H, CH_2), 3.01–3.07 (m, 2H, CH_2), 3.13–3.24 (t, 2H, CH_2), 3.42–4.07 (m, 2H, CH_2), 4.21–4.27, 4.71–4.81 (m, 2H, CH_2), 5.71–5.85 (m, 1H, CH), 6.93–7.07 (m, 1H, Ar-H), 7.18–7.21(m, 2H, Ar-H); MS (ESI) *m/z*: 291.4 [M + H]. Anal. calcd. for $C_{17}H_{23}FN_2O \cdot HCl$: C, 62.47; H, 7.40; N, 8.57. Found: C, 61.54; H, 7.31; N, 8.44.

*2-Heptyl-2,3,6,7-tetrahydro-1H-pyrazino[2,1-a] isoquinolin-4(11bH)-one **6a***

White solid; yield: 84.5%; m.p.: 261.7°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.90–0.94 (t, *J* = 6 Hz, 3H, CH_3), 1.29–1.47 (m, 8H, CH_2), 1.81–2.09 (m, 2H, CH_2), 2.84–3.20 (t, 4H, CH_2), 3.25–3.48 (t, 2H, CH_2), 4.03–4.33 (t, 2H, CH_2), 4.05–4.11, 4.81–4.85 (dd, 2H, CH_2), 5.80–5.87 (t, 1H, CH), 7.14–7.19 (m, 1H, Ar-H), 7.23–7.29 (t, *J* = 9 Hz, 3H, Ar-H); MS (ESI) *m/z*: 301.4 [M + H]. Anal. calcd. for

$C_{19}H_{28}N_2O \cdot HCl$: C, 67.74; H, 8.68; N, 8.32. Found: C, 67.68; H, 8.73; N, 8.30.

2-Heptyl-2,3,6,7-tetrahydro-9,10-dimethoxy-1H-pyrazino[2,1-a]isoquinolin-4(11bH)-one ***6b***

White solid; yield: 89.5%; m.p.: 260.4°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.85–0.95 (t, *J* = 6 Hz, 3H, CH_3), 1.22–1.41 (m, 8H, CH_2), 1.73–2.08 (m, 2H, CH_2), 2.69–2.94 (t, 4H, CH_2), 3.14–3.33 (t, 2H, CH_2), 3.87 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 4.13–4.19 (m, 2H, CH_2), 4.85 (m, 2H, CH_2), 5.72–5.76 (dd, *J* = 9.3 Hz, 1H, CH), 6.64 (s, 1H, Ar-H), 6.71 (s, 1H, Ar-H); MS (ESI) *m/z*: 361.5 [M + H]. Anal. calcd. for $C_{21}H_{32}N_2O_3 \cdot HCl$: C, 63.54; H, 8.38; N, 7.06. Found: C, 61.97; H, 8.21; N, 7.03.

2-Heptyl-2,3,6,7-tetrahydro-10-fluoro-1H-pyrazino[2,1-a]isoquinolin-4(11bH)-one ***6c***

Yellow solid; yield: 79.9%; m.p.: 270.6°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.86–0.96 (t, *J* = 6.5 Hz, 3H, CH_3), 1.25–1.42 (m, 8H, CH_2), 1.87–2.03 (t, 2H, CH_2), 2.80–3.05 (t, 4H, CH_2), 2.99–3.08 (m, 2H, CH_2), 3.42–3.49 (m, 2H, CH_2), 4.00–4.24 (m, 2H, CH_2), 5.81–5.85 (m, 1H, CH), 6.94–7.00 (m, 2H, Ar-H), 7.17–7.19 (m, 1H, Ar-H); MS (ESI) *m/z*: 319.4 [M + H]. Anal. calcd. for $C_{19}H_{27}FN_2O \cdot HCl$: C, 64.30; H, 7.95; N, 7.89. Found: C, 64.25; H, 7.81; N, 7.74.

2-Decyl-2,3,6,7-tetrahydro-1H-pyrazino[2,1-a]isoquinolin-4(11bH)-one ***7a***

White solid; yield: 82.9%; m. p.: 275.6°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.83–0.87 (t, *J* = 6.9 Hz, 3H, CH_3), 1.23–1.43 (m, 12H, CH_2), 1.68–1.85 (m, 2H, CH_2), 2.68–2.86 (t, 2H, CH_2), 2.94–3.36 (t, 4H, CH_2), 3.77–3.94, 3.97–4.08 (dd, 2H, CH_2), 4.31–4.72 (t, 2H, CH_2), 5.24–5.33 (t, 1H, CH), 7.22–7.28 (m, 3H, Ar-H), 7.37–7.40 (t, *J* = 9 Hz, 1H, Ar-H); MS (ESI) *m/z*: 343.4 [M + H]. Anal. calcd. for $C_{22}H_{34}N_2O \cdot HCl$: C, 69.72; H, 9.31; N, 7.39. Found: C, 69.67; H, 9.27; N, 7.32.

2-Decyl-2,3,6,7-tetrahydro-9,10-dimethoxy-1H-pyrazino[2,1-a]isoquinolin-4(11bH)-one ***7b***

White solid; yield: 88.6%; m.p.: 276.4°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.78–0.93 (t, *J* = 6 Hz, 3H, CH_3), 1.18–1.41 (m, 14H, CH_2), 1.86 (s, 2H, CH_2), 2.64–3.01 (t, 4H, CH_2), 3.20–3.24 (t, 2H, CH_2), 3.87 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 4.11–4.25 (m, 2H, CH_2), 4.87 (m, 2H, CH_2), 5.73–5.76 (dd, *J* = 9.3 Hz, 1H, CH), 6.64 (s, 1H, Ar-H), 6.69 (s, 1H, Ar-H); MS (ESI) *m/z*: 403.6 [M + H]. Anal. calcd. for $C_{24}H_{38}N_2O_3 \cdot HCl$: C, 65.66; H, 8.95; N, 6.38. Found: C, 64.63; H, 8.81; N, 6.27.

2-Decyl-2,3,6,7-tetrahydro-10-fluoro-1H-pyrazino[2,1-a]isoquinolin-4(11bH)-one ***7c***

Yellow solid; yield: 80.1%; m.p.: 216.1°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.88–0.91 (t, *J* = 6.5 Hz, 3H, CH_3), 1.17–1.40 (m, 14H, CH_2), 1.87–2.03 (m, 2H, CH_2), 2.86–3.01 (m, 4H, CH_2), 3.11–3.22 (m, 2H, CH_2), 3.91–4.04 (m, 2H, CH_2), 4.79–4.84 (m, 2H, CH_2), 5.82–5.85 (m, 1H, CH), 6.91–7.00 (s, 2H, Ar-H), 7.17–7.21 (t, 1H, Ar-H); MS (ESI) *m/z*: 361.5 [M + H]. Anal. calcd. for $C_{22}H_{33}FN_2O \cdot HCl$: C, 66.56; H, 8.63; N, 7.06. Found: C, 66.02; H, 8.52; N, 7.04.

2-(4-Fluorobenzyl)-2,3,6,7-tetrahydro-1H-pyrazino[2,1-a]isoquinolin-4(11bH)-one ***8a***

Yellowish solid; yield: 87.5%; m.p.: 278.1°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.81–2.96 (m, 4H, CH_2), 3.36–3.62 (m, 2H, CH_2),3.92–4.09 (m, 2H, CH_2), 4.11–4.28 (m, 2H, CH_2), 5.81–5.88 (m, 1H, CH), 7.11–7.31 (m, 4H, Ar-H), 7.65–7.78 (m, 4H, Ar-H); MS (ESI) *m/z*: 311.4 [M + H]. Anal. calcd. for $C_{19}H_{19}FN_2O \cdot HCl$: C, 65.80; H, 5.81; N, 8.08. Found: C, 65.1.6; H, 5.78; N, 8.01.

2-(4-Fluorobenzyl)-2,3,6,7-tetrahydro-9,10-dimethoxy-1H-pyrazino[2,1-a]isoquinolin-4(11bH)-one ***8b***

Yellowish solid; yield: 90.1%; m.p.: 301.2°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.35–2.62 (m, 2H, CH_2), 2.82–2.94 (m, 3H, CH_2), 3.42–3.52 (m, 3H, CH_2), 3.52–3.55, 3.64–3.71 (m, 2H, CH_2), 3.82 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 4.72–4.85 (m, 1H, CH), 6.49 (s, 1H, Ar-H), 6.632 (s, 1H, Ar-H), 7.01–7.08 (t, J_1 = 2.1 Hz, J_2 = 4.8 Hz, 2H, Ar-H), 7.26–7.38(t, J_1 = 2.1 Hz, J_2 = 4.8 Hz, 2H, Ar-H); MS (ESI) *m/z*: 371.4 [M + H]. Anal. calcd. for $C_{21}H_{23}FN_2O_3 \cdot HCl$: C, 61.99; H, 5.95; N, 6.88. Found: C, 61.67; H, 6.76; N, 6.84.

2-(4-Fluorobenzyl)-2,3,6,7-tetrahydro-10-fluoro-1H-pyrazino[2,1-a]isoquinolin-4(11bH)-one ***8c***

Yellowish solid; yield: 81.5%; m.p.: 315.3°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.74–3.02 (m, 4H, CH_2), 3.32–3.52 (m, 2H, CH_2), 3.90–4.48 (m, 2H, CH_2), 4.72–4.85 (m, 2H, CH_2), 5.84 (m, 1H, CH), 6.84 (s, 2H, Ar-H), 6.72 (s, 1H, Ar-H), 7.15–7.22 (m, 1H, Ar-H), 7.26 (s, 1H, Ar-H), 7.56–7.78 (t, 2H, Ar-H); MS (ESI) *m/z*: 330.6 [M + H]. Anal. calcd. for $C_{19}H_{18}F_2N_2O \cdot HCl$: C, 62.55; H, 5.25; N, 7.68. Found: C, 61.45; H, 5.21; N, 7.61.

General procedure of the preparation of ***9a–12c***

To a solution of **5a–8c** (3.89 mmol) in THF, $LiAlH_4$ (15.5 mmol) was added with stirring. After stirring for 3.5 h, the excess of the reducing agent was decomposed with water. The organic layer was decanted and the solid material was extracted with THF. The combined organic extracts were dried over anhydrous Na_2SO_4. After removal of the solvent, the residue was taken up in ether and treated with 1 M HCl in ether. The salt was filtered and dried to afford **9a–12c**.

2-Pentyl-2,3,6,7,11b-hetrahydro-1H-pyrazino[2,1-a]isoquinolin hydrochloride ***9a***

White solid; yield: 94.1%; m.p.: 234.5°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.89–0.94 (t, *J* = 6.1 Hz, 3H, CH_3), 1.30–1.37 (m, *J* = 21.9 Hz, 4H, CH_2), 1.74–1.85 (m, 2H, CH_2), 3.14–3.20 (m, 4H, CH_2), 3.61–3.89 (m, 8H, CH_2), 3.90–3.94 (m, 1H, CH), 7.21–7.48 (m, 4H, Ar-H); MS (ESI) *m/z*: 259.4 [M + H]. Anal. calcd. for $C_{17}H_{26}N_2 \cdot 2\ HCl$: C, 61.63; H, 8.53; N, 8.45. Found: C, 61.52; H, 8.49; N, 8.37.

2-Pentyl-2,3,6,7,11b-hetrahydro-9,10-dimethoxy-1H-pyrazino[2,1-a]isoquinolin hydrochloride ***9b***

White solid; yield: 89.3%; m.p.: 262.5°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.91–0.97 (t, *J* = 6.1 Hz, 3H, CH_3), 1.30–1.38 (m, *J* = 22.8 Hz, 4H, CH_2), 1.63–1.78 (m, 2H, CH_2), 3.11–3.21 (t, 2H, CH_2), 3.71–3.77 (m, 8H, CH_2), 3.78–3.80 (m, 1H, CH), 3.71 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 6.83 (m, 2H, Ar-H), 6.89 (m, 2H, Ar-H); MS (ESI) *m/z*: 319.5 [M + H]. Anal. calcd. for $C_{19}H_{30}N_2O_2 \cdot 2\ HCl$: C, 58.31; H, 8.24; N, 7.16. Found: C, 58.26; H, 8.21; N, 7.19.

2-Pentyl-2,3,6,7,11b-hetrahydro-10-fluoro-1H-pyrazino[2,1-a]isoquinolin hydrochloride ***9c***

Yellowish solid; yield: 92.3%; m.p.: 219.4°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.95–1.05 (t, *J* = 6.4 Hz, 3H, CH_3), 1.41–1.49 (m, *J* = 22.2 Hz, 4H, CH_2), 1.81–2.05 (m, 2H, CH_2), 2.62–2.84 (t, 4H, CH_2), 3.12–3.25 (t, 6H), 4.21–4.34 (m, 2H, CH_2), 4.50–4.63 (m, 1H, CH), 6.82–6.93 (m, 2H, Ar-H), 7.12–7.19 (t, 1H, Ar-H); MS (ESI) *m/z*: 277.4 [M + H]. Anal. calcd. for $C_{17}H_{25}FN_2 \cdot 2$ HCl: C, 58.45; H, 7.79; N, 8.02. Found: C, 58.41; H, 7.71; N, 7.97.

2-Heptyl-2,3,6,7,11b-hetrahydro-1H-pyrazino[2,1-a] isoquinolin hydrochloride ***10a***

White solid; yield: 90.1%; m.p.: 243.5°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.86–0.91 (t, *J* = 6.1 Hz, 3H, CH_3), 1.32–1.39 (m, 8H, CH_2), 1.68–1.79 (m, 2H, CH_2), 3.11–3.17 (m, 4H, CH_2), 3.54–3.79 (m, 8H, CH_2), 3.90–3.93 (m, 1H, CH), 7.18–7.36 (m, 4H, Ar-H); MS (ESI) *m/z*: 287.5 [M + H]. Anal. calcd. for $C_{19}H_{30}N_2 \cdot 2$ HCl: C, 63.50; H, 8.98; N, 7.79. Found: C, 63.47; H, 8.89; N, 7.72.

2-Heptyl-2,3,6,7,11b-hetrahydro-9,10-dimethoxy-1H-pyrazino[2,1-a]isoquinolin hydrochloride ***10b***

White solid; yield: 91.5%; m.p.: 284.1°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.93–0.99 (t, *J* = 6.1 Hz, 3H, CH_3), 1.30–1.37 (m, *J* = 22.8 Hz, 8H, CH_2), 1.62–1.79 (m, 2H, CH_2), 3.16–3.28 (t, 2H, CH_2), 3.69–3.75 (m, 8H, CH_2), 3.80–3.81 (m, 1H, CH), 3.75 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 6.83 (m, 2H, Ar-H), 6.88 (m, 2H, Ar-H); MS (ESI) *m/z*: 347.5 [M + H]. Anal. calcd. for $C_{21}H_{34}N_2O_2 \cdot 2$ HCl: C, 60.14; H, 8.65; N, 6.68. Found: C, 60.01; H, 8.61; N, 6.64.

2-Heptyl-2,3,6,7,11b-hetrahydro-10-fluoro-1H-pyrazino[2,1-a]isoquinolin hydrochloride ***10c***

Yellowish solid; yield: 88.1%; m.p.: 233.2°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.97–1.02 (t, *J* = 6.4 Hz, 3H, CH_3), 1.39–1.46 (m, *J* = 22.2 Hz, 8H, CH_2), 1.84–2.07 (m, 2H, CH_2), 2.64–2.86 (t, 4H, CH_2), 3.14–3.28 (t, 6H, CH_2), 4.22–4.34 (m, 2H, CH_2), 4.50–4.62 (m, 1H, CH), 6.86–6.94 (m, 2H, Ar-H), 7.12–7.19 (t, 1H, Ar-H); MS (ESI) *m/z*: 305.4 [M + H]. Anal. calcd. for $C_{19}H_{29}FN_2 \cdot 2$ HCl: C, 60.47; H, 8.28; N, 7.42. Found: C, 60.39; H, 8.21; N, 7.37.

2-Decyl-2,3,6,7,11b-hetrahydro-1H-pyrazino[2,1-a] isoquinolin hydrochloride ***11a***

White solid; yield: 89.7%; m.p.: 259.1°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.83–0.88 (t, *J* = 6.2 Hz, 3H, CH_3), 1.03–1.44 (m, 14H, CH_2), 1.74–1.85 (m, 2H, CH_2), 3.14–3.20 (m, 4H, CH_2), 3.51–3.53 (m, 8H, CH_2), 3.96–4.05 (t, 1H, CH), 7.20–7.45 (m, 4H, Ar-H); MS (ESI) *m/z*: 329.5 [M + H]. Anal. calcd. for $C_{22}H_{36}N_2 \cdot 2$ HCl: C, 65.82; H, 9.54,; N, 6.98. Found: C, 65.77; H, 9.51; N, 6.91.

2-Decyl-2,3,6,7,11b-hetrahydro-9,10-dimethoxy-1H-pyrazino[2,1-a]isoquinolin hydrochloride ***11b***

White solid; yield: 93.2%; m.p.: 309.6°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.86–0.90 (t, *J* = 6.3 Hz, 3H, CH_3), 1.26–1.35 (m, *J* = 26.1 Hz, 14H, CH_2), 1.84–2.06 (m, 2H, CH_2), 2.84–3.08 (m, 4H, CH_2), 3.51–3.53 (m, 8H, CH_2), 3.78 (s, 6H, OCH_3), 5.32–5.48 (m, 1H, CH), 6.66 (s, 2H, Ar-H); MS (ESI) *m/z*: 389.6 [M + H]. Anal. calcd. for $C_{24}H_{40}N_2O_2 \cdot 2$ HCl: C, 62.46; H, 9.17; N, 6.07. Found: C, 62.41; H, 9.11; N, 6.01.

2-Decyl-2,3,6,7,11b-hetrahydro-10-fluoro-1H-pyrazino[2,1-a]isoquinolin hydrochloride ***11c***

Yellowish solid; yield: 88.3%; m.p.: 244.5°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.91–0.96 (t, *J* = 6.2 Hz, 3H, CH_3), 1.29–1.44 (m, 14H, CH_2), 1.84–2.06 (m, 2H, CH_2), 2.91–3.08 (t, 4H, CH_2), 3.51–3.85 (t, 6H, CH_2), 4.22–4.81 (m, 2H, CH_2), 5.49–5.77 (t, 1H, CH), 6.87–7.03 (m, 2H, Ar-H), 7.11–7.16 (t, 1H, Ar-H); MS (ESI) *m/z*: 347.5 [M + H]. Anal. calcd. for $C_{22}H_{35}FN_2 \cdot 2$ HCl: C, 63.00; H, 8.89; N, 6.68;.Found: C, 62.97; H, 8.81; N, 6.63.

2-(4-Fluorobenzyl)-2,3,6,7,11b-hetrahydro-1H-pyrazino[2,1-a]isoquinolin hydrochloride ***12a***

White solid; yield: 92.5%; m.p.: 304.5°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.41–2.54 (m, 4H, CH_2), 2.84–2.95 (t, 2H, CH_2), 3.41–3.57 (m, 1H, CH), 3.78–3.99 (m, 2H, CH_2), 3.22–3.41 (m, 2H, CH_2), 3.51–3.71 (m, 2H, CH_2), 6.79 (s, 1H, Ar-H), 6.85–6.89 (d, 1H, Ar-H), 7.01–7.04 (d, 3H, Ar-H), 7.07–7.12 (t, 2H, Ar-H), 7.17 (s, 1H, Ar-H); MS (ESI) *m/z*: 297.4 [M + H]. Anal. calcd. for $C_{19}H_{21}FN_2 \cdot 2$ HCl: C, 61.79; H, 6.28; N, 7.59. Found: C, 61.67; H, 6.21; N, 7.50.

2-(4-Fluorobenzyl)-2,3,6,7,11b-hetrahydro-9,10-dimethoxy-1H-pyrazino[2,1-a]isoquinolin hydrochloride ***12b***

White solid; yield: 94.7%; m.p.: 351.5°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.43–2.51 (m, 4H, CH_2), 2.87–2.91 (t, 2H, CH_2), 3.41–3.57 (m, 1H, CH), 3.78–3.99 (m, 2H, CH_2), 3.22–3.41 (m, 2H, CH_2), 3.51–3.71 (m, 2H, CH_2), 3.75 (s, 6H, OCH_3), 6.79 (s, 1H, Ar-H), 6.98 (d, 1H, Ar-H), 7.01 (d, 1H, Ar-H), 7.07–7.12 (t, 2H, Ar-H), 7.19 (s, 1H, Ar-H); MS (ESI) *m/z*: 357.4 [M + H]. Anal. calcd. for $C_{21}H_{25}FN_2O_2 \cdot 2$ HCl: C, 58.74; H, 6.34; N, 6.52. Found: C, 58.67; H, 6.21; N, 6.47.

2-(4-Fluorobenzyl)-2,3,6,7,11b-hetrahydro-10-fluoro-1H-pyrazino[2,1-a]isoquinolin hydrochloride ***12c***

Yellowish solid; yield: 89.1%; m.p.: 299.5°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.74–2.82 (m, 2H, CH_2), 2.94–3.12 (m, 2H, CH_2), 3.34–3.68 (t, 2H, CH_2), 3.82–3.89 (m, 1H, CH), 4.04–4.27 (m, 2H, CH_2), 3.22–3.41 (m, 2H, CH_2), 3.51–3.71 (m, 2H, CH_2), 6.78–6.88 (s, 2H, Ar-H), 7.05–7.22 (m, 3H, Ar-H), 7.56–7.78 (t, 2H, Ar-H); MS (ESI) *m/z*: 315.4 [M + H]. Anal. calcd. for $C_{19}H_{20}F_2N_2 \cdot 2$ HCl: C, 58.92; H, 5.73; N, 7.23. Found: C, 58.87; H, 5.71; N, 7.21.

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