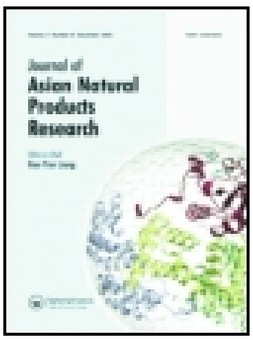

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Bioactivity-guided synthesis of gramine derivatives as new MT_1 and 5-HT_{1A} receptors agonists

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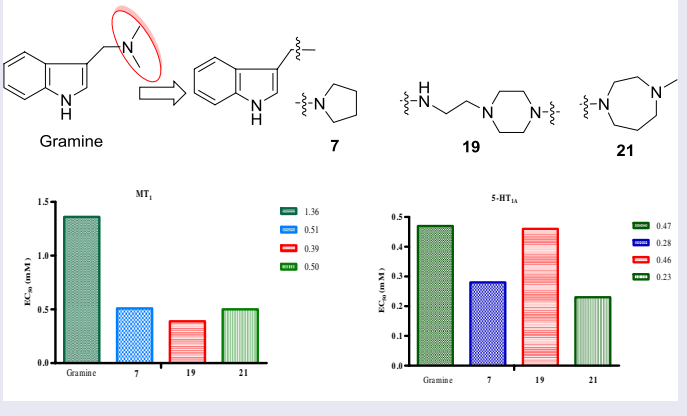
ABSTRACT

Twenty-four gramine derivatives were synthesized and evaluated on MT_1 and 5-HT_{1A} receptors *in vitro*. Among them, seven derivatives (**7**, **8**, **16**, **19**, **20**, **21,** and **24)** exhibited higher agonisting activities on MT_1 or 5-HT_{1A} receptors. Compared with gramine, derivatives **7**, **8**, **16**, **19**, **20**, **21,** and **24** displayed 1.6–3.5-fold increase in agonistic rates on 5-HT_{1A} receptor. Particularly, derivatives **7**, **19,** and **21** exhibited significant agonistic activities on MT_1 and 5-HT_{1A} receptors with EC_{50} values of 0.51, 0.39, 0.50 mM and 0.28, 0.46, 0.23 mM, respectively. The preliminary structure–activity relationships of gramine derivatives were summarized for further investigation on MT_1 and 5-HT_{1A} receptors as new potential agonists.



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1. Introduction

Melatonin (5-methoxy-*N*-acetyltryptamine, MT, Figure 1) is a neurohormone primarily secreted from the vertebrate pineal gland in response to the central switch of the circadian rhythm [1,2]. In 1917, the biological activity of melatonin was reported by McCord and Allan to discover that extracts of bovine pineal gland caused blanching of *Rana pipiens* tadpole skin in adjustment to environmental conditions [3,4]. Melatonin receptors were differentiated into MT_1, MT_2, and MT_3 subtypes based on their functions and pharmacological characteristics, which were viewed as potential targets for drug discovery [5–7]. The human melatonin receptor subtypes showed 60% homology at the amino acid level and distinct pharmacological profiles to partial agonists and antagonists. In mammals, MT_1 and MT_2 receptors had been cloned, which belonged to the G-protein-coupled receptor superfamily, sharing some specific short amino-acid sequences [8]. The MT_3 binding site was purified to homogeneity and identified by partial peptide sequencing as the homolog of the human quinone reductase 2 [9,10]. 5-Hydroxy-tryptamine (5-HT, Figure 1) was the immediate precursor of melatonin, and the conversion was realized by the sequential catalysis of two melatonin-synthesizing enzymes arylalkylamine *N*-acetyltransferase and hydroxyindole-*O*-methyl-transferase [11,12]. The known subtypes of 5-HT receptors, including 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7, had been identified. Selective agonists and antagonists for 5-HT receptor subtypes were characterized with modulating drugs in a vast variety of diseases [13]. The melatonin could potentiate the 5-HT_{1A} receptor

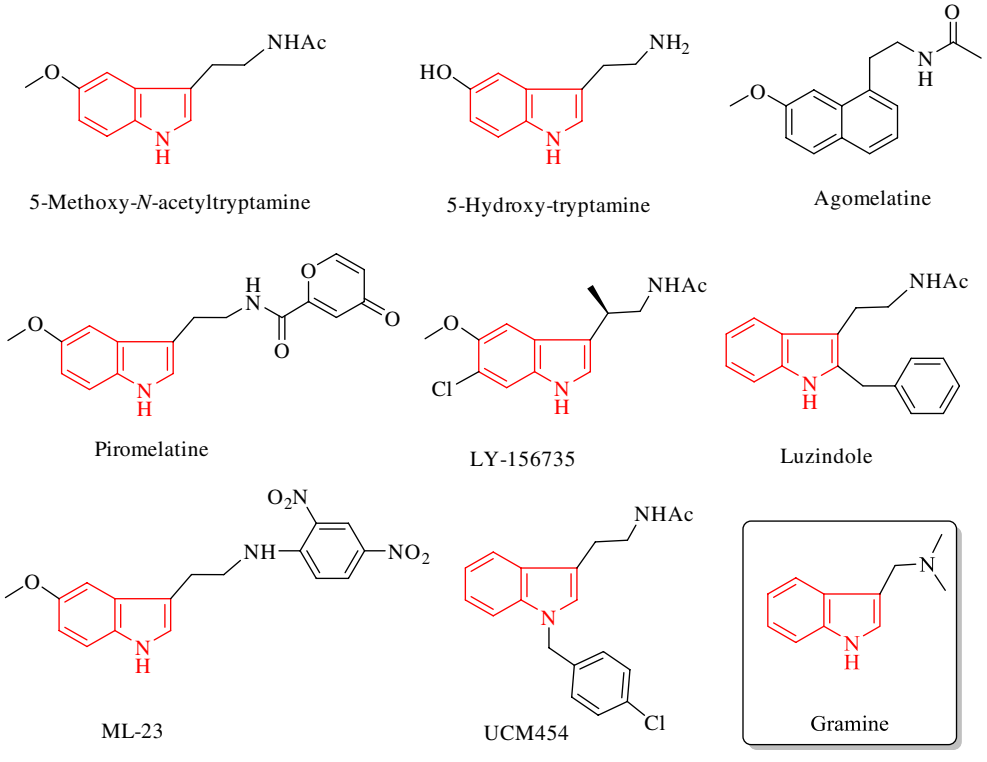


Figure 1. The clinical drugs and gramine.

activation in the hypothalamus, which was modulated by melatonin and thermoregulatory responses [14,15]. In recent years, several kinds of drugs which were capable of either selectively stimulating or inhibiting MT and 5-HT receptors were reported, some of which had been used for psychiatric purposes. For example, agomelatine (a MT_1 and MT_2 receptors agonist with 5-HT_{2C} antagonistic properties) was marketed for the treatment of depression [16], and piromelatine (Neu-P11, a novel MT_1, MT_2, 5-HT_{1A}, and 5-HT_{1D} receptor agonist) demonstrated efficacy for insomnia in the phase II clinical trial (Figure 1) [17].

Our previous studies proposed many natural small molecules and their derivatives had activities on MT and 5-HT receptors using the fluo-8 calcium assay *in vitro*. Indole alkaloids with privileged heterocyclic scaffolds provided tremendous opportunities to discover novel drugs [18], Many drugs curing central nervous system diseases have been on market or in clinical trial, including LY-156735, luzindole, ML-23 [19], UCM454, S-24014 (Figure 1) [20]. Gramine, a small molecule with fascinating indole ring, possessed many pharmaceutical and therapeutic applications, such as vasodilatation [21], antioxidant [22], inhibiting the early stage of enterovirus replication and treating neurodegenerative diseases [23]. However, gramine and their derivatives as MT_1 or 5-HT_{1A} receptor agonists were not reported. Our previous fluo-8 calcium assay suggested that gramine had activities on MT_1 and 5-HT_{1A} receptors with agitating rates of 182.12 ± 6.71 and 136.45 ± 6.96% at the concentration of 1.00 mM, respectively. This encouraged us to synthesize derivatives **1–24** and study their structure–activity relationships (SARs) in detail.

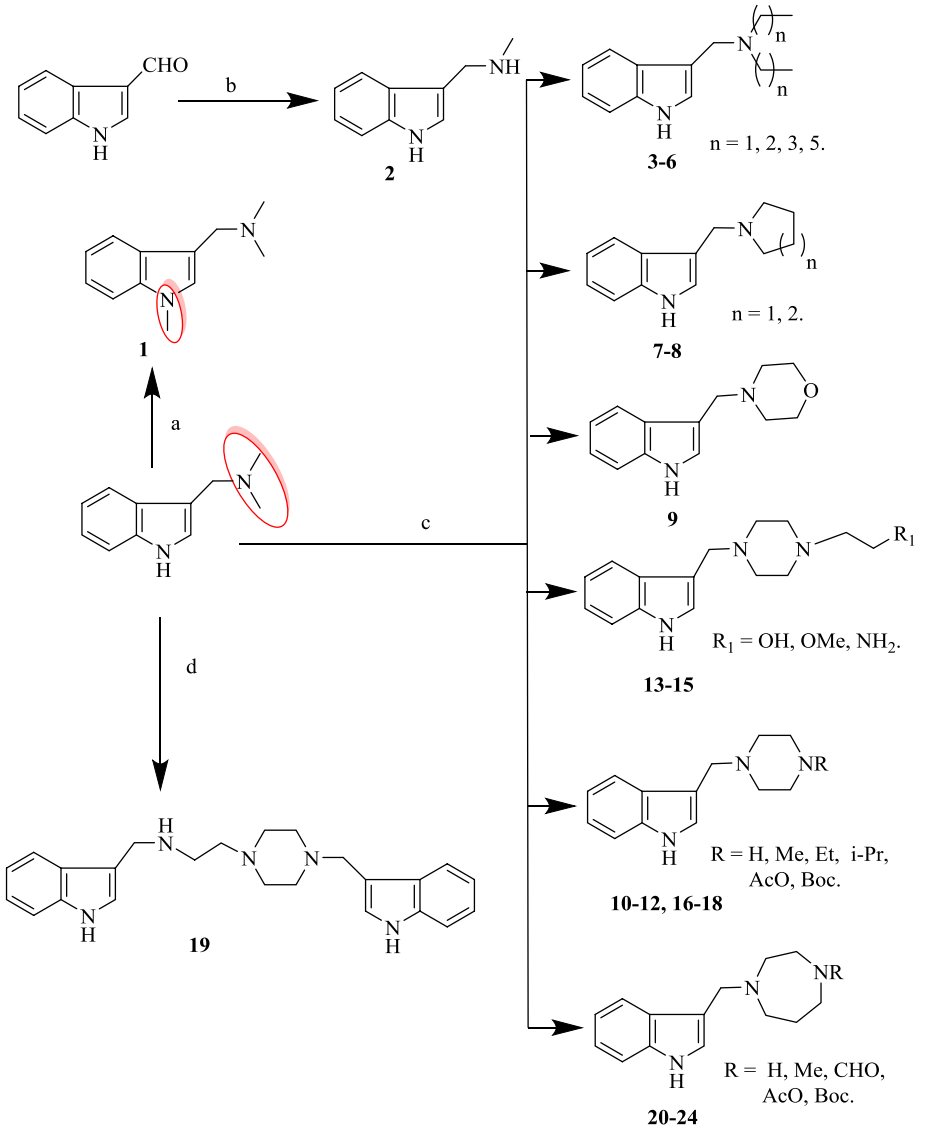
2. Results and discussion

2.1. Chemistry

In order to obtain new active compounds for MT_1 and 5-HT_{1A} receptors, the structure of gramine was modified through a flexible synthetic method (Scheme 1). *N*-Methylation derivative **1** was obtained from gramine and iodomethane in the presence of NaH. Derivative **2** with a single methyl group at *N*-1′ position was prepared by the reductive amination of 3-indole-carboxaldehyde with methylamine and $NaBH(AcO)_3$. To investigate whether different alkyl chains or heterocyclic ring could affect the potential agonistic activities, derivatives **3–24** were afforded by the reaction of gramine with secondary amines in anhydrous toluene. The results showed that the synthetic routes had the advantages of simple reaction and high yield.

2.2. Biological evaluation

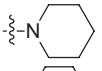
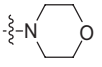
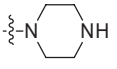
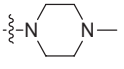
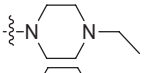
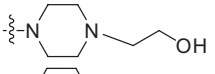
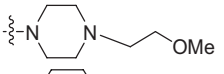
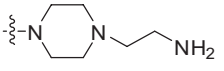
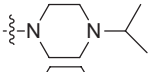
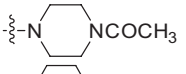
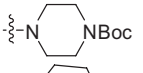
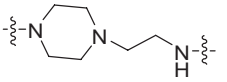
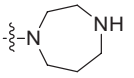
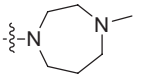
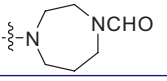
All the synthesized derivatives (**1–24**) were evaluated on MT_1 and 5-HT_{1A} receptors at the concentration of 1.00 mM (Table 1). Methylation derivative **1** possessed weak agonistic activity on MT_1 receptor with an agonistic rate of 61.55 ± 13.89%, suggesting that NH (*N*-1) in the indole ring was favorable for maintaining activity. Monomethylation derivative **2** had no activity, revealing that a trialkylamine group at *N*-1′ position played an important role for maintaining activity. Derivative **3** with two ethyl groups had a similar agonistic activity (184.19 ± 5.0%) on MT_1 receptor. The agonistic rates of homologs **3–6** decreased on MT_1 receptor in sequence from 184.19 ± 5.0 to 20.70 ± 2.99% (derivative **3** vs. **4** vs. **5** vs. **6**). Derivative **7** with a pyrrolyl group at *N*-1′ position indicated similar agonistic activity



Scheme 1. Synthesis of the gramine derivatives. Reagents and conditions: (a) NaH, CH_3I, DMF, 0 °C, 50–70%. (b) Methylamine, CH_3OH, r.t.; $NaBH(AcO)_3$, EtOH, r.t. 65%. (c) Corresponding amines, toluene, reflux, 75–97%. (d) 2-Amino-ethyl-piperazine, toluene, reflux, 70%.

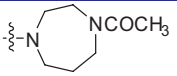
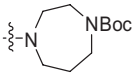
on MT_1 receptor, while the agonistic activities of piperidyl derivative **8** and morpholinyl derivative **9** decreased significantly. Piperazinyl derivative **10** exhibited moderate activity on MT_1 receptor. These results enlightened us to further explore the effects of derivatives **11–19** by introducing a piperazinyl group. Compared with derivative **10**, derivative **11** with a *N*-methylation piperazine group, and derivative **12** with a *N*-ethylation piperazine group obviously reduced activities on MT_1 receptor; derivatives **13**, **14,** and **15** with OH, OMe, and NH_2 groups, respectively, on C-2 position of *N*-ethyl piperazine possessed weak activities; derivative **16** with a *N*-isopropylation piperazine group and acylated derivatives **17–18** had no activities on MT_1 receptor; derivative **19** with bisindole structure showed a

Table 1. Agonistic activities of gramine derivatives on MT_1 and 5-HT_{1A} receptors.[a]

Compound	*R*	Agonistic rate (%)[b]	
		MT_1	5-HT_{1A}
Agomelatine	–	100 ± 7.83	–
5-Hydroxy-tryptamine	–	–	100 ± 3.43
Gramine	–	182.12 ± 6.71	136.45 ± 6.96
1	–	61.55 ± 13.89	0.77 ± 3.25
2		72.73 ± 14.59	1.84 ± 0.54
3		184.19 ± 5.0	275.40 ± 9.65
4		122.25 ± 5.17	161.56 ± 20.38
5		53.03 ± 3.83	165.71 ± 16.98
6		20.70 ± 2.99	−16.24 ± 2.20
7		193.66 ± 15.02	357.35 ± 17.47
8		34.32 ± 20.16	220.93 ± 2.88
9		71.59 ± 0.09	65.93 ± 1.91
10		148.07 ± 7.32	65.58 ± 2.01
11		83.26 ± 9.09	72.38 ± 1.57
12		106.38 ± 4.93	116.12 ± 21.38
13		49.52 ± 3.54	56.94 ± 0.59
14		68.99 ± 4.91	63.84 ± 3.12
15		110.08 ± 1.21	166.60 ± 21.82
16		127.11 ± 9.31	243.78 ± 11.46
17		−9.34 ± 20.16	21.30 ± 4.97
18		−2.44 ± 1.84	17.98 ± 4.61
19		220.97 ± 7.64	467.55 ± 3.12
20		175.23 ± 7.63	237.02 ± 15.38
21		204.10 ± 11.72	486.60 ± 17.62
22		62.63 ± 4.24	49.98 ± 0.71

(*Continued*)

Table 1. (Continued).

Compound	*R*	Agonistic rate (%)[b]	
		MT_1	5-HT_{1A}
23		78.66 ± 2.45	54.81 ± 2.78
24		78.50 ± 3.02	270.0 ± 23.32

[a]Agomelatine and 5-hydroxy-tryptamine were tested at the concentration of 1.11 and 6.67 μM, respectively. Other compounds were tested at the concentration of 1.00 mM.
[b]Agonist data on melatonin and 5-hydroxy-tryptamine receptors were the mean of triplicate experiments.

1.2-fold increase in agonistic activity on MT_1 receptor; derivative **20** with a homopiperzinyl group at *N*-1′ position proposed activity with an agonistic rate of 175.23 ± 7.63% on MT_1 receptor, indicating that the homopiperzinyl group was suitable for maintaining agonistic activity. Enlightened by these results, derivatives **21–24** with different substituents on the homopiperzinyl group were synthesized. The agonistic rates of derivatives **21–24** displayed from 204.10 ± 11.72 to 62.63 ± 4.24% on MT_1 receptor. The methylation derivative **21** exhibited significantly agonistic activity on MT_1 receptor with agonistic rate of 204.10% on MT_1 receptor. *N*-formyl (**22**), *N*-acetyl (**23**) and *N*-*tert*-butoxycarbonyl (**24**) showed weak activities on MT_1 receptor. The above analyses suggested that pyrrolyl, morpholinyl, piperidyl, piperazinyl, and homopiperazinyl groups at *N*-1′ position had a different effect for agonistic activities.

In Table 1, methylation derivative **1** had no agonistic activity on 5-HT_{1A} receptor, suggesting that NH was preferable for activity. Derivative **2** with a single methyl group at *N*-1′ position displayed no activity on 5-HT_{1A} receptor. Derivative **3** with two ethyl groups at *N*-1′ position increased activity with an agonistic rate of 275.40 ± 9.65% on 5-HT_{1A} receptor. The agonistic activities of homologs **3–6** decreased gradually with agonistic rates from 275.40 ± 9.65 to −16.24 ± 2.20% (derivative **3** vs. **4** ≈ **5** vs. **6**). The above analyses suggested that the trialkylamine moiety at *N*-1′ position was crucial for agonistic activity. Heterocyclic derivatives displayed attractive agonistic activities on 5-HT_{1A} receptor. Derivative **7** with a pyrrolyl group and derivative **8** containing a piperazinyl group at *N*-1′ position exhibited 2.7- and 1.6-fold increase in agonistic rates, respectively. Derivative **9** with a morpholinyl group and **10** with a piperazinyl group at *N*-1′ position showed no agonistic activities. Piperazinyl derivative **10** displayed weak activity on 5-HT_{1A} receptor. Compared with derivative **10**, the agonistic activities of derivative **11** with a *N*-methylation piperazine group and derivative **12** with a *N*-ethylation piperazine group decreased; derivatives **13**, **14,** and **15** with OH, OMe, and NH_2 groups at C-2 position of *N*-ethyl piperazine possessed weak activities; derivative **16** with a *N*-isopropylation piperazine group exhibited about 2-fold activity on 5-HT_{1A} receptor. Acylated derivatives **17** and **18** had no activities; derivative **19** with bis(indole) structure demonstrated an approximately 3.5-fold increase in agonistic activity on 5-HT_{1A} receptor. Derivative **20** with a homopiperzinyl group at *N*-1′ position proposed better agonistic activity on 5-HT_{1A} receptors (237.02 ± 15.38%, at 1.00 mM), indicating that the homopiperzinyl group was preferable for gaining high agonistic activity. The agonistic rates of homologs **21–24** with different substituents on the homopiperzinyl group were varied from 486.60 ± 17.62 to 49.98 ± 0.71% on 5-HT_{1A} receptor. Interestingly,

the *N*-methylation derivative **21** exhibited higher agonistic activity on 5-HT_{1A} receptor with an agonistic rate of 486.60% on 5-HT_{1A} receptor. Agonistic activities of *N*-formyl and *N*-acetyl substituted analogs **22** and **23** resulted in an obvious decrease on 5-HT_{1A} receptor. *N-tert*-butoxycarbonyl analog **24** displayed an approximate 2-fold increase in agonistic activity on 5-HT_{1A} receptor. The above analyses suggested the trialkylamine moiety at N-1′ position was important for agonistic activity.

The agonistic activities of derivatives **7**, **19,** and **21** were higher than other derivatives, which prompted us to evaluate the EC_{50} values of derivatives **7**, **19,** and **21** on MT_1 and 5-HT_{1A} receptors. It was noted that derivatives **7**, **19,** and **21** exhibited significant activities on MT_1 and 5-HT_{1A} receptors with EC_{50} values of 0.51, 0.39, 0.50 mM and 0.28, 0.46, 0.23 mM, respectively. The compared results were summarized in Table 2.

These results indicated the following SARs (Figure 2): (1) a secondary amine on indole ring played a significant role; (2) the single methylation at *N*-1′ position resulted in the decrease of agonistic activities; (3) the side chains at *N*-1′ position (less than 3 carbons) were crucial for agonistic activities; (4) the pyrrolyl, piperazinyl, and homopiperazinyl

Table 2. The EC_{50} (mM) values of derivatives **7**, **19,** and **21** on MT_1-HEK293 and 5-HT_{1A}- HEK293 cells *in vitro*.

	EC_{50} (mM)	
Compound	MT_1	5-HT_{1A}
Gramine	1.36	0.47
7	**0.51**	**0.28**
19	**0.39**	0.46
21	**0.50**	**0.23**

Notes: Dose-response of agonistic activity was performed in triplicate. EC_{50} values for the derivatives **7**, **19**, **21,** and gramine were determined from the dose-response curves obtained with seven concentrations from the range of 0.02–1.52 mM, and calculated by the software of Graphpad Prism 5.0. The activity data were marked in bold font.

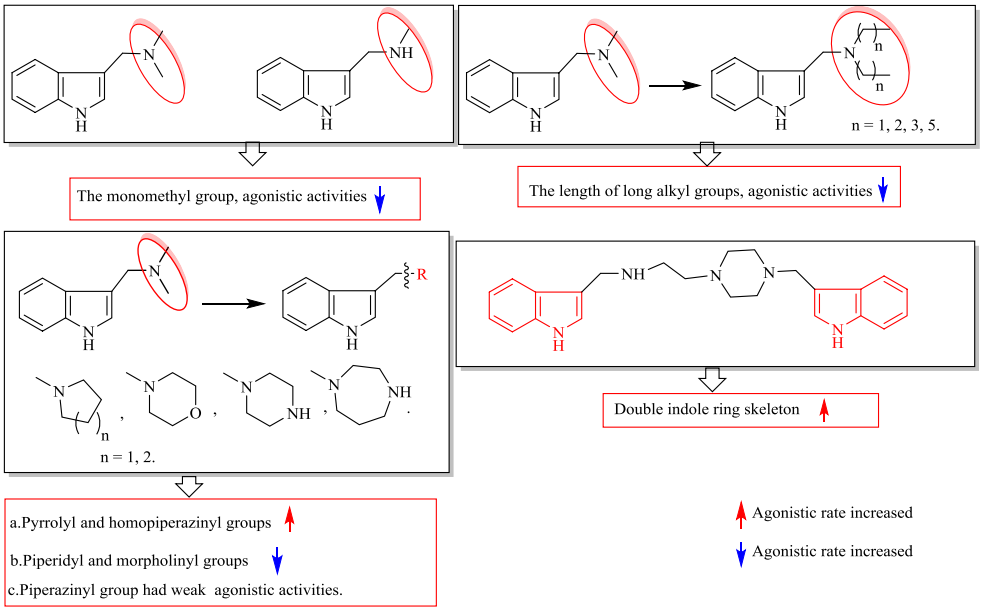


Figure 2. Structure–activity relationships of gramine derivatives.

groups were preferred for agonistic activities; (5) the piperidyl and morpholinyl groups were not favorable to maintain agonisting activities. This investigation will provide valuable information for further developing gramine derivatives as potential MT and 5-HT receptors agonists.

3. Conclusion

In summary, twenty-four gramine derivatives were synthesized and examined for their agonistic activities *in vitro*, of which, derivatives **7**, **8**, **16**, **19**, **20**, **21,** and **24** displayed higher agonisting activities on MT_1 and HT_{1A} receptors. In particular, derivatives **7**, **19,** and **21** exhibited significant agonistic activities on MT_1 and 5-HT_{1A} receptors with EC_{50} values of 0.51, 0.39, 0.50 mM and 0.28, 0.46, 0.23 mM, respectively. In addition, the SARs analyses indicated that a secondary amine on indole ring and the trialkylamine moiety at *N*-1′ position played significant roles for agonistic activities in the current series of compounds.

4. Experimentation

4.1. General experimental procedures

Melting points were measured on a micro-melting point apparatus SGW®X-4B (Shanghai Precision & Scientific Instrument Co., Ltd., Shanghai, China). ^{1}H NMR and ^{13}C NMR data were recorded in $CDCl_3$ on a 400 MHz spectrometer (Bruker, Bremerhaven, Germany) with tetramethylsilane as an internal standard. Low-resolution mass spectra and high-resolution mass spectra data were measured on Shimadzu liquid chromatography-mass spectrometry-ion trap-time of flight (Shimadzu, Kyoto, Japan). The purity of the target compounds was checked by three different solvent systems and HPLC methods. The solvents were dried according to standard procedures. Organic solvents were analytical grade reagents, purchased from Tianjin Chemical Reagent Co., Ltd (Tianjin, China). All reactions were carried out under an air atmosphere and monitored by using thin-layer chromatography (TLC, 200-300 mesh, Qingdao Makall Group Co., Ltd; Qingdao, China). Gramine and corresponding amines were purchased from Alfa Aesar or J&K Scientific Ltd (Bingjing, China).

The calcium flow assay was performed on MT_1-HEK293 and 5-HT_{1A}-HEK293 cell lines by Wash Free Fluo-8 Calcium Assay Kit (HD Biosciences Co. Ltd, Shanghai, China, HD03-0010), and the results were read with a FlexStation 3 Benchtop Multi-Mode Microplate Reader (Molecular Devices, Sunnyvale, California, U.S.A.) at room temperature. CO_2 incubator was purchased from Thermo Forma Co., Ltd (U.S.). MT_1 and 5-HT_{1A} receptor cell lines were purchased from HD Biosciences Co. Ltd (Shanghai, China). Agomelatine (CAS: 138112-76-2) and 5-hydroxytryptamine (CAS: 50-67-9) were purchased from Sahn Chemical Technology Co., Ltd (Shanghai, China) and Yunnan Zehao Trade Co., Ltd (Yunnan, China), respectively, which was used as the positive control.

4.2. Chemical synthesis

4.2.1. General procedure for preparation of compound 1

Gramine (3.0 mmol) was dissolved in *N*, *N*-dimethyl formamide (15.0 mL), and NaH (3.3 mmol) was slowly added into the above solution at 0 °C. The mixture were dropped by CH_3I (3.0 mmol) at room temperature, and monitored by TLC. Subsequently, the reaction

mixture were suspended in saturated NaCl (30.0 ml), extracted with EtOAc (3 × 30 ml) and washed with saturated NaCl. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to dryness *in vacuo*. Purification by column chromatography on silica gel using Et_2NH/MeOH/$CHCl_3$ (2/5/93, v/v/v) provided a target derivative.

N-Methylgramine (**1**): White amorphous powder, mp 113–114 °C, yield 70%.

4.2.2. General procedure for preparation of compound 2

CH_3NH_2 (6.0 mmol, MeOH 5 ml) methanol solution was slowly dropped into indole-3-carboxaldehyde (2.0 mmol, MeOH 10 ml) methanol solution. The mixture was stirred at room temperature for 3 h, and then concentrated to a light yellow oil. This oil and $NaBH(AcO)_3$ (2.2 mmol) were subsequently dissolved in EtOH (10 ml), and the mixture was stirred at room temperature for 6 h. The solvent was evaporated *in vacuo* to give crude products which were poured into 1 mol/L NaOH (1.5 ml), extracted with EtOAc (3 × 30 ml) and washed with saturated NaCl (30 ml). The organic layer was dried over anhydrous Na_2SO_4, concentrated to dryness *in vacuo* and purified by column chromatography on silica gel using Et_2NH/MeOH/$CHCl_3$ (2/4/94, v/v/v).

N-Methyl-3-indolylmethylamine (**2**): Yellow oil, yield 65%.

4.2.3. General procedure for preparation of compounds 3–18 and 20–24

The gramine (2 mmol) and the corresponding amine (2–10 mmol) were dissolved in dry toluene (15 ml), and the mixture was refluxed for 8–14 h and monitored by TLC. The solvent was condensed subsequently *in vacuo* to obtain crude products which was purified by column chromatography on silica gel using Et_2NH/MeOH/$CHCl_3$ (2/4/94–3/5/92, v/v/v).

N, N-Diethyl-3-indolylmethylamine **(3)**: White amorphous powder, mp 105-106 °C, yield 97%.

N, N-di(n-Propyl)-3-indolylmethylamine (**4**): Yellow oil, yield 98%.

N, N-di(n-Butyl)-3-indolylmethylamine (**5**): Yellow oil, yield 96%.

N, N-di(n-Hexyl)-3-indolylmethylamine (**6**): Yellow oil, yield 97%. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 8.75 (s, 1H, NH), 7.86-7.02 (m, 5H, H-2, 4, 5, 6, 7), 3.93 (s, 2H, CH_2 N), 2.64-2.60 (m, 4H, H-1′, 1″), 1.68-1.66 (m, 4H, H-2′, 2″), 1.45-1.38 (m, 12H, H-3′, 3″, 4′, 4″, 5′, 5″), 1.01-0.98 (m, 6H, H-6′, 6″). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 136.3 (C-8), 128.2 (C-9), 123.8 (C-2), 121.7 (C-6), 119.5 (C-5), 119.2 (C-4), 113.1 (C-3), 111.2 (C-7), 53.8 (C-1′, 1″), 49.1 (CH_2 N), 32.0 (C-2′, 2″), 27.5 (C-4′, 4″), 27.0 (C-3′, 3″), 22.8 (C-5′, 5″), 14.2 (C-6′, 6″). ESIMS: *m*/*z* 315 $[M + H]^+$; HRESIMS: *m*/*z* 315.2688 $[M + H]^+$ (calcd for $C_{21}H_{34}N_2$, 315.2795).

N-Pyrrolyl-3-indolylmethylamine (**7**): White amorphous powder, mp 123–124 °C, yield 96%.

N-Piperidyl-3-indolylmethylamine (**8**): White amorphous powder, mp 126–127 °C, yield 97%.

N-Morpholinyl-3-indolylmethylamine (**9**): White amorphous powder, mp 123.5–124.5 °C, yield 98%.

N-Piperazinyl-3-indolylmethylamine (**10**): White amorphous powder, mp 122–123 °C, yield 90%.

N-Methyl-*N*-Piperazinyl-3-indolylmethylamine (**11**): White amorphous powder, mp 137–138 °C, yield 92%.

N-Ethyl-*N*-Piperazinyl-3-indolylmethylamine (**12**): Yellow oil, yield 93%. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 9.60 (s, 1H, NH), 7.75-7.01 (m, 5H, H-2, 4, 5, 6, 7), 3.74 (s, 2H, CH_2 N), 2.96-2.70 (m, 4H, H-2′, 6′), 1.83-1.75 (m, 2H, $\underline{CH_2}CH_3$), 1.83–1.75 (m, 4H, H-3′, 5′), 1.17 (d, 3H, $CH_2\underline{CH_3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 136.3 (C-8), 128.2 (C-9), 124.3 (C-2), 121.7 (C-6), 119.4 (C-5), 119.3 (C-4), 111.5 (C-3), 111.2 (C-7), 61.1 (C-3′, 5′), 53.7 (CH_2 N), 53.4 (C-2′, 6′), 50.6 (C-1″), 20.1 (C-2″). ESIMS: *m*/*z* 244 [M + H]$^+$; HRESIMS: *m*/*z* 244.1705 [M + H]$^+$ (calcd for $C_{15}H_{21}N_3$, 244.1808).

N-(2″-Hydroxyethyl)-*N*-Piperazinyl-3-indolylmethylamine (**13**): White amorphous powder, mp 86–87 °C, yield 83%.

N-(2″-Methoxyethyl)-*N*-Piperazinyl-3-indolylmethylamine (**14**): Yellow oil, yield 85%. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 9.71 (s, 1H, NH), 7.73-6.94 (m, 5H, 5H, H-2, 4, 5, 6, 7), 3.76 (s, 2H, CH_2 N), 3.51-3.49 (m, 2H, $CH_2\underline{CH_2}OCH_3$), 3.32 (s, 3H, $CH_2CH_2O\underline{CH_3}$), 2.69-2.57 (m, 10H, H-1″, 2′, 3′, 5′, 6′). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 136.3 (C-8), 128.3 (C-9), 124.6 (C-2), 121.6 (C-6), 119.3 (C-5), 119.2 (C-4), 111.4 (C-3), 111.1 (C-7), 70.0 (C-2″), 58.8 ($O\underline{CH_3}$), 57.8 (C-3′, 5′), 53.5 (CH_2 N), 53.1 (C-2′, 6′), 52.6 (C-1″). ESIMS: *m*/*z* 274 [M + H]$^+$; HRESIMS: *m*/*z* 274.1810 [M + H]$^+$ (calcd for $C_{16}H_{23}N_3O$, 274.1914).

N-Aminoethyl-*N*-Piperazinyl-3-indolylmethylamine (**15**): Yellow oil, yield 80%. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 8.38 (s, 1H, NH), 7.73-7.10 (m, 5H, H-2, 4, 5, 6, 7), 3.74 (s, 2H, CH_2 N), 2.79-2.17 (m, 14H, 2-aminoethylpiperazinyl-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 136.1 (C-8), 128.0 (C-9), 123.8 (C-2), 121.9 (C-6), 119.4 (C-5), 119.4 (C-4), 112.1 (C-3), 111.0 (C-7), 60.7 ($\underline{CH_2}CH_2$ NH_2), 53.2 (CH_2N), 53.2 (C-3′, 5′), 52.9 (C-2′, 6′), 38.6 ($CH_2\underline{CH_2}NH_2$). ESIMS: *m*/*z* 259 [M + H]$^+$; HRESIMS: *m*/*z* 259.1841 [M + H]$^+$ (calcd for $C_{15}H_{22}N_4$, 259.1917).

N-Isopropyl-*N*-piperazinyl-3-indolylmethylamine (**16**): White amorphous powder, mp 118-119 °C, yield 93%. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 9.57 (s, 1H, NH), 7.73-6.94 (m, 5H, H-2, 4, 5, 6, 7), 3.78 (s, 2H, CH_2 N), 2.69-2.60 (m, 9H, H-1″, 2′, 3′, 5′, 6′), 1.05 (d, 6H, Me). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 136.2 (C-8), 128.3 (C-9), 124.4 (C-2), 121.7 (C-6), 119.3 (C-5), 119.3 (C-4), 111.3 (C-3), 111.2 (C-7), 54.5 (C-1″), 53.1 (CH_2 N), 53.1 (C-3′, 5′), 48.7 (C-2′, 6′), 18.7 (C-Me). ESIMS: *m*/*z* 258 [M + H]$^+$; HRESIMS: *m*/*z* 258.1862 [M + H]$^+$ (calcd for $C_{16}H_{23}N_3$, 258.1965).

N-Acetyl-*N*-Piperazinyl-3-indolylmethylamine (**17**): White amorphous powder, mp 116–116.5 °C, yield 97%.

N-*tert*-Butyloxycarbonyl-*N*-Piperazinyl-3-indolylmethylamine (**18**): White amorphous powder, mp 160–161 °C, yield 96%. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 8.93 (s, 1H, NH), 7.77-7.07 (m, 5H, H-2, 4, 5, 6, 7), 3.77 (s, 2H, CH_2 N), 3.48 (m, 4H, H-3′, 5′), 2.51-2.49 (m, 4H, H-2′, 6′), 1.50 (s, 9H, Me-Boc). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 154.9 (CO), 136.3 (C-8), 127.9 (C-9), 124.1 (C-2), 121.9 (C-6), 119.4 (C-5), 119.4 (C-4), 111.6 (C-3), 111.2 (C-7), 79.7 (C-Boc), 53.5 (CH_2 N), 52.7 (C-2′, 6′), 45.0 (C-3′, 5′), 28.4 (C-Boc). ESIMS: *m*/*z* 316 [M + H]$^+$; HRESIMS: *m*/*z* 316.1952 [M + H]$^+$ (calcd for $C_{18}H_{25}N_3O_2$, 316.2020).

N-Homopiperazinyl-3-indolylmethylamine (**20**): Yellow oil, yield 76%.

N-Methyl-*N*-homopiperazinyl-3-indolylmethylamine (**21**): Yellow oil, yield 80%.^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 10.05 (s, 1H, NH), 7.80-7.02 (m, 5H, H-2, 4, 5, 6, 7), 3.89 (s, 2H, CH_2 N), 2.89-2.85 (m, 4H, H-2′, 4′), 2.75-2.69 (m, 4H, H-6′, 7′), 2.40 (s, 3H, NMe), 1.89-1.85 (m, 2H, H-3′). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 136.4 (C-8), 128.1 (C-9), 124.3 (C-2), 121.6 (C-6), 119.4 (C-5), 119.1 (C-4), 112.6 (C-3), 111.3 (C-7), 57.5 (C-6′), 56.8 (C-7′),

54.3 (C-4′), 54.0 (C-2′), 53.6 (CH_2 N), 46.9 (NMe), 27.0 (C-3′). ESIMS: *m/z* 244 $[M + H]^+$; HRESIMS: *m/z* 244.1742 $[M + H]^+$ (calcd for $C_{15}H_{21}N_3$, 244.1808).

N-Formyl-*N*-homopiperazinyl-3-indolylmethylamine (**22**): White amorphous powder, mp 108–109.5 °C, yield 88%. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 8.71 (s, 1H, NH), 8.08-7.05 (m, 5H, H-2, 4, 5, 6, 7), 7.72 (m, 1H, CHO), 3.83 (s, 2H, CH_2 N), 3.54-3.39 (m, 4H, H-2′, 4′), 2.75-2.66 (m, 4H, H-6′, 7′), 1.90-1.88 (m, 2H, H-3′). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 162.8 (CHO), 136.3 (C-8), 127.6 (C-9), 123.6 (C-2), 121.8 (C-6), 119.4 (C-5), 119.2 (C-4), 112.6 (C-3), 111.1 (C-7), 56.7 (C-7′), 54.2 (C-2′), 53.4 (CH_2 N), 48.0 (C-6′), 43.7 (C-4′), 29.2 (C-3′). ESIMS: *m/z* 258 $[M + H]^+$; HRESIMS: *m/z* 258.1524 $[M + H]^+$ (calcd for $C_{15}H_{19}N_3O$, 258.1601).

N-Acetyl-*N*-homopiperazinyl-3-indolylmethylamine (**23**): White amorphous powder, mp 116-117 °C, yield 86%. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 8.71 (s, 1H, NH), 7.75-7.02 (m, 5H, H-2, 4, 5, 6,7), 3.82 (s, 2H, CH_2 N), 3.67-3.61 (m, 4H, H-2′, 4′), 2.72-2.66 (m, 4H, H-6′, 7′), 2.09 (s, 3H, CH_3), 1.85–1.84 (m, 2H, H-3′). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.5 (CO), 136.3 (C-8), 127.6 (C-9), 123.8 (C-2), 121.6 (C-6), 119.2 (C-5), 119.0 (C-4), 112.3 (C-3), 111.1 (C-7), 55.2 (C-7′), 54.0 (C-2′), 53.3 (CH_2 N), 48.5 (C-6′), 44.9 (C-4′), 28.0 (C-3′), 21.6 (Me). ESIMS: *m/z* 272 $[M + H]^+$; HRESIMS: *m/z* 272.1688 $[M + H]^+$ (calcd for $C_{16}H_{21}N_3O$, 272.1757).

N-tert-Butyloxycarbonyl-*N*-homopiperazinyl-3-indolylmethylamine (**24**): Yellow amorphous powder, mp 95–96 °C, yield 82%. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 8.91 (s, 1H, NH), 7.76-7.03 (m, 5H, H-2, 4, 5, 6, 7), 3.83 (s, 2H, CH_2 N), 3.55-3.43 (m, 4H, H-2′, 4′), 2.72-2.66 (m, 4H, H-6′, 7′), 1.85-1.82 (m, 2H, H-3′), 1.49 (s, 9H, Me-Boc). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 155.7 (CO), 136.4 (C-8), 127.9 (C-9), 123.8 (C-2), 121.8 (C-6), 119.5 (C-5), 119.3 (C-4), 112.8 (C-3), 111.2 (C-7), 79.4 (C-Boc), 55.77 (C-7′), 54.7 (C-2′), 53.5 (CH_2 N), 46.8 (C-6′), 46.2 (C-4′), 28.6 (C-Me-Boc), 27.9 (C-3′). ESIMS: *m/z* 330 $[M + H]^+$; HRESIMS: *m/z* $[M + H]^+$ 330.1284 (calcd for $C_{19}H_{27}N_3O_2$, 330.2176).

4.2.4. General procedure for preparation of compound 19

The gramine (4 mmol) and 2-amino-ethyl-piperazine (2 mmol) were dissolved in dry toluene (15 ml). The mixture were refluxed for 10 h monitored by TLC. The solvent was removed *in vacuo* to afford crude products which were purified by column chromatography on silica gel using Et_2NH /MeOH/$CHCl_3$ (3:5: 92, v/v/v).

N-[*N*-(3-Indolylmethyl)-aminoethyl]-*N*-piperazinyl-3-indolylmethylamine (**19**): Yellow oil, yield 70%. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 8.88 (s, 1H, NH), 8.85 (s, 1H, NH), 7.72-7.01 (m, 10H, H-2, 2′, 4, 4′, 5, 5′, 6, 6′, 7, 7′), 3.98 (s, 2H, CH_2 N), 3.72 (s, 2H, $CH_2CH_2NH\underline{CH}_2$), 2.80–2.28 (m, 13H, 2-aminoethylpiperazinyl-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 136.4 (C-8, 8′), 128.1 (C-9), 127.0 (C-9′), 124.1 (C-2), 122.8 (C-2′), 122.0 (C-6), 121.8 (C-6′), 119.4 (C-5), 119.4 (C-5′), 118.7 (C-4, 4′), 114.5 (C-3), 111.9 (C-3′), 111.3 (C-7), 111.1 (C-7′), 57.4 (CH_2 N), 53.1 (C-3″, 5″), 53.0 (CH_2 N), 52.7 (C-2″, 6″), 45.6 ($\underline{CH}_2CH_2NHCH_2$), 44.5 ($CH_2\underline{CH}_2NHCH_2$). ESIMS: *m/z* 388 $[M + H]^+$; HRESIMS: *m/z* 388.2477 $[M + H]^+$ (calcd for $C_{24}H_{29}N_5$, 388.2496).

4.3. Biological test methods

The agonistic rates of the tested compounds were performed according to the previous reports, [24,25] and the details of fluo-8 calcium assay were listed as follows. HEK293 cell

lines stably expressing the human MT_1 or 5-HT_{1A} receptor were cultivated in Dubecco's modified Eagle's medium supplemented with 10% fetal bovine serum, and cultured with 95% O_2/5% CO_2 at 37 °C. The cells were seeded in a matrigel coated 96-well black plate with a plating volume of 100 µl/well at a density of 4×10^4/well, and incubated in CO_2 incubator (Thermo Forma 3310, Gaithersburg, U.S.) for overnight. Then the cells were dyed by HDB wash free calcium assay kit, and placed in CO_2 incubator for 1 h. Tested compounds and the positive drug were dissolved in 10 µl dimethyl sulfoxide and 990 µl HBSS buffer, respectively, and extracted a plating volume of 100 µl/well in a Matrigel coated 96-well clear bottom plate. Two 96-well plates were put into Flexstation 3 Benchtop Multi-Mode Microplate Reader. The absorption values were read by Flexstation 3 Benchtop Multi-Mode Microplate Reader at room temperature with wavelength (excitation: 485 nm; emission: 525 nm; emission cut-off: 515 nm). The agonistic activity was performed in triplicate. EC_{50} values for the derivatives **7**, **19**, **21,** and gramine were determined from the dose-response curves obtained with seven concentrations from the range of 0.02 to 1.52 mM. The results were calculated by the software of Graphpad Prism 5.0.

Disclosure statement

No potential conflict of interest was reported by the authors.

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