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Polybenzyls from *Gastrodia elata*, their agonistic effects on melatonin receptors and structure-activity relationships

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ABSTRACT

Gastrodia elata is a famous traditional Chinese herb with medicinal and edible application. In this study, nine polybenzyls (**1**−**9**), including six new ones (**2**−**5**, **7** and **9**), were isolated from the EtOAc extract of *G. elata*. Five compounds **1**, **3**, **4**, **6** and **8** were found to activate melatonin receptors. Especially, compound **1** showed agonistic effects on MT_1 and MT_2 receptors with EC_{50} values of 237 and 244 μM. For better understanding their structure-activity relationships (SARs), ten polybenzyl analogs were further synthesized and assayed for their activities on melatonin receptors. Preliminary SARs study suggested that two *para*-hydroxy groups were the key pharmacophore for maintaining activity. Molecular docking simulations verified that compound **1** could strongly interact with MT_2 receptor by bonding to Phe 118, Gly 121, His 208, Try 294 and Ala 297 residues.

1. Introduction

Melatonin receptors, consisting of MT_1 and MT_2 subtypes, are vital targets for accommodating sleep disorder via regulating circadian rhythms.[1–4] Several melatonin receptor-mediated drugs have been on the market for treating insomnia and depression, involving prolonged release melatonin (Circadin®, Neurim, Israel, UK),[5] ramelteon (Rozerem®, Takeda Pharmaceutical Co, Osaka, Japan)[6] and tasimelteon (Hetlioz®, Vanda Pharmaceuticals, USA).[5] The currently published melatonin receptor agonists are almost synthetic compounds with highly structural and metabolic similarity to melatonin.[7–9] In contrast, only a few melatonin natural agonists, *e.g.* magnolol, tropine, dimeric isoechinulin-type alkaloid, peaoveitols and gramnie derivatives have been reported.[10–15] Regarding to the importance of natural products in drug development,[16] it is meaningful to discover new scaffolds of melatonin agonists from natural sources.

Gastrodia elata is a famous traditional Chinese medicine widely used for the treatment of hypertension, headache, convulsions and neurodegenerative diseases. Recent study manifested that gastrodin,[17] N^6-(4-hydroxybenzyl) adenosine (NHBA) and N^6-(3-methoxyl-4-hydroxybenzyl) adenine riboside (B2)[18] from *G. elata* had the potency of sedation and hypnosis, of which NHBA took effects by activating adenosine receptors.[19] However few literatures reported about the psychoactive effects of polybenzyls, a characteristic type of compounds in *G. elata*.

In this research, the EtOAc extract of *G. elata* was first found to activate MT_2 receptor with an agonistic rate of 105.4% at the concentration of 102.2 μg/mL. In order to characterize the active constituents, bioactivity-guided isolation yielded nine polybenzyls (**1**−**9**, Fig. 1). Compounds **1**, **6** and **8** showed agonistic effect on MT_2 receptor, among which compound **1** could obviously stimulate MT_1 and MT_2 receptors with EC_{50} values of 237 and 244 μM. For better understanding the structure-activity relationships, ten derivatives (**10**−**19**, Fig. 2) were further synthesized. Herein, we report their isolation, structural modification and agonistic effects on MT receptors.

2. Material and methods

2.1. General instruments and chemical reagents

NMR spectra were undertaken by Avance III-400 and III-600 spectrometers (Bruker, Bremerhaven, Germany). LCMS-IT-TOF (Shimadzu,

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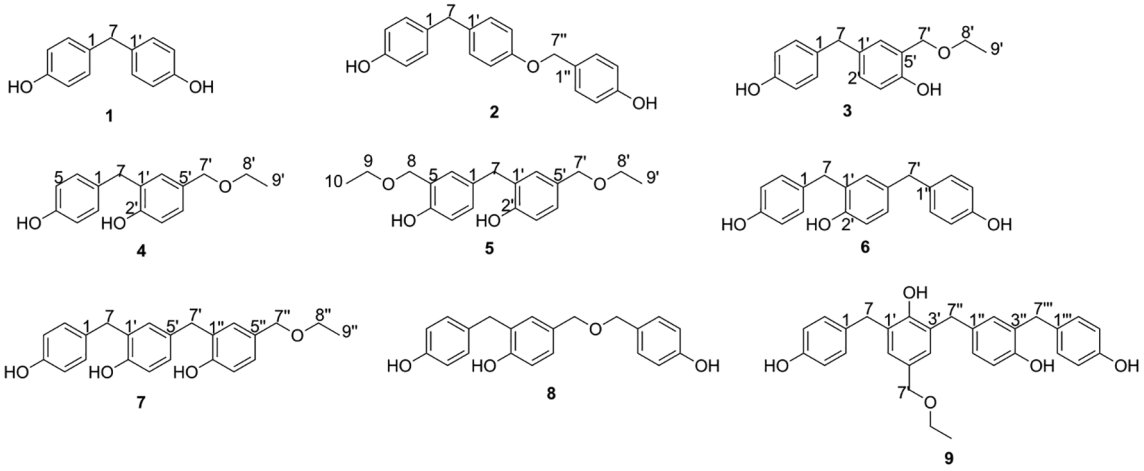


Fig. 1. Structures of compounds **1 – 9**.

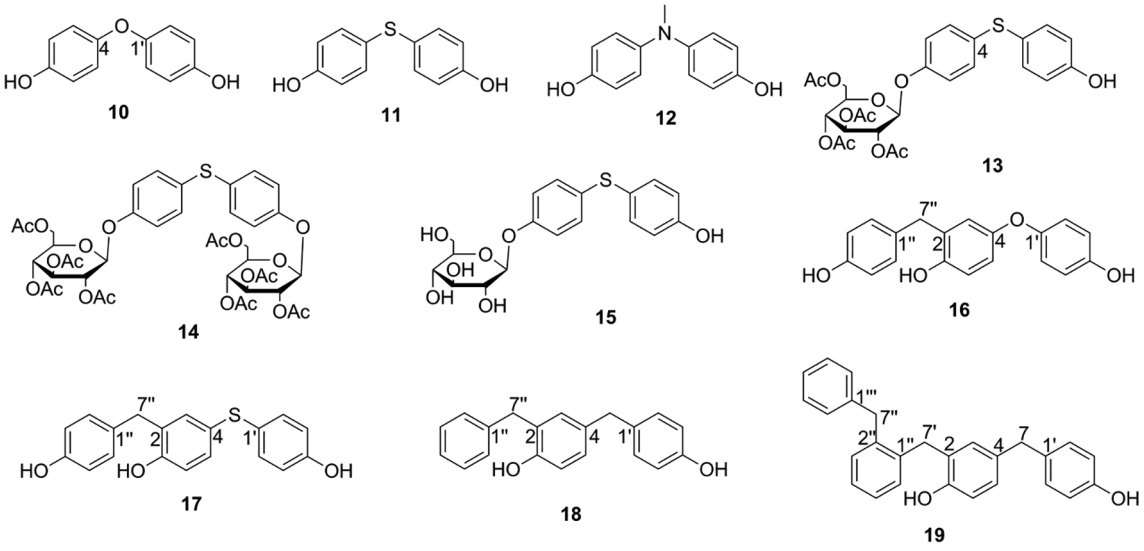


Fig. 2. Structures of synthetic compounds **10 – 19**.

Kyoto, Japan) was used for detecting molecular mass spectra. IR spectra were obtained on NICOLET iS10 (Thermo scientific, Waltham, USA) or Bio-Rad FTS-135 (Hercules, California, USA) spectrometers using ATR ITX-DIAMOND mode or KBr pellets. UV spectra were recorded on a UV-2401 equipment (Shimadzu, Kyoto, Japan). Thin-layer chromatography (TLC) analyses were performed on silica gel GF254 plates (Jiangyou, Chemical Co. Ltd., Yantai, China). Compounds were purified by silica gel (200–300 mesh, Qingdao Makall group Co. Ltd., Qingdao, China) and Sephadex LH-20 (Amersham Bioscience, Sweden) column chromatography. MPLC was performed on a Dr-Flash II apparatus (Lisui, Suzhou, China) using a CHP20P MCI gel column (Mitsubishi, Tokyo, Sweden), and HPLC was conducted on a Shimadzu LC-CBM-20 system (Shimadzu, Kyoto, Japan) with an Agilent XDB-C_{18} column (9.4 × 250 mm, Agilient, California, USA). Bioactive assay was conducted on a FlexStation3 Benchtop Multi-Mode Microplate Reader (Molecular Devices, Sunnyvale, California, USA). Acetonitrile (chromatographic grade, Merk Co. Ltd., Darmstadf, Germany) and formic acid (chromatographic grade, Aladdin Chemistry Co. Ltd., Shanghai, China) were used for HPLC. Chemical reagents for modification were purchased from J&K Co. Ltd. (Shanghai, China). Melatonin as the positive control was obtained from Damas-beta Co. Ltd. (Shanghai, China).

2.2. Plant material

Gastrodia elata Bl. was bought from Zhaotong in Yunnan Province of China, in September 2014, and was identified by Prof. Li-Gong Lei, Kunming Institute of Botany, CAS. A voucher specimen (No. 20141107) was deposited at the Laboratory of Anti-virus and Natural Medicinal Chemistry, Kunming Institute of Botany, CAS.

2.3. Extraction and isolation

Fresh rhizomes of *G. elata* (45.0 kg) were cut into slices and extracted with 90% aqueous ethanol (45 L × 3) for three times at room temperature. The combined extract was concentrated under reduced pressure and partitioned between H_2O and EtOAc. The EtOAc extract (88 g) was separated by silica gel column chromatography (CC) using gradient elution with EtOAc-$CHCl_3$ (0:100, 5:95, 10:90, 20:80, 40:60, 100:0 *v/v*) as the mobile phase to yield five fractions (Fr. 1-Fr. 5). Based on the bioassay, Fr. 2 (22 g) was separated by silica gel CC with EtOAc-petroleum ether system (5:95, 10:90, 20:80, 40:60, 100:0) to yield five fractions (Fr. 2.1-Fr. 2.5). Then, Fr. 2.4 (3.4 g) was further separated by silica gel CC (Me_2CO-petroleum ether, 3:97, 5:95, 10:90, 20:80, 50:50) to give five fractions (Fr. 2.4.1-Fr. 2.4.5). Fr. 2.4.4 was isolated by Sephadex LH-20 (MeOH-$CHCl_3$, 50:50) and silica gel CC (Me_2CO-$CHCl_3$, 10:90) to give compound **1** (1 g). Compounds **2** (12 mg), **5**

(6 mg) and **3** (120 mg) were purified from Fr. 2.4.5 by HPLC on an Agilent XDB-C_{18} column with the elution of MeCN-H_2O (40:60). Fr. 3 was subjected to silica gel CC (Me_2CO-petroleum ether, 5:95, 10:90, 20:80, 40:60, 100:0) to yield five fractions (Fr. 3.1-Fr. 3.5). Further purified by silica gel CC, Sephadex LH-20 CC (MeOH-$CHCl_3$ 50:50) and semi-preparative HPLC to yield compound **4** (1 g, MeCN-H_2O, 23:77, t_R = 11.5 min) from Fr. 3.1, compounds **6** (700 mg, MeCN-H_2O 45:55, *v/v*, t_R = 19 min) and **7** (11 mg, MeCN-H_2O, 45:55, t_R = 26 min) from Fr. 3.2, and compounds **8** (35 mg, MeCN-H_2O, 50:50, t_R = 20 min) and **9** (27 mg, MeCN-H_2O, 33:67, t_R = 20 min) from Fr. 3.5.

2.4. Spectroscopic data

The known compounds **1**, **6** and **8** were determined as bisphenol F (**1**),[20] 2,4-bis(4-hydroxybenzyl)phenol (**6**)[21] and gastrol (**8**)[22] by comparing their MS and NMR data with the previous reports.

2.4.1. Compound *2*

White powder; UV (MeOH) λ_{max} (log ε): 277 (3.59), 252 (2.91), 230 (4.38), 215 (1.21) nm; IR (KBr) ν_{max}: 3427, 1613, 1513, 1455, 1340, 1306, 1234, 1171 cm^{-1}; HRMS (ESI) *m/z* 305.1168 $[M-H]^-$ (calcd for 305.1183). ^1H NMR and ^{13}C NMR see Tables 1 and 2.

2.4.2. Compound *3*

White powder; UV (MeOH) λ_{max} (log ε): 281 (3.51), 252 (2.61), 229 (4.08), 220 (4.04) nm; IR (KBr) ν_{max}: 3424, 1614, 1598, 1512, 1444, 1553, 1248, 1076 cm^{-1}; HRMS (ESI) *m/z* 257.1171 $[M-H]^-$ (calcd for 257.1183). ^1H NMR and ^{13}C NMR see Tables 1 and 2.

2.4.3. Compound *4*

Pale yellow powder; UV (MeOH) λ_{max} (log ε): 280 (3.35), 251 (2.50), 225 (3.96), 217 (3.92) nm; IR (KBr) ν_{max}: 3405, 1613, 1512, 1441, 1352, 1261, 1232, 1067 cm^{-1}; HRMS (ESI) *m/z* 257.1166 $[M-H]^-$ (calcd for 257.1183). ^1H NMR and ^{13}C NMR see Tables 1 and 2.

2.4.4. Compound *5*

White powder; UV (MeOH) λ_{max} (log ε): 281 (3.45), 251 (2.68) nm; IR (KBr) ν_{max}: 3420, 1631, 1614, 1509, 1441, 1373, 1352, 1266, 1095, 1074 cm^{-1}; HRMS (ESI) *m/z* 315.1577 $[M-H]^-$ (calcd for 315.1602). ^1H NMR and ^{13}C NMR see Tables 1 and 2.

2.4.5. Compound *7*

Pale yellow colloidal solid; UV (MeOH) λ_{max} (log ε): 281 (3.77), 253 (2.98) nm; IR (KBr) ν_{max}: 3425, 1613, 1511, 1440, 1373, 1257, 1228, 1099, 1068 cm^{-1}; HRMS (ESI) *m/z* 363.1588 $[M-H]^-$ (calcd for 363.1602). ^1H NMR and ^{13}C NMR see Tables 1 and 2.

2.4.6. Compound *9*

Pale yellow colloidal solid; UV (MeOH) λ_{max} (log ε): 281 (3.84), 253 (3.12) nm; IR (KBr) ν_{max}: 3440, 1632, 1615, 1513, 1443, 1236, 1072,

Table 2
^{13}C NMR (150 MHz, CD_3OD) data of compounds **2–5** and **7**.

No	2	3	4	5	7
1	132.6	132.8	133.5	133.5	133.9
2	129.3	129.4	131.0	130.5	130.9
3	114.7	114.8	116.1	116.1	116.0
4	155.1	155.0	156.4	154.9	156.4
5	114.7	114.8	116.1	125.3	116.0
6	129.3	129.4	131.0	131.3	130.9
7	39.7	39.8	35.8	35.9	36.0
8	–	–	–	69.4	–
9	–	–	–	66.3	–
10	–	–	–	15.6	–
1′	134.2	132.9	129.8	129.8	129.4
2′	129.3	128.9	156.0	156.1	154.3
3′	114.5	114.8	115.9	115.9	116.0
4′	157.2	153.4	128.5	128.5	128.6
5′	114.5	123.9	130.3	130.3	133.4
6′	129.3	129.6	131.8	131.9	132.3
7′	–	67.9	73.8	73.9	35.8
8′	–	65.5	66.4	66.9	–
9′	–	14.1	15.5	15.5	–
1″	128.2	–	–	–	129.9
2″	129.1	–	–	–	156.0
3″	114.8	–	–	–	115.8
4″	156.9	–	–	–	128.4
5″	114.8	–	–	–	130.3
6″	129.1	–	–	–	131.8
7″	69.7	–	–	–	73.9
8″	–	–	–	–	66.4
9″	–	–	–	–	15.5

Table 1
^1H NMR (600 MHz, *J* in Hz, CD_3OD) data of compounds **2–5** and **7**.

No	2	3	4	5	7
2	6.98 (1H, d, 8.5)	6.97 (1H, d, 8.6)	7.03 (1H, d, 8.6)	6.98 (1H, m)	6.99 (1H, d, 8.5)
3	6.70 (1H, d, 8.5)	6.72 (1H, d, 8.6)	6.68 (1H, d, 8.6)	6.68 (1H, d, 8.2)	6.65 (1H, d, 8.5)
5	6.70 (1H, d, 8.5)	6.72 (1H, d, 8.6)	6.68 (1H, d, 8.6)		6.65 (1H, d, 8.5)
6	6.98 (1H, d, 8.5)	6.97 (1H, d, 8.6)	7.03 (1H, d, 8.6)	7.10 (1H, d, 2.1)	6.99 (1H, d, 8.5)
7	3.79 (2H, s)	3.74 (2H, s)	3.82 (2H, s)	3.83 (2H, s)	3.78 (2H, s)
8	–	–	–	4.50 (2H, s)	–
9		–	–	3.54 (2H, q, 7.0)	–
10	–	–	–	1.19 (3H, t, 7.0)	–
2′	7.06 (1H, d, 8.5)	6.92 (1H, dd, 8.2, 2.1)	–	–	–
3′	6.87 (1H, d, 8.5)	6.73 (1H, d, 8.2)	6.75 (1H, d, 8.1)	6.74 (1H, d, 8.0)	6.66 (1H, d, 8.1)
4′	–	–	6.98 (1H, dd, 8.1, 2.2)	6.99 (1H, m)	6.85 (1H, dd, 8.1, 2.0)
5′	6.87 (1H, d, 8.5)	–	–	–	–
6′	7.06 (1H, d, 8.5)	7.04 (1H, d, 2.1)	6.95 (1H, d, 2.2)	6.96 (1H, d, 2.1)	6.86 (1H, brs)
7′	–	4.50 (2H, s)	4.29 (2H, s)	4.33 (2H, s)	3.77 (2H, s)
8′	–	3.53 (2H, q, 7.0)	3.45 (2H, q, 7.0)	3.47 (2H, q, 7.0)	–
9′	–	1.19 (3H, t, 7.0)	1.15 (3H, t, 7.0)	1.16 (3H, t, 7.0)	–
2″	7.25 (1H, d, 8.5)	–	–	–	–
3″	6.79 (1H, d, 8.5)	–	–	–	6.73 (1H, d, 8.1)
4″	–	–	–	–	6.97 (1H, dd, 8.1, 2.1)
5″	6.79 (1H, d, 8.5)	–	–	–	–
6″	7.25 (1H, d, 8.5)	–	–	–	6.89 (1H, d, 2.1)
7″	4.89 (2H, s)	–	–	–	4.30 (2H, s)
8″	–	–	–	–	3.45 (2H, q, 7.0)
9″	–	–	–	–	1.15 (3H, t, 7.0)

Table 3
^{1}H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) data of compound **9**.

No	δ_H (J in Hz)	δ_C	No	δ_H (J in Hz)	δ_C
1	–	133.2	1″	–	132.9
2	7.03 (1H, d, 8.5)	130.9	2″	6.86 (1H, d, 2.1)	132.2
3	6.70 (1H, d, 8.5)	116.2	3″	–	132.6
4	–	156.5	4″	–	154.3
5	6.70 (1H, d, 8.5)	116.2	5″	6.70 (1H, d, 8.5)	116.0
6	7.03 (1H, d, 8.5)	130.9	6″	6.85 (1H, dd, 8.5, 2.1)	128.6
7	3.88 (2H, s)	36.2	7″	3.84 (2H, s)	36.2
1′	–	130.4	1‴	–	133.8
2′	–	153.2	2‴	7.01 (1H, d, 8.5)	131.0
3′	–	132.9	3‴	6.67 (1H, d, 8.5)	116.0
4′	6.81 (1H, d, 1.8)	129.6	4‴	–	156.3
5′	–	130.7	5‴	6.67 (1H, d, 8.5)	116.0
6′	6.76 (1H, d, 1.8)	129.7	6‴	7.01 (1H, d, 8.5)	131.0
7′	4.25 (2H, s)	73.8	7‴	3.80 (2H, s)	35.9
8′	3.43 (2H, q, 7.0)	66.4	–	–	–
9′	1.13 (3H, t, 7.0)	15.6	–	–	–

1047 cm^{-1}; HRMS (ESI) m/z 469.2024 $[M-H]^-$ (calcd for 469.2020). ^{1}H NMR and ^{13}C NMR see Table 3.

2.5. Schemes of synthetic compounds

The synthesis of compounds **12**−**19** were depicted in Schemes 1–3. In the first attempt, the methylene groups was replaced by different hetero atoms to speculate the influence of the methylene on activity (compound **12**, Scheme 1). Next, glycosylation was applied to see the influence of a hydroxy group (compounds **13**−**15**, Scheme 2). Additional phenyls were introduced into the structure to assess the effect of enlarged skeleton (compounds **16**−**19**, Scheme 3).

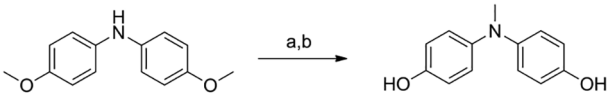
2.5.1. Compound *12*

To a stirred solution of 4,4′-dimethoxy diphenylamine (115 mg, 0.5 mmol) in DMSO (2 mL), sodium hydroxide (1 mL, 1 M) was added and the reaction mixture was stirred at room temperature for 30 min. Then methyl iodide was added and stirred for additional 1 h. The reaction was diluted with H_2O (10 mL) and extracted with ethyl acetate (10 mL × 3). The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated under *vacuum*. To a solution of the residue in dry CH_2Cl_2 (10 mL) at −78 °C, BBr_3 solution (2 mL, 5 M in THF) was added dropwise. The reaction was stirred for 1 h and slowly warmed to 0 °C for 3 h. The crude solution was quenched with saturated sodium bicarbonate and extracted with EtOAc (10 mL × 3). The organic phase was washed with brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. Column chromatography of the residue on silica gel CC (acetone-petroleum ether 20:80) provided compound **12**.

*2.5.1.1. 4-(4-Methylimino) bisphenol (**12**).* Pale purple powder, 66% yield. HRMS (ESI) m/z 214.0878 $[M-H]^-$ (calcd for 214.0874). UV (MeOH) λ_{max} (log ε): 282 (3.78), 261 (3.70), 247 (3.77), 230 (3.67) nm; IR (KBr) ν_{max}: 3424, 1636, 1509, 1451, 1235 cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD) δ_H: 6.72 (4H, d, J = 8.5 Hz), 6.43 (4H, d, J = 8.5 Hz), 3.2 (3H, s); ^{13}C NMR (100 MHz, CD_3OD) δ_C: 148.2, 142.1, 122.4, 116.8, 37.8.

2.5.2. Compounds *13* and *14*

To a solution of the β-D-glucose penta-acetate (390 mg, 1 mmol) in 10 mL CH_2Cl_2 was added HBr (2 mL, 2 M in AcOH) dropwise at 0 °C. After being stirred for 4 h, the mixture was pour into ice-cold water and extracted with CH_2Cl_2. The organic layer was washed with saturated sodium bicarbonate, brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure to yield of crude bromide. 4,4′-Thiobisphenol (109 mg, 0.5 mmol) was dissolved in potassium carbonate (10 mL, 2 M) and stirred for 30 min. Then, the mixture was added to the solution of crude bromide and TBAB (322 mg, 1 mmol) in CH_2Cl_2 (10 mL) and stirred for 3 h at room temperature. The resulting mixture was quenched with 5% HCl and extracted with EtOAc (10 mL × 3). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude was purified by silica gel CC (acetone-petroleum ether 20:80) to yield of compounds **13** and **14**.



Scheme 1. Synthesis of compound **12**. (a) CH_3I, DMSO, rt, 2 h; (b) BBr_3, CH_2Cl_2, −78 °C to 0 °C, 4 h.

*2.5.2.1. 4-S-(4-Phenol) phenol 1-O-penta-O-acetyl-β-D-glucopyranoside (**13**).* White powder, 11% yield. HRMS (ESI) m/z 547.1296 $[M-H]^-$ (calcd for 547.1280). UV (MeOH) λ_{max} (logε): 251 (4.13), 238 (4.04), 230 (4.07), 219 (4.01) nm; IR (KBr) ν_{max}: 3424, 1751, 1742, 1633, 1601, 1584, 1492, 1431, 1371, 1262, 1231, 1077, 1051 cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD) δ_H: 7.25 (2H, d, J = 8.5 Hz), 7.15 (2H, d, J = 8.5 Hz), 6.94 (2H, d, J = 8.5 Hz), 6.79 (2H, d, J = 8.5 Hz), 5.38 − 4.05 (7H, m), 2.01 − 2.04 (12H,$COCH_3$); ^{13}C NMR (100 MHz, CD_3OD) δ_C: 170.9–169.7 ($COCH_3$), 157.6, 155.5, 134.5, 130.2, 132.3, 123.6, 117.2, 116.0, 98.3, 72.7 − 61.6, 19.2 ($COCH_3$).

*2.5.2.2. 4-S-(4-Phenol) phenol 1,4′-O-di-penta-O-acetyl-β-D-glucopyranoside (**14**).* White powder, 10% yield. HRMS (ESI) m/z 923.2300 $[M + COOH]^-$ (calcd for 923.2285). UV (MeOH) λ_{max} (log ε): 252 (4.12), 225 (4.20), 239 (4.05), 219 (4.18) nm; IR ν_{max}: 1745, 1491, 1367, 1224, 1075, 1054, 1034 cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD) δ_H: 7.32 (4H, d, J = 8.7 Hz), 7.07 (4H, d, J = 8.7 Hz), 5.45–4.06 (14H), 2.02 − 1.79 (24H, $COCH_3$); ^{13}C NMR (100 MHz, CD_3OD) δ_C: 169.8 − 168.8 ($COCH_3$), 156.5, 132.7, 129.7, 117.7, 98.2, 72.4–61.8, 19.7 − 18.5 ($COCH_3$).

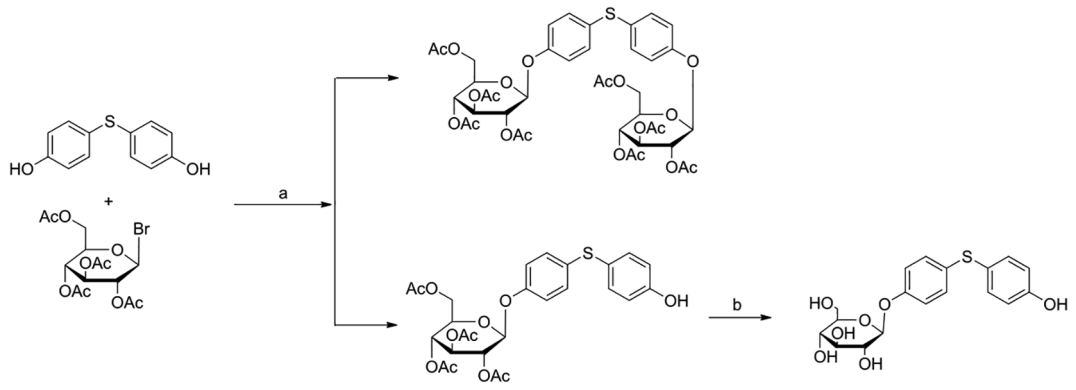
2.5.3. Compound *15*

Sodium hydroxide (5 mL, 1 M) was added to the solution of 4-*S*-(4-phenol) phenol 1-*O*-penta-*O*-acetyl-β-D-glucopyranoside (27 mg, 0.05 mmol) in DMSO (3 mL) at room temperature. After 1 h, the reaction mixture was quenched with 5% HCl and extracted with EtOAc (10 mL × 3). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Compound **15** was purified by silica gel CC (MeOH-$CHCl_3$ 10:90).

*2.5.3.1. 4-S-(4-Phenol) phenol 1-O-β-D-glucopyranoside (**15**).* White powder, 79% yield. HRMS (ESI) m/z 379.0866 $[M-H]^-$ (calcd for 379.0857). UV (MeOH) λ_{max} (log ε): 251 (4.19), 231 (4.16), 239 (4.12), 219 (4.07) nm; IR (KBr) ν_{max}: 3442, 1634, 1490, 1227, 1068, 1037 cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD) δ_H: 7.24 (2H, d, J = 8.6 Hz), 7.17 (2H, d, J = 8.6 Hz), 7.03 (2H, d, J = 8.6 Hz), 6.78 (2H, d, J = 8.6 Hz), 3.90 − 3.42 (7H, m); ^{13}C NMR (100 MHz, CD_3OD) δ_C: 157.4, 156.6, 134.0, 130.7, 130.8, 124.3, 117.1, 115.9, 100.9, 76.7 − 61.1.

2.5.4. Compounds *16* and *17*

To a stirred solution of 4,4′-oxybisphenol (404 mg, 2 mmol) or 4,4′-thiobisphenol (437 mg, 2 mmol) in 1,4-dioxane (7.5 mL) and toluene (2.5 mL) at room temperature was added sequentially *p*-hydroxybenzyl alcohol (124 mg, 1 mmol) and TsOH (0.4 mg, 0.2 mmol). The mixture was heated at 60 °C for 3 h. The reaction mixture was quenched with saturated sodium bicarbonate and extracted with EtOAc (10 mL × 3). The organic layers were washed with brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residues were purified by silica gel CC (EtOAc-petroleum ether, 10:90) and



Scheme 2. Synthesis of compounds **13 – 15**. (a) K_2CO_3, TBAB, CH_2Cl_2, rt, 3 h; (b) NaOH, DMSO, rt, 1 h.

delivered compounds **16** and **17**.

*2.5.4.1. 2-(4-Hydroxybenzyl)-4-O-(4-phenol)-1,4-bisphenol (**16**).* Pale yellow powder, 19% yield. HRMS (ESI) *m/z* 307.0963 $[M-H]^-$ (calcd for 307.0976). UV (MeOH) λ_{max} (log ε): 285 (3.76), 260 (3.20), 227 (4.27), 220 (4.25) nm; IR (KBr) ν_{max}: 3520, 3424, 1613, 1500, 1441, 1357, 1206, 1174 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ_H: 7.03 (2H, d, J = 8.4 Hz), 6.76–6.68 (8H, m), 6.61(1H, brs), 3.77 (2H, s); ^{13}C NMR (100 MHz, CD_3OD) δ_C: 155.0 – 150.3, 131.7, 129.7, 129.4, 120.2, 118.9, 116.4, 115.5, 115.1, 114.6, 34.4.

*2.5.4.2. 2-(4-Hydroxybenzyl)-4-S-(4-phenol) phenol (**17**).* Pale yellow powder, 13% yield. HRMS (ESI) *m/z* 323.0748 $[M-H]^-$ (calcd for 323.0747). UV (MeOH) λ_{max} (log ε): 277 (3.82), 273 (3.82), 228 (4.24), 221 (4.22) nm; IR (KBr) ν_{max}: 3442, 1636, 1615, 1513, 1492, 1413, 1239 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ_H: 7.10 (2H, d, 8.4), 7.01–7.00 (4H, m), 7.73 – 6.67 (5H, m), 3.78 (2H, s); ^{13}C NMR (100 MHz, CD_3OD) δ_C: 156.5, 155.0, 154.4, 133.3, 130.0, 132.3, 131.6, 129.5, 129.4, 126.4, 125.8, 115.6, 114.6, 115.2, 34.2.

*2.5.5. Compounds **18** and **19***

A mixture of 4,4′-methylenebisphenol (200 mg, 1 mmol), benzyl alcohol (0.2 mL, 2 mmol) and phosphoric acid (1 mL, 17 mmol) in toluene (10 mL) was kept at 100 °C for 6 h. The reaction was quenched with saturated sodium bicarbonate and extracted with EtOAc (10 mL × 3). The organic layers were washed with brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification of the crude by silica gel CC (EtOAc-petrleum ether 10:90) provided compounds **18** and **19**.

*2.5.5.1. 2-Benzyl-4-(4-hydroxybenzyl) phenol (**18**).* Pale yellow powder, 22% yield. HRMS (ESI) *m/z* 289.1214 $[M-H]^-$ (calcd for 289.1234). UV (MeOH) λ_{max} (log ε): 281 (3.63), 253 (2.90) nm; IR (KBr) ν_{max}: 3443, 1636, 1615, 1511, 1444, 1252 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ_H: 7.24–7.10 (5H, m), 6.94 (2H, d, J = 8.5 Hz), 6.83 (1H, brs), 6.81 (1H, m), 6.71 – 6.66 (3H, m), 3.90 (2H, s), 3.71 (2H, s); ^{13}C NMR (100 MHz, CD_3OD) δ_C: 155.0, 152.9, 141.1, 132.9, 132.8, 130.6, 129.3, 128.4, 127.7, 127.4, 127.0, 125.2, 114.6, 114.5, 39.7, 35.2.

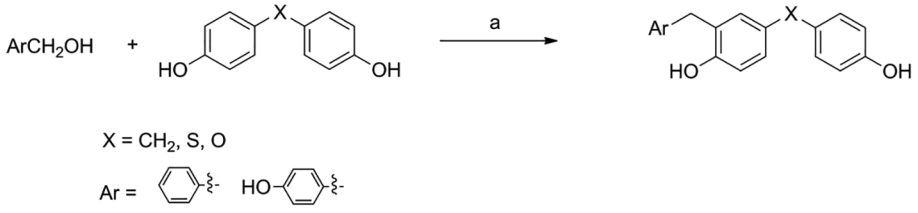
*2.5.5.2. 2-(2-Benzyl)benzyl-4-(4-hydroxybenzyl) phenol (**19**).* Pale yellow powder, 23% yield. HRMS (ESI) *m/z* 379.1706 $[M-H]^-$ (calcd for 379.1704). UV (MeOH) λ_{max} (log ε): 281 (3.21), 253 (2.61) nm; IR (KBr) ν_{max}: 3398, 1613, 1607, 1511, 1451, 1252, 1237, 1219, 1200, 1096 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ_H: 7.26 – 6.66 (16H, m), 3.90 (2H, s, H-7′), 3.85 (2H, s), 3.69 (2H, s); ^{13}C NMR (100 MHz, CD_3OD) δ_C: 154.9, 152.8, 141.6, 139.0, 138.5, 133.0, 132.8, 130.6, 129.3, 128.5, 128.3, 128.0, 127.6, 127.0, 125.5, 114.6, 41.0, 39.7, 35.2.

2.6. Molecular docking

The docking model of MT_2 receptor from Rivara[23] was chosen in this research because the crystal structure of melatonin receptors was not released yet, and docking model was validated by Rivara. Among these models, Rho_{ACT} model with ligand **7a**, a melatonin agonist,[24] was chosen as a docking pocket for its high $EF^{2\%}$ and ever usage in previous study.[23,25] AutoDock Vina, a free software (http://vina.scripps.edu), was applied for molecular docking[26] since the algorithms of AutoDock Vina for scoring function were widely applied.[26,27] In docking simulation study, the position of the original compound in Rho_{ACT} model was firstly redefined as a binding site of docking pocket. Then compound **1** and melatonin were docked into the model by AutoDock Vina. After docking, binding affinities were calculated by AutoDock Vina as a standard parameter to estimate whether compound **1** could bind to the central location as that of melatonin. The interactions between protein and compounds were analyzed by Discovery Studio 4.5.

2.7. Agonistic assay on MT_1 and MT_2 receptors

Agonistic activities of MT_1 and MT_2 receptors were assayed on HEK293 cell line in accordance with the previous study.[28,29] Cells were cultivated in 5% CO_2 incubator at 37 °C by adding Dulbecco's modified eagle medium, G418 (400 μg/mL) and fetal bovine serum (10%). Cells were put at a density of 4×10^4/well in a Matrigel® coated 96-well black plate and proliferated in CO_2 for 24 h. Subsequently, cells were dyed by Wash Free Fluo-8 Calcium Assay Kit (HD Biosicences Co. Ltd., Hd03-0010) and placed in CO_2 incubator for 1 h. Tested compounds and positive control were dissolved in 10 μL DMSO and 990 μL HBSS Buffer, respectively, and a volume of 100 μL/well was put in another 96-well plate. Then two 96-well plates were put into Flex Station 3



X = CH_2, S, O

Ar =

Scheme 3. Synthesis of compounds **16 – 19**. (a) TsOH, 1,4-dioxane-toluene, 60 °C, 3 h (compounds **16–17**) or H_3PO_4, toluene, 100 °C, 6 h (compounds **18 – 19**).

Benchtop Multi-Mode Micro plate Reader (Molecular Devices, Sunnyvale, California, USA), and compounds or positive control was added a volume of 50 μL/well in 96-well plate with cells automatically. Data were recorded and read via Flex Station 3 Benchtop Multi-Mode Micro plate Reader at room temperature using specified settings (excitation wave length: 485 nm, emission wave length, 525 nm, emission cut-off, 515 nm). EC_{50} values were calculated by GraphPad Prism 5 software.

3. Results and discussion

3.1. Structural elucidation

3.1.1. Compound 2

Compound **2** had a molecular formula of $C_{20}H_{18}O_3$ with twelve degrees of unsaturation, which was deduced from negative HR-ESI-MS at *m/z* 305.1168 (calcd. for 305.1183). In the ^{1}H NMR spectrum, three sets of AB coupled protons at δ_H 7.25 (2H, d, J = 8.5 Hz) and 6.79 (2H, d, J = 8.5 Hz), 7.06 (2H, d, J = 8.5 Hz) and 6.87 (2H, d, J = 8.5 Hz), and 6.98 (2H, d, J = 8.5 Hz) and 6.70 (2H, d, J = 8.5 Hz) indicating the presence of three *para*-substituted phenyls. In the shielded region, two methylenes at δ_H 4.89 (2H, s) and 3.79 (2H, s) were recognized. Compared with **1**, compound **2** had an additional 4-hydroxybenzyl moiety: δ_H 7.25 (2H, d, J = 8.5 Hz, H-2″ and H-6″), 6.79 (2H, d, J = 8.5 Hz, H-3″ and H-5″) and 4.89 (2H, s, H-7″); δ_C 156.9 (C-4″), 129.1 (C-2″ and C-6″), 128.2 (C-1″), 114.8 (C-3″ and C-5″) and 69.7 (C-7″). Based on the HMBC correlation from H-7″ (δ_H 4.89) to C-4′ (δ_C 157.2), the ether linkage between C-7″ and C-4′ was established (Fig. 3). Thus, compound **2** was elucidated as gastropolybenzylol A.

3.1.2. Compound 3

Compound **3** had a molecular formula of $C_{16}H_{18}O_3$ with eight degrees of unsaturation, which was speculated from $[M-H]^-$ ion at *m/z* 257.1171 (calcd for 257.1183). Compound **3** had similar NMR data with **1** besides an extra ethoxymethyl: δ_H 4.50 (2H, s, H-7′), 3.53 (2H, q, H-8′) and 1.19 (3H, t, H-9′); δ_C 67.9 (C-7′), 65.5 (C-8′), 14.1 (C-9′). The alkylation position at C-5′ (δ_C 123.9) was proved by the key HMBC correlations of H-7′ (δ_H 4.50, 2H, s) with C-4′ (δ_C 153.4), C-6′ (δ_C 129.6) and C-8′ (δ_C 65.5). Thus, compound **3** was defined as gastropolybenzylol B.

3.1.3. Compound 4

Compound **4** showed a molecular formula of $C_{16}H_{18}O_3$ with eight degrees of unsaturation according to the $[M-H]^-$ ion at *m/z* 257.1166 (calcd for 257.1183). Detailed analysis of their NMR data manifested the differences of δ_C 39.8 (C-7) and 67.9 (C-7′) in **3** *vs* δ_C 35.8 (C-7) and 73.8 (C-7′) in **4**. This analysis suggested that the hydroxyl group at C-4′ in **3** might migrate to C-2′ in **4**. Further evidence was established by the HMBC correlations of H-7 (δ_H 3.82, 2H, s) with C-2′ (δ_C 156.0), and of H-7′ (δ_H 4.29, 2H, s) with C-4′ (δ_C 128.5). Finally, compound **4** was established as gastropolybenzylol C.

3.1.4. Compound 5

The molecular formula of **5** was determined to be $C_{19}H_{24}O_4$ with eight degrees of unsaturation from the $[M-H]^-$ ion at *m/z* 315.1577 (calcd for 315.1602). Compared with the NMR spectra of **4**, compound **5** possessed one more ethoxymethyl: δ_H 4.50 (2H, s, H-8), 3.54 (2H, q, H-9) and 1.19 (3H, t, H-10); δ_C 69.4 (C-8), 66.3 (C-9) and 15.6 (C-10). The position of ethoxymethyl group was located at C-5 (δ_C 125.3) by the HMBC correlation of H-8 (δ_H 4.50, s) with C-5 (δ_C 125.3), C-4 (156.4) and C-6 (131.3). As a result, compound **5** was elucidated as gastropolybenzylol D.

3.1.5. Compound 7

Compound **7** was assigned a molecular formula of $C_{23}H_{24}O_4$ with 12 degrees of unsaturation based on the $[M-H]^-$ ion at *m/z* 363.1588 (calcd for 363.1602). Its ^{1}H NMR indicated the existence of two sets of ABX coupled protons [δ_H 6.97 (1H, dd, J = 8.1, 2.1 Hz), 6.89 (1H, d, J = 2.1 Hz) and 6.73 (1H, d, J = 8.1 Hz); 6.86 (1H, brs), 6.85 (1H, dd, J = 8.1, 2.0 Hz) and 6.66 (1H, d, J = 8.1 Hz)], one set of AB coupled protons [δ_H 6.99 (2H, d, J = 8.5 Hz) and 6.65 (2H, d, J = 8.5 Hz)] and four sets of methylenes [δ_H 4.30 (2H, s), 3.78 (2H, s), 3.77 (2H, s) and 3.45 (2H, q)], implying **7** with one more 4-hydroxybenzyl moiety than **4**. The HMBC correlations of H-7 (δ_H 3.78, 2H, s) with C-2 (δ_C 130.9), C-6 (δ_C 130.9), C-2′ (δ_C 154.3) and C-6′ (δ_C 132.3) proved that a 4-hydroxybenzyl moiety was attached to C-1′ (δ_C 129.4) (Fig. 3). As a result, compound **7** was determined as gastropolybenzylol E.

3.1.6. Compound 9

Compound **9** was assigned a molecular formula of $C_{30}H_{30}O_5$ with 16 degrees of unsaturation according to the $[M-H]^-$ ion at *m/z* 469.2024 (calcd for 469.2020). The ^{1}H NMR showed an ethoxyl [δ_H 3.43 (2H, q) and 1.13 (3H, t)] and five methylenes [δ_H 4.25(2H, s), 3.88 (2H, s), 3.84 (2H, s), 3.80 (2H, s) and 3.43 (2H, q)]. The remaining signals were all aromatic protons with two sets of AB coupled protons [δ_H 7.03 (2H, d, J = 8.5 Hz) and 6.70 (2H, d, J = 8.5 Hz); 7.01(2H, d, J = 8.5 Hz) and 6.67 (2H, d, J = 8.5 Hz)], one ABX coupled protons [δ_H 6.86 (1H, d,

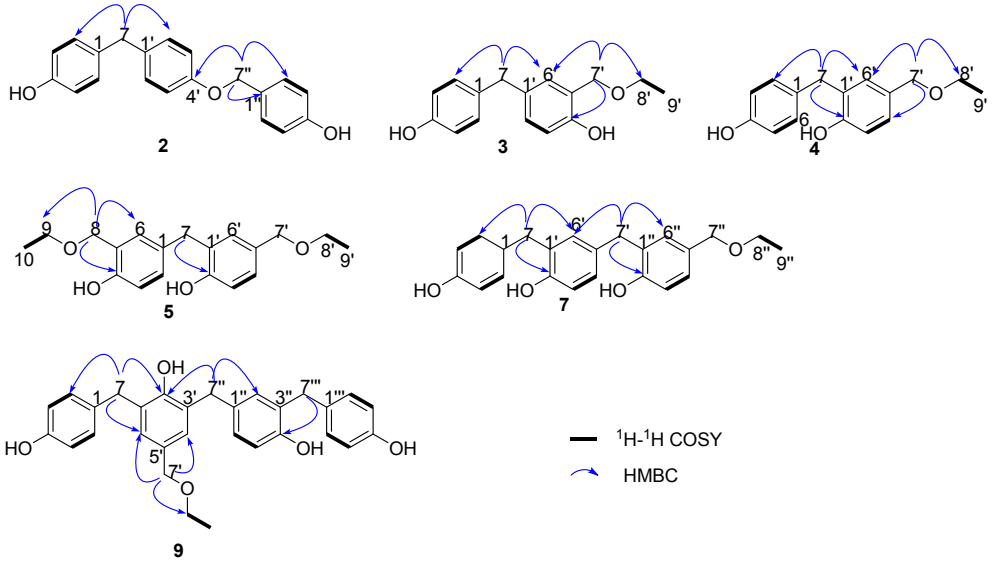


Fig. 3. Key ^{1}H − ^{1}H COSY and the HMBC correlation of compounds **2** − **5**, **7** and **9**.

Table 4
Agonistic rates of compounds **1–19** on MT_1 and MT_2 receptors (0.5 mM).

Comp.	Agonistic rates (%)		Comp.	Agonistic rates (%)	
	MT_1	MT_2		MT_1[2]	MT_2
1	80.3 ± 14.4	103.0 ± 7.1	**10**	45.9 ± 18.0	67.1 ± 13.0
2	−5.6 ± 1.6	15.3 ± 0.3	**11**	44.6 ± 6.4	94.1 ± 54.7
3	37.0 ± 18.1	117.1 ± 16.0	**12**	−9.5 ± 0.8	44.7 ± 7.6
4	3.3 ± 0.3	38.3 ± 13.3	**13**	0.3 ± 1.8	8.5 ± 1.8
5	−11.2 ± 1.7	5.3 ± 4.4	**14**	−0.4 ± 3.0	−3.6 ± 1.7
6	46.5 ± 4.5	134.2 ± 31.5	**15**	−2.3 ± 1.7	−2.2 ± 1.9
7	−5.7 ± 0.5	−10.4 ± 1.0	**16**	37.1 ± 28.8	51.2 ± 7.7
8	25.1 ± 16.8	59.4 ± 4.1	**17**	31.3 ± 16.4	48.4 ± 33.0
9	−5.3 ± 2.6	29.49 ± 1.6	**18**	−8.6 ± 1.7	−6.6 ± 1.3
			19	−2.3 ± 0.4	−6.7 ± 6.0

The agonistic activities were expressed as Mean ± SD (n = 3). Melatonin was used as the positive control with EC_{50} values of 1.0 nM (MT_1) and 25.0 nM (MT_2).

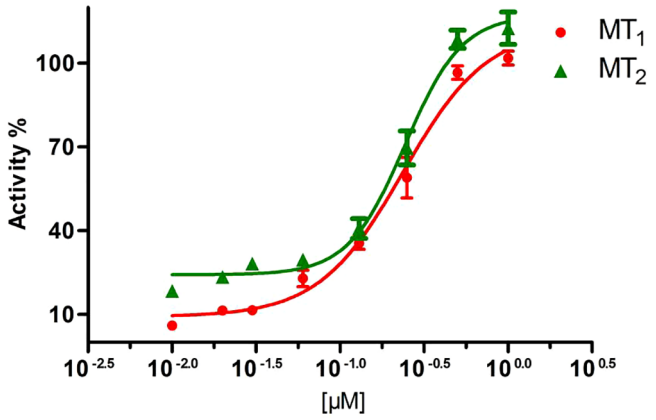


Fig. 4. The dose-dependent effects of compound **1** on MT_1 and MT_2 receptors, which provided the EC_{50} values of 237 μM and 244 μM, respectively. The agonistic activities were expressed as Mean ± SD (n = 3).

J = 2.1 Hz), 6.85 (1H, dd, J = 8.5, 2.1 Hz) and 6.70 (1H, d, J = 8.5 Hz)] and a 1,2,3,5-subtituted aromatic protons [δ_H 6.81 (1H, d, J = 1.8 Hz) and 6.76 (1H, d, J = 1.8 Hz)]. Compared with the NMR spectra of **7**, compound **9** had one more 4-hydroxybenzyl moiety: δ_H 7.03 (2H, d, J = 8.5 Hz, H-2 and H-6), 6.70 (2H, d, J = 8.5 Hz, H-3 and H-5) and 3.88 (2H, s, H-7); δ_C 156.5 (C-4), 133.2 (C-1), 130.9 (C-2 and C-6), 116.2 (C-3 and C-5) and 36.2 (C-7). The HMBC correlations of H-7 (δ_H 3.88, 2H, s) and H-7″ (δ_H 3.84, 2H, s) with C-2′ (δ_C 153.2) verified the connection. Accordingly, compound **9** was defined as gastropolybenzylol F.

3.2. Agonistic activity and structure-activity relationships

Agonistic activities of all compounds on melatonin receptors were evaluated at the concentration of 0.5 mM (Table 4). Compound **1** showed potent activities on MT_1 and MT_2 receptors with agonistic rates of 80.3 ± 14.4% and 103.0 ± 7.1%. Compounds **10**, **11** and **12** containing heteroatoms showed decreased activities, indicating the methylene moiety were crucial to the activity. When one of the hydroxyl group in **1** was etherified by 4-hydroxybenzyl moiety, the obtained derivative **2** lost activity. The same results were observed in **13**−**15** by comparing with **11**, which suggested that two hydroxyls were crucial for maintaining activity. Compounds **4** and **5** with an adjacent hydroxyl group (C-2′) were inactive in this tests, indicating the importance of two *para*-hydroxyl groups (C-4′). Based on the above results, two *para*-hydroxy groups were supposed as the vital site in binding to melatonin receptors. The trimer **6** showed activities on $MT_{1/2}$ receptors with agonistic rates of 46.5 ± 4.5% and 134.9 ± 31.5%, while the agonistic activities of **16** and **17** decreased when heteroatom was introduced into the structure. The tetramers **9** and **19** showed no activity, which might be due to the steric hindrance. The most active **1** was further investigated for the dose-response relationship on MT_1 and MT_2 receptors, which provided the EC_{50} values of 237 μM and 244 μM, respectively (Fig. 4).

3.3. Potential mechanisms of compound *1* for activating MT_2 receptor

Binding affinity could verify the interaction between compounds and receptors. The binding affinities of compound **1** and melatonin with MT_2 receptor were −6.9 kcal/mol and −6.4 kcal/mol, respectively, suggesting that **1** could interact with MT_2 receptor. To further predict interaction between the MT_2 receptor and ligands, pharmacophore analysis was performed by Discovery Studio 4.5. Compound **1** showed interaction with Phe 118, Gly 121, His 208, Try 294 and Ala 297 residues (Fig. 5), similar to melatonin (Fig. 6). Furthermore, two *para*-hydroxyl groups could form hydrogen bonds with the His 208 and Phe 118 residues of MT_2 receptor.

4. Conclusion

In this research, polybenzyls were first revealed with agonistic effects on melatonin receptors. Especially, compound **1** showed fascinating effects on MT_1 and MT_2 receptors with EC_{50} values of 237 and 244 μM. For better understanding their structure-activity relationships, both synthesis and docking simulation were conducted, which suggested that two *para*-hydroxy groups were the key pharmacophore for maintaining activity. Compound **1** was considered as a potential candidate for melatonergic agonists.

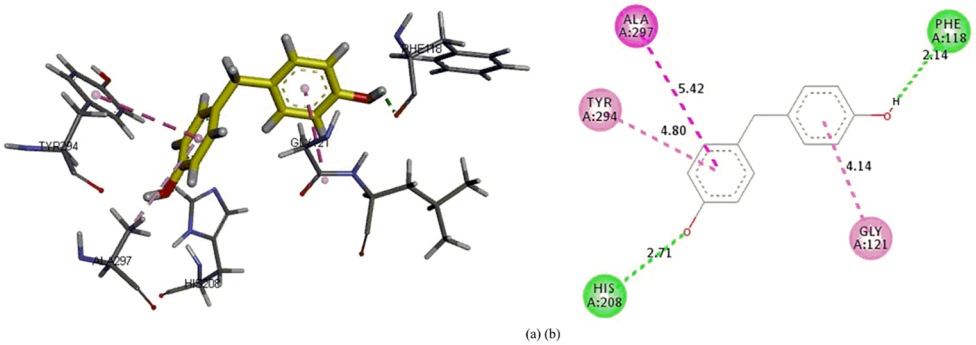


Fig. 5. Interaction between compound **1** (yellow) and MT_2 receptor in 3D diagram (a: left) and 2D diagram (b: right). Hydrogen bonds and hydrophobic effects were marked as green and pink balls, respectively. The binding affinity was −6.9 kcal/mol.

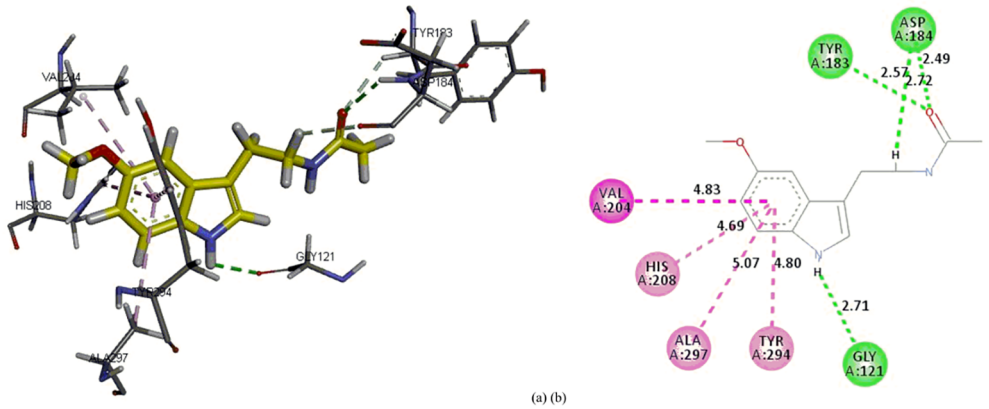


Fig. 6. Interaction between melatonin (yellow) and MT_2 receptor in 3D diagram (a: left) and 2D diagram (b: right). Hydrogen bonds and hydrophobic effects were marked as green and pink balls, respectively. The binding affinity was −6.4 kcal/mol.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data (1D, 2D NMR, UV, IR, HRESIMS spectra for compounds **2-5**, **7** and **9**; 1D NMR, UV, IR, HRESIMS spectra for the derivatives **12-19**) to this article can be found online at https://doi.org/10.1016/j.bmc.2019.06.008.

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