Synthesis and Biological Evaluation of Berberine Derivatives as IBS Modulator

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Abstract: Irritable bowel syndrome is the most common functional gastrointestinal disorder characterized by chronic abdominal pain or discomfort in association with a change in bowel habit. 5-HT receptor modulators have been developed as IBS therapeutic agents and proved to be effective in the treatment of the disease. In this letter, 12 berberine derivatives were designed and synthesized as 5-HT receptor modulators. Preliminary biological tests suggested that the new compounds exhibited promising activity for IBS therapy.

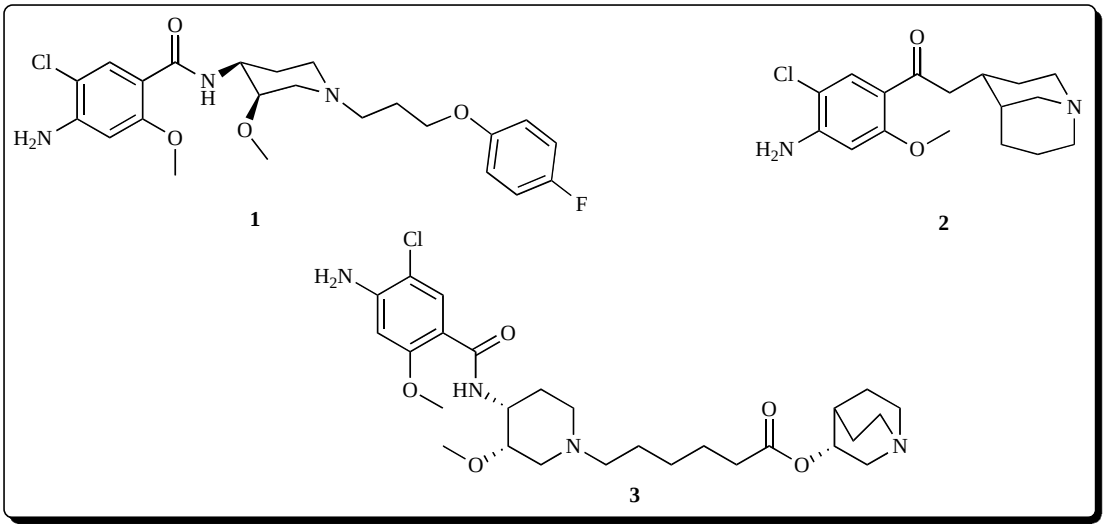
Keywords: 5-hydroxytryptamine, 5-hydroxytryptamine receptor modulators, Berberine, Derivatives, Gastrointestinal disorder, Irritable bowel syndrome.

INTRODUCTION

Irritable bowel syndrome (IBS) is the most common functional gastrointestinal (GI) disorder characterized by chronic abdominal pain or discomfort in association with a change in bowel habit [1,2]. According to the Rome II definition, the prevalence of IBS in North America is 4.7% to 11.4% and the rates vary from 1% to 8% in other western countries [3]. Although only a minority ever seek the health care for their symptoms, IBS is still one of the most common disorders in primary care or specialist clinics [4]. In addition, IBS can severely affect the individual who suffer's quality of life and is deemed to a major cause of work absenteeism [5,6].

Despite the prevalence and impact of IBS in the community, the aetiological mechanism of IBS remains unknown [7]. The pathophysiology of IBS is most likely multifactorial, such as abnormal GI motor function, visceral hypersensitivity, autonomic dysfunction, mucosal immune activation, alteration of intestinal flora and psychological factors [2,8,9]. 5-hydroxytryptamine (5-HT) is an important neurotransmitter in the GI tract. Recent studies demonstrate that 5-HT plays a crucial role in the modulation of gut function, such as motility, sensation, blood flow and secretion [10,11]. It has also been reported that the plasma 5-HT levels were abnormal in IBS patients [12].

Given the important role of 5-HT in IBS, 5-HT receptor modulators such as cisapride (**1**), renzapride (**2**), and ATI-7505 (**3**) have been developed as IBS therapeutic agents and proved to be effective in the treatment of the disease [8].



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These 5-HT receptor modulators have a similar structure and López-Rodríguez etc. have summarized their pivotal pharmacophores: 1) an aromatic moiety, 2) a carbonyl function, and 3) nitrogen heterocyclic ring (Fig. (**1**)) [13].

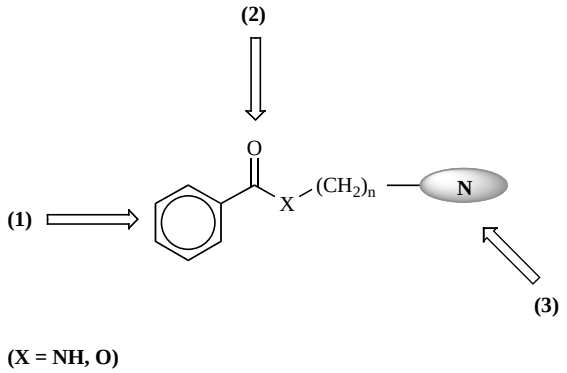
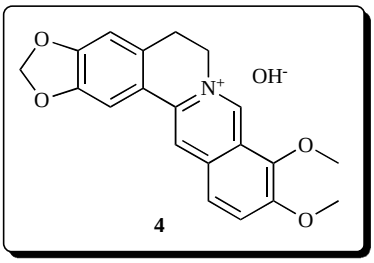


Fig. (1). The pharmacophore model for 5-HT receptor modulators. (1) aromatic moiety, (2) carbonyl function, (3) nitrogen heterocyclic ring.

Although 5-HT receptor modulators contribute to the therapy for IBS, the serious adverse effects, such as severe constipation, ischemic colitis, higher cerebrovascular and cardiac events, result in a number of 5-HT receptors being withdrawn from the market [14].

Berberine (**4**) is a natural isoquinoline alkaloid present in plants of the genus Coptis, Hydrastis and Berberis [15,16]. In China, berberine has been used for the treatment of gastroenteritis and diarrhea. Clinical trials have verified that berberine is an effective drug with lower toxicity [17]. It has been shown that berberine can greatly relieve the main symptoms of IBS patients [18]. Furthermore, berberine has poor intestinal absorption [19] implying that berberine and its derivatives might be difficult to enter into circulation and have less side effects.

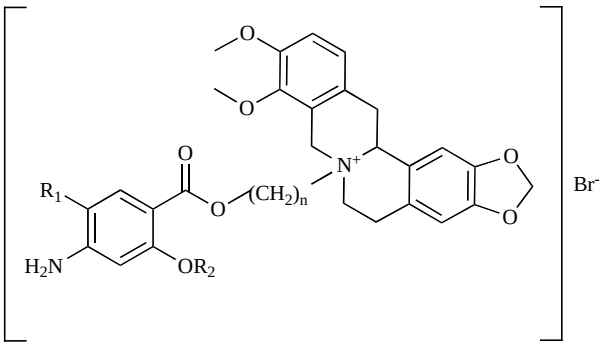


MATERIALS AND METHODS

Based on the information above, we have designed and synthesized a series of berberine derivatives (Table **1**) as novel 5-HT receptor modulators. Among these compounds, we employed tetrahydroberineper as the nitrogen heterocyclic ring and the berberine-like structure might inhibit the absorption in GI tract that attenuates the systemic side effects.

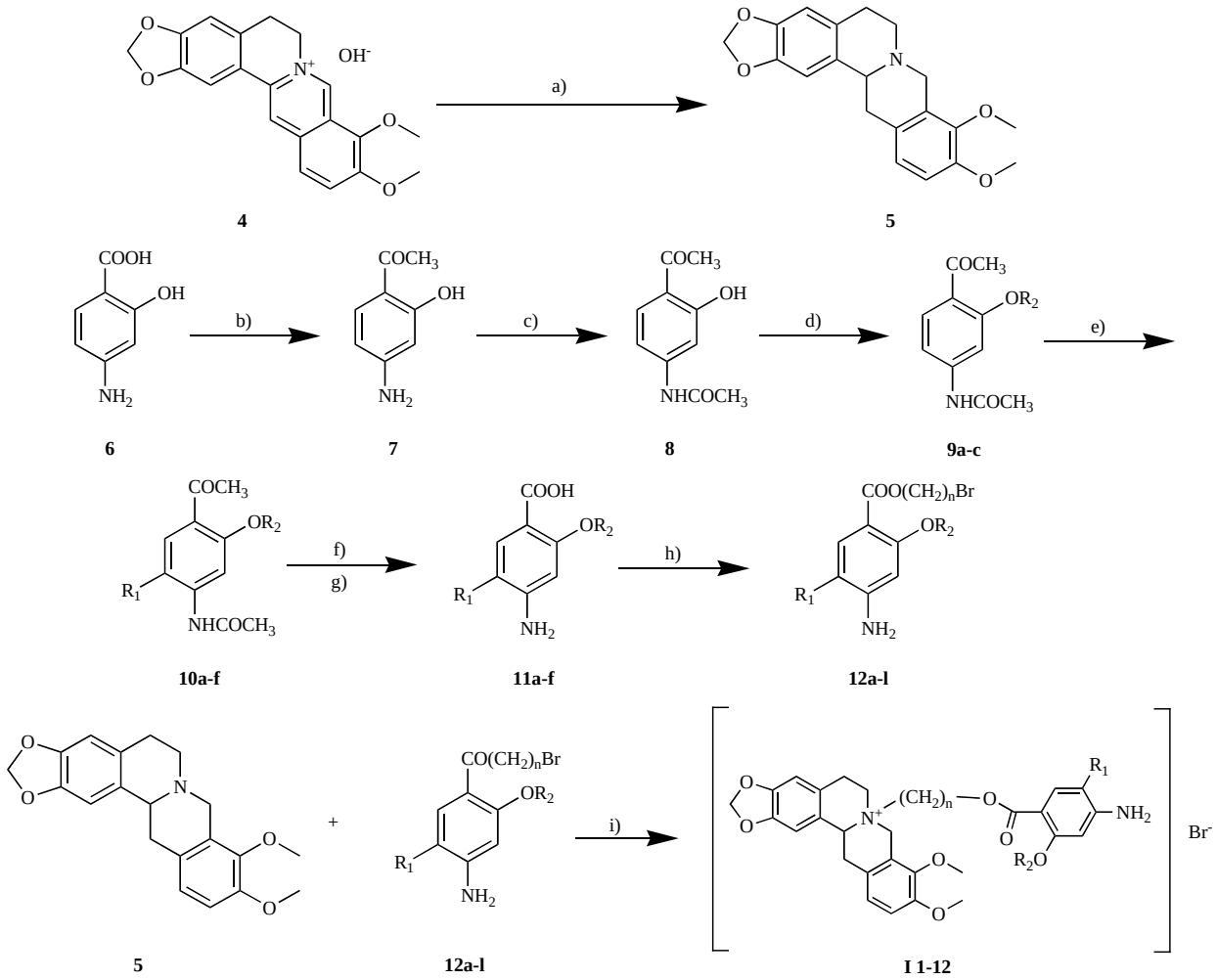
Twelve berberine derivatives were prepared as shown in Scheme **1**. Hydrogenation of commercially available berberine **4** by $NaBH_4$ resulted in tetrahydroberineper **5**. Compound **7** was converted from para-amino salicylic acid **6** with MeOH and concentrated sulfuric acid under room temperature for 2h. Then solid **7** was dissolved in acetic acid, acetic anhydride was added to react for 1h. After adding suitable amount of water, compound **8** was obtained. The phenolic hydroxyl group of **8** that was substituted by dimethyl sulphate, ethyl bromide and propyl bromide respectively yielded compound **9**. Halogenating **9** by NCS and NBS produced compound **10**. Hydrolization of **10** with aqueous NaOH and 95% C_2H_5OH, then neutralized by chlorhydric acid separate out **11**. Compound **11** reacted with bromoethanol and bromopropanol catalyzed by EDC and DMAP in dichlormethane provided **12**. Finally, the quaternary ammonium salt forming reaction was performed between compound **5** and **12** in nitromethanc under 100℃ [20], the target compounds **I 1-12** [21] were obtained after 24h.

Table 1. Structure of Target Compounds I 1-12



Compound	n	R_1	R_2
I 1	2	Cl	$-CH_3$
I 2	3	Cl	$-CH_3$
I 3	2	Cl	$-CH_2CH_3$
I 4	3	Cl	$-CH_2CH_3$
I 5	2	Cl	$-CH_2CH_2CH_3$
I 6	3	Cl	$-CH_2CH_2CH_3$
I 7	2	Br	$-CH_3$
I 8	3	Br	$-CH_3$
I 9	2	Br	$-CH_2CH_3$
I 10	3	Br	$-CH_2CH_3$
I 11	2	Br	$-CH_2CH_2CH_3$
I 12	3	Br	$-CH_2CH_2CH_3$

A rat model of IBS was induced by the method reported by Zou Baicang *et al.* and Jun-Ho La *et al.* Briefly, during a short anesthesia with ethylether, 4% acetic acid solution (40ml/L) was injected intrarectally *via* a polyethylene catheter inserted 8cm proximal to the anus. 6 days after acetic acid solution administration, then animals underwent constraint stimulation for 3 hours. Test agents were administered intragastrically for 7 days. Animals were assigned to the following treatment groups, each consisting of 8 rats: compounds **I 1-12** (5mg/kg) and berberine (5mg/kg). IBS-untreated group (blank group) received 0.3%



Scheme 1. Synthesis of **I 1-12**: **a**) $NaBH_4$, 90%C_2H_5OH, reflux; **b**) CH_3OH, 98%H_2SO_4; **c**) $(CH_3CO)_2O$, CH_3COOH; **d**) $(CH3)_2SO_4$ or C_2H_5Br or n-C_3H_7Br, NaOH, DMF; **e**) NCS or NBS, DMF; **f**) 30%NaOH, 95%C_2H_5OH; **g**) HCl; **h**) EDC, DMAP, $Br(CH_2)_nOH$; **i**) CH_3NO_2 100℃.

Table 2. Results of Water Ratio of IBS Rat Feces[a]

Compound	1d (%)	3d (%)	5d (%)	7d (%)
I 1	79.81±1.41	74.43±0.68*	74.00±0.81*	71.31±2.08**
I 2	79.69±1.91	80.28±0.61	73.41±0.80*	67.17±1.68***
I 3	80.50±0.70	78.31±0.90	77.36±0.90	76.79±1.65*
I 4	79.22±1.12	69.14±0.21**	63.37±0.59***	56.44±0.60***
I 5	80.63±0.96	73.43±2.15*	72.45±1.16*	70.31±1.03**
I 6	79.97±0.44	73.22±1.23*	70.75±1.00**	62.64±0.77***
I 7	81.45±0.21	72.60±2.08*	71.65±1.74**	68.56±1.85**
I 8	80.83±1.00	73.83±1.33*	69.13±2.35**	67.64±0.77***
I 9	81.29±2.07	79.54±0.54	72.53±0.40*	69.20±1.52**
I 10	80.19±1.11	74.31±0.78*	71.21±0.90**	68.83±0.91**
I 11	80.33±0.75	74.73±4.50	70.68±2.14**	67.26±1.89***
I 12	80.21±1.37	76.31±1.31*	73.52±0.73*	69.31±0.82**
berberine	80.03±1.62	73.46±2.75*	71.78±1.95**	69.43±0.77**
blank	80.50±1.11	80.56±2.87	79.27±0.75	80.82±1.56

*P < 0.05; **P < 0.01; ***P < 0.001. (Compare to the blank group).

[a]The water ratio of rat feces was evaluated at the first, third, fifth and seventh day during administration. The lower value of water ratio means the faster recovery of IBS.

Table 3. Results of Rectal Distension-Induced Nociception of IBS Rats[a]

Compound	1d (ml)	3d (ml)	5d (ml)	7d (ml)
I 1	0.1167±0.0152	0.1600±0.0100**	0.1733±0.0115*	0.2000±0.0100**
I 2	0.1125±0.0125	0.1750±0.0191**	0.1900±0.0258*	0.1950±0.0100**
I 3	0.1100±0.0100	0.1333±0.0115	0.1467±0.0115	0.1900±0.0141*
I 4	0.1109±0.0100	0.1800±0.0200**	0.1933±0.0230**	0.2367±0.0057***
I 5	0.1175±0.0035	0.1225±0.0035	0.1700±0.0141*	0.1800±0.0282*
I 6	0.1109±0.0100	0.1700±0.0200**	0.2050±0.0100***	0.2575±0.0170***
I 7	0.1100±0.0100	0.1517±0.0076**	0.2000±0.0200**	0.2200±0.0200**
I 8	0.1167±0.0057	0.1500±0.0100**	0.1733±0.0115*	0.2300±0.0100***
I 9	0.1097±0.0115	0.1600±0.0200**	0.1933±0.0152**	0.2000±0.0200**
I 10	0.1167±0.0082	0.1583±0.0075**	0.1883±0.0075*	0.2067±0.0082**
I 11	0.1100±0.0063	0.1550±0.0054**	0.1933±0.0152**	0.2100±0.0089**
I 12	0.1117±0.0075	0.1633±0.0052**	0.1900±0.6724*	0.2100±0.0063**
berberine	0.1167±0.0057	0.1467±0.0115*	0.1533±0.0230	0.1933±0.0503*
blank	0.1167±0.0057	0.1167±0.0057	0.1333±0.0015	0.1500±0.0100

*P < 0.05; **P < 0.01; ***P < 0.001. (Compare to the blank group).

[a]The thresholds of rectal distension-induced nociception of rats were tested at the first, third, fifth and seventh day during administration. The higher volume value indicates the lower visceral sensitivity.

CMC-Na solution to serve as a control. To evaluate the anti-IBS activities of berberine derivatives **I 1-12**, the feces water ratio and rectal distension-induced nociception of IBS rats were tested respectively during the period of administration [22,23]. The results are presented in Tables **2** and **3**. As it can be seen from the data, all of the new compounds exhibited favorable effect on IBS therapy, such as reduced the water ratio of feces, relieved rectal distension-induced nociception. Especially the compounds **I 4** and **I 6** showed higher activity than berberin. The results might provide insight into the further research on these compounds for IBS therapy.

CONCLUSION

In conclusion, twelve berberine derivatives were designed and synthesized. The biological evaluation tests suggested that all the novel compounds showed potential for IBS therapy, and two of which exhibited promising activity that were capable of further researching.

CONFLICT OF INTEREST

Declared none.

ACKNOWLEDGEMENTS

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[21] Chemical data of compound **I 1**: yield 29.1%, marron crystal, m.p.200-201℃, 1H NMR (DMSO-d6, 300MHz, δppm): 7.50(s, 1H, ArH), 6.83-7.11(m, 4H, ArH), 6.27(s, 1H, ArH), 6.03(s, 2H, OCH2O), 5.03(m, 1H, NCH), 4.69(m, 2H, OCH2CH2N), 4.46(m, 2H, OCH2CH2N), 4.08(s, 3H, ArOCH3), 3.79-3.82 (d, 6H, 9,10-OCH3), 2.50-3.50 (m, 8H); MS(ESI) m/z: [M-Br-H]+ 569.1; IR(KBr,υ): 3470, 3028, 1725, 1599, 1497, 1280, 873.

I 2: yield 27.5%, amber crystal, m.p.172-173℃, 1H NMR(DMSO-d6, 300MHz, δppm): δ: 7.51(s, 1H, ArH), 6.84-7.18(m, 4H, ArH), 6.28(s, 1H, ArH), 6.03(s, 2H, OCH2O), 5.06(m, 1H, NCH), 4.61(m, 2H, OCH2CH2N), 4.47(m, 2H, OCH2CH2N), 4.07(s, 3H, ArOCH3), 3.81-3.83 (d, 6H, 9, 10-OCH3), 2.17-3.50 (m, 10H); m/z: [M-Br-H]+ 581.2; IR(KBr,υ): 3453, 2975, 1723, 1600, 1456, 1182, 854.

I 3: yield 28.3%, amber crystal, m.p.204-205℃, 1H NMR(DMSO-d6, 300MHz, δppm): 7.92(d, 1H, ArH), 7.82(d, 1H, ArH), 7.53(s, 1H, ArH), 7.34(s, 1H, ArH), 7.10(s, 1H, ArH), 6.76(s, 1H, ArH), 6.44(s, 2H, OCH2O), 5.38(m, 1H, NCH), 4.82(m, 2H, OCH2CH2N), 4.09-4.10(d, 6H, 9,10-OCH3),2.28-3.86(m, 12H), 0.89(t, 3H, OCH2CH2CH3); m/z: [M-Br-H]+ 581.1; IR(KBr,υ): 3300, 2942, 1715, 1607, 1507, 1261, 888.

I 4: yield 31.1%, amber crystal, m.p.166-167℃, 1H NMR(DMSO-d6, 300MHz, δppm): 8.10(d, 1H, ArH), 7.90(d, 1H, ArH), 7.71(s, 1H, ArH), 7.39(s, 1H, ArH), 7.12(s, 1H, ArH), 6.44(s, 1H, ArH), 6.24(s, 2H, OCH2O), 5.39(m, 1H, NCH), 4.84(m, 2H, OCH2CH2N), 4.08-4.09(d, 6H, 9,10-OCH3),1.54-3.96(m, 14H), 0.86(t, 3H, OCH2CH2CH3); m/z: [M-Br-H]+ 595.2; IR(KBr,υ): 3344, 2937, 1711, 1600, 1504, 1275, 855.

I 5: yield 27.3%, amber crystal, m.p.197-198℃, 1H NMR(DMSO-d6, 300MHz, δppm): 8.21(d, 1H, ArH), 8.00(d, 1H, ArH), 7.80(s, 1H, ArH), 7.50(s, 1H, ArH), 7.10(s, 1H, ArH), 6.46(s, 1H, ArH), 6.18(s, 2H, OCH2O), 5.45(m, 1H, NCH), 4.96(m, 2H, OCH2CH2N), 4.07-4.09(d, 6H, 9,10-OCH3),2.00-3.90(m, 14H), 0.82(t, 3H, OCH2CH2CH3); m/z: [M-Br-H]+ 595.1; IR(KBr,υ): 3340, 2961, 1710, 1605, 1497, 1281, 873.

I 6: yield 21.5%, amber crystal, m.p.165-166℃, 1H NMR(DMSO-d6, 300MHz, δppm): 8.22(d, 1H, ArH), 7.92(d, 1H, ArH), 7.81(s, 1H, ArH), 7.51(s, 1H, ArH), 7.11(s, 1H, ArH), 6.76(s, 1H, ArH), 6.17(s, 2H, OCH2O), 5.41(m, 1H, NCH), 4.98(m, 2H, OCH2CH2N), 4.09-4.11(d, 6H, 9,10-OCH3), 1.34-3.87(m, 16H), 0.83(t, 3H, OCH2CH2CH3); m/z: [M-Br-H]+ 609.3; IR(KBr,υ): 3415, 2923, 1700, 1602, 1494, 1275, 854.

I 7: yield 24.0%, amber crystal, m.p.200-201℃, 1H NMR(DMSO-d6, 300MHz, δppm): δ: 7.90(s, 1H, ArH), 7.28-7.55(m, 4H, ArH), 6.83(s, 1H, ArH), 6.31(s, 1H, ArH), 6.03(s, 2H, OCH2O), 5.04(m, 1H, NCH), 4.71(m, 2H, OCH2CH2N), 4.50(m, 2H, OCH2CH2N), 4.05(s, 3H, ArOCH3), 3.79-3.82 (d, 6H, 9, 10-OCH3), 2.50-3.50 (m, 8H); m/z: [M-Br+H]+ 613.0; IR(KBr,υ): 3500, 2960, 1703, 1626, 1497, 1281, 887.

I 8: yield 26.5%, amber crystal, m.p.175-176℃, 1H NMR(DMSO-d6, 300MHz, δppm): δ: 7.84(s, 1H, ArH), 7.28-7.55(m, 4H, ArH), 6.84(s, 1H, ArH), 6.32(s, 1H, ArH), 6.03(s, 2H, OCH2O), 5.07(m, 1H, NCH), 4.62(m, 2H, OCH2CH2N), 4.48(m, 2H, OCH2CH2N), 4.05(s, 3H, ArOCH3), 3.79-3.84 (d, 6H, 9, 10-OCH3), 2.50-3.50 (m, 10H); m/z: [M-Br+H]+ 627.2; IR(KBr,υ): 3416, 2918, 1705, 1631, 1494, 1246.

I 9: yield 31.5%, amber crystal, m.p.165-166℃, 1H NMR(DMSO-d6, 300MHz, δppm): 8.22(d, 1H, ArH), 8.01(d, 1H, ArH), 7.81(s, 1H, ArH), 7.35(s, 1H, ArH), 7.09(s, 1H, ArH), 6.45(s, 1H, ArH), 6.30(s, 2H, OCH2O), 5.37(m, 1H, NCH), 4.95(m, 2H, OCH2CH2N), 4.07-4.10(d, 6H, 9,10-OCH3), 2.00-3.90(m, 12H), 0.87(t, 3H, OCH2CH3); m/z: [M-Br+H]+ 627.1; IR(KBr,υ): 3410, 2901, 1700, 1621, 1497, 1280, 872.

I 10: yield 27.9%, amber crystal, m.p.181-182℃, 1H NMR(DMSO-d6, 300MHz, δppm): 8.21(d, 1H, ArH), 8.00(d, 1H, ArH), 7.81(s, 1H, ArH), 7.34(s, 1H, ArH), 7.10(s, 1H, ArH), 6.45(s, 1H, ArH), 6.29(s, 2H, OCH2O), 5.35(m, 1H, NCH), 4.97(m, 2H, OCH2CH2N), 4.06-4.12(d, 6H, 9,10-OCH3), 1.46-3.90(m, 14H), 0.83(t, 3H, OCH2CH3); m/z: [M-Br+H]+ 641.2; IR(KBr,υ): 3571, 2926, 1711, 1594, 1503, 1273, 855.

I 11: yield 21.8%, amber crystal, m.p.195-196℃, 1H NMR(DMSO-d6, 300MHz, δppm): 8.22(d, 1H, ArH), 8.02(d, 1H, ArH), 7.81(s, 1H, ArH), 7.35(s, 1H, ArH), 7.11(s, 1H, ArH), 6.45(s, 1H, ArH), 6.27(s, 2H, OCH2O), 5.40(m, 1H, NCH), 4.96(m, 2H, OCH2CH2N), 4.00-4.07(d, 6H, 9,10-OCH3), 1.34-3.90(m, 14H), 0.84(t, 3H, OCH2CH3); m/z: [M-Br-H]+ 639.0; IR(KBr,υ): 3517, 2942, 1705, 1607, 1497, 1281, 887.

I 12: yield 22.6%, amber crystal, m.p.177-178℃, 1H NMR(DMSO-d6, 300MHz, δppm): 8.13(d, 1H, ArH), 7.91(d, 1H, ArH), 7.71(s, 1H, ArH), 7.41(s, 1H, ArH), 7.13(s, 1H, ArH), 6.64(s, 1H, ArH), 6.15(s, 2H, OCH2O), 5.41(m, 1H, NCH), 5.01(m, 2H, OCH2CH2N), 4.09-4.10(d, 6H, 9,10-OCH3), 1.37-3.86(m, 16H), 0.87(t, 3H, OCH2CH3); m/z: [M-Br+H]+ 653.0; IR(KBr,υ): 3504, 2943, 1710, 1600, 1505, 1275, 871.

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