European Journal of Medicinal Chemistry 45 (2010) 55–62

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and antitumor activity of *cis*-dichloridoplatinum(II) complexes of 1,1′-biisoquinolines

Chen-Yuan Kuo [a,*], Ming-Jung Wu [b], Chu-Chieh Lin [c]

[a] Department of Biological Science and Technology, Yung-Ta Institute of Technology and Commerce, 316 JhongShan Road, Pingtung 909, Taiwan, ROC
[b] Department of Chemistry, National Sun Yat-Sen University, Kaohsiung, Taiwan, ROC
[c] Department of Chemistry, National Chung-Hsing University, Taichung 402, Taiwan, ROC

ARTICLE INFO

Article history:
Received 23 March 2009
Received in revised form
27 July 2009
Accepted 10 September 2009
Available online 16 September 2009

Keywords:
Biisoquinoline
Antitumor activity
Bischler – Napieralski

ABSTRACT

A simple approach to 1,1′,2,2′,3,3′,4,4′-octahydro-1,1′-biisoquinolines is described. Reaction of phenethylamines with oxalyl chloride led to *N,N′*-bis(phenethyl) oxamides (**1**). Cyclization of oxamides by using Bischler – Napieralski conditions gave 3,3′,4,4′-tetrahydro-1,1′-biisoquinoline(**3**) and unusual products **2, 4, 5.** Reduction of 3,3′,4,4′-tetrahydro-1,1′-biisoquinolines with sodium boron hydride gave both *rac*-1,1′,2,2′,3,3′,4,4′-octahydro-1,1′-biisoquinolines(**6**) and *meso*-1,1′,2,2′,3,3′,4,4′ -octahydro-1,1′-biisoquinolines(**7**). Compound **6** was resolved to (1S, 1S′) **(8)** and (1R, 1R′) **(9)** furtherly. By treating all the biisoquinolines with K_2PtCl_4 afforded their cis-dichloridoplatinum (II) complexes (**12–18**). The antitumor activity of these complexes was evaluated.

1. Introduction

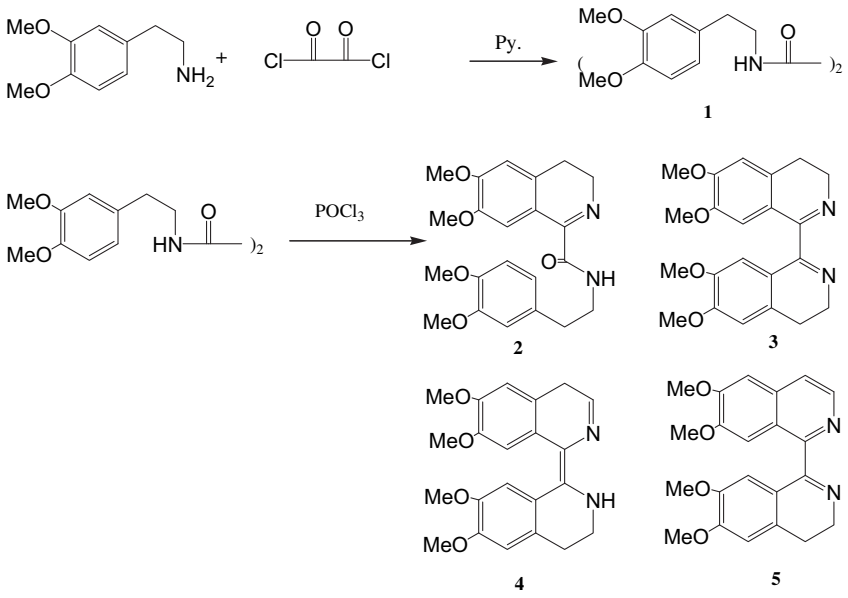
The adrenergic transmitters adrenaline and noradrenaline are the natural chemical messengers which on ‘switch on’ the receptors in the adrenergic nervous system. They have an alkylamine chain linked to a catechol ring (the 1,2-benzenediol ring) [1]. The backbone appears in isoquinolines which appear in numerous natural products [2,3], and most of them exhibit different kind of bioactivity [4–6]. Thus, the synthesis of a variety of isoquinolines, such as 1-substituented-isoquinolines [7,8] has been wildly attempted by many researchers. Biisoquinolines, a kind of isoquinolines, constituted a new class ligands for the synthesis of oligocyclic metal(II) complexes. Nielsen [9] afforded epimeric 2,2′-diacetyl-1,1′,2,2′-tetrahydro-1,1′-biisoquinolines by biomolecular reduction of isoquinoline with zinc-acetic anhydride, and the 1,1′,2,2′,3,3′,4,4′-octahydro-1,1′-biisoquinoline was obtained via four steps. That using biisoquinolines as ligands reacted with some metal ion (Pt, Fe, Ru,Os) to obtain the complexes of metal have been studied by Kato et al. [10]. Besides, these compounds contain chiral diamino groups which have numerous applications in asymmetric synthesis [11,12]. Recently, Judeh and his partners reported that the 6,6′,7,7′-tetramethoxy-3,3′,4,4′-tetrahydro-1,1′-biisoquinoline were synthesized by Bischler – Napieralski cyclization [13–15] in a room temperature ionic liquid gave excellent yields [16], but the costly ionic liquid is a disadvantage. Pt adducts containing the specially designed chiral diamine ligand, **Bip** = 2,2′-bipiperidine, are dramatically less fluxional [17]. On the other hand, cisplatin is a very useful antitumor agent for the treatment of testicular and ovarian cancers [18,19]. Since the antitumor activity of cisplatin was reported, various platinum complexes have been synthesized and tested for the antitumor activity [13]. In the present paper, we used the simple double Bischler – Napieralski cyclization followed by reduction of the resulting bis-imine to synthesize 1,1′,2,2′,3,3′,4,4′-octahydro-1,1′-biisoquinolines and investigated all possible products in different conditions. Furthermore, we synthesized cis-dichloridoplatinum(II) complexes of 1,1′,2,2′,3,3′,4,4′-octahydro-1,1′-biisoquinolines (OHBIIQs) and their antitumor activity of these complexes was evaluated.

2. Chemistry

The treatment of 3,4-dimethoxyphenethylamine with oxalyl chloride gave *N,N′*-Bis(3,4-dimethoxyphenethyl)oxamide **1** in good yield. The most direct route to **3 (2,4,5** also gave**)** would be via double Bischler – Napieralski cyclization from compound **1.** Treatment of bis-imine **3** with $NaBH_4$ gave a mixture of diastereomers **6** and **7.** *Rac*-**6** were resolved to **8** (1S, 1S′) and **9** (1R, 1R′). By treating all the biisoquinolines with K_2PtCl_4 afforded their cis-dichloridoplatinum (II) complexes (**12–18**).

* Corresponding author. Tel.: +886 4 2840412x718; fax: +886 8 7227024.
E-mail address: cchlin@mail.nchu.edu.tw (C.-Y. Kuo).

0223-5234/$ – see front matter © 2009 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2009.09.023



Scheme 1.

3. Pharmacology

Fifteen *cis*-dichloridoplatinum complexes have been synthesized by treatment of 1-(2-aminophenyl)-1,2,3,4 -tetrahydroisoquinolines (THIQs) with K_2PtCl_4. The antitumor activity of these compounds was examined against four different human tumor cell lines. Their structure-activity relationships for antitumor activity were reported [20]. All of these compounds exhibited activity against MCF-7 cell line and showed good activity. In order to understand the relationship between the structure of ligands of the complexes and the biological activity further, we synthesized a series of cis-dichloridoplatinum(II)

Table 1
Reaction of *N*,*N*′-bis(3,4-dimethoxyphenethyl)oxamide with $POCl_3$.

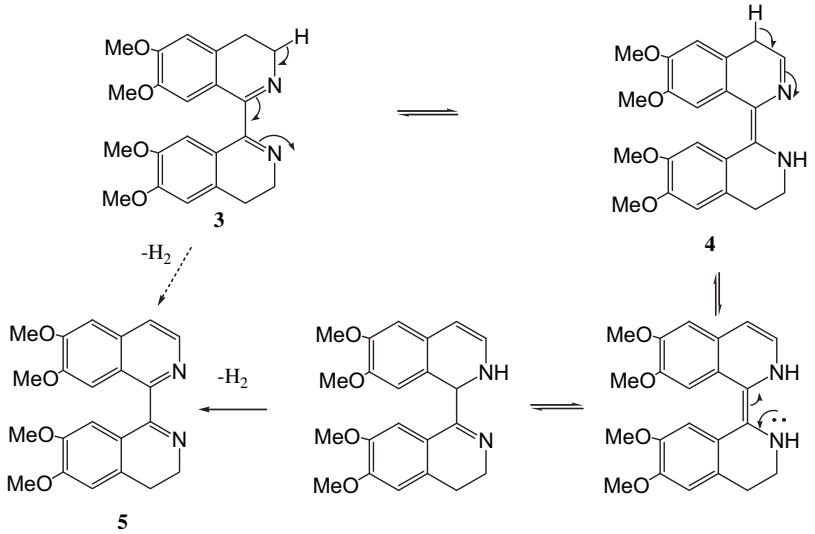
Entry	Method	Temp (°C)	Time (h)	Yields (%)			
				2	3	4	5
1	A1	111	5–6	5	18	34	17
2	A2	80	5–6	15	75	–	–
3	A2	80	12–18	6	50	10	8
4	A2	70	5–6	84	–	–	–

Method A1: Treated with $POCl_3$ and toluene.
Method A2: Treated with $POCl_3$ and CH_3CN.

Table 2
Reaction of 6,6′,7,7′-tetramethoxy-3,3′,4,4′-tetrahydro-1,1′-biisoquinoline (**3**) with reducing agents.

Entry	Reducing agent	Temp (°C)	Yields [%]	6/7 Ratio
1	**$NaBH_4$**	r.t[a]	68	18:50
2	**$NaBH_4$**	55[a]	76	19:67
3	**$NaBH_3CN$**	r.t[b]	75	75:0
4	**$NaBH_3CN$**	55[b]	82	82:0
5	**$LiAlH_4$**	r.t[c]	0	0:0
6	**$LiAlH_4$**	35[c]	0	0:0

[a] All reactions were carried out using General Procedure C.
[b] All reactions were carried out using General Procedure D.
[c] Reactions were carried out using General Procedure C. No products were obtained.



Scheme 2.

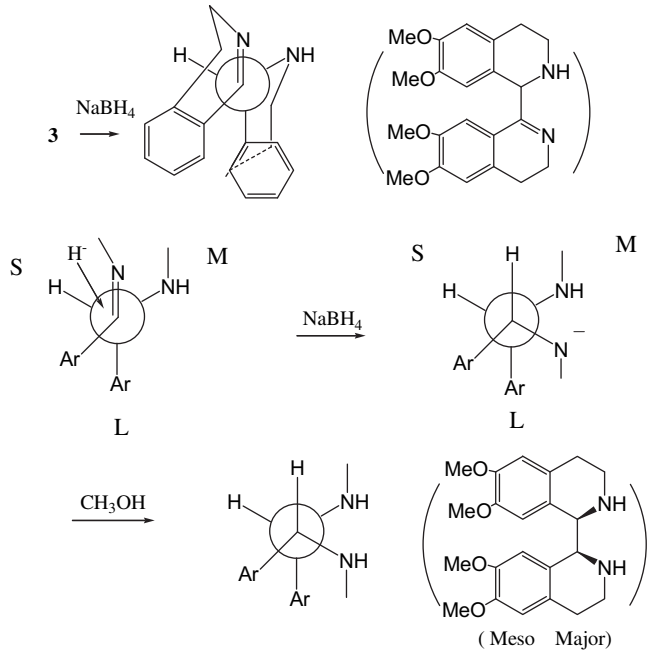


Fig. 1. Treating 3 with sodium borohydride.

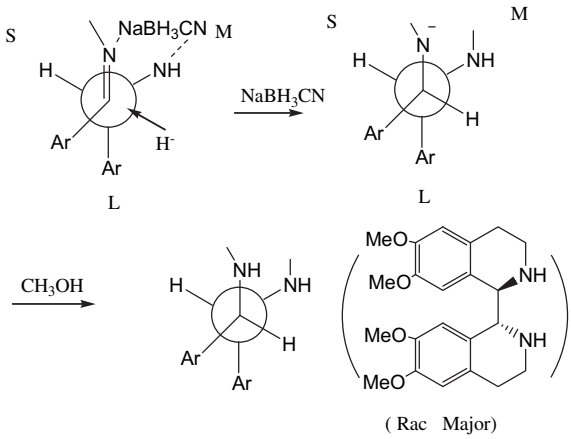
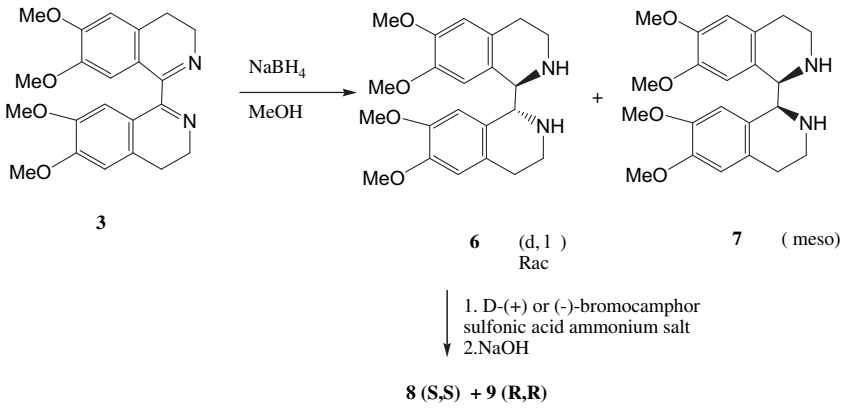


Fig. 2. Treating 3 with sodium cyanoborohydride.

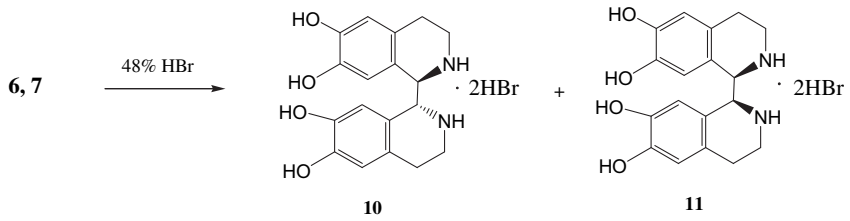
complexes of 1,1′,2,2′,3,3′,4,4′-octahydro-1,1′-biisoquinolines (OHBIIQs) and evaluated their antitumor activity in this paper.

4. Results and discussions

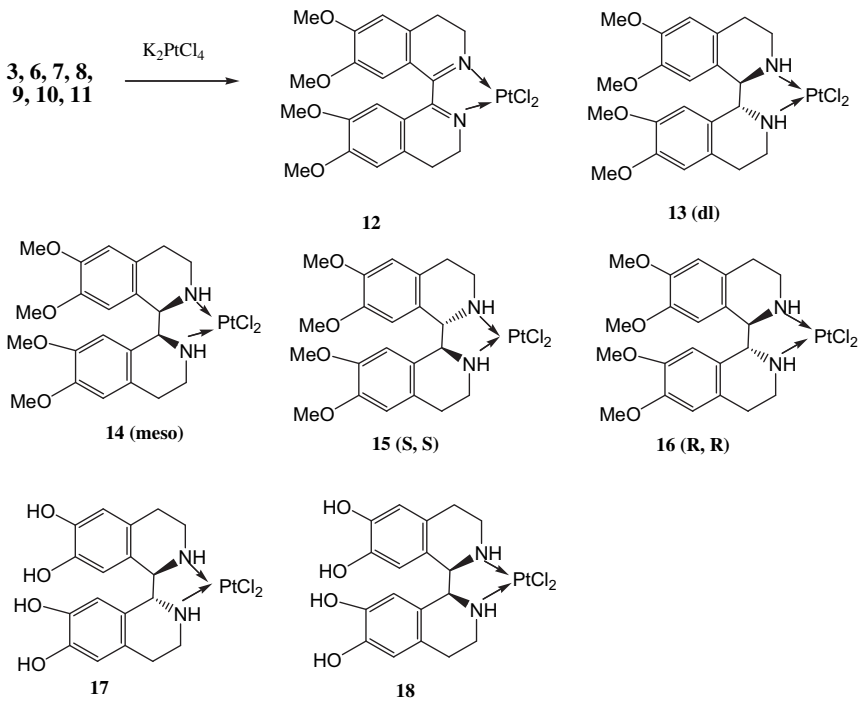
The most direct route to **3** would be via double Bischler – Napieralski cyclization from compound **1** (Scheme 1). However, the products were contaminated with an intensely red impurity that could not be easily removed by chromatography. The results are summarized in Table 1. Treatment of compound **1** with 5 equiv of $POCl_3$ at 111 °C for 6 h afforded a 18:5:34:17 mixtures of products, **3, 2**, **4**, and **5** (entry 1), which were separated by column chromatography. A better yield of **3** (75%) was obtained if compound **1** was treated with 5 equiv of $POCl_3$ at 80 °C for 6 h (entry 2). However, treatment with 5 equiv of $POCl_3$ at 70 °C for 6 h afforded compound **2** in high yield (84%) (entry 4). The relationship among **3, 4** and **5** are shown in Scheme 2. Treatment of bisimine **3** with $NaBH_4$ gave a mixture of diastereomers **6** and **7** in 18% and 50% (19% and 67%) yields, respectively. However, replacement of the $NaBH_4$ with $NaBH_3CN$ afforded only the *rac* reduction product **6**. The results are summarized in Table 2. Interestingly, replacement of the $NaBH_4$ with $LiAlH_4$ afforded no **6** and **7.** For the additions to the carbon-nitrogen double bond of imine (**3**) containing an asymmetric α-carbon, Cram's rule [21] may predict which diastereomer will predominate. The rule indicates that the presence of an asymmetric center in a molecule induces the formation of an asymmetric center adjacent to it based on steric hindrance. If the molecule is observed along its axis, it may be represented as in Fig. 1, where S, M, and L stand for small, medium, and large, respectively. The nitrogen of the imino orients itself between the small- and the medium-sized groups. The rule is that the incoming group preferentially attacks on the side of plan containing the small group. By the rule, it can be predicted that *meso*- will be formed in larger amounts than *rac*-. Treating **3** with sodium borohydride under different reaction conditions, gave compounds **6** (*rac*-) and **7** (*meso*-) were obtained in 1:3 ratios as Table 2 shown, respectively. The results follow the Cram's rule (Fig. 1). In contrasting, treating **3** with sodium cyanoborohydride gave compound **6** (*rac*-) only. That is because the sodium cyanoborohydride interacted with C═N and N-H groups that the incoming group (H^-) preferentially attacks on the other side (Fig. 2). *Rac*-**6** were resolved to (1S, 1S′) and (1R, 1R′) as outline in Scheme 3 [22]. Treatment of *Rac*-**6** with D-(+)-α-bromo camphor-π-sulfonic acid ammonium salt afforded the (1S, 1S′)-1,1′–biisoquino line sulfonic acid salt. The sulfonic acid salt was alkalinized then. Removal of drying agent and solvent gave solid, and the solid was recrystallised from ethanol to give (1S, 1S′) **8**. Similarly, replacement of the D-(+)-α-bromo camphor-π-sulfonic acid



Scheme 3.



Scheme 4.



Scheme 5.

ammonium salt with D-(-)-α- bromocamphor-π-sulfonic acid ammonium salt, using the same process, afforded (1R, 1R′) **9.** Converting methyl ether to hydroxyl groups will usually strengthen hydrogen bonding. The obvious explanation is that the proton of the hydroxyl group is involved in the hydrogen bond to the receptor and if it is removed the hydrogen bonding is lost. According to this reason, we converted compounds **6** and **7** to the compounds (**10, 11**), which may strengthen the biological activity. The title compounds were prepared as Scheme 4 shown. The BIIQs (**3,6,7,8,9**) and equimolar amounts of K_2PtCl_4 were dissolved in 0.1 M HCl at 60–65 °C. To this stirring solution was added with 0.1 N NaOH to neutrality at 60–65 °C. [BIIQs] dichloridoplatinums(II) (**12–16**) were obtained in good yields. On the other hand, The BIIQs (**10, 11**) were dissolved in water at 60–65 °C. To this stirring solution was added with 0.1 N NaOH to neutrality at 60–65 °C. [BIIQs] dichloridoplatinums(II) (**17,18**) were obtained in good yields (Scheme 5). Due to Cl-DMSO ligand exchange [19], the target products showed multiple sets of signals in ^{1}H NMR and ^{13}C NMR spectra, taken in DMSO (d6) that made assignment more difficult. The ^{1}H NMR spectroscopic data of compound **13** and **14** show the presence of multiple sets singlet signals at δ 4.37–4.88 corresponding to the methine (CH) group at the 1,1′ position. Elemental analysis confirmed the structure of the target products further. The cytotoxicities of the series of cis-dichloridoplatinum complexes (**12–18**) were examined with Hepa59T/VGH, WiDr, HeLa and MCF-7 cell lines. The results are summarized in Table 3. As shown in Table 3, all these compounds exhibited good activity against MCF-7 cell lines, except compound **12** which are planar due to the C═N bond. D'Ocon et al. [23] reported that the planarity of the isoquinoline ring decreases the affinity for α1-adrenoreceptor, whereas the more flexible THIQ ring increases the interaction with the α1-adrenoreceptor site. In our report, compound **12,** containing planar structure, exhibited similar result as D'Ocon et al. reported in against MCF-7 cell lines.

Table 3
Cytotoxicity of compounds **12–18** against Human Tumor Cells ($IC_{50} \pm SD$, μM).

Compound	Hepa59T/VGH[a]	WiDr[b]	HeLa[c]	MCF-7[d]
12	(-)	(-)	(-)	24.31 ± 0.67
13	29.05 ± 0.73	26.50 ± 1.09	(-)	10.12 ± 0.17
14	(-)	29.84 ± 1.15	(-)	8.63 ± 0.75
15	(-)	(-)	29.94 ± 1.07	5.37 ± 0.32
16	25.98 ± 0.40	27.55 ± 0.95	21.95 ± 0.32	5.19 ± 0.44
17	(-)	22.23 ± 1.39	(-)	7.83 ± 0.22
18	(-)	16.48 ± 0.61	23.67 ± 0.84	8.14 ± 0.37
cisplatin	3.52 ± 0.33	8.84 ± 0.52	12.06 ± 0.41	7.76 ± 0.30

[a] Human liver carcinoma.
[b] Human colon adenocarcinoma.
[c] Human cervical epitheloid carcinoma.
[d] Human breast adenocarcinoma (-) $IC_{50} > 50$ μM.

5. Conclusion

Double Bischler – Napieralski cyclization of compound **1** afforded a mixture of products, **3, 2**, **4**, and **5**. Treatment of bis-imine with $NaBH_4$ gave a mixture of diastereomers *rac* and *meso*. However, replacement of the $NaBH_4$ with $NaBH_3CN$ afforded only the *rac* reduction product. Seven platinum complexes of BIIQ have been synthesized. Most of these compounds exhibited good activity against MCF-7 cell lines.

6. Experimental

6.1. Chemistry

All melting points were determined on a Digital MEL-TEMP melting point apparatus, and are uncorrected. Infrared spectra were recorded on a Digilab FTIR spectrophotometer. The ^1H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively. High-resolution mass spectra were recorded on a double focusing magnetic sector mass spectrometer using EI at 70 eV. All reactions were monitored by analytical TLC (silica gel 60 F_{254}, Merck). The residues were purified by flash chromatography (230–400 mesh). Elemental analyses were recorded using a Heraeus CHNO-Rapid elemental analyzer.

6.2. *N,N′-Bis(3,4-dimethoxyphenethyl)oxamide (**1**)*

6.2.1. General procedure A

Oxalyl chloride (7.25 g, 57 mmol) was dissolved in dry benzene (30 mL) and added dropwise to a stirred solution of 3,4-dimethoxyphenethylamine (8.12 g, 44 mmol) and pyridine (4.53 g, 57 mmol) in dry benzene (100 mL). The mixture was continuously stirred at room temperature overnight and then poured into water (200 mL). Filterated the solution and the solid were washed with 1.0 N HCl (100 mL), 1.0 N NaOH (100 mL), and water (100 mL). Recrystallization of the crude product from EtOAc afforded **1** (5.91 g). The mother layer was evaporated and the residue was recrystallized from EtOAc to afford white crystals **1** (1.54 g). Totally, compound **1** was obtained in 83% yield (7.45 g).

M.p. 170–171 °C, ^1H NMR (400 MHz, $CDCl_3$) δ 7.51 (1H, br s) 6.71–6.75 (2H, *m*) 6.8 (1H, d) 3.87 (3H, s) 3.86 (3H, s) 3.54 (2H, q) 2.80 (2H, t); ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ159.6 (C═O), 149.1 (C-3), 147.8 (C-4), 130.5 (C-1), 120.6 (C-6), 111.7 (C-2), 111.4 (C-5), 55.9 (OCH_3), 55.8 (OCH_3), 40.9 (CH_2N), 35.0 (CH_2CH_2N). EI-MS *m*/*z* (%) = 416 (100) [M^+], Anal. Calcd for $C_{22}H_{28}N_2O_6$: C, 63.45; H, 6.78; N, 6.73. Found: C, 62.74; H, 6.68; N, 6.79.

6.3. *6,6′,7,7′-tetramethoxy-3,3′,4,4′ tetrahydro-1,1′-biisoquinoline and their relative compounds*

6.3.1. General procedure B (method A1)

A mixture of *N,N′*-diphenethyloxamide (6.7 mmol), $POCl_3$ (33 mmol), and dry toluene (100 mL) was refluxed for 3 h. The cooled mixture was poured into ice-water (100 mL) and stirred for 2 h. The organic layer was discarded. The water layer and precipitates were then basified with 2 N NaOH (100 mL) and extracted with CH_2Cl_2 (50 mL × 3). The extracts were washed with water (100 mL), dried (Na_2SO_4), and evaporated to give product as a solid. The crude product was purified by column chromatography using silica gel (EtOAc/n-hexane, 2:1) and then recrystallization of the products from ethanol, afforded compounds **2,3,4,5.** The products were contaminated with an intensely red impurity. The red impurity was separated through a silica gel pad (column 20 × 100 mm) using EtOAc-AcOH-MeOH 8:1:1. The resulting mixture was diluted with 50 mL of CH_2Cl_2 and 50 mL of water, basified with 2 N NaOH, and the layers were separated. The organic phase was washed with saturated Na_2CO_3 solution (3 × 10 mL), 10 mL of water, and 10 mL of brine and dried ($MgSO_4$). After filtration and evaporation, the residue was recrystallized from ethyl alcohol to give **3.**

6.3.2. General procedure B (method A2)

To a stirring solution of *N,N′*-diphenethyloxamide (12.9 mmol) in dry CH_3CN (80 mL) was added $POCl_3$ (32 mmol) dropwise, and the stirring was continued for 1 h at room temperature. The resulting mixture was heated to reflux and stirred for approximately 5–12 h. After removal of the solvent *in vacuo*, $CHCl_3$ (50 mL) and H_2O (50 mL) were added, and the aqueous phase was adjusted with aqueous 10 N NaOH to pH 12. The organic layer was washed with saturated aqueous $NaHCO_3$ (3 × 100 mL), dried over anhydrous Na_2SO_4, and the solvent was removed *in vacuo*. The crude product was purified by column chromatography using silica gel (EtOAc/n-hexane, 2:1) and then recrystallization of the products from ethanol, afforded compounds **2,3,4,5.**

*6.3.2.1. 1-(3,4-dimethoxyphenethyl carbamoyl) 6,7-dimethoxy-3,4 –dihydro isoquinoline **(2)**.* White powders, mp 118–120 °C, ^1H NMR (400 MHz, $CDCl_3$) δ 7.96 (1H, s, H-8), 7.63 (1H, br, NH), 6.76–6.80 (3H, *m*, H-1′,5′,6′), 6.67 (1H, s, H-5), 3.91 (3H, s, OMe), 3.90 (3H, s, OMe), 3.87 (3H, s, OMe), 3.86 (3H, s, OMe), 3.72 (2H, t, *J* = 7.6 Hz, ═NCH_2), 3.62 (2H, q, *J* = 7.2 Hz, $NHCH_2$), 2.85 (2H, t, *J* = 7.2 Hz, $NHCH_2$ CH_2), 2.64 (2H, t, *J* = 7.6 Hz, ═NCH_2CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ164.2 (C═O), 158.8 (C═N), 151.6 (C-6), 148.8 (C-7), 147.9 (C-4′), 147.3 (C-3′), 132.0 (C-4a), 131.4 (C-8a), 120.6 (C-8), 119.0 (C-1), 112.0 (C-5), 111.9 (C-6′), 111.3 (C-5′), 109.8 (C-2′), Anal. Calcd for $C_{22}H_{24}N_2O_4$: C, 69.46; H, 6.36; N, 7.36. Found: C, 69.71; H, 6.41; N, 7.25.

*6.3.2.2. 6,6′,7,7′-tetramethoxy-3,3′,4,4′-tetrahydro-1,1′-biisoquinoline (**3**).* Red powders, m.p. 198–200 °C, ^1H NMR (400 MHz, $CDCl_3$) δ 6.84 (2H, s, H-8,8′) 6.72 (2H, s, H-5,5′) 3.92 (6H, s, OMe-C7,7′) 3.91 (4H, t, *J* = 7.6 Hz, H-3) 3.71 (6H, s, OMe-C6,6′), 2.80 (4H, t, *J* = 7.6 Hz, H-4); ^{13}C NMR (100 MHz, $CDCl_3$), δ 164.6 (C═N), 51.6 (C-6,6′), 147.4 (C-7,7′), 131.5 (4a,4a′), 120.8 (C-8a,8a′), 110.6 (5,5′), 110.3 (8,8′), 56.0 (OMe), 55.9 (OMe) 47.1 (C-3,3′), 25.6 (C-4,4′); HREIMS *m*/*z* (%) = 380 (100) [M^+], Anal. Calcd for $C_{22}H_{24}N_2O_4 \cdot 0.5\ H_2O$: C, 67.85; H, 6.47; N, 7.19. Found: C, 68.51; H, 6.63; N, 6.69.

*6.3.2.3. 6,6′,7,7′-tetramethoxy-3,4-dihy dro-2H,4′H-[1,1′]biisoquinolinylidene (**4**).* Brown liquid, ^1H NMR (400 MHz, $CDCl_3$) δ 7.17 (1H, s, H-8) 6.94 (1H, s, H-8′) 6.70 (1H, s, H-5) 6.66 (2H, *m*, H-3 and H-2′) 6.63 (1H, s, H-5′) 4.03 (2H, s, H-4) 3.85 (3H, s, OCH_3-7) 3.80 (3H, s, OCH_3-6) 3.76 (3H, s, OCH_3-7′) 3.75 (3H, s, OCH_3-6′) 3.77 (2H, q, *J* = 6.8 Hz, H-3′) 2.83 (2H, t, *J* = 6.8 Hz, H-4′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.9 (C-6), 148.2 (C-7), 147.9 (C-6′), 147.5 (C-7′), 145.4 (C-3 C═N), 129.4 (C-1), 129.3 (C-1′), 122.5 (C-4a), 120.9 (C-4′a), 119.9 (C-8a), 119.9 (C-8′a), 111.2 (C-8′), 111.1, (C-8), 110.9 (C-5′), 105.8 (C-5), 55.8 (OMe), 55.7 (OMe), 55.6 (OMe), 40.7 (C-3′), ′33.0 (C-4), 25.6 (C-4′); HREIMS *m*/*z*(%) = 380 (100) [M^+], 379 (34) [M^+-H], 365 (30) [M^+- CH_3], 349 [M^+-H-2CH_3], Anal. Calcd for $C_{22}H_{24}N_2O_4$: C, 69.46; H, 6.36; N, 7.36. Found: C, 69.33; H, 6.31; N, 7.41.

*6.3.2.4. 6,6′,7,7′-tetramethoxy-3′,4′- dihydro-1,1′-biisoquinoline (**5**).* Yellowish powders, m.p. 184–186 °C, ^1H NMR (400 MHz, $CDCl_3$) δ 8.45 (1H, d, *J* = 7.0 Hz, H-3) 7.56 (1H, d, *J* = 7.0 Hz, H-4) 7.42 (1H, s, H-8) 7.11 (1H, s, H-5) 6.78 (1H, s, H-8′) 6.51 (1H, s, H-5′) 4.03 (3H, s, H-7) 4.03 (2H, t, *J* = 7.6 Hz, H-3′) 3.93 (3H, s, H-6′) 3.87 (3H, s, H-7′) 3.56 (3H, s, H-6′) 2.89 (2H, t, *J* = 7.6 Hz, H-4′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.4 (N═C′-1), 154.8 (N═C-1′), 152.9 (C-6), 151.5 (C-7), 150.2 (C-6′), 147.4 (C-7′), 140.7 (C-3), 133.9 (C-4), 131.7 (C-4a), 123.1 (C-8a), 122.0 (C-4′a), 119.9 (C-8a′), 111.2 (C-8′), 110.3 (C-8), 104.8 (C-5′), 104.7 (C-5), 56.0 (OMe), 55.9 (OMe), 55.7 (OMe), 47.6 (C-3,3′), 25.7 (C-4,4′);

HREIMS m/z (%) = 378 (36) [M^+], 377 (19) [M^+-H], 363 (100) [M^+-CH_3], 347 (99) [M^+-H-2CH_3], Anal. Calcd for $C_{22}H_{22}N_2O_4$ 1/2H_2O: C, 68.20; H, 5.98; N, 7.23. Found: C, 68.09; H, 5.83; N, 7.14.

6.4. 6,6′,7,7′-tetramethoxy-1,1′,2,2′,3, 3′,4,4′-octahydro-1,1′-biisoquinolines (**6,7**)

6.4.1. General procedure C

To a solution of 6,6′,7,7′-tetramethoxy- 3,3′,4,4′-tetrahydro-1,1′-biisoquinolines (3) (4.2 mmol) in methanol (80 mL) $NaBH_4$ (21 mmol) was added in portions, the mixture was continuously stirred at room temperature for approximately 4 h. After removal of the solvent *in vacuo*, CH_2Cl_2 (50 mL) and H_2O (50 mL) were added. The aqueous phase was extracted with CH_2Cl_2 (20 mL × 2), and the combined organic layers were washed with water (30 mL × 2) and dried over anhydrous $MgSO_4$. The solvent was evaporated *in vacuo*. The crude products were separated by a flash column (EtOAc/n-Hexane 2:1), afforded crystals **6, 7**.

*6.4.1.1. Rac-6,6′,7,7′-Tetramethoxy-1,1′, 2,2′,3,3′,4,4′-octahydro-1,1′-biisoquinline (**6**).* The title compound was obtained as a White powders in 56% yield from **3** following procedures C; mp 174–179 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.75 (2H, s, H-8,8′) 6.60 (2H, s, H-5,5′), 4.53 (2H, s, H-1,1′), 3.86 (6H, s, 2OMe), 3.85 (6H, s, 2OMe), 3.17–3.22 (2H, *m*, H-3e,3′e), 2.79–2.90 (4H, *m*, H- 3a, 3a′, H-4e, 4e′), 2.54–2.60 (2H, *m*, H-4a, 4a′), 2.05 (2H, br, 2 NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.6 147.5 (C-6,6′,7,7′), 129.4 (4a,4a′), 127.1 (C-8a,8a′), 111.9 (5,5′), 108.7 (8,8′), 59.4 (C-1), 56.0 (OMe), 55.8 (OMe) 42.4 (C-3,3′), 29.5 (C-4,4′); HREIMS m/z(%) = 384 (0) [M^+], 192 (100) [M^+/2], 176 (20) [M^+/2- H-CH_3], Anal. Calcd for $C_{22}H_{28}N_2O_4$: C, 68.73; H, 7.34; N, 7.29. Found: C, 68.81; H, 7.20; N, 7.01.

*6.4.1.2. Meso-6,6′,7,7′-Tetramethoxy-1,1′,2,2′,3,3′,4,4′-octahydro-1,1′-biisoquinoline (**7**).* The title compound was obtained as a white powders in 18% yield from **3** following procedures C; mp 165–168 °C (lip. 169–171), ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.55 (2H, s, H-8,8′) 6.41 (2H, s, H-5,5′), 4.58 (2H, s, H-1,1′), 3.83 (6H, s, 2OMe), 3.56 (6H, s, 2OMe), 3.08–3.14 (2H, *m*, H-3e,3′e), 2.83–2.90 (2H, *m*, H-3a), 2.63–2.70 (2H, *m*, H-4e), 2.54–2.59 (2H, *m*, H-4a), 2.04 (2H, br, 2 NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.4 147.3 (C-6,6′,7,7′), 129.0 (4a,4a′), 127.6*o* (C-8a,8a′), 111.8 (5,5′), 109.1 (8,8′), 60.1 (C-1), 55.8 (OMe), 55.5 (OMe) 42.2 (C-3,3′), 29.7 (C-4,4′); HREIMS m/z(%) = 384 (0) [M^+], 192 (100) [M^+/2], 176 (20) [M^+/2- H-CH_3], Anal. Calcd for $C_{22}H_{28}N_2O_4$: C, 68.73; H, 7.34; N, 7.29. Found: C, 68.35; H, 7.13; N, 6.97.

6.4.2. General procedure D

To a solution of 3,3′,4,4′-tetrahydro-1,1′-biisoquinolines (4.2 mmol) in methanol (80 mL), 3 N HCl (3 mL) and $NaBH_3CN$ (21 mmol) was added in portions, the mixture was continuously stirred at room temperature for approximately 4 h. After removal of the solvent *in vacuo*, CH_2Cl_2 (50 mL) and H_2O (50 mL) were added, and the aqueous phase was adjusted with aqueous 10 N NaOH to pH 12. The organic layer was washed with saturated aqueous $NaHCO_3$ (3 × 100 mL), dried over anhydrous Na_2SO_4, and the solvent was removed *in vacuo*. Recrystallization of the crude product from ethanol, afforded crystal **7** (81%).

6.5. (1S, 1S′) and (1R, 1R′) (-6,6′,7,7′-tetramethoxy-1,1′,2,2′3,3′,4,4′-octahydro-1,1′-biisoquinoline)

6.5.1. (1S, 1S′)-6,6′,7,7′-tetramethoxy- 1,1′,2,2′3,3′,4,4′-octahydro-1,1′–biiso- quinline D-(+)-α-bromocamphor-π- sulfonic acid salt

Rac-**6** (0.24 g, 0.625 mmol) and D-(+)-α-bromocamphor-π-sulfonic acid ammonium salt (0.21 g, 0.625 mmol) were recrystallized from ethanol (5 mL) to give the title compound in 82% yield.

White solid, m.p. 130–133 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.73 6.65 (2H, ss, H-8,8′) 6.37 6.35 (2H, ss, H-5,5′), 4.76 4.73 (2H, ss, H-1,1′), 4.47 (1H, d, J = 4.4 Hz, C*H*Br), 3.85 (6H, s, 2OMe), 3.71 (6H, s, 2OMe), 3.51–3.54 (2H, *m*, H-3e,3′e), 3.09–3.23 (2H, *m*, H-3a, 3a′), 2.83–3.02 (6H, *m*, H-4, CHCH_2), 2.58 (1H, d, J = 14.0 Hz, C*H*CHBr), 1.98 (2H, *m*, CHCH_2), 1.56 (1H, *m*, CCH_2-e), 1.36 (1H, *m*, CCH_2-a),1.06 (CH_3), 0.88 (CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.9 (C═O), 149.0 (C-7,7′), 147.7 (C-6,6′), 127.0 126.9(C-4a,4a′), 121(C-8a,8a′), 111.5 111.4 (C-5,5′), 110.7(C-8,8′), 59.5 (C-1), 57.6 (*C*C═O), 56.0 (4 × OMe), 53.9 (O_3SCH_2), 53.4 (CHBr), 47.2 (*C*), 46.8 (*C*HCHBr), 40.3 (C-3,3′), 30.1 (C*C*H_2), 26.8 (C-4,4′), 22.0 (CH*C*H_2), 17.4 (CH_3), 9.7 (CH_3);

6.5.2. (1S, 1S′)-6,6′,7,7′-tetramethoxy -1,1′,2,2′3,3′,4,4′-octahydro-1,1′ –biisoquinline (**8**)

D-(+)-α-bromocamphor-π- sulfonic acid salt (0.44 mmol) was stirred in CH_2Cl_2 (20 mL) and 5 M NaOH (20 mL) at rt for 2 h. The organic layer was separated and dried over NaOH (pellets). Removal of drying agent and solvent gave solid, recrystallization from ethanol to give ′the title compound in 77% yield.

White solid, mp 182–185 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.74 (2H, s, H-8,8′) 6.60 (2H, s, H-5,5′), 4.52 (2H, s, H-1,1′), 3.85 (6H, s, 2OMe), 3.84 (6H, s, 2OMe), 3.17–3.23 (2H, *m*, H-3e,3′e), 2.80–2.90 (4H, *m*, H- 3a, 3a′, H-4e, 4e′), 2.55–2.60 (2H, *m*, H-4a, 4a′), 2.07 (2H, br, 2 NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.7 147.5 (C-6,6′,7,7′), 129.3 (4a,4a′), 127.0 (C-8a,8a′), 111.9 (5,5′), 108.6 (8,8′), 59.5 (C-1), 56.0 (OMe), 55.9 (OMe) 42.3 (C-3,3′), 29.5 (C-4,4′); HREIMS m/z (%) = 384 (0) [M^+], 192 (100) [M^+/2], 176 (20) [M^+/2- H-CH_3], Anal. Calcd for $C_{22}H_{28}N_2O_4$: C, 68.73; H, 7.34; N, 7.29. Found: C, 68.55; H, 7.23; N, 7.35.

6.5.3. (1R, 1R′)-6,6′,7,7′-tetramethoxy -1,1′,2,2′3,3′,4,4′-octahydro-1,1′–biisoquinoline D-(-)-α-bromocamphor-π- sulfonic acid salt

Rac-6,6′,7,7′-tetramethoxy-1,1′,2,2′3,3′,4,4′-octahydro-1,1′-biisoquinoline (0.24 g, 0.625 mmol) and D-(-)-bromo camphor-sulfonic acid ammonium salt (0.22 g, 0.62 mmol) were recrystallized from ethanol (5 mL) to give the title compound in 89% yield.

White solid, m.p. 135–137 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.75 6.66 (2H, ss, H-8,8′) 6.38 6.35 (2H, ss, H-5,5′), 4.77 4.74 (2H, ss, H-1,1′), 4.48 (1H, d, J = 4.4 Hz, C*H*Br), 3.85 (6H, s, 2OMe), 3.72 (6H, s, 2OMe), 3.51–3.55 (2H, *m*, H-3e,3′e), 3.10–3.23 (2H, *m*, H-3a, 3a′), 2.83–3.04 (6H, *m*, H-4, CHCH_2), 2.59 (1H, d, J = 14.0 Hz, C*H*CHBr), 1.99 (2H, *m*, CHCH_2), 1.55 (1H, *m*, CCH_2-e), 1.36 (1H, *m*, CCH_2-a),1.05 (CH_3), 0.89 (CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.1 (C═O), 149.2 (C-7,7′), 147.8 (C-6,6′), 127.1 126.9(C-4a,4a′), 121.1 (C-8a,8a′), 111.5 111.4 (C-5,5′), 110.7(C-8,8′), 59.5 (C-1), 57.6 (*C*C═O), 56.0 (4 × OMe), 53.7 (O_3SCH_2), 53.4 (CHBr), 47.2 (*C*), 46.9 (*C*HCHBr), 40.4 (C-3,3′), 30.3 (C*C*H_2), 26.6 (C-4,4′), 22.0 (CH*C*H_2), 17.4 (CH_3), 9.8 (CH_3);

6.5.4. (1R, 1R′)-6,6′,7,7′-tetramethoxy -1,1′,2,2′3,3′,4,4′-octahydro-1,1′ –biisoquinoline (**9**)

The title compound was obtained as a white powders in 82% yield in a similar procedure for the preparation of **8;** m.p. 180–182 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.75 (2H, s, H-8,8′) 6.61 (2H, s, H-5,5′), 4.54 (2H, s, H-1,1′), 3.87 (6H, s, 2OMe), 3.85 (6H, s, 2OMe), 3.16–3.22 (2H, *m*, H-3e,3′e), 2.79–2.91 (4H, *m*, H- 3a, 3a′, H-4e, 4e′), 2.54–2.61 (2H, *m*, H-4a, 4a′), 2.06 (2H, br, 2 NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.6 147.4 (C-6,6′,7,7′), 129.3 (4a,4a′), 127.3 (C-8a,8a′), 111.9 (5,5′), 108.7 (8,8′), 59.3 (C-1), 55.9 (OMe), 55.8 (OMe) 42.6 (C-3,3′), 29.5 (C-4,4′); HREIMS m/z(%) = 384 (0) [M^+], 192 (100) [M^+/2], 176 (20) [M^+/2- H-CH_3], Anal. Calcd for $C_{22}H_{28}N_2O_4$: C, 68.73; H, 7.34; N, 7.29. Found: C, 69.01; H, 7.40; N, 7.32.

*6.6. 6,6′,7,7′-Tetrahydroxy-1,1′,2,2′, 3,3′,4,4′-octahydro-1,1′-biisoquinoline Dihydrobromides (**10,11**)*

6.6.1. General procedure E

A suspension of the, 6′,7,7′-tetrameth oxy-1,1′,2,2′,3,3′,4,4′-octahydro-1,1′-biisoquinoline (10 mmol) in 48% aqueous hydrobromic acid (3 mL) was refluxed until TLC showed no starting material (approximately 4–8 h). The reaction mixture was slowly cooled to 5 °C and stirred at this temperature for additional 12 h. The resulting yellow precipitate was filtered, immediately washed with Et_2O (3 × 5 mL) and then dried *in vacuo*.

*6.6.1.1. Rac-6,6′,7,7′-Tetrahydroxy-1,1′,2,2′,3,3′,4,4′-octahydro-1,1′-biisoquinoline Dihydrobromides (**10**).* Yellow powders, m.p. 228–230 °C, ^{1}H NMR (400 MHz, D_2O) δ6.63–6.85 (2H, *m*, H-8,8′) 5.68–6.526 (2H, *m*, H-5,5′), 5.01–5.20 (2H, *m*, H-1,1′), 3.4–.81 (6H, *m*, H-3e, H-3′e, H-3a, H-3a′, 2 × NH), 2.92–3.39 (4H, *m*, H-4e, H-4e′, H-4a, H-4a′), Anal. Calcd for $C_{18}H_{22}N_2O_4Br_2$: C, 44.10; H, 4.52; N, 5.71. Found: C, 43.89; H, 4.46; N, 5.84.

*6.6.1.2. Meso-6,6′,7,7′-Tetrahydroxy-1,1′,2,2′,3,3′,4,4′-octahydro-1,1′-biisoquino line Dihydrobromides (**11**).* Yellow powders, m.p. 236–238 °C, ^{1}H NMR (400 MHz, D_2O) δ 6.72–6.87 (2H, *m*, H-8,8′) 6.21–6.51 (2H, *m*, H-5,5′), 5.20–5.43 (2H, *m*, H-1,1′), 3.38–3.80 (6H, *m*, H-3e, H-3′e, H-3a, H-3a′, 2 × NH), 2.86–2.97 (4H, *m*, H-4e, H-4e′, H-4a, H-4a′); ^{13}C NMR (100 MHz, D_2O) δ 145.5 144.7 144.4 144.1 (C-6,6′,7,7′), 126.8 (4a,4a′), 125.9 (C-8a,8a′), 117.7 116.8 116.7 116.3 (C-5,5′), 113.5 113.3 112.1 109.3 (C-8,8′), 57.8 57.7 57.5 55.9 55.8 55.5 (C-1,1′), 41.8 41.6 (C-3,3′), 25.5 24.3 24.1 (C-4,4′), Anal. Calcd for $C_{18}H_{22}N_2O_4Br_2$: C, 44.10; H, 4.52; N, 5.71. Found: C, 44.36 H, 4.69; N, 5.58.

6.7. (6,6′,7,7′-tetramethoxy -1,1′-biiso quinoline)dichloridoplatinums (II)

6.7.1. General procedure F1

The BIIQs were dissolved at 60–65 °C in 0.05 M HCl (50 mL). After addition of the equimolar amount of K_2PtCl_4, the mixture was neutralized slowly with 0.1 M NaOH to pH 6. The complexes precipitated out, were washed with H_2O and with EtOH and were dried.

*6.7.1.1. (6,6′,7,7′-tetramethoxy-3,3′,4,4′-tetrahydro-1,1′-biisoquinoline)dichlorido platinum(II) (**12**).* H^1 NMR (400 MHz, $[D]_6$DMSO) δ 6.00–8.42 (4H, *m*, H-5,5′,8,8′), 3.89 3.88 3.85 3.84 3.82 3.80 3.77 3.74 3.72 3.71 3.70 3.69 3.68 3.67 3.58 3.56 (12H, 4 × OMe), 2.65–3.13 (4H, *m*, H-3,3′), 2.05–2.54 (4H, *m*, H-4,4′), Anal. Calcd for $C_{22}H_{24}N_2O_4PtCl_2 \cdot 2H_2O$: C, 38.72; H, 4.14; N, 4.10. Found: C, 38.41; H, 4.02; N, 3.94.

*6.7.1.2. (Rac-6,6′,7,7′-tetramethoxy-1,1′, 2,2′3,3′,4,4′-octahydro-1,1′-biisoquinoline)dichloridoplatinum(II) (**13**).* ^{1}H NMR (400 MHz, $[D]_6$DMSO) δ 7.01–7.75 (2H, 2 × NH), 5.45–6.95 (4H, *m*, H-5,5′,8,8′), 4.76 4.37 (2H, 2s, H-1,1′), 3.78 3.75 3.74 3.71 3.70 (12H, 4 × OMe), 3.03–3.19 (2H, *m*, H-3e,3′e), 2.64–2.93 (2H, *m*, H-3a, 3′a), 2.50–2.54 (2H, *m*, H-4e, 4′e), 2.08–2.33 (2H, *m*, H-4a, 4′a), 1.03 (t, CH_3CH_2OH), Anal. Calcd for $C_{22}H_{28}N_2O_4PtCl_2 \cdot C_2H_5OH$: C, 41.39; H, 4.92; N, 4.02. Found: C, 40.07; H, 4.74; N, 4.03.

*6.7.1.3. (Meso-6,6′,7,7′-tetramethoxy-1,1′,2,2′3,3′,4,4′-octahydro-1,1′-biiso quinoline)dichloridoplatinum(II) (**14**).* ^{1}H NMR (400 MHz, $[D]_6$DMSO) δ 6.95–8.15 (2H, 2 × NH), 5.55–6.95 (4H, *m*, H-5,5′,8,8′), 4.88 4.76 4.55 4.43 (2H, 4s, H-1,1′), 3.79 3.77 3.75 3.73 (6H, 2OMe), 3.71 (6H, 2OMe), 3.01–3.13 (2H, *m*, H-3e,3′e), 2.66–2.99 (2H, *m*, H-3a, 3′a), 2.08–2.54 (3H, *m*, H-4,4′), 1.03 (t, CH_3CH_2OH), Anal. Calcd for $C_{22}H_{28}N_2O_4PtCl_2 \cdot C_2H_5OH$: C, 41.39; H, 4.92; N, 4.02. Found: C, 41.34; H, 5.17; N, 4.26.

*6.7.1.4. (1S, 1S′)-6,6′,7,7′-tetramethoxy -1,1′,2,2′3,3′,4,4′-octahydro-1,1′-biiso quinoline)dichloridoplatinum(II) (**15**).* ^{1}H NMR (400 MHz, $[D]_6$DMSO) δ 7.02–7.75 (2H, 2 × NH), 5.44–6.95 (4H, *m*, H-5,5′,8,8′), 4.75 4.38 (2H, br, H-1,1′), 3.78 3.75 3.74 3.72 3.70 (12H, 4 × OMe), 3.05–3.20 (2H, *m*, H-3e,3′e), 2.63–2.93 (2H, *m*, H-3a, 3′a), 2.51–2.54 (2H, *m*, H-4e, 4′e), 2.09–2.33 (2H, *m*, H-4a, 4′a), 1.03 (t, CH_3CH_2OH), Anal. Calcd for $C_{22}H_{28}N_2O_4PtCl_2 \cdot C_2H_5OH$: C, 41.39; H, 4.92; N, 4.02. Found: C, 41.08; H, 4.84; N, 4.09.

*6.7.1.5. (1R, 1R′)-6,6′,7,7′-tetramethoxy -1,1′,2,2′3,3′,4,4′4′-octahydro-1,1′-biiso quinoline)dichloridoplatinum(II) (**16**).* ^{1}H NMR (400 MHz, $[D]_6$DMSO) δ 7.04–7.75 (2H, 2 × NH), 5.44–6.97 (4H, *m*, H-5,5′,8,8′), 4.75 4.39 (2H, br, H-1,1′), 3.79 3.75 3.74 3.72 3.70 (12H, 4 × OMe), 3.06–3.20 (2H, *m*, H-3e,3′e), 2.62–2.93 (2H, *m*, H-3a, 3′a), 2.50–2.54 (2H, *m*, H-4e, 4′e), 2.06–2.33 (2H, *m*, H-4a, 4′a), 1.03 (t, CH_3CH_2OH), Anal. Calcd for $C_{22}H_{28}N_2O_4PtCl_2 \cdot C_2H_5OH$: C, 41.39; H, 4.92; N, 4.02. Found: C, 41.11; H, 4.79; N, 4.05.

6.8. 6,6′,7,7′-tetrahydroxy-1,1′,2,2′ 3,3′, 4,4′-octahydro-1,1′-biisoquinoline)dichloridoplatinums(II)

6.8.1. General procedure F2

The BIIQs were dissolved at 60–65 °C in water (50 mL). After addition of the equimolar amount of K_2PtCl_4, the mixture was neutralized slowly with 0.1 M NaOH to pH 6. The complexes precipitated out, were washed with H_2O and with EtOH and were dried.

*6.8.1.1. (Rac-6,6′,7,7′-tetrahydroxy-1,1′, 2,2′3,3′,4,4′-octahydro-1,1′-biisoquinoline)dichloridoplatinum(II) (**17**).* IR (cm^{-1} neat) 3150–3600 (S, -OH) 2250–2700 (-NH$^+$-) 1250 (S, Ar-O); ^{1}H NMR (400 MHz, $[D]_6$DMSO) δ 8.60–9.20 (4H, *m*, OH), 7.01–7.75 (2H, *m*, 2 × NH), 5.45–6.95 (4H, *m*, H-5,5′,8,8′), 4.51 4.15 (2H, *m*, H-1,1′), 3.13–3.89 (4H, *m*, H-3), 2.63–2.98 (4H, *m*, H- 4), 1.03 (t, CH_3CH_2OH), Anal. Calcd for $C_{18}H_{20}N_2O_4PtCl_2 \cdot C_2H_5OH$: C, 37.39; H, 4.39; N, 4.36. Found: C, 37.01; H, 4.54; N, 4.23.

*6.8.1.2. (Meso-6,6′,7,7′-tetrahydroxy-1, 1′,2,2′3,3′,4,4′-octahydro-1,1′-biisoquinoline)dichloridoplatinum(II) (**18**).* IR (cm^{-1} neat) 3300–3600 (S, -OH) 2250–2700 (-NH$^+$-) 1250 (S, Ar-O); ^{1}H NMR (400 MHz, $[D]_6$DMSO) δ 7.80–9.20 (4H, OH), 7.05–7.20 (2H, 2 × NH), 5.50–6.80 (4H, *m*, H-5,5′,8,8′), 4.40–4.60 (2H, *m*, H-1,1′), 3.13–3.89 (4H, *m*, H-3), 2.01–2.90 (4H, *m*, H- 4), 1.03 (t, CH_3CH_2OH), Anal. Calcd for $C_{18}H_{20}N_2O_4PtCl_2 \cdot C_2H_5OH$: C, 37.39; H, 4.39; N, 4.36. Found: C, 37.54; H, 4.51; N, 4.26.

6.9. Cytotoxicity assay (rapid colorimetric assay) [24,25]

The assay using MTT [3-(4,5–di methylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] against Hepa59T/VGH (human liver carcinoma), WiDr (Human colon adenocarcinoma), Hela (human cervical epitheloid carcinoma) and MCF-7 (Human breast adenocarcinoma) tumor cells was based on the reported methods. The tumor cells were purchased from the American Type Culture Collection (ATCC). The tumor cell lines and culture conditions used were the same as previously described. Briefly, cells seeded at a density 5×10^{-3} cells per well (0.1 mL) were incubated for 24 h and 2-fold dilutions (0.1 mL) of tested samples were added. At the end of 72 h incubation, the medium was replaced with a fresh medium without fetal calf serum. The cells were then labeled with 10 μL of MTT stock solution (5 mg/mL) and the incubation continued at 37 °C for 2 h. The medium was removed and 0.2 mL of DMSO was

addes to each well to dissolve formazan crystals. Cytotoxic dose of 50% growth inhibition (The IC_{50} was defined by comparison with the untreated cells as the concentration of test sample resulting in 50% reduction of absorbance) was then estimated from optical density data at 550 nm. Negative control was also included and the tests were done quadruplicate and repeated at least twice.

Acknowledgements

We would like to thank Dr. Sun, researcher of the National Research Institute of Chinese Medicine, for helpful cytotoxicity assay.

References

[1] E.J. Shin, M.R. Hajaligol, F. Rasouli, Fuel 83 (2004) 1445–1453.
[2] A.R. Carroll, R.A. Davis, P.I. Forster, G.P. Guymer, R.J. Quinn, J. Nat. Prod. 64 (2001) 1572–1573.
[3] M.A. Rashid, K.R. Gustafson, M.R. Boyd, J. Nat. Prod. 64 (2001) 1249–1250.
[4] N. Sotomayeor, E. Dominguez, E. Lete, J. Org. Chem. 61 (1996) 4062–4072.
[5] S.A. Kim, Y. Kwon, J.H. Kim, M.T. Muller, I.K. Chung, Biochemistry 37 (1998) 16316–16324.
[6] K. Orito, M. Miyazawa, R. Kanbayashi, M. Tokuda, H. Suginome, J. Org. Chem. 64 (1999) 6583–6596.
[7] P.S. Charifson, J.P. Bowen, S.D. Wyrick, A.J. Hoffman, M. Cory, A.T. McPhail, R.B. Mailman, J. Med. Chem. 32 (1989) 2050–2058.
[8] D. Beaumont, R.D. Waigh, M. Sunbhanich, M.W. Nott, J. Med. Chem. 29 (1983) 507–515.
[9] A.H. Nielsen, J. Org. Chem. 35 (1970) 2498–2503.
[10] M. Kato, K. Sasano, C. Kosuge, M. Yamazaki, S. Yano, M. Kimura, Inorg. Chem. 35 (1996) 116–123.
[11] J.C. Plaquevent, T. Perrard, C. Cahard, Chemistry 8 (2002) 3300–3307.
[12] D. Lucet, L. Toupet, G.T. Le, C. Mioskowski, J. Org. Chem. 62 (1997) 2682–2683.
[13] E.R. Jamieson, S.L. Lippard, Chem. Rev. 99 (1999) 2467–2498.
[14] H. Ott, G.E. Hardtmann, M.D. Albert, A.J. Frey, J.H. Gogerty, G.H. Leslie, J.H. Trapold, Journal of Medicinal Chemistry (1968) 777–787.
[15] W.J. Gensler, in: R. Adams (Ed.), Organic Reactions, vol. 6, Wiley, New York, 1951, pp. 191–206.
[16] Z.M.A. Judeh, C.B. Ching, J. Bu, A. McCluskey, Tetrahedron. Lett. 29 (2002) 5089–5091.
[17] S.O. Ano, F.P. Intini, G. Natile, L.G. Marzilli, Inorg. Chem. 38 (1999) 2989–2999.
[18] M.J. Abrams, B.A. Murrer, Science 261 (1993) 725–731.
[19] M.A. Fuertes, C. Alonso, J.M. Perez, Chem. Rev. 103 (2003) 645–662.
[20] C.Y. Kuo, M.J. Wu, Y.H. Kuo, Eur. J. Med. Chem. 41 (2006) 940–949.
[21] D.J. Cram, F.A.A. Elhafez, J. Am. Chem. Soc. 74 (1952) 5828–5835.
[22] M.C. Elliott, E. Williams, Org. Biomol. Chem. 1 (2003) 3038–3047.
[23] P. D'Ocon, R. Blasco, L. Candenas, D. Ivorra, S. Lopez, C. Villaverde, L. Castedo, D. Cortes, Eur. J. Pharmacol. 196 (1991) 183–187.
[24] C.M. Sun, W.J. Syu, Y.T. Huang, C.C. Chen, J.C. Ou, J. Nat. Prod. 60 (1997) 382–384.
[25] T.J. Mosmann, J. Immunol. Meth. 65 (1983) 55–63.