Synthesis, Characterization and Biological Evaluation of Platinum(II) Complexes with a Chiral *N*-Monosubstituted 1,2-Cyclohexyldiamine Derivative

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Eight platinum(II) compounds with a new chiral ligand, 2-(((1*R*,2*R*)-2-aminocyclohexylamino)methyl)phenol (HL), were designed, prepared and spectrally characterized. All compounds showed better aqueous solubility than cisplatin and oxaliplatin. *In vitro* cytotoxicity of these compounds against human HepG-2, MCF-7, A549 and HCT-116 cell lines was evaluated. Results indicated that all compounds showed cytotoxicity against A549 and HepG-2 cell lines. Particularly, compounds B1 and B8, which have $CF_3SO_3^-$ and $(CH_3)_3COCH_2COO^-$ as leaving groups, respectively, exhibited better cytotoxicitiy than that of carboplatin in these two cell lines.

Key words 1*R*,2*R*-cyclohexyldiamine derivative; platinum(II) complex; cytotoxicity

Cisplatin is one of the most frequently used chemotherapeutics in the treatment of malignant tumors, nevertheless, the clinical application is greatly limited by two factors: intolerable side effects and intrinsic/acquired resistance.[1—5] So far, tremendous efforts have been devoted to developing new platinum compounds with improved pharmacological properties and broader range of antitumor activity. This leads to the clinical development of cisplatin analogues such as carboplatin and oxaliplatin which have been widely used for treating ovarian cancers and colorectal tumors, respectively.[6—9]

Oxaliplatin is the first platinum-based drug owning a chiral moiety, 1*R*,2*R*-diaminocyclohexane (DACH), as a carrier group, and now a preferred drug in treating advanced colorectal carcinoma (ACRC). Although oxaliplatin has supplanted cisplatin as the largest selling platinum therapeutic, its dose-limiting toxicity in the clinical practice is still insufferable. It has been reported that approximately 90% of patients treated with oxaliplatin suffered from acute neurotoxicity, while 10—15% of patients suffered from cumulative sensory.[10,11] Like other clinically available platinum anticancer drugs, oxaliplatin can not offer any substantial clinical advantages over the existing cisplatin, either.[12—14]

Considering the advantages of oxaliplatin in treating ACRC and its drawbacks, we have been devoting to studying new platinum compounds with chiral ligands in recent years. Since 1*R*,2*R*-diaminocyclohexane plays an important role in the molecular skeleton of oxaliplatin,[15,16] we have designed and prepared a number of 1*R*,2*R*-diaminocyclohexane based compounds, whose structures remain the chiral unit of DACH. Among those compounds, 2-(((1*R*,2*R*)-2-aminocyclohexylamino)methyl)phenol (HL) as a tridentate ligand has been prepared. A methylphenolic group was introduced to the DACH skeleton for two expectations. One is that the ligand can offer three coordination sites to bind a platinum atom that leaves an empty position to allow bridging ligands to form dinuclear platinum(II) complexes.[17] The other is that the aromatic ring in the ligand might yield steric hindrance like α-picoline in ZD0473[18] that shows no cross resistance with classical platinum-based drugs such as cisplatin and carboplatin. With HL as a carrier ligand and some monoacidic anions[19,20] as leaving groups, eight novel mononuclear platinum complexes (B1—B8) have been obtained. Herein reported are these platinum complexes and their *in vitro* cytotoxicity against four human cancer cell lines.

Experimental

Materials and Instruments K_2PtCl_4 was purchased from a local chemical company, mono-*tert*-butoxycarbonyl (Boc) protecting DACH was used as the starting material and prepared according to the procedure reported.[21]

All reagents were of high purity and used without any further purification. Elemental analysis for C, H and N was performed with a Perkin-Elmer 1400C instrument, while Pt was determined according to the method in EP5. Electrospray ionization-mass spectra (ESI-MS) spectra were carried out in Finnigan MAT SSQ 710 (120—1000 amu) apparatus and IR spectra were scanned by a Nicolet IR200 spectrophotometer in the range of 4000—400 cm^{-1} in KBr pellet. ^{1}H-NMR spectra were recorded on a Bruker DRX-500 spectrometer at 500 MHz in D_2O using tetramethylsilane (TMS) as an internal reference. Specific rotations were tested on a Shenguang WZZ-2B instrument.

Synthesis. Ligand HL: 2-(((1*R*,2*R*)-2-Aminocyclohexylamino)methyl)-phenol Fifty millimole mono-Boc protecting DACH and 60 mmol salicylaldehyde were dissolved in 100 ml of toluene and refluxed for 5 h. After concentrating the solution, white solids were obtained, which was dissolved in 200 ml of methanol and 100 mmol $NaBH_4$ was added in portions. The mixture was refluxed for 3 h, EtOAc was used to extract the product into the organic phase which was washed with water twice. The organic solution was mixed with excess HCl/EtOAc (5 mol/l), leading to the formation of HL hydrochloride, which was finally neutralized by aqueous Na_2CO_3 solution (1 mol/l) to give free HL 4.3 g. Yield, 40%. Found (Calcd for $C_{13}H_{20}N_2O$), C 71.08 (70.91), H 9.23 (9.09), N 12.58 (12.73). IR (KBr, cm^{-1}): 3418—3274 (m, ν_{N-H}), 2928, 2852 (m, ν_{C-H}), 1596 (m, ν_{Ar}), 750 (m, γ_{ArH}). ESI-MS *m*/*z*: $[M+H]^+$=221 (100%). ^{1}H-NMR ($CDCl_3$, ppm) δ: 1.06—2.38 (m, 8H, 4$\underline{CH_2}$ of DACH), 2.79—3.05 (m, 2H, 2$\underline{CH}$ of DACH), 3.79—4.02 (m, 2H, C_6H_4–$\underline{CH_2}$), 6.78—7.13 (m, 4H, $\underline{C_6H_4}$). $[\alpha]_D^{15}$ +95.0 (*c*=1.0, MeOH).

Intermediate [PtLI] Twelve millimole K_2PtCl_4 was added into a stirring aqueous solution containing 80 mmol KI in 100 ml water. The blending solution was stirred at 25 °C for 30 min under a nitrogen atmosphere to get a black solution of K_2PtI_4. Then a mixing aqueous solution of 12 mmol HL and 12 mmol NaOH in 30 ml water was added dropwisely under stirring in the dark at 25 °C. After 6 h, the dark yellow precipitate was filtered, washed sequentially with water, ethanol and ether, and finally dried in vacuum. Data for [PtLI]: 6.2 g, Yield: 95%, dark yellow solid. Found (Calcd for $C_{13}H_{19}IN_2OPt$), C 28.56 (28.83), H 3.65 (3.51), N 5.26 (5.18), Pt 35.87 (36.04). IR (KBr, cm^{-1}): 3418—3180 (m, ν_{N-H}), 2933, 2859 (m, ν_{C-H}), 1594 (m, ν_{Ar}), 748 (m, γ_{ArH}). ESI-MS *m*/*z*: $[M+H]^+$=542 (60%), $[M+Na]^+$=564 (100%). ^{1}H-NMR (DMSO, ppm) δ: 1.06—2.74 (m, 10H of DACH), 3.88—4.09 (m, 2H, C_6H_4–$\underline{CH_2}$), 5.83—6.54(m, 3H, $\underline{NH_2}$ and $\underline{NH}$), 6.72—7.09 (m, 4H, $\underline{C_6H_4}$). $[\alpha]_D^{15}$ +96.9 (*c*=1.0, MeOH).

Compound B1 Two millimole [PtLI] was first suspended in 100 ml pure

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water, and next a solution of 2 mmol $AgNO_3$ in 20 ml pure water was added. After stirring under a nitrogen atmosphere in the dark for 12 h at 40 °C, the deposit was filtered off. Filtrate was blended with 2 mmol CF_3SO_3Na and stirred at 60 °C for 24 h. The solution was concentrated and cooled to 0 °C. White solid was collected, washed with a small amount of chilled water and ethanol, and then dried at 60 °C in vacuum. Yield 21%, Found (Calcd for $C_{14}H_{19}F_3N_2O_4SPt$), C 29.61 (29.83), H 3.53 (3.37), N 5.10 (4.97), Pt 34.81 (34.64). IR (KBr, cm^{-1}): 3433—3107 (m, ν_{N-H}), 2936, 2864 (m, ν_{C-H}), 1566 (m, ν_{Ar}), 758 (m, γ_{ArH}). ESI-MS *m/z*: $[M-H]^-$=62 (50%). ^{1}H-NMR (D_2O, ppm) δ: 1.13—2.64 (m, 10H of DACH), 3.87—4.23 (m, 2H, C_6H_4–$\underline{CH_2}$), 6.66—7.15 (m, 4H, $\underline{C_6H_4}$).

The procedures for preparing compounds **B2**—**B8** were similar to **B1**.

Compound B2 Yield 27%, Found (Calcd for $C_{15}H_{21}ClN_2O_3Pt$), C 35.20 (35.46), H 4.27 (4.14), N 5.39 (5.52), Pt 38.29 (38.43). IR (KBr, cm^{-1}): 3427—3104 (m, ν_{N-H}), 2937, 2861 (m, ν_{C-H}), 1596 (s, $\nu_{C=O}$), 755 (m, γ_{ArH}). ESI-MS *m/z*: $[M-H]^-$=507 (100%). ^{1}H-NMR (D_2O, ppm) δ: 1.12—2.60 (m, 10H of DACH), 3.84—3.97 (m, 2H, Cl$\underline{CH_2}$COO), 4.18—4.28 (m, 2H, C_6H_4–$\underline{CH_2}$), 6.63—7.14 (m, 4H, $\underline{C_6H_4}$).

Compound B3 Yield 19%, Found (Calcd for $C_{15}H_{22}N_2O_3Pt$), C 37.90 (38.05), H 4.79 (4.65), N 5.76 (5.92), Pt 41.38 (41.23). IR (KBr, cm^{-1}): 3413—3102 (m, ν_{N-H}), 2936, 2861 (m, ν_{C-H}), 1596 (s, $\nu_{C=O}$), 751 (m, γ_{ArH}). ESI-MS$^-$ *m/z*: $[M-H]^-$=472 (100%). ^{1}H-NMR (D_2O, ppm) δ: 1.12—2.60 (m, 13H, 10H of DACH and 3H of $\underline{CH_3}$), 3.83—4.27 (m, 2H, C_6H_4–$\underline{CH_2}$), 6.24—7.15 (m, 4H, $\underline{C_6H_4}$).

Compound B4 Yield 31%, Found (Calcd for $C_{16}H_{24}N_2O_4Pt$), C 38.03 (38.17), H 4.90 (4.77), N 5.82 (5.57), Pt 38.93 (38.77). IR (KBr, cm^{-1}): 3418—3111 (m, ν_{N-H}), 2936, 2862 (m, ν_{C-H}), 1597 (s, $\nu_{C=O}$), 750 (m, γ_{ArH}). ESI-MS$^-$ *m/z*: $[M+CH_3OCH_2COO^-]^-$=592 (100%). ^{1}H-NMR (D_2O, ppm) δ: 1.06—2.74 (m, 10H of DACH), 3.19—3.43 (m, 5H of $\underline{CH_3O}\underline{CH_2}$COO), 3.76—4.04 (m, 2H, C_6H_4–$\underline{CH_2}$), 6.30—7.07 (m, 4H, $\underline{C_6H_4}$).

Compound B5 Yield 33%, Found (Calcd for $C_{17}H_{26}N_2O_4Pt$), C 39.21 (39.46), H 5.20 (5.03), N 5.58 (5.42), Pt 37.49 (37.74). IR (KBr, cm^{-1}): 3432—3112 (m, ν_{N-H}), 2934, 2863 (m, ν_{C-H}), 1597 (s, $\nu_{C=O}$), 749 (m, γ_{ArH}). ESI-MS$^-$ *m/z*: $[M+CH_3CH_2OCH_2COO^-]^-$=620 (100%). ^{1}H-NMR (D_2O, ppm) δ: 0.84—2.74 (m, 13H, 10H of DACH and 3H of $\underline{CH_3}$), 3.19—3.49 (m, 4H of $\underline{CH_2O}\underline{CH_2}$COO), 3.67—4.09 (m, 2H, C_6H_4–$\underline{CH_2}$), 6.25—7.07 (m, 4H, $\underline{C_6H_4}$).

Compound B6 Yield 23%, Found (Calcd for $C_{18}H_{28}N_2O_4Pt$), C 40.52 (40.68), H 5.11 (5.27), N 5.42 (5.27), Pt 36.88 (36.72). IR (KBr, cm^{-1}): 3428—3114 (m, ν_{N-H}), 2934, 2863 (m, ν_{C-H}), 1598 (s, $\nu_{C=O}$), 748 (m, γ_{ArH}). ESI-MS$^-$ *m/z*: $[M-H]^-$=530 (100%). ^{1}H-NMR (D_2O, ppm) δ: 1.01—2.72 (m, 16H, 10H of DACH and 6H of 2$\underline{CH_3}$)), 3.19—3.66 (m, 3H of $\underline{CHO}\underline{CH_2}$COO), 3.76—4.08 (m, 2H, C_6H_4–$\underline{CH_2}$), 6.29—7.07 (m, 4H, $\underline{C_6H_4}$).

Compound B7 Yield 34%, Found (Calcd for $C_{19}H_{30}N_2O_4Pt$), C 41.56 (41.83), H 5.33 (5.50), N 5.21 (5.14), Pt 35.91 (35.79). IR (KBr, cm^{-1}): 3419—3110 (m, ν_{N-H}), 2935, 2863 (m, ν_{C-H}), 1596 (s, $\nu_{C=O}$), 751 (m, γ_{ArH}). ESI-MS$^-$ *m/z*: $[M+CH_3(CH_2)_3OCH_2COO^-]^-$=676 (100%). ^{1}H-NMR ($D_2O$, ppm) δ: 0.84—2.73 (m, 17H, 10H of DACH and 7H of $\underline{CH_3CH_2CH_2}$), 3.34—3.49 (m, 4H of $\underline{CH_2O}\underline{CH_2}$COO), 3.80—4.04 (m, 2H, C_6H_4–$\underline{CH_2}$), 6.48—7.07 (m, 4H, $\underline{C_6H_4}$).

Compound B8 Yield 36%, Found (Calcd for $C_{19}H_{30}N_2O_4Pt$), C 41.52 (41.83), H 5.66 (5.50), N 5.06 (5.14), Pt 35.68 (35.79). IR (KBr, cm^{-1}): 3437—3111 (m, ν_{N-H}), 2936, 2862 (m, ν_{C-H}), 1597 (s, $\nu_{C=O}$), 751 (m, γ_{ArH}). ESI-MS$^-$ *m/z*: $[M+(CH_3)_3COCH_2COO^-]^-$=676 (100%). ^{1}H-NMR ($D_2O$, ppm) δ: 0.92—2.72 (m, 19H, 10H of DACH and 9H of 3$\underline{CH_3}$)), 3.35—3.74 (m, 2H of CO$\underline{CH_2}$COO), 3.80—4.09 (m, 2H, C_6H_4–$\underline{CH_2}$), 6.25—7.02 (m, 4H, $\underline{C_6H_4}$).

***In Vitro* Cytotoxicity** 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was carried out as described by Mosmann.[22] Tumor cells were plated onto 96-well sterile plates in 100 ml of medium at a density of 4×10^3—8×10^3 cells per well and incubated for 24 h at 37 °C in a 5% CO_2 containing incubator. Compounds B1—B8, oxaliplatin and carboplatin were added in final concentrations ranging from 0 to 100 μM. After 48 h, 50 μl MTT in phosphate buffered saline (PBS) (5 mg/ml) was added to each well and the plates were incubated for 3 h at 37 °C. Removing the liquid and adding dimethyl sulfoxide (DMSO) (100 ml) to dissolve the MTT formazan. The optical density (OD) for each well was measured on a microplate reader at a wavelength of 490 nm. All cytotoxicity tests were carried out 3 times parallelly, IC_{50} values were got from curves constructed by plotting cell survival (%) *versus* compound concentration (in μM).

Result and Discussion

For the synthesis of the ligand (HL), it is hard to directly get a *N*-monsubstituted derivative due to the equivalent reactivity of the two amino groups in DACH. Thus, mono-Boc protecting DACH[21] was used as the starting material, and the ligand was synthesized *via* several synthetic steps.

When preparing the targeted platinum compounds, we first synthesized the intermediate [PtLI] by adopting a similar method described literaturally.[23] And then $AgNO_3$ was used to remove the iodide anions of [PtLI] to form $[PtL(H_2O)]NO_3$, which was *in situ* reacted with sodium salts of the corresponding acids, respectively, to give compounds **B1** to **B8** (Chart 1) .

All platinum complexes were characterized by IR, ^{1}H-NMR, ESI-MS spectra and microanalyses. The elemental analysis results are in good agreement with the calculated values. The IR spectra of these complexes are similar, Pt–N

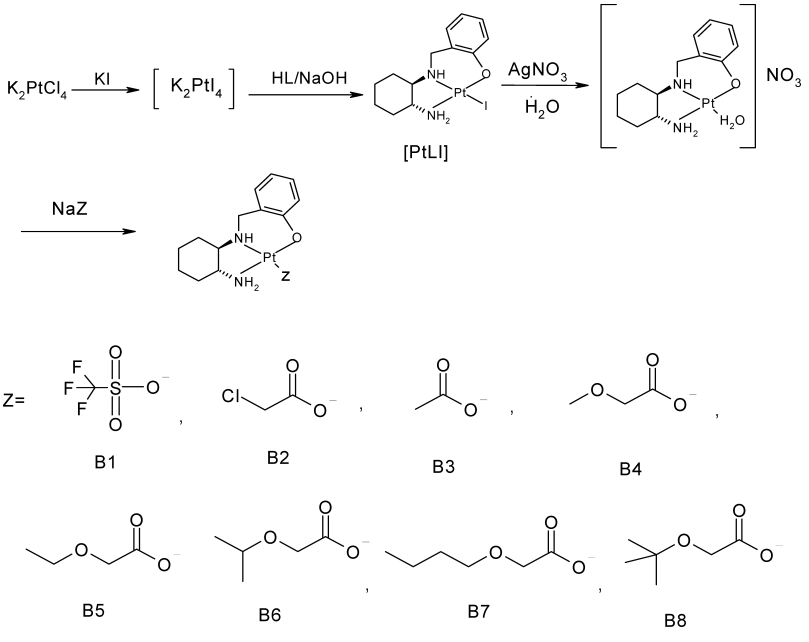

Chart 1. Synthetic Chart for Platinum(II) Compounds **B1**—**B8**

coordination bonds were confirmed by the examination of $\nu NH_2/\nu NH$ shifting to lower frequencies comparing with their free amino groups. On the other hand, the shifts of the C=O absorption from free carboxylic acids near 1700 cm^{-1} to a band near 1597—1596 cm^{-1} proved that carboxylate anions were coordinated to Pt(II) ions in each case (**B2**—**B8**).[24] The ^{1}H-NMR spectra of the complexes are consistent with their corresponding protons both in the chemical shifts and the number of hydrogens. All prepared complexes showed a peak of $[M+Z]^-$ or $[M-H]^-$ in their negative ESI mass spectra, which are consistent with expected molecular formula weights. The mass spectra of the compounds exhibited three typical molecular ion peaks due to the isotopes of ^{194}Pt (33%),^{195}Pt (34%) and ^{196}Pt (25%).

Poor aqueous solubility is a problem for some platinum anticancer drugs, such as cisplatin. Hence the aqueous solubility of our Pt compounds was measured at 25 °C (Table 1). Compared with cisplatin (1 mg/ml) and oxaliplatin (8 mg/ml), all prepared platinum compounds have rather good aqueous solubility ranging from 12.8 to 26.2 mg/ml. In addition, the optical rotations of all complexes were tested. All compounds exhibited analogous optical rotations (+35.2°—+67.9°) to that of oxaliplatin (+76.1°).

The *in vitro* cytotoxicity of these platinum compounds was evaluated by MTT colorimetric assay using HepG-2 human hepatocellular carcinoma cell, MCF-7 human breast cancer cell, A549 human lung cancer cell and HCT-116 human colorectal cancer cell, with carboplatin and oxaliplatin as positive controls. As we can see from Table 2, all platinum compounds showed activity against HepG-2 and A549 cell lines. Among them, compounds **B1** and **B8** gave lower IC_{50} values than carboplatin in these two cell lines. Compounds **B6** and **B7** also showed better antitumor activity than carboplatin against HepG-2. Further studies indicated that when alkoxyacetates were selected as leaving groups in these compounds against A549 cell lines,the longer the carbon chain was, the better the antitumor activity was. The activity order was **B4** ($CH_3OCH_2COO^-$)<**B5** ($CH_3CH_2OCH_2COO^-$)<**B6** ($(CH_3)_2CHOCH_2COO^-$)<**B7** ($CH_3(CH_2)_3OCH_2COO^-$)<**B8** ($(CH_3)_3COCH_2COO^-$). It is observed that compounds **B4** to **B8** have diminished their hydrophily but increased their lipotropy with the increase of the carbon chain in alkoxyacetate. Thus, B8 was assumed to be easier to enter the tumor cells and inhibit proliferation.[25—29] It is noted that compounds B7 and B8 have the same carbon numbers, but **B8** with a branched carbon chain showed better activity than **B7** with a linear carbon chain. All complexes exhibited analogous optical rotations to that of oxaliplatin, this may be one of reasons that they are of activity against HepG-2 and A549 cell lines.

When against MCF-7 and HCT-116, only compound **B1** exhibited close activity to positive controls. Despite of being less cytotoxicity than that of oxaliplatin against HCT-116, our compounds were assumed to have no cross resistance with classical platinum-based drugs due to the unique coordination mode of the deprotonated ligand with the metal atom. But further work needs to be done to support our assumption in future.

We have ever reported a number of Pt(II) complexes with several alkoxyacetates as leaving groups.[19] Among them, compounds **1a**, **2a** and **3a** with DACH as a carrier ligand showed better activity than those of cisplatin, carboplatin and oxaliplatin against a few of human tumor cell lines including A549. Although compounds **B1** and **B8** showed better activity than carboplatin, they did not surpass those of compounds **2a** and **3a** when treated with A549 cell line. As expected, HL has offered three donor atoms to bind a platinum atom strongly by forming three chelating rings. The methyl phenol group was assumed not to function as a leaving group but offer steric resistance to hinder the Pt-DACH species to bind with glutathione, however, this group has appreared to decrease the antitumor activity of the Pt-DACH species of our compounds in comparison to that of oxaliplatin. While in compounds **1a**, **2a** and **3a**, the Pt-DACH species with more empty space after the leaving of two alkoxyacetate anions could interact with DNA, leading to high cytotoxicity of these compounds.

Table 1. Aqueous Solubility and Optical Rotation of Complexes **B1**—**B8**

Complex	Aqueous solubility (mg/ml, 25 °C)	$[\alpha]_D^{15}$ (c=1.0, H_2O)
B1	12.8	+60.8°
B2	23.8	+48.8°
B3	20.5	+67.9°
B4	26.2	+39.7°
B5	21.3	+40.8°
B6	18.9	+35.2°
B7	13.6	+42.3°
B8	15.1	+41.8°

Table 2. Cytotoxicity of Compounds **B1**—**B8** against Four Tumor Cell Lines

Compd.	$IC_{50}/(\mu mol \cdot l^{-1})$			
	HepG-2	MCF-7	A549	HCT-116
B1	4.8	18.1	5.4	6.9
B2	31.3	>100	36.0	>100
B3	47.4	66.5	22.5	>100
B4	27.9	>100	50.1	>100
B5	13.1	>100	36.9	>100
B6	8.3	>100	25.5	59.2
B7	9.6	53.2	16.8	38.1
B8	7.1	27.6	8.3	13.6
Carboplatin	9.7	15.3	11.2	Not tested
Oxaliplatin	Not tested	Not tested	Not tested	4.3

Conclusion

We prepared a new compound of HL derived from 1*R*,2*R*-diaminocyclohexane. Using HL as a carrier ligand, we have designed and synthesized eight novel Pt(II) complexes with a number of caroxylate anions as leaving groups. All compounds showed better aqueous solubility than cisplatin and oxaliplatin. *In vitro* cytotoxicity tests showed that these compounds had certain antitumor activity against A549 and HepG-2 cell lines. Among them, compounds **B1** and **B8** showed better antitumor activity than carboplatin against these two cell lines. Besides, compound **B1** also exhibited close activity to positive controls against HCT-116 and MCF-7 cell lines. Consequently, the prepared platinum compounds, especially **B1**, may be deserved for further investigation as a leading compound.

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