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Synthesis of neuroprotective cyclopentenone prostaglandin analogs: Suppression of manganese-induced apoptosis of PC12 cells

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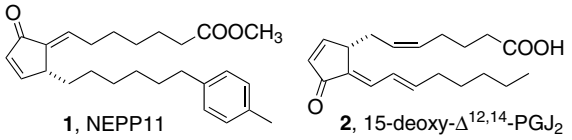
Abstract—The synthesis and evaluation for anti- and proapoptotic properties of cyclopentenone prostaglandin analogs are described. Novel J-type analogs of NEPP11 with a cross-conjugated cyclopentadienone moiety and a lipophilic ω-side chain suppressed manganese ion-induced apoptosis of PC12 cells at comparable levels to NEPP11, while monoenone derivatives were inactive. The proapoptotic activities of J-type analogs were much lower than that of NEPP11. Natural 15-deoxy-$\Delta^{12,14}$-PGJ_2 and Δ^{7}-PGA_1 methyl ester were highly toxic, inducing apoptosis at lower concentrations.

Prostaglandins (PGs) E and D are important lipid-signaling mediators that are metabolized to cyclopentenone PGs of the A and J series by both enzymatic and non-enzymatic processes.[1] Although cyclopentenone PGs have no membrane receptors, they penetrate cell membranes and bind directly to intracellular proteins, and thus regulate their activities. Cyclopentenone PGs are now attracting increasing interest as low-molecular-weight compounds that can modulate cellular proliferation, differentiation, and viability.[2,3]

In previous studies on the synthesis and biological activities of cyclopentenone PGs, we demonstrated that certain synthetic analogs with a cross-conjugated dienone structure exhibited neuroprotective activities.[4–7] These dienone PGs not only protected HT22 neuronal cells against oxidative glutamate toxicity, but also promoted neurite outgrowth from PC12 cells or from dorsal root ganglion explants induced by nerve growth factor. The most potent compound for both activities was analog **1** (designated NEPP11).[6] EPP11 was found to protect the brain in vivo against ischemic death caused by permanent middle cerebral artery occlusion in mice.[5] In contrast, natural 15-deoxy-$\Delta^{12,14}$-PGJ_2 (**2**), which possesses an extended trienone moiety, could not protect neuronal cells against glutamate toxicity.[5]



1, NEPP11 **2**, 15-deoxy-$\Delta^{12,14}$-PGJ_2

More recently, we found that low concentrations of NEPP11 (<10 μM) inhibited manganese ion-induced apoptosis of PC12 cells,[8] which has been used as a cellular model of Parkinson's disease.[9] These findings suggested that NEPP11 may act as a therapeutic agent against neurodegenerative diseases including Alzheimer's and Parkinson's diseases. At high concentrations (≥10 μM), however, NEPP11 was toxic to PC12 cells, as well as inducing apoptosis,[8] suggesting the need for less toxic compounds with sufficient antiapoptotic activity. The rational design of better neuroprotective compounds thus requires investigation of the relationships

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between the structure of NEPP11 analogs and their anti- and proapoptotic effects. This paper describes the synthesis of modified NEPP11 analogs and evaluation of their effects on manganese ion-treated/untreated PC12 cells.

NEPP11 can protect neuronal cells from manganese ion-induced apoptosis as well as glutamate-mediated cell death (necrosis).[5–7] We recently reported that induction of hemeoxygenase-1 (HO-1) through activation of the Keap1/Nrf2 pathway by NEPP11 is crucial for suppressing glutamate-mediated necrotic death of HT22 cells.[7] HO-1 is a phase II enzyme that serves as a defense system against oxidative stress. In contrast, the antiapoptotic effect of NEPP11 on manganese ion-treated PC12 cells is associated with prevention of phosphorylation of c-Jun N-terminal kinase (JNK).[8] Since MKK4, an upstream kinase of JNK, is activated by manganese ion in the presence of NEPP11, one target of NEPP11 may be the phosphorylation stage of JNK by activated MKK4. Taken together, these data suggest that NEPP11 targets different molecules in each of the neuroprotective effects. This implies that the structural features of compounds required for the suppression of manganese-induced apoptosis may differ from those required for protection against glutamate toxicity.

We reported previously that two structural aspects of cyclopentenone PGs are essential for the suppression of glutamate-induced neuronal cell death: a cross-conjugated dienone moiety and a modestly lipophilic ω-side chain.[5] To explore simple relationships between the structure and antiapoptotic effects of cyclopentenone PGs on manganese ion-treated PC12 cells, we designed and synthesized several NEPP11 analogs, particularly focusing on the enone and ω-side-chain moieties.[10] The structures of the compounds investigated are presented in Figure 1.

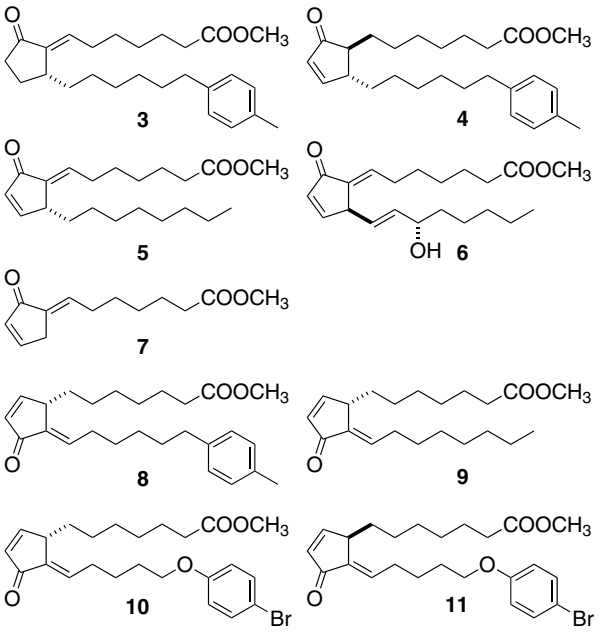
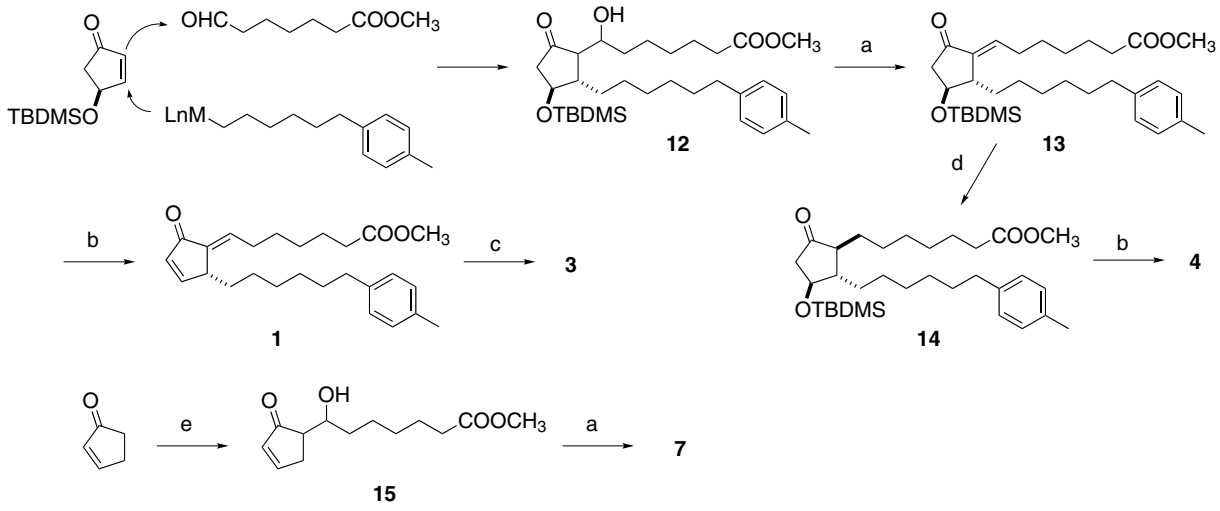


Figure 1. Structures of cyclopentenone PGs.

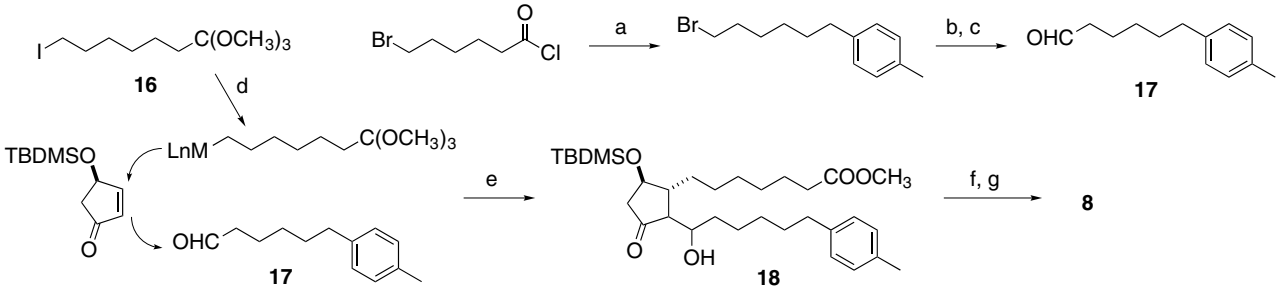
Two different types of monoenone analogs, an alkyliden cyclopentanone type compound **3** and an endo cyclopentenone type compound **4**, can be seen in the structures. Compounds **5**, **6**, and **7** are ω-side-chain variants, in which **5** has a simple octyl chain, **6** has the same ω-side chain as natural primary prostaglandins, and **7** does not have an ω-side chain. The remaining compounds in Figure 1 represent the topological isomers of A-type PGs at the cyclopentenone ring and are categorized as J-type PGs. Thus, **8** and **9** are the upside-down isomers of NEPP11 and **5**, respectively. We also designed **10** and its enantiomer **11**, each of which contains a bromophenyl group at the terminal end of the ω-side chain. These brominated compounds are intended to apply to positron emission tomography studies as ^{76}Br-incorporated tracers.

The designed cyclopentenone PG analogs were generally synthesized based on the established three-component coupling method.[11] We have already described the syntheses of NEPP11, **5**, and **6**.[6,12] The monoenone derivatives **3** and **4** were prepared from NEPP11 and **13** as illustrated in Scheme 1. Thus, conjugate reduction of NEPP11 with DIBAL–CuI–CH_3Li complex[13] proceeded selectively at C10–C11 to yield the alkyliden-type analog **3**. In contrast, treatment of **13** with tributylstannane in the presence of di-*tert*-butylperoxide gave the saturated derivative **14**,[14] which was then converted to the desired product **4** by acidic elimination of the oxygen functional group at C11. The remaining A-type analog **7** was synthesized by aldol condensation of cyclopent-2-en-1-one with 7-oxoheptanoate followed by the mesylation-elimination sequence.

The J-type derivatives were synthesized by employing a modified three-component coupling method, in which the roles of the side-chain units were reversed. This process involves nucleophilic attack of the organocuprate or organozincate derived from the α-side-chain unit and subsequent trapping of the resulting enolate with the ω-side-chain aldehyde. Scheme 2 demonstrates the synthesis of **8** by this method. The orthoester **16**, selected as the α-side-chain unit, was prepared as described.[15] The ω-side-chain aldehyde **17** was synthesized by (1) Friedel–Crafts acylation of toluene with 6-bromohexanoyl chloride, followed by reductive deoxygenation of the resulting ketone with triethylsilane in one pot, (2) conversion of the bromide, 6-(4-methylphenyl)-1-bromohexane, to the corresponding iodide with sodium iodide, and (3) DMSO oxidation of the iodide to the desired aldehyde **17**. The three-component coupling of the cuprate generated from **16**, (*R*)-4-(*tert*-butyldimethylsiloxy)cyclopent-2-en-1-one, and **17** afforded the aldol adduct **18** in the ester form through concomitant hydrolysis of the orthoester moiety during workup procedures. The adduct **18** was dehydrated with methanesulfonyl chloride and 4-(dimethylamino)pyridine, followed by elimination of the siloxy moiety under acidic conditions to produce **8**. The use of octanal or 5-(4-bromophenoxy)pentanal as the ω-side-chain unit yielded the derivatives **9**[16] (38% in three steps) and **10** (26% in three steps), respectively. Compound **11**, the antipode of **10**, was similarly synthesized using the enantiomeric



Scheme 1. Synthesis of A-type PG analogs. Reagents and conditions: (a) MsCl, 4-(dimethylamino)pyridine, CH_2Cl_2, rt, 79% for **13** (16 h) and 87% for **7** (2 h); (b) CH_3COOH–THF–H_2O, 70 °C, 73% for **1** (22 h) and 87% for **4** (24 h); (c) CuI, CH_3Li, DIBAL, THF–HMPA, −50 °C, 1 h, 92%; (d) Bu_3SnH, $(CH_3)_3COOC(CH_3)_3$, 110 °C, 15 m, 74%; (e) LTMP, THF, −78 °C, 45 m, then methyl 7-oxoheptanoate, −78 °C, 1.75 h, 58%.



Scheme 2. Synthesis of J-type PG analogs. Reagents and conditions: (a) $AlCl_3$, toluene, CH_2Cl_2, rt, 14 h, then Et_3SiH, rt, 40 m, 93%; (b) NaI, acetone, rt, 14 h, 94%; (c) DMSO, $NaHCO_3$, 150 °C, 10 m, 73%; (d) *t*-BuLi, CuI, PBu_3; (e) ether–THF, −78 °C (0.5 h) to −40 °C (0.5 h), 30%; (f) MsCl, 4-(dimethylamino)pyridine, CH_2Cl_2, rt, 24 h, 56%; (g) CH_3COOH–THF–H_2O, 70 °C, 12 h, 72%.

cyclopentenone unit with the 4*S* configuration (32% in three steps). All compounds synthesized were fully characterized by 400 MHz ^1H NMR and 100 MHz ^{13}C NMR.

The ability of the cyclopentenone PGs to inhibit manganese ion-induced apoptosis of PC12 cells was evaluated by DNA fragmentation.[8] Briefly, PC12 cells (ca. 2×10^7 were preincubated with each PG (10 and 20 μM) at 37 °C for 30 min in Dulbecco's modified Eagle's medium supplemented with 5% fetal bovine serum, followed by the addition of 0.25 mM $MnCl_2$ and further incubation for 20 h at the same temperature. The soluble DNA was extracted from the cells, electrophoresed on agarose gels, and visualized with an ultraviolet transilluminator. Proapoptotic activity of the PGs was evaluated under the same assay conditions, but in the absence of manganese ion. The results are summarized in Figure 2 and Table 1.

Compound **5** suppressed manganese-induced DNA fragmentation to a similar extent as NEPP11. At 10 μM, compound **5** was less toxic than NEPP11, whereas at 20 μM, compound **5** induced a considerable degree of DNA fragmentation (Fig. 2B). The monoenone analogs, **3** and **4**, were completely inactive, indicating that the cross-conjugated cyclopentadienone structure is a prerequisite for exerting antiapoptotic effects. However, possession of the cross-conjugated dienone structure alone is not sufficient, inasmuch as Δ^7-PGA_1 methyl ester (**6**) and **7**, both of which possess the dienone moiety, showed no antiapoptotic activity. These results indicate that the lipophilic interaction of the ω-side chain with the target protein is important for binding. Thus, the hydroxy group in the ω-side chain

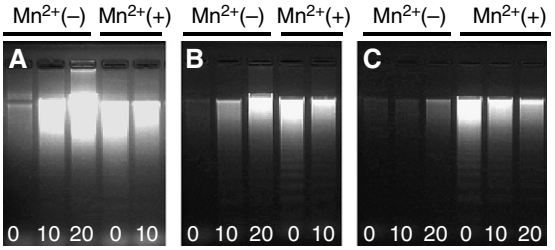


Figure 2. DNA fragmentation patterns observed with compounds **2** (A), **5** (B), and **9** (C). PC12 cells were preincubated with each compound (0, 10, and 20 μM) for 30 min and incubated with medium in the presence (Mn^{2+}(+)) or absence (Mn^{2+}(−)) of $MnCl_2$. After 20 h, DNA fragmentation was measured.

Table 1. Anti- and proapoptotic activities of cyclopentenone PGs

Compound	Antiapo[a]	Toxicity[b]
1	++	++
2	–	++
3	–	–
4	–	–
5	++	+
6	–	++
7	–	–
8	++	–
9	++	–
10	+	–
11	–	++

[a] Antiapoptotic activities: ca. 50% suppression of DNA fragmentation at 10 μM (++); less than 50% (+); no suppression up to 20 μM (–).
[b] Proapoptotic activities: induction of extensive DNA fragmentation at 10 μM (++); slight fragmentation at 10 μM (+); no fragmentation up to 20 μM (–).

may interfere with its interaction with the target protein, and deletion of the ω-side chain results in a complete loss of binding affinity. In addition, since the octyl derivative **5** retains antiapoptotic activity, the terminal tolyl group in the ω-side chain is probably not an essential component, but it may also not impair binding.

Natural 15-deoxy-$\Delta^{12,14}$-PGJ2 (**2**), which seems to meet the above requirements except for differences in topology and conjugation of the cyclopentenone structure, strongly induced DNA fragmentation by itself, even at low concentrations; thus, any antiapoptotic effect on manganese ion-treated cells was not detected across the range of concentrations examined (Fig. 2A). By contrast, compound **9**, which possesses a conjugation system saturated at the distal double bond of the extended trienone structure of **2**, showed a concentration-dependent inhibition of DNA laddering, but was not toxic, even at 20 μM (Fig. 2C). The NEPP11-type analog **8** also displayed similar activities. These results indicate that the topological feature of the cyclopentadienone structure is not important, at least for inhibition of DNA fragmentation, whereas the extension of conjugation leads to enhanced toxicity. The bromophenoxy analog **10** only weakly inhibited manganese-induced apoptosis, whereas its enantiomer **11**, which did not suppress DNA fragmentation, strongly induced apoptosis at 10 μM, suggesting the inadequacy of the bulky or excessively lipophilic bromophenoxy group. These findings emphasize the regulatory role of the ω-side chain in binding.

Cyclopentenone PGs commonly induce cytotoxicity with increasing concentrations. Conjugated enones are electron-deficient at their olefinic moiety, and thus easily react with nucleophiles such as thiols to form 1,4-adducts under physiological conditions.[17] Furthermore, cross-conjugated cyclopentadienones are more reactive to thiols than are simple cyclopentenones.[17] Therefore, cyclopentenone PGs with a cross-conjugated dienone moiety may react with a sulfhydryl group of a cysteine residue in various proteins in the cells, forming covalent complexes at lower concentrations than monoenone PGs. This covalent adduct formation may impair protein function, thus affecting cellular viability. Actually, 15-deoxy-$\Delta^{12,14}$-PGJ_2 has been reported to target mitochondria respiratory complex I by covalent binding, inhibiting the function of the protein and resulting in the induction of reactive oxygen species.[18] Although the molecular mechanisms underlying the proapoptotic effects of cyclopentenone PGs on PC12 cells are unclear, differences in toxicity may partially be attributable to the reactivity of the enone structures. This hypothesis is supported by our finding, that compounds **3** and **4**, which have a less reactive monoenone structure, did not exhibit cytotoxicity at the concentrations tested. However, compound **6** was more toxic than NEPP11 and **5**, suggesting that not only the cyclopentenone moiety but also the structure of the ω-side chain affected the proapoptotic effects of these compounds. Conversely, these findings suggest that appropriate modification of the ω-side chain and cyclopentenone moieties may eliminate the undesirable cytotoxicity of cyclopentenone PGs. To achieve the desired functionality, it is necessary to identify the target molecule(s) associated with the proapoptotic effects and to establish structure–activity relationships by rational structural modification. Analysis of intracellular binding proteins using photoaffinity labeling probes will be addressed in our future studies.

In summary, we have assayed the simple structure–activity relationship of cyclopentenone analogs. These experiments have provided a greater understanding of the importance of the ω-side chain for both anti- and proapoptotic effects. Moreover, we have succeeded in elaborating novel J-type analogs with reduced cytotoxicity. These compounds may not only be powerful molecular tools to elucidate the mechanisms of Parkinson's disease but may also provide important clues to the development of drugs for the treatment of such neurodegenerative diseases.

Acknowledgments

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