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Incorporation of a coumarin unit by nucleophilic addition reaction into a PPARγ ligand

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ABSTRACT

We examined the incorporation of a coumarin unit into model compounds using nucleophilic addition reaction. Then we applied these conditions to synthesize coumarin-incorporated Rosiglitazone. This fluorescent labeled compound maintained transcriptional activity for PPARγ.

Introduction

The coumarin skeleton is found in a variety of heterocyclic compounds in plants and exhibits a wide range of biological activities. In addition, it is widely used as a fluorescent probe or pigments [1]. Strategies for constructing the coumarin skeleton are well established and were recently reviewed [2] and include Perkin condensation, Knoevenagel condensation, Pechmann reactions, Baylis-Hillman reactions, and reactions using metal catalysts.

We recently reported a Turn-on type fluorescent probe [3] containing a coumarin precursor that formed the coumarin skeleton by conjugating to nucleophilic amino acid residues in a protein. This reaction promoted by proximity effect through ligand – biomolecule interaction. It was not intended for organic synthesis. We planned to investigate whether this nucleophilic cyclization reaction is applicable to ligand synthesis in the areas of medicinal chemistry or chemical biology. This strategy for synthesizing receptor ligands has several advantages: i) the fluorescence wavelength would likely change depending on the nucleophile, allowing imaging with different colors, ii) the coumarin ligand would be readily constructed in one step. iii) introduction of the coumarin would be facile if precursor of ligand has a nucleophilic functional group.

In this study we first examined the best reaction conditions for forming the coumarin skeleton from 7-methoxy coumarin precursors as reported [3], using model substrates containing a nucleophile. The fluorescence intensity of coumarin and wavelength can be modified by modulating electron donating property at the 7-position [4]. We selected thiols and amines to make the strategy applicable to various receptor ligands. Because these nucleophiles are used ligand synthesis process. Specifically, we chose PPARγ as the ligand target [5]. PPARγ is a member of the nuclear receptor superfamily, and they influence in glucose, lipid, and lipoprotein homeostasis. PPARγ is a platform receptor used for demonstration studies in medicinal chemistry and thus our aim was to synthesize a Rosiglitazone derivative incorporating a coumarin (Figure 1).

Results and discussion

Investigation using model substrates

Using reported coumarin precursors [3] and model substrates with different heteroatoms (X = SH, NH_2, N(H)Me, OH), we investigated appropriate conditions for synthesizing coumarins. The model substrates comprised thiols and amino groups and were commercially available, except for substrates with aminomethyl groups [6]. The coumarin precursor **1** was synthesized based on previously reported synthetic scheme [3]. We then investigated the reaction conditions using various bases, reaction temperatures and solvents. The results are shown in Table 1. The bases tested included inorganic weak bases, inorganic strong bases, and organic bases. The best yield with a thiol group is entry 4 (quant.), with an amino group it is entry 9 (88%), and with an aminomethyl groups it is entry 11 (30%). Then we used Et_3N as a solvent under reflux condition, in this reaction, dimer formation was promoted (entry 12,

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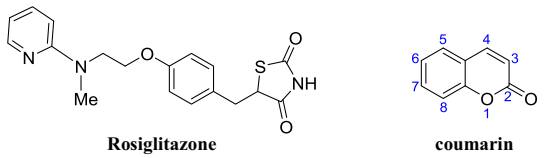


Figure 1. Structure of Rosiglitazone and coumarin.

31%). When we applied PTSA salt, X = NMe·PTSA, reduced the dimer formation and improved the yield (entry 13, 70%). In all cases which were obtained target compounds, Et_3N was used as the base. On the other hands, when we use 2-phenylethanol (X = OH), or phenol (Table S1) as the nucleophile, the reaction results were no reaction (entry 14), decomposition (entries 15, 16, 18 and 20), or obtaining almost dimerization product, monitored by TLC (entries 17 and 19). The best yields were obtained using a thiol group.

Synthesis of the PPARγ ligand

Next, we synthesized the coumarin contained PPARγ ligand (Scheme 1). The ligand unit was synthesized referring previous study [7], starting with 2-(methylamino)ethanol. After that, coumarin was conjugated to Rosiglitazone unit, which the reaction using Et_3N as solvent under reflux condition (Table 1, entry 13). With the Rosiglitazone unit in hand, we attempted to incorporate coumarin into compound **7** by using the coumarin precursor (compound **1**). Table S2 shows the conditions tested and the results obtained using Rosiglitazone units. We synthesized compound **8** in the yield 54%, when Et_3N was used as solvent under reflux condition. Then we attempted to use DIPEA with a higher boiling point as a solvent. The yield of compound **8** was slightly improved to 57% (Table S2, entry 8).

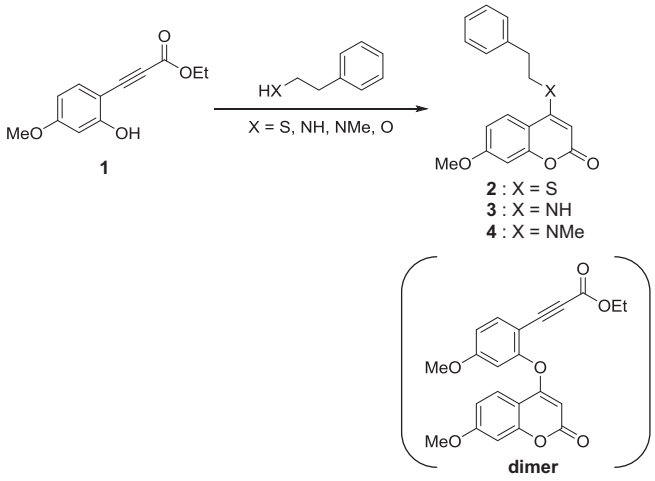
Transcriptional activity

The transcriptional activity of compound **8** was evaluated by the dual luciferase assay in COS-7 cells using the agonist Rosiglitazone as a positive control (Table 2). The results showed that compound **8** has comparable activity to Rosiglitazone in COS-7 cells,

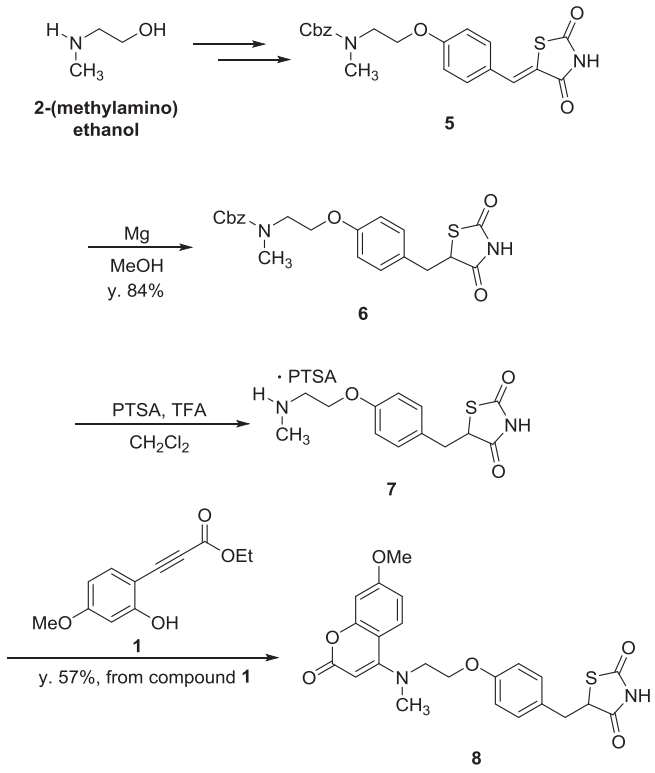
Table 2
EC_{50}s of the transcriptional activities of compound **8** and Rosiglitazone.

	Rosiglitazone	compound **8**
EC_{50} (nM)	0.13	0.45

Table1
Investigation of reaction conditions using model substrates.



Entry	X (1.5 equiv.)	Base (2.0 equiv.)	Solvent	Temp (°C)	Results
1	S	–	1,4-dioxane	80	no reaction
2	S	K_2CO_3	DMF	60	y. 91%
3	S	KOH	DMF	60	y. 8%
4	S	Et_3N	DMF	60	quant.
5	S	DBU	DMF	60	y. 93%
6	NH	–	1,4-dioxane	80	no reaction
7	NH	K_2CO_3	DMF	60	decomposition
8	NH	KOH	DMF	60	decomposition
9	NH	Et_3N	DMF	60	y. 88%
10	NH	DBU	DMF	60	decomposition
11	NMe	Et_3N	DMF	60	y. 30%
12	NMe	–	Et_3N	reflux	y. 31%
13	NMe·PTSA	–	Et_3N	reflux	y. 70%
14	O	–	1,4-dioxane	80	no reaction
15	O	K_2CO_3	DMF	60	decomposition
16	O	KOH	DMF	60	decomposition
17	O	Et_3N	DMF	60	dimerization
18	O	DBU	DMF	60	decomposition
19	O	Et_3N	toluene	100	dimerization
20	O	NaH	DMF	60	decomposition



Scheme 1. Synthesis of the ligand unit.

Table 3
Fluorescence properties in several solvents.

Compound	λ (nm)	CH_2Cl_2	THF	MeOH	H_2O	PBS	hexane
2	λ^{abs}_{max}	286	283	296	298	303	278
	λ^{f}_{max}	397	398	400	398	391	348
3	λ^{abs}_{max}	309	309	305	303	304	309
	λ^{f}_{max}	369	339	370	400	394	340
4	λ^{abs}_{max}	304	305	313	312	312	313
	λ^{f}_{max}	471	474	392	396	399	364
8	λ^{abs}_{max}	316	314	314	315	313	–
	λ^{f}_{max}	469	467	392	444	389	–
Rosiglitazone	λ^{abs}_{max}	312	312	313	315	312	–
	λ^{f}_{max}	362	360	369	379	380	–

indicating that the incorporation of coumarin does not affect ligand agonistic activity.

Fluorescence properties

We measured the fluorescence spectra of model compounds **2**, **3** and **4**, and of the receptor ligand, Rosiglitazone and compound **8** in various solvents to evaluate their fluorescence properties. Six solvents were used: hexane, CH_2Cl_2, THF, MeOH, H_2O and phosphate buffered saline (PBS). The results are shown in Table 3.

Rosiglitazone and compound **8** could not be measured in hexane due to their insolubility. The greatest shifts to longer fluorescence maximum wavelengths of model compounds **2**, **3** and **4**, and of the receptor ligands Rosiglitazone and compound **8**, were observed in CH_2Cl_2 and THF.

Conclusion

Incorporation of coumarin into a ligand is achieved using conjugate addition reaction of alkynone **1**. The lactone ring is constructed by the geometric transformation from alkyne to alkene to produce coumarin. It does not require extreme conditions and is considered applicable to the last stage of the synthesis. Since coumarin is small molecule in fluorescent probe, compound **8** showed biological activity comparable to Rosiglitazone.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tetlet.2020.151842.

References

[1] (a) E. Sekino, T. Kumamoto, T. Tanaka, T. Ikeda, T. Ishikawa, J. Org. Chem. 69 (2004) 2760–2767;
(b) N. Kitamura, S. Kohtani, R. Nakagaki, J. Photochem. Photobiol. C: Photochemistry Reviews. 6 (2005) 168–185.
[2] (a) D. Cao, Z. Liu, P. Verwilst, S. Koo, P. Jangjili, J. S. Kim, W. Lin, Chem. Rev. 119 (2019), 10403−10519.
(b) Priyanka, R. K. Sharma, D. Katiyar, Synthesis, 48 (2016) 2303–2322.
(c) M. M. Heravi, S. Khaghaninejad, M. Mostofi, Advances in Heterocyclic Chemistry, First Edition, 112 (2014) 1−50.
[3] H. Kojima et al. in press DOI: https://doi.org/10.1016/j.chembiol.2020.01.006.
[4] N. Kitamura, T. Fukagawa, S. Kohtani, S. Kitoh, K. Kunimoto, R. Nakagaki, J. Photochem. Photobiol. A: Chem. 188 (2007) 378–386.
[5] (a) M. Ohashi, T. Oyama, I. Nakagome, M. Satoh, Y. Nishio, H. Nobusada, S. Hirono, K. Morikawa, Y. Hashimoto, H. Miyachi, J. Med. Chem. 54 (2011) 331–341;
(b) S. Ban, T. Oyama, J. Kasuga, K. Ohgane, Y. Nishio, K. Morikawa, Y. Hashimoto, H. Miyachi, Bioorg. Med. Chem. 20 (2012) 3460−3464.
(c) S. Kanda, R. Nakashima, K. Takahashi, J. Tanaka, J. Ogawa, T. Ogata, M. Yachi, K. Araki, J. Ohsumi, J. Pharmacol. Sci., 111 (2009) 155–166.
[6] C.G. McPherson, N. Caldwell, C. Jamieson, I. Simpsonb, A.J.B. Watsona, Org. Biomol. Chem. 15 (2017) 3507–3518.
[7] (a) D.L. Mohler, G. Shen, Org. Biomol. Chem. 4 (2006) 2082–2087;
(b) A.G. Chittiboyina, M.S. Venkatraman, C.S. Mizuno, P.V. Desai, A. Patny, S.C. Benson, C.I. Ho, T.W. Kurtz, H.A. Pershadsingh, M.A. Avery, J. Med. Chem. 49 (2006) 4072–4084.