Bioorganic & Medicinal Chemistry Letters 20 (2010) 5143–5146

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Fluorescent retinoid X receptor ligands for fluorescence polarization assay

Shoya Yamada [a], Fuminori Ohsawa [a,b], Shuji Fujii [a], Ryosuke Shinozaki [a], Makoto Makishima [c], Hirotaka Naitou [d], Shuichi Enomoto [a,b], Akihiro Tai [e], Hiroki Kakuta [a,*]

[a] Division of Pharmaceutical Sciences, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 1-1-1, Tsushima-Naka, Okayama 700-8530, Japan
[b] Multiple Molecular Imaging Research Laboratory, RIKEN Center for Molecular Imaging Science, Minatojima-minamimachi 6-7-3, Chuo-ku, Kobe, Hyogo 650-0047, Japan
[c] Division of Biochemistry, Department of Biomedical Sciences, Nihon University School of Medicine, Itabashi-ku, Tokyo 173-8610, Japan
[d] Graduate School of Nutritional and Environmental Sciences and Global COE Program, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan
[e] Faculty of Life and Environmental Sciences, Prefectural University of Hiroshima, 562 Nanatsuka, Shobara, Hiroshima 727-0023, Japan

ARTICLE INFO

Article history:
Received 23 May 2010
Revised 25 June 2010
Accepted 6 July 2010
Available online 8 July 2010

Keywords:
Retinoid X receptor
Fluorescence polarization
Molecular design
Carbostyril

ABSTRACT

Retinoid X receptor (RXR) agonists are candidate agents for the treatment of metabolic syndrome and type 2 diabetes via activation of peroxisome proliferator-activated receptor (PPAR)/RXR or liver X receptor (LXR)/RXR-heterodimers, which control lipid and glucose metabolism. Reporter gene assays or binding assays with radiolabeled compounds are available for RXR ligand screening, but are unsuitable for high-throughput screening. Therefore, as a first step towards stabilizing a fluorescence polarization (FP) assay system for high-throughput RXR ligand screening, we synthesized fluorescent RXR ligands by modification of the lipophilic domain of RXR ligands with a carbostyril fluorophore, and selected the fluorescent RXR agonist 6-[ethyl(1-isobutyl-2-oxo-4-trifluoromethyl-1,2-dihydroquinolin-7-yl)amino]nicotinic acid **8d** for further characterization. Compound **8d** showed FP in the presence of RXR and the FP was decreased in the presence of the RXR agonist LGD1069 (**2**). This compound should be a lead compound for use in high-throughput assay systems for screening RXR ligands.

Retinoid X receptors (RXRs) are nuclear receptors that function as homodimers or heterodimers with other nuclear receptors, such as peroxisome proliferator-activated receptors (PPARs), liver X receptors (LXRs), farnesoid X receptor (FXR), retinoic acid receptors (RARs), thyroid hormone receptors (TRs), and so on.[1,2] In RXR-heterodimers, RXRs are involved in stabilization of the binding of the heterodimer partner to DNA.[2] RXR agonists can stabilize the heterodimeric structures and sometimes potentiate the agonistic activity of the heterodimer partners. For example, an RAR agonist at a low concentration which does not induce cell differentiation by itself may induce cell differentiation upon synergistic activation of RAR/RXR with an RXR agonist.[3] Moreover, PPARs, which are target molecules for treatment of metabolic syndrome and type 2 diabetes, and LXRs, which are involved in lipid and glucose metabolism, can be activated synergistically by RXR agonists in combination with PPAR or LXR agonists, respectively, and also can be activated by RXR agonists alone (permissive mechanism) to promote transcription of downstream genes.[4] Thus, RXRs are attractive targets for the treatment of metabolic syndrome and type 2 diabetes.[5,6]

Known RXR agonists include 9-*cis* retinoic acid (**1**), an endogenous ligand of RXRs, and LGD1069 (**2**), which is approved in the USA for treatment of cutaneous T cell lymphoma, HX630 (**3**), PA024 (**4**), and so on (Fig. 1).[2,3,7–11] We have also created less lipophilic RXR agonists, NEt-3IP (**5**), and NEt-3IB (**6**).[12–14] Interestingly, **3** and **4** have different characteristics in activation of permissive heterodimers PPAR/RXR and LXR/RXR,[15] although we have obtained similar results with carboxy bioisosteric derivatives.[16] In other words, RXR agonists can show a structure-dependent activation profile towards RXR-heterodimers, and thus are attractive candidates for application as RXR-heterodimer modulators.

For the screening of RXR ligands, reporter gene assays based on gene transactivation or binding assays using radioisotopes are generally used.[17] Though the former approach is useful for the verification of RXR-agonistic or antagonistic activities, it takes several days. On the other hand, although the latter approach offers high sensitivity, the use of radioisotopes is inconvenient. We considered that safe, simple, and inexpensive high-throughput RXR ligand screening might be attained by using fluorescence polarization (FP) assay systems. However, no fluorescent RXR ligand has yet been reported. Here we describe the molecular design, synthesis, and characterization of fluorescent RXR ligands suitable for fluorescence polarization-based screening of RXR ligands.

Fluorescent ligands for FP assay are generally created by introduction of a fluorophore, such as fluorescein or rhodamine, into an existing ligand via a spacer.[18–20] However, X-ray structure analysis of RXRs indicates that there is insufficient space at the ligand-binding site for the introduction of a fluorophore or a spacer (e.g., 1FBY). Thus, we considered that a part of the RXR ligand would have to be converted to a fluorophore. Focusing on RXR agonist

* Corresponding author. Tel./fax: +81 (0)86 251 7963.
E-mail address: kakuta@pharm.okayama-u.ac.jp (H. Kakuta).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.07.011

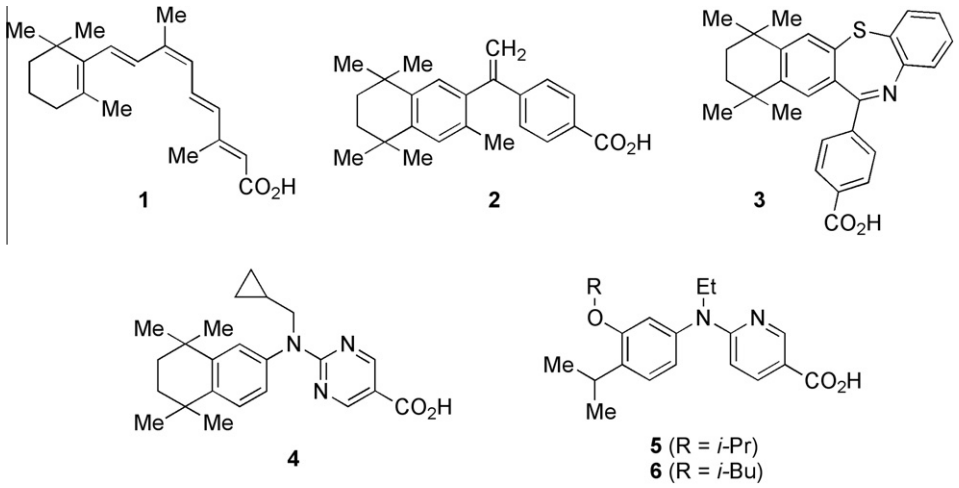


Figure 1. Chemical structures of known RXR agonists.

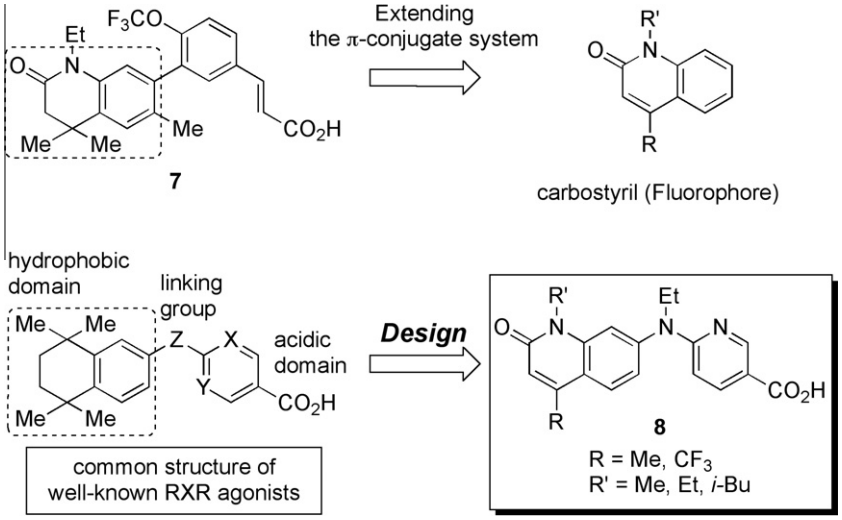
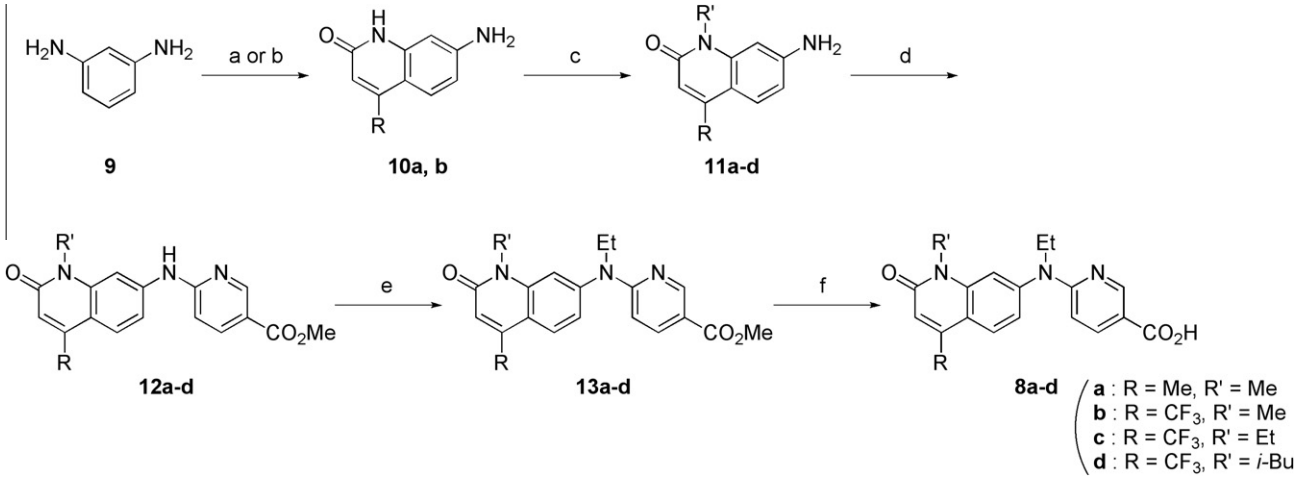


Figure 2. Design strategy of fluorescent RXR ligands.



Scheme 1. Reagents and conditions: (a) $CH_3COCH_2CO_2Et$, water, microwave, 150 °C, 46%. (b) $CF_3COCH_2CO_2Et$, *p*-TsOH, EtOH, reflux, 42%. (c) RI (R = Me, Et), NaH, DMF or *i*-BuBr, NaH, KI, DMF, rt 22–72%. (d) 6-Chloronicotinic acid methyl ester, *p*-TsOH, dioxane, DMSO, reflux, 20–87%. (e) EtI, NaH, DMF, rt 13–67%. (f) $NaOH_{aq}$, MeOH, 60 or 65 °C, 19–97%.

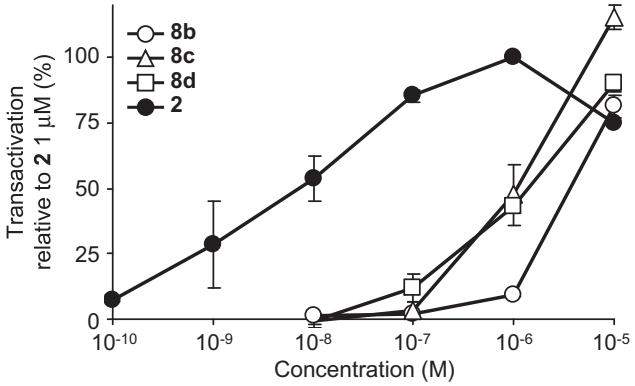


Figure 3. Co-transfection data (RXRα) for synthetic compounds and known RXR agonist LGD1069 (**2**) in COS-1 cells. Data are the average ($n = 3$) ±SEM.

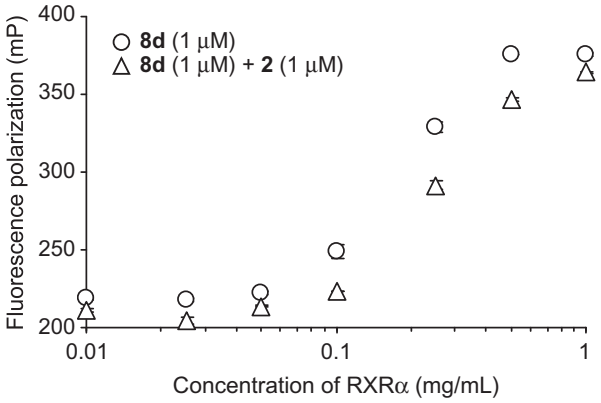
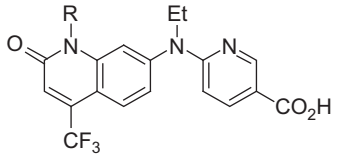


Figure 5. Fluorescence polarization values upon addition of RXRα to **8d** (1 μM) in HEPES buffer. Data are the average ($n = 3$) ±SEM.

Table 1
Fluorescence properties of **8b–d** (10 μM) in PBS



Compound	R	Ex[a] (nm)	Em[b] (nm)	Intensity[c] (arb. units)
8b	Me	372	494	5.60
8c	Et	368	496	7.90
8d	*i*-Bu	368	493	14.5

[a] Excitation maximum wavelength.
[b] Emission maximum wavelength.
[c] Fluorescent intensity shown by arbitrary units.

7, in which the structure of the so-called lipophilic domain resembles a fluorescent carbostyril structure, we designed **8**, which possesses a carbostyril structure as its lipophilic domain in the general RXR structure (Fig. 2).[21]

The synthetic scheme is shown in Scheme 1, starting from *m*-phenylenediamine. 7-Amino-4-methyl-1*H*-quinolin-2-one (**10a**: R = methyl) was synthesized by the use of microwave irradiation at 150 W and 150 °C for 80 min,[22] while 7-amino-4-trifluoromethyl-1*H*-quinolin-2-one (**10b**: R = trifluoromethyl) was prepared by Knorr condensation with ethyl acetoacetate or ethyl 1,1,1-trifluoroacetoacetate and a catalytic amount of *p*-tosylic acid in ethanol.[23] *N*-Alkylation at the lactam ring of **10** was performed with corresponding alkyl halides and sodium hydride in dry DMF to afford anilino derivatives **11**. After coupling reaction of the obtained anilino derivatives and methyl 6-chloronicotinate with a catalytic amount of *p*-tosylic acid in dioxane and DMSO, *N*-alkylation at the linking group and deprotection of the carboxylic group afforded the target molecules **8**.

RXR-agonistic activity of **8a–d**, as an indicator of RXR binding activity, was evaluated by reporter gene assay. While **8a** showed no RXR-agonistic activity (data not shown), **8b–d** show RXR-agonistic activity with EC_{50} values of about 1 μM, although they are less potent than **2** (Fig. 3).

Next, the fluorescence properties of **8b–d** were evaluated (Table 1). Each compound showed a fluorescence excitation maximum at around 370 nm, and emission at around 490 nm. As the *N*-alkyl chain at the lactam ring was extended, the relative fluorescence intensity tended to increase. Thus, we selected **8d** as the most potent RXR agonist, with the highest fluorescence intensity, and evaluated its FP characteristics according to the procedure reported by Peterson et al.[19] Figure 4a shows the FP of **8d** in glycerol solution, a viscous liquid, that impedes solute motion. As the ratio of glycerol in the solution was increased, increasing FP of **8d** was observed. The fluorescence intensity of **8d** increased in proportion to the concentration of **8d**, while the FP value remained constant, at a fixed ratio of glycerol (Fig. 4b).

In order to evaluate the binding of **8d** to RXR, we added RXRα protein to a solution of 1 μM **8d** in PBS buffer (Fig. 5). With increasing RXR concentration, the FP value of **8d** was increased, indicating that **8d** was bound to RXR. Moreover, addition of **2** to the solution decreased the FP value, indicating that binding of **8d** is competitive

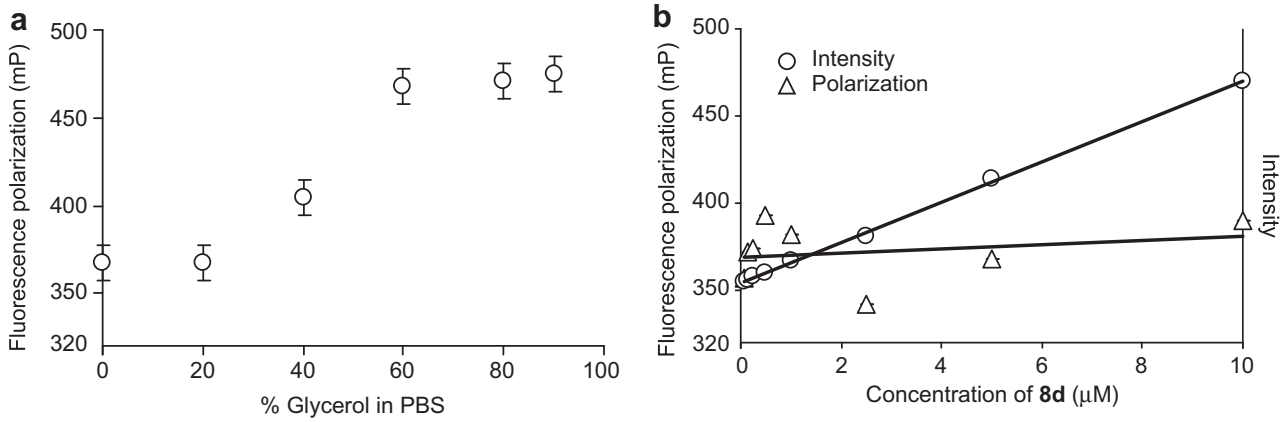


Figure 4. Fluorescence polarization characteristics of **8d**. (a) Fluorescence polarization calibration curve of **8d** (1 μM) in glycerol/PBS buffer. (b) Intensity and polarization values as a function of the concentration of **8d** in PBS. Data are the average ($n = 3$) ±SEM.

with that of **2**, and therefore presumably occurs at the same site in RXR.

We designed, synthesized, and characterized fluorescent RXR agonists that were expected to be suitable for high-throughput FP screening of RXR ligands. One of them, 6-[ethyl(1-isobutyl-2-oxo-4-trifluoromethyl-1,2-dihydroquinolin-7-yl)amino]nicotinic acid **8d**, was concluded to be suitable for this purpose. Further work is planned to increase the RXR-agonistic activity and fluorescence intensity of **8d**. The strategy presented here is expected to be useful for creation and development of other fluorescent RXR agonists for RXR ligand screening systems.

Acknowledgments

The authors are grateful to the SC-NMR Laboratory of Okayama University for the NMR experiments. This work was partially supported by a Grant-in Aid for Scientific Research on Priority Areas from the Ministry of Education, Science, Culture and Sports of Japan. The authors are also grateful to Mr. Ryosuke Fukai, Ms. Mariko Nakayama, and Mr. Kohei Kawata for helpful discussions during the preparation of this manuscript.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.07.011.

References and notes

1. Rowe; Eager, N. S. C.; Brickell, P. M. *Development* **1991**, *111*, 771.
2. Umemiya, H.; Kagechika, H.; Fukasawa, H.; Kawachi, E.; Ebisawa, M.; Hashimoto, Y.; Eisenmann, G.; Erb, C.; Pornon, A.; Chambon, P.; Gronemeyer, H.; Shudo, K. *Biochem. Biophys. Res. Commun.* **1997**, *233*, 121.
3. Ohta, K.; Kawachi, E.; Inoue, N.; Fukasawa, H.; Hashimoto, Y.; Itai, A.; Kagechika, H. *Chem. Pharm. Bull.* **2000**, *48*, 1504.
4. Mukherjee, R.; Davies, P. J.; Crombie, D. L.; Bischoff, E. D.; Cesario, R. M.; Jow, L.; Hamann, L. G.; Boehm, M. F.; Mondon, C. E.; Nadzan, A. M.; Paterniti, J. R., Jr.; Heyman, R. A. *Nature* **1997**, *386*, 407.
5. Pinaire, J. A.; Reifel-Miller, A. *PPAR Res.* **2007**, *2007*, 604.
6. Leibowitz, M. D.; Ardecky, R. J.; Boehm, M. F.; Broderick, C. L.; Carfagna, M. A.; Crombie, D. L.; D'Arrigo, J.; Etgen, G. J.; Faul, M. M.; Grese, T. A.; Havel, H.; Hein, N. I.; Heyman, R. A.; Jolley, D.; Klausing, K.; Liu, S.; Mais, D. E.; Mapes, C. M.; Marschke, K. B.; Michellys, P. *Endocrinology* **2006**, *147*, 1044.
7. Heyman, R. A.; Mangelsdorf, D. J.; Dyck, J. A.; Stein, R. B.; Eichele, G.; Evans, R. M.; Thaller, C. *Cell* **1992**, *68*, 397.
8. Boehm, M. F.; Zhang, L.; Badea, B. A.; White, S. K.; Mais, D. E.; Berger, E.; Suto, C. M.; Goldman, M. E.; Heyman, R. A. *J. Med. Chem.* **1994**, *37*, 2930.
9. Gottardis, M. M.; Bischoff, E. D.; Shirley, M. A.; Wagoner, M. A.; Lamph, W. W.; Heyman, R. A. *Cancer Res.* **1996**, *56*, 5566.
10. Rizvi, N. A.; Marshall, J. L.; Dahut, W.; Ness, E.; Truglia, J. A.; Loewen, G.; Gill, G. M.; Ulm, E. H.; Geiser, R.; Jaunakais, D.; Hawkins, M. J. *Clin. Cancer Res.* **1999**, *5*, 1658.
11. Cohen, M. H.; Hirschfeld, S.; Honig, S. F.; Ibrahim, A.; Johnson, J. R.; O'leary, J. J.; White, R. M.; Williams, G. A.; Pazdur, R. *Oncologist* **2001**, *6*, 4.
12. Takamatsu, K.; Takano, A.; Yakushiji, N.; Morishita, K.; Matsuura, N.; Makishima, M.; Ali, H. I.; Akeho, E.; Tai, A.; Sasaki, K.; Kakuta, H. *ChemMedChem* **2008**, *3*, 454.
13. Takamatsu, K.; Takano, A.; Yakushiji, N.; Morohashi, K.; Morishita, K.; Matsuura, N.; Makishima, M.; Tai, A.; Sasaki, K.; Kakuta, H. *ChemMedChem* **2008**, *3*, 780.
14. Morishita, K.; Yakushiji, N.; Ohsawa, F.; Takamatsu, K.; Matsuura, N.; Makishima, M.; Kawahata, M.; Yamaguchi, K.; Tai, A.; Sasaki, K.; Kakuta, H. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1001.
15. Nishimaki-Mogami, T.; Tamehiro, N.; Sato, Y.; Okuhira, K.; Sai, K.; Kagechika, H.; Shudo, K.; Abe-Dohmae, S.; Yokoyama, S.; Ohno, Y.; Inoue, K.; Sawada, J. *Biochem. Pharmacol.* **2008**, *76*, 1006.
16. Fujii, S.; Ohsawa, F.; Yamada, S.; Shinozaki, R.; Fukai, R.; Makishima, M.; Enomoto, S.; Tai, A.; Kakuta, H. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5139.
17. Boehm, M. F.; McClurg, M. R.; Pathirana, C.; Mangelsdorf, D.; White, S. K.; Hebert, J.; Winn, D.; Goldman, M. E.; Heyman, R. A. *J. Med. Chem.* **1994**, *37*, 408.
18. Parker, G. J.; Law, T. L.; Lenoch, F. J.; Bolger, R. E. *J. Biomol. Screen.* **2000**, *5*, 77.
19. DeGrazia, M. J.; Thompson, J.; Heuvel, J. P. V.; Peterson, B. R. *Bioorg. Med. Chem.* **2003**, *11*, 432.
20. Han, K.; Kim, J. H.; Kim, K.; Kim, E. E.; Seo, J.; Yang, E. G. *Anal. Biochem.* **2010**, *398*, 185.
21. Saroja, G.; Sankaran, N. B.; Samanta, A. *Chem. Phys. Lett.* **1996**, *249*, 392.
22. Lee, H.; Cao, H.; Rana, T. M. *J. Comb. Chem.* **2005**, *7*, 279.
23. Oeveren, A.; Pio, B. A.; Tegley, C. M.; Higuchi, R. I.; Wu, M.; Jones, T. K.; Marschke, K. B.; Negro-Vilar, A.; Zhi, L. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1523.