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Synthesis and biological evaluation of 1-[2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl]-4-ethynylimidazole. A novel and highly potent anti-inflammatory and cytoprotective agent

Tadashi Honda [a,b,*], Albena T. Dinkova-Kostova [c,d], Emilie David [b], Eric M. Padegimas [b], Chitra Sundararajan [b], Melean Visnick [e], Ron Bumeister [e], W. Christian Wigley [e]

[a] Department of Chemistry, Institute of Chemical Biology and Drug Discovery, State University of New York at Stony Brook, Stony Brook, NY 11794, USA
[b] Department of Chemistry, Dartmouth College, Hanover, NH 03755, USA
[c] Biomedical Research Institute, University of Dundee, Dundee DD1 9SY, Scotland, UK
[d] Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
[e] Reata Pharmaceuticals Inc., Irving, TX 75063, USA

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ABSTRACT

To explore more potent *N*-acylimidazole analogues of CDDO than CDDO-Im, which is one of the most potent compounds in several widely used bioassays related to protection against inflammation and carcinogenesis; we have synthesized and evaluated five new *N*-acyl(acetylenic)imidazole analogues. Among them, 4-ethynylimidazole **4** is nearly equivalent to CDDO-Im in potency in these bioassays. Remarkably, the solid form of **4** is more stable than that of CDDO-Im. These findings suggest that **4** is a very promising anti-inflammatory and cytoprotective agent and its further preclinical evaluation is warranted.

Over the past decade, we have been engaged in the improvement of anti-inflammatory and cytoprotective activity of oleanolic acid, a naturally occurring triterpenoid. This led to the discovery of 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO, bardoxolone).[1,2] Its methyl ester (CDDO-Me, bardoxolone methyl) is presently being developed in late Phase II clinical trials for the treatment of severe chronic kidney disease in type 2 diabetes mellitus patients, for which there exists a significant unmet medical need. Bardoxolone methyl significantly increases eGFR (estimated glomerular filtration rate) in more than 90% of the patients.[3]

We have been exploring the mechanism of action of CDDO and its derivatives. Towards that end, the identification of the protein targets of CDDO is very important. The *N*-acylimidazole is known to be very reactive towards various nucleophiles.

Therefore, we envisioned that the *N*-acylimidazole derivative of CDDO (CDDO-Im) would be a useful tool for identification of the protein targets. Unfortunately, formation of protein adducts with CDDO-Im was not observed, most likely because the *N*-acylimidazole group is located at C17, a much hindered position. However, introduction of the *N*-acylimidazole group greatly enhanced potency, and CDDO-Im is now one of the most potent compounds in our pool of semisynthetic triterpenoids which we have previously evaluated in various bioassays in vitro and in vivo.[4–6] CDDO-Im is more potent and less toxic in rodents than CDDO-Me. However, during feasibility studies on CDDO-Im, it was found that it is unstable upon incubation in human plasma[7] and its solid form undergoes decomposition within one year when stored at 4 °C.

To explore more potent and stable *N*-acylimidazole analogues of CDDO, we have synthesized various *N*-acylimidazoles of CDDO using commercially available imidazoles (e.g., 2-methyl, 4-methyl, 2,4-dimethyl, 2-ethyl, 4-bromo-, 2-phenyl, etc.) However, these analogues did not show any satisfactory results when tested for potency and stability. Recently, we found that TBE-31 (Fig. 1) is the most potent compound amongst semisynthetic triterpenoids and synthetic tricycles,[7–10] and that the acetylene group is essential for strong potency. Therefore, we reasoned that *N*-acyl(acetylenic)imidazoles of CDDO, **1**–**5** might be interesting and important (Fig. 2). Indeed, these compounds are more potent than

* Corresponding author at present address: Department of Chemistry, Institute of Chemical Biology & Drug Discovery, State University of New York at Stony Brook, Stony Brook, NY 11794 USA. Tel.: +1 631 632 7162; fax: +1 631 632 7942.
E-mail address: tadashi.honda@stonybrook.edu (T. Honda).

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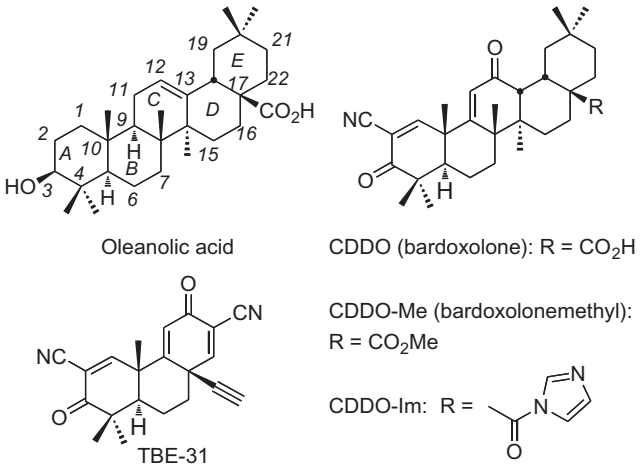


Figure 1. Structures of oleanolic acid, CDDO, CDDO-Me, CDDO-Im, and TBE-31.

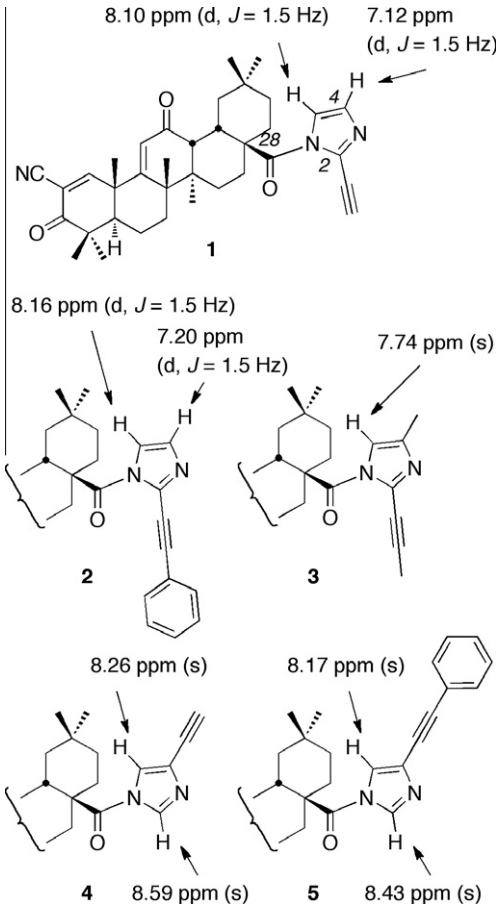
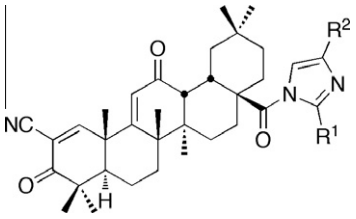


Figure 2. Structures of **1–5** and chemical shifts (δ) of their imidazole protons. ^{1}H NMR spectra of **1–4** were measured in DMSO-d_6 and **5** in CD_3OD.

CDDO for inhibition of nitric oxide (NO) production in RAW 264.7 cells and induction of the cytoprotective enzymes, NAD(P)H: quinone oxidoreductase 1(NQO1)[11] and heme oxygenase-1 (HO-1)[12] (Table 1 and Figs. 3 and 4). Particularly, 4-ethynylimidazole **4** is nearly equivalent to CDDO-Im in potency in these bioassays. Moreover, the solid state of **4** is more stable than that of CDDO-Im. Herein we report the synthesis, anti-inflammatory and cytoprotective potency of these new derivatives.

Table 1
Inhibitory activity of new compounds **1–5** on NO production induced by IFN-γ in RAW cells and their NQO1–inducing potency in Hepa1c1c7 cells



Compound	R^1	R^2	IC_{50}[a] (nM)	CD[b] (nM)
1	–C≡CH	H	2.3 ± 0.7	0.9
2	–C≡C–Ph	H	4.0 ± 1.0	1.2
3	–C≡C–Me	Me	2.0 ± 0.1	0.4
4	H	–C≡CH	1.4 ± 0.3	0.4
5	H	–C≡CPh	7.8 ± 1.0	
CDDO-Im[c]	H	H	0.6 ± 0.2	0.2
CDDO			11.0 ± 1.0	3.0

[a] RAW 264.7 mouse macrophages were plated in 96-well plates at 30,000 cells/well in triplicate. On the next day cells were pre-treated with DMSO or test compounds (0–200 nM dose range) for 2 h, followed by IFN-γ treatment for an additional 24 h. NO concentration in media was determined using the Griess reagent system (Promega). Cell viability was assessed using WST-1 reagent (Roche). IC_{50} values were determined based on suppression of IFN-γ-induced NO production normalized to cell viability.

[b] Hepa1c1c7 cells (10,000 cells/well) were grown in 96-well plates for 24 h and then treated with serial dilutions of compounds for 48 h. The concentration required to double (CD) the specific enzyme activity of NQO1 was used to quantity inducer potency. The value is based on the activity from eight replicate wells at each concentration. The standard deviation in each case was between 5 and 10%.

[c] Freshly prepared CDDO-Im was used for this evaluation. Its CD value (0.2 nM) is 16 times lower than the one that was previously reported (3.3 nM).[7,17] The latter assay was done with a DMSO solution of CDDO-Im that had been stored, and the discrepancy in inducer potency can be explained by the now known instability of CDDO-Im. The new CD value is in good agreement with the potency in the iNOS assay.

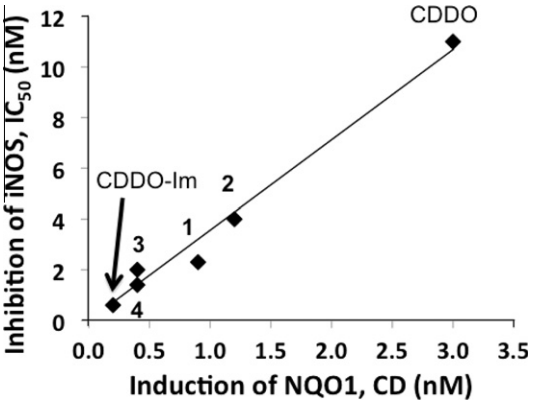


Figure 3. Correlation of potencies of new compounds **1–4**, CDDO and CDDO-Im as inducers of NQO1 in Hepa1c1c7 murine hepatoma cells, expressed as CD values, and for suppression of iNOS induction by IFN-γ in RAW cells, expressed as IC_{50} values. The linear correlation coefficient is $r^2 = 0.98$.

The acetylenic-1*H*-imidazoles **6**–**10** are not commercially available, and were previously unknown except for **10**[13] (structure, see Scheme 2). These acetylenic-1*H*-imidazoles were synthesized by the sequence shown in Scheme 1. 2-Ethynyl-1*H*-imidazole (**6**)[14] could not be obtained from the known imidazole **11**[13] under two classic conditions, tetra(*n*-butyl)ammonium fluoride (TBAF) in THF and 49% aqueous hydrofluoric acid in acetonitrile. After survey of various conditions, we have found that trifluoroacetic acid (TFA) in THF (at room temperature, overnight) is very efficient for the

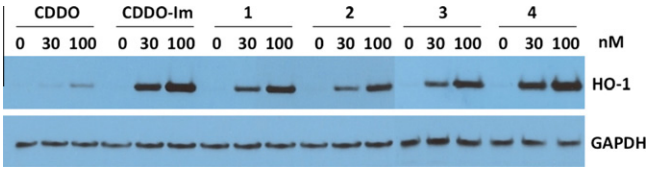
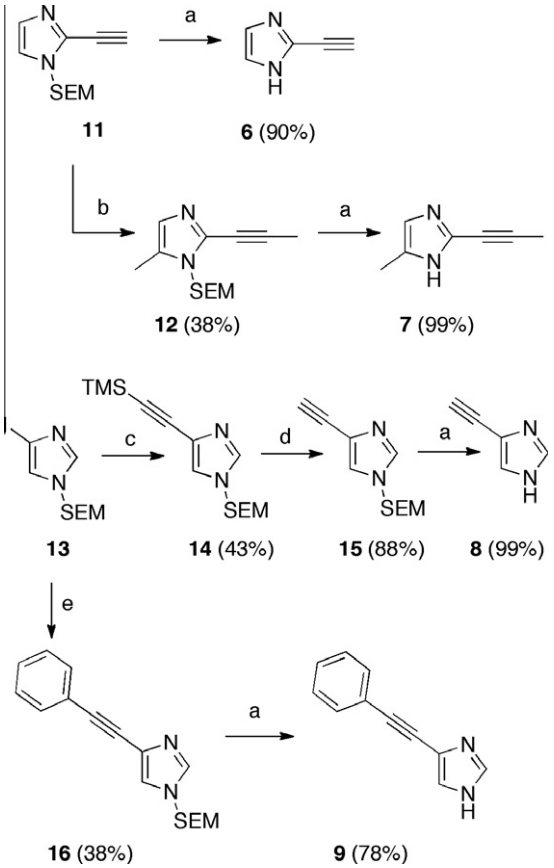
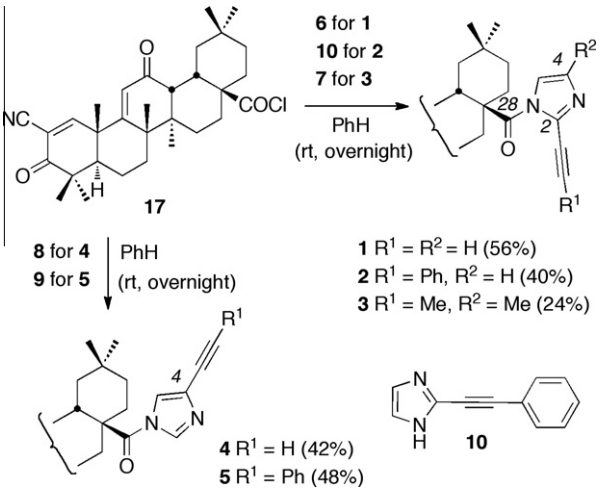


Figure 4. Compounds **1–4** induce HO-1. RAW 264.7 cells were plated in a 24-well dish at 100,000 cells/well. On the next day cells were treated with DMSO or test compounds overnight for 16 h and harvested in tricine sample buffer with β-ME. Lysates were separated by SDS–PAGE and probed with the antibodies against HO-1 (Santa Cruz) or GAPDH (Sigma).



Scheme 1. Synthesis of new imidazoles **6–9**. Reagents and conditions: (a) TFA, THF (rt, overnight); (b) MeI, MeLi, THF; (c) HC≡C-TMS, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N (60 °C, overnight); (d) aqueous NaOH, MeOH (rt); (e) PhC≡CH, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N (60 °C, overnight).

cleavage of trimethylsilylethoxymethyl (SEM) group from *N*-SEM-imidazoles. Imidazole **6** was obtained in 90% yield from **11** by this method. 4-Methyl-2-(1-propynyl)-1*H*-imidazole (**7**) was synthesized as follows. Imidazole **12** was prepared in 38% yield by the treatment of **11** with MeI and MeLi. The removal of SEM group by TFA in THF gave **7** in 99% yield. 4-Acetylenic-1*H*-imidazoles **8** and **9** were synthesized as follows. Sonogashira coupling between known **13**[15] and trimethylsilylacetylene (TMS-acetylene) in the presence of $Pd(PPh_3)_2Cl_2$ and CuI in triethylamine afforded **14** in 51% yield. The TMS group of **14** was removed in a mixture of aqueous NaOH solution and methanol to give **15** in 88% yield. The removal of SEM group yielded 4-ethynyl-1*H*-imidazole (**8**) in 99% yield. Imidazole **16** was prepared in 38% yield by Sonogashira coupling between **13** and phenylacetylene under the same conditions as for **14**. Removal of the SEM group gave 4-(phenylethynyl)-1*H*-imidazole (**9**) in 78% yield.



Scheme 2. Synthesis of new imidazole analogues **1–5**.

New imidazole analogues **1–5**[16] were synthesized by condensation of known acyl chloride of CDDO **17** (1 equiv) and the corresponding imidazoles **6–10** (2 equiv) in benzene (at room temperature, overnight) (Scheme 2, yields are shown in the scheme).[4] Regiochemistry of the ethynyl group at imidazole moiety of **4** was determined by comparison of chemical shifts of imidazole protons of **4** with those of **1** (Fig. 2). The C4 imidazole proton of **1** appears at δ 7.12 ppm (d, J = 1.5 Hz) and the C5 proton appears at δ 8.10 ppm (d, J = 1.5 Hz) due to deshielding effect of carbonyl group. On the other hand, two imidazole protons of **4** appear at δ 8.26 and 8.59 ppm (broad singlet each) due to deshielding effect of carbonyl group. Therefore, the ethynyl group is placed at C4 of imidazole moiety of **4**. Because two imidazole protons of **5** show similar chemical shift (δ 8.17 and 8.43 ppm) to those of **4**, we have assigned the structure of **5** as shown in Figure 2. This regiochemistry is kinetically supported because the acyl chloride moiety of **17** would preferentially attack the less hindered nitrogen of imidazoles. An imidazole proton of **3** is observed at δ 7.74 ppm. We have assigned the structure of **3** as shown in Figure 2 for the following reasons: (1) The chemical shift value is close to C5 protons on the imidazoles of **1** and **2** (δ8.10 and 8.16 ppm) rather than the C4 protons (δ7.12 and 7.20 ppm). (2) The regiochemistry is kinetically supported as described above.

We evaluated the potency of the new compounds **1–5** for inhibition of NO production in RAW 264.7 cells stimulated with interferon-γ. The IC_{50} values are shown in Table 1. All compounds **1–5** are more potent than CDDO. Notably, **4** is nearly equivalent to CDDO-Im in potency. Compounds **1** and **3** are slightly less potent than **4**. Two (phenylethynyl)imidazoles **2** and **5** are much less potent than **4**. These results indicate that a bulky phenyl group decreases potency.

We evaluated compounds **1–4** for induction of the phase 2 cytoprotective enzyme NQO1 in Hepa1c1c7 murine hepatoma cells. All compounds **1–4** are more potent than CDDO. Notably, compounds **3** and **4** are nearly as potent as CDDO-Im. We previously demonstrated a linear correlation between inhibitory activity on NO production (IC_{50}) and NQO1 inducer potency (CD) of oleanolic acid derivatives.[17] In this series of *N*-acylimidazole derivatives of CDDO, we observed an even more striking correlation (r^2 = 0.98, Fig. 3) than the previously reported one (r^2 = 0.91).[17]

We have also evaluated compounds **1–4** for induction of the anti-inflammatory and cytoprotective enzyme, heme oxygenase-1 (HO-1) in RAW 264.7 cells (Fig. 4). There is major interest in stimulating HO-1 as a protective enzyme in many chronic disease conditions in which inflammation and oxidative stress play a key

role.[8] Even in this assay, compounds **1**–**4** are much more potent than CDDO. Particularly, compound **4** is as potent as or slightly more potent than CDDO-Im.

Notably, the solid forms of compounds **1**–**4** are more stable than that of CDDO-Im. Whilst the solids of **1**–**4** were stable for at least three years at 4 °C, the solid of CDDO-Im was decomposed one year after storage under the same conditions. We speculate that the acetylenicimidazoles of these compounds can prevent nucleophilic attack to a greater extent than the imidazole of CDDO-Im, which has no substituents.

In summary, we have found a new promising compound, 1-[2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl]-4-ethynylimidazole (**4**), which is nearly as potent as CDDO-Im in three bioassays and whose solid is more stable than that of CDDO-Im. Presently further preclinical studies of this compound are in progress.

Acknowledgments

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References and notes

1. Honda, T.; Gribble, G. W.; Suh, N.; Finlay, H. J.; Rounds, B. V.; Bore, L.; Favaloro, F. G., Jr.; Wang, Y.; Sporn, M. B. *J. Med. Chem.* **2000**, *43*, 1866.
2. Honda, T.; Rounds, B. V.; Bore, L.; Finlay, H. J.; Favaloro, F. G., Jr.; Suh, N.; Wang, Y.; Sporn, M. B.; Gribble, G. *J. Med. Chem.* **2000**, *43*, 4233.
3. Schwartz, S. L.; Denham, D. S.; Hurwitz, C. A.; Meyer, C. J.; Pergola, P.E. American Diabetes Association Meeting, New Orleans, LA, Abstract No. 112-OR (2009).
4. Honda, T.; Honda, Y.; Favaloro, F. G., Jr.; Gribble, G. W.; Suh, N.; Place, A. E.; Rendi, M. H.; Sporn, M. B. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1027.
5. Place, A. E.; Suh, N.; Williams, C. R.; Risingsong, R.; Honda, T.; Honda, Y.; Gribble, G. W.; Leesnitzer, L. M.; Stimmel, J. B.; Wilson, T. M.; Rosen, E.; Sporn, M. B. *Clin. Cancer Res.* **2003**, *9*, 2798.
6. Yates, M. S.; Kwak, M.-K.; Egner, P. A.; Groopman, J. D.; Bodreddigari, S.; Sutter, T. R.; Baumgartner, K. J.; Roebuck, B. D.; Liby, K. T.; Yore, M. M.; Honda, T.; Gribble, G. W.; Sporn, M. B.; Kensler, T. W. *Cancer Res.* **2006**, *66*, 2488.
7. Liby, K.; Yore, M. M.; Roebuck, B. D.; Baumgartner, K. J.; Honda, T.; Sundararajan, C.; Yoshizawa, H.; Gribble, G. W.; Williams, C. R.; Risingsong, R.; Royce, D. B.; Dinkova-Kostova, A. T.; Stephenson, K. K.; Egner, P. A.; Yates, M. S.; Groopman, J. D.; Kensler, T. W.; Sporn, M. B. *Cancer Res.* **2008**, *68*, 6727.
8. Honda, T.; Sundararajan, C.; Yoshizawa, H.; Su, X.; Honda, Y.; Liby, K. T.; Sporn, M. B.; Gribble, G. W. *J. Med. Chem.* **2007**, *50*, 1731.
9. Dinkova-Kostova, A. T.; Talalay, P.; Sharkey, J.; Zhang, Y.; Holtzclaw, W. D.; Xiu Jun Wang, X. J.; David, E.; Schiavoni, K. H.; Finlayson, S.; Dale, F.; Mierke, D. F.; Honda, T. *J. Biol. Chem.* **2010**, *285*, 33747.
10. Honda, T.; Yoshizawa, H.; Sundararajan, C.; David, E.; Lajoie, M. J.; Favaloro, F. G. Jr.; Janosik, T.; Su, X.; Honda, Y.; Roebuck, B. D.; Gribble, G. W. J. Med. Chem., in press. doi:10.1021/jm101445p.
11. Ross, D.; Kepa, J. K.; Winski, S. L.; Beall, H. D.; Anwar, A.; Siegel, D. *Chem. Biol. Interact.* **2000**, *129*, 77.
12. Ryter, S. W.; Alam, J.; Choi, A. M. *Physiol. Rev.* **2006**, *86*, 583.
13. Paul, D.; Wytko, J. A.; Koepf, M.; Weiss, J. *Inorg. Chem.* **2002**, *41*, 3699.
14. Cosford, N. D.; Kamenecka, T.; Roppe, J. R. U.S. Patent 0085523 A1, 2005. The inventors prepared an inseparable mixture of **6** and 2-formyl-1*H*-imidazole from 2-formyl-1*H*-imidazole with dimethyl (1-diazo-2-oxopropyl)phosphonate.
15. He, Y.; Chen, Y.; Du, H.; Schmid, L. A.; Lovely, C. J. *Tetrahedron Lett.* **2004**, *45*, 5529.
16. All new compounds **1**–**5** provided acceptable HRMS data (±5 ppm) and ^{1}H NMR spectra that exhibit no discernible impurities. ^{13}C NMR was not measured because of the low solubility in various solvents (e.g., $CDCl_3$, DMSO-d_6 etc.). Instead of the optical rotations ($[\alpha]_D$), circular dichroism (CD) values were measured. 1-(2-Cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl)-2-ethynylimidazole (**1**): CD: Δe_{228} = +1.39, Δe_{237} = −0.58, and Δe_{247} = +1.32 (c = 0.0027, CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 8.65 (1H, s), 8.10 (1H, d, J = 1.5 Hz), 7.12 (1H, d, J = 1.5 Hz), 6.26 (1H, s), 4.50 (1H, s), 3.09 (1H, m), 2.82 (1H, d, J = 4.8 Hz), 1.43, 1.26, 1.16, 1.05, 0.96, 0.95, 0.91 (each 3H, s); MS (ESI+) m/z 566 [M+H]$^+$; HRMS: (ESI+) calcd for $C_{36}H_{43}N_3O_3$ + H 566.3383, found 566.3372. 1-(2-Cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl)-2-phenylethynylimidazole (**2**): CD: Δe_{224} = −3.40 and Δe_{247} = +3.69 (c = 0.00078, CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 8.62 (1H, s), 8.16 (1H, d, J = 1.5 Hz), 7.48 (5H, m), 7.20 (1H, d, J = 1.5 Hz), 6.23 (1H, s), 3.11 (1H, d, J = 6.0 Hz), 2.88 (1H, d, J = 3.0 Hz), 1.31, 1.18, 1.13, 1.01 (each 3H, s), 0.96 (6H, s), 0.92 (3H, s); MS (ESI+) m/z 642 [M+H]$^+$; HRMS (ESI+) calcd for $C_{42}H_{48}N_3O_3$ + H 642.3696, found 642.3711. 1-(2-Cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl)-4-methyl-2-(1-propynyl)imidazole (**3**): CD: Δe_{217} = −2.34 and Δe_{235} = +1.75 (c = 0.0013, CH_2Cl_2); ^{1}H NMR (DMSO-d_6) d 8.66 (1H, s), 7.74 (1H, s), 6.25 (1H, s), 3.05 (1H, d, J = 13 Hz), 2.87 (1H, d, J = 5.0 Hz), 2.10, 2.00, 1.43, 1.28, 1.16, 1.05, 0.95, 0.94, 0.91 (each 3H, s); MS (ESI+) m/z 594 [M+H]$^+$; HRMS: (ESI+) calcd for $C_{38}H_{47}N_3O_3$ + H 594.3696, found 594.3680. 1-(2-Cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl)-4-ethynylimidazole (**4**): CD: Δe_{248} = +1.08 (c = 0.0030, CH_2Cl_2); ^{1}H NMR (DMSO-d_6) d 8.66 (1H, s), 8.59 (1H, s), 8.26 (1H, s), 6.24 (1H, s), 4.25 (1H, s), 3.09 (1H, d, J = 4.5 Hz), 2.96 (1H, d, J = 13 Hz), 1.42, 1.19, 1.16, 1.05, 0.97 (each 3H, s), 0.91 (6H, s); MS (ESI+) m/z 566 [M+H]$^+$; HRMS: (ESI+) calcd for $C_{36}H_{43}N_3O_3$ + H 566.3383, found 566.3391. 1-(2-Cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl)-4-phenylethynylimidazole (**5**): CD: Δe_{274} = −0.76 (c = 0.0034, CH_2Cl_2); ^{1}H NMR (CD_3OD) δ 8.61 (1H, s), 8.43 (1H, s), 8.17 (1H, s), 7.53 (2H, m), 7.42 (3H, m), 6.18 (1H, s), 3.17 (1H, m), 1.54, 1.34, 1.24 1.17, 1.11, 1.03, 0.97 (each 3H, s); MS (ESI+) m/z 642 [M+H]$^+$; HRMS: (ESI+) calcd for $C_{42}H_{47}N_3O_3$ + H 642.3696, found 642.3684.
17. Dinkova-Kostova, A. T.; Liby, K. T.; Stephenson, K. K.; Holtzclaw, W. D.; Gao, X.; Suh, N.; Williams, C.; Risingsong, R.; Honda, T.; Gribble, G. W.; Sporn, M. B.; Talalay, P. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 4584.