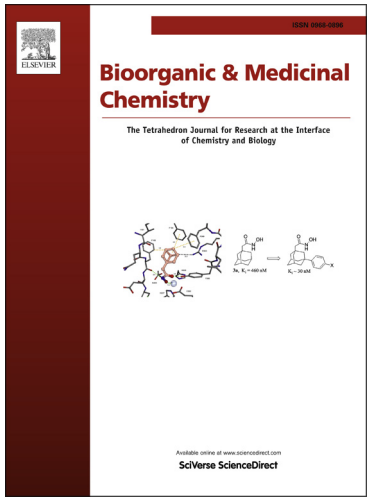

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Design, Synthesis and Evaluation of Retinoids with Novel Bulky Hydrophobic Partial Structures

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Abstract

Many synthetic retinoids contain an aromatic structure with a bulky hydrophobic fragment. In order to obtain retinoids with therapeutic potential that do not bind to or activate retinoic acid X receptors (RXRs), we focused on the introduction of novel hydrophobic moieties, *i.e.*, metacyclophane, phenalene and benzoheptalene derivatives. The designed compounds were synthesized and their agonistic activities towards RARs and RXRs were evaluated. Most of the active compounds showed selectivity for RAR and RAR over RAR , and higher RAR transactivating activity seemed to correlate with higher cell differentiation-inducing activity towards promyelocytic leukemia cell line HL-60. These compounds showed no agonistic activity towards RXRs.

1. Introduction

All-*trans*-retinoic acid (ATRA) is an active metabolite of vitamin A, and its chemistry and biology have been intensively investigated[1]. ATRA and related compounds with similar activities (generally called retinoids) regulate various critical biological functions, including cell proliferation and morphogenesis, as well as differentiation of embryonic stem cells and committed cells such as blood, dermal, immunological and neuronal cells, by activating retinoic acid receptors (RARs) [2-7].

Figure 1

Many synthetic agonists and antagonists of ATRA's activities have been reported[8-10]. These compounds are ligands of nuclear receptors, RARs and/or RXRs, each of which has three subtypes, *i.e.*, RAR , RAR , RAR and RXR , RXR , RXR , respectively[11-21]. RXRs dimerize with many other nuclear receptors, so for therapeutic purposes (*e.g.*, to treat immunological and neurodegenerative disorders such as Alzheimer's disease), it is important to find compounds that bind selectively to RARs, and not to RXRs[22-25]. Among existing compounds, tamibarotene (Am80) is a very potent, RAR-selective, non-irritative, and synthetically accessible agent[26]. Therefore, it is a significant challenge to develop new compounds superior to Am80, even though it has been known for a long time.

Among aromatic retinoids, Am80[27], Am555S (amsilarotene)[28] and their derivatives[29] contain an amide group which links two aromatic rings, one of which bears a bulky hydrophobic fragment. Generally, such compounds are selective for RAR and RAR , while they only slightly activate RAR , and do not bind to RXRs, which is a therapeutically desirable profile[30-33]. Thus, in order to find structurally new receptor-selective retinoids as candidates for further clinical application, we focused on the bulky hydrophobic fragment of synthetic retinoids and designed a series of compounds with novel hydrophobic moieties, *i.e.*, metacyclophane, phenalene and benzoheptalene derivatives. The

activities, including cell differentiation-inducing activity, of the synthesized compounds were evaluated.

2. Chemistry

As described above, structure-activity studies suggested that we should focus on the bulky hydrophobic fragment of synthetic retinoids.[27-33] Thus, we designed and synthesized a series of metacyclophane, phenalene and benzoheptalene derivatives.

2.1. Metacyclophane derivatives

Among [n]metacyclophane derivatives, [10]metacyclophane has been prepared from 1,3-dichlorobenzene by Tamao[34], though yields were poor. Smaller metacyclophanes are synthetically inaccessible, so we focused on [10]metacyclophane derivatives. Nitration of [10]metacyclophane gave 12-nitro (15%) and 12,14-dinitro derivatives (61%), but not the 13-nitro derivative. 13-Substituted derivatives (**1**, **2**), which structurally correspond to Am80, were obtained through multistep reactions from the 12,14-dinitro or 12-nitro compound. The 12,14-dinitro compound was first reduced to diamine, then the 13-nitro group was introduced, and the two amino groups were reductively removed to afford the 13-nitro derivative, which was reduced to 13-amino[10]metacyclophane. This was derivatized to the benzoic acid derivative (**1**). The 12-nitro compound was converted via multistep processes to the 13-carboxy compound and then reacted with methyl 4-aminobenzoate followed by hydrolysis to afford compound (**2**).

Figure 2

2.2. Phenalene derivatives

Although the [10]metacyclophane derivatives showed significant biological activity, further derivatization proved impractical. Instead, we designed a series of compounds that may be regarded as mimics of the macrocyclic ring, *i.e.*, 5,6,6a,7,8,9-hexahydro-4*H*-phenalene derivatives.

Figure 3

Nitration of the hexahydro-4*H*-phenalene, synthesized from 1-tetralone[35], did not proceed at the desired position, affording only the 1-substituted compound. However, acetylation gave a mixture of 1- and 2-acetyl derivatives. Various reaction conditions were examined, but did not increase the ratio of the 2-derivative. The products were separated by chromatography, and oxidized with hypobromide to the corresponding carboxylic acids (see Supplementary data). The 2-carboxylic acid was converted to 2-amine, then reacted with several methyl terephthalic acid chlorides to afford

4-[(5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenenyl)carbamoyl-4-benzoic acid derivatives (**3a-6a**). The 2-carboxylic acid was also condensed with various aminobenzoic acid esters to give 4-[(5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)carboxamide]-4-benzoic acid derivatives (**7a-14a**).

An angular methyl-substituted hexahydrophenalene was synthesized from 3-bromophenylpropane[36]. Acetylation of the phenalene proceeded smoothly to afford the 2-acetyl compound, and this was treated as above to give the desired phenalenecarbamoylbenzoic acids (**3b-6b**) and phenalenecarboxamide-4-benzoic acids (**7b-14b**)

2.3. Benzo[ef]heptalene derivatives

The benzo[*ef*]heptalene skeleton was prepared from 1-benzosuberone by means of two successive Horner-Emmons reactions and cyclization to ketone, and then the carbonyl group was removed with Et_3SiH-trifluoroacetic acid to give 5,6,7,7a,8,9,10,11-octahydro-4*H*-benzo[*ef*]heptalene (see Supplementary data).

The acetylation proceeded smoothly to give the desired 2-acetyl derivative as the major product with 1-acetyl heptalene as a minor product. The 2-acetyl derivative was readily converted to carboxylic acid and then to the corresponding amine. The carbamoyl derivatives (**15**, **16**) and carboxamide derivatives (**17-22**) were prepared in the manner described above.

Figure 4

3. Biological Activity

3.1. Transcriptional activity

The functional profiles of the novel compounds were evaluated by means of reporter assay utilizing COS-1 cells with expression vectors containing GAL4-RAR (, , and) and full length RXR (, , and). GAL4-RARs were produced by using the CheckMate™ Mammalian Two-Hybrid System (Progema code #E2440). They were composed of pBIND vector and each ligand-binding domain. When retinoids interact with this domain, GAL4-RAR vector binds to the reporter plasmid, pG5*luc* vector, and firefly luciferase is expressed[37]. The results were expressed as fold activation with respect to the blank control value. Am80 and ATRA were used as positive controls for RARs and HX630 was used as positive control for RXRs.

Table 1

Figure 5

3.2. Differentiation-inducing assay

Human promyelocytic leukemia cell line HL-60 was cultured in RPMI-1640 medium supplemented with 5% FBS in a humidified atmosphere of 5% CO_2 in air at 37°C. Test compounds were added to the cells, which were seeded at about 8.0×10^4 cells/ml. The final DMSO concentration was kept below 0.1%. Control cells were given only the same volume of DMSO. Am80 and ATRA (positive controls) were assayed at the same time. The cells were incubated for 4 days and differentiated cells were evaluated by NBT reduction assay. Cells were incubated for 20 min at 37°C in RPMI-1640 medium (5%) and an equal volume of phosphate-buffered saline (PBS) containing NBT (0.2%) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) as described[26]. The percentage of cells differentiated to monocytes (containing blue-black formazan) was determined on a minimum of 200 cells.

Table 2

4. Results and discussion

The metacyclophane derivatives (**1**, **2**) showed significant agonistic activity towards RARs (about 1/10 of that of retinoic acid towards RAR and RAR without activity towards RXRs, but synthetic difficulty meant that we could not obtain a wider range of derivatives for further evaluation.

Tricyclic phenalene and heptalene derivatives both showed similar patterns of activity to other corresponding amides such as tamibarotene. The preferred linking position of the amide (carbamoyl or carboxamide) benzoic acid group is *meta* (8-position of phenalene, and 2-position of heptalene). Generally, carboxamide-type benzoic acid derivatives have higher activities in transactivation assay than carbamoyl-type compounds. Introduction of fluorine as a substituent on the benzoic acid seems to increase the activity towards RAR without affecting that towards RAR (cf. **7b** vs **11b**; **17** vs **19**). Introduction of a nitrogen atom in the benzoic acid moiety (nicotinic acid) resulted in higher RAR activity (**7a** vs **8a**; **7b** vs **8b**; **17** vs **18**). It might be possible to utilize this finding to obtain RAR antagonists. Thiophene carboxylic acid showed a lower activity than benzoic acid. Among the compounds listed in Scheme, **11a**, **11b**, **12b** and **19** are the most selective, showing high selectivity for RAR , lower selectivity for RAR , and very low (at 1000 nM) activity towards RAR . The ratio of activities (EC_{50}) towards RAR and RAR is more than 100. Compounds **8a**, **8b** and **18** showed potent RAR transactivation activity. In particular, **18** is a potent pan-RAR agonist. Compounds **4a** and **4b** are relatively selective for RAR among these compounds. Absence of RAR activity is preferable for clinical application, because activation of RAR is associated with skin irritation and other unfavorable events[30-33].

All of the compounds were inactive towards RXRs at 1000 nM, and so these amide-type retinoids can be classified as strongly RAR-specific. This is very important, because RXR dimerizes with many other nuclear receptors, including TR, VDR, Nurr and PPAR. Although it was believed that

ATRA activates all three RARs, but not RXRs, while 9-*cis*-retinoic acid (9cRA) activates both RARs and RXRs, recent work has shown that ATRA activates RXRs as well as RARs, at least under conditions of cell culture. For example, ATRA activates RXR in CAT–reporter assay at the concentration (EC_{50}) of 50 nM[15], and its agonistic potency for RARs is in the range of only 3 to 8 times[17] or 40 times[19] greater than that for RXRs. Therefore, it seems to have been wrongly concluded that the biological activities of ATRA are mediated *only* by RARs[14, 21], and other nuclear receptors that may be activated by RXRs could also play a significant role[37-41]. Therefore, the present RAR-specific ligands, which do not activate RXRs, could be very useful in medicinal and biological research. It should be noted that the level of 9-*cis*-retinoic acid in tissues is very low, and may not be high enough to activate endogenous RXR receptors[42, 43].

We also examined the effects of our compounds on the differentiation of promyelocytic leukemia cell line, HL-60 cells, to mature myeloid cells, which is thought to involve RAR [44, 45]. Among the present compounds, those that activate both RAR and RAR showed high differentiating activities. The highest cell-differentiating activity was observed with **8a** and **8b**. Interestingly, **19**, **20** and **21** showed weaker activity than Am80, even though their agonistic activity towards RAR seems to be equal to or higher than that of Am80. This may suggest that HL-60 differentiation is more influenced by RAR activation than by RAR activation. This idea is supported by the absence of inhibition of HL-60 proliferation by **19** at concentrations up to 10^{-6} M, even though the agonistic activity of RAR of the compound is higher than that of Am80. However, it is important to note that RAR activation often leads to induction of RAR , so RAR activation may be necessary as a prerequisite to the RAR activation. It is also important that at least some of the present compounds are less cytotoxic than ATRA or Am80.

Finally, the activity of compounds in reporter assays such as those used here may depend on many factors, including plasmid construction, the kind of cells used, the presence of various transcriptional cofactors, and incubation conditions, so it will be important to evaluate the biological activity of our compounds more extensively *in vivo*.

5. Conclusion

Retinoids containing metacyclophane, phenalene and benzoheptalene as bulky hydrophobic structures were synthesized and their agonistic activities towards RARs and RXRs were evaluated. These compounds were generally selective for RAR and RAR over RAR while they showed no agonistic activity towards RXRs. Among these compounds, low RAR -selective activity was correlated with weak HL-60 differentiation-inducing activity, suggesting that RAR activation may play a prominent role in the differentiation of HL-60 cells.

6. Experimental

6.1. General

Melting points were determined on a Yanagimoto micro-melting point apparatus (hot plate), and are not corrected. ^{1}H NMR spectra were obtained on a Varian Mercury 300 at 300 MHz in $CDCl_3$ or in DMSO-d_6. The chemical shifts are calculated on the basis of tetramethylsilane (0 ppm in $CDCl_3$) or $(C\underline{H}_3)_2SO$ (2.49 ppm in DMSO-d_6). High-resolution ESI-MS (HR-ESI-MS) was recorded on a Shimadzu LCMS-IT-TOF spectrometer. Column chromatography was conducted on silica gel (Fuji Davison BW 200). Usual work-up refers to washing of the organic phase with water or brine, drying over anhydrous Na_2SO_4, and removing the solvent(s) by evaporation under reduced pressure.

*6.2. Synthesis of metacyclophane derivatives (**1** and **2**).*

4-[13-([10]Metacyclophanyl)carbamoyl]benzoic acid (1)

To a solution of 13-amino[10]metacyclophane (0.090 g, 0.389 mmol) in benzene (10 ml) and pyridine (2 ml) was added terephthalic acid monomethyl ester chloride (0.116 g, 0.584 mmol). The reaction mixture was stirred at rt for 3 h, then poured into 2 M aqueous HCl and extracted with AcOEt. The organic phase was washed with 10% aqueous Na_2CO_3. Usual work-up gave a residue, which was purified by silica gel column chromatography (AcOEt:*n*-hexane = 1:20) to afford methyl 4-[13-([10]metacyclophanyl)carbamoyl]benzoate (0.116 g, 76%). To a solution of the above ester (0.094 g, 0.239 mmol) in EtOH (8 ml) was added 2 M aqueous NaOH (3 ml). The reaction mixture was stirred at rt for 3 h, then acidified by adding 2 M aqueous HCl and extracted with $CHCl_3$. Usual work-up gave a residue, which was purified by recrystallization from AcOEt/*n*-hexane to afford **1** (0.080 g, 88%). **1**: Colorless plates (AcOEt/*n*-hexane); mp 234-235°C; ^{1}H NMR (DMSO-d_6) : 0.86-1.00 (4H, m), 1.04-1.11 (4H, m), 1.12-1.23 (4H, m), 1.60-1.70 (4H, m), 2.61 (4H, t, *J*=5.7 Hz), 6.93 (1H, s), 7.44 (2H, s), 8.04 (4H, s), 10.26 (1H, s); HR-ESI-MS *m/z*: 378.2069 ($[M-H]^+$, calcd for $C_{24}H_{28}NO_3$, 378.2069).

4-[13-([10]Metacyclophanyl)carboxamido]benzoic acid (2)

To a suspension of [10]metacyclophane-13-carboxylic acid (0.175 g, 0.672 mmol) in benzene (3 ml) was added $SOCl_2$ (1 ml). The reaction mixture was refluxed for 4 h, then evaporated, and the residue was dissolved in benzene (2 ml) and pyridine (5 ml). Methyl 4-aminobenzoate (0.106 g, 0.701 mmol) and a small amount of 4-DMAP were added to the resulting solution. The reaction mixture was stirred at rt overnight, poured into 2 M aqueous HCl and extracted with AcOEt. The organic phase was washed with 10% aqueous Na_2CO_3. Usual work-up gave a residue, which was purified by silica gel column chromatography (AcOEt:*n*-hexane=1:30) to afford methyl 4-[13-([10]metacyclophanyl)carboxamido]benzoate (0.179 g, 68%). To a solution of the above ester

(0.175 g, 0.445 mmol) in EtOH (10 ml) was added 2 M aqueous NaOH (3 ml). The reaction mixture was stirred at rt for 6 h, then acidified by adding 2 M aqueous HCl and extracted with $CHCl_3$. Usual work-up gave a residue, which was purified by recrystallization from AcOEt/*n*-hexane to afford **2** (0.146 g, 86%). **2**: Colorless needles (AcOEt/*n*-hexane); mp 273-274°C; ^{1}H NMR (DMSO-d_6) : 0.86-1.00 (4H, m), 1.04-1.12 (4H, m), 1.13-1.23 (4H, m), 1.72 (4H, br s), 2.73 (4H, t, *J*=5.7 Hz), 7.42 (1H, s), 7.64 (2H, s), 7.93 (4H, s), 10.44 (1H, s); HR-ESI-MS *m/z*: 378.2070 ([M-H]$^+$, calcd for $C_{24}H_{28}NO_3$, 378.2069).

*6.3. Synthesis of phenalene derivatives (**3a, b**-**14a, b**)*

4-[(5,6,6a,7,8,9-Hexahydro-4*H*-2-phenalenyl)carbamoyl]benzoic acid (3a) and analogs (4a-6a)

To a solution of 5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenylamine[46] (0.260 g, 1.39 mmol) in benzene (10 ml) and pyridine (3 ml) was added terephthalic acid monomethyl ester chloride (0.304 g, 1.53 mmol). The reaction mixture was stirred at rt for 3 h, then poured into 2 M aqueous HCl and extracted with AcOEt. The organic phase was washed with 10% aqueous Na_2CO_3. Usual work-up gave a residue, which was purified by recrystallization from $CHCl_3$/*n*-hexane give methyl 4-[(5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)carbamoyl]benzoate (0.460 g, 95%). To a suspension of the above ester (0.093 g, 0.266 mmol) in EtOH (10 ml) was added 2 M aqueous NaOH (3 ml). The reaction mixture was stirred at rt for 3 h, then acidified by adding 2 M aqueous HCl and extracted with $CHCl_3$. Usual work-up gave a residue, which was purified by recrystallization from AcOEt/*n*-hexane to afford **3a** (0.080 g, 90%). **3a**: Colorless prisms (AcOEt/*n*-hexane); mp 283-284°C; ^{1}H NMR (DMSO-d_6) : 1.21-1.30 (2H, m), 1.25-1.78 (2H, m), 1.81-1.94 (4H, m), 2.43-2.47 (1H, m), 2.73-2.76 (4H, m), 7.30 (2H, s), 8.01 (2H, d, *J*=8.6 Hz), 8.05 (2H, *d*, J=8.6 Hz), 10.15 (1H, s); HR-ESI-MS *m/z*: 334.1430 ([M-H]$^+$, calcd for $C_{21}H_{20}NO_3$, 334.1443).

6-[(5,6,6a,7,8,9-Hexahydro-4*H*-2-phenalenyl)carbamoyl]nicotinic acid (**4a**): 83% yield. Pale yellow needles (AcOEt/*n*-hexane); mp 252-253°C; ^{1}H NMR ($CDCl_3$) : 1.26-1.40 (2H, m), 1.72-1.86 (2H, m), 1.88-2.00 (4H, m), 2.51-2.61 (1H, m), 2.82-2.87 (4H, m), 7.36 (2H, s), 8.42 (1H, d, *J*=8.1 Hz), 8.58 (1H, dd, *J*=8.1, 2.1 Hz), 9.26 (1H, d, *J*=2.1 Hz), 9.88 (1H, s); HR-ESI-MS *m/z*: 335.1380 ([M-H]$^+$, calcd for $C_{20}H_{19}N_2O_3$, 335.1396).

5-[(5,6,6a,7,8,9-Hexahydro-4*H*-2-phenalenyl)carbamoyl]pyridine-2-carboxylic acid (**5a**): 89% yield. Pale yellow needles (AcOEt/*n*-hexane); mp 239-240°C; ^{1}H NMR (DMSO-d_6) : 1.14-1.28 (2H, m), 1.63-1.76 (2H, m), 1.78-1.83 (2H, m), 1.84-1.92 (2H, m), 2.40-2.42 (1H, m), 2.71-2.74 (4H, m), 7.27 (2H, s), 8.13 (1H, d, *J*=8.1 Hz), 8.40 (1H, dd, *J*=8.1, 2.1 Hz), 9.13 (1H, d, *J*=2.1 Hz), 10.32 (1H, s); HR-ESI-MS *m/z*: 335.1372 ([M-H]$^+$, calcd for $C_{20}H_{19}N_2O_3$, 335.1396).

5-[(5,6,6a,7,8,9-Hexahydro-4*H*-2-phenalenyl)carbamoyl]thiophene-2-carboxylic acid (**6a**): 65% yield. Pale yellow powder (AcOEt/*n*-hexane); mp 281-283°C; ^{1}H NMR (DMSO-d_6) : 1.18-1.27 (2H,

m), 1.66-1.76 (2H, m), 1.77-1.92 (4H, m), 2.44-2.45 (1H, m), 2.71-2.73 (4H, m), 7.22 (2H, s), 7.26 (1H, d, *J*=4.2 Hz), 7.95 (1H, d, *J*=4.2 Hz), 10.17 (1H, s); HR-ESI-MS *m/z*: 340.1004 ($[M-H]^+$, calcd for $C_{19}H_{18}NO_3S$, 340.1007).

4-[(5,6,6a,7,8,9-Hexahydro-4*H*-2-phenalenyl)carboxamido]benzoic acid (7a) and analogs (8a-14a)

To a suspension of 5,6,6a,7,8,9-hexahydro-4*H*-phenalene-2-carboxylic acid[46] (0.338 g, 1.56 mmol) in benzene (7.5 ml) was added $SOCl_2$ (2.5 ml). The reaction mixture was refluxed for 4 h, then evaporated, and the residue was dissolved in benzene (3 ml) and pyridine (10 ml). Methyl 4-aminobenzoate (0.259 g, 1.71 mmol) and 4-DMAP (0.020 g, 0.164 mmol) were added to the resulting solution. The reaction mixture was stirred at rt for 15 h, then poured into 2 M aqueous HCl and extracted with AcOEt. The organic phase was washed with 10% aqueous Na_2CO_3. Usual work-up gave a residue, which was purified by silica gel column chromatography (AcOEt:*n*-hexane=1:10) to afford methyl 4-[(5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)carboxamido]benzoate (0.478 g, 88%). To a suspension of the above ester (0.385 g, 1.10 mmol) in EtOH (10 ml) was added 2 M aqueous NaOH (7.5 ml). The reaction mixture was stirred at 60°C for 1 h, then cooled, acidified by adding 2 M aqueous HCl and extracted with $CHCl_3$. Usual work-up gave a residue, which was purified by recrystallization from EtOH to afford **7a** (0.348 g, 94%). **7a**: Colorless needles (EtOH); mp >300°C; ^{1}H NMR (DMSO-d_6) : 1.21-1.34 (2H, m), 1.73-1.99 (6H, m), 2.55-2.66 (1H, m), 2.80-2.85 (4H, m), 7.49 (2H, s), 7.90 (4H, s), 10.33 (1H, s); HR-ESI-MS *m/z*: 334.1430 ($[M-H]^+$, calcd for $C_{21}H_{20}NO_3$, 334.1443).

6-[(5,6,6a,7,8,9-Hexahydro-4*H*-2-phenalenyl)carboxamido]nicotinic acid (**8a**): 61% yield. Colorless powder (EtOH/$CHCl_3$); mp > 300°C; ^{1}H NMR (DMSO-d_6) : 1.20-1.34 (2H, m), 1.68-1.80 (2H, m), 1.82-1.99 (4H, m), 2.55-2.62 (1H, m), 2.78-2.83 (4H, m), 7.58 (2H, s), 8.30 (1H, s), 8.31 (1H, s), 8.88 (1H, d, *J*=1.8 Hz), 10.93(1H, s); HR-ESI-MS *m/z*: 335.1390 ($[M-H]^+$, calcd for $C_{20}H_{19}N_2O_3$, 335.1396).

5-[(5,6,6a,7,8,9-Hexahydro-4*H*-2-phenalenyl)carboxamido]pyridine-2-carboxylic acid (**9a**): 69% yield. Pale yellow powder (EtOH); mp 219-220°C; ^{1}H NMR (DMSO-d_6) : 1.19-1.32(2H, m), 1.71-1.78 (2H, m), 1.82-1.87 (2H, m), 1.88-1.97 (2H, m), 2.53-2.60 (1H, m), 2.78-2.83 (4H, m), 7.50 (2H, s), 8.04 (1H, d, *J*=8.4 Hz), 8.37 (1H, dd, *J*=8.4, 2.7 Hz), 9.01 (1H, d, *J*=2.7 Hz), 10.55 (1H, s); HR-ESI-MS *m/z*: 335.1384 ($[M-H]^+$,

calcd for $C_{20}H_{19}N_2O_3$, 335.1396).

5-[(5,6,6a,7,8,9-Hexahydro-4*H*-2-phenalenyl)carboxamido]thiophene-2-carboxylic acid (**10a**): 36% yield. Colorless powder (AcOEt/*n*-hexane); mp 259-261°C; ^{1}H NMR (DMSO-d_6) : 1.20-1.32 (2H, m), 1.71-1.78 (2H, m), 1.79-1.87 (2H, m), 1.89-1.98 (2H, m), 2.53-2.61 (1H, m), 2.78-2.83 (4H, m), 6.90 (1H, d, *J*=4.2 Hz), 7.52 (2H, s), 7.54 (1H, d, *J*=4.2 Hz), 11.74 (1H, s); HR-ESI-MS *m/z*:

340.1007([M-H]$^+$, calcd for $C_{19}H_{18}NO_3S$, 340.1007).

2-Fluoro-4-[(5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)carboxamido]benzoic acid (**11a**): 80% yield. Colorless needles (AcOEt/*n*-hexane); mp >300°C; ^{1}H NMR (DMSO-d_6) : 1.21-1.34 (2H, m), 1.74-1.80 (2H, m), 1.83-1.89 (2H, m), 1.92-1.99 (2H, m), 2.53-2.62 (1H, m), 2.80-2.85 (4H, m), 7.49 (2H, s), 7.65 (1H, dd, *J*=8.7, 2.1 Hz), 7.81-7.91 (2H, m), 10.51 (1H, s); HR-ESI-MS *m/z*: 352.1351([M-H]$^+$, calcd for $C_{21}H_{19}FNO_3$, 352.1349).

2-Chloro-4-[(5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)carboxamido]benzoic acid (**12a**): 72% yield. Colorless needles (AcOEt/*n*-hexane); mp 281-282°C; ^{1}H NMR (DMSO-d_6) : 1.21-1.34 (2H, m), 1.73-1.80 (2H, m), 1.82-1.89 (2H, m), 1.92-1.99 (2H, m), 2.56- 2.62 (1H, m), 2.80-2.85 (4H, m), 7.49 (2H, s), 7.80-7.87 (2H, m), 8.05 (1H, d, *J*=2.1 Hz), 10.43 (1H, s); HR-ESI-MS *m/z*: 368.1035 ([M-H]$^+$, calcd for $C_{21}H_{19}ClNO_3$, 368.1053).

2-Hydroxy-4-[(5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)carboxamido]benzoic acid (**13a**): 79% yield. Colorless prisms (AcOEt/*n*-hexane); mp 267-267.5°C; ^{1}H NMR (DMSO-d_6) : 1.15-1.33 (2H, m), 1.68-1.80 (2H, m), 1.82-1.89 (2H, m), 1.91-1.99 (2H, m), 2.53-2.61 (1H, m), 2.79-2.85 (4H, m), 7.32 (1H, dd, *J*=8.7, 2.1 Hz), 7.47 (2H, s), 7.53 (1H, d, *J*=2.1 Hz), 7.74 (1H, d, *J*=8.7 Hz), 10.29 (1H, s); HR-ESI-MS *m/z*: 350.13906 ([M-H]$^+$, calcd for $C_{21}H_{20}NO_4$, 350.1392).

2-Methoxy-4-[(5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)carboxamido]benzoic acid (**14a**): 64% yield. Colorless needles (AcOEt/*n*-hexane); mp 206-208°C; ^{1}H NMR (DMSO-d_6) : 1.21-1.34 (2H, m), 1.74-1.80 (2H, m), 1.84-1.89 (2H, m), 1.92-1.99 (2H, m), 2.52-2.56 (1H, m), 2.80-2.85 (4H, m), 3.78 (3H, s), 7.35 (1H, d, *J*=8.1Hz), 7.50 (2H, s), 7.56-7.62 (2H, m), 10.22 (1H, s); HR-ESI-MS *m/z*: 364.1535 ([M-H]$^+$, calcd for $C_{22}H_{22}NO_4$, 364.1549).

4-[(6a-Methyl-5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)carbamoyl]benzoic acid (3b) and analogs (4b-6b)

Following the procedure used for synthesizing **3a** from 5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenylamine, **3b** was obtained from 6a-methyl-5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenylamine[46] in 71% yield. **3b**: Colorless needles (AcOEt/*n*-hexane); mp 282-283°C; ^{1}H NMR (DMSO-d_6) : 1.11 (3H, s), 1.42 (2H, td, *J*=12.8, 4.9 Hz), 1.64 (2H, dt, *J*=12.8, 4.0 Hz), 1.71- 1.80 (2H, m), 1.93-2.09 (2H, m), 2.72 (2H, dt, *J*=17.4, 8.7 Hz), 2.85 (2H, ddd, *J*=17.1, 7.8, 3.0 Hz), 7.28 (2H, s), 8.01 (2H, d, *J*=8.4 Hz), 8.05 (2H, d, *J*=8.4 Hz), 10.13(1H, s); HR-ESI-MS *m/z*: 348.1600 ([M-H]$^+$, calcd for $C_{22}H_{22}NO_3$, 348.1600).

6-[(6a-Methyl-5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)carbamoyl]nicotinic acid (**4b**): 73% yield. Pale yellow needles (AcOEt/*n*-hexane); mp 212-213°C; ^{1}H NMR ($CDCl_3$) : 1.17 (3H, s), 1.51 (2H, td, *J*=12.9, 5.1 Hz), 1.69 (2H, dt, *J*=12.9, 3.9 Hz), 1.77-1.88 (2H, m), 2.00-2.16 (2H, m), 2.83 (2H, dt, *J*=17.4, 8.7 Hz), 2.96 (2H, ddd, *J*=17.3, 8.1, 3.2 Hz), 7.31 (2H, s), 8.42 (1H, d, *J*=8.1 Hz), 8.57 (1H, dd, *J*=8.1, 1.8 Hz), 9.26 (1H, d, *J*=1.8 Hz), 9.87(1H, s); HR-ESI-MS *m/z*: 349.1536 ([M-H]$^+$, calcd

for $C_{21}H_{21}N_2O_3$, 349.1552).

5-[(6a-Methyl-5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)carbamoyl]pyridine-2-carboxylic acid (**5b**): 86% yield. Pale yellow powder (AcOEt/*n*-hexane); mp 199-200°C; ^{1}H NMR (DMSO-d_6) : 1.09(3H, s), 1.39 (2H, td, *J*=12.9, 5.1 Hz), 1.62 (2H, dt, *J*=12.6, 3.6 Hz), 1.68-1.78 (2H, m), 1.91-2.06 (2H, m), 2.70 (2H, dt, *J*=17.1, 8.7 Hz), 2.79-2.88 (2H, m), 7.25 (2H, s), 8.13 (1H, d, *J*=8.1 Hz), 8.40 (1H, dd, *J*=8.1, 2.1 Hz), 9.12 (1H, d, *J*=2.1 Hz), 10.30 (1H, s); HR-ESI-MS *m/z*: 349.1527 ([M-H]$^+$, calcd for $C_{21}H_{21}N_2O_3$, 349.1552).

5-[(6a-Methyl-5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)carbamoyl]thiophene-2-carboxylic acid (**6b**): 69% yield. Pale yellow powder (AcOEt/*n*-hexane); mp 271-272°C; ^{1}H NMR (DMSO-d_6) : 1.08 (3H, s), 1.38 (2H, td, *J*=12.9, 5.1 Hz), 1.61 (2H, dt, *J*=12.6, 3.9 Hz), 1.68-1.79 (2H, m), 1.88-2.04 (2H, m), 2.68 (2H, dt, *J*=17.4, 8.7 Hz), 2.82 (2H, ddd, *J*=17.7, 8.1, 3.3 Hz), 7.20 (2H, s), 7.72 (1H, d, *J*=3.9 Hz), 7.94 (1H, d, *J*=3.9 Hz), 10.16 (1H, s); HR-ESI-MS *m/z*: 354.1158 ([M-H]$^+$, calcd for $C_{20}H_{20}NO_3S$, 354.1164).

4-[(6a-Methyl-5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)carboxamido]benzoic acid (7b) and analogs (8b-14b)

Following the procedure used for synthesizing **7a** from 5,6,6a,7,8,9-hexahydro-4*H*-phenalene-2-carboxylic acid, **7b** was obtained from 6a-methyl-5,6,6a,7,8,9-hexahydro-4*H*-phenalene-2-carboxylic acid[46] in 55% yield. **7b**: Colorless prisms (AcOEt/*n*-hexane); mp 283.5-285°C; ^{1}H NMR (DMSO-d_6) : 1.14 (3H, s), 1.45 (2H, td, *J*=12.8, 4.8 Hz), 1.69 (2H, dt, *J*=12.8, 3.8 Hz), 1.74-1.83 (2H, m), 1.95-2.11 (2H, m), 2.81 (2H, dt, *J*=17.4, 8.7 Hz), 2.96 (2H, ddd, *J*=17.0, 7.8, 2.9 Hz), 7.45 (2H, s), 7.87 (2H, d, *J*=8.9 Hz), 7.92 (2H, d, *J*=8.9 Hz), 10.34(1H, s); HR-ESI-MS *m/z*: 348.1582 ([M-H]$^+$, calcd for $C_{22}H_{22}NO_3$, 348.1600).

6-[(6a-Methyl-5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)carboxamido]nicotinic acid (**8b**): 69% yield. Colorless powder (EtOH/$CHCl_3$); mp >300°C; ^{1}H NMR (DMSO-d_6) : 1.14 (3H, s), 1.48 (2H, td, *J*=12.9, 5.1 Hz), 1.68 (2H, dt, *J*=12.9, 4.4 Hz), 1.75- 1.84 (2H, m), 1.94-2.11 (2H, m), 2.80 (2H, dt, *J*=17.3, 8.6 Hz), 2.95 (2H, ddd, *J*=17.4, 7.7, 3.2 Hz), 7.57 (2H, s), 8.29-8.32 (2H, m), 8.87 (1H, s), 10.88 (1H, s); HR-ESI-MS *m/z*: 349.1534 ([M-H]$^+$, calcd for $C_{21}H_{21}N_2O_3$, 349.1552).

5-[(6a-Methyl-5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)carboxamido]pyridine-2-carboxylic acid (**9b**): 54% yield. Colorless needles (EtOH); mp 222-223°C; ^{1}H NMR (DMSO-d_6) : 1.11 (3H, s), 1.43 (2H, td, *J*=12.9, 4.8 Hz), 1.66 (2H, dt, *J*=12.9, 3.6 Hz), 1.71-1.81 (2H, m), 1.93-2.09 (2H, m), 2.80 (2H, dt, *J*=17.4, 8.7 Hz), 2.94 (2H, ddd, *J*=17.4, 8.1, 3.3 Hz), 7.47 (2H, s), 8.04 (1H, d, *J*=8.7 Hz), 8.36 (1H, dd, *J*=8.7, 2.4 Hz), 9.00 (1H, d, *J*=2.4 Hz), 10.54 (1H, s); HR-ESI-MS *m/z*: 349.1538 ([M-H]$^+$, calcd for $C_{21}H_{21}N_2O_3$, 349.1552).

5-[(6a-Methyl-5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)carboxamido]thiophene-2-carboxylic acid (**10b**): 44% yield. Colorless powder (AcOEt/*n*-hexane); mp 259-260°C; ^{1}H NMR (DMSO-d_6) : 1.11

(3H, s), 1.42 (2H, td, *J*=12.9, 4.5 Hz), 1.62-1.69 (2H, m), 1.72-1.80 (2H, m), 1.92- 2.08 (2H, m), 2.79 (2H, dt, *J*=17.3, 8.7 Hz), 2.94 (2H, ddd, *J*=17.7, 8.1, 3.3 Hz), 6.91 (1H, d, *J*=4.2 Hz), 7.48 (2H, s), 7.61 (1H, d, *J*=4.2 Hz), 11.80 (1H, s); HR-ESI-MS *m/z*: 354.1153 ([M-H]$^+$, calcd for $C_{20}H_{20}NO_3S$, 354.1164).

2-Fluoro-4-[(6a-methyl-5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)carboxamido]benzoic acid (**11b**): 74% yield. Colorless prisms (AcOEt/*n*-hexane); mp 276-278°C; ^{1}H NMR (DMSO-d_6) : 1.14 (3H, s), 1.45 (2H, td, *J*=12.9, 5.1 Hz), 1.67 (2H, dt, *J*=12.9, 3.9 Hz), 1.74-1.85 (2H, m), 1.95-2.11 (2H, m), 2.82 (2H, dt, *J*=17.3, 8.7 Hz), 2.96 (2H, ddd, *J*=17.3, 7.8, 3.3 Hz), 7.45 (2H, s), 7.63 (1H, d, *J*=8.7 Hz), 7.79-7.91 (2H, m), 10.50 (1H, s); HR-ESI-MS *m/z*: 366.1491([M-H]$^+$, calcd for $C_{22}H_{21}FNO_3$, 366.1505.

2-Chloro-4-[(6a-methyl-5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)carboxamido]benzoic acid (**12b**): 79% yield. Colorless prisms (AcOEt/*n*-hexane); mp 244-246°C; ^{1}H NMR (DMSO-d_6) : 1.13 (3H, s), 1.45 (2H, td, *J*=12.9, 5.1 Hz), 1.68 (1H, dt, *J*=12.9, 3.9 Hz), 1.75-1.83 (2H, m), 1.95-2.10 (2H, m), 2.81 (2H, dt, *J*=17.3, 8.7 Hz), 2.96 (2H, ddd, *J*=17.1, 7.6, 3.0 Hz), 7.46 (2H, s), 7.81 (1H, dd, *J*=8.7, 1.8 Hz), 7.87 (1H, d, *J*=8.7 Hz), 8.04 (1H, d, *J*=1.8 Hz), 10.43 (1H, s); HR-ESI-MS *m/z*: 382.1203 ([M-H]$^+$, calcd for $C_{22}H_{21}ClNO_3$, 382.1210).

2-Hydroxy-4-[(6a-methyl-5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)carboxamido]benzoic acid (**13b**): 87% yield. Colorless prisms (AcOEt/*n*-hexane); mp 244-245°C; ^{1}H NMR (DMSO-d_6) : 1.13 (3H, s), 1.45 (2H, td, *J*=12.9, 5.1 Hz), 1.68 (2H, dt, *J*=12.9, 3.9 Hz), 1.74-1.84 (2H, m), 1.95-2.11 (2H, m), 2.81 (2H, dt, *J*=17.3, 8.4 Hz), 2.96 (2H, ddd, *J*=17.4, 7.8, 3.0 Hz), 7.31 (1H, dd, *J*=8.7, 1.8 Hz), 7.43 (2H, s), 7.51 (1H, d, *J*=1.8 Hz), 7.74 (1H, d, *J*=8.7 Hz), 10.29 (1H, s); HR-ESI-MS *m/z*: 364.1539 ([M-H]$^+$, calcd for $C_{22}H_{22}NO_4$, 364.1549).

2-Methoxy-4-[(6a-methyl-5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenyl)carboxamido]benzoic acid (**14b**): 68% yield. Colorless needles (AcOEt/*n*-hexane); mp 184-186°C; ^{1}H NMR (DMSO-d_6) : 1.14 (3H, s), 1.46 (2H, td, *J*=12.9, 5.4 Hz), 1.69 (2H, dt, *J*=12.5, 3.8 Hz), 1.75-1.85 (2H, m), 1.95-2.01 (2H, m), 2.82 (2H, dt, *J*=17.4, 8.7 Hz), 2.96 (2H, ddd, *J*=17.3, 7.7, 3.3 Hz), 3.77 (3H, s), 7.41 (1H, dd, *J*=8.6, 2.1 Hz), 7.46 (2H, s), 7.55-7.57 (2H, m), 10.19 (1H, s); HR-ESI-MS *m/z*: 378.1696 ([M-H]$^+$, calcd for $C_{23}H_{24}NO_4$, 378.1705).

*6.3. Synthesis of benzo[ef]heptalene derivatives (**15-22**)*

4-[(5,6,7,7a,8,9,10,11-Octahydro-4*H*-2-benzo[*ef*]heptalenyl)carbamoyl]benzoic acid (15) and analog (16)

Following the procedure used for synthesizing **3a** from 5,6,6a,7,8,9-hexahydro-4*H*-2-phenalenylamine, **15** was obtained from 5,6,7,7a,8,9,10,11-octahydro-4*H*-2-benzo[*ef*]heptalenylamine[46] in 67% yield. **15**: Colorless needles

(AcOEt/*n*-hexane); mp 280-281°C; ^{1}H NMR (DMSO-d_6) : 1.34-1.59 (6H, m), 1.69-1.88 (6H, m), 2.71-2.80 (4H, m), 3.10-3.21 (1H, m), 7.27 (2H, s), 7.95 (2H, d, *J*=8.1 Hz), 8.05 (2H, d, *J*=8.1 Hz), 10.17 (1H, s); HR-ESI-MS *m/z*: 362.1736 ([M-H]$^+$, calcd for $C_{23}H_{24}NO_3$, 362.1756).

6-[(5,6,7,7a,8,9,10,11-Octahydro-4*H*-2-benzo[*ef*]heptalenyl)carbamoyl]nicotinic acid (**16**): 81% yield. Pale yellow needles (AcOEt/*n*-hexane); mp 239-240°C; ^{1}H NMR ($CDCl_3$) : 1.53-1.66 (6H, m), 1.79-1.91 (6H, m), 2.84-2.88 (4H, m), 3.19-3.26 (1H, m), 7.38 (2H, s), 8.39 (1H, d, *J*=8.1 Hz), 8.54 (1H, d, *J*=8.1 Hz), 9.24 (1H, s), 9.86 (1H, s); HR-ESI-MS *m/z*: 363.1694 ([M-H]$^+$, calcd for $C_{22}H_{23}N_2O_3$, 363.1709).

4-[(5,6,7,7a,8,9,10,11-Octahydro-4*H*-2-benzo[*ef*]heptalenyl)carboxamido]benzoic acid (17) and analogs (18-22)

Following the procedure used for synthesizing **7a** from 5,6,6a,7,8,9-hexahydro-4*H*-phenalene-2-carboxylic acid, **17** was obtained from 5,6,7,7a,8,9,10,11-octahydro-4*H*-benzo[*ef*]heptalene-2-carboxylic acid[46] in 58% yield. **17**: Colorless needles (AcOEt/*n*-hexane); mp 275-276°C; ^{1}H NMR (DMSO-d_6) : 1.37-1.60 (6H, m), 1.70-1.90 (6H, m), 2.84-2.87 (4H, m), 3.20-3.31 (1H, m), 7.48 (2H, s), 7.83 (2H, d, *J*=8.7 Hz), 7.89 (2H, d, *J*=8.7 Hz), 10.32 (1H, s); HR-ESI-MS *m/z*: 362.1747 ([M-H]$^+$, calcd for $C_{23}H_{24}NO_3$, 362.1756).

6-[(5,6,7,7a,8,9,10,11-Octahydro-4*H*-2-benzo[*ef*]heptalenyl)carboxamido]nicotinic acid (**18**): 6% yield. Colorless needles (EtOH/$CHCl_3$); mp >300°C; ^{1}H NMR (DMSO-d_6) : 1.44-1.62 (6H, m), 1.73-1.93 (6H, m), 2.87-2.91 (4H, m), 3.25-3.68 (1H, m), 7.63 (2H, s), 8.28-8.91 (2H, m), 8.86 (1H, s), 10.87 (2H, s); HR-ESI-MS *m/z*: 363.1709 ([M-H]$^+$, calcd for $C_{22}H_{24}N_2O_3$, 363.1709).

2-Fluoro-4-[(5,6,7,7a,8,9,10,11-octahydro-4*H*-2-benzo[*ef*]heptalenyl)carbamoyl]benzoic acid (**19**): 23% yield. Colorless needles (AcOEt/*n*-hexane); mp 266-267°C; ^{1}H NMR (DMSO-d_6) : 1.43-1.56 (6H, m), 1.74-1.91 (6H, m), 2.88 (4H, t, *J*=5.7 Hz), 3.11-3.19 (1H, m), 7.50 (2H, s), 7.62 (1H, dd, *J*=8.7, 2.1 Hz), 7.78-7.89 (2H, m), 10.47 (1H, s); HR-ESI-MS *m/z*: 380.1648 ([M-H]$^+$, calcd for $C_{23}H_{23}FNO_3$, 380.1662).

2-Chloro-4-[(5,6,7,7a,8,9,10,11-octahydro-4*H*-2-benzo[*ef*]heptalenyl)carbamoyl]benzoic acid (**20**): 43% yield. Colorless needles (AcOEt/*n*-hexane); mp 243-244°C; ^{1}H NMR (DMSO-d_6) : 1.43-1.59 (6H, m), 1.72-1.90 (6H, m), 2.87 (4H, t, *J*=5.4 Hz), 3.12-3.18 (1H, m), 7.50 (2H, s), 7.79 (1H, dd, *J*=8.4, 1.8 Hz), 7.84 (1H, d, *J*=8.4 Hz), 8.02 (1H, d, *J*=1.8 Hz), 10.38 (1H, s); HR-ESI-MS *m/z*: 396.1355 ([M-H]$^+$, calcd for $C_{23}H_{23}ClNO_3$, 396.1366).

2-Hydroxy-4-[(5,6,7,7a,8,9,10,11-octahydro-4*H*-2-benzo[*ef*]heptalenyl)carbamoyl]benzoic acid (**21**): 17% yield. Colorless needles (AcOEt/*n*-hexane); mp 242-243°C; ^{1}H NMR (DMSO-d_6) : 1.43-1.59 (6H, m), 1.71-1.91 (6H, m), 2.87 (4H, t, *J*=5.4 Hz), 3.06-3.13 (1H, m), 7.29 (1H, dd, *J*=8.7, 1.8 Hz), 7.48 (2H, s), 7.49 (1H, d, *J*=1.8 Hz), 7.72 (1H, d, *J*=8.7 Hz), 10.25 (1H, s); HR-ESI-MS

m/z: 378.1702 ([M-H]$^+$, calcd for $C_{23}H_{24}NO_4$, 378.1705).

2-Methoxy-4-[(5,6,7,7a,8,9,10,11-octahydro-4*H*-2-benzo[*ef*]heptalenyl)carbamoyl]benzoic acid (**22**): 41% yield. Colorless needles (AcOEt/*n*-hexane); mp 233-234°C; ^{1}H NMR (DMSO-d_6) : 1.43-1.56 (6H, m), 1.72-1.91 (6H, m), 2.88 (4H, t, *J*=5.4 Hz), 3.06-3.15 (1H, m), 3.78 (3H, s), 7.43 (1H, d, *J*=8.7 Hz), 7.50 (2H, s), 7.56-7.63 (2H, m), 10.21(1H, s); HR-ESI-MS *m/z*: 392.1844 ([M-H]$^+$, calcd for $C_{24}H_{26}NO_4$, 392.1862).

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Figure Legends

Figure 1.

Structures of ATRA, 9cRA, and Am80.

Figure 2.

Synthesis of metacyclophane derivatives (**1**, **2**). Reagents: (i) $NaNO_3$, H_2SO_4; (ii) H_2, 10% Pd/C; (iii) Ac_2O, pyridine; (iv) HNO_3, H_2SO_4; (v) HCl; (vi) $NaNO_2$, H_2SO_4, EtOH; (vii) terephthalic acid monomethyl ester chloride, pyridine; (viii) 2 M aq. NaOH, EtOH; (ix) a) CH_3CN, BCl_3, $AlCl_3$ b) 2 M aq. HCl ; (x) 2.5 M aq. NaOH, Br_2; (xi) a) $SOCl_2$ b) methyl 4-aminobenzoate, 4-DMAP, pyridine.

Figure 3.

Synthesis of phenalene derivatives (**3a**, **b**-**14a**, **b**). Reagents: (i) AcCl, $AlCl_3$ (ii) 2.5 M aq. NaOH, Br_2; (iii) a) $SOCl_2$ b) aq. NaN_3 c) aq. AcOH d) HCl, MeOH; (iv) ClOC-Ar-COOMe, pyridine; (v) 2 M aq. NaOH, EtOH; (vi) a) $SOCl_2$ b) H_2N-Ar-COOMe; 4-DMAP, pyridine.

Figure 4.

Synthesis of benzo[*ef*]heptalene derivatives (**15**-**22**). Reagents: (i) AcCl, $AlCl_3$ (ii) 2.5 M aq. NaOH, Br_2; (iii) a) $SOCl_2$ b) aq. NaN_3 c) aq. AcOH d) HCl, MeOH; (iv) ClOC-Ar-COOMe, pyridine; (v) 2 M aq. NaOH, EtOH; (vi) a) $SOCl_2$ b) H_2N-Ar-COOMe; 4-DMAP, pyridine.

[a] Ar-COOHs are the same as shown in Figure 3.

Figure 5.

Transcriptional activities measured by means of reporter gene assay in Cos-1 cells expressing GAL4-RAR (●), GAL4-RAR (□), and GAL4-RAR (▲). Data are mean values of fold increase (vs DMSO) measured by two or more experiments. The horizontal scale is the molar concentration of added compound.

Table 1.

Transcriptional activation assay data.[a]

[a] Data are mean values of fold induction (vs DMSO) at 100 nM measured by two or more experiments.

[b] Metacyclophane derivatives (**1**, **2**); Phenalene derivatives (**3a**,**b**-**14a**,**b**); Benzo[*ef*]heptalene derivatives (**15**-**22**).

[c] Ar-COOHs are the same as shown in Figure 3.

[d] One experiment.

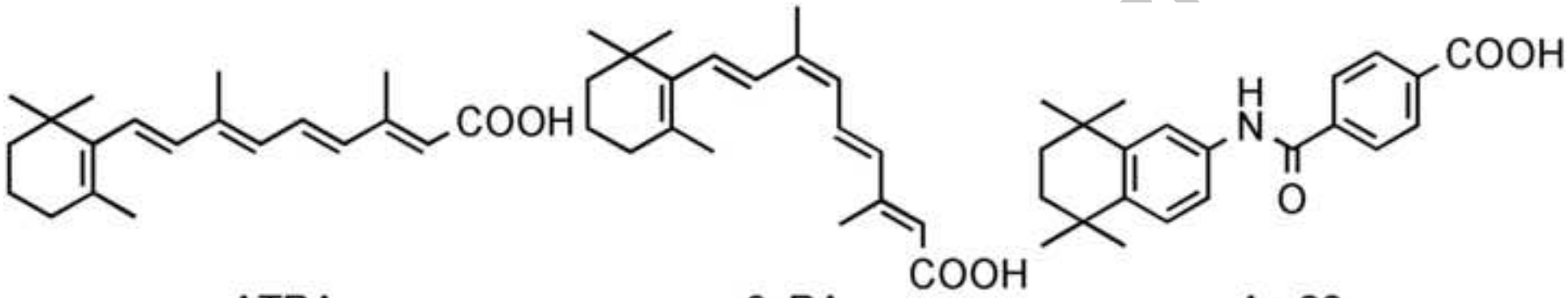
[e] Not determined.

Table 2.

HL-60 cell differentiation-inducing activity.[a]

[a] Data are mean values of the percentage of differentiated cells evaluated from NBT reduction assay at 10 nM and 100 nM.

[b] Not determined.



ATRA
(All-*trans*-retinoic acid)

9cRA
(9-*cis*-retinoic acid)

Am80
(tamibarotene)

Figure 1. Structures of ATRA, 9cRA, and Am80.

Fig2.tif

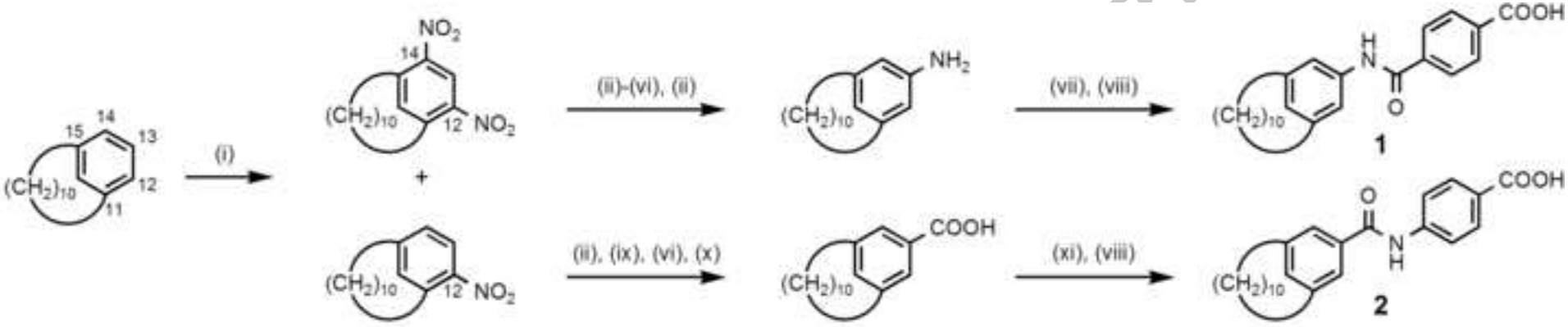
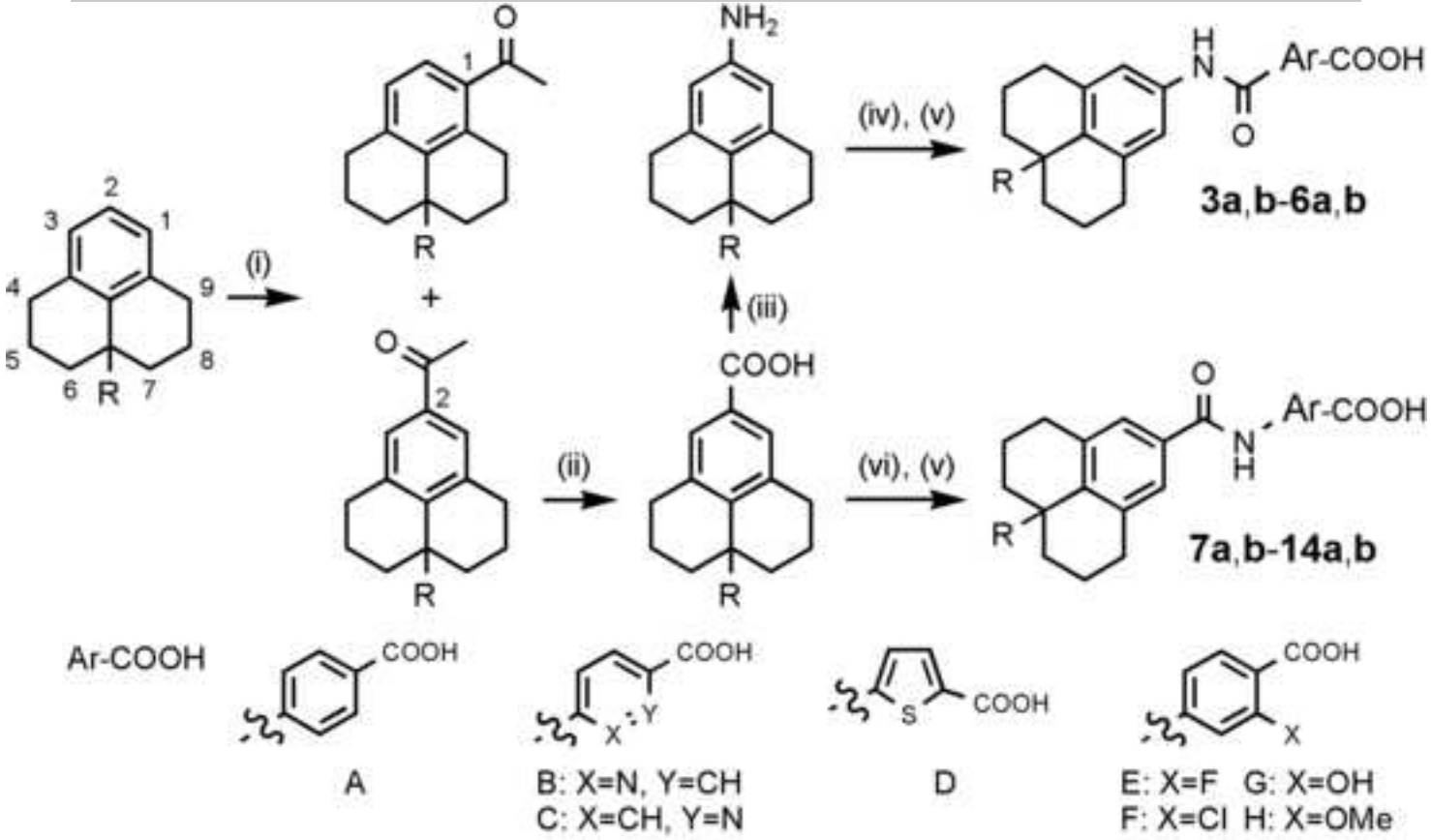


Figure 2. Synthesis of metacyclophane derivatives (**1**, **2**).

Fig3.tif

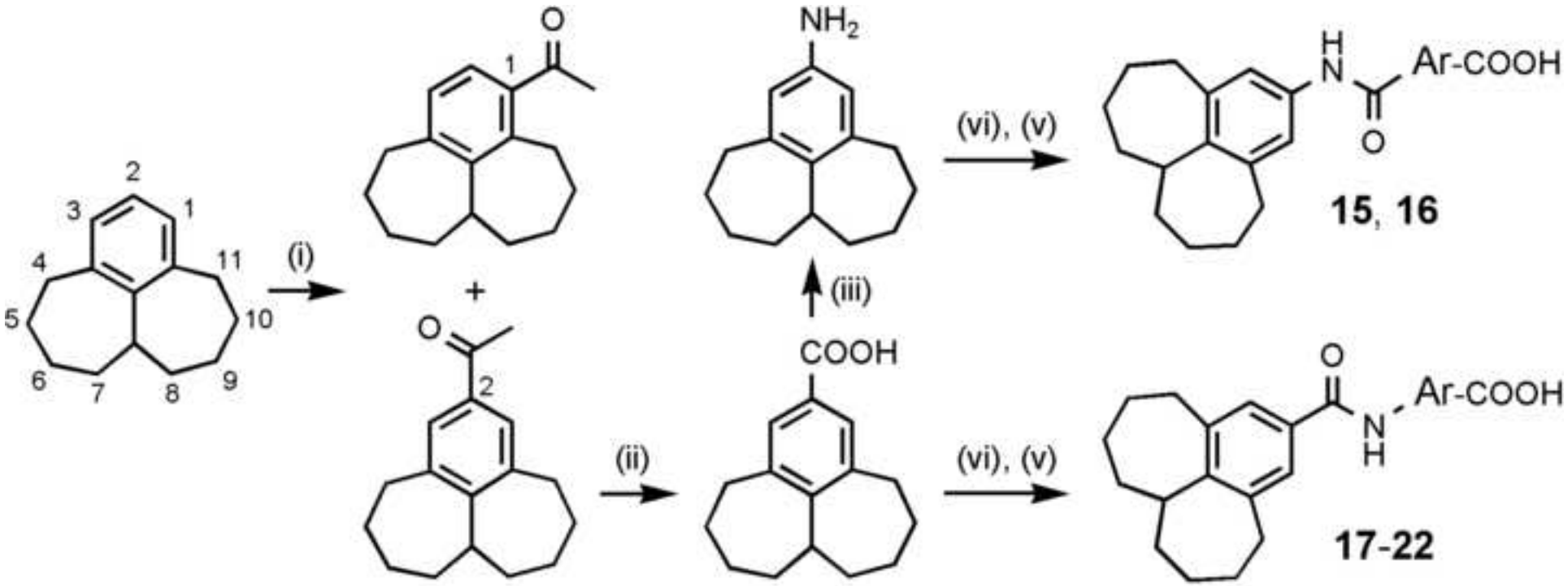


R=H		R=Me	
Compound	Ar-COOH	Compound	Ar-COOH
3a	A	**3b**	A
4a	B	**4b**	B
5a	C	**5b**	C
6a	D	**6b**	D

R=H		R=Me	
Compound	Ar-COOH	Compound	Ar-COOH
7a	A	**7b**	A
8a	B	**8b**	B
9a	C	**9b**	C
10a	D	**10b**	D
11a	E	**11b**	E
12a	F	**12b**	F
13a	G	**13b**	G
14a	H	**14b**	H

Figure 3. Synthesis of phenalene derivatives (**3a**,**b-14a**,**b**).

Fig4.tif



Compound	Ar-COOH	Compound	Ar-COOH
15	A	**17**	A
16	B	**18**	B
		19	E
		20	F
		21	G
		22	H

Figure 4. Synthesis of benzo[*ef*]heptalene derivatives (**15-22**).

[a] Ar-COOHs are the same as shown in Figure 3.

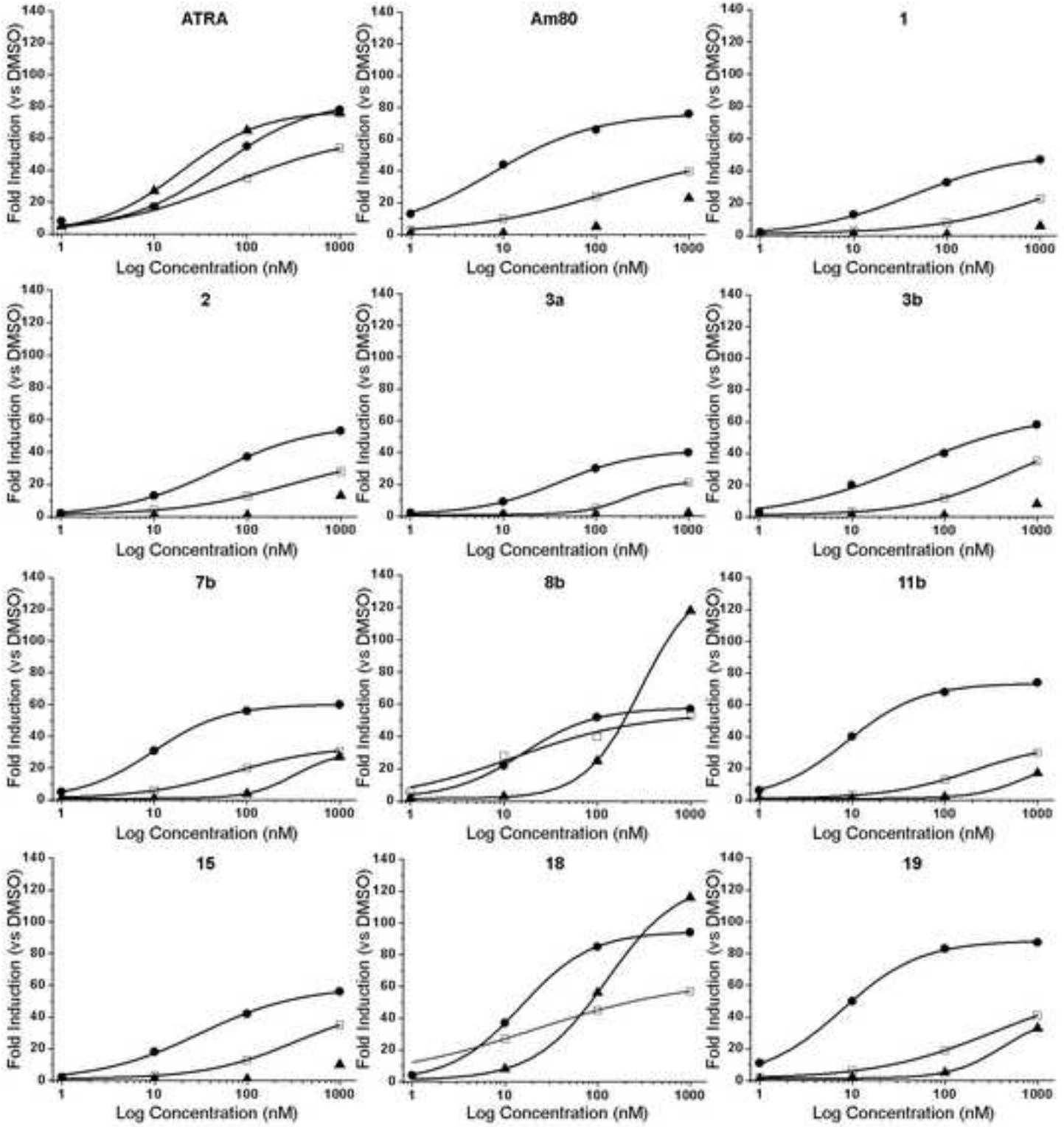


Figure 5. Transcriptional activities measured by means of reporter gene assay in Cos-1 cells with GAL4-RARα (●), GAL4-RARβ (□), and GAL4-RARγ (▲). Data are mean values of fold increase (vs DMSO) measured by two or more experiments. The horizontal scale is the molar concentration of added compound.

Table 1. Transcriptional activation assay data.[a]

Compound[b]	Ar-COOH[c]	Relative fold induction		
		RAR	RAR	RAR
ATRA		55	35	65
Am80		66	24	5
1	A	33	8	1
2	A	37	13	1
3a	A	30	5	1
3b	A	40	12	1
4a	B	34	16	7
4b	B	34[d]	26	2[d]
5a	C	1	N. D.[e]	1[d]
5b	C	2	N. D.[e]	1[d]
6a	D	1[d]	N. D.[e]	1[d]
6b	D	3[d]	N. D.[e]	1[d]
7a	A	51	18	3
7b	A	56	20	4
8a	B	50	40	20
8b	B	52	40	25
9a	C	24	N. D.[e]	1[d]
9b	C	23	N. D.[e]	1[d]
10a	D	20[d]	N. D.[e]	1[d]
10b	D	22	1[d]	1
11a	E	51	7[d]	2
11b	E	68	13	2
12a	F	45	6	1
12b	F	59[d]	8[d]	1[e]
13a	G	38	6	2
13b	G	51[d]	7[e]	1[d]
14a	H	28[d]	2[d]	1[d]
14b	H	38[d]	3[d]	1[d]
15	A	42	13	1
16	B	40[d]	17	2[d]
17	A	62	27	10
18	B	85	45	56
19	E	83	19	5
20	F	63	13	2
21	G	63	11	2
22	H	43[d]	1[d]	2[d]

[a] Data are mean values of fold induction (vs DMSO) at 100 nM measured by two or more experiments.

[b] Metacyclophane derivatives (**1**, **2**); Phenalene derivatives (**3a**,**b**-**14a**,**b**); Benzo[*ef*]heptalene derivatives (**15**-**22**).

[c] Ar-COOHs are the same as shown in Figure 3.

[d] One experiment.

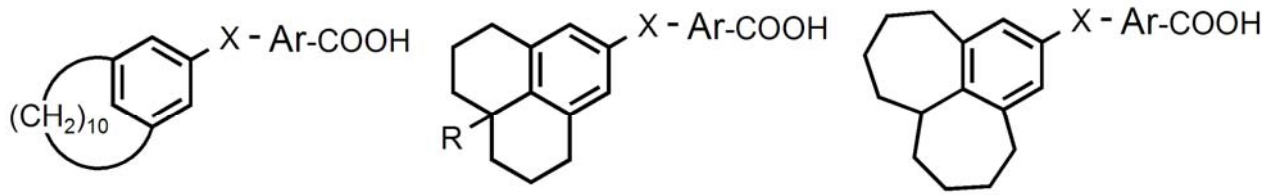
[e] Not determined.

Table 2. HL-60 cell differentiation-inducing activity.[a]

Compound	NBT reduction (%)	
	10 nM	100 nM
ATRA	46	71
Am80	60	68
1	16	37
2	30	38
3a	7	18
3b	12	N. D.[b]
4a	2	5
4b	5	16
7a	27	38
7b	36	N. D.[b]
8a	54	59
8b	41	61
11a	49	60
11b	18	29
12a	16	37
12b	19	21
13a	24	42
13b	17	32
14a	7	10
14b	5	17
15	13	53
17	34	40
19	28	44
20	18	35
21	29	41

[a] Data are mean values of the percentage of differentiated cells evaluated from NBT reduction assay at 10 nM and 100 nM.

[b] Not determined.



X: -NHCO- or -CONH-