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9-*cis*-Retinoic acid analogues with bulky hydrophobic rings: new RXR-selective agonists

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Abstract—Stille cross-coupling of aryltriflates **10** and dienylstannane **11**, oxidation and Horner–Wadsworth–Emmons reaction afforded stereoselectively retinoates **15**. Saponification provided the carboxylic acids **8a** and **8b**, retinoids that incorporate a bulky hydrophobic ring while preserving the 9-*cis*-geometry of the parent system. In contrast to the pan-RAR/RXR agonistic profile of the lower homologue of **8a**, compound **7** (LG100567), retinoids **8** showed selective binding and transactivation of RXR, devoid of significant RAR activation. In PLB985 leukemia cells that require RXR agonists for differentiation compounds **8** induced maturation in the presence of the RAR-selective pan-agonist TTNPB; this effect was blocked by an RXR-selective antagonist.

1. Introduction

The hormone action of retinoic acid **1** and its 9-*cis*-isomer **2** in multicellular organisms is expressed through the activation of genetic networks governed by the binding of the ligand–cognate receptor complexes[1] to promoter regions of DNA. The retinoid families of nuclear proteins (RARs-retinoic acid receptors- and RXRs-retinoid X receptors-, both comprising isotypes α,β,γ) act as ligand-inducible transcription factors, regulating physiological processes essential for embryonic development as well as for cell growth, differentiation, and death. *trans*-Retinoic acid (**1**) is the natural ligand for RAR, and its isomer 9-*cis*-retinoic acid (**2**) has high affinity for, and activates, both RAR and RXR. It is believed that RARs and RXRs act mainly as RAR–RXR heterodimers, the functional units that also affect other cell signaling pathways, albeit RXR can also function autonomously.[2]

The ability of retinoids[3,4] to regulate cell growth and induce differentiation throughout life has been demonstrated by the response of a large number of cancer cell lines in culture to retinoids and the therapeutic success of *trans*-retinoic acid **1** in the treatment of acute promielocytic leukemia (APL).[5]

Concerns about secondary effects of retinoid therapy (retinoic acid syndrome, teratogenicity) has stimulated the search for novel retinoids incorporating structural modifications at the hydrophobic ring and/or the side chain, and these modifications oftentimes contribute to improve chemical stability.[4] Commonly the trimethylcyclohexenyl ring is replaced by the lipophilic bioisostere 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-naphthalene. In addition, the presence of aryl rings as substitutes of terminal dienes imparts greater stability in some of the most active analogues, thus increasing the chances for therapeutical application. LGD1069 (bexarotene, Targretin®) **3** is the first synthetic RXR-selective agonist (rexinoid) approved for the treatment of cutaneous T-cell lymphoma.[6]

Another common substitute of the hydrophobic ring is a 3,5-di-*tert*-butylbenzene, and this structure is present in some selected antiproliferative retinoids (Fig. 1).[7] Amide **4** and chalcone **5** are selective RAR ligands, and show induction of differentiation on the HL-60 promyelocytic cell line.

Keywords: 9-*cis*-Retinoic acid; RXR agonist; Nuclear receptor.
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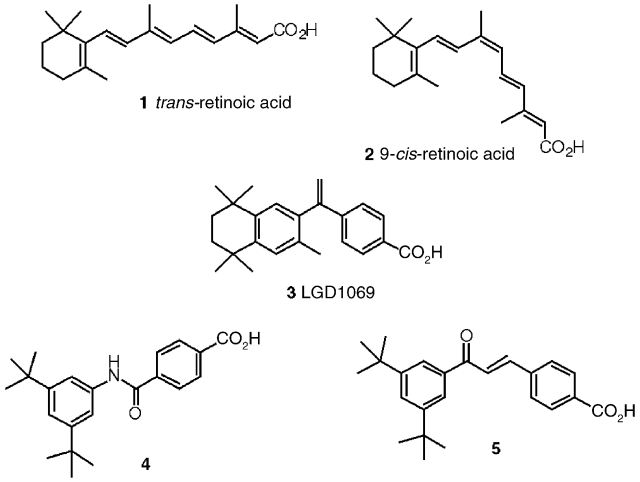


Figure 1.

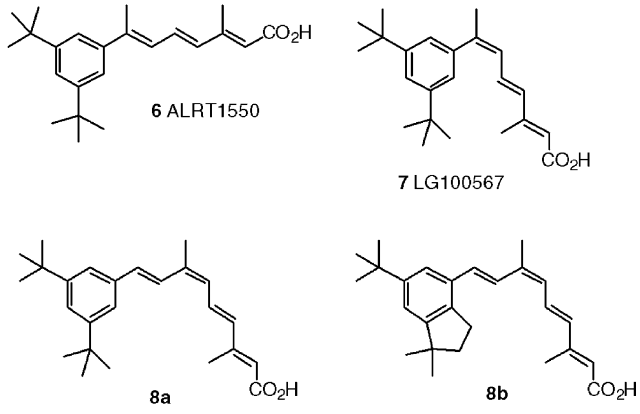


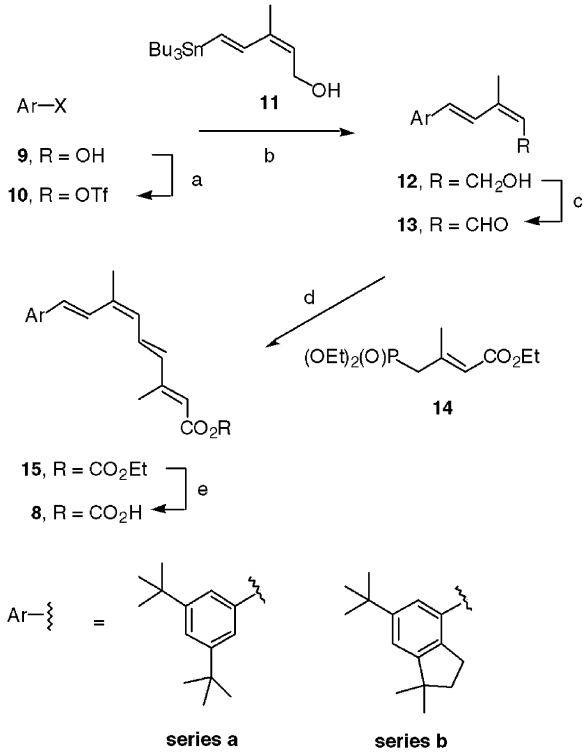
Figure 2.

2. Design and synthesis of novel 9-*cis*-retinoic acid analogues with bulky hydrophobic rings

Within the group of 3,5-di-*tert*-butylbenzene-based retinoids, ALRT1550 (**6**) is receiving increased attention, because it is a highly potent and selective activator of the RARs in binding and cotransfection assays, and inhibits proliferation of human cervical carcinoma cells.[8] Although less active than the *E* isomer in inhibiting the proliferation of ME-180 cervical tumor, the *Z* isomer **7** (LG100567), a pan-RAR/RXR agonist, showed a ca. 25 times greater potency than 9-*cis*-retinoic acid **2** (cf. **6** is 300 times more potent than *trans*-retinoic acid **1**). The pan-RAR/RXR agonistic profile of retinoid **7** is likely due to its flexibility,[9] that allows the conformational adaptation to the topologically distinct binding pockets of both RARs[9] and RXRs,[10] in a manner similar to 9-*cis*-retinoic acid **2**. We considered that this conformational adaptability could be challenged by the incorporation of an additional ethenyl group to the side chain of **7** (compound **8a**). Binding of **8a** to the more elongated I-shaped binding pocket of RAR9 would be disfavored, whereas the ability to fit into the L-shaped LBP of RXR10 would be preserved. In addition, analogue **8b**, having a 6-*tert*-butyl-1,1-dimethylindanyl group as a bioisostere of the retinoid hydrophobic cyclohexenyl ring,[11] was anticipated to exhibit RXR selectivity, since the indanyl ring forces the bending of the side-chain relative to the ring, a structural feature observed in the crystal structure of 9-*cis*-retinoic acid **2** bound to RXR[10] (Fig. 2).

We report herein stereoselective approaches to retinoids **8** using metal-catalyzed cross-coupling reactions[12] and confirm the structure-based predictions by transactivation studies and rexinoid-dependent leukemia cell differentiation assays.

We envisioned a stepwise construction tactic to retinoid polyene side-chains[12] starting with the Stille coupling reaction[13] of geometrically homogeneous dienylstannane **11**[12h] and the triflate[14] derived from 3,5-di-*tert*-butylphenol **10a** or 6-*tert*-butyl-1,1-dimethylindanol **10b**,[15] and completing the polyene chain with the Horner–Wadsworth–Emmons condensation.[16] The pseudohalides **10** were obtained from **9** in virtually quantitative yield by treatment with *N*-phenyltriflimide and Et_3N (Scheme 1).[14] The coupling of aryltriflates to stannanes is considered to require LiCl in order to stabilize the oxidative addition products and avoid the formation of catalytically inactive Pd species.[17] In the presence of LiCl, coupling of **10** and known[12f] **11** took place at 60 °C in NMP under the mediation of $Pd_2(dba)_3/AsPh_3$ to afford dienols **12** in 56% and 47%



Scheme 1. Reagents and reaction conditions: (a) *N*-phenyltriflimide, Et_3N, CH_2Cl_2, 48 h (**10a**: 98%; **10b**: 98%); (b) **11**, $Pd_2(dba)_3$ (cat), $AsPh_3$, LiCl, NMP, 60 °C (**12a**: 56%; **12b**: 47%); (c) TPAP (cat)/NMO, CH_2Cl_2, 0 °C (**13a**: 71%; **13b**: 60%); (d) i. *n*-BuLi, **14**, DMPU, 0 °C; ii. aldehyde **13**, −78 °C (**15a**: 86%; **15b**: 74%); (e) 5 M KOH, MeOH, 80 °C, 2 h (**8a**: 93%; **8b**: 81%).

yield, respectively. Addition of the alcohol **12** to NMO and 4 Å MS in CH_2Cl_2, followed by TPAP at 0 °C, and then stirring at ambient temperature delivered the aldehydes **13** in 71% and 60% yield, respectively (Scheme 1). The chain was then extended by treatment of these unstable aldehydes with the anion (*n*-BuLi, THF, DMPU, −78 °C) of ethyl (*E*)-3-(ethoxycarbonyl)-2-methylprop-2-enyl phosphonate **14**, as previously reported,[16] affording esters **15** (86% for **15a**, 74% for **15b**). Finally, basic hydrolysis provided the corresponding acids **8a** (93% yield) and **8b** (81% yield).[18]

3. Modeling reveals structural basis of RXR selectivity

Crystal structure-based modeling provided initial evidence that compounds **8** could have gained selectivity compared with the parent 9-*cis*-retinoic acid. While the structural details of 9-*cis*-retinoic acid binding in the ligand binding pockets are well understood from the corresponding crystal structures of the 9-*cis* RA-RARγ ligand binding domain (LBD) complex (PDB ID, 3LBD) and the 9-*cis*-RXR LBD complex (PDB ID, 1FM6), modeling reveals that **8b** cannot be equally accommodated in the RARγ binding pocket (Fig. 3, top panel; note that one of the two methyl groups attached to the indanyl ring extends outside the cavity, thus incurring in steric clashes). Similar results were obtained for **8a** (data not shown). In contrast, due to the alternate shape of the RXRα ligand binding pocket **8b** fits well inside (Fig. 3, middle and bottom panels show two views, the middle panel corresponds to the view of RARγ). Thus, modeling suggests that compounds **8** may have acquired selective RXR binding activity compared to 9-*cis*-retinoic acid.

4. Biological activity of novel rexinoids

To differentiate RAR and RXR-selective ligand-mediated transactivation we used transient assays with chimeric Gal4-receptor fusion proteins and the cognate reporter gene carrying five Gal4 response elements in front of the β-globin promoter used to drive the luciferase reporter (schematically illustrated in Fig. 4, top). No significant transactivation was seen with the rexinoids **8**, while the pan-RAR/RXR agonist 9-*cis*-retinoic acid parent molecule displayed a strong transcriptional activity (Fig. 4, top panel). In striking contrast, using the equivalent RXR reporter system both **8a** and **8b** activated transcription through RXR with an activity about 10 times lower than 9-*cis*-retinoic acid (Fig. 4, bottom panel). Note, however, that RXR selectivity is not absolute, as at high concentrations (>1 μM) a weak RAR agonist activity becomes apparent (Fig. 4, top panel).

Competition assays done with all three RARs confirmed that compounds **8a** and **8b** do not substantially interact with RARα and RARβ, while there is a weak interaction with RARγ at high concentrations (Fig. 5). In all cases both **8a** and **8b** act as very weak antagonists with apparent binding affinities about 1000 times lower than

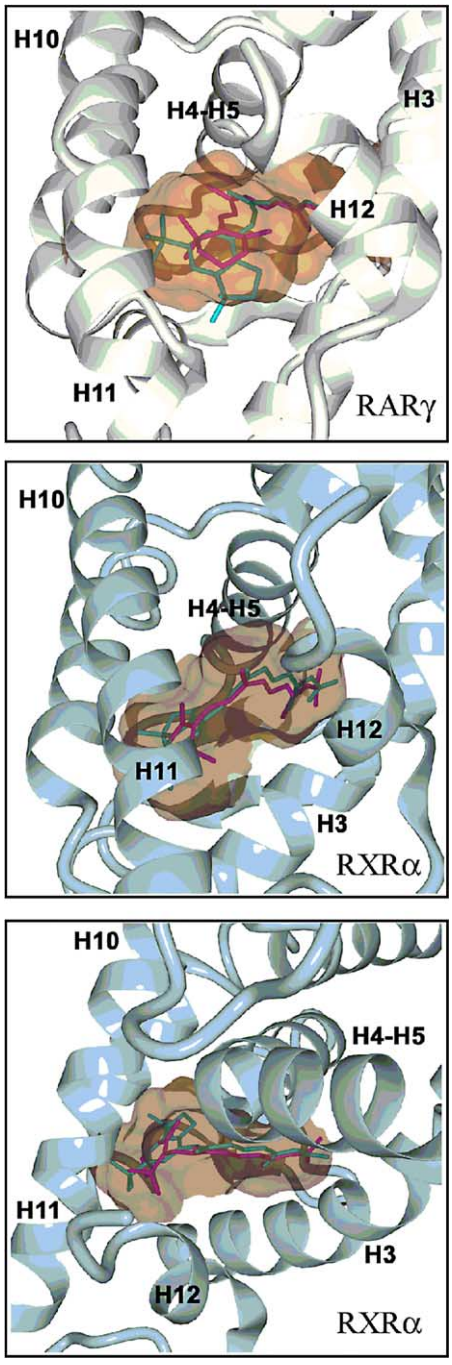


Figure 3. Modeling of **8b** in the ligand binding pockets of RAR γ and RXRα. The ligands **8** (green), used for docking experiments including all hydrogen atoms, were built with the QUANTA package using the force field from CHARMM.[19] The protein structures used for the docking experiments were the X-ray structures of either the 9-*cis* RA (red)-RARγ LBD complex (PDB ID, 3LBD) or the 9-*cis* RA (red)-RXR LBD complex (PDB ID, 1FM6). The cavity volume of the ligand binding pockets was calculated in the program O8[20] with VOIDOO[21] and used as a guide during the docking process. One of the cavities calculated by VOIDOO gives the volume accessible to the center of the probe-sphere (1.4 Å radius) that is in contact with the protein van der Waals surface. In this representation of the cavity most of the apolar heavy atoms should lie inside the calculated volume. The ligands were then manually fitted in the ligand binding pocket using the 9-*cis* RA, the cavity volume and the favored conformations as landmarks. The force field from the Quanta/CHARMM package was used for the energy minimization.

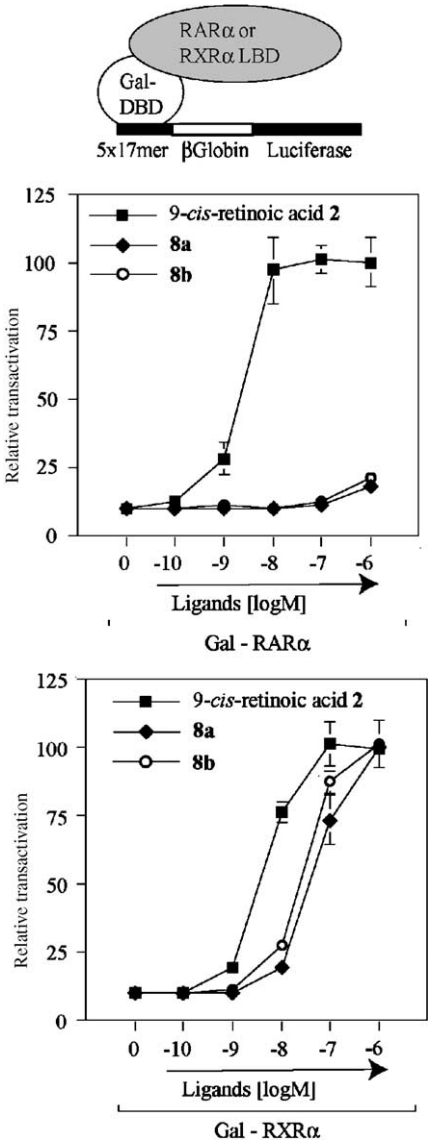


Figure 4. Transient transactivation studies to assess RAR or RXR activity of **8a** and **8b**. Transiently transfected COS cells expressing chimeric proteins containing the GAL4 DNA-binding domain (Gal-DBD) fused to the ligand binding domain (LBD) of either RARα (Gal-RARα, top panel) or RXRα (Gal-RXRα bottom panel), and a luciferase gene driven by a pentamer of the Gal4 recognition sequence ('17m') in front of the β-globin promoter, as illustrated at the top. This reporter system is insensitive to endogenous receptors, which cannot recognize the GAL4 binding site. Cells were incubated with increasing concentrations of 9-*cis*-retinoic acid **2** (closed squares), **8a** (closed diamonds) or **8b** (open circles).

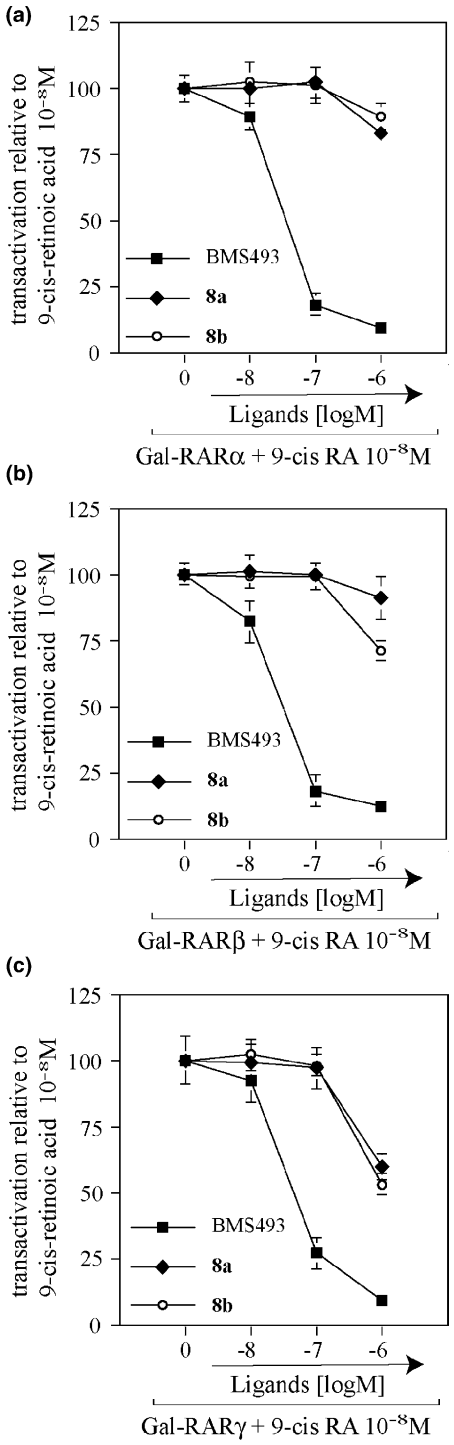


Figure 5. Transient transactivation studies to assess RAR antagonist potential of **8a** and **8b**. Transiently transfected COS cells expressing chimeric proteins containing the GAL4 DNA-binding domain (Gal-DBD) fused to the ligand binding domain (LBD) of either RARα (Gal-RARα, (a) panel) or RARβ (Gal-RARβ, (b) panel) or RARγ (Gal-RARγ, (c) panel), and the luciferase gene driven by the 17m sequence in front of the β-globin promoter, as illustrated in Figure 4. Cells were incubated with 10nM 9-*cis*-retinoic **2** and increasing concentrations of the RAR pan-antagonist BMS493 (closed squares), **8a** (closed diamonds) or **8b** (open circles).

that of the RAR agonist 9-*cis*-retinoic. As control the strong RAR pan-antagonist BMS493 is shown for comparison.

To investigate if the rexinoid activity of **8a** and **8b** would suffice to exert a biological activity in intact cells we used leukemic PLB985 cells that display a strong synergy between RAR and RXR agonists for induction of differentiation along the granulocyte lineage (our unpublished observation). We chose conditions (four days exposure to the indicated ligand concentrations) under which neither the RAR pan-agonist TTNPB nor compounds **8a** or **8b** would induce significant differentiation, as deter-

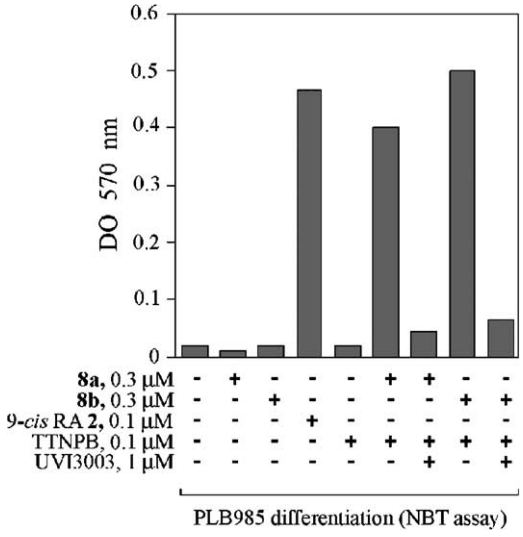


Figure 6. Differentiation-inducing activity of rexinoids **8**. PLB985 cells were exposed to the indicated retinoids and/or rexinoids and differentiation was assessed at day 4 by NBT staining. Shown is a representative of three independent experiments.

mined by NBT staining (see Fig. 6). Notably, under those conditions the combination of TTNPB and either **8a** or **8b** strongly induced cell differentiation revealing a highly synergistic action of the two types of ligands (Fig. 6). As expected, 9-*cis*-retinoic acid, which is agonist for both RARs and RXRs, is on its own competent to induce cell maturation already at a three-times lower concentration than that used for compounds **8** when tested alone. Importantly, UVI3003 a potent selective rexinoid antagonist (our unpublished observations) efficiently blocked the synergistic induction of differentiation by TTNPB and rexinoids **8**. As could be expected from their residual RAR agonist activity, compounds **8** when used at concentrations at or above 1 μM did induce PLB985 cell differentiation, indicating that the separation of RAR and RXR activities is not absolute.

Taken together we have demonstrated here how to modify the pan-RAR/RXR agonist selectivity of 9-*cis*-retinoic acid toward selective rexinoid activity by ligand design, provide a structural rationale for this selectivity and verify it in biological readouts.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2004.08.072.

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18. The purity of all new compounds was judged by a combination of HPLC and ^{1}H and ^{13}C NMR analysis before mass spectra were recorded. Data for selected compounds: **8a**: yellow solid (mp 196–198 °C, Et_2O/hexane). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.37 (s, 18H), 2.11 (s, 3H), 2.41 (s, 3H), 5.81 (s, 1H), 6.18 (d, J = 11.4 Hz, 1H), 6.32 (d, J = 15.0 Hz, 1H), 6.77 (d, J = 15.8 Hz, 1H), 7.2–7.4 (m, 5H). ^{13}C NMR (100.62 MHz, $CDCl_3$): δ 13.9 (q), 21.1 (q), 31.4 (q, 6×), 34.8 (s, 2×), 118.3 (d), 121.0 (d, 2×), 122.5 (d), 124.4 (d), 129.3 (d), 130.2 (d), 132.2 (d), 134.9 (d), 136.5 (s), 138.5 (s), 151.1 (s, 2×), 154.8 (s), 172.2 (s). FTIR (NaCl): υ 3600–3100 (br, OH), 2964 (s, C–H), 1674 (s, C=O), 1585 (s), 1363 (w), 1256 (m), 1183 (m), 960 (m) cm^{-1}. UV (MeOH): λ_{max} 326 nm. MS: *m/z* (%) 366 (M^+, 90), 351 (11), 321 (32), 309 (34), 265 (16), 257 (21), 219 (24), 203 (25), 163 (34), 149 (39), 97 (39), 86 (100), 69 (72). HRMS: calcd for $C_{25}H_{34}O_2$: 366.2559; found: 366.2563. Anal. Calcd: C, 81.97; H, 9.38. Found: C, 81.88; H, 9.40. For **8b**: yellow solid (mp 203–204 °C, Et_2O/hexane). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.29 (s, 6H), 1.38 (s, 9H), 1.98 (t, J = 7.2 Hz, 2H), 2.12 (s, 3H), 2.40 (s, 3H), 2.97 (t, J = 7.2 Hz, 2H), 5.85 (s, 1H), 6.19 (d, J = 11.5 Hz, 1H), 6.32 (d, J = 15.0 Hz, 1H), 6.81 (d, J = 15.9 Hz, 1H), 7.13 (d, J = 1.5 Hz, 1H), 7.26 (dd, J = 15.0, 11.5 Hz, 1H), 7.34 (d, J = 15.9 Hz, 1H), 7.38 (d, J = 1.5 Hz, 1H) ppm. ^{13}C NMR (100.62 MHz, $CDCl_3$): δ 21.0 (q), 23.8 (q), 28.7 (q, 2×), 28.8 (t), 31.6 (q, 3×), 34.8 (s), 41.3 (t), 44.0 (s), 117.6 (d), 118.8 (d), 120.9 (d), 125.9 (d), 129.3 (d), 130.1 (d), 130.4 (d), 132.9 (s), 134.8 (d), 138.3 (s), 138.8 (s), 150.2 (s), 153.2 (s), 155.0 (s), 170.5 (s) ppm. FTIR (NaCl): υ 3600–2900 (br, OH), 2956 (s, C–H), 1677 (s, C=O), 1578 (s), 1258 (m) cm^{-1}. MS: *m/z* (%) 379 (M^++1, 30), 378 (M^+, 100), 321 (37), 261 (27), 215 (36), 214 (38), 187 (28), 159 (23). HRMS: calcd for $C_{26}H_{34}O_2$, 378.2559; found, 378.2549. Anal. Calcd C, 82.55; H, 8.98. Found: C, 82.44; H, 9.01.
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