Identification of Gibberellic Acid Derivatives that Deregulate Cholesterol Metabolism in Prostate Cancer Cells

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ABSTRACT: The naturally occurring pentacyclic diterpenoid, gibberellic acid (**1**) was used in the generation of a drug-like amide library using parallel-solution-phase synthesis. Prior to the synthesis, a virtual library was generated and prioritized based on drug-like physicochemical parameters such as log P, hydrogen bond donor/acceptor counts, and molecular weight. The structures of the synthesized analogues (**2**–**13**) were elucidated following analysis of the NMR, MS, UV and IR data. Compound **12** afforded crystalline material, and its structure was confirmed by X-ray crystallographic analysis. All compounds were evaluated in vitro for cytotoxicity and deregulation of lipid metabolism in LNCaP prostate cancer cells. While no cytotoxic activity was identified at the concentrations tested, synthesized analogues **3**, **5**, **7**, **10**, and **11** substantially reduced cellular uptake of free cholesterol in prostate cancer cells, suggesting a novel role of gibberellic acid derivatives in deregulating cholesterol metabolism.

Natural products (NPs) have been a major source of chemical diversity and biological activity, and a driving force for the pharmaceutical industry over the past century.[1,2] Their contributions to drug discovery have been significant, since they have provided therapeutic agents for the treatment of cancer, microbial infections, hypercholesteremia, and immune suppression, to name a few.[3,4] In fact, numerous drugs on the market are of NP origin, either as unmodified or modified NPs.[5]

From computational studies, NPs have been shown to occupy complementary areas of chemical space when compared with their synthesized counterparts, which indicates that NPs can be used to increase and diversify the chemistry of screening libraries.[6-10] It is reasoned that by increasing the chemical complexity and diversity of compound screening collections, this will translate into higher hit rates during HTS campaigns that will subsequently give researchers more opportunities for progressing a hit compound into a lead or drug. [6-11]

Various synthetic strategies that focus on NP drug discovery have been used to generate new drugs or leads. Examples include, the *de novo* synthesis of a NP followed by analogue generation, the use of simplified core motifs found in NPs for library generation, diverted total synthesis, and diversity-orientated synthesis, all of which explore chemical scaffolds from Nature that might prove to be useful from a pharmaceutical point of view.[9,10] While chemists have used motifs derived from natural sources as starting points or scaffolds for the synthesis of structurally complex bioactive compounds, this semi-synthetic approach is gaining popularity as the target scaffolds become easier to obtain. Acquiring NP scaffolds in adequate quantities from commercial suppliers or from NP compound and biota collections, such as Griffith University's NatureBank,[12-14] is becoming more commonplace. The semi-synthetic strategy has the advantage of saving time and money, since it circumvents the *de novo* synthetic strategy for scaffold production.[11] Based on this premise, and our belief that drug discovery will profit from this approach, our current research focuses on the design

and synthesis of drug discovery libraries (10–20 analogues) that use unique and/or complex NP scaffolds.[12-17] Other examples of the use of NP scaffolds for the generation of screening libraries and/or lead optimization have also been reported. For example, Dias *et al.* reported the synthesis of a six-membered plant-derived guttiferone–A library,[18] while, Li *et al.* used the plant-based template, oridonin to generate a 14–membered *O*-diterpenoid library.[19]

To further contribute to knowledge in this area of research, gibberellic acid (GA_3, **1**) was chosen as NP scaffold for medicinal chemistry studies and library generation. Scaffold **1** is a hormone found in plants and fungi, and was first identified as a metabolic by-product of the plant pathogen *Gibberella fujikuroi* in 1935.[20] GA_3 is an attractive NP scaffold for the synthesis of screening libraries due to its high-degree of structural complexity [i.e. multiple stereogenic centres (n = 11)], potential chemical handles (i.e. the hydroxycarbonyl and hydroxy groups) that are available for synthetic modifications, and its commercial availability.

It is highly important to give the design of the NP screening library due consideration before the synthesis is undertaken, thus, adherence to important physicochemical parameters itemized in Lipinski's "Rule of Five" for drug-like compounds, was of utmost importance in order to hasten the progression of any potential hit.

We herein report the design and parallel solution-phase synthesis of compounds **2**–**13** based on the NP scaffold, gibberellic acid (**1**), together with their cytotoxicity and deregulation of lipid metabolism in human prostate cancer cell line (LNCaP).

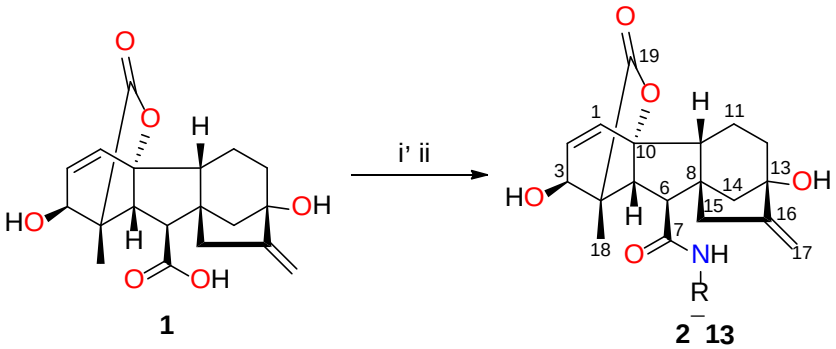
RESULTS AND DISCUSSION

The amide bond is widely prevalent in both NPs and synthesized compounds. Its importance has been described in the pharmaceutical industry, being present in ~25% of available drugs, with amidation reactions being one of the most commonly used

transformations in medicinal chemistry.[21] The commercially available GA_3 (**1**) was modified at the hydroxycarbonyl moiety to generate a library of amides.

Prior to commencing the modification on scaffold **1**, 26 commercially available amines were initially selected, and a virtual analogue library generated (VA1–VA26), which was subsequently analyzed using ChemDraw Ultra.[22] Physicochemical parameters relating to Lipinski's "Rule of Five" for drug-like compounds (HBD ≤ 5, HBA ≤ 10, MW ≤ 500 and Log P ≤ 5)[23] were calculated *in silico* on VA1–VA26 (Figure S71, Supporting information), with the intention of selecting the most suitable reaction partners for subsequent synthesis. On the basis of the *in silico* data, VA1–VA13 were prioritized for synthesis, because they all had minimal or no "Rule of Five" violations.

Narrowing down to an appropriate coupling agent to use for amidation reaction is a significant challenge, due to the vast numbers of coupling reagents that have been reported.[24,25] Adam *et al*. reported the amidation reactions between the NP scaffold (**1**) and various amines using the coupling reagent *N*,*N*′-dicyclohexylcarbodiimide (DCC), with yields ranging from 19–77%.[26] However, the disadvantage of this coupling reagent is that it is not user-friendly as it cannot be partitioned into the H_2O layer during the reaction work-up. Acyl chlorides are one of the simplest activated carboxylic acid derivatives to form; amide coupling in this context is usually a two-step process, involving first the conversion of the acid into the acyl halide, followed by its coupling with an amine.[24] Thus, we opted to use the coupling reagent, oxalyl chloride $(COCl)_2$ (Scheme 1).



Scheme 1. Reagents and Conditions. (i) Anhydrous CH_2Cl_2, Pyridine, $(COCl)_2$, DMF, rt (ii) Amine, Pyridine, CH_2Cl_2, 20 min, 0 °C.

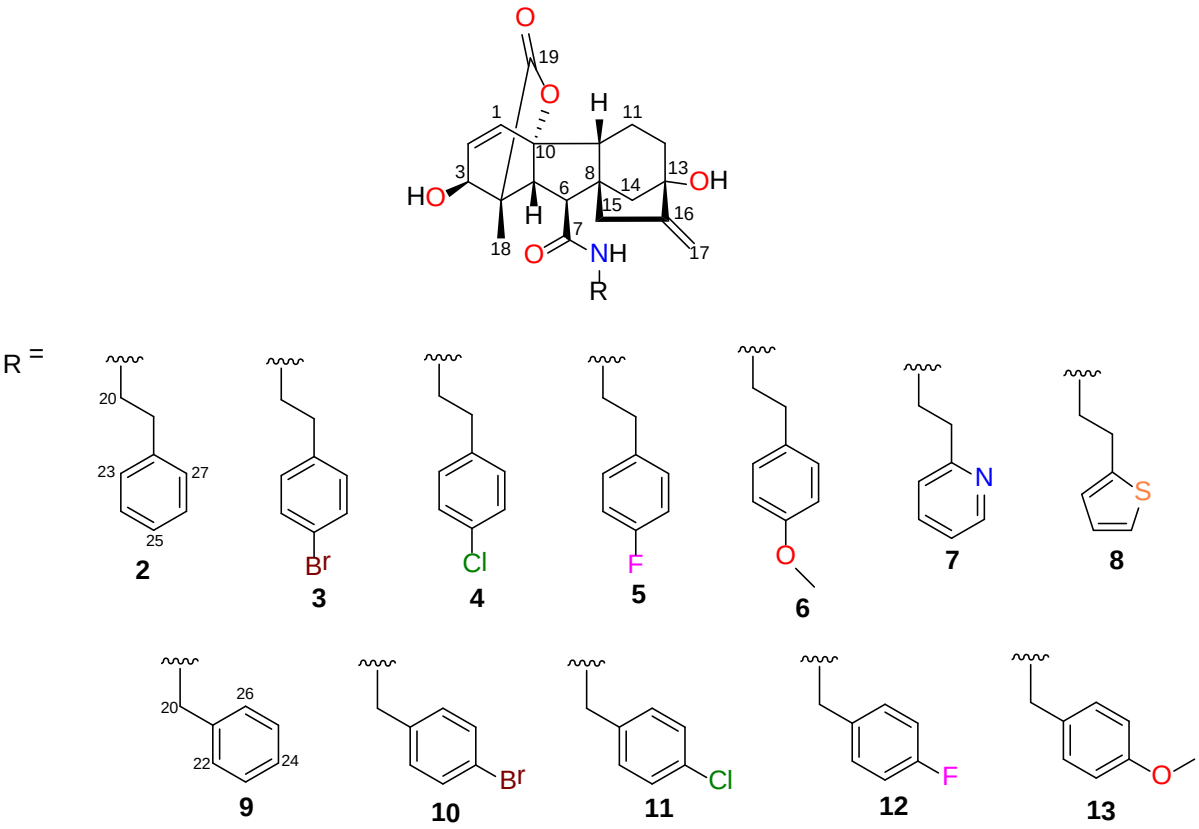


Figure 1. Chemical structures of the amide library **2**–**13**

Treatment of NP scaffold **1** with 12 primary amines afforded the secondary amides (**2**–**13**), with yields ranging from 4 to 49% (Figure 1). All compounds were analyzed for purity by ^{1}H NMR spectroscopy and shown to be > 95% pure. Excess amine was removed under N_2, after which C_{18} bonded silica or phenyl bonded silica was used for pre-adsorption work before HPLC separations (see Experimental Section).

The synthesis of compounds **2, 6** and **9** have previously been reported,[26] but these three compounds were only partially characterized using ^{1}H NMR and LR-MS data. We report the first synthesis of all other amide library members, and the full characterization of

all compounds (**2**–**13**) by 1D (^{1}H, ^{13}C) and 2D NMR (COSY, HMBC, HSQC and ROESY) spectroscopy and (+)-HRESIMS data analysis.

For example, the ^{1}H NMR spectrum of compound **2** in DMSO-d_6 showed a signal at δ_H 8.13, which corresponded to the NH moiety coupling to the methylene protons at C-20. Analysis of the COSY spectrum of compound **2** identified five spin systems. The first spin system contained the methine protons [δ_H 6.32, d, (H-1) and 5.77, dd, (H-2)] and a proton at 3.83, d, (H-3). The second spin system was located between δ_H 3.10, d, (H-5) and 2.50, d, (H-6). A further spin system was identified between δ_H 1.79 (H-9), 1.62, (H-11a), 1.78, (H-11b), and, 1.58, (H-12a) and 1.87, (H-12b). The two methylene protons [δ_H 3.28, m, (H-20a), 3.36, m, (H-20b)] and 2.70, m, (H-21) comprised another spin system, while the last spin system was located in the aromatic part of the molecule. The intactness of the structure was also supported by the key HMBC correlations, for example Me-18 (δ_H 1.01) showed HMBC correlations to C-3 (δ_C 68.6), C-4 (53.1) and C-19 (179.2), H-11 (δ_H 1.62, 1.78) exhibited HMBC correlations to C-8 (δ_C 49.8), C-10 (90.9), and C-13 (76.8), while, H-17 (δ_H 4.78, 5.07) displayed HMBC correlations to C-13 (δ_C 76.8), C-15 (43.3), and C-16 (158.0). Further confirmation of the amidation position was supported by the HMBC correlations from the NH (δ_H 8.13) to C-7 (δ_C 170.6) and C-20 (40.2), in conjunction with the ROESY correlations (Figure 2). The relative configuration of compound **2** was established to be identical to the NP scaffold following the analysis of the ROESY and ^{1}H-^{1}H coupling constant data, and the magnitude of the ^{1}H NMR chemical shifts. For example, key ROESY correlations for compound **2** are shown in Figure 2. The observed ROESY correlations from H_3-18 to H-3 is explained by the fact that the Me-18 and H-3 assume quasi-equatorial positions when in solution.[27] This was further supported by the X-ray diffraction studies of a similar analogue, compound **12** (Figure 3) which shows that the OH-3 and the C-7 amide moiety are β-oriented. These data enabled the chemical structure of **2** to be unequivocally assigned.

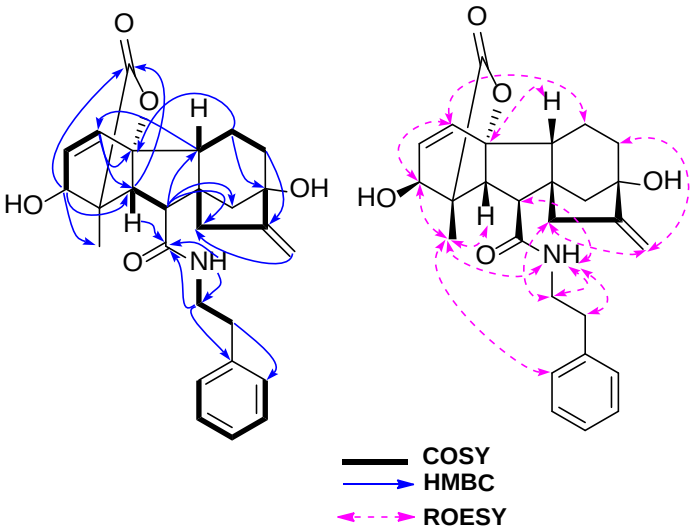


Figure 2. COSY, key HMBC (⟶), and ROESY (⇠⇢) correlations for **2**

Slow evaporation of a solution of compound **12** in MeOH resulted in crystals suitable for X-ray diffraction. Therefore, the structure of compound **12** was also confirmed by X-ray crystallography (Figure 3 and Figure S75, Supporting information).

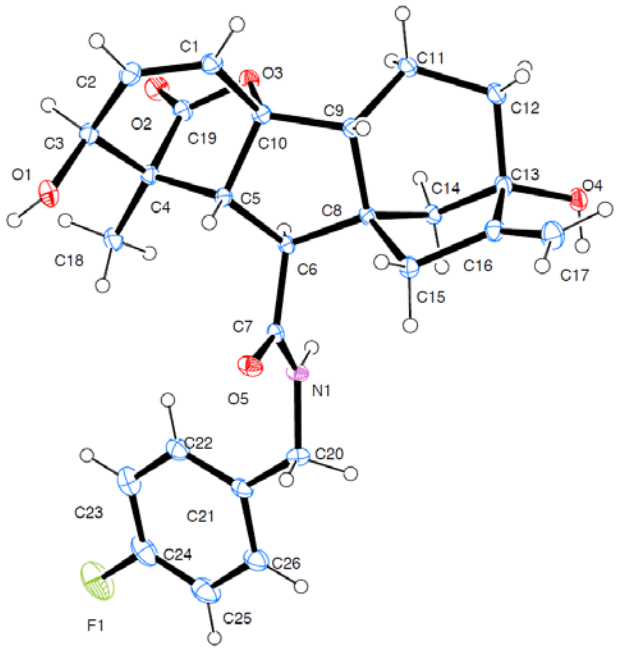


Figure 3. ORTEP drawing for one of the two independent molecules of compound **12**, the water and methanol solvates of crystallization have been omitted for clarity

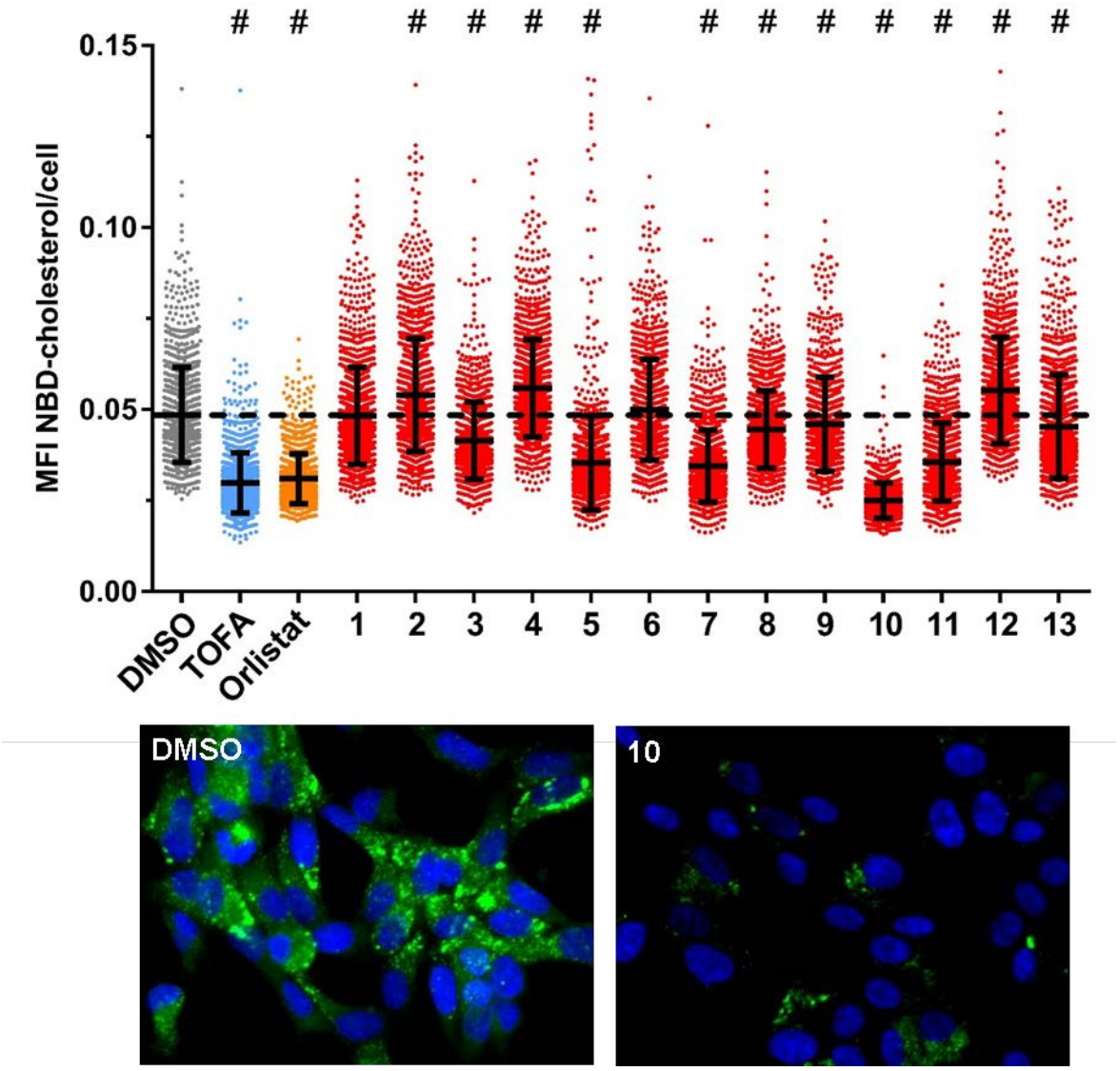


Figure 4. High-content imaging and quantitative image analysis of free cholesterol uptake in LNCaP cells treated with compounds **1**–**13** (30 µM) for 48 h. As controls, cells were treated with vehicle control (DMSO), TOFA (10 µM) and orlistat (20 µM). The cellular mean fluorescence intensity (MFI) of NBD-cholesterol was quantified with CellProfiler software (n~ 3000 cells per treatment, mean ±SD, # = one-way ANOVA with Dunnett's multiple comparison test relative to DMSO $p<0.01$). A representative graph of three independent experiments is shown. Representative images for DMSO and compound **10** at a 10x magnification are shown (bottom panel, blue=DNA, green= NBD-cholesterol).

Compounds **1–13** have been recently added to the Davis open-access natural product-based library, which is curated by Compounds Australia, Griffith University. Currently this library consists of 512 distinct structures, the majority of which have been obtained from

Australian natural sources, such as endophytic fungi,[28] plants,[29] macrofungi,[30] and marine invertebrates.[31]

To date all the gibberellic acid semi-synthesized analogues (**2**−**13**) together with the NP scaffold (**1**), have been evaluated for their cytotoxicity against a human prostate cancer cell line LNCaP (lymph node metastasis, androgen-sensitive) using a metabolic assay (alamarBlue). Compounds **1**−**13** did not show any cell viability inhibition towards the LNCaP cell line, with less than 30% inhibition at 10 μM (Figure S72, Supporting information). Additional assays measuring metabolic parameters of LNCaP cells (lipid uptake, lipid content, and mitochondrial activity) by high-content imaging revealed that compounds **3**, **5**, **7**, **10,** and **11** (Figure 4 and Figure S73, Supporting information) substantially decreased the uptake of free cholesterol when compared to the parent compound (**1**) (Figure 4), while fatty acid uptake and cellular lipid content remained unchanged (data not shown). The most active compound (**10**) suppressed cholesterol uptake to levels similar to the positive controls TOFA [5-(tetradecyloxy)-2-furoic acid)] and orlistat (Figure S76, Supporting Information). These fatty acid synthesis inhibitors were reported to cause an upregulation in the *de novo* synthesis of cholesterol,[32] leading to reduced uptake of exogenous cholesterol from the media. Furthermore, we observed that **3**, **5**, **7**, **10,** and **11** also substantially reduced the uptake of acetylated low density lipoprotein, a critical source of exogenous cholesterol (data not shown), suggesting that reduced cholesterol uptake is caused by deregulation of intracellular cholesterol metabolism and feedback inhibition rather than direct inhibition of different cholesterol uptake pathways. No clear SAR trend were identified, however this is not unexpected, since, in this cell-based assay, changes in the structure can influence many factors, such as permeability in addition to ligand-target(s) interactions.

In conclusion, a 12-membered amide library was designed and synthesized using the plant derived natural product scaffold, gibberellic acid. The structures of all the synthesized

analogues were fully characterized by NMR, MS, UV, and IR data. In addition, the structure of one of the semi-synthesized compounds was confirmed by single crystal X-ray crystallography. This unique library has been added to an open-access compound repository for future drug discovery and chemical biology screening. The library has been tested for cytotoxicity and deregulation of lipid metabolism in LNCaP prostate cancer cells. The importance of dysregulated cholesterol metabolism in prostate cancer is well documented.[33] Although, there are only a few studies describing the inhibition of cellular cholesterol uptake/absorption in cancer with natural products (grape seed, red wine polyphenol extracts, curcumin, etc), however, none in prostate cancer. Targeting cholesterol metabolism in prostate cancer has gained interest as therapeutic intervention (e.g. statins inhibiting de novo cholesterol synthesis),[34] but candidate drugs that reduce cholesterol uptake are rare and understudied as potential cancer therapeutics.[35] While no cytotoxic activity was identified, synthesized analogues **3**, **5**, **7**, **10,** and **11** reduced cellular uptake of free cholesterol in prostate cancer cells. The most active compound (**10**) suppressed cholesterol uptake to levels observed with the positive controls TOFA and orlistat. Both controls have been reported to increase the expression of the *de novo* cholesterol synthesis pathway enzymes,[32] however, this is the first report that describes the effect of TOFA and orlistat on cholesterol uptake in prostate cancer cells.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined on a Cole-Palmer melting point apparatus and are uncorrected. Optical rotations were determined on a JASCO P-1020 polarimeter. UV spectra were obtained using a JASCO V-650 UV/vis spectrophotometer. IR data were acquired using an attached Universal 335 Attenuated Total Reflectance (UATR) Two module on a PerkinElmer 336 spectrophotometer. NMR spectra

were recorded at 25 °C on a Bruker 500 MHz Oxford magnet AVANCE III HD NMR spectrometer. The ^{1}H and ^{13}C NMR chemical shifts were referenced to the solvent peak for DMSO-d_6 at δ_H 2.50 and δ_C 39.5. LRESIMS data were recorded on a Waters ZQ mass spectrometer. HRESIMS data were recorded on a 12 T SolariX XR FT-ICR-MS. FTIR data were recorded on a Universal Attenuated Total Reflectance Two (UATR) attachment on a PerkinElmer spectrophotometer. A Waters 600 pump equipped with a Waters 996 PDA detector, and Gilson 215 liquid handler was used for semi-preparative HPLC separations. An Alltech stainless steel guard cartridge (10 mm × 30 mm) was used for loading pre-adsorbed synthetic reaction products onto the semi-preparative HPLC columns. Alltech Davisil C_{18} bonded silica (35–75 µm 150 Å) or Activon phenyl bonded silica was used for pre-adsorption work before HPLC separations. TLC was carried out on Merck gel 60 F_{254} pre-coated aluminium plates and was observed using UV light. A Thermo Electron C_{18} Betasil column (5 µm, 143 Å, 21.2 mm × 150 mm), Phenomenex Phenyl-hexyl column (5 µm, 100 Å, 250 mm × 10 mm) were used for semi-preparative HPLC separation. All solvents used for chromatography were Lab-Scan HPLC grade, and the H_2O was Millipore Milli-Q PF filtered. All synthetic reagents were purchased from Sigma-Aldrich and used without further purification.

Generation of the Amide Library. Gibberellic acid (**1**, 50 mg, 0.14 mmol) was dissolved in dry CH_2Cl_2 (2 mL) under nitrogen. Anhydrous $(COCl)_2$ (0.2 mL, 0.43 mmol) was added, followed by the dropwise addition of DMF (33 µL, 0.43 mmol), on which gas evolution was observed. In another vial, the relevant primary amine (1.45 mmol) and anhydrous pyridine (100 µL) were stirred in dry CH_2Cl_2 (2 mL) under nitrogen. This vial was cooled to 0 °C and the generated acid chloride was added dropwise after which the reaction mixture was stirred for 20 min.[12] Reaction workup involved quenching with 2N HCl (1 × 10 mL), followed by extraction with CH_2Cl_2 (3 ×10 mL). The organic phase was dried under

nitrogen to give the crude product. The crude product was purified by RP-HPLC using a semi-preparative Betasil C_{18} HPLC column at a flow rate of 9 mL/min or Phenyl-hexyl column at a flow rate of 4 mL/min, and isocratic conditions of 10% MeOH (0.1% TFA) : 90% H_2O (0.1% TFA) for 20 min, followed by a linear gradient to 100% MeOH (0.1% TFA) over 80 min, and then isocratic conditions of 100% MeOH (0.1% TFA) for 20 min. One hundred and twenty fractions (120 ×1 min) were collected from the start of the HPLC run. Fractions containing UV-active material from each separate HPLC run were analyzed by ^{1}H NMR spectroscopy and LRMS, and relevant fractions with purity levels >95% were combined to afford the targeted products. Yields for each reaction are shown below.

Compound 2: Yellow gum (31.8 mg, 49%); $[\alpha]_D^{25}$ +104 (*c* 0.3, EtOH); literature value: $[\alpha]^{25}_D$ +81.2 (*c* 0.26, EtOH); UV (MeOH) λ_{max} (log ε) 205 (5.15), 257 (4.31) nm; IR (UATR) ν_{max} 3286, 1756, 1647, 1023, 996, 746, 496 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.01 (3H, s, H-18), 1.56 (1H, overlap, H-14a), 1.58 (1H, overlap, H-12a), 1.62 (1H, overlap, H-11a), 1.66 (1H, overlap, H-14b), 1.78 (1H, overlap, H-11b) 1.79 (1H, overlap, H-9) 1.87 (1H, overlap, H-12b), 2.00 (1H, m, H-15a), 2.04 (1H, m, H-15b), 2.50 (1H, d, *J* = 10.0 Hz, H-6), 2.70 (2H, m, H-21), 3.10 (1H, d, *J* = 10.2 Hz, H-5), 3.28 (1H, m, H-20a), 3.36 (1H, m, H-20b), 3.83 (1H, d, *J* = 3.6 Hz, H-3), 4.78 (1H, m, H-17a), 5.07 (1H, m, H-17b), 5.51 (1H, d, *J* = 6.7 Hz, 3-OH), 5.77 (1H, dd, *J* = 9.3, 3.6 Hz, H-2), 6.32 (1H, d, *J* = 9.3 Hz, H-1), 7.18 (1H, m, H-25), 7.20 (2H, m, H-23, H-27), 7.27 (2H, m, H-24, H-26), 8.13 (1H, dd, *J* = 5.7, 5.7 Hz, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 14.3 (C-18), 16.6 (C-11), 35.2 (C-21), 38.9 (C-12), 40.2 (C-20), 43.3 (C-15), 44.9 (C-14), 49.8 (C-8), 50.4 (C-9), 51.4 (C-6), 52.4 (C-5), 53.1 (C-4), 68.6 (C-3), 76.8 (C-13), 90.9 (C-10), 105.6 (C-17), 126.1 (C-25), 128.3 (2C, C-24, C-26), 128.6 (2C, C-23, C-27), 131.5 (C-1), 133.3 (C-2), 139.3 (C-22), 158.0 (C-16), 170.6 (C-7), 179.2 (C-19); (+)-LRESIMS *m/z* (rel. int.) 450 (100) $[M + H]^+$, 899 (40) $[2M + H]^+$; (+)-HRESIMS *m/z* 472.2089 $[M + Na]^+$ (calcd for $C_{27}H_{31}NO_5Na$, 472.2094).

Compound 3: Yellow gum (32.5 mg, 43%); $[\alpha]_D^{25}$ +21 (*c* 0.3, EtOH); UV (MeOH) λ_{max} (log ε) 203 (4.51), 221 (4.11) nm; IR (UATR) ν_{max} 3301, 2925, 1752, 1648, 1011, 512 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ_H 0.99 (3H, s, H-18), 1.54 (1H, overlap, H-14a), 1.58 (1H, overlap, H-12a), 1.61 (1H, overlap, H-11a), 1.66 (1H, overlap, H-14b), 1.77 (1H, overlap, H-11b), 1.78 (1H, overlap, H-9) 1.87 (1H, overlap, H-12b), 1.95 (1H, m, H-15a), 2.00 (1H, m, H-15b), 2.48 (1H, d, *J* = 10.2 Hz, H-6), 2.67 (2H, m, H-21), 3.09 (1H, d, *J* = 10.2 Hz, H-5), 3.32 (2H, m, H-20), 3.83 (1H, d, *J* = 3.6 Hz, H-3), 4.77 (1H, m, H-17a), 5.08 (1H, m, H-17b), 5.50 (1H, d, *J* = 6.7 Hz, 3-OH), 5.76 (1H, dd, *J* = 9.3, 3.6 Hz, H-2), 6.31 (1H, dd, *J* = 9.3, 1.0 Hz, H-1), 7.17 (2H, m, H-23, H-27), 7.44 (2H, m, H-24, H-26), 8.08 (1H, t, *J* = 5.6 Hz, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 14.3 (C-18), 16.6 (C-11), 34.5 (C-21), 38.9 (C-12), 40.0 (C-20), 43.2 (C-15), 44.9 (C-14), 49.7 (C-8), 50.4 (C-9), 51.4 (C-6), 52.4 (C-5), 53.1 (C-4), 68.6 (C-3), 76.8 (C-13), 90.9 (C-10), 105.5 (C-17), 119.2 (C-25), 131.0 (2C, C-23, C-27), 131.1 (2C, C-24, C-26), 131.5 (C-1), 133.3 (C-2), 138.7 (C-22), 157.9 (C-16), 170.6 (C-7), 179.2 (C-19); (+)-LRESIMS *m/z* (rel. int.) 528 (90) $[^{79}Br: M + H]^+$, 530 (100) $[^{81}Br: M + H]^+$; (+)-HRESIMS *m/z* 550.1221 $[M + Na]^+$ (calcd for $C_{27}H_{30}BrN\ O_5Na$, 550.1200).

Compound 4: Yellow gum (30.6 mg, 44%); $[\alpha]_D^{25}$ +21 (*c* 0.3, EtOH); UV (MeOH) λ_{max} (log ε) 203 (4.56), 220 (4.34) nm; IR (UATR) ν_{max} 3288, 1756, 1651, 1023, 519 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ_H 0.98 (3H, s, H-18), 1.54 (1H, overlap, H-14a), 1.58 (1H, overlap, H-12a), 1.62 (1H, overlap, H-11a), 1.65 (1H, overlap, H-14b), 1.77 (1H, overlap, H-11b), 1.78 (1H, overlap, H-9) 1.86 (1H, overlap, H-12b), 1.94 (1H, m, H-15a), 2.00 (1H, m, H-15b), 2.47 (1H, d, *J* = 10.2 Hz, H-6), 2.69 (2H, m, H-21), 3.09 (1H, d, *J* = 10.2 Hz, H-5), 3.32 (2H, m, H-20), 3.83 (1H, dd, *J* = 6.7, 3.6 Hz, H-3), 4.77 (1H, m, H-17a), 5.07 (1H, m, H-17b), 5.50 (1H, d, *J* = 6.7 Hz, 3-OH), 5.77 (1H, dd, *J* = 9.3, 3.6 Hz, H-2), 6.31 (1H, d, *J* = 9.3 Hz, H-1), 7.22 (2H, m, H-23, H-27), 7.31 (2H, m, H-24, H-26), 8.08 (1H, t, *J* = 5.6 Hz, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 14.3 (C-18), 16.6 (C-11), 34.4 (C-21), 38.9 (C-12), 40.0

(C-20), 43.2 (C-15), 44.9 (C-14), 49.7 (C-8), 50.4 (C-9), 51.4 (C-6), 52.4 (C-5), 53.1 (C-4), 68.6 (C-3), 76.8 (C-13), 90.9 (C-10), 105.5 (C-17), 128.2 (2C, C-24, C-26), 130.6 (2C, C-23, C-27), 130.7 (C-25), 131.5 (C-1), 133.3 (C-2), 138.3 (C-22), 157.9 (C-16), 170.6 (C-7), 179.1 (C-19); (+)-LRESIMS m/z (rel. int.) 484 (100) $[M + H]^+$, 967 (40) $[2M + H]^+$; (+)-HRESIMS *m/z* 506.1714 $[M + Na]^+$ (calcd for $C_{27}H_{30}ClNO_5Na$, 506.1705).

Compound 5: Yellow gum (32.1 mg, 48%); $[\alpha]_D^{25}$ +64 (*c* 0.3, EtOH); UV (MeOH) λ_{max} (log ε) 204 (4.88), 259 (4.07) nm; IR (UATR) ν_{max} 3286, 1756, 1645, 1509, 1220, 1024, 500 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ_H 0.98 (3H, s, H-18), 1.52 (1H, overlap, H-14a), 1.58 (1H, overlap, H-12a), 1.60 (1H, overlap, H-11a), 1.68 (1H, overlap, H-14b), 1.77 (1H, overlap, H-11b), 1.77 (1H, overlap, H-9) 1.86 (1H, overlap, H-12b), 1.91 (1H, m, H-15a), 2.00 (1H, m, H-15b), 2.46 (1H, d, *J* = 10.2 Hz, H-6), 2.68 (2H, m, H-21), 3.07 (1H, d, *J* = 10.2 Hz, H-5), 3.31 (2H, m, H-20), 3.82 (1H, d, *J* = 3.6 Hz, H-3), 4.76 (1H, m, H-17a), 5.06 (1H, m, H-17b), 5.76 (1H, dd, *J* = 9.3, 3.6 Hz, H-2), 6.31 (1H, d, *J* = 9.3 Hz, H-1), 7.07 (2H, m, H-24, H-26), 7.22 (2H, m, H-23, H-27), 8.09 (1H, t, *J* = 5.6 Hz, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 14.5 (C-18), 16.9 (C-11), 34.4 (C-21), 39.0 (C-12), 40.3 (C-20), 43.4 (C-15), 45.1 (C-14), 50.0 (C-8), 50.6 (C-9), 51.6 (C-6), 52.7 (C-5), 53.4 (C-4), 68.8 (C-3), 77.1 (C-13), 91.3 (C-10), 105.9 (C-17), 115.2 (2C, d, $^2J_{CF}$ = 21.1 Hz, C-24, C-26), 130.7 (2C, d, $^3J_{CF}$ = 7.9 Hz, C-23, C-27), 131.8 (C-1), 133.5 (C-2), 135.5 (d, $^4J_{CF}$ = 3.1 Hz, C-22), 157.9 (C-16), 161.0 (d, $^1J_{CF}$ = 241.5 Hz, C-25), 171.0 (C-7), 179.5 (C-19); (+)-LRESIMS *m/z* (rel. int.) 468 (100) $[M + H]^+$, 935 (40) $[2M + H]^+$; (+)-HRESIMS *m/z* 490.1989 $[M + Na]^+$ (calcd for $C_{27}H_{30}FNO_5Na$, 490.2000).

Compound 6: Yellow gum (7.0 mg, 10%); $[\alpha]_D^{25}$ +21 (*c* 0.3, EtOH); literature value: $[\alpha]^{25}_D$ +61.8 (*c* 0.25, EtOH); UV (MeOH) λ_{max} (log ε) 203 (4.33), 224 (3.92) nm; IR (UATR) ν_{max} 3370, 2981, 1758, 1653, 1513, 1248, 1023, 455 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ_H 1.01 (3H, s, H-18), 1.53 (1H, overlap, H-14a), 1.57 (1H, overlap, H-12a), 1.61 (2H, overlap, H-

11), 1.66 (1H, overlap, H-14b), 1.78 (1H, overlap, H-9) 1.86 (1H, overlap, H-12b), 1.97 (1H, m, H-15a), 2.02 (1H, m, H-15b), 2.48 (1H, m, H-6), 2.63 (2H, m, H-21), 3.09 (1H, d, *J* = 10.2 Hz, H-5), 3.28 (2H, m, H-20), 3.70 (3H, s, H-28), 3.83 (1H, d, *J* = 3.6 Hz, H-3), 4.77 (1H, br s, H-17a), 5.06 (1H, br s, H-17b), 5.77 (1H, dd, *J* = 9.3, 3.6 Hz, H-2), 6.32 (1H, dd, *J* = 9.3, 0.9 Hz, H-1), 6.83 (2H, m, H-24, H-26), 7.11 (2H, m, H-23, H-27), 8.08 (1H, t, *J* = 5.6 Hz, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 14.3 (C-18), 16.7 (C-11), 34.4 (C-21), 39.0 (C-12), 40.0 (C-20), 43.3 (C-15), 44.9 (C-14), 49.8 (C-8), 50.4 (C-9), 51.4 (C-6), 52.5 (C-5), 53.2 (C-4), 55.0 (C-28), 68.6 (C-3), 76.8 (C-13), 91.0 (C-10), 105.6 (C-17), 113.8 (2C, C-24, C-26), 129.6 (2C, C-23, C-27), 131.1 (C-22), 131.6 (C-1), 133.3 (C-2), 157.7 (C-25), 157.9 (C-16), 170.6 (C-7), 179.2 (C-19); (+)-LRESIMS *m/z* (rel. int.) 480 (100) $[M + H]^+$, 959 (40) $[2M + H]^+$; (+)-HRESIMS *m/z* 502.2183 $[M + Na]^+$ (calcd for $C_{28}H_{33}NO_6Na$, 502.2200).

Compound 7: Light yellow gum (16.9 mg, 26%); $[\alpha]_D^{25}$ +98 (*c* 0.3, EtOH); UV (MeOH) λ_{max} (log ε) 204 (4.87), 261 (4.12) nm; IR (UATR) ν_{max} 3283, 1755, 1651, 1596, 1439, 1044, 997, 506 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 0.98 (3H, s, H-18), 1.53 (1H, overlap, H-14a), 1.58 (1H, overlap, H-12a), 1.61 (1H, overlap, H-11a), 1.64 (1H, overlap, H-14b), 1.77 (1H, overlap, H-11b), 1.78 (1H, overlap, H-9) 1.86 (1H, overlap, H-12b), 1.98 (2H, m, H-15a), 2.02 (2H, m, H-15b), 2.47 (1H, d, *J* = 10.2 Hz, H-6), 2.86 (2H, m, H-21), 3.09 (1H, d, *J* = 10.2 Hz, H-5), 3.45 (1H, m, H-20), 3.82 (1H, dd, *J* = 6.7, 3.6 Hz, H-3), 4.78 (1H, m, H-17a), 5.07 (1H, m, H-17b), 5.49 (1H, d, *J* = 6.7 Hz, 3-OH), 5.77 (1H, dd, *J* = 9.3, 3.6 Hz, H-2), 6.31 (1H, dd, *J* = 9.3, 1.0 Hz, H-1), 7.21 (1H, ddd, *J* = 7.7, 4.9, 1.0 Hz, H-25), 7.24 (1H, brd, *J* = 7.7 Hz, H-23), 7.70 (1H, ddd, *J* = 7.7, 7.7, 1.9 Hz , H-24), 8.11 (1H, t, *J* = 5.7 Hz, NH), 8.49 (1H, ddd, *J* = 4.9, 1.9, 1.0 Hz, H-26); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 14.3 (C-18), 16.6 (C-11), 37.3 (C-21), 38.5 (C-20), 38.9 (C-12), 43.3 (C-15), 44.8 (C-14), 49.7 (C-8), 50.4 (C-9), 51.4 (C-6), 52.4 (C-5), 53.1 (C-4), 68.6 (C-3), 76.8 (C-13), 90.9 (C-10), 105.6 (C-17), 121.6 (C-25), 123.3 (C-23), 131.5 (C-1), 133.3 (C-2), 136.6 (C-24), 148.9 (C-26), 158.0 (C-

16), 158.7 (C-22), 170.6 (C-7), 179.1 (C-19); (+)-LRESIMS *m/z* (rel. int.) 451 (100) [M + H]$^+$; (+)-HRESIMS *m/z* 451.2213 [M + H]$^+$ (calcd for $C_{26}H_{31}N_2O_5$, 451.2227).

Compound 8: White powder (3.6 mg, 7%); $[\alpha]_D^{25}$ +20 (*c* 0.2, EtOH); UV (MeOH) λ_{max} (log ε) 203 (4.45), 234 (4.16) nm; IR(UATR) ν_{max} 3315, 2924, 1755, 1646, 1539, 1041, 889, 697 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.03 (3H, s, H-18), 1.56 (1H, overlap, H-14a), 1.58 (1H, overlap, H-12a), 1.62 (1H, overlap, H-11a), 1.66 (1H, overlap, H-14b), 1.79 (1H, overlap, H-9), 1.79 (1H, overlap, H-11b), 1.86 (1H, overlap, H-12b), 2.03 (2H, m, H-15), 2.52 (1H, d, 10.2 Hz , H-6), 2.91 (2H, m, H-21), 3.11 (1H, d, *J* = 10.2 Hz, H-5), 3.29 (1H, m, H-20a), 3.35 (1H, m, H-20b), 3.84 (1H, d, *J* = 3.6 Hz, H-3), 4.79 (1H, m, H-17a), 5.07 (1H, m, H-17b), 5.77 (1H, dd, *J* = 9.3, 3.6 Hz, H-2), 6.32 (1H, dd, *J* = 9.3, 1.0 Hz, H-1), 6.87 (1H, dd, *J* = 3.4, 1.2 Hz, H-23), 6.94 (1H, dd, *J* = 5.2, 3.4 Hz, H-24), 7.32 (1H, dd, *J* = 5.2, 1.2 Hz, H-25), 8.22 (1H, dd, *J* = 5.6, 5.6 Hz, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 14.3 (C-18), 16.7 (C-11), 29.4 (C-21), 38.9 (C-12), 40.5 (C-20), 43.3 (C-15), 44.9 (C-14), 49.8 (C-8), 50.4 (C-9), 51.4 (C-6), 52.5 (C-5), 53.1 (C-4), 68.6 (C-3), 76.8 (C-13), 90.9 (C-10), 105.6 (C-17), 124.0 (C-25), 125.2 (C-23), 126.9 (C-24), 131.5 (C-1), 133.3 (C-2), 141.4 (C-22), 157.9 (C-16), 170.8 (C-7), 179.2 (C-19); (+)-LRESIMS *m/z* (rel. int.) 456 (100) [M + H]$^+$, 911 (25) [2M + H]$^+$; (+)-HRESIMS *m/z* 478.1659 [M + Na]$^+$ (calcd for $C_{25}H_{29}NO_5SNa$, 478.1658).

Compound 9: White powder (23.7 mg, 38%); $[\alpha]_D^{25}$ +54 (*c* 0.3, MeOH); literature value: $[\alpha]^{25}_D$ +90.9 (*c* 0.26, EtOH); UV (MeOH) λ_{max} (log ε) 205 (4.77), 258 (2.96) nm; IR (UATR) ν_{max} 3288, 1755, 1643, 1557, 1040, 890, 528 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.06 (3H, s, H-18), 1.59 (1H, overlap, H-12a), 1.60 (1H, overlap, H-14a), 1.64 (1H, overlap, H-11a), 1.71 (1H, overlap, H-14b), 1.80 (1H, overlap, H-11b), 1.82 (1H, overlap, H-9) 1.86 (1H, overlap, H-12b), 2.08 (2H, m, H-15), 2.62 (1H, d, *J* = 10.2 Hz, H-6), 3.15 (1H, d, *J* = 10.2 Hz, H-5), 3.85 (1H, dd, *J* = 6.7, 3.6 Hz, H-3), 4.19 (1H, dd, *J* = 15.2, 5.4 Hz, H-20a), 4.38 (1H, dd, *J* = 15.2, 6.4 Hz, H-20b), 4.78 (1H, m, H-17a), 4.82 (1H, s, 13-OH), 5.07 (1H, m,

H-17b), 5.52 (1H, d, *J* = 6.7 Hz, 3-OH), 5.78 (1H, dd, *J* = 9.3, 3.6 Hz, H-2), 6.33 (1H, dd, *J* = 9.3, 0.8 Hz, H-1), 7.25 (2H, m, H-22, H-26), 7.23 (1H, m, H-24), 7.31 (2H, m, H-23, H-25), 8.57 (1H, dd, *J* = 6.4, 5.4 Hz, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 14.5 (C-18), 16.7 (C-11), 38.8 (C-12), 42.3 (C-20), 43.3 (C-15), 45.0 (C-14), 49.8 (C-8), 50.5 (C-9), 51.4 (C-6), 52.6 (C-5), 53.2 (C-4), 68.6 (C-3), 76.8 (C-13), 91.0 (C-10), 105.5 (C-17), 126.8 (C-24), 127.3 (2C, C-22, C-26), 128.3 (2C, C-23, C-25), 131.5 (C-1), 133.5 (C-2), 139.5 (C-21), 157.9 (C-16), 170.8 (C-7), 179.1 (C-19); (+)-LRESIMS *m/z* (rel. int.) 436 (100) $[M + H]^+$, 871 (40) $[2M + H]^+$; (+)-HRESIMS *m/z* 458.1932 $[M + Na]^+$ (calcd for $C_{26}H_{29}NO_5Na$, 458.1938).

Compound 10: White powder (5.6 mg, 8%); $[\alpha]_D^{25}$ +17 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.63), 219 (4.29) nm; IR (UATR) ν_{max} 3281, 1761, 1643, 1561, 1099, 1008, 530 cm^{-1};^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.04 (3H, s, H-18), 1.57 (1H, overlap, H-14a), 1.58 (1H, overlap, H-12a), 1.63 (1H, overlap, H-11a), 1.70 (1H, overlap, H-14b), 1.81 (1H, overlap, H-11b), 1.81 (1H, overlap, H-9) 1.87 (1H, overlap, H-12b), 2.03 (1H, m, H-15a), 2.06 (1H, m, H-15b), 2.59 (1H, d, *J* = 10.3 Hz, H-6), 3.13 (1H, d, *J* = 10.3 Hz, H-5), 3.84 (1H, dd, *J* = 6.7, 3.6 Hz, H-3), 4.17 (1H, dd, *J* = 15.2, 5.4 Hz, H-20a), 4.33 (1H, dd, *J* = 15.3, 6.4 Hz, H-20b), 4.77 (1H, m, H-17a), 4.81 (1H, s, 13-OH), 5.06 (1H, m, H-17b), 5.51 (1H, d, *J* = 6.7 Hz, 3-OH), 5.78 (1H, dd, *J* = 9.3, 3.6 Hz, H-2), 6.33 (1H, dd, *J* = 9.3, 0.9 Hz, H-1), 7.21 (2H, m, H-22, H-26), 7.50 (2H, m, H-23, H-25), 8.60 (1H, dd, *J* = 6.4, 5.4 Hz, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 14.4 (C-18), 16.6 (C-11), 38.8 (C-12), 41.7 (C-20), 43.3 (C-15), 44.9 (C-14), 49.8 (C-8), 50.5 (C-9), 51.4 (C-6), 52.5 (C-5), 53.1 (C-4), 68.6 (C-3), 76.8 (C-13), 90.9 (C-10), 105.5 (C-17), 119.8 (C-24), 129.5 (2C, C-22, C-26), 131.1 (2C, C-23, C-25), 131.5 (C-1), 133.3 (C-2), 139.0 (C-21), 157.9 (C-16), 170.8 (C-7), 179.1 (C-19); (+)-LRESIMS *m/z* (rel. int.) 514 (100) $[^{79}Br: M + H]^+$, 516 (100) $[^{81}Br: M + H]^+$; (+)-HRESIMS *m/z* 536.1057 $[M + Na]^+$ (calcd for $C_{26}H_{28}BrNO_5Na$, 536.1043).

Compound 11: White powder (9.0 mg, 13%); $[\alpha]_D^{25}$ +83 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 204 (5.20), 221 (4.97) nm; IR (UATR) ν_{max} 3283, 1760, 1644, 1558, 1093, 1008, 533 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ_H 1.03 (3H, s, H-18), 1.57 (1H, overlap, H-14a), 1.59 (1H, overlap, H-12a), 1.64 (1H, overlap, H-11a), 1.69 (1H, overlap, H-14b), 1.81 (1H, overlap, H-11b), 1.81 (1H, overlap, H-9) 1.87 (1H, overlap, H-12b), 2.03 (1H, m, H-15a), 2.06 (1H, m, H-15b), 2.59 (1H, d, *J* = 10.2 Hz, H-6), 3.12 (1H, d, *J* = 10.2 Hz, H-5), 3.84 (1H, dd, *J* = 6.7, 3.6 Hz, H-3), 4.18 (1H, dd, *J* = 15.3, 5.4 Hz, H-20a), 4.35 (1H, dd, *J* = 15.3, 6.3 Hz, H-20b), 4.77 (1H, m, H-17a), 4.81 (1H, s, 13-OH), 5.06 (1H, m, H-17b), 5.51 (1H, d, *J* = 6.7 Hz, 3-OH), 5.78 (1H, dd, *J* = 9.3, 3.6 Hz, H-2), 6.33 (1H, dd, *J* = 9.3, 0.9 Hz, H-1), 7.26 (2H, m, H-22, H-26), 7.37 (2H, m, H-23, H-25), 8.60 (1H, dd, *J* = 6.3, 5.4 Hz, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 14.4 (C-18), 16.6 (C-11), 38.8 (C-12), 41.7 (C-20), 43.3 (C-15), 44.9 (C-14), 49.8 (C-8), 50.5 (C-9), 51.4 (C-6), 52.5 (C-5), 53.1 (C-4), 68.6 (C-3), 76.8 (C-13), 90.9 (C-10), 105.5 (C-17), 128.2 (2C, C-23, C-25), 129.2 (2C, C-22, C-26), 131.3 (C-24), 131.5 (C-1), 133.3 (C-2), 138.6 (C-21), 157.9 (C-16), 170.8 (C-7), 179.1 (C-19); (+)-LRESIMS *m/z* (rel. int.) 470 (100) $[M + H]^+$, 492 (20) $[M + Na]^+$; (+)-HRESIMS *m/z* 492.1531 $[M + Na]^+$ (calcd for $C_{26}H_{28}ClNO_5Na$, 492.1548).

Compound 12: White needles (31.0 mg, 47%); mp 232–236 °C; $[\alpha]_D^{25}$ +72 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.80), 264(3.32) nm; IR (UATR) ν_{max} 3282, 1757, 1642, 1563, 1509, 1224, 1028, 496 cm^{-1};^1H NMR (500 MHz, DMSO-d_6) δ_H 1.03 (3H, s, H-18), 1.57 (1H, overlap, H-14a), 1.57 (1H, overlap, H-12a), 1.63 (1H, overlap, H-11a), 1.70 (1H, overlap, H-14b), 1.81 (1H, overlap, H-11b), 1.82 (1H, overlap, H-9) 1.88 (1H, overlap, H-12b), 2.04 (1H, m, H-15a), 2.06 (1H, m, H-15b), 2.60 (1H, d, *J* = 10.2 Hz, H-6), 3.14 (1H, d, *J* = 10.2 Hz, H-5), 3.85 (1H, dd, *J* = 6.7, 3.6 Hz, H-3), 4.17 (1H, dd, *J* = 15.0, 5.4 Hz, H-20a), 4.35 (1H, dd, *J* = 15.0, 6.4 Hz, H-20b), 4.77 (1H, m, H-17a), 4.81 (1H, s, 13-OH), 5.07 (1H, m, H-17b), 5.51 (1H, d, *J* = 6.7 Hz, 3-OH), 5.78 (1H, dd, *J* = 9.3, 3.6 Hz, H-2), 6.33 (1H, dd, *J* =

9.3, 0.9 Hz, H-1), 7.13 (2H, m, H-23, H-25), 7.28 (2H, m, H-22, H-26), 8.60 (1H, dd, *J* = 6.4, 5.4 Hz, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 14.4 (C-18), 16.6 (C-11), 38.8 (C-12), 41.6 (C-20), 43.3 (C-15), 44.9 (C-14), 49.8 (C-8), 50.5 (C-9), 51.4 (C-6), 52.5 (C-5), 53.1 (C-4), 68.6 (C-3), 76.8 (C-13), 91.0 (C-10), 105.5 (C-17), 115.0 (2C, d, $^2J_{CF}$ = 21.2 Hz, C-23, C-25), 129.3 (2C, d, $^3J_{CF}$ = 8.2 Hz, C-22, C-26), 131.5 (C-1), 133.5 (C-2), 135.7 (d, $^4J_{CF}$ = 2.9 Hz, C-21), 157.9 (C-16), 161.2 (d, $^1J_{CF}$ = 242.7 Hz, C-24), 170.7 (C-7), 179.1 (C-19); (+)-LRESIMS *m/z* (rel. int.) 454 (100) $[M + H]^+$, 907 (50) $[2M + H]^+$; (+)-HRESIMS *m/z* 476.1828 $[M + Na]^+$ (calcd for $C_{26}H_{28}FNO_5Na$, 476.1844).

Compound 13: White powder (2.9 mg, 4%); $[\alpha]_D^{25}$ +37 (*c* 0.3, EtOH); UV (MeOH) λ_{max} (log ε) 203 (4.61), 226 (4.31) nm; IR (UATR) ν_{max} 3273, 1757, 1639, 1567, 1513, 1244, 1030, 889, 486 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 1.04 (3H, s, H-18), 1.58 (1H, overlap, H-14a), 1.58 (1H, overlap, H-12a), 1.63 (1H, overlap, H-11a), 1.68 (1H, overlap, H-14a), 1.81 (1H, overlap, H-11b), 1.81 (1H, overlap, H-9) 1.86 (1H, overlap, H-12b), 2.06 (2H, m, H-15), 2.59 (1H, d, *J* = 10.2 Hz, H-6), 3.13 (1H, d, *J* = 10.2 Hz, H-5), 3.72 (3H, s, H-27), 3.84 (1H, dd, *J* = 6.7, 3.6 Hz, H-3), 4.12 (1H, dd, *J* = 14.8, 5.4 Hz, H-20a), 4.30 (1H, dd, *J* = 14.8, 6.4 Hz, H-20b), 4.77 (1H, m, H-17a), 4.80 (1H, s, 13-OH), 5.06 (1H, m, H-17b), 5.51 (1H, d, *J* = 6.7 Hz, 3-OH), 5.78 (1H, dd, *J* = 9.3, 3.6 Hz, H-2), 6.33 (1H, dd, *J* = 9.3, 1.0 Hz, H-1), 6.87 (2H, m, H-23, H-25), 7.16 (2H, m, H-22, H-26), 8.48 (1H, dd, *J* = 6.4, 5.4 Hz, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 14.4 (C-18), 16.6 (C-11), 38.8 (C-12), 41.7 (C-20), 43.3 (C-15), 44.9 (C-14), 49.8 (C-8), 50.5 (C-9), 51.4 (C-6), 52.5 (C-5), 53.1 (C-4), 55.1 (C-27), 68.6 (C-3), 76.8 (C-13), 91.0 (C-10), 105.5 (C-17), 113.6 (2C, C-23, C-25), 128.6 (2C, C-22, C-26), 131.5 (C-1), 133.3 (C-2), 157.9 (C-16), 158.2 (C-24), 170.6 (C-7), 179.1 (C-19); (+)-LRESIMS *m/z* (rel. int.) 466 (100) $[M + H]^+$, 931 (25) $[2M + H]^+$; (+)-HRESIMS *m/z* 488.2048 $[M + Na]^+$ (calcd for $C_{27}H_{31}NO_6Na$, 488.2044).

X-ray Crystallography Studies on Compound 12. Intensity data for **12** were collected with an Oxford Diffraction SuperNova CCD diffractometer using Cu-Kα radiation, the temperature during data collection was maintained at 130.0(1) K using an Oxford Cryosystems cooling device. The structure was solved by direct methods and difference Fourier Synthesis.[36] Hydrogen atoms bound to the carbon atom were placed at their idealized positions using appropriate HFIX instructions in SHELXL, and included in subsequent refinement cycles. Hydrogen atoms attached to nitrogen and oxygen were located from difference Fourier maps and refined freely with isotropic displacement parameters. Thermal ellipsoid plots were generated using the program ORTEP-3[37] integrated within the WINGX suite of programs.[38] Full details of the data collection and refinement and tables of atomic coordinates, bond lengths and angles, and torsion angles have been deposited with the Cambridge Crystallographic Data Centre (CCDC 1474250). Copies can be obtained free of charge on application at the following address: http://www.ccdc.cam.ac.uk/cgi-bin/catreq.cgi.

Crystal data for 12. $(C_{26}H_{28}FNO_5)_2$. $(H_2O.\ CH_3OH)$, M = 957.04, T = 130.0(2) K, λ = 1.5418 Å, Monoclinic, space group $P2_1$, a = 9.4144(2), b = 25.4932(4), c = 10.9393(2) Å, β = 115.504(2)° V = 2369.63(8) Å^3, Z = 4, Z' = 2 D_c = 1.341 Mg M^{-3} μ = 0.827 mm^{-1}, *F(000)* = 1016, crystal size 0.57 x 0.13 x 0.09 mm. θ_{max} = 76.8°, 17242 reflections measured, 9500 independent reflections (R_{int} = 0.0397) the final R = 0.0355 [I > 2σ(I), 9048 data] and *w*R(F^2) = 0.0918 (all data) GOOF = 1.024, Absolute Structure Parameter (Flack) -0.01(7).

Assessment of Cell Viability and Cholesterol Uptake. LNCaP cells were obtained from the American Type Culture Collection (ATCC) and were cultured in phenol-red free Roswell Park Memorial Institute (RPMI)-1640 medium (Thermo Fisher Scientific) supplemented with 5% FBS (Thermo Fisher Scientific) at 37 °C in an atmosphere containing 5% CO_2 and maintained in log phase growth. Cell viability as a function of metabolic activity was measured using the alamarBlue reagent, according to the manufacturer's instructions

(Thermo Fisher Scientific) and as previously described.[29] Briefly, LNCaP (4000 cells per well) were seeded for 24 h into 96-well tissue culture plates (Corning) and treated with the indicated compounds for 72 h. Compounds were dissolved in DMSO and diluted in growth medium (final concentration 0.3%). Control cells were treated with the equivalent dose of DMSO (negative control, Sigma Aldrich) or vinblastine (50 nM, Sigma Aldrich) as positive control. Each data point was performed in triplicate, and repeated in at least three independent experiments.

For the assessment of cholesterol uptake, LNCaP cells were seeded for 72 h as described above into poly-L-ornithine (Sigma-Aldrich) coated optical 96-well plates (ibidi). After treatment of three replicate wells with vehicle control (DMSO) or 30 µM of the indicated compounds for 48 h, RPMI-1640 5% FBS was replaced with serum-free RPMI-1640 supplemented with 0.2% BSA (Sigma) and 15 µM NBD-cholesterol {22-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-23,24-bisnor-5-cholen-3β-ol} (Thermo-Fisher, N1148) (Figure S76, Supporting Information), and cells were incubated for 3 h at 37 °C in an atmosphere containing 5% CO_2. As positive controls, cells were treated with10 µM TOFA [5-(tetradecyloxy)-2-furoic acid] (Sigma, T6575) or 20 µM orlistat *N*-formyl-L-leucine {(1S)-1-[(2S,3S)-3-hexyl-4-oxo-2-oxetanyl)methyl]dodecyl ester} (Sigma, 04139) for 48 h, which was previously reported to increase *de novo* cholesterol synthesis,[32] leading to reduced uptake of free cholesterol. Cells were washed with PBS, fixed for 15 min at room temperature in the dark with 4% (v/v) paraformaldehyde, and unreacted aldehyde was inactivated with PBS containing 30 mM glycine for 30min. Nuclear DNA and F-actin (cell mask) was counterstained for 2 h with 1 µg/mL DAPI [2-(4-amidinophenyl)-1*H*-indole-6-carboxamidine] (Sigma Aldrich, D9542) and Alexa Fluor 633 Phalloidin (Thermo-Fisher, A22284), respectively. Images in the DAPI, FITC (NBD-cholesterol) and CY5 channels (Alexa Fluor 633 Phalloidin) at a 10x magnification were acquired with an InCell Analyzer

(GE Healthcare) until 1000 cells/well were imaged. Automated image segmentation based on DNA and F-actin staining and quantification of cellular NBD-cholesterol of ~3,000 cells/treatment were performed with CellProfiler software (Broad Institute, Cambridge, USA).[39] Statistical significance was analyzed with GraphPad Prism (GraphPad Software) by one-way ANOVA with Dunnett's multiple comparison test.

ASSOCIATED CONTENT

Supporting Information

Supporting information (including 1D and 2D NMR spectra of all compounds (**2**–**13**), structures of virtual analogues VA1–VA26, orlistat, TOFA, and NBD-cholesterol, cytotoxicity and high-content imaging and quantitative image analysis of free cholesterol uptake in LNCaP for all compounds associated with this article can be found in the online version http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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