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Synthesis and SAR of α-Acylaminoketone Ligands for Control of Gene Expression

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Abstract—A lead discovery library and a follow-up focused library of α-acylaminoketones were designed based on known dibenzoylhydrazine ecdysone agonists, including GS™–E. The compounds were assayed in mammalian cells expressing the ecdysone receptor from *Bombyx mori* for their ability to cause expression of a reporter gene downstream of an ecdysone response element. The most potent α-acylaminoketones were comparable to GS™–E in this assay.

The ability to control the level and timing of gene expression is a powerful tool in biological systems and has potential applications in human gene therapy and the production of therapeutic proteins.[1–3] A number of such 'gene-switch' systems have been described, among them some based on the insect ecdysone receptor (EcR).[4–8] These have the advantage that they are intrinsically orthogonal to mammalian steroid hormone receptors and can be activated by certain ecdysteroids and by non-steroidal ecdysone agonist ligands.[9,10] As part of our effort to develop the RheoPlex™ system of orthogonal 'gene switches' based on natural and mutated ecdysone receptors, we sought novel chemotypes with ecdysone agonist activity.[11]

Dibenzoyl derivatives of *t*-butylhydrazine e.g., RH-5849 (**1a**) and GS™–E (**1d**) are well known as ecdysone agonists (Fig. 1).[10] Examination of structure **1** suggested that replacement of the *N*-*t*-butyl moiety with an appropriately chosen dialkylmethylene group (CR^1R^2) to afford α-acylaminoketone **2** might lead to a new class of ecdysone agonists. To test the validity of this concept, we prepared a lead discovery library of α-acylaminoketones **2** in which X was varied, R^1 and R^2 were either both methyl or were joined to form a cyclopentane ring, and Y was fixed as hydrogen. The geminal dimethyl compounds were prepared from Boc-Aib–OH (**3**) as shown in Scheme 1.[12] Thus, **3** was coupled with *N*,*O*-dimethylhydroxylamine to afford Weinreb amide **4**. Reaction of **4** with PhMgBr gave aminoketone **5a** directly in modest yield, rather than the expected Boc derivative. Coupling of **5a** with benzoic acids using standard protocols afforded the desired α-acylaminoketones **2aa**–**2af** (Table 1).[13] Similarly, acylation of 1-amino-1-benzoylcyclopentane (**5b**), which is available by amination of cyclopentyl phenyl ketone,[14] afforded compounds **2ba**–**2bf**.

The compounds were assayed at a single dose in a cell line engineered to express *Bombyx mori* EcR and to contain a β-galactosidase gene under the control of an ecdysone response element.[5,15] The assay results are presented as fold induction relative to a DMSO control (Table 1). No increase in the expression of β-galactosidase above background levels was observed with the geminal dimethyl compounds **2aa**–**2ac** or **2ae**; however, a 2-fold increase in expression was observed with **2ad** and **2af**. These compounds bear the X = 4-ethyl and 2-methyl-3-methoxy substitution patterns seen in the commercial ecdysone agonist insecticides tebufenozide (**1b**) and methoxyfenozide (**1c**). Similarly, in the cyclopentane

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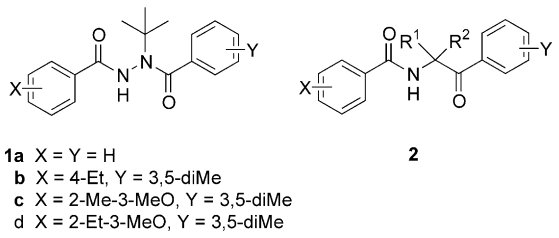
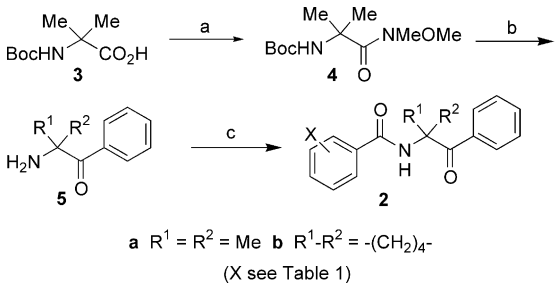


Figure 1. Dibenzoylhydrazine ecdysone agonists **1a–d** and isosteric α-acylaminoketones **2**.

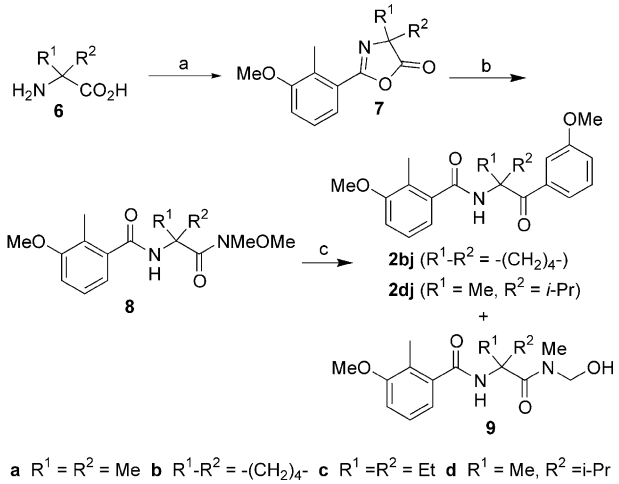


Scheme 1. Synthesis of lead discovery library of α-acylaminoketones. (a) MeNHOMe, EDC, *i*-Pr_2NEt, CH_2Cl_2, rt, 24 h; (b) R^4MgBr (5 equiv), THF, rt, 5 h; (c) PS–HOBt–O_2CPhX, *i*-Pr_2NEt, CH_2Cl_2, 12 h, rt.

Table 1. Single dose transactivation assay results[a]

			Fold Induction[b,c] (33 μM)	
			2a	**2b**
	X	Y	Me, Me	$-(CH_2)_4-$
a	H	H	1	2
b	2-Me	H	1	2
c	3-MeO	H	1	2
d	4-Et	H	2	133
e	3,4-OCH_2O	H	1	1
f	2-Me–3-MeO	H	2	71

[a]See ref 15 for assay protocol.
[b]Ratio of light measured in treated cells versus a DMSO control.
[c]Average of two replicates.



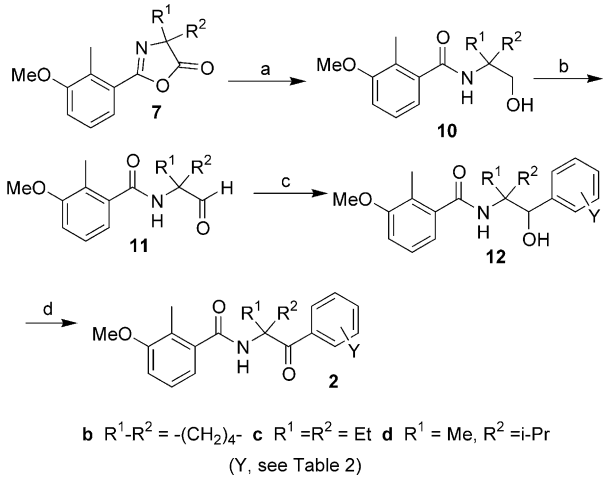
Scheme 2. Weinreb amide route to α-acylaminoketones. (a) 2-Me–3-MeO–PhCOCl (2.5 equiv), pyridine, rt; (b) MeNHOMe.HCl, pyridine, CH_2Cl_2, rt; (c) 3-MeOPhMgBr (4 equiv), THF, rt, 5 h.

series, **2bd** and **2bf** stood out, causing substantial increases in gene expression. These results suggested that, for **2** to effectively promote gene expression, greater bulk was necessary at R^1 and R^2 than was provided by the geminal dimethyl moiety and, possibly, that R^1 and R^2 should be constrained in a ring.

To further optimize potency, a focused library of α-acylaminoketones **2** in which X was fixed as 2-Me-3–MeO was designed. The substitution pattern X = 2-Me-3–MeO, rather than X = 4-Et, was used in the focused library to render the resulting target compounds **2** somewhat less lipophilic. R^1 and R^2 were selected to be $-(CH_2)_4-$; Et, Et; or Me, *i*-Pr. Finally Y was chosen to include methyl and methoxy at the 2-, 3- and 4-positions as well as the 3,5-dimethyl group that is present in the commercial ecdysone agonist insecticides **1b** and **1c**, and in GS™–E (**1d**).

Initially Weinreb amides **8** (Scheme 2) were anticipated to be pivotal intermediates in this synthesis. Thus, treatment of the commercially available α,α-disubstituted amino acids **6b–d** with 2.5 equiv of 2-methyl-3-methoxybenzoyl chloride in pyridine afforded azlactones **7b–d**. These were ring opened by treatment with *N*,*O*-dimethylhydroxylamine in the presence of pyridine to afford **8b–d**.[16] Treatment of **8b** with 3-methoxyphenylmagnesium bromide in THF at room temperature afforded the desired α-acylaminoketone **2bj** in 63% yield; however, when the more sterically congested **8d** was treated under similar conditions the major product was *N*-(hydroxymethyl)-*N*-methylamide **9d**; ^{13}C NMR indicated that little or none of the desired ketone **2dj** was present.[17] Given this result the reaction of Weinreb amides **8** with Grignard reagents was deemed insufficiently general for library production.

As an alternative, we undertook the longer sequence depicted in Scheme 3. The previously prepared azlactones **7b–d** were reduced with sodium borohydride in THF to provide the primary alcohols **10b–d**.[18] These



Scheme 3. Amidoaldehyde route to α-acylaminoketones. (a) $NaBH_4$, THF, rt: (b) Dess–Martin periodinane, CH_2Cl_2, rt; (c) Y–PhMgBr (4 equiv), THF, −70 °C → rt; (d) Dess–Martin periodinane, CH_2Cl_2, rt.

Table 2. Single dose transactivation assay results[a]

	X	Y	Fold Induction[b,c] (33 μM) 2b –$(CH_2)_4$–	2c Et,Et	2d Me, *i*-Pr
f	2-Me–3-MeO	H	71	11	71
g	2-Me–3-MeO	2-Me	30	5	452
h	2-Me–3-MeO	2-MeO	1196	384	599
i	2-Me–3-MeO	3-Me	291	525	370
j	2-Me–3-MeO	3-MeO	127	256	35
k	2-Me–3-MeO	4-Me	21	8	31
l	2-Me–3-MeO	4-MeO	5	4	[d]
m	2-Me–3-MeO	4-F	218	60	[d]
n	2-Me–3-MeO	3-Me-4-F	487	223	265
o	2-Me–3-MeO	3,5-diMe	660	1005	751

[a]See ref 15 for assay protocol.
[b]Ratio of light measured in treated cells versus a DMSO control.
[c]Average of two replicates.
[d]Compound not made.

were cleanly oxidized to the corresponding aldehydes **11b–d** using the Dess–Martin periodinane. The overall yields of aldehydes **11b–d** from the corresponding aminoacids **6b–d** were 62, 47 and 88%, respectively. Aldehydes **11b–d** were reacted with a variety of phenylmagnesium bromides in THF at low temperature to afford the secondary alcohols **12**, which were once again oxidized with the Dess–Martin periodinane to afford the desired α-acylaminoketones **2** (Table 2).[19] The last two steps of the sequence proved to be amenable to manual parallel synthesis. Aqueous workups were performed using Varian Chem-Elut cartridges[20] and polymer supported tosyl hydrazide was used to scavenge unreacted aldehyde from ketone product. Fractionation of the final products on silica gel SPE cartridges routinely afforded material of >85% purity. Yields for conversion of aldehydes **11** to α-acylaminoketones **2** were not optimized and ranged from 9 to 60%. The lowest yields were encountered with products derived from hindered aldehyde **11d**. All compounds were characterized by ^1H and ^{13}C NMR and selected compounds were more fully characterized.[21]

Table 3. Dose response transactivation assay results[a]

	X	Y	LC_{50} (μM)[b]/Rel Max FI[c] 2b –$(CH_2)_4$–	2c Et,Et	2d Me, *i*-Pr
f	2-Me–3-MeO	H	2.7/0.14	[d]	33.3/0.04
g	2-Me–3-MeO	2-Me	[d]	[d]	18.0/0.26
h	2-Me–3-MeO	2-MeO	5.2/0.70	6.2/0.29	15.0/0.20
i	2-Me–3-MeO	3-Me	3.4/0.46	6.6/0.25	8.6/0.47
j	2-Me–3-MeO	3-MeO	8.8/0.12	33.3/0.01	25.5/0.12
k	2-Me–3-MeO	4-Me	[d]	[d]	20.0/0.05
l	2-Me–3-MeO	4-MeO	[d]	[d]	[e]
m	2-Me–3-MeO	4-F	15.0/0.14	13.7/0.04	[e]
n	2-Me–3-MeO	3-Me-4-F	3.5/0.43	10.4/0.07	41.8/0.24
o	2-Me–3-MeO	3,5-diMe	1.9/0.85	4.5/0.60	2.1/0.75

[a]See ref 15 for assay protocol.
[b]Dose affording 50% of maximum transactivation.
[c]Ratio of maximum level of gene expression of compound to maximum level of gene expression with **1d**.
[d]Compound not tested.
[e]Compound not made.

The initial screening results of the focused library of α-acylaminoketones are shown in Table 2. Library members which caused >50-fold induction of β-galactosidase expression at 33 μM were screened in a dose response assay in the same cell line. The results are reported in Table 3 in terms of LC_{50} and maximum fold induction compared to GS™–E (**1d**). An effective ligand must combine a low LC_{50} value with a high maximum fold induction. By these criteria, α-acylaminoketones **2bo** and **2do**, which can be considered as isosteres of the commercial insecticide methoxyfenozide (**1c**), were the best compounds in the library.

In conclusion, we have described the discovery of a new class of ecdysone agonists useful for the control of gene expression in ecdysone responsive systems. Levels of reporter gene expression induced by the most potent compounds **2bo** and **2do** approached those seen with the GS™–E (**1d**).

Acknowledgements

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15. A bulk transformed HEK-293 cell line, created as described in ref 5, was provided by Gage and Suhr. Dilution cloning was used to isolate individual clones. Clone Z3 was selected using 450 μg mL^{-1} G418 and 100 ng mL^{-1} puromycin. Cells

were trypsinized and diluted to a concentration of 2.5×10^4 cells mL^{-1}. One hundred microliters of cell suspension was placed in each well of a 96 well plate (Dynex, 14-245-182) and incubated at 37 °C under 5% CO_2 for 24 h. Ligand stock solutions were prepared (10 mM in DMSO) and diluted 100-fold in media. 50 μL of diluted ligand solution was added to each well. The final concentration of DMSO was maintained at 0.03% in both controls and treatments. β-Galactosidase reporter gene expression was measured 48 h after treatment of the cells using Gal Screen™ bioluminescent reporter gene assay system from Tropix (GSY1000). Luminescence was detected at room temperature using a Dynex MLX microtiter plate luminometer. Fold inductions were calculated by dividing relative light units (RLU) in ligand treated cells with RLU in DMSO treated cells.

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19. The following procedure for the preparation of **2di** is typical. (1) **Preparation of 7d**: To a stirred suspension of α-methylvaline (2.62 g, 20 mmol) in pyridine (40 mL) cooled to ~5 °C was added solid 3-methoxy-2-benzoyl chloride (8.31 g, 45 mmol). The mixture was allowed to warm to rt, stirred for 1 week, evaporated under reduced pressure to remove pyridine and taken up in ether (150 mL) and water (50 mL). The organic layer was separated, washed with 5% aq HCl (50 mL) and satd aq $NaHCO_3$ (50 mL), and dried over $MgSO_4$. Removal of the solvent left **7d** (6.41 g, 122%) as an oil. ^1H NMR (300 MHz, $CDCl_3$) δ 0.97 (d, $J = 6.6$ Hz, 3H), 1.09 (d, $J = 6.6$ Hz, 3H), 1.51 (s, 3H), 2.12 (m, 1H), 2.50 (s, 3H), 3.85 (s, 3H), 7.02 (m, 1H), 7.24 (m, 1H), 7.40 (m, 1H). (2) **Preparation of 10d**: To a stirred solution of **7d** (1.76 g, 6.7 mmol) in THF (30 mL) at rt was added solid sodium borohydride (0.15 g, 4.0 mmol). The mixture was stirred for 16 h. Removal of the solvent under reduced pressure left a white glassy solid which was taken up in CH_2Cl_2 (150 mL), washed with 1% aq HCl (50 mL) and satd aq $NaHCO_3$ (50 mL) and dried over $MgSO_4$. Removal of the solvent left **10d** (1.25 g, 69%) as a white solid, mp 142–145 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 0.97 (d, $J = 6.6$ Hz, 3H), 0.98 (d, $J = 6.6$ Hz, 3H), 1.11 (s, 3H), 2.26 (s, 3H), 2.50 (m, 1H), 3.75 (m, 2H), 3.84 (s, 3H), 5.39 (br s, 1H), 5.83 (br s, 1H), 6.90 (m, 2H), 7.18 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.4, 16.8, 17.0, 18.3, 31.1, 55.5, 62.4, 68.1, 111.2, 118.1, 124.0, 126.6, 138.2, 157.9, 171.3. (3) **Preparation of 11d**: To a stirred solution of **10d** (285 mg, 1.1 mmol) in CH_2Cl_2 (10 mL) at rt was added Dess–Martin periodinane solution (15% by weight, 2.4 mL, ~1.1 mmol). The mixture was stirred at rt for 4 h and poured into satd aq $NaHCO_3$ (50 mL). Solid $Na_2S_2O_3$ (2.13 g, 8.6 mmol) was added and the mixture was stirred for 0.5 h. The mixture was extracted with ether (150 mL). The ether extract was washed with satd aq $NaHCO_3$ (50 mL), dried and evaporated under reduced pressure to afford **11d** (293 mg, 103%) as an oil. ^1H NMR (300 MHz, $CDCl_3$) δ 0.98 (d, $J = 6.6$ Hz, 3H), 1.04 (d, $J = 6.6$ Hz, 3H), 1.51 (s, 3H), 2.27 (s, 3H), 2.29 (m, 1H), 3.84 (s, 3H), 6.30 (br s, 1H), 6.91 (m, 1H), 6.96 (m, 1H), 7.18 (m, 1H), 9.60 (s, 1H). (4) **Preparation of 2di**: An oven dried vial equipped with a stir bar was flushed with N_2, charged with a stock solution of **11d** in dry THF (1 mL of 0.5 M, 0.5 mmol) and cooled in dry ice acetone. 3-Methyl-phenylmagnesium bromide in THF (1.0 M, 2 mL, 2.0 mmol) was added and the mixture was stirred at rt for 2 h. The reaction was quenched by addition of satd aq $NaHCO_3$ (5 mL), applied to a 10-mL ChemElut cartridge, allowed to stand for 5 min and eluted with CH_2Cl_2 (25 mL). The eluate was evaporated to leave crude carbinol **12di** (180 mg). To a stirred solution of crude **12di** in CH_2Cl_2 (2 mL) was added Dess–Martin periodinane (1.4 mL, 15 wt.% in CH_2Cl_2, ~0.65 mmol). The mixture was stirred at rt for 6 h, diluted with satd aq $NaHCO_3$ (5 mL) and treated with solid $Na_2S_2O_3$ (~1 g, 6.3 mmol). The mixture was stirred for 0.5 h, applied to a 10-mL ChemElut cartridge, allowed to stand for 5 min and eluted with CH_2Cl_2 (20 mL). The eluate was evaporated to leave crude ketone **2di** (95 mg). The crude ketone was taken up in CH_2Cl_2 (4 mL) treated with Argonaut PS-TsNHNH$_2$ resin (0.20 g, 2.9 mmol g^{-1}, 0.58 mmol) and allowed to stand for 6 h. The mixture was filtered and washed with CH_2Cl_2 and ether. The filtrate was evaporated to leave a solid which was fractionated on a 2 g silica cartridge eluted sequentially with 0, 25, 50, 75 and 100% ether in hexanes (10 mL of each). The 4th fraction (75% ether in hexanes) contained ketone **2di** (27 mg, 15%) as an off-white solid. ^1H NMR ($CDCl_3$) δ 0.94 (d, $J = 6.8$ Hz, 3H), 1.09 (d, $J = 6.7$ Hz, 3H), 1.67 (s, 3H), 2.01 (s, 3H), 2.37 (s, 3H), 2.50 (m, 1H), 6.36 (br s, 1H), 6.76 (d, $J = 7.6$ Hz, 1H), 6.84 (d, $J = 8.1$ Hz, 1H), 7.12 (m, 1H), 7.40 (m, 2H), 7.78 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.1, 17.1, 17.4, 17.5, 21.5, 34.3, 55.6, 67.5, 111.3, 118.3, 125.0, 125.1, 126.5, 127.7, 129.2, 132.5, 136.7, 137.6, 137.9, 158.0, 168.9, 201.0. The 3rd fraction (50% ether in hexanes) contained additional **2di** (34.5 mg, 20%) of lower purity.

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21. Characterization of **2bo**: mp 130–132 °C. ^1H NMR ($CDCl_3$) δ 1.80 (m, 4H), 1.85 (s, 3H), 2.06 (m, 2H), 2.31 (s, 6H), 2.58 (m, 2H), 3.77 (s, 3H), 6.46 (br s, 1H), 6.52 (d, $J = 7.6$ Hz, 1H), 6.79 (d, $J = 8.1$ Hz, 1H), 7.04 (t, $J = 7.9$ Hz, 1H), 7.10 (s, 1H), 7.46 (s, 2H)). ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.7, 21.3, 24.9, 37.8, 55.6, 70.9, 111.2, 118.2, 124.8, 126.1, 126.3, 133.2, 136.7, 137.3, 137.4, 157.9, 169.0, 201.7. IR ($CDCl_3$) 3431, 1684, 1664 cm^{-1}. MS (ESI, +ve ion) m/z 366 (M + 1).