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Synthesis and SAR of *cis*-1-Benzoyl-1,2,3,4-tetrahydroquinoline Ligands for Control of Gene Expression in Ecdysone Responsive Systems

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Abstract—A library of 35 *cis*-1-benzoyl-2-methyl-4-(phenylamino)-1,2,3,4-tetrahydroquinolines was prepared. The compounds bore various substitutuents on the benzoyl ring, at the 4-position of the phenylamino ring and at the 6-position of the tetrahydroquinoline ring. The compounds were assayed for their ability to cause expression of a reporter gene downstream of an ecdysone response element in a mammalian cell line engineered to express the ecdysone receptor from *Aedes aegypti*. In general, compounds with small lipophilic substituents at the *meta* and *para*-positions of the benzoyl ring and hydrogen or fluorine at the 4-position of the phenylamino ring and the 6-position of the tetrahydroquinoline ring were the most potent.

The ecdysone receptor (EcR)[1] is a nuclear hormone receptor (NR) that plays a pivotal role in insect metamorphosis and development.[2] Despite similar domain organization and homology to mammalian NRs, EcRs and their ligands are orthogonal to the mammalian receptors. This property has made EcR an attractive target for environmentally benign insecticides[3] and, more recently, for use in systems to control gene expression in transgenic organisms.[4–8] As part of our effort to develop the RHeoPlex™ system of orthogonal 'gene switches' based on natural and mutated ecdysone receptors, we sought novel synthetic ecdysone agonists (Fig. 1) with distinctly different structures than the natural ecdysteroids for example, **1** and the synthetic diacylhydrazines for example, **2**.[9,10]

Cullen, Dixson and co-workers recently reported that *cis*-1-benzoyl-4-(phenylamino)-1,2,3,4-tetrahydroquinolines

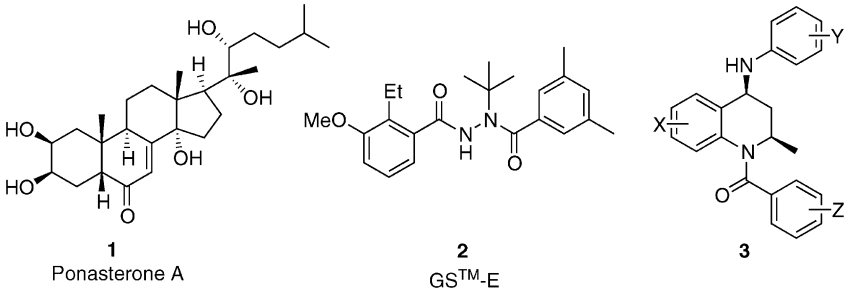

Figure 1. Ecdysone agonists.

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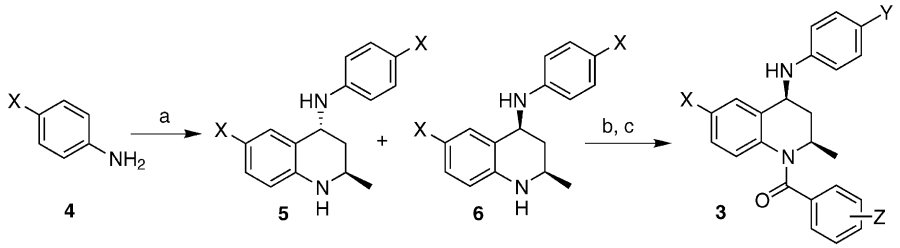
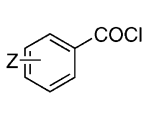


Scheme 1. Synthesis of *cis*-1-benzoyl-2-methyl-4-phenylamino-1,2,3,4-tetrahydroquinolines. (a) MeCHO, (+/−) 1,2,3-benzotriazole, EtOH, rt, ≥4 days; (b) Separate **5** and **6** by flash chromatography; (c) Z-C_6H_4COCl, PS-NMM, CH_2Cl_2, rt, 1 day followed by PS-trisamine, 3 h.

of general structure **3** bind to the *Drosophila melanogaster* EcR and possess weak insecticidal activity against *Heliothis virescens*.[11,12] Compounds of general structure **3** have also been claimed to promote the secretion of soluble β-amyloid precursor protein[13] and the formation of apolipoprotein A-I.[14] Below we report the synthesis and SAR of a library of compounds of general structure **3** for the control of gene expression in ecdysone responsive systems.

Compounds of general structure **3**, in which the substituents X and Y are identical and located at the 6- and 4′ positions, were readily prepared as shown in Scheme 1. Thus, reaction of aniline **4a** with an equimolar amount of acetaldehyde for 4 days at room temperature gave a mixture of *trans*- and *cis*-diamines **5a** and **6a** in 17% yield; similarly, **4b** and **4c** afforded diamine products **5b** + **6b** and **5c** + **6c** in 15 and 33% yields, respectively.[15] The conversion of **4b** to **5b** + **6b** was studied in somewhat greater detail. Addition of 0.2 equiv of 1,2,3-benzotriazole to the reaction was shown to give a cleaner crude product and a slightly improved overall yield of 18% after 4 days.[16,17] Extended reaction times (30 days) afforded **5b** + **6b** in 38% yield but attempts to accelerate the reaction by heating lead to reduced yields and formation of numerous byproducts. Careful chromatography separated the desired *cis* isomers **6a–c** from the *trans* isomers **5a–c**. The stereochemical assignments of **5** and **6** were based on the work of Funabashi et al.[18]

The *cis* diamine isomers **6a–c** were treated with benzoyl chlorides **7a–m** (Fig. 2) to produce the library of 35 compounds shown in Table 1. To streamline the execution of the benzoylation reactions, they were performed at room temperature with 1.2 equiv of the benzoyl chloride in the presence of morpholinomethylpolystyrene. Trisamine functionalized polystyrene scavenger resin was used to remove unreacted benzoyl chloride.[19,20] In all cases **6a–c** were selectively monobenzoylated on the ring nitrogen even under these operationally straightforward conditions; subsequent acylation of the exocyclic nitrogen proceeded only sluggishly on treatment with excess benzoyl chloride in the presence of pyridine.[21] Initial purification on silica SPE cartridges was followed by reverse phase preparative HPLC. Yields of target compounds ranged from 11% (**3aa**) to 67% (**3ce**). Overall derivatives of diamine **6c** were formed in the best yields. All compounds were characterized by ^{1}H NMR and by ^{19}F NMR when fluorine was present. Selected compounds were more fully characterized.[22]



a Z = H **b** 2-F **c** 2-Me **d** 2-MeO **e** 2-CF_3 **f** 3-F **g** 3-Me **h** 3-MeO **i** 3-CF_3 **j** 4-Cl **k** 4-Me **l** 4-MeO **m** 4-CF_3

Figure 2. Benzoyl chlorides used for library production.

The 35-compound library was screened at 33 μM in a cell line which expressed the EcR derived from *Aedes aegypti* (AaEcR) and a luciferase gene under the control of an ecdysone response element.[23] The results are presented in Table 1 as fold induction relative to a DMSO control. All but two of the library members (**3ae**, **3cd**) showed measurable increases in luciferase expression; however, the increases were most pronounced for those compounds in which X = Y = F. To gain further insight into the activity and SAR trends of these compounds, 33 of them were advanced to a dose response version of the same assay. The results are reported in Table 2 in terms of EC_{50} and maximum fold induction compared to GS™-E (**2**) as a positive control. An effective ligand must combine a low EC_{50} value with a high relative

Table 1. Single dose assay results using AaEcR[a]

		Fold Induction[b,c] (33 μM)		
	Z	**3a** (X, Y = H)	**3b** (X, Y = F)	**3c** (X, Y = Me)
a	H	20	283	35
b	2-F	[d]	78	5
c	2-Me	[d]	158	19
d	2-MeO	[d]	4	0
e	2-CF_3	1	64	12
f	3-F	75	204	102
g	3-Me	[d]	686	6
h	3-MeO	9	119	3
i	3-CF_3	28	89	12
j	4-Cl	130	212	43
k	4-Me	64	91	121
l	4-MeO	64	110	57
m	4-CF_3	89	230	69

[a]See ref 23 for assay protocol.
[b]Ratio of light measured in treated cells versus a DMSO control.
[c]Average of two replicates.
[d]Compound not made.

Table 2. Dose response assay results using AaEcR[a]

		EC_{50} (μM)[b]/Rel Max FI[c]		
	Z	**3a** (X = H)	**3b** (X = F)	**3c** (X = Me)
a	H	1.12/0.07	1.73/0.76	5.40/0.19
b	2-F	[d]	3.00/0.28	33.30/0.01
c	2-Me	[d]	3.19/0.50	8.71/0.04
d	2-MeO	[d]	33.30/0.01	[e]
e	2-CF_3	[e]	10.99/0.32	33.30/0.04
f	3-F	0.75/0.44	0.99/0.57	3.00/0.27
g	3-Me	[d]	1.36/0.55	33.30/0.01
h	3-MeO	0.70/0.15	1.48/0.98	33.30/0.01
i	3-CF_3	1.21/0.42	1.00/0.93	1.84/0.47
j	4-Cl	0.98/0.43	0.64/0.70	2.15/0.39
k	4-Me	0.99/0.42	0.87/0.67	5.00/0.26
l	4-MeO	1.43/0.44	1.14/0.87	5.32/0.09
m	4-CF_3	1.18/0.64	0.92/0.63	4.41/0.23

[a]See ref 23 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 **2**.
[d]Compound not made.
[e]Compound not tested.

maximum fold induction. Library members derived from **6c** were much less effective than those derived from **6a** and **6b**. Introduction of substituents at the *ortho* position on the benzoyl ring reduced activity compared to the parent compounds while substition at the *meta* and *para* positions generally improved potency. The *meta*-CF_3 (**3bi**) and *para*-Cl compounds (**3bj**) derived from difluorodiamine **6b** were of particular interest; however, none of the library compounds equaled the potency of the standard ecdysone agonist ligand **2**, whose $EC_{50} = 0.44\,\mu M$ in this assay.

We have described the synthesis and SAR of a first generation optimization library of *cis*-1-benzoyl-1,2,3,4-tetrahydroquinolines of general structure **3**. These compounds are promising leads for use as inducers in systems to control gene expression based on AaEcR.

Acknowledgements

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17. Preparation of ***cis*-6-fluoro-2-methyl-4-(4-fluorophenylamino)-1,2,3,4-tetrahydroquinoline** (**6b**): To a stirred solution of 4-fluoroaniline (**4b**) (3.79 mL, 40.0 mmol) and benzotriazole (0.95 g, 8.0 mmol, 0.2 equiv) in absolute ethanol (40 mL) was added acetaldehyde (2.24 mL, 40.0 mmol). The mixture was stirred at room temperature for 4 days. The solvent was removed under reduced pressure. The oily crude product was taken up in ether (175 mL), washed with 1% aq HCl (50 mL) and immediately with saturated aqueous $NaHCO_3$ (50 mL). The ether solution was dried over Na_2SO_4 and the solvent was removed under reduced pressure to leave an oily solid (2.93 g) which was chromatographed on a 40-g silica cartridge eluted sequentially with 0, 10, 20, 30, 40 and 50% ether in hexanes (100 mL of each) to afford a ca. 1:1 mixture of **5b** and **6b** (1.03 g, 18%). A second chromatography on a 40-g silica gel cartridge eluted sequentially with 0, 5, 10, 15, 20, 25, 30, 40 and 50% ether in hexanes (100 mL of each) afforded, in order of elution, **5b** (0.30 g, 5%) as an oil, a mixture of **5b** and **6b** (0.34 g, 6%) as an oily solid and **6b** (0.23 g, 4%) as a beige solid. *trans*-6-fluoro-2-methyl-4-(4-fluorophenylamino)-1,2,3,4-tetrahydroquinoline (**5b**): ^1H NMR ($CDCl_3$) δ 1.22 (d, $J = 6.2$ Hz, 3H), 1.56 (m, 1H), 2.12 (m, 1H), 3.38 (m, 1H), 3.78 (br s, 2H), 4.43 (br s, 1H), 6.47 (m, 1H), 6.57 (m, 2H), 6.80 (m, 1H), 6.92 (m, 3H); ^{19}F NMR ($CDCl_3$) δ −127.9, −128.2; ^{13}C NMR ($CDCl_3$) δ 22.0, 34.9, 42.6, 49.5, 113.7, 115.5, 115.9, 116.3, 116.4, 122.1, 141.3, 142.6, 154.7, 156.6. IR ($CDCl_3$) 3427 cm^{-1}. MS (EI) *m*/*z* 274, 164, 148. *cis*-6-fluoro-2-methyl-4-(4-fluorophenyl amino)-1,2,3,4-tetrahydroquinoline (**6b**): Mp. 120–122 °C. ^1H NMR ($CDCl_3$) δ 1.22 (d, $J = 6.3$ Hz, 3H), 1.44 (m, 1H), 2.29 (m, 1H), 3.55 (m, 3H), 4.67 (m, 1H), 6.42 (m, 1H), 6.58 (m, 2H), 6.73 (m, 1H), 6.88 (m, 2H), 7.11 (m, 1H); ^{19}F NMR ($CDCl_3$) δ-127.3,-128.0; ^{13}C NMR ($CDCl_3$) δ 22.4, 37.5, 47.2, 51.1, 113.3, 114.2, 114.7, 114.9, 115.8, 124.6, 141.2, 143.8, 154.9, 156.8. IR ($CDCl_3$) 3419 cm^{-1}. MS (EI) *m*/*z* 274, 164, 148. Anal. calcd for $C_{16}H_{16}F_2N_2$: C, 70.06; H, 5.88; F, 13.85; N, 10.21. Found: C, 70.08; H, 5.67; N, 10.16.
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20. Preparation of ***cis*-2,6-dimethyl-1-(4-methoxy benzoyl)-4-(4-methylphenylamino)-1,2,3,4-tetrahydro quinoline** (**3cl**) To a vial was added PS-NMM resin (400 mg, 1.87 mmol g^{-1},

0.75 mmol) and *cis*-diamine **6c** (69 mg, 0.25 mmol) in CH_2Cl_2 (4 mL). A solution of 4-methoxybenzoyl chloride (**7l**, 51 mg, 0.3 mmol) in CH_2Cl_2 (2 mL) was added and the reaction stirred at rt for 18 h. AP-trisamine resin (100 mg, 2.71 mmol g^{-1}, 0.27 mmol) was added and the mixture was stirred for 3 h. The resins were removed by filtration and washed with CH_2Cl_2 and ether. The filtrate was evaporated and the residue was chromatographed on a 2 g silica gel SPE cartridge eluted sequentially with 0, 10, 25, 50, 75, and 100% ether/hexanes (10 mL of each) to give an oil which was further purified by reverse phase preparative HPLC (C-18 column, H_2O: MeCN gradient) to give **3cl** as a white foam (51 mg, 51% yield). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.25 (d, J = 6.2 Hz, 3H), 1.33 (m, 1H), 2.25 (s, 3H), 2.28 (s, 3H), 2.78 (m, 1H), 3.74 (s, 1H), 3.78 (s, 3H), 4.39 (m, 1H), 4.86 (m, 1H), 6.45 (d, J = 8.0 Hz, 1H), 6.64 (d, J = 8.0 Hz, 2H), 6.74 (m, 3H), 7.06 (d, J = 8.0 Hz, 2H), 7.16 (s, 1H), 7.23 (d, J = 8.5 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 20.4, 21.2, 21.3, 41.4, 48.2, 50.0, 55.2, 113.1, 113.4, 124.3, 126.7, 127.3, 127.5, 128.1, 130.0, 130.7, 134.8, 135.1, 136.1, 145.0, 160.9, 168.9.

21. By contrast, treatment of the *trans*-diamines **5a**–**c** with one equivalent of a benzoyl chloride, even under carefully controlled conditions, always afforded a mixture of starting diamine, product that was monoacylated on the ring nitrogen and diacylated product. In no case was the product of monoacylation on the exocyclic nitrogen isolated.

22. Compound **3aj**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.28 (d, J = 6.3 Hz, 3H, CHCH_3), 1.39 (m, 1H), 2.82 (m, 1H), 3.88 (d, J = 6.7 Hz, 1H, ArNH), 4.46 (m, 1H, ArNHCH), 4.90 (m, 1H, CHCH_3), 6.53 (d, J = 7.8 Hz, 1H), 6.70 (d, J = 8.0 Hz, 2H), 6.80 (t, J = 7.3 Hz, 1H), 6.96 (t, J = 7.4 Hz, 1H), 7.10 (t, J = 7.4 Hz, 1H), 7.23 (m, 6H), 7.34 (d, J = 8.1 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 21.2, 41.2, 48.4, 49.6, 113.2, 118.2, 124.1, 125.8, 126.9, 127.0, 128.3, 129.5, 130.2, 134.3, 136.2, 136.4, 136.7, 147.1, 168.2; IR ($CDCl_3$) 3100, 1631, 1311; MS (ESI, + ve ion) m/z 377 (M + 1).

23. 3T3 cells were trypsinized and plated at 2.5×10^3 cells/well on a 96-well plate. After incubation for 24 h at 37 °C under 5% CO_2, cells were transfected with the inducible expression system in serum free media using Superfect (Qiagen). The expression system consisted of: Gal4DBD/AaEcR (DEF), encoding the D, E and F domains of AaEcR and the GAL4DBD under the control of the CMV promoter; VP16βRXR (E), encoding the E domain from mouse (*Mus musculus*) RXR fused to the carboxyl terminus of the activation domain from VP16 under the control of the SV40 promoter; and the reporter plasmid, pFRLuc, containing 5XGAL4 response element and the firefly luciferase gene. After transfection for 4 h at 37 °C, the cells were treated with ligand in serum media. Ligand stock solutions were prepared in DMSO and diluted 300-fold for all treatments. Single dose testing was performed at 33 μM, while dose response testing was run at 8 concentrations ranging from 33 to 0.01 μM. Luciferase reporter gene expression was measured 48 h after cell treatment using Bright-Glo™ Luciferase Assay System from Promega (E2650). Luminescence was detected at room temperature using a Dynex MLX microtiter plate luminometer. Fold inductions were calculated from single dose testing by dividing relative light units (RLU) in ligand treated cells by RLU in DMSO treated cells. EC_{50}s were calculated from dose response data using a three parameter logistic model.