An Automated, Cell-based Platform for the Rapid Detection of Novel Androgen Receptor Modulators

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Introduction

The Androgen Receptor (AR) is a member of the family of nuclear receptors responsive to steroid hormones. AR activity is regulated through the binding of androgenic hormones (eg., testosterone and di-hydroxy testosterone). In turn, AR functions to regulate the expression of a myriad of genes involved in metabolic processes, cell proliferation/apoptosis, and male sexual differentiation and development. AR dysfunction often has severe health implications, such as androgen insensitivity syndrome and prostate cancer. Consequently, AR commands much attention as a target for the discovery and development of improved drugs. In particular, anti-androgens comprise an important class of drugs used in the treatment prostate cancer.

The aim of the present work was to devise, validate and perform a preliminary (small-scale) automated HTS screening campaign to identify novel modulators of AR activity. The study incorporated a new whole-cell, luciferase-based assay to quantify human Androgen Receptor activity. The performance and specificity of this AR assay were first confirmed using a small commercial library of known nuclear receptor modulators. The validated AR assay was then scaled-up and deployed to screen a library comprising 506 diverse small-molecule compounds of natural origin. The natural compound library was functionally interrogated to identify both activators and inhibitors of AR. Chemicals identified in the primary screen as putative AR inhibitors were further analyzed using a live cell multiplex assay. The multiplexed assay incorporates a fluorescent Calcein-AM assay to monitor viable cell number, as well as a luminescent AR assay, which were performed sequentially in the same assay well. Using this format, cytotoxic compounds that elicited false-positive 'hits' were eliminated from further consideration. Test compounds displaying novel bio-activities to AR, either agonist or antagonist, were further analyzed to establish relative potency values.

Automation of the initial library screens was carried out using an easy-to-use, robust instrumentation. An 8-channel liquid handler was used to prepare serial dilutions of library and reference compounds, followed by their transfer into 384-well assay plates. Subsequent additions of AR reporter cells and detection reagent were performed using a high-speed non-contact reagent dispenser. The small footprint of the instrument, as well as capability to autoclave the dispensing pathway, allowed for sterile manipulations during the assay process. A combination washer/dispenser, capable of media and reagent removal, as well as dispensing cells and other components was then used to perform the live cell multiplex assay. Results demonstrate how the combination of the novel, cell-based chemistries and automation can be used to rapidly, and accurately identify modulators of this important drug target.

BioTek Instrumentation

Liquid Handling

The **Precision™ Microplate Pipetting System** combines an 8-channel pipetting head and an 8-channel bulk reagent dispenser in one instrument. The instrument was used to dilute the library compounds, create compound dose response curves for validation and secondary screening, and transfer all compounds to the 96- and 384-well assay plates.

The **EL406™ Combination Washer Dispenser** offers fast, accurate media removal and plate washing capabilities through its Dual-Action™ Manifold. It also offers reagent dispensing capabilities through the use of its peristaltic or syringe pumps. The instrument was used for cell dispensing, media removal, buffer wash, as well as LCMA and detection substrate addition in the LCM assay.

Detection

The **Synergy™ H4 Hybrid Multi-Mode Microplate Reader** combines a filter-based and monochromator-based detection system in the same unit. A dedicated luminescence detection system was used to quantify the luminescent signal from the Androgen Receptor Assay, while the filter-based system was used to detect the fluorescent cell viability signal, which is part of the Live Cell Multiplex Assay.

INDIGO AR Assay: Primary and Secondary Screening

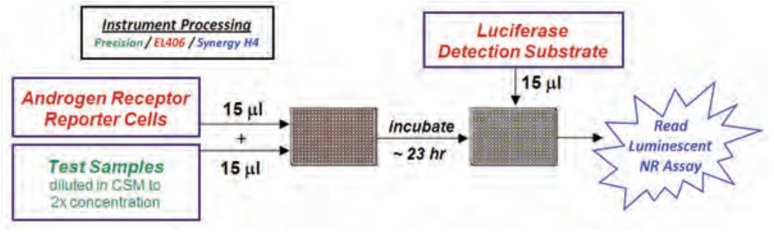


Figure 1 – INDIGO Human Androgen Receptor Reporter Assay System. Reporter cells include the luciferase reporter gene functionally linked to an AR-responsive promoter. Therefore modulators of AR activity will lead to an increase or decrease in luminescent output, relative to a basal signal.

Diluted compounds and reporter cells are added to the 384-well assay plates. Following incubation at 37°C/5% CO_2, detection substrate is then added, and the luminescent signal is quantified.

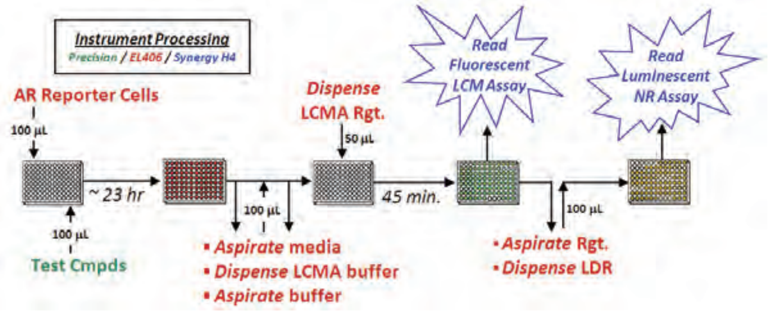

Instrument Processing
Precision / EL406 / Synergy H4
AR Reporter Cells
100 µl
~23 hr
100 µl
Test Cmpds
Read Fluorescent LCM Assay
Dispense LCMA Rgt.
50 µL
100 µL
Aspirate media
Dispense LCMA buffer
Aspirate buffer
45 min.
Read Luminescent NR Assay
100 µL
Aspirate Rgt.
Dispense LDR

Figure 2 – INDIGO 96-well format LIVE Cell Multiplex (LCM) Reporter Assay allows secondary screening of test compounds to assess cell toxicity. In addition to the AR reporter system, the assay also utilizes the fluorogenic substrate Calcein-AM (LCMA Reagent) to provide a quantitative measure of the relative number of live cells remaining in the assay wells following exposure to test compounds. In this way, "False-positive" antagonist hits are identified and eliminated from further analyses.

Following cell and compound addition and incubation, media is washed from the wells and the LCMA Reagent is added. The cell-permeable molecule, once taken into the cells, is hydrolyzed to generate the fluorescent molecule Calcein by endogenous esterases found in intact living cells. Following incubation at 37°C/5% CO_2 the fluorescent signal is quantified. LCMA reagent is then removed from the wells and LDR is added, as with the AR Reporter Assay System. The luminescent signal is then quantified, which is proportional to AR activity in the well.

Automated AR Assay Validation

A Z'-factor test was performed to validate the automated 384-well AR assay. 6α-FIT was used as the control compound. Twenty-four replicates of 10 µM or 0 µM compound were used as the positive and negative control, respectively. Dose response curves were also created using the control agonist, 6α-Fluoro-Testosterone (6α-FIT), and the control antagonist, Bicalutamide (BIC), to validate the ability of the automated assay to generate correct compound pharmacology.

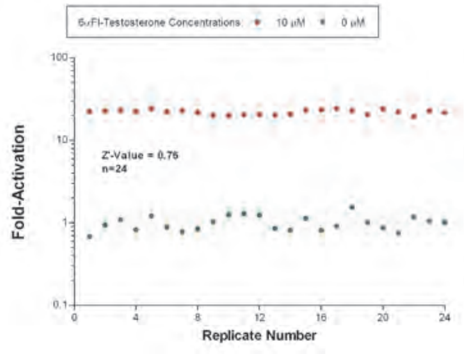


Figure 3 – Z'-factor validation data. Z' values ≥0.5 are indicative of a robust assay for HTS (Zhang et al., 1999).

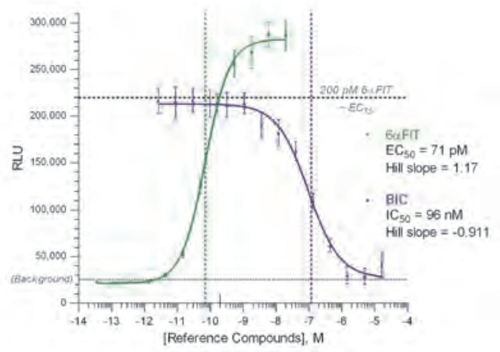


Figure 4 – EC_{50} value of 71 pM for 6α-FIT, and IC_{50} value of 96 nM for BIC are equivalent to values previously generated using manual methods.

NR Ligand Library Primary Screen

The 76 member Screen-Well® Nuclear Receptor Ligand Library (generously contributed by ENZO Biochem), containing compounds with known bioactivity to Nuclear Receptors, was used to validate the automated 384-well Human Androgen Receptor Assay setup for screening purposes.

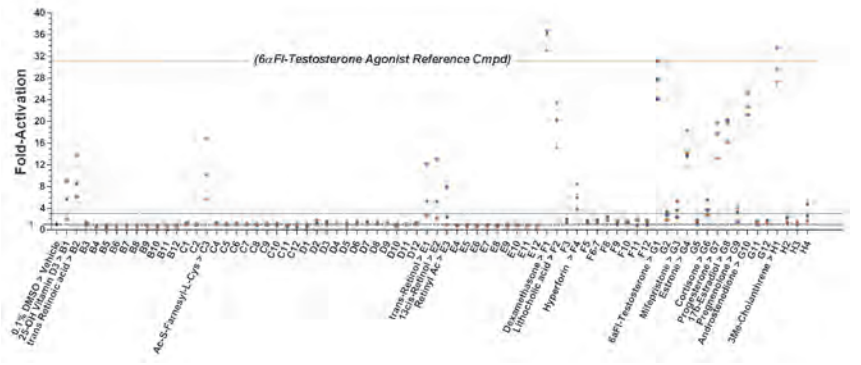


Figure 5 – Human Androgen Receptor Assay, Agonist Validation Screen using Screen-Well® Nuclear Receptor Ligand Library, BML-2802.

Test compounds were prepared at three sub-concentrations, then screened (*n*=6) at the following concentrations for agonist activities against AR: • 10 µM, • 2.0 µM, and • 0.40 µM. 0.1% DMSO (◆) was included as the vehicle control. 6α–Fluoro-Testosterone, a common reference agonist for AR, is resident within the library (▪; Well G1). Fold-activation is defined as [$RLU^{Test\ Cmpd}$ / $RLU^{Vehicle\ Control}$].

Table 1. Summary of Positive Hits from the Androgen Receptor AGONIST Screen. Screen-Well® Nuclear Receptor Ligand Library, BML-2802 Version 2.2

Well location	Test Compound Common Name	Fold-ACTIVATION of Human AR at these concentrations			Known BioActivities
		10 µM	2.0 µM	0.40 µM	
Vehicle	DMSO, 0.1%	1.00	1.00	1.00	
B1	25-OH-Vitamin D3	9.06	5.71	2.06	Calcifediol; prohormone of calcitriol, a major circulating form of vitamin D; VDR agonist
B2	Retinoic acid, all *trans*	13.8	8.61	6.09	Derivative of Vitamin A; Endogenous agonist for retinoic acid receptors (RAR's)
C3	Ac-S-Farnesyl-L-Cysteine	16.8	10.1	5.76	Inhibits S-farnesylcysteine methyl transferase
E1	Retinol, all-*trans*	12.1	5.30	2.64	Vitamin A; RXR beta agonist
E2	13-*cis*-Retinol	13.0	5.27	2.14	Catabolic metabolite possibly involved in regulating 11-cis-retinol concentrations
E3	Retinyl acetate	7.87	2.40	1.00	Vitamin A Acetate; a natural form of vitamin A
F1	Dexamethasone	36.6	36.2	33.1	GR ligand agonist with anti-inflammatory activity
	Fold activaiton of luciferase in 'Mock' Reporter Cells →	2.6	2.2	1.2	
F2	Lithocholic acid	20.2	23.4	15.2	Natural ligand for VDR and the PXR; activates VDR without raising calcium levels
F4	Hyperforin	5.88	8.49	3.86	Activator of the pregnane X receptor (PXR) a key regulator of CYP3A4 transcription
G1	6α-Fluro-Testosterone	24.2	27.7	31.2	Common Reference Agonist for Androgen Receptor (AR)
	CV =	6.1%	5.5%	4.2%	
	Z' =	0.800	0.819	0.862	
G3	Mifepristone	2.42	3.63	5.30	Antagonist at progesterone (PR) and glucocorticoid (GR) receptors
G4	Estrone	13.7	18.3	14.2	Natural estrogen and agonist for the estrogen receptors
G6	Cortisone	3.63	5.52	2.71	Adrenal glucocorticoid released during stress; increases gluconeogenesis
G7	Progesterone	17.7	19.7	13.1	Endogenous progesterone receptor (PR) agonist
G8	17β-Estradiol	20.2	19.7	16.2	Endogenous estrogen receptor (ER) agonist
G9	Pregnenolone	1.47	3.28	3.94	PXR Ligand, Cytochrome P-450 inducer
G10	Androstenedione	21.2	22.7	25.2	Precursor to testosterone and estradiol; Weak selective receptor modulator for AR
H1	3-Me-Cholanthrene	33.7	29.6	27.3	Aryl hydrocarbon receptor (AhR) agonist

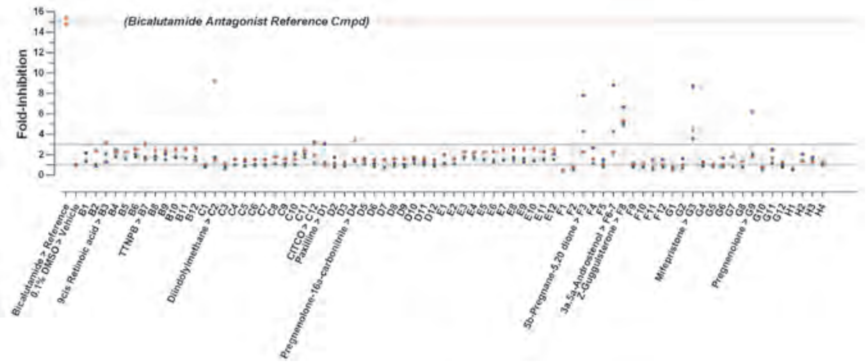


Figure 6 – Human Androgen Receptor Assay, Antagonist Validation Screen using Screen-Well® Nuclear Receptor Ligand Library, BML-2802.

Test compounds were prepared at three sub-concentrations, then screened (*n*=6) at the following concentrations for antagonist activities: • 10 µM, • 2.0 µM, and • 0.40 µM. AR Reporter cells were pre-treated with 200 pM (~EC_{75}) of 6α–Fluoro-Testosterone as the challenge agonist. 0.1% DMSO (◆,vehicle) and 5.0 µM Bicaluatamide (▪, Reference antagonist) were tested as controls. Mifepristone, another common reference antagonist for AR, is resident within the library (▪; Well G3). Fold-inhibition is defined as [$RLU^{Vehicle\ Cmpd}$ / $RLU^{Test\ Control}$].

Table 2. Summary of Positive Hits from the Androgen Receptor ANTAGONIST Screen. Screen-Well® Nuclear Receptor Ligand Library, BML-2802 Version 2.2

Plate 1 Well	Test Compound Common Name	Fold-INHIBITION of Human AR at these concentrations			Known BioActivities
		10 µM	2.0 µM	0.40 µM	
Vehicle	DMSO, 0.1%	1.00	1.00	1.00	
	CV =	6.4%	6.6%	6.5%	
	6αFIT at EC_{75} Z' =	0.782	0.799	0.781	
Reference Antagonist	Bicalutamide, 5 µM	15.4	14.8	20.3	Non-steroidal androgen receptor antagonist; common Reference Antagonist for AR
F3	5β-Pregnan-3,20-dione	7.82	4.29	2.25	Converted to progesterone via Progesterone 5α Reductase
F6-7	3α, 5α-Androstenol	8.82	4.23	2.22	Inverse agonist of the murine constitutive androstane receptor, CAR3
F8	Z-Guggulsterone	6.69	5.00	5.36	Broad spectrum steroid receptor ligand: MR, PR, GR & FXR antagonists
G3	Mifepristone	8.71	3.58	4.39	Antagonist of progesterone (PR) and glucocorticoid (GR) receptors

The luminescent signal from library compounds was compared to those generated from vehicle control wells containing 0.1% DMSO only, to generate fold-activation and fold-inhibition values. Hits were defined as compounds having fold values ≥3 for two of the three concentrations tested. Results from known AR agonists and antagonists demonstrate the utility of the automated 384-well Human Androgen Receptor Assay to accurately detect AR modulators.

Natural Product Library Primary Screen

The 506 compound Screen-Well® Natural Product Library (generously contributed by ENZO Biochem) was then screened using the automated 384-well Human Androgen Receptor Assay. The library was chosen to examine whether novel AR modulators could be identified within the compound set.

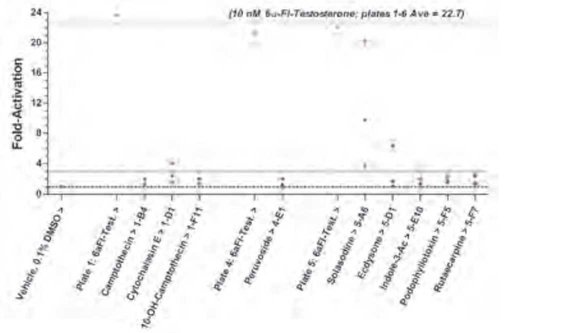


Figure 7 – Summary of agonist "Positive" compounds from the 506 member Screen-Well® Natural Product Library, BML-2865, Version 7.2.

Test compounds were prepared at three sub-concentrations, then screened (*n*=1) at the following concentrations for agonist activities: • 1:1,000, • 1:5,000 and • 1:25,000. 0.1% DMSO (◆, vehicle) and 10 nM 6α–Fluoro-Testosterone (▪, Reference agonist) were added to each of the six 384-well assay plates.

Table 3. Summary of Positive Hits from the Androgen Receptor AGONIST Screen. Screen-Well® Natural Product Library, BML-2865 Version 7.2

Library Plate	Well Location	Test Compound Common Name	Highest Conc. Tested (µM)	Fold-ACTIVATION of AR at these dilutions			Known BioActivities
				1:1,000	1:5,000	1:25,000	
Plates 1-6	Vehicle	DMSO	0.10%	1.00	-	-	
Plate 1	Reference Agonist	6α-Fl-Testosterone	10.0 nM	23.6	CV = 6.9 % Z' = 0.756		Common Reference Agonist for Androgen Receptor (AR)
	1-D1	Cytochalasin E	4.04 µM	4.10	2.42	1.60	Actin microfilament depolymerizing agent
	Fold activaiton of luciferase in 'Mock' Reporter Cells →			3.20	3.96	2.38	
Plate 5	Reference Agonist	6α-Fl-Testosterone	10.0 nM	22.1	CV = 6.7 % Z' = 0.777		
	5-A6	Solasodine	4.64 µM	20.2	9.76	3.77	Poisonous glycoalkaloid from Solanaceae plants

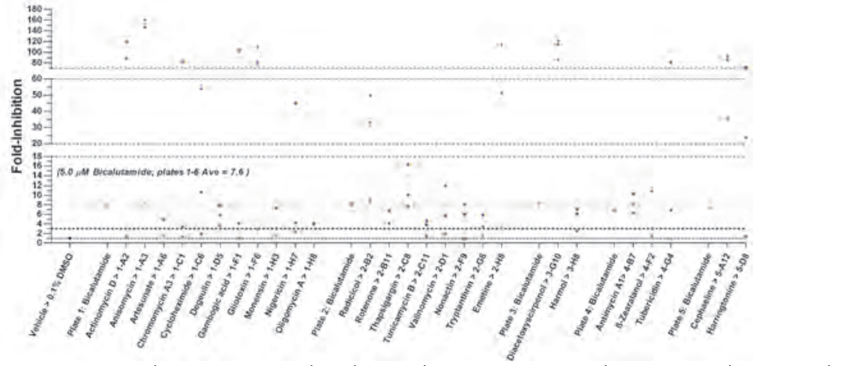


Figure 8 – Summary of antagonist "Positive" compounds from the 506 member Screen-Well® Natural Product Library, BML-2865, Version 7.2.

Test compounds were prepared at three sub-concentrations, then screened (*n*=1) at the following concentrations for antagonist activities: • 1:1,000, • 1:5,000 and • 1:25,000. AR Reporter cells were pre-treated with 200 pM (~EC_{75}) of 6α–Fluoro-Testosterone as the challenge agonist. 0.1% DMSO (◆, vehicle) and 5.0 µM Bicaluatamide (▪, Reference agonist) were added to each of the six 384-well assay plates.

Table 4. Summary of Positive Hits from the Androgen Receptor ANTAGONIST Screen. Screen-Well® Natural Product Library, BML-2865 Version 7.2

Library Plate	Well Location	Test Compound Common Name	Highest Conc. Tested	Fold-INHIBITION of AR at these dilutions			Known BioActivities
				1:1,000	1:5,000	1:25,000	
Plates 1-6	Vehicle	DMSO	0.10%	1.00	6αFIT at EC_{75} Ave Z' = 0.723		
Plate 1	Reference Antagonist	Bicalutamide	5.0 µM	7.70	CV = 7.8 %		Non-steroidal androgen receptor antagonist; common Reference Antagonist for AR
	1-A2	Actinomycin D	4.31 µM	119	88.2	132	DNA intercalating agent and transcription inhibitor; Induces apoptosis
	1-A3	Anisomycin	7.54 µM	146	160	8.39	Activates numerous kinases; Inhibits peptidyl transferase; can induce apoptosis
	1-A6	Artesunate	5.20 µM	5.08	4.85	1.62	CDC25 inhibitor; displays a profound cytotoxic action against 55 tumor cell lines
	1-C1	Chromomycin A3	1.69 µM	82.9	3.38	1.24	Chromomycin A3 is a GC-specific DNA ligand which inhibits transcription
	1-C6	Cycloheximide	7.11 µM	53.8	10.7	1.90	Inhibits protein synthesis in eukaryotes; may induce apoptosis
	1-D5	Degeulin	5.07 µM	7.90	5.92	3.65	Inhibits cyclooxygenase-2 (COX-2) expression; Inhibitor of Akt, may induces apoptosis
	1-F1	Gambogic acid	3.18 µM	103	4.11	1.08	Apoptosis induction proceeds via upregulation of bax and inhibition of bcl-2 expression
	1-F6	Gliotoxin	6.13 µM	80.0	110	2.86	Blocks membrane thiol groups; Induces Ca2+ release from intact rat liver mitochondria
	1-H3	Monensin	2.89 µM	7.37	3.08	1.61	Na⁺ ionophore. Blocks glycoprotein secretion. Inhibits proliferation through cell cycle arrest and apoptosis
	1-H7	Nigericin-Na	2.68 µM	45.1	4.29	2.32	Disrupts membrane potential; Stimulates Ca²⁺ release from mitochondrial stores; Inhibits Golgi functions
	1-H8	Oligomycin A	2.53 µM	4.12	3.94	4.14	Inhibits mitochondrial ATPases; Stimulates lysosome acidification; Induces apoptosis
Plate 2	Reference Antagonist	Bicalutamide	5.0 µM	8.21	CV = 7.4 %		
	2-B2	Radicicol	5.48 µM	33.4	49.9	8.99	Potent inhibitor of HSP90
	2-B11	Rotenone	5.07 µM	6.72	4.20	4.15	Inhibits mammalian cell proliferation by inhibiting microtubule assembly *via* tubulin binding
	2-C8	Thapsigargin	3.07 µM	16.4	10.1	7.66	ATPase inhibitor; Induces the release of intracellular stored Ca2+
	2-C11	Tunicamycin B	2.41 µM	4.60	3.82	1.45	Inhibits protein N-glycosylation & induces ER-stress; Arrests cell cycle in late G1
	2-D1	Valinomycin	1.80 µM	5.70	12.0	1.86	A potassium ionophore. Induces mitochondrial swelling. Induces apoptosis
	2-F9	Nonactin	2.71 µM	6.04	8.12	0.89	Monovalent cation ionophore, blocks processing of precursor proteins destined for the mitochondria
	2-G8	Tryptanthrin	8.06 µM	5.86	3.42	1.67	Potent inhibitor of prostaglandin and leukotriene synthesis; Selective inhibitor of COX-2
	2-H8	Emetine-2HCl	3.61 µM	113	51.3	3.27	Binding to ribosomal 40S subunit blocks protein synthesis; drug to induce vomiting, see "Cephaeline"
Plate 3	Reference Antagonist	Bicalutamide	5.0 µM	8.29	CV = 7.6 %		
	3-G10	Diacetoxyscirpenol	4.31 µM	114	121	86.4	Mycotoxin inhibits protein synthesis; causes death of rapidly proliferating cells; anti-cancer properties
	3-H8	Harmol-HCl-2H2O	7.54 µM	7.09	6.16	2.60	
Plate 4	Reference Antagonist	Bicalutamide	5.0 µM	6.89	CV = 6.7 %		
	4-B7	Antimycin A1	3.65 µM	10.3	6.33	8.17	Antibiotic, inhibits mitochondrial respiration and may deplete cellular levels of ATP
	4-F2	β-Zearalanol	6.20 µM	10.8	3.04	1.47	Mycotoxin agonist of ERs; suspected phytoestrogen endocrine-disrupting chemical; toxic to livestock
	4-G4	Tubericidin	7.51 µM	80.6	6.90	0.90	Toxic adenosine analog that blocks purine biosynthesis; inhibits mammalian SAH hydrolase
Plate 5	Reference Antagonist	Bicalutamide	5.0 µM	7.54	CV = 7.2 %		
	5-A12	Cephaeline-HBr	3.65 µM	92.9	84.9	35.3	Blocks protein synthesis; effective emergency use to induce vomiting; precursor to Emetine
	5-D8	Harringtonine	3.76 µM	70.6	23.9	1.44	Inhibits the formation of diphenylalanine. Lowers the levels of telomerase. Induces apoptosis

Hit Confirmation and Secondary Screening

Compounds demonstrating positive AR agonist or antagonist activity were confirmed using two different methods. Positive agonists were further tested using "Mock" reporter cells, which contained the luciferase reporter gene construct without expressed Androgen Receptor. In this way, compounds which induced luciferase expression in the absence of receptor activation could be removed from further consideration.

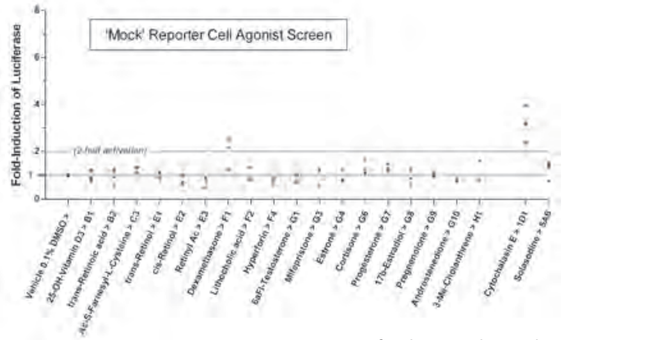


Figure 9 – Secondary screen of Agonist-Positive compounds against 'Mock' Reporter Cells. Screen-Well® Nuclear Receptor Ligand Library, BML-2802 (• 1 µM, • 2.0 µM, • 0.40 µM) Screen-Well® Natural Product Library BML-2865 Version 7.2 (• 1:1,000, • 1:5,000, • 1:25,000)

Putative "positive" antagonists were further evaluated using INDIGO's Live Cell Multiplex (LCM) Assay. A compounds were tested at their highest HTS assay concentrations.

Table 5. Results of LIVE Cell Multiplex & AR Antagonist Assays.

Library	Plate	Well	Compound	Test Conc.	Percent LIVE Cells in assay	Fold-inhibition of AR
100% LIVE + 6αFIT @ EC_{75}			DMSO	0.10%	100%	1.0
Cyto-Tox "+" Control			Staurosporin	4.0 µM	4.6%	7.714
AR Antagonist "+" Control			Bicalutamide	5.0 µM	94%	10.2
Screen-Well Nuclear Receptor Ligand Library BML-2802 Version 2.2		F3	5βPregnan-3,20-dione	10 µM	88%	24.8
		F6-7	3α, 5α-Androstenol	10 µM	84%	25.1
		F8	Z-Guggulsterone	10 µM	73%	27.6
		G3	Mifepristone	10 µM	81%	25.1
Screen-Well Natural Product Library BML-2865 Version 7.2 (Test Conc. = 1:1,000 dilution of library stocks)	Plate 1	1A2	Actinomycin D	4.3 µM	24%	2.144
		1A3	Anisomycin	7.5 µM	13%	6.547
		1A6	Artesunate	5.2 µM	60%	26.6
		1C1	Chromomycin A3	1.7 µM	34%	1.305
		1C6	Cycloheximide	7.1 µM	40%	124.5
		1D5	Degeulin	5.1 µM	67%	21.5
		1F1	Gambogic acid	3.2 µM	4.5%	4.629
		1F6	Gliotoxin	6.1 µM	6.9%	7.629
		1H3	Monensin	2.9 µM	72%	14.1
		1H7	Nigericin Na	2.7 µM	40%	445
		1H8	Oligomycin A	2.5 µM	68%	12.4

Library	Plate	Well	Compound	Test Conc.	Percent LIVE Cells in assay	Fold-inhibition of AR
100% LIVE + 6αFIT @ EC_{75}			DMSO	0.10%	100%	1.0
Cyto-Tox "+" Control			Staurosporin	4.0 µM	4.4%	4.417
AR Antagonist "+" Control			Bicalutamide	5.0 µM	98%	12.0
Screen-Well Natural Product Library BML-2865 Version 7.2 (Test Conc. = 1:1,000 dilution of library stocks)	Plate 2	2B2	Radicicol	5.5 µM	47%	142
		2B11	Rotenone	5.1 µM	57%	26.8
		2C8	Thapsigargin	3.1 µM	51%	59.6
		2C11	Tunicamycin B	2.4 µM	69%	8.3
		2D1	Valinomycin	1.8 µM	62%	23.4
		2F9	Nonactin	2.7 µM	64%	22.8
		2G8	Tryptanthrin	8.1 µM	33%	117.2
		2H8	Emetine 2HCl	3.6 µM	30%	2.324
	Plate 3	3G10	Diacetoxyscirpenol	5.5 µM	12%	4.052
		3H8	Harmol	7.4 µM	91%	5.6
	Plate 4	4B7	Antimycin A1	3.6 µM	64%	17.1
		4F2	β-Zearalanol	6.2 µM	94%	20.9
		4G4	Tubericidin	7.5 µM	10%	8.595
	Plate 5	5A12	Cephaeline HBr	3.7 µM	15%	2.969
		5D8	Harringtonine	3.8 µM	15%	1.137

Compounds yielding greater than 90% Live cells at the assay endpoint are scored as true inhibitors of AR activity. Compounds yielding between 90-50% Live cells may be cytostatic, and are included in additional analyses. Compounds yielding <50% Live cells are cyto-toxic, scored as 'false positive' antagonists, and removed from further testing.

Next, fill dose-response curves were generated using the confirmed positive AR agonists and antagonists to determine EC_{50} or IC_{50} values.

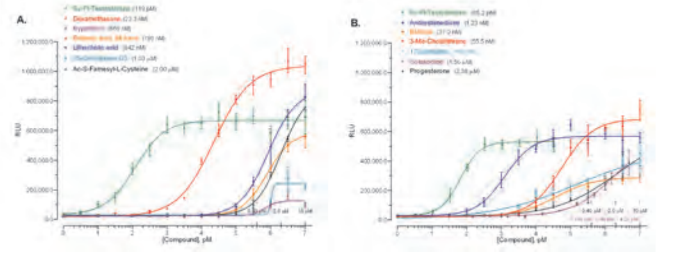
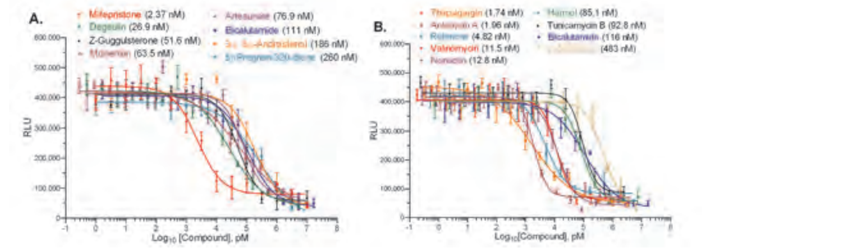


*Figure 10 – Summary of agonist "Positive" compound dose response test following "Mock" Receptor Cell confirmation. Titrations were begun at 10 µM, with the exception of 6α–Fluoro-Testosterone in panel B (33.3 nM) and Solasodine (4.8 µM). Points on each curve represent Avg ± SD of n=3 points. EC_{50} values are reported for each dose response curve. Original primary screen concentrations represented below curves in BLACK, except for Solasodine which are represented in **PURPLE**.*

Figure 11 – Summary of antagonist "Positive" compound dose response test following Live Cell Multiplex Assay confirmation. Titrations were begun at a 1:1000 dilution of the original stock compound concentration. Points on each curve represent Avg ± SD of n=3 points. IC_{50} values are reported for each dose response curve.



Conclusions

1. The cell-based Human Androgen Receptor Assay from INDIGO provides a streamlined, robust HTS assay
2. INDIGO's Live Cell Multiplex Assay is an efficient method to assess cytotoxic properties of test compounds while confirming their AR-inhibitory activity
3. Cryopreserved reporter cells further simplify the assay procedure and provide increased repeatability during the primary and secondary screening process
4. The BioTek Precision and EL406 are able to easily titrate and transfer library compounds, dispense cells and reagents, and perform the media and buffer removals necessary during the LCM assay
5. BioTek's Synergy H4 is capable of detecting the luminescent and fluorescent signals from each of the assays, without the need to change the optical setup of the instrument
6. This combination of AR assay and instrumentation provides an ideal platform for primary HTS and secondary screening of library test compounds