Ms. No.: JBSC-12-0035
Title: A High-Content Biosensor Based Screen Identifies Cell Permeable Activators and Inhibitors of EGFR Function: Implications in Drug Discovery
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Supplementary methods

Microscopy image acquisition and analysis

Cell images in 384-well format were acquired using the IN Cell Analyzer 2000 (INCA2000) automated microscope as previously described.[8] Briefly, 4 images per well were collected at 20X objective magnification using a 20x ASAC objective (0.45NA). GFP images of granule formation were acquired at 490/20 nm excitation and 525/36 nm emission with a 1.2 second exposure time. DRAQ5 images of stained nuclei were acquired at 645/30 nm excitation and 705/72 nm emission with a 0.6 second exposure time. GFP granules and nuclei were identified using object-based segmentation on each channel using a customized image analysis protocol in Developer Toolbox 1.7 software (GE Healthcare, Piscataway, NJ). Automated image analysis using our custom protocol yielded granule and nuclei count that were used for the quantification of EGFR activity and cell count, respectively.

Chemical libraries

The library used for the screen combines 6,912 chemicals obtained from MicroSource, Prestwick, Tocris, Sigma-Aldrich and other commercial sources as previously described.[9] Briefly, the MicroSource Library contains 2,000 biologically active and structurally diverse compounds from known drugs, experimental bioactives, and pure natural products. The Prestwick Chemical Library is a unique collection of 1,119 bioactive chemical compounds, all

off patent and selected for structural diversity and broad spectrum. Approximately 90% of the library consists of marketed drugs and 10% of bioactive alkaloids or related substances. The Tocris collection includes 1,280 high purity compounds active at GPCRs, kinases, ion channels, nuclear receptors and transporters. The LOPAC^{1280} library from Sigma-Aldrich consists of 1,280 well-characterized, high-purity compounds representing all major target classes.

Confirmation of positives using the EGFRB assay

Positives selected from the pilot screen were resupplied from vendors and their activity was confirmed in dose response studies using 12 doubling dilutions in duplicate with a 10 μM and 1 μM compound concentration as the upper limit. Controls were identical as those used in screening plates and consisted of 1% DMSO (v/v) (high control) and 10 μM gefitinib in 1% DMSO (v/v) (low control). The assay was performed according to the same workflow used for control run and screening. Following automated image analysis, dose response data files were uploaded onto the HTS Core Screening Data Management System for curve fitting and IC_{50} calculation.

Assessment of compound optical interference

The potential optical interference of selected initial positives from the screen in the GFP channel was assessed in white 384 well microtiter plates (Corning #3570, Corning, NY) by measuring the fluorescence intensity of wells containing 45 μL PBS, pre- and post-addition of 5 μL of the resupplied compounds in solution in 10% DMSO (v/v) with a PP-384-M Personal Pipettor (Apricot Designs, Monrovia, CA) using a LEADseeker™ multimodality imaging system (GE Healthcare, Piscataway, NJ) equipped with fluorescein excitation, emission and dichroic

filters. Each compound was tested in dose response using 12 doubling dilutions in triplicate with a 10 μM compound concentration in 1% DMSO (v/v) as the upper limit. Compounds inducing a dose-dependent increase in fluorescence post- versus pre-addition were flagged as autofluorescent and potentially interfering with the GFP granule readout. In addition, A549 parental cells were imaged in the GFP channel after treatment with the identified activators of granule formation in the same conditions as A549-EGFRB cells to rule out the possibility that the observed increase in granule results from compound auto-fluorescence.

Assessment of compound solubility limit

The solubility limit of selected initial positives from the screen was assessed in UV-transparent 384 well microtiter plates (#781801, Greiner Bio-One, Monroe, NC) using a NEPHELOstar microplate laser nephelometer (BMG LABTECH GmbH, Ortenberg, Germany). The turbidity of wells containing 45 μL PBS was measured pre- and post-addition of 5 μL of the resupplied compounds in solution in 10% DMSO (v/v) with the PP-384-M Personal Pipettor. Each compound was tested in dose response using 12 doubling dilutions in triplicate with a 10 μM compound concentration in 1% DMSO (v/v) as the upper limit. Controls consisted of standards of 0.1, 1, 10, 20, 100, 200, 800 and 1,000 Nephelometric Turbidity Units (Hach, Loveland, CO). The compound solubility limit was defined as the compound concentration yielding turbidity values greater than the average turbidity values for the 100 NTU standard. Compounds with a solubility limit lower than 10 μM in 1% DMSO (v/v) could potentially interfere with the EGFRB assay due to low solubility in the conditions of the assay.

FIGURES & TABLE LEGENDS

Supplementary Figure 1. Dose response of confirmed positives in the EGFRB assay

A) Dose response studies of ZM-306416 result in a calculated IC_{50} of 0.67±0.2 µM for granule count formation and no effect on cell number. Representative images at 0.04, 0.6 and 10, 0.6 and 10 µM show a decrease in granule formation without any observed cytotoxicity effects. **B**) Dose response studies of flurandrenolide resulted in a calculated EC_{50} of 0.023±0.02 µM for granule count and no effect on cell number. Representative images at 0.04, 0.6 and 10, 0.6 and 10 µM show an increase in granule formation without any cytotoxicity or increase in cell number. **A)** and **B)** Granule segmentation resulting from automated image analysis is overlaid on images in yellow.

Supplementary Table 1. Performance of the 82 inhibitors of granule formation identified in the EGFR biosensor screen.

The compound name, supplier, described biological property, performance in the EGFRB assay and in the follow up confirmatory workflow is summarized for the 82 inhibitors of granule formation identified in the EGFR biosensor screen. O.I.: optical interference. sol.: solubility limit. HTS %I GRAN: percentage inhibition in granule count in the EGFRB assay during the screen. HTS %I NUC: percentage inhibition in nuclei count in the EGFRB assay during the screen.

Supplementary Table 2. Performance of the 66 activators of granule formation identified in the EGFR biosensor screen.

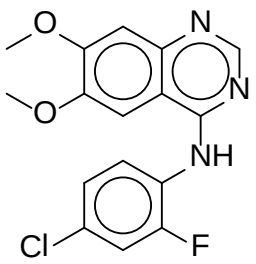
The compound name, supplier, described biological property, performance in the EGFRB assay and in the follow up confirmatory workflow is summarized for the 66 activators of granule formation identified in the EGFR biosensor screen. O.I.: optical interference. sol.: solubility limit. HTS %I GRAN: percentage inhibition in granule count in the EGFRB assay during the screen. HTS %I NUC: percentage inhibition in nuclei count in the EGFRB assay during the screen.

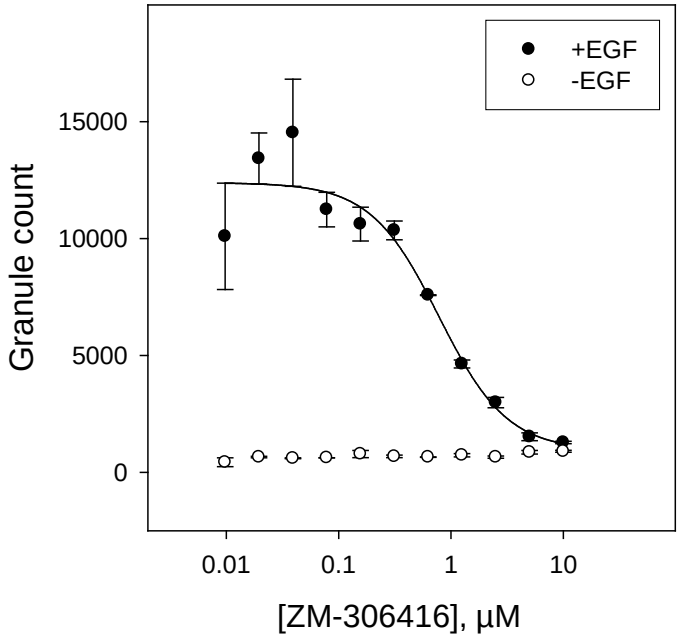
Supplementary Table 3. Chemical structure, image and summary table of data in the EGFRB assay of the 16 hits and the erbstatin analog

The chemical structure, representative image out of 4 fields imaged during the screen and summary table of data performance of 16 confirmed positives from the EGFR biosensor screen and of the described EGFR kinase inhibitor erbstatin analog included in the confirmation studies is summarized. Granule segmentation resulting from automated image analysis is overlaid on images in yellow.

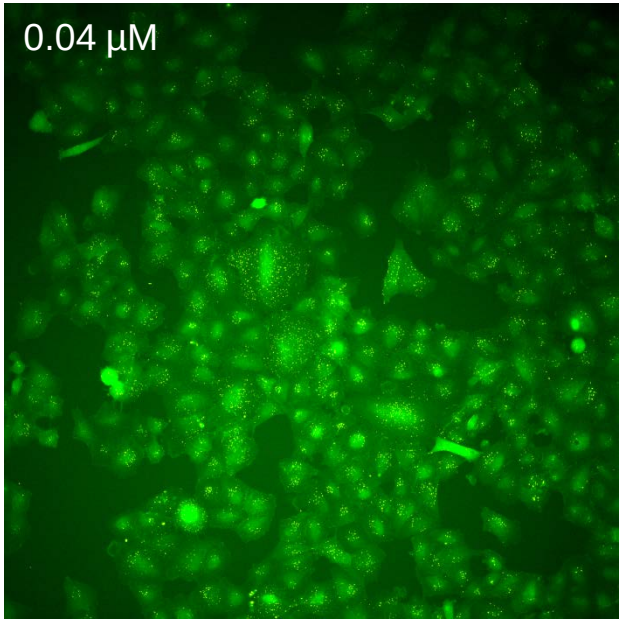
A

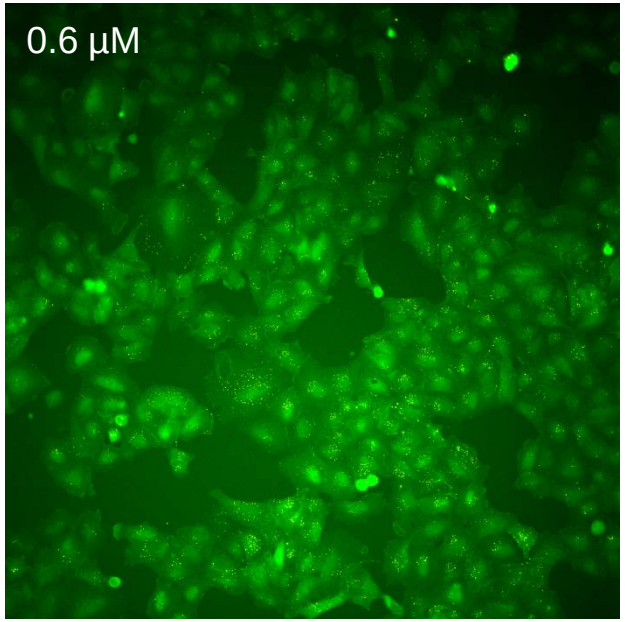
ZM-306416

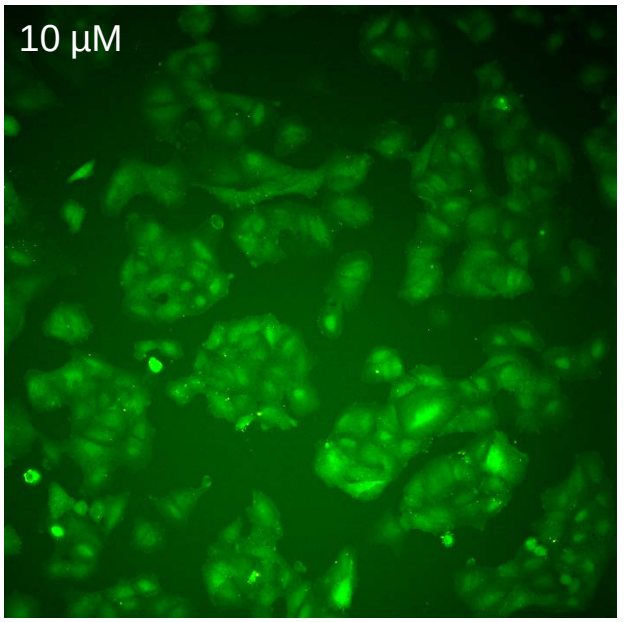




Granule count: IC_{50} = 0.67 0.2 µM
Nuclei count: IC_{50} > 10 µM



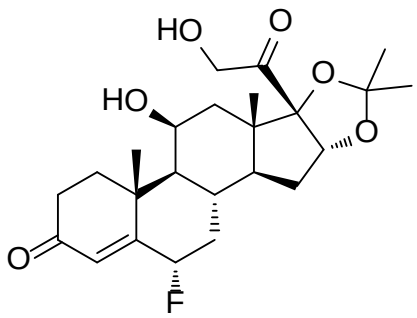




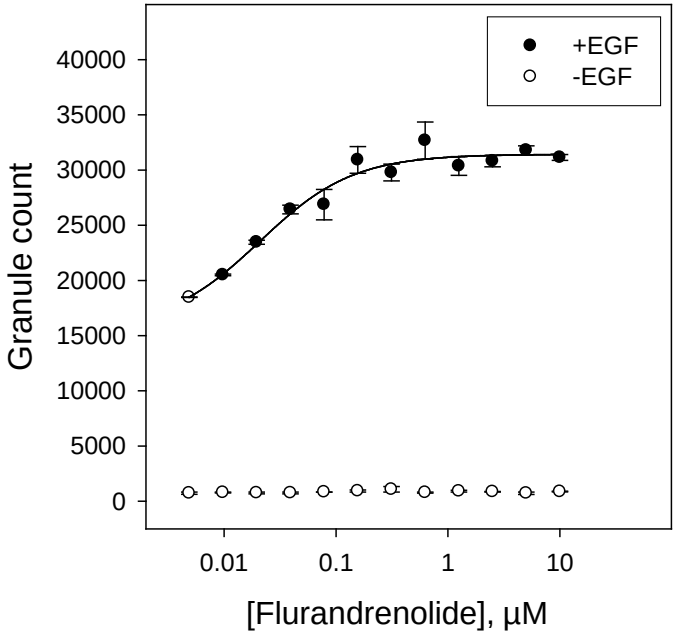
Suppl. Figure 1 – Antczak et al.

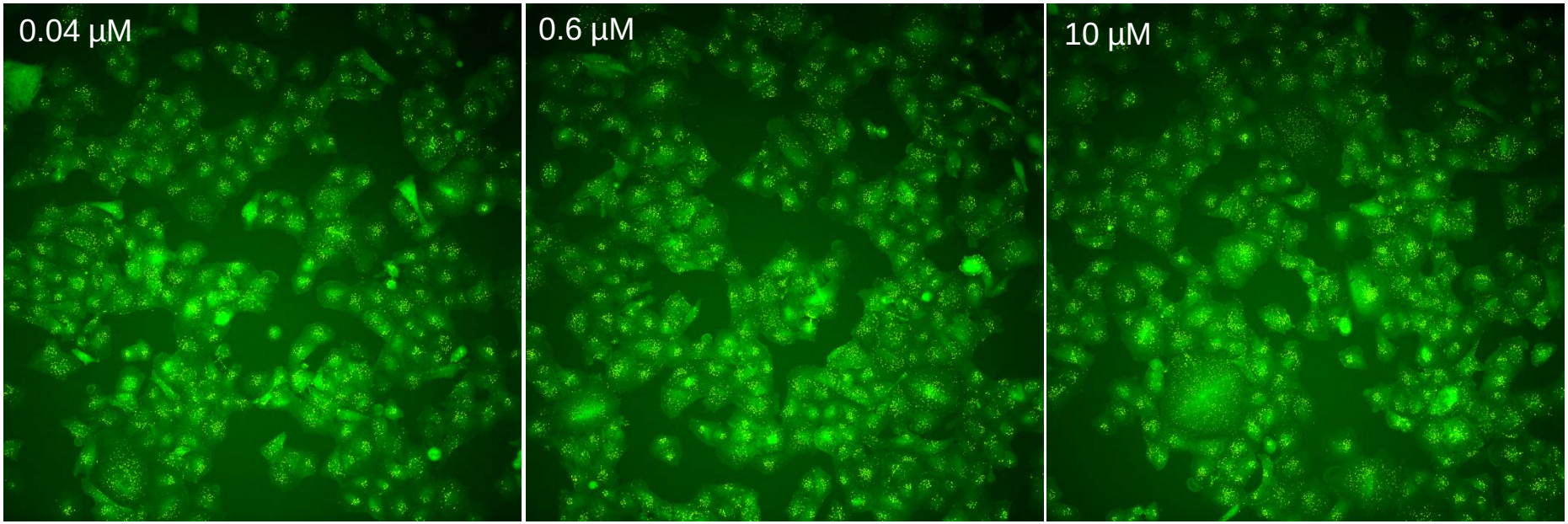
B

Flurandrenolide



Granule count: EC_{50} = 0.023 0.02 µM
Nuclei count: IC_{50} > 10 µM





Suppl. Figure 1 – Antczak et al.

Compound name	Supplier	resupplied	confirmed	O.I.	sol.	Biological property	HTS %I GRAN	HTS %I NUC
Erlotinib mesylate	Sequoia Research Products	yes	yes	no	> 10 µM	EGFR kinase inhibitor	69	14
Gefitinib	LC Laboratories	yes	yes	no	> 10 µM	EGFR kinase inhibitor	62	12
Lapatinib	LC Laboratories	yes	yes	no	> 10 µM	EGFR kinase inhibitor	98	34
PD 153035	Biaffin GmbH	yes	yes	no	> 10 µM	EGFR kinase inhibitor	88	49
GW 2974	Sigma-Aldrich	yes	yes	no	> 10 µM	EGFR kinase inhibitor	84	20
Tyrphostin AG 1478	Sigma-Aldrich	yes	yes	no	> 10 µM	EGFR kinase inhibitor	65	30
BIBU 1361 dihydrochloride	Tocris Bioscience	yes	yes	no	> 10 µM	EGFR kinase inhibitor	63	5
GW 583340 dihydrochloride	Tocris Bioscience	yes	yes	no	> 10 µM	EGFR kinase inhibitor	62	22
ZM-306416 hydrochloride	Tocris Bioscience	yes	yes	no	> 10 µM	VEGFR tyrosine kinase inhibitor	84	47
PKC412	LC Laboratories	yes	yes	no	> 10 µM	Tyrosine kinase inhibitor	68	43
Aminopurvalanol A	Tocris Bioscience	yes	yes	no	> 10 µM	Cyclin-dependent kinase inhibitor	77	46
17-DMAG hydrochloride	LC Laboratories	yes	yes	no	> 10 µM	Hsp90 inhibitor	61	31
Camptothecin	Prestwick Chemicals	yes	yes	no	> 10 µM	DNA topoisomerase inhibitor	92	22
Bromothioetrazole	Internal Supplier	yes	no	no	> 10 µM	Unknown	62	18
Furopyrroledione	Internal Supplier	yes	no	no	> 10 µM	Unknown	67	33
N-Boc-L-valine	Internal Supplier	yes	no	no	> 10 µM	Unknown	78	41
Cyclopentyphenylpyrimidinylamine	Sigma-Aldrich	yes	no	no	> 10 µM	Lck tyrosine kinase inhibitor	96	14
BAY 61-3606 hydrochloride hydrate	Sigma-Aldrich	yes	no	no	> 10 µM	Syk inhibitor	97	11
BIO	Sigma-Aldrich	yes	no	no	> 10 µM	ATP-competitive GSK-3α/β inhibitor	82	15
Diacylglycerol Kinase Inhibitor II	Sigma-Aldrich	yes	no	no	> 10 µM	Diacylglycerol kinase inhibitor	68	22
Naloxone hydrochloride	Sigma-Aldrich	yes	no	no	> 10 µM	Opioid antagonist	87	48
Parthenolide	Sigma-Aldrich	yes	no	no	> 10 µM	Antiproliferative agent	83	50
TBBz	Sigma-Aldrich	yes	no	no	> 10 µM	CK2 inhibitor	63	32
GW 1929	Tocris Bioscience	yes	no	no	> 10 µM	PPARγ agonist	70	26
INCA-6	Tocris Bioscience	yes	no	no	> 10 µM	Calcineurin-NFAT signaling inhibitor	64	12
CCT 018159	Tocris Bioscience	yes	no	no	> 10 µM	Hsp90 inhibitor	82	15
17-AAG	LC Laboratories	no	N/A	no	> 10 µM	Hsp90 inhibitor	65	27
Geldanamycin	Sequoia Research Products	no	N/A	no	> 10 µM	Hsp90 inhibitor	94	31
Tyrphostin AG 1478 hydrochloride	Tocris Bioscience	no	N/A	no	> 10 µM	EGFR kinase inhibitor	98	3
PD 153035 hydrochloride	Tocris Bioscience	no	N/A	no	> 10 µM	EGFR kinase inhibitor	65	47
BIBX 1382 dihydrochloride	Tocris Bioscience	no	N/A	no	> 10 µM	EGFR kinase inhibitor	69	6
PD 158780	Tocris Bioscience	no	N/A	no	> 10 µM	EGFR kinase inhibitor	62	34
Camptothecin	MicroSource	no	N/A	no	> 10 µM	DNA topoisomerase inhibitor	62	36
Camptothecin	Tocris Bioscience	no	N/A	no	> 10 µM	DNA topoisomerase inhibitor	69	33
10-Hydroxycamptothecin	MicroSource	no	N/A	no	> 10 µM	DNA topoisomerase inhibitor	81	38
SN 38	Tocris Bioscience	no	N/A	no	> 10 µM	DNA topoisomerase inhibitor	74	23
Mitoxantrone dihydrochloride	Prestwick Chemicals	no	N/A	no	> 10 µM	DNA topoisomerase inhibitor	66	28
Teniposide	MicroSource	no	N/A	no	> 10 µM	DNA topoisomerase inhibitor	68	47
Cycloheximide	MicroSource	no	N/A	no	> 10 µM	Protein synthesis inhibitor	92	2
Cycloheximide	Prestwick Chemicals	no	N/A	no	> 10 µM	Protein synthesis inhibitor	92	32
Emetine	MicroSource	no	N/A	no	> 10 µM	Protein synthesis inhibitor	91	5
Emetine dihydrochloride	Prestwick Chemicals	no	N/A	no	> 10 µM	Protein synthesis inhibitor	63	46
Emetine dihydrochloride hydrate	Sigma-Aldrich	no	N/A	no	> 10 µM	Protein synthesis inhibitor	68	39
Lycorine	MicroSource	no	N/A	no	> 10 µM	Protein synthesis inhibitor	87	47
Lycorine hydrochloride	Prestwick Chemicals	no	N/A	no	> 10 µM	Protein synthesis inhibitor	62	42
Tetrahydrobenzopyrimidinamine	Internal Supplier	no	N/A	no	> 10 µM	Unknown	64	29
Tetrahydrobenzothienopyrimidinamine	Internal Supplier	no	N/A	no	> 10 µM	Unknown	76	43
Tetrahydropyrandiyldiacetate	Internal Supplier	no	N/A	no	> 10 µM	Unknown	75	36
Tetrahydrotolybenzothienopyrimidinamine	Internal Supplier	no	N/A	no	> 10 µM	Unknown	82	47
Atracurium besylate	Sequoia Research Products	no	N/A	no	> 10 µM	Acetylcholine receptor blocker	79	49
5-Iodotubercidin	Biaffin GmbH	no	N/A	no	> 10 µM	Adenosine kinase nhibitor	91	39
Pifithrin- µ	Tocris Bioscience	no	N/A	no	> 10 µM	Antiapoptotic agent	79	42
Acrisorcin	MicroSource	no	N/A	no	> 10 µM	Antimicrobial	66	41
Resorcinol	MicroSource	no	N/A	no	> 10 µM	Antimicrobial	77	41
Parthenolide	Prestwick Chemicals	no	N/A	no	> 10 µM	Antiproliferative agent	70	42
Andrographolide	MicroSource	no	N/A	no	> 10 µM	Diterpenoid lactone	63	44
Sulpiride	Prestwick Chemicals	no	N/A	no	> 10 µM	Dopamine receptor antagonist	71	49
Rhoifolin	MicroSource	no	N/A	no	> 10 µM	Flavonoid	65	9
Tracazolate	Sigma-Aldrich	no	N/A	no	> 10 µM	GABA receptor modulator, anxiolytic	61	9
Scriptaid	Tocris Bioscience	no	N/A	no	> 10 µM	Histone deacetylase inhibitor	62	11
Crassin acetate	MicroSource	no	N/A	no	> 10 µM	HO-1 inducer	61	25
Doxorubicin hydrochloride	Prestwick Chemicals	no	N/A	no	> 10 µM	Intercalating agent	93	31
Piperlongumine	Prestwick Chemicals	no	N/A	no	> 10 µM	Natural product	64	28
Pyrrolidinedithiocarbamate ammonium	Tocris Bioscience	no	N/A	no	> 10 µM	NF-κB inhibitor	91	0
(±)-Clopidogrel hydrochloride	Tocris Bioscience	no	N/A	no	> 10 µM	P2Y receptor antagonist	66	39
Paradichlorobenzene	MicroSource	no	N/A	no	> 10 µM	Pesticide	91	42
Bortezomib	LC Laboratories	no	N/A	no	> 10 µM	Proteasome inhibitor	94	25
Meclofenamic acid	Prestwick Chemicals	no	N/A	no	> 10 µM	Prostaglandin synthesis inhibitor	97	21
Venlafaxine	MicroSource	no	N/A	no	> 10 µM	Serotonin reuptake inhibitor	89	17
Alosetron hydrochloride	Sequoia Research Products	no	N/A	no	> 10 µM	Serotonin receptor antagonist	68	44
BNTX maleate	Tocris Bioscience	no	N/A	no	> 10 µM	Opioid receptor antagonist	62	39
Guggulsterone	Tocris Bioscience	no	N/A	no	> 10 µM	Steroid receptor antagonist	83	44
Cephaeline	Prestwick Chemicals	no	N/A	no	> 10 µM	Emetic	69	26
Pyrromycin	MicroSource	no	N/A	no	> 10 µM	Antiproliferative agent	83	46
Triptonide	MicroSource	no	N/A	no	> 10 µM	Natural product	67	45
Benzylmethylindolacetamide	Internal Supplier	no	N/A	no	> 10 µM	Unknown	64	35
Dimethylpentanoic acid methyl ester	Internal Supplier	no	N/A	no	> 10 µM	Unknown	90	48
Nitrophenylpyrazole	Internal Supplier	no	N/A	no	> 10 µM	Unknown	73	38
N/A	Internal Supplier	no	N/A	no	> 10 µM	Unknown	73	27
N/A	Internal Supplier	no	N/A	no	> 10 µM	Unknown	77	36
N/A	Internal Supplier	no	N/A	no	> 10 µM	Unknown	94	12
N/A	Internal Supplier	no	N/A	no	> 10 µM	Unknown	67	27

Suppl. Table 1 – Antczak et al.

Compound name	Supplier	resupplied	confirmed	O.I.	sol.	Biological property	HTS %I GRAN	HTS %I NUC
Flurandrenolide	Prestwick Chemicals	yes	yes	no	> 10 µM	Corticosteroid	-106	10
Beclomethasone	Sigma-Aldrich	yes	yes	no	> 10 µM	Corticosteroid	-111	8
Ebastine	Sigma-Aldrich	yes	yes	no	> 10 µM	H1 receptor antagonist	-112	7
R-(-)-Desmethyldeprenyl hydrochloride	Sigma-Aldrich	yes	no	no	> 10 µM	MAO-B inhibitor	-108	8
L-allylglycine	Sigma-Aldrich	yes	no	no	> 10 µM	Glutamic acid decarboxylase inhibitor	-109	12
Epiafzelechin trimethylether	MicroSource	yes	no	no	> 10 µM	Flavonoid	-115	12
Ropinirole hydrochloride	Sigma-Aldrich	yes	no	no	> 10 µM	Dopamine receptor agonist	-102	15
SB 218078	Tocris Bioscience	yes	no	yes	> 10 µM	Chk1 kinase inhibitor	-107	28
Ursocholanic acid	MicroSource	yes	no	no	> 10 µM	Bile acid	-129	5
GBR-12935	Tocris Bioscience	yes	no	no	> 10 µM	Dopamine reuptake inhibitor	-118	10
Dicloxyacillin sodium salt	Prestwick Chemicals	yes	no	no	> 10 µM	Antibacterial	-137	7
Scopoletin	MicroSource	yes	no	no	> 10 µM	Acetylcholinesterase inhibitor	-117	13
Tropisetron hydrochloride	Tocris Bioscience	yes	no	no	> 10 µM	5-HT receptor antagonist	-141	5
Nonanoic acid methyl ester	Internal Supplier	yes	no	no	> 10 µM	Unknown	-118	3
2,6-dimethoxyquinone	MicroSource	yes	no	no	> 10 µM	Unknown	-138	-2
Flurandrenolide	MicroSource	no	N/A	no	> 10 µM	Corticosteroid	-110	18
Beclomethasone dipropionate	MicroSource	no	N/A	no	> 10 µM	Corticosteroid	-112	3
Betamethasone 17,21-dipropionate	MicroSource	no	N/A	no	> 10 µM	Corticosteroid	-145	17
Clobetasol propionate	Prestwick Chemicals	no	N/A	no	> 10 µM	Corticosteroid	-138	7
Clobetasol propionate	Sequoia Research Products	no	N/A	no	> 10 µM	Corticosteroid	-120	8
Dexamethasone	MicroSource	no	N/A	no	> 10 µM	Corticosteroid	-100	6
Dexamethasone	Tocris Bioscience	no	N/A	no	> 10 µM	Corticosteroid	-113	-1
Dexamethasone	Prestwick Chemicals	no	N/A	no	> 10 µM	Corticosteroid	-114	6
Hydrocortisone base	Prestwick Chemicals	no	N/A	no	> 10 µM	Corticosteroid	-112	20
Hydrocortisone butyrate	MicroSource	no	N/A	no	> 10 µM	Corticosteroid	-106	10
Hydrocortisone hemisuccinate	MicroSource	no	N/A	no	> 10 µM	Corticosteroid	-105	16
Flumethasone	Prestwick Chemicals	no	N/A	no	> 10 µM	Corticosteroid	-123	11
Flumethasone pivalate	MicroSource	no	N/A	no	> 10 µM	Corticosteroid	-130	5
Methylprednisolone, 6-alpha	Prestwick Chemicals	no	N/A	no	> 10 µM	Corticosteroid	-110	10
Prednisolone	MicroSource	no	N/A	no	> 10 µM	Corticosteroid	-132	4
Prednisolone acetate	MicroSource	no	N/A	no	> 10 µM	Corticosteroid	-128	2
Fluticasone propionate	Prestwick Chemicals	no	N/A	no	> 10 µM	Corticosteroid	-111	13
Fluticasone propionate	Tocris Bioscience	no	N/A	no	> 10 µM	Corticosteroid	-107	5
Fluocinolone acetonide	MicroSource	no	N/A	no	> 10 µM	Corticosteroid	-116	2
Fluocinonide	Prestwick Chemicals	no	N/A	no	> 10 µM	Corticosteroid	-130	1
Triamcinolone	Prestwick Chemicals	no	N/A	no	> 10 µM	Corticosteroid	-116	15
Triamcinolone acetonide	MicroSource	no	N/A	no	> 10 µM	Corticosteroid	-110	-6
Clocortolone pivalate	Prestwick Chemicals	no	N/A	no	> 10 µM	Corticosteroid	-121	12
Diflucortolone pivalate	MicroSource	no	N/A	no	> 10 µM	Corticosteroid	-107	7
Fludrocortisone	Prestwick Chemicals	no	N/A	no	> 10 µM	Corticosteroid	-163	2
Flunisolide	Prestwick Chemicals	no	N/A	no	> 10 µM	Corticosteroid	-129	8
Halcinonide	Prestwick Chemicals	no	N/A	no	> 10 µM	Corticosteroid	-100	11
Rimexolone	Prestwick Chemicals	no	N/A	no	> 10 µM	Corticosteroid	-105	9
Triflupromazine hydrochloride	MicroSource	no	N/A	no	> 10 µM	Dopamine receptor antagonist	-124	4
Itopride hydrochloride	Sequoia Research Products	no	N/A	no	> 10 µM	Dopamine receptor antagonist	-103	13
Trihexyphenidyl	Prestwick Chemicals	no	N/A	no	> 10 µM	Muscarinic receptor ligand	-113	5
Isamoltane hemifumarate	Tocris Bioscience	no	N/A	no	> 10 µM	5-HT receptor antagonist	-105	9
Cisplatin	Tocris Bioscience	no	N/A	no	> 10 µM	Alkylating agent	-101	13
Cefamandole sodium	MicroSource	no	N/A	no	> 10 µM	Antibacterial	-106	8
Chloramphenicol hemisuccinate	MicroSource	no	N/A	no	> 10 µM	Antimicrobial	-101	13
Merbromin	MicroSource	no	N/A	no	> 10 µM	Antimicrobial	-165	7
Ketoconazole	Tocris Bioscience	no	N/A	no	> 10 µM	Antifungal	-121	6
AFMK	Sigma-Aldrich	no	N/A	no	> 10 µM	Antioxidant	-119	8
Mesna	Prestwick Chemicals	no	N/A	no	> 10 µM	Antioxidant	-105	8
Isradipine	Prestwick Chemicals	no	N/A	no	> 10 µM	Calcium channel blocker	-104	18
GW 405833	Tocris Bioscience	no	N/A	no	> 10 µM	CB2 receptor agonist	-101	12
RS 504393	Tocris Bioscience	no	N/A	no	> 10 µM	CCR2 chemokine receptor antagonist	-116	13
Purvalanol B	Tocris Bioscience	no	N/A	no	> 10 µM	Cyclin-dependent kinase inhibitor	-112	5
Clopamide	MicroSource	no	N/A	no	> 10 µM	Ion channel inhibitor	-121	9
5,7-dihydroxyisoflavone	MicroSource	no	N/A	no	> 10 µM	Isoflavonoid	-115	5
T 0901317	Tocris Bioscience	no	N/A	no	> 10 µM	Liver X receptor (LXR) agonist	-103	9
Luzindole	Tocris Bioscience	no	N/A	no	> 10 µM	Melatonin receptor antagonist	-138	-1
Aminohippuric acid	Prestwick Chemicals	no	N/A	no	> 10 µM	Metabolite	-101	8
Dihydrofolic acid	MicroSource	no	N/A	no	> 10 µM	Nucleic acid precursor	-108	8
1-Aminocyclobutane carboxylic acid	MicroSource	no	N/A	no	> 10 µM	Unknown	-116	-1
Carbonic acid ester phenyl ester	Internal Supplier	no	N/A	no	> 10 µM	Unknown	-109	17

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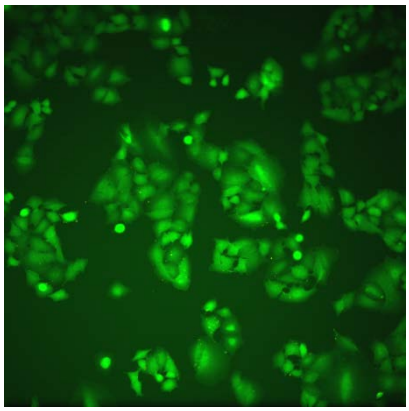
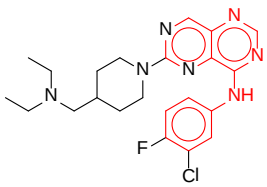
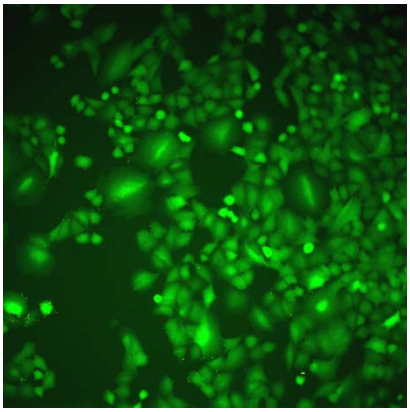
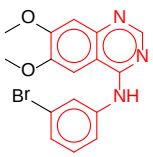


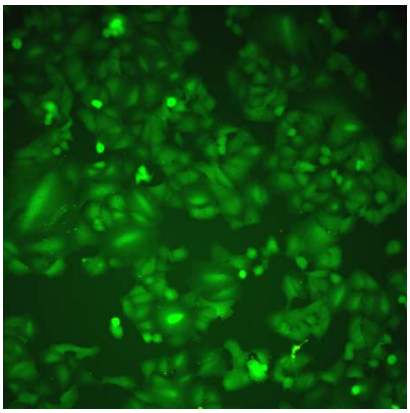
tyrphostin AG 1478	HTS %I	IC_{50}, µM
granule count	94	0.065±0.008
nuclei count	12	> 10
molecular target	EGFR kinase	



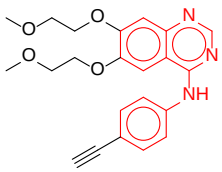


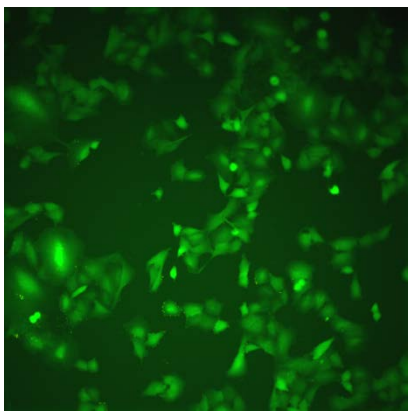
BIBU 1361	HTS %I	IC_{50}, µM
granule count	98	0.038±0.007
nuclei count	3	> 10
molecular target	EGFR kinase	



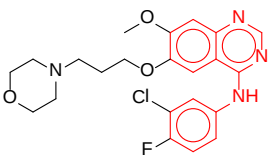


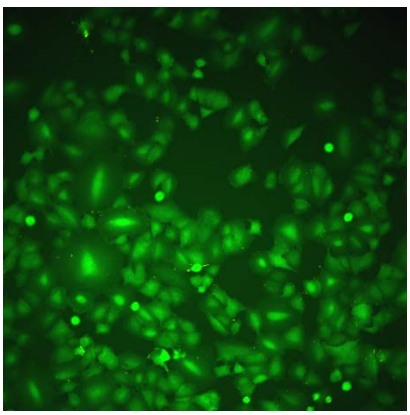
PD 153035	HTS %I	IC_{50}, µM
granule count	97	0.091±0.02
nuclei count	12	> 10
molecular target	EGFR kinase	



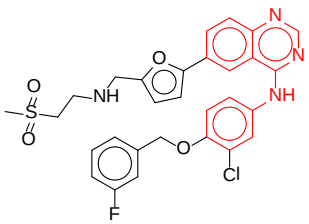


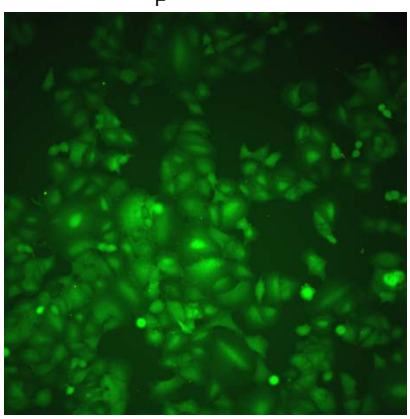
erlotinib	HTS %I	IC_{50}, µM
granule count	98	0.21±0.03
nuclei count	36	> 10
molecular target	EGFR kinase	





gefitinib	HTS %I	IC_{50}, µM
granule count	91	0.38±0.04
nuclei count	5	> 10
molecular target	EGFR kinase	





lapatinib	HTS %I	IC_{50}, µM
granule count	91	0.59±0.03
nuclei count	0	> 10
molecular target	EGFR kinase	

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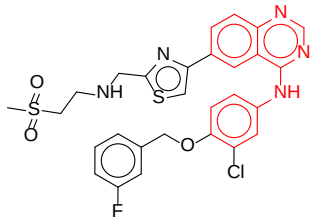
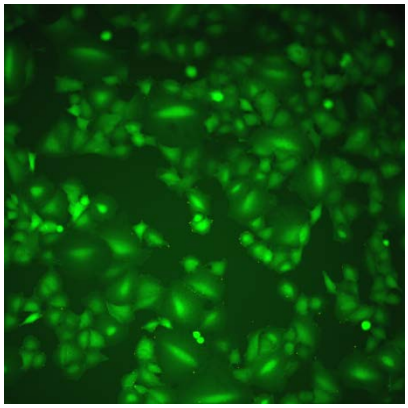
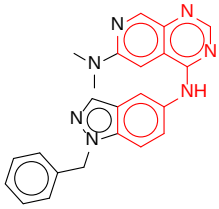
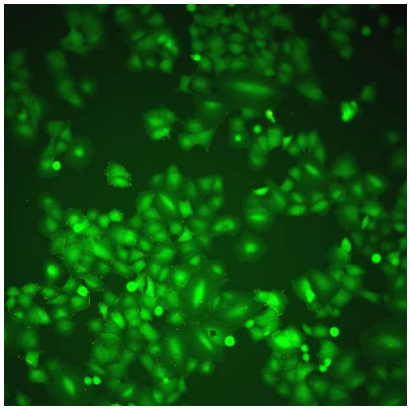


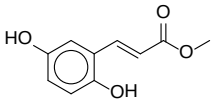


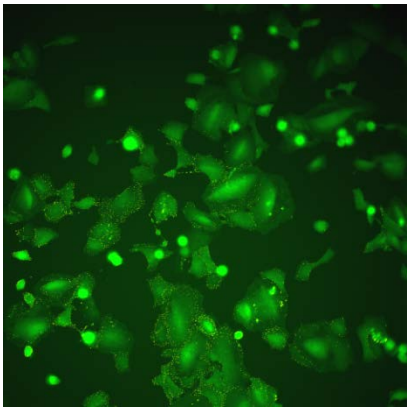
GW 583340	HTS %I	IC_{50}, µM
granule count	94	2.0±0.4
nuclei count	26	> 10
molecular target	EGFR kinase	



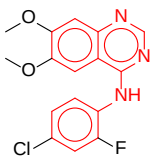


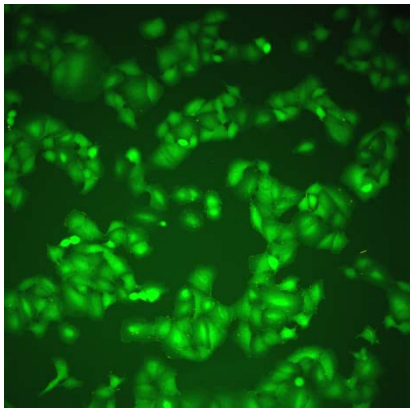
GW 2974	HTS %I	IC_{50}, µM
granule count	89	1.3±0.5
nuclei count	18	> 10
molecular target	EGFR kinase	



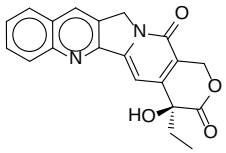


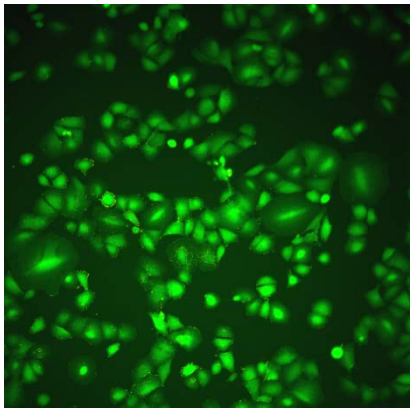
erbstatin analog	HTS %I	IC_{50}, µM
granule count	-24	> 10
nuclei count	2	2.3
molecular target	EGFR kinase	



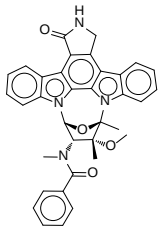


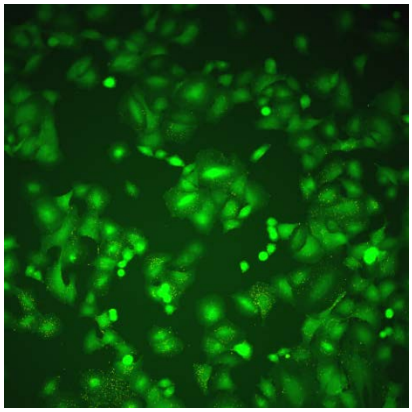
ZM 306416	HTS %I	IC_{50}, µM
granule count	97	0.67±0.2
nuclei count	21	> 10
molecular target	VEGFR kinase	





camptothecin	HTS %I	IC_{50}, µM
granule count	81	0.72±0.1
nuclei count	40	> 10
molecular target	topoisomerase I	





PKC 412	HTS %I	IC_{50}, µM
granule count	68	1.3±0.7
nuclei count	35	> 10
molecular target	pan-kinase inhibitor	

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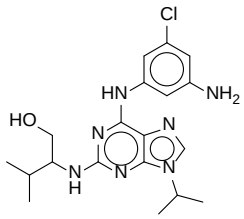
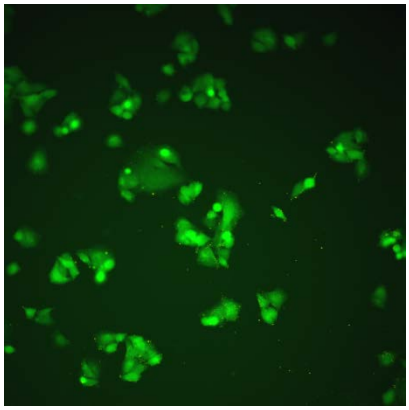
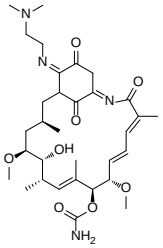
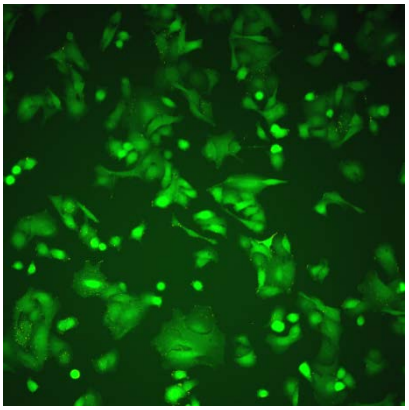


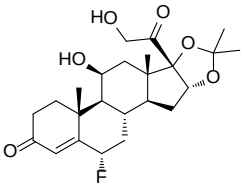


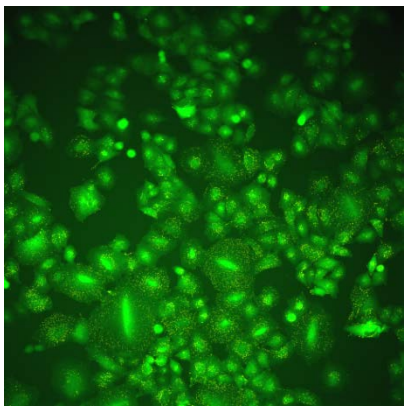
aminopurvalanol A	HTS %I	IC_{50}, µM
granule count	62	0.91±0.4
nuclei count	40	7.7
molecular target	CDKs	



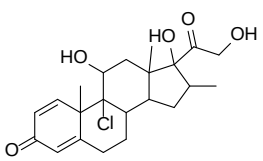


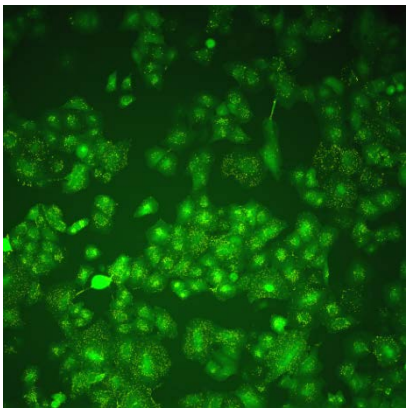
17-DMAG	HTS %I	IC_{50}, µM
granule count	92	0.029±0.003
nuclei count	33	> 10
molecular target	Hsp90	



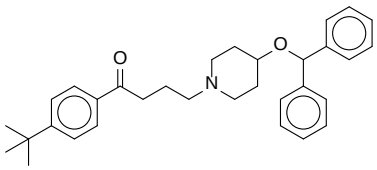


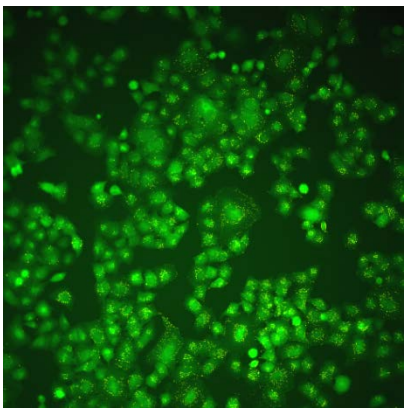
flurandrenolide	HTS %I	EC_{50}, µM
granule count	-165	0.023±0.02
nuclei count	7	> 10
molecular target	steroid	





beclomethasone	HTS %I	EC_{50}, µM
granule count	-116	0.042±0.007
nuclei count	16	> 10
molecular target	glucocorticoid	





ebastine	HTS %I	EC_{50}, µM
granule count	-110	1.5±0.3
nuclei count	6	> 10
molecular target	H1 receptor	

Suppl. Table 3 (3/3) – Antczak et al.