Innopharma Screening Platform

Examples of our services

Please contact us for custom services : biofarma.group@usc.es

ASSAYS AVAILABLE

Radioligand binding assays

Screening can be carried out for binding activity at the receptors listed bellow, as well as at many other receptors (as several cell lines expressing diferent receptors are available on the market). Given the Innopharma Screening Platform´s wide experience in screening, the fine tuning of these assays will not suppose a significant increase in the result reporting time.

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Radioligand binding assays

Adenosine Receptors

A_1 A_{2A} A_{2B} A_3

Dopamine Receptors

D_1 D_2 D_3 D_4 D_5

Leukotrienes Receptors

LTB_4

Histamine Receptors

H_1 H_2 H_3

Transporters

Dopamine transporter
Noradrenaline transporter
Serotonin transporter

Adrenergic Receptors

α_1 α_2 α_{2C}
β_1 β_2 β_3

GABA Receptors

$GABA_B$

Muscarinic Receptors

M_1 M_2 M_3 M_4 M_5

Transporters

Calcium channels
$GABA_A$
NMDA
PCP
Potasium chanels (hERG)
Serotonin 5-HT_3
Sodium channel

Cannabinoid Receptors

CB_1 CB_2

Serotonin Receptors

5-HT_{1A}
5-HT_{1B}
5-HT_{1D}
5-HT_{2A}
5-HT_{2B}
5-HT_{2C}
5-HT_4
5-HT_{4C}
5-HT_{4D}
5-HT_{4E}
5-HT_{5A}
5-HT_6
5-HT_7

Functional Assays

Second messenger assays

Arachidonic acid metabolism:
Phospholipase A2

Nitric oxide synthase:
Inducible and Constitutive

Adenilate cyclase
Guanilate Cyclase

Inositol exchange:
Chromatographic assay for measurement of inositol phsphates

Isolated organ assays

Adenosine:
A_{2A}, A_{2B}, A_1
Adrenergic:
α_1, α_2, β_1
Histamine:
H_1, H_2
Muscarinic:
M_1, M_2, M_3
Serotonin:
$5\text{-}HT_{2A}$,
$5\text{-}HT_{2B}$, $5\text{-}HT_3$,
$5\text{-}HT_4$

Cytotoxicity Assays

These assays are performed with the cell lines listened below, and the viability measured by means of crystal violet, MTT reduction of sulforhodamine B methodologie. The available cell lines are:

- Human hepatocarcinoma cells (HepG2)
- Human kidney cells(LLC-PK1)
- Human cervix cancer cells (HeLa 229)
- Human ovarian cancer cells (A2780)
- Cisplatin-resitant human ovarian cancer cells (A2780cis)
- Human lung cancer cells (NCI-H460)
- Human breast cancer cells (Hs 578T)
- Human breast cancer cells(MCF7)
- Human breast cancer cells(T47D)
- Human promyelocytic leukaemia cells(HL-60)
- Human fibroblast cells (MRC-5)

Preliminary Safety Assays

CytochromeP450 inhibition (CYP1A2,CYP2C9,CYP2C19,CYP2D6,CYP3A4)

Enzymatic Assays

Phosphatases :
(1B, 2B, CD45, pNPP, alkaline phosphatase, acid phophatase, calcineurin, protein tyrosine phosphatase)

Kinases:
(More than 75 serin/threonin kinases and more than 40 tyrosine kinases)

Phosphodiesterases:
(PDE1, PDE2, PDE3, PDE4, PDE5, PDE6)

Glucose metabolism studies

Preliminary ADME Assays

Caco2 studies

Solubility assays

SCREENING BATTERIES AVALIABLE

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General characterization of hits/candidates

These tests include characterization of the affinity of a compound in a series of more than 50 studied (including the current group of drug targets) in addition to safety of antitargets/targets, cytotoxicity, metabolism and preliminary pharmacokinetic studies. Diverse targets are evaluated:

- Receptors
- Ion channels
- Transporters
- Pumps
- Structural proteins
- Enzymes (Phosphodiesterases, kinases, phosphatases, hydrolases)

Adenosine Platform

Adenosine receptors are involved in different pathological processes: inflammation, renal insufficiency, Parkinson's disease, asthma and COPD, allergic rhinitis. The battery of tests includes:

- Studies of binding activity at human adenosine receptors (A_1, A_{2A}, A_{2B} and A_3).
- Functional studies of the mobilization of second messengers at human adenosine receptors (A_1, A_{2A}, A_{2B} and A_3).
- Functional studies in tissues isolated from experimental animals (A_1, A_{2A} and A_{2B}).

Serotonin Receptors

Serotonin receptors are involved in numerous physiological and pathological processes, for example, the 5-HT_{2B} serotonin receptor has recently been suggested to be involved in cardiac valvular pathologies. The battery of tests include:

- Studies of binding activity at human receptors (5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_{7}).
- Functional studies of the mobilization of second messengers at human serotonin receptors (5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_{7}).
- Functional studies in tissues isolated from experimental animals (5-HT_{2A}, 5-HT_{2B}, 5-HT_{7}).

Aminergic Receptors - antipsychotics

Although the mechanism of action of antipsychotics is still unclear, it has been suggested that these drugs exert their effect by interaction with different receptor subtypes (Payne A. Abstracts of the XIX Symposium on Medicinal Chemistry 2006. Drugs of the future 31 (suppl A) L-33; Roth et al., Nat Rev Drug Discov 2004; 3:353-9). In this battery of tests, chemical libraries of compounds are evaluated for binding activity at a selection of aminergic receptors involved in the mechanism of action of antipsychotic drugs:

- •5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_6 and 5-HT_7 serotonin receptors
- •D_1, D_2 D_3 and D_4 dopaminergic receptors
- •α_{1A} and α_{2A} adrenergic receptors
- •M_1, M_2, M_3 and M_4 muscarinic receptors
- • H_1 histaminergic receptor
- •σ_1 opioid receptor

Once compounds that display activity at some of these binding sites have been identified (% of displacement of the specific binding > 80%) the K_i for these receptors is calculated.

Cytotoxicity

The cytotoxicity of compounds with possible tumour activity is determined in three human cell lines:

- Lung tumour line (NCI-H460)
- Breast tumour line (MCF-7)
- Glioma tumour line (SF-268).

If more than 50% inhibition of cell growth is observed in any of these three cell lines, the cytotoxic potency (IC_{50}) is calculated from concentration-response curves (6 concentrations in duplicate).

Once an active compound is identified in any of these three cell lines, its toxicity is evaluated in other cell lines that the Innopharma Screening Platform has available (kidney, liver, ovarian, uterine cell and colon tumour cell lines, and leukaemia cell lines).

Preliminary safety

The preliminary safety of the compounds is evaluated in an initial battery of tests:

- Inhibition and induction of cytochromes: as indicators of possible alterations in the metabolism of other drugs.
- Cytotoxicity to fibroblasts: as an indicator of proapoptotic compounds.
- Alteration of the function of K^+ channels (hERG): as an indicator of compounds that induce cardiovascular alterations. We have available a rapid and economic technique currently validated in the pharmaceutical industry in *patch-clamp* and *in vivo* studies.

The potency of each compound in each of the assays is evaluated (6 concentrations in duplicate). Once this study is completed further safety studies can be carried out if the client wishes.

Preliminary pharmacokinetic tests in screening cascades

Evaluation of: passage through biological membranes (Caco-2), inhibition of P-glycoprotein and solubility of the compounds (turbidimetric study).

Programmes for discovery of preclinical candidate compounds

The Innopharma Screening Platform assesses and carries out *screening* cascades for clients, aimed at obtaining preclinical candidate compounds. The design consists of different stages:

- Definition of the clinical profile of the future drug: Must include the reasoning, needs not covered, differentiation of competitors, revision of the current market, potential peaks in sales and a clinical development plan.
- Creation of a permanent scientific committee formed by experts in external preclinical programmes who periodically evaluate the progress of the project.
- Selection of a Project Manager and team.
- Definition of the profile of the candidate preclinical compound and backups.
- Definition of leads: aimed at obtaining the profile required in leads from which the candidate will be obtained.
- Definition of the screening cascade and the critical route: establish studies to be carried out for complete pharmacological characterization of the candidate and the backups and those studies that are critical in this process for the scaffold of each of the hits.
- Fine-tuning of the screening cascade techniques
- Establishment of a timetable for monitoring the project.

Fine tuning and miniaturization of assays

The assays required in each programme are miniaturized and validated. The validation is carried out by inclusion of commercial and internal standards to correlate them with the values described in the literature and/or in internal low-throughput assays.

Large scale production of the biological reagent

The amounts of candidate biological reagents requested by the client are obtained. The client must provide the Innopharma Screening Platform with the original biological reagent of which large quantities are required.

Production of cell lines expressing human targets

The cDNA of the target of interest is transfected in cell lines. Those clones in which the transfection has been positive are selected and the expression of the target is confirmed by radioligand binding studies.

Biologically annotated library of chemical compounds

The Innopharma Screening Platform has its own chemical library containing approximately 12000 structurally diverse compounds that have been annotated and selected by virtual screening to guarantee the maximum diversity of biological interactions (Cellular Mapping).

High throughput functional screening of GPCRs for chemical libraries

The Innopharma Screening Platform has developed a technique for the simultaneous evaluation of the mobilization of inositol phosphates, arachidonic acid and cAMP. The assays involved enable identification of inverse neutral/agonists, as well as allosteric modulators of receptors to study in HTS and at a single concentration of the compound. These assays also enable evaluation of possible phenomena of *agonist trafficking/collateral efficacy/conformational selectivity*.

Pharmacological regulation of gene expression

The target cells are treated with the compounds selected in the programme and the regulation of gene expression induced by the treatment is evaluated by microarrays. Primary tissues are available for the prediction of clinical efficacy from the gene regulation exerted by the compounds.

Studies of protein regulation in *ex vivo* studies with experimental animals.

Regulation of the expression and conformational distribution exerted by the compounds under study is evaluated at the receptors of interest. Acute and subchronic treatments are carried out with therapeutic doses of the compounds in experimental animals; the animals are then sacrificed and a comparative study is carried out of the regulation exerted by the compounds relative to a control group (animals only treated with vehicle).

Proteomics studies to identify signalling routes

The intracellular signalling routes that mobilize the compounds under study are identified by phosphorylation/dephosphorylation studies.

Oligomerization, localization and translocation of proteins

Fluorescence microscope studies are carried out to evaluate the oligomerization, localization and translocation of the target receptors and the changes induced in these by the treatment.

Studies of protein-protein interactions

Carried out by different methodological approaches ranging from fluorescence microscopy to enzyme immunoassays with marked proteins.

Genotoxicity studies

The formation of micronuclei after treatment with compounds in the target cells is evaluated by fluorescence microscope studies.

Studies with human samples

The Innopharma Screening Platform has access to human samples from patients in the Santiago de Compostela Hospital Complex, which enables the validation of possible new targets of interest in samples of patients and/or controls.

Studies with panels of kinases

The efficacy of compounds on recombinant human kinases is evaluated by homogeneous time-resolved fluorescence (hTRF) measurements.

Studies of allosteric modulators of GPCR

Study of the modulation of GPCR conformations in the presence of an orthosteric ligand is carried out at the Innopharma Screening Platform by radioligand binding studies (kinetic and competition assays), as well as by functional assays to measure the formation of second messengers to evaluate modifications in the power and/or efficacy of the orthosteric ligand.

Tests with orphan GPCRs

Identification of agonists and antagonists of orphan GPCRs is a challenge for the identification of new therapeutic targets. The identification is carried out at the Innopharma Screening Platform by evaluation of the activation of orphan GPCRs, by use of different experimental techniques: GTPγ[^{35}S] binding, measurement of second messengers (cAMP, cGMP, Ca^{2+}, IP_3, etc), measurement of enzyme activity (Rho, ERK, etc), and use of chimeric G proteins that increase IP_3 or CAMP.

Client directed screening of chemical libraries

•Evaluation of chemical libraries with targets selected by the client. The compounds in the chemical library are evaluated at a single concentration agreed on with the client. The activity of the selected compounds as hits is confirmed by a second assay. Finally, the affinity (K_i) of the confirmed hits is tested with a concentration response curve (6 concentrations in duplicate).

•Evaluation of the affinity of hits in groups of targets/antitargets. Each compound is evaluated at a particularly concentration on the targets (> 50) chosen on the basis of programme (chemical/biological), by radioligand binding assays. The activity of the selected compounds as hits is confirmed in a second assay. Finally, the affinity (K_i) of the confirmed hits is calculated from a concentration response curve (6 concentrations in duplicate).

Client directed screening of chemical libraries

•Studies of functional characterization of compounds in human receptors: The agonist/antagonist behaviour of the compounds under study is determined at GPCR by evaluating the mobilization of second messengers (IP_3, cAMP, cGMP, Ca^{2+}).

The following are evaluated:

Agonist compounds: Potency (EC_{50}) and efficacy (Emax).
Antagonist compounds: Potency (K_B).

All evaluations are carried out by use of concentration-response curves for the compound under study (6 concentrations per point, in duplicate).

Client directed screening of chemical libraries

Studies of the functional characterization of compounds at receptors in experimental animals: The agonist/antagonist behaviour of the compounds under study is determined at different receptors in experimental animals, by studies with isolated tissues (aorta, stomach fundus, auricle, colon and ileum) from the animals (different species of mouse and guinea pig). (Tests are currently available for A_1, A_{2A} and A_{2B} adenosine receptors, α_1, α_2 and β adrenergic receptors, H_1 and H_2 histamine receptors, M_1, M_2 and M_3 muscarinic receptors, 5-HT_{2A}, 5-HT_{2B}, 5-HT_3 and 5-HT_4 serotonin receptors.

The following are evaluated:

•Agonist compounds: Potency (EC_{50}) and efficacy (Emax), by concentration-response curves for the compounds.

•Antagonist compounds: Potency (K_B), by concentration-response curves for an agonist in the absence and presence of the compound under study.

Client directed screening of chemical libraries

For more information, please visit us at:

For any question or quotation inquiries please contact us at: biofarma.group@usc.es