***FINAL REPORT***

*In Vitro* Pharmacology

# Study of HC-122608

STUDY NUMBER
100004002

Annie OTTO-BRUC, Ph.D.

September 6, 2012

***CONFIDENTIAL***

# STUDY REFERENCES

| | | |
|---|---|---|
| **Study title** | ***In Vitro* Pharmacology Study of HC-122608** | |
| **Cerep study number** | **100004002** | **FINAL REPORT September 6, 2012** |
| **Experimental period** | August 10, 2012 -August 31, 2012 | |
| **Client personal study number** | - | |
| **PO number** | HBS14359 | |

# PERSONS INVOLVED IN THE STUDY

| | | |
|---|---|---|
| **Investigator** | **Cerep**<br>Le Bois l'Evêque<br>B.P. 30001<br>86 600 Celle l'Evescault<br>France<br>Tel: +33 (0)5 49 89 30 00<br>Fax: +33 (0)5 49 43 21 70 | **Annie OTTO-BRUC, Ph.D.**<br>Principal Scientist, Pharmacology<br><br>Tel: +33 (0)5 49 89 33 87<br>Fax: +33 (0)5 49 43 21 70 |
| **Study sponsor** | **HYDRA BIOSCIENCES**<br>Target Discovery-Channel group<br>790 Memorial Drive<br><br>CAMBRIDGE, MA 02139<br>U.S.A. | **Dr. Magdalene MORAN**<br>Senior Scientist and Director |
| **Testing facilities** | **Cerep**<br>Le Bois l'Evêque<br>B.P. 30001<br>86 600 Celle l'Evescault<br>France | **Cerep**<br>15318 NE 95th Street<br>Redmond, WA 98052<br>U.S.A. |

# APPROVAL

| | | |
|---|---|---|
| **Investigator** | **Cerep**<br>Le Bois l'Evêque<br>B.P. 30001<br>86 600 Celle l'Evescault<br>France<br>Tel: +33 (0)5 49 89 30 00<br>Fax: +33 (0)5 49 43 21 70 | **Annie OTTO-BRUC, Ph.D.**<br>Principal Scientist, Pharmacology<br><br>Tel: +33 (0)5 49 89 33 87<br>Fax: +33 (0)5 49 43 21 70<br><br>Date September 6, 2012<br>Signature<br>  |
| **Quality assurance statement** | Cerep's Quality Unit certifies that results presented in this report were generated using the materials and methods mentioned and that these results accurately reflect the raw data. | Date Septembre 07, 2012<br>Signature<br>  <br>Nadine Pasquier. |

# CONTENTS

# 1. SUMMARY

The purpose of this study was to test HC-122608 in binding and enzyme assays.

# 2. COMPOUNDS

## 2.1. Test Compounds

From: **HYDRA BIOSCIENCES**

| Client Compound ID | Cerep ID | Reference Number | Batch Number | FW | MW | Purity | Received Form | Stock solution | Flag |
|---|---|---|---|---|---|---|---|---|---|
| HC-122608 | 100004002-1 | - | 5 | 525.0 | 525.0 | - | Liquid | 1.E-02 M DMSO* | - |

*FW: Formula Weight - MW: Molecular Weight*

*: For *in vitro* pharmacology assays, depending on the assay volume and solvent tolerance, the stock solutions were diluted to [100x], [333x] or [1000x] in 100% solvent, then either added directly or further diluted to [10x] or [5x] in $H_2O$ or assay buffer before addition to the assay well (final solvent concentration kept constant).

## 2.2. Reference Compounds

In each experiment and if applicable, the respective reference compound was tested concurrently with HC-122608, and the data were compared with historical values determined at Cerep. The experiment was accepted in accordance with Cerep's validation Standard Operating Procedure.

# 3. RESULTS

## 3.1. *In Vitro* Pharmacology: Binding Assays

### 3.1.1. Test Compound Results

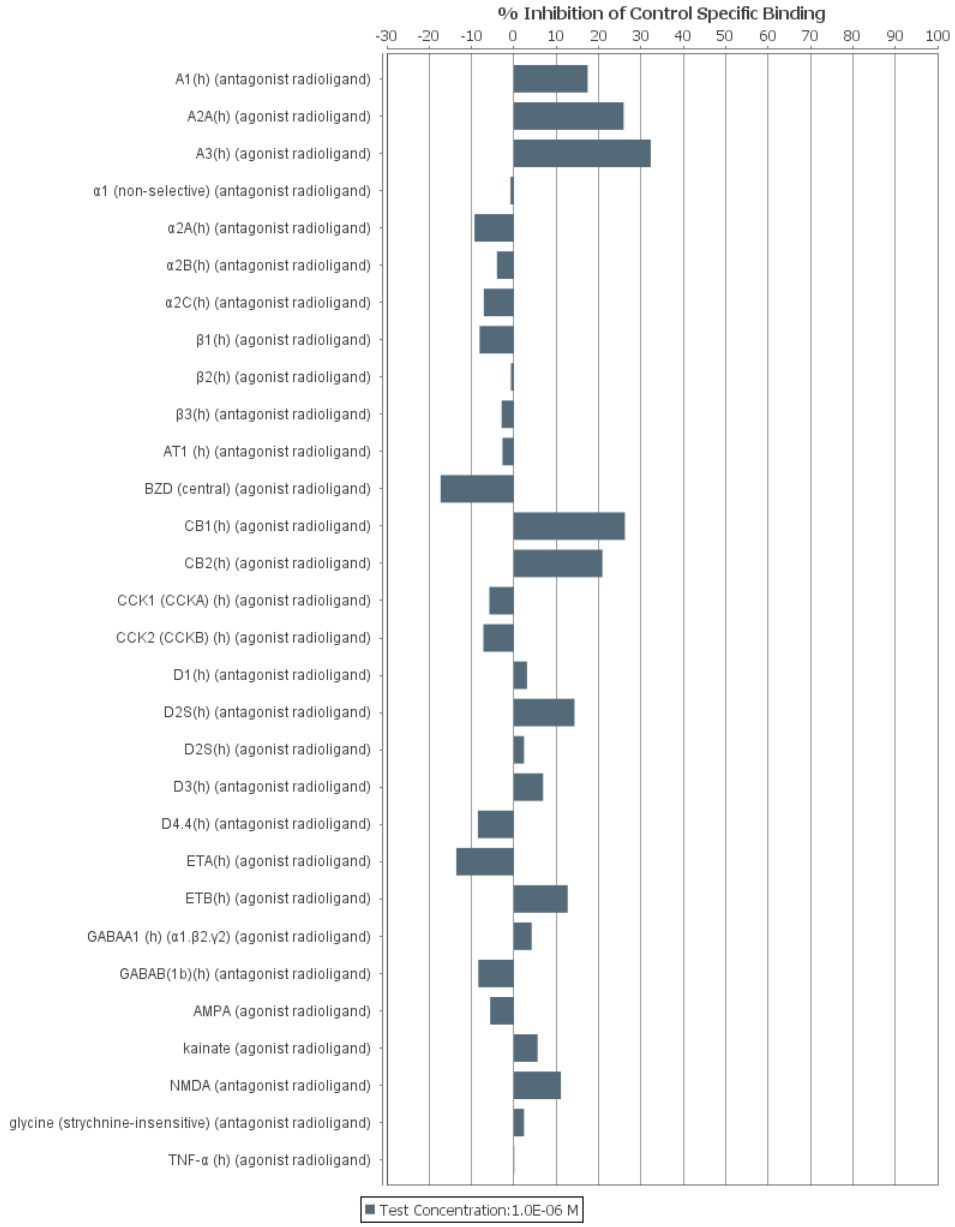


**Figure 1. Histogram for HC-122608 [1/3]**

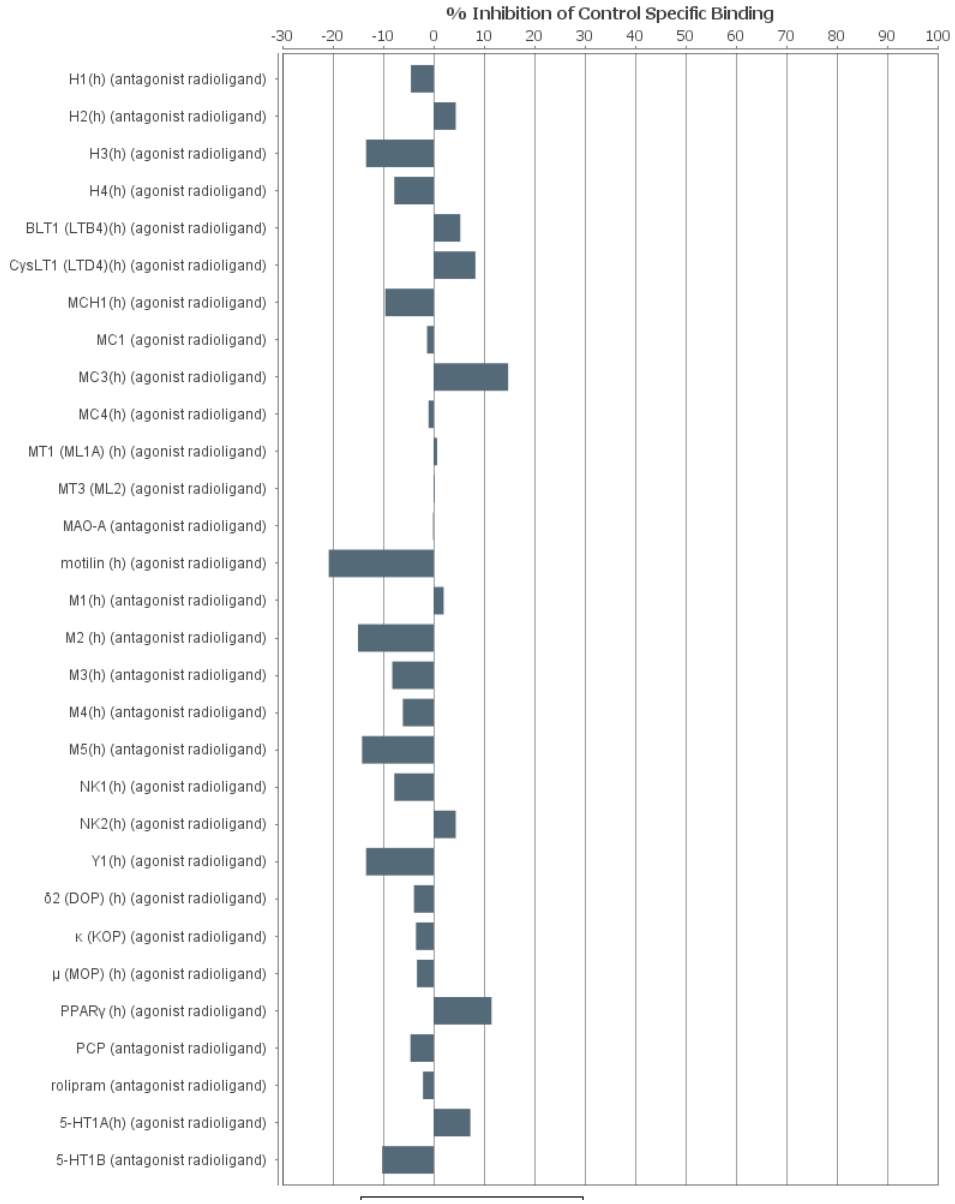


**Figure 2. Histogram for HC-122608 [2/3]**


**Figure 3. Histogram for HC-122608 [3/3]**

| Cerep Compound I.D. | Client Compound I.D. | Test Concentration | % Inhibition of Control Specific Binding | | |
|---|---|---|---|---|---|
| | | | 1st | 2nd | Mean |
| **$A_1$ *(h)* (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 22.5 | 12.3 | 17.4 |
| **$A_{2A}$ *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 20.8 | 31.1 | 25.9 |
| **$A_3$ *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 28.3 | 36.4 | 32.3 |
| **$\alpha_1$ (non-selective) (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -2.8 | 1.1 | -0.8 |
| **$\alpha_{2A}$ *(h)* (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -14.9 | -3.7 | -9.3 |
| **$\alpha_{2B}$ *(h)* (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -7.4 | -0.7 | -4.0 |

| Cerep Compound I.D. | Client Compound I.D. | Test Concentration | % Inhibition of Control Specific Binding | | |
|---|---|---|---|---|---|
| | | | 1st | 2nd | Mean |
| **$\alpha_{2C}$ *(h)* (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -4.1 | -10.2 | -7.1 |
| **$\beta_1$ *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -14.5 | -1.8 | -8.1 |
| **$\beta_2$ *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -1.9 | 0.5 | -0.7 |
| **$\beta_3$ *(h)* (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 0.9 | -6.7 | -2.9 |
| **$AT_1$ *(h)* (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 0.5 | -6.0 | -2.7 |
| **BZD (central) (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -3.0 | -31.5 | -17.3 |
| **$CB_1$ *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 27.0 | 25.5 | 26.2 |
| **$CB_2$ *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 16.6 | 25.1 | 20.9 |
| **$CCK_1$ ($CCK_A$) *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -11.2 | -0.4 | -5.8 |
| **$CCK_2$ ($CCK_B$) *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -13.8 | -0.7 | -7.2 |
| **$D_1$ *(h)* (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 1.1 | 5.0 | 3.1 |
| **$D_{2S}$ *(h)* (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 19.2 | 9.4 | 14.3 |
| **$D_{2S}$ *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 4.6 | 0.2 | 2.4 |
| **$D_3$ *(h)* (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 4.0 | 9.7 | 6.9 |
| **$D_{4.4}$ *(h)* (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -20.4 | 3.4 | -8.5 |
| **$ET_A$ *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -33.9 | 6.7 | -13.6 |
| **$ET_B$ *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 25.5 | -0.1 | 12.7 |
| **$GABA_{A1}$ *(h)* (α1,β2,γ2) (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 0.6 | 7.9 | 4.2 |
| **$GABA_{B(1b)}$ *(h)* (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -9.0 | -7.8 | -8.4 |
| **AMPA (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -2.1 | -9.2 | -5.6 |
| **kainate (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 5.9 | 5.3 | 5.6 |
| **NMDA (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 19.0 | 3.2 | 11.1 |
| **glycine (strychnine-insensitive) (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 1.7 | 3.1 | 2.4 |
| **TNF-α *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 8.8 | -8.6 | 0.1 |
| **$H_1$ *(h)* (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -8.1 | -0.9 | -4.5 |
| **$H_2$ *(h)* (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 1.1 | 7.7 | 4.4 |
| **$H_3$ *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -25.9 | -0.8 | -13.4 |
| **$H_4$ *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -13.6 | -1.9 | -7.8 |

| Cerep Compound I.D. | Client Compound I.D. | Test Concentration | % Inhibition of Control Specific Binding | | |
|---|---|---|---|---|---|
| | | | 1st | 2nd | Mean |
| **$BLT_1$ ($LTB_4$) *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -2.9 | 13.4 | 5.3 |
| **$CysLT_1$ ($LTD_4$) *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 13.2 | 3.4 | 8.3 |
| **$MCH_1$ *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 7.9 | -27.2 | -9.6 |
| **$MC_1$ (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 1.0 | -3.6 | -1.3 |
| **$MC_3$ *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 14.4 | 15.3 | 14.8 |
| **$MC_4$ *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 2.9 | -4.8 | -1.0 |
| **$MT_1$ ($ML_{1A}$) *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 2.2 | -0.9 | 0.7 |
| **$MT_3$ ($ML_2$) (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -0.7 | 1.2 | 0.2 |
| **MAO-A (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -3.8 | 3.6 | -0.1 |
| **motilin *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -25.4 | -16.2 | -20.8 |
| **$M_1$ *(h)* (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -1.6 | 5.5 | 2.0 |
| **$M_2$ *(h)* (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -15.9 | -14.2 | -15.0 |
| **$M_3$ *(h)* (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -9.0 | -7.5 | -8.2 |
| **$M_4$ *(h)* (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -16.1 | 3.9 | -6.1 |
| **$M_5$ *(h)* (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -15.6 | -12.8 | -14.2 |
| **$NK_1$ *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -2.6 | -13.0 | -7.8 |
| **$NK_2$ *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -10.0 | 18.9 | 4.4 |
| **$Y_1$ *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -12.8 | -14.1 | -13.4 |
| **$\delta_2$ (DOP) *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -10.8 | 3.1 | -3.9 |
| **κ (KOP) (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -8.1 | 1.1 | -3.5 |
| **μ (MOP) *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 0.1 | -6.8 | -3.3 |
| **PPARγ *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 6.7 | 16.2 | 11.5 |
| **PCP (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -9.0 | -0.1 | -4.6 |
| **rolipram (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -4.6 | 0.5 | -2.1 |
| **5-$HT_{1A}$ *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -0.2 | 14.8 | 7.3 |
| **5-$HT_{1B}$ (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -11.0 | -9.4 | -10.2 |
| **5-$HT_{1D}$ (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -4.2 | -8.8 | -6.5 |
| **5-$HT_{2A}$ *(h)* (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -4.3 | 6.9 | 1.3 |

| Cerep Compound I.D. | Client Compound I.D. | Test Concentration | % Inhibition of Control Specific Binding | | |
|---|---|---|---|---|---|
| | | | 1st | 2nd | Mean |
| **5-HT$_{2A}$ *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 1.8 | -14.3 | -6.2 |
| **5-HT$_{2B}$ *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 15.9 | 15.8 | 15.9 |
| **5-HT$_{2C}$ *(h)* (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 12.1 | 12.6 | 12.3 |
| **5-HT$_{2C}$ *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -8.5 | -4.7 | -6.6 |
| **5-HT$_3$ *(h)* (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -4.1 | 9.5 | 2.7 |
| **5-HT$_{4\ e}$ *(h)* (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -3.7 | 10.6 | 3.4 |
| **5-HT$_6$ *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -5.1 | 7.3 | 1.1 |
| **5-HT$_7$ *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 12.1 | 10.9 | 11.5 |
| **sigma (non-selective) (h) (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 28.0 | 30.6 | 29.3 |
| **sst$_4$ *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -27.0 | 9.5 | -8.8 |
| **GR *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -5.0 | -3.8 | -4.4 |
| **AR *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -3.8 | -9.0 | -6.4 |
| **TR (TH) (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 3.2 | -1.6 | 0.8 |
| **UT *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 11.5 | 7.0 | 9.3 |
| **VPAC$_1$ (VIP$_1$) *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -9.0 | -8.4 | -8.7 |
| **V$_{1\ a}$ *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -9.9 | 12.5 | 1.3 |
| **V$_2$ *(h)* (agonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 8.6 | 5.7 | 7.1 |
| **$Ca^{2+}$ channel (L, diltiazem site) (benzothiazepines) (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -1.9 | 8.8 | 3.4 |
| **$Ca^{2+}$ channel (L, verapamil site) (phenylalkylamine) (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 2.3 | 14.5 | 8.4 |
| **$Ca^{2+}$ channel (N) (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -5.9 | -13.1 | -9.5 |
| **SK$_{Ca}$ channel (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -2.5 | -4.7 | -3.6 |
| **$Na^+$ channel (site 2) (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 12.0 | -7.3 | 2.3 |
| **$Cl^-$ channel (GABA-gated) (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 24.7 | 23.2 | 23.9 |
| **norepinephrine transporter *(h)* (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 4.8 | 18.1 | 11.5 |
| **dopamine transporter *(h)* (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 22.8 | 37.1 | 29.9 |
| **GABA transporter (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -13.1 | -18.4 | -15.7 |
| **choline transporter (CHT1) *(h)* (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -5.8 | -1.9 | -3.9 |
| **5-HT transporter *(h)* (antagonist radioligand)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -6.2 | 4.6 | -0.8 |

## 3.1.2. Reference Compound Results

| Reference Compound | $IC_{50}$ (M) | $K_i$ (M) | nH |
|---|---|---|---|
| **$A_1$ *(h)* (antagonist radioligand)** | | | |
| DPCPX | 7.8E-10 M | 4.9E-10 M | 1.2 |
| **$A_{2A}$ *(h)* (agonist radioligand)** | | | |
| NECA | 1.7E-08 M | 1.4E-08 M | 0.6 |
| **$A_3$ *(h)* (agonist radioligand)** | | | |
| IB-MECA | 1.8E-10 M | 1.1E-10 M | 0.8 |
| **$\alpha_1$ (non-selective) (antagonist radioligand)** | | | |
| prazosin | 2.1E-10 M | 5.5E-11 M | 1.1 |
| **$\alpha_{2A}$ *(h)* (antagonist radioligand)** | | | |
| yohimbine | 3.4E-09 M | 1.5E-09 M | 1.1 |
| **$\alpha_{2B}$ *(h)* (antagonist radioligand)** | | | |
| yohimbine | 5.9E-09 M | 3.9E-09 M | 1.0 |
| **$\alpha_{2C}$ *(h)* (antagonist radioligand)** | | | |
| yohimbine | 2.3E-09 M | 7.4E-10 M | 1.1 |
| **$\beta_1$ *(h)* (agonist radioligand)** | | | |
| atenolol | 1.5E-07 M | 8.6E-08 M | 1.0 |
| **$\beta_2$ *(h)* (agonist radioligand)** | | | |
| ICI 118551 | 5.2E-10 M | 1.7E-10 M | 0.9 |
| **$\beta_3$ *(h)* (antagonist radioligand)** | | | |
| cyanopindolol | 5.9E-08 M | 3.6E-08 M | 0.5 |
| **$AT_1$ *(h)* (antagonist radioligand)** | | | |
| saralasin | 1.2E-09 M | 6.2E-10 M | 0.9 |
| **BZD (central) (agonist radioligand)** | | | |
| diazepam | 8.3E-09 M | 6.9E-09 M | 0.8 |
| **$CB_1$ *(h)* (agonist radioligand)** | | | |
| CP 55940 | 5.0E-10 M | 4.4E-10 M | 0.7 |
| **$CB_2$ *(h)* (agonist radioligand)** | | | |
| WIN 55212-2 | 3.2E-10 M | 2.1E-10 M | 0.5 |
| **$CCK_1$ ($CCK_A$) *(h)* (agonist radioligand)** | | | |
| CCK-8s | 8.2E-11 M | 6.2E-11 M | 1.0 |
| **$CCK_2$ ($CCK_B$) *(h)* (agonist radioligand)** | | | |
| CCK-8s | 1.1E-10 M | 4.5E-11 M | 0.8 |
| **$D_1$ *(h)* (antagonist radioligand)** | | | |
| SCH 23390 | 2.4E-10 M | 9.5E-11 M | 0.7 |
| **$D_{2S}$ *(h)* (antagonist radioligand)** | | | |
| (+)butaclamol | 2.5E-09 M | 8.4E-10 M | 1.1 |
| **$D_{2S}$ *(h)* (agonist radioligand)** | | | |
| 7-OH-DPAT | 1.7E-09 M | 6.8E-10 M | 0.9 |
| **$D_3$ *(h)* (antagonist radioligand)** | | | |
| (+)butaclamol | 1.3E-09 M | 3.0E-10 M | 0.8 |
| **$D_{4.4}$ *(h)* (antagonist radioligand)** | | | |
| clozapine | 4.6E-08 M | 1.8E-08 M | 0.9 |
| **$ET_A$ *(h)* (agonist radioligand)** | | | |
| endothelin-1 | 6.6E-11 M | 3.3E-11 M | 1.3 |
| **$ET_B$ *(h)* (agonist radioligand)** | | | |
| endothelin-3 | 1.1E-11 M | 6.4E-12 M | 1.0 |
| **$GABA_{A1}$ *(h)* (α1,β2,γ2) (agonist radioligand)** | | | |
| muscimol | 8.0E-08 M | 5.3E-08 M | 0.8 |
| **$GABA_{B(1b)}$ *(h)* (antagonist radioligand)** | | | |
| CGP 54626 | 8.2E-10 M | 4.1E-10 M | 0.9 |
| **AMPA (agonist radioligand)** | | | |
| L-glutamate | 3.1E-07 M | 2.8E-07 M | 0.9 |
| **kainate (agonist radioligand)** | | | |
| kainic acid | 2.0E-08 M | 1.6E-08 M | 0.7 |
| **NMDA (antagonist radioligand)** | | | |
| CGS 19755 | 2.0E-06 M | 1.6E-06 M | 1.4 |

| Reference Compound | $IC_{50}$ (M) | $K_i$ (M) | nH |
|---|---|---|---|
| **glycine (strychnine-insensitive) (antagonist radioligand)** | | | |
| glycine | 2.6E-07 M | 2.4E-07 M | 0.6 |
| **TNF-α *(h)* (agonist radioligand)** | | | |
| TNF-alpha | 1.3E-10 M | 4.4E-11 M | 0.8 |
| **$H_1$ *(h)* (antagonist radioligand)** | | | |
| pyrilamine | 2.3E-09 M | 1.5E-09 M | 1.2 |
| **$H_2$ *(h)* (antagonist radioligand)** | | | |
| cimetidine | 3.3E-07 M | 3.2E-07 M | 0.9 |
| **$H_3$ *(h)* (agonist radioligand)** | | | |
| (R)α-Me-histamine | 2.2E-09 M | 5.3E-10 M | 0.9 |
| **$H_4$ *(h)* (agonist radioligand)** | | | |
| imetit | 4.4E-09 M | 1.9E-09 M | 1.0 |
| **$BLT_1$ ($LTB_4$) *(h)* (agonist radioligand)** | | | |
| $LTB_4$ | 2.2E-10 M | 1.1E-10 M | 0.8 |
| **$CysLT_1$ ($LTD_4$) *(h)* (agonist radioligand)** | | | |
| $LTD_4$ | 2.7E-10 M | 1.2E-10 M | 1.1 |
| **$MCH_1$ *(h)* (agonist radioligand)** | | | |
| human MCH | 7.6E-11 M | 6.9E-11 M | 0.9 |
| **$MC_1$ (agonist radioligand)** | | | |
| NDP-α-MSH | 1.0E-10 M | 5.1E-11 M | 1.1 |
| **$MC_3$ *(h)* (agonist radioligand)** | | | |
| NDP-α-MSH | 7.3E-11 M | 6.2E-11 M | 0.6 |
| **$MC_4$ *(h)* (agonist radioligand)** | | | |
| NDP-α-MSH | 4.0E-10 M | 3.7E-10 M | 0.8 |
| **$MT_1$ ($ML_{1A}$) *(h)* (agonist radioligand)** | | | |
| melatonin | 1.8E-10 M | 1.4E-10 M | 1.0 |
| **$MT_3$ ($ML_2$) (agonist radioligand)** | | | |
| melatonin | 2.6E-07 M | 2.5E-07 M | 0.3 |
| **MAO-A (antagonist radioligand)** | | | |
| clorgyline | 1.0E-09 M | 6.0E-10 M | 1.5 |
| **motilin *(h)* (agonist radioligand)** | | | |
| [$Nleu^{13}$]-motilin | 2.8E-09 M | 2.3E-09 M | 0.7 |
| **$M_1$ *(h)* (antagonist radioligand)** | | | |
| pirenzepine | 1.7E-08 M | 1.5E-08 M | 0.8 |
| **$M_2$ *(h)* (antagonist radioligand)** | | | |
| methoctramine | 3.0E-08 M | 2.1E-08 M | 0.9 |
| **$M_3$ *(h)* (antagonist radioligand)** | | | |
| 4-DAMP | 5.7E-10 M | 4.1E-10 M | 1.2 |
| **$M_4$ *(h)* (antagonist radioligand)** | | | |
| 4-DAMP | 3.5E-10 M | 2.1E-10 M | 0.8 |
| **$M_5$ *(h)* (antagonist radioligand)** | | | |
| 4-DAMP | 4.5E-10 M | 2.2E-10 M | 0.8 |
| **$NK_1$ *(h)* (agonist radioligand)** | | | |
| [$Sar^9$,$Met(O_2)^{11}$]-SP | 2.2E-10 M | 9.8E-11 M | 1.1 |
| **$NK_2$ *(h)* (agonist radioligand)** | | | |
| [$Nleu^{10}$]-NKA (4-10) | 5.8E-09 M | 3.2E-09 M | 0.8 |
| **$Y_1$ *(h)* (agonist radioligand)** | | | |
| NPY | 1.4E-10 M | 9.6E-11 M | 0.9 |
| **$δ_2$ (DOP) *(h)* (agonist radioligand)** | | | |
| DPDPE | 4.0E-09 M | 2.4E-09 M | 0.9 |
| **κ (KOP) (agonist radioligand)** | | | |
| U 50488 | 8.1E-10 M | 5.4E-10 M | 1.1 |
| **μ (MOP) *(h)* (agonist radioligand)** | | | |
| DAMGO | 1.0E-09 M | 4.1E-10 M | 0.9 |
| **PPARγ *(h)* (agonist radioligand)** | | | |
| rosiglitazone | 1.1E-08 M | 5.8E-09 M | 0.9 |
| **PCP (antagonist radioligand)** | | | |
| MK 801 | 6.2E-09 M | 3.5E-09 M | 1.2 |

| Reference Compound | $IC_{50}$ (M) | $K_i$ (M) | nH |
|---|---|---|---|
| **rolipram (antagonist radioligand)** | | | |
| rolipram | 7.1E-10 M | 3.6E-10 M | 0.7 |
| **5-$HT_{1A}$ *(h)* (agonist radioligand)** | | | |
| 8-OH-DPAT | 4.3E-10 M | 2.7E-10 M | 1.1 |
| **5-$HT_{1B}$ (antagonist radioligand)** | | | |
| serotonin | 6.9E-09 M | 4.2E-09 M | 0.7 |
| **5-$HT_{1D}$ (agonist radioligand)** | | | |
| serotonin | 1.4E-09 M | 4.7E-10 M | 1.1 |
| **5-$HT_{2A}$ *(h)* (antagonist radioligand)** | | | |
| ketanserin | 1.2E-09 M | 6.6E-10 M | 1.4 |
| **5-$HT_{2A}$ *(h)* (agonist radioligand)** | | | |
| (±)DOI | 1.5E-10 M | 1.2E-10 M | 0.6 |
| **5-$HT_{2B}$ *(h)* (agonist radioligand)** | | | |
| (±)DOI | 5.5E-09 M | 2.7E-09 M | 1.1 |
| **5-$HT_{2C}$ *(h)* (antagonist radioligand)** | | | |
| RS 102221 | 2.3E-09 M | 7.8E-10 M | 0.8 |
| **5-$HT_{2C}$ *(h)* (agonist radioligand)** | | | |
| (±)DOI | 6.8E-10 M | 6.1E-10 M | 1.0 |
| **5-$HT_3$ *(h)* (antagonist radioligand)** | | | |
| MDL 72222 | 1.0E-08 M | 7.1E-09 M | 1.1 |
| **5-$HT_{4\ e}$ *(h)* (antagonist radioligand)** | | | |
| serotonin | 5.0E-07 M | 1.7E-07 M | 0.9 |
| **5-$HT_6$ *(h)* (agonist radioligand)** | | | |
| serotonin | 9.3E-08 M | 4.4E-08 M | 1.0 |
| **5-$HT_7$ *(h)* (agonist radioligand)** | | | |
| serotonin | 3.2E-10 M | 1.2E-10 M | 1.1 |
| **sigma (non-selective) (h) (agonist radioligand)** | | | |
| haloperidol | 5.4E-08 M | 4.3E-08 M | 0.8 |
| **$sst_4$ *(h)* (agonist radioligand)** | | | |
| somatostatin-14 | 3.5E-09 M | 3.5E-09 M | 0.9 |
| **GR *(h)* (agonist radioligand)** | | | |
| dexamethasone | 4.1E-09 M | 2.1E-09 M | 1.1 |
| **AR *(h)* (agonist radioligand)** | | | |
| mibolerone | 2.6E-09 M | 1.1E-09 M | 1.0 |
| **TR (TH) (agonist radioligand)** | | | |
| $T_3$ | 2.1E-10 M | 1.5E-10 M | 1.0 |
| **UT *(h)* (agonist radioligand)** | | | |
| urotensin-II | 1.2E-09 M | 8.9E-10 M | 1.0 |
| **$VPAC_1$ ($VIP_1$) *(h)* (agonist radioligand)** | | | |
| VIP | 2.3E-10 M | 1.3E-10 M | 0.9 |
| **$V_{1\ a}$ *(h)* (agonist radioligand)** | | | |
| $[d(CH_2)_5{}^1,Tyr(Me)_2]$-AVP | 5.1E-10 M | 3.2E-10 M | 0.9 |
| **$V_2$ *(h)* (agonist radioligand)** | | | |
| AVP | 1.2E-09 M | 8.8E-10 M | 0.7 |
| **$Ca^{2+}$ channel (L, diltiazem site) (benzothiazepines) (antagonist radioligand)** | | | |
| diltiazem | 1.6E-08 M | 1.3E-08 M | 1.2 |
| **$Ca^{2+}$ channel (L, verapamil site) (phenylalkylamine) (antagonist radioligand)** | | | |
| D 600 | 1.2E-08 M | 5.9E-09 M | 0.6 |
| **$Ca^{2+}$ channel (N) (antagonist radioligand)** | | | |
| ω-conotoxin GVIA | 1.2E-12 M | 5.1E-13 M | 1.9 |
| **$SK_{Ca}$ channel (antagonist radioligand)** | | | |
| apamin | 1.2E-11 M | 5.9E-12 M | 1.7 |
| **$Na^+$ channel (site 2) (antagonist radioligand)** | | | |
| veratridine | 3.9E-06 M | 3.5E-06 M | 1.0 |
| **$Cl^-$ channel (GABA-gated) (antagonist radioligand)** | | | |
| picrotoxinin | 9.1E-08 M | 7.5E-08 M | 0.9 |
| **norepinephrine transporter *(h)* (antagonist radioligand)** | | | |
| protriptyline | 2.4E-09 M | 1.8E-09 M | 1.0 |

| Reference Compound | $IC_{50}$ (M) | $K_i$ (M) | nH |
|---|---|---|---|
| **dopamine transporter *(h)* (antagonist radioligand)** | | | |
| BTCP | 7.1E-09 M | 3.8E-09 M | 0.9 |
| **GABA transporter (antagonist radioligand)** | | | |
| nipecotic acid | 1.6E-06 M | 1.6E-06 M | 0.8 |
| **choline transporter (CHT1) *(h)* (antagonist radioligand)** | | | |
| hemicholinium-3 | 6.5E-09 M | 3.6E-09 M | 0.7 |
| **5-HT transporter *(h)* (antagonist radioligand)** | | | |
| imipramine | 1.5E-09 M | 6.9E-10 M | 1.1 |

## 3.2. *In Vitro* Pharmacology: Enzyme and Uptake Assays

### 3.2.1. Test Compound Results

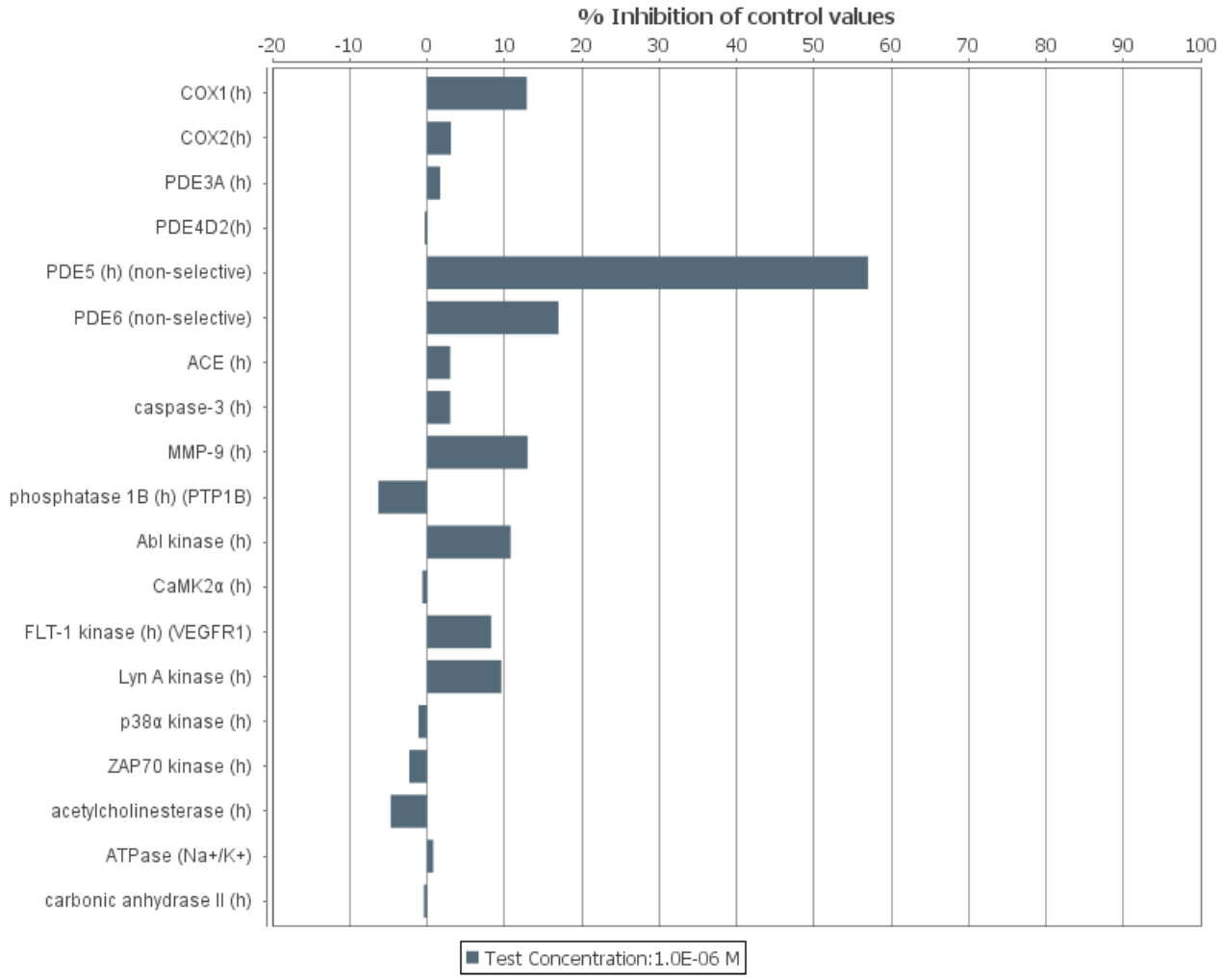


**Figure 4. Histogram for HC-122608**

| Cerep Compound I.D. | Client Compound I.D. | Test Concentration | % Inhibition of control values | | |
|---|---|---|---|---|---|
| | | | 1st | 2nd | Mean |
| **$COX_1$ *(h)*** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -1.5 | 27.2 | 12.9 |
| **$COX_2$ *(h)*** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -5.6 | 11.8 | 3.1 |
| **PDE3A *(h)*** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 3.1 | 0.2 | 1.7 |
| **$PDE4D_2$ *(h)*** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 1.3 | -2.0 | -0.3 |
| **PDE5 *(h)* (non-selective)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 54.6 | 59.4 | **57.0** |
| **PDE6 (non-selective)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 25.3 | 8.8 | 17.0 |

| Cerep Compound I.D. | Client Compound I.D. | Test Concentration | % Inhibition of control values | | |
|---|---|---|---|---|---|
| | | | 1st | 2nd | Mean |
| **ACE *(h)*** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 0.0 | 6.0 | 3.0 |
| **caspase-3 *(h)*** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 3.7 | 2.3 | 3.0 |
| **MMP-9 *(h)*** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 8.7 | 17.2 | 13.0 |
| **phosphatase 1B *(h)* (PTP1B)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -6.7 | -5.9 | -6.3 |
| **Abl kinase *(h)*** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 11.4 | 10.2 | 10.8 |
| **CaMK2α *(h)*** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -4.0 | 2.8 | -0.6 |
| **FLT-1 kinase *(h)* (VEGFR1)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 9.3 | 7.3 | 8.3 |
| **Lyn A kinase *(h)*** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 13.7 | 5.5 | 9.6 |
| **p38α kinase *(h)*** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -0.9 | -1.2 | -1.1 |
| **ZAP70 kinase *(h)*** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -1.6 | -3.0 | -2.3 |
| **acetylcholinesterase *(h)*** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -5.9 | -3.5 | -4.7 |
| **ATPase ($Na^+/K^+$)** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | 3.0 | -1.5 | 0.8 |
| **carbonic anhydrase II *(h)*** | | | | | |
| 100004002-1 | HC-122608 | 1.0E-06 M | -0.9 | 0.2 | -0.4 |

## 3.2.2. Reference Compound Results

| Reference Compound | $IC_{50}$ (M) | nH |
|---|---|---|
| **$COX_1$ *(h)*** | | |
| diclofenac | 1.1E-08 M | 1.8 |
| **$COX_2$ *(h)*** | | |
| NS 398 | 1.6E-07 M | 1.4 |
| **PDE3A *(h)*** | | |
| milrinone | 3.5E-07 M | 1.0 |
| **$PDE4D_2$ *(h)*** | | |
| rolipram | 4.8E-08 M | 0.7 |
| **PDE5 *(h)* (non-selective)** | | |
| dipyridamole | 8.8E-07 M | 1.0 |
| **PDE6 (non-selective)** | | |
| zaprinast | 1.7E-07 M | 0.7 |
| **ACE *(h)*** | | |
| captopril | 1.6E-09 M | 1.2 |
| **caspase-3 *(h)*** | | |
| Ac-DEVD-CHO | 1.5E-09 M | 1.2 |
| **MMP-9 *(h)*** | | |
| GM6001 | 3.2E-10 M | 1.6 |
| **phosphatase 1B *(h)* (PTP1B)** | | |
| ammonium molybdate | 5.4E-07 M | 2.9 |
| **Abl kinase *(h)*** | | |
| staurosporine | 8.3E-08 M | 1.3 |
| **CaMK2α *(h)*** | | |
| AIP | 8.1E-08 M | 1.0 |
| **FLT-1 kinase *(h)* (VEGFR1)** | | |
| staurosporine | 1.5E-08 M | 1.4 |

| Reference Compound | $IC_{50}$ (M) | nH |
|---|---|---|
| **Lyn A kinase *(h)*** | | |
| staurosporine | 2.6E-08 M | 2.1 |
| **p38α kinase *(h)*** | | |
| SB202190 | 2.3E-08 M | 1.4 |
| **ZAP70 kinase *(h)*** | | |
| staurosporine | 9.0E-08 M | 1.2 |
| **acetylcholinesterase *(h)*** | | |
| neostigmine | 1.6E-08 M | 1.3 |
| **ATPase ($Na^+/K^+$)** | | |
| ouabain | 1.2E-06 M | 1.3 |
| **carbonic anhydrase II *(h)*** | | |
| acetazolamide | 3.9E-08 M | 1.8 |

# 4. RESULTS INTERPRETATION GUIDE

## *In Vitro* Pharmacology

Results showing an inhibition (or stimulation for assays run in basal conditions) higher than 50% are considered to represent significant effects of the test compounds. 50% is the most common cut-off value for further investigation (determination of $IC_{50}$ or $EC_{50}$ values from concentration-response curves) that we would recommend.
Results showing an inhibition (or stimulation) between 25% and 50% are indicative of weak to moderate effects (in most assays, they should be confirmed by further testing as they are within a range where more inter-experimental variability can occur).
Results showing an inhibition (or stimulation) lower than 25% are not considered significant and mostly attributable to variability of the signal around the control level.
Low to moderate negative values have no real meaning and are attributable to variability of the signal around the control level. High negative values (≥ 50%) that are sometimes obtained with high concentrations of test compounds are generally attributable to non-specific effects of the test compounds in the assays. On rare occasion they could suggest an allosteric effect of the test compound.

# 5. STORAGE AND RETENTION OF RECORDS

All documents generated during the performance of the study (raw data, various records such as QA audit reports, an original copy of the study report, study plan…) will be archived by Cerep for a 10-year period after study completion. The access to the archives is restricted to authorized employees only.

The original final report provided to the sponsor will be kept by the sponsor under its sole responsibility.

# 6. MATERIALS AND METHODS

## 6.1. Experimental Conditions

### 6.1.1. *In Vitro* Pharmacology: Binding Assays

| Assay | Source | Ligand | Conc. | Kd | Non Specific | Incubation | Detection Method | Bibl. |
|---|---|---|---|---|---|---|---|---|
| **Receptors** | | | | | | | | |
| **$A_1$*(h)* (antagonist radioligand)** | human recombinant (CHO cells) | [$^3$H]DPCPX | 1 nM | 1.7 nM | DPCPX (1 µM) | 60 min RT | Scintillation counting | 245 |
| **$A_{2A}$*(h)* (agonist radioligand)** | human recombinant (HEK-293 cells) | [$^3$H]CGS 21680 | 6 nM | 27 nM | NECA (10 µM) | 120 min RT | Scintillation counting | 141 |
| **$A_3$*(h)* (agonist radioligand)** | human recombinant (HEK-293 cells) | [$^{125}$I]AB-MECA | 0.15 nM | 0.22 nM | IB-MECA (1 µM) | 120 min RT | Scintillation counting | 206 |
| **$\alpha_1$ (non-selective) (antagonist radioligand)** | rat cerebral cortex | [$^3$H]prazosin | 0.25 nM | 0.09 nM | prazosin (0.5 µM) | 60 min RT | Scintillation counting | 88 |
| **$\alpha_{2A}$ *(h)* (antagonist radioligand)** | human recombinant (CHO cells) | [$^3$H]RX 821002 | 1 nM | 0.8 nM | (-)epinephrine (100 µM) | 60 min RT | Scintillation counting | 542 |
| **$\alpha_{2B}$*(h)* (antagonist radioligand)** | human recombinant (CHO cells) | [$^3$H]RX 821002 | 2.5 nM | 5 nM | (-)epinephrine (100 µM) | 60 min RT | Scintillation counting | 56 |
| **$\alpha_{2C}$ *(h)* (antagonist radioligand)** | human recombinant (CHO cells) | [$^3$H]RX 821002 | 2 nM | 0.95 nM | (-)epinephrine (100 µM) | 60 min RT | Scintillation counting | 56 |
| **$\beta_1$ *(h)* (agonist radioligand)** | human recombinant (HEK-293 cells) | [$^3$H](-)CGP 12177 | 0.3 nM | 0.39 nM | alprenolol (50 µM) | 60 min RT | Scintillation counting | 548 |
| **$\beta_2$ *(h)* (agonist radioligand)** | human recombinant (CHO cells) | [$^3$H](-)CGP 12177 | 0.3 nM | 0.15 nM | alprenolol (50 µM) | 120 min RT | Scintillation counting | 794 |
| **$\beta_3$*(h)* (antagonist radioligand)** | SK-N-MC cells (endogenous) | [$^{125}$I]CYP (+ 0.1 µM (-)propranolol) | 0.6 nM | 0.7 nM | (-)propranolol (1 mM) | 90 min 37°C | Scintillation counting | 52 |
| **$AT_1$ *(h)* (antagonist radioligand)** | human recombinant (HEK-293 cells) | [$^{125}$I][Sar$^1$,Ile$^8$]-AT-II | 0.05 nM | 0.05 nM | angiotensin-II (10 µM) | 120 min 37°C | Scintillation counting | 776 |
| **$CB_1$*(h)* (agonist radioligand)** | human recombinant (CHO cells) | [$^3$H]CP 55940 | 0.5 nM | 3.5 nM | WIN 55212-2 (10 µM) | 120 min 37°C | Scintillation counting | 657 |
| **$CB_2$*(h)* (agonist radioligand)** | human recombinant (CHO cells) | [$^3$H]WIN 55212-2 | 0.8 nM | 1.5 nM | WIN 55212-2 (5 µM) | 120 min 37°C | Scintillation counting | 165 |
| **$CCK_1$ ($CCK_A$) *(h)* (agonist radioligand)** | human recombinant (CHO cells) | [$^{125}$I]CCK-8s | 0.08 nM | 0.24 nM | CCK-8s (1 µM) | 60 min RT | Scintillation counting | 562 |
| **$CCK_2$ ($CCK_B$) *(h)* (agonist radioligand)** | human recombinant (CHO cells) | [$^{125}$I]CCK-8s | 0.08 nM | 0.054 nM | CCK-8s (1 µM) | 60 min RT | Scintillation counting | 134 |
| **$D_1$*(h)* (antagonist radioligand)** | human recombinant (CHO cells) | [$^3$H]SCH 23390 | 0.3 nM | 0.2 nM | SCH 23390 (1 µM) | 60 min RT | Scintillation counting | 281 |
| **$D_{2S}$*(h)* (antagonist radioligand)** | human recombinant (HEK-293 cells) | [$^3$H]methyl-spiperone | 0.3 nM | 0.15 nM | (+)butaclamol (10 µM) | 60 min RT | Scintillation counting | 87 |
| **$D_{2S}$*(h)* (agonist radioligand)** | human recombinant (HEK-293 cells) | [$^3$H]7-OH-DPAT | 1 nM | 0.68 nM | butaclamol (10 µM) | 60 min RT | Scintillation counting | 87 |

| Assay | Source | Ligand | Conc. | Kd | Non Specific | Incubation | Detection Method | Bibl. |
|---|---|---|---|---|---|---|---|---|
| **$D_3$*(h)* (antagonist radioligand)** | human recombinant (CHO cells) | [$^3$H]methyl-spiperone | 0.3 nM | 0.085 nM | (+)butaclamol (10 µM) | 60 min RT | Scintillation counting | 145 |
| **$D_{4.4}$*(h)* (antagonist radioligand)** | human recombinant (CHO cells) | [$^3$H]methyl-spiperone | 0.3 nM | 0.19 nM | (+)butaclamol (10 µM) | 60 min RT | Scintillation counting | 252 |
| **$ET_A$*(h)* (agonist radioligand)** | human recombinant (CHO cells) | [$^{125}$I]endothelin-1 | 0.03 nM | 0.03 nM | endothelin-1 (100 nM) | 120 min 37°C | Scintillation counting | 30 |
| **$ET_B$ *(h)* (agonist radioligand)** | human recombinant (CHO cells) | [$^{125}$I]endothelin-1 | 0.03 nM | 0.04 nM | endothelin-1 (0.1 µM) | 120 min 37°C | Scintillation counting | 541 |
| **$GABA_{A1}$ *(h)* (α1,β2,γ2) (agonist radioligand)** | human recombinant (CHO cells) | [$^3$H]muscimol | 15 nM | 30 nM | muscimol (10 µM) | 120 min RT | Scintillation counting | 1096 |
| **$GABA_{B(1b)}$*(h)* (antagonist radioligand)** | human recombinant (CHO cells) | [$^3$H]CGP 54626 | 1 nM | 1 nM | CGP 52432 (100 µM) | 120 min RT | Scintillation counting | 508 |
| **TNF-α *(h)* (agonist radioligand)** | U-937 cells | [$^{125}$I]TNF-α | 0.1 nM | 0.05 nM | TNF-α (10 nM) | 120 min 4°C | Scintillation counting | 26 |
| **$H_1$*(h)* (antagonist radioligand)** | human recombinant (HEK-293 cells) | [$^3$H]pyrilamine | 1 nM | 1.7 nM | pyrilamine (1 µM) | 60 min RT | Scintillation counting | 492 |
| **$H_2$*(h)* (antagonist radioligand)** | human recombinant (CHO cells) | [$^{125}$I]APT | 0.075 nM | 2.9 nM | tiotidine (100 µM) | 120 min RT | Scintillation counting | 540 |
| **$H_3$*(h)* (agonist radioligand)** | human recombinant (CHO cells) | [$^3$H]N$^α$-Me-histamine | 1 nM | 0.32 nM | (R)α-Me-histamine (1 µM) | 60 min RT | Scintillation counting | 563 |
| **$H_4$*(h)* (agonist radioligand)** | human recombinant (HEK-293 cells) | [$^3$H]histamine | 10 nM | 7.6 nM | imetit (1 µM) | 60 min RT | Scintillation counting | 631 |
| **$BLT_1$ ($LTB_4$) *(h)* (agonist radioligand)** | human recombinant (CHO cells) | [$^3$H]$LTB_4$ | 0.2 nM | 0.2 nM | $LTB_4$ (0.2 µM) | 60 min RT | Scintillation counting | 616 |
| **$CysLT_1$ ($LTD_4$) *(h)* (agonist radioligand)** | human recombinant (CHO cells) | [$^3$H]$LTD_4$ | 0.3 nM | 0.24 nM | $LTD_4$ (1 µM) | 60 min RT | Scintillation counting | 618 |
| **$MCH_1$*(h)* (agonist radioligand)** | human recombinant (CHO cells) | [$^{125}$I][Phe$^{13}$,Tyr$^{19}$]-MCH | 0.1 nM | 1 nM | human MCH (0.1 µM) | 60 min RT | Scintillation counting | 526 |
| **$MC_1$ (agonist radioligand)** | B16-F1 cells (endogenous) | [$^{125}$I]NDP-α-MSH | 0.05 nM | 0.05 nM | NDP-α-MSH (1 µM) | 90 min RT | Scintillation counting | 390 |
| **$MC_3$*(h)* (agonist radioligand)** | human recombinant (CHO cells) | [$^{125}$I]NDP-α-MSH | 0.075 nM | 0.4 nM | NDP-α-MSH (1 µM) | 60 min 37°C | Scintillation counting | 211 |
| **$MC_4$*(h)* (agonist radioligand)** | human recombinant (CHO cells) | [$^{125}$I]NDP-α-MSH | 0.05 nM | 0.54 nM | NDP-α-MSH (1 µM) | 120 min 37°C | Scintillation counting | 211 |
| **$MT_1$ ($ML_{1A}$) *(h)* (agonist radioligand)** | human recombinant (CHO cells) | [$^{125}$I]2-iodomelatonin | 0.01 nM | 0.04 nM | melatonin (1 µM) | 60 min RT | Scintillation counting | 639 |
| **$MT_3$ ($ML_2$) (agonist radioligand)** | hamster brain | [$^{125}$I]2-iodomelatonin | 0.1 nM | 4.8 nM | melatonin (30 µM) | 60 min 4°C | Scintillation counting | 186 |
| **motilin *(h)* (agonist radioligand)** | human recombinant (CHO cells) | [$^{125}$I]motilin | 0.05 nM | 0.26 nM | [Nleu$^{13}$]-motilin (1 µM) | 120 min RT | Scintillation counting | 285 |
| **$M_1$*(h)* (antagonist radioligand)** | human recombinant (CHO cells) | [$^3$H]pirenzepine | 2 nM | 13 nM | atropine (1 µM) | 60 min RT | Scintillation counting | 59 |
| **$M_2$ *(h)* (antagonist radioligand)** | human recombinant (CHO cells) | [$^3$H]AF-DX 384 | 2 nM | 4.6 nM | atropine (1 µM) | 60 min RT | Scintillation counting | 59 |
| **$M_3$ *(h)* (antagonist radioligand)** | human recombinant (CHO cells) | [$^3$H]4-DAMP | 0.2 nM | 0.5 nM | atropine (1 µM) | 60 min RT | Scintillation counting | 546 |

| Assay | Source | Ligand | Conc. | Kd | Non Specific | Incubation | Detection Method | Bibl. |
|---|---|---|---|---|---|---|---|---|
| **$M_4$*(h)* (antagonist radioligand)** | human recombinant (CHO cells) | [$^3$H]4-DAMP | 0.2 nM | 0.32 nM | atropine (1 µM) | 60 min RT | Scintillation counting | 59 |
| **$M_5$*(h)* (antagonist radioligand)** | human recombinant (CHO cells) | [$^3$H]4-DAMP | 0.3 nM | 0.3 nM | atropine (1 µM) | 60 min RT | Scintillation counting | 59 |
| **$NK_1$*(h)* (agonist radioligand)** | U-373MG cells (endogenous) | [$^{125}$I]BH-SP | 0.15 nM | 0.12 nM | [$Sar^9$,$Met(O_2)^{11}$]-SP (1 µM) | 60 min RT | Scintillation counting | 104 |
| **$NK_2$*(h)* (agonist radioligand)** | human recombinant (CHO cells) | [$^{125}$I]NKA | 0.1 nM | 0.12 nM | [$Nleu^{10}$]-NKA (4-10) (300 nM) | 60 min RT | Scintillation counting | 3 |
| **$Y_1$*(h)* (agonist radioligand)** | SK-N-MC cells (endogenous) | [$^{125}$I]peptide YY | 0.025 nM | 0.06 nM | NPY (1 µM) | 120 min 37°C | Scintillation counting | 391 |
| **$\delta_2$ (DOP) *(h)* (agonist radioligand)** | human recombinant (CHO cells) | [$^3$H]DADLE | 0.5 nM | 0.73 nM | naltrexone (10 µM) | 120 min RT | Scintillation counting | 501 |
| **κ (KOP) (agonist radioligand)** | rat recombinant (CHO cells) | [$^3$H]U 69593 | 1 nM | 2 nM | naloxone (10 µM) | 60 min RT | Scintillation counting | 771 |
| **µ (MOP) *(h)* (agonist radioligand)** | human recombinant (HEK-293 cells) | [$^3$H]DAMGO | 0.5 nM | 0.35 nM | naloxone (10 µM) | 120 min RT | Scintillation counting | 260 |
| **PPARγ *(h)* (agonist radioligand)** | human recombinant (*E. coli*) | [$^3$H]rosiglitazone | 5 nM | 5.7 nM | rosiglitazone (10 µM) | 120 min 4°C | Scintillation counting | 567 |
| **5-$HT_{1A}$*(h)* (agonist radioligand)** | human recombinant (HEK-293 cells) | [$^3$H]8-OH-DPAT | 0.3 nM | 0.5 nM | 8-OH-DPAT (10 µM) | 60 min RT | Scintillation counting | 164 |
| **5-$HT_{1B}$ (antagonist radioligand)** | rat cerebral cortex | [$^{125}$I]CYP (+ 30 µM isoproterenol) | 0.1 nM | 0.16 nM | serotonin (10 µM) | 120 min 37°C | Scintillation counting | 111 |
| **5-$HT_{1D}$ (agonist radioligand)** | rat recombinant (CHO cells) | [$^3$H]serotonin | 1 nM | 0.5 nM | serotonin (10 µM) | 60 min RT | Scintillation counting | 777 |
| **5-$HT_{2A}$*(h)* (antagonist radioligand)** | human recombinant (HEK-293 cells) | [$^3$H]ketanserin | 0.5 nM | 0.6 nM | ketanserin (1 µM) | 60 min RT | Scintillation counting | 20 |
| **5-$HT_{2A}$*(h)* (agonist radioligand)** | human recombinant (HEK-293 cells) | [$^{125}$I](±)DOI | 0.1 nM | 0.3 nM | (±)DOI (1 µM) | 60 min RT | Scintillation counting | 288 |
| **5-$HT_{2B}$*(h)* (agonist radioligand)** | human recombinant (CHO cells) | [$^{125}$I](±)DOI | 0.2 nM | 0.2 nM | (±)DOI (1 µM) | 60 min RT | Scintillation counting | 571 |
| **5-$HT_{2C}$*(h)* (antagonist radioligand)** | human recombinant (HEK-293 cells) | [$^3$H]mesulergine | 1 nM | 0.5 nM | RS 102221 (10 µM) | 120 min 37°C | Scintillation counting | 543 |
| **5-$HT_{2C}$*(h)* (agonist radioligand)** | human recombinant (HEK-293 cells) | [$^{125}$I](±)DOI | 0.1 nM | 0.9 nM | (±)DOI (10 µM) | 60 min 37°C | Scintillation counting | 288 |
| **5-$HT_{4e}$*(h)* (antagonist radioligand)** | human recombinant (CHO cells) | [$^3$H]GR 113808 | 0.3 nM | 0.15 nM | serotonin (100 µM) | 60 min 37°C | Scintillation counting | 309 |
| **5-$HT_6$ *(h)* (agonist radioligand)** | human recombinant (CHO cells) | [$^3$H]LSD | 2 nM | 1.8 nM | serotonin (100 µM) | 120 min 37°C | Scintillation counting | 161 |
| **5-$HT_7$ *(h)* (agonist radioligand)** | human recombinant (CHO cells) | [$^3$H]LSD | 4 nM | 2.3 nM | serotonin (10 µM) | 120 min RT | Scintillation counting | 217 |
| **sigma (non-selective) (h) (agonist radioligand)** | cellules Jurkat (endogène) | [3H]DTG | 10 nM | 41 nM | Haloperidol (10 µM) | 120 min RT | Scintillation counting | 1136 |
| **$sst_4$*(h)* (agonist radioligand)** | human recombinant (CHO cells) | [$^{125}$I]$Tyr^{11}$-somatostatin-14 | 0.1 nM | 5.9 nM | somatostatin-14 (1 µM) | 120 min RT | Scintillation counting | 296 |

| Assay | Source | Ligand | Conc. | Kd | Non Specific | Incubation | Detection Method | Bibl. |
|---|---|---|---|---|---|---|---|---|
| **GR *(h)* (agonist radioligand)** | IM-9 cells (cytosol) | [$^{3}$H]dexamethasone | 1.5 nM | 1.5 nM | triamcinolone (10 µM) | 6 hr 4°C | Scintillation counting | 283 |
| **AR *(h)* (agonist radioligand)** | LNCaP cells (cytosol) | [$^{3}$H]methyltrienolone | 1 nM | 0.8 nM | mibolerone (1 µM) | 24 hr 4°C | Scintillation counting | 498 |
| **TR (TH) (agonist radioligand)** | rat liver | [$^{125}$I]$T_3$ | 0.1 nM | 0.24 nM | $T_3$ (1 µM) | 20 hr 4°C | Scintillation counting | 114 |
| **UT *(h)* (agonist radioligand)** | human recombinant (CHO cells) | [$^{125}$I]urotensin-II | 0.1 nM | 0.29 nM | urotensin-II (3 µM) | 120 min RT | Scintillation counting | 622 |
| **$VPAC_1$ ($VIP_1$) *(h)* (agonist radioligand)** | human recombinant (CHO cells) | [$^{125}$I]VIP | 0.04 nM | 0.05 nM | VIP (1 µM) | 60 min RT | Scintillation counting | 50 |
| **$V_{1a}$*(h)* (agonist radioligand)** | human recombinant (CHO cells) | [$^{3}$H]AVP | 0.3 nM | 0.5 nM | AVP (1 µM) | 60 min RT | Scintillation counting | 343 |
| **$V_2$*(h)* (agonist radioligand)** | human recombinant (CHO cells) | [$^{3}$H]AVP | 0.3 nM | 0.76 nM | AVP (1 µM) | 120 min RT | Scintillation counting | 343 |
| **Ion channels** | | | | | | | | |
| **BZD (central) (agonist radioligand)** | rat cerebral cortex | [$^{3}$H]flunitrazepam | 0.4 nM | 2.1 nM | diazepam (3 µM) | 60 min 4°C | Scintillation counting | 227 |
| **AMPA (agonist radioligand)** | rat cerebral cortex | [$^{3}$H]AMPA | 8 nM | 82 nM | L-glutamate (1 mM) | 60 min 4°C | Scintillation counting | 166 |
| **kainate (agonist radioligand)** | rat cerebral cortex | [$^{3}$H]kainic acid | 5 nM | 19 nM | L-glutamate (1 mM) | 60 min 4°C | Scintillation counting | 160 |
| **NMDA (antagonist radioligand)** | rat cerebral cortex | [$^{3}$H]CGP 39653 | 5 nM | 23 nM | L-glutamate (100 µM) | 60 min 4°C | Scintillation counting | 221 |
| **glycine (strychnine-insensitive) (antagonist radioligand)** | rat cerebral cortex | [$^{3}$H]MDL 105,519 | 0.5 nM | 5 nM | glycine (1 mM) | 45 min 0°C | Scintillation counting | 219 |
| **PCP (antagonist radioligand)** | rat cerebral cortex | [$^{3}$H]TCP | 10 nM | 13 nM | MK 801 (10 µM) | 120 min 37°C | Scintillation counting | 257 |
| **5-$HT_3$*(h)* (antagonist radioligand)** | human recombinant (CHO cells) | [$^{3}$H]BRL 43694 | 0.5 nM | 1.15 nM | MDL 72222 (10 µM) | 120 min RT | Scintillation counting | 109 |
| **$Ca^{2+}$ channel (L, diltiazem site) (benzothiazepines) (antagonist radioligand)** | rat cerebral cortex | [$^{3}$H]diltiazem | 15 nM | 52 nM | diltiazem (10 µM) | 120 min RT | Scintillation counting | 212 |
| **$Ca^{2+}$ channel (L, verapamil site) (phenylalkylamine) (antagonist radioligand)** | rat cerebral cortex | [$^{3}$H]D888 | 3 nM | 3 nM | D 600 (10 µM) | 120 min RT | Scintillation counting | 194 |
| **$Ca^{2+}$ channel (N) (antagonist radioligand)** | rat cerebral cortex | [$^{125}$I]ω-conotoxin GVIA | 0.001 nM | 0.0007 nM | ω-conotoxin GVIA (10 nM) | 30 min RT | Scintillation counting | 259 |
| **$SK_{Ca}$ channel (antagonist radioligand)** | rat cerebral cortex | [$^{125}$I]apamin | 0.007 nM | 0.007 nM | apamin (100 nM) | 60 min 4°C | Scintillation counting | 112 |
| **$Na^+$ channel (site 2) (antagonist radioligand)** | rat cerebral cortex | [$^{3}$H]batrachotoxinin | 10 nM | 91 nM | veratridine (300 µM) | 60 min 37°C | Scintillation counting | 28 |
| **$Cl^-$ channel (GABA-gated) (antagonist radioligand)** | rat cerebral cortex | [$^{35}$S]TBPS | 3 nM | 14.6 nM | picrotoxinin (20 µM) | 120 min RT | Scintillation counting | 136 |
| **Transporters** | | | | | | | | |
| **norepinephrine transporter *(h)* (antagonist radioligand)** | human recombinant (CHO cells) | [$^{3}$H]nisoxetine | 1 nM | 2.9 nM | desipramine (1 µM) | 120 min 4°C | Scintillation counting | 180 |
| **dopamine transporter *(h)* (antagonist radioligand)** | human recombinant (CHO cells) | [$^{3}$H]BTCP | 4 nM | 4.5 nM | BTCP (10 µM) | 120 min 4°C | Scintillation counting | 190 |
| **GABA transporter (antagonist radioligand)** | rat cerebral cortex | [$^{3}$H]GABA (+ 10 µM isoguvacine) (+ 10 µM baclofen) | 10 nM | 4600 nM | GABA (1 mM) | 30 min RT | Scintillation counting | 214 |
| **choline transporter (CHT1) *(h)* (antagonist radioligand)** | human recombinant (CHO cells) | [$^{3}$H]hemicholinium-3 | 3 nM | 3.9 nM | hemicholinium-3 (10 µM) | 60 min RT | Scintillation counting | 648 |

| Assay | Source | Ligand | Conc. | Kd | Non Specific | Incubation | Detection Method | Bibl. |
|---|---|---|---|---|---|---|---|---|
| **5-HT transporter *(h)* (antagonist radioligand)** | human recombinant (CHO cells) | [$^3$H]imipramine | 2 nM | 1.7 nM | imipramine (10 µM) | 60 min RT | Scintillation counting | 566 |
| **Other enzymes** | | | | | | | | |
| **MAO-A (antagonist radioligand)** | rat cerebral cortex | [$^3$H]Ro 41-1049 | 10 nM | 14 nM | clorgyline (1 µM) | 60 min 37°C | Scintillation counting | 36 |
| **rolipram (antagonist radioligand)** | mouse brain | [$^3$H]rolipram | 1 nM | 1 nM | rolipram (10 µM) | 60 min 4°C | Scintillation counting | 60 |

## 6.1.2. *In Vitro* Pharmacology: Enzyme and Uptake Assays

| Assay | Source | Substrate/Stimulus/Tracer | Incubation | Measured Component | Detection Method | Bibl. |
|---|---|---|---|---|---|---|
| **Kinases** | | | | | | |
| **Abl kinase *(h)*** | human recombinant (insect cells) | ATP + Ulight-TK peptide (100 nM) | 60 min RT | phospho-Ulight-TK peptide | LANCE | 556 |
| **CaMK2α *(h)*** | human recombinant | ATP + Ulight-CGSGSGRPRTSSFAEG (50 nM) | 30 min RT | phospho-Ulight-CGSGSGRPRTSSFAEG | LANCE | 647 |
| **FLT-1 kinase *(h)* (VEGFR1)** | human recombinant (Sf9 cells) | ATP + Ulight-TK peptide (100 nM) | 15 min RT | phospho-Ulight-TK peptide | LANCE | 650 |
| **Lyn A kinase *(h)*** | human recombinant (insect cells) | ATP + biotinyl-βAβAβAKVEKIGEGTYGVVYK (400 nM) | 90 min RT | phospho-biotinyl-βAβAβAKVEKIGEGTYGVVYK | HTRF | 41 |
| **p38α kinase *(h)*** | human recombinant (*E. coli*) | ATP + Ulight-CFFKNIVTPRTPPPSQGK-amide (100 nM) | 60 min RT | phospho-Ulight-CFFKNIVTPRTPPPSQGK-amide | LANCE | 620 |
| **ZAP70 kinase *(h)*** | human recombinant (insect cells) | ATP + biotinyl-βAβAβADEEEYFIPP (2 µM) | 15 min RT | phospho-biotinyl-βAβAβADEEEYFIPP | HTRF | 556 |
| **Other enzymes** | | | | | | |
| **$COX_1$*(h)*** | human recombinant (Sf9 cells) | arachidonic acid (4 µM) | 5 min RT | $PGE_2$ | HTRF | 486 |
| **$COX_2$*(h)*** | human recombinant (Sf9 cells) | arachidonic acid (2 µM) | 5 min RT | $PGE_2$ | HTRF | 486 |
| **PDE3A *(h)*** | human recombinant (Sf9 cells) | cAMP (40 nM) | 30 min RT | residual cAMP | HTRF | 902 |
| **$PDE4D_2$*(h)*** | human recombinant (Sf9 cells) | cAMP (40 nM) | 15 min 37°C | residual cAMP | HTRF | 906 |
| **PDE5 *(h)* (non-selective)** | human platelets | [$^3$H]cGMP + cGMP (1 µM) | 60 min RT | [$^3$H]5'GMP | Scintillation counting | 263 |
| **PDE6 (non-selective)** | bovine retina | [$^3$H]cGMP + cGMP (2 µM) | 60 min RT | [$^3$H]5'GMP | Scintillation counting | 306 |
| **caspase-3 *(h)*** | human recombinant (*E.coli*) | benzyloxycarbonyl-Asp-Glu-Val-Asp-AFC (3.6 µM) | 60 min RT | AFC | Fluorimetry | 476 |
| **MMP-9 *(h)*** | human recombinant | NFF-2 (10 µM) | 90 min 37°C | Mca-Arg-Pro-Lys-Pro-Tyr-Ala | Fluorimetry | 297 |
| **phosphatase 1B *(h)* (PTP1B)** | human recombinant (*E. coli*) | DIFMUP (100 µM) | 30 min RT | DIFMU | Fluorimetry | 928 |
| **acetylcholinesterase *(h)*** | human recombinant (HEK-293 cells) | AMTCh (400 µM) | 30 min RT | 5 thio 2 nitrobenzoic acid | Photometry | 63 |
| **ATPase ($Na^+/K^+$)** | porcine cerebral cortex | ATP (2 mM) | 60 min 37°C | Pi | Photometry | 71 |
| **carbonic anhydrase II *(h)*** | human erythrocytes | 4-nitrophenyl acetate (450 µM) | 20 min RT | 4-nitrophenol | Photometry | 923 |

## 6.2. Analysis and expression of results

### 6.2.1. *In Vitro* Pharmacology: Binding Assays

The results are expressed as a percent of control specific binding

$$\frac{\text{measured specific binding}}{\text{control specific binding}} *100$$

and as a percent inhibition of control specific binding

$$100-\left(\frac{\text{measured specific binding}}{\text{control specific binding}} *100\right)$$

obtained in the presence of HC-122608.

REDACTED

### 6.2.2. *In Vitro* Pharmacology: Enzyme and Uptake Assays

The results are expressed as a percent of control specific activity

$$\frac{\text{measured specific activity}}{\text{control specific activity}} *100$$

and as a percent inhibition of control specific activity

$$100-\left(\frac{\text{measured specific activity}}{\text{control specific activity}} *100\right)$$

obtained in the presence of HC-122608.

REDACTED

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