Arruda, Maria Augusta and Stoddart, Leigh A. and Gherbi, Karolina and Briddon, Stephen J. and Kellam, Paul and Hill, Stephen J. (2017) A non-imaging high throughput approach to chemical library screening at the unmodified adenosine-A3 receptor in living cells. Frontiers in Pharmacology . ISSN 1663-9812 (In Press)

A non-imaging high throughput approach to chemical library screening at the unmodified adenosine-A_3 receptor in living cells

Maria Augusta Arruda[1,2,+], Leigh A Stoddart[1,+], Karolina Gherbi[1], Stephen J Briddon[1*], Barrie Kellam[3], Stephen J Hill[1*]

[1]Cell Signalling Research Group, School of Life Sciences & Centre of Membrane Proteins and Receptors, Medical School, Queen's Medical Centre, University of Nottingham, Nottingham, UK, [2]Vice-Diretoria de Ensino, Pesquisa e Inovacao, Farmanguinhos, Fiocruz, Rio de Janeiro, Brazil and [3]School of Pharmacy, Centre for Biomolecular Sciences, University of Nottingham, University Park, Nottingham, UK.

+These authors contributed equally to this work.

***Correspondence:**
Stephen J Hill or Stephen J Briddon
Cell Signalling Research Group & Centre of Membrane Proteins and Receptors
School of Life Sciences
Medical School
Queen's Medical Centre
University of Nottingham
Nottingham NG7 2UG, UK.
E-mail: stephen.hill@nottingham.ac.uk
E-Mail: Stephen.briddon@nottingham.ac.uk

ABSTRACT (346 words).

Recent advances in fluorescent ligand technology have enabled the study of GPCRs in their native environment without the need for genetic modification such as addition of N-terminal fluorescent or bioluminescent tags. Here, we have used a non-imaging plate reader (PHERAstar FS) to monitor the binding of fluorescent ligands to the human adenosine-A_3 receptor (A_3AR; CA200645 and AV039), stably expressed in CHO-K1 cells. To verify that this method was suitable for the study of other GPCRs, assays at the human adenosine-A_1 receptor, and β_1 and β_2 adrenoceptors (β_1AR and β_2AR; BODIPY-TMR-CGP-12177) were also carried out. Affinity values determined for the binding of the fluorescent ligands CA200645 and AV039 to A_3AR for a range of classical adenosine receptor antagonists were consistent with A_3AR pharmacology and correlated well (R^2=0.94) with equivalent data obtained using a confocal imaging plate reader (ImageXpress Ultra). The binding of BODIPY-TMR-CGP-12177 to the β_1AR was potently inhibited by low concentrations of the β_1-selective antagonist CGP 20712A (pK_i 9.68) but not by the β_2- selective antagonist ICI 118551(pK_i 7.40). Furthermore, in experiments conducted in CHO K1 cells expressing the β_2AR this affinity order was reversed with ICI 118551 showing the highest affinity (pK_i 8.73) and CGP20712A (pK_i 5.68) the lowest affinity.

To determine whether the faster data acquisition of the non-imaging plate reader (~3 min per 96-well plate) was suitable for high throughput screening, we screened the LOPAC library for inhibitors of the binding of CA200645 to the A_3AR. From the initial 1263 compounds evaluated, 67 hits (defined as those that inhibited the total binding of 25nM CA200645 by ≥40%) were identified. All compounds within the library that have medium to high affinity for the A_3AR (pK_i ≥6) were successfully identified. We found three novel compounds in the library that displayed unexpected sub-micromolar affinity for the A_3AR. These were K114 (pK_i 6.43), retinoic acid p-hydroxyanilide (pK_i 6.13) and SU 6556 (pK_i 6.17). Molecular docking of these latter three LOPAC library members provided a plausible set of binding poses within the vicinity of the established orthosteric A_3AR binding pocket. A plate reader based library screening at an untagged receptor is therefore possible using fluorescent ligand opening the possibility of its use in compound screening at natively expressed receptors.

Keywords

Adenosine receptors, fluorescent ligands, Adenosine A_3 receptor, high throughput screening, LOPAC library.

INTRODUCTION

G protein-coupled receptors (GPCRs) represent the largest family of cell surface receptors and account for approximately 4% of the entire protein-coding human genome. There are approximately 700 separate GPCRs of which over 300 are non-olfactory receptors (Kuder *et al.*, 2014). Based on sequence homology, five distinct families of non-olfactory receptors have been classified: Family A/Rhodopsin, Family B/secretin, Adhesion GPCRs, Family C/Glutamate, and Family F/frizzled (Guo *et al.*, 2012). Family A receptors contains the largest number of the non-olfactory GPCRs and including many of the most widely studied receptors, each of which acts to translate extracellular signals into intracellular effects by activating both heterotrimeric G protein-dependent and –independent signalling cascades (Castro *et al.*, 2005; Guo *et al.*, 2012). Importantly, these family A GPCRs are also currently targeted by a large number of clinically used drugs and are validated targets for a significant number of drug discovery programmes.

Adenosine is one biological transmitter which plays a vital homeostatic role and acts via a family of Class A GPCRs comprising four distinct subtypes: namely the adenosine-A_1 receptor (AR), A_{2A}AR, A_{2B}AR, and A_3AR (Fredholm et al., 2011). Both the A_1AR and A_3ARs inhibit intracellular cAMP formation by activating inhibitory G_i proteins, whilst the A_{2A}AR and A_{2B}ARs generally stimulate cAMP formation via stimulatory G_s proteins. Adenosine-mediated signalling has been implicated in a number of pathological states. For instance, the signalling pathways regulated by these receptors can promote angiogenesis (Headrick et al., 2013) and reduce inflammation (Antonioli et al., 2014). Within this family, the A_3AR is a promising molecular target for the control of a range of pathological conditions including cancer (Cao et al., 2017; Joshaghani et al., 2017; Nakamura et al., 2015; Montinaro et al., 2013), inflammation (Yoshida et al., 2017; Cohen et al., 2014), autoimmune diseases (Ravani et al., 2017), ischaemia (Ohana et al., 2016; González-Fernández et al., 2014; Hussain et al., 2014; Mulloy et al., 2013) and chronic neuropathic pain (Tosh et al., 2015), making it an important target for drug development (Borea et al., 2015). As a consequence, identifying new screening methods for discovery of novel chemical scaffolds which bind to the A_3AR would be beneficial.

With this in mind, it is of note that recent advances in fluorescent ligand technology have enabled unlabelled GPCRs to be studied in their native environment without any need for genetic modification through the addition of a bioluminescent or fluorescent tag. For instance fluorescent ligands have been used to study various aspects of GPCR pharmacology including ligand binding, receptor-ligand kinetics, receptor localisation and trafficking (Stoddart *et al.*, 2015b). Of particular relevance to purinergic drug discovery, Stoddart *et al.* (2012) developed a competitive binding assay for the human A_3AR and A_1AR in live cells, using a high content screening (HCS) platform that allowed the screening of small fragment libraries. This assay system was also used to validate the pharmacology of A_3AR selective compounds that were identified from virtual screening of homology models (Ranganathan *et al.*, 2015). However, a disadvantage of this technique is that it involves the acquisition and analysis of a large number of images which can impose severe time, data handling and storage limitations at the early stages of drug discovery, particularly in hit discovery, when very large libraries (>100,000 compounds) are used in initial screening campaigns (Tomasch *et al.*, 2012). In this work, we show that such a competitive fluorescent based binding screen is possible on a higher throughput, non-imaging-based platform using two structurally unrelated fluorescent antagonists. The suitability of this assay for higher throughput screens has been demonstrated by screening a library of pharmacological active compounds (LOPAC) against the native human A_3AR in living cells, with a view to identifying potential novel scaffolds for A_3AR ligands.

RESULTS.

Comparison of high content (HCS) and high throughput (HTS) screening platforms for measuring competition binding to the A_3AR.

As previously described, competition binding assays have been performed on cells expressing the wild type human A_3AR using the fluorescent adenosine receptor antagonist CA200645 by automated image acquisition using an ImageXpress (IX) Ultra confocal imaging plate reader (Stoddart *et al.*, 2012). In order to see if this method could be translated into a faster non-imaging format, we directly compared HCS and plate reader based CA200645 binding by sequentially reading the same samples on the PHERAstar FS (BMG techonologies) then the IX Ultra. As shown in the IX Ultra plate image in Figure 1A, binding of 25 nM CA200645 was clearly seen, and was subsequently displaced by increasing concentrations of competing (unlabelled) antagonists. The same 96-well plate was also measured on a standard non-imaging fluorescence plate reader (PHERAstar FS), with 81 separate repeat reads per well to take into account variation in cell density, and a similar pattern of fluorescence was observed (Figure 1B). The montage images from both instruments show that the high affinity A_3AR antagonist MRS1220, AV019 (compound 1 in Vernall *et al.*, (2012)) and the non-selective adenosine receptor antagonist xanthine amine congener (XAC) caused a concentration-dependent reduction in the fluorescence intensity observed with 25 nM CA200645 alone. Competition binding curves were generated from the quantified data (Figure 1C), and pK_i values for the five adenosine receptor antagonists obtained, which were comparable to values reported in the literature (Table 1). Comparison of the affinity values from the HTS platform (PHERAstar) to those from the HCS platform (IX Ultra) showed a high degree of correlation (R^2=0.94) (Figure 1E) and we have previously shown that affinity values obtained from the HCS platform correlated well with values obtained in a functional assay (Stoddart *et al.*, 2012). In addition to the XAC based fluorescent ligand CA200645, a structurally distinct and highly selective fluorescent A_3AR antagonist was also used in both assays (AV039; compound 19 in Vernall *et al.*, 2012). As with CA200645, using 5 nM AV039 as label, competition binding experiments produced the expected rank order of antagonist affinity for the A_3AR (Figure 1D, Table 1).

Application to A_1AR and β-adrenoceptors.

To verify that the experimental approach used for the A_3AR was suitable for the study of other GPCRs, we conducted the same experimental design with CA200645 on CHO cells expressing the human A_1AR, since this fluorescent ligand also binds with high affinity to this receptor (Stoddart *et al.*, 2012). This is important, since being able to screen for compound selectivity is an important aspect of developing a screening methodology. As with the A_3AR, a clear concentration-dependent decrease in fluorescence intensity was detected on the HTS plate reader in the presence of four different adenosine receptor antagonists (Figure 2A). The affinity values from these data were consistent with A_1AR pharmacology with CGS 15943 showing the highest affinity and MRS1220 exhibiting a lower affinity than at the A_3AR. In addition, ZM241385, an A_{2A}AR selective antagonist showed the expected low affinity at the A_1AR (Table 1).

The confocal based fluorescent ligand binding assay has also been recently applied to study the pharmacology of the β_1AR using BODIPY-TMR labelled CGP 12177 (BODIPY-TMR-CGP; Gherbi *et al.*, 2014) and we therefore also tested whether ligand binding to the β_1AR and β_2AR could also be monitored using the HTS platform in order to develop a counter screen for the A_3AR. As shown in Figure 2B, in CHO cells expressing either the β_1AR or β_2AR, binding of BODIPY-TMR-CGP could be clearly detected, and clear competition binding was observed with all three βAR ligands at both receptors. Importantly, the β_1AR selective antagonist CGP 20712A displayed the highest affinity at the β_1AR and the β_2AR selective antagonist ICI 118551 the lowest (Table 2), whilst this rank order was reversed at

the β_2AR, with ICI 118551 showing the highest affinity and CGP20712A the lowest affinity (Figure 2C, Table 2).

Screening of a focussed library of pharmacologically active ligands at the A_3AR.

To determine whether the HTS version of the competitive fluorescent binding assay was suitable for the screening of large compound libraries, we chose to screen the Library of Pharmacologically Active Compounds (LOPAC) against the A_3AR. The LOPAC library is considered to be a recognised standard for assay validation as it is based on an extensive number of bioactive compounds. Many of these are known to affect targets involved in adenosine receptor signalling (Iturrioz *et al.*, 2010). CHO cells expressing the A_3AR were grown to confluency in 96-well plates and incubated with a single concentration (10 μM) of the known A_3AR antagonist MRS1220 as a positive control or one of the 1263 compounds (10 μM) from the LOPAC library and CA200645 (25 nM) and the fluorescence intensity of each well determined on the PHERAstar plate reader as described in *Experimental Procedures*. Hits were defined as those compounds which inhibited the binding of CA200645 by >40%, and of the initial 1263 compounds evaluated, 67 hits were identified (Supporting Information Table 1; Figure 3). Inhibition data for all the compounds tested in the initial screen can be found in Supporting Information Table 1. Among the hits, all the compounds within the library with medium to high affinity for the A_3AR ($pK_i \geq 6$; Figure 3; Table 3) were identified along with four low affinity adenosine-related molecules (1,3-dipropyl-8-p-sulfophenylxanthine, DMPX, etazolate hydrochloride and 2-phenylaminoadenosine; Table 3). This confirmed the utility of this approach to identify compounds with known A_3AR binding affinity. Importantly, the assay Z' factor was 0.47 ± 0.03 (mean ± SEM, n = 97), demonstrating its suitability for screening larger libraries in living cells.

Ten hits from the initial screen which demonstrated the biggest inhibition of CA200645 binding to the A_3AR were investigated further and full inhibition curves for each compound were generated. We were unable to further test reactive blue 2 (position 4 in the full screen) as it is currently not available commercially. As shown in Table 4 and Figure 4, four of the top ten compounds showed low- to sub-micromolar affinity for the A_3AR. As expected the adenosine receptor antagonist CGS15943 displaced the binding of CA200645 at both the A_3AR and A_1AR in a concentration-dependent manner with the expected affinity (Table 1, Figure 4). As CGS15943 was one of the top ten hits from the initial screen it was also tested in cells expressing the β_2AR and had no effect on the binding of BODIPY-TMR-CGP (Figure 4). Three further compounds, retinoic acid p-hydroxyanilide (fenretinide), K114 and SU 6656, were found to inhibit the binding of CA20065 to the A_3AR in a concentration-dependent manner with affinity values in the sub-micromolar range, roughly 10-fold lower than CGS15943 (Figure 4 and Table 4). Five further hits (BIO, rottlerin, quercetin, PD173952 and kenpaullone) only displaced the binding of CA200645 at the highest concentration tested (10 μM), prohibiting an accurate affinity determination. For those four compounds showing micromolar affinity, the selectivity of their interaction with the A_3AR was determined by investigating their ability to bind to A_1AR and β_2AR. Both K114 and retinoic acid p-hydroxyanilide inhibited the binding of CA200645 at the A_1AR with similar affinity to that observed at the A_3AR. SU 6656 only inhibited binding at the highest concentration tested and the affinity was not calculated. None of the other compounds showed any measureable activity at the A_1AR. When tested in CHO cells expressing β_2AR, no significant inhibition of BODIPY-TMR-CGP binding was observed for any of the ten compounds screened but the control β_2AR antagonist propranolol had the expected affinity ($pK_i = 8.72 \pm 0.14$, n = 3) . There was an increase in fluorescence in the presence of 10 μM SU 6656 (128.4 ± 18.4%). However this was small compared to the increase seen with 10 nM BODIPY-TMR-CGP and the large increase in fluorescence in the presence of BIO ($pEC_{50} = 5.84 \pm 0.13$). This is likely to be due to these compounds interfering with the

BODIPY-TMR fluorescence signal, which was not observed when using the more red-shifted BODIPY 630/650 fluorophore in the A_1AR and A_3AR binding assays.

Molecular modelling of selected LOPAC hits at the A_3AR.

Using our previously established homology model of the human A_3AR (Vernall *et al.,* 2013) we sought to investigate potential binding poses for the three sub-micromolar compounds (retinoic acid p-hydroxyanilide (fenretinide), K114 and SU 6656) identified in the LOPAC screen which did not have previous literature precedent for interacting with this receptor sub-type. Using the commercially available docking software, CLC Drug Discovery Workbench, ligand and receptor binding pocket preparation was followed by targeted ligand docking. The highest scoring docked poses for K114, SU 6656 and retinoic acid p-hydroxyanilide were selected and are illustrated in Fig 5. All three compounds were able to engage via plausible poses to the A_3R within the vicinity of the orthosteric binding pocket of this receptor.

DISCUSSION

Fluorescent ligands for GPCRs are a valuable tool in the study of multiple aspects of receptor pharmacology and they are a potential replacement for radiolabelled ligands in saturation and equilibrium binding studies to determine the affinity of labelled and unlabelled ligands (Stoddart *et al.*, 2016). In this study, we aimed to further develop a previously described fluorescence based live cell binding assay that used a high content screening (HCS) system (Stoddart *et al.*, 2012) to an assay that could be performed with un-tagged receptors on a high throughput screening (HTS) system. To this end, we chose the PHERAstar FS fluorescent plate reader since it allowed the determination of the optimal focal height for the fluorescence read and multiple scans per well. Use of the HTS system to obtain data resulted in a marked reduction in the time each 96-well plate took to process; from around 40 minutes per plate on the confocal HCS system for data collection and analysis to less than 3 minutes for the HTS system. This also produced a significant reduction in the amount of data that needed to be stored; 500 Mb per plate for HCS versus 160 Kb for HTS. Using the A_3AR as a model system, we demonstrated that the data generated on the HTS system was in close agreement to that obtained on the HCS system, validating this system as a higher throughput methodology that would be essential for screening large compound libraries using fluorescence-based binding assays in whole cells.

Various methods using fluorescent ligands to measure ligand binding at GPCRs have been recently developed, each using a different approaches to measure the fluorescence of the bound ligand, including flow cytometry (Hara *et al.*, 2009; Kozma *et al.*, 2013; Young *et al.*, 2005), fluorescence polarization (Cornelius *et al.*, 2009; Kecskes *et al.*, 2010) and resonance energy transfer based systems (Stoddart *et al.*, 2015a; Zwier *et al.*, 2010). Each method has advantages and disadvantages, for instance ligand depletion (fluorescence polarization) and the need to tag the receptor of interest (BRET and FRET). One limitation of the simple fluorescent intensity measurement used in the system described here is the potential for a low signal/noise ratio as a result of high levels of non-specific binding and the use of whole cells. As this technique measures total well fluorescence intensity it will be affected by both high levels of non-specific membrane binding and also non-specific uptake of the fluorescent ligand into the cells. As an example of this, for the A_3AR the maximal reduction in the levels of CA200645 fluorescence measured in the presence of unlabelled ligands was 60% whilst that with BODIPY-TMR-CGP for the β_1AR was only 20% (Figure 1C and 2B). This small signal/noise ratio for this ligand at the β_1AR has been observed previously (Gherbi *et al.*, 2014), although it is notable that even under these conditions, the method described here still allowed us to generate robust data within this small signal/noise window. The proximity-based assays (e.g. NanoBRET; Stoddart *et al.*, 2015) overcome this issue but they obviously require genetic modification of the extracellular N-terminus of the receptor with a fluorescent or luminescent protein, which precludes their use on native receptors – a main aim of the assay developed in this study. What is also clear from this point of view, is that the limit of this signal to noise ratio is likely to be highly dependent on both the pharmacological and photophysical properties of the fluorescent ligand, as we have previously demonstrated (Vernall *et al.*, 2013). To progress the use of this assay to use with endogenously expressed untagged receptors, consideration should also be given to fluorescent ligand selectivity in situations where multiple receptor subtypes are often co-expressed; this is particularly true for adenosine receptors. To this end, the demonstration that this assay also works with a highly A_3AR selective ligand, AV039 (Vernall *et al.*, 2012) is important.

To demonstrate the utility of this assay system for compound screening, we investigated if we could identify known ligands for the A_3AR within a library of pharmacologically active

compounds (LOPAC). Within the LOPAC library there were 37 compounds identified as ligands for adenosine receptors. For the 1263 compounds screened, we defined a hit as a compound that inhibited more than 40% of the total CA200645 binding. Using these criteria, we identified 67 hits, of which 14 had previously described activity at adenosine receptors (Table 3). Of these, four were the known A_3R selective agonists, 2-Cl-IB-MECA (Gallo-Rodriguez *et al.*, 1994), IB-MECA (Klotz *et al.*, 1998), AB-MECA (Klotz *et al.*, 1998) and HEMADO (Klotz *et al.*, 2007), and the A_3R selective antagonist MRS1523 (Li *et al.*, 1998). A further five compounds were known to be non-selective at this adenosine receptor subtype (CGS15943 (Ongini *et al.*, 1999), NECA (Gao *et al.*, 2004), APNEA (Gao *et al.*, 2004), 2-CADO (van Galen *et al.*, 1994) and 1,3-dipropyl-8-p-sulfophenylxanthine (Daly *et al.*, 1985)). The remaining four compounds were SCH 58261, CV1808, DPCPX and FSCPX. SCH 58261 is widely described as an A_{2A} selective and DPCPX as an A_1AR-selective antagonist, and both retain affinity in the μM range for the A_3AR (Jacobson *et al.*, 2006; Stoddart *et al.*, 2012). FSCPX is an irreversible antagonist at the A_1AR (van Muijlwijk-Koezen *et al.*, 2001) but to date it had not been tested at other adenosine receptor subtypes. Our data from this screen indicates that FSCPX is likely to retain activity at the A_3R at least in the low μM range and this is also true for CV1808 that has been described as an agonist at the A_{2A}AR (Dionisotti *et al.*, 1997). A variety of different compounds that act at different (i.e. non-A_3AR) adenosine receptors were included in the library and as expected were not identified as hits in our screen (Supplementary Table 1). These included A_1AR selective agonists and antagonists such as R-PIA (Klotz *et al.*, 1998) and CPT (Dalpiaz *et al.*, 1998), A_{2A}AR selective agonists and antagonists such as CGS 21680 (Klotz *et al.*, 1998) and CSC (Jacobson *et al.*, 1993), and the A_{2B}AR selective antagonist alloxazine (Ji *et al.*, 2001). A variety of low affinity non-selective antagonists and agonists were also present in the library including adenosine, theophylline, caffeine and paraxanthine that have reported affinity at the A_3AR in the 13-100 μM range (Fredholm *et al.*, 2001; Jacobson *et al.*, 1999). Due to the concentration of CA200645 (25nM) used in the primary screen only compounds with an affinity of <10μM would be expected to be identified as a hit. Overall, the assay performed well at identifying all the compounds with known activity at the A_3AR.

We found three compounds in the library that displayed unexpected sub-micromolar affinity at the A_3AR (Figure 4 and Table 4). These were K114, retinoic acid p-hydroxyanilide and SU 6556. K114 is used to identify amyloid lesions from Aβ peptide, α-synuclien and tau through an increase in its fluorescence upon binding to these lesions. It is has minimal fluorescence in aqueous solution and has emission maxima of 550 nm that is unlikely to interfere with the emission of BY630 at 650 nm (Crystal *et al.*, 2003). In addition, the assay described here monitors a decrease in fluorescence in the presence of inhibitors that would mean it would be more likely to give false-negatives rather than false-positives. Retinoic acid p-hydroxyanilide, also known as fenretinide or 4-HPR, is an analogue of retinoic acid and is a potential therapy in the treatment of cancer due to its ability to induce apoptosis (Wu *et al.*, 2001). It is possible that it was causing apoptosis of the cells in our assay system leading to a concurrent decrease in fluorescence but as the presence of retinoic acid p-hydroxyanilide had no effect in cells expressing the β_2AR this is unlikely to be the case (Figure 4). SU6556 is a Src kinase inhibitor that has also been found to inhibit a variety of other kinases including Aurora C and AMPK (Bain *et al.*, 2007). It also displayed slight selectivity for the A_3AR over A_1AR.

Docking of the sub-micromolar compounds identified in the LOPAC screen provided a plausible set of binding poses within the vicinity of the established orthosteric A_3AR binding pocket (Figure 5). K114 bound in a fully extended form with one of the terminal phenols optimally positioned to engage in a hydrogen bond interaction with the side-chain of Thr94. Meanwhile, the remaining vinyl-linked aromatic moieties pass through a hydrophobic

channel created by Ile76, Val169, Leu90, Leu246, Ile249, Leu264, Ile268 and Phe168; the latter engaging via a face-to-face pi-stacking interaction. SU 6656 favoured binding higher up in the orthosteric pocket with the 4,5,6,7-tetrahydroindolyl portion of the molecule engaging in a face-to-face interaction with Phe168, with the hydrophobic interactions predominating with Leu90, Val65, Ile268 and Leu246. Finally, retinoic acid *p*-hydroxyanilide displayed a binding pose passing through the same hydrophobic channel observed with K114. The 1,3,3-trimethylcyclohex-1-enyl region of the molecule was positioned deepest into the binding pocket engaging in hydrophobic interactions with residues Leu246, Ile249, Met177 and Phe168. The *p*-hydroxyanilde region of the molecule was positioned in such a way as to allow a face-to-edge interaction with Tyr265 at the top of transmembrane helix 7. With the predominance of aromatic and hydrophobic interactions observed between the receptor and the three ligands discussed, this would seem to correlate well with the experimental binding affinities whilst also offering the potential to undertake productive modifications of these compounds to potentially enhance their overall binding interactions.

In conclusion, we have shown that a simple intensity based fluorescent ligand binding assay can be modified to work in a potentially high throughput format, giving significant advances in both speed and data volume compared to previous high content versions. The assay allows screening of a small compound library in live cells, and can assess binding to the unmodified native receptors. The assays performed well under test conditions, identifying both known adenosine receptor ligands in a focussed library as well as novel potential ligand scaffolds. Further work on establishing this assay to screen at endogenous A_3AR in a mixed receptor background will be important to allow subsequent screens to be performed under more physiological conditions.

EXPERIMENTAL PROCEDURES

Chemicals

Known GPCR antagonists were purchased from Tocris Bioscience and G418 was obtained from Invitrogen. Fetal calf serum was obtained from PAA Laboratories and L-glutamine from Lonza. All other biological reagents were obtained from Sigma-Aldrich. CA200645 was obtained from CellAura Technologies. BODIPY-TMR-CGP (BODIPY-TMR-(±)-CGP 12177) was purchased from Molecular Probes. AV039 was synthesized in house as previously described (Vernall *et al.*, 2012). The LOPAC library was obtained from Sigma-Aldrich.

Cell Culture

CHO-K1 cells stably expressing the human A_3AR (Vernall *et al.*, 2012), β_1AR (Guo *et al.*, 2012), β_2AR (Baker *et al.*, 2002) or the human A_1AR (May *et al.*, 2010) were maintained in DMEM/F12 medium containing 10% foetal calf serum and 2 mM L-glutamine at 37°C in a humidified atmosphere of air/CO_2 (19:1).

Fluorescence Competition Binding Assay

CHO cells stably expressing the A_3AR, A_1AR, β_1AR or β_2AR were seeded into the central 60 wells (for high content confocal analysis) or every well (high throughput analysis) of a 96-well clear-bottomed, black-walled plate (Greiner BioOne) and grown to confluency. On the day of experiment, normal growth medium was removed and cells washed twice with HEPES-buffered saline solution (HBSS; 25 mM HEPES, 10 mM glucose, 145 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 2 mM sodium pyruvate, 1.3 mM $CaCl_2$, pH 7.4) pre-warmed to 37°C. Fresh HBSS was added to each well followed by the addition of the required concentration of unlabelled compound and the respective fluorescent ligands (25 nM CA200645, 5 nM AV039 or 10 nM BODIPY-TMR-CGP). Cells were incubated for 1h at 37°C/5% CO_2. Buffer was then removed from each well, cells washed once in HBSS and fresh HBSS added at room temperature. Plates were then immediately subjected to high content or high throughput screening analysis as detailed below.

High content screening

High content analysis was conducted as previously described (Stoddart *et al.*, 2012). Briefly, plates were imaged using an ImageXpress Ultra confocal plate reader, which captured four central images per well using a Plan Fluor 40x NA0.6 extra-long working distance objective. CA200645 was excited at 635 nm and emission collected through a 640-685 nm band pass filter. Total image intensity was obtained using a modified multi-wavelength cell scoring algorithm within the MetaXpress software (MetaXpress 2.0, Molecular Devices).

High throughput screening

High throughput analysis was performed using a PHERAstar FS plate reader (BMG Technlogies). Fluorescent intensity of each well was assessed by bottom scanning using the following optical modules: excitation 540 nm and emission 590 nm (for BODIPY-TMR-CGP-labelled cells), or excitation 630 nm and emission 650 nm (for the BY630 compounds CA200645 and AV039). Optimal focal height was determined automatically and total fluorescence intensity was assessed by taking 81 reads per well.

Screening of the LOPAC library of pharmacological active compounds

The LOPAC compound library contained 1263 compounds and each compound was provided as a pre-dissolved solution in 10 mM in DMSO. Compound plates containing 2 µl of compound per well were provided by the University of Nottingham Managed Compound Collection. Each plate contained 40 compounds from the LOPAC library together with positive and blank control samples. For the blank controls, 2 µl of DMSO was added per well and for the positive controls the A_3AR antagonist MRS1220 (10µM final concentration)

was used. The compounds were diluted to 100 μM in HBSS prior to assay. Each compound was tested in duplicate at a final concentration of 10μM on three separate experimental days. Experiment was carried out as detailed above using the A_3AR expressing cell line and 25 nM CA200645 as the tracer ligand. Data were normalised on a per plate basis to the fluorescence observed in blank control wells.

The 67 compounds that inhibited by more than 40% the total binding of CA200645 compared to blank controls were classed as hits. From this list 16 compounds were selected for secondary screening to determine their IC_{50} values and binding affinity. This was achieved by investigating the effect of increasing concentrations of each inhibitor on the specific binding of 25 nM CA200645 or 10 nM BODIPY-TMR-CGP in cells expressing the A_3AR, A_1AR or β_2AR.

Molecular Modelling

Using our previously reported homology model of the human A_3AR (Vernall *et al.*, 2013) and the CLC Drug Discovery Workbench software package (Version 3.0.2, Qiagen, Netherlands), the protein target was prepared with no water molecules present. Before setting up the docking experiments, the binding site was generated as a 13 Å sphere centred around the established orthosteric pocket. All small molecules were constructed using ChemDraw Professional 16.0 (CambridgeSoft, Cambridge, MA, USA) and imported into the docking programme using the Balloon PlugIn (http://users.abo.fi/mivainio/balloon) (Vainio *et al.*, 2007) to afford the lowest energy conformer for each ligand. During the docking process, each ligand underwent 1000 individual iterations, with the conformation of each ligand set as flexible, allowing full movement around all rotatable bonds, whilst the protein was held as a rigid structure. The best scoring pose for each ligand was returned using the $PLANTS_{PLP}$ algorithm to determine that docking score (Korb *et al.*, 2009) and the best ranked compounds were selected and their binding residues observed using the CLC Drug Discovery Workbench visualization tool.

Data analysis

Competition binding curves were fitted to the following equation using GraphPad Prism 5 (GraphPad Software):

$$\%\ inhibition\ of\ specific\ binding = \frac{100\times[A]}{[A] + IC_{50}}$$

where [A] is the concentration of competing drug and IC_{50} is the molar concentration of ligand required to inhibit 50% of the specific binding of a fixed concentration [L] of the appropriate fluorescent ligand. The IC_{50} values obtained were converted to K_i values using the following equation:

$$K_i = \frac{IC_{50}}{1 + \frac{[L]}{K_D}}$$

where [L] is the concentration and K_D is the equilibrium dissociation constant of the fluorescent ligand . The K_D values for the fluorescent ligands used were 11.0 nM and 3.11 nM for CA200645 at the A_1AR and A_3AR respectively (Stoddart et al., 2012). K_D values for BODIPY-TMR-CGP were taken from Baker et al., (2003).

The Z' values were calculated on a per plate basis using the following equation:

$$Z' = 1 - \frac{3(\sigma_p + \sigma_n)}{\mu_p - \mu_n}$$

where μ_p and σ_p are the mean and standard deviation from the control wells (DMSO only) and μ_n and σ_n are the mean and standard deviation from the MRS1220 treated wells.

FIGURE LEGENDS

Figure 1. Competition binding at the A_3AR using fluorescent ligands. CHO cells expressing the A_3AR were incubated with 25 nM CA200645 and increasing concentrations of MRS1220, XAC or AV019. **(A)** Four images per well were obtained on the ImageXpress confocal plate reader and resulting images shown as a montage. **(B)** Montage fluorescence intensity measurement of the same plate obtained using the BMG PheraStar FS where blue, green, yellow and red pixels represents increasing intensity of fluorescence. **(C)** Competition curves at the A_3AR generated from the total fluorescence intensity measured on the PHERAstar FS microplate reader for five adenosine receptor antagonists. **(D)** CHO A_3AR cells were incubated with increasing concentrations of antagonist and 5 nM AV039 for 1h, 37^{o}C, washed and fluorescence intensity assessed using the PHERAstar FS. **(E)** Correlation between pKi values obtained using the IX Ultra (high content screening; HCS) and the PHERAstar FS (high throughput screening; HTS) for the data obtained using CA200645 as fluorescent ligand. Data were normalized to the maximal intensity observed per experiment and each data point represents the mean ± SEM from *n* number of experiments (See Table 1) performed in triplicate.

Figure 2. Competition binding assays at the adenosine A_1 and β_1/β_2-adrenoceptors. CHO cell lines stably expressing A_1AR **(A)**, β_1AR **(B)** or the β_2AR **(C)** were incubated with 25 nM CA200645 (A_1AR) or 10 nM BODIPY-TMR-CGP (β_1AR and β_2AR), in the absence or the presence of increasing concentrations of antagonists. Fluorescence intensity in each well was monitored using the PHERAstar FS. Values are mean ± SEM from 3 - 6 independent experiments performed in triplicate.

Figure 3. Screening the LOPAC library against the A_3AR. Example of the data generated from one plate of compounds from the LOPAC library. Each plate contained 40 compounds (each at 10μM final concentration) from the LOPAC library in duplicate along with four basal and four MRS1220 (10 μM) controls, also in duplicate. The fluorescence intensities obtained on the PHERAstar FS from this plate are shown as mean and range of duplicates with the hits highlighted in red and adenosine indicated in blue. The plate shown is a representative plate of one of the three experiments performed using these compounds and the inhibition data for all compounds screened can be found in Supporting Table 1.

Figure 4. Competition binding curves at the A_1AR, A_3AR and β_2AR for three hits identified from the LOPAC library. CHO cell lines stably expressing A_1AR **(A)**, A_3AR **(B)** or β_2AR **(C)** were incubated with 25 nM CA200645 (A_3AR and A_1AR) or 10 nM BODIPY-TMR-CGP (β_2AR) in the absence or in the presence of increasing concentrations of the indicated compounds. Values are mean ± SEM from three independent experiments performed in triplicate.

Figure 5. Molecular modelling simulation of K114, SU 6656 and retinoic acid *p*-hydroxyanilide binding to the A_3AR. A side-on (**A**, **C** and **E**) and top-down (**B**, **D** and **F**) view of the top scoring binding poses for K114, SU 6656 and retinoic acid *p*-hydroxyanilide (dark grey liquorice colouring) respectively, bound into our previously reported A_3AR receptor homology model (Vernall *et al.,* 2013). Previously identified amino acid side chain residues associated with the orthosteric binding pocket (Squarcialupi *et al.*, 2013) are represented in light grey liquorice colouring and labelled alongside the TM loop regions for clarity.

1

2

TABLES

Table 1. Affinity of compounds measured at the A_1AR and A_3AR: Affinity values from the PHERAstar HTS assay for unlabelled ligands measured on CHO cells expressing the A_3AR or the A_1AR using 25 nM CA200645 or 5 nM AV039. Values represent mean ± SEM from *n* number of experiments performed in triplicate. ND = not determined. Literature values for both A_3AR and A_1AR taken from Stoddart et al., 2012.

	A_3AR					**A_1AR**		
	CA200645		**AV039**			**CA200645**		
	pK_i	*n*	pK_i	*n*	Literature Values	pK_i	*n*	Literature Values
MRS1220	9.30 ± 0.32	5	9.21 ± 0.12	6	9.02	7.35 ± 0.19	5	7.14
AV019	8.82 ± 0.28	4	ND	-	8.51	ND	-	5.93
XAC	8.06 ± 0.16	5	8.04 ± 0.22	4	7.85	7.70 ± 0.08	4	7.54
CGS15943	7.91 ± 0.20	3	7.91 ± 0.01	3	8.18	8.35 ± 0.16	3	8.95
ZM241385	6.63 ± 0.20	3	6.32 ± 0.28	3	6.74	6.54 ± 0.04	3	6.68

Table 2. Affinity of compounds measured at the β_1AR and β_2AR: Affinity values for β-adrenoceptor ligands measured in CHO cells expressing the β_1AR or the β_2AR using 10 nM of BODIPY-TMR-CGP in the HTS format fluorescent ligand binding assay. Values represent mean ± SEM from three experiments performed in triplicate.

	β_1AR		**β_2AR**	
	pK_i	*n*	pK_i	*N*
Propranolol	8.89 ± 0.16	3	9.00 ± 0.09	3
CGP 20712A	9.68 ± 0.12	3	5.68 ± 0.06	3
ICI 118,551	7.40 ± 0.03	3	8.73 ± 0.07	3

Table 3. Known A_3AR ligands in the LOPAC library: Compounds within the LOPAC library that have known activity at adenosine receptors, their rank order in the full screen and the % of 25 nM CA200645 binding in the presence of 10 μM of these compounds

Name	Agonist or Antagonist	LOPAC description	& Total CA200645 binding	Rank
CGS 15943	Antagonist	Potent non-selective adenosine receptor antagonist	30.0 ± 3.0	9
2-Cl-IB-MECA	Agonist	A_3 adenosine receptor agonist	32.3 ± 6.1	12
IB-MECA	Agonist	Selective A_3 adenosine receptor agonist	36.3 ± 4.0	18
NECA	Agonist	Adenosine receptor agonist	38.1 ± 4.3	20
HEMADO	Agonist	A_3 adenosine receptor agonist	40.1 ± 10.5	24
APNEA	Agonist	Non-selective adenosine receptor agonist	41.0 ± 7.2	26
1,3-dipropyl-8-p-sulfophenylxanthine	Antagonist	Adenosine receptor antagonist (slight selectivity for A_1 over A_2)	42.3 ± 4.8	29
AB-MECA	Agonist	High affinity A_3 adenosine receptor agonist	49.5 ± 5.8	38
2-CADO	Agonist	Adenosine receptor agonist with selectivity for A_1 over A_2	51.0 ± 6.7	43
SCH 58261	Antagonist	A_{2A} adenosine receptor antagonist	52.2 ± 5.4	47
CV1808	Agonist	Selective A_2 adenosine receptor agonist	53.3 ± 19.9	56
DPCPX	Antagonist	Selective A1 adenosine receptor antagonist	56.3 ± 3.4	58
FSCPX	Antagonist	Irreversible A_1 adenosine receptor antagonist	57.5 ± 23.0	63
MRS 1523	Antagonist	Selective A_3 adenosine receptor antagonist in rat	58.3 ± 11.4	64

Table 4. Affinity of selected hits from the LOPAC library at the A_3AR, A_1AR and β_2AR: Compounds were tested on CHO cells expressing the A_3AR, A_1AR and β_2AR in the HTS format fluorescent ligand binding assay using 25 nM CA200645 as the tracer for A_3AR and A_1AR and 10 nM of BODIPY-TMR-CGP for β_2AR. Data represents mean ± SEM from three experiments performed in triplicate. ND = not determined as accurate curve could not be generated.

		A_3AR	**A_1AR**	**β_2AR**
Position in primary screen	**Compound**	**pK_i**	**pK_i**	**% Total binding at 10 µM**
2	SU 6656	6.17 ± 0.08	ND	128.4 ± 18.4
5	K114	6.43 ± 0.04	6.56 ± 0.11	95.8 ± 5.5
8	Retinoic acid p-hydroxyanilide	6.13 ± 0.18	6.04 ± 0.21	102.7 ± 5.1
9	CGS 15943	7.24 ± 0.14	8.14 ± 0.09	115.4 ± 5.0

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by the Medical Research Council [grant numbers G0800006 and MR/N020081/1].

Author contributions

SH, SB and BK conceived the study. MA, LS, SB, BK and SH participated in research design. MA and LS performed the experiments and data analysis. KG performed the beta receptor screening experiments and analysed the data. BK performed the molecular docking studies. MA, LS, BK, SB and SH all wrote or contributed to the writing and editing of the manuscript.

References.

Antonioli L, Csóka B, Fornai M, Colucci R, Kókai E, Blandizzi C et al., (2014) Adenosine and inflammation: what's new on the horizon? *Drug Discov Today*. 19(8):1051-68.

Bain J, Plater L, Elliott M, Shpiro N, Hastie CJ, McLauchlan H*, et al.* (2007). The selectivity of protein kinase inhibitors: a further update. *Biochem J* 408(3): 297-315.

Baker JG, Hall IP, Hill SJ (2002). Pharmacological characterization of CGP 12177 at the human beta(2)-adrenoceptor. *Br J Pharmacol* 137(3): 400-408.

Borea PA, Varani K, Vincenzi F, Baraldi PG, Tabrizi MA, Merighi S et al. (2015) The A3 adenosine receptor: history and perspectives. *Pharmacol Rev*. 67(1):74-102.

Cao HL, Liu ZJ, Chang Z (2017) Cordycepin induces apoptosis in human bladder cancer cells via activation of A3 adenosine receptors. *Tumour Biol*. 39(7):1010428317706915.

Castro M, Nikolaev VO, Palm D, Lohse MJ, Vilardaga J-P (2005). Turn-on switch in parathyroid hormone receptor by a two-step parathyroid hormone binding mechanism. *PNAS* 102(44): 16084-16089.

Cohen S, Barer F, Bar-Yehuda S, IJzerman AP, Jacobson KA, Fishman P (2014) A_3 adenosine receptor allosteric modulator induces an anti-inflammatory effect: in vivo studies and molecular mechanism of action. *Mediators Inflamm*. 2014:708746.

Cornelius P, Lee E, Lin W, Wang R, Werner W, Brown JA*, et al.* (2009). Design, synthesis, and pharmacology of fluorescently labeled analogs of serotonin: application to screening of the 5-HT2C receptor. *J Biomol Screen* 14(4): 360-370.

Crystal AS, Giasson BI, Crowe A, Kung MP, Zhuang ZP, Trojanowski JQ*, et al.* (2003). A comparison of amyloid fibrillogenesis using the novel fluorescent compound K114. *J Neurochem* 86(6): 1359-1368.

Dalpiaz A, Townsend-Nicholson A, Beukers MW, Schofield PR, AP IJ (1998). Thermodynamics of full agonist, partial agonist, and antagonist binding to wild-type and mutant adenosine A1 receptors. *Biochem Pharmacol* 56(11): 1437-1445.

Daly JW, Padgett W, Shamim MT, Butts-Lamb P, Waters J (1985). 1,3-Dialkyl-8-(p-sulfophenyl)xanthines: potent water-soluble antagonists for A1- and A2-adenosine receptors. *J Med Chem* 28(4): 487-492.

Dionisotti S, Ongini E, Zocchi C, Kull B, Arslan G, Fredholm BB (1997). Characterization of human A2A adenosine receptors with the antagonist radioligand [3H]-SCH 58261. *Br J Pharmacol* 121(3): 353-360.

Fredholm BB, Irenius E, Kull B, Schulte G (2001). Comparison of the potency of adenosine as an agonist at human adenosine receptors expressed in Chinese hamster ovary cells. *Biochem Pharmacol* 61(4): 443-448.

Fredholm BB, IJzerman AP, Jacobson KA, Linden J, Müller CE (2011) International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors--an update. *Pharmacol Rev*. 63(1):1-34.

Gallo-Rodriguez C, Ji XD, Melman N, Siegman BD, Sanders LH, Orlina J*, et al.* (1994). Structure-activity relationships of N6-benzyladenosine-5'-uronamides as A3-selective adenosine agonists. *J Med Chem* 37(5): 636-646.

Gao ZG, Mamedova LK, Chen P, Jacobson KA (2004). 2-Substituted adenosine derivatives: affinity and efficacy at four subtypes of human adenosine receptors. *Biochem Pharmacol* 68(10): 1985-1993.

Gherbi K, Briddon SJ, Hill SJ (2014). Detection of the secondary, low-affinity beta1 - adrenoceptor site in living cells using the fluorescent CGP 12177 derivative BODIPY-TMR-CGP. *Br J Pharmacol* 171(23): 5431-5445.

González-Fernández E, Sánchez-Gómez MV, Pérez-Samartín A, Arellano RO, Matute C (2014) A3 Adenosine receptors mediate oligodendrocyte death and ischemic damage to optic nerve. *Glia*. 62(2):199-216.

Guo J, Song L, Liu M, Mahon MJ (2012b). Fluorescent ligand-directed co-localization of the parathyroid hormone 1 receptor with the brush-border scaffold complex of the proximal tubule reveals hormone-dependent changes in ezrin immunoreactivity consistent with inactivation. *Biochim Biophys Acta* 1823(12): 2243-2253.

Hara T, Hirasawa A, Sun Q, Koshimizu TA, Itsubo C, Sadakane K*, et al.* (2009). Flow Cytometry-Based Binding Assay for GPR40 (FFAR1; Free Fatty Acid Receptor 1). *Mol Pharmacol* 75(1): 85-91.

Headrick JP, Ashton KJ, Rose'meyer RB, Peart JN (2013) Cardiovascular adenosine receptors: expression, actions and interactions. *Pharmacol Ther*: 140(1):92-111.

Hussain A, Gharanei AM, Nagra AS, Maddock HL (2014) Caspase inhibition via A3 adenosine receptors: a new cardioprotective mechanism against myocardial infarction. *Cardiovasc Drugs Ther*. 28(1):19-32.

Iturrioz X, Alvear-Perez R, De Mota N, Franchet C, Guillier F, Leroux V*, et al.* (2010). Identification and pharmacological properties of E339-3D6, the first nonpeptidic apelin receptor agonist. *FASEB J* 24(5): 1506-1517.

Jacobson KA, Gallo-Rodriguez C, Melman N, Fischer B, Maillard M, van Bergen A*, et al.* (1993). Structure-activity relationships of 8-styrylxanthines as A2-selective adenosine antagonists. *J Med Chem* 36(10): 1333-1342.

Jacobson KA, Gao Z-G (2006). Adenosine receptors as therapeutic targets. *Nat Rev Drug Discov* 5(3): 247-264.

Jacobson KA, Ijzerman AP, Linden J (1999). 1,3-dialkylxanthine derivatives having high potency as antagonists at human A2B adenosine receptors. *Drug Dev Res* 47: 45-53.

Joshaghani HR, Jafari SM, Aghaei M, Panjehpour M, Abedi H (2017) A3 adenosine receptor agonist induce G1 cell cycle arrest via Cyclin D and cyclin-dependent kinase 4 pathways in OVCAR-3 and Caov-4 cell lines. *J Cancer Res Ther*: 13(1):107-112.

Ji X, Kim YC, Ahern DG, Linden J, Jacobson KA (2001). [3H]MRS 1754, a selective antagonist radioligand for A(2B) adenosine receptors. *Biochem Pharmacol* 61(6): 657-663.

Kecskes M, Kumar TS, Yoo L, Gao ZG, Jacobson KA (2010). Novel Alexa Fluor-488 labeled antagonist of the A(2A) adenosine receptor: Application to a fluorescence polarization-based receptor binding assay. *Biochem Pharmacol* 80(4): 506-511.

Klotz K-N, Falgner N, Kachler S, Lambertucci C, Vittori S, Volpini R*, et al.* (2007). [3H]HEMADO-- a novel tritiated agonist selective for the human adenosine A3 receptor. *Eur J Pharmacol* 556(1-3): 14-18.

Klotz KN, Hessling J, Hegler J, Owman C, Kull B, Fredholm BB*, et al.* (1998). Comparative pharmacology of human adenosine receptor subtypes - characterization of stably transfected receptors in CHO cells. *Naunyn-Schmiedebergs Arch Pharmacol* 357(1): 1-9.

Korb O, Stutzle T, Exner TE (2009). Empirical scoring functions for advanced protein-ligand docking with PLANTS. *J Chem Inf Model* 49(1): 84-96.

Kozma E, Gizewski ET, Tosh DK, Squarcialupi L, Auchampach JA, Jacobson KA (2013). Characterization by flow cytometry of fluorescent, selective agonist probes of the A(3) adenosine receptor. *Biochem Pharmacol* 85(8): 1171-1181.

Kuder KJ, Kiec-Kononowicz K (2014). Fluorescent GPCR ligands as new tools in pharmacology-update, years 2008-early 2014. *Curr Med Chem* 21(34): 3962-3975.

Li AH, Moro S, Melman N, Ji XD, Jacobson KA (1998). Structure-activity relationships and molecular modeling of 3,5-diacyl-2,4-dialkylpyridine derivatives as selective A(3) adenosine receptor antagonists. *J Med Chem* 41(17): 3186-3201.

Little JW, Ford A, Symons-Liguori AM, Chen Z, Janes K, Doyle T et al (2015) Endogenous adenosine A3 receptor activation selectively alleviates persistent pain states. *Brain*. 138 (1):28-35.

May LT, Self TJ, Briddon SJ, Hill SJ (2010). The Effect of Allosteric Modulators on the Kinetics of Agonist-G Protein-Coupled Receptor Interactions in Single Living Cells. *Mol Pharmacol* 78(3): 511-523.

Montinaro A, Iannone R, Pinto A, Morello S (2013) Adenosine receptors as potential targets in melanoma. Pharmacol Res. 76:34-40.

Mulloy DP, Sharma AK, Fernandez LG, Zhao Y, Lau CL, Kron IL et al (2013) Adenosine A3 receptor activation attenuates lung ischemia-reperfusion injury. *AnnThorac Surg*. 95(5):1762-7.

Nakamura K, Shinozuka K, Yoshikawa N (2015) Anticancer and antimetastatic effects of cordycepin, an active component of Cordyceps sinensis. *J Pharmacol Sci.*127(1):53-6.

Ohana G, Cohen S, Rath-Wolfson L, Fishman P (2016) A3 adenosine receptor agonist, CF102, protects against hepatic ischemia/reperfusion injury following partial hepatectomy. *Mol Med Rep*. 14(5): 4335-4341.

Ongini E, Dionisotti S, Gessi S, Irenius E, Fredholm BB (1999). Comparison of CGS 15943, ZM 241385 and SCH 58261 as antagonists at human adenosine receptors. *Naunyn-Schmiedeberg's Arch Pharmacol* 359(1): 7-10.

Ranganathan A, Stoddart LA, Hill SJ, Carlsson J (2015). Fragment-Based Discovery of Subtype-Selective Adenosine Receptor Ligands from Homology Models. *J Med Chem* 58(24): 9578-9590.

Ravani A, Vincenzi F, Bortoluzzi A, Padovan M, Pasquini S, Gessi S et al. (2017) Role and Function of A(2A) and A_3 Adenosine Receptors in Patients with Ankylosing Spondylitis, Psoriatic Arthritis and Rheumatoid Arthritis. *Int J Mol Sci*.18(4). pii: E697.

Squarcialupi L, Colotta V, Catarzi D, Varano F, Filacchioni G, Varani K*, et al.* (2013). 2-Arylpyrazolo[4,3-d]pyrimidin-7-amino derivatives as new potent and selective human A3 adenosine receptor antagonists. Molecular modeling studies and pharmacological evaluation. *J Med Chem* 56(6): 2256-2269.

Stoddart LA, Johnstone EK, Wheal AJ, Goulding J, Robers MB, Machleidt T*, et al.* (2015a). Application of BRET to monitor ligand binding to GPCRs. *Nat Methods* 12(7): 661-663.

Stoddart LA, Kilpatrick LE, Briddon SJ, Hill SJ (2015b). Probing the pharmacology of G protein-coupled receptors with fluorescent ligands. *Neuropharmacol* 98: 48-57.

Stoddart LA, Vernall AJ, Denman JL, Briddon SJ, Kellam B, Hill SJ (2012). Fragment screening at adenosine-A(3) receptors in living cells using a fluorescence-based binding assay. *Chem Biol* 19(9): 1105-1115.

Stoddart LA, White CW, Nguyen K, Hill SJ, Pfleger KD (2016). Fluorescence- and bioluminescence-based approaches to study GPCR ligand binding. *Br J Pharmacol* 173(20): 3028-3037.

Tomasch M, Schwed JS, Kuczka K, Meyer Dos Santos S, Harder S, Nusing RM*, et al.* (2012). Fluorescent Human EP3 Receptor Antagonists. *ACS Med Chem Lett* 3(9): 774-779.

Tosh DK, Padia J, Salvemini D, Jacobson KA. (2015) Efficient, large-scale synthesis and preclinical studies of MRS5698, a highly selective A3 adenosine receptor agonist that protects against chronic neuropathic pain. *Purinergic Signal.*11(3):371-87

Vainio MJ, Johnson MS (2007). Generating conformer ensembles using a multiobjective genetic algorithm. J Chem Inf Model 47(6): 2462-2474.

van Galen PJ, van Bergen AH, Gallo-Rodriguez C, Melman N, Olah ME, AP IJ*, et al.* (1994). A binding site model and structure-activity relationships for the rat A3 adenosine receptor. *Mol Pharmacol* 45(6): 1101-1111.

van Muijlwijk-Koezen JE, Timmerman H, van der Sluis RP, van de Stolpe AC, Menge WM, Beukers MW*, et al.* (2001). Synthesis and use of FSCPX, an irreversible adenosine A1 antagonist, as a 'receptor knock-down' tool. *Bioorg Med Chem Lett* 11(6): 815-818.

Vernall AJ, Stoddart LA, Briddon SJ, Hill SJ, Kellam B (2012). Highly Potent and Selective Fluorescent Antagonists of the Human Adenosine A(3) Receptor Based on the 1,2,4-Triazolo 4,3-a quinoxalin-1-one Scaffold. *J Med Chem* 55(4): 1771-1782.

Vernall AJ, Stoddart LA, Briddon SJ, Ng HW, Laughton CA, Doughty SW*, et al.* (2013). Conversion of a non-selective adenosine receptor antagonist into A3-selective high affinity fluorescent probes using peptide-based linkers. *Org Biomol Chem* 11(34): 5673-5682.

Wu JM, DiPietrantonio AM, Hsieh TC (2001). Mechanism of fenretinide (4-HPR)-induced cell death. *Apoptosis* 6(5): 377-388.

Yoshida K, Ito M, Matsuoka I (2017) Divergent regulatory roles of extracellular ATP in the degranulation response of mouse bone marrow-derived mast cells. *Int Immunopharmacol*. 43:99-107.

Young SM, Bologa C, Prossnitz ER, Oprea TI, Sklar LA, Edwards BS (2005). High-throughput screening with HyperCyt (R) flow cytometry to detect small molecule formylpeptide receptor ligands. *J Biomol Screen* 10(4): 374-382.

Zwier JM, Roux T, Cottet M, Durroux T, Douzon S, Bdioui S*, et al.* (2010). A Fluorescent Ligand-Binding Alternative Using Tag-lite (R) Technology. *J Biomol Screen*15(10): 1248-1259.

Figure 1

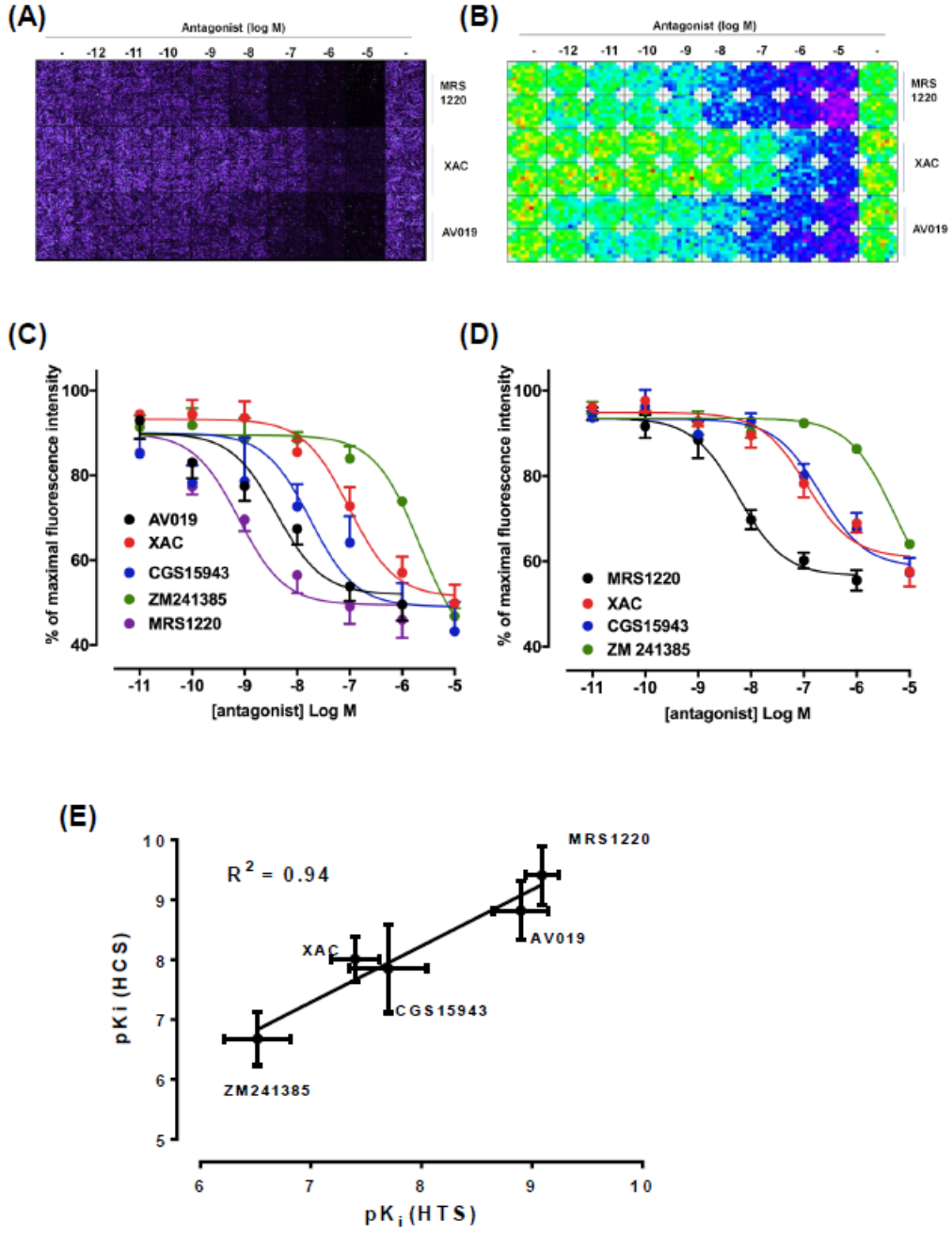
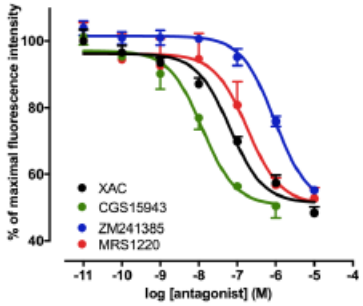
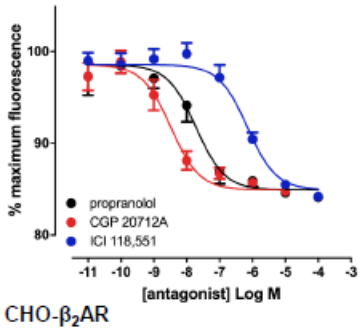

(A)
Antagonist (log M)
- -12 -11 -10 -9 -8 -7 -6 -5 -
MRS 1220
XAC
AV019
(B)
Antagonist (log M)
- -12 -11 -10 -9 -8 -7 -6 -5 -
MRS 1220
XAC
AV019
(C)
% of maximal fluorescence intensity
100
80
60
40
AV019
XAC
CGS15943
ZM241385
MRS1220
-11 -10 -9 -8 -7 -6 -5
[antagonist] Log M
(D)
% of maximal fluorescence intensity
100
80
60
40
MRS1220
XAC
CGS15943
ZM 241385
-11 -10 -9 -8 -7 -6 -5
[antagonist] Log M
(E)
R2 = 0.94
MRS1220
AV019
XAC
CGS15943
ZM241385
pKi (HCS)
10
9
8
7
6
5
6 7 8 9 10
pKi (HTS)

Figure 2

(A) CHO-A_1AR



(B) CHO-β_1AR



(C) CHO-β_2AR

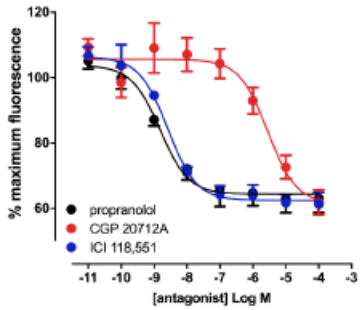
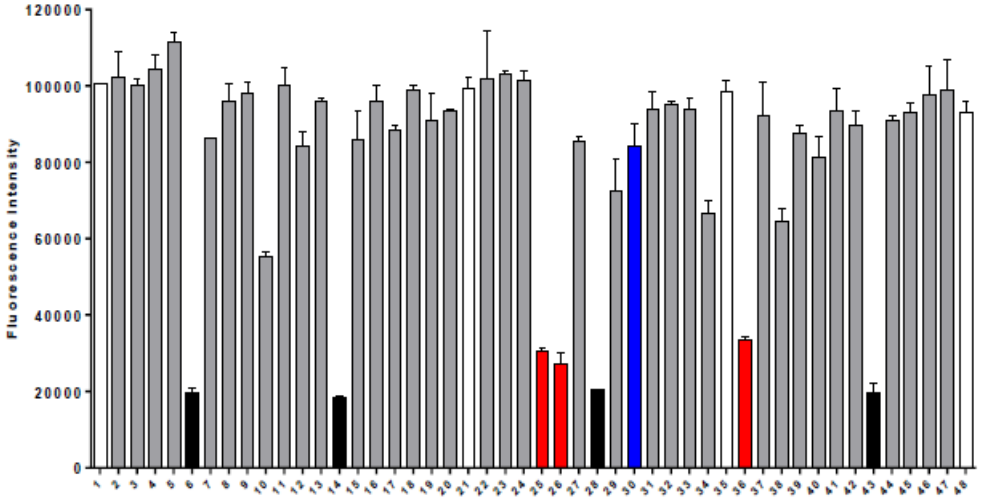


Figure 3



1. Basal
2. Amoxapine
3. 5'-Amino-5'-deoxyadenosine p-toluenesulfonate
4. Amiloride
5. 6-Aminohexanoic acid
6. MRS1220
7. Trovafloxacin mesylate
8. Antozoline
9. Aniracetam
10. 1-benzoyl-5-methoxy-2-methylindole-3-acetic acid
11. (±)-AMT hydrochloride
12. CBIQ
13. L-2-aminoadipic acid
14. MRS1220
15. 5-(N,N-hexamethylene)amiloride
16. L(-)-Norepinephrine bitartrate
17. Fulvestrant
18. 2-(Methylthio)adenosine 5'-diphosphate
19. Altretamine
20. Paroxetine
21. Basal
22. N-Acetyl-L-Cysteine
23. Benzamide
24.SKF-89145
25. HEMADO
26. AS-252424
27. (±)-p-Aminoglutethimide
28. MRS1220
29. Psora-4
30. Adenosine
31. Opipramol
32. AIDA
33. Allopurinol
34. SB 200646
35. Basal
36. N6-2-(4-Aminophenyl)ethyladenosine
37. Amsacrine
38. Lercanidipine
39. N-(4-Amino-2-chlorophenyl)phthalimide
40. Sildenafil
41.Azithromycin
42. S(-)-Atenolol
43. MRS1220
44. (±)-HA-966
45. Astaxanthin
46. L-Aspartic acid
47. Alaproclate
48.Basal

Figure 4

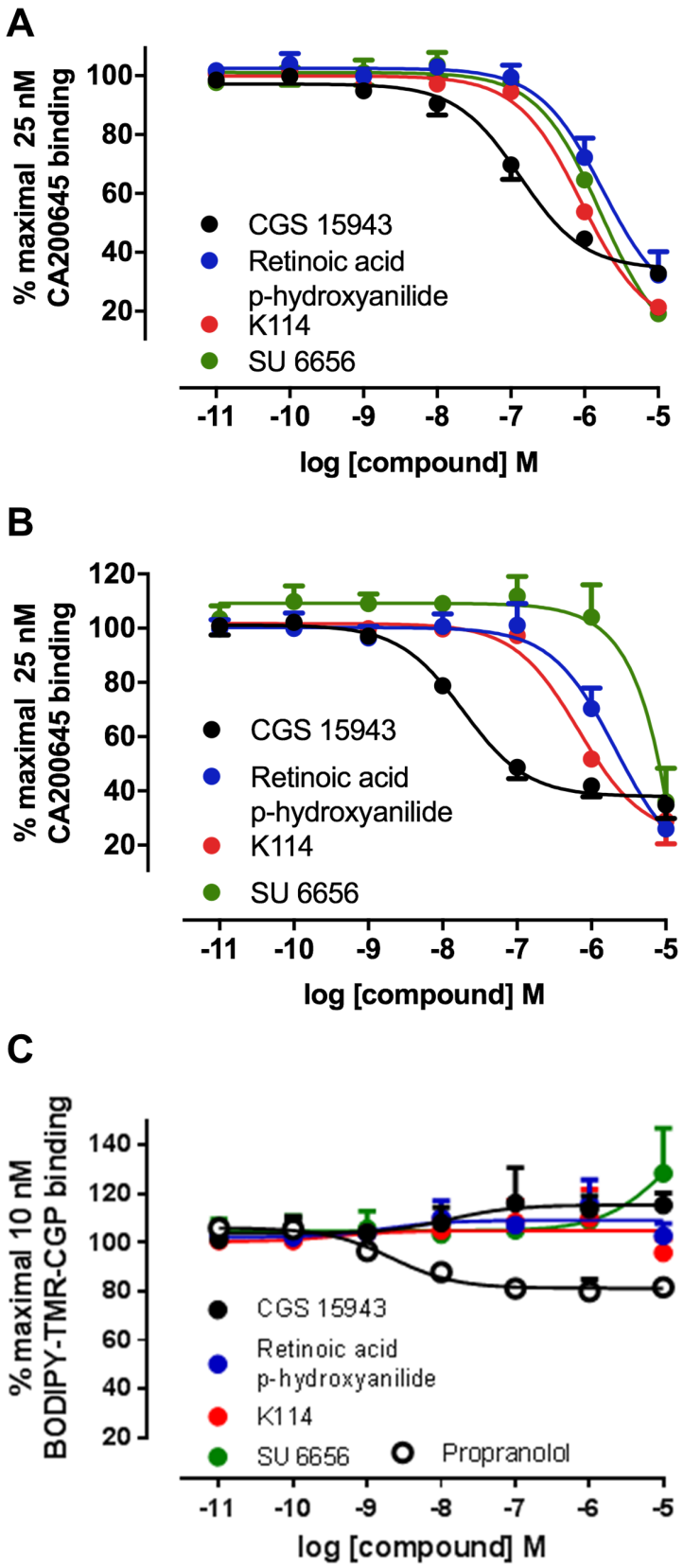
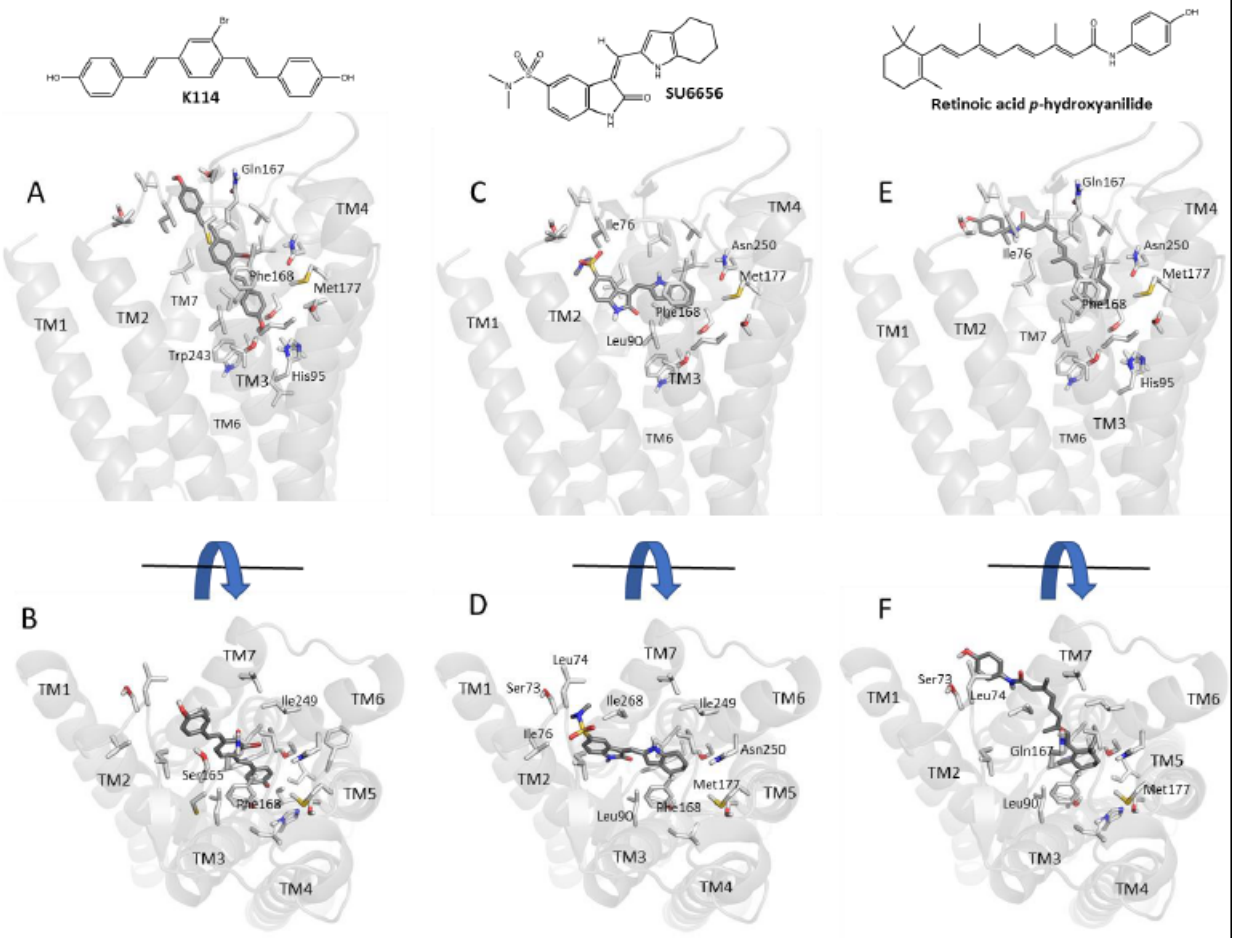

A
% maximal 25 nM CA200645 binding
100
80
60
40
20
CGS 15943
Retinoic acid
p-hydroxyanilide
K114
SU 6656
-11
-10
-9
-8
-7
-6
-5
log [compound] M
B
% maximal 25 nM CA200645 binding
120
100
80
60
40
20
CGS 15943
Retinoic acid
p-hydroxyanilide
K114
SU 6656
-11
-10
-9
-8
-7
-6
-5
log [compound] M
C
% maximal 10 nM BODIPY-TMR-CGP binding
140
120
100
80
60
40
20
CGS 15943
Retinoic acid
p-hydroxyanilide
K114
SU 6656
Propranolol
-11
-10
-9
-8
-7
-6
-5
log [compound] M

Figure 5


K114
SU6656
Retinoic acid p-hydroxyanilide
A
B
C
D
E
F
Gln167
TM4
Phe168
Met177
TM7
TM1
TM2
Trp243
TM3
His95
TM6
Ile76
Asn250
Leu90
Ile249
TM5
Ser165
Leu74
Ser73
Ile268
TM1
TM2
TM3
TM4
TM5
TM6
TM7

Supporting Information

for

A non-imaging high throughput approach to chemical library screening at the unmodified adenosine-A_3 receptor in living cells

Maria Augusta Arruda*, Leigh A Stoddart*, Karolina Gherbi, Stephen J Briddon, Barrie Kellam, Stephen J Hill

Table 1. Inhibition of CA200645 binding at the A_3AR by the LOPAC library of compounds.

Values obtained in a fluorescent adenosine receptor antagonist binding assay using whole, live cells expressing the A_3AR. Values quoted are % of control wells (wells containing 1% DMSO and 25 nM CA200645). All compounds were tested at 10 μM. Data shown represents mean ± SD from three separate experiments performed in duplicate. ND = not determined as compounds were not included in the screen.

Rank	Compound Name	% 25 nM CA200645 binding		Rank	Compound Name	% 25 nM CA200645 binding
1	BIO	16.1 ± 3.2		641	1-(4-Chlorobenzyl)-5-methoxy-2-methylindole-3-acetic acid	97.1 ± 10.6
2	SU 6656	17.3 ± 1.8		642	Etodolac	97.1 ± 13.7
3	Rottlerin	22.8 ± 1.4		643	Anisotropine methyl bromide	97.1 ± 12.3
4	Reactive Blue 2	24.5 ± 2.7		644	Metrazoline oxalate	97.1 ± 2.0
5	K114	24.6 ± 5.8		645	Ebastine	97.2 ± 6.4
6	Quercetin dihydrate	25.2 ± 6.9		646	(+)-Brompheniramine maleate	97.2 ± 7.4
7	PD173952	25.6 ± 5.3		647	Citalopram hydrobromide	97.2 ± 4.4
8	Retinoic acid p-hydroxyanilide	26.5 ± 2.0		648	1,5-Isoquinolinediol	97.2 ± 4.1
9	CGS-15943	30.0 ± 3.0		649	Paroxetine hydrochloride hemihydrate	97.2 ± 4.3
10	Kenpaullone	30.6 ± 6.6		650	S(-)-Atenolol	97.2 ± 11.6
11	DAPH	31.8 ± 9.9		651	(±)-CPP	97.2 ± 1.5
12	Chloro-IB-MECA	32.3 ± 6.1		652	Captopril	97.2 ± 6.3
13	PD-166866	34.3 ± 9.7		653	U0126	97.2 ± 19.0
14	Rutaecarpine	34.3 ± 2.1		654	8-(p-Sulfophenyl)theophylline	97.2 ± 13.7
15	PD 169316	35.7 ± 3.3		655	Nisoxetine hydrochloride	97.3 ± 6.5
16	1,3,5-tris(4-hydroxyphenyl)-4-propyl-1H-pyrazole	35.7 ± 9.6		656	Imiloxan hydrochloride	97.3 ± 10.2
17	AGK2	35.8 ± 5.6		657	CHM-1 hydrate	97.3 ± 5.3
18	IB-MECA	36.3 ± 4.0		658	IMID-4F hydrochloride	97.3 ± 5.7
19	U-74389G maleate	36.9 ± 10.8		659	SKF-89145 hydrobromide	97.3 ± 9.6
20	5'-N-Ethylcarboxamidoadenosine	38.1 ± 4.3		660	(±)-Methoxyverapamil hydrochloride	97.3 ± 10.6

21	CL 316,243	38.7 ± 18.1		661	Venlafaxine hydrochloride	97.3 ± 8.5
22	Calcimycin	38.9 ± 3.3		662	CGS-12066A maleate	97.3 ± 10.3
23	Sanguinarine chloride	38.9 ± 20.6		663	Vinpocetine	97.3 ± 11.5
24	HEMADO	40.1 ± 10.4		664	Sunitinib malate	97.4 ± 12.4
25	SP600125	41.0 ± 10.4		665	Imazodan	97.4 ± 12.7
26	N6-2-(4-Aminophenyl)ethyladenosine	41.0 ± 7.1		666	Atropine sulfate	97.4 ± 1.9
27	6(5H)-Phenanthridinone	41.6 ± 14.1		667	DL-Cycloserine	97.4 ± 8.2
28	Apigenin	41.8 ± 13.4		668	(±)-Vanillylmandelic acid	97.4 ± 12.9
29	1,3-Dipropyl-8-p-sulfophenylxanthine	42.3 ± 4.8		669	Sepiapterin	97.4 ± 20.7
30	SU 5416	43.3 ± 10.8		670	Albuterol hemisulfate	97.4 ± 12.0
31	DL-Stearoylcarnitine chloride	44.2 ± 6.0		671	4-Aminobenzamidine dihydrochloride	97.4 ± 8.5
32	Roscovitine	45.3 ± 8.6		672	Diltiazem hydrochloride	97.4 ± 8.9
33	AS-252424	45.8 ± 20.9		673	CGP-13501	97.4 ± 7.1
34	Etazolate hydrochloride	46.2 ± 5.3		674	L-741,626	97.5 ± 15.5
35	Eupatorin	47.2 ± 12.7		675	Sematilide monohydrochloride monohydrate	97.5 ± 2.4
36	Imperatorin	47.6 ± 2.3		676	Tomoxetine	97.5 ± 8.3
37	AB-MECA	48.5 ± 9.1		677	1-Allyl-3,7-dimethyl-8-p-sulfophenylxanthine	97.5 ± 9.3
38	Furafylline	49.5± 5.8		678	Gabaculine hydrochloride	97.5 ± 8.4
39	SB 242084 dihydrochloride hydrate	49.6± 12.4		679	Eprosartan mesylate	97.5 ± 15.0
40	MNS	50.1 ± 14.8		680	Labetalol hydrochloride	97.5 ± 13.9
41	Indirubin-3'-oxime	50.6 ± 24.7		681	Cantharidic Acid	97.5 ± 13.0
42	PD-184161	50.8 ± 14.3		682	SCH-28080	97.5 ± 14.8
43	2-Chloroadenosine	51.0 ± 6.7		683	Bendamustine hydrochloride	97.6 ± 4.1
44	SB 218795	51.1 ± 8.4		684	Chlorpropamide	97.6 ± 7.8
45	Diacylglycerol Kinase Inhibitor II	51.5 ± 9.4		685	Oxaprozin	97.6 ± 6.3
46	(±)-2-Amino-7-phosphonoheptanoic acid	52.2 ± 10.8		686	Agmatine sulfate	97.6 ± 11.6
47	UCL 2077	52.2 ± 5.4		687	PMEG hydrate	97.6 ± 14.8
48	SCH 58261	52.5 ± 9.7		688	gamma-Acetylinic GABA	97.6 ± 3.0
49	Emodin	52.8± 4.4		689	Carboplatin	97.7 ± 5.9
50	SU 4312	53.2 ± 16.7		690	DBO-83	97.7 ± 11.0
51	N-Oleoyldopamine	53.5 ± 8.3		691	L(-)-Norepinephrine bitartrate	97.7 ± 6.2
52	NU2058	53.9 ± 7.1		692	loxoprofen	97.7. ± 0.7
53	Gossypol	54.1 ± 12.3		693	Podophyllotoxin	97.7 ± 17.3
54	Calmidazolium chloride	54.4 ± 19.1		694	5-Hydroxy-L-tryptophan	97.7 ± 1.5
55	PF-573228	54.7 ± 30.2		695	Atorvastatin calcium salt trihydrate	97.7 ± 2.2
56	2-Phenylaminoadenosine	55.3 ± 19.9		696	Moclobemide	97.8 ± 6.0
57	GW7647	55.8 ± 12.2		697	Piribedil maleate	97.8 ± 1.7
58	8-Cyclopentyl-1,3-dipropylxanthine	56.3 ± 3.4		698	(-)-Naproxen sodium	97.8 ± 4.0
59	Nifedipine	56.6 ± 11.1		699	5-Aminovaleric acid hydrochloride	97.9 ± 5.1
60	FSCPX	57.1 ± 9.1		700	SKF 83959 hydrobromide	97.9 ± 6.2

61	MRS 1523	57.3 ± 10.9		701	N-Bromoacetamide	97.9 ± 5.0
62	GW2974	57.3 ± 11.9		702	BIX 01294 trihydrochloride hydrate	97.9 ± 21.2
63	Tyrphostin AG 879	57.5 ± 23.0		703	Oxiracetam	97.9 ± 12.4
64	AS 604850	58.3 ± 11.4		704	S(-)-Pindolol	98.0 ± 8.4
65	7-Cyclopentyl-5-(4-phenoxy)phenyl-7H-pyrrolo[2,3-d]pyrimidin-4-ylamine	58.8 ± 13.2		705	Amisulpride	98.0 ± 5.3
66	1-benzoyl-5-methoxy-2-methylindole-3-acetic acid	59.0 ± 9.2		706	L-Cycloserine	98.0 ± 1.4
67	AMG 9810	59.0 ± 6.4		707	(±)-7-Hydroxy-DPAT hydrobromide	98.0 ± 1.6
68	(+)-Bromocriptine methanesulfonate	60.6 ± 9.2		708	3-Isobutyl-1-methylxanthine	98.0 ± 7.1
69	SB 206553 hydrochloride	60.7 ± 9.3		709	SB-215505	98.1 ± 14.8
70	N6-Methyladenosine	61.1 ± 11.7		710	Fluphenazine dihydrochloride	98.1 ± 12.0
71	IRAK-1/4 Inhibitor I	61.3 ± 9.4		711	Demeclocycline hydrochloride	98.1 ± 10.5
72	TNP	61.8 ± 11.7		712	L-Buthionine-sulfoximine	98.1 ± 5.3
73	Myricetin	62.7 ± 9.0		713	cis(+/-)-8-OH-PBZI hydrobromide	98.1 ± 5.1
74	IPA-3	63.1 ± 15.2		714	Cytosine-1-beta-D-arabinofuranoside hydrochloride	98.1 ± 3.7
75	LY-367,265	64.8 ± 25.8		715	EBPC	98.2 ± 13.5
76	O6-benzylguanine	65.0 ± 6.0		716	Quinacrine dihydrochloride	98.2 ± 4.9
77	Thapsigargin	65.1 ± 10.7		717	Vinblastine sulfate salt	98.2 ± 16.0
78	YC-1	65.3 ± 4.7		718	N-Oleoylethanolamine	98.2 ± 10.8
79	Mecamylamine hydrochloride	65.4 ± 10.0		719	Guanabenz acetate	98.2 ± 11.7
80	CGS-21680 hydrochloride	65.7 ± 8.4		720	Tetrahydrozoline hydrochloride	98.2 ± 6.4
81	Genistein	66.1 ± 12.2		721	BRL 37344 sodium	98.2 ± 9.4
82	Psora-4	66.4 ± 9.2		722	CP-346086 dihydrate	98.2 ± 12.5
83	Mephetyl tetrazole	66.4 ± 18.5		723	(±)-8-Hydroxy-DPAT hydrobromide	98.2 ± 1.8
84	G15	66.5 ± 16.0		724	Tyrphostin AG 537	98.3 ± 17.8
85	Fusaric acid	66.5 ± 29.0		725	BU99006	98.3 ± 5.1
86	Cilnidipine	67.0 ± 19.0		726	Actinonin	98.3 ± 4.3
87	WIN 62,577	67.3 ± 5.6		727	HA-100	98.3 ± 9.4
88	(-)-Bicuculline methbromide, 1(S), 9(R)	67.4 ± 5.9		728	Ammonium pyrrolidinedithiocarbamate	98.3 ± 7.2
89	TBB	67.4 ± 13.4		729	Famotidine	98.3 ± 15.4
90	Phloretin	67.7 ± 15.2		730	Pancuronium bromide	98.3 ± 10.5
91	7,8-Dihydroxyflavone hydrate	68.2 ± 13.0		731	1,10-Diaminodecane	98.3 ± 12.0
92	CCT007093	68.4 ± 3.4		732	Sodium Taurocholate hydrate	98.3 ± 7.3
93	SB 202190	68.5 ± 12.1		733	Bestatin hydrochloride	98.3 ± 9.7
94	S(-)-p-Bromotetramisole oxalate	68.6 ± 44.8		734	Clodronic acid	98.4 ± 3.5
95	CyPPA	68.8 ± 15.9		735	Betaxolol hydrochloride	98.4 ± 4.9
96	Cisplatin	69.0 ± 8.2		736	N-Desmethylclozapine	98.4 ± 14.7
97	R(-)-N6-(2-Phenylisopropyl)adenosine	69.2 ± 26.4		737	D-ribofuranosylbenzimidazole	98.4 ± 15.0
98	N6-Cyclopentyladenosine	69.6 ± 6.1		738	ATPO	98.4 ± 3.3
99	AA-861	69.6 ± 8.4		739	RepSox	98.5 ± 5.4
100	6-Hydroxy-DL-DOPA	69.7 ± 9.9		740	Parthenolide	98.5 ± 15.3

101	KRM-III	70.4 ± 12.2		741	SIB 1757	98.5 ± 4.5
102	R(-)-Apocodeine hydrochloride	70.5 ± 34.0		742	DL-erythro-Dihydrosphingosine	98.5 ± 11.2
103	I-OMe-Tyrphostin AG 538	71.3 ± 34.4		743	Thiolactomycin	98.5 ± 7.2
104	1-(1-Naphthyl)piperazine hydrochloride	71.5 10.7		744	p-Fluoro-L-phenylalanine	98.5 ± 8.4
105	PD-156707	71.9 ± 8.4		745	LE 300	98.5 ± 4.5
106	Morin	72.1 ± 9.6		746	1-Deoxynojirimycin hydrochloride	98.5 ± 11.6
107	Ro 90-7501	72.1 ± 6.0		747	Disopyramide phosphate	98.5 ± 7.6
108	(±)-Chloro-APB hydrobromide	72.6 ± 20.5		748	(-)-Scopolamine,n-Butyl-, bromide	98.5 ± 15.4
109	Celecoxib	72.6 ± 20.4		749	CP-100263 dihydrochloride hydrate	98.5 ± 6.9
110	Indomethacin	72.9 ± 18.3		750	L-allylglycine	98.5 ± 1.7
111	U-73122	73.1 ± 7.4		751	Nomifensine maleate	98.5 ± 14.7
112	Tyrphostin AG 835	73.1 ± 6.8		752	Succinylcholine chloride	98.6 ± 15.7
113	Chelerythrine chloride	73.9 ± 3.7		753	EGTA	95.6 ± 5.9
114	Clotrimazole	74.0 ± 15.0		754	4-Imidazoleacrylic acid	95.6 ± 8.4
115	FPL 64176	74.2 ± 6.7		755	Cetirizine dihydrochloride	98.6 ± 19.3
116	TBBz	74.5 ± 15.0		756	(+)-Butaclamol hydrochloride	98.6 ± 1.9
117	AL-8810	75.0 ± 17.3		757	(-)-Isoproterenol hydrochloride	98.6 ± 14.4
118	Flupirtine maleate	75.3 ± 9.1		758	Y-27632 dihydrochloride	98.6 ± 10.3
119	Dephostatin	75.4 ± 19.6		759	Zonisamide sodium	98.6 ± 10.8
120	Cilostamide	75.9 ± 3.4		760	L-3,4-Dihydroxyphenylalanine methyl ester hydrochloride	98.6 ± 13.3
121	10058-F4	75.9 ± 8.5		761	Naftopidil dihydrochloride	98.6 ± 14.2
122	WB-4101 hydrochloride	76.0 ± 5.8		762	(±)-threo-1-Phenyl-2-decanoylamino-3-morpholino-1-propanol hydrochloride	98.6 ± 11.0
123	SB-525334	76.3 ± 9.5		763	S(+)-Raclopride L-tartrate	98.6 ± 4.9
124	alpha-Guanidinoglutaric acid	76.4 ± 9.5		764	Rolipram	98.7 ± 10.9
125	Olvanil	76.7 ± 2.8		765	Tropicamide	98.7 ± 3.0
126	SB 222200	76.8 ± 5.5		766	Histamine, R(-)-alpha-methyl-, dihydrochloride	98.7 ± 8.4
127	FAUC 213	76.8 ± 3.4		767	5alpha-Pregnan-3alpha-ol-11,20-dione	98.7 ± 9.4
128	Betamethasone	77.0 ± 7.6		768	Felbamate	98.7 ± 4.1
129	L-798106	77.1 ± 10.4		769	Nilutamide	98.7 ± 10.0
130	p-Iodoclonidine hydrochloride	77.2 ± 42.2		770	4-Hydroxyphenethylamine hydrochloride	98.7 ± 19.0
131	CP-154526 hydrochloride	77.3 ± 23.1		771	N-(3,3-Diphenylpropyl)glycinamide	98.7 ± 7.8
132	Nelfinavir mesylate hydrate	77.3 ± 17.3		772	MK-886	98.7 ± 12.2
133	TG003	77.3 ± 17.0		773	Semicarbazide hydrochloride	98.7 ± 22.6
134	6-Fluoronorepinephrine hydrochloride	77.6 ± 28.4		774	Ciprofibrate	98.7 ± 5.2
135	CP-64434 hydrate	77.6 ± 21.9		775	CP-471474	98.7 ± 17.5
136	Hispidin	77.8 ± 19.6		776	Eliprodil	98.8 ± 8.5
137	R(+)-6-Bromo-APB hydrobromide	77.8 ± 20.1		777	5-Fluorouracil	98.8 ± 8.7
138	7-Chloro-4-hydroxy-2-phenyl-1,8-naphthyridine	77.8 ± 8.8		778	Ro 41-0960	98.8 ± 6.8
139	GR 79236X	78.0 ± 19.3		779	Benazoline oxalate	98.8 ± 14.3

140	Ellipticine	78.2 ± 23.8		780	Tryptamine hydrochloride	98.8 ± 5.7
141	GYKI 52466 hydrochloride	78.2 ± 10.4		781	Dicyclomine hydrochloride	98.9 ± 10.8
142	Pimozide	78.2 ± 10.9		782	Supercinnamaldehyde	98.9 ± 0.6
143	Gallamine triethiodide	78.3 ± 38.7		783	Tracazolate	98.9 ± 4.3
144	BF-170 hydrochloride	78.6 ± 20.5		784	Azithromycin dihydrate	98.9 ± 6.9
145	Betaine hydrochloride	78.7 ± 15.6		785	Phentolamine mesylate	98.9 ± 10.7
146	Dipyridamole	78.8 ± 14.8		786	Tiapride hydrochloride	98.9 ± 15.2
147	Disopyramide	78.9 ± 27.0		787	4-Amidinophenylmethanesulfonyl fluoride hydrochloride	98.9 ± 8.1
148	PNU-282987	78.9 ± 33.0		788	Oleic Acid	98.9 ± 11.8
149	Nocodazole	79.0 ± 5.7		789	Bupropion hydrochloride	98.9 ± 4.9
150	Piceatannol	79.1 ± 22.1		790	Phosphomycin disodium	98.9 ± 12.7
151	L-165,041	79.1 ± 19.7		791	Benserazide hydrochloride	98.9 ± 5.3
152	Felodipine	79.1 ± 13.7		792	Ketoconazole	98.9 ± 9.0
153	Cyclophosphamide monohydrate	79.2 ± 18.9		793	2-Methylthioadenosine triphosphate tetrasodium	99.0 ± 0.7
154	Cefaclor	79.3 ± 20.7		794	Triflupromazine hydrochloride	99.0 ± 16.0
155	Caffeic acid phenethyl ester	79.3 ± 10.7		795	N-Acetyltryptamine	99.0 ± 14.1
156	Nordihydroguaiaretic acid from Larrea divaricata (creosote bush)	79.3 ± 28.3		796	Benzamide	99.1 ± 4.7
157	Ritanserin	79.4 ± 12.5		797	Moxonidine hydrochloride	99.1 ± 3.2
158	8-(3-Chlorostyryl)caffeine	79.6 ± 7.9		798	L-3,4-Dihydroxyphenylalanine	99.1 ± 9.5
159	Loxapine succinate	80.2 ± 12.7		799	Theophylline	99.1 ± 10.8
160	Phorbol 12-myristate 13-acetate	80.3 ± 5.6		800	3-(1H-Imidazol-4-yl)propyl di(p-fluorophenyl)methyl ether hydrochloride	99.1 ± 1.1
161	NU6027	80.6 ± 9.9		801	Altretamine	99.1 ± 9.2
162	ET-18-OCH3	80.6 ± 4.5		802	8-Methoxymethyl-3-isobutyl-1-methylxanthine	99.2 ± 10.1
163	Promazine hydrochloride	80.6 ± 8.4		803	Formoterol	99.2 ± 6.5
164	erythro-9-(2-Hydroxy-3-nonyl)adenine hydrochloride	80.7 ± 17.0		804	Aminoguanidine hemisulfate	99.2 ± 8.4
165	PD 98,059	80.7 ± 2.6		805	Diethylenetriaminepentaacetic acid	99.2 ± 10.9
166	Gabapentin	80.7 ± 22.8		806	Imipramine hydrochloride	99.2 ± 4.6
167	Debrisoquin sulfate	81.0 ± 16.3		807	(±)-Chlorpheniramine maleate	99.2 ± 9.9
168	Phenserine	81.1 ± 29.6		808	PF-4708671	99.2 ± 13.5
169	3-Bromo-7-nitroindazole	81.2 ± 6.7		809	Dihydroergotamine methanesulfonate	99.2 ± 11.1
170	CGP 57380	81.2 ± 23.0		810	(±)-6-Chloro-PB hydrobromide	99.3 ± 4.1
171	Fenspiride hydrochloride	81.2 ± 5.0		811	Hydroxylamine hydrochloride	99.3 ± 3.4
172	cDPCP	81.3 ± 5.4		812	Guvacine hydrochloride	99.3 ± 14.1
173	Clofibrate	81.3 ± 24.9		813	(-)-Quinpirole hydrochloride	99.3 ± 13.8
174	Esomeprazole magnesium dihydrate	81.5 ± 16.8		814	2,3-Dimethoxy-1,4-naphthoquinone	99.3 ± 8.8
175	Tyrphostin 1	81.6 ± 2.9		815	(-)-Physostigmine	99.3 ± 6.1
176	SB 200646 hydrochloride	81.7 ± 19.9		816	Imidazole-4-acetic acid hydrochloride	99.3 ± 14.9
177	Arecoline hydrobromide	81.8 ± 27.0		817	L-Aspartic acid	99.3 ± 3.7
178	N-Succinyl-L-proline	81.8 ± 11.2		818	CP-335963	99.3 ± 11.5

179	Staurosporine aglycone	81.9 ± 6.1		819	Mexiletene hydrochloride	99.4 ± 6.9
180	Pentoxifylline	81.9 ± 17.4		820	Ritodrine hydrochloride	99.4 ± 8.5
181	AMN082	81.87813367		821	(±)-cis-Piperidine-2,3-dicarboxylic acid	99.4 ± 1.9
182	Fenoterol hydrobromide	81.9 ± 17.1		822	Trihexyphenidyl hydrochloride	99.4 ± 5.7
183	Fenobam	81.9 ± 15.0		823	Artemether	99.4 ± 9.9
184	Auranofin	82.1 ± 31.0		824	(±)-SKF-38393 hydrochloride	99.4 ± 9.0
185	SANT-1	82.1 ± 15.9		825	Hexamethonium bromide	99.4 ± 1.1
186	2',3'-didehydro-3'-deoxythymidine	82.1 ± 11.9		826	Phenelzine sulfate	99.4 ± 7.6
187	Ro 04-6790 dihydrochloride	82.1 ± 15.5		827	N-Methylhistaprodifen dioxalate salt	99.4 ± 7.3
188	3'-Azido-3'-deoxythymidine	82.3 ± 18.1		828	S-(+)-PD 123177 trifluoroacetate salt hydrate	99.4 ± 7.4
189	S-(p-Azidophenacyl)glutathione	82.4 ± 8.6		829	AIDA	99.4 ± 5.4
190	Wortmannin from Penicillium funiculosum	82.6 ± 17.7		830	Clomipramine hydrochloride	99.4 ± 3.8
191	BRL 50481	82.8 ± 17.4		831	Lorglumide sodium	99.4 ± 5.5
192	BMY 7378 dihydrochloride	82.8 ± 23.0		832	(+)-Norfenfluramine hydrochloride	99.5 ± 11.3
193	Pergolide methanesulfonate	82.8 ± 15.4		833	S-Nitrosoglutathione	99.5 ± 12.8
194	Ibudilast	82.8 ± 12.0		834	8-Bromo-cAMP sodium	99.5 ± 9.1
195	Palmitoyl-DL-Carnitine chloride	82.9 ± 21.8		835	Flumazenil	99.5 ± 3.5
196	Lercanidipine hydrochloride hemihydrate	82.9 ± 16.7		836	NCS-382	99.5 ± 31.9
197	R(-)-2,10,11-Trihydroxyaporphine hybrobromide	83.0 ± 14.1		837	O-(Carboxymethyl)hydroxylamine hemihydrochloride	99.5 ± 10.8
198	MRS 2159	83.0 ± 11.6		838	Domperidone	99.6 ± 10.5
199	R-(+)-8-Hydroxy-DPAT hydrobromide	83.0 ± 17.5		839	DL-Homatropine hydrobromide	99.6 ± 8.1
200	Tamoxifen	83.1 ± 23.3		840	(±)-Baclofen	99.6 ± 6.9
201	(±)-Octoclothepin maleate	83.2 ± 25.2		841	Sandoz 58-035	99.6 ± 12.7
202	L-701,324	83.2 ± 4.8		842	(S)-(+)-Camptothecin	99.7 ± 12.1
203	Clozapine	83.2 ± 22.9		843	TPMPA	99.7 ± 3.7
204	SC-57461A	83.2 ± 13.1		844	Clemizole hydrochloride	99.7 ± 8.2
205	(±)-Metoprolol (+)-tartrate	83.3 ± 9.2		845	(±)-SKF 38393, N-allyl-, hydrobromide	99.7 ± 11.6
206	AS605240	83.4 ± 13.4		846	(±)-alpha-Lipoic Acid	99.7 ± 5.6
207	SCH-202676 hydrobromide	83.4 ± 13.1		847	Trandolapril	99.7 ± 12.5
208	CPNQ	83.4 ± 4.6		848	Trimethoprim	99.7 ± 13.3
209	1-Aminobenzotriazole	83.5 ± 18.8		849	(-)-Scopolamine hydrobromide	99.7 ± 17.0
210	Kynurenic acid	83.5 ± 10.7		850	Thioperamide maleate	99.7 ± 10.4
211	Urapidil, 5-Methyl-	83.5 ± 22.8		851	1-Methylhistamine dihydrochloride	99.7 ± 10.5
212	Mifepristone	83.5 ± 5.2		852	Allopurinol	99.8 ± 7.2
213	CP-226269	83.6 ± 17.8		853	Corticosterone	99.8 ± 5.3
214	Ganaxolone	83.6 ± 13.4		854	N-Ethylmaleimide	99.8 ± 13.3
215	Amitriptyline hydrochloride	83.6 ± 19.8		855	(-)-cis-(1S,2R)-U-50488 tartrate	99.8 ± 10.5
216	AC-55649	83.6 ± 13.2		856	Valproic acid sodium	99.8 ± 9.6
217	trans-(±)-ACPD	83.6 ± 13.4		857	Doxazosin mesylate	99.9 ± 9.5

218	L-Cysteinesulfinic Acid	83.6 ± 21.3		858	Amsacrine hydrochloride	99.8 ± 8.3
219	BAY 61-3606 hydrochloride hydrate	83.8 ± 10.8		859	(±)-2-Amino-4-phosphonobutyric acid	99.9 ± 8.9
220	Reserpine	83.8 ± 14.9		860	Imetit dihydrobromide	99.9 ± 2.2
221	NF 023	83.8 ± 25.8		861	Tulobuterol hydrochloride	99.9 ± 4.7
222	K 185	84.1 ± 1.4		862	U-73343	99.9 ± 5.3
223	Hydrocortisone	84.4 ± 24.1		863	Acyclovir	99.9 ±13.7
224	Flutamide	84.4 ± 23.0		864	BTO-1	99.9 ± 23.7
225	Sulindac sulfone	84.5 ± 13.2		865	L-Glutamine	99.9 ± 14.1
226	Pyrilamine maleate	84.6 ± 34.1		866	Lithium Chloride	99.9 ± 5.2
227	JX401	84.6 ± 8.9		867	Diclofenac sodium	100.0 ± 12.4
228	Cefmetazole sodium	84.6 ± 22.3		868	DL-Thiorphan	100.0 ± 12.5
229	Pindolol	84.7 ± 11.8		869	Quipazine, 6-nitro-, maleate	100.0 ± 4.7
230	Ziprasidone hydrochloride monohydrate	84.7 ± 3.4		870	Choline bromide	100.0 ± 6.6
231	Chlormethiazole hydrochloride	84.7 ± 21.7		871	L-Tryptophan	100.0 ± 3.7
232	N-Methyl-beta-carboline-3-carboxamide	84.9 ± 8.4		872	3,5-Dinitrocatechol	100.0 ± 11.8
233	4-DAMP methiodide	84.9 ± 19.8		873	SKF 96365	100.0 ± 16.7
234	Tyrphostin 23	84.9 ± 6.4		874	AFMK	100.0 ± 2.7
235	Loratadine	85.0 ± 1.8		875	Caffeic Acid	100.0 ± 4.2
236	SB 415286	85.0 ± 3.4		876	R(-)-Me5	100.1 ± 8.0
237	DNQX	85.0 ± 6.8		877	Leflunomide	100.1 ± 5.3
238	Spiperone hydrochloride	85.1 ± 18.3		878	Methotrexate hydrate	100.1 ± 8.9
239	5alpha-Pregnan-3alpha-ol-20-one	85.2 ± 19.4		879	Tranylcypromine hydrochloride	100.1 ± 11.2
240	13-cis-retinoic acid	85.2 ± 2.9		880	Ketanserin tartrate	100.1 ± 15.2
241	Cyclobenzaprine hydrochloride	85.3 ± 24.5		881	Avridine	100.2 ± 21.9
242	5'-Amino-5'-deoxyadenosine p-toluenesulfonate salt	85.5 ± 26.7		882	Neostigmine bromide	100.2 ± 23.4
243	5-Carboxamidotryptamine maleate	85.7 ± 14.5		883	NS 2028	100.2 ± 8.6
244	Tetracaine hydrochloride	85.8 ± 26.7		884	(S)-Propranolol hydrochloride	100.2 ± 8.9
245	p-Benzoquinone	85.9 ± 13.7		885	9-Amino-1,2,3,4-tetrahydroacridine hydrochloride	100.2 ± 15.6
246	(R,R)-cis-Diethyl tetrahydro-2,8-chrysenediol	85.9 ± 6.0		886	D-Serine	100.2 ± 11.0
247	Dequalinium chloride hydrate	86.1 ± 10.0		887	THIP hydrochloride	100.3 ± 3.4
248	Etoposide	86.36471682		888	PRE-084	100.3 ± 14.3
249	SMER28	86.4 ± 8.7		889	Lansoprazole	100.3 ± 13.0
250	N-Acetylprocainamide hydrochloride	86.4 ± 17.7		890	Resveratrol	100.3 ± 16.3
251	Danazol	86.4 ± 9.1		891	Ketoprofen	100.3 ± 5.0
252	Papaverine hydrochloride	86.6 ± 22.0		892	7,7-Dimethyl-(5Z,8Z)-eicosadienoic acid	100.3 ± 12.4
253	Dihydrocapsaicin	86.7 ± 9.6		893	2-(Methylthio)adenosine 5'-diphosphate trisodium salt hydrate	100.3 ± 5.3
254	(±)-3-(3,4-dihydroxyphenyl)-2-methyl-DL-alanine	86.8 ± 7.1		894	N-Acetyl-L-Cysteine	100.3 ± 0.2
255	Biperiden hydrochloride	86.9 ± 6.4		895	Pentamidine isethionate	100.3 ± 6.3
256	Cephalosporin C zinc salt	86.9 ± 28.2		896	Fulvestrant	100.4 ± 12.1

257	SC-51322	86.9 ± 5.7		897	(-)-alpha-Methylnorepinephrine	100.4 ± 6.1
258	SDZ-205,557 hydrochloride	87.0 ± 9.3		898	PPNDS tetrasodium	100.4 ± 12.5
259	Me-3,4-dephostatin	87.0 ± 11.0		899	L-Histidine hydrochloride	100.4 ± 6.4
260	CBIQ	87.1 ± 7.0		900	(±)-2,3-Dichloro-alpha-methylbenzylamine hydrochloride	100.4 ± 9.9
261	(±)-Norepinephrine (+)bitartrate	87.1 ± 8.1		901	6,7-ADTN hydrobromide	100.4 ± 8.1
262	1-(4-Hydroxybenzyl)imidazole-2-thiol	87.3 ± 21.6		902	Phenamil methanesulfonate	100.4 ± 3.8
263	A-77636 hydrochloride	87.3 ± 8.5		903	Granisetron hydrochloride	100.4 ± 14.5
264	Isoguvacine hydrochloride	87.4 ± 16.5		904	N-Acetyl-5-hydroxytryptamine	100.5 ± 7.6
265	Brefeldin A from Penicillium brefeldianum	87.5 ± 5.2		905	Opipramol dihydrochloride	100.5 ± 7.4
266	5-(N,N-hexamethylene)amiloride	87.5 ± 4.4		906	(-)-Epinephrine bitartrate	100.5 ± 6.3
267	CP-91149	87.6 ± 14.6		907	Linezolid	100.5 ± 2.1
268	Fenofibrate	87.6 ± 17.1		908	Praziquantel	100.5 ± 15.0
269	CGP-7930	87.6 ± 4.9		909	Ceftriaxone sodium	100.5 ± 11.9
270	XCT790	87.7 ± 9.0		910	Hydralazine hydrochloride	100.6 ± 8.6
271	beta-Estradiol	87.8 ± 13.4		911	(±)-AMT hydrochloride	100.6 ± 12.5
272	1-(2-Chlorophenyl)-1-(4-chlorophenyl)-2,2-dichloroethane	87.8 ± 23.1		912	L-655,708	100.6 ± 12.8
273	DCEBIO	87.8 ± 8.9		913	Uridine 5'-diphosphate sodium	100.6 ± 5.7
274	Isoliquiritigenin	88.0 ± 9.1		914	Yohimbine hydrochloride	100.6 ± 0.5
275	CP-380736	88.0 ± 4.4		915	Hydroquinone	100.7 ± 6.6
276	SB 204741	88.1 ± 14.4		916	E-64	100.7 ± 6.0
277	Sildenafil citrate salt	88.1 ± 8.0		917	Olprinone hydrochloride	100.7 ± 4.5
278	Edrophonium chloride	88.1 ± 20.0		918	L-azetidine-2-carboxylic acid	100.7 ± 4.1
279	Tetraethylthiuram disulfide	88.1 ± 9.2		919	N-Methyl-1-deoxynojirimycin	100.7 ± 20.9
280	Doxycycline hydrochloride	88. 2 ± 13.0		920	Hexamethonium dichloride	100.8 ± 9.2
281	Trequinsin hydrochloride	88.3 ± 40.0		921	BU224 hydrochloride	100.8 ± 7.2
282	1-Aminocyclopropanecarboxylic acid hydrochloride	88.3 ± 14.5		922	Z-L-Phe chloromethyl ketone	100.8 ± 7.1
283	CPCCOEt	88.3 ± 3.9		923	Carvedilol	100.8 ± 14.2
284	Ethosuximide	88.4 ± 5.5		924	Iofetamine hydrochloride	100.8 ± 7.1
285	R(+)-3PPP hydrochloride	88.4 ± 12.1		925	Vancomycin hydrochloride from Streptomyces orientalis	100.8 ± 8.6
286	Tyrphostin AG 698	88.4 ± 5.8		926	Cefsulodin sodium salt hydrate	100.8 ± 6.6
287	SIB 1893	88.4 ± 9.6		927	1,7-Dimethylxanthine	100.9 ± 19.4
288	Icilin	88.5 ± 5.8		928	Forskolin	100.9 ± 7.2
289	N,N-Dihexyl-2-(4-fluorophenyl)indole-3-acetamide	88.5 ± 10.2		929	BW 284c51	100.9 ± 3.8
290	Isonipecotic acid	88.5 ± 19.5		930	Rilmenidine hemifumarate	100.9 ± 6.4
291	Amiloride hydrochloride	88.5 ± 23.0		931	5,7-Dichlorokynurenic acid	100.9 ± 8.8
292	Mitoxantrone	88.5 ± 12.7		932	Rufinamide	100.9 ± 12.8
293	(-)-Scopolamine methyl bromide	88.5 ± 14.0		933	Aminoguanidine hydrochloride	100.9 ± 6.1
294	Pirenperone	88.6 ± 5.4		934	GR 46611	100.9 ± 13.0
295	Dofetilide	88.8 ± 14.7		935	Pregnenolone sulfate sodium	100.9 ± 23.1
296	Perphenazine	88.8 ± 23.6		936	Fluvoxamine maleate	100.9 ± 16.7
297	Nefiracetam	88.8 ± 23.8		937	3-n-Propylxanthine	101.0 ± 10.1

298	IC 261	88.8 ± 5.2		938	R-(-)-Desmethyldeprenyl hydrochloride	101.0 ± 11.7
299	Daidzein	88.9 ± 6.0		939	Cephalexin hydrate	101.0 ± 8.0
300	Pyrazinecarboxamide	88.9 ± 26.1		940	Propionylpromazine hydrochloride	101.0 ± 0.8
301	p-Aminoclonidine hydrochloride	89.0 ± 23.3		941	RX 821002 hydrochloride	101.0 ± 17.5
302	R(-)-2,10,11-Trihydroxy-N-propylnoraporphine hydrobromide	89.0 ± 14.4		942	Piroxicam	101.0 ± 12.2
303	(S)-MAP4 hydrochloride	89.0 ± 15.4		943	Oxybutynin Chloride	101.0 ± 12.8
304	Alloxazine	89.0 ± 4.9		944	Sertraline hydrochloride	101.1 ± 11.7
305	DPO-1	89.1 ± 3.8		945	L-Canavanine	101.1 ± 4.7
306	Orphenadrine hydrochloride	89.1 ± 18.3		946	Oxolinic acid	101.1 ± 12.4
307	Sulfaphenazole	89.1 ± 34.9		947	S(+)-Isoproterenol (+)-bitartrate	101.2 ± 2.4
308	Aminophylline ethylenediamine	89.2 ± 8.4		948	1-[2-(Trifluoromethyl)phenyl]imidazole	101.2 ± 9.4
309	Cantharidin	89.3 ± 25.6		949	N^G,N^G-Dimethylarginine hydrochloride	101.2 ± 10.7
310	Cysteamine hydrochloride	89.3 ± 6.0		950	P1,P4-Di(adenosine-5')tetraphosphate triammonium	101.2 ± 10.2
311	L-Glutamic acid, N-phthaloyl-	89.4 ± 32.8		951	Droperidol	101.2 ± 18.5
312	CI-976	89.4 ± 17.7		952	Phosphoramidon disodium	101.2 ± 7.1
313	2-Chloroadenosine triphosphate tetrasodium	89.5 ± 23.0		953	Tetradecylthioacetic acid	101.3 ± 12.1
314	(-)-Scopolamine methyl nitrate	89.5 ± 24.4		954	2,3-Butanedione	101.3 ± 15.0
315	Procainamide hydrochloride	89.6 ± 33.7		955	U-99194A maleate	101.3 ± 8.9
316	NBI 27914	89.7 ± 7.0		956	S-(-)-Carbidopa	101.3 ± 7.8
317	Carbamazepine	89.8 ± 14.0		957	Oxotremorine methiodide	101.3 ± 18.6
318	2-Chloro-2-deoxy-D-glucose	89.9 ± 5.0		958	Thio-NADP sodium	101.4 ± 10.4
319	Furegrelate sodium	89.9 ± 4.6		959	Chlormezanone	101.4 ± 10.9
320	AC-93253 iodide	89.8 ± 11.3		960	Acetohexamide	101.4 ± 13.1
321	3-Aminopropylphosphonic acid	90.0 ± 2.1		961	4-Imidazolemethanol hydrochloride	101.5 ± 8.2
322	1,4-Dideoxy-1,4-imino-D-arabinitol	90.0 ± 6.2		962	(±)-Brompheniramine maleate	101.5 ± 4.3
323	SKF 89626	90.14026278		963	L-2-aminoadipic acid	101.5 ± 9.7
324	Tyrphostin AG 538	90.2 ± 14.0		964	(E)-4-amino-2-butenoic acid	101.5 ± 12.6
325	Triprolidine hydrochloride	90.2 ± 0.5		965	Chlorzoxazone	101.5 ± 2.4
326	Tyrphostin AG 1478	90.3 ± 8.9		966	Diazoxide	101.5 ± 12.2
327	alpha-Lobeline hydrochloride	90.3 ± 6.6		967	Protriptyline hydrochloride	101.6 ± 7.3
328	Centrophenoxine hydrochloride	90.4 ± 28.8		968	Mizoribine	101.6 ± 4.8
329	Prochlorperazine dimaleate	90.4 ± 18.3		969	MDL 105,519	101.6 ± 19.9
330	Varenicline tartrate	90.5 ± 2.6		970	Niclosamide	101.6 ± 22.5
331	Metolazone	90.5 ± 39.1		971	5-Bromo-2'-deoxyuridine	101.6 ± 8.3
332	B-HT 933 dihydrochloride	90.5 ± 9.0		972	(6R)-5,6,7,8-Tetrahydro-L-biopterin hydrochloride	101.6 ± 17.7
333	Capsazepine	90.6 ± 11.8		973	Theobromine	101.7 ± 4.5
334	Fenoldopam bromide	90.6 ± 5.5		974	(±)-PPHT hydrochloride	101.7 ± 5.1
335	(±)-Synephrine	90.7 ± 28.9		975	Vanillic acid diethylamide	101.7 ± 2.4
336	PD-161570	90.7 ± 9.1		976	Minocycline hydrochloride	101.7 ± 17.9

337	1,10-Phenanthroline monohydrate	90.7 ± 13.0		977	Bepridil hydrochloride	101.8 ± 6.3
338	Acepromazine maleate	90.7 ± 4.5		978	Diphenhydramine hydrochloride	101.8 ± 15.9
339	(±)-2-Amino-5-phosphonopentanoic acid	90.8 ± 6.2		979	Tolbutamide	101.8 ± 7.4
340	WAY-100635 maleate	90.8 ± 17.8		980	Dipropyldopamine hydrobromide	101.8 ± 8.0
341	Atropine methyl nitrate	90.8 ± 8.8		981	Dobutamine hydrochloride	101.9 ± 15.9
342	Benzamidine hydrochloride	90.8 ± 8.3		982	(±)-Nipecotic acid	101.9 ± 5.9
343	Raloxifene hydrochloride	90.8 ± 14.0		983	Oxotremorine sesquifumarate salt	101.9 ± 13.3
344	SC-236	90.8 ± 15.2		984	Iodoacetamide	101.9 ± 8.2
345	Estrone	91.0 ± 8.5		985	ABT-418 hydrochloride	101.9 ± 4.7
346	Kainic acid	90.1 ± 18.1		986	L-Hyoscyamine	101.9 ± 12.00
347	Pyrocatechol	91.0 ± 7.4		987	Clonidine hydrochloride	101.9 ± 9.8
348	N-(4-Amino-2-chlorophenyl)phthalimide	91.1 ± 7.1		988	Terfenadine	101.9 ± 5.3
349	Aminopterin	91.1 ± 3.5		989	Ouabain	102.0 ± 3.5
350	5HPP-33	91.1 ± 9.2		990	Tocainide hydrochloride	102.0 ± 15.0
351	NAN-190 hydrobromide	91.1 ± 29.0		991	S-Methyl-L-thiocitrulline acetate	102.0 ± 13.0
352	L-732,138	91.1 ± 5.0		992	S-(+)-Fluoxetine hydrochloride	102.0 ± 9.9
353	R(+)-Butylindazone	91.2 ± 11.8		993	N-p-Tosyl-L-phenylalanine chloromethyl ketone	102.0 ± 13.7
354	ML-9	91.2 ± 7.1		994	Histamine dihydrochloride	102.0 ± 10.2
355	Molindone hydrochloride	91.2 ± 7.2		995	Daphnetin	102.0 ± 13.1
356	NS8593 hydrochloride	91.2 ± 11.5		996	Dextromethorphan hydrobromide monohydrate	102.0 ± 12.4
357	Tetrabenazine	91.3 ± 18.4		997	Metaproterenol hemisulfate	102.0 ± 5.4
358	Acetyl-beta-methylcholine chloride	91.4 ± 19.2		998	Topotecan hydrochloride hydrate	102.0 ± 7.8
359	(±)-Ibuprofen	91.4 ± 12.3		999	Isotharine mesylate	102.1 ± 6.6
360	Tyrphostin AG 494	91.5 ± 7.4		1000	(±)-Sulpiride	102.1 ± 6.1
361	Pheniramine maleate	91.5 ± 14.9		1001	U-101958 maleate	102.1 ± 9.0
362	S-Ethylisothiourea hydrobromide	91.5 ± 10.5		1002	UK 14,304	102.1 ± 10.4
363	2-(2-Aminoethyl)isothiourea dihydrobromide	91.5 ± 12.4		1003	Flunarizine dihydrochloride	102.1 ± 8.8
364	Amiodarone hydrochloride	91.5 ± 10.5		1004	CP-93129 dihydrochloride hydrate	102.1 ± 6.9
365	3-aminobenzamide	91.6 ± 2.5		1005	Ranitidine hydrochloride	102.1 ± 11.6
366	Methylergonovine maleate	91.6 ± 8.0		1006	Levetiracetam	102.2 ± 9.6
367	Azelaic acid	91.8 ± 5.2		1007	Phenylephrine hydrochloride	102.2 ± 12.7
368	Molsidomine	91.8 ± 17.4		1008	Spermidine trihydrochloride	102.2 ± 19.0
369	8-(4-Chlorophenylthio)-cAMP sodium	91.8 ± 2.6		1009	Carmustine	102.2 ± 1.0
370	1,3-Dimethyl-8-phenylxanthine	91.8 ± 4.8		1010	BW 723C86	102.2 ± 11.4
371	3-Aminopropionitrile fumarate	91.9 ± 6.3		1011	Atropine methyl bromide	102.2 ± 7.3
372	S-(4-Nitrobenzyl)-6-thioguanosine	91.9 ± 3.2		1012	9-cyclopentyladenine	102.2 ± 2.7
373	Mianserin hydrochloride	92.0 ± 7.7		1013	5-Hydroxyindolacetic acid	102.3 ± 8.2
374	Pyridostigmine bromide	92.0 ± 6.7		1014	CNS-1102	102.3 ± 11.4
375	SB-366791	92.0 ± 7.1		1015	Enoximone	102.3 ± 5.2
376	5-azacytidine	92.0 ± 10.7		1016	alpha,beta-Methylene adenosine 5'-triphosphate dilithium	102.3 ± 5.3

377	Cortisone 21-acetate	92.1 ± 5.7		1017	Alfuzosin hydrochloride	102.3 ± 4.9
378	ML-7	92.1 ± 9.6		1018	4-Methylpyrazole hydrochloride	102.3 ± 3.8
379	Chlorpromazine hydrochloride	92.2 ± 10.3		1019	Cinnarizine	102.3 ± 5.4
380	Adenosine	92.3 ± 7.9		1020	Ranolazine dihydrochloride	102.3 ± 13.7
381	Pifithrin-mu	92.3 ± 34.4		1021	CP-101537	102.3 ± 6.7
382	Methysergide maleate	92.3 ± 21.2		1022	8-Bromo-cGMP sodium	102.4 ± 7.4
383	Rotenone	92.4 ± 12.1		1023	DL-alpha-Methyl-p-tyrosine	102.4 ± 5.8
384	Stevioside	92.4 ± 13.9		1024	Lidocaine hydrochloride	102.4 ± 8.9
385	Acetazolamide	92.4 ± 4.0		1025	Dihydroouabain	102.4 ± 11.9
386	PD 168,077 maleate	92.4 ± 5.3		1026	Ciproxifan hydrochloride	102.4 ± 6.2
387	Dihydrokainic acid	92.4 ± 30.0		1027	Thioridazine hydrochloride	102.5 ± 9.6
388	BWB70C	92.4 ± 13.0		1028	Cytidine 5'-diphosphocholine sodium salt hydrate	102.5 ± 15.7
389	5-Fluoroindole-2-carboxylic acid	92.5 ± 8.9		1029	Acetylsalicylic acid	102.5 ± 9.1
390	Zimelidine dihydrochloride	92.5 ± 9.4		1030	Amoxapine	102.6 ± 1.7
391	Fiduxosin hydrochloride	92.6 ± 13.4		1031	Naltrexone hydrochloride	102.6 ± 6.5
392	L-alpha-Methyl DOPA	92.6 ± 21.6		1032	1,1-Dimethyl-4-phenyl-piperazinium iodide	102.6 ± 13.3
393	Salmeterol xinafoate	92.6 ± 8.3		1033	(S)-3,5-Dihydroxyphenylglycine	102.7 ± 20.4
394	A-315456	92.6 ± 5.2		1034	Emetine dihydrochloride hydrate	102.7 ± 9.1
395	Diphenyleneiodonium chloride	92.7 ± 5.6		1035	SQ 22536	102.7 ± 4.9
396	Aminobenztropine	92.7 ± 5.2		1036	Terbutaline hemisulfate	102.8 ± 10.1
397	2-Hydroxysaclofen	92.7 ± 0.2		1037	Tyrphostin AG 112	102.8 ± 4.7
398	Budesonide	92.7 ± 8.6		1038	Trifluperidol hydrochloride	102.8 ± 9.3
399	Glybenclamide	92.8 ± 16.7		1039	MHPG sulfate potassium	102.8 ± 6.8
400	GR 113808	92.8 ± 8.0		1040	BRL 54443 maleate	102.8 ± 6.2
401	6-Chloromelatonin	92.8 ± 24.3		1041	Pargyline hydrochloride	102.9 ± 12.8
402	GR 55562 dihydrobromide	92.8 ± 13.3		1042	Bromoacetyl alprenolol menthane	102.8 ± 4.8
403	Pilocarpine nitrate	92.9 ± 2.4		1043	Naratriptan hydrochloride	102.9 ± 17.6
404	TTNPB	92.9 ± 11.5		1044	Fluoxetine hydrochloride	102.9 ± 7.1
405	N6-Cyclohexyladenosine	92.9 ± 12.9		1045	1,3-Dipropyl-7-methylxanthine	102.9 ± 21.6
406	Amperozide hydrochloride	92.9 ± 0.6		1046	Buspirone hydrochloride	102.9 ± 6.0
407	Dopamine hydrochloride	93.0 ± 11.1		1047	Epibestatin hydrochloride	102.9 ± 13.0
408	ODQ	93.0 ± 5.7		1048	cis-4-Aminocrotonic acid	103.0 ± 10.1
409	Fusidic acid sodium	93.0 ± 9.1		1049	Indatraline hydrochloride	103.0 ± 8.6
410	Maprotiline hydrochloride	93.0 ± 35.9		1050	(±)-Octopamine hydrochloride	103.0 ± 1.6
411	Bezafibrate	93.0 ± 7.9		1051	SKF 86466	103.0 ± 12.5
412	LY-310,762 hydrochloride	93.0 ± 7.5		1052	Iproniazid phosphate	103.0 ± 5.6
413	beta-Lapachone	93.1 ± 5.0		1053	(-)-Sulpiride	103.0 ± 8.9
414	(±)-Ibotenic acid	93.1 ± 9.6		1054	Carbachol	103.1 ± 3.5
415	Tyrphostin A9	93.2 ± 14.5		1055	SR 2640	103.1 ± 17.1
416	PK 11195	93.2 ± 6.1		1056	DL-alpha-Difluoromethylornithine hydrochloride	103.2 ± 12.1
417	Cyclothiazide	93.2 ± 4.7		1057	ARL 67156 trisodium salt	103.2 ± 16.2
418	L-703,606 oxalate salt hydrate	93.3 ± 4.4		1058	(±)-PD 128,907 hydrochloride	103.2 ± 2.1
419	O-Phospho-L-serine	93.4 ± 14.4		1059	Ribavirin	103.3 ± 14.0

420	Ofloxacin	93.4 ± 20.0		1060	S(-)-Timolol maleate	103.3 ± 4.5
421	Quazinone	93.4 ± 5.4		1061	Phosphonoacetic acid	103.4 ± 12.3
422	ICI 63,137	93.4 ± 5.4		1062	4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride	103.5 ± 4.8
423	Suramin sodium salt	93.4 ± 9.0		1063	(±)-alpha-Methyl-4-carboxyphenylglycine	103.5 ± 10.0
424	6-Methoxy-1,2,3,4-tetrahydro-9H-pyrido[3,4b] indole	93.4 ± 1.1		1064	Moxisylyte hydrochloride	103.5 ± 2.3
425	(±)-Sotalol hydrochloride	93.5 ± 12.0		1065	YS-035 hydrochloride	103.5 ± 12.5
426	YM 976	93.5 ± 3.0		1066	SR-95531	103.5 ± 3.4
427	Meloxicam sodium	93.6 ± 6.8		1067	Methoctramine tetrahydrochloride	103.6 ± 10.6
428	SB 269970 hydrochloride	93.6 ± 22.9		1068	1-(m-Chlorophenyl)-biguanide hydrochloride	103.6 ± 13.0
429	4-Aminopyridine	93.6 ± 7.2		1069	(±)-Atenolol	103.6 ± 10.0
430	Meclofenamic acid sodium	93.6 ± 9.6		1070	2',3'-dideoxycytidine	103.6 ± 6.0
431	Lamotrigine	93.6 ± 9.2		1071	3-alpha,21-Dihydroxy-5-alpha-pregnan-20-one	103.6 ± 6.1
432	Retinoic acid	93.6 ± 14.4		1072	Nylidrin hydrochloride	103.7 ± 6.3
433	Beclomethasone	93.7 ± 1.6		1073	Dilazep hydrochloride	103.7 ± 12.7
434	LP 12 hydrochloride hydrate	93.7 ± 9.5		1074	Quinolinic acid	103.7 ± 10.0
435	TCPOBOP	93.7 ± 8.4		1075	Sulindac	103.7 ± 16.6
436	Nimodipine	83.7 ± 4.5		1076	R(-)-Isoproterenol (+)-bitartrate	103.8 ± 21.4
437	CB 1954	93.7 ± 2.8		1077	LP44	103.8 ± 8.7
438	Aurintricarboxylic acid	93.7 ± 12.0		1078	PHA-543613	103.8 ± 13.6
439	Ketorolac tris salt	93.7 ± 13.0		1079	Phenytoin sodium	103.8 ± 12.2
440	Colchicine	93.8 ± 5.1		1080	1-(5-Isoquinolinylsulfonyl)-2-methylpiperazine dihydrochloride	103.8 ± 5.9
441	3-deazaadenosine	93.8 ± 11.9		1081	Na-p-Tosyl-L-lysine chloromethyl ketone hydrochloride	103.9 ± 14.0
442	McN-A-343	93.8 ± 10.4		1082	Oxymetazoline hydrochloride	103.9 ± 10.0
443	Ketotifen fumarate	93.8 ± 4.0		1083	(+)-Pilocarpine hydrochloride	103.9 ± 1.7
444	BBMP	93.9 ± 9.9		1084	Tyrphostin 47	104.0 ± 4.7
445	CP-66713	93.9 ± 10.2		1085	5-hydroxydecanoic acid sodium	104.0 ± 7.6
446	Azathioprine	93.9 ± 5.0		1086	L-Canavanine sulfate	104.0 ± 8.6
447	Guanidinyl-naltrindole di-trifluoroacetate	93.9 ± 7.0		1087	(+)-Cyclazocine	104.1 ± 10.3
448	Fexofenadine hydrochloride	93.9 ± 3.6		1088	Cyclosporin A	104.1 ± 16.2
449	1-Phenyl-3-(2-thiazolyl)-2-thiourea	94.0 ± 11.6		1089	2,4-Diamino-6-pyrimidinone	104.1 ± 13.8
450	Dihydro-beta-erythroidine hydrobromide	94.0 ± 5.4		1090	Alprenolol hydrochloride	104.1 ± 15.8
451	Cimetidine	94.0 ± 4.0		1091	Nemadipine-A	104.1 ± 11.6
452	Cortisone	94.0 ± 5.3		1092	(-)-MK-801 hydrogen maleate	104.1 ± 10.2
453	JS-K	94.0 ± 5.4		1093	Tamoxifen citrate	104.2 ± 20.7
454	CGP-74514A hydrochloride	94.1 ± 9.9		1094	U-69593	104.2 ± 10.1
455	5-(N-Ethyl-N-isopropyl)amiloride	94.1 ± 7.3		1095	GR 127935 hydrochloride hydrate	104.2 ± 8.3
456	Metergoline	94.1 ± 8.5		1096	Trimipramine maleate	104.3 ± 7.1
457	6-Hydroxymelatonin	94.1 ± 9.8		1097	L-alpha-Methyl-p-tyrosine	104.3 ± 4.3
458	Chloroquine diphosphate	94.1 ± 9.1		1098	Pirenzepine dihydrochloride	104.3 ± 2.0

459	(±)-p-Aminoglutethimide	94.1 ± 8.7		1099	GR-89696 fumarate	104.3 ± 9.0
460	BMS-193885	94.2 ± 1.0		1100	2-methoxyestradiol	104.4 ± 10.2
461	Cefotaxime sodium	94.2 ± 6.2		1101	Desipramine hydrochloride	104.4 ± 9.0
462	Loperamide hydrochloride	94.2 ± 11.6		1102	Harmane	104.5 ± 8.0
463	Org 24598 lithium salt	94.3 ± 5.8		1103	Carbetapentane citrate	104.5 ± 6.7
464	N-(2-[4-(4-Chlorophenyl)piperazin-1-yl]ethyl)-3-methoxybenzamide	94.3 ± 6.0		1104	Hemicholinium-3	104.5 ± 15.9
465	Bumetanide	94.3 ± 2.5		1105	Caroverine hydrochloride	104.5 ± 21.3
466	BTCP hydrochloride	94.3 ± 14.0		1106	Procaine hydrochloride	104.5 ± 8.1
467	(+)-Catechin Hydrate	94.3 ± 10.9		1107	Phenylbutazone	104.5 ± 10.1
468	Trovafloxacin mesylate	94.4 ± 17.2		1108	Bay 11-7082	104.6 ± 21.3
469	Lumefantrine	94.4 ± 10.9		1109	Cephalothin sodium	104.6 ± 12.0
470	GW9508	94.4 ± 0.8		1110	Amantadine hydrochloride	104.6 ± 3.5
471	Clemastine fumarate	94.4 ± 2.5		1111	ICI 204,448 hydrochloride	104.6 ± 7.5
472	NBQX disodium	94.5 ± 3.8		1112	Trazodone hydrochloride	104.7 ± 11.3
473	Fluspirilene	94.5 ± 10.0		1113	2-Methyl-5-hydroxytryptamine maleate	104.7 ± 14.7
474	Spironolactone	94.5 ± 9.9		1114	17alpha-hydroxyprogesterone	104.8 ± 10.6
475	SB 216763	94.5 ± 10.2		1115	(+)-MK-801 hydrogen maleate	104.8 ± 18.5
476	2-Cyclooctyl-2-hydroxyethylamine hydrochloride	94.5 ± 6.6		1116	Famciclovir	104.8 ± 9.2
477	Lonidamine	94.6 ± 7.2		1117	Hexahydro-sila-difenidol hydrochloride, p-fluoro analog	104.8 ± 7.7
478	(±) trans-U-50488 methanesulfonate	94.6 ± 5.3		1118	Alaproclate hydrochloride	104.8 ± 8.1
479	Hypotaurine	94.6 ± 26.1		1119	SC 19220	105.0 ± 9.4
480	LY-294,002 hydrochloride	94.6 ± 11.0		1120	DM 235	105.1 ± 10.7
481	Amifostine	94.6 ± 2.0		1121	Pinacidil	105.1 ± 8.3
482	Isoxanthopterin	94.7 ± 15.0		1122	2,2'-Bipyridyl	105.2 ± 7.5
483	CNQX disodium	94.7 ± 15.8		1123	U-62066	105.2 ± 12.6
484	Tetraethylammonium chloride	94.7 ± 8.6		1124	Naphazoline hydrochloride	105.3 ± 13.5
485	Cambinol	94.8 ± 8.3		1125	4-Hydroxybenzhydrazide	105.3 ± 8.4
486	SID7969543	94.8 ± 24.3		1126	Linopirdine	105.3 ± 3.6
487	3,7-Dimethyl-1-propargylxanthine	94.8 ± 1.8		1127	PAC-1	105.3 ± 4.5
488	SR 59230A oxalate	94.8 ± 26.7		1128	Cirazoline hydrochloride	105.5 ± 7.7
489	Dantrolene sodium	94.8 ± 9.5		1129	Adenosine 3',5'-cyclic monophosphate	105.5 ± 3.5
490	DFB	94.8 ± 1.4		1130	L-745,870 hydrochloride	105.7 ± 4.1
491	SNC80	94.8 ± 9.3		1131	Rhodblock 6	105.7 ± 10.7
492	(±)-Muscarine chloride	94.8 ± 15.8		1132	Quinelorane dihydrochloride	105.7 ± 9.9
493	Paliperidone	94.9 ± 1.6		1133	Cilostazol	105.8 ± 41.7
494	NS-1619	94.9 ± 11.8		1134	Spermine tetrahydrochloride	105.8 ± 16.0
495	(±)-p-Chlorophenylalanine	94.9 ± 9.4		1135	ML 10302	105.8 ± 6.4
496	Tyrphostin 51	94.9 ± 2.9		1136	(-)-Eseroline fumarate	105.8 ± 9.3
497	4-Hydroxy-3-methoxyphenylacetic acid	94.9 ± 9.6		1137	Levallorphan tartrate	105.9 ± 7.3
498	Apomorphine hydrochloride hemihydrate	95.0 ± 6.4		1138	5,5-Dimethyl-1-pyrroline-N-oxide	106.0 ± 5.3
499	Betaine aldehyde chloride	95.0 ± 5.8		1139	Gemcitabine hydrochloride	106.0 ± 4.9

500	D-Cycloserine	94.5 ± 6.4		1140	(±)-Propranolol hydrochloride	106.0 ± 10.5
501	Ivermectin	94.5 ± 8.2		1141	Vincristine sulfate	106.1 ± 21.2
502	TMB-8 hydrochloride	95.0 ± 0.4		1142	Nortriptyline hydrochloride	106.1 ± 17.3
503	MHPG piperazine	95.1 ± 7.6		1143	Nalidixic acid sodium	106.1 ± 13.7
504	Idarubicin	95.1 ± 1.7		1144	PPADS	106.1 ± 11.3
505	Bromoacetylcholine bromide	95.1 ± 8.8		1145	Putrescine dihydrochloride	106.2 ± 11.7
506	S-(4-Nitrobenzyl)-6-thioinosine	95.1 ± 33.3		1146	Haloperidol	106.2 ± 9.5
507	SB 205384	95.1 ± 6.2		1147	Paromomycin sulfate	106.3 ± 7.8
508	TMPH hydrochloride	95.1 ± 13.7		1148	Pentolinium di[L(+)-tartrate]	106.3 ± 2.7
509	Tetraisopropyl pyrophosphoramide	952 ± 1.5		1149	Xylazine hydrochloride	106.3 ± 5.8
510	N-Phenylanthranilic acid	95.2 ± 6.4		1150	CGP 20712A methanesulfonate	106.4 ± 13.7
511	Nimustine hydrochloride	95.2 ± 13.6		1151	(+)-Quisqualic acid	106.4 ± 10.3
512	Cibenzoline succinate	95.2 ± 7.2		1152	Decamethonium dibromide	106.4 ± 21.3
513	Aconitine	95.2 ± 4.4		1153	H-8 dihydrochloride	106.4 ± 6.7
514	BP 897	95.2 ± 9.6		1154	Metoclopramide hydrochloride	106.5 ± 2.9
515	Efaroxan hydrochloride	95.2 ± 6.3		1155	(-)-Cotinine	106.5 ± 4.3
516	Bay 11-7085	95.2 ± 7.7		1156	L-Mimosine from Koa hoale seeds	106.5 ± 13.7
517	SC-51089 hydrate	95.2 ± 13.2		1157	Melatonin	106.6 ± 11.6
518	Benzamil hydrochloride	95.3 ± 5.6		1158	S(-)-UH-301 hydrochloride	106.6 ± 12.1
519	(±)-Isoproterenol hydrochloride	95.3 ± 10.4		1159	Ipratropium bromide	106.7 ± 26.2
520	(±)-Bay K 8644	95.3 ± 3.4		1160	Xylometazoline hydrochloride	106.7 ± 10.4
521	SKF-525A hydrochloride	95.3 ± 9.0		1161	Taurine	106.8 ± 10.4
522	Triamterene	95.3 ± 10.4		1162	Prilocaine hydrochloride	106.9 ± 9.7
523	1-(5-Isoquinolinylsulfonyl)-3-methylpiperazine dihydrochloride	95.4 ± 14.7		1163	Naltriben methanesulfonate	106.9 ± 8.0
524	4-Amino-1,8-naphthalimide	95.4 ± 12.1		1164	MG 624	106.9 ± 11.5
525	Pentylenetetrazole	95.4 ± 8.7		1165	Ancitabine hydrochloride	106.9 ± 5.2
526	5-fluoro-5'-deoxyuridine	95.4 ± 4.3		1166	Bisoprolol hemifumarate salt	106.9 ± 6.1
527	Ifenprodil tartrate	95.4 ± 10.6		1167	Telenzepine dihydrochloride	107.0 ± 15.2
528	Ruthenium red	95.4 ± 2.3		1168	Proglumide	107.1 ± 0.6
529	R(+)-IAA-94	95.5 ± 13.0		1169	L-Methionine sulfoximine	107.1 ± 5.4
530	(±)-Normetanephrine hydrochloride	95.5 ± 24.8		1170	Mevastatin	107.1 ± 10.0
531	D-609 potassium	95.5 ± 4.1		1171	Ro 8-4304	107.2 ± 11.5
532	A3 hydrochloride	95.5 ± 8.6		1172	Phaclofen	107.2 ± 18.9
533	5-(N,N-Dimethyl)amiloride hydrochloride	95.5 ± 2.7		1173	Tizanidine hydrochloride	107.2 ± 6.1
534	Propantheline bromide	95.5 ± 4.8		1174	O-Methylserotonin hydrochloride	107.2 ± 4.7
535	Ibandronate sodium	95.5 ± 10.4		1175	Stattic	107.3 ± 19.6
536	CX 546	95.5 ± 26.6		1176	Doxylamine succinate	107.4 ± 0.7
537	Tetramisole hydrochloride	95.5 ± 22.8		1177	Ropinirole hydrochloride	107.5 ± 15.4
538	GABA	95.6 ± 8.2		1178	Muscimol hydrobromide	107.5 ± 2.4
539	Cephradine	95.6 ± 1.1		1179	Mibefradil dihydrochloride	107.6 ± 26.6
540	Enalaprilat dihydrate	95.6 ± 2.1		1180	Hydroxyurea	107.6 ± 23.3
541	3-Tropanylindole-3-carboxylate methiodide	95.6 ± 4.9		1181	S(+)-Ibuprofen	107.7 ± 17.0

542	Cyproterone acetate	95.6 ± 2.1		1182	(2S,1'S,2'S)-2-(carboxycyclopropyl)glycine	107.7 ± 15.6
543	PAPP	95.6 ± 9.9		1183	Chlorothiazide	107.7 ± 8.4
544	A-68930 hydrochloride	95.6 ± 9.3		1184	VER-3323 hemifumarate salt	107.7 ± 7.9
545	Hydrochlorothiazide	95.6 ± 3.9		1185	6-Nitroso-1,2-benzopyrone	107.9 ± 12.7
546	DL-p-Chlorophenylalanine methyl ester hydrochloride	95.7 ± 6.7		1186	PD-166285 hydrate	108.1 ± 4.5
547	GBR-12909 dihydrochloride	95.7 ± 6.3		1187	Ethopropazine hydrochloride	108.3 ± 15.2
548	Acetylthiocholine chloride	95.7 ± 4.8		1188	1-Amino-1-cyclohexanecarboxylic acid hydrochloride	108.3 ± 1.1
549	Furosemide	95.7 ± 5.9		1189	S(-)-3PPP hydrochloride	108.4 ± 3.1
550	Tranilast	95.7 ± 1.4		1190	MRS 2179	108.4 ± 2.3
551	(±)-Epinephrine hydrochloride	95.7 ± 7.0		1191	Norcantharidin	108.5 ± 19.2
552	IMS2186	95.7 ± 10.2		1192	L-687,384 hydrochloride	108.6 ± 4.6
553	Benoxathian hydrochloride	95.8 ± 4.4		1193	Hydroxytacrine maleate	108.6 ± 13.0
554	3,4-Dichloroisocoumarin	95.8 ± 9.3		1194	N2-Ethyl-2'-deoxyguanosine	108.7 ± 6.3
555	Caffeine	95.8 ± 4.7		1195	(±)-Thalidomide	108.7 ± 11.6
556	Serotonin hydrochloride	95.8 ± 9.3		1196	Ro 20-1724	108.8 ± 2.1
557	6-Methyl-2-(phenylethynyl)pyridine hydrochloride	95.8 ± 6.2		1197	(-)-trans-(1S,2S)-U-50488 hydrochloride	108.9 ± 5.7
558	(+)-Hydrastine	95.9 ± 10.1		1198	alpha-Methyl-DL-tyrosine methyl ester hydrochloride	108.9 ± 8.2
559	L-Beta-threo-benzyl-aspartate	95.9 ± 9.2		1199	6-Aminohexanoic acid	109.0 ± 9.6
560	Aniracetam	95.9 ± 3.1		1200	Picotamide	109.0 ± 15.4
561	SKF 89976A hydrochloride	95.9 ± 25.1		1201	NG-Nitro-L-arginine methyl ester hydrochloride	109.0 ± 16.7
562	N,N,N',N'-Tetramethylazodicarboxamide	95.9 ± 2.2		1202	CR 2249	109.1 ± 19.6
563	Ro 25-6981 hydrochloride	95.9 ± 5.0		1203	Tolazamide	109.2 ± 9.0
564	Steviol	95.9 ± 19.9		1204	Prazosin hydrochloride	109.3 ± 23.7
565	Triamcinolone	95.9 ± 21.3		1205	Zaprinast	109.4 ± 6.4
566	5,5-Diphenylhydantoin	95.9 ± 12.2		1206	D(-)-2-Amino-5-phosphonopentanoic acid	109.4 ± 11.9
567	Arecaidine propargyl ester hydrobromide	96.0 ± 12.2		1207	N-Methyl-D-aspartic acid	109.5 ± 4.3
568	Benztropine mesylate	96.0 ± 5.8		1208	3-Tropanyl-indole-3-carboxylate hydrochloride	109.6 ± 9.2
569	Clorgyline hydrochloride	96.0 ± 4.2		1209	NNC 55-0396	109.8 ± 31.5
570	MDL 28170	96.0 ± 2.8		1210	2,6-Difluoro-4-[2-(phenylsulfonylamino)ethylthio]phenoxyacetamide	109.8 ± 13.1
571	Cyproheptadine hydrochloride	96.0 ± 5.5		1211	BNTX maleate salt hydrate	109.9 ± 5.7
572	Riluzole	96.0 ± 3.6		1212	Memantine hydrochloride	109.9 ± 10.5
573	(±)-2-Amino-3-phosphonopropionic acid	96.0 ± 3.9		1213	2,3-Butanedione monoxime	109.9 ± 13.3
574	Propofol	96.0 ± 3.0		1214	Piracetam	110.0 ± 4.3
575	8-Cyclopentyl-1,3-dimethylxanthine	96.1 ± 9.6		1215	Doxepin hydrochloride	110.0 ± 13.2
576	Acetamide	96.1 ± 7.6		1216	Chelidamic acid	110.2 ± 8.8
577	Arcaine sulfate	96.1 ± 6.4		1217	BIA 2-093	110.6 ± 5.0
578	Nitrendipine	96.1 ± 17.4		1218	Cortexolone	110.6 ± 19.2
579	R(-)-Propylnorapomorphine	96.1 ± 7.1		1219	T0070907	110.9 ± 6.0

	hydrochloride					
580	Voriconazole	96.1 ± 10.4		1220	R-(+)-7-Hydroxy-DPAT hydrobromide	110.9 ± 7.0
581	Primidone	96.1 ± 4.9		1221	Naltrindole hydrochloride	111.0 ± 9.1
582	CP-135807	96.1 ± 6.8		1222	(±)-Taxifolin	111.1 ± 7.5
583	N-Methyldopamine hydrochloride	96.1 ± 15.9		1223	Propafenone hydrochloride	111.3 ± 10.1
584	(±)-AMPA hydrobromide	96.2 ± 10.6		1224	3-Nitropropionic acid	111.3 ± 13.2
585	JL-18	96.2 ± 7.5		1225	Methapyrilene hydrochloride	111.4 ± 10.2
586	Lidocaine N-ethyl bromide quaternary salt	96.2 ± 12.5		1226	Sobuzoxane	111.5 ± 7.6
587	Phenylbenzene-omega-phosphono-alpha-amino acid	96.2 ± 4.8		1227	Quinidine sulfate	111.6 ± 16.6
588	1-Phenylbiguanide	96.2 ± 2.3		1228	N-omega-Methyl-5-hydroxytryptamine oxalate salt	111.6 ± 33.8
589	R(+)-SCH-23390 hydrochloride	96.2 ± 12.3		1229	CP-31398 dihydrochloride hydrate	111.8 ± 1.4
590	Ganciclovir	96.3 ± 6.5		1230	NADPH tetrasodium	112.0 ± 13.1
591	NSC 95397	96.3 ± 15.0		1231	S-Methylisothiourea hemisulfate	112.2 ± 21.2
592	Glipizide	96.3 ± 1.2		1232	Methiothepin mesylate	112.2 ± 9.4
593	Cefazolin sodium	96.3 ± 4.6		1233	NG-Monomethyl-L-arginine acetate	112.2 ± 7.1
594	Nicardipine hydrochloride	96.3 ± 24.2		1234	BRL 52537 hydrochloride	112.4 ± 4.2
595	Droxinostat	96.3 ± 1.9		1235	Spiroxatrine	112.7 ± 3.0
596	Genipin	96.3 ± 9.0		1236	Idazoxan hydrochloride	112.7 ± 7.0
597	L-N6-(1-Iminoethyl)lysine hydrochloride	96.4 ± 1.7		1237	Metolazone	112.9 ± 23.7
598	Sorbinil	96.4 ± 14.0		1238	(±)-Vesamicol hydrochloride	112.9 ± 7.4
599	Pirfenidone	96.4 ± 15.8		1239	(-)-Tetramisole hydrochloride	112.9 ± 6.8
600	Sodium Oxamate	96.5 ± 14.4		1240	L-Glutamic acid hydrochloride	113.0 ± 19.3
601	NO-711 hydrochloride	95.5 ± 5.2		1241	Niflumic acid	113.3 ± 19.3
602	Rauwolscine hydrochloride	96.5 ± 8.7		1242	3-Morpholinosydnonimine hydrochloride	114.0 ± 15.4
603	cis-(Z)-Flupenthixol dihydrochloride	96.5 ± 4.1		1243	(±)-Verapamil hydrochloride	114.0 ± 7.1
604	3-Amino-1-propanesulfonic acid sodium	96.5 ± 4.6		1244	Nimesulide	114.0 ± 22.1
605	SC-58125	96.5 ± 13.6		1245	(±)-CGP-12177A hydrochloride	114.1 ± 10.3
606	Sivelestat sodium salt hydrate	96.6 ± 6.4		1246	Naloxone hydrochloride	114.3 ± 14.2
607	Epinastine hydrochloride	96.6 ± 14.2		1247	GW9662	114.5 ± 11.5
608	Cystamine dihydrochloride	96.6 ± 12.8		1248	Noscapine hydrchloride	114.6 ± 20.4
609	Chlorprothixene hydrochloride	96.6 ± 7.7		1249	1-(2-Methoxyphenyl)piperazine hydrochloride	114.8 ± 8.3
610	(±)-HA-966	96.6 ± 6.4		1250	alpha-Methyl-5-hydroxytryptamine maleate	115.5 ± 12.2
611	ATPA	96.6 ± 6.2		1251	ZM 39923 hydrochloride	115.5 ± 23.0
612	SD-169	96.7 ± 5.5		1252	1-Methylimidazole	115.7 ± 16.4
613	Minoxidil	96.7 ± 6.2		1253	(-)-Perillic acid	115.8 ± 16.6
614	Promethazine hydrochloride	96.7 ± 11.4		1254	Quinine sulfate	116.2 ± 16.5
615	Imipenem monohydrate	96.8 ± 2.2		1255	p-MPPF dihydrochloride	117.8 ± 19.4
616	Piperaquine tetraphosphate tetrahydrate	96.8 ± 7.2		1256	SKF 95282 dimaleate	120.1 ± 40.0
617	3-Iodo-L-tyrosine	96.8 ± 8.8		1257	5-Nitro-2-(3-phenylpropylamino)benzoic acid	120.8 ± 13.5

618	Aprindine hydrochloride	96.8 ± 13.3		1258	NG-Nitro-L-arginine	120.0 ± 6.8
619	S-(-)-Eticlopride hydrochloride	96.8 ± 1.1		1259	MDL 26,630 trihydrochloride	124.5 ± 16.5
620	(+)-Chlorpheniramine maleate	96.8 ± 6.2		1260	7-Nitroindazole	126.0 ± 15.1
621	Astaxanthin	96.8 ± 4.6		1261	S-Nitroso-N-acetylpenicillamine	127.5 ± 10.1
622	Ara-G hydrate	96.8 ± 6.9		1262	Methoxamine hydrochloride	132.3 ± 57.0
623	Picrotoxin	96.8 ± 4.1		1263	JFD00244	113.9 ± 48.7
624	Nialamide	96.8 ± 7.4		1264	(±)-Butaclamol hydrochloride	ND
625	Lomefloxacin hydrochloride	96.9 ± 10.1		1265	(±)-Quinpirole dihydrochloride	ND
626	Eletriptan hydrobromide	96.9 ± 5.3		1266	Aurothioglucose	ND
627	nor-Binaltorphimine dihydrochloride	96.9 ± 14.7		1267	Bethanechol chloride	ND
628	Bicalutamide (CDX)	96.9 ± 5.3		1268	DL-Buthionine-[S,R]-sulfoximine	ND
629	Cinoxacin	96.9 ± 2.7		1269	GBR-12935 dihydrochloride	ND
630	(±)-gamma-Vinyl GABA	96.9 ± 7.5		1270	Guanfacine hydrochloride	ND
631	3-Tropanyl-3,5-dichlorobenzoate	96.9 ± 4.1		1271	GW5074	ND
632	DL-threo-beta-hydroxyaspartic acid	97.0 ± 9.5		1272	L-162,313	ND
633	3,4-Dihydroxyphenylacetic acid	97.0 ± 9.9		1273	m-Iodobenzylguanidine hemisulfate	ND
634	Olomoucine	97.0 ± 10.1		1274	MK-912	ND
635	Milrinone	97.0 ± 4.9		1275	PD-407824	ND
636	Antozoline hydrochloride	97.0 ± 6.0		1276	Progesterone	ND
637	S15535	97.0 ± 1.3		1277	Propentofylline	ND
638	Urapidil hydrochloride	97.1 ± 1.5		1278	Protoporphyrin IX disodium	ND
639	Trifluoperazine dihydrochloride	97.1 ± 25.1				
640	L-Arginine	97.1 ± 5.5				