Introduction

Acetylcholine receptors, also known as Muscarinic receptors, are classified into 5 different groups, M1, M2, M3, M4 and M5. In order to investigate the pharmacological behavior of various agonists or antagonists, Cisbio has developed a comprehensive platform including ligand binding assays for determining the affinity for the receptors, and functional assays (cAMP, IP-One and phospho-Erk) to assess compounds' efficacy. For the purpose of the present study, Muscarinic M1 receptor, a key target in pulmonary diseases (asthma and COPD), metabolic, cardiovascular and CNS pathologies, was selected and assessed for both binding (Tag-1™) and function studies (IP-One) with different Cisbio liquid handling devices for small and high throughput. Both HTRF assays were performed with the semi-automatic pipettor CyBi®-SELM 96/25 µl which is a reliable tool to handle low sample numbers.

Material & methods

Serial dilution plate preparation

Compound serial dilution plates were prepared in parallel with the CyBi®-SELM 96/25 µl or with the CyBio robotic workstation (1) as described below. The CyBi®-SELM 96/25 µl was equipped with either a 96 tip tray for buffer and reagent transfers or with an 8-channel magazine for serial dilution.

• Transfer of 3 x 20 µl buffer from a 12 column robotic reservoir to the serial dilution plate. Conning 500 µl 96 well v-bottom plate (W-96-4500-C) column 3 remained empty.

• Manual transfer of 80 µl compound in column 3 of the serial dilution plate.

• 9 x 14 serial dilution steps with 20 µl transfers and 3 mixing cycles (from the last dilution 20 µl were discarded).

• 3 x 20 µl transfer of buffer or antagonist from an 8 line robotic reservoir to the serial dilution plate according to the plate layout.

• 5 or 7 µl transfer from the serial dilution plate to the Greiner 384vs white assay plate (#784080), in quadruplicates according to the assay description (Fig. 1).

Assay protocol

According to the assay description, the different reagents were dispensed as follows:

• The cells were aspirated from a reservoir with 96-bottom profile and homogenized by repeated resuspension prior the transfer in the assay plate.

• The red antagonist or the IP1-D2 was transferred from a 12 column robotic reservoir.

• The anti-IP1 cryptate was transferred from a 96 reservoir.

Binding assay results

The fluorescent ligand used for the binding assay was a Tag-labile muscarinic red antagonist (derived Telenzepin labeled with a red HTRF fluorescent probe). Because M1 is a Gq coupled receptor, HTRF IP-One kit was used to assess the production of inositol monophosphate as a readout of the PLC pathway activation. The assay is based on a competitive immunassay using a monoclonal cryptate labeled anti-IP1 antibody and D2 labeled IP1.

Functional assay results

The CyBi®-SELM is a semi-automatic pipettor for precise and reliable liquid handling in microplates (Fig. 2). It operates via a touch screen where all liquid handling parameters and heights can be adjusted and methods can be stored. The pipetting technology of the CyBi®-SELM 96/25 µl head is exactly the same as in the CyBi®-Well vario 96/25 µl head, which is integrated in the CyBio robotic workstation (Fig. 3).

Serial dilutions can be performed with the CyBi®-SELM 96/25 µl using the corresponding 8-channel magazine. The microplate adapter 384 supports the reliable liquid transfer in 384 well plates.

Conclusions

The present study illustrates how different M1-related binding and functional HTRF assays were implemented and run successfully using the CyBi®-SELM 96/25 µl and a completely integrated CyBio robotic workstation. With both liquid handling systems, comparable pharmacological results were achieved: it could be demonstrated that:

• The associations of HTRF assays and CyBio robotic systems are ideally suited for smooth transfer from assay development to screening.

• Cisbio HTRF assays and CyBio liquid handling solutions form a perfect match to offer a straightforward platform for small throughput analysis as well as for drug discovery at the HTS level.

References

