

7 September 2020

The Manager Companies
ASX Limited
20 Bridge Street
SYDNEY NSW 2000

(3 pages by email)

Dear Madam,

BIOTRON COMPOUNDS SHOW ACTIVITY AGAINST SARS-CoV-2 VIRUS IN PRELIMINARY ASSAYS

The Directors are pleased to announce the conclusion of the first stage of screening selected Biotron compounds against SARS-CoV-2, the coronavirus that causes COVID-19.

Several compounds have been shown in laboratory cell-culture studies to have antiviral activity against SARS-CoV-2. The assays were run under contract by an Australian NATA accredited clinical trial speciality laboratory, 360biolabs, based in Melbourne, Victoria.

Biotron's Managing Director, Michelle Miller, said "The results to date are encouraging. There is a need for new ways to treat this disease, and Biotron believes that these results open up a promising new therapeutic pathway. The results underscore the versatility of Biotron's approach to designing and developing drugs to target serious virus infections".

Forty-seven Biotron compounds have been screened in an industry standard cytopathic effect (CPE) cell-culture assay. Compounds that demonstrated promising activity in that first assay then underwent confirmatory testing in a second anti-SARS-CoV-2 assay.

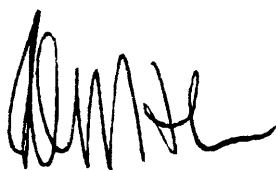
This screening program has successfully identified a subset of 15 compounds that had activity against SARS-CoV-2.

Details of methods used and criteria for scoring activity are set out below in an Addendum to this announcement.

The Company's focus will now be on building on this preliminary stage screening program to include a new series of recently designed and synthesised compounds. Screening of these additional new compounds is expected to conclude before the end of 2020. It is hoped that within these new compounds there will be potent, druggable compound(s) that can be progressed to testing in animal models of COVID-19 disease and ultimately clinical trials.

This announcement has been approved by the Board of Biotron Limited.

Yours sincerely



Peter J. Nightingale
Company Secretary

ADDENDUM

In vitro susceptibility of viruses to an antiviral agent is usually assessed using a quantitative assay to measure virus replication in the presence of increasing concentrations of the product compared to replication in the absence of the product. The effective concentration is the concentration of product at which virus replication is inhibited by 50 percent (EC₅₀ for cell-based assays).

The primary screening assay utilised in this compound screening program was a cytopathic CPE inhibition assay against SARS-CoV-2 hCoV-19/Australia/VIC01/2020 obtained from Melbourne's Peter Doherty Institute for Infection and Immunity, using the Vero E6 cell line. Compounds were diluted in a 3-fold, 9-point dilution series in a 96-well plate. SARS-CoV-2 was then added and plates incubated for four days. Additionally, compounds were assessed for cellular toxicity in Vero E6 cells.

At the conclusion of 4 days incubation, viable cells were measured via MTT staining, representing cell protection from virus infection. EC₅₀ and cytotoxic concentration 50 (CC₅₀) were calculated by linear regression. The endpoint for the CPE assay was cell protection from virus infection.

The secondary assay was a yield reduction (YR) assay, in which 3 concentrations of compound were added to wells of a 24-well plate, preceded with Vero E6 cells. SARS-CoV-2 virus was added and plates incubated for 48 hours. After that time, virus titre was quantified as a median tissue culture infective dose (TCID₅₀) value. TCID₅₀ is a measure of virus titre and it represents the titre of a virus that produces infection in 50% of the tissue culture samples exposed. The endpoint for the YR assay was reduction in yield of infectious virus released from cells after 48 hours. A reduction of virus titre indicates a compounds effect on the production of infectious virus. Compounds with >20% reduction in virus titre of virus in this assay were considered as having confirmed activity.

Forty seven Biotron compounds were selected for screening based on past observed activity against a range of different animal and human coronaviruses. All 47 compounds were tested in the primary screening CPE assay. The criteria for advancement into the secondary YR assay was greater or equal to 30% inhibition of virus, at any concentration and >70% viability of cells, indicating no cytotoxicity at the concentration where virus inhibition was observed. Seventeen of the 47 compounds showed sufficient activity fell into this category and progressed to testing in the YR assay. The antiviral activity of 15/17 compounds was confirmed in this assay.

The number of replicates (3) used in the secondary YR assay is too small to determine statistical significance (p-value) and is not a standard calculation used in early development and antiviral screening.

This screening study is the first round of screening of Biotron's extensive compound library. Data from this study will inform the selection of additional compounds for the next round of screening and enable the development of structure-activity relationships (SAR) which drive the development of new therapeutic agents.

About Biotron

Biotron Limited is engaged in the research, development, and commercialisation of drugs targeting significant viral diseases with unmet medical need. The Company has BIT225 in clinical development for HIV-1 and a promising preclinical program for HBV. In addition, Biotron has several earlier stage programs designing drugs that target a class of virus protein known as viroporins which have a key role in the virus life cycle of a very broad range of viruses, many of which have caused worldwide health issues such as Coronavirus, Dengue, Ebola, Middle East Respiratory virus, Influenza and Zika viruses.

Enquiries

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