Screening Anticancer Activities of *Spirulina platensis* Extracts on Various Cancer Cell Lines

Summary

*Spirulina platensis* crude extract and C–phycocyanin (C–PC) were screened in order to discover anticancer agents from natural sources in 13 cancer cell lines together with two non-cancerogenic cell lines in this study. To determine the growth inhibition and colony formation efficiencies of the cells treated with crude extract and C–PC, MTT and soft agar assays were performed. Due to the complex nature of crude extract, it generally exhibited higher cytotoxic activity than C–PC. Crude extract were effective in SW480, Raji, NCI–H82, cell lines with IC$_{50}$ values ranging between 0.006 and 0.030μg/mL. C–PC had greater activity both in MCF7 and Raji cell lines with IC$_{50}$ value of 1.7μg/mL. Among the cancer cell lines, NCI–H82, Raji, SW480, and MCF7 turned out to be the most susceptible cell lines inhibited by crude extract and C–PC. In this study, *S. platensis* crude extract generally exhibited higher activity than C–PC, for this reason only the colony formation capacities of the cell lines treated with IC$_{50}$ concentrations of crude extract were determined. It is found that crude extract inhibits the colony formation in leukemia, lymphoma and neuroblastoma cell lines.

Keywords: *Spirulina platensis*, crude extract, C–phycocyanin, anti-cancer activities, soft agar colony formation.
Özet
Doğal kaynaklardan anti-kanser ajanların belirlenmesi kapsamında, Spirulina platensis ham ekstraktı ve fikosiyanın 13 kanser hücre hattında ve iki normal hücre hattında incelemenmiştir. Büyüme inhibisyonu ve koloni formasyon özelliklerini baskılamıştır. MTT ve çift tabakalı yumuşak agar testleri ile belirlenmiştir. Ham ekstrakt kompleks doğasından dolayı, fikosiyaninden daha yüksek sitotoksik aktivite göstermiştir. Ham ekstrakt, SW480, Raji ve NCI–H82 hücre hatlarında 0.006 ve 0.030μg/mL arasında bir inhibisyon konsantrasyonuna sahiptir, fikosiyanın ise MCF7 ve Raji hücre hatlarında 1.7μg/mL değerinde bir inhibisyon konsantrasyonuna sahiptir. Bütün kanser hücre hatları arasında, NCI–H82, Raji, SW480, ve MCF7 ham ekstrakt ve fikosiyaninden en fazla etkilenen hücre hatları olmuştur. Bu çalışmada, ham ekstrakt fikosiyanından daha yüksek aktivite gösterdiğini için çift tabakalı yumuşak agar testi sadece ham ekstrakt ile muamele edilmiş hücrelerde yapılmış ve ham ekstraktın lösemi, lenfoma ve nöroblastoma hücrelerinde koloni oluşumunu inhibe ettiği belirlenmiştir.

Anahtar Sözcükler: Spirulina platensis, ham ekstrakt, fikosiyanın, anti–kanser aktivite, çift tabakalı yumuşak agar üzerinde koloni formasyonu.
INTRODUCTION

Cancer is one of the daunting diseases which jeopardize human life in our current world. Many searches have been made and are still made, aiming at the etiology and treatment of this deadly disease that is also known as abnormal cell proliferation in the organism. With all thrusts made in recent years, scientific world has been exerting tremendous efforts in order to be able to eliminate negative repercussions of this disease on life. That is why, most part of the studies carried out on bioactive plants have been focused on cancer treatment. In recent years, traditional medical practices have become a topic which gains universal importance. Possibilities of utilizing many plant species and marine natural products which are thought to have “medicinal herb” property in medical practices have been studied scientifically too. Wide interest in “green treatment” and the success of antitumorigenic pharmaceuticals of vegetable origin like taxol, etoposide, vincristine, topotecan designate that this inclination will continue 1-7.

As part of our ongoing search for researching anticancer agents from natural sources, we analyzed Spirulina (Arthospira). Spirulina is a microscopic filamentous alga that is rich in proteins, vitamins, essential amino acids, minerals and essential fatty acids like γ-linolenic acid (GLA). It is produced commercially and sold as a food supplement in health food stores around the world. Just recently, the interest in Spirulina used to be mainly in its nutritive value. Currently, however, numerous people are looking for possible therapeutic effects of Spirulina. Many pre-clinical studies and a few clinical studies suggest several therapeutic effects ranging from reduction of cholesterol and cancer to enhancing the immune system, increasing intestinal lactobacilli, reducing nephrotoxicity by heavy metals and drugs and radiation protection 8, 9. Recently, much more attention has been paid to the study of the therapeutic effects of Spirulina. In addition to its effectiveness in reducing hyperlipidemia, diabetes and high blood pressure in human beings and animals, anti-viral effects of orally given S. platensis involving immune functions have also been reported 10. C–PC is one of the major biliproteins in S. platensis, being composed of two different subunits: α– and β–subunits, and functioning as a light–harvesting protein in cyanobacteria. C–PC, a characteristic photosynthesis pigment protein and an antioxidant in Spirulina, has been known to increase the growth of a human myeloid cell line, RPMI 8226. Recently, Liu et al. (11) reported that C–PC inhibited growth of human leukemia K562 cells and enhanced the arrest of the cell growth at G1 phase, suggesting enhancement of differentiation of the cells 10, 11. Because C–PC has characteristic stability and solubility in aqueous solution, in addition to its high content in cyanobacteria (up to 15% of proteins in S. platensis) and non–toxicity, it has been used in many research applications. The C–PC from cyanobacteria has similar features, including a hepatoprotective effect and anti–inflammatory properties. Daily ingestion of a small dosage of C–PC could maintain or accelerate lymphocytic functions to prevent malignancy such as cancer or to inhibit its growth or recurrence. C–PC enhanced the laser cytotoxic effect for cancer laser therapy after tumor cells had been cultured with 250 mg L–1 C–PC 11. The purpose of our study was to screen and compare anticarcinogenic activity of crude aqueous S. platensis extract and C–PC isolated from PSE, against fourteen cancer cell lines including Caco–2 (human epithelial colorectal adenocarcinoma), SW–48 (human colon adenocarcinoma), HT–29 (human colon adenocarcinoma), HBL–100 (human breast carcinoma), MCF7(human breast adenocarcinoma), HeLa–S3 (human cervical carcinoma), HepG2 (human hepatocellular liver carcinoma), NCI–H–82 (small cell lung cancer), HL–60 (human promyelocytic leukemia), K–562 (human erythromyeloblastoid...
leukemia), Raji (human Burkitt’s lymphoma), DOHH (human B cell lymphoma), CA–46 (human Burkitt lymphoma), Hep3B (human hepatocellular carcinoma); one animal cancer cell line Neuro–2A (murin neuroblastoma) and two normal cell lines L929 (mouse connective tissue fibroblast) and MDCK (Madin–Darby canine kidney).

**MATERIAL and METHODS**

**Production of Spirulina platensis**

The origin of the strain of *S. platensis* used in this study goes back to Peru Parachas. The pure culture was obtained from Ege University microalgae culture collection–Ref. No: EGE–MACC–31. The cyanobacteria were produced in raceway ponds agitated by a paddle wheel under sunlight in Zarrouk’s medium and harvested by filtration method. The bioreactor used for growing the inoculum culture of pond production was illuminated by 8 florescent lamps, two on the top, two at the bottom and four at both sides of the bioreactor. When the culture reached to 140 L in volume, it was transferred to the open raceway ponds located in the plant. The cyanobacteria were harvested by filtration lay out on the drying trays and were dried in an aerated dryer.

**Crude Extract Preparation**

The crude extract of the cells was prepared by freezing–thawing method. Filtrated cells produced in the raceway ponds were frozen and thawed, in order to disrupt their membrane, and afterwards it was extracted by hot water. The extract frozen at 24°C was thawed quickly in phosphate buffer saline (PBS, pH 7) at 4°C. Three cycles of freezing–thawing were performed and the process was followed by centrifugation (9000 rpm, 30 min, Hettich Rotina 35 R Germany), in order to precipitate the solid particles. The aliquot was sterilized by filtration gradually (0.8 µm, 0.45 µm and 0.22 µm, Sartorius Germany). The supernatant was aliquoted into 1.5 ml of sterile bottles, lyophilized (Christ 1.8 B–Plus, Germany) and kept at –20°C until used.

**Extraction and Purification of C–PC**

Extraction and purification processes were carried out according to the method laid out by Zhang et. al. (14) 10 g of lyophilized *S. platensis* powder was suspended in 1000 ml of 1.5% CaCl₂·2H₂O (w/v) aqueous solution. The suspension was agitated over night at 4°C and centrifuged at 9000 rpm for 45 min (Hettich Rotina 35 R Germany). The precipitate was removed and then left for saturation with 40% ammonium sulphate overnight at 4°C. The precipitate was removed by centrifugation for 20 min at 13 000 rpm. The same process was repeated with 70% ammonium sulphate saturation step. The final precipitate was dissolved in 20 ml of 0.05 M PBS (pH 7) and dialyzed against the same buffer solution overnight. C–PC containing dialyzed samples was chromatographed through DEAE–sepharose CL–6B (Amersham Biosciences, Switzerland) columns (2.5 X 15 cm). The C–PC containing samples were eluted with the ion concentration gradient (0–0.25 M NaCl, 1ml/min). The salt was removed by washing with pH 7 0.002 M sodium phosphate buffer solution (0.5ml/min) in the Sephadex–G 100 (Sigma– Aldrich, Germany). A₆₂₀/A₃₈₀ ratios were considered for choosing the fractions; the ratios above 4 were collected in 1 ml of tubes.

The UV visible spectrums of the C–PC samples were measured with the Varian Carry 300 Bio UV/Vis spectrophotometer (Australia). 620 nm is the maximum wavelength that C–PC can absorb (15). A₆₂₀/A₃₈₀ ratios were evaluated for the purity of the
fractions; where by resulting values of 4 and higher were considered as pure (5) and were collected. After lyophilization, the samples were kept at –20°C.

Dry Weight Determination

The “P1–P3–P2” equation was used to calculate the dry weight of crude S. platensis extract in these experiments, where P1 refers to dry weight (mg), P2 refers to the weight of the bottles and P3 is the weight of the bottle with lyophilized extract.

Cell Culture

The PSE and C–PC were dissolved in DMSO a final concentration of 1000X. Subsequent dilutions were made in culture medium. The same proportion of DMSO/culture medium was added to the controls. The final DMSO content was never above 0.1%. MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] was purchased from Sigma Chemical Company (St. Louis, MO). The cell lines [NCI–H82 (human small cell lung cancer), K–562 (human erythroleukemia), Raji (human Burkitt’s lymphoma), DOHH–2 (human B cell lymphoma), CA–46 (human Burkitt’s lymphoma), SW480 (human colon adenocarcinoma), HeLa S3 (human cervical carcinoma), Caco–2 (human epithelial colorectal adenocarcinoma), Hep G2 (human hepatocellular liver carcinoma), Hep 3B (human hepatocellular), MCF7 (human breast adenocarcinoma), HT–29 (human colon adenocarcinoma) and Neuro–2A (murine neuroblastoma cell line), MDCK (Madin–Darby Canine Kidney) and L–929 (mouse connective tissue fibroblast) and Caco–2 (human epithelial colorectal adenocarcinoma) were obtained from American Cell Culture Collection (ATCC Manassas, VA, USA). The cells were cultured in any one of RPMI 1640 or DMEM–Ham’s F12 depending on the requirement of the cell lines supplemented with 10% fetal bovine serum (FBS), L–glutamine (2mmol/L), penicillin (100U/mL) and streptomycin (100µg/mL) and were maintained at 37°C with 5% CO2 in a humidified atmosphere.

Cytotoxic Activity Assay

The cytotoxic activity of S. platensis crude extract and C–PC on 12 human cancer cell lines and two normal cell lines was measured by MTT assay. Cells in the exponential growth phase were placed in 96–well plates to make 6000 cells/wells. After 24 h of incubation and adding ten fold dilutions of the sample solutions in the growth medium ranging from 0.001 to 100µg/mL, respectively, in each well, they were incubated for 48 h. Cell proliferation was determined by adding 0.5mg/mL S. platensis crude extract per well, prepared as a sterile stock solution of 5mg/mL in Dulbecco’s phosphate–buffered saline (DPBS, Gibco, USA), diluted 1:10 with medium prior to use. Medium was removed 3 h later, and blue formazan crystals were dissolved in 200µL of 100% DMSO per well. Quantities of the blue formazan product were measured at 570–690nm using a microplate reader (Versamax, tunable microplate reader, USA). As for cells, strong correlations between numbers of cells present and amounts of MTT formazan product were observed. The data were obtained from three independent assays, using three sets of wells for each assay. Cytotoxic effects of the compounds were determined based on the percent cell viability.

Soft Agar Colony Formation Assay

Briefly, 0.5% low temperature melting agar was dissolved in ultrapure water and was
sterilized. Bottom agar layer was prepared with RPMI–1640 with 10% FBS at the ratio
of 2:8 respectively 6mL of bottom agar was added to 60 mm diameter grid bottom
culture plates. After the solidification of the agar 1.5mL of top agar containing 0.5%
agar, RPMI–1640 with 10% FBS, cell suspension consisting 10⁵ cells were applied at a
ratio of 1:8.5:0.5 Cell suspensions were prepared from the cells pre–treated with the
determined IC₅₀ values for S. platensis crude extract for 72 h. 4 replicates of soft agar
plates for each cell line and non–treated control groups were incubated at 37°C and 5%
CO₂ for 120 h. Those colonies formed, which contained at least 18–20 cells were
counted.
The percentage of transformation index was determined as colony number on one
culture plate (consisting 18–20 cells) formed in 100.000 cells seeded, as given in the
following formula:
Transformation index (%) = (Colony number on one culture plate/100.000 cells seeded) ×100

RESULTS
Recent studies have demonstrated antioxidant, antimutagenic, antiviral, anticancer, anti–
allergic, immune enhancing, hepato–protective, blood vessel relaxing, blood lipid–
lowering effects and immune functions by promoting immune competent–cell
proliferation or differentiation of S. platensis crude extract. C–PC is one of the
major light harvesting biliproteins of Spirulina and is of great importance because of its
various biological and pharmacological properties. This pigment is known to be
hepatoprotective, antioxidant, radical scavenger, anti–arthritic, anti–inflammatory, anti–
tumor and immunostimulating activities and is used not only as nutrient ingredient and
natural dyes in food and cosmetics industries but also as fluorescent markers in
biomedical research. In this study, we examined the inhibitory effects of
crude Spirulina aqueous extracts and the active component C–PC in human colorectal,
colon, breast, neuroblastoma, liver, lung, leukemia, lymphoma, cervical cancer cells.
Cytotoxic activities of PSE, together with pure compound C–PC, were analyzed by an
MTT assay. The cytotoxic effects in terms of cell death were determined by treating
NCI–H82, K–562, Raji, DOHH–2, CA–46, SW480, HeLa S3, Caco–2, Hep G2, Hep 3B,
MCF7, HT–29, MDCK, Neuro–2A, L929 cell lines with doses ranging between 0.001
and 100μg/mL for 48 h.
PSE and C–PC pure compound demonstrated cytotoxic activity against the entire cell
tables Table 1. PSE generally exhibited superior activity compared to that of C–PC. PSE
was more effective on SW480, Raji and NCI–H82, cell lines with IC₅₀ values ranging
between 0.006 and 0.030μg/mL. C–PC had greater activity in MCF7 and Raji cell lines
with IC₅₀ value of 1.7μg/mL. Among the cancer cell lines, NCI–H82 (small cell lung
cancer), Raji (Burkitt’s lymphoma), SW480 (colon adenocarcinoma), and MCF7(breast
adenocarcinoma) turned out to be the most susceptible cell lines inhibited by crude
extract and C–PC. Antiestrogen drugs work by competing with the hormone estrogen to
bind to the receptors in estrogen dependent cancer cells. The drug slows the growth and
reproduction of breast cancer cells by blocking estrogen in the breast. Administration of
estrogen suppressing injections is becoming a common practice. Comparatively, less
non–steroidal treatments are also available against breast cancer cell lines, where C–CP
can be a potential candidate for further clinical trials. Interestingly, three of the most
sensitive cell lines (NCI–H82, Raji and MCF7) were lung, lymphoma and breast
cancers. Although crude extract and C–PC exhibited various cytotoxic effects against
different cell lines, no correlation could be found between suspension (NCI–H82, Raji) and solid (SW480, MCF7) tumor lines. *S. platensis* crude extract and C–PC were also cytotoxic against the normal cell line L929 with IC$_{50}$ values of 0.277 and 10μg/mL respectively Table 1.

Regarding our findings, *S. platensis* crude extract generally exhibited superior activity compared to that of C–PC, as crude extract contains other biological and pharmacological active compounds such as calcium–spirulan. For this reason, only the colony formation capacities of the cell lines treated with IC$_{50}$ concentrations of *S. platensis* crude extract were determined in this study. Transformation indexes of the cell lines calculated based on the colony numbers formed on soft agar are listed in Table 2. For colon adenocarcinoma cell line; HT–29 transformation index could not be determined because of the very high colony forming efficiencies of the cells on soft agar; treatment of *S. platensis* crude extract, could not reduce the colony numbers. However, *S. platensis* crude extract affected Caco2 epithelial colorectal cell line, HeLa S3 epithelial carcinoma cell line, K 562 leukemia cell line, Burkitt’s lymphoma cell lines Raji and Ca–46, human B cell lymphoma cell line, DOHH and mouse neuroblastoma cell line; Neuro2A. For these cell lines, reductions of transformation indexes were higher than 2% as listed in Table 2. In this study, epithelial colon adenocarcinoma, Caco–2 transformation indexes were reduced by 7.15% which was attributed to the C–PC component in *S. platensis* crude extract. It was shown that C–PC selectively inhibits the COX–2 enzyme for prostoglandine synthesis which is highly expressed in colon adenocarcinoma cells and is related to tumorogenicity. In addition, leukemia and lymphoma cell lines, K–562, Raji, CA–46 and DOHH transformation indexes were reduced by 4.9%, 7.57%, 3.85% and 2.8% respectively. Liu et. al. (11), reported that *S. platensis* C–PC content significantly inhibited the growth of K562 cells in a dose–dependent manner.

**DISCUSSION**

Subhashini et al. (23) reported that C–PC exhibited IC$_{50}$ values of 50mM in leukemia cells and induced apoptosis in leukemia cells by cytochrome c release from mitochondria into the cytosol, PARP cleavage and down regulation of Bcl–2. They concluded that C–PC was capable of antiproliferative action in leukemic cells, which supports our findings where by C–PC showed cytotoxic effect against K562 with IC$_{50}$ value of lower than 1 μg/mL.

Based on the results of this study, it can be suggested that new anticancer molecules can be found by bioactivity guided isolation of crude extract in subsequent studies. These findings are in compliance with our results where anti proliferative effects of crude extract and C–PC were observed for human cancer cell lines, but on the other hand slight differences was found out between crude extract and C–PC. Moreover, crude extract contains a mixture of proteins, vitamins, carotenoids especially polysaccharides which interact synergistically in mediating anti proliferation of cancer cells.

**ACKNOWLEDGEMENTS**

This work was financially supported by a grant from Republic of Turkey Prime Ministry State Planning Organization (DPT) (2002–DPT–040) and Ege University, Directorate of Administrative and Financial Affairs (2001–BIL–020).
References


**Table List**

**Tabla Listesi**

**Table 1.** Estimated \( IC_{50} \) values of *S. platensis* crude extract and *C–PC*

**Tablo 1.** *S. platensis* ham ekresi ve *C–PC*’nin tahmini \( IC_{50} \) değerleri.

**Table 2.** Transformation indexes determined by \( IC_{50} \) values of *S. platensis* crude extract

**Tablo 2.** *S. platensis* ham ekresinin \( IC_{50} \) değerleri ile sapтанmış transformasyon indeksleri
**Table 1.** Estimated IC$_{50}$ values of *S. platensis* crude extract and C–PC

**Tablo1.** *S. platensis* ham ektresi ve C–PC’nin tahmini IC$_{50}$ değerleri.

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>IC$_{50}$ (μg/ml)</th>
<th>IC$_{50}$ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude Extract</td>
<td>C–PC</td>
</tr>
<tr>
<td>NCI–H82</td>
<td>0.030 (±0.002)</td>
<td>2.8 (±0.081)</td>
</tr>
<tr>
<td>K562</td>
<td>0.554 (±0.029)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Raji</td>
<td>0.009 (±0.003)</td>
<td>1.7 (±0.030)</td>
</tr>
<tr>
<td>DOHH–2</td>
<td>0.360 (±0.023)</td>
<td>–</td>
</tr>
<tr>
<td>CA–46</td>
<td>0.277 (±0.016)</td>
<td>–</td>
</tr>
<tr>
<td>SW480</td>
<td>0.006 (±0.001)</td>
<td>2.8 (±0.067)</td>
</tr>
<tr>
<td>HeLa S3</td>
<td>0.935 (±0.037)</td>
<td>–</td>
</tr>
<tr>
<td>Caco–2</td>
<td>0.277 (±0.016)</td>
<td>6.7 (±0.133)</td>
</tr>
<tr>
<td>Hep G2</td>
<td>0.277 (±0.016)</td>
<td>6.7 (±0.211)</td>
</tr>
<tr>
<td>Hep 3B</td>
<td>0.277 (±0.016)</td>
<td>6.7 (±0.211)</td>
</tr>
<tr>
<td>MCF7</td>
<td>0.277 (±0.016)</td>
<td>1.7 (±0.054)</td>
</tr>
<tr>
<td>HT–29</td>
<td>0.554 (±0.029)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>MDCK</td>
<td>0.554 (±0.029)</td>
<td>–</td>
</tr>
<tr>
<td>Neuro–2A</td>
<td>0.554 (±0.029)</td>
<td>&gt;10</td>
</tr>
<tr>
<td>L929</td>
<td>0.277 (±0.016)</td>
<td>10 (±0.241)</td>
</tr>
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</table>
Table 2. Transformation indexes determined by IC\(_{50}\) values of S. platensis crude extract

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Non–treated</th>
<th>IC(_{50}) of crude extract treated cells</th>
<th>Difference % from non–treated cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCI–H82</td>
<td>15.42 (±0.01)</td>
<td>15.4 (±0.01)</td>
<td>0.02 (±0.013)</td>
</tr>
<tr>
<td>K562</td>
<td>14.1 (±0.1)</td>
<td>9.2 (±0.1)</td>
<td>4.9 (±0.026)</td>
</tr>
<tr>
<td>Raji</td>
<td>9.62 (±0.1)</td>
<td>2.05 (±0.1)</td>
<td>7.57 (±0.18)</td>
</tr>
<tr>
<td>DOHH</td>
<td>9.37 (±0.1)</td>
<td>6.57 (±0.1)</td>
<td>2.8 (±0.241)</td>
</tr>
<tr>
<td>CA–46</td>
<td>8.15 (±0.1)</td>
<td>4.3 (±0.1)</td>
<td>3.85 (±0.23)</td>
</tr>
<tr>
<td>SW–480</td>
<td>1.25 (±0.05)</td>
<td>0.425 (±0.05)</td>
<td>0.825 (±0.024)</td>
</tr>
<tr>
<td>HeLa S3</td>
<td>13.5 (±0.1)</td>
<td>9.25 (±0.1)</td>
<td>4.25 (±0.241)</td>
</tr>
<tr>
<td>CaCO(_2)</td>
<td>22.3 (±0.2)</td>
<td>15.15 (±0.2)</td>
<td>7.15 (±0.245)</td>
</tr>
<tr>
<td>HEP–G(_2)</td>
<td>1.07 (±0.01)</td>
<td>0.32 (±0.01)</td>
<td>0.75 (±0.0183)</td>
</tr>
<tr>
<td>Hep 3B</td>
<td>1.37 (±0.01)</td>
<td>0.2 (±0.01)</td>
<td>1.17 (±0.0178)</td>
</tr>
<tr>
<td>MCF7</td>
<td>0.6 (±0.02)</td>
<td>0.27 (±0.02)</td>
<td>0.33 (±0.01)</td>
</tr>
<tr>
<td>HT–29</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>–</td>
</tr>
<tr>
<td>MDCK</td>
<td>0.3 (±0.01)</td>
<td>0.1 (±0.01)</td>
<td>0.2 (±0.0352)</td>
</tr>
<tr>
<td>Neuro2A</td>
<td>3.47 (±0.01)</td>
<td>0.07 (±0.01)</td>
<td>3.4 (±0.0323)</td>
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<tr>
<td>L–929</td>
<td>0.3 (±0.01)</td>
<td>0.2 (±0.01)</td>
<td>0.1 (±0.0274)</td>
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