**Candidate drugs on Josh’s list and related literature:**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Index | Drug | Paper | Incubation time in the paper | LD15 | LD30 | LD50 | LD65 | LD80 | LD95 |
| 1 | (+)-Griseofulvin | https://onlinelibrary.wiley.com/doi/epdf/10.1002/jcb.21240?saml\_referrer | 8h | 10μM | 20μM | 30μM | 40μM | 50μM | 60μM |
| 2 | (S)-(+)-Camptothecin | https://www.nature.com/articles/2400734.pdf?proof=tr | 3h | 1μM | 2μM | 3μM | 4μM | 5μM | 6μM |
| ~~3~~ | ~~5,5-Diphenylhydantoin~~ | ~~file:///Users/lideyi/Downloads/IJEB549553-559.pdf~~ | ~~48h~~ | ~~25μM~~ | ~~50μM~~ | ~~75μM~~ | ~~100μM~~ | ~~150μM~~ | ~~200μM~~ |
| 4 | Chloramphenicol | https://journals.sagepub.com/doi/pdf/10.1191/096032700672635987 | 48h | 1mM | 2mM | 3mM | 4mM | 5mM | 6mM |
| 5 | Metformin | https://www.sciencedirect.com/science/article/pii/S0378111918300246?via%3Dihub |  |  |  |  |  |  |  |

**Experimental concentrations (or serial dilution plate, unit: μM):**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Drug | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| (+)-Griseofulvin | 128 | 64 | 32 | 16 | 8 | 4 | 2 | 1 | 0.5 | 0.25 | 0.125 | 0 |
| ~~96~~ | ~~48~~ | ~~24~~ | ~~12~~ | ~~6~~ | ~~3~~ | ~~1.5~~ | ~~0.75~~ | ~~0.375~~ | ~~0.1875~~ | ~~0.09375~~ | ~~0~~ |
| (S)-(+)-Camptothecin | 32 | 16 | 8 | 4 | 2 | 1 | 0.5 | 0.25 | 0.125 | 0.0625 | 0.03125 | 0 |
| ~~96~~ | ~~48~~ | ~~24~~ | ~~12~~ | ~~6~~ | ~~3~~ | ~~1.5~~ | ~~0.75~~ | ~~0.375~~ | ~~0.1875~~ | ~~0.09375~~ | ~~0~~ |
| Chloramphenicol | 3200 | 1600 | 800 | 400 | 200 | 100 | 50 | 25 | 12.5 | 6.25 | 3.125 | 0 |
| ~~4800~~ | ~~2400~~ | ~~1200~~ | ~~600~~ | ~~300~~ | ~~150~~ | ~~75~~ | ~~37.5~~ | ~~18.75~~ | ~~9.375~~ | ~~4.6875~~ | ~~0~~ |
| Metformin | 512 | 256 | 128 | 64 | 32 | 16 | 8 | 4 | 2 | 1 | 0.5 | 0 |
|  |  |  |  |  |  |  |  |  |  |  |  |
| ~~5,5-Diphenylhydantoin~~ | ~~256~~ | ~~128~~ | ~~64~~ | ~~32~~ | ~~16~~ | ~~8~~ | ~~4~~ | ~~2~~ | ~~1~~ | ~~0.5~~ | ~~0.25~~ | ~~0~~ |
| ~~192~~ | ~~96~~ | ~~48~~ | ~~24~~ | ~~12~~ | ~~6~~ | ~~3~~ | ~~1.5~~ | ~~0.75~~ | ~~0.375~~ | ~~0.1875~~ | ~~0~~ |
| **Molecular weight of each drug:** | | (+)-Griseofulvin:  352.76 g/mol | | | (S)-(+)-Camptothecin:  348.352 g/mol | | | Chloramphenicol:  323.132 g/mol | | | Metformin:  129.164 g/mol | |
| **Initial amount of each drug (dissolve in 200 ml medium)** | | 36.12mg | | | 8.918 mg | | | 827.2 mg | | | 52.91 mg | |

**Plate design (100μL/well, 2 replicates, same for test plates):**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A |  |  |  |  |  |  |  |  |  |  |  |  |
| B |  |  |  |  |  |  |  |  |  |  | Positive 1 |  |
| C |  |  |  |  |  |  |  |  |  |  | Positive 2 |  |
| D |  |  |  |  |  |  |  |  |  |  | Positive 3 |  |
| E |  |  |  |  |  |  |  |  |  |  | Positive 4 |  |
| F |  |  |  |  |  |  |  |  |  |  | Negative |  |
| G |  |  |  |  |  |  |  |  |  |  | Blank |  |
| H |  |  |  |  |  |  |  |  |  |  |  |  |

1. **Positive control:**

|  |  |  |  |
| --- | --- | --- | --- |
|  |  |  |  |

6-fold high concentration of each drug + Medium + cells

1. **Negative control:**

|  |
| --- |
| **Neg Ctr** |

Cells + Medium

1. **Blank well:**

|  |
| --- |
| Blank |

Only Medium

**CCK-8 assay:**

1. Seed cells in a 96-well plate (100μL/well).
2. Replace the original medium with the medium containing various concentrations of drugs to be tested to the plate.
3. Incubate the plate for 48 hours in the incubator.
4. Add 10 μL of CCK8 solution to each well of the plate using a repeating pipettor (Experimental wells + Positive control wells + Negative control wells + Blank wells should all be tested). Be careful not to introduce bubbles to the wells, since they interfere with the O.D. reading.
5. Incubate the plate for **1.5** hours in the incubator.
6. Before reading the plate, it is important to mix gently on an orbital shaker for 1 minute to ensure homogeneous distribution of color.
7. Measure the absorbance at 450 nm using a microplate reader.

8. Cell viability (%) = [(As-Ab) / (Ac-Ab)] × 100

As = absorbance of the experimental well (absorbance of cells, medium, CCK8 and wells of the test drugs).

Ab = blank well absorbance (absorbance of wells containing medium and CCK8).

Ac = control well absorbance (absorbance of wells containing cells, medium and CCK8).