Neuraminidase (NA) Inhibition Assay

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Abstract:

In this research article, *A microplate-based screening assay for neuraminidase inihibitos*, substrate, 2‘-(4-Methylumbelliferyl)-alpha-D-N-acetylneuraminic acid, 4-MUNANA, is used to assess the inhibition ability of the influenza enzyme. This neuraminidase experiment applies the same method using MUNANA as substrate to examine whether the checmial compound C can be used for potential drug candidate for influenza.The inhibition value is determined by a comparison value with the positive control, without the addition of compound C. The electromagnetic spectroscopy is used to read the enzyme activity after the system is being set up. The flourecense shows the enzyme binding ability in terms of wavelength, and the value is then used for inhibition % calculation. The result shows that compound C has a high inhibition value of 97.8%, so it can be listed as a drug candidate.

Experiment:

The purpose of this neuraminidase assay is to search for potential drug candidaates by testing chemical compound C, an extract from a fruit named mangosteen, to see if it can inhibit the influenenza enzyme activity.

The influenza enzyme is first being optimized, using the enzyme-substrate (E-S) graph. The E-S graph is used to determine the minimum concentration required for the maximum enzyme performance in the assay. When the concentration of the substrate increases, the slope reaches zero, the point is then selected as the optimum enzyme concentration for the experiment. A 96 micro-wells plate is used for this NA assay, which applies the method of using 4-MUNANA as substrate. The chemical compound used for this testing is labeled as compound C, which is one of the chemical extracts from mangosteen. A measurement of 1 mg of compound C is first being dissolved in 1ml of DMSO in an eppendorf tube.

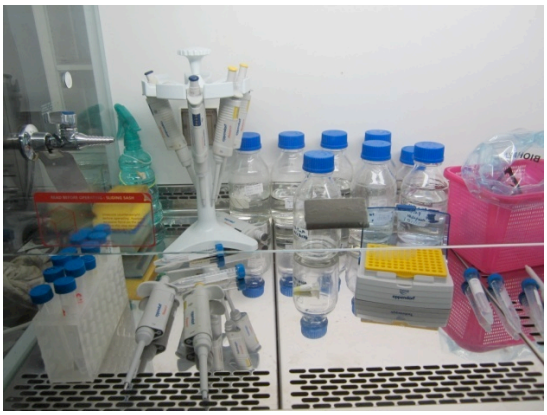


Figure 1: The micro pipettes and the 96 micro-wells plate.

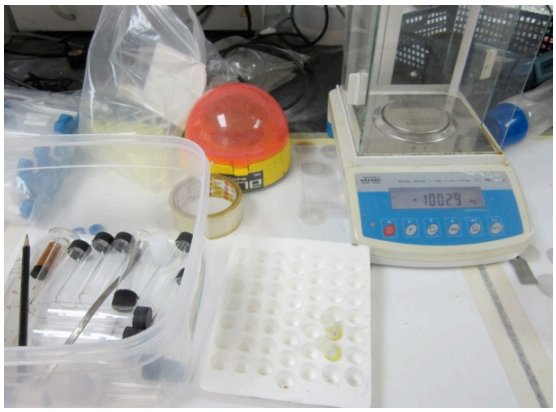


Figure 2: The chemical extract, compound C, in the eppendorf tube.

To prepare a 2.5% of DMSO mixed with compound C, 25 µL of solution is diluted with 975 µL of 200 µU/mL MES using dilution factor. Starting with a concentration of 1250 µg/mL, the solution is first added into the first well, and then it is diluted into the next well by

adding an additional 25 µl of DMSO. The dilution step repeats until reaching the last well in the same row. The entire micro plate is then incubated for 30 minutes. In preparation of the substrate, 100 µM of 4-MUNANA is prepared from 51.03 mM of 4-MUNANA and MES. After the dilution factor calculation, 10 µl of 4-MUNANA is used to dilute with 4990 µl of MES. The amount of 50 µl of 4-MUNANA is added into each well on the 96-micro plate, from left to right, in a chronological order.

After the addition of the substrate in each well, the plate is covered with aluminum foil and placed in a dry bath incubator under a set temperature of 37 ºC and stirring at 200 rmp for an hour.

Figure 3: Dry bath incubator.

Figure 4: The electromagnetic spectroscopy.

After an hour of incubation, the enzyme activity is then stopped by the addition of a buffer with a high pH value of 10.4 due to the drastic change in pH. The enzyme is then placed into the fluorescence machine to see how much light is being absorbed and reflected.

Result:

The result of compound C is shown in Table 1. It shows a high inhibition value of 97.8% with a florescence value of 1798.1 over the value for the control system, which is 82607.7. In the first three dilutions, the % inhibition values have an average of 94.4%, which shows that

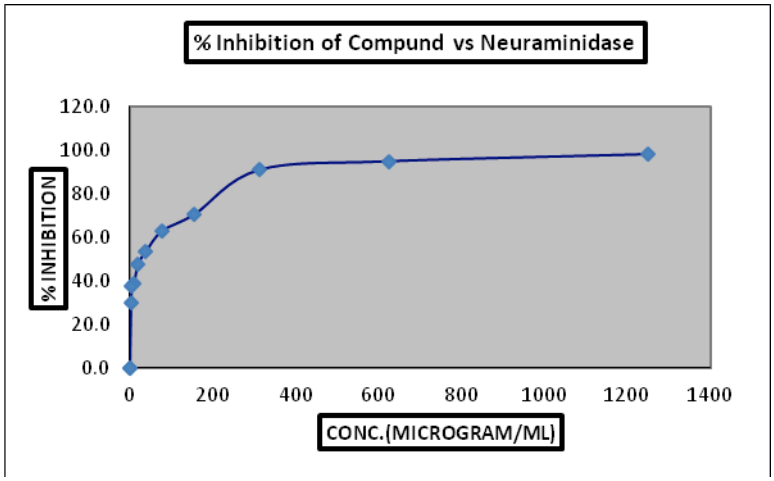
compound C has high inhibition ability, and it can be listed as a candidate.

Table 1: Fluorescence result for compound C.

Conc. (µg/mL)	Trial 1	Trial 2	Mean	% Activity trial 1	% Activity trial 2	% Activity mean	S.D.	% error	% Inhibition
1250.0	3155.4	440.9	1798.1	3.8	0.5	2.2	2.1	0.7	97.8
625.0	5550.3	3419.6	4484.9	6.7	4.1	5.4	3.4	1.1	94.6
312.5	9386.8	5948.1	7667.5	11.4	7.2	9.3	5.8	1.8	90.7
156.3	30798.9	18087.1	24443.0	37.3	21.9	29.6	18.7	5.9	70.4
78.1	44487.5	16890.8	30689.2	53.9	20.4	37.2	27.2	8.6	62.8
39.1	54472.7	22314.4	38393.6	65.9	27	46.5	33.2	10.5	53.5
19.5	64290.5	22346.1	43318.3	77.8	27.1	52.4	39.5	12.5	47.6
9.8	69945.6	31018.4	50482.0	84.7	37.5	61.1	42.4	13.4	38.9
4.9	66922.0	36402.7	51662.4	81.0	44.1	62.5	40.6	12.8	37.5
2.4	74184.2	41572.9	57878.6	89.8	50.3	70.1	45.0	14.2	29.9
Positive control			82607.7						

% Activity Mean = (Trial 1/Positive control) x 100 (Eq.1)

% Inhibition = 1 - % Activity mean (Eq.2)



Graph 1: Inhibition % v.s. Neuraminidase.

Future work:

In this neuronamidase assay, compound C shows a high inhibition value of 97.8%. The next step after this experiment is to identify the composition of compound C using the NMR.

Reference

Li, A. F., Wang, W. H., Xu, W. F., & Gong, J. Z. (January 01, 2009). A microplate-based screening assay for neuraminidase inhibitors. *Drug Discoveries & Therapeutics, 3,* 6, 260-5.