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Huawei Pharmaceutical Com. Ltd.

Experiment Report of New Drug Screening

Study of the Compounds from YuePeng Gao Research Team of
Shi He Si Medical university

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1. Materials and methods

1.1. Compounds

1.1.1 compounds: see Table 1

1.1.2 LOT:

1.1.3 purity:

1.1.4 pack: 1.5 ml tube

1.1.5 M.W. and weight: see Table 1

1.1.6 Solution preparation: using DMSO

1.1.7 storage: -20°C

1.1.8 validity: 2015-12

1.1.9 provider: Study of the Compounds from YuePeng Gao Research Team of Shi He Si Medical university

Compound I.D.	Stock Concentration (mM)	Solvent
AP501/42026328	10	DMSO
AT-156/43461756	10	DMSO
Gefitinib	10	DMSO

1.2. Reference compounds

1.2.1 name:

1.2.2 catalog:

1.2.3 purity: >98%

1.2.4 character:

1.2.5 validity: 2015-12

1.2.6 storage: -20°C

1.2.7 provider: Selleck Chemicals Co.,Ltd

1.3. Solvent

1.3.1 name: DMSO

1.3.2 catalog: D100627

1.3.3 specification: 500 mL

1.3.4 character: transparent liquid;

1.3.5 storage: room temperature

1.3.6 validity: 2015-12

1.3.7 provider: Sangon Biotech Co.,Ltd

1.4. Dosage designation

According to the literature

1.5. Methods

These assays were carried out as described previously.[01] All of the enzymatic reactions were conducted at 30°C for 40 minutes. The 50µl reaction mixture contains 40 mM Tris, pH 7.4, 10 mM MgCl₂, 0.1 mg/ml BSA, 1 mM DTT, 10 µM ATP, Kinase and the enzyme substrate. The compounds were diluted in 10% DMSO and 5µl of the dilution was added to a 50µl reaction so that the final concentration of DMSO is 1% in all of reactions. The assay was performed using Kinase-Glo Plus luminescence kinase assay kit. It measures kinase activity by quantitating the amount of ATP remaining in solution following a kinase reaction. The luminescent signal from the assay is correlated with the amount of ATP present and is inversely correlated with the amount of kinase activity. The IC₅₀ values were calculated using nonlinear regression with normalized dose-response fit using Prism GraphPad software.

[01] Kashem, M.A.et al . (2006) Three mechanistically distinct kinase assays compared: Measurement of intrinsic ATPase activity identified the most comprehensive set of ITK inhibitors. J. Biomol. Screen . 12, 70–83.

Assay	Enzyme Used/ Reaction	Substrate
EGFR(WT)	25ng	0.2 mg/ml Poly (Glu, Tyr)/ 10 u M ATP

1.6. Quality control

All the experimental results will be considered to be credible when reference compound's result is similar as the literature result

1.7. Statistical

2. Results

2.1. Enzyme EGFR IC50 results table

Compound	Repeat 1 (nM)	Repeat 2 (nM)	Average (nM)	SD
AP501/42026328	>100uM	>100uM	>100uM	>100uM
AT-156/43461756	>100uM	>100uM	>100uM	>100uM
Gefitinib	2	3	2	1

2.2. Compound AP501/42026328 result of EGFR enzyme activity influence

AP501/42026328	EGFR		Activity %	
	Repeat1	Repeat2	Repeat1	Repeat2
No compound	23913	24116		
10 nM	24228	24759	99	96
33 nM	24547	23811	97	101
100 nM	25039	25681	94	91
333 nM	24079	25422	100	92
1 μ M	24950	24288	95	98
3.3 μ M	25252	24633	93	96
10 μ M	25918	25354	89	92
33 μ M	26650	26099	85	88
100 μ M	27401	26729	81	85
Background	40707	42529		

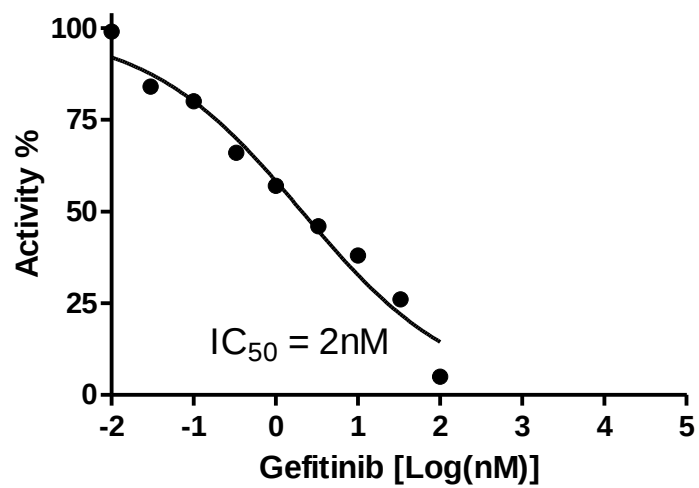
2.3. Compound AT-156/43461756 result of EGFR enzyme activity influence

AT-156/43461756	EGFR		Activity %	
	Repeat1	Repeat2	Repeat1	Repeat2
No compound	23913	24116		
10 nM	24557	23804	97	101
33 nM	23953	25092	100	94
100 nM	24785	24105	96	99
333 nM	25471	24920	92	95
1 μ M	26173	25551	88	91
3.3 μ M	27042	26369	83	87
10 μ M	27520	26829	80	84
33 μ M	28291	27684	76	79
100 μ M	29247	28615	70	74
Background	40707	42529		

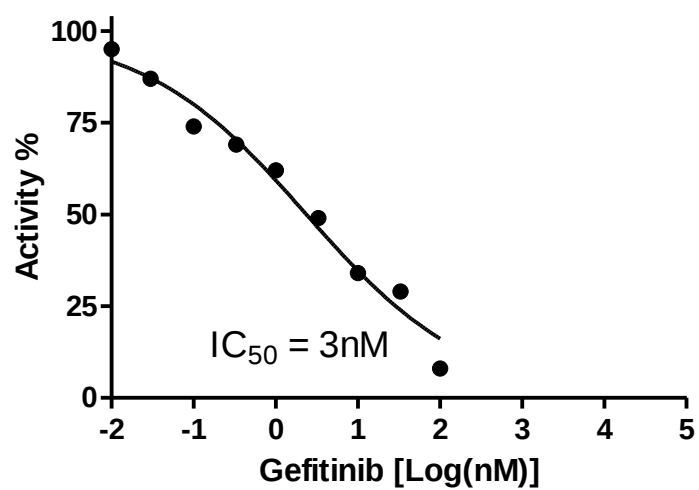
2.4. Compound Gefitinib result of EGFR enzyme activity influence

Gefitinib	EGFR		Activity %	
	Repeat1	Repeat2	Repeat1	Repeat2
No compound	23913	24116		
0.01 nM	24248	24980	99	95
0.03 nM	26811	26248	84	87
0.1 nM	27539	28654	80	74
0.33 nM	29989	29555	66	69
1 nM	31632	30740	57	62
3.3 nM	33605	32933	46	49
10 nM	34862	35688	38	34
33 nM	37050	36519	26	29
100 nM	40793	40172	5	8
Background	40707	42529		

EGFR



EGFR



3. Conclusion

4. Appendix

4. 1. Experimental data document

All experimental records are kept in HuaWei Pharmaceutical Com. archives room, including paper documents and electronic document; By default, the original document data will be saved about ten years in my company.

4.2. Compounds storage

All the experimental samples were stored in the HuaWei Pharmaceutical Com. sample library, and storage conditions is - 20 °C avoiding light. The default time of saving the samples is one year, and samples will be disposed one year later, in order to protect the customer's project secret.