

Searching for novel cyclooxygenase II inhibitors by virtual screening and evaluating their inhibitory effects on cyclooxygenase II activity
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Introduction: Cyclooxygenase-2 (COX-2) enzyme bind to arachidonic acid and release metabolites that are use to induce pain and inflammatory response. Resent study was shown that strong COX-2 expression is highly correlated with increas tumor risk. Therefor there is still a need to develop more potent COX-2 inhibitors, for both relief the pain and cancers.

Methods: after downloading near 2000000 compounds from ZINC and SIGMA databases, about 12000 compounds with similar structure to selective COX-2 inhibitor, celecoxib, were selected by ChemOffice program. Then different scripts were written and autodock 3 in conjunction with MGL Tools was used to screen above databases, against COX-2 X-ray structure. SC-558 was used as a positive control. The calculation by virtual screening took about 2.5 days to run under linux cluster including six Pentium 3GHz CPUs. We selected four at the best compounds with lower docking energies, including:

1. 5-bromotryptophan, 2. phar-95239, 3. T0511-4424, 4. Zu-4280011

For in vitro screening (effect of compounds on the CoX-2 compared with COX-1) using colorimetric COX(ovine) screening assay kit(from Cayman company), the selectivity index according the following formula was calculated.

$$\text{Selectivity index} = \frac{\text{IC}_{50} \text{ for inhibition of COX-1}}{\text{IC}_{50} \text{ for inhibition of COX-2}}$$

Then MTT assay for cytotoxic activity with different dilutions (25 μ M , 50 μ M, 75 μ M , 100 μ M) from analogues was investigated. Absorbance of the wells was read at 545nm by ELISA-Reader. Then IC₅₀ was calculated by calcsyn program.

Results and Discussion: Docking energy of the four analogues (-29.62 kcal/mol to -14 kcal/mol) was better than positive controls: SC-558(-10.38 kcal/mol) and celecoxib (-8.02 kcal/mol) in spite of other three compounds, 5-bromo tryptophan didn't show any COX-2 inhibitory effect. According to MTT assay, the IC₅₀ for compound 2,3 and 4 was found to be 134.81, 122, 80.21 kcal/mol and the selectivity index was found to be 11.36, 12.20 and 20.03 respectively. Therfor there was a relationship between selectivity index and anti-cancer activity. Compound 4 (Zu-4280011) with highest selectivity index showed the best result in MTT assay.

Keywords: Cyclooxygenase. Zinc, Sigma, AutoDock, Cluster, Virtual Screening, MTT.