DOCKING STUDIES OF CURCUMIN ANALOGS AGAINST METHIONINE AMINOPETIDASE 2 AND BIOLOGICAL EVALUATION OF LEAD MOLECULES IN COLON CELLS

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Abstract

Colorectal cancer is one of the major causes of death worldwide. In Canada, colon cancer is the third most commonly diagnosed cancer. [1] Fortunately, 90% of deaths and cases of colon cancer are preventable. If detected at an early stage, it can be cured. There are different challenges in developing drugs for cancer treatments, these include tumor selectivity, target specificity, drug efficacy and multidrug resistance (MDR).

Enzymes such as methionine aminopeptidases (MetAPs) play a major role in being a anticancer drug target. MetAP2 has been found highly expressed in several tumours. Continuous efforts have been made to develop structurally divergent new MetAP2 inhibitors to enhance drug specificity and to treat drug resistant colon cancers. NC 2213, a drug design strategy by Pati et al. [4] to undertake structural modification of curcumin to produce novel synthetic analogs (enones were designed as thiol alkylators) with MetAP2 inhibitory properties as anticancer agents.

The purpose of the present study is to identify and investigate promising curcumin analogs displaying both MetAP2 inhibitory and cytotoxic properties for further development as potent anticancer drug candidates.

Methods

1) Literature survey for identifying potent curcumin analogs
2) Creation of a database of potent cytotoxic curcumin analogs: comprised of 130 compounds
3) MetAP2-ligand docking studies using the database of curcumin analogs: Compounds with docking scores of -8.5 and below were taken for further evaluation.
4) Pharmacokinetic and drug-likeness studies
5) The biological properties of these compounds was evaluated by
   - Cytotoxic evaluation of compounds
   - Western Blot analysis
6) Statistical analysis performed by Student's t-test for paired data and Dunett's Post-Hoc test.

Results

- A library of 130 compounds were docked against MetAP2 enzyme and top 10 compounds that showed good results for their physicochemical properties assessment were taken for cytotoxic evaluation.
- Among the 10 compounds, 2 compounds (NC 1768 and NC 1773) had good IC_{50} and GI_{50} profiles.
- NC 1773 and NC 1768 on western blot indicated that protein expression was significantly reduced for NC 1773 at IC_{50} concentration against HT-29.
- The reduced protein expression on western blot is indicative of NC 1773 potential for inhibiting the MetAP2 enzyme in multidrug resistant HT-29 colon cancer cell line.
- The statistical analysis performed using Student's t-test for paired data was not significant with $p>0.05$ in all 3 bivariate linear models.
- Dunnett's post hoc test showed significance at $p<0.05$ for the treatment protocol of NC 1773 in comparison with control (cells with absence of treatment).

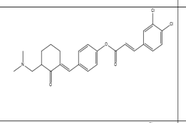
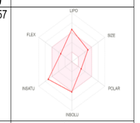
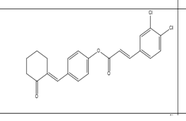
NC number	Docking Score	Structure	HT-29 IC_{50} in µM (mean± sd)	HCT-116 IC_{50} in µM (mean± sd)	SwissADME
1773	-9.606		5.51± 0.11	4.21± 0.57	
1768	-9.523		0.24± 0.14	0.57± 0.59	

Table 1: Docking scores against MetAP2 enzyme, IC50 profile and physicochemical properties of NC 1773 and NC 1768

	5FU	NC 1773	NC 1768
CRL1790 IC_{50} in µM (mean± sd)	14.74± 0.88	4.70± 0.33	0.02± 0.01
HT29 IC_{50} in µM (mean± sd)	15.36±0.65	5.51± 0.11	4.21± 0.57
HCT116 IC_{50} in µM (mean± sd)	4.02±0.48	0.24± 0.14	0.57± 0.59

Table 2: Comparison of IC50 profile of NC 1773 and NC 1768 with %-flurouracil (5FU) as standard

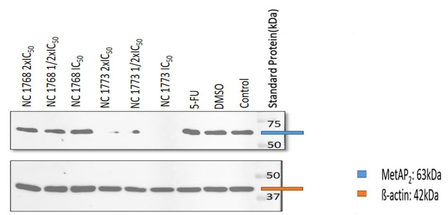


Fig 1: Western Blot Analysis of NC 1773 and NC 1768 against HT-29 for effects on protein expression

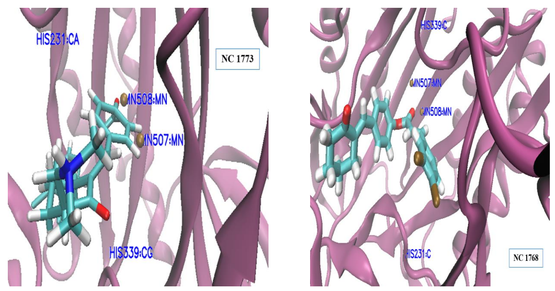


Fig 2: Docked view of NC 1773 and NC 1768 at the active site of MetAP2

Conclusion

The compound NC 1773 has shown excellent results in docking and Western blot analysis. The western blot indicated that protein expression was reduced for NC 1773 against HT29 cell line. Dunnett's post hoc test showed significance at $p<0.05$ for the treatment protocol of NC 1773 in comparison to control (cells with absence of treatment). It has an ideal IC_{50} profile and predicted bioavailability which warrants further investigation for the mechanism of action.

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