In-Silico and *In-Vitro* Study of Benzanilide Derivatives as a Potent Anti-Cancer Agent

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Abstract:

Cancer is one of the leading causes of death in the current world. Among numerous anticancer drug targets, EGFR is a potential and one of the highly studied drug target. Benzanilide scaffold and its derivatives are the promising groups of compounds with several biological activities including antifungal, antimycotic, antibacterial, spasmolytic, and anticancer. Here, we have tried to develop an improve anticancer compound with benzanilide scaffold. A list of *in-silico* based benzanilide derivatives was designed and evaluated using molecular docking energy and pharmacophoric interactions in comparison to known EGFR inhibitor. The two optimum compounds A and B were synthesized and tested for their *in-vitro* anticancer activity by MTT Assay against MCF-7 cell line. Blood lymphocytes were used for studying the effect of the compounds on non-cancerous cells within the human body. The IC_{50} value of compound A and compound B against MCF-7 was calculated to be 122.3 µM and 101.9 µM respectively. Evaluation of cytotoxic studies of synthesized molecules reveals that both the compounds show cytotoxic activity. The results suggested that compounds A and B could be further explored and studied with more molecular assays to understand its detail activity as an anti-cancer agent.

Background

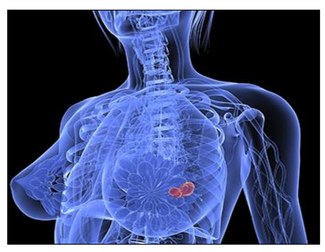

Fig 1: Breast Cancer

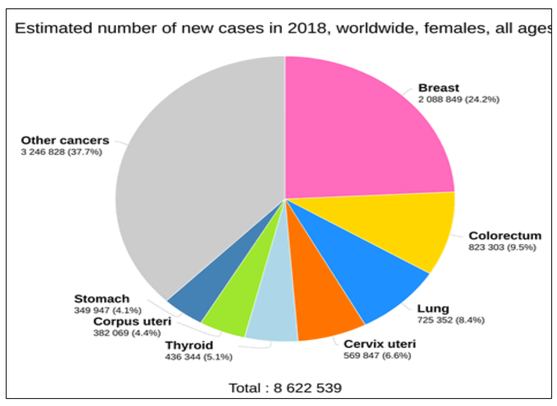

Fig 2: Breast Cancer statistical data

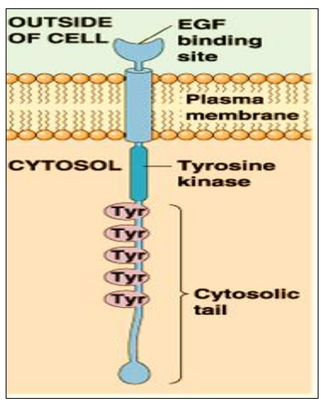

Fig 3: EGFR structure

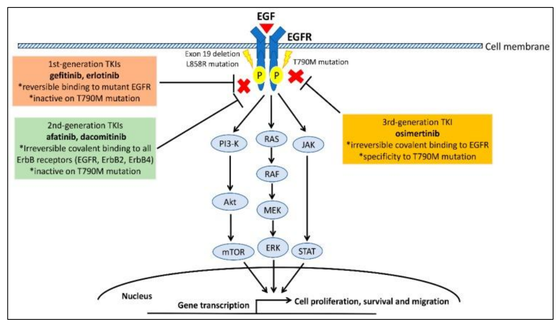

Fig 4: EGFR Mechanism

Methodology

In-Silico Modelling

- Sketch and design compounds
- ADME-T properties calculation
- Modelling of EGFR protein kinase domain
- Energy minimization
- Structure validation
- Molecular docking

Synthesis of Compounds

- Melting point analysis
- TLC

In-Vitro Analysis

- MTT assay on MCF-7 and lymphocytes
- IC50 value and statistical validation.

Fig 5: Methodology

Results

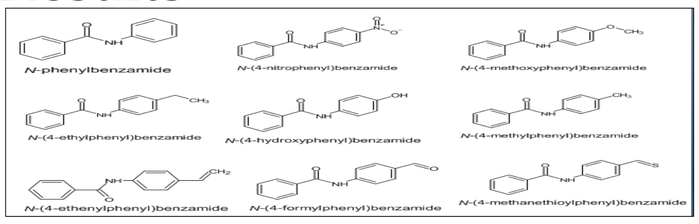

Fig 6: Designed and sketched compounds

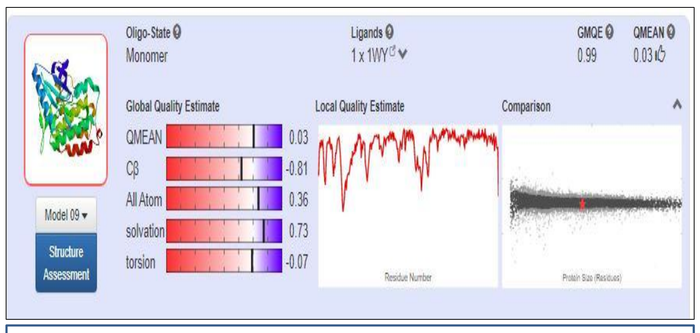

Fig 7: Homology Modelling of EGFR kinase domain

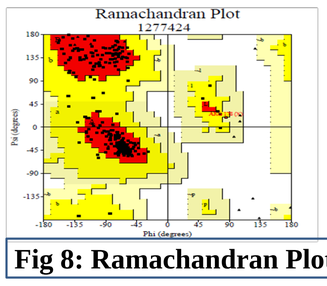

Fig 8: Ramachandran Plot

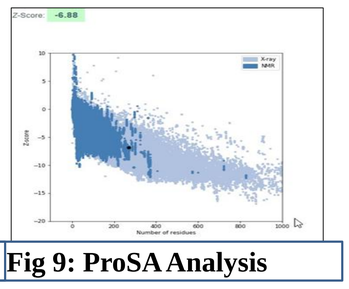

Fig 9: ProSA Analysis

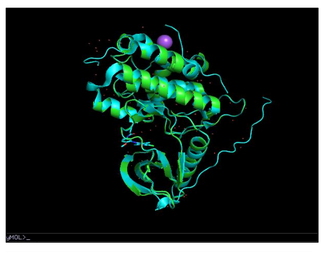

Fig 10: RMSD Template Vs Model = 0.197A

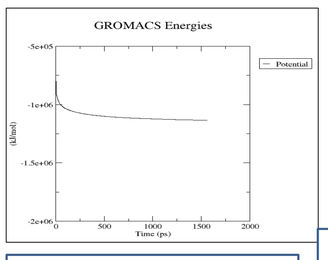

Fig 11: Energy minimization of model

Table: 1.0 – Molecular Docking result with Compound Standard , A and B

Ligand	Protein	Binding Energy (Kcal/Mol)	Conventional Hydrogen bond	Pi-Pi stacked	Van der waals
Standard molecule	EGFR Tyrosine Kinase	-9.5	MET A-793	PHE A: 723	THR A -790 THR A : 854 GLY A- 719 LEU A- 792
Compound A	EGFR Tyrosine Kinase	-7	-	PHE A: 723	ASN A:842 THR A: 854 ASP A : 855 LYS A :745
Compound B	EGFR Tyrosine Kinase	-7.8	MET A- 793	PHE A: 723	ASP A : 855 LYS A :745 GLY A: 796 GLY A: 719

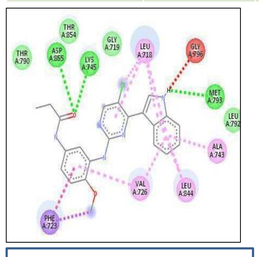

Fig 12: Molecular docking standard compound

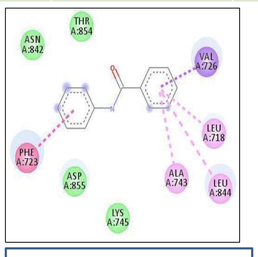

Fig 13: Molecular docking compound A

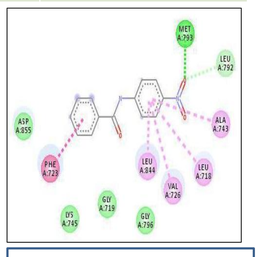

Fig 14: Molecular docking compound B

Table: 2.0 – Compound Synthesized A and B

COMPOUND	WATER	ETHANOL	METHANOL	DMSO
COMPOUND A	Not soluble	Soluble	Soluble	Soluble
COMPOUND B	Partially soluble	Soluble	Soluble	Soluble

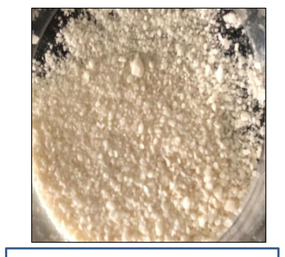

Fig 15: Synthesized compound A – N-phenylbenzamide

Fig 16: Synthesized compound B – N-(4-nitrophenyl)benzamide

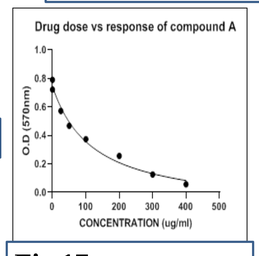

Fig 17: Cytotoxicity result of compound A with MCF-7

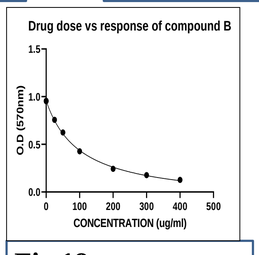

Fig 18: Cytotoxicity result of compound B with MCF-7

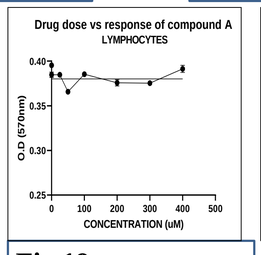

Fig 19: Cytotoxicity result of compound B with Lymphocytes

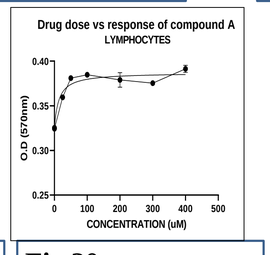

Fig 20: Cytotoxicity result of compound B with Lymphocytes

- The statistical analysis of compounds on MCF- 7 cell line shows, 95% confidence level or profile likelihood. IC50 value was calculated to be **122.3** for compound A and **101.9** for compound B. The R2 value for compounds A and B was **0.9853** and 0.9991 respectively
- In lymphocytes, IC50 value in the range of **300-400** which indicates that much higher concentration of drug is required to inhibit non-cancerous cells making them less harmful. The R2 value for compound A and B was **0.2362 and 0.9356**
- The cells exhibited altered morphology and a change in adherence patterns was reported.
- The MTT assay results for lymphcytes show no significant changes in cell density even with increase in concentration of the drug, indicating that it has no toxic effect on non-cancerous cells in human body.

Conclusion: Benzanilide scaffold is majorly used as drugs for various infectious diseases and very less information is available about their anti-cancer activity. The results suggested that compounds A and B could be further explored and studied with more molecular assays to understand its detail activity as an anti-cancer agent.

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