Design, synthesis and biological evaluation of Novel *1H*-benzo[d]imidazole derivatives as Fatty Acid Synthase (FASN) inhibitors for cancer treatment

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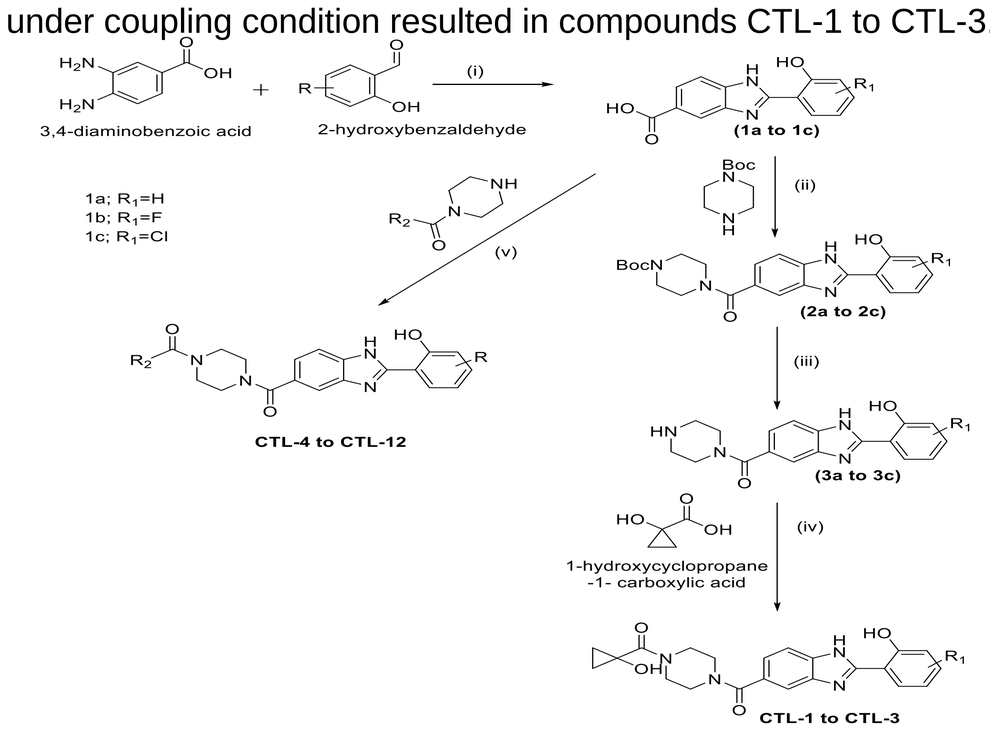
INTRODUCTION

- Fatty acid synthase (FASN) enzyme plays an important role in cellular mechanism by synthesizing higher fatty acids. In normal cells the *de novo* fatty acid synthesis is minimal However hyperactivation of this enzyme contribute in pathogenesis of several diseases like cancer, NAFLD, Obesity[1]. Targeting this enzyme by rational design small molecules is a valuable strategy in treatment of these diseases. In the present study we designed novel FASN inhibitor by opting *de-novo* Drug discovery approach for targeting breast and colorectal cancer.

MATERIALS AND METHODS

- **Synthesis of *1H*-benzo[d]imidazole derivatives (CTL-1 to CTL-12):** The compounds were synthesized by condensation of 3,4 diamino benzoic acid with substituted hydroxy benzaldehyde in presence of sodium metabisulphite to provide substituted benzimidazole acid which further reacted with substituted piperazines under amide coupling conditions to afford compounds CTL-4 to CTL-12 **(Scheme 1)**. The compounds CTL-1 to CTL-3 is formed by coupling the substituted benzimidazole acid with Boc-piperazine under standard coupling condition to afford piperazine amide. Further Boc-protecting group was removed by treating with trifluoroacetic acid to yield piperazine salt. Subsequent reacting the substituted piperazine salt with hydroxycyclopropane-1-carboxylic acid under coupling condition resulted in compounds CTL-1 to CTL-3.



Scheme 1 : Reagents and Condition : (i) $Na_2S_2O_5$, DMAc , 100 ^{0}C, 8hr (ii) tert- butylpiperazine-1-carboxylate, EDC·HCl, HOBt, NMM, DMF, 4 h, 56 % (iii) Trifluoro acetic acid, DCM, 2 h, 98%; (iv) EDC·HCl, HOBt, NaOH EtOH, 24 h, 47 %. (v) DMF, DIPEA and HATU stirr at room temperature for 24 hrs

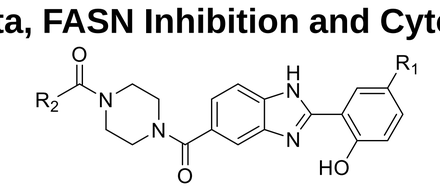
- **Cell Cytotoxicity & FASN Inhibition Assay:** The synthesized compounds (CTL-1 to CTL-12) were investigated for cytotoxicity studies against colon (HCT-116 and CaCO2) and breast (MCF-7 and MDA-MB-231) cancer cell line and a non-cancerous cell line (HEK-293). Further FASN inhibitory potency of the synthesized compounds were evaluated against HCT-116 cells as per protocol described previously[2].
- **Cell cycle Analysis, Apoptosis Assay & Western Blot Analysis :** The most active obtained from cytotoxicity studies and FASN inhibition assay i.e. CTL-1 ad CTL-7 were screened for mechanistic studies.
- **Molecular Docking, *Molecular Dynamics (MD) simulations and MM/PBSA :*** The molecular docking study of synthesized compounds against protein (PDBID 6NNA) carried out using the Autodock 4.2 software. Amber 18 simulation package was employed to carry out the optimizations and MD simulations
- **Statistical analysis:** Statistical analysis was carried out using GraphPad Prism 5.0 software, USA.

CONCLUSION

- We designed a series of 1*H*-benzo[d]imidazole derivatives and investigated for FASN inhibition and cytotoxic potency against four cancer cell line HCT-116, CaCO2, MDA-MB-231 and MCF-7 and non-cancerous cell line HEK-293. All compounds shows cytotoxicity towards anticancer cell lines at less than 12 µM concentration and shows more than 30 µM concentration against HEK-293. Among all the compounds, CTL-1 and CTL-7 shows potent FASN inhibition at < 3 µM and arrested the cell cycle progression at S-phase inducing apoptosis. The western blot analysis reveals that CTL-1 and CTL-7 causes apoptosis in HCT-116 cells significantly in dose dependent manner by inhibiting the FASN pathway. The molecular dynamics simulation studies of CTL-1 and CTL-7 against the FASN enzyme reveal the binding mechanism of these inhibitors within the KR domain of the enzyme.

RESULTS

Table 1. Structural Data, FASN Inhibition and Cytotoxicity assay data



S.No	R_1	R_2	IC_{50} (µM)					
			FASN Inhibition	HCT-116	CaCO2	MCF-7	MDA-MB-231	HEK293
CTL-1	-Cl	Hydroxy cyclopropyl	2.5	4.0	5.0	3.5	4.0	31.2
CTL-2	-F	Hydroxy cyclopropyl	2.5	6.1	6.5	4.5	5.6	36.5
CTL-3	-H	Hydroxy cyclopropyl	3.75	7.2	8.1	5.8	7.2	38.8
CTL-4	-F	Cyclopropyl	2.5	4.5	5.6	4.3	4.6	32.3
CTL-5	-Cl	Cyclopropyl	3.5	4.2	5.2	4.5	4.8	33.5
CTL-6	-H	Cyclopropyl	3.75	5.8	6.6	5.7	6.4	36.0
CTL-7	-Cl	C_2H_5O-	3	3.0	4.1	3.5	4.4	30.1
CTL-8	-F	C_2H_5O-	3	4.5	5.6	4.4	5.8	33.8
CTL-9	-H	C_2H_5O-	3.25	5.5	6.6	5.5	6.2	34.5
CTL-10	-Cl	$-CH_3$	3.75	5.5	6.4	6.0	6.5	34.6
CTL-11	-F	$-CH_3$	3.25	6.3	7.2	6.2	6.8	36.8
CTL-12	-H	$-CH_3$	6.75	10.0	11.6	9.8	10.6	42.4

Figure 1. Cell Cycle Analysis and Apoptosis Assay of CTL 1 & CTL-7

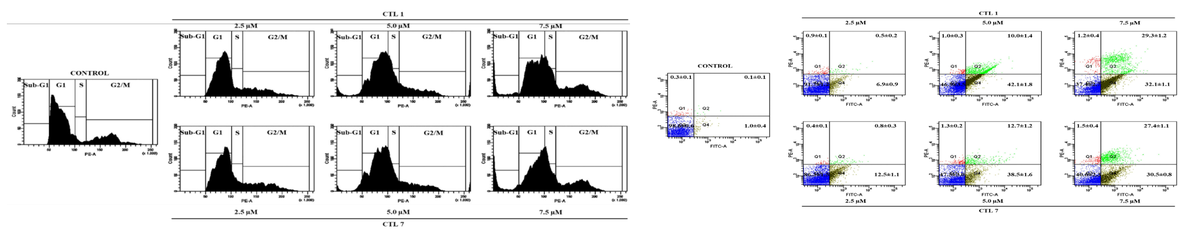


Figure 2. Docking Studies of CTL-1 and CTL-7

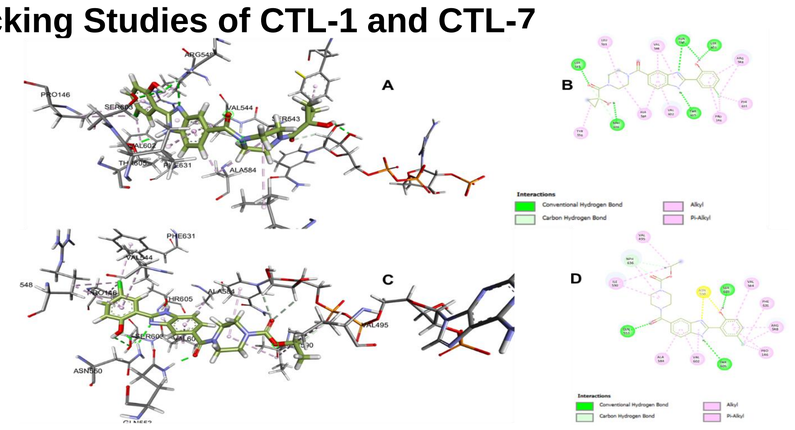


Figure 3. Western blot analysis of CTL-1 and CTL-7 at different concentrations

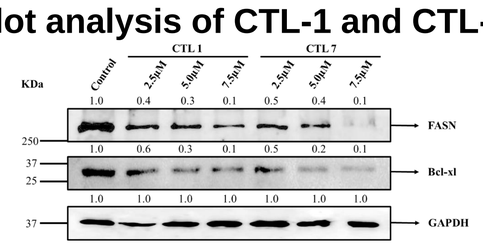
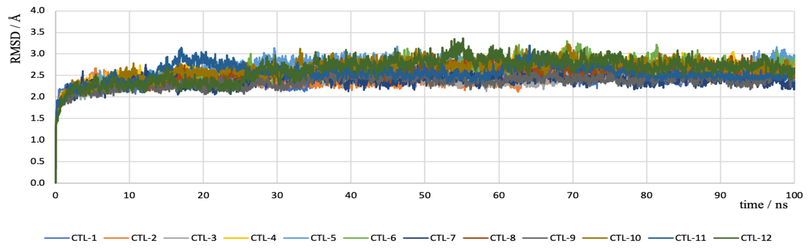


Figure 4. Root mean square-deviation (RMSD) values for backbone atoms of the various systems studied by MD simulations.



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