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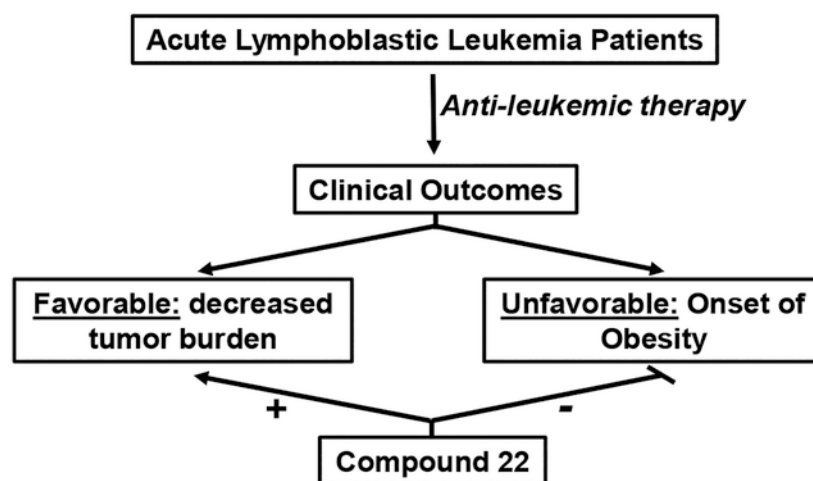
Abstract

Over the past decade, the therapeutic strategies employed to treat B-precursor acute lymphoblastic leukemia (ALL) have been progressively successful in treating the disease. Unfortunately, the treatment associated dyslipidemia, either acute or chronic, is very prevalent and a cause for decreased quality of life in the surviving patients. To overcome this hurdle, we tested a series of cylopropanecarboxamides, a family demonstrated to target lipid metabolism, for their anti-leukemic activity in ALL. Several of the compounds tested showed anti-proliferative activity, with one, compound 22, inhibiting both Philadelphia chromosome negative REH and Philadelphia chromosome positive SupB15 ALL cell division. The novel advantage of these compounds is the potential synergy with standard chemotherapeutic agents, while concomitantly blunting the emergence of dyslipidemia. Thus, the cylopropanecarboxamides represent a novel class of compounds that can be potentially used in combination with the present standard-of-care to limit treatment associated dyslipidemia in ALL patients.

Graphical Abstract

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B lineage acute lymphoblastic leukemia (ALL) is the most prevalent cancer diagnosed in pediatric patients. Fortunately, due to advances in treatment strategies approximately 90% of these patients will recover from the disease[1, 2]. The etiology of the disease involves differentiation arrest followed by proliferation of the immature B cells leading to their accumulation within the bone marrow[3]. Proliferation is generally attributed to a fusion oncogene created by chromosomal translocation with BCR-ABL, called the Philadelphia chromosome, being studied the most[4]. At present, patients with ALL are typically treated with an induction dose followed by a consolidation therapy and finally the patients are kept on a maintenance therapy along with CNS prophylaxis, if specifically warranted, based on tumor phenotype[5, 6]. However, with great success with treatment, obesity has been identified as an unanticipated potential long-term harmful effect of the cancer therapy in ALL survivors[7, 8]. The cause of obesity has been attributed to treatment-induced alteration of altered cholesterol metabolism leading to elevated triglycerides and very-low-density lipoprotein (VLDL) and low levels of high-density lipoprotein (HDL)[9]. Specifically, the hyperlipidemia during treatment with chemotherapy has been shown to be correlated with decreased lipoprotein lipase activity[10]. In light of this, the present study was undertaken to identify novel compounds that could target lipid metabolism pathway and be used in combination with chemotherapy to curb the obesity effect of therapy in ALL.

Recently we identified a series of cyclopropanecarboxamides that modulate lipid metabolism *in vivo*[11–13]. These compounds shared a common fluorinated carboxamide moiety with the R-groups studied for structure-activity relationships. One of these compounds, the lead structure (Figure 1), was found to be a modulator of triglyceride metabolism. Since it has been shown that dysregulated lipid metabolism may be a key factor in the causation of obesity in ALL survivors[14], we screened the cyclopropanecarboxamides for potential leads in an ALL drug discovery program.

We screened a series of cyclopropanecarboxamides in two different ALL cell lines, including Philadelphia chromosome negative cell line, REH (peripheral blood derived) and Philadelphia chromosome positive, SupB15, (bone marrow derived) (Figure 2). Because, the oncogenes driving proliferation are different in both cell lines, with BCR-ABL oncogene driving proliferation of SupB15 and TEL-AML1 oncogene driving proliferation of REH, it is critical to identify novel compounds that are effective in these diverse cell phenotypes. The physico-chemical properties of the tested compounds are shown in table 1. All compounds were obtained from the Chembridge Chemical company (www.hit2lead.com).

Figure 2 shows the results of the screen. Both types of cells were grown in 96-well plates at a density of 50,000 cells per well. Cells were treated with the compounds at final concentration (50 μM), and the cell proliferation was tested after 72 hours of incubation with the compounds. A cell counting kit was utilized according to the manufacturer's instructions (Cell counting kit-8, catalog NoCK04, Dojindo Molecular Technologies Inc.). Briefly, 10 uL of the assay reagent was added to each well and incubated for 2 hours at 37° C, after which the plates were read on a BioTek Cynergy 5 plate reader at 450 nm absorbance. Untreated cells were used as controls.

As shown in Figure 2, all of the compounds tested decreased cell proliferation in the REH cell line, with compound 22 showing the largest inhibition (>50% compared to control). SupB15 cells were more resistant to the compounds, and fewer of the compounds, including compound 22, were able to prevent cell proliferation as compared to the REH cells. Further testing of compound 22 in a dose-response study demonstrated the IC_{50} for the REH cells was 16.5 μM, and 44.66 μM for SupB15 (Figure 3). To further validate the efficacy of compound 22, we utilized a co-culture model of drug resistance that has been well-characterized and previously reported by our lab[15–18]. Specifically, we have defined a suspended leukemic cell population in the co-culture with bone marrow stromal cells, called floaters (FL), and a leukemic cell population that is buried underneath the bone marrow stromal layer, called phase dim (PD) and demonstrated that PD cells are very resistant to the present standard-of-care[16, 17]. As seen in figure 4, compound 22 was very effective in decreasing the live cell population of FL and PD in both REH and SupB15 when co-cultured with bone marrow stromal cells. This decrease in live cells was co-related with increased cell death found in REH and SupB15 cells. Interestingly, compound 22 was more effective in inducing death in the drug resistant PD cells compared to the FL cells and furthermore compound 22 was more sensitive in SupB15 than the REH cells. Initial structure-activity relationships seen from this pilot screen suggests that a hydrophobic aromatic moiety seems to be preferred for activity in SubB15 cells. Future studies will be focused on exploring this attribute of compound 22.

The structure of compound 22 was evaluated in silico for drug-likeness (ADME/T) by submitting it to the SwissADMET server. As shown in Figure 5, compound 22 is predicted to adhere to the Lipinski Rule of 5, suggesting that the structure would serve as a druggable lead structure. Furthermore, the serum albumin binding of compound 22 and the lead compound were evaluated using a fluorescent plate reader assay as described previously[19]. Plasma binding is important for determining the ability of a compound's distribution in the body and the availability for systemic circulation, as well as the free-concentration of drug in

the serum. Only the free/unbound fraction of the drug is able to have a pharmacodynamic effect. In Figure 6 it can be seen that compound 22 has less than 5% binding to serum albumin. This suggests a large fraction of the drug will be free for interacting pharmacodynamically. An additional parameter is the predicted permeability using the parallel artificial membrane assay (PAMPA)[19]. We found that compound 22 had a logPe of −4.40 while the lead compound had a logPe of −4.53. These finding suggest that further optimization will be needed for optimum cellular permeability.

To gain insight into the ligand interactions with the enzyme, docking studies were done using the homology model as previously described[13] with the MOE 2016 (www.chemcom.com) cheminformatics suite. The binding pocket was identified with the SiteFinder function, which located a suitable binding pocket. As can be seen in Figure 7, our docking data show that the lead compound interacts via hydrogen bonding with the amino acids Ser216 and Thr213. On the other hand, compound 22 interacts via primarily a hydrogen bond with the amino acid Lys294 and secondary there are several hydrophobic interactions with the aromatic rings in the pocket. These data suggest that optimization of compound 22 would be driven towards campaigns focused at increasing direct hydrogen-bond interactions with the LPL enzyme, while still being able to maintain the lead-likeness as calculated in figure 5.

In conclusion, the lipid dysregulation of cancer cells led us to test a novel series of cyclopropanecarboxamides for anti-leukemic activity in B-cell ALL to consider possible dual effects on tumor cell proliferation and lipid metabolism. Interestingly, one of the compounds inhibited proliferation of both the Philadelphia chromosome negative REH cells as well as Philadelphia chromosome positive SupB15 cells. Taken together, compound 22 represents a novel chemical class of anti-leukemic compounds focused on targeting both lipid dysregulation of the tumor cells and proliferative capacity.

Acknowledgements

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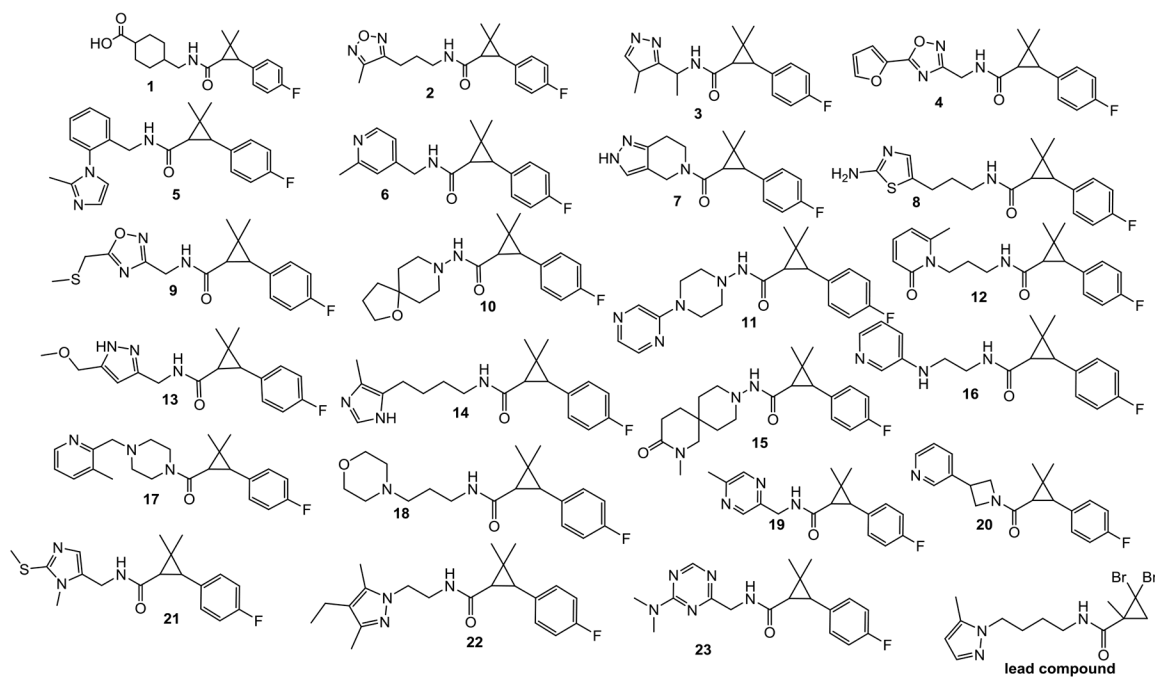
Figure 1.
Structures of the compounds tested in the B-cell ALL cells.
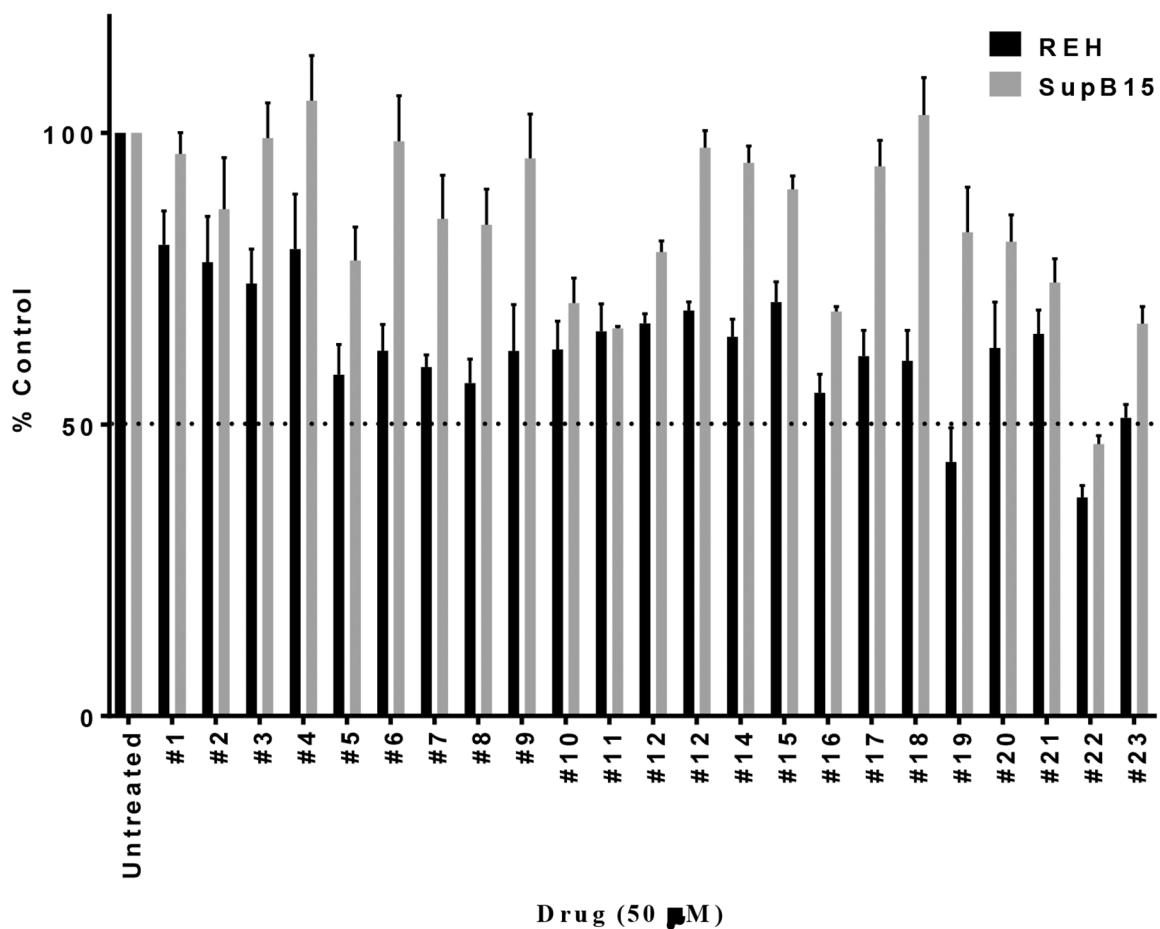
Figure 2.
Results from the cell proliferation screening assay of the compounds in the REH and SupB15 specific cells in the cell proliferation assay. Each bar represents mean ± SEM where N = 3.
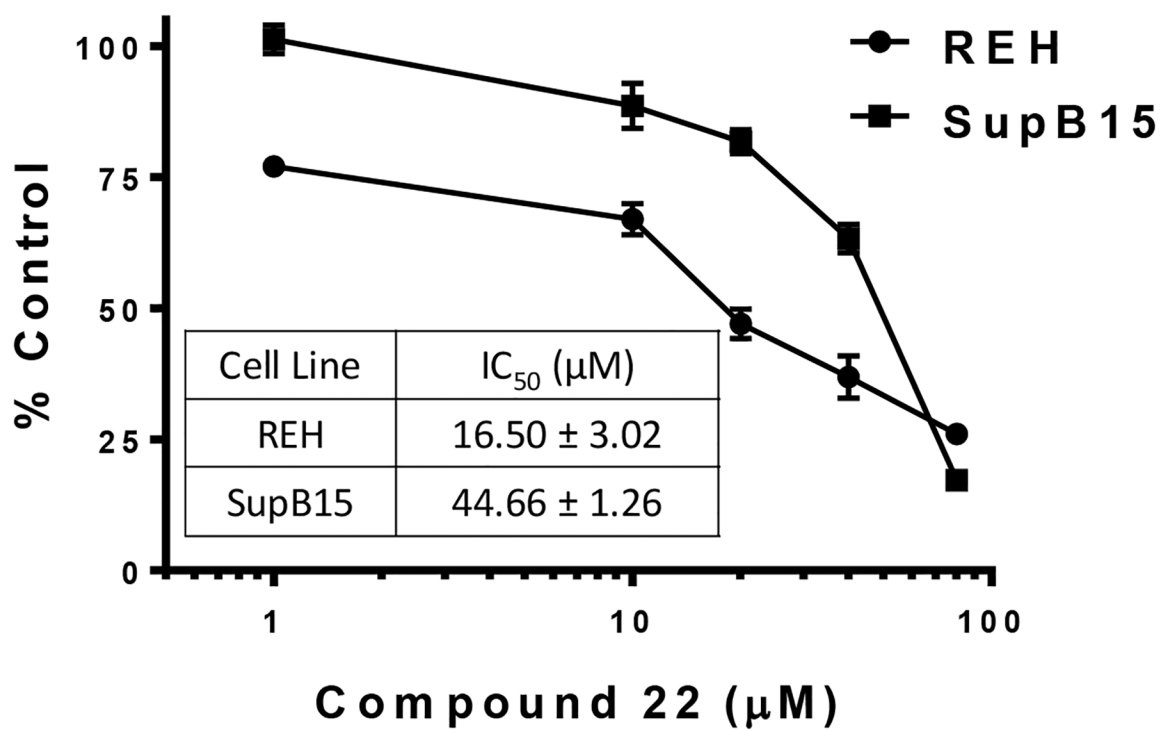
Figure 3.
Concentration-dependent curve of compound 22 in ALL humans cell lines. Data is represented as mean ± SEM, where N = 3.
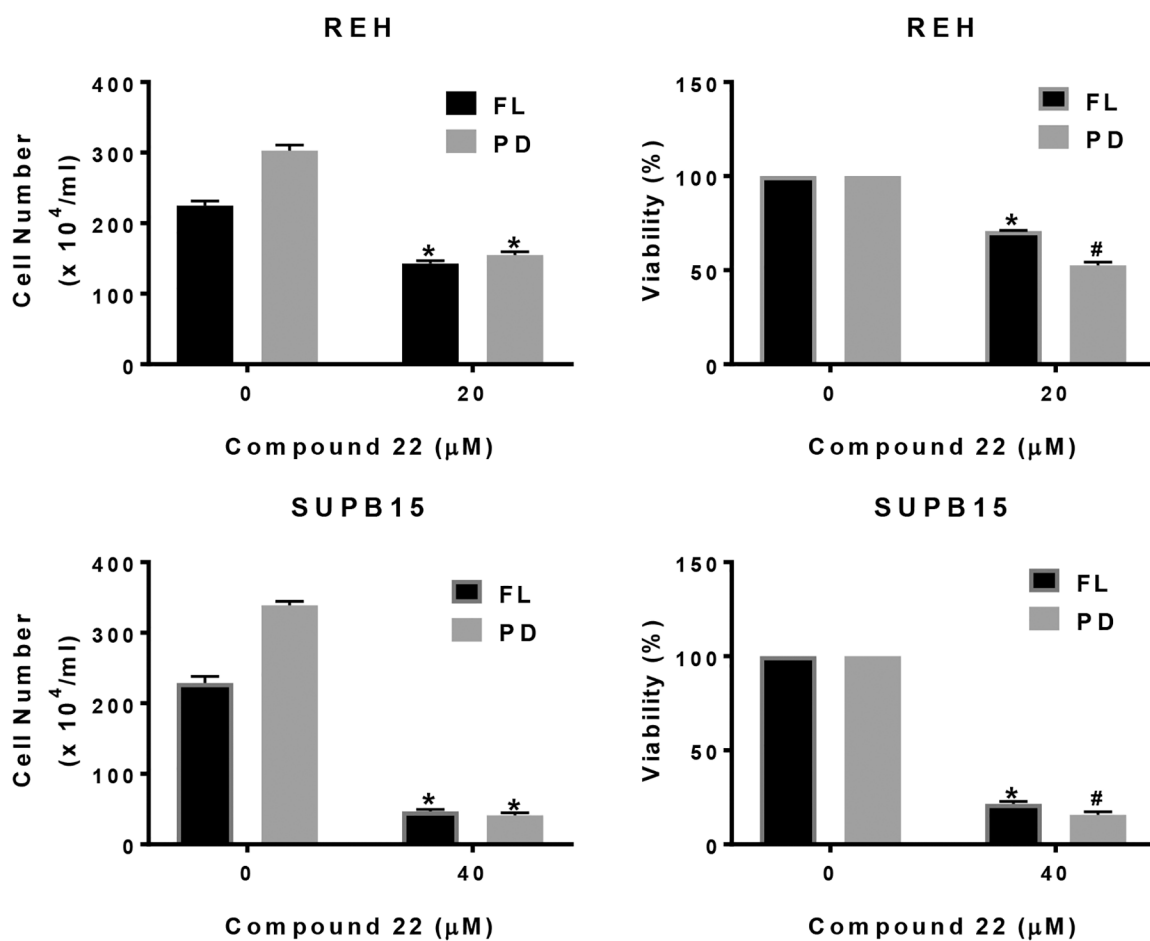
Figure 4.
Compound 22-mediated death in ALL cells grown in co-culture with bone marrow stromal cells. Each bar represents mean ± SEM where N = 3. *p<0.05, when compared to untreated and #p<0.05, when compared to all other treatment groups.

SMILES CCc1c(C)nn(c1C)CCNC(=O)C1(CC1(C)C)c1ccc(cc1)F

Physicochemical Properties

Formula	C21H28FN3O
Molecular weight	357.46 g/mol
Num. heavy atoms	26
Num. arom. heavy atoms	11
Fraction Csp3	0.52
Num. rotatable bonds	7
Num. H-bond acceptors	3
Num. H-bond donors	1
Molar Refractivity	101.80
TPSA	46.92 Å²

Lipophilicity

Log $P_{o/w}$ (iLOGP)	3.24
Log $P_{o/w}$ (XLOGP3)	3.95
Log $P_{o/w}$ (WLOGP)	4.11
Log $P_{o/w}$ (MLOGP)	3.51
Log $P_{o/w}$ (SILICOS-IT)	5.17
Consensus Log $P_{o/w}$	4.00

Water Solubility

|	
Log S (ESOL)	-4.40
Solubility	1.44e-02 mg/ml ; 4.02e-05 mol/l
Class	Moderately soluble
Log S (Ali)	-4.64
Solubility	8.28e-03 mg/ml ; 2.32e-05 mol/l
Class	Moderately soluble
Log S (SILICOS-IT)	-7.12
Solubility	2.71e-05 mg/ml ; 7.59e-08 mol/l
Class	Poorly soluble

Pharmacokinetics

GI absorption	High
BBB permeant	Yes
P-gp substrate	Yes
CYP1A2 inhibitor	No
CYP2C19 inhibitor	Yes
CYP2C9 inhibitor	Yes
CYP2D6 inhibitor	Yes
CYP3A4 inhibitor	Yes
Log K_p (skin permeation)	-5.68 cm/s

Druglikeness

Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability Score	0.55

Medicinal Chemistry

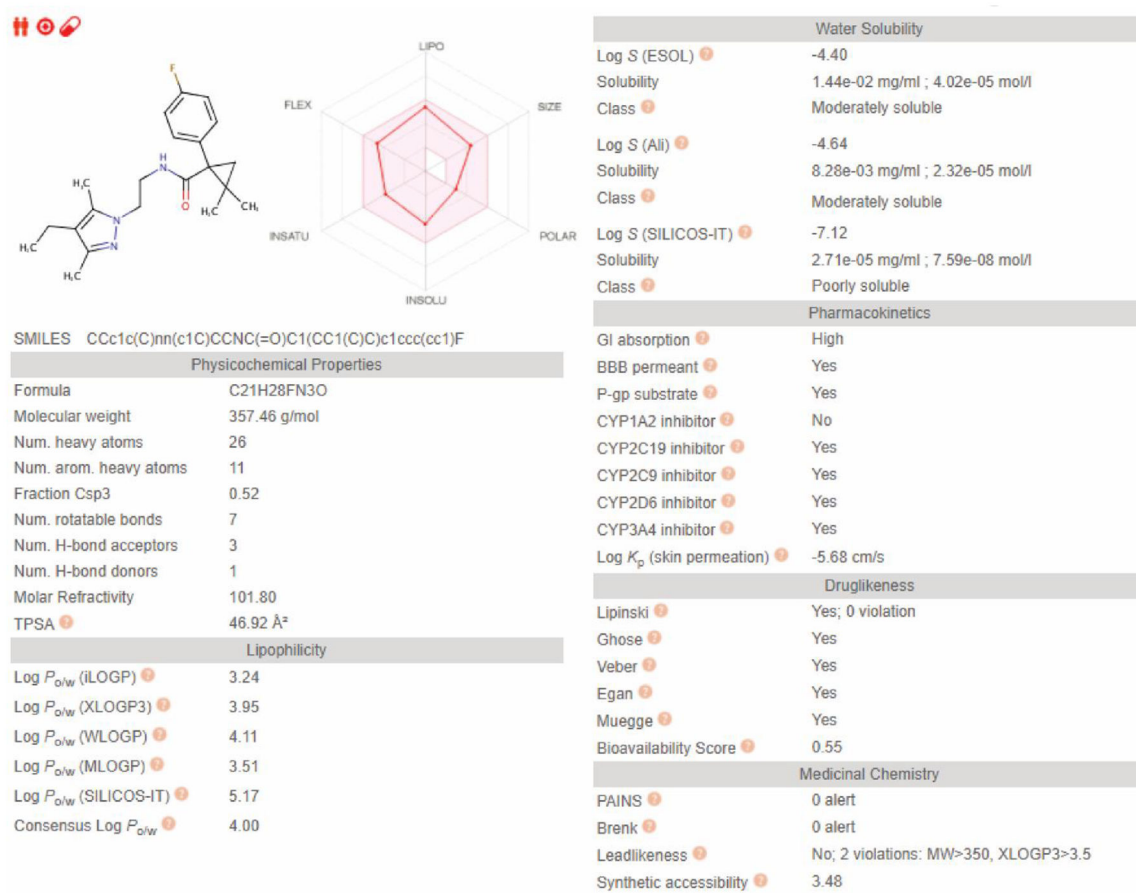|	
PAINS	0 alert
Brenk	0 alert
Leadlikeness	No; 2 violations: MW>350, XLOGP3>3.5
Synthetic accessibility	3.48

Figure 5.
ADMET property prediction for compound 22 identified from this study. The online server SwissADMET was used for the prediction.[20]
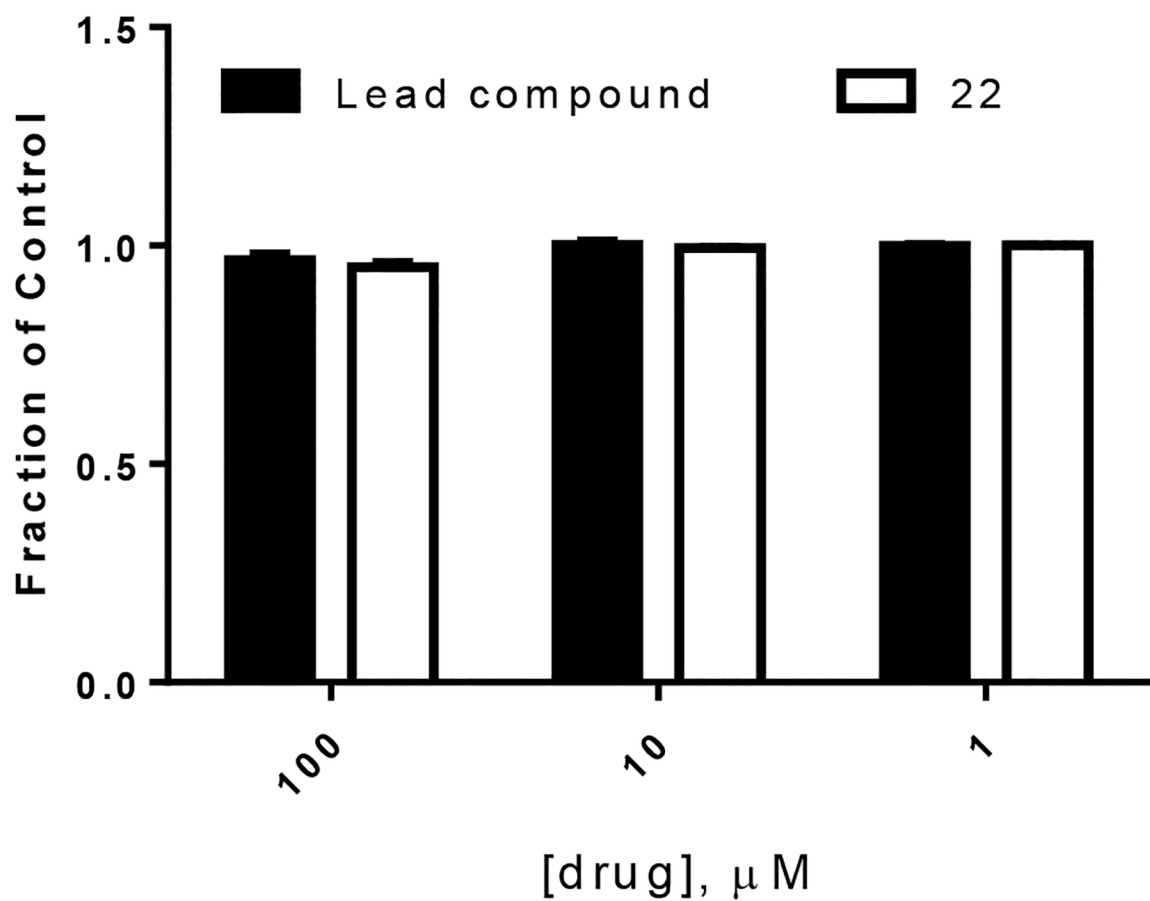
Figure 6.
Serum albumin binding of the lead compound of this study and compound 22 shows low level of binding. Each bar represents average + S.D. where N = 3.
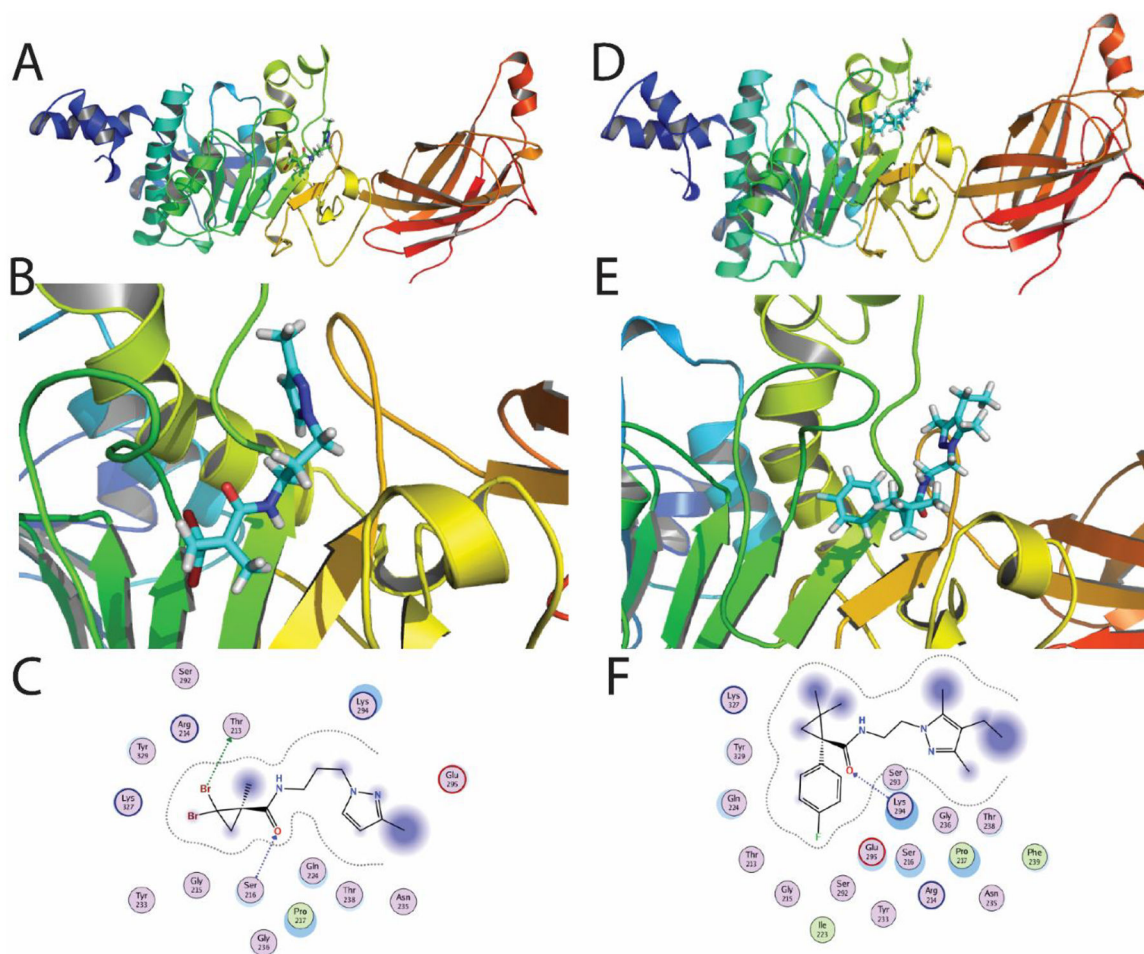
Figure 7.
Docking studies of the lead compound A-C and compound 22 D-F. As can be seen, the lead compound interacts with Ser216 and Thr213 via hydrogen bonding, while compound 22 interacts via Lys294 and a hydrophobic interaction with the aromatic rings. Docking studies were done using MOE 2016, while the figures were rendered in PyMOL (v99).

Table 1.

Structures of the cyclopropanecarboxamide compounds screened in the ALL cell proliferation assay and their physico-chemical properties

	ID[*]	Mol Weight	cLogP	LogSW	RB	tPSA	hDon	hAcc
1	43625552	347.4	3	−4.295	5	66.4	2	3
2	28137555	333.4	2.4	−3.681	6	77.25	1	5
3	61604264	316.4	1.439	−4.872	4	59.81	1	4
4	17179599	355.4	2.75	−4.14	5	81.16	1	5
5	17823051	377.5	3.84	−5.231	5	46.92	1	2
6	95033174	312.4	2.79	−2.561	4	41.99	1	2
7	11487776	349.4	2.41	−3.806	6	68.02	1	4
8	46595938	313.4	2.8	−3.877	2	48.99	1	2
9	54059102	347.5	2.8	−4.125	6	68.01	2	2
10	60479521	331.4	3.41	−4.529	2	29.54	0	2
11	21398303	354.4	2.5	−3.919	3	49.33	0	3
12	77681436	356.4	2.69	−4.096	6	51.1	1	2
13	67761960	331.4	1.66	−3.034	6	67.01	2	3
14	21281178	361.5	2.26	−3.766	7	57.78	2	2
15	25151041	372.5	2.99	−4.469	2	40.62	0	2
16	21127878	327.4	1.43	−2.809	6	54.02	2	2
17	15812955	381.5	3.19	−3.406	4	36.44	0	3
18	56656615	334.4	2	−3.347	6	41.57	1	3
19	65401844	313.4	1.79	−3.014	4	54.88	1	3
20	10636932	324.4	2.83	−2.682	3	33.2	0	2
21	81879452	347.5	2.68	−4.022	5	46.92	1	2
22	83163475	357.5	4.19	−5.385	6	46.92	1	2
23	27444028	358.4	0.24	−3.328	5	97.03	2	4
Lead Compound	8817367	379.1	2.062	−3.725	5	46.92	1	2

[*] hit2lead.com ID number