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Anti-cancer Investigation of Newly Derived Alicyclic ring and Morpholine Supported Hybrid Molecules by Industrially viable Route

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In an attempt to design and synthesize a new class of anticancer molecules, we have reported coupling of aryl aldehyde, *p*-toluidine and morpholine based isocyanide with cyclopropane carboxylic acid to generate a small library of 08 compounds (5a-5h) by ugi multicomponent reaction in a single step manner. Structures of the newly synthesized compounds were recognized on the basis of spectral data *i.e.* ^{1}H NMR, ^{13}C NMR, IR and Mass. These compounds were screened for their anticancer activity against nine basic panels as well as NCI-60 cell-lines. *In vitro* anticancer studies revealed that the compound 5a showed maximum potency against HCT-116 in colon cancer cell lines with GI_{50} values 46.27 µg/ml.

Keywords: Cyclopropane, Anticancer Screening, Ugi Multicomponent Reaction, Morpholinoethyl Isocyanide

Introduction

Cancer is a class of diseases characterized by out-of-control cell growth. According to WHO reports, there are over 100 different types of cancer, and each is classified by the type of cell that is initially affected[1,2]. Ivar Ugi in 1959 first time stated one of the most imperative MCRs that create peptide-like structures was Ugi four-component reaction (U-4CR) and gave α-acylamino amides types products[3]. Many ugi reactions were reported starting by using alicyclic or aliphatic components as one of subtracts and combination of it with heterocyclic system were synthesized as potent medicinal agents as well as produced most versatile organic synthetic intermediates[4,5]. In advance, another important pharmacophoric group is the morpholine core incorporated in a wide variety of therapeutically key drugs and key structural component in some natural products and clinical drugs[6]. To move ahead from our projected work to formulate newer nitrogen and halogen based molecules[7], a new approach was put forward, *i.e.* Ugi multicomponent reaction to elucidate a method for better anti-cancer agent against NCI-60 cell-lines.

Material and Method

Reagents and Chemicals

"Chemicals, solvents and reagents were purchased from the Sigma-Aldrich Chemical Co., Merck Chemical, Finar and Spectrochem Ltd. All the chemicals were used without further purification unless and until it required. Thin-layer chromatography (TLC) was accomplished on 0.2 mm precoated plates of silica gel G60 F254 (Merck). Visualization was made under UV light (254 and 365nm) or with an iodine vapor. IR spectra were recorded on an IR Affinity-1S spectrophotometer (Shimadzu). ^{1}H NMR (400 MHz) and ^{13}C NMR (101.1 MHz) spectra were recorded on a Bruker AVANCE II spectrometer in DMSO-d_6. Chemical shifts are expressed in δ ppm downfield from TMS as an internal standard. Mass spectra were determined using a direct inlet probe on a GCMS-QP 2010 mass spectrometer (Shimadzu). Solvents were evaporated using a Medica rotary evaporator. Melting points were measured in open capillaries tube."

General procedure

Synthesis of N - (1- (substitutedphenyl) -2- ((2-morpholinoethyl) amino) -2-oxoethyl) -2- (3, 4-di fluorophenyl) -N- (p-tolyl) cyclopropane -1- carboxamide (5a-5h)

In a polar solvent (methanol), add 2-(3,4-difluoro-phenyl)cyclopropane-1-carboxylic acid (2.50 gm, 0.012 mole), *p*-toluidine (1.35 gm, 0.012 mole), aromatic aldehyde (0.012 mole) and 4-(2-isocya-noethyl)morpholine (2.65 gm, 0.018 mole) were taken and stirred it at room temperature (RT) for 12.0 hrs in the presence of N_2 atmosphere and under anhydrous condition (Anhd. $CaCl_2$). Completion of the reaction was monitored by TLC using three different mobile

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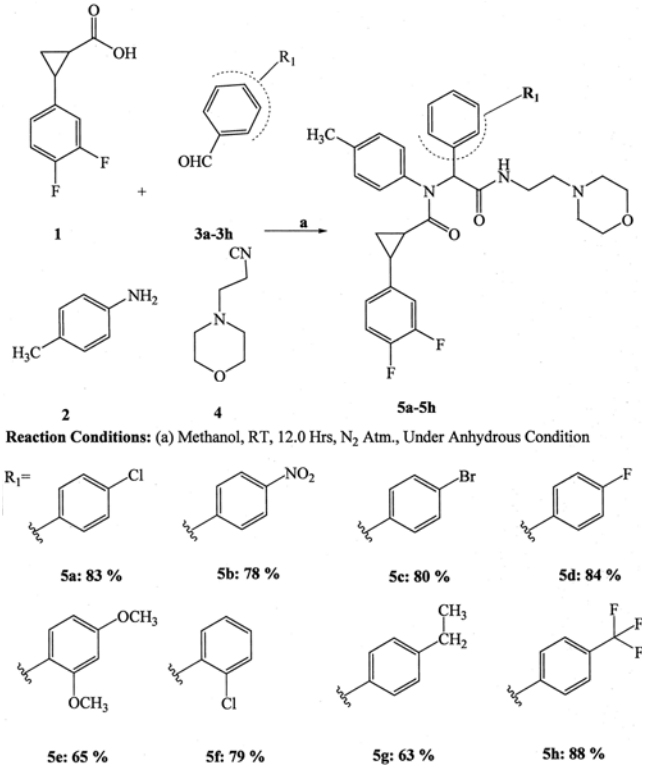
phase system to identified product spot in comparison to starting material due to minor difference in Rf values.

After completion of the reaction, the reaction mixture was poured onto crushed ice water and stirred at RT for 12.0 hrs. It was filtered, washed with distilled water and dried using a vacuum dryer. Purification of the final product was carried out by silica gel column chromatography (60-120 mesh) by using ethyl acetate and hexane as mobile phase. Recrystallization of the dried product was carried out using ethanol: DMF (9:1) mixture in order to receive an analytically pure product (Reaction Scheme 1). All the compounds of the series 5a-5h were synthesized according to the above cited method and structural confirmations were done by spectral analysis.

N - (1-(4-Chlorophenyl) -2- ((2-morpholinoethyl) amino) -2-oxoethyl) -2- (3,4-difluorophenyl) -N- (p-tolyl)cyclopropane -1-carboxamide (5a)

Yield: 85 %; mp 282 °C; IR (cm^{-1}): 3342.64 (N-H stretching), 3028.84 (Aromatic C-H stretching), 2930 (Aliphatic C-H stretching), 1725.35 (Ketonic group), 1624.06, 1589.34, 1470.25 (Aromatic ring skeleton), 1359.82 (Aliphatic C-H bending), 1320 (C-N bending), 1085.92 (C-O-C Stretching), 827.46 (*p*-substituted aromatic ring), 771.53 (C-Cl stretching); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.63 (s, 1H), 7.88 (d, 2H), 7.76 (d, 2H), 7.50 (d, 1H), 7.41 (d, 1H), 7.34 (d, 1H), 7.21 (d, 2H), 6.90 (d, 2H), 4.35 (s, 1H), 3.23 (t, 2H), 2.77 (t, 2H), 2.65 (t, 4H), 2.35 (m, 1H), 2.13 (s, 3H), 1.82 (q, 1H), 1.12 (d, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.16, 159.12, 148.25, 135.29, 130.87, 128.35, 127.01, 125.95, 121.09, 119.12, 110.30, 68.42, 58.56, 53.90, 39.23, 27.71; MS: *m/z* 568 (M^+); Elemental analysis of $C_{31}H_{32}ClF_2N_3O_3$: Calculated= C, 65.55; H, 5.68; Cl, 6.24; F, 6.69; N, 7.40; O, 8.45 and Found= C, 65.56; H, 5.70; Cl, 6.26; F, 6.66; N, 7.38; O, 8.44.



Reaction Scheme 1: Synthesis of multi-component ugi adducts (5a-5h)

2 - (3,4-Difluorophenyl) – N - (2 - ((2-morpholinoethyl) amino) -1-(4-nitrophenyl) -2- oxoethyl) -N- (p-tolyl)cyclopropane -1-carboxamide (5b)

Yield: 89 %; mp 270 °C; IR (cm^{-1}): 3340.58 (N-H stretching), 3032.88 (Aromatic C-H stretching), 2919.01 (Aliphatic C-H stretching), 1715.12 (Ketonic group), 1625.16, 1581.55, 1480.56 (Aromatic ring skeleton), 1356.58 (Aliphatic C-H bending), 1350.11 (C-N bending), 1082.75 (C-O-C Stretching), 831.40 (*p*-substituted aromatic ring), 682.80 (C-F stretching); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.65 (s, 1H,), 7.78 (d, 2H), 7.69(d, 2H), 7.49 (d, 1H), 7.35 (d, 1H), 7.29 (d, 1H), 7.17 (d, 2H), 6.89 (d, 2H), 4.28 (s, 1H), 3.68 (t, 2H), 2.84 (t, 2H), 2.71 (t, 4H), 2.41 (m, 1H), 2.10 (s, 3H), 1.80 (q, 1H), 1.24 (d, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.87, 169.45, 158.12, 144.98, 135.26, 132.45, 129.58, 126.10, 118.78, 114.65, 67.54, 59.56, 54.89, 38.21, 27.12; MS: *m/z* 579 (M^+); Elemental analysis of $C_{31}H_{32}F_2N_4O_5$: Calculated= 84C, 64.35; H, 5.57; F, 6.57; N, 9.68; O, 13.83 and Found= C, 64.34; H, 5.54; F, 6.58; N, 9.69; O, 13.

N - (1-(4-Bromophenyl) -2- ((2-morpholinoethyl) amino) -2-oxoethyl) -2- (3,4-difluorophenyl) -N- (p-tolyl)cyclopropane -1-carboxamide (5c)

Yield: 87 %; mp 274 °C; IR (cm^{-1}): 3350.04 (N-H stretching), 3020.11 (Aromatic C-H stretching), 2929.59 (Aliphatic C-H stretching), 1720.99 (Ketonic group), 1625.59, 1585.09, 1481.22 (Aromatic ring skeleton), 1350.80 (Aliphatic C-H bending), 1328.55 (C-N bending), 1080.64 (C-O-C Stretching), 835.50 (*p*-substituted aromatic ring), 570.07 (C-Br stretching); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.88 (s, 1H,), 7.87 (d, 2H), 7.72(d, 2H), 7.69 (d, 1H), 7.51 (d, 1H), 7.42 (d, 1H), 7.35 (d, 2H), 7.04 (d, 2H), 4.46 (s, 1H), 3.71 (t, 2H), 2.89 (t, 2H), 2.78 (t, 4H), 2.54 (m, 1H), 2.21 (s, 3H), 1.94 (q, 1H), 1.32 (d, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.12, 159.78, 149.54, 140.98, 136.12, 132.98, 129.91, 122.02,116.93, 67.23, 60.82, 58.20, 52.69, 39.01, 20.85; MS: *m/z* 613 (M^+); Elemental analysis of $C_{31}H_{32}BrF_2N_3O_3$: Calculated= C, 60.79; H,

5.27; Br, 13.05; F, 6.20; N, 6.86; O, 7.84 and Found= C, 60.75; H, 5.28; Br, 13.06; F, 6.22; N, 6.85; O, 7.84.

2 - (3,4-Difluorophenyl) -N- (1-(4-fluorophenyl) -2- ((2-morpholinoethyl) amino) -2- oxoethyl) -N- (p-tolyl)cyclopropane -1- carboxamide (5d)

Yield: 80 %; mp 261 °C; IR (cm^{-1}): 3350.03 (N-H stretching), 3020.56 (Aromatic C-H stretching), 2950.21 (Aliphatic C-H stretching), 1726.93 (Ketonic group), 1623.79, 1585.01, 1475.08 (Aromatic ring skeleton), 1358.45 (Aliphatic C-H bending), 1356.21 (C-N bending), 1059.78 (C-O-C Stretching), 826.89 (*p*-substituted aromatic ring), 751.96 (C-F stretching); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.78 (s, 1H,), 7.74 (d, 2H), 7.62 (d, 2H), 7.59 (d, 1H), 7.46 (d, 1H), 7.32 (d, 1H), 7.29 (d, 2H), 7.10 (d, 2H), 4.23 (s, 1H), 3.67 (t, 2H), 2.74 (t, 2H), 2.68 (t, 4H), 2.42 (m, 1H), 2.11 (s, 3H), 1.75 (q, 1H), 1.09 (d, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.98, 163.83, 157.13, 148.09, 136.45, 133.59, 131.27, 130.43, 124.75, 121.06, 117.30, 114.08, 67.89, 56.23, 34.95, 28.80, 15.69; MS: *m/z* 552 (M^+); Elemental analysis of $C_{31}H_{32}F_3N_3O_3$: Calculated= C, 67.50; H, 5.85; F, 10.33; N, 7.62; O, 8.70 and Found= C, 67.53; H, 5.83; F, 10.32; N, 7.60; O, 8.72.

Anticancer screening methodology (NIH/NCI)

"Anticancer screening was accomplished at the National Cancer Institute, a division of the National Institute of Health (NCI/NIH), USA (https://dtp.cancer.gov/compsub/news.xhtml). The human tumor cell lines of the cancer screening panel were grown-up in RPMI 1640 medium having 5% fetal bovine serum and 2 mM L-glutamine, inoculated into 96 well microtiter plates. The microtiter plates are incubated at standard conditions at 37 °C, 5 % CO_2, 95 % air and 100 % relative humidity for 24 hours. Experimental molecules were solubilized in DMSO solvent at 400-fold to find out the desired final maximum test concentration. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 µg/ml of gentamicin. An additional four, 10-fold or ½ log serial dilutions were made to provide a total of five drug concentrations plus control. Aliquots of 100 µl of these different drug dilutions were added to the appropriate microtiter wells already containing 100 µl of medium, resulting in the required final drug concentrations. The plates were incubated for an additional 48 hours at the same conditions. Addition was carried out in each well by sulforhodamine B (SRB) solution (100 µl) at 0.4 % (w/v) in 1 % acetic acid. After 10 minutes staining, unbound dye was removed by washing with 1 % acetic acid and the plates were air dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515 nm. Growth inhibition of 50 % (GI_{50}) was calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50[8]."

Results and Discussion

General

For the projected work, *N*-((2-morpholinoethylcarbamoyl)(substitutedphenyl)methyl)-2-(3,4-difluorophenyl)-*N*-*p*-tolylcyclopropanecarboxamide analogues were chosen for synthesis and analysis. Thus, eight compounds 5a-5h (Reaction Scheme 1) became the choices for the initial experiments. The overall goal was not the determination of the best conditions for reactions of each substrate, but rather to find a hybrid molecule having a cyclic aliphatic ring indirectly coupled with morpholine base and also a screen that synthesized molecules for their anticancer activity against NCI-60 cell-lines. We are therefore interested in investigating the introduction of the aliphatic system into a morpholine coupled heterocycles to synthesized *N*-((2-morpholinoethylcarbamoyl)(substituted phenyl) methyl)-2-(3,4-difluorophenyl)-*N*-*p*-tolylcyclopropanecarboxamide (5a-5h) with the expectation that the products would be of potential biological interest. Thus, when compound 1 (2-(3,4-difluoro phenyl)cyclopropanecarboxylic acid) react with *p*-toluidine (2), aryl aldehyde (3a-3h) and 4-(2-isocyanoethyl)morpholine (4), a small library of hybrid molecules were synthesized by a well-known Ugi four component condensation reaction (U-4CCR's) (Reaction Scheme 1). It's a combination of four components ***i.e.*** A carboxylic acid, an amine, aldehyde and isocyanide, in a simple and economically viable route by using methanol as a solvent and at room temperature (RT).

Spectroscopic analysis

The structure of synthesized compounds 5a-5h was confirmed on the basis of its spectral data. The IR spectrum of the reaction product showed an absorption band for the amidic >C=O group at 1735 cm^{-1} and the >C=O near to cyclopropane ring show stretching band at 1685 cm^{-1}. The absorption band of secondary –NH gives a signal at 3699 cm^{-1} and absence of primary amine signal confirmed the formation of final adducts. Furthermore, absence of signal near 2210-2260 cm^{-1} confirms the –NC to –NHCO– conversion. The ^{1}H

NMR spectrum displayed no amino protons ($–NH_2$) and no –COOH proton in their respective δppm value, and the presence of a singlet of a chiral proton at δ= 6.0 ppm, assignable to the -NH at 8.45 δppm identified the desired products. Furthermore, the structure assigned for this reaction product was fully supported by its mass spectrum, which showed a molecular ion peak and its fragments peak with their respective abundance ration.

Anti-cancer screening

The anticancer activities of newly synthesized derivatives were evaluated against nine human cancer cell line panels, *i.e.* Leukemia cell line, the non-small lung cancer cell line, the colon cancer cell-lines, CNS cancer cell-lines, melanoma cancer cell-lines, the ovarian cancer cell-lines, the renal cancer cell-lines, the prostate cancer cell lines and the breast cancer cell-line using cisplatin as the reference drug. NCI uses 60 different cell-lines to evaluate single compounds to find a better anti-tumor agent. Out of all derived molecules only five of them (5a, 5b, 5c, 5d, and 5e) were selected for anticancer screening for single dose response study. The anti-tumor results for the hybrids are summarized in Table 1. The biological results from Table 1 suggested that the existence of a chloro- substituent played an important role in the anti-cancer activity of the compounds. It's surprising that the other halogen substituents and most donating groups did not showed better pharmacological enhancement. In all synthesized derivatives, hybrid 5a molecule is having more potent cytotoxic activity against colon cancer panel (HCT-116; GI_{50}= 46.27), prostate cancer panel (PC-3; GI_{50}= 58.99) and breast cancer panel (MDA-MB-468; GI_{50}= 52.78) could be identified as the most potent anti-tumor agent among those studied.

Structural diversity to enhance anti-cancer activity

From the anti-cancer screening, it was summarized that the scaffolds which contains electron withdrawing group gives better biological response against GI_{50} values. The most active compounds (5a) have been shown comparable response against colon cancer, prostate cancer and breast cancer, which may due to the negative resonance effect of –Cl atom. Molecules 5b, 5c, 5d and 5e behaviors were also related to their substituents to address their biological response. The single dose response result of 5a is summarized in Figure 1. Overall, even though only a few compounds were found to exhibit excellent antitumor activities, we could study the tendency of alicyclic hybrid compounds and conduct further medicinal importance on the basis of our current research results.

Application in the field of industry and researchers

Firstly, it performed without any catalyst, under room temperature and using less hazardous solvent. It proceeds in a single step manner and reaction is stereoselective as compared to other multi-component reaction, so it can be transferred for the production purpose. It formulates a huge molecule, generally these types of molecules is formed in multi-step process. But by applying Ugi-4CCR's, we can frame a drug like molecules in the field of pharma industry. Thus, it is strongly recommended for mass production.

Conclusion

In summary, we have successfully applied Ugi 4-CCR's synthetic approach in order to prepare analogs of fluorinated cyclopropane skeletons (5a-5h). We have incorporated acidic terminal of fluorinated cyclopropane, base end of p-toluidine with morpholine ethyl isocyanide as prompt molecule. The *invitro* anti-

Table 1 — Conclusion of anti-cancer activity of selected molecules

Sr. No.	Substituents (R)	Panels and Cell-lines (In the range of GI_{50}: > 70 µg/ml)		
1	–Ph, 4-Cl	Leukemia	#*RPMI-8226*	62.37
		Non-small cell lung cancer	#*HOP-92*	66.27
		Colon cancer	**HCT-116*	46.27
		Prostate cancer	#*PC-3*	58.99
		Breast cancer	**MDA-MB-468*	52.78
			#*T-47D*	67.60
2	–Ph, 4-$NO_2$$	-	-	-
3	–Ph, 4-Br	Non-small cell lung cancer	#*NCI-H522*	68.16
4	–Ph, 4-F$	-	-	-
5	–Ph, 2,5- di OMe$	-	-	-

Note: *: Shows promising single dose response, #: Moderate activity as compared to HCT-116 and $: Very weak anti-cancer activity in which GI_{50} values were above 90.

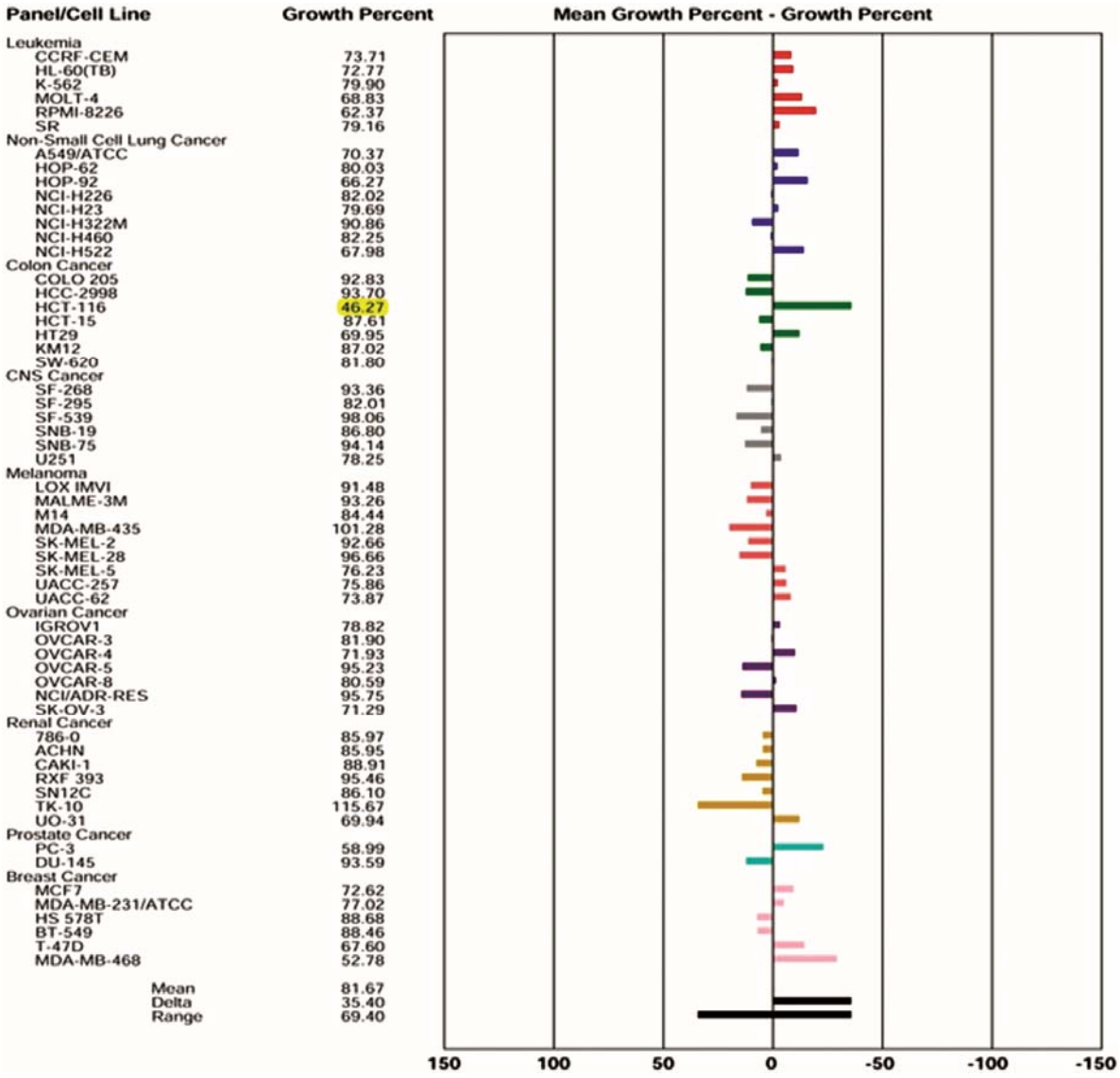


Fig.1 — Anti-cancer study of compound 5a against nine cancer cell panels

cancer investigation of newly synthesized molecules indicates that most of these compounds exhibited significant cytotoxicity with the lowest tested concentration 46.27 µg/ml (5a). In particular, a compound 5a was extremely potent against HCT-116 cell-lines in colon cancer cell panel.

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Conflict of Interest

The authors confirm that this article content has no any conflict of interest.

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