Anticancer Activity of Novel Unnatural Synthetic Isoprenoids

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Abstract. *Background: The KRAS oncogene has a high prevalence in solid malignancies. Targeting KRAS and inappropriate activation of the MAPK pathway with novel drugs is of interest. This study developed and screened a library of compounds designed to inhibit KRAS signaling by altering prenyl function. Materials and Methods: To screen a library of novel farnesyl analogs for their anticancer activity in human lung cancer and breast cancer cell lines. To evaluate if the designed and actual pharmacology are congruent. Results: Sixty-seven novel compounds were tested and 70% of them screened positive for activity in at least one cell line. Two active compounds inhibited phosphorylation of MAP kinase consistent with KRAS inhibition. Conclusion: Although 47 of the 67 novel agents screened positive for activity, none of them were highly potent. However, targeting RAS with compounds that compete with farnesyl and geranylgeranyl modification of the protein remains viable and further work is already underway to create second generation molecules.*

Cancer of the lung and breast are two of the most common and fatal malignancies in the United States (1). Patients who are diagnosed with metastatic disease are rarely cured with systemic therapy (2, 3). The ineffective nature of systemic therapy is associated with the complexity of cancer biology. The most common histology for these tumors is adenocarcinoma, which commonly features a central role for KRAS activation of the MAP kinase (MAPK) signaling cascade (4, 5). Approximately 70% of non-small cell lung cancers have activation of the RAS-MAPK pathway and activating mutations in the *KRAS* oncogene are present in approximately 40% of lung adenocarcinomas (6). Targeting KRAS and the MAPK pathway with new agents is of significant interest and could have a broad spectrum of activity.

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Key Words: RAS protein farnesylation, farnesyl diphosphate analogues, breast cancer, lung cancer.

RAS proteins must be processed through the prenylation pathway for both membrane localization and to participate in cellular transformation (7). Farnesylation is the first and obligatory step in an ordered series of posttranslational modifications that direct this membrane localization and potentially protein-protein interactions (8, 9). FTase inhibitors (FTIs) were developed to target the *RAS* oncogene and have been tested in clinical trials for the treatment of cancer (10-12). Unfortunately, FTI clinical trial results to date have shown poor activity (13-16). These results can be explained by the fact that *KRAS* is geranylgeranylated by the related prenyltransferase, protein geranylgeranyl transferase type I (GGTase-I) (13, 17-19). Both farnesylated and geranylgeranylated KRAS have similar biological functions (20). Cells transformed with *KRAS* are more resistant to FTIs than *HRAS*-transformed cells (17). *KRAS* prenylation is blocked in *KRAS*-transformed cells treated with both an FTI and a GGPTase-I inhibitor (GGTI) (21). However, toxicity associated with GGTI treatment *in vivo* limits the duration of treatment and potential benefits of dual prenyltransferase inhibitor therapy (22).

Lung adenocarcinoma is heterogeneous with diverse combinations of mutations, yet shows strong commonality in affected signaling pathways (5). Therefore, new anti-RAS therapies may be useful against a wide range of these tumors. FPP analogues have been used to study the physical interactions between the lipid, FTase, and the Ca_1a_2X peptide as well as the biological function of the modification (17, 18, 23). The analogue 8-anilinogeranyl diphosphate (AGPP, 2a, Figure 1) is transferable to Ca_1a_2X substrates with apparent steady-state kinetics nearly identical to FPP, and the aniline moiety appears to act as an isostere for the FPP terminal isoprene (24, 25). AGPP has been used to probe the endogenous modification of proteins by FTase and is competitive with FPP *in vitro* and in cell culture (24, 26, 27). FTase catalyzes lipid transfer of a wide range of AGPP analogues to Ca_1a_2X peptides corresponding to the HRAS and *KRAS* Ca_1a_2X motifs. The reactivity of the analogues depends on the Ca_1a_2X sequence as well as the size and shape of the lipid (28, 29). The normal biological function of HRAS in the MAPK signaling cascade is blocked when modified with analogues that are three-to-five orders of

magnitude less hydrophobic than the farnesyl group (30). These observations suggest that unnatural FPP analogues may act as prenyl function inhibitors (PFIs) by blocking important interactions downstream of the *C*-terminal processed H-RAS protein. The unnatural FPP analogues may overcome alternative prenylation by GGTase-I by forming a pharmacological dominant negative analogue modified RAS that is neither functional nor a substrate for GGTase-I.

This paper explores whether *KRAS* can be targeted for cancer therapy with FPP analogues. It is shown that unnatural FPP analogues that are transferable substrates for FTase have activity against human cancer cells in culture. To this end, a library of 67 FPP analogues were examined for cytotoxicity and cytostasis in lung cancer and breast cancer cell lines. The synthesis of these compounds and assessment of their ability to act as alternative substrates for FTase is described elsewhere (24, 25, 30, 31).

Materials and Methods

Cell culture. The A549, MDA-MB-231, MCF-7 and NCI-H460 (H460) cell lines were obtained from the American Type Culture Collection (Manassas, MD, USA) and grown in DMEM supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/ml) /streptomycin (100 µg/ml), 2 mM glutamine and 1 mM sodium pyruvate. All cell culture reagents were purchased from Invitrogen (Carlsbad, CA, USA).

Compounds. Farnesyl diphosphate analogues were synthesized as previously published (24, 25, 31, 32). These compounds were suspended in DMSO and stored at –20˚C prior to being diluted with DMEM plus 10% FBS to a concentration of 10 mM stock solution. Lovastatin was purchased from Sigma. Commercial source paclitaxel was obtained from Mayne Pharma Inc. (Paramus, NJ, USA).

Activity assay. Cells were harvested from exponential phase cultures by 0.05% trypsin, counted, and seeded into 96-well plates. Seeding densities were 7.5×10^3, 1.5×10^4, 7.5×10^3, and 1×10^4 cells per well for H460, MDA-MB-231, A549, and MCF-7, respectively. Cells were allowed to adhere overnight, before being exposed to compounds at seven ½ log dilutions beginning at 100 µM. Cells were then incubated in test-drug for 48 hours. Cell viability was assessed by the colorimetric sulforhodamine B (SRB) assay as previously described (33). In short, after the 48 hour incubation period, cells were fixed with cold 10% w/v trichloroacetic acid (TCA) then stained with 0.4% SRB in 1% v/v acetic acid for 30 minutes. KC4 software (Bio-Tek Instruments, Winooski, VT, USA) was utilized for data analysis. Mean absorbance at 490 nm for each drug concentration was expressed as a percentage of the control untreated well absorbance.

Activity analysis. The growth inhibitory/cytotoxicity action was determined in a two step process. The first step tested agents at a single 100 µM concentration across cell lines described above. Compounds were defined as active if the treated/control percent was less than 32% in one or more of the cell lines. Selected active agents were further characterized with three response parameters; concentration resulting in growth inhibition of 50% (GI_{50}), total growth inhibition (TGI), and the 50% lethal concentration (LC_{50}).

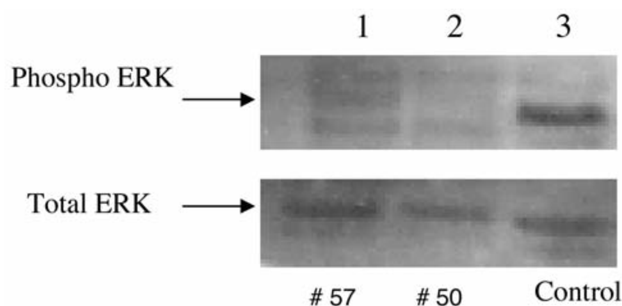


Figure 1. *RAS function assessed via ERK 1/2 phosphorylation in the A549 cell line treated with compounds 50 and 57 for 24 hours with 140 µM.*

These parameters were calculated as previously described (34). Two of the most active compounds were then selected to evaluate the pharmacology *via* MAPK phosphorylation, sensitivity in the presence of lovastatin, and synergy with paclitaxel.

MAPK phosphorylation. Exponentially growing A549 cells were seeded into T-25 flasks and allowed to adhere overnight. The selected compound was added to cells on day zero. After 24 hours cells were harvested, lysed in Laemmli buffer, heated, and stored at –20˚C until analysis by Western blot.

Aliquots containing equal amounts of total cell extract were resolved by a 10% SDS-PAGE gel. Following electrophoresis, the proteins were transferred to a PDVF membrane. The membrane was blocked with 5% BSA in Tris-buffered saline with Tween (TBST) for 1 hour at room temperature. The membrane was incubated in phosphor-p44/42 MAPK antibody (1:1000 dilution; Cell Signaling, Cambridge, MA, USA) and in parallel with p44/42 MAPK antibody (1:2000 dilution; Cell Signaling) in 5%-BSA TBST overnight at 4˚C. The membrane was washed three times for 10 minutes each with TBST and then incubated for 1 hour at room temperature with rabbit-antihuman (1:10,000 dilution; Cell Signaling) conjugated to horseradish peroxidase. After washing the membrane three times with TBST (10 minutes per washing), the proteins were visualized using the ECL detection system (Amersham, Arlington Heights, IL, USA). The bands were quantified using GeneSnap software (Synoptics, Cambridge, UK).

Synergy assays. Synergy studies were performed with paclitaxel and selected active compounds at a 1:10 ratio of drugs (respectively). This is near the ratio of IC_{50}s for the respective compounds. Results were analyzed using CalcuSyn software (Biosoft, Cambridge, UK), which utilizes the median effect method described by Chou and Talalay (35).

Results

The library of 67 novel prenyl function inhibitors was screened against a human non-small cell lung cancer cell line (NCI-H460) that contains oncogenic mutations in *KRAS*, and breast cancer cell lines (MCF-7 or MDA-MB-231). MCF-7 cells are the traditional cell line used to screen agents; however, it is estrogen dependent and has wild-type *RAS*, while the MDA-MB-231 cell line overexpresses the

epidermal growth factor receptor (EGFR) and has mutated *KRAS* (36). Forty-seven of the 67 compounds screened meet the criteria of being active (see Table I). Fourteen of the 47 active agents were further characterized with a full cytoxicity assay. These were perceived to be the most active of the group tested in the initial single concentration screening. By evaluating the cell number at the beginning of the assay (Tz), GI_{50}, TGI, LC_{50} were calculated (Table II). Additionally, selected compounds were screened for activity against a second non-small cell lung cancer cell line (A549) that has mutated *KRAS* and high background phosphorylation of the MAPK pathway. Through this process, compounds 50 and 57 were identified as the selected active compounds.

Active selected compounds were then tested for cytotoxicity with or without the presence of an HMG-Co reductase inhibitor to deplete the endogenous isoprenoid pools. Depletion of native isoprenoids is expected to increase the relative concentrations of the unnatural isoprenoids, making them more effective. However, co-administration of lovastatin at pharmacologically achievable concentrations (1 μmol) did not significantly improve the cytoxic activity of the selected agents (Table III). The lack of enhanced activity suggests that isoprenoid biosynthesis is not completely blocked by lovastatin at this concentration or that the observed cytotoxicity of the compounds is through some other mechanism.

The downstream effects of RAS inhibition were then tested to examine whether treatment with compounds 50 and 57 affected signal transduction downstream of RAS. Phosphorylation of ERK1/2 in A549 cells was decreased after 24 hours of exposure with both compounds (Figure 1). These data support the hypothesis that analogues 50 and 57 act as prenyl function inhibitors.

Farnesyl transferase inhibitors consistently show synergy with taxanes (37). To determine if farnesyl analogues had similar effects, synergy experiments testing compound 53 in combination with paclitaxel were performed in H460 cells and MDA-MB-231 cells. The Chou and Talalay method was used (35), which quantifies the effect of two drugs (synergism, additive effect, and antagonism) by a term known as the combination index (CI). If the CI equals one, then the effects of the agents are additive, if it is less than one they are synergistic, while a CI greater than one indicates antagonism. In these studies, synergy was seen at all concentrations tested, except the lowest, relatively non-toxic concentration and the highest, very toxic concentration (Table IV) (35).

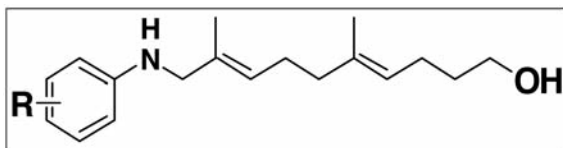
Discussion

Based on the National Cancer Institute developmental therapeutics program (NCI DTP) criteria (34), 47 compounds (70%) examined in this study were considered active. The NCI DTP estimates that approximately 50% of candidates can be removed from further consideration without a significant loss of active compounds by discarding those molecules that fail to achieve a T/C percent cut-off of 32% after a 48 hour treatment of cells with 100 μM compound. The assignment of activity to 70% of the compounds in this library indicated that the library was active. However, characterization of the dose response relationship shows that no highly potent compounds were present. The screening method used in this study varied from the NCI three cell line screening process in that the SF-268 cell line (human glioblastoma cell line) was not screened. Initial screening with lung cancer (H460) and breast cancer (MCF-7) cell lines was consistent with the NCI three cell line screening process. However, the MCF-7 cell line was replaced with the MDA-MB-231 cell line as the study progressed, because the MCF-7 cells are estrogen dependent and have much lower p-MAPK levels and less Ras activation. (38) Since the proposed mechanism of these compounds is to inhibit the RAS-MAPK pathway cell lines were changed to maximize the potential sensitivity to changes in RAS activity. Despite the differences in cell lines employed, the molecules in this library that screened negative, were believed to be truly inactive.

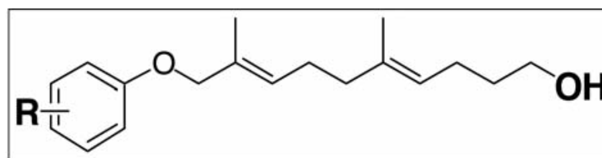
Others have prepared unnatural FPP analogs to prevent prenylation or alter protein prenyl function. Unnatural isoprenoids prepared by Zhou *et al.* have been demonstrated to inhibit FTase or to serve as a alternative FTase substrate based on enzymatic assays (39). To the best of the Authors' knowledge, cytotoxic screening of compounds that are transferable substrates for FTase against human lung cancer and breast cancer cell lines has not been reported. FTIs are another class of drugs that target Ras. Lonafarnib and tipafarnib are FTIs that are in clinical trials and have been tested against human breast and lung cancer cell lines. Preclinical studies with lonafarnib have shown anti-tumor activity against NCI-H460 (ED_{50} 1 μM), A549 (IC_{50} 2 μM), and MCF-7 (IC_{50} 25 nM) (40, 41). Similarly, tipifarnib has shown activity against MCF-7 (IC_{50} 46 nM) and MDA-MB-231 (IC_{50} 5.9 μM) (42). These studies report IC_{50}s comparable or slightly lower than our GI_{50}s in lung cancer, but are significantly more potent against MCF-7 breast cancer cell lines. Although these agents have not reached the evidence needed for FDA approval, they continue to be studied.

The physical properties and rates of *in vitro* FTase catalyzed transfer rates to model Ca_1a_2X peptides show substantial variation for this library (17, 21, 22, 24). Unexpectedly, the cytotoxic activity of these compounds did not vary widely and there were no simple correlations with any of their physical properties, structures or *in vitro* FTase catalyzed transfer rates. A more sophisticated analysis was not performed because of the lack of highly potent isoprenoid analogues in the library, as well as the absence of obvious structure-related activity

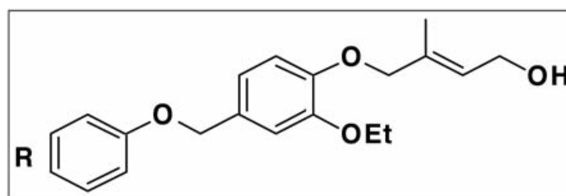
Table I. *Activity and structure of analogs screened. Compounds were screened at 100 μM concentration against human cancer cell lines. The treated/control percentage (T/C %) is reported (+/– standard deviation) for compounds and when the results are greater than 32 in all cell lines tested the compound is considered inactive.*



Compound #	R group	H460	MCF-7	A549
1	R=H	77.7±18.2	61.3±6	n/a
2	R=4-OPh	3.1±1.5	3.4±2.6	5.3±1.2
3	R=3-OCF$_3$	9.6±5.9	3.8±0.5	28.3±6.1
4	R=2-OCF$_3$	14.5±6.6	4.6±1.0	31.7±6.8
5	R=4-OCF$_3$	3.3±2.6	4.4±1.5	26.7±4.7
6	R=4-Br	30.1±5.2	11.7±7.8	30.3±5.0
7	R=3-I	29.9±11.8	16.9±8.2	28.3±3.8
8	R=4-CN	58.4±4.8	42.9±6.6	n/a
9	R=2-Ph	2±1.4	1.5±0.7	25.5±2.1
10	R=2-tBu	33±4.2	16±11.3	31.5±0.7
11	R=2-OMe	93±17	47.5±7.8	32.5±7.8
12	R=2-OPh	10.5±0.7	7.5±6.4	18.0±8.5
13	R=2-Bn	39.5±3.5	18.5±13.4	29.0±1.4
14	R=4-OMe	51.5±9.2	21.5±7.8	29.0±5.7
15	R=2-CF$_3$	83.5±3.5	40±21.2	55.0±1.4
16	R=3-CF$_3$	26±8.5	6.5±7.8	22.0±4.2
17	R=4-Bn	1±0	1±0	14.0±7.1
18	R=3-Cl	33±5.7	17.5±13.4	25.5±4.9
19	R=4-F	85±2.8	36.5±14.8	68.5±0.7
20	R=3-Bn	5.9±1.9	5.7±0.6	41.5±3.5
21	R=3-F	40.3±2.5	28.1±0.5	67.5±20.5
22	R=2-Cl	61.9±0.8	61.4±1.3	94.5±9.2
23	R=3-OPh	5.5±0.1	5.1±1.4	36.0±1.4
24	R=2-F	28.5±0.8	19.5±1.9	58.0±4.2
25	R=3-OMe	37.6±2.4	34.2±1.7	57.5±4.9
26	R=4-iPr	2.6±2.3	6.1±4.0	9.0±11.3
27	R=2-iPr	2.6±2.1	6.1±4.0	33.0±2.8
28	R=2-Me	40.2±1.5	19.7±6.7	64.5±4.9
29	R=3-Me	26.3±0.6	23.2±1.5	44.0±1.4
30	R=4-Me	28.5±2.5	29.5±0.2	46.5±4.9
31	R=4-Ph	109.7±16	110.4±8.6	103.0±4.2
32	4-Cl	4.2±1.9	5.7±1.5	35.0±2.8
33	R=3-CN	26.3	n/a	n/a



Compound #	R group	H460	MDA-MB-231
34	R=4-OPh	6.0±2.6	15.4±6.7
35	R=4-I	26.0±27.6	29.8±24.6
36	R=4-F	32.2±23.7	46.4±5.7
37	R=4-iPr	30.8	n/a
38	R=2,5-Dichloro	23.5±29.4	63.6
39	R=2-Br	43.3±2.1	50.9±2.4
40	R=2-F	32.1±20.6	32.8±26.0
41	R=2-I	53.7	54.1
42	R=4-NO$_2$	20.7±15.8	n/a
43	R=3-F	20.6±3.9	32.2±2.2
44	R=3-CN	25.8	33.8
45	R=4-CN	21.6±6.1	39.0±13.1
46	R=2-CH$_2$OH	73.9	n/a
47	R=3-CH$_2$OH	48.6	n/a
48	R=4-CH$_2$OH	20.9	n/a
49	R=4-Et	14.5	34
50	R=4-OCF$_3$	1.6±1.0	0.7
51	R=3-OCF$_3$	15.1	30.7
52	R=3-Et	15.5	30.5
53	R=4-SCF$_3$	3.6±2.2	2.7±1.7



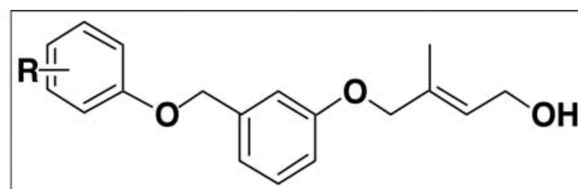
Compound #	R group	H460	MDA-MB-231
54	R=3-F	15.9±3.0	30.5

Table I. continued

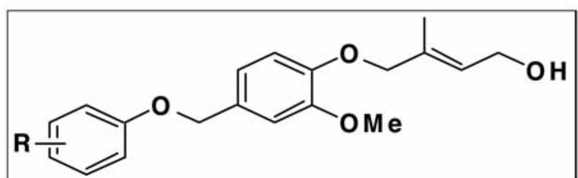
One explanation for the difference in transfer rates and cytotoxic activity may have to do with molecular features facilitating cell entry. The unnatural isoprenoid analogues tested in the current study were prepared as alcohol pro-drugs of the diphosphates intended to facilitate entry of the analogues into cells. This strategy assumes that once in the cell they are converted to the diphosphate form usable by FTase as a substrate. Detection of proteins modified by the unnatural anilinogeranyl group in an FTase-dependent fashion introduced by treatment of cells with AGOH suggest that this is a viable strategy (26, 27, 43-46). The activity of these compounds suggests that the other analogues of AGOH may become phosphorylated once inside the cell. However, this study did not provide definitive proof of conversion to diphosphate nor the rates and determinants of the kinase reactions.

Before moving to a second generation of prenyl function inhibitors, the study sought to determine if the compounds worked as designed. Interestingly, the co-administration of selective active compounds with lovastatin did not result in

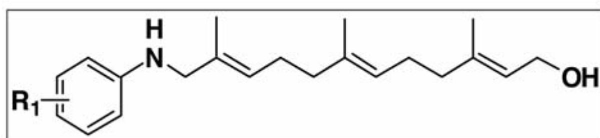
Table I. *continued*



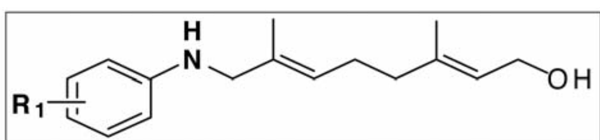
Compound #	R group	H460	MDA-MB-231
55	R=2-F	22	45.9
56	R=3-F	14.7	40.9
57	R=3-F	12.9±5.5	38
58	R=H	14.7	n/a



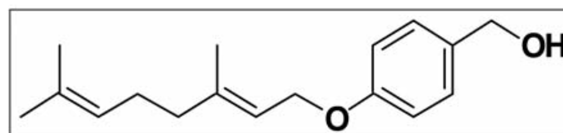
Compound #	R group	H460	MDA-MB-231
59	R=3-F	27	43.9
60	R=4-F	n/a	38.7



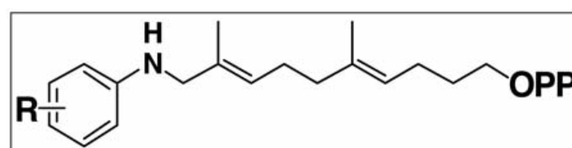
Compound #	R group	H460	MCF-7	A549
61	R1= H	6.4±5.2	1.2±1.4	n/a
62	R1=4-NO_2	6.6±5.0	16.1±3.8	2.7±2.1



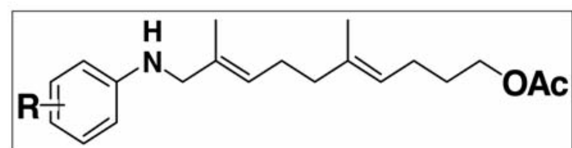
Compound #	R group	H460	MCF-7
63	R1=4-NO_2	46.8±5.3	33±7.6



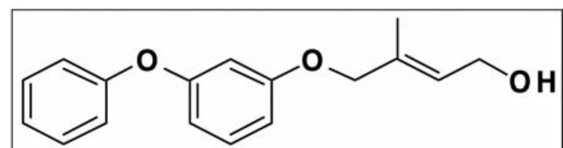
Compound #	R group	H460	MDA-MB-231
64	See above	40.3±7.4	50.2±8.2



Compound #	R group	H460	MDA-MB-231
65	R=H	57.5	68.3



Compound #	R group	H460	MCF-7
66	R=H	57.0±0	75.0±0



Compound #	R group	H460	MDA-MB-231
67	See above	41.1±5.4	64.1±9.3

Table II. *Cytotoxic activity of selected compounds characterized by the concentration required to inhibit growth by 50% (GI50), total growth inhibition (TGI), and the 50% lethal concentration (LC50) in different cell lines.*

H460 cell line

Compound #	GI_{50} (μM±STD)	TGI (μM±STD)	LC_{50} (μM±STD)
2	48.9±19.0	65.0±22.1	82.3±19.6
3	52.2±4.2	77.5±8.5	96.0±4.9
4	72.4±4.3	92.9±8.0	99.4±1.0
5	37.9±17.2	61.2±12.9	84.5±9.8
6	55.3±16.3	>100	>100
7	79.8±7.8	>100	>100
34	20.0±2.4	94.8±7.4	>100
43	59.5±4.2	>100	>100
45	41.4±3.5	>100	>100
50	10±1.0	48.9±8.1	71.2±9.0
53	41.7±15.4	87.7±8.9	95.5±4.3
57	28.8±4.9	>100	>100
61	52.1±6.2	71.3±5.2	88.0±3.2
62	59.6±4.3	79.0±5.9	94.3±5.1

MCF-7 cell line

Compound #	GI_{50} (μM±STD)	TGI (μM±STD)	LC_{50} (μM±STD)
2	24.5±5.2	46.7±15.1	64.8±16.7
3	41.6±7.7	59.7±10.1	79.8±8.4
4	60.1±9.2	76.0±5.6	89.8±2.6
5	21.1±17.8	60.0±14.7	76.4±18.6
6	31.0±4.9	44.8±13.5	62.7±23.3
7	51.1±16.0	76.2±13.1	95.3±7.6
61	24.4±5.5	34.9±8.7	48.9±16.8
62	33.8±10.7	53.5±14.8	84.1±13.3

A549 cell line

Compound #	GI_{50} (μM±STD)	TGI (μM±STD)	LC_{50} (μM±STD)
50	40.4±5.5	98.1±2.2	>100
57	45.0±3.9	>100	>100

MDA-MB-231 cell line

Compound #	Gi_{50} (μM)	TGI (μM)	LC_{50} (μM)
34	35.5±19.9	90.6±13.3	>100
43	68.9±0.6	>100	>100
45	62.0±2.6	>100	>100
53	36.2±9.8	72.9±20.9	85.7±15.6

Table III. *Enhanced effects with lovastatin (concurrent exposure at 1 μmol) in lung (H460) and breast cancer (MDA-MB-231) cell lines.*

H460 cell line	(–) Lovastatin	(+) Lovastatin
Compound # 34		
GI_{50} μM	20.0±2.4	16.7±3.5
TGI μM	94.8±7.4	71.6±5.4
LC_{50} μM	>100	>100
Compound # 43		
GI_{50} μM	59.5±4.2	41.9±2.6
TGI μM	>100	>100
LC_{50} μM	>100	>100
Compound # 45		
GI_{50} μM	41.4±3.5	31.0±0.8
TGI μM	>100	>100
LC_{50} μM	>100	>100
Compound # 53		
GI_{50} μM	41.7±15.4	35.0±14.7
TGI μM	87.7±8.9	87.5±7.8
LC_{50} μM	95.5±4.3	96.8±3.6
Compound # 50		
GI_{50} μM	10.0±1.0	8.5±1.1
TGI μM	48.9±8.1	42.7±8.1
LC_{50} μM	71.2±9.0	65.0±7.6
Compound # 57		
GI_{50} μM	28.8±4.9	23.2±3.3
TGI μM	>100	76.8±21.9
LC_{50} μM	>100	>100

MDA-MB-231 cell line	(–) Lovastatin	(+) Lovastatin
Compound # 34		
GI_{50} μM	35.5±19.9	33.2±20.3
TGI μM	90.6±13.3	84±22.7
LC_{50} μM	>100	>100
Compound # 43		
GI_{50} μM	68.9±0.6	60.2±8.1
TGI μM	>100	>100
LC_{50} μM	>100	>100
Compound # 45		
GI_{50} μM	62.0±2.6	44.4±0.7
TGI μM	>100	>100
LC_{50} μM	>100	>100
Compound # 53		
GI_{50} μM	36.2±9.8	35.4±11.7
TGI μM	72.9±20.9	71.5±25.6
LC_{50} μM	85.7±15.6	81.0±23.0

improved sensitivity. A high, but non-toxic, pharmacologically achievable lovastatin concentration that could be translated into clinical trials was tested (47). Cells were exposed to lovastatin and the study compounds concurrently for approximately two doublings, which should deplete endogenous farnesyl and geranylgernyl pools. However, the native pools of prenyl diphosphates may not have been sufficiently depleted to see an effect. The study compounds do inhibit MAPK phosphorylation, which is consistent with Ras inhibition. High concentrations of farnesol increase MAPK phosphorylation, which is different from the findings of the prenyl alcohols. Although not proven, the Authors believe this library of compounds work by serving as substrates for prenyl transferases, leading to aberrant protein anchoring to the cell membrane and impaired signaling.

Table IV. *Evaluation of synergy with paclitaxel utilizing the median effect model to calculate a combinatorial index (synergy when less than 1, additive at 1, and antagonism if greater than 1).*

Compound # 53 in H460 cell line

Compound # 53 conc. (μM)	Taxol conc. (μM)	Combinatorial index (CI)	Antagonism (–) or synergy (+)
0.14	0.014	81.5±83.2	–
0.41	0.041	0.577±0.23	+
1.2	0.12	0.133±0.01	+
3.7	0.37	0.142±0.03	+
11.1	1.11	0.305±0.12	+
33.3	3.33	0.976±0.19	+
100	10	1.272±0.31	–

Compound # 53 in MDA-MB-231 cell line

Compound # 53 conc. (μM)	Taxol conc. (μM)	Combinatorial index (CI)	Antagonism (–) or synergy (+)
0.14	0.014	92.2±113.2	–
0.41	0.041	0.396±0.04	+
1.2	0.12	0.140±0.06	+
3.7	0.37	0.247±0.14	+
11.1	1.11	0.411±0.32	+
33.3	3.33	0.964±0.67	+
100	10	2.014±2.5	–

In conclusion, the library of prenyl function inhibitors demonstrated activity against human lung and breast cancer cell lines. Experiments designed to shed light on potential mechanisms of action returned ambiguous results. It is unclear if the analogues function as designed due to absent synergy with lovastatin. However, inhibition of the MAPK pathway supports the designed pharmacology. The approach of targeting RAS with compounds that compete with farnesyl and geranylgeranyl diphosphate remains attractive despite the low potency of this current library.

Acknowledgements

This work was supported in part by the Kentucky Lung Cancer Research Program (HPS), the National Institutes of Health (GM66152 to HPS), and the NMR instruments used in this work were obtained with support from NSF CRIF Grant No. CHE-9974810.

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Received April 1, 2010
Revised May 14, 2010
Accepted May 20, 2010