Bioorganic & Medicinal Chemistry Letters 22 (2012) 5707–5713

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design and synthesis of Pictet–Spengler condensation products that exhibit oncogenic-*RAS* synthetic lethality and induce non-apoptotic cell death

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ARTICLE INFO

Article history:
Received 30 May 2012
Revised 22 June 2012
Accepted 25 June 2012
Available online 30 June 2012

Keywords:
Pictet–Spengler
RAS
Synthetic lethal
Structure–activity relationship (SAR)
Non-apoptotic
Tetrahydro-β-carboline

ABSTRACT

A series of Pictet–Spengler condensation derivatives (tetrahydro-β-carbolines) was designed, synthesized and evaluated for lethality against a panel of seven cancer cell lines. Seven compounds (**2a**, **13**, **20**, **21**, **27**, **29** and **34**) showed lethality in at least five cell lines. Among these, compound **27** showed a unique selectivity towards oncogenic-*RAS* expressing BJ-TERT/LT/ST/RAS^{V12} tumor cells, compared to non-transformed BJ-TERT cells. Further investigation revealed that **27** induces cell death without activation of caspases. This represents a useful new probe of non-apoptotic cell death and oncogenic-*RAS* synthetic lethality.

Pictet–Spengler condensation derivatives, known as tetrahydro-β-carbolines, have been investigated as agents for treating human diseases, including cancer. The tricyclic tetrahydro-β-carboline ring structure is a motif in both natural and synthetic cytotoxic compounds that can act through multiple mechanisms.[1] Among the tetrahydro-β-carboline compounds that display potent cytotoxicity against numerous cancer cell lines are harman and norharman derivatives,[2] manzanine,[3] eudistomine K,[4] azatoxin,[5] fascaplysine[6] and picrasidine L.[7]

During the last two decades, there has been a tremendous increase in interest in developing more effective treatment strategies for cancer, as traditional chemotherapeutic agents exhibit toxic side effects and induce drug resistance.[8] In particular, numerous small molecules have been discovered that inhibit specific kinases. Some of these compounds have been successfully developed for treatment of cancers, including Gleevec,[9] Iressa,[10] Tarceva,[11] Tykerb,[12]and Sutent.[13] Chemotherapeutic agents are much sought that selectively target tumor cells while having minimal affect on normal cells, as this property decreases the toxicity associated with treatment and assists in understanding genetic networks governing tumors. The rat sarcoma (*RAS*) oncogenes were discovered over three decades ago with the ability to immortalize mammalian cells.[14] The *RAS* oncogenes are mutated in 25% of all human tumors, and so are attractive targets for anti-cancer agents. Despite extensive efforts, there are currently no treatments that directly target Ras proteins. Instead, several groups, including ours, have focused on identifying compounds that selectively kill tumor cells expressing oncogenic-*RAS* genes. Increased lethality in the presence of oncogenic-*RAS* is termed synthetic lethality.[15] We have been interested in identifying compounds that induce lethality selectively in cancer cells with defined genetic changes. Examples of such small molecules include erastin (EC_{50} (BJ-TERT) = 23.7 μM; EC_{50} (BJ-TERT/LT/ST/RAS^{V12}) = 3.9 μM)[16] and RSL3 (EC_{50} (BJ-TERT) = 2.8 μM; EC_{50} (BJ-TERT/LT/ST/RAS^{V12}) = 0.365 μM),[17] both of which are synthetically lethal with oncogenic-*RAS* genes (*HRAS*, *KRAS* and *NRAS*).

Recently, we identified an inhibitor, which we named MEL24, of the oncoprotein complex Mdm2–MdmX.[18] MEL24 is selectively lethal to immortalized cancer cells and induces cell death by stabilizing the tumor-suppressor p53. Considering that both MEL24 and RSL3 contain the privileged 1,2,3,4-tetrahydro-β-carboline moiety, but quite different substitution patterns, we wondered whether it would be possible to transform the MEL24 scaffold from an Mdm2–MdmX inhibitor to a oncogenic-*RAS* selective lethal compound, by altering the substitution around the tetrahydro-β-carboline moiety. Towards this end, we designed and synthesized novel tetrahydro-β-carboline derivatives including: (i) 5-(2,9-dihydro-1*H*-pyrido[3,4-*b*]indol-1-yl)-1,3-dimethylpyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (semi-oxidized) and 1,3-dimethyl-5-(9*H*-pyrido[3,4-*b*]indol-1-yl) pyrimidine-

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http://dx.doi.org/10.1016/j.bmcl.2012.06.077

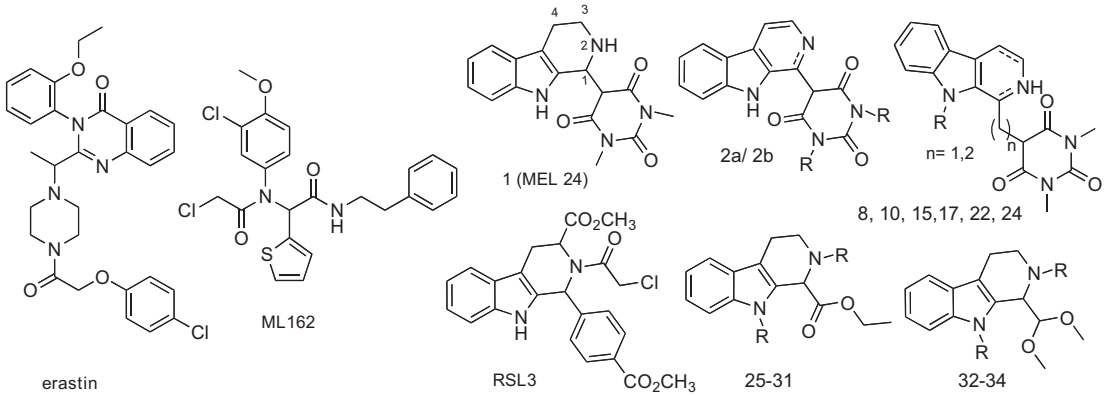
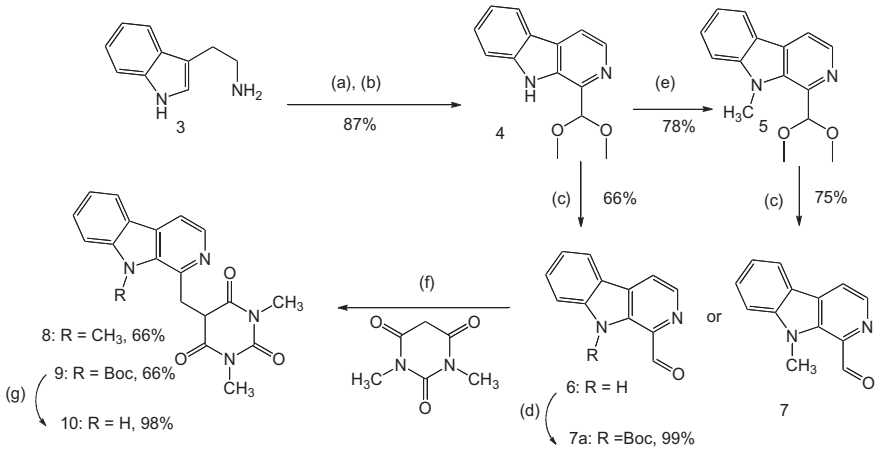


Figure 1. Structures of small molecules explored in this study.



Scheme 1. Synthesis of barbituric acid analogs 8 and 10 linked through 1 carbon spacer, (a) $OHCCH(OMe)_2$, 5% TFA, CH_2Cl_2, rt, 17 h. (b) $KMnO_4$, THF, rt, 17 h. (c) $AcOH/H_2O$ (2:3 ratio), 120 °C, 1 h. (d) $(Boc)_2O$, DMAP, THF, rt, 17 h. (e) CH_3I, NaH, DMF, rt, 4 h. (f) Diludine, proline (10 mol %), CH_3CN, rt, 48 h. (g): HCl 4.0 M, dioxane, 80 °C, 17 h.

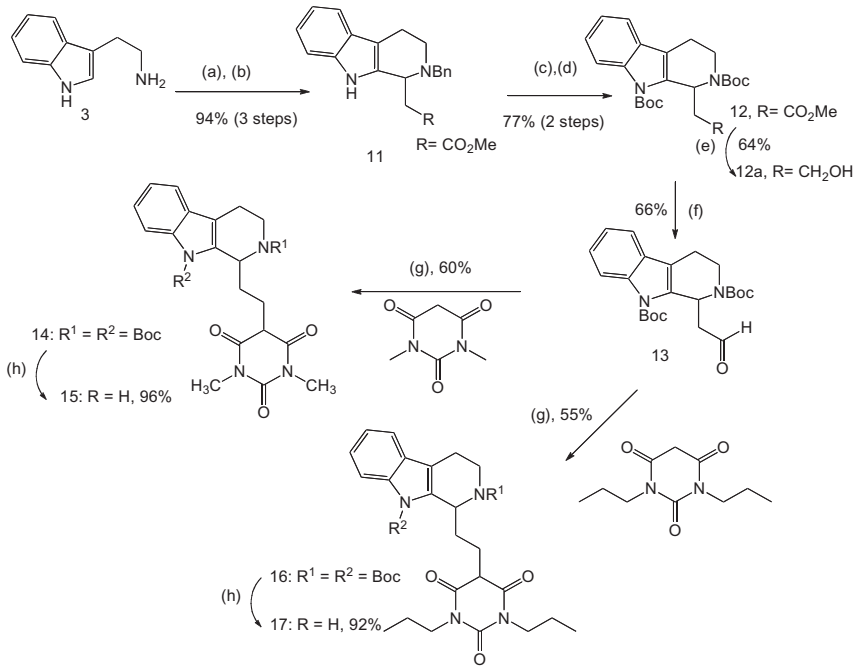
2,4,6(1*H*,3*H*,5*H*)-trione (fully oxidized) analogs with a barbituric acid moiety at the 1-position (Fig. 1, compounds **2a**/**2b**); (ii) those with carbon spacers between the tetrahydro-1*H*-pyrido[3,4-*b*]indole (tetrahydro-β-carboline) and the barbituric acid moiety, in order to improve their stability and avoid rearrangement and opening of the tricyclic moiety upon protonation of the secondary amine, thus reducing acid lability (Fig. 1, compounds **8**, **10**, **15**, **17**, **22** and **24**) and (iii) tetrahydro-1*H*-pyrido[3,4-*b*]indole (tetrahydro-β-carboline) analogs without a barbituric acid moiety (Fig. 1, compounds **25–31** and **32–34**).

Below, we present the results of these studies, which led to the discovery of a small molecule that (i) exhibits *RAS* synthetic lethality similar to RSL3, but without acting as a covalent inhibitor, and (ii) induces cell death without activation of caspases. This new compound was discovered through the synthesis of a new series of Pictet–Spengler condensation derivatives, analysis of their structure–activity relationships (SAR), and evaluation of their lethal mechanisms in a panel of seven cancer cell lines.

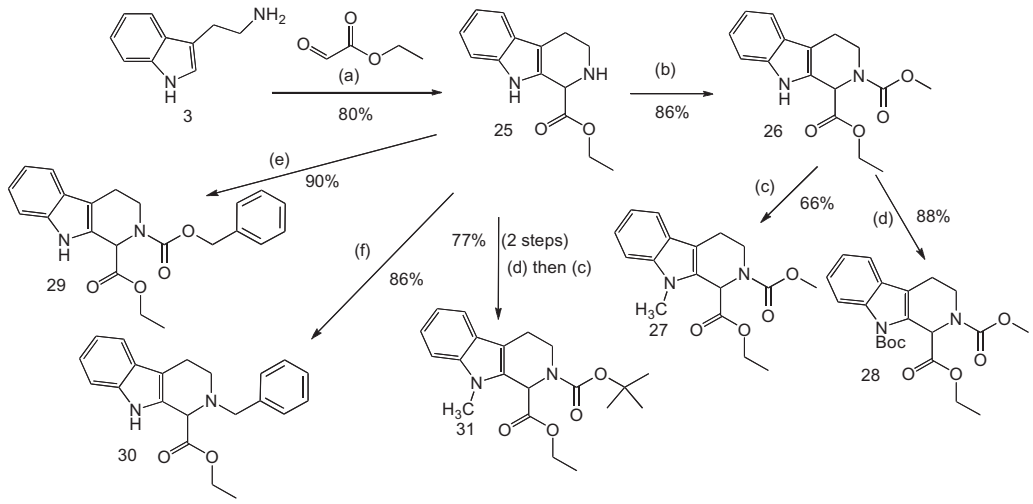
We were interested in understanding the effect of saturation of the β-carboline nucleus on activity. We started our investigation by oxidizing MEL24 (compound **1**, Fig. 1) in DMF in the presence of $KMnO_4$. We isolated the semi-oxidized compound **2a** and the fully oxidized compound **2b** in modest yields (25% and 36% yields, respectively) (see Supplementary Scheme 1).

Next, the fully unsaturated β-carbolines **8** and **10** were synthesized in five and six steps, respectively (Scheme 1). Our synthetic strategy (Scheme 1) started with the Pictet–Spengler reaction of tryptophan **3** with dimethoxyglyoxal (60% solution in water) to afford the tetrahydro-β-carboline derivative, which was oxidized with $KMnO_4$ at room temperature overnight, resulting in acetal **4** (87%, in 2 steps).[19] Compound **4** was methylated in DMF in the presence of NaH and methyl iodide to generate acetal analog **5** in 78% yield. The deprotection of both acetals (**4** and **5**) in aqueous acetic acid gave aldehydes **6** and **7** in 66% and 75% yield, respectively. The indole nitrogen of aldehyde **6** was protected using di-*tert*-butyl dicarbonate $(Boc)_2O$ and 4-(dimethylamino)pyridine (DMAP) to generate compound **7a** in quantitative yield (99%). Aldehydes **7** and **7a** were coupled with barbituric acid using proline as a catalyst and diludine (Hantzsch Ester) as a source of hydrogen to provide the desired products **8** and **9** in 60% and 55% yields, respectively. Finally, treatment of compound **9** with dilute HCl in dioxane gave the desired compound **10** in quantitative yield (Scheme 1).

Previous studies suggested that both the tetrahydro-β-carboline and barbituric acid moieties are important for the activity of MEL 24.[18] Krasnov and coworkers reported a possible rearrangement (in the presence of proton source) between the 1,2,3,4-tetrahydro-β-carboline and barbituric acid moieties, accompanied by opening of the tetrahydropyridine ring.[20] Thus, new analogs with a carbon spacer between the tetrahydro-β-carboline and the barbituric acid moieties were designed in order to avoid this rearrangement. The synthesis of tetrahydro-β-carbolines **15** and **17** is shown in Scheme 2. We prepared 1,2,3,4-tetrahydro-β-carboline **11** in three steps,[21] as follows: tryptamine **3** was converted into its N-protected derivative through a reductive amination (benzaldehyde,



Scheme 2. Synthesis of barbituric acid analogs 15 and 17 linked through 2 carbon spacer, (a): C_6H_5CHO, toluene, then $NaBH_4$, MeOH, rt, 17 h. (b) $HCCCO_2Me$, MeOH then 5% TFA, CH_2Cl_2, rt, 17 h. (c) H_2, Pd/C, AcOH, 120 °C, 1 h. (d) $(Boc)_2O$, DMAP, THF, rt, 17 h. (e) Dibal-H, THF, −78 °C to rt, 17 h. (f) DMP, $NaHCO_3$, CH_2Cl_2, 2 h, rt, 17 h. (g) Diludine, proline (10 mol %), CH_3CN, rt, 48 h. (h): HCl 4.0 M, dioxane, 80 °C, 17 h.

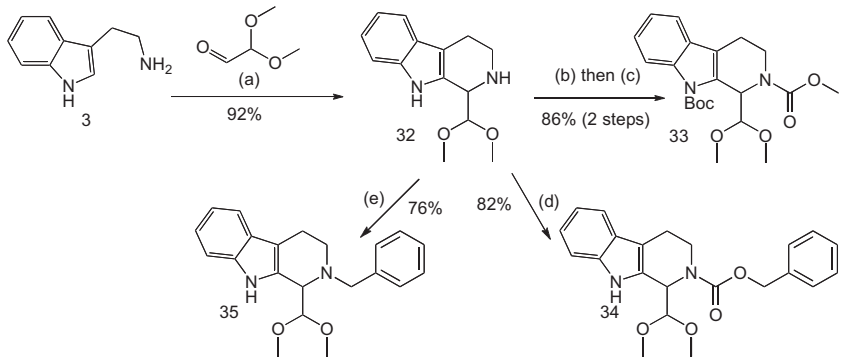


Scheme 3. Synthesis of tetrahydro-β-carboline analogs **25**–**31**, (a): CH_2Cl_2, 45 °C, 4 h, then 5% TFA at −78 °C to rt, 17 h. (b) DIPEA, methyl chloroformate, CH_2Cl_2, rt, 17 h. (c) CH_3I, NaH, DMF, rt, 17 h. (d) $(Boc)_2O$ (1 equiv), DMAP, THF, rt, 17 h. (e) DIPEA, benzyl chloroformate, CH_2Cl_2, rt, 17 h. (f) BnBr, NaH, DMF, rt, 17 h.

$NaBH_4$) in two steps, followed by Pictet–Spengler cyclization of the N-protected tryptamine with methyl propiolate in the presence of a catalytic amount of trifluoroacetic acid (TFA) at room temperature, providing tetrahydro-β-carboline **11** in 94% yield (3 steps). Protecting the two nitrogens of **11** was necessary in order to obtain aldehyde **13** in good yield. Thus, aldehyde **13** was prepared in four steps starting from **11**: hydrogenolysis of the benzyl group in the presence of Pd/C followed by protection of the two nitrogens of tetrahydro-β-carboline **11** using $(Boc)_2O$/DMAP (77% yield, 2 steps). Ester **11** was reduced to the primary alcohol **12a**, in the presence of diisobutylaluminum hydride (2.2 equiv of DIBAL-H, 64% yield), which was oxidized to the desired aldehyde **13** using the Dess–Martin reagent (66% yield) (Scheme 2).

Having aldehyde **13** in hand, we decided to explore the Knoevenagel condensation reaction with barbituric acids, followed by reduction of the double bond in the presence of proline as a catalyst, and the Hantzsch Ester as a source of hydrogen. The desired N-Boc protected products **14** and **16** were isolated in 60% and 55% yields, respectively. Finally, treatment of **14** and **16** with HCl 4.0 N in dioxane under reflux gave the final compounds **15** and **17** in 96% and 92% yields, respectively (Scheme 2). Compounds **22** and **24** (see Supplementary Scheme 2), which have a branched spacer, were successfully prepared in 77% and 75% yields, respectively, using an analogous synthetic route (Scheme 2).

Previously, study of MEL24 analogs revealed that both the tetrahydro-β-carboline and the barbituric acid moieties were necessary to inhibit the Mdm2–MdmX complex. We synthesized 1,2,3,4-tetrahydroisoquinone derivatives without the barbituric acid moiety (Scheme 3) in order to test the notion that this could eliminate the Mdm2–MdmX-targeting activity, but still allow for anti-tumor



Scheme 4. Synthesis of tetrahydro-β-carboline analogs **33**–**35**, (a) glyoxal dimethyl acetal, 60% aqueous solution, CH_2Cl_2, 45 °C, 4 h, then 5% TFA at −78 °C to rt, 17 h. (b) Methyl chloroformate, DIPEA, CH_2Cl_2, rt, 17 h. (c) $(Boc)_2O$ (1 equiv), DMAP, THF, rt, 17 h. (d) DIPEA, benzyl chloroformate, CH_2Cl_2, rt, 17 h. (e) BnBr, K_2CO_3, DMF, rt, 17 h.

Table 1
Lethality of compounds (LC_{50}, μM)

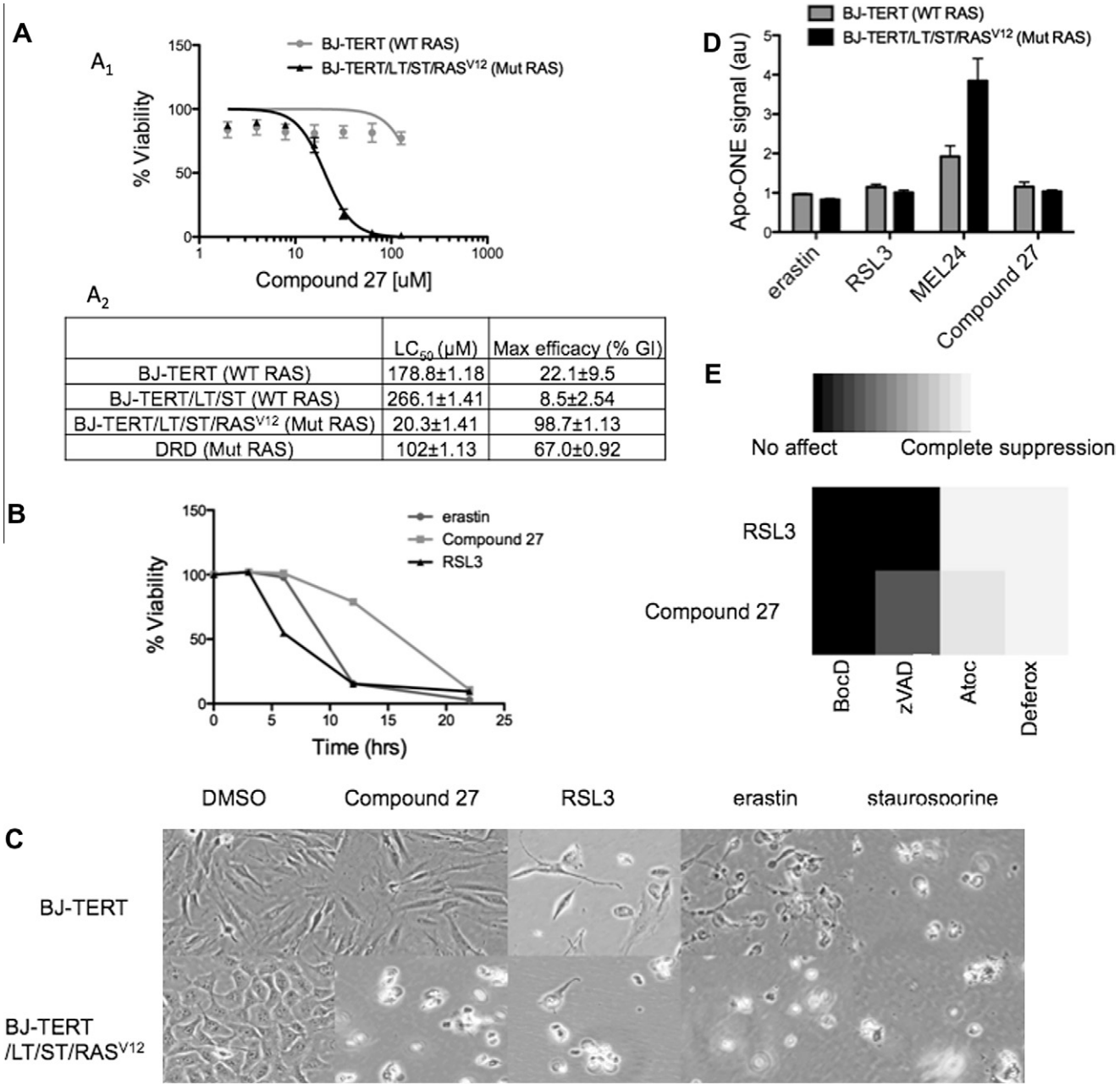
Compound	HT1080	RKO	MCF7	U2OS	HT1299	BJ-TERT	BJ-TERT/LT/ST/RAS^{V12}
1 (MEL24)	16.5	30.9	31.2	29.4	32.8	32.2	23.3
2a[a]	16.0	25.0	61.7	—	61.7	—	12.9
2b[a]	—	—	—	—	—	—	—
4	—	—	—	—	—	82.6	7.8
6	67.5	11.1	—	—	67.5	—	32.4
8	57.1	15.4	57.1	14.2	57.5	—	—
9	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—
11	—	—	—	—	—	—	59.8
12a	—	49.7	—	49.7	—	—	—
13	15.2	7.0	7.0	10.3	11.8	48.3	23.4
15	—	—	—	—	—	—	—
17	—	—	—	—	—	—	—
18[a]	—	—	—	—	—	73.5	31.6
18a[a]	53.7	27.9	23.6	53.7	53.7	—	—
18b[a]	46.0	16.0	22.1	17.8	24.8	60.66	—
19[a]	25.2	9.9	9.5	14.1	42.3	—	—
20[a]	13.7	7.7	11.4	10.0	46.7	—	46.7
21[a]	14.6	11.4	9.8	12.1	16.9	—	35.2
21a[a]	—	—	—	—	—	—	42.7
22[a]	—	54.3	—	54.3	—	54.3	54.3
24[a]	—	—	—	—	—	—	—
25	81.9	—	—	81.9	—	71.7	71.7
26	—	—	—	—	—	—	—
27	63.2	31.6	—	63.2	30.0	63.2	6.3
28	—	—	—	—	—	—	—
29	52.9	14.8	52.9	12.9	52.9	52.9	12.9
30	—	—	—	—	—	—	—
31	—	—	—	—	—	—	—
32	—	—	—	—	—	—	81.3
33	—	—	—	48.7	—	—	—
34	52.6	22.3	6.5	10.2	52.6	52.6	10.2
35	59.5	59.5	59.5	59.5	—	—	—

Breast cancer (MCF7), fibrosarcoma (HT1080), osteosarcoma (U2OS), colon cancer (RKO), lung cancer (H1299) and a pair of engineered isogenic cell lines, BJeH and BJeLR, were treated with compound in a 2-fold, 5-point dilution series starting at 20 μg/mL. '—' represents no activity up to 20 μg/mL.

[a] See the Supplementary data for the synthesis of this compound.

activity through other mechanisms making use of the tetrahydro-β-carboline moiety. Pictet–Spengler cyclization of tryptamine **3** with ethyl glyoxalate provided the ethyl ester tetrahydro-β-carboline **25** in 80% yield, which was reacted with methyl chloroformate in the presence of DIPEA to provide the N-formate **26** in 86% yield. The indole nitrogen of compound **26** was protected by methylation (**27**) or with Boc (**28**) in 66% and 88% yields, respectively. Tetrahydro-β-carboline **25** was reacted with chlorobenzylformate/DIPEA or benzyl bromide/K_2CO_3, respectively, to provide compounds **29** and **30** in 90% and 86%, respectively. Finally, tetrahydro-β-carboline **31** was prepared by selective protection of the secondary amine using $(Boc)_2O$/DMAP followed by methylation of the indole nitrogen in 77% yield (2 steps).

In order to test a variety of tetrahydro-β-carboline analogs, we also synthesized analogs bearing an acetal in the one position instead of an ester (Scheme 4). Once again, Pictet–Spengler cyclization of tryptamine **3** with aqueous glyoxal dimethyl acetal provided the ethyl ester tetrahydro-β-carboline derivative **32** in 92% yield. The secondary amine of **32** was reacted with methylchloroformate in the presence of diisopropyethylamine (DIPEA) followed by Boc protection of the indole nitrogen in the presence of dimethylaminopyridine (DMAP), providing compound **33** in 86% (2 steps). Similarly, compound **34** was obtained in 82% yield by the reaction of the tetrahydro-β-carboline **32** with benzylchloroformate in the presence of diisopropyethylamine (DIPEA). Finally, compound **35** was successfully prepared in 76% yield by



	LC_{50} (μM)	Max efficacy (% GI)
BJ-TERT (WT RAS)	178.8±1.18	22.1±9.5
BJ-TERT/LT/ST (WT RAS)	266.1±1.41	8.5±2.54
BJ-TERT/LT/ST/RAS^{V12} (Mut RAS)	20.3±1.41	98.7±1.13
DRD (Mut RAS)	102±1.13	67.0±0.92

Figure 2. Compound **27** exhibits RAS synthetic lethality and induces non-apoptotic cell death.[24]

reaction of **32** with benzylbromide in the presence of potassium carbonate.

All compounds were tested for their Mdm2–MdmX inhibitory activity.[18] As predicted, none of the compounds showed any inhibitory activity (Supplementary Fig. 1). In order to determine the potential anti-cancer activity of these Pictet–Spengler condensation derivatives through other mechanisms, all compounds were tested in a 2-fold, 12-point dilution series starting at 20 μg/mL in seven cell lines. The cell lines used represent breast cancer (MCF7), fibrosarcoma (HT-1080), osteosarcoma (U2OS), colon cancer (RKO), lung cancer (H1299), and a pair of engineered isogenic cell lines, BJ-TERT and BJ-TERT/LT/ST/RAS^{V12} (Table 1). Cells were treated with each compound for 48 h and viability determined with Alamar blue. Among the seven cell lines, the original compound **1** (bearing a barbituric acid at the C1 position without spacer) showed only modest activity in HT-1080 (LC_{50} = 16.5 μM), BJ-TERT/LT/ST/RAS^{V12} (LC_{50} = 23.3 μM), U2OS (LC_{50} = 29.4 μM), RKO (LC_{50} = 30.9 μM), MCF7 (LC_{50} = 31.2 μM), BJ-TERT (LC_{50} = 32.2 μM), H1299 (LC_{50} = 32.8 μM). On the other hand, compound **2a** (the semi-oxidized version of **1**) was two-fold more lethal in BJ-TERT/LT/ST/RAS^{V12} (LC_{50} = 12.9 μM). Most analogs containing a carbon spacer between the tetrahydro-β-carboline and barbituric acid moieties were not active or only weakly active and not selective in most of the cell lines. For example, compound **8** with an *N*-methylindole, was slightly more active compared to compound **9** (N-Boc indole) and **10** (NH indole). Analogs with a 2 carbon spacer between the tetrahydro-β-carboline and barbituric acid moieties were inactive (**15**, **17, 21a, 22 and 24**) except for compound **21**, which showed modest to good potency in six cell lines. On the other hand, the Pictet–Spengler condensation analogs without a barbituric acid moiety, such as **18a**, **18b**, **19**, **20**, and **35** showed activity in at least four human cancer cells. Compounds **2a**, **13**, **20**, **21, 27**, **29** and **34** showed lethality in at least five cell lines (Table 1). Among these, compound **27** showed the most potent and

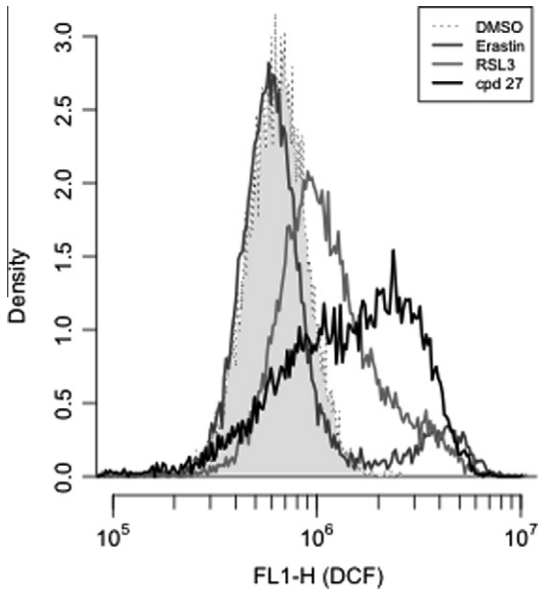


Figure 3. Reactive oxygen species (ROS) levels in BJ-TERT/LT/ST/RAS^{V12} cells.[26]

selective lethality in BJ-TERT/LT/ST/RAS^{V12}, expressing oncogenic-*RAS*, compared to BJ-TERT cells lacking oncogenic-*RAS*. Compound **27**, bearing an ethyl ester instead of a barbituric acid moiety, showed high lethality (four fold enhancement compared with compound **1**) in BJ-TERT/LT/ST/RAS^{V12} (LC_{50} = 6.3 μM, Table 1). From testing in the seven cell lines, compound **27** showed the greatest selectivity towards BJ-TERT/LT/ST/RAS^{V12} and was effective in inducing cell death in six out of seven cell lines. Thus, despite the micromolar potency, we decided to further examine compound **27**'s mechanism of action.

To examine the selectivity of compound **27** in inducing cell death in the presence of oncogenic-*RAS*, four engineered isogenic cell lines from BJ foreskin fibroblasts were used.[16,22] BJ-TERT and BJ-TERT/LT/ST are not transformed with oncogenic-*HRAS*, while BJ-TERT/LT/ST/RAS^{V12} and DRD cells (BJ-TERT/p53DD/CDK4^{R24C}/CyclinD1/ST/RAS^{V12}) contain oncogenic-*HRAS*^{G12V}. Compound **27** exhibited 10-fold selectivity in BJ-TERT/LT/ST/RAS^{V12} cells over BJ-TERT cells, and less (~3-fold) selectivity in DRD cells over BJ-TERT cells (Fig. 2A). Compounds that induce synthetic lethality with oncogenic-*RAS* have been found to induce rapid cell death.[17] Indeed, compound **27**, when treated at the LC_{90}, started to kill BJ-TERT/LT/ST/RAS^{V12} cells within 12 h and complete cell death was observed by 24 h (Fig. 2B), similar to RSL3 and erastin. This cell death was observed only in BJ-TERT/LT/ST/RAS^{V12} cells and not BJ-TERT (Fig. 2C) cells. Staurosporine, an inducer of apoptosis, induced cell death in both BJ-TERT and BJ-TERT/LT/ST/RAS^{V12} (Supplementary Fig. 2). Both RSL3 and erastin induce a form of non-apoptotic, caspase-independent cell death termed *ferroptosis*,[23] so we examined the ability of compound **27** to induce caspase 3/7 activation during cell death (Fig. 2D). Even at the highest concentrations tested, compound **27** induced cell death in BJ-TERT/LT/ST/RAS^{V12} cells, but failed to induced caspase 3/7 activation. On the other hand, MEL24, an established tetrahydro-β-carboline that induces caspase-dependent apoptosis,[18] activated caspase 3/7 in both BJ-TERT and BJ-TERT/LT/ST/RAS^{V12} (Supplementary Fig. 3).

To study the mechanism of action of compound **27**, we tested the ability of several biologically active compounds to suppress the lethality of compound **27**, using our recently reported modulatory profiling approach.[25] Similar to RSL3, compound **27**'s lethality was not inhibited by the caspase inhibitors carbobenzoxy-valyl-alanyl-aspartyl-[*O*-methyl]-fluoromethylketone (z-VAD-fmk) or Boc-Asp(OMe)-FMK (Boc-D-fmk), but was suppressed by antioxidants, iron chelators and MEK1/2 inhibitors (Fig. 2E). This suggests reactive oxygen species (ROS) are involved in **27**-induced cell death. To validate this hypothesis, ROS levels in cells was measured with 2′,7′-dichlorodihydrofluorescein diacetate (DCF) (Fig. 3 and Supplementary Fig. 5). Compound **27**, erastin and RSL3 induced increases in DCF fluorescence before cells died, which indicated that ROS are generated in the course of cell death.

Previously, Weiwer et al.[27] reported that ML162, which contains a chloroacetamide moiety, is an oncogenic-*RAS* selective lethal compound in the BJ cell series (EC_{50} (BJ-TERT) = 0.58 μM; EC_{50} (BJ-TERT/LT/ST/RAS^{V12}) = 0.025 μM). ML162 was discovered in a manner similar to RSL3 and erastin. Mass spectrometry studies revealed that ML162 reacted in vitro with glutathione (GSH) in the presence of triethylamine, forming an ML162-GSH adduct. The -chloroacetamide moiety is required for this electrophilic activity. The chloro group is a good leaving group, and these compounds may react non-selectively in cells with proteins, DNA and glutathione, leading to undesired off-target effects.[28] We therefore examined whether **27** generates, or is, an electophile, by measuring compound-glutathione adduct formation, signifying the ability of the compound to react non-specifically. MEL24 did not form an adduct with GSH as determined by mass spectrometry,[18] while a known electrophile, phenylisothiocyanate, formed a glutathione adduct under the same reaction conditions (Supplementary Fig. 6). A **27**-glutathione adduct was not observed after 48 h of reaction (Supplementary Fig. 7). Compound **27**, unlike ML162, does not contain an electrophilic group and does not act as an electrophile experimentally. This result suggests that compound **27** is likely to be more specific, as it is a non-covalent oncogenic-*RAS* selective lethal compound. Taken together, these data suggest that compound **27** is selectively lethal to tumorigenic cells and induces non-apoptotic cell death and may be more specific in cells than other electrophilic oncogenic-*RAS* synthetic lethal compounds.

In conclusion, we have taken advantage of the privileged nature of the tetrahydro-β-carboline scaffold to create a non-electrophilic compound with oncogenic-*RAS* selective lethality. To do this, we designed and synthesized a novel series of substituted Pictet–Spengler condensation products. These compounds were tested for their lethality against a panel of seven cancer cell lines. Among these compounds, compound **27** showed a unique selectivity towards tumorigenic versus non-tumorigenic cells. Further investigations revealed that this compound exhibits (1) synthetic lethality with oncogenic-*RAS* and (2) caspase-independent cell death. Based on these data, we suggest that compound **27** induces caspase-independent cell death selectively in cancerous cells. These studies have revealed a potentially useful probe for studying caspase-independent ferroptotic cell death and oncogenic-*RAS* signaling, and have highlighted the utility of the tetrahydro–carboline scaffold.

Acknowledgments

This research was supported by Grants from the US National Institutes of Health (R01CA097061, R01GM085081 and R01CA161061), the Bioaccelerate Award from the New York City Investment Fund and NYSTAR. B.R.S. is an Early Career Scientist of the Howard Hughes Medical Institute.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.06.077.

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24. (A) Compound **27** selectively reduces the viability of oncogenic-RAS expressing cells. A_1-cells were treated in a two fold, 7-point dilution series for 48 h before Alamar blue reading of viability. SEM of triplicates are shown. A_2-table of LC_{50} and maximum growth inhibitory efficacy in four cell lines ± SEM of triplicates. (B) Kinetic-analysis of compound **27** induced cell death. BJ-TERT/LT/ST/RAS^{V12} were treated for designated times with erastin (10 μM), compound **27** (30 μM) or RSL3 (0.57 μM). Data are representative of duplicate experiments. (C) Photograph of BJ-TERT and BJ-TERT/LT/ST/RAS^{V12} cells treated with compound **27** (30 μM), RSL3 (568 nM), erastin (10 μM) or staurosporine (1 μM) for 24 h. (D) Compound **27** does not induce caspase activation. BJ-TERT and BJ-TERT/LT/ST/RAS^{V12} cells were treated with Compound **27** for 28 h in a two-fold, 14-point dilution series before Alamar blue addition and detection of caspase activation by Apo-ONE. For the viability of RSL3, MEL24 and Erastin in BJ-TERT and BJ-TERT/LT/ST/RAS^{V12} cells see Supplementary Figure 4. (E) Compound **27** lethality is rescued by deferoxamine and -tocopherol (ATOC), but not z-VAD-fmk or Boc-D-fmk. HT1080 cells were treated with compound **27** in the presence of deferoxamine (152 μM), -tocopherol (100 μM), z-VAD-fmk (45 μM) or Boc-D-fmk (50 μM).
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26. Using 2′,7′-dichlorodihydrofluorescein diacetate (DCF), BJ-TERT/LT-/ST/RAS^{V12} cells were treated with followings for indicated time: DMSO (18 h), erastin (6 h), RSL3 (6 h), compound **27** (18 h). The scatterplots of the same data are shown in the Supplementary data (Supplementary Fig. 5).
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