Bioorganic & Medicinal Chemistry Letters 26 (2016) 1471–1474

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Evaluation of synthetic coumarins for antiausterity cytotoxicity against pancreatic cancers

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ARTICLE INFO

Article history:
Received 2 November 2015
Revised 14 January 2016
Accepted 19 January 2016
Available online 22 January 2016

Keywords:
Antiausterity agents
Pancreatic cancer
Coumarins
Apoptosis
Starvation-resistance

ABSTRACT

A series of functionalized coumarins were synthesized and evaluated for their capacity to inhibit the resistance to starvation of pancreatic cancer cells. This form of cytotoxicity, termed 'antiausterity' activity, was evaluated using a preferential cytotoxicity assay that compared cell survival in nutrient poor and nutrient rich conditions. Six of the seventeen compounds showed weak antiausterity activity against PANC-1. Compound **34** was active against PANC-1, MIA PaCa-2, and Capan-1 cancer cell lines. All of the compounds tested were simplified structural analogs of previously reported natural product leads. Six of the compounds, including **34**, contain functionalized triazoles as novel potential bioisosteres of the side chain of the natural product angelmarin. Overall, the analogs were found to have low antiausterity activity relative to the corresponding natural products.

With a five year survival rate of less than 5% and no effective chemotherapy options available, a pressing need exists for medicines to combat pancreatic cancer.[1,2] In 2000, Esumi and co-workers showed that pancreatic cancer cells demonstrate an atypical capacity to survive for up to 3 days when deprived of serum, glucose, and amino acids; an incubation medium that kills human fibroblast cells and other cancers much more rapidly.[3] Given the fact that fast-growing pancreatic tumors are characterized by nutrient-deficient microenvironments in which these cancer cells continue to thrive,[4] the inhibition of starvation-resistance became a potential therapeutic strategy against this disease. This was coined the 'antiausterity' approach with inhibitors described as having 'preferential cytotoxicity' (PC_{50} or PC_{100}) indicating that they induce cell death upon 24 incubation in a nutrient deprived medium with no corresponding cytotoxicity in under standard, nutrient rich, incubation conditions.[5,6] Various potential biochemical mechanisms for cellular resistance to starvation have been investigated.[7–11] Meanwhile, antiausterity bioassay guided-isolation of natural products has identified numerous promising inhibitors,[12–21] or antiausterity agents, several of which share the coumarin structural scaffold. The first coumarin-based inhibitor was angelmarin (**1**), a phenolic furanocoumarin isolated from *Angelica pubescens* in 2006, which kills 100% of PANC-1 cells under nutrient-deprived conditions at a concentration of 0.01 μg/mL (PC_{100} = 0.03 μM).[13] In 2008, more than a dozen densely substituted hydroxycoumarins from *Kayea assamica* were found to be active against PANC-1.[16,17] The most potent of these were kayeassamins A, B, D, E, and G (compounds **2–6,** Fig. 1) all with PC_{100} concentrations of 1 μM. The geranylated coumarin ostruthin (**7**), from *Rhizoma et Radix Notopterygii*, was also found to be active with a PC_{50} of 7.2 μM.[21]

Anti-austerity active natural products continue to be isolated,[22,23] and the intriguing bioactivity of these compounds has also inspired the efforts of synthetic chemists.[24–32] In 2012, Coster and co-workers synthesized a series of active analogs of angelmarin the most potent of which were two trifluoromethyl-substituted derivatives **8** and **9** with PC_{50} values of 23 and 79 nM, respectively (Fig. 2).[26] Carrico-Moniz and co-workers have made a number of polyisoprenyl ethers including coumarins **10–13** (similar to ostruthin, **7**) the most active of which was the farnesyl ether **12** with a PC_{50} of 4 μM.[31,32]

Here we report our evaluation of the antiausterity activity of seventeen coumarins synthesized with the aim of structural simplification of the natural products illustrated in Figure 1. First, we tested eleven coumarins functionalized with simple *n*-alkyl ethers (**14**) intended to mimic the lipophilicity of polyisoprenyl ether side chains (**15**) of the kayeassamins (Fig. 3). Secondly we evaluated six coumarins with readily accessible triazole-containing side chains (**16**) intended as alternatives to the more synthetically challenging scaffold of angelmarin (**17** and **1**).

Synthetic compounds **18–34** were tested for preferential cytotoxicity against PANC-1 human pancreatic cancer using a previ-

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http://dx.doi.org/10.1016/j.bmcl.2016.01.054
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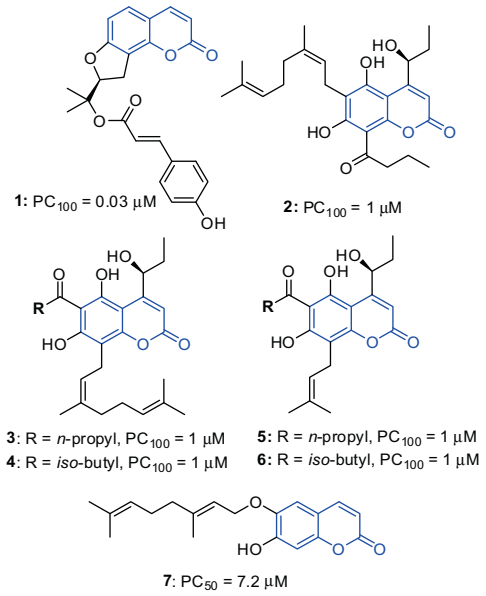


Figure 1. Coumarin natural products with anti-austerity activity against PANC-1.

ously reported antiausterity cytotoxicity assay (Table 1).[12] Briefly, cell survival was measured after incubation for 24 h under nutrient deprived medium (NDM) as well as incubation in Dulbecco's modified Eagle medium (DMEM) in the presence of the test compounds (see Supporting information). Of the eleven simple alkyl ethers evaluated (**18**–**28**) only four compounds, **18**, **23**, **24**, and **25,** showed weak preferential cytotoxicity against PANC-1 under nutrient deprived conditions. These included the three *n*-octyloxy coumarins, **18, 23** and **25,** which were each more active than their analogous compounds with longer *n*-alkyl chains (Table 1). Five of the triazoles tested, **28**–**33,** demonstrated no antiausterity activity against PANC-1 while a sixth, the *meta*-trifluoromethylphenyl derivative **34**, was active with a PC_{50} of 29 μM.

The difference in antiausterity activity between the *para*- and *meta*-trifluoromethylphenyl derivatives **33** and **34** was surprising given the opposite trend evident in angelmarin analogs **8** and **9** (see Fig. 2) reported by Coster and co-workers.[26] This prompted us to evaluate both compounds **33** and **34** against two additional pancreatic cancer cell lines, Capan-1 and MIA PaCa-2 (Fig. 4). Compound **34** showed preferential cytotoxicity against both Capan-1 and MIA PaCa-2 cells with PC_{50} concentrations of 8.5 and 18 μM, respectively. In contrast, compound **33** was selectively cytotoxic only to MIA PaCa-2 cells (PC_{50} = 9.6 μM) without antiausterity

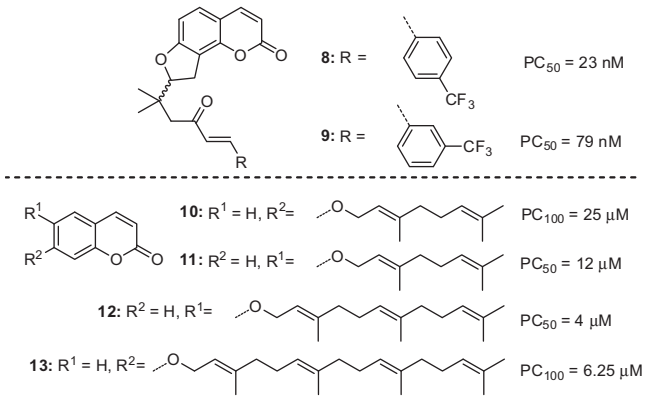


Figure 2. Anti-austerity active synthetic analogs of natural products.

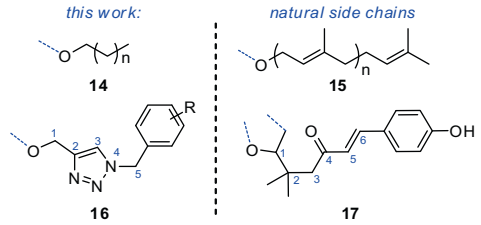
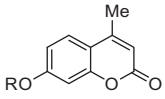
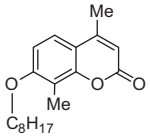
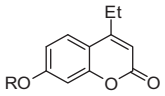
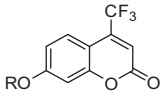
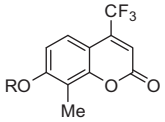
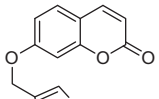
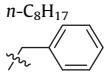
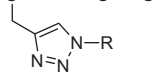
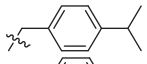
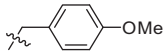
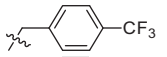
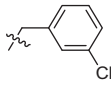


Figure 3. Naturally occurring coumarin substituents, **15** and **17**, and their simplified mimics, **14** and **16**, that were synthesized and evaluated in this study.

Table 1
Preferential cytotoxicity of synthetic coumarins **18**–**35** against PANC-1

Entry	Structure	R	Compound #	PC_{50}[a] (μM)
1		n-C_8H_{17}	**18**	48
2		n-$C_{12}H_{25}$	**19**	>100
3		n-$C_{14}H_{29}$	**20**	>100
5			**21**	>100
6			**22**	>100
7		n-C_8H_{17}	**23**	29
8		n-$C_{12}H_{25}$	**24**	51
9		n-C_8H_{17}	**25**	63
10		n-$C_{16}H_{33}$	**26**	>100
11		n-$C_{12}H_{25}$	**27**	>100
12		n-$C_{16}H_{33}$	**28**	>100
13		n-C_8H_{17}	**29**	>100
14			**30**	>100
15			**31**	>100
16			**32**	>100
17			**33**	>100
18			**34**	29

[a] PC_{50} describes cytotoxicity against PANC-1 (i.e., LC_{50} estimated using the graphical method) after 24 h incubation in nutrient deprived medium (NDM) with no corresponding cytotoxicity at 100 μM in Dulbecco's modified Eagle medium (DMEM) (see Supporting information).

activity against PANC-1 or Capan-1. The mechanism of cell death was determined to be apoptotic (PANC-1, compound **34**) based on a modified ethidium bromide and acridine orange (EB/AO) staining assay (Fig. 5).[33]

All of the compounds evaluated above were readily accessible in one or two synthetic operations from inexpensive substrates. Compounds **18**–**28** were products of the alkylation of the corresponding 7-hydroxycoumarins with alkyl halides or alkyl sulfonates under typical conditions of K_2CO_3 in refluxing acetone. Compounds **29**–**34** were prepared by assembly of the triazole rings