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Natural product coumarins that inhibit human carbonic anhydrases

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ABSTRACT

Natural products (NPs) have proven to be an invaluable source of new chemotherapies yet very few have been explored to source small molecule carbonic anhydrase (CA) inhibitors. CA enzymes underpin physiological pH and are critical to the progression of several diseases including cancer. The present study is the first to more widely investigate NP coumarins for CA inhibition following the recent discovery of a NP coumarin CA inhibitor. We assembled a NP library comprising 24 plant coumarins (compounds **4**–**27**) and three ascidian coumarins (compounds **28**–**30**) that together provide a diverse collection of structures containing the coumarin pharmacophore. This library was then evaluated for inhibition of six human CA isozymes (CAs I, II, VII, IX, XII and XIII) and a broad range of inhibition and isozyme selectivity profiles were evident. Our findings provide a platform to support further evaluation of NPs for the discovery of new chemotypes that inhibit disease relevant CA enzymes.

1. Introduction

Carbonic anhydrase enzymes (CA, EC 4.2.1.1) catalyze the reversible hydration of carbon dioxide (CO_2) to generate bicarbonate anion (HCO_3^-) and a proton (H^+).[1,2] Our knowledge of the physiological impact of this reaction has grown significantly in recent years, with CA now validated as a target for cancer therapy intervention by inhibition of extracellular CA IX[3] and XII.[4] Several CA isozymes, including cytosolic CA VII[5] and CA XIII,[6,7] are potential targets in other conditions where pH homeostasis is critical.[8] The CA enzyme active site comprises a tetrahedral Zn^{2+} cation that plays both a structural and catalytic role for the enzyme[9] and this metal is the implied target for medicinal chemistry. As a consequence almost all reported small molecule CA inhibitors comprise a zinc binding group (ZBG) of which the primary sulfonamide moiety ($-SO_2NH_2$) is the foremost example for CA inhibition. A core role of medicinal chemists in the CA field is to develop novel, drug-like, isozyme selective small molecule inhibitors for human CA isozymes. Natural products (NPs) comprise a vast collection of diverse chemical structures and have proven an invaluable source of new chemotherapies[10–13] yet very few NPs have been explored to source novel CA inhibitors.[14] NPs that comprise a primary sulfonamide moiety in their structure are rare and have not been investigated for CA inhibition, Figure 1.[15,16] As the primary sulfonamide moiety is poorly represented in NP chemical space, this space presents a rich source of potential new chemotypes towards discovery of alternate CA inhibitors.

Plant NPs have been the basis of traditional medicine for thousands of years and continue to actively contribute to contemporary drug discovery.[13] The significance of NPs in drug discovery is most evident in the anti-cancer[17] and antibiotic[18,19] therapeutic areas. Coumarin compounds are abundant secondary metabolites in plants and are found in lesser amounts in microorganisms and animal sources. Plant coumarins are phytoalexins, defense compounds produced when the plant is under threat from other organisms, and have attracted interest owing to a range of biological activities including anti-microbial, anti-viral, anti-cancer, anti-oxidant and anti-inflammatory properties.[20] A recent review highlights the growing interest in the coumarin class of compound to

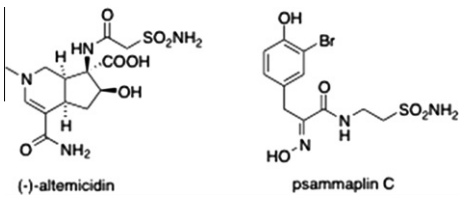

Figure 1. NPs that comprise a primary sulfonamide moiety in their structure, such as (−) altemicidin and psammaplin C, are rare and have not been investigated for CA inhibition.

Abbreviations: NP, natural product; CA, carbonic anhydrase; ZBG, zinc binding group; SAR, structure–activity relationship; ADME, absorption distribution metabolism and elimination; ESI MS, electrospray ionization mass spectrometry.

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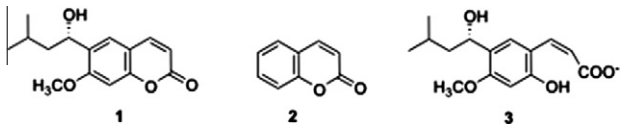

Figure 2. Compound **1**, the first reported natural product coumarin CA inhibitor; compound **2**, coumarin—the simplest structure of the coumarin compound class and a weak CA inhibitor; the CA-catalyzed hydrolysis product of coumarin **1**, the cinnamic acid derivative compound **3**.

deliver new therapeutics.[21] In a study of plant NPs ten alkaloid enriched *Leionema ellipticum* (Rutaceae) extracts were screened using electrospray ionization mass spectrometry (ESI MS) for binding to bovine CA II (bCA II).[22] From this study the coumarin, 6-(1*S*-hydroxy-3-methylbutyl)-7-methoxy-2*H*-chromen-2-one (**1**) was identified as a ligand for bCA II as it formed a noncovalent complex that could be detected by ESI MS (Fig. 2). It was subsequently demonstrated that **1** differentially inhibits a spectrum of human CAs in a time dependent manner, and with much higher activity than coumarin **2**, the simplest NP coumarin (Fig. 2).[23] As coumarin compounds lack the classic sulfonamide ZBG of known small molecule CA inhibitors it was of interest to understand how this NP binds to CAs. Using protein X-ray crystallography we observed the hydrolysis product of **1**, the cinnamic acid derivative **3**, spanning the entrance to the hCA II active site following incubation of **1** with hCA II (Fig. 2).[23] Esterase activity is known for CAs[24,25] and the observation of cinnamic acid **3** rather than coumarin **1**, although unexpected, could be rationalized as a consequence of hCA II esterase activity leading to hydrolysis of the lactone of **1**.

Since the discovery of the NP coumarin **1** synthetic libraries of coumarins and thiocoumarins have been prepared and evaluated as CA inhibitors.[26–28] These synthetic compounds have supported our findings with the NP coumarin, namely maximum inhibition is observed following 6 h incubation with the CA enzyme (while 15 min incubation is usual for classical sulfonamide CA inhibitors). The extended incubation period is related to the kinetics of coumarin hydrolysis. Recently a series of substituted coumarins incorporating a selection of glycosyl moieties were synthesized.[3] These glycoconjugates were very weak inhibitors of off-target CA I and II, while several strongly inhibited tumor-associated CA IX and XII.[3] One glycosyl coumarin inhibited the growth of primary tumors in the highly aggressive 4T1 syngeneic mouse mammary

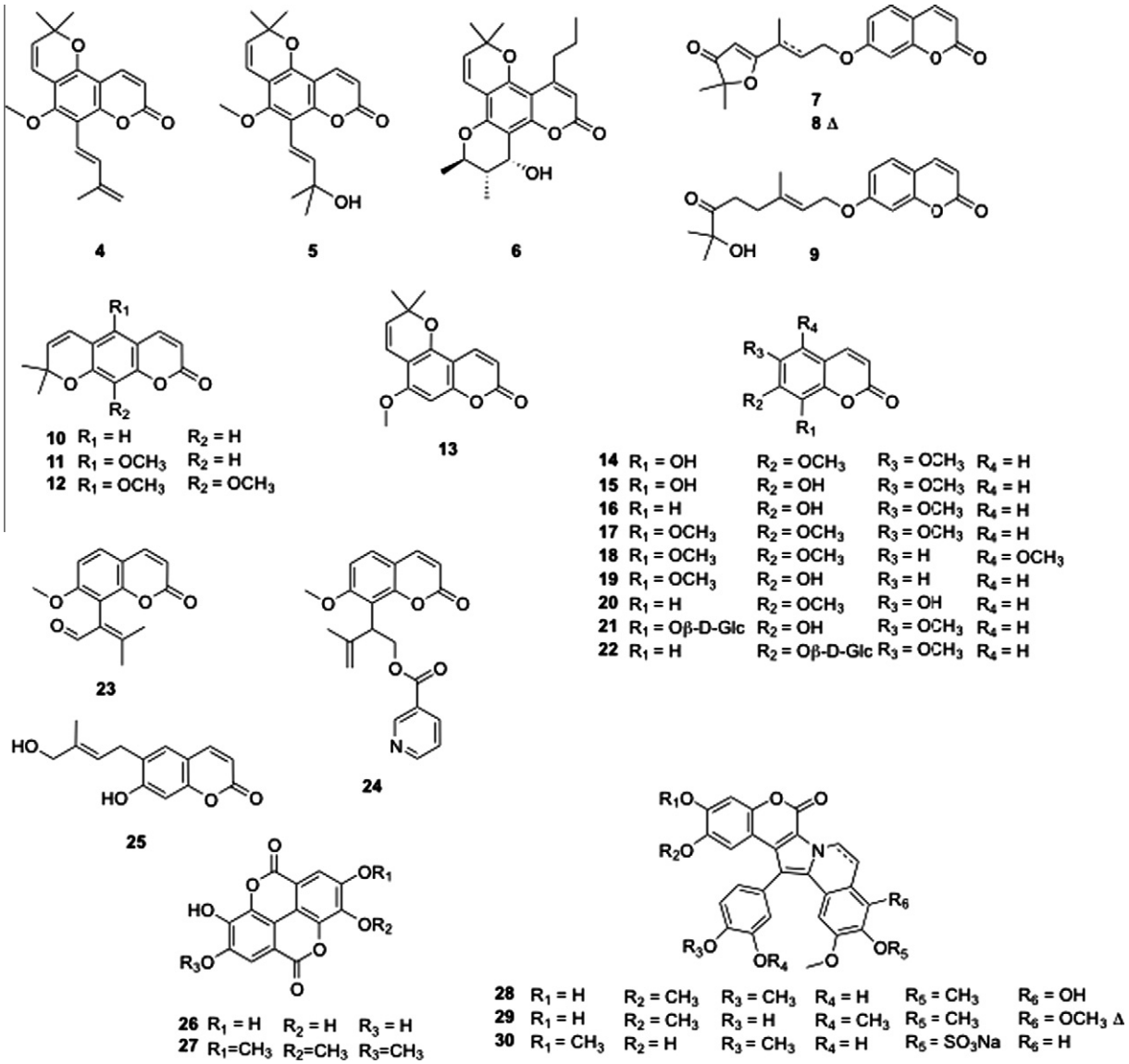

Figure 3. Natural product coumarin library sourced from Nature Bank,[33] compounds **4**–**30**.

tumor model, where 4T1 cells overexpress CA IX in hypoxia in vitro.[3] Finally, a small selection of other plant NPs have been evaluated as CA inhibitors, most notably phenolic NPs[14,29–32] that displayed interesting CA inhibition characteristics.

Inspired by the success of NPs in contributing new small molecule therapeutics together with the promising findings that the coumarin class of compounds act as CA inhibitors and have antitumor activity[3,23] we accessed Nature Bank (from which coumarin **1** was discovered) to source further NP coumarins. Nature Bank is a unique drug discovery resource that consists of >50,000 biota samples collected from Australia, China and Papua New Guinea along with biota extracts, semi-purified fractions and pure compounds.[33] A substructure search of the Nature Bank pure compound repository against the bare coumarin scaffold **2** was performed and a set of 81 coumarins were initially identified. From this set a subset of 27 coumarins were sourced in sufficient quantity and purity for follow up evaluation as CA inhibitors. These NP coumarins, compounds **4**–**30** (Fig. 3), form the basis of the present manuscript wherein we describe the CA inhibition against six human CA isozymes and evaluate the drug-like properties of this coumarin library.

2. Results and discussion

2.1. Compound library

The NP coumarin library of this study comprises 24 plant coumarins (compounds **4**–**27**) and three ascidian coumarins (compounds **28**–**30**), all of which have previously been characterized. Specifically, the plant NPs comprise avicennin **4**,[34,35] trans-avicennol **5**,[36,37] calanolide B **6**,[33,38] dihydrogeiparvarin **7**,[39] geiparvarin **8**,[39,40] dehydromarmin **9**,[39] xanthyletin **10**,[41] xanthoxyletin **11**,[36,41] ceylantin **12**,[42] alloxanthoxyletin **13**,[41] fraxidin **14**,[43] fraxin **15**,[44] scopoletin **16**,[45] 6,7,8-trimethoxycoumarin **17**,[46] 5,7,8-trimethoxycoumarin **18**,[46] 7-hydroxy-8-methoxycoumarin **19**,[45] isoscopoletin **20**,[47] fraxoside **21**,[48] scopolin **22**,[49] murralongin **23**,[50] (+)-isomurralonginol nicotinate **24**,[51] isophellodenol C **25**,[52] ellagic acid **26**,[53] and nasutin B **27**.[54] The ascidian NP coumarins include lamellarins E **28**,[55] B **29**,[56] and G 8-sulfate **30**.[57] A variety of bioactivities have been reported for these coumarins, for example calanolide B **6**, isolated from the tropical rainforest tree *Calophyllum lanigerum*, displayed protection against HIV-1 replication and cytopathicity (EC_{50} = 0.4 μM).[38] Dihydrogeiparvarin **7** and geiparvan **8**, both isolated from *Geijera paruiflora*,[39,40] possessed significant in vitro activity against human carcinoma of the nasopharynx.[58,59] Xanthoxyletin **11**,[36,41] purified from a variety of *Citrus* species, acted as a DNA-damaging agent,[60] while several synthetic derivatives have been shown to exhibit toxicity towards L-1210 leukemia cells with IC_{50} values ranging from 0.9 to 60.3 μM.[60]

2.2. Carbonic anhydrase inhibition

The inhibition activity data for coumarin **2** and NP coumarins **4–30** against human CA I and II (off-target isozymes), as well CA VII, IX, XII and XIII (isozymes of interest in therapeutic drug development) is presented in Table 1.

Coumarin **2**, the simplest coumarin, is not an appreciable inhibitor of CA VII, IX, XII or XIII however it is a weak inhibitor of off-target CA I and CA II with K_is of 3.1 and 9.2 μM, respectively. The complexity and diversity of structures within this NP coumarin library far exceeds that described for synthetic coumarins that have been assessed for CA inhibition. These NP coumarins are substituted at any of six available sites, with many fused to form tricyclic, tetracyclic or larger ring systems. This diversity does not readily allow simple structure–activity relationships (SARs) to be defined,

Table 1
Inhibition data for coumarin **2** and NP coumarins **4**–**30** against human CA isozymes (CA I, II, VII, IX, XII and XIII) following a 6 h incubation time with enzyme

Compd	K_i (μM)[a,b]					
	CA I	CA II	CA VII	CA IX	CA XII	CA XIII
2	3.10	9.20	>1000	>1000	>1000	>1000
4	7.66	>100	0.65	0.62	0.79	45.0
5	8.46	>100	8.98	0.78	0.77	29.3
6	9.31	50.7	8.87	0.83	0.81	21.0
7	59.2	63.4	9.03	0.89	0.60	27.4
8	9.75	>100	7.82	0.60	0.83	9.62
9	7.81	>100	3.69	4.03	0.70	6.10
10	21.5	>100	9.18	7.51	25.7	8.36
11	7.71	>100	6.27	0.74	0.96	3.15
12	9.21	49.3	9.31	0.86	8.35	>100
13	5.60	>100	8.11	3.50	9.10	5.91
14	9.89	>100	5.56	0.85	7.84	95.7
15	4.86	94.3	4.32	0.61	7.70	9.73
16	10.56	>100	8.71	0.96	4.05	17.8
17	0.0097	>100	9.28	6.58	18.2	4.24
18	4.31	9.65	7.01	0.76	0.83	3.32
19	36.4	>100	4.53	0.85	9.12	7.26
20	14.0	>100	23.8	7.37	4.14	5.27
21	5.04	>100	3.87	0.37	7.45	9.80
22	5.93	>100	9.11	8.72	0.78	8.43
23	9.11	>100	8.85	8.12	7.44	8.89
24	5.84	>100	>100	0.67	7.39	4.06
25	7.52	78.9	6.92	9.75	0.77	6.35
26	68.2	>100	8.79	79.8	8.15	4.24
27	44.1	>100	8.58	17.4	7.42	5.97
28	6.45	>100	14.5	3.22	9.07	4.63
29	40.1	>100	58.3	6.33	8.51	3.70
30	6.55	>100	78.4	3.27	1.79	4.24

[a] This inhibition data was acquired using a stopped-flow assay that monitors the CA-catalyzed hydration of CO_2.[61] Errors in the range of ±5% of the reported value, from three determinations.
[b] All proteins were recombinant.

however several trends surrounding CA inhibition are evident. Most obvious is that the NP coumarin library members are very weak CA II inhibitors, most have K_is > 100 μM, the only exception being the trimethoxycoumarin **18** (K_i = 9.65 μM). When compared to the structurally related methoxy/hydroxy coumarins **14–17**, **19** and **20**, compound **18** differs only in the pattern of substituents, this SAR indicates that it may be a combination of interacting substituents that directs the CA inhibition profile at CA II. At CA I, VII, IX, XII and XIII many of the NP coumarins have K_is in the range of 1–10 μM, this tight grouping of K_is reflects minimal isozyme selectivity for these coumarins, however there are a few outliers to this general trend and these compounds represent interesting structures owing to their CA isozyme selectivity characteristics. At CA I there was one stand out compound being compound **17**, a nanomolar CA I inhibitor. This trimethoxy coumarin is the most potent of any of the NP coumarins across the six CA isozymes of the present study. At CA XIII there were no submicromolar inhibitors while the dimethoxy coumarin **12** was the weakest of the CA XIII inhibitors (K_i > 100 μM). Similar to CA I and II the relationship of methoxy substituents for CA XIII binding appeared important for defining the inhibition characteristics, with the structurally related coumarins **10** and **11** 10-fold better inhibitors than the methoxy coumarin **12**. At CA VII three coumarins, compounds **24**, **29** and **30**, exhibited weaker inhibition (K_is 58 to >100 μM) than the remainder, interestingly these include three of the four nitrogen containing coumarins. Around half of the NP coumarins have submicromolar inhibition of the validated cancer-associated isozymes CA IX and XII, some of these coumarins (**4**, **5**, **6**, **7**, **8**, **11** and **18**) are submicromolar at both CA IX and XII, while the remainder are submicromolar at either CA IX (**12**, **14–16**, **19**, **21** and **24**) or CA XII (**9**, **22** and **25**). This subset of NP coumarins have viable CA IX and/or

XII selectivity characteristics that warrant further studies in cell-based models of CA in cancer.

2.3. Property profiling

Software tools for multiparameter profiling are inexpensive and provide rapid feedback, and when used with an awareness of the limitations around accuracy of prediction may provide insight into drug-like properties of a compound class to guide the direction of follow-on studies and flag potential compound liabilities.[62] QikProp (Schrödinger Suite 2009) is a Pharma industry gold standard software package that allows the calculation of compound properties and prediction of absorption, distribution, metabolism and elimination (ADME) properties.[63] For the NP coumarins **4**–**30** a selection of predicted property values generated by QikProp are provided in the supplementary data. Of note is that these coumarin NPs have predicted property and descriptor values that are all within the 95% range of values for known drugs.[64] Reactive Michael acceptors are a general structural alert in drug discovery, however it has been demonstrated that simple coumarins exhibit poor protein binding characteristics compared to other carbonyl containing Michael acceptors.[65,66] This poorer reactivity of the coumarin double bond compared with other Michael acceptors, has been attributed to it being part of a pseudoaromatic system.[67]

3. Conclusions

Following from the recent discovery that coumarins are time dependent CA inhibitors (owing to CA-mediated lactone hydrolysis) this study is the first to more fully investigate NP coumarins for CA inhibition. A NP coumarin library, compounds **4–30**, comprising a diverse collection of structures containing the coumarin pharmacophore was sourced from Nature Bank.[33] This coumarin library provided a rich collection of stereochemistry and structural diversity, yet significantly the compounds lack the sulfonamide ZBG that is typical of classical CA inhibitors. These coumarins exhibited CA inhibition profiles consistent with compounds useful for the development of small molecules that act with an alternate mechanism of CA enzyme inhibition compared to classical CA inhibitors. Our findings provide a platform to support the further evaluation of NPs for discovery of novel chemotypes with improved drug-like properties for inhibition of disease relevant CA enzymes.

4. Experimental

4.1. Chemistry

Compounds **4**–**30** were all isolated from plant or marine invertebrates archived in Nature Bank,[33] which is located at the Eskitis Institute, Griffith University. All compounds were identified as previously reported NPs following spectroscopic and spectrometric data analysis and comparison with literature values.[33–57] Prior to biological evaluation all compounds were subjected to purity analysis by ^{1}H NMR spectroscopy and shown to be >95%.

4.2. CA inhibition assay

An SX.18MV-R Applied Photophysics stopped-flow instrument has been used for assaying the CA I, II, VII, IX, XII and XIII CO_2 hydration activity.[61] Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nM, with 10 mM Hepes (pH 7.5) as buffer, 0.1 M $NaClO_4$ (for maintaining constant the ionic strength—this anion is not inhibitory), following the CA-catalyzed CO_2 hydration reaction for a period of 10–100 s. Saturated CO_2 solutions in water at 20 °C were used as substrate. Stock solutions of inhibitors were prepared at a concentration of 10–50 mM (in the assay buffer) and dilutions up to 1 nM were done with the assay buffer mentioned above. Inhibitor and enzyme solutions were preincubated together for 6 h at room temperature prior to assay, in order to allow for the formation of the enzyme-inhibitor complex. The inhibition constants were obtained by non-linear least-squares methods using PRISM 3. The curve-fitting algorithm allowed us to obtain the IC_{50} values, working at the lowest concentration of substrate of 1.7 mM), from which K_i values were calculated by using the Cheng–Prusoff equation. The catalytic activity (in the absence of inhibitors) of these enzymes was calculated from Lineweaver–Burk plots and represents the mean from at least three different determinations. Enzyme concentrations were CA I, 15; CA II, 8.6; CA VII, 12; CA IX, 6.9; CA XII, 14; CA XII, 16 nM. All enzymes in this study were recombinant.

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References and notes

1. Supuran, C. T. *Nat. Rev. Drug Disc.* **2008**, *7*, 168.
2. Krishnamurthy, V. M.; Kaufman, G. K.; Urbach, A. R.; Gitlin, I.; Gudiksen, K. L.; Weibel, D. B.; Whitesides, G. M. *Chem. Rev.* **2008**, *108*, 946.
3. Touisni, N.; Maresca, A.; McDonald, P. C.; Lou, Y.; Scozzafava, A.; Dedhar, S.; Winum, J.-Y.; Supuran, C. T. *J. Med. Chem.* **2011**, *54*, 8271.
4. Neri, D.; Supuran, C. T. *Nat. Rev. Drug Disc.* **2011**, *10*, 767.
5. Bootorabi, F.; Haapasalo, J.; Smith, E.; Haapasalo, H.; Parkkila, S. *Health* **2011**, *3*, 6.
6. Lehtonen, J. M.; Shen, B.; Vihinen, M.; Casini, A.; Scozzafava, A.; Supuran, C. T.; Parkkila, A.-K.; Saarnio, J.; Kivela, A. J.; Waheed, A.; Sly, W. S.; Parkkila, S. *J. Biol. Chem.* **2004**, *279*, 2719.
7. Di Fiore, A.; Monti, S. M.; Hilvo, M.; Parkkila, S.; Romano, V.; Scaloni, A.; Pedone, C.; Scozzafava, A.; Supuran, C. T.; De Simone, G. *Proteins* **2008**, 164.
8. Hassan, M. I.; Shajee, B.; Waheed, A.; Ahmad, F.; Sly, W. S. *Bioorg. Med. Chem.* **2012**. Epub ahead of print.
9. Alterio, V.; Di Fiore, A.; D'Ambrosio, K.; Supuran, C. T.; De Simone, G. *Chem. Rev.* 2012, Articles ASAP, doi: http://dx.doi.org/10.1021/cr200176r.
10. Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2007**, *70*, 461.
11. Newman, D. J.; Cragg, G. M. *Future Med. Chem.* **2009**, *1*, 1415.
12. Lam, K. S. *Trends Microbiol.* **2007**, *15*, 279.
13. Lachance, H.; Wetzel, S.; Kumar, K.; Waldmann, H. *J. Med. Chem.* **2012**, *55*, 5989.
14. Supuran, C. T. *Mol. Div.* **2011**, *15*, 305.
15. Takahashi, A.; Kurasawa, S.; Ikeda, D.; Okami, Y.; Takeuchi, T. *J. Antibiot.* **1989**, *42*, 1556.
16. Jimenez, C.; Crews, P. *Tetrahedron* **1991**, *47*, 2097.
17. Mann, J. *Nat. Rev. Cancer* **2002**, *2*, 143.
18. Butler, M. S.; Cooper, M. A. *J. Antibiot.* **2011**, *64*, 413.
19. Butler, M. S.; Buss, A. D. *Biochem. Pharmacol.* **2006**, *71*, 919.
20. Borges, F.; Roleira, F.; Milhazes, N.; Santana, L.; Uriarte, E. *Curr. Med. Chem.* **2005**, *12*, 887.
21. Kontogiorgis, C.; Detsi, A.; Hadjipavlou-Litina, D. *Expert Opin. Ther. Pat.* **2012**, *22*, 437.
22. Vu, H.; Pham, N. B.; Quinn, R. J. *J. Biomol. Screen.* **2008**, *13*, 265.
23. Maresca, A.; Temperini, C.; Vu, H.; Pham, N. B.; Poulsen, S.-A.; Scozzafava, A.; Quinn, R. J.; Supuran, C. T. *J. Am. Chem. Soc.* **2009**, *131*, 3057.
24. Lopez, M.; Vu, H.; Wang, C. K.; Wolf, M. G.; Groenhof, G.; Innocenti, A.; Supuran, C. T.; Poulsen, S.-A. *J. Am. Chem. Soc.* **2011**, *133*, 18452.
25. Krebs, J. F.; Ippolito, J. A.; Christianson, D. W.; Fierke, C. A. *J. Biol. Chem.* **1993**, *268*, 27458.
26. Carta, F.; Maresca, A.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem.* **2012**, *20*, 2266.
27. Maresca, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4511.
28. Maresca, A.; Temperini, C.; Pochet, L.; Masereel, B.; Scozzafava, A.; Supuran, C. T. *J. Med. Chem.* **2009**, *53*, 335.
29. Davis, R. A.; Hofmann, A.; Osman, A.; Hall, R. A.; Mühlschlegel, F. A.; Vullo, D.; Innocenti, A.; Supuran, C. T.; Poulsen, S. A. *J. Med. Chem.* **2011**, *54*, 1682.

30. Davis, R. A.; Innocenti, A.; Poulsen, S.-A.; Supuran, C. T. *Bioorg. Med. Chem.* **2010**, *18*, 14.
31. Innocenti, A.; Sarikaya, S. B. O.; Gulcin, I.; Supuran, C. T. *Bioorg. Med. Chem.* **2010**, *18*, 2159.
32. Poulsen, S.-A.; Davis, R. A.; Keys, T. G. *Bioorg. Med. Chem.* **2006**, *14*, 510.
33. http://www.nature-bank.com.au.
34. Sarker, S. D.; Armstrong, J. A.; Gray, A. I.; Waterman, P. G. *Biochem. Syst. Ecol.* **1994**, *22*, 641.
35. Sarker, S. D.; Armstrong, J. A.; Waterman, P. G. *Biochem. Syst. Ecol.* **1994**, *22*, 863.
36. Gray, A. I.; Waigh, R. D.; Waterman, P. G. *Phytochemistry* **1977**, *16*, 1017.
37. Sultana, N.; Sarker, S. D.; Armstrong, J. A.; Wilson, P. G.; Waterman, P. G. *Biochem. Syst. Ecol.* **2003**, *31*, 681.
38. Kashman, Y.; Gustafson, K. R.; Fuller, R. W.; Cardellina Ii, J. H.; McMahon, J. B.; Currens, M. J.; Buckheit, R. W., Jr.; Hughes, S. H.; Cragg, G. M.; Boyd, M. R. *J. Med. Chem.* **1992**, *35*, 2735.
39. Dreyer, D. L.; Lee, A. *Phytochemistry* **1972**, *11*, 763.
40. Lahey, F. N.; MacLeod, J. K. *Aust. J. Chem.* **1967**, *20*, 1943.
41. Mujumdar, R. B.; Rathi, S. S.; Rao, A. V. R. *Indian J. Chem., Sect B* **1977**, *15B*, 200.
42. Ahmad, J.; Shamsuddin, K. M.; Zaman, A. *Phytochemistry* **1984**, *23*, 2098.
43. Spath, E.; Dobrovolny, E. *Ber. Dtsch. Chem. Ges. B* **1938**, *71B*, 1831.
44. Morikawa, T.; Tao, J.; Toguchida, I.; Matsuda, H.; Yoshikawa, M. *J. Nat. Prod.* **2003**, *66*, 86.
45. Barbera, O.; Alberto Marco, J.; Sanz, J. F.; Sanchez-Parareda, J. *Phytochemistry* **1986**, *25*, 2357.
46. Gonzalez, A. G.; Diaz Chico, E.; Lopez Dorta, H.; Medina, J. M.; Rodriguez Luis, F. *An. Quim.* **1977**, *73*, 1015.
47. Zhang, W.-D.; Kong, D.-Y.; Li, H.-T.; Gu, Z.-B.; Qin, L.-P. *Zhongguo Yiyao Gongye Zazhi* **1998**, *29*, 498.
48. Lin, S.; Wang, S.; Liu, M.; Gan, M.; Li, S.; Yang, Y.; Wang, Y.; He, W.; Shi, J. *J. Nat. Prod.* **2007**, *70*, 817.
49. Wu, T. S.; Tsang, Z. J.; Wu, P. L.; Lin, F. W.; Li, C. Y.; Teng, C. M.; Lee, K. H. *Bioorg. Med. Chem.* **2001**, *9*, 77.
50. Raj, K.; Misra, S. C.; Kapil, R. S.; Popli, S. P. *Phytochemistry* **1976**, *15*, 1787.
51. Ito, C.; Furukawa, H. *Heterocycles* **1987**, *26*, 2959.
52. Nakamori, T.; Taniguchi, M.; Shibano, M.; Wang, N.-H.; Baba, K. *J. Nat. Med.* **2008**, *62*, 403.
53. Bhatia, I. S.; Bhatia, M. S.; Sharma, R. S.; Bajaj, K. L. *Indian J. Chem.* **1972**, *10*, 959.
54. Rao, K. V. *Planta Med.* **1974**, *25*, 166.
55. Lindquist, N.; Fenical, W.; Van Duyne, G. D.; Clardy, J. *J. Org. Chem.* **1988**, *53*, 4570.
56. Andersen, R. J.; Faulkner, D. J.; He, C. H.; Van Duyne, G. D.; Clardy, J. *J. Am. Chem. Soc.* **1985**, *107*, 5492.
57. Davis, R. A.; Carroll, A. R.; Pierens, G. K.; Quinn, R. J. *J. Nat. Prod.* **1999**, *62*, 419.
58. Padmawinata, K. *Acta Pharm. Hung.* **1973**, *4*, 1.
59. Jerris, P. J.; Smith, A. B., III *J. Org. Chem.* **1981**, *46*, 577.
60. Gunatilaka, L.; Kingston, D.; Wijeratne, K.; Bandara, R.; Hofmann, G.; Johnson, R. *J. Nat. Prod.* **1994**, *57*, 518.
61. Khalifah, R. G. *J. Biol. Chem.* **1971**, *246*, 2561.
62. Kerns, E. H.; Di, L. *Drug-like Properties: Concepts, Structure Design and Methods: from ADME to Toxicity Optimization*; Academic Press: London, 2008.
63. Schrödinger. Schrödinger LLC: New York, 2005; http://www.schrodinger.com.
64. Schrödinger. QikProp 3.5 User Manual, Schrödinger Press, 2012.
65. Schultz, T. W.; Rogers, K.; Aptula, A. O. *Contact Dermatitis* **2009**, *60*, 21.
66. Wolan, D. W.; Zorn, J. A.; Gray, D. C.; Wells, J. A. *Science* **2009**, *326*, 853.
67. Aptula, A. O.; Patlewicz, G.; Roberts, D. W. *Chem. Res. Toxicol.* **2005**, *18*, 1420.