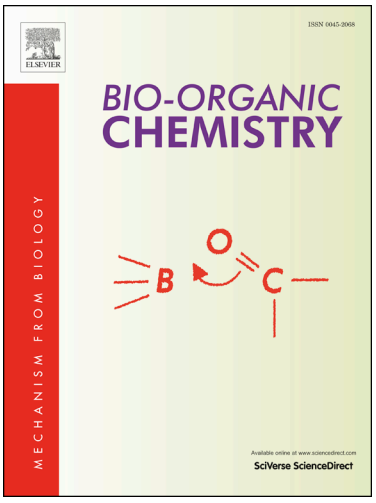

Heterocyclic cellular lipid peroxidation inhibitors inspired by the marine antioxidant barettin

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Heterocyclic cellular lipid peroxidation inhibitors inspired by the marine antioxidant barettin

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Abstract

The marine environment remains a rich source for the discovery and development of novel bioactive compounds. The present paper describes the design, synthesis and biological evaluation of a library of small molecule heterocyclic mimetics of the marine 2,5-diketopiperazine barettin which is a powerful natural antioxidant. By mainly focusing on the influence from the brominated indole and heterocyclic core of barettin, a library of 19 compounds was prepared. The compounds comprised a heterocyclic core, either a 2,5 diketopiperazine, an imidazolidinedione or a thioxothiazolidinone, which were mainly monosubstituted with ranging bulky substituents. The prepared compounds were screened for activity in a cellular lipid peroxidation assay using HepG2 cells. Several of the synthetic compounds showed antioxidant properties superior to the positive control barettin. Two of the prepared compounds displayed inhibitory activity similar to commercial antioxidants with significant inhibition at low μg/mL concentrations. The toxicity of the compounds was also investigated against MRC-5 lung fibroblasts and none of the included compounds displayed any toxicity at 50 μg/mL.

Keywords

2,5-diketopiperazine, barettin, heterocycle, CLPAA, structure–activity relationship, marine natural products

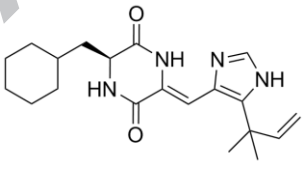
Abbreviations

Diketopiperazine (DKP), cellular lipid peroxidation antioxidant assay (CLPAA), *N*-methyl-2-pyrrolidone (NMP), Methyl tert-butyl ether (MTBE)

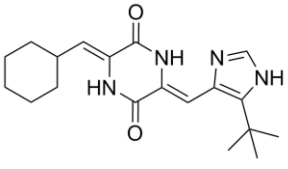
Short title: Antioxidant barettin mimetics

Introduction

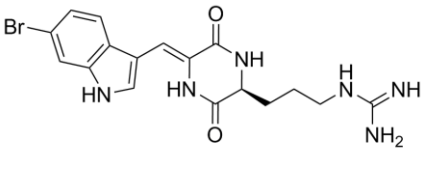
The 2,5-diketopiperazine (DKP) motif is found in numerous bioactive natural products, terrestrial and marine alike and represent the smallest cyclic peptide. The bioactivity of natural DKPs and their synthetic mimics differs vastly and was recently extensively reviewed by Borthwick[1]. A highly relevant example from a drug discovery perspective is the 2,5-DKP phenylahistin initially isolated from *Aspergillus ustus* NSC-F038 by Fukumoto and co-workers[2]. Phenylahistin exhibits strong cell cycle inhibitory properties and it was modified synthetically to yield the improved analog plinabulin which has been investigated in a range of clinical studies against several types of tumors[3-7]. Plinabulin and analogs thereof are potent microtubuli depolymerisation agents with low nanomolar inhibitory concentrations[4]. A related and also particularly interesting 2,5-DKP is barettin which was originally isolated from the marine boreal deep-water sponge *Geodia barretti*[8]. Barettin (cyclo-Arg-(6-Br)-dehydro-Trp) has been ascribed numerous bioactivities of which its ability to prevent settlement of other marine micro- and macro organisms is the most thoroughly studied and reported[9-10].



Phenylahistin



Plinabulin



Barettin

In addition, barettin was recently shown by Lind and co-workers to display potent antioxidant and anti-inflammatory effects and it was suggested that the compound may have an

atheroprotective effect[11]. Only barettin and a debrominated analog were studied in the paper by Lind et.al. which limited the insights into the structure activity relationship of the structural contributions to activity.

Antioxidants can prevent the damage induced by oxidative stress and free radicals and several diseases such as cardiovascular disease, diabetes, cancer and inflammation have been associated with oxidative damage[12-13]. Several studies have reported seemingly beneficial effects with regards to the prevention or delayed progression of these diseases by antioxidants but this has rarely been supported by larger scale clinical studies[14-15]. While observations from clinical trials into the actual effects of antioxidants remain inconsistent, several observational epidemiologic studies still imply that antioxidants can lower the prevalence of certain diseases where oxidative damage is involved[16-17].

Based on the potent antioxidant activity of barettin we decided to investigate this natural scaffold further with simplified synthetic compounds in analogy to our ongoing work on transferring natural pharmacophores onto DKP scaffolds[18]. The initial focus was on the influence of the heterocyclic core of barettin and also the role of the brominated indole substituent. In Nature Trp is often found in DKPs and several hundred 2,5-DKPs natural product have been reported in the literature[1]. For this purpose, a library of 19 analogs of ranging structural complexity was designed and the compounds were evaluated as inhibitors of the cellular lipid peroxidation in HepG2 cells. In addition, the potential cytotoxic effect of the prepared compounds was further investigated using MRC-5 cells.

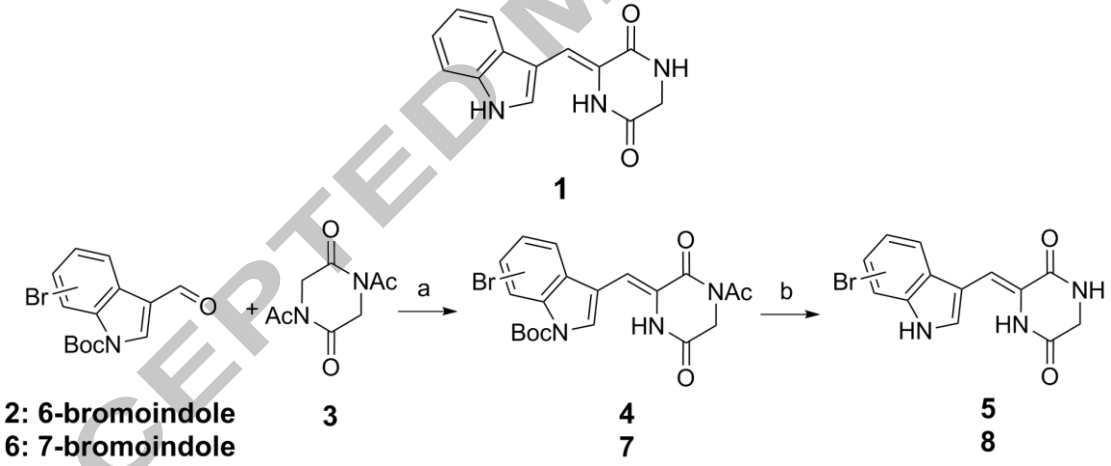
Results and discussion

Compound design and synthesis

During the course of the present study, 19 heterocyclic compounds were designed and synthesised. Their preparation (yields reported solely for novel compounds) and biological evaluation is presented in the following sections. While barettin represents a natural promising antioxidant lead it still remains a complicated compound to produce synthetically in significant amounts. Relying on the isolation of natural products from natural sources is rarely a viable option for extensive studies and the synthesis of barettin was reported by Johnson et al. in 2004[19]. However, despite recent synthetic improvements such as those recently reported by Kelley et al.[20] who employed iterative aldol condensations to

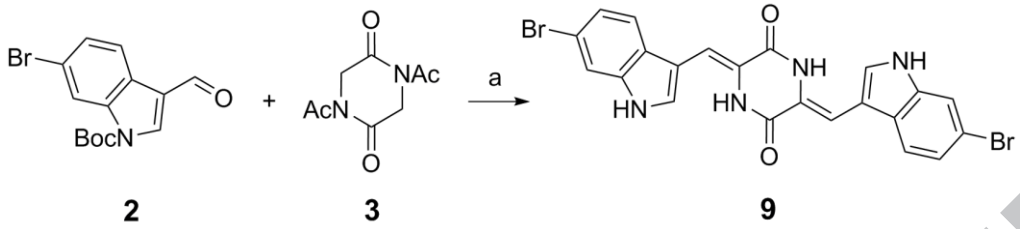
effectively generate monoalkylidene diketopiperazines, the enantioselective synthesis of barettin remains a low yielding synthesis. From a rational drug design perspective a removal of the chiral component of barettin would simplify the preparation significantly. This strategy was also successfully employed during the transformation of phenylahistin into plinabulin[1]. As a removal of the charged guanidine of barettin may also increase potential passive uptake properties[21] it was decided to focus on compounds without a charged chiral component with the main focus on the nature of the brominated indole substituent. To further probe the structure activity relationship of the prepared compounds, both imidazolidinedione and thioxothiazolidinone cores where also investigated in addition to the original 2,5-DKP scaffold.

Compound **1** was synthesised as described in the literature[22]. Compounds **4** and **7** were prepared from the reaction between the Boc-protected aldehyde **2** or **6** and the 1,4-diacetylpiperazine-2,5-dione **3**[23]. Deprotection was carried out using hydrazine hydrate to afford **5** and **8**[24].



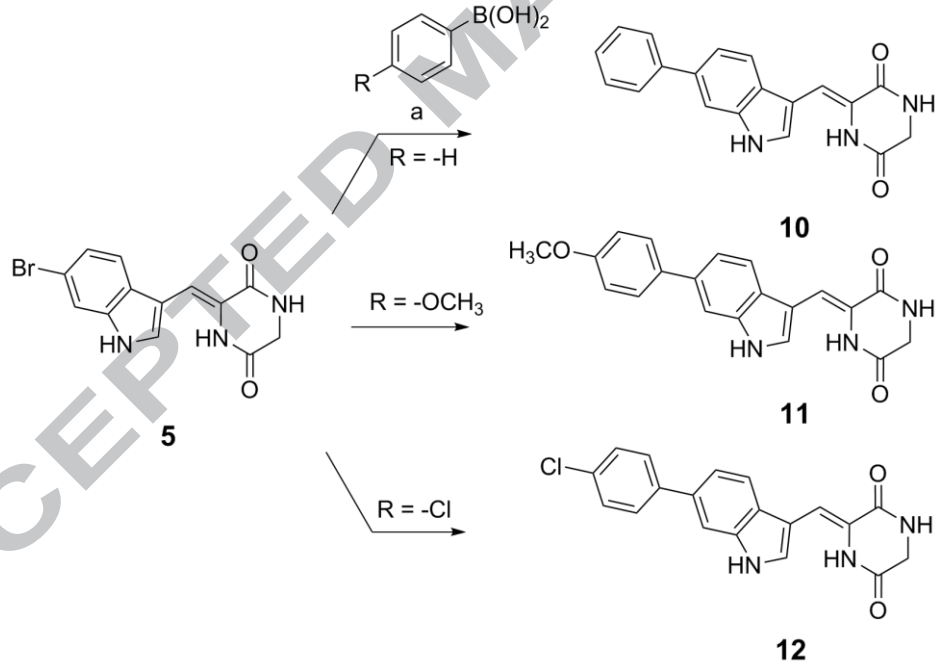
Scheme 1. Structure of compound **1**. Preparation of compound **5** and **8**. Reagents and conditions: (a) t-BuOK in THF, t-BuOH, NMP, RT, 24 h (**4**: 76 %, **7**: 50 %); (b) hydrazine hydrate, RT, 24 h (**5**: 73 %, **8**: 67 %).

By changing the reaction conditions and also the number of equivalents of **2**, it was possible to also readily obtain the bis-indole derivative **9**[25]**.**



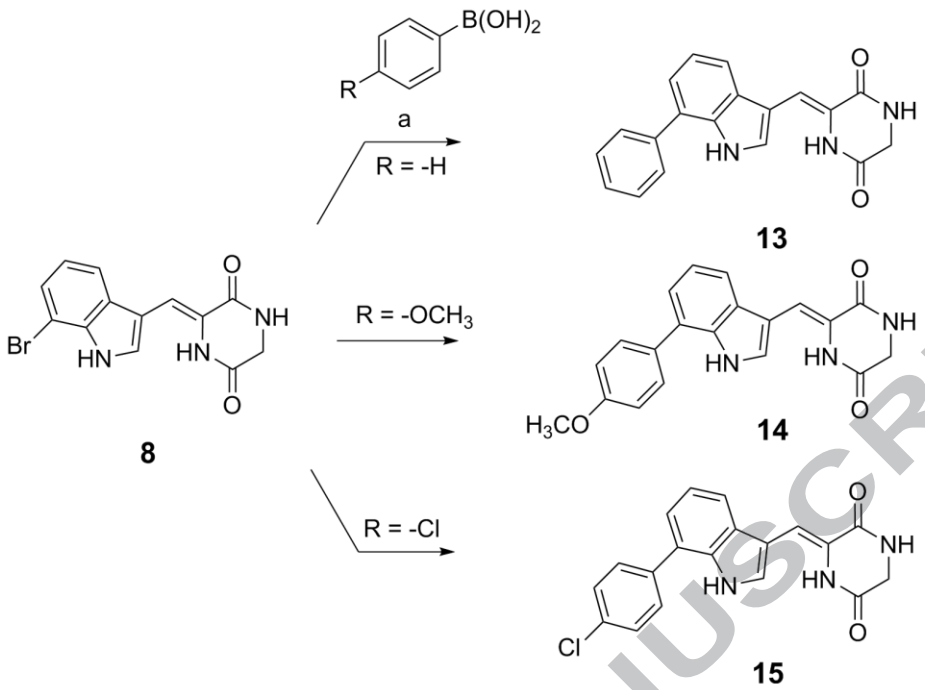
Scheme 2. Preparation of compound **9**. Reagents and conditions: (a) Cs_2CO_3, NMP, RT, 36 h; TFA, CH_2Cl_2, RT, 2 h (63 %).

In order to add additional hydrophobic bulk to the indole, compounds **5** and **8** were further functionalised using Suzuki coupling[26]. Compound **5** was reacted with phenylboronic acid, 4-methoxyphenylboronic acid and 4-chlorophenylboronic acid to yield **10**, **11** and **12** respectively. Brominated boronic acids were not included to avoid potential polymerization reactions.



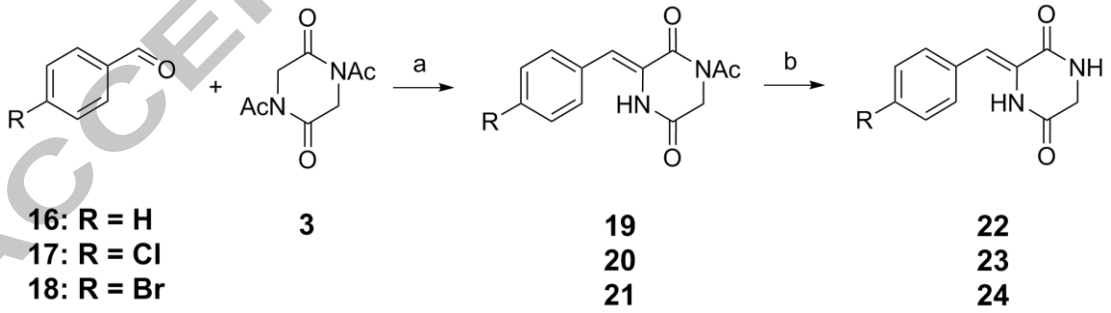
Scheme 3. Preparation of compound **10**, **11** and **12**. Reagents and conditions: (a) $PdCl_2(PPh_3)_2$, K_2CO_3, THF/H_2O, 60 °C, 16 h (**10**: 79 %, **11**: 72 %, **12**: 44 %).

In a similar fashion, **8** was reacted with the same set of boronic acids to afford compounds **13**, **14** and **15** substituted in the indolic 7-position.



Scheme 4. Preparation of compound **13**, **14** and **15**. Reagents and conditions: (a) $PdCl_2(PPh_3)_2$, K_2CO_3, THF/H_2O, 60 °C, 48 h (**13**: 79 %, **14**: 72 %, **15**: 89 %).

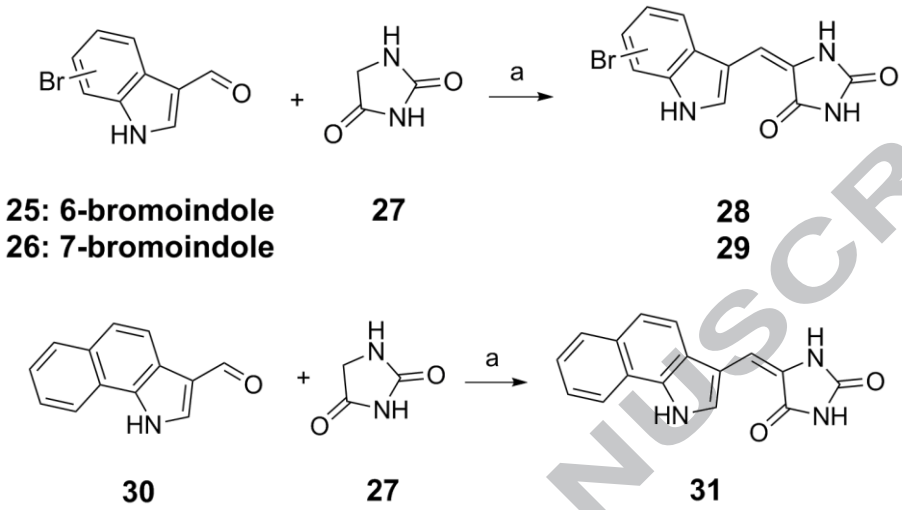
Structurally simpler analogs of the brominated indole of barettin were also included and prepared using **3** and a series of simple benzaldehydes. Compound **3** was reacted with the aldehydes **16, 17** and **18** to afford the mono-acetyl derivatives **19**[27-28]**, 20** and **21** which were deacetylated using hydrazine hydrate[29] to finally give **22**[28], **23** and **24** in high yields.



Scheme 5. Preparation of compounds **19-21** and **22-24**. Reagents and conditions: (a) NEt_3, NMP, RT, 16 h (**20**: 45 %, **21**: 80 %); (b) hydrazine hydrate, RT, 2 h (**23**: 99 %, **24**: 92 %).

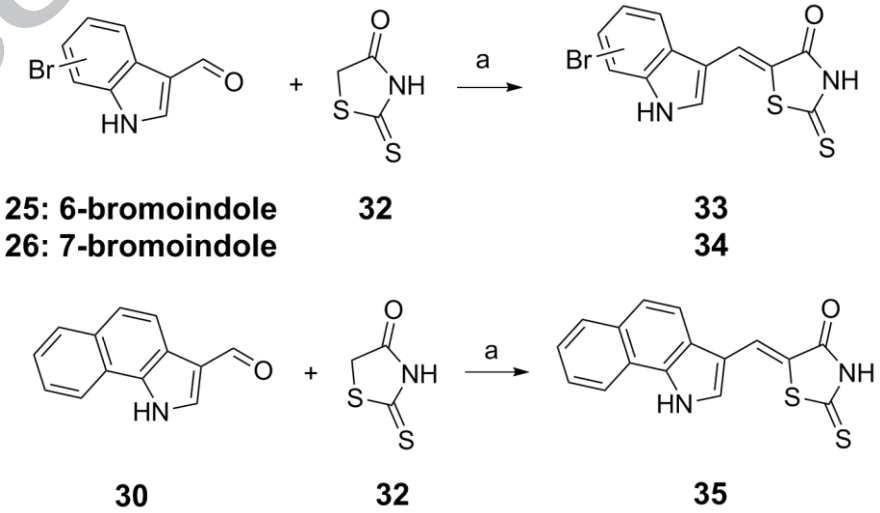
By reacting the bromoindole aldehydes **25** and **26** with hydantoin (**27**), the central core of the barettin was replaced with a 2,4-imidazolidinedione core, to yield the derivatives **28** and **29** in a single step condensation[30]. Indole hydantoin derivatives represent interesting compounds

and they have recently been shown to be promising p53-MDM2 binding inhibitors with IC_{50} values down to 33 nM reported[31]. Reaction of the same hydantoin **27** with the aldehyde **30** afforded the derivative **31**.



Scheme 6. Preparation of compounds **28**, **29** and **31**. Reagents and conditions: (a) piperidine, reflux, 1 h (**28**: 18 %, **29**: 10 %, **31**: 28 %).

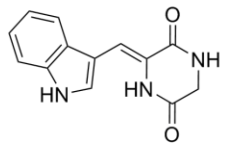
In addition, the thioxothiazolidinone derivatives **33** and **34** were prepared as described in the literature by condensation of the aldehydes **25** and **26** with rhodanine **32**[32]. The thioxothiazolidinone core represents a versatile scaffold that can be reacted with both nucleophiles and electrophiles, that has been used to generate a range of different biologically active compounds[33-34]. Following the same procedure, the thioxothiazolidinone **35** was obtained by condensation of the aldehyde **30** with rhodanine **32**[35].



Scheme 7. Preparation of compound **33**, **34** and **35**. Reagents and conditions: (a) AcONa, ACOH, reflux, 2 h, (**35**: 47 %).

The prepared compounds were generally prepared in acceptable yields (reported only for the novel compounds) and their structures are summarised in the Figure 1. below.

2,5-Diketopiperazines



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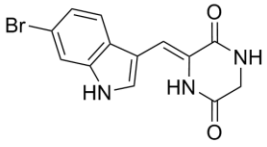

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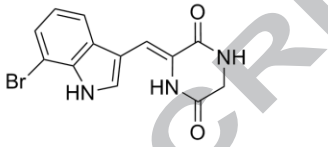

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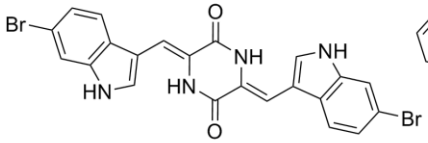

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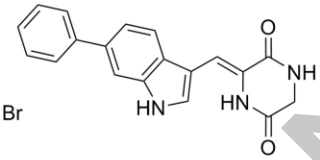

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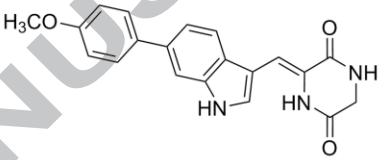

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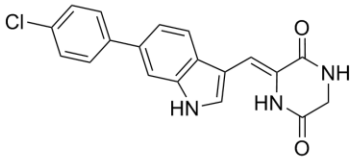

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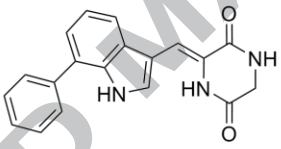

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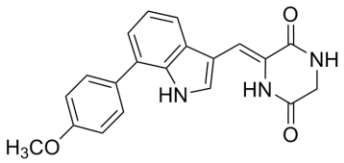

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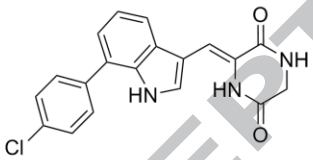

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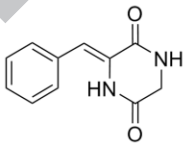

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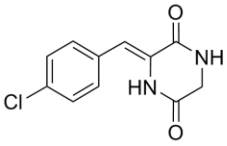

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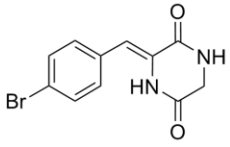

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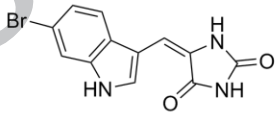
Imidazolidinediones



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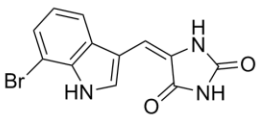

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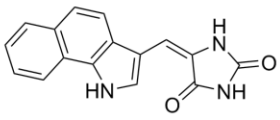

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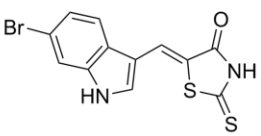
Thioxothiazolidinones



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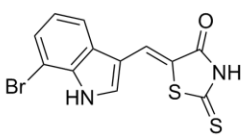

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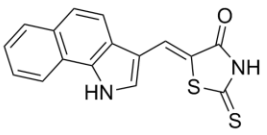

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Figure 1. Structures of the prepared compounds.

Bioactivity testing

Following synthesis and purification, the compounds were investigated as inhibitors in a cellular lipid peroxidation antioxidant assay employing HepG2 cells. In the CLPAA assay the lipid peroxidation is initiated by the addition of cumene hydroperoxide and the inhibition by the compounds can be evaluated using fluorescence after the addition of the reactive oxygen species marker C-11-BODIPY[36]. Initially the compounds were screened at a concentration of 50 μg/mL to assess their inhibitory potential. Compounds displaying sufficient inhibition (<50 % remaining activity) were considered as active and were included in a dose-response analysis to establish their EC_{50} values as summarised in Table 1 and Figure 2.

Table 1. Physicochemical properties of the compounds and their CLPAA inhibitory properties

Compound	Mw	ClogP[1]	Activity [50 μg/mL]	50 % inhibition [μg/mL][3]
2,5-Diketopiperazines				
1	241.25	0.419	na[2]	nt[4]
5	320.15	1.450	**a**[2]	**10**
8	320.15	1.450	na	nt
9	526.19	3.436	**a**	12
10	317.35	2.307	**a**	25
11	347.37	2.226	na	nt
12	351.79	3.010	**a**	**5**
13	317.35	2.307	na	nt
14	347.37	2.227	na	nt
15	351.79	3.010	**a**	**10**
22	202.21	0.429	na	nt
23	236.66	1.142	na	nt
24	281.11	1.292	**a**	>75
Barettin	419.28	0.299	**a**	30
Imidazolidinediones				
28	306.12	2.023	**a**	20
29	306.12	2.023	**a**	>75
31	277.28	2.166	**a**	**2.5**
Thioxothiazolidinones				
33	339.23	2.823	na	nt
34	339.23	2.823	na	nt
35	310.39	2.966	na	nt

[1]Calculated values using ChemBioDraw Ultra

[2]Not active or active. Active defined as > 50 % inhibition and not active as < 50 % inhibition in the CLPAA assay

[3]Inhibition data generated from dose response analysis up to 75 μg/mL

[4]Not tested.

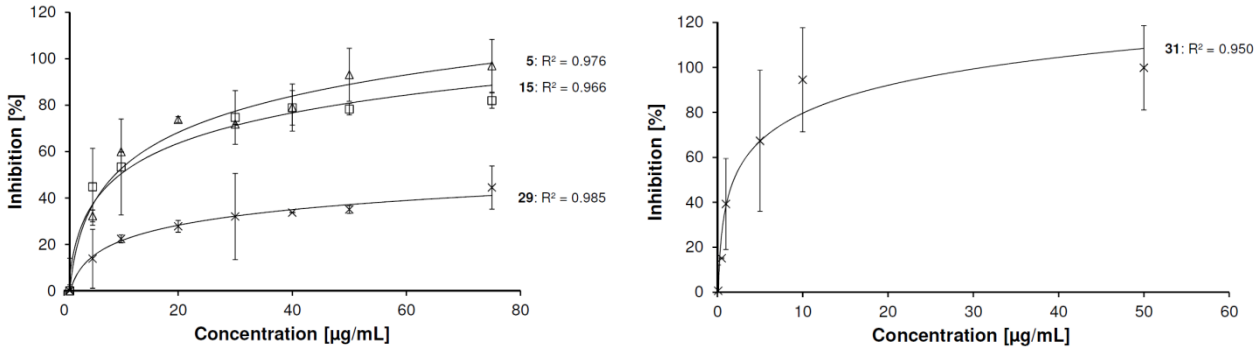

Figure 2. Left: Dose-response analysis of the antioxidant properties of selected compounds in the CLPAA assay; -Δ- compound **5**, -□- compound **15** and -×- compound **29**. Right: Dose-response analysis of the antioxidant properties of compound **31** at a lower concentration range.

The potential toxicity on human lung fibroblasts (MRC-5 cells) was also evaluated using a commonly employed MTT-assay. Barettin has previously been reported to display no reduction in viability at concentrations up to 100 µg/mL, (maximum concentration investigated) against both HepG2 and MRC-5 cells. The prepared compounds were thus investigated for toxic activity against MRC-5 cells in an analogous fashion, initially at a concentration of 50 µg/mL. At 50 µg/mL, none of the compounds induced a decrease in MRC-5 viability (data not shown) and subsequently none of the compounds were evaluated in further dose-response studies.

Structure-activity-relationship

The recent study on barettin and its debromo analog debromobarettin revealed a CLPAA inhibition of 50 % at 30 µg/mL (relative to the positive control cumene hydroperoxide)[11]. Debromobarettin was inactive as inhibitor implying that the influence of the halogen substituent is of major importance for the inhibitory ability of the disubstituted 2,5-DKP scaffold. Removal of the bromine has also been observed to negatively affect the antifouling properties of barettin derivatives[37]. The two compounds were also compared to the commercially employed synthetic antioxidant butylated hydroxytoluene (BHT) which was significantly more potent with a 75 % reduction of oxidation at a concentration of 10 µg/mL[11].

The current library includes significantly simplified and rational structures inspired by the structure of the naturally occurring barettin. The prepared compounds are achiral and none of the

compounds bear the cationic arm of barettin. Instead focus lies on hydrophobic substituents, halogenation and alternative heterocyclic cores.

As shown in table 1, 10 out of the 20 analysed compounds (including barettin) displayed inhibition of cumene hydroperoxide induced lipid peroxidation. It is interesting to see that several of these simplified compounds are active despite the absence of the chiral cationic side chain found in natural barettin. Dose response analysis revealed that several of the compounds (compounds **5**, **9**, **10**, **12**, **15**, **28** and **31**) displayed improved antioxidative activities in comparison to those of barettin. The most potent inhibitors were compounds **12** and **31** which displayed low EC_{50} values of 5 and 2.5 μg/mL respectively. Both of those compounds also revealed a 75 % inhibition at 10 μg/mL, which is similar to that of the commercial antioxidant BHT indicating a particularly strong antioxidant activity[11]. These two compounds are also superior in inhibition to the positive antioxidant control luteolin which was included at 50 μg/mL (Table S1 in Supporting information) in the CLPAA assay.

All compounds in the prepared library are noncharged, small, with theoretical ClogP values ranging from 0.419 to 3.436. Compound **9** substituted with two 6-bromoindoles stands out as both the largest and most hydrophobic compound in the library. Compound **9** is indeed an active inhibitor but several of the other compounds display similar activities while being both smaller and more hydrophilic so there appears to be no clear-cut link between size and polarity and activity.

As three different heterocyclic cores have been used to generate the libraries it is also relevant to consider the SAR from this perspective. Active compounds **5**, **9**, **10**, **12**, **15** and **24** are all 2,5-DKPs. Upon dose-response analysis of compound **24** an EC_{50}-value >75 μg/mL was obtained suggesting that this compound is not very active. The remaining five active 2,5-DKPs display EC_{50} values between 5 and 25 μg/mL. Of the smaller compounds **1**, **5**, **8**, **22**, **23** and **24** only compound **5** displays a pronounced activity with 10 μg/mL EC_{50}-values. Compound **5** is brominated in the 6-position whereas inactive compound **8** is brominated in the 7-position. In barettin, the 6-position is brominated and the clear difference between **5** and **8** implies that this is a beneficial position for activity. In analogy to **8**, and also previous studies on debromobarettin, the non brominated analog **1** is also inactive[11,37]. Compounds **22**, **23**, **24** were designed with smaller substituted phenyls instead of indoles and none of them

are regarded as active. The larger compounds (**10**-**15**) prepared from **5** and **8** by additional Suzuki couplings contained several active compounds. Both compounds **10** and **12** were active with a pronounced activity seen for the *para*-chlorinated analog **12** (EC_{50} = 5 μg/mL). Compounds **13**-**15**, substituted in the indole 7-position from **8** are less active than their indole 6-analogs. *Para*-chlorinated compound **15** still remains highly active (EC_{50} = 10 μg/mL) but inferior to its indole 6-analog (**12**). Three brominated boronic acids (2-, 3- and 4-bromophenylboronic acid) were initially evaluated in the Suzuki couplings with an aim to generate brominated analogs. However due to multiple side-reactions and polymerisations, the strategy was abandoned in favor of the chlorinated alternatives (**12** and **15**). To conclude the 2,5-DKP library, it appears that halogenated compounds are more active. With regards to substitution patterns it also appears that the 6-position on the indole is beneficial as actives **5**, **9**, **10** and **12** all share this substitution pattern while the chlorinated **15** represents the only active 2,5-DKP substituted in the 7-position. It also evident that the larger compounds are more active than the smaller ones in this library.

Moving over to hydantoin derivatives **28**, **29** and **31**, all displaying an imidazolidinedione moiety, these derivatives all displayed antioxidant activity in the initial screen at 50 μg/mL. This illustrates that this scaffold is also suited for the generation of effective inhibitors. Both the 2,5-DKP and the imidazolidinedione scaffold display a comparable ability to engage in potential hydrogen bonding interactions and they appear equally suited for the generation of this type of compounds. For compound **29**, the activity initially observed in the screen was determined to be somewhat lower in the dose-response analysis in a similar fashion as for **24** with an EC_{50}-value >75 μg/mL. Worth noting is that compound **24** also is substituted in the 7-position on the indole which is in agreement with the lower activity seen for the DKP library discussed in the previous section. The 6-indole analog **28** was an active inhibitor (EC_{50} = 20 μg/mL) which also correlates well with the SAR for the DKPs. Introduction of the bulky aldehyde **30** generated compound **31** which is not halogenated. Despite this, compound **31** represent the most active of all the prepared compounds with a low EC_{50} of 2.5 μg/mL. This is superior to compound **12** and suggest that these two compounds display antioxidant activity equal to BHT in this assay[11].

Compounds **33**-**35**, represent thioxothiazolidinone derivatives of **28**, **29** and **31**, and were prepared from rhodanine. Interestingly none of these compounds were active in the initial

screen which was unexpected given that all the imidazolidinedione derivatives were active in the initial screen and also generated the most active compound (**31**) in this study. This observation implies that the sulfur containing thioxothiazolidinone ring impairs the antioxidant capacity of the compounds in comparison to the DKP and the imidazolidinedione scaffolds.

Conclusions

In the present study 19 synthetic heterocyclic antioxidants have been prepared and evaluated for their ability to inhibit cumene hydroperoxide induced lipid peroxidation. The library contains compounds inspired by the natural brominated marine antioxidant and antifoulant barettin all of which are considerably simplified structurally. Several of the prepared compounds displayed potent inhibitory activities superior to barettin with two compounds exhibiting inhibitory properties comparable to commercially employed antioxidants. The high inhibitory potency observed for the most active compounds suggest that these compounds should be further studied and refined in the future. For the current library, the inhibitory activity is linked to the nature and position of the indole substituents. The imidazolidinedione scaffold compares well with natural 2,5-DKP scaffold while none of the compounds incorporating a thioxothiazolidinone ring were active. The study highlights how it is possible to generate highly active and structurally simple synthetic antioxidants by employing a natural marine template.

Experimental section

General

NMR spectra were acquired on a Varian 7000e 400 MHz spectrometer and HRMS was performed employing an LTQ Orbitrap XL Hybrid Fourier Transform mass spectrometer from Thermo Scientific. Reagents and solvents were obtained from commercial suppliers and used without further purification. Air-sensitive reactions were carried out under an argon atmosphere. Reaction were monitored by thin-layer chromatography which was carried out on aluminum-backed plates coated with silica gel and visualised under UV light at 254 nm and ethanolic vanillin dip.

Synthesis

***tert*-butyl (Z)-3-[(4-acetyl-3,6-dioxopiperazin-2-ylidene)methyl]-6-bromo-1H-indole-1-carboxylate (4)**

To a solution of 1,4-diacetylpiperazine-2,5-dione (1.4 g, 6.8 mmol, 2.0 eq.) and *tert*-butyl 6-bromo-3-formyl-1H-indole-1-carboxylate (1.1 g, 3.4 mmol, 1.0 eq.) in a mixture of anhydrous *tert*-butanol (5 mL) and NMP (10 mL) was added at 0 °C a solution of potassium tert-butoxide in THF (1M, 5.1 mL, 5.1 mmol, 1.5 eq.). The mixture was stirred at room temperature for 24 h and then, diluted with water. The resulting precipitate was filtered, washed with ethanol and diethyl ether to afford **4** as a pale yellow solid (1.2 g, 76 %). ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm): 2.51 (s, 3H), 4.38 (s, 2H), 7.09 (s, 1H), 7.50 (dd, J = 8.4 Hz, 1H), 7.73 (d, J = 8.4 Hz, 1H), 8.18 (s, 1H), 8.24 (d, 1H), 10.42 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm): 27.2, 28.0, 46.2, 85.7, 109.0, 113.1, 118.0, 118.2, 126.6, 127.6, 127.9, 128.9, 135.3, 148.9, 161.7, 164.6, 172.3. FTMS (-ESI): calcd for $C_{20}H_{19}O_5N_3{}^{79}Br^-$: m/z = 460.0503, found: 460.0513.

(*Z*)-3-[(6-bromo-1H-indol-3-yl)methylene]piperazine-2,5-dione (5)

To a solution of **4** (280 mg, 0.60 mmol, 1.0 eq.) in NMP (5 mL) was added hydrazine hydrate (294 µL, 6.0 mmol, 10.0 eq.). The mixture was stirred at room temperature for 24 h and then, diluted with water. The precipitate was filtered, washed with ethanol, acetone and MTBE to afford **5** as a pale yellow solid (140 mg, 73 %). ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm): 4.00 (s, 2H), 6.94 (s, 1H), 7.22 (d, J = 8.0 Hz, 1H), 7.60 (m, 1H), 7.61 (s, 1H), 7.94 (s, 1H), 8.94 (s, 1H), 9.54 (s, 1H), 11.74 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm): 44.9, 106.7, 108.1, 114.3, 114.6, 120.0, 122.6, 123.3, 126.0, 127.1, 136.5, 160.5, 164.5. FTMS (-ESI): calcd for $C_{13}H_9N_3O_2{}^{79}Br^-$: m/z = 317.9884, found: 317.9876.

***tert*-butyl (Z)-3-[(4-acetyl-3,6-dioxopiperazin-2-ylidene)methyl]-7-bromo-1H-indole-1-carboxylate (7)**

To a solution of 1,4-diacetylpiperazine-2,5-dione (2.5 g, 12.6 mmol, 1.5 eq.) and *tert*-butyl 7-bromo-3-formyl-1H-indole-1-carboxylate (2.7 g, 8.3 mmol, 1.0 eq.) in a mixture of anhydrous *tert*-butanol (20 mL) and NMP (20 mL) was added at 0 °C a solution of potassium tert-butoxide in THF (1M, 13 mL, 13.0 mmol, 1.6 eq.). The mixture was stirred at room temperature for 24 h and then, diluted with water. The resulting cloudy mixture was extracted

with acetone and ethyl acetate, and then, washed with a saturated ammonium chloride solution. The organic layer was dried over Na_2SO_4, filtered through a silica gel pad and evaporated under reduced pressure to afford, after trituration with MTBE, **7** (1.9 g, 50 %) as a pale yellow solid. ^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ (ppm): 1.65 (s, 9H), 2.52 (s, 3H), 4.39 (s, 2H), 7.08 (s, 1H), 7.27 (t, J = 7.6 Hz, 1H), 7.63 (d, J = 7.6 Hz, 1H), 7.77 (d, J = 7.6 Hz, 1H), 8.15 (s, 1H), 10.42 (s, 1H). ^{13}C NMR (DMSO-*d*$_6$, 100 MHz): δ (ppm): 26.8, 27.4, 45.8, 85.5, 107.3, 108.7, 112.3, 118.8, 124.9, 127.2, 129.9, 130.2, 132.7, 148.0, 161.3, 164.2, 171.9. FTMS (-ESI): calcd for $C_{20}H_{19}O_5N_3{}^{79}Br^-$: m/z = 460.0514, found: 460.0523.

(*Z*)-3-[(7-bromo-1H-indol-3-yl)methylene]piperazine-2,5-dione (8)

To a solution of **7** (900 mg, 2.0 mmol, 1.0 eq.) in NMP (4 mL) was added hydrazine hydrate (0.76 mL, 15.6 mmol, 8.0 eq.). The mixture was stirred at room temperature for 24 h and then, diluted with water. The precipitate was filtered, washed with ethanol and MTBE to afford **8** as a yellow solid (140 mg, 67 %). ^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ (ppm): 4.00 (s, 2H), 6.94 (s, 1H), 7.05 (m, 1H), 7.39 (d, J = 6.4 Hz, 1H), 7.66 (d, J = 6.4 Hz, 1H), 7.96 (s, 1H), 8.16 (s, 1H), 9.67 (s, 1H), 11.82 (s, 1H). ^{13}C NMR (DMSO-*d*$_6$, 100 MHz): δ (ppm): 44.8, 104.5, 106.7, 109.1, 111.7, 121.2, 123.6, 124.6, 127.2, 128.7, 134.1, 160.5, 164.6. FTMS (-ESI): calcd for $C_{13}H_9N_3O_2{}^{79}Br^-$: m/z = 317.9884, found: 317.9887.

(3Z,6Z)-3,6-bis[(6-bromo-1H-indol-3-yl)methylene]piperazine-2,5-dione (9)

To a solution of 1,4-diacetylpiperazine-2,5-dione (0.24 g, 1.2 mmol, 1.0 eq.) and *tert*-butyl 6-bromo-3-formyl-1H-indole-1-carboxylate (1.0 g, 3.1 mmol, 2.6 eq.) in NMP (6 mL) was added cesium carbonate (1.3 g, 3.6 mmol, 3.0 eq.). The mixture was stirred at room temperature for 36 h and then, diluted with water. The precipitate that formed was filtered, taken up in acetone and the volatiles were removed under reduced pressure. The residue was taken up in dichloromethane (10 mL) and TFA (6 mL), and the resulting solution was stirred at room temperature for 2 h. The mixture was quenched with water, basified with NaOH 1M and extracted with ethyl acetate and Et_2O. The organic layer was filtered and the precipitate washed with MTBE to afford **9** as a dark yellow solid (400 mg, 63 %). ^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ (ppm): 7.03 (s, 2H), 7.25 (dd, J = 8.5 Hz, J = 1.5 Hz 2H), 7.63 (d, J = 1.5 hz, 2H), 7.66 (d, J = 8.5 Hz, 2H), 8.12 (s, 2H). ^{13}C NMR (DMSO-*d*$_6$, 100 MHz): δ (ppm): 107.0, 108.3, 114.4, 114.8, 120.1, 122.8, 1296.1, 126.3, 127.6, 136.6, 158.2. FTMS (-ESI): calcd for $C_{22}H_{13}N_4O_2{}^{79}Br_2^-$: m/z = 522.9411, found: 522.9407.

(Z)-3-[(6-phenyl-1H-indol-3-yl)methylene]piperazine-2,5-dione (10)

To a mixture of **5** (50 mg, 0.16 mmol, 1.0 eq.) in a mixture of degassed THF (4 mL) and water (2 mL) were added, under an argon atmosphere, phenylboronic acid (29 mg, 0.24 mmol, 1.5 eq.), K_2CO_3 (65 mg, 0.48 mmol, 3.0 eq.) and $PdCl_2(PPh_3)_2$ (11 mg, 0.016 mmol, 0.1 eq.). The reaction was stirred at 60 °C for 16 h and then, diluted with water after cooling to room temperature. The mixture was extracted with ethyl acetate, filtered and the precipitate was washed with ethanol, dichloromethane and MTBE to afford **10** (40 mg, 79 %) as a dark yellow solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm): 4.01 (s, 2H), 7.02 (s, 1H), 7.34 (t, J = 7.0 Hz, 1H), 7.45 (m, 3H), 7.70 (m, 4H), 7.98 (s, 1H), 8.12 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm): 44.9, 107.4, 107.9, 109.7, 118.6, 119.2, 122.8, 126.7, 127.0, 128.9, 134.5, 136.3, 141.2, 160.6, 164.4. FTMS (-ESI): calcd for $C_{19}H_{14}O_2N_3^-$: m/z = 316.1081, found: 316.1098.

(Z)-3-{[6-(4-methoxyphenyl)-1H-indol-3-yl]methylene}piperazine-2,5-dione (11)

To a mixture of **5** (50 mg, 0.16 mmol, 1.0 eq.) in a mixture of degassed THF (4 mL) and water (2 mL) were added, under an argon atmosphere, 4-methoxyphenylboronic acid (36 mg, 0.23 mmol, 1.5 eq.), K_2CO_3 (65 mg, 0.47 mmol, 3.0 eq.) and $PdCl_2(PPh_3)_2$ (11 mg, 0.016 mmol, 0.1 eq.). The reaction was stirred at 60 °C for 16 h and then, diluted with water after cooling to room temperature. The mixture was extracted with ethyl acetate, filtered and the precipitate was washed with ethanol, dichloromethane and MTBE to afford **11** (40 mg, 72 %) as a grey solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm): 3.80 (s, 3H), 4.01 (s, 2H), 7.02 (m, 3H), 7.38 (d, J = 8.0 Hz, 1H), 7.61 (m, 3H), 7.68 (d, J = 8.0 Hz, 1H), 7.95 (s, 1H), 8.12 (s, 1H), 9.49 (s, 1H), 11.67 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm): 44.9, 55.2, 107.5, 107.8, 109.1, 114.3, 118.5, 119.0, 122.7, 125.9, 126.8, 127.8, 133.6, 134.3, 136.4, 158.4, 160.7, 164.5. FTMS (-ESI): calcd for $C_{20}H_{16}O_3N_3^-$: m/z = 346.1197, found: 346.1194.

(Z)-3-{[6-(4-chlorophenyl)-1H-indol-3-yl]methylene}piperazine-2,5-dione (12)

To a mixture of **5** (50 mg, 0.16 mmol, 1.0 eq.) in a mixture of degassed THF (4 mL) and water (2 mL) were added, under an argon atmosphere, 4-chlorophenylboronic acid (38 mg, 0.23 mmol, 1.5 eq.), K_2CO_3 (65 mg, 0.47 mmol, 3.0 eq.) and $PdCl_2(PPh_3)_2$ (11 mg, 0.016 mmol, 0.1 eq.). The reaction was stirred at 60 °C for 16 h and then, diluted with water after cooling to room temperature. The mixture was extracted with ethyl acetate, filtered and the

precipitate was washed with ethanol, dichloromethane and MTBE to afford **12** (25 mg, 44 %) as a grey solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm): 4.02 (s, 2H), 7.02 (s, 1H), 7.42 (d, J = 8.0 Hz, 1H), 7.51 (d, J = 8.0 Hz, 2H), 7.68 (s, 1H), 7.73 (d, J = 8.0 Hz, 3H), 8.00 (s, 1H), 9.54 (s, 1H), 11.76 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm): 44.9, 107.3, 107.9, 109.8, 118.7, 119.1, 122.9, 126.7, 127.3, 128.5, 128.8, 131.5, 133.1, 136.3, 140.0, 160.7, 164.5. FTMS (-ESI): calcd for $C_{19}H_{13}O_2N_3{}^{35}Cl^-$: m/z = 350.0702, found: 350.0699.

(Z)-3-[(7-phenyl-1H-indol-3-yl)methylene]piperazine-2,5-dione (13)

To a mixture of **8** (100 mg, 0.32 mmol, 1.0 eq.) in a mixture of degassed THF (8 mL) and water (4 mL) were added, under an argon atmosphere, phenylboronic acid (78 mg, 0.64 mmol, 2.0 eq.), K_2CO_3 (130 mg, 0.47 mmol, 3.0 eq.) and $PdCl_2(PPh_3)_2$ (22 mg, 0.031 mmol, 0.1 eq.). The reaction was stirred at 60 °C for 48 h and then, diluted with water after cooling to room temperature. The precipitate was filtered and the residue was washed with ethanol, dichloromethane and MTBE to afford **13** (80 mg, 79 %) as a grey solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm): 4.00 (s, 2H), 7.02 (s, 1H), 7.21 (m, 2H), 7.44 (m, 1H), 7.55 (d, J = 7.4 Hz, 2H), 7.63 (m, 3H), 7.90 (s, 1H), 8.12 (s, 1H), 9.64 (s, 1H), 11.49 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm): 44.9, 107.3, 108.2, 117.3, 120.5, 122.3, 122.9, 125.7, 127.0, 127.4, 128.0, 128.3, 129.0, 132.8, 138.3, 160.8, 164.6. FTMS (-ESI): calcd for $C_{19}H_{14}O_2N_3^-$: m/z = 316.1092, found: 316.1085.

(Z)-3-{[7-(4-methoxyphenyl)-1H-indol-3-yl]methylene}piperazine-2,5-dione (14)

To a mixture of **8** (100 mg, 0.32 mmol, 1.0 eq.) in a mixture of degassed THF (8 mL) and water (4 mL) were added, under an argon atmosphere, 4-methoxyphenylboronic acid (97 mg, 0.64 mmol, 2.0 eq.), K_2CO_3 (130 mg, 0.47 mmol, 3.0 eq.) and $PdCl_2(PPh_3)_2$ (22 mg, 0.031 mmol, 0.1 eq.). The reaction was stirred at 60 °C for 48 h and then, diluted with water after cooling to room temperature. The precipitate was filtered and the residue was washed with ethanol, dichloromethane and MTBE to afford **14** (80 mg, 72 %) as a dark yellow solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm): 3.85 (s, 3H), 4.00 (s, 2H), 7.03 (s, 1H), 7.12 (d, J = 8.6 Hz, 2H), 7.15 (m, 1H), 7.20 (t, J = 7.4 Hz, 1H), 7.57 (d, J = 8.6 Hz, 2H), 7.61 (d, J = 7.4 Hz, 1H), 7.90 (s, 1H), 8.12 (s, 1H), 9.63 (s, 1H), 11.44 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm): 44.9, 55.3, 107.5, 108.2, 114.4, 116.9, 120.5, 122.0, 122.8, 125.6, 127.0, 127.9, 129.5, 132.9, 158.7, 160.9, 164.6. FTMS (-ESI): calcd for $C_{20}H_{16}O_3N_3^-$: m/z = 346.1197, found: 346.1192.

(Z)-3-{[7-(4-chlorophenyl)-1H-indol-3-yl]methylene}piperazine-2,5-dione (15)

To a mixture of **8** (100 mg, 0.32 mmol, 1.0 eq.) in a mixture of degassed THF (8 mL) and water (4 mL) were added, under an argon atmosphere, 4-chlorophenylboronic acid (100 mg, 0.64 mmol, 2.0 eq.), K_2CO_3 (130 mg, 0.47 mmol, 3.0 eq.) and $PdCl_2(PPh_3)_2$ (22 mg, 0.031 mmol, 0.1 eq.). The reaction was stirred at 60 °C for 48 h and then, diluted with water after cooling to room temperature. The precipitate was filtered and the residue was washed with ethanol, dichloromethane and MTBE to afford **15** (100 mg, 89 %) as a grey solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm): 4.00 (s, 2H), 7.02 (s, 1H), 7.23 (m, 2H), 7.60 (d, J = 8.2 Hz, 2H), 7.66 (m, 3H), 7.93 (s, 1H), 8.13 (s, 1H), 9.64 (s, 1H), 11.53 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm): 44.9, 107.2, 108.4, 117.9, 120.5, 122.3, 123.0, 124.5, 127.1, 128.1, 129.0, 130.2, 132.2, 132.8, 137.2, 160.7, 164.6. FTMS (-ESI): calcd for $C_{19}H_{13}O_2N_3{}^{35}Cl^-$: m/z = 350.0702, found: 350.0697.

(Z)-1-acetyl-3-(4-chlorobenzylidene)piperazine-2,5-dione (20)

To a solution of 1,4-diacetylpiperazine-2,5-dione (1.0 g, 5.0 mmol, 1.0 eq.) and 4-chlorobenzaldehyde (703 mg, 5 mmol, 1.0 eq.) in NMP (3 mL) was added triethylamine (0.7 mL, 5.0 mmol, 1.0 eq.). The mixture was stirred at room temperature for 16 h and then, diluted with water. The resulting precipitate was filtered, washed with acetone and MTBE to afford **20** as a beige solid (800 mg, 45 %). ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm): 4.36 (s, 2H), 6.93 (s, 1H), 7.49 (d, J = 8.4 Hz, 2H), 7.60 (d, J = 8.4 Hz, 2H), 10.43 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm): 26.7, 45.6, 117.4, 127.5, 128.6, 131.3, 132.0, 133.0, 161.5, 164.1, 171.8. FTMS (-ESI): calcd for $C_{13}H_{10}O_3N_2{}^{35}Cl^-$: m/z = 277.0385, found: 277.0387.

(Z)-1-acetyl-3-(4-bromobenzylidene)piperazine-2,5-dione (21)

To a solution of 1,4-diacetylpiperazine-2,5-dione (1.0 g, 5.0 mmol, 1.0 eq.) and 4-bromobenzaldehyde (925 mg, 5 mmol, 1.0 eq.) in NMP (3 mL) was added triethylamine (0.7 mL, 5.0 mmol, 1.0 eq.). The mixture was stirred at room temperature for 16 h and then, diluted with water. The resulting precipitate was filtered, washed with acetone and MTBE to afford **21** as a yellow solid (1.3 g, 80 %). ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm): 4.36 (s, 2H), 6.93 (s, 1H), 7.49 (d, J = 8.4 Hz, 2H), 7.60 (d, J = 8.4 Hz, 2H), 10.28 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm): 26.7, 45.6, 117.4, 127.5, 128.6, 131.3, 132.0, 133.0, 161.5, 164.1, 171.8. FTMS (-ESI): calcd for $C_{13}H_{10}O_3N_2{}^{79}Br^-$: m/z = 320.9880, found: 320.9880.

(Z)-3-(4-chlorobenzylidene)piperazine-2,5-dione (23)

To a mixture of **20** (200 mg, 0.72 mmol, 1.0 eq.) in NMP (2 mL) was added hydrazine hydrate (52 µL, 1.08 mmol, 1.5 eq.). The reaction was stirred at room temperature for 2 h. Water was added and the insoluble was filtered, washed with water and triturated with acetone and MTBE to afford **23** as a pale yellow solid (170 mg, 99 %). ^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ (ppm): 4.00 (s, 2H), 6.65 (s, 1H), 7.44 (m, 2H), 7.50 (m, 2H), 8.32 (s, 1H), 10.04 (s, 1H). ^{13}C NMR (DMSO-*d*$_6$, 100 MHz): δ (ppm): 44.8, 112.6, 127.6, 128.6, 131.0, 132.2, 132.4, 159.8, 164.7. FTMS (-ESI): calcd for $C_{11}H_8O_2N_2{}^{35}Cl^-$: m/z = 235.0280, found: 235.0279.

(Z)-3-(4-bromobenzylidene)piperazine-2,5-dione (24)

To a mixture of **21** (200 mg, 0.62 mmol, 1.0 eq.) in NMP (2 mL) was added hydrazine hydrate (45 µL, 0.93 mmol, 1.5 eq.). The reaction was stirred at room temperature for 2 h. Water was added and the insoluble was filtered, washed with water and triturated with acetone and MTBE to afford **24** as pale yellow solid (160 mg, 92 %). ^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ (ppm): 4.00 (s, 2H), 6.63 (s, 1H), 7.43 (d, J = 8.2 Hz, 2H), 7.58 (d, J = 8.2 Hz, 2H), 8.32 (s, 1H), 10.05 (s, 1H). ^{13}C NMR (DMSO-*d*$_6$, 100 MHz): δ (ppm): 45.2, 113.1, 121.2, 128.0, 131.7, 131.9, 133.2, 160.2, 165.1. FTMS (-ESI): calcd for $C_{11}H_8O_2N_2{}^{79}Br^-$: m/z = 278.9775, found: 278.9774.

(E)-5-[(6-bromo-1H-indol-3-yl)methylene]imidazolidine-2,4-dione (28)

A mixture of hydantoin (446 mg, 4.46 mmol, 2.5 eq.) and 6-bromoindole-3-carboxaldehyde (400 mg, 1.78 mmol, 1.0 eq.) in piperidine (2 mL) was refluxed during 1 h. The mixture was cooled to room temperature, then diluted with acetone and ethyl acetate. The organic layer was washed with a 1 M HCl, dried over Na_2SO_4 and then evaporated under reduced pressure. The residue was triturated with ethyl acetate, ethanol and MTBE to afford **28** as an orange solid (250 mg, 18 %). ^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ (ppm): 6.70 (s, 1H), 7.22 (dd, J = 8.4 Hz, J = 1.6 Hz, 1H), 7.61 (d, J = 1.6 Hz, 1H), 7.75 (d, J = 8.4 Hz, 1H), 8.13 (s, 1H), 10.14 (bs, 1H), 11.01 (bs, 1H), 11.92 (s, 1H). ^{13}C NMR (DMSO-*d*$_6$, 100 MHz): δ (ppm): 100.9, 108.6, 114.4, 115.0, 120.1, 122.9, 124.3, 125.9, 127.5, 136.6, 155.2, 165.3. MS: FTMS (-ESI): calcd for $C_{12}H_7N_3O_2{}^{79}Br^-$: m/z = 303.9727, found: 303.9726.

(*E*)-5-[(7-bromo-1H-indol-3-yl)methylene]imidazolidine-2,4-dione (29)

A mixture of hydantoin (223 mg, 2.23 mmol, 2.5 eq.) and 7-bromoindole-3-carboxaldehyde (200 mg, 0.89 mmol, 1.0 eq.) in piperidine (1 mL) was refluxed during 1 h. The mixture was cooled to room temperature, then diluted with acetone and ethyl acetate. The organic layer was washed with a 1 M HCl, dried over Na_2SO_4 and then evaporated under reduced pressure. The residue was triturated with ethanol, dichloromethane, heptane and acetone to afford **29** as a dark yellow solid (70 mg, 10 %). ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm): 6.70 (s, 1H), 7.07 (d, J = 7.8 Hz, 1H), 7.41 (d, J = 7.8 Hz, 1H), 7.80 (d, J = 7.8 Hz, 1H), 8.21 (s, 1H), 10.25 (s, 1H), 11.08 (s, 1H), 11.99 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm): 100.8, 104.5, 109.6, 117.7, 121.5, 124.5, 124.9, 127.7, 128.6, 134.3, 155.3, 165.4. FTMS (-ESI): calcd for $C_{12}H_7N_3O_2{}^{79}Br^-$: m/z = 303.9727, found: 303.9727.

(E)-5-[(1H-benzo[g]indol-3-yl)methylene]imidazolidine-2,4-dione (31)

A mixture of hydantoin (256 mg, 2.56 mmol, 2.5 eq.) and 1H-benzo[g]indole-3-carbaldehyde (200 mg, 1.02 mmol, 1.0 eq.) in piperidine (1 mL) was refluxed during 2 h. The mixture was cooled to room temperature, then diluted with acetone and ethyl acetate. The organic layer was washed with water, dried over Na_2SO_4 and then evaporated under reduced pressure. The residue was triturated with ethanol and MTBE to afford **31** as a dark red solid (80 mg, 28 %).^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm): 6.84 (s, 1H), 7.45 (t, J = 7.8 Hz, 1H), 7.57 (m, 1H), 7.59 (d, J = 8.8 Hz, 1H), 7.89 (d, J = 8.8 Hz, 1H), 7.97 (d, J = 7.8 Hz, 1H), 8.20 (m, 1H), 8.38 (d, J = 7.8 Hz, 1H), 10.18 (s, 1H), 11.07 (s, 1H), 12.66 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm): 101.7, 110.2, 118.1, 120.6, 120.9, 121.8, 122.8, 124.1, 124.2, 124.5, 127.8, 128.4, 130.1, 130.5, 155.3, 165.4. FTMS (-ESI): calcd for $C_{16}H_{10}N_3O_2^-$: m/z = 276.0778, found: 276.0778.

(Z)-5-[(1H-benzo[g]indol-3-yl)methylene]-2-thioxothiazolidin-4-one (35)

A mixture of rhodanine (136 mg, 1.02 mmol, 1.0 eq.), sodium acetate (251 mg, 3.06 mmol, 3.0 eq.) and 1H-benzo[g]indole-3-carbaldehyde (200 mg, 1.02 mmol, 1.0 eq.) in acetic acid (2 mL) was refluxed during 2 h. The mixture was cooled to room temperature, then diluted with water and the precipitate was filtered to afford, after trituration with ethanol and MTBE, **35** as a red solid (150 mg, 47 %). ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm): 7.50 (t, J = 7.4 Hz, 1H), 7.62 (t, J = 7.4 Hz, 1H), 7.68 (d, J = 8.2 Hz, 1H), 7.86 (s, 1H), 8.01 (d, J = 8.2 Hz, 1H),

8.04 (m, 2H), 8.47 (d, J = 7.4 Hz, 1H), 13.15 (s, 1H), 13.61 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm): 112.5, 118.1, 119.0, 120.8, 121.8, 122.1, 123.1, 124.7, 126.1, 127.2, 128.5, 130.5, 131.3, 169.1, 194.8. FTMS (-ESI): calcd for $C_{16}H_9N_2OS_2^-$: m/z = 309.0162, found: 309.0159.

Cellular lipid peroxidation method

HepG2 cells were cultured and assayed in MEM supplemented with Earle's salt (E-MEM, Biochrom) further supplied with fetal bovine serum (10 % FBS, Merck), L-alanyl-L-glutamine (2 mM, Merck) and gentamycin (10 µg/mL Biochrom, A2712). The cells were seeded in black 96-well microtiter plates with clear bottom (Corning, 3603) at approximately 90000 cells/well and incubated overnight in 5 % CO_2 at 37 °C. The cell media was replaced and cells were washed with PBS. Total reaction volume was 100 µL. The cells were subsequently labelled with C11-BODIPY (Invitrogen, Eugene, OR, USA) for 30 min and incubated with ranging concentrations of the test compounds for 1 h. The compounds were prepared from a DMSO stock by addition of MQ-water, from which 20 µL were added to 80 µL of MEM to reach the desired final concentration (maximum final DMSO concentration was 0.5 %) in the wells. Cumene hydroperoxide (50 µM CumOOH final concentration, Sigma-Aldrich) was added to initiate lipid peroxidation and the plate was immediately installed in a Victor3 Plate Reader. Both red (590/7 nm (excitation), 632/45 nm (emission)) and green (485/14 nm, 520/10 nm) fluorescence were recorded to follow the lipid peroxidation. Percent inhibition was calculated relative to the positive control (50 µM CumOOH without test compound). The antioxidant effect of known antioxidant luteolin at 50 µg/mL was used as positive antioxidant control and Hank's saline solution without CumOOH was used as negative control to ensure the quality of the *in vitro* assay.

Cytotoxicity testing

The potential cytotoxic activity of the compounds was investigated at a concentration of 50 µg/mL against human normal lung fibroblasts (MRC5). MRC5 cells were cultured and assayed in MEM (Biochrom) supplied with fetal bovine serum (10 % FBS, Merck), L-alanyl-L-glutamine (2 mM, Biochrom), non-essential amino acids (Biochrom), sodium pyruvate (1 mM Biochrom), $NaHCO_3$ (0.15 % Biochrom) and gentamycin (10 µg/mL Biochrom). The cells were seeded in 96-well microtiter plates (Thermo Fisher Scientific) at 4000 cells/well

and incubated for 24 h in 5 % CO_2 at 37 °C to allow the cells to adhere before the cell media was replaced and the compounds added. The plates were left to incubate with the compounds for 72 h before 10 µL of MTS solution (CellTiter 96 Aqueous One Solution Reagent, Promega) was added to each well, and the cells were additionally incubated for 1 h at 37 °C. The absorbance at 485 nm was measured using a DTX multimode detector (Beckman Coulter). The negative control was defined as cells assayed with their respective cell media with supplemented with 1 % DMSO (1% was the highest DMSO concentration used in wells with fraction/compound), and positive control as cells assayed with 0.5 % Triton X-100 (Sigma-Aldrich). Cell viability was calculated as follows: Cell survival (%) = (Absorbance treated wells – absorbance positive control)/(absorbance negative control – absorbance positive control) × 100.

Supporting information

The ^{1}H NMR and ^{13}C NMR data of all evaluated compounds can be viewed in the online version of this article at the publisher's web site.

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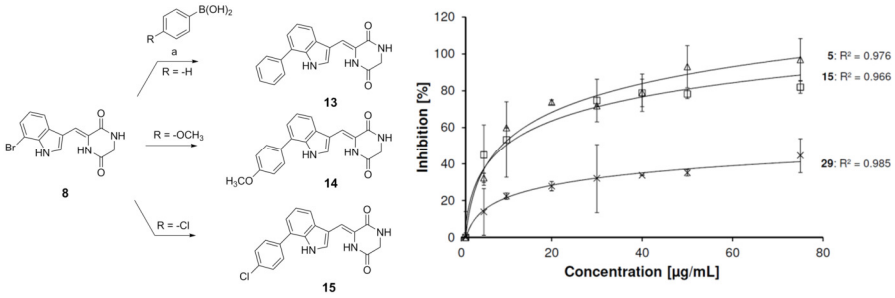
Graphical abstract



Highlights

- Synthetic analogs of barretin are powerful antioxidants
- Compound prepared display comparable activities as commercial antioxidants
- Changing the hetereocyclic core alters the antioxidative properties
- Simplifed analogs of marine natural products display improved bioactivity