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Pro-apoptotic carboxamide analogues of natural fislatifolic acid targeting Mcl-1 and Bcl-2

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ABSTRACT

A library of 26 novel carboxamides deriving from natural fislatifolic acid has been prepared. The synthetic strategy involved a bio-inspired Diels-Alder cycloaddition, followed by functionalisations of the carbonyl moiety. All the compounds were evaluated on Bcl-xL, Mcl-1 and Bcl-2 proteins. In this series of cyclohexenyl chalcone analogues, six compounds behaved as dual Bcl-xL/Mcl-1 inhibitors in micromolar range and one exhibited sub-micromolar affinities toward Mcl-1 and Bcl-2. The most potent compounds evaluated on A549 and MCF7 cancer cell lines showed moderate cytotoxicities.

Mcl-1 and Bcl-2 are pro-survival members of the Bcl-2-like proteins family that play a key role in the mitochondrial apoptosis pathway. This family of proteins is divided in three classes including multi-domain pro-apoptotic proteins such as Bak and Bax; BH3-only pro-apototic proteins like Bid, Bim, Bad and Noxa; and anti-apopotic proteins as Mcl-1, Bcl-2 and Bcl-xL. These anti-apoptotic proteins are frequently upregulated in cancer cells by inhibiting apoptosis after the sequestration of BH3-only pro-apototic proteins.[1] Therefore, the development of anti-apopotic proteins antagonist has become a promising alternative for the treatment of various cancers.[2] In recent years several therapeutic agents have been developed with different selectivity toward Mcl-1 and Bcl-2. ABT-737[3] and ABT-263[4] (Fig 1), active at subnanomolar level on Bcl-xL and Bcl-2 but not on Mcl-1, were found to cause severe thrombocytopenia.[5] Moreover, resistance to ABT-737 has been observed due to up-regulation of Mcl-1. A series of clinical candidate targeting Bcl-2 have been discovered including ABT-199[6] (Fig 1) launched on the market. Some potent Mcl-1 inhibitors have also entered in clinical trial.[7] Recent studies revealed that combined inhibition of Mcl-1 and Bcl-2 may be a relevant strategy for the treatment of cancers, and especially by developing Mcl-1/Bcl-2 dual inhibitor.[8]

Our group has a longstanding interest in the isolation[9] and synthesis[10] of natural compounds that target anti-apopotic proteins. Recently, a bioassay-guided purification of *Fissistigma latifolium* bark extract using an in-vitro assay based on the modulation of Bcl-xL/Bak and Mcl-1/Bid interactions led to the isolation of new cyclohexenyl chalcones including fislatifolic acid **1** and fislatifolione **3** (Fig 2).[9h] While the latter was not active on Bcl-2 family proteins, fislatifolic acid **1** revealed to be a good Bcl-xL/Mcl-1 dual inhibitor on micromolar range. Later on, an asymmetric total synthesis of both enantiomers of these natural myrcene-derived cyclohexenyl chalcones has been developed based on a bio-inspired asymmetric Diels-Alder cycloaddition using a chiral Evans oxazolidinone.[10f] The affinity of the synthetic cyclohexyl chalcones **1** and **3** toward Bcl-2 family protein has been confirmed and the Weinreb amide intermediate **2** proved to be an excellent Mcl-1/Bcl-2 dual inhibitor on submicromolar level (Fig. 2). This work has shown that minor modifications on the carbonyl moiety could have a significant impact on the activity of the compounds. These first results encouraged us to explore pharmacomodulations on this part of the molecule. In this study, we have developed a series of carboxamide analogues of (+)- and (−)-fislatifolic acid **1**. These compounds have been evaluated by in-vitro affinity displacement assays based on the modulation of Bcl-xL/Bak, Mcl-1/Bid, and Bcl-2/Bim interactions.

The synthesis of (+)- and (−)-fislatifolic acid **1** was achieved on 5 g scale in 3 steps in 54-57% overall yield (Scheme 1). First, the

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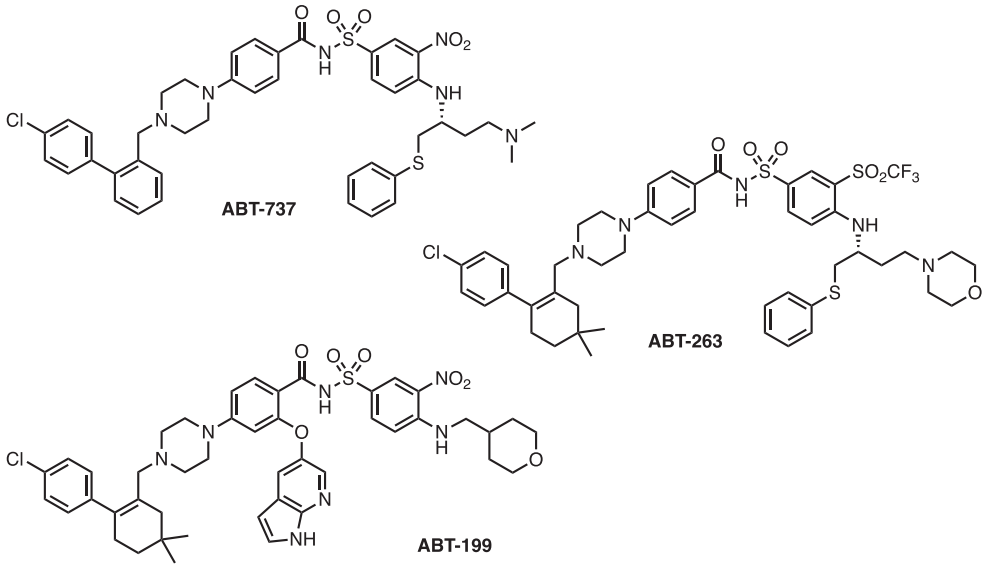


Fig. 1. Structures of ABT-737, ABT-263 and ABT-199.

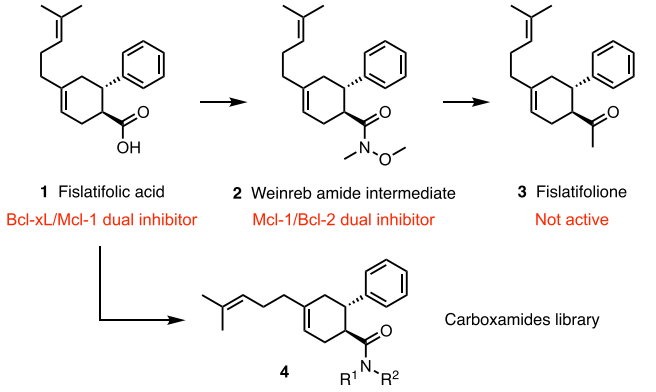


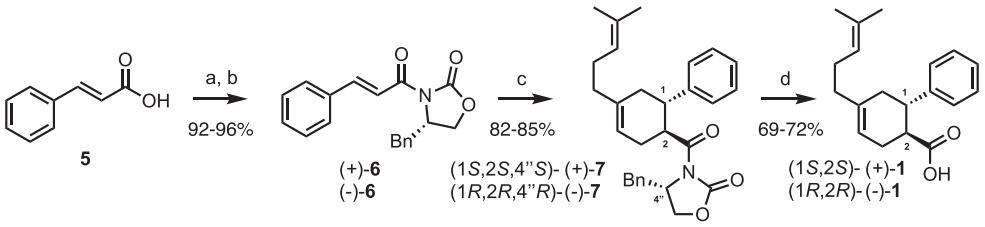
Fig. 2. From fislatifolic acid **1** toward the synthesis of carboxamides **4** as Mcl-1/Bcl-2 dual inhibitors.

oxazolidinones (+)-**6** and (−)-**6** were prepared separately from commercially available *trans*-cinnamic acid **5** and either (4*S*)- or (4*R*)-4-benzyl-1,3-oxazolidin-2-one respectively. Then a dimethylaluminium chloride-mediated Diels-Alder cycloaddition in presence of myrcene gave the expected cycloadducts (+)-**7** and (−)-**7** in 82-85% yield with a complete regio-, diastereo- and endo/exo-selectivity (> 95:5). Finally, (+)- and (−)-fislatifolic acid **1** were obtained after removal of chiral auxiliary (Scheme 1).

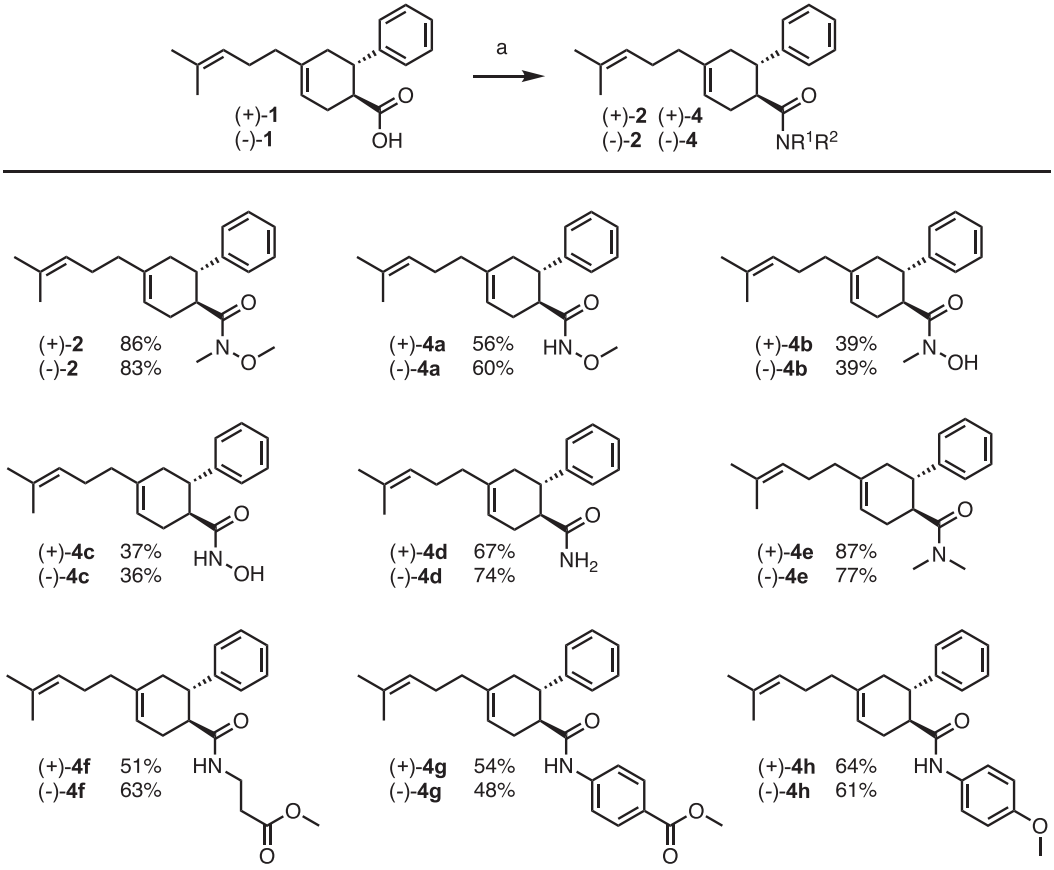
With carboxylic acid (+)- and (−)-**1** in hands, various analogues were prepared following the procedure used to obtain the Weinreb amide intermediates (+)-**2** and (−)-**2** throughout the synthesis of natural (+)- and (−)-fislatifolione **3**.[10f] First, to understand the role of the amides deriving from *N,O*-dimethylhydroxylamine in the interaction with the target proteins and to modulate their affinity, various Weinreb amide analogues (+)- and (−)-**4a-e** were prepared from methoxyamine, *N*-methylhydroxylamine, hydroxylamine, ammonia, and dimethylamine (Scheme 2). Larger groupments were then installed to mimic other natural myrcene-derived cyclohexenyl chalcones like ecarlottone[9h] or nicolaioidesin C.[11] Thus, the use of β-alanine methyl ester, methyl 4-aminobenzoate, and 4-anisidine led to carboxamide analogues (+)- and (−)-**4f-h** in acceptable yields.

Saponification of ester function of (+)- and (−)-**4f** and **4g** in presence of lithium hydroxide furnished the corresponding carboxylic acids (+)- and (−)-**4i** and **4j** in decent yields (Scheme 3). The release of the phenol moiety of carboxamides (+)-**4h** was performed using stoechiometric amount of boron tribromide (Scheme 4). Although demethylation was observed by proton NMR analysis, the expected product (+)-**4k** was not formed. Indeed a cyclization of the 1,5-diene occurred leading mainly to the bicylo compound (+)-**8**. This reaction mediated by Brönsted or Lewis acid was studied more than 60 years by Eschenmoser, Stork and others[12] and is still employed nowadays.[13] In order to enrich our library of analogues, we decided to apply this cyclisation reaction on (+)- and (−)-fislatifolic acid **1**. In presence of boron trifluoride diethyl etherate, the cyclisation products (+)- and (−)-**9** could be isolated in 69-73% yield (Scheme 4). The corresponding Weinreb amides (+)- and (−)-**9** were then synthesized to compare their affinity to Bcl-2 family proteins to the most active compound (+)-**2** of our series. The demethylated compounds (+)- and (−)-**4k** were finally obtained by coupling between 4-aminophenol to (+)- and (−)-fislatifolic acid **1** using the same conditions described above (Scheme 5).

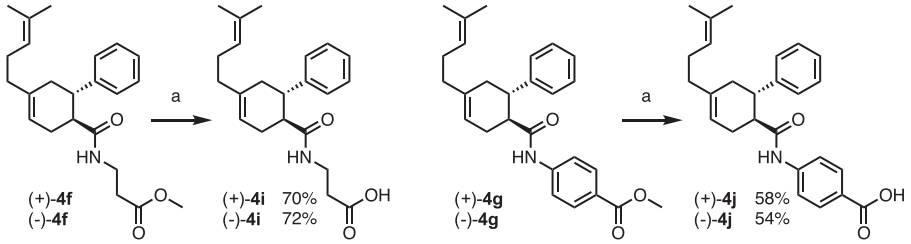
All the products synthesized were evaluated by in-vitro affinity displacement assays based on the modulation of Bcl-xL/Bak, Mcl-1/Bid, and Bcl-2/Bim interactions (Table 1). Although less active, the carboxamides of methoxyamine, *N*-methylhydroxylamine and hydroxylamine (+)- and (−)-**4a-c** showed the same selectivity toward Mcl-1 and Bcl-2 than Weinreb amides (+)-**2** and (−)-**2**. As previously noticed, in this series the (+) enantiomers were found as or more active than the (−) enantiomers. Compounds (+)- and (−)-**4c** even exhibited sub-micromolar affinity to Bcl-2. On the contrary, both enantiomers of compounds **4d** and **4e** revealed almost no activity on the three target



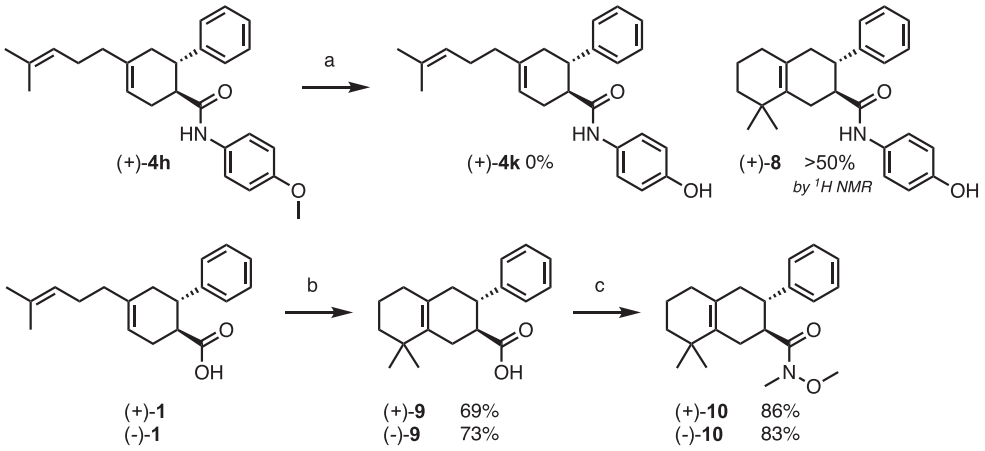
Scheme 1. Asymmetric synthesis of both enantiomers of fislatifolic acid (+)- and (−)-**1**: a) NEt_3 (3 equiv.), trimethylacetyl chloride (1.2 equiv.), THF, -40 °C, 2 h; b) LiCl (1.2 equiv.), (4*S*)- or (4*R*)-4-benzyl-1,3-oxazolidin-2-one (1.2 equiv.), r.t., 18 h; c) myrcene (2 equiv.), Me_2AlCl 1 M in hexanes (2 equiv.), CH_2Cl_2, 0 °C to r.t., 16 h; d) $LiOH.H_2O$ (4 equiv.), H_2O_2 30% (3 equiv.), THF/H_2O 2:1, r.t., 16 h.



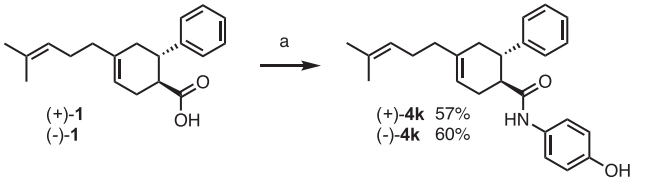
Scheme 2. Synthesis of carboxamide derivatives (+)- and (−)-**4a-h** by peptidic coupling on (+)- and (−)-fislatifolic acid **1**: a) HNR^1R^2 (1.1 equiv.), HATU (1.1 equiv.), *N,N*-diisopropylethylamine (2 equiv.), DMF, r.t., 2 h. (+) Enantiomers correspond to 1*S*,2*S* stereochemistry and (−) enantiomers to 1*R*,2*R*.



Scheme 3. Synthesis of carboxylic acid derivatives (+)- and (−)-**4i** and **4j** by saponification of (+)- and (−)-**4f** and **4g** respectively: a) $LiOH.H_2O$ (4 equiv.), THF/H_2O 2:1, r.t., 16 h. (+) Enantiomers correspond to 1*S*,2*S* stereochemistry and (−) enantiomers to 1*R*,2*R*.



Scheme 4. Attempt of demthylation of carboxamide (+)-**4h** and cyclisation of 1,5-diene of fislatifolic acid (+)- and (−)-**1**: a) BBr_3 1 M in CH_2Cl_2 (2 equiv.), CH_2Cl_2, -78 °C to r.t., 3 h; b) $BF_3.OEt_2$ (2 equiv.), CH_2Cl_2, 0 °C to r.t., 4 h; c) MeONHMe·HCl (1.1 equiv.), HATU (1.1 equiv.), *N,N*-diisopropylethylamine (2 equiv.), DMF, r.t., 2 h. (+) Enantiomers correspond to 1*S*,2*S* stereochemistry and (−) enantiomers to 1*R*,2*R*.



Scheme 5. Synthesis of carboxamide derivatives (+)- and (−)-**4k** by peptidic coupling on (+)- and (−)-fislatifolic acid **1**: a) 4-Aminophenol (1.1 equiv.), HATU (1.1 equiv.), *N,N*-diisopropylethylamine (2 equiv.), DMF, r.t., 2 h. (+) Enantiomers correspond to 1*S*,2*S* stereochemistry and (−) enantiomers to 1*R*,2*R*.

proteins. Activity of the analogues with a longer chain was also studied. While compound (+)-**4f** was found to be a Mcl-1/Bcl-2 dual inhibitor with micromolar range affinities on both proteins, its enantiomer (−)-**4f** expressed only a little affinity toward Mcl-1. Compounds **4g-i** and **4k** were completely inactive against all three Bcl-2 family proteins. In contrast, (+)-**4j** revealed to be Mcl-1 selective and its enantiomer (−)-**4j** Bcl-2 selective. Finally, both enantiomers of the cyclized products **9** and **10** lost their activity in comparison with fislatifolic acid **1** and Weinreb amide **2**.

In terms of cytotoxicity on A549 and MCF7 cancer cell lines, some of the most potent compounds (+)-**1**, (+)-**2**, (+)-**4a**, (+)-**4c** and (+)-**4f** according to *in vitro* protein assays revealed moderate cytotoxic activities (Table 2), in the same range as compound (+)-**4k**, not active on Bcl-2 proteins. Thus, no direct correlation could be established between protein affinity and cytotoxicity on cancer cell lines. This result indicates that another mechanism could be involved in the cell death. However, the Weinreb amide (+)-**2** is still the most active analogue on these two cancer cell lines with 31.2 and 42.1 μM on A549 and MCF7 respectively, thus approaching the result obtained with the cisplatin on this latter cell line (43.2 μM).

In conclusion, a library of 26 novel carboxamides deriving from synthetic fislatifolic acid (+)- and (−)-**1** has been prepared based on the results previously obtained. Their evaluation on the modulation of Bcl-xL/Bak, Mcl-1/Bid, and Bcl-2/Bim interactions have shown the incidence of tiny modifications on the carboxamide part and confirmed than the (+) enantiomers were as or more active than the (–) enantiomers. In this series, six compounds behaved as dual Bcl-xL/Mcl-1 inhibitors in micromolar range but the previously prepared Weinreb amide (+)-**2** is still the most promising compound deriving from natural cyclohexenyl chalcones with sub-micromolar affinities toward Mcl-1 and Bcl-2 but with moderate cytotoxicities on A549 and MCF7 cancer cell lines.

Table 1
Biological evaluation of synthesized compounds on Bcl-xL/Bak, Mcl-1/Bid and Bcl-2/Bim displacement assays.

Cpd.	Bcl-xL/Bak binding affinity[a] K_i[b] (μM)	Mcl-1/Bid binding affinity[a] K_i[b] (μM)	Bcl-2/Bim binding affinity[a] K_i[b] (μM)
(+)-**1**	5.7 ± 0.7	4.0 ± 0.5	> 23
(−)-**1**	9.6 ± 0.6	8.6 ± 0.6	> 23
(+)-**2**	> 23	0.9 ± 0.1	0.3 ± 0.1
(−)-**2**	> 23	> 33	> 23
(+)-**4a**	> 23	16.6 ± 1.7	2.4 ± 0.1
(−)-**4a**	> 23	> 33	2.7 ± 0.7
(+)-**4b**	> 23	7.2 ± 0.6	2.1 ± 0.2
(−)-**4b**	> 23	9.2 ± 0.6	2.3 ± 0.3
(+)-**4c**	19.0 ± 1.0	7.3 ± 0.4	0.6 ± 0.1
(−)-**4c**	17.4 ± 0.6	8.2 ± 0.4	0.7 ± 0.1
(+)-**4d**	> 23	> 33	> 23
(−)-**4d**	> 23	> 33	> 23
(+)-**4e**	20.1 ± 1.9	5.4 ± 0.3	> 23
(−)-**4e**	> 23	> 33	> 23
(+)-**4f**	> 23	5.5 ± 0.3	3.1 ± 2.2
(−)-**4f**	> 23	15.0 ± 1.0	> 23
(+)-**4g**	> 23	> 33	> 23
(−)-**4g**	> 23	> 33	> 23
(+)-**4h**	> 23	> 33	> 23
(−)-**4h**	> 23	> 33	> 23
(+)-**4i**	> 23	> 33	> 23
(−)-**4i**	> 23	> 33	> 23
(+)-**4j**	> 23	8.5 ± 0.4	> 23
(−)-**4j**	> 23	> 33	2.2 ± 0.3
(+)-**4k**	> 23	> 33	> 23
(−)-**4k**	> 23	> 33	> 23
(+)-**9**	> 23	9.0 ± 0.3	> 23
(−)-**9**	> 23	> 33	> 23
(+)-**10**	> 23	18.9 ± 2.4	> 23
(−)-**10**	> 23	15.0 ± 3.8	> 23
Ref.[c]	8.3 ± 1.2	5.2 ± 1.2	1.5 ± 0.1

[a] Binding affinities were measured by fluorescence polarization[14] after competition between the ligand and a fluorescein-labeled peptide.
[b] *Ki* is the concentration of the ligand corresponding to 50% of the binding of the labelled reference compound, corrected for experimental conditions.[15]
[c] Meiogynin A was used as reference compound.[9h]

Acknowledgments

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Table 2
Cytotoxicity assays on A549 and MCF7 cell lines.

Cpd.	A549 IC_{50} (μM)[a]	MCF7 IC_{50} (μM)[a]
(+)-**1**	53.7 ± 0.2	64.3 ± 7.7
(+)-**2**	31.2 ± 3.6	42.1 ± 1.2
(+)-**4a**	42.2 ± 2.6	45.2 ± 1.3
(+)-**4c**	71.7 ± 8.7	72.3 ± 1.2
(+)-**4f**	87.9 ± 5.7	96.8 ± 1.4
(+)-**4k**	66.8 ± 3.1	66.7 ± 1.2
Gefitinib	10.5 ± 4.3	–
Cisplatin	–	43.2 ± 8.1
Antimycin A	6.5 ± 1.7	–

[a] IC_{50} measures the drug concentrations required for 50% cell death.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127003.

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