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Discovery of indolizine lactones as anticancer agents and their optimization through late-stage functionalization†

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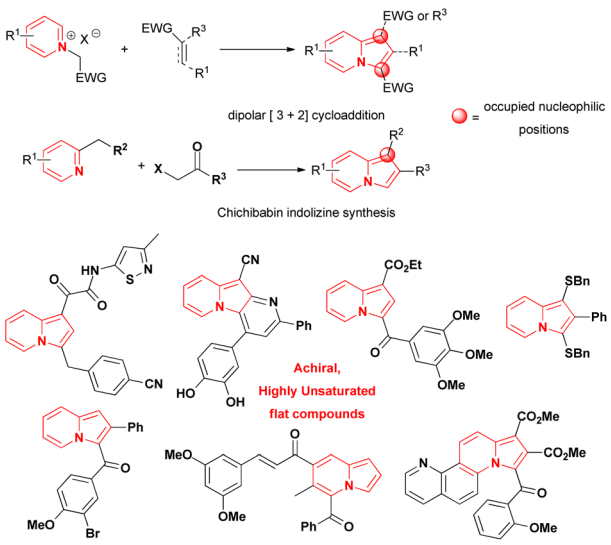
Indolizines fused with a seven-member lactone ring were identified as a promising scaffold in the search for new anticancer agents. Through a modular synthetic sequence, a library of *cis* and *trans* indolizines lactones had their antiproliferative activity evaluated against hormone-refractory prostate DU-145 and triple-negative breast MDA-MB-231 cancer cell lines. A methoxylated analogue was identified as an initial hit against MDA-MB-231 and late-stage functionalization of the indolizine core led to analogues within potencies up to twenty times higher than the parent precursor.

Introduction

Indolizines, which are indole isomers and bioisoesters,[1] are present in numerous compounds that exhibit biological activity.[2,3] They are considered privileged scaffolds for drug development,[4] but no indolizine-based drugs have been marketed so far.[1] The best studied indolizines in terms of biological activity were primarily assessed through dipolar cycloadditions involving pyridinium ylides and electron deficient alkenes/alkynes or *via* the wide-spread Chichibabin reaction from 2-methylpyridinium salts (Scheme 1).[1–3,5–15] The mild conditions, the ready access to starting materials and the modular synthetic approach that characterised those methods were some of the reasons leading to their extensive use in the search for indolizines with biological activities. Consequently, indolizines obtained by those methods were typically characterised by a high degree of planarity and by the absence of stereogenic centers, leading to an overall low level of molecular complexity, a property associated with improvements in the physicochemical proprieties, selectivity and safety of compounds during the drug discovery process.[16–19] The multi-substituted character of these indolizines, especially at the nucleophilic positions 1 and 3 of the heterocyclic core or/and the presence of electron-withdrawing groups directly attached to them, potentially precluded the evaluation of late-stage functionalization (LSF) strategies as suitable option to increase the drug profile of these molecules. This is another point that should be addressed during the design of new indolizines for biological activity evaluation.

The development of synthetic methods that would lead to more complex indolizines is therefore of great significance, and the introduction of chiral centers, fused rings or increases in the fraction of sp^3 carbons (Fsp^3 – the number of sp^3 hybridised carbons/total carbon count) could be used to assess compounds with different topologies and increased molecular complexity.[16] The functionalization of indolizines, rather than their *de novo* synthesis, appears to represent a suitable way to achieve this



Scheme 1 Examples of indolizine motifs founded in compounds with anticancer activity and common strategies for their synthesis.[1,10–15]

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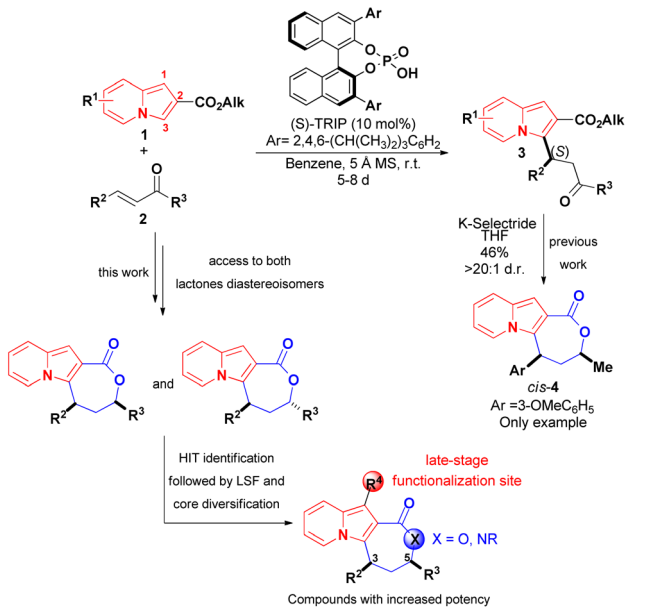


Scheme 2 Reported asymmetric synthesis of an indolizine lactone (Coelho and List, 2017)[20] and the molecular sites available for further modifications during the investigation of biological activities of **4**.

goal. Along these lines, we recently described an organocatalytic asymmetric and diastereoselective synthesis of an indolizine fused to a lactone ring bearing two stereocenters, starting from the pre-formed 2-carboxyindolizine core **1** (Scheme 2).[20] The synthetic sequence of regioselective conjugate addition of **1** to enone **2**, followed by carbonyl reduction/ring closure, offers a modular approach for synthesis of indolizine lactone **4** with different structural patterns, allowing a rapid evaluation of the structure–activity relationship (SAR) towards a biological target. Additionally, the nucleophilic C1 position of the indolizine remains available on **4** and could represent a site for late-stage functionalization after the identification of promising compounds.

The exquisite indolizine lactone **4** has a seven-membered ring that resembles the one encountered in colchicine, a potent cytotoxic natural product extensively investigated

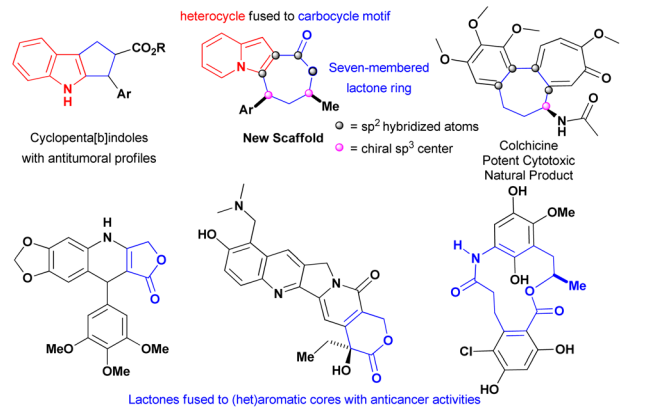


Fig. 1 Structural features of indolizines lactones founded in compounds with anticancer proprieties.

against different types of cancer,[21] and its fused heterocyclic–carbocycle motif could be viewed as an ring-expanded version of cyclopenta[*b*]indoles that display excellent cytotoxicity against multiple cancer cell lines (Fig. 1).[22] These factors, associated with the findings that indolizines[1,10–15] and lactones[23–25] were encountered in compounds with antitumoural proprieties, prompted our evaluation of this new core against triple-negative breast (MDA-MB-231) and hormone-refractory prostate metastatic (DU-145) cancer cells-both of which are metastatic.

We anticipated that the flexible approach used to construct more complex indolizine lactone derivatives, together with the late-stage functionalization strategy, would be fundamental to finding compounds with increased antiproliferative activity.

Results and discussion

Chemistry

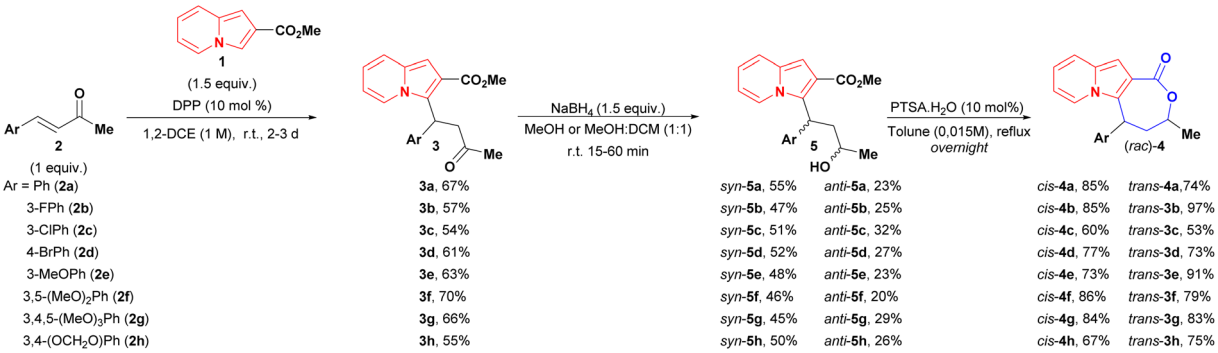
Despite the existence of an enantio- and diastereoselective route for the synthesis of *cis*-indolizine lactones **4**, the evaluation of racemates and *trans* isomers potentially expands the possibilities of finding a hit compound on a first biological screening. Targeted *cis* and *trans* (*rac*)-lactones were obtained using the following 3-step sequence (Scheme 3): (1) diphenyl phosphate (DPP) catalysed conjugate addition between 2-carboxymethyl indolizine (**1**) and methyl cinnamyl ketones **2**; (2) reduction of conjugate adducts **3** using $NaBH_4$ (no lactonization was observed in this step), followed by chromatography separation of the *syn* and *anti*-hydroxy esters **5**; (3) lactonization of alcohols **5** catalysed by *para*-toluenesulfonic acid (*p*-TsOH).

As general remarks, the reduction of conjugate adducts **3** using the non-hindered reducing agent $NaBH_4$ favoured the *syn* isomer, as observed in our previous work.[20] It showed moderated stereoselectivity, which allowed the isolation of *anti* alcohols **5**, as well as access to *trans* lactones **4**. Alternatively, the key hydroxy esters could be obtained in a one-pot two-step conjugate addition/reduction way, thereby obviating the need for the one chromatography step, as exemplified in the synthesis of *syn* and *anti* **5f** analogues in a slightly better global yield of 55% yield (Scheme 4).

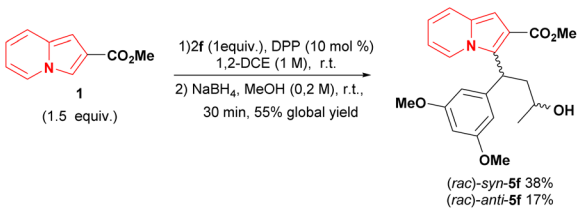
Indolizine lactone *cis*-**4f** was identified as an initial hit on the first biological screening (see Table 1) and was selected for further synthetic manipulations. Modifications to the backbone of the seven-membered ring and to the indolizine motif were realised first. Lactam analogues of **4f** were synthesised *via* reductive amination of conjugate adduct **3** to afford a separable mixture of *syn* and *anti* aminoesters **6**, which cyclized into the corresponding *cis* and *trans* lactams (Scheme 5). Intriguingly, the majority product after the reduction step was the *anti* diastereoisomer **6** rather than the *syn* diastereoisomer observed after reduction of **3** with $NaBH_4$ or K-Selectride.[20]

Another straightforward modification of the scaffold of *cis*-**4f** was achieved through the partial hydrogenation of its indolizine core using platinum oxide (PtO_2) as the catalyst. This led to the 5,6,7,8-tetrahydro-indolizine lactone *cis*-**8** in 65% yield (Scheme 6).[26]

In addition to changes to the backbone of *cis*-**4f**, late-stage functionalization of the available indolizine C1 position opened up a new chemical space for further investigations. The



Scheme 3 Synthetic sequence to both diastereoisomers of indolizines lactones. [a]The relative configurations of **5** were determined after the lactonization step.

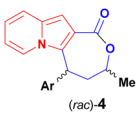


Scheme 4 One-pot synthesis of **5f** hydroxy esters.

C1 reactivity of indolizines has scarcely been explored since it is less reactive than C3 and few methods are available for the synthesis of C1-free, C3-substituted indolizines.[27] Here, we investigated the reactivity of *cis*-**4f** in reactions of thiolation,[15,28] trifluoroacetylation and oxidative coupling,[29] to further evaluate the impact of these peripheral modifications on the anti-proliferative activity (Scheme 7).

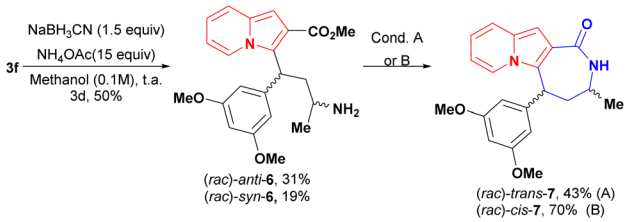
Thiolation of *cis*-**4f** in the presence of thiophenol, *tert*-butyl hydroperoxide (TBHP), and catalytic potassium iodide (KI) afforded *cis*-**9** in 57% yield (Scheme 7). Previously,[28] only

Table 1 IC_{50} and CC_{50} values in μM for *cis* and *trans* lactones **4**[a]

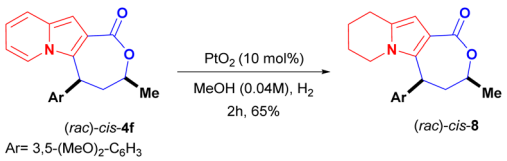


		IC_{50}		CC_{50}[f]	
		Cancer cell lines		Non-tumor cell lines (systemic)	
Entry	Ar	DU-145[b]	MDA-MB-231[c]	FC3H[d]	HFF-1[e]
1	Ph (*cis*-**4a**)	>100	67.86 ± 6.61	ND	ND
2	Ph (*trans*-**4a**)	>100	>100	ND	ND
3	3-FPh (*cis*-**4b**)	>100	79.51 ± 21.84	ND	ND
4	3-FPh (*trans*-**4b**)	>100	52.87 ± 0.84	ND	ND
5	3-ClPh (*cis*-**4c**)	54.71 ± 2.59	35.99 ± 0.69	ND	ND
6	3-ClPh (*trans*-**4c**)	>100	>100	ND	ND
7	**4-BrPh (*cis*-4d)**	**34.41 ± 2.68 (0.03)[g]**	**16.72 ± 0.80 (0.06)[g]**	**>100**	**0.92 ± 0.22**
8	4-BrPh (*trans*-**4d**)	46.65 ± 7.29 (>2)[g]	21.99 ± 3.44 (>5)[g]	>100	>100
9	**3-MeOPh (*cis*-4e)**	**>100**	**21.05 ± 2.22 (>5)[g]**	**>100**	**>100**
10	3-MeOPh (*trans*-**4e**)	>100	53.03 ± 2.31	ND	ND
11	**3,5-MeOPh (*cis*-4f)**	**52.41 ± 0.45 (>2)[g]**	**20.47 ± 0.79 (>5)[g]**	**>100**	**>100**
12	3,5-MeOPh (*trans*-**4f**)	>100	27.64 ± 0.99	ND	ND
13	3,4,5-MeOPh (*cis*-**4g**)	36.93 ± 2.58	>100	ND	ND
14	3,4,5-MeOPh (*trans*-**4g**)	>100	>100	ND	ND
15	Piperonyl (*cis*-**4h**)	52.28 ± 1.46	31.95 ± 2.72	ND	ND
16	Piperonyl (*trans*-**4h**)	35.67 ± 2.24	29.41 ± 5.46	ND	ND

[a] Mean values ± standard error were expressed as the result of three independent experiments. DMSO was used as a negative control and doxorubicin and colchicine were used as positive controls (see ESI for further details). [b] Human prostate epithelial tumour cells. [c] Human breast epithelial tumour cells. [d] Mouse liver fibroblast cells. [e] Human skin fibroblast cells. [f] Only molecules with $IC_{50} \leq 20$ μM in cancer cell lines were tested against the nontumour ones. [g] Values in parentheses are the selectivity indexes ($SI = CC_{50}/IC_{50}$) towards the HFF-1 cell lines. ND: not determined.



Scheme 5 Synthesis of lactams **7** and partial hydrogenation of *cis*-**4f**. Condition A: NEt_3 (0.022 M) reflux, overnight B: toluene (0.025 M), NEt_3 (12 equiv.) reflux, overnight.

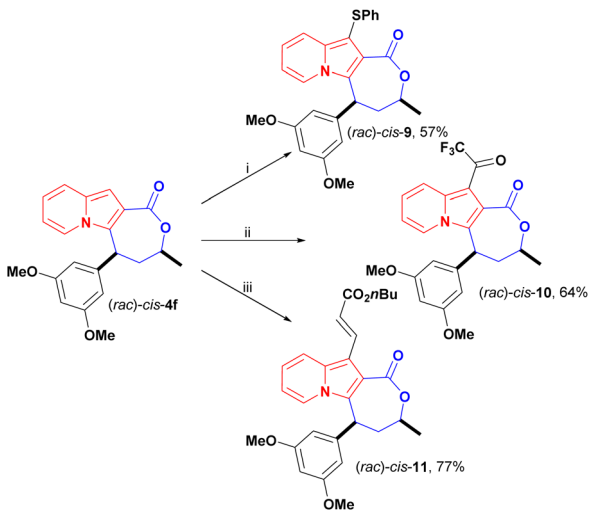


Scheme 6 Synthesis of lactams **7** and partial hydrogenation of *cis*-**4f**.

unsubstituted 2-aryl indolizines were reported as substrates for this transformation, but indolizine lactone *cis*-**4f** proved to be a suitable substrate for this transformation.

Trifluoroacetylation was achieved under mild conditions using trifluoroacetic anhydride (TFFA) and triethylamine. The trifluoromethyl ketone (TFMK) derivative *cis*-**10** was obtained in 64% yield (Scheme 7).

Olefination of *cis*-**4f** with *n*-butyl acrylate was carried out under Rh(II) catalysis using the lactone motif as a directing group for C–H activation at the C1 position, and *cis*-**11** was obtained in good yield, at 77%, in these conditions (Scheme 7). Notably, C–H activation of the C8 and C2 positions of indolizines was previously achieved employing amides, esters, ketones and aldehydes as directing groups.[29,30] Here, we successfully expanded this strategy to activate the C-1 position within the carboxyl lactone motif.



Scheme 7 Late-stage functionalization of *cis*-**4f**: (i) PhSH (2 equiv.), KI (10%), TBHP (2 equiv.), DMSO (0.1 M), 60 °C, 22 h. (ii) TFFA (1.25 equiv.), NEt_3 (1.5 equiv.), DCM (0.02 M), 0 °C, 1 h. (iii) *n*-butyl acrylate (4 equiv.), $[Cp^*RhCl_2]_2$ (4 mol%), $AgSbF_6$ (0.16 equiv.), $Cu(OAc)_2$. H_2O (2.1 equiv.), DCE (0.1 M), 100 °C.

Biological activity

The antiproliferative activities of lactones **4** were tested *in vitro* against two human cancer cell lines (DU-145, ATCC: HTB-81, hormone-refractory prostate cancer; and MDA-MB-231, ATCC: HTB-26, triple-negative breast cancer) purchased from American Type Culture Collection (ATCC, Manassas, Virginia, EUA) or Rio de Janeiro Cell Bank (BCRJ, Rio de Janeiro, Brazil), using resazurin assays. DMSO was used as a negative control, and colchicine and doxorubicin were employed as positive controls (Table 1).

Except for the *trans* lactones **4a**, **4c**, and **4g**, all compounds exhibited antiproliferative activity against at least one of the human cancer cell lines tested. As a general trend, indolizines **4** showed more cytotoxicity towards breast cancer cells than towards the prostate cancer cells. The exception was *cis*-**4g** (Table 1, entry 13), which seemed to be promisingly selective for the DU-145 cell lines ($IC_{50} = 36.93 \pm 2.58$ μM). Fortunately, the diastereoisomer *cis* was more active than the *trans* isomers, and could be synthesised diastereoselectively using a hindered reducing agent.[20] This difference in activity highlights the important role of chirality in determining biological effectiveness.

The cytotoxic activities of *cis* halogenated indolizines (Table 1, entries 3, 5 and 7) against MDA-MB-231 cell lines were inversely proportional to the electronegativity of the substituents, suggesting a possible 'halogen bonding effect'.[31,32] The presence of a fluorine atom on *cis*-**4b** [IC_{50} (MDA-MB-231) = 79.51 ± 21.84 μM] had a negative impact on its antiproliferative capacity when compared with unsubstituted phenyl analogue *cis*-**4a** [IC_{50} (MDA-MB-231) = 67.86 ± 6.61 μM]. Conversely, the presence of a bromine atom appeared to increase the antiproliferative activities of both the *cis* and *trans* lactones **4d** (entries 6 and 7).

Lactone *cis*-**4d** (entry 7) exhibited the highest cytotoxicity against both cancer lines [IC_{50} (DU-145) = 34.41 ± 2.68 μM and IC_{50} (MDA-MB-231) = 16.72 ± 0.80 μM] in this first evaluation and was almost inactive against non-tumoural mouse liver fibroblast cell lines [CC_{50} (FC3H, BCRJ: 0082) > 100 μM] (Table 1, entry 7). Unfortunately, *cis*-**4d** was more cytotoxic against healthy human skin fibroblast cell lines [CC_{50} (HFF-1, ATCC: SCRC-1041) = 0.92 ± 0.22 μM] than it was against cancer lines, thereby ruling out further investigations and structural modifications of this compound. The *trans* isomer of lactone **4d** (entry 8) was slightly less potent against cancer cells [IC_{50} (DU-145) = 46.65 ± 7.29 μM and IC_{50} (MDA-MB-231) = 21.99 ± 3.44 μM)] than *cis*-**4d**; nevertheless, it displayed good selectivity index (SI) values [SI (HFF-1/DU-145) ≥ 2.14 and SI (HFF-1/MDA-MB-231 ≥ 4.55)] when compared with the observed cytotoxicity against both systemic cell types evaluated (CC_{50} > 100 μM for both cell lines).

Among the alkoxylated analogues (Table 1, entries 9–16), mono-methoxylated *cis*-**4e** and bis-methoxylated *cis*-**4f** (entries 9 and 11) displayed lower IC_{50} values against breast cancer cell

lines (21.05 ± 2.22 μM and 20.47 ± 0.79 μM, respectively) and good SI values (both compounds showed CC_{50} values higher than 100 μM for FC3H and HFF-1 cells). Increasing the number of methoxy groups (entries 13 and 14) or using piperonyl derivatives (entries 15 and 16) affected the antiproliferative activity of the lactones.

Indolizine lactone *cis*-**4f** showed the best balance between cytotoxicity and selectivity and was therefore selected for further synthetic manipulations that might improve these attributes (Fig. 2). The presence of alkoxylated motifs in a series of natural and synthetic compounds with observed antitumoral activities[21,33–36] and in other studies with indolizine derivatives as well,[10,11,37,38] reinforced the choice of *cis*-**4f** as our first hit.

Changes to the backbone of **4f**, such as the substitution of the seven-member lactone ring for a lactam ring, could give important insights into the molecular proprieties associated with biological activity. The N–H motif acts as a hydrogen-bond donor and could therefore positively impact the molecular recognition of a compound by a biological target, while also improving its water solubility. In addition, lactams are less susceptible to hydrolysis and might improve compound bioavailability compared with lactones derivatives.[39,40] However, the lactam analogues of **4f**, *cis*-**7** [IC_{50} (DU-145) ≥ 100 μM and IC_{50} (MDA-MB-231) = 57.17 ± 3.99 μM] and *trans*-**7** [IC_{50} (DU-145) ≥ 100 μM and IC_{50} (MDA-MB-231) = 52.44 ± 0.81 μM] were considerably less cytotoxic than the parent compound to cancer cell lines (Fig. 2), proving that the presence of oxygen on the seven-membered ring was essential to biological activity.

The role of the fully aromatised indolizine core on the biological outcome was evaluated by synthesising a 5,6,7,8-tetrahydro-indolizine *cis*-**8** (Scheme 6). This new compound possesses a higher fraction of sp^3 carbon atoms (where Fsp^3 = the number of sp^3 hybridised carbons/total carbon count), at

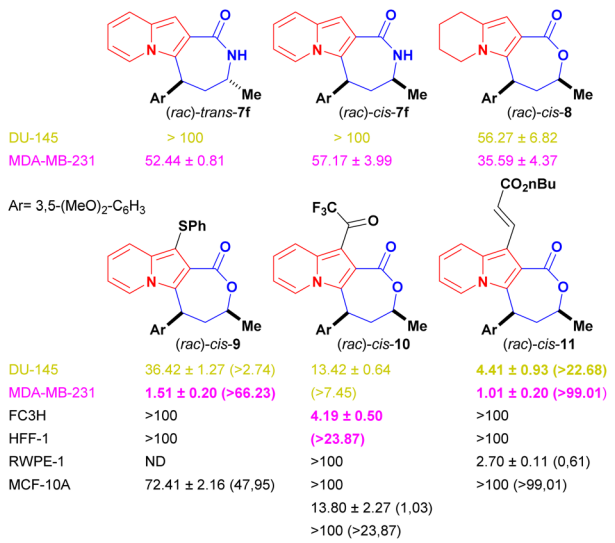


Fig. 2 IC_{50} values (μM) of *cis*-**4f** analogues. Selectivity index values (SI = CC_{50}/IC_{50}) between cancer cell lines (DU-154 and MDA-MB-231) and systemic (FC3H and HFF-1) and local non-tumour cells [RWPE-1 (prostate) and MCF-10A (breast)] are listed in parentheses. ND: not determined.

$Fsp^3 = 0.476$ *versus* $Fsp^3 = 0.285$ for the parent compound *cis*-**4f**, a property that is used to measure molecular complexity.[16,17] Derivative *cis*-**8** was, nevertheless, less active [IC_{50} (DU-145) ≥ 56.27 ± 6.82 μM and IC_{50} (MDA-MB-231) = 35.59 ± 4.37 μM] than *cis*-**4f**, demonstrating the important role of the indolizine aromatised core (Fig. 2).

Peripherical modifications of *cis*-**4f** were then performed through late-stage functionalization of the indolizine C1 position (see Scheme 7). Fortuitously, the thiolated, trifluoroacetylated and olefinated analogues were more cytotoxic than their precursor against both cancer cell lines without being harmful to the systemic non-tumour FC3H and HFF-1 cells (CC_{50} > 100 μM for both lines) (Fig. 2). Remarkably, all the new analogues were more active against breast cancer cells.

Thiolated *cis*-**9** displayed an impressive 13.5-fold cytotoxicity increase against the breast cancer cells (IC_{50} = 1.51 ± 0.20 μM) and a modest 1.4-fold against the prostate cancer cells (IC_{50} = 36.42 ± 1.27 μM) when compared with the activity of the parent compound *cis*-**4f**. Decoration of *cis*-**4f** with the covalent warheads trifluoromethyl ketone and acrylate resulted in compounds with increased antiproliferative activity (Fig. 2). TFMK *cis*-**10** [IC_{50} (DU-145) = 13.42 ± 0.64 μM and IC_{50} (MDA-MB-231) = 4.19 ± 0.50 μM] showed a 4.9-fold increased cytotoxicity against the breast cancer cells and a 3.9-fold increase against the prostate cancer cells. Olefinated analogue *cis*-**11** displayed the highest antiproliferative values against both cancer lines among all compounds tested, with an impressive 12-fold increase against prostate cancer cells (IC_{50} = 4.41 ± 0.93 μM) and a 20-fold cytotoxicity increase against breast cancer cells (1.01 ± 0.20 μM).

Finally, with the aim of broadening the analysis of the specificity of the selected compounds regarding the site of the primary manifestation of prostate and breast cancer, *cis*-**9** to **11**, which displayed IC_{50} values lower than 20 μM and systemic selectivity indexes higher than 5, had their cytotoxicity evaluated against local tissue non-tumoural RWPE-1 (BCRJ: 0389) and MCF-10A (ATCC: CRL-10317) cells (Fig. 2). Indolizine lactone *cis*-**9**, which showed markedly better cytotoxicity against MDA-MB-231 cell lines than against DU-145 lines, displayed a promising local SI value for breast tissues (SI = 47.95). Low or no local selectivity was observed for prostate cells when *cis*-**10** and **11** were evaluated (SI = 1.03 and 0.61 respectively); nevertheless, they showed good to excellent local SI values towards breast cell lines (SI ≥ 24 and ≥99 respectively).

Conclusions

The modular ring synthesis of *cis* and *trans* decorated lactones **4** emphasised the role of stereochemistry in biological activity, as the *cis* compounds were systematically more cytotoxic than the *trans* compounds for both tested cancer cell lines DU-145 (prostate) and MDA-MB-231 (breast) and were selectively more potent towards breast cancer cells. By screening both diastereoisomers, we doubled the number of identities tested and found bis-methoxylated *cis*-**4f** as an initial hit. Late-stage functionalization of *cis*-**4f** proved to be a suitable strategy for increasing the antiproliferative activity of the core while still

maintaining good selectivity index values. Decoration of the *cis*-**4f** motif with covalent warheads, such as trifluoromethyl ketone and acrylate, resulted in up to a 20-fold cytotoxicity increment.

Although the introduction of covalent warheads would be expected to lead to an increase in cytotoxicity, we found that the thiolated analogue *cis*-**9** potentially avoided the toxicity issues associated with irreversible covalent inhibitors due their promiscuous reactivity.

Considering the availability of an organocatalytic, asymmetric and diastereoselective synthesis of *cis*-indolizine lactones **4**, we can expect that the evaluation of enantiopure compounds will lead to IC_{50} values even lower than the ones observed here. The modular synthetic approach towards these cores also offers other sites for further modifications, such as the indolizine pyridine moiety or the thioether substitution pattern of *cis*-**9** derivatives.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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Notes and references

1 D. A. James, K. Koya, H. Li, G. Liang, Z. Xia, W. Ying, Y. Wu and L. Sun, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 1784.
2 K. M. Dawood and A. A. Abbas, *Expert Opin. Ther. Pat.*, 2020, **30**, 695.
3 G. S. Singh and E. E. Mmatli, *Eur. J. Med. Chem.*, 2011, **46**, 5237.
4 M. E. Welsch, S. A. Snyder and B. R. Stockwell, *Curr. Opin. Chem. Biol.*, 2010, **14**, 347.
5 V. Sharma and V. Kumar, *Med. Chem. Res.*, 2014, **23**, 3593.
6 C. R. de Souza, A. C. Gonçalves, M. F. Z. J. Amaral, A. A. Dos Santos and G. C. Clososki, *Targets Heterocycl. Syst.*, 2016, **20**, 365.
7 B. Sadowski, J. Klajn and D. T. Gryko, *Org. Biomol. Chem.*, 2016, **14**, 7804.
8 S. Dong, X. Fu and X. Xu, *Asian J. Org. Chem.*, 2020, **9**, 1133.
9 J. Hui, Y. Ma, J. Zhao and H. Cao, *Org. Biomol. Chem.*, 2021, **19**, 10245.
10 A. Boot, A. Brito, T. van Wezel, H. Morreau, M. Costa and F. Proença, *Anticancer Res.*, 2014, **34**, 1673.
11 A. Ghinet, C. M. Abuhaie, P. Gautret, B. Rigo, J. Dubois, A. Farce, D. Belei and E. Bîcu, *Eur. J. Med. Chem.*, 2015, **89**, 115.
12 R. Danac, C. M. Al Matarneh, S. Shova, T. Daniloaia, M. Balan and I. I. Mangalagiu, *Bioorg. Med. Chem.*, 2015, **23**, 2318.
13 S. Park, E. H. Kim, J. Kim, S. H. Kim and I. Kim, *Eur. J. Med. Chem.*, 2018, **144**, 435.
14 A. V. Aksenov, N. A. Arutiunov, N. K. Kirilov, D. A. Aksenov, I. Y. Grishin, N. A. Aksenov, H. Wang, L. Du, T. Betancourt, S. C. Pelly, A. Kornienko and M. Rubin, *Org. Biomol. Chem.*, 2021, **19**, 7234.
15 G. Li, X. Wu, P. Sun, Z. Zhang, E. Shao, J. Mao, H. Cao and H. Huang, *Biomed. Pharmacother.*, 2021, **133**, 110961.
16 F. Lovering, J. Bikker and C. Humblet, *J. Med. Chem.*, 2009, **52**, 6752.
17 F. Lovering, *Medchemcomm*, 2013, **4**, 515.
18 O. Méndez-Lucio and J. L. Medina-Franco, *Drug Discovery Today*, 2017, **22**, 120.
19 S. L. Schreiber, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 6699.
20 J. T. M. Correia, B. List and F. Coelho, *Angew. Chem., Int. Ed.*, 2017, **56**, 7967.
21 Y. Lu, J. Chen, M. Xiao, W. Li and D. D. Miller, *Pharm. Res.*, 2012, **29**, 2943.
22 M. S. Santos, D. C. Fernandes, M. T. Rodrigues, T. Regiani, A. D. Andricopulo, A. L. T. G. Ruiz, D. B. Vendramini-Costa, J. E. de Carvalho, M. N. Eberlin and F. Coelho, *J. Org. Chem.*, 2016, **81**, 6626.
23 Y. Hitotsuyanagi, M. Fukuyo, K. Tsuda, M. Kobayashi, A. Ozeki, H. Itokawa and K. Takeya, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 315.
24 Y. Pommier, *Nat. Rev. Cancer*, 2006, **6**, 789.
25 M. Wang, G. Shen and B. S. J. Blagg, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 2459.
26 B. V. M. Teodoro, J. T. M. Correia and F. Coelho, *J. Org. Chem.*, 2015, **80**, 2529.
27 L. A. Zeoly, L. V Acconcia, M. T. Rodrigues, H. Santos, R. A. Cormanich, J. C. Paniagua, A. Moyano and F. Coelho, *Org. Biomol. Chem.*, 2023, **21**, 3567.
28 B. Li, Z. Chen, H. Cao and H. Zhao, *Org. Lett.*, 2018, **20**, 3291.
29 X. Feng, J. Tian, Y. Sun, H. Hu, M. Lu, Y. Kan, D. Fang and C. Wang, *Chin. Chem. Lett.*, 2021, **32**, 470.
30 P. P. Jadhav, N. M. Kahar and S. G. Dawande, *Eur. J. Org. Chem.*, 2019, **2019**, 7831.
31 L. A. Hardegger, B. Kuhn, B. Spinnler, L. Anselm, R. Ecabert, M. Stihle, B. Gsell, R. Thoma, J. Diez, J. Benz, J. M. Plancher, G. Hartmann, D. W. Banner, W. Haap and F. Diederich, *Angew. Chem., Int. Ed.*, 2011, **50**, 314.
32 Y. Lu, Y. Liu, Z. Xu, H. Li, H. Liu and W. Zhu, *Expert Opin. Drug Discovery*, 2012, **7**, 375.
33 J. M. Andreu, B. Perez-Ramirez, M. J. Gorbunoff, D. Ayala and S. N. Timasheff, *Biochemistry*, 1998, **37**, 8356.
34 M. Niu, J. Qin, C. Tian, X. Yan, F. Dong, Z. Cheng, G. Fida, M. Yang, H. Chen and Y. Gu, *Acta Pharmacol. Sin.*, 2014, **35**, 967.
35 L. Li, S. Jiang, X. Li, Y. Liu, J. Su and J. Chen, *Eur. J. Med. Chem.*, 2018, **151**, 482.

36 W. Shuai, G. Wang, Y. Zhang, F. Bu, S. Zhang, D. D. Miller, W. Li, L. Ouyang and Y. Wang, *J. Med. Chem.*, 2021, **64**, 7963.
37 L. Lucescu, A. Ghinet, D. Belei, B. Rigo, J. Dubois and E. Bîcu, *Bioorg. Med. Chem. Lett.*, 2015, **25**, 3975.
38 M.-C. Sardaru, A. M. Craciun, C.-M. Al Matarneh, I. A. Sandu, R. M. Amarandi, L. Popovici, C. I. Ciobanu, D. Peptanariu, M. Pinteala, I. I. Mangalagiu and R. Danac, *J. Enzyme Inhib. Med. Chem.*, 2020, **35**, 1581.
39 I. Chourpa, J. M. Millot, G. D. Sockalingum, J. F. Riou and M. Manfait, *Biochim. Biophys. Acta, Gen. Subj.*, 1998, **1379**, 353.
40 K. C. Nicolaou, D. Vourloumis, N. Winssinger and P. S. Baran, *Angew. Chem., Int. Ed.*, 2000, **39**, 44.