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Discovery of indolizines containing triazine moiety as new leads for the development of antitumoral agents targeting mitotic events

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ABSTRACT

A new family of 3-aroylindolizines bearing a dimethoxytriazine unit in their position 1 was designed, synthesized and evaluated for their ability to inhibit tubulin polymerization and cellular growth in vitro. Compound **39** was the best candidate in the current study with a GI_{50} value of 870 nM on SNB-75 CNS cancer cells and of 920 nM on MDA-MB-231/ATCC breast cancer cells. The standard NCI Compare results indicated that indolizine **39** may target PLK1 (polo-like kinase 1).

Indolizines are important chemical structures having an important place both in organic synthesis and in biochemistry as they represent promising molecules with potential applications in the fields of pharmaceuticals,[1–3] total synthesis,[1] organic materials as novel fluorescent sensors[4] and as key intermediates in organic synthesis.[1–3] In particular, 3-aroylindolizine systems have attracted considerable interest from our research group in recent years for their antitumoral potential.[5,6] The reported biological activity for similar indolizines also highlights efficiency on cardiovascular disorders[7–10] or on degenerative joints diseases[11] as osteoarthritis or spondyloses. However, the existing literature on 3-aroylindolizines bearing a heterocyclic unit in their position 1 is extremely scarce and mainly concerns the synthetic procedures.[7,8,12–18] Only three of such indolizines have been reported and patented for their biological activity, particularly as potential antagonists of the binding of FGFs (fibroblast growth factors) to their receptors (compounds **I**–**III**, Fig. 1).[7,8]

Because of this limited number, the general development of new methodologies that can target these heterocycles and especially when they allow their diversification and discovery of new bioactive agents, is a valuable ambition. In this perspective, we propose in this manuscript the development of a new family of 3-aroylindolizines substituted by a dimethoxytriazine unit targeting mitotic events (target compounds **27**–**45**, Fig. 1). Tubulin is the structural protein of microtubules, a major component of the cytoskeleton. Since cancer cells divide more frequently than normal cells, and since mitotic microtubule dynamics is 4–100 times greater than that of microtubules during interphase, tubulin is a target for antitumor compounds.[19] Inhibitors of tubulin polymerization will therefore alter the cytoskeleton and inhibit the mitotic spindle formation, resulting in a high cytotoxic effect. Phenstatin (compound **IV**, Fig. 1) is one of the most potent tubulin polymerization inhibitor that binds to the colchicine site of the protein.[20] We have already obtained microtubule-interacting agents, phenstatin analogs, with indolizine skeleton as B-ring that showed excellent in vitro antiproliferative effect in the nanomolar range on MDA-MB-435 melanoma cell lines (GI_{50} = 30 nM) (e.g., compound **VI**, Fig. 1).[5] In order to develop new anti-cancer agents and enrich the structure–activity relationships in this field, we designed and synthesized new phenstatin analogs with dimethoxytriazine–indolizine core as B-ring and diversely substituted phenyl unit as A-ring (Fig. 1).

The chemical strategy started with the synthesis of the key dipolarophile **3**. In this light, propiolic acid **1**, after activation as acid chloride **2**, then treatment with zinc

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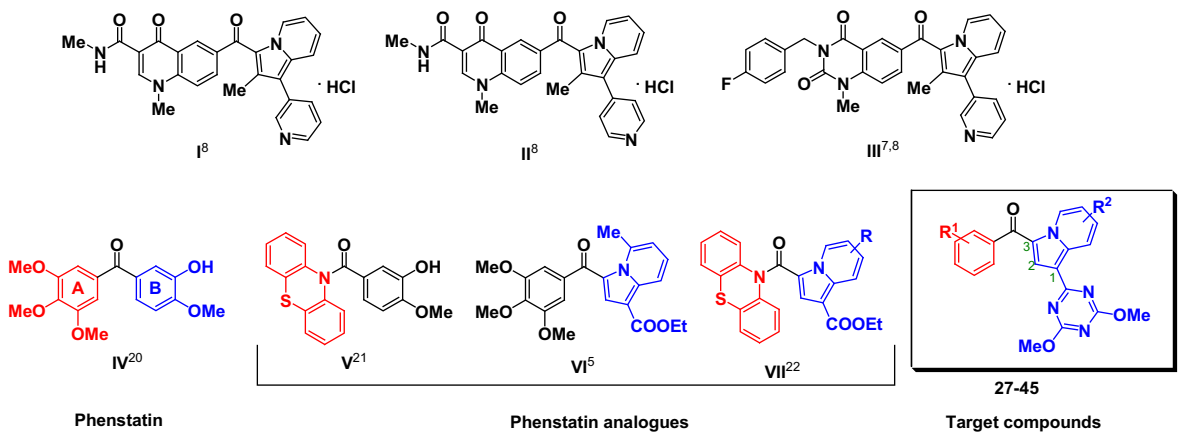
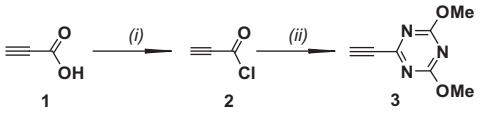


Figure 1. Structure of some biologically active 3-aroylindolizines **I–III**,[7,8] of phenstatin **IV**,[20] phenstatin analogs **V–VII**[5,21,22] and of target compounds **27–45**.

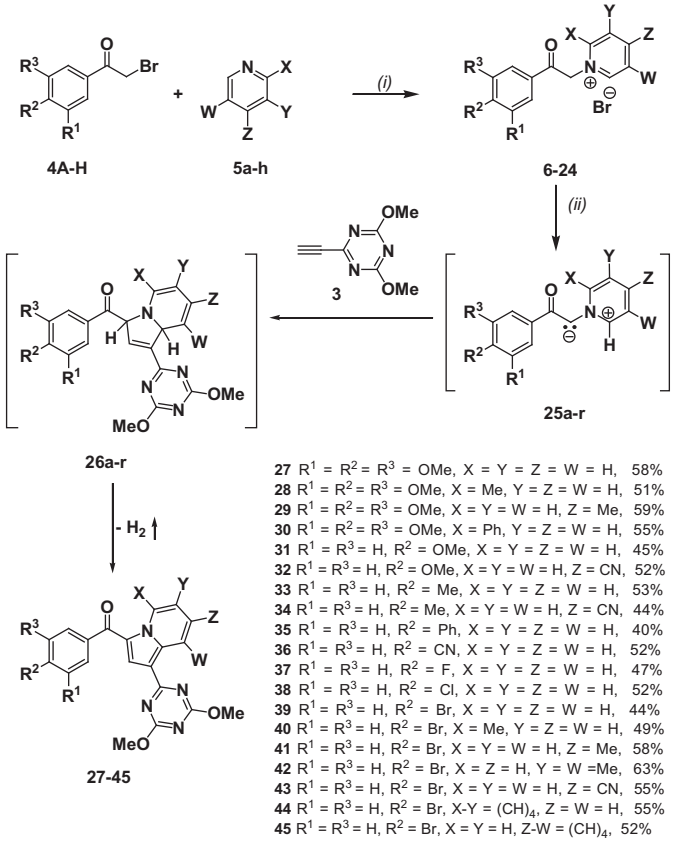


Scheme 1. Reagents and conditions: (i) 1 equiv PCl_5, dichloromethane, N_2, rt, 1 h, 90% yield; (ii) 0.7 equiv zinc dimethylimidodicarbonimidate, molecular sieves 4 Å, dichloromethane, N_2, rt, 24 h, 55% yield.[23]

dimethylimidodicarbonimidate salt,[23] furnished acetylenic derivative **3** (Scheme 1), used further for all the cycloaddition reactions.

Target indolizines containing triazine **27–45** were next obtained by [3+2] cycloaddition reaction of the corresponding ylide **25a–r**, generated in situ by triethylamine treatment of pyridinium salts **6–24**, with 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **3**, followed by spontaneous aromatization of intermediates **26a–r** (Scheme 2). The cycloimonium salts **6–24** have been easily obtained in good yields by reacting commercially available pyridine derivatives **5a–h** with ω-bromoacetophenones **4A–H**.

In order to investigate the importance of the dimethoxytriazine ring of compound **39** on antitumoral activity, additional modulations have been envisaged and realized. In this light, we decided to use the chemical strategy discovered previously on *N*-substituted pyrrolidinones linked to a 4,6-dimethoxy-1,3,5-triazine[24] and transpose it to indolizines containing triazine developed in the current manuscript. Thus, refluxing indolizine **39** in 2% aqueous HCl for 24 h resulted in a total O-demethylation of methoxy groups and furnished triazinedione **46** in 94% yield. A mono



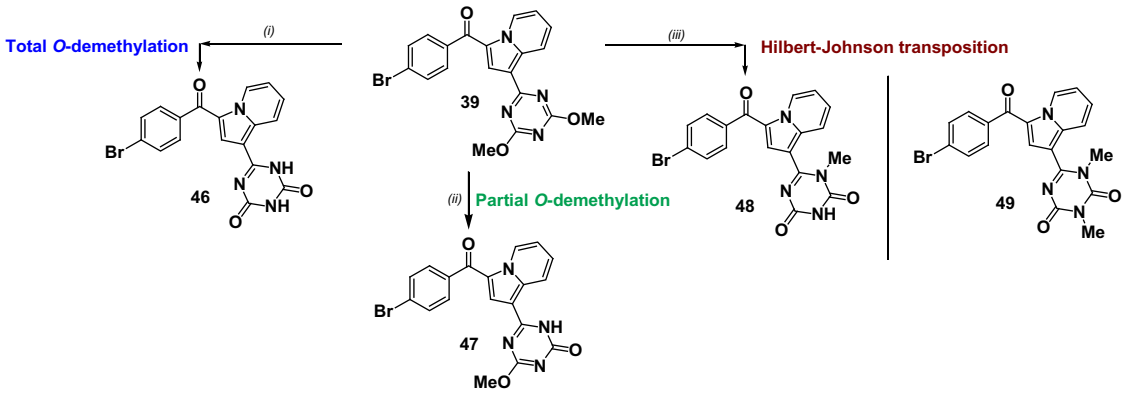
6 $R^1 = R^2 = R^3$ = OMe, X = Y = Z = W = H, 70%
7 $R^1 = R^2 = R^3$ = OMe, X = Me, Y = Z = W = H, 96%
8 $R^1 = R^2 = R^3$ = OMe, X = Y = W = H, Z = Me, 65%
9 $R^1 = R^2 = R^3$ = OMe, X = Ph, Y = Z = W = H, 20%
10 $R^1 = R^3$ = H, R^2 = OMe, X = Y = Z = W = H, 93%
11 $R^1 = R^3$ = H, R^2 = OMe, X = Y = W = H, Z = CN, 23%
12 $R^1 = R^3$ = H, R^2 = Me, X = Y = Z = W = H, 94%
13 $R^1 = R^3$ = H, R^2 = Me, X = Y = W = H, Z = CN, 84%
14 $R^1 = R^3$ = H, R^2 = Ph, X = Y = Z = W = H, 70%
15 $R^1 = R^3$ = H, R^2 = CN, X = Y = Z = W = H, 93%
16 $R^1 = R^3$ = H, R^2 = F, X = Y = Z = W = H, 70%
17 $R^1 = R^3$ = H, R^2 = Cl, X = Y = Z = W = H, 92%
18 $R^1 = R^3$ = H, R^2 = Br, X = Y = Z = W = H, 99%
19 $R^1 = R^3$ = H, R^2 = Br, X = Me, Y = Z = W = H, 89%
20 $R^1 = R^3$ = H, R^2 = Br, X = Y = W = H, Z = Me, 97%
21 $R^1 = R^3$= H, R^2 = Br, X = Z = H, Y = W =Me, 96%
22 $R^1 = R^3$ = H, R^2 = Br, X = Y = W = H, Z = CN, 56%
23 $R^1 = R^3$ = H, R^2 = Br, X-Y = $(CH)_4$, Z = W = H, 97%
24 $R^1 = R^3$ = H, R^2 = Br, X = Y = H, Z-W = $(CH)_4$, 89%

27 $R^1 = R^2 = R^3$ = OMe, X = Y = Z = W = H, 58%
28 $R^1 = R^2 = R^3$ = OMe, X = Me, Y = Z = W = H, 51%
29 $R^1 = R^2 = R^3$ = OMe, X = Y = W = H, Z = Me, 59%
30 $R^1 = R^2 = R^3$ = OMe, X = Ph, Y = Z = W = H, 55%
31 $R^1 = R^3$ = H, R^2 = OMe, X = Y = Z = W = H, 45%
32 $R^1 = R^3$ = H, R^2 = OMe, X = Y = W = H, Z = CN, 52%
33 $R^1 = R^3$ = H, R^2 = Me, X = Y = Z = W = H, 53%
34 $R^1 = R^3$ = H, R^2 = Me, X = Y = W = H, Z = CN, 44%
35 $R^1 = R^3$ = H, R^2 = Ph, X = Y = Z = W = H, 40%
36 $R^1 = R^3$ = H, R^2 = CN, X = Y = Z = W = H, 52%
37 $R^1 = R^3$ = H, R^2 = F, X = Y = Z = W = H, 47%
38 $R^1 = R^3$ = H, R^2 = Cl, X = Y = Z = W = H, 52%
39 $R^1 = R^3$ = H, R^2 = Br, X = Y = Z = W = H, 44%
40 $R^1 = R^3$ = H, R^2 = Br, X = Me, Y = Z = W = H, 49%
41 $R^1 = R^3$ = H, R^2 = Br, X = Y = W = H, Z = Me, 58%
42 $R^1 = R^3$ = H, R^2 = Br, X = Z = H, Y = W =Me, 63%
43 $R^1 = R^3$ = H, R^2 = Br, X = Y = W = H, Z = CN, 55%
44 $R^1 = R^3$ = H, R^2 = Br, X-Y = $(CH)_4$, Z = W = H, 55%
45 $R^1 = R^3$ = H, R^2 = Br, X = Y = H, Z-W = $(CH)_4$, 52%

4A $R^1 = R^2 = R^3$ = OMe
4B $R^1 = R^3$ = H, R^2 = OMe
4C $R^1 = R^3$ = H, R^2 = Me
4D $R^1 = R^3$ = H, R^2 = Ph
4E $R^1 = R^3$ = H, R^2 = CN
4F $R^1 = R^3$ = H, R^2 = F
4G $R^1 = R^3$ = H, R^2 = Cl
4H $R^1 = R^3$ = H, R^2 = Br

5a X = Y = Z = W = H
5b X = Me, Y = Z = W = H
5c X = Y = W = H, Z = Me
5d X = Ph, Y = Z = W = H
5e X = Z = H, Y = W =Me
5f X = Y = W = H, Z = CN
5g X-Y = $(CH)_4$, Z = W = H
5h X = Y = H, Z-W = $(CH)_4$

Scheme 2. Reagents and conditions: (i) EtOAc, reflux, 24 h; (ii) 1.5 equiv TEA, acetonitrile, rt, 24 h.



Scheme 3. Reagents and conditions: (i) HCl aq 2%, dioxane, reflux, 24 h, 94% yield; (ii) 9 equiv KOH, dioxane, reflux, 48 h, 85% yield; (iii) 3.75 equiv Me_2SO_4, toluene, reflux, 72 h, 34% yield.

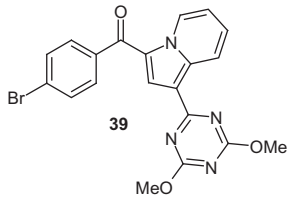
O-demethylation of indolizine–triazine **39** was next accomplished in basic conditions and provided indolizine **47**. Finally, the Hilbert–Johnson oxygen to nitrogen transposition of the methyl groups[24] was tested by refluxing dimethoxytriazine **39** in toluene in presence of dimethyl sulfate for 72 h. Unexpectedly, only the transposition of one methyl group has been observed and we were able to isolate indolizine **48** as unique reaction product. No traces of expected transposition product **49** have been detected. This underlines a different chemical behavior for the dimethoxytriazine unit when linked to a sp^2 carbon compared to dimethoxytriazines linked to a sp^3 carbon in previously described pyrrolidin-2-ones[24] (Scheme 3).

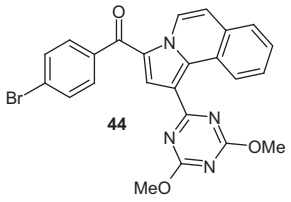
Table 1
In vitro percentage of growth inhibition (GI%) caused by the compounds **27**–**34**, **38**–**41**, and **43**–**45** against some tumor cell lines in the single-dose assay[a]

Panel	Cell line	Compound														
		27	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**38**	**39**	**40**	**41**	**43**	**44**	**45**
Leukemia	CCRF-CEM	–[b]	10	26	62	–	–	13	–	11	–	–	–	–	–	–
	HL-60(TB)	–	33	60	–	–	–	–	–	–	12	–	–	–	–	–
	K-562	10	45	56	29	–	–	–	–	–	22	–	–	–	–	–
	RPMI-8226	–	10	30	52	–	–	26	–	13	26	–	–	–	–	–
	SR	20	67	74	29	19	13	22	–	26	36	–	–	–	–	–
Non-small cell lung cancer	A549/ATCC	–	17	36	28	21	–	–	11	21	62	–	–	–	55	49
	HOP-62	–	34	28	–	97	25	–	60	77	96	–	–	–	–	79
	HOP-92	–	–	–	–	–	–	–	11	–	59	–	–	–	–	–
	NCI-H226	–	–	–	–	12	–	–	–	–	75	–	–	–	28	35
	NCI-H460	–	–	16	14	–	–	–	–	20	70	–	–	–	71	68
	NCI-H522	–	56	43	11	60	–	–	21	47	40	–	–	–	–	–
Colon cancer	HCT-116	–	15	27	–	36	–	12	–	26	65	–	–	–	–	–
CNS cancer	SF-295	–	15	–	40	47	–	–	47	33	87	22	20	–	76	59
	SF-539	–	14	29	–	32	–	–	42	39	76	–	–	–	–	–
	SNB-75	–	67	58	23	–**17**	**46**	18	–**14**	–**3**	–5	**75**	–	**27**	75	69
	U251	–	–	15	–	56	–	–	56	44	61	–	–	–	–	–
Melanoma	MALME-3M	11	37	61	–	–	–	–	17	30	37	18	73	–	67	58
	MDA-MB-435	**35**	**86**	**100**	–	–	–	–	–	–	45	–	–	–	–	–
	SK-MEL-28	–	14	40	–	–	–	–	–	12	63	14	30	–	52	43
	SK-MEL-5	–	41	63	**67**	–	–	19	–	–	27	–	–	–	–	–
	UACC-257	–	–	38	–	–	–	–	16	–	62	–	31	11	32	30
	UACC-62	–	30	63	39	11	–	–	14	14	30	–	–	–	–	–
Ovarian cancer	OVCAR-3	–	19	75	28	–	–	–	–	–	44	15	–	–	51	31
	OVCAR-4	–	–	21	18	–	–	–	–	–	88	18	**95**	–	33	30
	OVCAR-8	–	12	15	41	16	–	–	14	24	52	29	–	–	50	50
	NCI/ADR-RES	–	15	24	38	19	–	–		–	50	14	–	–	46	43
	SK-OV-3	–	18	29	–	54	–	–	42	55	–**11**	20	–	–	–	–
Renal cancer	786-0	–	–	20	–	27	–	–	40	31	59	14	–	–	81	45
	ACHN	–	30	47	30	54	–	–	15	56	70	30	–	–	29	22
	CAKI-1	–	11	25	43	26	–	**43**	–	–	56	–	–	–	44	33
	RXF 393	–	31	41	15	59	14	–	52	37	75	36		14	81	61
	TK-10	–	–	–	–	18	–	–	–	12	78	–	–	–	–	–
Prostate cancer	PC-3	–	19	36	37	18	–	13	–	19	55	–	–	–	58	37
Breast cancer	MCF7	–	55	73	22	13	–	10	–	19	18	24	38	–	60	44
	MDA-MB-231/ATCC	–	17	25	23	50	18	35	39	43	48	27	–	11	**96**	**80**
	HS 578T	–	17	35	14	25	16	–	42	46	96	22	–	–	65	56
	T-47D	–	–		32	17	–	15	–	19	52	19	–	–	38	31

The bold values highlight the best activities (the most representative results).
[a] Data obtained from NCI's in vitro disease-oriented human tumor cell screen at 10 μM concentration.
[b] No inhibitory activity.

Table 2
Results of the 5-dose in vitro 60-cell-lines screen[a] for compounds **39** and **44**





Cell type	Cell line	GI_{50}[b] (μM) **39**	GI_{50} (μM) **44**
Breast cancer	MDA-MB-231/ATCC	**0.92**	nd[c]
Melanoma	SK-MEL-28	83.8	nd
Prostate cancer	DU-145	29.1	nd
CNS cancer	SNB-75	**0.87**	**2.58**
	U251	**1.87**	nd
Renal cancer	TK-10	3.32	nd
	786-0	2.6	**4.06**
	RXF 393	4.74	**3.57**
Non-small cell lung cancer	HOP-62	2.18	22.8
	HOP-92	**1.08**	13.2

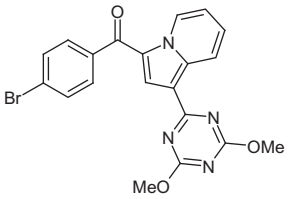
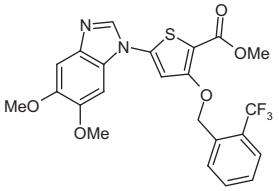
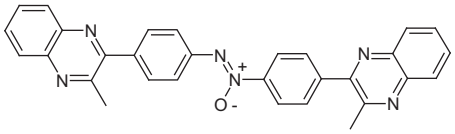
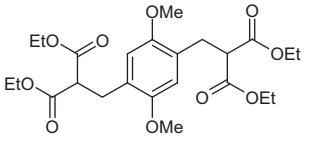
The bold values highlight the best activities (the most representative results).
[a] Data obtained from NCI's in vitro 60 cell 5-dose screening.[26]
[b] GI_{50} is the molar concentration of indolizine–triazine causing 50% growth inhibition of tumor cells.
[c] Not determined.

All synthesized indolizines containing triazine **27**–**48** have been tested in vitro for their ability to inhibit tubulin polymerization.[25] Unpredictably, no active molecule was detected at a 100 μM concentration. The absence of inhibitory potential of analogs **27**–**30** bearing the classical 3,4,5-trimethoxyphenyl unit of parent phenstatin **IV** as A ring was the most astonishing and confirms that the biological target of this series is different than tubulin.

The biological evaluation continued on fifteen indolizines containing triazine **27**–**34**, **38**–**41**, and **43**–**45**, selected by the National Cancer Institute (NCI) for initial biological screening on the NCI-60 cell lines panel at a single dose of 10 μM.[26] The representative results are shown in Table 1. Most indolizines containing triazine showed the best cellular growth inhibition on SNB-75 CNS cancer cell line. Additionally, indolizines **31**, **34**, **38** and **39** exhibited a cytotoxic effect at this concentration. 3,4,5-Trimethoxyphenylindolizines **27**–**29** displayed the best cellular inhibitory activity on MDA-MB-435 melanoma cell line in the same way as reference compound **VI** (GI% (MDA-MB-435) = 100)[5] (Fig. 1). However, these derivatives showed the best inhibitory potential on leukemia cancer cell lines among the tested compounds. The replacement of the trimethoxyphenyl unit by a *para*-bromophenyl resulted in superior biological potential and, particularly, in a different inhibition profile (indolizines **27** vs **39**, Table 1), suggesting a different biological mode of action. Thus, an interesting cytotoxic potential on SK-OV-3 ovarian cancer cells, overexpressing Pgp (permeability glycoprotein) has been registered for indolizine–triazine **39** (Table 1). On non-small cell lung cancer cells, indolizines **31** and **39** were the most active, *para*-methoxyphenyl substituted indolizine **31** having similar inhibitory percentage as *para*-bromophenyl derivative **39**. Again, compound **39** was the most active, inhibiting the growth of 65% of HCT-116 colon cancer cells. The growth of renal cancer cell lines (786-0 and RXF 393) was mainly inhibited by compound **44**, a sterically hindered analog of indolizine **39** bearing a pyrrolo[2,1-*a*]isoquinolinyl unit. The same compound was the best inhibitor of the growth of PC-3 prostate cancer cells. Finally, on breast cancer cells, the most active molecules were again indolizines **39** (96% inhibition of HS 578T cells) and **44** (96% inhibition of MDA-MB-231/ATCC cells). Compound **45**, the position isomer of indolizine **44**, showed decreased biological potency.

The best candidates issued from this single-dose assay, indolizines **39** and **44**, progressed in the 5-dose in vitro 60-cell-lines screen in order to evaluate their GI_{50} values. Results are reported in Table 2 and confirm that compound **39** is the best candidate in the current study with a GI_{50} value of 870 nM on SNB-75 CNS cancer cells and of 920 nM on MDA-MB-231/ATCC breast cancer

Table 3
Standard NCI COMPARE results obtained for indolizine **39**

Compound ID	Structure	NSC number	Rank	Correlation	Count common cell lines
39		S763415	—	1	42
GSK1030058A		S756135	1	0.942	42
63875		S63875	2	0.865	42
492247		S625157	3	0.859	42

cells. The bulky derivative **44** was globally less active with a GI_{50} value in the low micromolar range on SNB-75 cells.

In our search for the biological target of the newly synthesized indolizines containing triazine, we decided to use the NCI COMPARE program. The NCI COMPARE is an online database and a comparison tool that analyzes the profile of cellular growth inhibition from the NCI-60 cell line panel of tested compounds to assist in the identification of molecules with similar activity profiles, previously screened by the DTP (Developmental Therapeutics Program).[27] Thus, strong correlation with drugs from the DTP database may provide guidance on the biological target. Interestingly, the standard compare results for the most active molecule in the current study (indolizine **39**), indicated a correlation of 0.942 with molecule GSK1030058A (Tables 3 and S1 available in 'Supplementary data' section), a PLK1 (polo-like kinase 1) inhibitor.[28] PLK1 belongs to PLK family, proteins that associate with tubulin and the mitotic spindle.[29,30] They are also called mitotic kinases and have important roles in centrosome separation and chromosome segregation.[30] Mitotic events are thus targeted and could provide more effective cancer therapeutics.

A new family of 3-aroylindolizines bearing a dimethoxytriazine unit in their position 1 was designed, synthesized and evaluated for their ability to inhibit tubulin polymerization and cellular growth in vitro. The tubulin studies revealed that all compounds were devoid of inhibitory properties. However, the biological evaluation on the growth of cancer cell lines highlighted promising activity for *para*-bromobenzoylindolizines **39** and **44**. The best inhibitor in the current study was definitely indolizine **39** with cellular activity in the submicromolar range on SNB-75 (CNS cancer) and MDA-MB-231/ATCC (breast cancer) cell lines. Some structure–activity relationships raise from this study: the replacement of the bromo substituent in *para*-position of the phenyl unit by methyl (indolizine **33**) or chloro (indolizine **38**) groups was detrimental for the biological activity. The *para*-methoxy substitution in indolizine **31** resulted in diminished potency. Substitutions on the indolizine ring in position 5, 6 or 7 similarly furnished analogs with reduced inhibitory properties.

The seeking of new scientific collaborators in order to conclude on the real potential of indolizine **39** family on PLK1 will be realized in due course. Additional modulations will be realized in order to reinforce the biological potential and open research for new indolizine families with unprecedented antitumoral activity.

In terms of contribution to the field of organic chemistry, we have developed an advantageous and rapid synthetic pathway to access 3-aroylindolizines substituted by a dimethoxytriazine unit in position 1. Similar compounds are scarcely developed in the literature and deserve further improvement. Moreover, the complete and partial O-demethylations and Hilbert–Johnson transposition realized on the dimethoxytriazine unit of indolizine **39** emphasize new points of chemical modulation to explore in the future.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.07.025.

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