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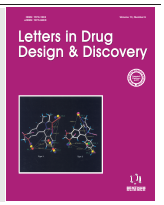
Synthesis and Biological Evaluation of Some New Indolizine Derivatives as Antitumoral Agents



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Abstract: A new series of indolizine derivatives were synthesized and screened for the antiproliferative potential against NCI 60 tumor cell line panel. The results of the study revealed a selective and good antitumor growth inhibitory activity against SNB-75 CNS cancer cell line for 1-cyanoindolizine derivative **10b**. Moreover, a supplementary *in vitro* biological evaluation showed that compound **9d** exhibited a significant farnesyltransferase inhibition activity (IC_{50} = 1.07 ± 0.34 μM) and could represent a lead for the development of new antitumoral chemical entities.

Keywords: Indolizine, farnesyltransferase, anticancer agent.

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1. INTRODUCTION

The indolizine ring system is an important structural moiety frequently found in valuable biologically active compounds. Synthetic indolizine derivatives have been found to have a variety of biological potencies such as antitumoral [1-4], antitubercular [5-7], antioxidant [8, 9], anti-inflammatory [10] and antibacterial [11, 12] activities. Our research group is interested in the study of substituted indolizin-3-yl (phenyl /heteroaryl) methanones with anticancer properties. Related structural indolizines were described as phosphatase inhibitors (e.g. compound **I**) [13] or apoptosis inducers (e.g. compound **II**) [14] (Fig. **1**). In this light, we reported new microtubule-interacting agents with indolizine skeleton (**III** [15] and **IV** [16]) and we have also described for the first time indolizine-containing inhibitors of human protein farnesyltransferase (FTase) (**V**) [17]. As part of our ongoing research focused on the synthesis of novel antitumoral compounds with indolizine skeleton, we report here the design, synthesis and *in vitro* evaluation for antiproliferative, farnesyltransferase inhibition and antitubulin activity of some new 3-(*p*-bromobenzoyl) substituted indolizine derivatives (Fig. **1**).

2. RESULTS AND DISCUSSION

2.1. Synthesis

The target indolizine derivatives were synthesized as presented in Scheme (**1**). The key intermediates, the pyridinium salts **3a-g** were prepared in good yields, by refluxing a solution of 2,4′-dibromoacetophenone **1** and an appropriate pyridine derivative **2a-g** in ethyl acetate. The construction of the indolizine unit in products **6a-g**, **9a-e** and **10a**,**b** was then achieved by [3+2] cycloaddition reaction of the corresponding ylide **4a-g**, generated *in situ* by base treatment of pyridinium salts **3a-g**, with ethyl propiolate, acrylonitrile, dimethylacetylenedicarboxylate (DMAD), followed by spontaneous aromatization of intermediates (Scheme **1**). The target 1-carboxyethylindolizines **6a-g** and 1,2-dicarboxymethyl indolizines **9a-e** were isolated in good to moderate yields (Scheme **1** and Table **1**). The use of acrylonitrile as dipolarophile provided the 1-cyanoindolizines **10a**, **b** in lower yields (Scheme **1** and Table **1**). After cycloaddition reaction of ylide **4f** with dimethylacetylenedicarboxylate as dipolarophile, besides indolizine **9e**, we were able to separate by column chromatography, the partial aromatized intermediate **11**. Another exception was observed for the reaction of 4-methoxy-pyridine substituted cycloimonium salt **3e** with acrylonitrile, when only a tricyclic derivative **12** was isolated, in place of the expected indolizine (Scheme **1**). This type of double dipolar cycloaddition product has been observed and described previously by our research group by reacting olefinic dipolarophiles with *p*-dimethylamino pyridinium ylides [18]. We were then interested to study the influence on bioactivity of free carboxylic acids analogues of indolizines **6c** and **6f**. In this light, saponification of the ethyl ester group of the title compounds was accomplished and straightforwardly furnished corresponding acids **7a** and **7b** in very good yields (Scheme **1** and Table **1**).

The structures of the newly synthesized compounds were confirmed on the basis of IR, ^{1}H-NMR, and ^{13}C-NMR spectra. In the IR spectra of the synthesized indolizines, the characteristic C=O stretching bands due to the ketone group were

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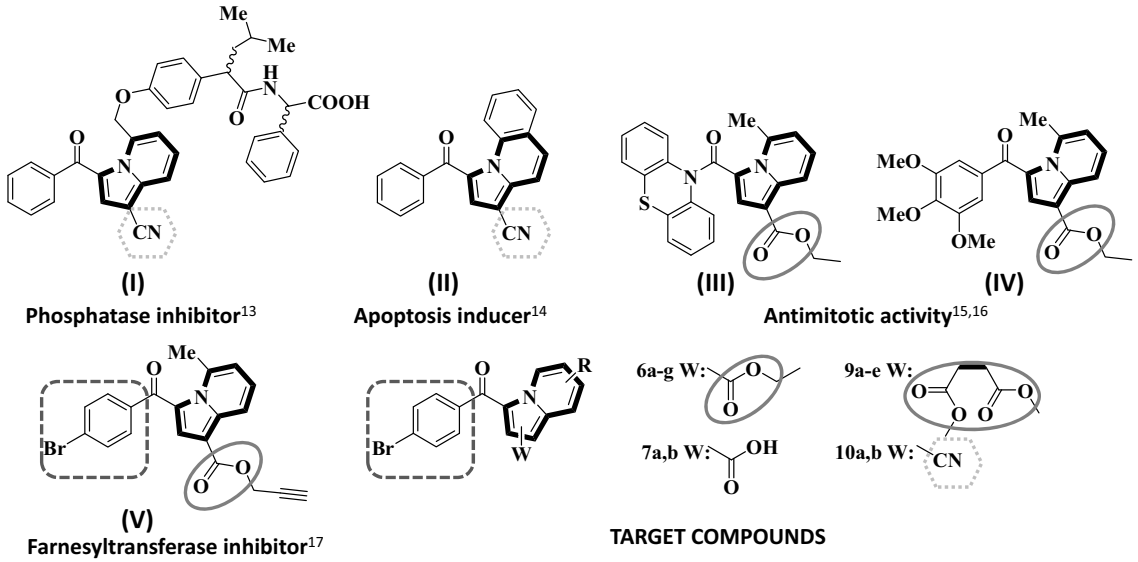


Fig. (1). Structure of some biologically active indolizine derivatives and of target compounds **6**, **7**, **9** and **10**.

observed at about 1615-1644 cm^{-1}. The characteristic C=O stretching bands due to the ester group of compounds **6a-g** were observed at about 1697-1716 cm^{-1} and, as expected, these bands do not appear in the IR spectra of carboxylic compounds **7a**,**b**. For the dicarboxymethyl derivatives **9a-e** two characteristic C=O stretching bands due to the ester group were observed at about 1697-1745, and 1732-1745 cm^{-1} respectively. The characteristic C≡N stretching bands due to the nitrile group of compounds **10a**,**b** and **12** appear in the IR spectra at about 2225-2235 cm^{-1}. In the ^{1}H-NMR spectra, depending on the structure, the characteristic H_5 of indolizine cycle were observed as a doublet signal at about 9.20-9.93 ppm. This signal disappears in the ^{1}H-NMR of 5-substituted indolizines. Another characteristic signal attributed to H_2 proton of the indolizine cycle, appears in the ^{1}H-NMR at about 7.70-8.14 ppm for compounds **6a-g**, **7a**,**b** and **10a**,**b**, while for the dicarboxymethyl derivatives this signal is not observed. The characteristic chemical shifts of the *p*-bromophenyl ring were found in the aromatic region, at about 7.53-8.10 ppm. The ^{13}C-NMR spectra were in accordance with the proposed structures, and the most significant chemical shifts were attributed to the quaternary carbon from ketone group at 183-186 ppm. Some physicochemical properties of the synthesized compounds are presented in Table **1**.

2.2. Biological Activity

Seven of the synthesized indolizines **6c**, **g**, **f**, **7a**,**b** and **10a**,**b** were selected by the National Cancer Institute (NCI) for screening against 60 human tumor cell lines. The most significant results are summarized in Table **2**. As it can be observed, the best antiproliferative potential was registered for compound **10a** on the SNB-75 CNS cancer cell line, with 52% cell growth inhibition at a 10μM dose. Similar cellular inhibition profile was observed for the other 1-cyanoindolizine derivative **10b**. The replacement of the nitrile group with a carboxyethyl unit in compounds **6f, g**, lead to complete loss of antiproliferative activity. However, the 1-carboxyethyl substituted indolizine **6c**, showed a moderate activity, with the best cell growth inhibition on HT29 colon cancer cell line. The saponification of the ester group in compounds **7a** and **7b** resulted in diminished inhibitory activity.

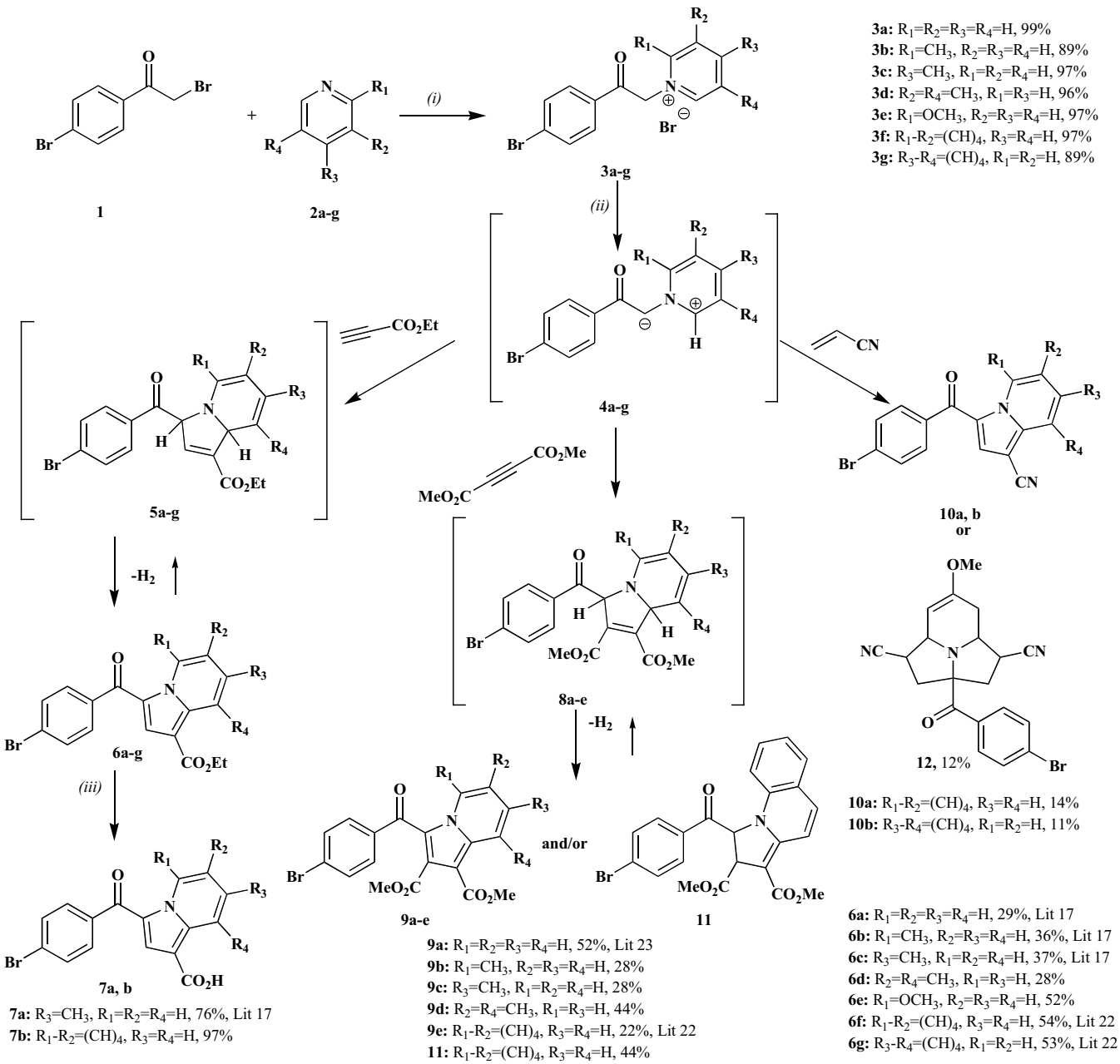
In order to identify the biological target of our synthesized indolizines, compounds **6a-g**, **7a**,**b**, **9a-e** and **10a**,**b** were evaluated for their inhibitory potential on human farnesyltransferase (FTase) protein. Surprisingly, only indolizine derivative **6d** presented a strong inhibitory potential, with a value of IC_{50} (FTase) = 1.07 ± 0.34 μM, while the other tested compounds were completely inactive.

These preliminary results showed that the cellular target of the compounds **10a** and **10b** with the best antiproliferative potential is different from FTase. Our previous results concerning the ability of indolizine derivatives as tubulin polymerization inhibitors [15, 16], encouraged us to test the newly synthesized compounds for this type of inhibitory activity and the results are described in Table **3**. Again, the results were modest, compound **10b** showed a lower inhibitory activity, while compound **10a** was inactive. The most significant inhibitory profile of the series, with an inhibition ratio of 31%, was registered for compound **9c**, a 7-methyl substituted indolizine, with two ester functional groups in the structure. Any other modification realized on this structure as the position change of the methyl group on the indolizine unit in compound **9b**, a 6,8-dimethyl substitution in compound **9d**, the absence of substituents in compound **9a** or replacement by a pyrrolo [2,1-a]quinoline in compound **9e** lead to a lower or a completely loss of inhibitory activity.

3. EXPERIMENTAL

3.1. Materials and Methods

The material and methods utilized are described in a precedent publication [19].



Scheme (1). Reagents and conditions: (i) EtOAc, reflux, 24h; (ii) TEA (1.5 equiv), acetonitrile, dipolarophile (acrylonitrile, ethyl propiolate, DMAD) (1.3 equiv), rt, 24 h; (iii) 2N aqueous NaOH (29-32 equiv), MeOH, reflux, 4-8 h.

Table 1. Physicochemical properties of synthesized compounds 6 – 12.

Compound	Molecular Formula	Molecular Weight (g/mol)	Yield[a] (%)	mp (°C)[b]	R_f Value[c]
6a	$C_{18}H_{14}BrNO_3$	372.21	29	122-125	0.74
6b	$C_{19}H_{16}BrNO_3$	386.24	36	120-122	0.56
6c	$C_{19}H_{16}BrNO_3$	386.24	37	118-121	0.55
6d	$C_{20}H_{18}BrNO_3$	400.27	28	129-130	0.8
6e	$C_{19}H_{16}BrNO_4$	402.24	52	180-183	0.86
6f	$C_{22}H_{16}BrNO_3$	422.27	54	199-202	0.72
6g	$C_{22}H_{16}BrNO_3$	422.27	53	195-197	0.72

Table **1**. contd…

Compound	Molecular Formula	Molecular Weight (g/mol)	Yield[a] (%)	mp (°C)[b]	R_f Value[c]
7a	$C_{17}H_{12}BrNO_3$	358.19	76	257-277	0.0
7b	$C_{20}H_{12}BrNO_3$	394.22	97	267-269	0.0
9a	$C_{19}H_{14}BrNO_5$	416.22	52	146-150	0.39
9b	$C_{20}H_{16}BrNO_5$	430.25	28	210-213	0.84
9c	$C_{20}H_{16}BrNO_5$	430.25	28	151-154	0.7
9d	$C_{21}H_{18}BrNO_5$	444.28	44	126-128	0.8
9e	$C_{23}H_{16}BrNO_5$	466.28	22	193-196	0.78
11	$C_{23}H_{18}BrNO_5$	468.3	44	121-125	0.84
10a	$C_{20}H_{11}BrN_2O$	375.22	14	282-284	0.8
10b	$C_{20}H_{11}BrN_2O$	375.22	11	282-285	0.78
12	$C_{20}H_{18}BrN_3O_2$	412.28	12	268-269	0.36

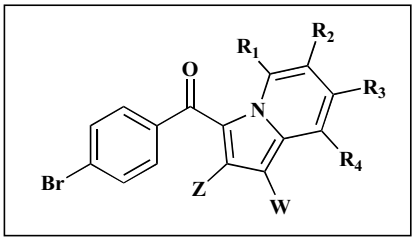
[a]Solvent used for recrystallization: ethanol.
[b]Melting point.
[c]Retention factor; eluting system – *n*-hexane:ethyl acetate (1:1).

Table 2. ***In vitro*** **percentage growth inhibition (GI%) caused by the compounds 6c, 6f, 6g, 10a, 10b, 7a and 7b against some tumor cell lines in the single-dose assay.**

	Compound	6c	7a	7b	10a	10b
Panel	Cell line	Cell growth inhibition, GI% (10^{-5} M)[a]				
Leukemia	K-562	20	-	-	-[b]	-
	SR	20	-	11	12	-
Non-Small Cell Lung Cancer	HOP-62	-	-	-	20	29
	HOP-92	-	21	22	-	-
	NCI-H322M	-	-	13	-	-
	NCI-H522	31	-	-	29	14
Colon Cancer	HCT-116	14	-	11	-	-
	HCT-15	18	-	12	-	-
	HT29	35	-	-	-	-
CNS Cancer	SNB-75	-		-	43	52
	U251	17	10	-	-	
Melanoma	MALME-3M	-	-	-	-	17
	UACC-62	14	-	-	-	
	SK-OV-3	-	-	-	16	16
	A498	-	16	-	-	
	ACHN	-	-	-	-	15
Ovarian Cancer	MDA-MB-231/ATCC	12	-	-	12	-
	T-47D	24	11	-	13	-

[a]Data obtained from NCI's in vitro disease-oriented human tumor cell screen at 10 μM concentration
[b]GI% (10^{-5} M) < 10

Table 3. Inhibitory activities on tubulin polymerization.



Compound	R_1	R_2	R_3	R_4	W	Z	% TPI[a,b]
6a	H	H	H	H	CO_2Et	H	21.9
6b	Me	H	H	H	CO_2Et	H	15.3
6c	H	H	Me	H	CO_2Et	H	3.8
6d	H	Me	H	Me	CO_2Et	H	0
6e	H	H	MeO	H	CO_2Et	H	0
6f	R_1-R_2=$(CH)_4$		H	H	CO_2Et	H	21.3
6g	H	H	R_3-R_4=$(CH)_4$		CO_2Et	H	23.6
7a	H	H	Me	H	CO_2H	H	0
7b	R_1-R_2=$(CH)_4$		H	H	CO_2H	H	19.7
9a	H	H	H	H	CO_2Me	CO_2Me	21.9
9b	Me	H	H	H	CO_2Me	CO_2Me	0
9c	H	H	Me	H	CO_2Me	CO_2Me	31.0
9d	H	Me	H	Me	CO_2Me	CO_2Me	0
9e	R_1-R_2=$(CH)_4$		H	H	CO_2Me	CO_2Me	0
10a	R_1-R_2=$(CH)_4$		H	H	CN	H	0
10b	H	H	R_3-R_4=$(CH)_4$		CN	H	17.6

[a]Inhibition of tubulin polymerization at a 100 μM concentration.
[b]Values represent mean of two experiments.

General Procedure for the Synthesis of Pyridinium Salts 3a-g

The pyridine derivative **2a-g** (1.5 equiv) was added to a solution of 2,4′-dibromoacetophenone **1** (1 equiv) in ethyl acetate. The resulting mixture was stirred at reflux for 24 h. After cooling the reaction medium to room temperature, the obtained precipitate was filtered, washed with ethyl acetate to give the corresponding pure salt which was used further in cycloaddition reactions.

1-[2-(4-Bromophenyl)-2-oxoethyl]pyridinium bromide (3a)

The general procedure was followed using 2,4′-dibromoacetophenone **1** (15.0 g, 54.0 mmol, 1 equiv), pyridine **2a** (6.5 mL, 80.9 mmol, 1.5 equiv) and ethyl acetate (60 mL) to provide pure salt **3a** as a white solid with the same physico-chemical properties as described in the literature [20]; 99% yield; mp>250 ^oC.

1-[2-(4-Bromophenyl)-2-oxoethyl]-2-methylpyridinium bromide (3b)

The general procedure was followed using 2,4′-dibromoacetophenone **1** (0.7 g, 2.52 mmol, 1 equiv), 2-methylpyridine **2b** (0.33 mL, 3.76 mmol, 1.5 equiv) and ethyl acetate (20 mL) to provide pure salt **3b** as a white solid with the same physico-chemical properties as described in the literature [21]; 89% yield; mp 179-182^oC.

1-[2-(4-Bromophenyl)-2-oxoethyl]-4-methylpyridinium bromide (3c)

The general procedure was followed using 2,4′-dibromoacetophenone **1** (0.7 g, 2.52 mmol, 1 equiv), 4-methylpyridine **2c** (0.37 mL, 3.76 mmol, 1.5 equiv) and ethyl acetate (20 mL) to provide pure salt **3c** as a white solid with the same physico-chemical properties as described in the literature [22]; 97% yield; mp 272-274^oC.

1-[2-(4-Bromophenyl)-2-oxoethyl]-3,5-dimethylpyridinium bromide (3d)

The general procedure was followed using 2,4′-dibromoacetophenone **1** (1.5 g, 5.39 mmol, 1 equiv), 3,5-dimethylpyridine **2** (1.16 mL, 8.12 mmol, 1.5 equiv) and ethyl acetate (20 mL). White solid; 96% yield; mp 215-217°C. IR ν cm^{-1}: 1696, 1582, 1502, 1486, 1395, 1351, 1323, 1310, 1225, 1203, 1180, 1065, 1008, 985, 939, 843, 829, 720, 668, 614, 578, 535, 495, 456.^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 2.51 (s, 6H, 2CH_3), 6.42 (s, 2H, CH_2), 7.90 (d, J = 8.4 Hz, 2H, Ar*H*), 8.02 (d, J = 8.4 Hz, 2H, Ar*H*), 8.45 (s, 1H, Ar*H*), 8.77 (s, 2H, Ar*H*). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 17.9 ($2CH_3$), 65.9 (CH_2), 129.0 (C), 130.3 (2CH), 132.4 (2CH), 132.7 (C), 137.8 (2C), 143.1 (2CH), 147.4 (CH), 190.2 (C).

1-[2-(4-Bromophenyl)-2-oxoethyl]-4-methoxypyridinium bromide (3e)

The general procedure was followed using 2,4′-dibromoacetophenone **1** (1.0 g, 3.60 mmol, 1 equiv), 4-methoxypyridine **2f** (0.55 mL, 5.38 mmol, 1.5 equiv) and ethyl acetate (25 mL) to provide pure salt **3e** as a white solid; 97% yield; mp 204-206°C. IR ν cm^{-1}: 1695, 1642, 1586, 1569, 1518, 1396, 1352, 1297, 1239, 1205, 1177, 1070, 1001, 867, 849, 816, 767. ^{1}H RMN (DMSO-d_6, 400 MHz): δ (ppm) 4.16 (s, 3H, OCH_3), 6.29 (s, 2H, CH_2), 7.76 (d, J = 7.6 Hz, 2H; Ar*H*), 7.90 (d, J = 8.4 Hz, 2H; Ar*H*), 7.99 (d, J = 8.4 Hz, 2H; Ar*H*), 8.79 (d, J = 7.6 Hz, 2H, Ar*H*). ^{13}C RMN (DMSO-d_6, 100 MHz): δ 58.2 (CH_3), 66.0 (CH_2), 113.2 (2CH), 128.7 (C), 130.1 (2CH), 130.3 (2CH), 132.7 (C), 145.5 (2CH), 171.1 (C), 190.8 (C).

1-[2-(4-Bromophenyl)-2-oxoethyl]quinolinium bromide (3f)

The general procedure was followed using 2,4′-dibromoacetophenone **1** (1.5 g, 5.40 mmol, 1 equiv), quinoline **5g** (0.96 mL, 8.09 mmol, 1.5 equiv) and ethyl acetate (25 mL) to provide pure salt **3f** as a white solid with the same physico-chemical properties as described in the literature [23]; 97% yield; mp 260-263°C.

2-[2-(4-Bromophenyl)-2-oxoethyl]isoquinolinium bromide (3g)

The general procedure was followed using 2,4′-dibromoacetophenone **1** (1.0 g, 2.52 mmol, 1 equiv), isoquinoline **5h** (0.43 mL, 3.78 mmol, 1.5 equiv) and ethyl acetate (20 mL) to provide pure salt **3g** as a white solid with the same physico-chemical properties as described in the literature [23]; 89% yield; mp 255-257°C.

General Procedure for the Preparation of Indolizines 6a-g, 9a-e, 11, 10a,b and 12

Triethylamine (1.5 equiv) was added to a suspension of cycloimonium salt **3a-g** (1 equiv) in acetonitrile. The dipolarophile (ethyl propiolate, DMAD or acrylonitrile) (1.3 equiv) was then added, after the formation of the intermediate ylide **4a-g** in situ (yellow coloration of the reaction medium). The resulting mixture was stirred at room temperature for 24 h. The residue obtained upon evaporation was purified by recrystallization from EtOH or by chromatography (EtOAc: *n*-heptane) and then recrystallized from EtOH to give pure indolizines.

Ethyl 3-(4-bromobenzoyl)indolizine-1-carboxylate (6a)

The general procedure was followed using 1-[2-(4-bromophenyl)-2-oxoethyl]pyridinium bromide **3a** (0.7 g, 1.96 mmol, 1 equiv), ethyl propiolate (0.26 mL, 2.55 mmol, 1.3 equiv), triethylamine (0.41 mL, 2.94 mmol, 1.5 equiv) and acetonitrile (15 mL) to provide pure indolizine **6a** as a yellow solid with the same physico-chemical properties as described in the literature [17]; 29% yield; mp (EtOH) 122-125°C.

Ethyl 3-(4-bromobenzoyl)-5-methylindolizine-1-carboxylate (6b)

The general procedure was followed using 1-[2-(4-bromophenyl)-2-oxoethyl]-2-methylpyridinium bromide **3b** (1.0 g, 2.69 mmol, 1 equiv), ethyl propiolate (0.35 mL, 3.5 mmol, 1.3 equiv), triethylamine (0.56 mL, 4.04 mmol, 1.5 equiv) and acetonitrile (15 mL) to provide pure indolizine **6b** as a yellow solid with the same physico-chemical properties as described in the literature [17]; 36% yield; mp (EtOH) 120-122°C.

Ethyl 3-(4-bromobenzoyl)-7-methylindolizine-1-carboxylate (6c)

The general procedure was followed using 1-[2-(4-bromophenyl)-2-oxoethyl]-4-methylpyridinium bromide **3c** (1.0 g, 2.69 mmol, 1 equiv), ethyl propiolate (0.35 mL, 3.5 mmol, 1.3 equiv), triethylamine (0.56 mL, 4.04 mmol, 1.5 equiv) and acetonitrile (15 mL) to provide pure indolizine **6c** as a cream solid with the same physico-chemical properties as described in the literature [17]; 37% yield; mp (EtOH) 118-121°C.

Ethyl 3-(4-bromobenzoyl)-6,8-dimethylindolizine-1-carboxylate (6d)

The general procedure was followed using 1-[2-(4-bromophenyl)-2-oxoethyl]-3,5-dimethylpyridinium bromide **3d** (0.4 g, 1.04 mmol, 1 equiv), ethyl propiolate (0.13 mL, 1.35 mmol, 1.3 equiv), triethylamine (0.22 mL, 1.55 mmol, 1.5 equiv) and acetonitrile (15 mL). White solid; 28% yield; mp (EtOH) 129-130°C; *Rf*=0.8 (EtOAc:*n*-hexane, 1:1). IR ν cm^{-1}: 1716, 1622, 1520, 1479, 1351, 1172, 1053, 935, 847, 836, 775, 748, 694, 520, 451, 414. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 1.36 (t, J = 7.2 Hz, 3H, CH_3), 2.39 (s, 3H, CH_3), 2.78 (s, 3H, CH_3), 4.31 (q, J = 7.2 Hz, 2H, CH_2), 7.09 (s, 1H, Ar*H*), 7.66 (s, 4H, Ar*H*), 7.70 (s, 1H, Ar*H*), 9.74 (s, 1H, Ar*H*). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.4 (CH_3), 18.3 (CH_3), 21.8 (CH_3), 60.6 (CH_2), 108.3 (C), 121.2 (C), 125.1 (CH), 125.2 (C), 126.0 (C), 129.2 (C), 130.6 (2CH), 130.7 (CH), 131.6 (2CH), 131.9 (C), 132.3 (CH), 137.8 (C), 139.1 (C), 164.0 (C), 183.8 (C). Anal. calcd for $C_{20}H_{18}BrNO_3$: C, 60.1; H, 4.53; N, 3.50. Found: C, 60.52; H, 5.02; N, 3.96.

Ethyl 3-(4-bromobenzoyl)-7-methoxyindolizine-1-carboxylate (6e)

The general procedure was followed using 1-[2-(4-bromophenyl)-2-oxoethyl]-4-methoxypyridinium bromide **3e** (0.25 g, 0.65 mmol, 1 equiv), ethyl propiolate (0.09 mL, 0.84 mmol, 1.3 equiv), triethylamine (0.13 mL, 0.97 mmol, 1.5 equiv) and acetonitrile (15 mL). Cream solid; 52% yield; mp (EtOH) 180-183°C. IR ν cm^{-1}: 1697, 1648, 1605, 1538, 1524, 1476, 1457, 1344, 1301, 1283, 1252, 1226, 1203, 1171, 1084, 1068, 1041, 1020, 926, 848, 832, 805, 776, 749, 689, 597, 426, 449.^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 1.39 (t, *J*=7.2 Hz, 3H, C*H*$_3$), 3.97 (s, 3H, OC*H*$_3$), 4.36 (q, *J*=7.2 Hz, 2H, C*H*$_2$), 6.78 (dd, *J*=7.6, 2.4 Hz, 1H, Ar*H*), 7.63-7.69 (m, 4H, Ar*H*), 7.75 (d, *J*=2.4 Hz, 1H, Ar*H*), 9.79 (d, *J*= 7.6 Hz, 1H, Ar*H*).^{13}C NMR ($CDCl_3$, 100 MHz): δ14.6 (CH_3), 55.8 (CH_3), 60.1 (CH_2), 97.6 (CH), 104.9 (C), 109.4 (CH), 121.5 (C), 126.1 (C), 129.7 (CH), 130.4 (2CH), 130.7 (CH), 131.6 (2CH), 138.7 (C), 143.1 (C), 160.1 (C), 164.3 (C), 183.6 (C). Anal. calcd for $C_{19}H_{16}BrNO_4$: C, 56.73; H, 4.01; N, 3.48. Found: C, 57.64; H, 4.74; N, 3.91.

Ethyl 1-(4-bromobenzoyl)pyrrolo[1,2-a]quinoline-3-carboxylate (6f)

The general procedure was followed using 1-[2-(4-bromophenyl)-2-oxoethyl]quinolinium bromide **3f** (1.0 g, 2.45 mmol, 1 equiv), ethyl propiolate (0.32 mL, 3.19 mmol, 1.3 equiv), triethylamine (0.51 mL, 3.68 mmol, 1.5 equiv) and acetonitrile (15 mL) to provide pure indolizine **6f** as a cream solid with the same physico-chemical properties as described in the literature [24]; 54% yield; mp (EtOH) 199-202°C.

Ethyl 3-(4-bromobenzoyl)pyrrolo[2,1-a]isoquinoline-1-carboxylate (6g)

The general procedure was followed using 2-[2-(4-bromophenyl)-2-oxoethyl]isoquinolinium bromide **3g** (1.0 g, 2.45 mmol, 1 equiv), ethyl propiolate (0.32 mL, 3.19 mmol, 1.3 equiv), triethylamine (0.51 mL, 3.68 mmol, 1.5 equiv) and acetonitrile (15 mL) to provide pure indolizine **6g** as a pale pink solid with the same physico-chemical properties as described in the literature [24]; 53% yield; mp (EtOH) 195-197°C.

Dimethyl 3-(4-bromobenzoyl)indolizine-1,2-dicarboxylate (9a)

The general procedure was followed using 1-[2-(4-bromophenyl)-2-oxoethyl]pyridinium bromide **3a** (1.0 g, 2.8 mmol, 1 equiv), DMAD (0.45 mL, 3.64 mmol, 1.3 equiv), triethylamine (0.58 mL, 4.2 mmol, 1.5 equiv) and acetonitrile (15 mL) to provide pure indolizine **9a** as a yellow solid with the same physico-chemical properties as described in the literature [25]; 52% yield; mp (EtOH) 146-150°C.

Dimethyl 3-(4-bromobenzoyl)-5-methylindolizine-1,2-dicarboxylate (9b)

The general procedure was followed using 1-[2-(4-bromophenyl)-2-oxoethyl]-2-methylpyridinium bromide **3b** (1.0 g, 2.69 mmol, 1 equiv), DMAD (0.43 mL, 3.5 mmol, 1.3 equiv), triethylamine (0.56 mL, 4.04 mmol, 1.5 equiv) and acetonitrile (15 mL). Yellow solid; 28% yield; mp (EtOH) 210-213°C. IR ν cm^{-1}: 1746, 1689, 1638, 1583, 1542, 1514, 1458, 1370, 1315, 1200, 1164, 1096, 1008, 925, 873, 850, 821, 791, 754, 724, 652, 632, 517, 461. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 2.42 (s, 3H, C*H*$_3$), 3.40 (s, 3H, OC*H*$_3$), 3.88 (s, 3H, OC*H*$_3$), 6.90 (d, *J* = 7.2 Hz, 1H, Ar*H*), 7.40 (t, *J* = 7.2 Hz, 1H, Ar*H*), 7.64 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.81 (d, *J* = 8.4 Hz, 2H, Ar*H*), 8.36 (d, *J* = 8.8 Hz, 1H, Ar*H*). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 23.2 (CH_3), 51.6 (CH_3), 52.3 (CH_3), 103.0 (C), 117.3 (CH), 117.9 (CH), 122.7 (C), 127.2 (CH), 128.5 (C), 130.2 (C), 131.5 (2CH), 131.7 (2CH), 137.0 (C), 138.4 (C), 139.7 (C), 163.5 (C), 165.5 (C), 184.0 (C). Anal. calcd for $C_{20}H_{16}BrNO_5$: C, 55.83; H, 3.75; N, 3.26. Found: C, 56.41; H, 4.16; N, 3.88.

Dimethyl 3-(4-bromobenzoyl)-7-methylindolizine-1,2-dicarboxylate (9c)

The general procedure was followed using 1-[2-(4-bromophenyl)-2-oxoethyl]-4-methylpyridinium bromide **3c** (0.96 g, 2.58 mmol, 1 equiv), DMAD (0.45 mL, 3.36 mmol, 1.3 equiv), triethylamine (0.54 mL, 3.88 mmol, 1.5 equiv) and acetonitrile (20 mL). Orange solid; 28% yield; mp (EtOH) 151-154°C. IR ν cm^{-1}: 1746, 1697, 1615, 1582, 1500, 1442, 1375, 1341, 1294, 1253, 1203, 1152, 1094, 1006, 920, 873, 801, 781, 760, 662, 470, 435. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 2.50 (s, 3H, C*H*$_3$), 3.38 (s, 3H, OC*H*$_3$), 3.87 (s, 3H, OC*H*$_3$), 6.96 (dd, *J* = 7.2, 1.6 Hz, 1H, Ar*H*), 7.53 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.59 (d, *J* = 8.8 Hz, 2H, Ar*H*), 8.16 (s, 1H, Ar*H*), 8.54 (d, *J* = 7.2 Hz, 1H, Ar*H*). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 21.7 (CH_3), 51.6 (CH_3), 52.3 (CH_3), 103.2 (C), 118.6 (CH), 118.7 (CH), 120.0 (C), 126.4 (C), 128.1 (CH), 130.2 (2CH), 131.3 (2CH), 132.1 (C), 138.5 (C), 138.9 (C), 140.2 (C), 163.4 (C), 165.3 (C), 185.2 (C). Anal. calcd for $C_{20}H_{16}BrNO_5$: C, 55.83; H, 3.75; N, 3.26. Found: C, 56.32; H, 4.29; N, 3.48.

Dimethyl 3-(4-bromobenzoyl)-6,8-dimethylindolizine-1,2-dicarboxylate (9d)

The general procedure was followed using 1-[2-(4-bromophenyl)-2-oxoethyl]-3,5-dimethylpyridinium bromide **3d** (0.7 g, 1.82 mmol, 1 equiv), DMAD (0.29 mL, 2.35 mmol, 1.3 equiv), triethylamine (0.38 mL, 2.73 mmol, 1.5 equiv) and acetonitrile (20 mL). Cream solid; 44% yield; mp (EtOH) 126-128°C. IR ν cm^{-1}: 1736, 1710, 1624, 1587, 1489, 1443, 1373, 1289, 1200, 1172, 1067, 1013, 926, 833, 789, 766, 726, 679, 659, 535, 452. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 2.34 (s, 3H, C*H*$_3$), 2.57 (s, 3H, C*H*$_3$), 3.30 (s, 3H, OC*H*$_3$), 3.85 (s, 3H, OC*H*$_3$), 6.99 (s, 1H, Ar*H*), 7.57 (s, 1H, Ar*H*), 9.20 (s, 1H, Ar*H*). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 18.4 (CH_3), 20.2 (CH_3), 52.1 (CH_3), 52.3 (CH_3), 108.0 (C), 119.7 (C), 123.4 (CH), 125.6 (C), 126.7 (C), 128.9 (C), 129.0 (C), 130.2 (2CH), 130.8 (CH), 131.4 (2CH), 134.3 (C), 139.3 (C), 164.8 (C), 164.9 (C), 185.5 (C). Anal. calcd for $C_{21}H_{18}BrNO_5$: C, 56.77; H, 4.08; N, 3.15. Found: C, 57.32; H, 4.57; N, 3.48.

Dimethyl 3-(4-bromobenzoyl)pyrrolo[2,1-a]quinoline-1,2-dicarboxylate (9e)

The general procedure was followed using 1-[2-(4-bromophenyl)-2-oxoethyl]quinolinium bromide **3f** (1.0 g, 2.46 mmol, 1 equiv), DMAD (0.39 mL, 3.19 mmol, 1.3 equiv), triethylamine (0.51 mL, 3.69 mmol, 1.5 equiv) and acetonitrile (20 mL) to provide pure indolizine **9e** as a cream solid with the same physico-chemical properties as described in the literature [24]; 22% yield;mp (EtOH) 193-196°C.

Dimethyl 1-(4-bromobenzoyl)-1,3a-dihydropyrrolo[1,2-a]quinolone-2,3-dicarboxylate (11a)

By-product from the synthesis of indolizine **9e**; yellow solid; 44% yield; mp (EtOH) 121-125°C. IR ν cm^{-1}: 1733, 1695, 1678, 1625, 1583, 1556, 1470, 1426, 1353, 1250, 1067, 1009, 771, 760. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 3.49 (s, 3H, C*H*$_3$), 3.90 (s, 3H, C*H*$_3$), 4.56 (d, *J* = 12.8 Hz, 1H, C*H*), 5.44 (d, *J* = 12.8 Hz, 1H, C*H*), 5.80 (d, *J* = 6.8 Hz, 1H, Ar*H*), 6.22 (d, *J* = 6.8 Hz, 1H, Ar*H*), 7.04 (s, 1H, Ar*H*), 7.26 (s, 1H, Ar*H*), 7.36 (s, 1H, Ar*H*), 7.52-7.55 (m, 3H, Ar*H*), 7.86 (d, *J* = 6.8 Hz, 2H, Ar*H*). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 51.3 (CH_3), 53.0 (CH_3), 53.2 (CH), 65.4 (CH), 104.2 (C), 109.4 (CH), 123.6 (CH), 123.7 (CH), 124.8 (CH), 127.3 (CH), 128.4 (CH), 130.0 (C), 130.4 (C), 130.6 (2CH), 131.4 (C), 132.6 (2CH), 133.7 (C), 150.5 (C), 164.2 (C), 173.8 (C), 188.1 (C). Anal. calcd for $C_{23}H_{18}BrNO_5$: C, 58.99; H, 3.87; N, 2.99. Found: C, 59,23; H, 4.47; N, 3.26.

3-(4-Bromobenzoyl)pyrrolo[2,1-a]quinoline-1-carbonitrile (10a)

The general procedure was followed using 1-[2-(4-bromophenyl)-2-oxoethyl]quinolinium bromide **3f** (0.7 g, 1.72 mmol, 1 equiv), acrylonitrile (0.14 mL, 2.24 mmol, 1.3 equiv), triethylamine (0.36 mL, 2.58 mmol, 1.5 equiv) and acetonitrile (15 mL). Cream solid; 14% yield; mp (EtOH) 282-284°C. IR ν cm^{-1}: 3126, 2225, 1626, 1588, 1535, 1467, 1423, 1324, 1235, 1170, 1069, 1010, 968, 879, 833, 801, 749, 737, 685, 562, 504, 466, 408. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.26 (d, *J* = 7.6 Hz, 1H, Ar*H*), 7.60 (s, 1H, Ar*H*), 7.69 (d, *J* = 8.8 Hz, 2H, ArH), 7.72 (d, *J* = 8.8 Hz, 2H, ArH), 7.74-7.77 (m, 2H, Ar*H*), 7.83-7.86 (m, 1H, Ar*H*), 9.01 (d, *J* = 7.2Hz, 1H, Ar*H*), 9.46 (d, *J* = 7.2 Hz, 1H, Ar*H*). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 103.0 (C), 115.6 (CH), 117.3 (C), 120.6 (CH), 124.5 (C), 125.3 (CH), 126.7 (C), 127.0 (CH), 128.5 (C), 128.9 (CH), 129.9 (CH), 130.9 (C), 131.2 (2CH), 131.9 (2CH), 134.1 (C), 136.4 (CH), 137.4 (C), 179.2 (C). Anal. calcd for $C_{20}H_{11}BrN_2O$: C, 64.02; H, 2.95; N, 7.47. Found: C, 64.61; H, 3.56; N, 7.84.

3-(4-Bromobenzoyl)pyrrolo[2,1-a]isoquinoline-1-carbonitrile (10b)

The general procedure was followed using 2-[2-(4-bromophenyl)-2-oxoethyl]isoquinolinium bromide **3g** (0.7 g, 1.72 mmol, 1 equiv), acrylonitrile (0.14 mL, 2.24 mmol, 1.3 equiv), triethylamine (0.36 mL, 2.58 mmol, 1.5 equiv) and acetonitrile (15 mL). Cream solid; 11% yield; mp (EtOH) 282-285°C. IR ν cm^{-1}: 3127, 2225, 1626, 1588, 1535, 1467, 1452, 1423, 1362, 1342, 1236, 1171, 1069, 1010, 968, 879, 833, 801, 776, 749, 737, 685, 562, 467, 408. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.33 (d, *J* = 7.6 Hz, 1H, Ar*H*), 7.56 (s, 1H, Ar*H*), 7.67-7.77 (m, 6H, Ar*H*), 7.82-7.87 (m, 1H, Ar*H*), 8.98-9.03 (m, 1H, Ar*H*), 9.55 (d, *J* = 7.6 Hz, 1H, Ar*H*). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 86.1 (C), 116.0 (CH), 116.9 (C), 123.6 (C), 124.1 (CH), 124.1 (C), 125.1 (CH), 127.3 (C), 127.3 (CH), 128.8 (CH), 129.0 (CH), 129.9 (C), 130.2 (CH), 130.6 (2CH), 131.9 (2CH), 137.8 (C), 138.1 (C), 184.3 (C).Anal. calcd for $C_{20}H_{11}BrN_2O$: C, 64.02; H, 2.95; N, 7.47. Found: C, 64.71; H, 3.62; N, 7.69.

2a-(4-Bromobenzoyl)-6-methoxy-1,2,2a,3,4,4a,5,7a-octahydropyrrolo[2,1,5-cd]indolizine-1,4-dicarbonitrile (12)

The general procedure was followed using 1-[2-(4-bromophenyl)-2-oxoethyl]-4-methoxypyridinium bromide **3e** (0.4 g, 1.0 mmol, 1 equiv), acrylonitrile (0.09 mL, 1.34 mmol, 1.3 equiv), triethylamine (0.22 mL, 1.55 mmol, 1.5 equiv) and acetonitrile (15 mL). White crystals; 12% yield; mp (EtOH) 268-269°C. IR ν cm^{-1}: 2942, 2910, 2325, 2235, 1678, 1663, 1583, 1566, 1462, 1395, 1367, 1309, 1279, 1218, 1160, 1134, 1071, 1035, 993, 971, 851, 830, 808, 761, 722, 664, 578, 466. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 2.31-2.42 (m, 2H, C*H*$_2$), 2.60-2.65 (m, 1H, C*H*), 2.72-2.82 (m, 2H, C*H*$_2$), 3.18-3.24 (m, 1H, C*H*), 3.34-3.4 (m, 3H, C*H*$_2$C*H*), 3.65 (s, 3H, OC*H*$_3$), 3.92-3.96 (m, 1H, C*H*), 4.71-4.72 (m, 1H, C*H*), 7.61 (d, *J* = 9.2 Hz, 2H, Ar*H*), 8.10 (d, *J* = 8.4 Hz, 2H, Ar*H*). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 31.9 (CH_2), 32.7 (CH), 34.5 (CH), 39.5 (CH_2), 40.7 (CH_2), 55.3 (CH_3), 58.7 (CH), 59.9 (CH), 79.3 (C), 90.1 (CH), 118.9 (C), 121.3 (C), 128.8 (C), 131.4 (2CH), 131.9 (2CH), 132.8 (C), 157.0 (C), 198.2 (C). Anal. calcd for $C_{20}H_{18}BrN_3O_2$: C, 58.26; H, 4.40; N, 10.19. Found: C, 59.12; H, 4.72; N, 10.64.

General Procedure for the Preparation of 3-(4-substituted benzoyl)indolizine-1-carboxylic acids 7a and 7b

Aqueous 2N NaOH was added to a suspension of ethyl ester **6c** and **6f** (1 equiv) in MeOH. The reaction mixture was stirred at reflux, until complete solubilization. The resulting mixture was cooled to room temperature, and then citric acid was added until complete precipitation. The carboxylic acid obtained **7a** and **7b** was washed with hot ethanol and collected by filtration.

3-(4-Bromobenzoyl)indolizine-1-carboxylic acid (7a)

The general procedure was followed using ethyl 3-(4-bromobenzoyl)-7-methylindolizine-1-carboxylate **6c** (0.1 g, 0.28 mmol, 1 equiv), NaOHaq 2N (4 mL) and MeOH (4 mL) to provide pure carboxylic acid **7a** as a white solid with the same physico-chemical properties as described in the literature [20]; 76% yield; mp (EtOH) 275-277°C.

3-(4-Bromobenzoyl)pyrrolo[1,2-a]quinoline-1-carboxylic acid (7b)

The general procedure was followed using ethyl 1-(4-bromobenzoyl)pyrrolo[1,2-*a*]quinoline-3-carboxylate **6f** (0.1 g, 0.25 mmol, 1 equiv), NaOHaq 2N (4 mL) and MeOH (4 mL). White solid; 97% yield; mp (EtOH) 267-269°C. IR ν cm^{-1}: 3150, 1692, 1621, 1589, 1518, 1459, 1356, 1338, 1238, 1195, 1159, 1140, 965, 870, 748.^{1}H NMR (DMSO-d_6, 400

MHz): δ (ppm) 7.53 (d, *J* = 7.6 Hz, 1H, Ar*H*), 7.66 (s, 1H, Ar*H*), 7.69-7.81 (m, 6H, Ar*H*), 9.50 (d, *J* = 7.6 Hz, 1H, Ar*H*), 9.84 (d, *J* = 7.6 Hz, 1H, Ar*H*), 12.72 (s, 1H, O*H*).^{13}C NMR (DMSO-d_6, 100 MHz): δ 110.7 (C), 115.5 (CH), 122.4 (C), 123.8 (C), 124.7 (CH), 125.7 (C), 127.0 (CH), 127.6 (CH), 127.8 (CH), 129.5 (CH), 129.6 (CH), 130.1 (C), 130.9 (2CH), 131.5 (2CH), 135.8 (C), 138.2 (C), 165.2 (C), 183.8 (C). Anal. calcd for $C_{20}H_{12}BrNO_3$: C, 60.93; H, 3.07; N, 3.55. Found: C, 61.34; H, 3.29; N, 3.68.

Biological Evaluation

1. Farnesyltransferase assay: These assays were realized as described in a previous article [19, 26].

2. Tubulin studies: These studies were realized as described in a preceding publication [27], according to reported procedure [28, 29] using DAPI as fluorescent molecule.

3. Cell proliferation assay: The cell proliferation assay was realized with indolizines **6c**,**f**,**g**, **7a**,**b** and **10a**,**b** on a panel of 60 human cancer cell lines at the National Cancer Institute, Germantown, MD. The cytotoxicity studies were carried out using the sulforhodamine B assay [30-32].

CONCLUSION

A new series of 3-(*p*-bromobenzoyl) substituted indolizine derivatives was synthesized by [3+2] cycloaddition reaction, fully characterized and evaluated for cytotoxicity on an NCI-60 human cancer cell lines panel and tested for farnesyltransferase and tubulin polymerization inhibitory activity. The best results after these investigations were registered for compound **10b** which showed a selective antiproliferative activity against SNB-75 CNS cancer cell line, being a promising candidate for future modulations. Furthermore, farnesyltransferase assay revealed a strong inhibitory potential for 1-carboxyethyl-6,8-dimethyl substituted derivative **6d**, with an IC_{50} value of 1.07 ± 0.34 μM.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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