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Design, synthesis and preliminary biological evaluation of acridine compounds as potential agents for a combined targeted chemo-radionuclide therapy approach to melanoma

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ABSTRACT

Various iodo-acridone and acridine carboxamides have been prepared and evaluated as agents for targeted radionuclide and/or chemotherapy for melanoma, due to their structural similarity to benzamides which are known to possess specific affinity for melanin. Three of these carboxamides selected for their in vitro cytotoxic properties were radioiodinated with [^{125}I]NaI at high specific activity. Biodistribution studies carried out in B16F0 murine melanoma tumour-bearing mice highlighted that acridone **8f** and acridine **9d**, presented high, long-lasting tumour concentrations together with an in vivo kinetic profile favourable to application in targeted radionuclide therapy.

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1. Introduction

Malignant melanoma is the most serious forms of skin cancer and has a high rate of dissemination. Patients with distant metastases show a very low response rate to chemotherapy, monochemotherapy or polychemotherapeutic regimens, and there has been no progress in systemic palliative melanoma treatment for three decades.[1] Multiple randomised studies have been unable to demonstrate any significant advantage for survival.[2] Melanoma is described as intrinsically resistant to many chemotherapeutic drugs, which makes it exceptionally difficult to treat this neoplasm. The increasing incidence of skin melanoma and the lack of effective therapy have intensified the search for new methods of early disease detection and treatment. One approach explored is to use selective tissue-targeted chemotherapy or radionuclide therapy, which has led to the development of new carriers suitable for targeting melanoma. The carriers already investigated can be grouped into four classes: monoclonal antibodies (MAbs),[3] alpha-melanocyte stimulating hormone (α-MSH),[4,5] false melanin precursors[6,7] or agents with a high affinity for melanin. This fourth group constitutes an heterogeneous drug family that includes polyamines,[8] benzamides[9–12] and polycyclic aromatic compounds.[13]

Drug–melanin binding is a complex process involving various interactions. The polyanionic feature of melanin is an important factor in the affinity of these drugs.[8,14] Moreover, in vitro studies of the binding of many compounds to melanin[13] showed that several polycyclic aromatic compounds, especially those with coplanar fused rings, bind strongly to melanin. This class of compounds has been used for melanoma targeted chemotherapy or radionuclide therapy. Wolf et al., in a study on *N*-(2-diethylaminoethyl)-benzamides conjugated with alkylating cytostatics, reported higher melanin affinity and toxicity against B16F0 melanoma cells than with the parent drugs.[15] Otherwise, radiolabelled methylene blue (MTB) with astatine-211, an alpha emitter, has also shown promising results for the treatment of cutaneous tumours and their metastases.[16]

Our institution has recently developed aromatic and heteroaromatic analogues of *N*-(2-diethylaminoethyl)-4-iodobenzamide (**1**, BZA) (Fig. 1), which is a radiotracer already successfully used in humans for the diagnosis of malignant melanoma,[17] as part of a targeted radionuclide therapy approach to melanoma treatment.

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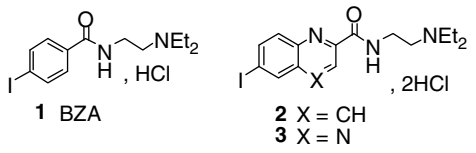


Figure 1.

In most cases, the aromatic moiety was changed without loss of specific tumoural affinity. Quinoline **2** and quinoxaline **3** analogues (Fig. 1) showed significantly higher tumoural uptakes after 72 h, respectively, peaking at 7- and 16-fold higher values than with the BZA parent compound.[18]

These results led us to attempt to combine the benefits of chemotherapy and radionuclide therapy by conjugating an effective radionuclide to cytotoxic heteroaromatic analogues of compounds **1**–**3**. Such compounds could be used for radionuclide therapy using a beta or Auger electron emitter and/or for chemotherapy of melanoma. We selected an acridine analogue, a decision based on several considerations: (a) these molecules are well known as cytotoxic DNA-intercalating agents[19–21]; (b) the acridine derivatives such as *N*-(2-dimethylaminoethyl)-acridine-4-carboxamide (DACA: NSC 601316) (**4**), SN 16713 (**5**) and the acridone derivative **6** possess a basic side chain similar to the BZA compound (Fig. 2); (c) the acridine moiety shows structural similarity with compounds **2** and **3,** and acridine orange (**5**) has a documented affinity for melanin and has been reported to bind strongly to synthetic melanin (98% binding with synthetic or uveal melanin).[22]

Furthermore, DACA (**4**) showed toxicity against human melanoma cell lines and their tumour xenografts in mice.[23,24] These effects are in accordance with low capacity of DACA to induce any significant resistance compared with other anti-tumoural agents.[25] Moreover, DNA-intercalating agents would appear suitable for application in radionuclide therapy using iodine-125, an Auger electron emitter, and it has been demonstrated that intracellular ^{125}I has a much higher cell-killing effect when located in the close vicinity of DNA than when in the cytoplasm.[26–28] The range of the Auger electron (1–1.5 nm) causes double-strand breaks in DNA and induces less surrounding tissue damage than ^{131}I because of that short-range biological effectiveness.[29] The beta particles emitted from ^{131}I have a maximum range of 3 mm and are better dedicated to neoadjuvant therapy, whereas ^{125}I, which is only highly toxic if it is located inside the cell nucleus, appears more suitable for adjuvant therapy or for the treatment of melanoma micrometastases.

In the present work, we describe the method for the synthesis of a panel of iodo-acridone or iodo-acridine-carboxamide compounds **8**–**11** (Fig. 3) and report the results of preliminary in vitro and in vivo biological tests designed to select a leading candidate for

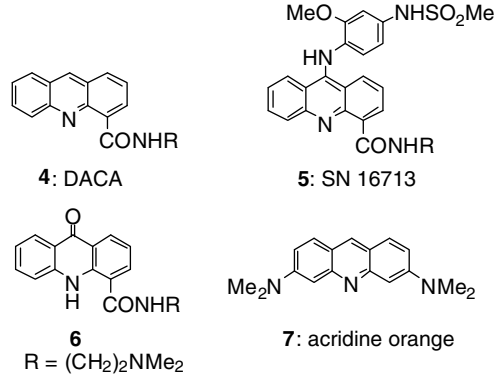


Figure 2. Structures of compounds studied in the text.

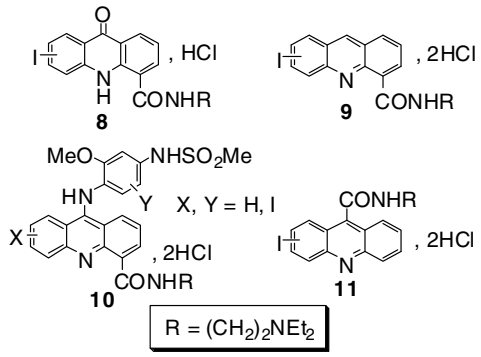


Figure 3. Chemical structures proposed.

use in radionuclide therapy and/or chemotherapy of melanoma. The structure–activity relationships and particularly the positions of iodine and carboxamide substituents were evaluated while the lipophilic side chain was kept identical to the parent compound BZA.

Given that we working towards an approach to melanoma treatment combining targeted chemotherapy and radionuclide therapy, this pharmacomodulation program gave rise to two initial questions: (i) is the toxicity of the chemical compounds conserved? (ii) Is there a specific affinity for melanoma tumour with a kinetic profile favourable to application in targeted radionuclide therapy when administered in vivo? Biological experiments were developed using in vitro and in vivo tumour models to assess these issues.

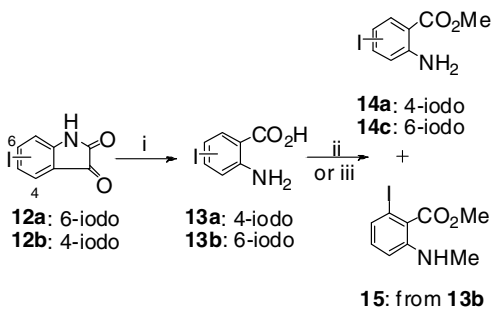
2. Results and discussion

2.1. Chemistry

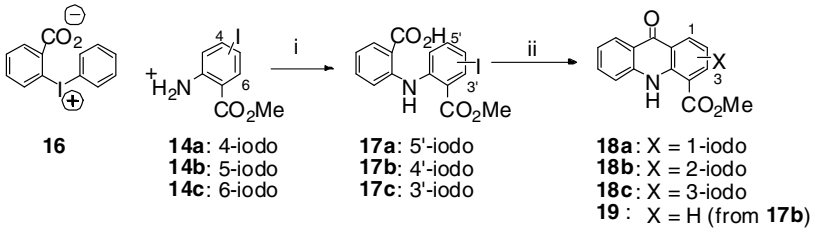
Acridones are important starting materials for the synthesis of a wide range of acridine derivatives. Furthermore, acridine compounds **9** and 9-anilino derivatives **10** were prepared from the same acridones **8**, whereas acridine-9-carboxamide products **11** were synthesised from the commercially available acridine-9-carboxylic acid hydrate.

2.1.1. Synthesis of acridone derivatives 8a–g

The (1-3)-iodo-acridones **18a**–**c** were achieved by a method reported previously,[30] starting from methyl iodoanthranilate **14** (Schemes 1 and 2). Methyl 5-iodoanthranilate (**14b**) was prepared following the procedure described by Sy.[31] Methyl 4- and 6-iodoanthranilates **14a** and **14c**, previously described by Allison[32] and Seltzman,[33] were prepared by a more convenient procedure from 4 and 6-iodoisatin (**12a–b**)[34] obtained from the same commercially



Scheme 1. Reagents and conditions: (i) a—1.5 N NaOH, 30% H_2O_2, 50 °C; b—6.5 N HCl; (ii) 17% HCl, MeOH, reflux from **13a**; (iii) NaH, DMF, MeI, rt, from **13b**.



Scheme 2. Reagents and conditions: (i) $Cu(OAc)_2$, DMF, 90–100 °C; (ii) H_2SO_4, 80 °C.

available precursor 3-iodoaniline (Scheme 1). Oxidation of 6-iodoisatin (**12a**) with alkaline hydrogen peroxide afforded 4-iodoanthranilic acid (**13a**) according to the procedure describe by Snow[35] for the 6-iodoanthranilic acid (**13b**). Esterification of the 4-iodoanthranilic acid (**13a**) with anhydrous methanol in the presence of a 17% HCl/MeOH solution afforded methyl anthranilate **14a**. In the case of acid **13b**, esterification using the usual conditions of 17% HCl/MeOH or $SOCl_2$/MeOH gave an unequivocal decarboxylation reaction to yield 3-iodoaniline. We then developed a less sensitive reaction by treating acid **13b** with sodium hydride and methyl iodide in dry dimethylformamide. Using these conditions, esterification gave the expected compound **14c** (44%) which was accompanied with methyl *N*-methyl-6-iodoanthranilate (**15**) in 13% yield.

According to the reaction described by Rewcastle,[28] diphenyliodonium-2-carboxylate (**16**)[36] reacted with methyl iodoanthranilates **14a–c** to give 2-(iodo-2′-methoxycarbonylphenyl-amino)benzoic acids **17a–c** with good yields (59–80%) (Scheme 2). Ring closure of acids **17a–c** was performed using sulphuric acid, as previously described,[37] to give methyl 9,10-dihydro-iodo-9-oxoacridine-4-carboxylates **18a–c**. During the synthesis of compound **18b**, the deiodinated by-product **19** was isolated with moderated yield (8%).

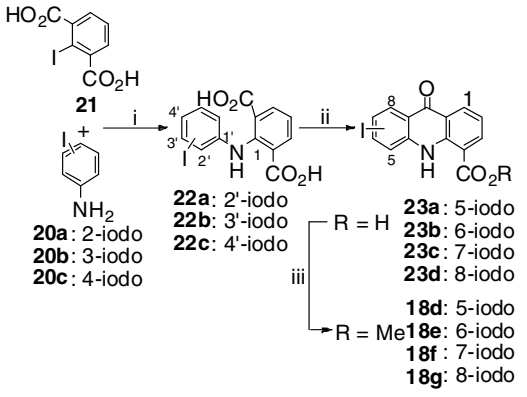
Preparation of (5–8)-iodo-9-oxoacridine-4-carboxylates **18d–g** required a different synthetic approach starting from 2-(iodophenylamino)isophthalic acids **22a–c** (Scheme 3) prepared via a Jourdan–Ullmann reaction. The cross-coupling reaction of *o*-iodoisophthalic[38,39] acid (**21**) with iodoanilines **20a–c** gave compounds **22a–c**, which were then cyclised using polyphosphoric acid (PPA) to yield 9,10-dihydro-9-oxoacridine-4-carboxylic acids **23a–d**, according to the procedure described by Rewcastle et al.[36] Diacids **22a** and **22c** substituted in the 2′- or 4′-position cyclised unequivocally in PPA to give the 5-iodo-9,10-dihydro-9-oxoacridine-4-carboxylic acid (**23a**) and 7-iodo-9,10-dihydro-9-oxoacridine-4-carboxylic acid (**23c**), respectively. For diacid **22b** substituted in the 3′-position, two available directions of ring closure were possible, and a mixture of both iodo-isomers **23b** and **23d** (1/1 as observed by ^1H NMR) was obtained. These isomers were separated by chromatographic procedures at the next step as ester derivatives **18e** and **18g** obtained from the same methodology developed for compound **14b**. We also prepared methyl 9,10-dihydro-5 and 7-iodo-9-oxoacridine-4-carboxylates (**18d** and **18f**) by known sequential treatment with thionyl chloride and methanol from an equimolar mixture of acids **23a** and **23c**.

The basic side chain was easily installed on the carboxylate function of **18a–g** by condensation of *N*,*N*-diethylethylenediamine in the presence of trimethylaluminium to give amides **24a–g** in 38–98% yields (Scheme 4). In order to carry out the in vitro and in vivo biological evaluation of these compounds, the free base forms of **24a–g** were converted into their water-soluble hydrochloride salts **8a–g**.

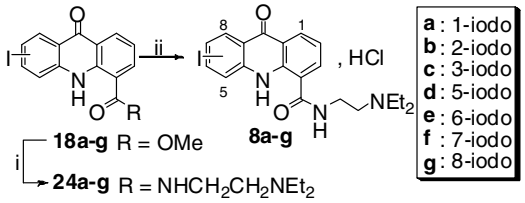
2.1.2. Synthesis of acridine derivatives 9a–g

There are several methods available that can be adapted to the synthesis of DACA analogues (**27**). The acridine **27** already reported was generally prepared[40] from acridine-4-carboxylic acids **26** generated by reduction of the corresponding acridones **25** by aluminium/mercury amalgam,[41] followed by oxidation of the resulting acridans with $FeCl_3$ (Scheme 5). It was noted that dehalogenation took place with the chlorinated compounds.[40]

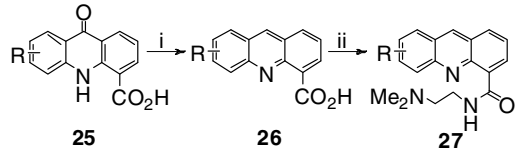
This prompted us to develop an original method for yield iodo analogues of DACA using borane tetrahydrofuran complex as reducing agent. A solution of BH_3–THF was added to reflux the tetrahydrofuran solution of compounds **18a–g** (Scheme 6). The reactions were maintained at reflux for 45 min before being hydrolysed. The resulting acridans **28a–g** were oxidised to acridines **29a–g** with $FeCl_3$ in yields ranging from 87% to 97%. These esters



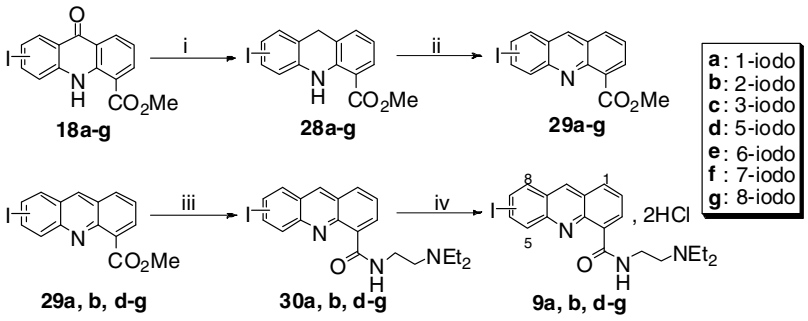
Scheme 3. Reagents and conditions: (i) CuCl, *N*-ethylmorpholine, butan-2,3-diol, benzene, 120 °C; (ii) PPA, 120 °C; (iii) a—$SOCl_2$, DMF, Δ; b—MeOH, $CaCO_3$ from **23a**,**c**; NaH, MeI, DMF, rt, from **23b**,**d**.



Scheme 4. Reagents and conditions: (i) $AlMe_3$, $NH_2(CH_2)_2NEt_2$, CH_2Cl_2 or PhMe, 0 °C then reflux; (ii) 2 N HCl, ether, rt.



Scheme 5. Reagents and conditions: (i) a—Al(Hg); b—$FeCl_3$; (ii) $SOCl_2$, $NH_2(CH_2)_2NEt_2$.



Scheme 6. Reagents and conditions: (i) BH_3–THF (1 M), THF, reflux; (ii) $FeCl_3$, H_2O, EtOH, 50 °C; (iii) $AlMe_3$, $NH_2(CH_2)_2NEt_2$, CH_2Cl_2 or PhMe, 0 °C then reflux; (iv) 2 N HCl, ether, rt.

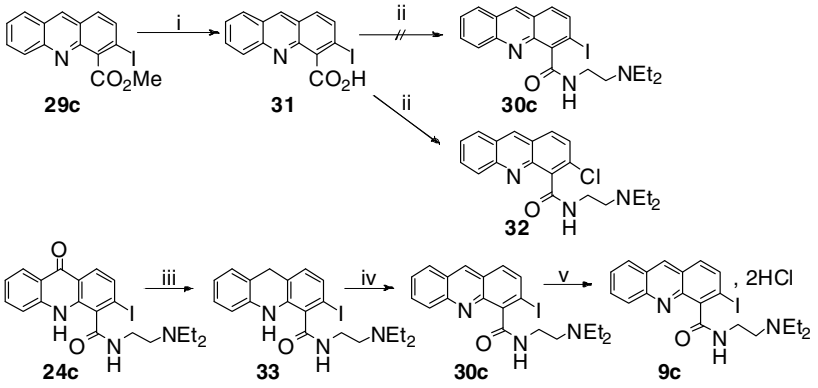
29a–**g** were very unstable to oxidation and afforded, in our case, the corresponding acridones **18a**–**g**. For this reason, the esters **29a**,**b**,**d**–**g** had to be immediately treated with *N*,*N*-diethylethylenediamine to give stable amides **30a**,**b**,**d**–**g** before being converted into dihydrochloride salts **9a**,**b**,**d**–**g** using the same procedure as developed for compounds **8a**–**g**.

Some difficulties were encountered with the more hindered methyl 3-iodoacridine-4-carboxylate (**29c**). Attempts to prepare compound **30c** by treatment of ester **29c** under the usual conditions (previously reported for compounds **24** and **30a**,**b**,**d**–**g**) only recovered starting material. This prompted us to follow another route for synthesising amide **30c** via acid intermediate **31** as outlined in Scheme 7. Treatment of acid **31**, obtained by saponification of ester **29c**, with thionyl chloride and *N*,*N*-diethylethylenediamine afforded the undesired chloramide **32**. One unexpected problem was the lability of iodine group in the presence of an ortho electron-withdrawing group, and we observed iodine/chloride exchange during activation with thionyl chloride. An alternative route to obtain amide **30c** was the reduction of the corresponding acridone-4-carboxamide **24c** with borane tetrahydrofuran complex, followed by $FeCl_3$ oxidation of the resulting acridan **33**. It was noted that secondary amides could be reduced rapidly and quantitatively into the corresponding amines by borane in refluxed tetrahydrofuran.[42] In our case, the presence of hindered 3-iodo-group prevents probably the reduction of 4-amido function. Finally, the dihydrochloride salt **9c** was obtained by treatment with a 2 N HCl/ether solution, as described for compounds **8** with an overall yield of 35% from **24c**.

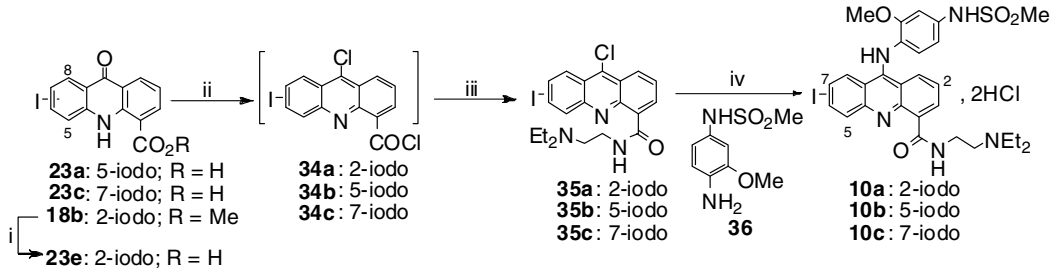
2.1.3. *m*-AMSA derivatives 10a–d syntheses

To obtain *m*-AMSA derivatives, the more convenient method was direct nucleophilic substitution of 9-choroacridines **35a–c** by anilino compound **36** (Scheme 8). One problem, as mentioned earlier for the synthesis of compound **30c** from **29c**, was the lability of the halogen substituent in the presence of thionyl chloride.[43] This prompted us not to use the 1, 3 and 6-iodo derivatives **18a**,**c**,**e** and the unstable 8-iodo compound 18 g for this series. In the case of 2-iodo compound **18b**, conversion in acid **23e** was performed using a methanolic 2 N sodium hydroxide solution. The iodo-9-chloroacridine carboxamides **35a**–**c** were prepared in two steps from 9,10-dihydroiodo-9-oxoacridine-4-carboxylic acids **23a**,**c**,**e**. Treatment of acids **23a**,**c**,**e** with thionyl chloride afforded 9-chloroacridine-4-carbonyl chlorides **34a**–**c**. Reacting these crude compounds with *N*,*N*-diethylethylenediamine in anhydrous dichloromethane gave excellent yields (80–96%) of the unstable carboxamide derivatives **35a**–**c**. Typically, *m*-AMSA analogues **10a**–**c** were obtained from **35a**–**c** by amine **36**[44] substitution at the 9-position under acidic conditions followed by treatment with a 2 N HCl/ether solution. There are two tautomeric forms of 9-substituted acridines,[45–47] but no isomers have been observed for 9-aminoacridine dihydrochloride salts **10a**–**c**.

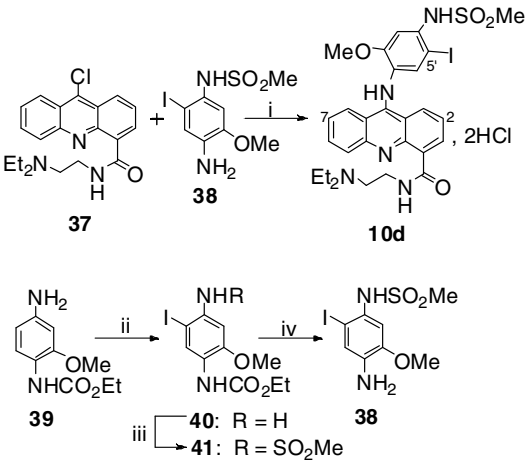
Preparation of *m*-AMSA derivative **10d** was performed with *N*-(2-diethylaminoethyl)-9-chloroacridine-4-carboxamide[48] (**37**) as previously reported, and iodoamino derivative **38** by a similar procedure to that used for compounds **10a–c** (Scheme 9). Intermediate derivative **38** was prepared from ethyl *N*-(2-methoxy-4-aminobenzene)carbamate[44] (**39**) using a three steps procedure. Monoiodinated aniline **40** was obtained in good yield by the reaction of benzyltriethylammonium dichloroiodate[49] with aniline **39** in the presence of calcium carbonate and methanol. Mesylation of the amino group afforded compound **41** and basic hydrolysis of the ethyl carbamate protecting group gave anilino compound **38** in good yield (89%). Regiospecific iodination reaction was confirmed by NMR studies (HSQC and HMBC ^1H–^{13}C NMR sequences performed on sulfamide **41**).



Scheme 7. Reagents and conditions: (i) a—4 N NaOH, MeOH, reflux; b—AcOH; (ii) a—$SOCl_2$, DMF, reflux; b—5% NEt_3/THF, $NH_2(CH_2)_2NEt_2$, rt; c—10% Na_2CO_3; (iii) BH_3–THF (1 M), THF, reflux; (iv) a—$FeCl_3$, H_2O, EtOH, 50 °C; (v) 2 N HCl, ether, rt.



Scheme 8. Reagents and conditions: (i) a—2 N NaOH, MeOH, reflux; b—AcOH; (ii) $SOCl_2$, DMF, reflux; (iii) a—CH_2Cl_2, $NH_2(CH_2)_2Et_2$, rt; b—10% Na_2CO_3; (iv) a—EtOH, HCl, reflux, b—2 N HCl, ether, rt.



Scheme 9. Reagents and conditions: (i) a—EtOH, HCl, reflux; b—2 N HCl, ether, rt; (ii) $BTEAlCl_2$, $CaCO_3$, CH_2Cl_2, MeOH; (iii) CH_3SO_2Cl, pyridine, 0 °C; (iv) 10% NaOH, reflux.

2.1.4. Acridine derivatives syntheses 11a–b

The dihydrochloride iodoacridine-9-carboxamide salts **11a**,**b** were prepared in three steps from methyl 9-acridinecarboxylate (**42**)[50] (Scheme 10). 2- and 2,7-diodoacridine **43a**,**b** (the more favourable position for electrophilic aromatic substitution) were directly synthesised from acridine **42** by the reaction with iodine and periodic acid dihydrate. The method used was the only one which gave a positive result. Regiospecific iodination was confirmed by NMR studies (^1H–^1H COSY, ^1H–^{13}C HSQC and ^1H–^{13}C HMBC NMR sequences) performed for monoiodinated compound **43a**. Coupling esters **43a**,**b** with *N*,*N*-diethylethylenediamine to give amides **44a**,**b** and the synthesis of dihydrochloride salts as reported previously for compounds **8** gave the expected amides **11a**,**b**.

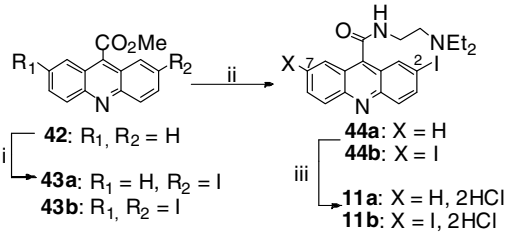
2.2. Cytotoxic activity

IC_{50} values were determined for compounds **8–11** in a panel of human cancer cell lines (M4Beu melanoma, Jurkat leukaemia and DLD-1 colon adenocarcinoma), a murine cell line (B16F0 melanoma) and human fibroblasts in primary culture, using amsacrine and DACA (**4**) as reference compounds. Table 1 gives the results for acridone and acridine derivatives (**8**, **9** and **11**) and Table 2 gives the results for amsacrine derivatives **10**.

The results reported in Table 1 highlighted variation of IC_{50} values depending on the position of iodo substituent. Substitution of iodine at the 3-position (**8c** and **9c**) decreased the cytotoxicity compared with parent compound **4** in all cell lines. *Ortho* substitution to the carboxamide function is likely to interfere with the side chain conformation, as was observed in the DACA series where 3-substitution led to a marked drop in DNA binding and cytotoxicity.[40] Analogues bearing a side chain at the 9-position (**11a**,**b**) were also less potent than DACA (**4**). In contrast, substitution of iodine at the 5-position (**9d**) led to markedly increased potency, especially on human melanoma cell line M4Beu, with a fivefold lower IC_{50} value than DACA. It should be underlined that such results have been shown with compounds of the DACA series.[51] For the other acridine compounds studied (**9**), there were no marked differences in effects compared with the parent compound. Among acridone analogues (**8**), all the compounds except **8c** exhibited a globally higher cytotoxicity than DACA on human melanoma but globally lower cytotoxicity on the other cell lines.

Our results (Table 2) showed that the compounds **10a**–**d** exhibited a significant in vitro cytotoxic activity, with IC_{50} values ranging from 0.61 to 3.5 μM on M4Beu cells. The most cytotoxic of these compounds was **10c**, which exhibited a similar effect to the amsacrine compound. However, all of these compounds showed lower activity was than amsacrine on Jurkat leukaemia, murine melanoma B16F0 and colon adenocarcinoma DLD-1 cell lines.

To conclude, from acridone derivatives **8**, which with the exception of **8c** expressed the same range of efficacy, we chose to select compound **8f** for several reasons. First, due to the chemical stability of its carbon-iodine bond comparatively to isomeric derivatives **8a**, **f** and **g** for which a deiodation took place in solution as previously observed by Denny[43] for 6- and 8-halo-4-carboxyacridones. Second, compound **8f** was obtained with better overall yield than **8d** and in few steps than **8b**. Based on their cytotoxic activity on human cell line M4Beu, the acridine compounds **9d** and **10c** were also selected as leads for further biological evaluation.



Scheme 10. Reagents and conditions: (i) HIO_4 dihydrate, I_2, AcOH, H_2O, H_2SO_4, reflux; (ii) $AlMe_3$, $NH_2(CH_2)_2NEt_2$, PhMe, 0 °C then reflux; (iii) 2 N HCl, ether, rt.

2.3. Radiolabelling of stannane compounds

The selected compounds **8f**, **9d** and **10c** were radioiodinated using electrophilic substitution methods with high specific activity, as depicted in Scheme 11. The iodo derivatives **24f**, **30d** and **48** (obtained from hydrochloride salt **10c**) were converted to the corresponding tributylstannane compounds **45**, **46** and **49** using a palladium(0)-catalysed cross-coupling reaction. It was noted that for compound **30d** a dehalogenation reaction took place and the desired stannane **46** was obtained with the by-product compound

Table 1
Cytotoxicity of compounds **8**, **9** and **11** in comparison to DACA (**4**)

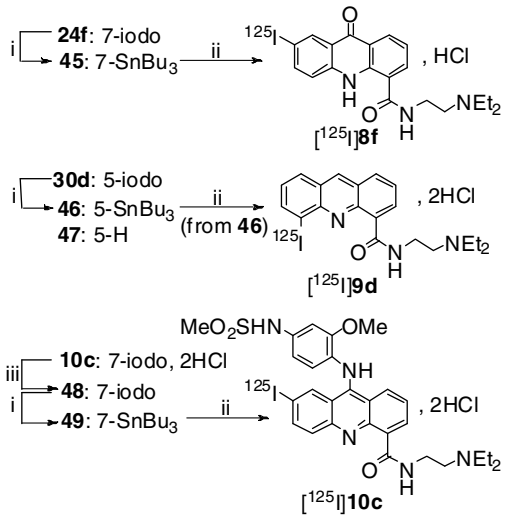
Compound	Iodine position	IC_{50}[a] (μM)				
		M4Beu[b]	B16F0[c]	DLD-1[d]	JURKAT[e]	Fibroblast[f]
DACA (**4**)		4.5 ± 0.6 (1.8 ± 0.2)	0.42 ± 0.3 (0.19 ± 0.1)	3.1 ± 0.3 (2.3 ± 1.6)	0.56 ± 0.2 (0.3 ± 0.1)	4.4 ± 0.4 (7.1 ± 0.2)
8a	1	2.9 ± 0.04 (3.9 ± 0.04)	2.2 ± 0.6 (1.8 ± 0.4)	2.6 ± 1.0 (2.3 ± 0.8)	0.80 ± 0.06 (1.2 ± 0.04)	2.3 ± 0.8 (2.4 ± 0.3)
8b	2	3.5 ± 1.4 (3.5 ± 1.0)	1.6 ± 0.4 (1.5 ± 0.1	3.1 ± 0.5 (2.2 ± 0.1)	1.5 ± 0.1 (1.4 ± 0.2)	3.7 ± 2.1 (1.8 ± 0.2)
8c	3	11 ± 1.6 (29 ± 4.5)	14 ± 5.9 (12 ± 5.4)	37 ± 5.1 (35 ± 7.4)	5.0 ± 1.9 (4.4 ± 0.4)	23 ± 8.7 (54 ± 2.1)
8d	5	5.4 ± 0.5 (3.9 ± 0.5)	3.6 ± 1.1 (3.0 ± 1.3)	7.0 ± 1.0 (4.1 ± 0.5)	1.8 ± 1.2 (2.6 ± 0.3)	4.2 ± 0.1 (3.6 ± 0.2)
8e	6	2.7 ± 0.3 (3.4 ± 0.4)	1.9 ± 0.8 (1.6 ± 0.6)	9.4 ± 0.2 (6.2 ± 0.1)	1.3 ± 0.1 (0.85 ± 0.1)	2.0 ± 1.0 (2.7 ± 1.4)
8f	7	4.7 ± 0.5 (3.8 ± 0.5)	4.1 ± 1.8 (3.7 ± 1.4)	5.4 ± 0.2 (3.9 ± 0.6)	2.8 ± 0.6 (0.88 ± 0.5)	4.2 ± 0.1 (3.1 ± 0.2)
8g	8	3.4 ± 0.4 (3.4 ± 0.3)	6.3 ± 0.5 (5.3 ± 0.1)	7.5 ± 0.3 (3.3 ± 2.7)	2.7 ± 1.3 (7.7 ± 0.5)	9.8 ± 0.2 (9.7 ± 0.2)
9a	1	1.5 ± 0.2 (2.4 ± 0.2)	1.4 ± 0.8 (1.1 ± 0.9)	4.4 ± 1.0 (2.3 ± 0.2)	1.1 ± 0.1 (0.9 ± 0.1)	2.0 ± 0.3 (2.2 ± 0.6)
9b	2	1.5 ± 0.1 (1.8 ± 0.2)	0.49 ± 0.2 (0.14 ± 0.1)	2.4 ± 0.5 (1.4 ± 0.2)	1.3 ± 0.2 (0.6 ± 0.1)	1.8 ± 0.1 (1.2 ± 0.05)
9c	3	52.9 ± 16.2 (52.0 ± 11.3)	39.3 ± 0.2 (22.5 ± 8.9)	79.2 ± 5.6 (47.6 ± 11.0)	22.9 ± 4.3 (32.3 ± 10.2)	27.1 ± 2.8 nd
9d	5	0.9 ± 0.05 (0.76 ± 0.2)	0.6 ± 0.2 (0.42 ± 0.2)	1.1 ± 0.4 (0.27 ± 0.1)	0.4 ± 0.1 (0.6 ± 0.1)	0.9 ± 0.1 (0.69 ± 0.6)
9e	6	2.4 ± 0.1 (3.6 ± 0.4)	1.8 ± 0.2 (1.4 ± 0.4)	2.2 ± 0.4 (1.8 ± 0.1)	1.2 ± 0.1 (1.4 ± 0.3)	2.3 ± 1.2 (2.1 ± 1.1)
9f	7	3.2 ± 0.3 (2.7 ± 0.3)	2.7 ± 0.8 (2.0 ± 0.3)	2.4 ± 0.4 (1.8 ± 0.4)	3.4 ± 1.4 (4.5 ± 0.5)	2.6 ± 0.1 (3.1 ± 0.2)
9g	8	2.1 ± 0.2 (3.0 ± 0.3)	1.9 ± 0.8 (1.1 ± 0.5)	4.5 ± 0.4 (2.2 ± 0.1)	1.7 ± 0.2 (1.1 ± 0.4)	2.5 ± 0.7 (2.8 ± 1.1)
11a	2	40 ± 16.3 (28 ± 5.1)	11 ± 0.9 (9.5 ± 1.4)	16 ± 0.9 (9.8 ± 0.5)	23 ± 5.4 (3.4 ± 3.1)	32 ± 11.8 nd
11b	2 and 7	5.9 ± 0.9 (5.6 ± 1.3)	20 ± 1.3 (21 ± 1.4)	5.3 ± 1.4 (4.3 ± 1.2)	6.3 ± 0.8 (7.5 ± 0.9)	6.7 ± 0.7 (3.9 ± 0.5)

[a] Results are expressed as IC_{50} obtained with the resazurin test with the Hoechst test in brackets. The values given are averages of at least two independent determinations.
[b] Human melanoma.
[c] Murine melanoma.
[d] Human colon adenocarcinoma.
[e] Human leukaemia.
[f] Normal human fibroblasts.

Table 2
Cytotoxicity of compounds **10** in comparison to amsacrine

Compound	Iodine position	IC_{50}[a] (μM)				
		M4Beu[b]	B16F0[c]	DLD-1[d]	JURKAT[e]	Fibroblast[f]
Amsacrine		0.74 ± 0.3 (0.40 ± 0.2)	0.08 ± 0.01 (0.04 ± 0.001)	0.57 ± 0.4 (0.08 ± 0.02)	0.08 ± 0.05 (0.02 ± 0.006)	4.2 ± 0.1 (2.0 ± 0.3)
10a	2	1.9 ± 1.1 (1.9 ± 0.9)	0.81 ± 0.2 (0.95 ± 0.4)	2.9 ± 1.4 (2.5 ± 0.6)	0.72 ± 0.1 (1.1 ± 0.1)	2.0 ± 1.0 (1.9 ± 1.6)
10b	5	3.5 ± 0.3 (3.0 ± 0.2)	0.58 ± 0.3 (0.42 ± 0.2)	3.0 ± 2.2 (1.7 ± 0.8)	0.24 ± 0.01 (0.15 ± 0.4)	1.2 ± 0.7 (0.83 ± 0.7)
10c	7	0.61 ± 0.1 (0.65 ± 0.1)	1.3 ± 0.5 (0.98 ± 0.3)	1.7 ± 1.1 (1.2 ± 0.6)	0.89 ± 0.3 (0.37 ± 0.01)	1.4 ± 0.5 (1.0 ± 0.4)
10d	5′	2.5 ± 0.2 (2.3 ± 0.2)	1.2 ± 0.5 (0.99 ± 0.4)	2.4 ± 0.3 (2.0 ± 0.2)	1.4 ± 0.1 (0.49 ± 0.5)	1.9 ± 0.2 (1.5 ± 0.2)

[a] Results are expressed as IC_{50} obtained with the rezazurin test with the Hoechst test in brackets. The values given are averages of at least two independent determinations.
[b] Human melanoma.
[c] Murine melanoma.
[d] Human colon adenocarcinoma.
[e] Human leukaemia.
[f] Normal human fibroblasts.



Scheme 11. Reagents and conditions: (i) $Pd(PPh_3)_4$, $(Bu_3Sn)_2$, PhMe, reflux; (ii) a—chloramine-T, [^{125}I]NaI, 1% AcOH/EtOH for **45** and **49** or citrate buffer solution for **46**; b—2 N HCl, ether, rt; (iii) saturated Na_2CO_3.

Table 3
HPLC data, radiochemical yields and purities for compounds [^{125}I]**8f**, [^{125}I]**9d** and [^{125}I]**10c**

Entry	CAT (mg/mL)	Retention time (min) [^{125}I]compounds	Retention time (min)Stannane compounds	Yield[a] (%)	Purity[b] (%)
1	0.4	Rt [^{125}I]**8f** = 9.8	Rt **45** = 17.8	57	99
2	0.5	Rt [^{125}I]**9d** = 8 11.9	Rt **46** = 17.3	66	99
3	0.25	Rt [^{125}I]**10c** = 9.9	Rt **49** = 18.5	53	97

[a] Radiochemical yields were calculated by dividing the radioactivity in the final HPLC product by the initial amount of radioactive sodium iodide.
[b] Radiochemical purities were determined by HPLC.

47 in 25% and 29% yields, respectively. The radioiodo-destannylation of compounds **45**, **46** and **49** with [^{125}I]NaI was performed using chloramine-T (CAT) as oxidant to give, after HPLC purification and conversion into hydrochloride salts, the desired [^{125}I]**8f**, [^{125}I]**9d** and [^{125}I]**10c** labelled compounds with high specific activity (81.4 TBq/mmol). The parameters such as amount of substrate, amount of oxidant and reaction time were all optimised for each reaction. The [^{125}I]-labelled compounds were obtained with good radiochemical conversion (53–66%) in a 10-min reaction time according to radio-TLC analysis. The radiochemical data of the three compounds [^{125}I]**8f**, [^{125}I]**9d** and [^{125}I]**10c** are summarised in Table 3.

2.4. In vitro binding to melanin and partition coefficients for compounds 8f, 9d and 10c

In our approach, the melanin affinity of the studied compounds had to be a consistent parameter. Synthesised (hetero)aromatic analogues **8f** and **9d** displayed the same strong affinity to synthetic melanin in water as BZA (see Table 4). When melanin was suspended in PBS, the melanin affinity of **8f** and **9d** was slightly less modified than with BZA. While this decrease in value for the parent compound BZA[52] is probably due to the ionic strength of PBS,[14] the lower variations in PBS for compounds **8f** and **9d**, with identical *N*-alkyl side chains to BZA, could result from a higher pi stacking interaction between the aromatic rings of melanin and acridine(one) derivatives, which was independent of both pH and ionic strength. In contrast, compound **10c** exhibited a weak melanin affinity that was highly dependent on the ionic charges. The lipophilicity values (log*P*) of the studied compounds (summarised in Table 4) were all lower than that obtained for BZA.

Table 4
In vitro binding to melanin and log*P* of **8f**, **9d** and **10c** derivatives compared to BZA

Compounds	% Bound to synthetic melanin H_2O/PBS	log*P*[a]
BZA[b]	78.6/13.8	1.34
8f	50.6/33.3	0.27
9d	69.2/56.8	0.8
10c	27.2/6.3	0.64

[a] P, partition coefficient between octanol and phosphate buffer solution (pH 7.4).
[b] BZA values were obtained from previous research.[52]

2.5. Biodistribution studies of compounds 8f, 9d and 10c

The three selected compounds also presented major differences in excretion (Table 5). Eliminations were almost complete in 72 h for **9d** with fairly equivalent rates for both urinary and faecal routes. Compounds **8f** and **10c** were mainly eliminated very slowly by faecal route, and after 72 h only 49% and 37% of radioactivity, respectively, had been excreted. The radioactivity present in one mouse 8 days after **8f** administration was still equal to 19% of the injected dose. This is an original pattern of kinetic behaviour compared with BZA and with the majority of benzamide analogues previously studied, which are mainly eliminated via the urinary tract and are almost completely eliminated at 72 h.

For two [^{125}I]-labelled compounds, **8f** and **9d**, tissue distribution evaluated in melanoma-bearing mice rapidly demonstrated a high tumoural activity, whereas there was no significant tumoural uptake for compound **10c**. This result was almost certainly related to the low affinity measured for synthetic melanin (Table 4). No melanoma targeting was observed and the weak tumoural uptake value remained stable throughout the study, while higher concentrations were measured in the liver and the elimination organs. This result is unexpected, given the high affinity for melanotic tumour tissue already showed with m-AMSA.[53]

With [^{125}I]**8f** and [^{125}I]**9d**, there was uptake in B16F0 melanoma graft cells from 1 h post-injection (pi) (>12.6% ID/g) which went on to peak at between 6 and 24 h and was still very high after 72 h. Tumoural concentrations of **9d** and **8f** at 72 h post-injection were 9- and 23-fold the BZA values, respectively. These values are suggestive of strong tumoural irradiation if used in doses geared to radionuclide therapy. The kinetic profile of compound **8f** justified the large amount of radioactivity present in the mouse body after a long delay.

Compared with our reference [^{125}I]BZA, the tumoural fixation values (% ID/g) of compounds [^{125}I]**8f** and [^{125}I]**9d** appeared not only significantly higher at each timepoint in the study, but also much more durable. While many organs and tissues concentrated the radioactivity at 1 h pi, a preferential affinity for the tumour compared with liver or lungs was observed from 3 h pi,

Table 5
Urinary and faecal excretions[a] of radioactivity following injection of [^{125}I]compounds **8f**, **9d** and **10c** in B16F0 melanoma-bearing mice compared to BZA

Compound	Urinary % ID 0–72 h	Faecal % ID 0–72 h
BZA[b]	83.1	4.8
[^{125}I]**8f**	10.4	38.4
[^{125}I]**9d**	52.8	46.1
[^{125}I]**10c**	4.2	33.2

[a] Cumulative excretions (two mice for each compound).
[b] BZA values were obtained from previous research.[52]

Table 6
Biodistribution of [^{125}I]compounds **8f**, **9d** and **10c** compared to BZA at various times after iv administration in B16F0 melanoma-bearing mice

Compound	Time (h)	Melanoma[a]	Brain[a]	IC[a,b]	SC[a,c]	Liver[a]	Muscle[a]	Lung[a]	Spleen[a]	Kidney[a]	Blood[a]	Uvea[a]
BZA[d]	1	9.5 ± 1.9	1.5 ± 0.3	7.4 ± 3.4	10.1 ± 3.2	5.9 ± 1.9	1.3 ± 0.3	5.5 ± 1.4	4.0 ± 0.7	7.3 ± 2.2	1.1 ± 0.3	13.1 ± 4.1
	3	9.1 ± 1.6	0.8[e]	3.7 ± 0.8	4.3 ± 1.5	3.3 ± 0.5	0.4 ± 0.2	2.0 ± 0.5	0.8 ± 0.3	3.1 ± 0.6	0.2 ± 0.2	12.0 ± 2.0
	6	7.7 ± 3.2		3.3 ± 2.2	4.5 ± 1.1	2.6 ± 0.5	0.1 ± 0.1	1.0 ± 0.4		1.6 ± 0.5		13.0 ± 4.5
	24	3.7 ± 1.2				0.4 ± 0.1						6.1 ± 2.5
	72	0.88 ± 0.3										3.8±0.9
8f	1	12.6 ± 2.8	1.6 ± 0.2	22.7 ± 19.6	23.1 ± 5.1	14.0 ± 82.8	1.9 ± 0.4	23.9 ± 2.8	13.9 ± 1.4	17.6 ± 2.3	2.08 ± 0.6	15.2 ± 12.3
	3	21.0 ± 8.8	1.2 ± 0.3	27.8 ± 10.9	13.4 ± 3.1	9.3 ± 0.6	1.4 ± 0.2	14.0 ± 2.7	9.9 ± 0.6	13.0 ± 2.0	1.4 ± 0.4	16.9 ± 6.2
	6	26.3 ± 8.6	0.8 ± 0.2	32.9 ± 8.8	24.4 ± 4.9	7.4 ± 0.7	0.9 ± 0.2	10.0 ± 1.5	7.2 ± 0.9	8.9 ± 1.4	1.1 ± 0.3	19.5 ± 2.1
	24	27.4 ± 8.3	0.1 ± 0.1	5.1 ± 2.8	3.1 ± 1.3	1.2±0.2	0.2[e]	0.7 ± 0.3	0.7 ± 0.3	0.7±0.4	0.2 ± 0.1	27.5 ± 7.1
	72	17.5 ± 2.7		0.3[e]		0.6 ± 0.2						26.4 ± 4.2
9d	1	18.8 ± 5.9	1.0 ± 0.4	155.7 ± 134.5	87.29 ± 55.9	17.9 ± 1.8	2.3 ± 0.5	9.6 ± 1.7	21.6 ± 4.6	16.8 ± 3.0	4.1 ± 0.9	20.0 ± 6.6
	3	21.4 ± 5.0	0.5 ± 0.2	177.1 ± 125.0	51.7 ± 23.04	11.3 ± 1.4	1.3 ± 0.5	6.8 ± 1.0	16.0 ± 11.5	8.2 ± 1.1	5.4 ± 0.7	20.1 ± 4.5
	6	23.6 ± 7.6	0.4 ± 0.1	109.2 ± 64.7	60.5 ± 3.6	7.6 ± 1.2	1.4 ± 0.6	6.2 ± 1.2	6.2 ± 0.6	6.4 ± 1.6	6.5 ± 1.9	16.2 ± 4.9
	24	13.8 ± 2.5		4.6 ± 1.9	3.5 ± 1.8	1.1 ± 0.2						14.4 ± 3.1
	72	6.9 ± 2.9										16.6 ± 5.3
10c	1	0.9 ± 0.4	0.1 ± 0.1	43.2 ± 34.2	10.5 ± 6.0	8.9 ± 1.0	0.8 ± 0.2	3.4±0.3	6.3 ± 1.0	13.8 ± 4.1	0.53 ± 0.1	1.8 ± 0.5
	3	1.5 ± 0.1	0.1[e]	57.7 ± 45.9	3.5 ± 0.9	4.9 ± 0.5	0.5 ± 0.1	2.9 ± 0.3	7.4 ± 5.0	8.6 ± 3.1	0.2 ± 0.1	1.4[e]
	6	1.2 ± 0.3		68.5 ± 45.3	3.8 ± 2.2	4.9 ± 0.3	0.4 ± 0.1	2.5 ± 0.3	4.1 ± 0.5	9.8 ± 4.5	0.2±0.1	1.4 ± 0.4
	24	1.2 ± 0.2		2.8 ± 1.0	1.2 ± 0.5	1.5 ± 0.2		0.7 ± 0.0	1.6 ± 0.3	3.6 ± 2.1		1.4 ± 0.3
	72	1.0 ± 0.4			0.9[e]	1.1 ± 0.1			0.7[e]	0.5 ± 0.2		2.1 ± 0.6

Absence of value: concentration in the organ equal to background value.
[a] Radioactive concentration values are expressed as means of % ID/g to ± SD (two mice, *n* determinations for each compound at each time point).
[b] IC, intestinal content.
[c] SC, stomach content.
[d] BZA values were obtained from previous research.[52]
[e] No standard deviation when only one determination was made per organ.

as illustrated in Table 6 and Figure 4. From 24 h p.i., only the tumour remained labelled, due to its selective affinity and the long-lasting binding, whereas the radioactivity had been eliminated from the other tissues.

Based on this in vivo data, melanoma targeting was confirmed for the compounds **8f** and **9d**, with favourable kinetic profiles for application in radionuclide therapy. The biological half life within the tumour, calculated using MIRD software was much higher with **8f** and **9d,** at 269 h and 43 h, respectively, than the 19.6 h for BZA. The kinetic profile of **8f** in one mouse is illustrated by the scintigraphic imaging reported in Figure 5, showing the specific long-lasting affinity for the tumour and the rapid clearance from non-target organs.

3. Conclusion

We synthesised 20 novel iodo-acridone or iodoa-cridine–carboxamide compounds designed by pharmacomodulation of known anti-tumoural agents. Three of these compounds were selected for their in vitro cytotoxicity, which was close to the parent molecules, and for their chemical stability. Their in vivo distribution profiles from studies performed on murine B16F0 melanoma-bearing mice after ^{125}I-radiolabelling showed: (1) that the 7-iodo amsacrine **10c** derivative displayed a lack of affinity for melanoma tumour; (2) that the 7-iodo-acridone **8f** and the 5-iodo acridine **9d** derivatives possesses a specific and durable in vivo affinity for melanoma, with for **8f**, a 14-fold longer biological half life within the tumour than BZA that was compatible with clinical application in targeted radionuclide therapy. The next step will be to study the individual in vivo anti-tumoural effects, the chemotoxicity of the stable compound, the radiotoxicity of the labelled compound, and the association of these two factors with a view to designing a bi-modal treatment using the same, single molecule.

4. Experimental

4.1. Chemistry

Column chromatography was performed with Merck neutral aluminium oxide 90 standardised (63–200 μm) or silica gel A normal phase (35–70 μm). Thin layer chromatography was performed on Merck neutral aluminium oxide 60 F_{254} plates or Merck silica gel 60 F_{254} plates. The plates were visualised with UV light (254 nm). Melting points were determined on an electrothermal IA9300 (capillary) or a Reichert-Jung-Koffler apparatus and were not corrected. NMR spectra (400 or 200 MHz for ^{1}H or 100 or 50 MHz for ^{13}C) were recorded on a Bruker Avance 400 or Bruker AM 200 instruments using $CDCl_3$, CD_3OD or DMSO-d_6 as solvent. Infrared spectra were recorded in KBr pellets or in CCl_4 on a FTIR Nicolet impact 410 or an FT vector 22 instrument (ν expressed in cm^{-1}). Mass spectra (MS) were obtained in electron impact mode on 5989A instruments (Agilent Technologies). Electrospray ionization mass spectra (ESI-MS) were obtained on an Esquire-LC spectrometer (Bruker Daltonics). The samples were analysed in CH_3OH/H_2O (1:1, containing 1% HCOOH) at a final concentration of 2–10 pmol/μL. Each ESI-MS spectrum was recorded by averaging 10 spectra. For organostannane compounds, theoretical masses were calculated based on the mass of the most abundant isotope (^{120}Sn, isotopic abundance, 32.59%). Microanalyses were performed by the Analytical Laboratory of the CNRS (Vernaison, France) for the elements indicated and were within 0.4% of the theoretical values unless indicated. All air-sensitive reactions were run under argon atmosphere. All solvents were dried using common techniques.[54]

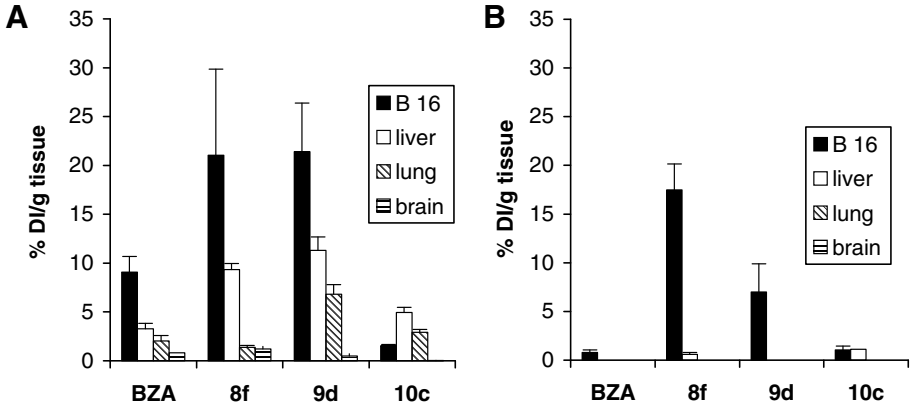


Figure 4. Concentrations of compounds [^{125}I]**8f**, [^{125}I]**9d** and [^{125}I]**10c** compared with BZA in selected tissues after iv administration in B16F0 melanoma-bearing mice. (A) At 3 h. (B) At 72 h. Mean ± SD (two mice, *n* determinations for each compound at each timepoint). BZA values were obtained from previous research.[50]

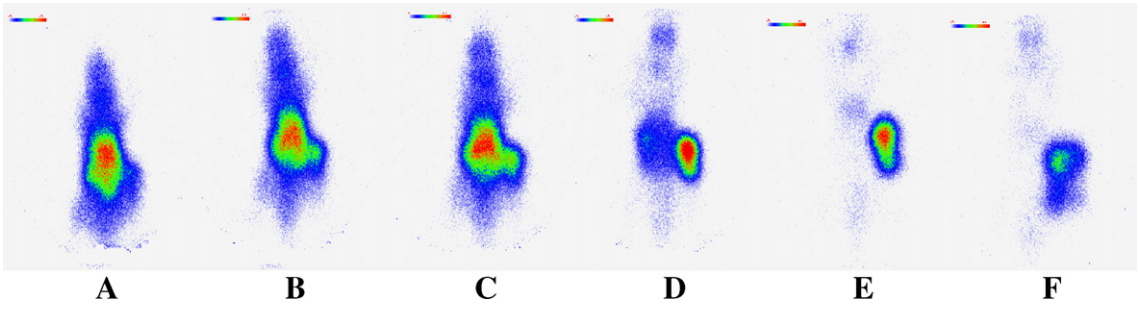


Figure 5. In vivo kinetics of compound [^{125}I]**8f** in a B16F0 melanoma-bearing mouse illustrated by repeated planar scintigraphic images using a γ imager dedicated to small animal imaging, at 1 h (A), 3 h (B), 6 h (C), 24 h (D), 72 h (E) and **8d** (F) after iv injection of a 3 MBq dose (acquisition time: 10 min).

4.1.1. 4-Iodoanthranilic acid[54] (13a)

To a mechanically stirred solution of 6-iodoisatin[34] (**12a**) (1.01 g, 3.70 mmol) in 1.5 N sodium hydroxide solution (8.6 mL) heated at 50 °C, was added an aqueous 30% hydrogen peroxide solution (0.9 mL) (kept temperature below 65 °C). After standing overnight at room temperature, the reaction mixture was carefully neutralised with an aqueous 12 N hydrochloric acid solution, treated with charcoal, and filtered through a pad of Celite® 545. The volume was reduced to 7 mL under vacuum and acidified to pH 4 with an aqueous 6.5 M hydrochloric acid solution. The resulting precipitate was collected by filtration, taken in dry ethanol (50 mL) and dried under vacuum. Yield 84%; mp 204–206 °C (lit.[55] 214 °C).

4.1.2. Methyl 4-iodoanthranilate[32] (14a)

A solution of 4-iodoantranilic acid (**13a**) (0.50 g, 1.90 mmol) in anhydrous methanol (5 mL) was stirred, at room temperature, under argon gas, for 10 min. An anhydrous 17% HCl/MeOH solution (5 mL) was added and heated gently under reflux for 21 h. After cooling to room temperature, the reaction mixture was made alkaline with a saturated aqueous sodium carbonate solution (30 mL) and extracted with dichloromethane (3× 50 mL). The organic layers were dried ($MgSO_4$), filtered and concentrated under vacuum. The product was used without purification. Yield 40%; mp 71–73 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.84 (s, 3H), 6.98 (dd, 1H, J = 8.5, 1.5 Hz), 7.10 (d, 1H, J = 1.5 Hz), 7.50 (d, 1H, J = 8.5 Hz).

4.1.3. Methyl 6-iodoanthranilate[33] (14c)

6-Iodoanthranilic acid[35] (**13b**) (4.50 g, 17.1 mmol) was dissolved in dry dimethylformamide (10 mL), sodium hydride (1.03 g, 25.67 mmol, 60% in mineral oil) was added, and the mixture was stirred for 30 min. Methyl iodide (1.60 mL, 25.7 mmol) was then added and the reaction mixture was stirred for 19 h at room temperature. The solvent was evaporated and water was added (50 mL). The reaction mixture was made alkaline to pH 8 with an aqueous saturated sodium carbonate solution and extracted with ethyl acetate (3× 80 mL). The organic layers were dried ($MgSO_4$), filtered and concentrated under vacuum. The crude product was chromatographed (SiO_2 and CH_2Cl_2), to give in order of elution: *methyl N-methyl-6-iodoanthranilate* (***15***); yield 13%; R_f = 0.73 (SiO_2 and CH_2Cl_2); mp 48–50 °C; IR (KBr) 3413, 2947, 1703, 1588, 1562, 1504, 1259 cm^{-1}; MS *m/z* 291 (M^+, 100), 259 (74), 231 (73), 104 (100), 77 (84); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.82 (s, 3 H), 3.92 (s, 3H), 6.00 (m, 1H), 6.66 (d, 1H, J = 8 Hz), 6.94 (t, 1H, J = 8 Hz), 7.24 (d, 1H, J = 8 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 30.2, 51.8, 94.8, 110.6, 120.2, 128.4, 132.9, 149.7, 168.6. *Methyl 6-iodoanthranilate*[33] (***14c***) as an oil; yield 44%; R_f = 0.51 (SiO_2 and dichloromethane); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.08 (s, 3H), 6.80 (d, 1H, J = 8 Hz), 6.98 (t, 1H, J = 8 Hz), 7.43 (d, 1H, J = 8 Hz).

4.1.4. General procedure for the synthesis of 2-(iodo-2′-methoxycarbonylphenylamino)benzoic acids 17a–c

A mixture of methyl iodoanthranilate **14a–c** (0.50 g, 1.80 mmol) diphenyliodonium-2-carboxylate[36] (**16**) (0.88 g, 2.72 mmol), and copper(II) acetate monohydrate (12 mg) was suspended in dimethylformamide (30 mL) and heated at 90–100 °C for 12 h. The solvent was removed under vacuum and the oily residue was dissolved in ethyl acetate (18 mL). This solution was washed with an aqueous 0.1 N hydrochloric acid solution (18 mL). The organic layers were extracted with an aqueous 0.1 N ammonia solution (2× 9 mL) and the aqueous extract was acidified to pH 6 with an aqueous

0.1 N hydrochloric acid solution. The precipitated product was filtered and washed well with hot water (5 mL). The precipitate was taken in dry ethanol (50 mL) and was dried under vacuum.

4.1.4.1. 2-(5′-Iodo-2′-methoxycarbonylphenylamino)benzoic acid (17a). From **14a**.[32] Yield 80%; mp 206–208 °C; IR (KBr) 3600–3000, 1685, 1575, 1254, 1220 cm^{-1}; MS *m/z* 397 (M^+, 100), 321 (88), 194 (59), 166 (95), 139 (62), 63 (77), 55 (61); ^1H NMR (DMSO-d_6, 400 MHz) 3.83 (s, 3H), 7.04 (t, 1H, *J* = 7.5 Hz), 7.30 (d, 1H, *J* = 8.5 Hz), 7.47 (m, 2H), 7.62 (d, 1H, *J* = 8.5 Hz), 7.74 (s, 1H), 7.92 (d, 1H, *J* = 7.5 Hz), 10.70 (s, 1H), 13.20 (m, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 52.1, 101.9, 115.6, 118.2 (2C), 121.1, 125.3, 128.5, 131.8, 132.8, 133.5, 142.4, 144.4, 166.5, 168.3.

4.1.4.2. 2-(4′-Iodo-2′-methoxycarbonylphenylamino)benzoic acid (17b). From **14b**.[31] Yield 59%; mp 230–232 °C; IR (KBr) 3200–2800, 1719, 1683, 1577, 1508, 1269, 1209 cm^{-1}; MS *m/z* 397 (M^+, 100), 320 (67), 194 (54), 166 (77), 139 (59), 63 (72); ^1H NMR (DMSO-d_6, 400 MHz) δ 3.86 (s, 3H), 7.00 (m, 1H), 7.33 (d, 1H, *J* = 9 Hz), 7.46 (m, 2H), 7.74 (d, 1H, *J* = 9 Hz), 7.93 (d, 1H, *J* = 8 Hz), 8.14 (s, 1H), 10.78 (s, 1H), 13.21 (m, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 52.2, 81.5, 117.8, 118.0, 118.7, 119.9, 120.7, 131.7, 133.4, 139.2, 141.8, 142.7, 143.1, 165.5, 168.4.

4.1.4.3. 2-(3′-Iodo-2′-methoxycarbonylphenylamino)benzoic acid (17c). From **14c**.[33] Yield 67%; mp 191–193 °C; IR (KBr) 3318, 3200–2700, 1740, 1657, 1575, 1444, 1269, 1247 cm^{-1}; MS *m/z* 397 (M^+, 73), 321 (100), 194 (36), 166 (41), 139 (18); ^1H NMR (DMSO-d_6, 200 MHz) δ 3.85 (s, 3 H), 6.85 (t, 1H, *J* = 8 Hz), 7.18 (m, 2H), 7.40 (t, 1H, *J* = 8 Hz), 7.50 (d, 1H, *J* = 8 Hz), 7.59 (d, 1H, *J* = 8 Hz), 7.90 (d, 1H, *J* = 8 Hz), 9.93 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 52.6, 93.6, 113.6, 114.4, 118.7, 121.1, 131.8, 131.9, 133.0, 133.4, 134.2, 139.0, 145.8, 167.6, 169.9.

4.1.5. General procedure for the synthesis of methyl 9,10-dihydro-iodo-9-oxoacridine-4-carboxylates 18a–c

2-(Iodo-2′-methoxycarbonylphenylamino)benzoic acids **17a–c** (1.00 g, 2.52 mmol) was stirred in concentrated sulphuric acid (2 mL) at 80 °C for 30 min. The cooled mixture was diluted with water (15 mL) and the resulting precipitate was collected and well washed with water (4 mL). The solid was taken in ethanol (50 mL) and dried under vacuum. The crude product was chromatographed (SiO_2, CH_2Cl_2/EtOH, 99.5:0.5, v/v).

4.1.5.1. Methyl 9,10-dihydro-1-iodo-9-oxoacridine-4-carboxylate (18a). From **17a**. Yield 85%; R_f = 0.65 (SiO_2, CH_2Cl_2/EtOH, 99.5:0.5, v/v); mp 189–191 °C; IR (KBr) 3217, 1685, 1640, 1613, 1508, 1273 cm^{-1}; MS *m/z* 379 (M^+, 71), 347 (81), 192 (24), 164 (100), 127 (24), 88 (24), 76 (24), 63 (24); ^{1} H NMR ($CDCl_3$, 400 MHz) δ 4.01 (s, 3 H), 7.29 (m, 2H), 7.67 (t, 1H, *J* = 8.5 Hz), 7.91 (m, 2H), 8.42 (d, 1H, *J* = 8.5 Hz), 12.07 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 52.8, 102.4, 113.4, 117.3, 119.8, 121.4, 122.9, 127.5, 134.1, 135.1, 135.3, 138.7, 142.4, 168.6, 176.2.

4.1.5.2. Methyl 9,10-dihydro-2-iodo-9-oxoacridine-4-carboxylate (18b) and methyl 9,10-dihydro-9-oxoacridine-4-carboxylate (19).[30] From **17b** afforded in order of elution: compound **18b**. Yield 85%; R_f = 0.41 (SiO_2, CH_2Cl_2/EtOH, 99.5:0.5, v/v); mp 275–277 °C; IR (KBr) 3262, 1694, 1508, 1430, 1282 cm^{-1}; MS *m/z* 379 (M^+, 100), 347 (52), 164 (30); ^1H NMR ($CDCl_3$, 200 MHz) δ 4.03 (s, 3H), 7.32 (t, 1H, *J* = 8.5 Hz), 7.38 (d, 1H, *J* = 8.5 Hz), 7.70 (t, 1 H, *J* = 8.5 Hz), 8.42 (d, 1H, *J* = 8.5 Hz), 8.65 (d, 1H, *J* = 2 Hz), 8.99 (d, 1H, *J* = 2 Hz), 11.65 (br s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 52.9, 81.9, 115.6, 117.8, 121.7, 122.8, 124.2, 127.3, 134.4, 139.9, 140.9, 142.4, 144.5, 167.3, 176.5. Compound **19**. Yield 8%; R_f = 0.21 (SiO_2, CH_2Cl_2/EtOH, 99.5:0.5, v/v); mp 170–172 °C (lit.[30] 172 °C).

4.1.5.3. Methyl 9,10-dihydro-3-iodo-9-oxoacridine-4-carboxylate (18c). From **17c**. Yield 65%; R_f = 0.41 (SiO_2, CH_2Cl_2/EtOH, 99.5:0.5, v/v); mp 202–204 °C; IR (KBr) 3600–3000, 1617, 1583, 1431, 1281 cm^{-1}; MS *m/z* 379 (M^+, 60), 347 (100), 164 (54), 139 (25), 63 (29); ^1H NMR ($CDCl_3$, 200 MHz) δ 4.07 (s, 3H), 7.31 (m, 2H), 7.70 (t, 1H, *J* = 7 Hz), 7.88 (d, 1H, *J* = 8.5 Hz), 8.23 (d, 1H, *J* = 8.5 Hz), 8.41 (d, 1H, *J* = 8 Hz), 10.56 (br s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 53.3, 102.0, 118.1, 120.4, 120.5, 122.2, 125.8, 127.8, 128.9, 131.1, 134.1, 137.4, 140.7, 167.0, 176.1.

4.1.6. General procedure for the synthesis of 2-(iodophenylamino)isophthalic acids 22a–c

A mixture of 2-iodoisophthalic acid[38] (**21**) (6.00 g, 20.5 mmol), commercial iodoaniline (**20a–c**) (6.74 g, 30.8 mmol), copper (I) chloride (2.03 g, 20.5 mmol), dry 2,3-butanediol (25 mL) and dry benzene (20 mL) was heated and stirred until the benzene being allowed to boil off. When the internal temperature reached 100 °C, distilled *N*-ethylmorpholine (5.88 mL, 45.9 mmol) was added, and the mixture was stirred at 120 °C for 4 h before being diluted with an aqueous 0.5 M ammonia solution (100 mL) and treated with charcoal (6 g). After filtration through Celite® 521 (washing with water (2× 50 mL)), the dark coloured solution was acidified with an aqueous 2 N hydrochloric acid solution (80 mL) and extracted with ethyl acetate (2× 100 mL). An insoluble inorganic precipitate was removed by filtration through Celite® 521 and the organic layers were extracted with an aqueous 0.5 M ammonia solution (2× 100 mL). The aqueous extract was acidified with concentrated hydrochloric acid and concentrated to 100 mL under *vacuum*. The product was collected by filtration and washed with hot water (15 mL). The precipitate was taken in dry ethanol (50 mL) and was dried under vacuum.

4.1.6.1. 2-(2′-Iodophenylamino)isophthalic acid[38] (22a). From **20a**. Yield 36%; mp 225–227 °C (lit.[38] 235–238 °C).

4.1.6.2. 2-(3′-Iodophenylamino)isophthalic acid (22b). From **20b**. Yield 26%; mp 214–216 °C; IR (KBr) 3200–2800, 1693, 1665, 1584, 1432, 1249 cm^{-1}; MS *m/z* 383 (M^+, 100), 321 (38), 194 (48), 166 (53), 139 (21); ^1H NMR (DMSO-d_6, 400 MHz) δ 6.89 (d, 1 H, *J* = 8 Hz), 6.98 (t, 1H, *J* = 8 Hz), 7.08 (t, 1 H, *J* = 8 Hz), 7.21 (d, 1H, *J* = 8 Hz), 7.25 (s, 1H), 7.95 (d, 2H, *J* = 8 Hz), 9.54 (br s, 1H), 13.10 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 95.1, 116.4, 120.2, 122.0 (2C), 125.2, 129.6, 130.9, 135.0 (2C), 142.8, 145.5, 168.4 (2C).

4.1.6.3. 2-(4′-Iodophenylamino)isophthalic acid (22c). From **20c**. Yield 62%; mp 241–243 °C; IR (KBr) 3200–2800, 1689, 1592, 1505, 1408, 1243 cm^{-1}; MS *m/z* 383 (M^+, 100), 194 (86), 166 (35), 139 (22); ^1H NMR (DMSO-d_6, 200 MHz) δ 6.75 (d, 2H, *J* = 9 Hz), 7.04 (t, 1H, *J* = 8 Hz), 7.49 (d, 2H, *J* = 9 Hz), 7.94 (d, 2H, *J* = 8 Hz), 9.60 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 83.8, 119.7 (2C), 119.8, 121.4 (2C), 135.1 (2C), 137.4 (2C), 143.3, 143.9, 168.5 (2C).

4.1.7. General procedure for the synthesis of 9,10-dihydro-iodo-9-oxoacridine-4-carboxylic acids 23a–d

2-(iodophenylamino)isophthalic acid **22a–c** (0.89 g, 23.2 mmol) was dissolved in polyphosphoric acid (PPA) (26 g) at 120 °C, and the resulting solution was stirred at this temperature for 2 h and then poured into boiling water (86 mL). The yellow precipitate was collected by filtration and dissolved in a mixture of methanol (70 mL) and an aqueous 1 N sodium hydroxide solution (70 mL). The hot solution (60 °C) was filtered, acidified with glacial acetic acid (pH 5), concentrated, and cooled to 0 °C. The resulting precipitate was collected by filtration and washed with water. The pure

product was finally obtained as yellow solid after recrystallization from methanol.

4.1.7.1. 9,10-Dihydro-5-iodo-9-oxoacridine-4-carboxylic acid[38] (23a). From **22a**. [38] Yield 77%; mp > 400 °C (lit.[38] > 360 °C).

4.1.7.2. 9,10-Dihydro-7-iodo-9-oxoacridine-4-carboxylic acid (23c). From **22c**. Yield 63%; mp 355–357 °C; IR (KBr) 3200–2800, 1693, 1609, 1518, 1446, 1148 cm^{-1}; MS *m/z* 365 (M^+, 95), 347 (100), 319 (23), 164 (33); ^1H NMR (DMSO-d_6, 200 MHz) δ 7.25 (t, 1H, *J* = 8 Hz), 7.46 (d, 1H, *J* = 9 Hz), 7.86 (d, 1H, *J* = 9 Hz), 8.34 (m, 3H), 11.93 (br s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 85.8, 115.9, 120.4, 121.0, 121.4, 122.2, 131.9, 134.0, 136.9, 139.0, 140.9, 141.6, 169.0, 175.1.

4.1.7.3. 9,10-Dihydro-6-iodo-9-oxoacridine-4-carboxylic acid (23b) and 9,10-dihydro-8-iodo-9-oxoacridine-4-carboxylic acid (23d). From **22b** which gave a mixture of acid **23b** and **23d** in ratio of 1:1 observed by ^1H NMR spectroscopy.

4.1.8. General procedure for the synthesis of methyl 9,10-dihydro-iodo-9-oxoacridine-4-carboxylates 18d–g

Method A: A sample of the appropriate acid **23** (1.00 g, 27.4 mmol) was warmed in thionyl chloride (30 mL) in the presence of three drops of dry dimethylformamide until total dissolution. The solution was maintained at the boiling temperature of thionyl chloride for 15 min. The solvent was evaporated under reduced pressure and the residue was suspended in dry toluene (20 mL). After evaporation to dryness, the product was dissolved in dry methanol (30 mL) and stirred at room temperature with calcium carbonate (1.20 g) for 3 h. The excess of calcium carbonate was filtered off and the methanol was evaporated under reduce pressure. An aqueous 1 N hydrochloric acid solution (25 mL) was added to the residue and the mixture was extracted with dichloromethane (3× 50 mL). The organic layers were dried (Na_2SO_4), concentrated under vacuum and the crude product was chromatographed (Al_2O_3 and CH_2Cl_2).

Method B: A mixture of acids **23b** and **23d** (95.0 mg, 0.26 mmol) was dissolved in dry dimethylformamide (5 mL), sodium hydride (10.4 mg, 0.26 mmol, 60% in mineral oil) was added, and the mixture was stirred for 30 min. Methyl iodide (16.2 μL, 0.26 mmol) was added and the reaction mixture was stirred for 18 h at room temperature. The solvent was evaporated and water (10 mL) was added. The resulting solution was made alkaline with an aqueous saturated sodium carbonate solution and extracted with ethyl acetate (3× 20 mL). The organic layers were dried ($MgSO_4$), filtered and concentrated under vacuum. The crude product was chromatographed (SiO_2 and CH_2Cl_2).

4.1.8.1. Methyl 9,10-dihydro-5-iodo-9-oxoacridine-4-carboxylate (18d). Method A, from **23a**.[38] Yield 57%; R_f = 0.78 (Al_2O_3 and CH_2Cl_2); mp 174–176 °C; IR (KBr) 3197, 1608, 1518, 1430, 1279, 1141 cm^{-1}; MS *m/z* 379 (M^+, 80), 347 (100), 164 (99), 75 (71), 63 (40); ^1H NMR ($CDCl_3$, 400 MHz) δ 4.07 (s, 3H), 7.06 (t, 1H, *J* = 7.5 Hz), 7.30 (t, 1H, *J* = 8 Hz), 8.17 (d, 1H, *J* = 7.5 Hz), 8.46 (m, 2 H), 8.69 (d, 1H, *J* = 8 Hz), 12.11 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 52.9, 86.2, 114.3, 120.8, 121.9, 122.5, 123.6, 127.7, 133.8, 137.0, 140.7, 141.5, 144.0, 168.0, 177.6.

4.1.8.2. Methyl 9,10-dihydro-7-iodo-9-oxoacridine-4-carboxylate (18f). Method A, from **23c**. Yield 60%; R_f = 0.85 (Al_2O_3 and CH_2Cl_2); mp 224–226 °C; IR (KBr) 3247, 1692, 1614, 1589, 1518, 1440, 1137 cm^{-1}; MS *m/z* 379 (M^+, 91), 347 (100), 220 (29), 164 (94), 75 (44), 63 (34); ^1H NMR ($CDCl_3$, 200 MHz) δ 4.01 (s, 3H), 7.14 (d, 1H, *J* = 8.5 Hz), 7.25 (t, 1H, *J* = 8 Hz), 7.89 (dd, 1H, *J* = 8.5, 2 Hz), 8.41 (d, 1H, *J* = 8 Hz), 8.67 (d, 1H, *J* = 8 Hz), 8.72 (d, 1H, *J* = 2 Hz), 11.76 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 52.7, 85.4, 113.9, 119.6, 120.4, 122.5, 123.2, 134.0, 136.0, 136.9, 139.3, 141.6, 142.3, 168.4, 176.5.

4.1.8.3. Methyl 9,10-dihydro-6-iodo-9-oxoacridine-4-carboxylate (18e) and methyl 9,10-dihydro-8-iodo-9-oxoacridine-4-carboxylate (18g). Method B, from a mixture of 23 b and **23d** afforded in order of elution: compound **18g**; yield 51%; R_f = 0.58 (SiO_2 and CH_2Cl_2); mp 239–241 °C; IR (KBr) 2961, 2926, 1692, 1611, 1592, 1261 cm^{-1}; MS *m/z* 379 (M^+, 79), 347 (82), 220 (28), 192 (34), 164 (100), 75 (43), 63 (33); ^1H NMR ($CDCl_3$, 400 MHz) δ 4.01 (s, 3H), 7.23 (m, 2H), 7.34 (d, 1H, *J* = 8 Hz), 7.92 (d, 1H, *J* = 7.5 Hz), 8.37 (d, 1H, *J* = 7 Hz), 8.67 (d, 1H, *J* = 7.5 Hz), 11.64 (br s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 52.6, 92.5, 113.3, 118.6, 119.0, 120.5, 122.3, 133.8, 134.4, 136.6, 137.2, 140.7, 141.3, 168.4, 176.1. Compound **18e**; yield 32%; R_f = 0.38 (SiO_2 and CH_2Cl_2); mp 258–260 °C; IR (KBr) 3271, 1685, 1640, 1609, 1589, 1280 cm^{-1}; MS *m/z* 379 (M^+, 95), 347 (100), 220 (32), 192 (29), 164 (99), 137 (26), 75 (58), 63 (42); ^1H NMR ($CDCl_3$, 200 MHz) δ 4.03 (s, 3H), 7.30 (t, 1H, *J* = 8 Hz), 7.61 (d, 1H, *J* = 8.5 Hz), 7.85 (s, 1H), 8.13 (d, 1H, *J* = 8.5 Hz), 8.46 (d, 1H, *J* = 7.5 Hz), 8.71 (d, 1H, *J* = 7.5 Hz), 11.77 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 52.7, 101.5, 120.5, 120.8, 121.3, 122.7, 126.4, 128.5, 131.6, 134.0, 136.9, 140.7, 141.7, 168.5, 174.6.

4.1.9. General procedure for the synthesis of *N*-(2-diethylaminoethyl)-9,10-dihydro-iodo-9-oxoacridine-4-carboxamides 24a–g

Diethylethylenediamine (77 μL, 0.54 mmol) and trimethylaluminium (2.0 M solution in hexanes) (0.36 mL, 0.72 mmol) were added dropwise, at 0 °C, to a stirred solution of dry dichloromethane or dry toluene (10 mL). After 10 min, the appropriate iodo compound **18** (0.15 g, 0.40 mmol) in dry dichloromethane solution (2 mL) or dry toluene solution (4 mL) was added and the mixture was refluxed. After cooling to room temperature, water (15 mL) was added and the mixture was made alkaline with a saturated aqueous sodium carbonate solution (20 mL) and extracted with dichloromethane (3× 30 mL). The organic layers were dried (Na_2SO_4) and concentrated under vacuum. The crude product was chromatographed (Al_2O_3, CH_2Cl_2/EtOH, 99:1, v/v).

4.1.9.1. *N*-(2-Diethylaminoethyl)-9,10-dihydro-1-iodo-9-oxoacridine-4-carboxamide (24a). From **18a**. The mixture was refluxed in dry dichloromethane for 5 h. Yield 64%; R_f = 0.40 (Al_2O_3, CH_2Cl_2/EtOH, 99:1, v/v); mp 248–250 °C; IR (KBr) 3281, 1615, 1513 cm^{-1}; MS *m/z* 463 (M^+, 1), 86 (100), 58 (16); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.04 (t, 6H, *J* = 6.5 Hz), 2.60 (q, 4H, *J* = 6.5 Hz), 2.70 (m, 2H); 3.48 (m, 2H), 7.19 (t, 1H, *J* = 7 Hz), 7.25 (d, 1H, *J* = 8.5 Hz), 7.36 (d, 1H, *J* = 8 Hz), 7.57 (m, 2H), 7.81 (d, 1H, *J* = 8 Hz), 8.34 (d, 1H, *J* = 8 Hz), 12.75 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.7 (2C), 37.1, 46.9 (2C), 51.2, 99.0, 117.5 (2C), 119.9, 121.3, 122.5, 127.4, 130.9, 134.0, 134.8, 139.1, 142.1, 168.4, 176.5.

4.1.9.2. *N*-(2-Diethylaminoethyl)-9,10-dihydro-2-iodo-9-oxoacridine-4-carboxamide (24b). From **18b**. The mixture was refluxed in dry toluene for 1.5 h. Yield 88%; R_f = 0.43 (Al_2O_3, CH_2Cl_2/EtOH, 99:1, v/v); mp 250–252 °C; IR (KBr) 3422, 1617, 1512 cm^{-1}; MS *m/z* 463 (M^+, 6), 348 (18), 320 (14), 193 (11), 164 (11), 86 (100), 58 (23); ^1H NMR ($CDCl_3$, 200 MHz) δ 1.08 (t, 6H, *J* = 7 Hz), 2.67 (q, 4H, *J* = 7 Hz), 2.75 (t, 2H, *J* = 6 Hz), 3.56 (m, 2H), 7.21 (t, 1H, *J* = 8 Hz), 7.30 (d, 1H, *J* = 8 Hz), 7.61 (t, 1H, *J* = 8 Hz), 7.65 (br s, 1H), 8.08 (d, 1H, *J* = 2 Hz), 8.32 (d, 1H, *J* = 8 Hz), 8.75 (d, 1H, *J* = 2 Hz), 12.15 (br s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.8 (2C), 37.4, 46.9 (2C), 51.2, 82.0, 117.9, 120.0, 121.2, 122.3, 124.0, 126.8, 134.0, 139.7, 139.8, 140.0 (2C), 166.9, 176.5.

4.1.9.3. *N*-(2-Diethylaminoethyl)-9,10-dihydro-3-iodo-9-oxoacridine-4-carboxamide (24c). From **18c**. The mixture was refluxed in dry dichloromethane for 6 h. Yield 38%; R_f = 0.40 (Al_2O_3, CH_2Cl_2/EtOH, 99:1, v/v); mp 215–217 °C; IR (KBr) 3230–2960, 1614, 1556 cm^{-1}; MS *m/z* 463 (M^+, 1), 86 (100), 58 (12); ^1H NMR ($CDCl_3$, 200 MHz) δ 1.49 (t, 6H, *J* = 7 Hz), 3.37 (m, 4H), 3.52 (m, 2H), 4.08 (m, 2H), 7.00 (d, 1H, *J* = 8 Hz), 7.19 (t, 1H, *J* = 8 Hz), 7.59 (m, 3H), 8.21 (d, 1H, *J* = 8 Hz), 8.77 (br s, 1H), 10.09 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 10.8 (2C), 39.5, 46.8 (2C), 50.3, 102.3, 118.2, 120.3, 120.4, 121.9, 125.7, 127.6, 130.9, 132.0, 133.9, 137.2, 140.9, 166.5, 176.3.

4.1.9.4. *N*-(2-Diethylaminoethyl)-9,10-dihydro-5-iodo-9-oxoacridine-4-carboxamide (24d). From **18d**. The mixture was refluxed in dry toluene for 2 h. Yield 73%; R_f = 0.23 (Al_2O_3, CH_2Cl_2/EtOH, 99:1, v/v); mp 148–150 °C; IR (KBr) 3500–3200, 1610, 1521 cm^{-1}; MS *m/z* 463 (M^+, 1), 86 (100), 58 (7); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.05 (t, 6H, *J* = 7 Hz), 2.61 (q, 4H, *J* = 7 Hz), 2.72 (m, 2H), 3.56 (m, 2H), 6.95 (t, 1H, *J* = 8 Hz), 7.21 (t, 1H, *J* = 8 Hz), 7.58 (br s, 1H), 7.92 (d, 1H, *J* = 8 Hz), 8.08 (d, 1H, *J* = 8 Hz), 8.36 (d, 1H, *J* = 8 Hz), 8.53 (d, 1H, *J* = 8 Hz), 12.80 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.8 (2C), 37.3, 46.9 (2C), 51.2, 86.3, 118.1, 120.6, 122.2, 122.3, 123.2, 127.6, 131.8, 132.0, 141.1, 141.2, 143.9, 167.9, 177.8.

4.1.9.5. *N*-(2-Diethylaminoethyl)-9,10-dihydro-6-iodo-9-oxoacridine-4-carboxamide (24e). From **18e**. The mixture was refluxed in dry dichloromethane for 2 h. Yield 65%; R_f = 0.25 (Al_2O_3, CH_2Cl_2/EtOH, 99:1, v/v); mp 208–210 °C; IR (KBr) 3343, 2966, 1610, 1580, 1560, 1522, 1449, 1301 cm^{-1}; MS *m/z* 463 (M^+, 1), 86 (100), 58 (12); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.25 (t, 6H, *J* = 7 Hz), 2.95 (m, 4H), 3.04 (m, 2H), 3.72 (m, 2H), 7.22 (t, 1H, *J* = 7.5 Hz), 7.47 (d, 1H, *J* = 8.5 Hz), 7.70 (s, 1H), 8.00 (d, 1H, *J* = 8.5 Hz), 8.25 (d, 1H, *J* = 7 Hz), 8.51 (d, 1H, *J* = 8 Hz), 8.67 (br s, 1H), 12.40 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 9.9 (2C), 35.9, 47.9 (2C), 52.5, 101.3, 116.7, 120.5, 120.6, 122.7, 126.5, 128.3, 131.0, 132.0, 133.0, 140.7, 141.2, 168.7, 177.7.

4.1.9.6. *N*-(2-Diethylaminoethyl)-9,10-dihydro-7-iodo-9-oxoacridine-4-carboxamide (24f). From **18f**. The mixture was refluxed in dry dichloromethane for 6 h. Yield 69%; R_f = 0.27 (Al_2O_3, CH_2Cl_2/EtOH, 99:1, v/v); mp 140–142 °C; IR (KBr) 3500–2800, 1647, 1613, 1516, 1300 cm^{-1}; MS *m/z* 463 (M^+, 4), 86 (100), 58 (7); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.30 (t, 6H, *J* = 7 Hz), 3.01 (m, 4H), 3.10 (m, 2H), 3.76 (m, 2H), 7.07 (d, 1H, *J* = 9 Hz), 7.24 (t, 1H, *J* = 8 Hz), 7.81 (d, 1H, *J* = 9 Hz), 8.25 (d, 1H, *J* = 8 Hz), 8.53 (d, 1H, *J* = 8 Hz), 8.65 (m, 2H), 12.48 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 10.0 (2C), 35.9, 47.6 (2C), 52.2, 84.9, 116.8, 119.7, 120.4, 122.4, 122.8, 131.9, 132.9, 135.6, 139.2, 141.0, 141.9, 168.6, 176.5.

4.1.9.7. *N*-(2-Diethylaminoethyl)-9,10-dihydro-8-iodo-9-oxoacridine-4-carboxamide (24g). From **18g**. The mixture was refluxed in dry toluene for 4 h. Yield 63% (unstable); IR (KBr) 3500–2800, 1645, 1606, 1514, 1298 cm^{-1}; MS *m/z* 463 (M^+, 1), 86 (100), 58 (12); ^1H NMR ($CDCl_3$, 200 MHz) δ 1.15 (t, 6H, *J* = 7 Hz), 2.76 (q, 4H, *J* = 7 Hz), 2.86 (t, 2H, *J* = 6 Hz), 3.59 (m, 2H), 7.17 (m, 2H), 7.31 (d, 1H, *J* = 8 Hz), 7.80 (br s, 1H), 7.85 (d, 1H, *J* = 7.5 Hz), 7.95 (d, 1H, *J* = 7.5 Hz), 8.54 (d, 1H, *J* = 7 Hz), 11.30 (br s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.3 (2C), 36.6, 47.0 (2C), 51.3, 92.2, 116.8, 118.5, 118.7, 120.3, 122.4, 131.8, 132.1, 133.6, 136.6, 140.1, 141.3, 168.1, 176.1.

4.1.10. General procedure for the synthesis of *N*-(2-diethylaminoethyl)-9,10-dihydro-iodo-9-oxoacridine-4-carboxamides hydrochloride salts 8a–g

Compound **24** (100 mg, 0.22 mmol) was diluted with dry dichloromethane (0.5 mL) and treated with an anhydrous 2 N hydrochloric acid–ether solution (2 mL). The solution was concentrated under vacuum and started again with dry ether (3 mL). The suspension was stirred overnight and filtered.

4.1.10.1. *N*-(2-Diethylaminoethyl)-9,10-dihydro-1-iodo-9-oxoacridine-4-carboxamide hydrochloride salt (8a). From **24a**. Yield 81%; mp 269–271 °C; IR (KBr) 3500–3100, 3057, 1613, 1577, 1513 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.26 (t, 6H, *J* = 7 Hz), 3.22 (m, 4H), 3.33 (m, 2H, *J* = 7 Hz), 3.75 (m, 2H), 7.33 (t, 1H, *J* = 7 Hz), 7.62 (d, 1H, *J* = 8 Hz), 7.76 (d, 1H, *J* = 7 Hz), 7.96 (m, 2H), 8.20 (d, 1H, *J* = 8 Hz), 9.49 (br s, 1H), 10.39 (br s, 1H), 12.70 (br s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 8.3 (2C), 34.3, 46.5 (2C), 49.6, 99.0, 117.7, 118.0, 118.8, 120.4, 122.3, 126.3, 133.0, 134.1, 134.3, 138.6, 141.3, 168.2, 175.2. Anal. Calcd for $C_{20}H_{22}IN_3O_2$, HCl, $3H_2O$: C, 43.38; H, 5.28; N, 7.59. Found: C, 43.74; H, 4.99; N, 7.68.

4.1.10.2. *N*-(2-Diethylaminoethyl)-9,10-dihydro-2-iodo-9-oxoacridine-4-carboxamide hydrochloride salt (8b). From **24b**. Yield 71%; mp 298–300 °C; IR (KBr) 3400–3200, 1617, 1560, 1517, 1300, 1168 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.27 (m, 6H), 3.23 (m, 4H), 3.33 (m, 2H) 3.75 (m, 2H), 7.33 (t, 1H, *J* = 7 Hz), 7.75 (m, 2H), 8.23 (d, 1H, *J* = 8 Hz), 8.60 (s, 1H), 8.68 (s, 1H), 9.42 (br s, 1H), 10.27 (br s, 1H), 12.19 (br s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 8.3 (2C), 34.2, 46.4 (2C), 49.5, 83.2, 118.6, 120.5 (2C), 122.3, 123.3, 125.9, 134.3, 138.2, 139.3, 139.8, 140.7, 166.8, 175.3. Anal. Calcd for $C_{20}H_{22}IN_3O_2$, HCl, $1.75H_2O$: C, 45.21; H, 5.03; N, 7.91. Found: C, 44.91; H, 4.66; N, 8.07.

4.1.10.3. *N*-(2-Diethylaminoethyl)-9,10-dihydro-3-iodo-9-oxoacridine-4-carboxamide hydrochloride salt (8c). From **24c**. Yield 87%; mp 262–264 °C; IR (KBr) 3400–3200, 1618, 1590, 1427 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.27 (t, 6H, *J* 8 7 Hz), 3.23 (m, 4H), 3.32 (m, 2H), 3.76 (m, 2H), 7.32 (t, 1H, *J* 8 8 Hz), 7.75 (m, 2H), 7.95 (d, 1H, *J* 8 8.5 Hz), 7.99 (d, 1H, *J* = 8.5 Hz), 8.21 (d, 1H, *J* = 8 Hz), 9.05 (m, 1H), 10.17 (br s, 1H), 10.79 (br s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 8.4 (2C), 34.5, 46.6 (2C), 49.3, 102.6, 118.3, 120.4 (2C), 121.9, 125.7, 127.8, 130.9, 131.6, 133.8, 137.2, 141.0, 166.9, 176.3. Anal. Calcd for $C_{20}H_{22}IN_3O_2$, HCl, H_2O: C, 46.39; H, 4.87; N, 8.12. Found: C, 46.59; H, 4.89; N, 8.06.

4.1.10.4. *N*-(2-Diethylaminoethyl)-9,10-dihydro-5-iodo-9-oxoacridine-4-carboxamide hydrochloride salt (8d) from 24d. From **24d.** Yield 82%; mp 251–253 °C; IR (KBr) 3400–3200, 2946, 2650, 1611, 1522, 1428, 1301 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.27 (t, 6H, *J* = 7 Hz), 3.24 (m, 4H), 3.36 (m, 2H), 3.79 (m, 2H), 7.12 (t, 1H, *J* = 8 Hz), 7.43 (t, 1H, *J* = 7.5 Hz), 8.26 (d, 1H, *J* = 8 Hz), 8.32 (d, 1H, *J* = 7 Hz), 8.46 (d, 1H, *J* = 8 Hz), 8.55 (d, 1H, *J* = 7 Hz), 9.55 (br s, 1H), 10.34 (br s, 1H), 12.97 (br s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 8.3 (2C), 34.2, 46.5 (2C), 49.8, 87.5, 117.4, 120.7, 121.1, 121.3, 123.5, 126.5, 130.8, 133.8, 140.5 (2C), 144.0, 168.1, 176.3. Anal. Calcd for $C_{20}H_{22}IN_3O_2$, HCl, H_2O: C, 46.39; H, 4.87; N, 8.12. Found: C, 46.03; H, 4.80; N, 8.06.

4.1.10.5. *N*-(2-Diethylaminoethyl)-9,10-dihydro-6-iodo-9-oxoacridine-4-carboxamide hydrochloride salt (8e). From **24e**. Yield 46%; mp 266-268 °C; IR (KBr) 3450-3250, 3066, 1611, 1577, 1523, 1450, 1309 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.27 (t, 6H, *J* = 7 Hz), 3.23 (m, 4H), 3.34 (m, 2H), 3.76 (m, 2H), 7.37 (t, 1H, *J* = 7.5 Hz), 7.61 (t, 1H, *J* = 8.5 Hz), 7.93 (d, 1H, *J* = 8.5 Hz), 8.26 (s, 1H), 8.38 (d, 1H, *J* = 7.5 Hz), 8.42 (d, 1H, *J* = 8 Hz), 9.43 (m, 1H), 10.41 (br s, 1H), 12.21 (br s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 8.3 (2C), 34.2, 46.5 (2C), 49.7, 102.2, 118.6, 119.5, 120.4, 121.7, 126.8, 127.4, 130.2, 130.6, 133.6, 139.9, 140.6, 167.9, 176.2. Anal. Calcd for $C_{20}H_{22}IN_3O_2$, HCl, $1.5H_2O$: C, 45.60; H, 4.97; N, 7.98. Found: C, 46.80; H, 4.71; N, 7.92.

4.1.10.6. *N*-(2-Diethylaminoethyl)-9,10-dihydro-7-iodo-9-oxoacridine-4-carboxamide hydrochloride salt (8f). From **24f**. Yield 99%; mp 220–222 °C; IR (KBr) 3400–3200, 1617, 1517, 1303 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.27 (t, 6H, J = 7 Hz), 3.23 (m, 4H), 3.34 (m, 2H), 3.76 (m, 2H), 7.38 (t, 1H, J = 8 Hz), 7.63 (d, 1H, J = 9 Hz), 8.00 (d, 1H, J = 9 Hz), 8.44 (m, 2H), 8.49 (s, 1H), 9.46 (br s, 1H), 10.40 (br s, 1H), 12.38 (br s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 8.3 (2C), 34.2, 46.5 (2C), 49.7, 85.7, 118.5, 120.4, 121.0, 121.6, 122.2, 130.4, 133.7, 134.1, 139.2, 140.1, 141.7, 168.0, 175.2. Anal. Calcd for $C_{20}H_{22}IN_3O_2$, HCl, $2H_2O$: C, 44.83; H, 5.08; N, 7.84. Found: C, 44.67; H, 4.43; N, 7.76.

4.1.10.7. *N*-(2-Diethylaminoethyl)-9,10-dihydro-8-iodo-9-oxoacridine-4-carboxamide hydrochloride salt (8g). From **24g**. Yield 91%; mp 228–230 °C; IR (KBr) 3400–3200, 2925, 1607, 1560, 1517, 1458, 1295 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.25 (t, 6H, J = 7 Hz), 3.30 (m, 6H), 3.71 (m, 2H), 7.37 (m, 2H), 7.71 (d, 1H, J = 8 Hz), 7.90 (d, 1H, J = 7.5 Hz), 8.28 (d, 1H, J = 7.5 Hz), 8.43 (d, 1H, J = 7 Hz), 9.23 (br s, 1H), 9.44 (m, 1H), 12.26 (br s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 8.3 (2C), 34.2, 46.5 (2C), 49.7, 92.4, 117.5, 117.8, 119.3, 120.4, 121.6, 130.9, 133.4, 134.2, 136.3, 139.3, 141.2, 168.0, 175.0. Anal. Calcd for $C_{20}H_{22}IN_3O_2$, HCl, $0.5H_2O$: C, 47.21; H, 4.74; N, 8.26. Found: C, 47.70; H, 5.12; N, 8.29.

4.1.11. General procedure for the synthesis of methyl iodoacridan-4-carboxylates 28a–g

The appropriate methyl 9,10-dihydro-iodo-9-oxoacridine-4-carboxylate **18** (0.26 g, 0.69 mmol) was dissolved in anhydrous tetrahydrofuran (10 mL) under a slowly flowing argon gas. A 1 M borane tetrahydrofuran complex solution (0.82 mL, 0.82 mmol) was added dropwise to the stirred reflux reaction. The mixture was refluxed for 45 min. After cooling to room temperature, an aqueous 3 N hydrochloric acid solution (5 mL) was added and the reaction mixture was made alkaline with an aqueous saturated sodium carbonate solution (20 mL). The mixture was extracted with dichloromethane (3× 20 mL). The organic phases collected were dried ($MgSO_4$), filtered and the solvent removed under reduce pressure. The crude product was chromatographed (SiO_2 and CH_2Cl_2) except for unstable compounds **28c** and **28e** which were used in the next step without purification.

4.1.11.1. Methyl 1-iodoacridan-4-carboxylate (28a). From **18a**. Yield 75%; R_f = 0.91 (SiO_2 and CH_2Cl_2); mp 119–121 °C; IR (KBr) 3304, 2948, 1690, 1585, 1498, 1431, 1269, 1252 cm^{-1}; MS m/z 365 (M^+, 100), 332 (78), 206 (52), 178 (53), 151 (23); ^1H NMR ($CDCl_3$, 400 MHz) δ 3.91 (s, 3H), 4.13 (s, 2H), 6.74 (d, 1H, J = 7.5 Hz), 6.88 (t, 1H, J = 7.5 Hz), 7.10 (m, 2H), 7.26 (d, 1H, J = 8.5 Hz), 7.41 (d, 1H, J = 8.5 Hz), 9.93 (br s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 38.1, 52.0, 109.0, 109.8, 114.5, 119.5, 121.8, 124.3, 127.6, 128.6, 128.8, 130.1, 137.9, 143.6, 168.8.

4.1.11.2. Methyl 2-iodoacridan-4-carboxylate (28b). From **18b**. Yield 58%; R_f = 0.93 (SiO_2 and CH_2Cl_2); mp 148–150 °C; IR (KBr) 3344, 2941, 1676, 1501, 1263, 1253 cm^{-1}; MS m/z 365 (M^+, 100), 332 (23), 178 (58), 151 (20), 127 (22); ^1H NMR ($CDCl_3$, 400 MHz) δ 3.92 (s, 3H), 4.06 (s, 2H), 6.77 (d, 1H, J = 7.5 Hz), 6.90 (t, 1H, J = 7.5 Hz), 7.06 (d, 1H, J = 7.5 Hz), 7.12 (t, 1H, J = 7.5 Hz), 7.48 (s, 1H), 8.08 (s, 1H), 9.77 (br s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 31.0, 52.2, 79.2, 112.3, 114.9, 119.4, 121.9, 124.4, 127.5, 128.4, 137.5, 138.3, 141.3, 143.3, 167.7.

4.1.11.3. Methyl 3-iodoacridan-4-carboxylate (28c). From **18c**. Very instable precipitate; Yield 78%; IR (KBr) 3442, 2946, 1730, 1234 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.98 (s, 3H), 4.00 (s, 2H), 6.72 (d, 1H, J = 8 Hz), 6.80 (d, 1H, J = 8 Hz), 6.91 (t, 1H, J = 7.5 Hz), 7.10 (m, 2H), 7.39 (d, 1 H, J = 8 Hz), 8.33 (br s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 31.2, 51.8, 91.7, 114.7, 118.1, 119.4, 121.7 (2C), 127.3, 128.2, 132.2, 132.5, 138.5, 142.4, 168.1.

4.1.11.4. Methyl 5-iodoacridan-4-carboxylate (28d). From **18d**. Yield 98%; R_f = 0.94 (SiO_2 and CH_2Cl_2); mp 105–107 °C; IR (KBr) 3260, 2924, 1689, 1491, 1443, 1259 cm^{-1}; MS m/z 365 (M^+, 100), 332 (56), 206 (53), 177 (81), 151 (63), 103 (36), 89 (37), 75 (46); ^1H NMR ($CDCl_3$, 400 MHz) δ 3.97 (s, 3H), 4.08 (s, 2H), 6.61 (t, 1H, J = 7.5 Hz), 6.83 (t, 1H, J = 7.5 Hz), 7.03 (d, 1H, J = 7 Hz), 7.24 (d, 1H, J = 7 Hz), 7.58 (d, 1H, J = 8 Hz), 7.84 (d, 1H, J = 8 Hz), 10.16 (br s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 32.2, 52.2, 84.3, 111.3, 119.6, 121.1, 121.7, 122.7, 128.3, 129.5, 133.3, 137.3, 140.1, 143.0, 168.5.

4.1.11.5. Methyl 6-iodoacridan-4-carboxylate (28e). From **18e**. Very instable precipitate; Yield 76%; IR (KBr) 3342, 2942, 1684, 1594, 1507, 1464, 1429, 1266 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.94 (s, 3H), 4.01 (s, 2H), 6.80 (m, 2H), 7.14 (s, 1H), 7.18 (d, 1H, J = 8 Hz), 7.22 (d, 1H, J = 7.5 Hz), 7.79 (d, 1H, J = 8 Hz), 9.82 (br s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 31.2, 52.0, 91.6, 110.8, 119.2, 119.5, 121.3, 123.3, 129.4, 129.9, 130.2, 133.6, 140.5, 143.0, 168.8.

4.1.11.6. Methyl 7-iodoacridan-4-carboxylate (28f). From **18f**. Yield 95%; R_f = 0.90 (SiO_2 and CH_2Cl_2); mp 122–124 °C; IR (KBr) 3320, 2948, 1682, 1594, 1502, 1466, 1430, 1269, 1194 cm^{-1}; MS m/z 365 (M^+, 100), 333 (50), 206 (40), 177 (65), 151 (44), 127 (30), 103 (29), 89 (33), 75 (42); ^1H NMR ($CDCl_3$, 400 MHz) δ 3.91 (s, 3H), 4.04 (s, 2 H), 6.54 (d, 1H, J = 8.5 Hz), 6.79 (t, 1H, J = 7.5 Hz), 7.22 (d, 1H, J = 7 Hz), 7.37 (m, 2H), 7.77 (d, 1H, J = 7.5 Hz), 9.83 (br s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 30.4, 52.0, 82.9, 110.7, 116.7, 119.1, 121.1, 122.4, 129.4, 133.7, 136.0, 136.8, 138.8, 143.1, 168.9.

4.1.11.7. Methyl 8-iodoacridan-4-carboxylate (28g). From **18g**. Yield 92%; R_f = 0.97 (SiO_2 and CH_2Cl_2); mp 108–110 °C; IR (KBr) 3319, 2960, 1685, 1603, 1501, 1442, 1262 cm^{-1}; MS m/z 365 (M^+, 100), 333 (53), 332 (55), 206 (81), 178 (90), 177 (95), 151 (71), 127 (42), 103 (42), 89 (32), 75 (66), 63 (42); ^1H NMR ($CDCl_3$, 400 MHz) δ 3.90 (s, 3H), 4.09 (s, 2 H), 6.69 (d, 1H, J = 8 Hz), 6.77 (m, 2H), 7.24 (d, 1H, J = 7.5 Hz), 7.36 (d, 1H, J = 8 Hz), 7.77 (d, 1H, J = 8 Hz), 9.77 (br s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 37.5, 51.9, 101.2, 110.3, 114.8, 115.7, 119.0, 122.5, 128.9, 129.4, 131.4, 133.9, 139.5, 142.8, 168.9.

4.1.12. General procedure for the synthesis of methyl iodoacridine-4-carboxylates 29a–g

Methyl iodoacridan-4-carboxylate **28** (140 mg, 0.38 mmol) was stirred in a mixture of ethanol (10 mL), water (2 mL) and iron chloride hexahydrate (0.30 g) for 30 min at 50 °C. After cooling to room temperature, an aqueous saturated sodium bicarbonate solution (20 mL) was added and the mixture was extracted with dichloromethane (3× 30 mL). The organic layers were dried ($MgSO_4$) and filtered. Evaporation of the solvent gave a very instable precipitate which was used without purification.

4.1.12.1. Methyl 1-iodoacridine-4-carboxylate (29a). From **28a**. Yield 87%; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.00 (s, 3H), 7.53 (t, 1 H, J = 7 Hz), 7.66 (d, 1H, J = 7 Hz), 7.76 (t, 1H, J = 7 Hz), 7.99 (d, 1H, J = 8 Hz), 8.07 (d, 1H, J = 7 Hz), 8.21 (d, 1H, J = 8 Hz), 8.91 (s, 1H).

4.1.12.2. Methyl 2-iodoacridine-4-carboxylate (29b). From 28 b. Yield 93%; ^1H NMR ($CDCl_3$, 200 MHz) δ 4.14 (s, 3H), 7.62 (t, 1H, J = 7.5 Hz), 7.88 (t, 1H, J = 7.5 Hz), 8.05 (d, 1H, J = 8 Hz), 8.36 (m, 2H), 8.59 (s, 1H), 8.86 (s, 1H).

4.1.12.3. Methyl 3-iodoacridine-4-carboxylate (29c). From **28c**. Yield 88%; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.19 (s, 3H), 7.55 (t, 1H,

J = 7.5 Hz), 7.68 (d, 1H, J = 8.5 Hz), 7.77 (m, 2H), 7.93 (d, 1H, J = 8.5 Hz), 8.20 (d, 1H, J = 8.5 Hz), 8.69 (s, 1H).

4.1.12.4. Methyl 5-iodoacridine-4-carboxylate (29d). From **28d**. Yield 97%; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.20 (s, 3H), 7.17 (dd, 1H, J = 8.5, 7 Hz), 7.51 (dd, 1H, J = 8.5, 7 Hz), 7.87 (d, 1H, J = 8.5 Hz), 8.03 (d, 1H, J = 8.5 Hz), 8.11 (d, 1H, J = 7 Hz), 8.39 (d, 1H, J = 7 Hz), 8.60 (s, 1H).

4.1.12.5. Methyl 6-iodoacridine-4-carboxylate (29e). From **28e**. Yield 88%; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.12 (s, 3H), 7.54 (dd, 1H, J = 8.5, 7 Hz), 7.67 (m, 2H), 8.06 (d, 1H, J = 8.5 Hz), 8.12 (d, 1H, J = 7 Hz), 8.70 (s, 1H), 8.77 (s, 1H).

4.1.12.6. Methyl 7-iodoacridine-4-carboxylate (29f). From **28f**. Yield 95%; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.13 (s, 3H), 7.48 (dd, 1H, J = 8.5, 7 Hz), 7.98 (m, 3H), 8.09 (d, 1H, J = 7 Hz), 8.29 (s, 1H), 8.53 (s, 1H).

4.1.12.7. Methyl 8-iodoacridine-4-carboxylate (29g). From 26 g. Yield 90%; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.11 (s, 3H), 7.51 (dd, 1H, J = 8.5, 7 Hz), 7.62 (dd, 1H, J = 8.5, 7 Hz), 8.22 (m, 3H), 8.32 (d, 1 H, J = 8.5 Hz), 9.02 (s, 1H).

4.1.13. *N*-(2-diethylaminoethyl)-iodoacridine-4-carboxamides 30a,b,d–g

Were prepared as described for the synthesis of carboxamides **24a–g**.

4.1.13.1. *N*-(2-Diethylaminoethyl)-1-iodoacridine-4-carboxamide (30a). From **29a**. The mixture was refluxed in dry dichloromethane for 4 h. Yield 80%; R_f = 0.40 (Al_2O_3, CH_2Cl_2/EtOH, 99:1, v/v); mp 103–105 °C; IR (KBr) 3500–3300, 3186, 2967, 1654, 1649, 1517 cm^{-1}; MS m/z 447 (M^+, 8), 332 (18), 177 (17), 86 (100), 58 (15); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.15 (t, 6H, J = 7 Hz), 2.78 (q, 4H, J = 7 Hz), 2.88 (t, 2H, J = 7 Hz), 3.83 (m, 2H), 7.63 (t, 1H, J = 7 Hz), 7.85 (t, 1H, J = 8 Hz), 8.07 (d, 1H, J 8 8.5 Hz), 8.24 (d, 1H, J = 8 Hz), 8.29 (d, 1H, J = 8.5 Hz), 8.60 (d, 1H, J = 7.5 Hz), 9.04 (s, 1H), 11.82 (br s, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.6 (2C), 38.0, 47.1 (2C), 51.9, 103.8, 126.9, 127.0, 128.0, 128.3, 128.7, 129.4, 131.9, 135.4, 137.1, 142.6, 145.8, 148.1, 165.4.

4.1.13.2. *N*-(2-diethylaminoethyl)-2-iodoacridine-4-carboxamide (30b). From **29b**. The mixture was refluxed in dry dichloromethane for 4 h. Yield 80%; R_f = 0.41 (Al_2O_3, CH_2Cl_2/EtOH, 99:1, v/v); mp 108–110 °C; IR (KBr) 3182, 2965, 1637, 1516 cm^{-1}; MS m/z 447 (M^+, 1), 177 (8), 86 (100), 58 (13); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.19 (t, 6H, J = 7 Hz), 2.85 (m, 4H), 2.96 (m, 2H), 3.88 (m, 2H), 7.61 (t, 1H, J = 7 Hz), 7.87 (t, 1 H, J = 8 Hz), 8.01 (d, 1H, J = 8.5 Hz), 8.29 (d, 1H, J = 8.5 Hz), 8.51 (s, 1H), 8.71 (s, 1H), 9.10 (s, 1H), 11.88 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.2 (2C), 37.7, 47.2 (2C), 51.7, 91.0, 126.2, 127.1, 128.2, 128.4, 129.4, 129.6, 131.8, 136.3, 140.7, 143.4, 145.0, 147.9, 164.8.

4.1.13.3. *N*-(2-Diethylaminoethyl)-5-iodoacridine-4-carboxamide (30d). From **29d**. The mixture was refluxed in dry toluene for 3 h. Yield 73%; R_f = 0.17 (Al_2O_3, CH_2Cl_2/EtOH, 99:1, v/v); mp 64–66 °C; IR (KBr) 3500–3300, 3200, 2925, 1654, 1648 cm^{-1}; MS m/z 447 (M^+, 1), 177 (9), 86 (100), 58 (12); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.11 (t, 6H, J = 7 Hz), 2.69 (q, 4H, J = 7 Hz), 2.92 (m, 2H), 3.82 (m, 2H), 7.19 (dd, 1H, J = 8.5, 7 Hz), 7.56 (dd, 1H, J = 8.5, 7 Hz), 7.86 (d, 1H, J = 8.5 Hz), 7.98 (d, 1H, J = 8.5 Hz), 8.32 (d, 1H, J = 7 Hz), 8.60 (s, 1H), 8.88 (d, 1H, J = 7 Hz), 11.76 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.8 (2C), 38.4, 47.5 (2C), 52.4, 102.6, 125.8, 126.1, 127.0, 127.1, 128.2, 129.5, 132.9, 136.0, 138.6, 141.6, 145.8, 146.3, 165.4.

4.1.13.4. *N*-(2-Diethylaminoethyl)-6-iodoacridine-4-carboxamide (30e). From **29e**. The mixture was refluxed in dry dichloromethane for 4 h. Yield 52%; R_f = 0.37 (Al_2O_3, CH_2Cl_2/EtOH, 99:1, v/v); mp 80–82 °C; IR (KBr) 3500–3300, 2924, 1654, 1559, 1458 cm^{-1}; MS m/z 447 (M^+, 3), 177 (9), 86 (100), 58 (15), ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.16 (t, 6H, J = 7 Hz), 2.79 (m, 6H), 3.77 (q, 2H, J = 6 Hz), 7.65 (dd, 1H, J = 8.5, 7 Hz), 7.70 (d, 1H, J = 9 Hz), 7.78 (d, 1H, J = 9 Hz), 8.06 (d, 1H, J = 8.5 Hz), 8.78 (s, 1H), 8.81 (s, 1 H), 8.97 (d, 1H, J = 7 Hz), 11.90 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 12.0 (2C), 38.0, 47.0 (2C), 51.9, 98.1, 124.6, 126.0, 127.0, 128.9, 129.0, 132.1, 134.8, 135.8, 137.6, 138.4, 146.6, 147.7, 165.5.

4.1.13.5. *N*-(2-Diethylaminoethyl)-7-iodoacridine-4-carboxamide (30f). From **29f**. The mixture was refluxed in dry dichloromethane for 6 h. Yield 53%; R_f = 0.33 (Al_2O_3, CH_2Cl_2/EtOH, 99:1, v/v); mp 176–178 °C; IR (KBr) 3500–3300, 2965, 1665, 1562, 1515, 1457 cm^{-1}; MS m/z 447 (M^+, 2), 332 (10), 305 (12), 177 (8), 86 (100), 58 (8); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.26 (t, 6H, J = 7 Hz), 2.98 (m, 6H), 3.98 (m, 2 H), 7.70 (dd, 1H, J = 8.5, 7 Hz), 8.06 (d, 1H, J = 9 Hz), 8.16 (m, 2H), 8.47 (s, 1H), 8.78 (s, 1H), 8.98 (d, 1H, J = 7 Hz), 12.01 (br s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 10.8 (2C), 37.2, 47.2 (2C), 51.6, 92.2, 126.1, 127.0, 127.5, 128.3, 130.9, 132.6, 135.8, 136.4, 136.8, 139.8, 146.3, 146.5, 166.1.

4.1.13.6. *N*-(2-Diethylaminoethyl)-8-iodoacridine-4-carboxamide (30g). From **29g**. The mixture was refluxed in dry dichloromethane for 16 h. Yield 55%; R_f = 0.35 (Al_2O_3, CH_2Cl_2/EtOH, 99:1, v/v); mp 78–80 °C; IR (KBr) 3200, 2925, 1654, 1546, 1508 cm^{-1}; MS m/z 447 (M^+, 1), 177 (11), 86 (100), 58 (11); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.16 (t, 6H, J = 7 Hz), 2.80 (q, 4H, J = 7 Hz), 2.91 (m, 2H), 3.85 (m, 2H), 7.50 (t, 1H, J = 8 Hz), 7.69 (t, 1H, J = 7.5 Hz), 8.16 (m, 2H), 8.27 (d, 1H, J = 8.5 Hz), 8.96 (s, 1H), 8.98 (d, 1H, J = 7 Hz), 11.76 (br s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.4 (2C), 37.7, 47.1 (2C), 51.8, 98.4, 126.1, 127.5, 127.7, 128.2, 130.2, 131.6, 132.4, 135.9, 137.4, 142.4, 146.7, 147.4, 165.6.

4.1.14. 3-Iodoacridine-4-carboxylic acid (31)

The ester **29c** (0.15 g, 0.36 mmol) was suspended in a mixture of methanol (30 mL) and an aqueous 4 N sodium hydroxide solution (30 mL). The solution was heated under reflux for 12 h to give a clear yellow solution which was filtered and acidified to pH 5 with glacial acetic acid. Concentration and cooling to 0 °C gave a yellow precipitate which was collected by filtration and washed with water (2 mL). The precipitate was taken in dry ethanol (50 mL) and dried under vacuum to give 3-iodoacridine-4-carboxylic acid (**31**). Yield 44%; mp 139–141 °C; IR (KBr) 3500–3200, 1577, 1421 cm^{-1}; ESI-MS m/z 349.4 $[M+H]^+$; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.61 (t, 1H, J = 7 Hz), 7.68 (d, 1H, J = 8.5 Hz), 7.82 (m, 2H), 8.07 (d, 1H, J = 8 Hz), 8.12 (d, 1H, J = 8.5 Hz), 9.02 (s, 1H); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 93.1, 125.1, 125.9 (2C), 126.3, 128.3, 129.0, 130.5, 135.2, 136.0, 145.4, 147.9, 149.6, 177.3.

4.1.15. *N*-(2-Diethylaminoethyl)-3-chloroacridine-4-carboxamide (32)

A suspension of 3-iodoacridine-4-carboxylic acid (**31**) (40 mg, 0.11 mmol) in thionyl chloride (5 mL) containing dimethylformamide (1 drop) was heated gently under reflux with stirring until homogeneous and then for a further 45 min. The solution was evaporated to dryness under vacuum below 40 °C, and residual traces of thionyl chloride were removed by addition of dry toluene (5 mL) and complete reevaporation of all solvents. The above product in dry dichloromethane solution (2 mL) was cooled to −5 °C and was added an ice-cold solution of *N*,*N*-diethylethylenediamine (20 μL, 0.14 mmol) in dry dichloromethane (1 mL). The solution was stirred at room temperature for 2 h. The organic layers were washed twice with a 10% aqueous sodium carbonate solution

(20 mL) and once with saturated aqueous sodium chloride solution (20 mL). The organic phase was dried ($MgSO_4$), filtered and evaporated to dryness. The product **32** obtained was chromatographed (Al_2O_3, EtOAc/cyclohexane, 8:2, v/v). Yield 79%; R_f = 0.40 (Al_2O_3, EtOAc/cyclohexane, 8:2, v/v); mp 229–231 °C; IR (KBr) 3500–3300, 2924, 1654 cm^{-1}; MS *m/z* 357 (M^++2, 3), 355 (M^+, 1), 240 (11), 177 (15), 86 (100), 58 (12); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.03 (t, 6H, *J* = 6.5 Hz), 2.68 (m, 4H), 2.89 (m, 2H), 3.80 (m, 2H), 7.05 (br s, 1H), 7.42 (d, 1H, *J* = 8.5 Hz), 7.52 (t, 1H, *J* = 7 Hz), 7.75 (t, 1H, *J* = 7 Hz), 7.88 (d, 1H, *J* = 9 Hz), 7.93 (d, 1H, *J* = 8 Hz), 8.16 (d, 1H, *J* = 9 Hz), 8.68 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 11.3 (2C), 37.3, 47.0 (2C), 51.8, 124.7, 126.4, 126.5, 127.3, 128.2, 130.0, 130.1, 130.9, 133.3, 134.8, 136.1, 146.5, 149.6, 166.6.

4.1.16. *N*-(2-Diethylaminoethyl)-3-iodoacridan-4-carboxamide (33)

Compound **33** was prepared from compound **24c** as described for the synthesis of methyl iodoacridan-4-carboxylates **28**. The product was chromatographed (Al_2O_3, CH_2Cl_2/EtOH, 99.5:0.5, v/v). Yield 60%; R_f = 0.27 (Al_2O_3, CH_2Cl_2/EtOH, 99.5:0.5, v/v); mp 106–108 °C; IR (KBr) 3268, 2346, 1636, 1449, 1164 cm^{-1}; MS *m/z* 449 (M^+, 1), 86 (100), 58 (10); ^1H NMR ($CDCl_3$, 200 MHz) δ 1.26 (t, 6H, *J* = 7 Hz), 2.99 (m, 6H), 3.91 (q, 2 H, *J* = 6.5 Hz), 3.98 (s, 2H), 6.48 (m, 1H), 6.71 (d, 1H, *J* = 8 Hz), 6.79 (t, 1H, *J* = 8 Hz), 6.89 (m, 1H), 7.09 (m, 2H), 7.26 (m, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 8.5 (2C), 31.1, 35.1, 53.4 (2C), 57.0, 90.1, 114.6, 119.2, 121.3, 121.5, 124.5, 127.3, 128.3, 130.9, 131.3, 138.8, 139.6, 168.8.

4.1.17. *N*-(2-Diethylaminoethyl)-3-iodoacridine-4-carboxamide (30c)

was prepared from **33** as described for the synthesis of methyl iodoacridine-4-carboxylates **29**. The product was chromatographed (Al_2O_3, EtOAc/pentane, 5:5, v/v). Yield 73%; R_f = 0.13 (Al_2O_3, EtOAc/pentane, 5/5, v/v); mp 81–83 °C; IR (KBr) 3500–3200, 2925, 1654, 1458 cm^{-1}; MS *m/z* 447 (M^+, 1), 177 (11), 86 (100), 58 (12); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.04 (t, 6H, *J* = 7 Hz), 2.69 (m, 4H), 2.91 (m, 2H), 3.81 (m, 2H), 6.95 (br s, 1H), 7.54 (d, 1H, *J* = 8 Hz), 7.65 (d, 1H, *J* = 9 Hz), 7.76 (t, 1H, *J* = 8 Hz), 7.80 (d, 1H, *J* = 9 Hz), 7.94 (d, 1H, *J* = 8.5 Hz), 8.17 (d, 1H, *J* = 9 Hz), 8.68 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.3 (2C), 37.2, 46.8 (2C), 51.6, 97.1, 125.3, 126.5, 126.6, 128.0, 129.5, 130.1, 130.7, 135.1, 136.0, 142.5, 146.2, 149.2, 169.2.

4.1.18. *N*-(2-Ddiethylaminoethyl)iodoacridine-4-carboxamides dihydrochloride salts 9a–g

Compounds **9a**–**g** were prepared as described for the synthesis of hydrochloride salts **8a–g**.

4.1.18.1. *N*-(2-Diethylaminoethyl)-1-iodoacridine-4-carboxamide dihydrochloride salt (9a).

From **30a**. Yield 71%; mp 219–221 °C; IR (KBr) 3500–3200, 2975, 2638, 2472, 1624, 1577, 1549, 1394 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.27 (t, 6 H, *J* = 7 Hz), 3.27 (m, 4 H), 3.43 (m, 2 H), 4.00 (m, 2 H), 7.80 (t, 1 H, *J* = 7.5 Hz), 8.06 (d, 1H, *J* = 7.5 Hz), 8.40 (d, 1H, *J* = 7.5 Hz), 8.46 (m, 2H), 8.58 (d, 1 H, *J* = 9 Hz), 9.34 (s, 1H), 10.25 (s, 1H), 11.30 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 8.5 (2C), 34.5, 46.9 (2C), 54.9, 105.4, 126.6, 127.4, 127.5, 128.3, 128.8, 132.7, 134.8, 136.8, 142.9, 144.7, 147.4, 165.4, one quaternary carbon not observed. Anal. Calcd for $C_{20}H_{22}IN_3O$, 2HCl: C, 46.18; H, 4.65; N, 8.08. Found: C, 46.38; H, 4.79; N, 7.99.

4.1.18.2. *N*-(2-Diethylaminoethyl)-2-iodoacridine-4-carboxamide dihydrochloride salt (9b).

From **30b**. Yield 76%; mp 215–217 °C; IR (KBr) 3500–3200, 2975, 2651, 1649, 1625, 1572 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 1.26 (t, 6H, *J* = 7 Hz), 3.24 (m, 4H), 3.41 (m, 2H), 3.83 (m, 2H), 7.73 (m, 1H), 8.01 (t, 1H, *J* = 8 Hz), 8.23 (d, 1H, *J* = 8.5 Hz), 8.52 (d, 1H, *J* = 8.5 Hz), 8.79 (s, 1H), 8.90 (s, 1H), 9.27 (s, 1H), 10.49 (m, 1H), 11.33 (br s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 8.5 (2C), 34.5, 46.9 (2C), 49.6, 91.2, 125.8, 127.4, 128.0, 128.6, 128.7, 129.1, 132.4, 138.0, 141.0, 142.0, 143.5, 146.9, 164.5. Anal. Calcd for $C_{20}H_{22}IN_3O$, 2HCl, 1.5H_2O: C, 43.90; H, 4.97; N, 7.68. Found: C, 43.77; H, 4.77; N, 7.54.

4.1.18.3. *N*-(2-Dethylaminoethyl)-3-iodoacridine-4-carboxamide dihydrochloride salt (9c).

From **30c**. Yield 83%; mp 134–136 °C; IR (KBr) 3500–3200, 2924, 1654, 1636, 1560, 1458 cm^{-1}; ^1H NMR (CD_3OD, 200 MHz) δ 1.47 (t, 6 H, *J* = 7 Hz), 3.49 (q, 4H, *J* = 7 Hz), 3.62 (t, 2H, *J* = 7 Hz), 3.97 (t, 2H, *J* = 7 Hz), 7.79 (m, 1H), 8.06 (m, 3H), 8.35 (m, 2H), 9.40 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 8.8 (2C), 34.1, 46.9 (2C), 49.4, 97.9, 124.9, 126.2, 126.6, 128.7, 128.9, 130.0, 131.6, 135.0, 137.0, 142.3, 145.6, 148.3, 169.0. Anal. Calcd for $C_{20}H_{22}IN_3O$, 2HCl, H_2O: C, 44.63; H, 4.87; N, 7.81. Found: C, 44.58; H, 4.92; N, 7.42.

4.1.18.4. *N*-(2-Diethylaminoethyl)-5-iodoacridine-4-carboxamide dihydrochloride salt (9d).

From **30d**. Yield 69%; mp 144–146 °C; IR (KBr) 3500–3300, 2928, 2660, 1654, 1578, 1546 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.27 (t, 6 H, *J* = 7 Hz), 3.26 (m, 4H), 3.43 (m, 2H), 4.10 (m, 2H), 7.48 (t, 1H, *J* = 7.5 Hz), 7.83 (t, 1H, *J* = 7.5 Hz), 8.27 (d, 1H, *J* = 8.5 Hz), 8.46 (d, 1H, *J* = 8 Hz), 8.60 (d, 1H, *J* = 7 Hz), 8.84 (d, 1H, *J* = 7 Hz), 9.36 (s, 1H), 10.72 (br s, 1H), 12.27 (br s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 8.4 (2C), 34.8, 46.4 (2C), 49.9, 104.0, 125.9, 126.0, 126.9, 127.0, 127.8, 129.3, 133.2, 135.7, 140.1, 141.8, 145.6, 145.8, 165.2. Anal. Calcd for $C_{20}H_{22}IN_3O$, 2HCl, H_2O: C, 44.63; H, 4.87; N, 7.81. Found: C, 44.80; H, 4.73; N, 7.63.

4.1.18.5. *N*-(2-Diethylaminoethyl)-6-iodoacridine-4-carboxamide dihydrochloride salt (9e).

From **30e**. Yield 72%; mp 201–203 °C; IR (KBr) 3500–3300, 2970, 1637, 1609 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.27 (t, 6 H, *J* = 7 Hz), 3.24 (m, 4 H), 3.38 (m, 2H), 3.97 (q, 2H, *J* = 6 Hz), 7.79 (dd, 1H, *J* 8 8.5, 7 Hz), 7.95 (d, 1H, *J* = 9 Hz), 8.02 (d, 1H, *J* = 9 Hz), 8.40 (d, 1H, *J* = 8.5 Hz), 8.77 (d, 1H, *J* = 7 Hz), 9.11 (s, 1H), 9.37 (s, 1H), 10.47 (br s, 1H), 11.33 (t, 1H, *J* = 6 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 8.5 (2C), 34.4, 46.9 (2C), 49.8, 100.5, 124.5, 125.6, 126.5, 127.7, 129.8, 133.4, 134.9, 135.5, 137.2, 139.5, 145.3, 147.2, 165.6. Anal. Calcd for $C_{20}H_{22}IN_3O$, 2HCl, 0.5H_2O: C, 45.39; H, 4.76; N, 7.94. Found: C, 45.23; H, 4.70; N, 7.72.

4.1.18.6. *N*-(2-Diethylaminoethyl)-7-iodoacridine-4-carboxamide dihydrochloride salt (9f).

From **30f**. Yield 67%; mp 213–215 °C; IR (KBr) 3500–3200, 3222, 2953, 2665, 1628, 1585, 1403 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.27 (t, 6H, *J* = 7 Hz), 3.25 (m, 4H), 3.41 (m, 2H), 3.92 (m, 2H), 7.79 (t, 1H, *J* = 7.5 Hz), 8.19 (d, 1H, *J* = 9 Hz), 8.36 (d, 1H, *J* = 9 Hz), 8.41 (d, 1H, *J* = 8.5 Hz), 8.74 (m, 2H), 9.28 (s, 1H), 10.57 (br s, 1H), 11.32 (br s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 8.5 (2C), 34.4, 46.9 (2C), 49.7, 93.3, 125.8, 126.5, 127.2, 128.0, 130.6, 133.3, 135.1, 136.7, 137.8, 139.8, 145.3, 145.7, 165.6. Anal. Calcd for $C_{20}H_{22}IN_3O$, 2HCl, 2.5H_2O: C, 42.50; H, 5.17; N, 7.43. Found: C, 42.78; H, 4.96; N, 7.35.

4.1.18.7. *N*-(2-Diethylaminoethyl)-8-iodoacridine-4-carboxamide dihydrochloride salt (9g).

From **30g**. Yield 72%; mp 128–130 °C; IR (KBr) 3500–3200, 1653, 1559, 1540, 1507 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.28 (t, 6 H, *J* = 7 Hz), 3.25 (m, 4 H), 3.41 (m, 2H), 3.99 (m, 2H), 7.71 (t, 1H, *J* = 7.5 Hz), 7.82 (t, 1H, *J* = 7 Hz), 8.33 (d, 1H, *J* = 7 Hz), 8.60 (m, 2H), 8.76 (d, 1H, *J* = 7 Hz), 9.25 (s, 1H), 10.73 (m, 1H), 11.26 (br s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 8.5 (2C), 34.4, 46.9 (2C), 49.7, 99.2, 125.9, 127.1, 127.3, 127.6, 130.0, 132.6, 133.4, 135.3, 137.8, 142.7, 145.6, 147.1, 165.5. Anal. Calcd for $C_{20}H_{22}IN_3O$, 2HCl, 1.5H_2O: C, 43.90; H, 4.97; N, 7.68. Found: C, 43.87; H, 4.77; N, 7.38.

4.1.19. 9,10-Dihydro-2-iodo-9-oxoacridine-4-carboxylic acid (23e)

Compound **23e** was prepared as described for the synthesis of 3-iodoacridine-4-carboxylic acid (**31**) with an aqueous 2 N sodium hydroxide solution and heated under reflux for 10 min. Yield 90%; mp > 400 °C; IR (KBr) 3415–3000, 1615, 1514, 1165 cm^{-1}; MS *m/z* 365 (M^+, 5), 164 (10), 84 (27), 69 (64), 55 (100); ^1H NMR (DMSO-d_6, 400 MHz) δ 7.31 (t, 1H, *J* = 7.5 Hz), 7.64 (d, 1H, *J* = 7.5 Hz), 7.77 (t, 1H, *J* = 7.5 Hz), 8.25 (d, 1H, *J* = 7.5 Hz), 8.59 (m, 2H), 14.45 (br s, 1 H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 83.3, 118.4, 120.4, 121.5, 123.1, 124.8, 126.0, 133.9, 136.5, 139.9, 140.7, 143.2, 167.8, 175.5.

4.1.20. General procedure for the synthesis of *N*-(2-diethylaminoethyl)-9-chloro-iodoacridine-4-carboxamides 35a–c

A suspension of 9,10-dihydro-iodo-9-oxoacridine-4-carboxylic acid **23** (100 mg, 0.27 mmol) in thionyl chloride (5 mL) containing dimethylformamide (1 drop) was heated gently under reflux with stirring until homogeneous and then for a further 45 min. The solution was evaporated to dryness under vacuum below 40 °C, and residual traces of thionyl chloride were removed by addition of dry toluene (5 mL) and complete reevaporation of all solvents to give 3-chloroacridine-4-carbonyl chloride **34a–c**. The above product in a 5% NEt_3/THF solution (2 mL) was cooled to −5 °C and to this was added in one portion an ice-cold solution of *N*,*N*-diethylethylenediamine (40 μL, 0.28 mmol) in dry dichloromethane (1 mL). The solution was stirred at room temperature for 2 h. The organic layers were washed twice with an aqueous 10% sodium carbonate solution (20 mL), once with a saturated aqueous sodium chloride solution (20 mL) and evaporated under vacuum. The unstable precipitate obtained was used without purification.

4.1.20.1. *N*-(2-Diethylaminoethyl)-9-chloro-2-iodoacridine-4-carboxamide (35a). From **23e**. Yield 80%; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.23 (t, 6H, *J* = 7 Hz), 2.95 (m, 6H), 3.93 (m, 2H), 7.70 (t, 1H, *J* = 7.5 Hz), 7.91 (t, 1H, *J* = 7.5 Hz), 8.38 (m, 2H), 8.98 (d, 1H, *J* = 2 Hz), 9.12 (d, 1H, *J* = 2 Hz), 11.77 (br s, 1H).

4.1.20.2. *N*-(2-Diethylaminoethyl)-9-chloro-5-iodoacridine-4-carboxamide (35b). From **23a** Yield 93%; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.38 (t, 6H, *J* = 7 Hz), 3.17 (q, 4H, *J* = 7 Hz), 3.37 (m, 2H), 4.22 (m, 2H), 7.41 (dd, 1H, *J* = 9, 7 Hz), 7.78 (dd, 1H, *J* = 8.5, 7 Hz), 8.44 (d, 1H, *J* = 8.5 Hz), 8.51 (d, 1H, *J* = 7 Hz), 8.60 (d, 1H, *J* = 8.5 Hz), 8.99 (d, 1H, *J* = 7 Hz), 12.40 (m, 1H).

4.1.20.3. *N*-(2-Diethylaminoethyl)-9-chloro-7-iodoacridine-4-carboxamide (35c). From **23c**. Yield 96%; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.12 (t, 6H, *J* 8 7 Hz), 2.75 (m, 6 H), 3.77 (q, 2H, *J* = 6 Hz), 7.72 (dd, 1H, *J* = 8.5, 7 Hz), 8.00 (m, 2H), 8.49 (d, 1H, *J* = 8.5 Hz), 8.74 (s, 1H), 8.98 (d, 1H, *J* = 7 Hz), 11.60 (m, 1H).

4.1.21. General procedure for the synthesis of *N*-(2-diethylaminoethyl)-iodo-9-(4′-methanesulfonamido-2′-methoxyanilino)acridine-4-carboxamides dihydrochloride salt 10a–c

To a solution of the appropriate 9-chloroacridine **35** (0.22 g, 0.46 mmol) in dry ethanol (10 mL), under argon atmosphere, was added 4-methanesulfonamido-2-methoxyaniline[43] (**36**) (97 mg, 0.45 mmol) and concentrated hydrochloric acid (40 μL). The mixture was refluxed for 17 h and allowed to room temperature. An aqueous saturated sodium carbonate solution (20 mL) was added and the mixture was extracted with dichloromethane (3× 50 mL). The combinated organic layers were dried ($MgSO_4$), filtered and evaporated under *vacuum*. The product was chromatographed (Al_2O_3, CH_2Cl_2/EtOH, 99:1, v/v). The red precipitate was diluted with dichloromethane (0.5 mL) and treated with an anhydrous 2 N hydrochloric acid–ether solution (2 mL). The solution was concentrated under vacuum started again with dry ether (3 mL). The suspension was stirred overnight and filtered.

4.1.21.1. *N*-(2-Diethylaminoethyl)-2-iodo-9-(4′-methanesulfonamido-2′-methoxyanilino)acridine-4-carboxamide dihydrochloride salt (10a). From **35a**. Yield 28%; mp 235–237 °C; IR (KBr) 3500–3200, 2933, 1618, 1510, 1323, 1150 cm^{-1}; MS (base) *m/z* 661 (M^+, 1), 582 (11), 509 (11), 86 (100), 58 (21); ^1H NMR (DMSO-d_6, 400 MHz) δ 1.29 (t, 6H, *J* = 7 Hz), 3.14 (s, 3H), 3.24 (m, 4H), 3.37 (m, 2H), 3.48 (s, 3H), 3.80 (m, 2H), 7.04 (d, 1H, *J* = 8.5 Hz), 7.09 (s, 1H), 7.46 (t, 1H, *J* = 7.5 Hz), 7.52 (d, 1H, *J* = 8 Hz), 8.00 (t, 1H, *J* = 7.5 Hz), 8.16 (d, 1H, *J* = 8.5 Hz), 8.20 (br s, 1H), 8.85 (s, 1H), 8.89 (br s, 1H), 9.80 (br s, 1H), 10.20 (s, 1H), 10.75 (br s, 1H), 11.75 (m, 1H), 13.83 (br s, 1H); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 8.5 (2C), 34.7, 39.5, 46.6 (2C), 49.8, 56.0, 87.0, 104.1, 112.0, 113.7, 116.0, 120.4, 121.8, 123.8, 124.8, 125.2, 127.7, 136.2, 137.3, 137.6, 139.1, 140.4, 142.7, 153.6, 155.3, 166.3. Anal. Calcd for $C_{28}H_{32}IN_5O_4S$, 2HCl, 2.5H_2O: C, 43.14; H, 5.04; N, 8.98. Found: C, 43.06; H, 4.74; N, 8.90.

4.1.21.2. *N*-(2-Diethylaminoethyl)-5-iodo-9-(4′-methanesulfonamido-2′-methoxyanilino)acridine-4-carboxamide dihydrochloride salt (10b). From **35b**. Yield 38%; mp 212–214 °C; IR (KBr) 3500–3200, 3100–2900, 1613, 1585, 1513, 1325, 1151 cm $^{-1}$; MS (base) *m/z* 661 (M^+, 1), 582 (9), 509 (15), 446 (13), 383 (12), 86 (100); ^1H NMR (DMSO-d_6, 400 MHz) δ 1.28 (t, 6H, *J* = 7 Hz), 3.13 (s, 3H), 3.25 (q, 4H, *J* = 7 Hz), 3.40 (m, 2H), 3.48 (s, 3H), 3.85 (m, 2H), 7.02 (m, 2H), 7.21 (t, 1H, *J* = 8 Hz), 7.55 (m, 2H), 8.39 (m, 1H), 8.54 (m, 2H), 8.89 (d, 1H, *J* = 7 Hz), 10.06 (s, 1H), 10.25 (br s, 1H), 10.81 (m, 1H), 12.00 (br s, 1H), 14.70 (br s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 8.3 (2C), 34.4, 39.5, 46.4 (2C), 49.5, 55.7, 89.5, 103.7, 111.6, 113.8, 114.2, 117.8, 122.7, 123.5, 125.2, 125.4, 127.5, 129.6, 135.9, 138.9, 139.3, 140.0, 146.0, 153.2, 156.9, 167.6. Anal. Calcd for $C_{28}H_{32}IN_5O_4S$, 2HCl, 3H_2O: C, 42.65; H, 5.11; N, 8.88. Found: C, 42.72; H, 4.81; N, 8.68.

4.1.21.3. *N*-(2-Diethylaminoethyl)-7-iodo-9-(4'-methanesulfonamido-2′-methoxyanilino)acridine-4-carboxamide dihydrochloride salt (10c). From **35c**. Yield 33%; mp 208–210 °C; IR (KBr) 3500–3300, 2926, 1618, 1586, 1511, 1324, 1150 cm^{-1}; MS (base) *m/z* 661 (M^+, 7), 582 (47), 509 (62), 466 (29), 439 (21), 383 (17), 86 (100), 58 (13); ^1H NMR (DMSO-d_6, 400 MHz) δ 1.28 (t, 6H, *J* = 7 Hz), 3.13 (s, 3H), 3.24 (m, 4H), 3.37 (m, 2H), 3.49 (s, 3H), 3.80 (m, 2H), 7.06 (d, 1H, *J* = 8.5 Hz), 7.09 (s, 1H), 7.54 (m, 2H), 7.97 (d, 1H, *J* = 9 Hz), 8.19 (d, 1H, *J* = 9 Hz), 8.58 (m, 2H), 8.70 (d, 1H, *J* = 7.5 Hz), 9.95 (t, 1H, *J* = 6 Hz), 10.26 (s, 1H), 10.85 (m, 1H), 11.80 (m, 1H), 14.04 (br s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 8.3 (2C), 34.3, 39.5, 46.4 (2C), 49.5, 55.7, 89.2, 103.7, 111.7, 114.2, 114.8, 119.9, 121.9, 122.7, 123.4, 127.5, 129.1, 133.3, 135.7, 137.9, 138.2, 140.2, 143.2, 153.3, 155.0, 167.2. Anal. Calcd for $C_{28}H_{32}IN_5O_4S$, 2HCl, 2H_2O: C, 43.65; H, 4.97; N, 9.09. Found: C, 43.37; H, 4.64; N, 8.93.

4.1.22. Ethyl *N*-(4-amino-5-iodo-2-methoxybenzene)carbamate (40)

To a solution of *N*-(2-methoxy-4-aminobenzene)carbamate (**39**)[44] (0.40 g, 1.90 mmol) in a mixture of methanol (15 mL) and dichloromethane (40 mL) was added benzyltriethylammonium dichloroiodate ($BTEAICl_2$)[49] (0.81 g, 2.09 mmol) and calcium carbonate (0.32 mg, 3.08 mmol). The solution was stirred at room temperature for ¾ h. After return back to room temperature, the mixture was filtered on Celite® 521 and concentrated to 1/3. An aqueous 5% sodium bicarbonate solution (10 mL) was added and the layers were separated. The organic phase was washed with an aqueous saturated sodium bicarbonate solution (10 mL), water

(10 mL) and an aqueous saturated sodium chloride solution (10 mL). The organic layers were dried ($MgSO_4$), filtered and evaporated under vacuum. The product was chromatographed (Al_2O_3, EtOAc/pentane, 7:3, v/v). Yield 16%; R_f = 0.79 (Al_2O_3, EtOAc/pentane, 7:3, v/v); viscous oil; IR (CCl_4) 3500–3200, 1529, 1221 cm^{-1}; MS *m/z* 336 (M^+, 100), 290 (16), 264 (18), 249 (18), 108 (32), 94 (20), 52 (20); ^1H NMR ($CDCl_3$, 200 MHz) δ 1.29 (t, 3H, *J* = 7 Hz), 3.78 (s, 3H), 4.01 (s, 2H), 4.19 (q, 2H, *J* = 7 Hz), 6.31 (s, 1H), 6.84 (br s, 1H), 8.25 (br s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.7, 55.8, 61.2, 72.8, 97.8, 120.5, 128.4, 142.8, 149.8, 153.7.

4.1.23. Ethyl *N*-(5-iodo-4-methanesulfonamido-2-methoxybenzene)carbamate (41)

To a solution of amine **40** (0.20 g, 1.90 mmol) in dry pyridine (5 mL), was added over 30 min, at −15 °C, under argon gas, methane sulfonyl chloride (0.16 mL, 2.09 mmol) keeping the temperature below −5 °C. The mixture was stored at 4 °C overnight. The solution was concentrated to 2/3. The excess of pyridine was neutralised by concentrated hydrochloric acid. The aqueous solution was extracted with dichloromethane (3× 30 mL). The combinated organic layers were dried ($MgSO_4$), filtered and evaporated under vacuum. The crude product was chromatographed (Al_2O_3, CH_2Cl_2). Yield 55%; R_f = 0.08 (Al_2O_3 and CH_2Cl_2); mp 153–155 °C; IR (KBr) 3353, 3195, 1708, 1526, 1337, 1150 cm^{-1}; MS *m/z* 414 (M^+, 40), 335 (100), 291 (13), 263 (40), 136 (12); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.31 (t, 3H, *J* = 7 Hz), 2.94 (s, 3H), 3.87 (s, 3H), 4.21 (q, 2H, *J* = 7 Hz), 6.45 (s, 1H), 7.14 (s, 1H), 7.19 (s, 1H), 8.53 (br s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.5, 39.8, 56.1, 61.6, 82.5, 106.8, 126.9, 127.5, 132.2, 148.6, 153.3.

4.1.24. 5-Iodo-4-methanesulfonamido-2-methoxyaniline (38)

A solution of carbamate **41** (0.60 g, 1.45 mmol) in an aqueous 10% sodium hydroxide solution was refluxed for 1.5 h. After cooling to room temperature the reaction mixture was acidified to pH 7–8 with concentrated hydrochloric acid and extracted with dichloromethane (4× 50 mL). The organic layers were dried ($MgSO_4$) and evaporated under vacuum to dryness to give the anilino compound **38** which was used without purification. Yield 89%; mp 110–112 °C; IR (KBr) 3430, 3356, 1723, 1529, 1221 cm^{-1}; MS *m/z* 342 (M^+, 19), 263 (100), 136 (18), 121 (12); ^1H NMR (DMSO-d_6, 400 MHz) δ 2.96 (s, 3H), 3.75 (s, 3H), 5.07 (s, 2H), 6.76 (s, 1H), 7.08 (s, 1H), 8.94 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 40.9, 55.5, 89.7, 111.7, 121.7, 126.0, 138.8, 146.3.

4.1.25. *N*-(2-Diethylaminoethyl)-9-(5′-iodo-4′-methanesulfonamido-2′-methoxyanilino)acridine-4-carboxamide hydrochloride salt (10d)

This compound was prepared from **37** [48] and **38** by similar procedure using for **10a–c**. Yield 33%; mp 213–215 °C; IR (KBr) 3500–3300, 2927, 1623, 1572, 1522, 1320, 1151 cm^{-1}; MS (base) *m/z* 661 (M^+, 2), 582 (24), 509 (25), 456 (74), 383 (73), 340 (40), 128 (27), 86 (100), 58 (19); ^1H NMR (DMSO-d_6, 400 MHz) δ 1.28 (t, 6H, *J* = 7 Hz), 3.16 (s, 3H), 3.24 (q, 4H, *J* = 7 Hz), 3.38 (m, 2H), 3.47 (s, 3H), 3.82 (m, 2H), 7.15 (s, 1H), 7.48 (m, 1H), 7.57 (m, 1H), 8.02 (m, 1H), 8.11 (s, 1H), 8.19 (d, 1H, *J* = 8 Hz), 8.28 (d, 1H, *J* = 8 Hz), 8.61 (d, 1H, *J* = 8 Hz), 8.74 (d, 1H, *J* = 7 Hz), 9.50 (br s, 1H), 9.95 (br s, 1H, NH), 10.86 (br s, 1H), 11.80 (br s, 1H), 14.20 (br s, 1H); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 8.3 (2C), 34.3, 41.4 (2C), 46.4, 49.5, 56.0, 86.4, 111.1, 113.6, 114.5, 119.7, 120.2, 122.6, 124.5, 124.9, 128.0, 129.1, 133.6, 135.9 (2C), 138.3, 138.8, 139.2, 153.0, 156.0, 167.3. Anal. Calcd for $C_{28}H_{32}IN_5O_4S$, 2HCl, $2H_2O$: C, 43.65; H, 4.97; N, 9.09. Found: C, 43.51; H, 4.76; N, 9.03.

4.1.26. Iodation of methyl acridine-9-carboxylate (42)

To a solution of methyl acridine-9-carboxylate[50] (**42**) (400 mg, 1.69 mmol) in a mixture of concentrated sulfuric acid (80 μL), water (320 μL) and glacial acetic acid (1 mL), were added periodic acid dihydrate (76 mg, 0.33 mmol) and iodine (216 mg, 0.85 mmol). The mixture was refluxed for 8 h. After cooling to room temperature, the solution was made alkaline with an aqueous saturated sodium carbonate solution and extracted with dichloromethane (3× 50 mL). The organic phases were dried ($MgSO_4$), filtered and the solvent removed under reduced pressure. The crude product was chromatographed (SiO_2, CH_2Cl_2) to give in order of elution. *Methyl 2,7-diiodoacridine-9-carboxylate* (***43b***); yield 6%; R_f = 0.17 (SiO_2 and CH_2Cl_2); mp 201–203 °C; IR (KBr) 1720, 1220 cm^{-1}; MS *m/z* 489 (M^+, 100), 458 (29), 430 (18), 347 (10), 303 (17), 164 (14); ^1H NMR ($CDCl_3$, 400 MHz) δ 4.24 (s, 3H), 8.04 (m 4H), 8.42 (d, 2H, *J* = 2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 53.5, 94.7 (2C), 123.9 (2C), 130.8 (2C), 134.1 (2C), 139.8, 146.8 (2C), 166.7. *Methyl 2-iodoacridine-9-carboxylate* (***43a***); yield 16%; R_f = 0.11 (SiO_2 and CH_2Cl_2); mp 106–108 °C; IR (KBr) 1728, 1218 cm^{-1}; MS *m/z* 363 (M^+, 100), 332 (33), 304 (28), 177 (33); ^1H NMR ($CDCl_3$, 400 MHz) δ 4.20 (s, 3H), 7.58 (m, 1H), 7.79 (m, 1H), 7.95 (m, 2H), 7.98 (d, 1H, *J* = 8 Hz), 8.20 (d, 1H, *J* = 8.5 Hz), 8.39 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 53.2, 93.6, 122.5, 123.7, 125.3, 127.8, 130.0, 130.7, 131.3, 134.0, 135.4, 138.9, 147.1, 148.8, 167.4.

4.1.27. *N*-(2-Diethylaminoethyl)iodoacridine-9-carboxamides 44a,b

Compound **44a**,**b** were prepared as described for the synthesis of carboxamides **24a–g**.

4.1.27.1. *N*-(2-Diethylaminoethyl)-2-iodoacridine-9-carboxamide (44a). From **43a**. Yield 58%; R_f = 0,61 (Al_2O_3, EtOAc/EtOH, 99:1, v/v); mp 83–85 °C; IR (KBr) 3400–3200, 2956, 1636 cm^{-1}; MS *m/z* 447 (M^+, 1), 177 (3), 86 (100), 58 (9); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.00 (t, 6H, *J* = 7 Hz), 2.58 (q, 4H, *J* = 7 Hz), 2.78 (t, 2H, *J* = 6 Hz), 3.79 (q, 2H, *J* = 6 Hz), 6.85 (m, 1H), 7.55 (m, 1H), 7.80 (m, 1H), 7.91 (d, 1H, *J* = 9 Hz), 7.96 (d, 1H, *J* = 9 Hz), 8.08 (d, 1H, *J* = 9 Hz), 8.17 (d, 1H, *J* = 8.5 Hz), 8.48 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 11.6 (2C), 37.6, 46.8 (2C), 51.8, 93.1, 122.3, 123.6, 125.6, 127.3, 129.7, 130.7, 131.1, 134.2, 138.9, 140.1, 147.2, 148.7, 166.5.

4.1.27.2. *N*-(2-Diethylaminoethyl)-2,7-diiodoacridine-9-carboxamide (44b). From **43b**. Yield 57%; R_f = 0,72 (Al_2O_3, EtOAc/EtOH, 99:1, v/v); mp 209–211 °C; IR (KBr) 3500-3300, 2924, 1637 cm^{-1}; MS *m/z* 573 (M^+, 1), 458 (2), 430 (3), 303 (7), 86 (100), 58 (12); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.03 (t, 6H, *J* = 7 Hz), 2.63 (q, 4H, *J* = 7 Hz), 2.80 (t, 2H, *J*– 6 Hz), 3.81 (q, 2H, *J* = 6 Hz), 6.90 (m, 1H), 7.88 (d, 2H, *J* = 9 Hz), 7.98 (d, 2H, *J* = 9 Hz), 8.46 (s, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.7 (2C), 37.8, 46.7 (2C), 51.7, 94.1 (2C), 123.6 (2C), 130.9 (2C), 134.4 (2C), 138.9, 139.3 (2C), 147.1 (2C), 165.9.

4.1.28. *N*-(2-Diethylaminoethyl)iodoacridine-9-carboxamides dihydrochloride salts 11a,b

Copmound **11a**,**b** were prepared as described for the synthesis of hydrochloride salts **8a–g**.

4.1.28.1. *N*-(2-Diethylaminoethyl)-2-iodoacridine-9-carboxamide dihydrochloride salt (11a). From **44a**. Yield 78%; mp 121–123 °C; IR (KBr) 3500–3200, 2976, 2600–2400, 1654, 1464, 1420 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.29 (t, 6H, *J* = 7 Hz), 3.22 (m, 4H), 3.41 (m, 2H), 3.96 (m, 2H), 7.79 (m, 1H), 8.06 (t, 1H, *J* = 7.5 Hz), 8.13 (m, 2H), 8.24 (d, 1H, *J* = 9 Hz), 8.33 (d, 1H, *J* = 9 Hz), 8.45 (s, 1H), 9.55 (t, 1H, *J* = 6 Hz), 10.79 (m, 1H); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 8.5 (2C), 34.3, 46.6 (2C), 49.3, 94.6, 122.0, 123.2, 126.2, 126.7, 128.8, 128.5, 133.0, 133.9, 140.6, 142.9, 144.4, 146.0, 165.3. Anal. Calcd for $C_{20}H_{22}IN_3O$, 2HCl, $1.5H_2O$: C, 43.90; H, 4.97; N, 7.68. Found: C, 43.73; H, 4.72; N, 7.58.

4.1.28.2. *N*-(2-Diethylaminoethyl)-2,7-diiodoacridine-9-carboxamide dihydrochloride salt (11b). From **44b**. Yield 92%; mp 224–226 °C; IR (KBr) 3500–3200, 2600–2300, 2575, 1663, 1420 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 1.31 (t, 6H, J = 7 Hz), 3.24 (m, 4H), 3.42 (m, 2H), 3.96 (m, 2H), 8.03 (d, 2H, J = 9 Hz), 8.21 (d, 2H, J = 9 Hz), 8.42 (s, 2H), 9.56 (m, 1H), 10.74 (br s, 1H); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 8.6 (2C), 34.4, 46.6 (2C), 49.3, 95.3 (2C), 123.4 (2C), 129.6 (2C), 133.9 (3C), 140.3 (2C), 145.5 (2C), 165.0. Anal. Calcd for $C_{20}H_{22}IN_3O$, 2HCl: C, 37.18; H, 3.59; N, 6.50. Found: C, 37.57; H, 3.76; N, 6.18.

4.1.29. General procedure for the synthesis of tributylstannyl compounds 45, 46 and 49

A mixture of the appropriate iodo compound **24f**, **30d** or **48** (0.22 mmol) in dry toluene (5 mL) was degassed and hexabutylditin (351 μL, 0.66 mmol) and freshly prepared tetrakis(triphenylphosphine)palladium(0) ($Pd(PPh_3)_4$)[56] (20 mg) were added under an argon atmosphere. The resulting solution was stirred under reflux, until no starting material was observed by TLC. The black reaction mixture was evaporated.

4.1.29.1. *N*-(2-Diethylaminoethyl)-9,10-dihydro-9-oxo-7-tributylstannylacridine-4-carboxamide (45). From **24f**. Reaction time at reflux 4 h. The crude product was chromatographed (Al_2O_3 and EtOAc). Yield 69%; R_f = 0.20 (Al_2O_3 and EtOAc); viscous oil; IR (CCl_4) 2960, 2928, 1652, 1608, 1508, 1150 cm^{-1}; ESI-MS m/z 628,2 $[M+H]^+$; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.88 (t, 9H, J = 7 Hz), 1.05 (m, 12H), 1.24 (m, 6H), 1.55 (m, 6H), 2.57 (q, 4H, J = 7 Hz), 2.70 (t, 2H, J = 7 Hz), 3.52 (m, 2H), 7.19 (t, 1H, J = 8 Hz), 7.37 (d, 1H, J = 8 Hz), 7.52 (br s, 1H), 7.75 (d, 1H, J = 8 Hz), 7.90 (d, 1H, J = 8 Hz), 8.54 (s, 1H), 8.65 (d, 1H, J = 8 Hz), 12.40 (br s, 1H);^{13}C NMR ($CDCl_3$, 50 MHz) δ 9.9, 12.1 (2C), 13.7, 27.4 ($^3J_{^{119}Sn-^{13}C}$ = 20 Hz), 29.2 ($^2J_{^{119}Sn-^{13}C}$ = 20 Hz), 37.3, 46.9 (2C), 51.1, 117.2, 117.8, 119.5, 121.1, 123.3, 131.3, 131.8, 134.8, 135.1, 140.4, 141.3, 141.4, 168.4, 178.2.

4.1.29.2. *N*-(2-Diethylaminoethyl)-5-tributylstannylacridine-4-carboxamide (46). From **30d**. Reaction time at reflux 32 h. The crude product was chromatographed (Al_2O_3, EtOAc/pentane, 5:5, v/v) to give in order of elution: stannane **46**; yield 25%; R_f = 0.79 (Al_2O_3, EtOAc/pentane, 5:5, v/v); viscous oil; IR (CCl_4) 2959, 2927, 1662, 1286 cm^{-1}; ESI-MS m/z 612,3 $[M+H]^+$; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.79 (t, 9H, J = 7 Hz), 1.3 (m, 18H), 1.45 (m, 6H), 2.76 (m, 4H), 2.88 (m, 2H), 3.73 (m, 2H), 7.54 (t, 1H, J = 7.5 Hz), 7.64 (dd, 1H, J = 7.5, 8.5 Hz), 8.00 (m, 2H), 8.15 (d, 1H, J = 8.5 Hz), 8.88 (s, 1H), 8.93 (d, 1H, J = 7.5 Hz), 10.59 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 10.1, 12.2, 13.7, 27.4 ($^3J_{^{119}Sn-^{13}C}$ = 60 Hz), 29.3 ($^2J_{^{119}Sn-^{13}C}$ = 20 Hz), 39.1, 47.8, 52.1, 125.1, 126.0, 126.5, 127.0, 128.8, 129.3, 132.7, 135.3, 139.3, 141.0, 145.0, 146.4, 153.0, 166.7. N-(2-diethylaminoethyl)-acridine-4-carboxamide[40] (***47***); yield 29%; R_f = 0.33 (Al_2O_3, EtOAc/pentane, 5:5, v/v)); mp 148–150 °C.

4.1.29.3. *N*-(2-Diethylaminoethyl)-7-tributylstannyl-9-(4′-methanesulfonamido-2′-methoxyanilino)acridine-4-carboxamide (49). The dihydrochloride **10c** (0.13 g, 0.50 mmol) was diluted with water (5 mL), and treated with an aqueous saturated sodium carbonate solution (20 mL). The aqueous solution was extracted with dichloromethane (3× 10 mL). The organic layers were dried (Na_2SO_4) and concentrated under vacuum to give compound **48**. Product **49** was obtained from **48** according to the procedure developed for compound **45** with reaction time at reflux 4.5 h. The crude product was chromatographed (Al_2O_3, EtOAc/EtOH, 96:4, v/v). Yield 34%; R_f = 0.48 (Al_2O_3, EtOAc/EtOH, 96:4, v/v), mp 139–141 °C; IR (KBr) 2924, 1592, 1507, 1457, 1152 cm^{-1}; ESI-MS m/z 826.5 $[M+H]^+$.

4.2. Radiolabelling

4.2.1. General methods

[^{125}I]NaI (3.7 GBq/mL, 644 MBq/μg) was purchased from Amersham Int. Plc. (Little Chalfont, Buckinghamshire, UK) as a 'no carrier added' solution in reductant free 0.1 N aqueous sodium hydroxide solution. Extrelut and citrate buffer solution (pH 4) were purchased from Merck (Darmstadt, Germany). The radio-TLC strips (Merck neutral aluminium oxide 60F_{254} plates) were developed with (Al_2O_3 and EtOAc) for [^{125}I]**8f**, (Al_2O_3, EtOAc/pentane, 5:5, v/v) for [^{125}I]**9d** or (Al_2O_3, EtOAc/EtOH, 96:4, v/v) for [^{125}I]**10c**, and measured on an AMBIS 400 system (Scanalytics, CSPI, San Diego, CA, USA). Analytical HPLC was run on a system consisting of an HP1100 chromatograph (Hewlett–Packard, Les Ulis, France) and a Flow one A_{500} Radiomatic detector (Packard, Canberra, Australia). Separation was carried out on a C_{18} column (Purospher RP_{18} e, 5 μm) under the following conditions: gradient time = 10 min, flow rate = 0.5 mL/min, H_2O/MeOH/(30:70 → 0:100) (NH_4OH 0.2%), λ = 254 nm. HPLC purification was performed on a system including a Shimadzu LC 6A pump, an SLC 6B controller, a CR_5A integrator, a SPD 6AV UV detector and a flow-through Raytest Steffi gamma detector. The separation was carried out on a C_{18} column (ZORBAX 80 Å, 4.6×150 mm) under the following conditions: gradient time = 10 min, flow rate = 1 mL/min, H_2O/MeOH/(30:70 → 0:100) (NH_4OH 0.2%), λ = 254 nm. All radiolabelled compounds were shown identical by TLC or HPLC to the authentic non-radioactive material and to be free of significant chemical and radiochemical impurities.

4.2.2. Preparation of radioiodinated compounds [^{125}I]8f, [^{125}I]9d and [^{125}I]10c

The selected [^{125}I]-labelled compounds were prepared using iododestannylation reactions with tributyltin precursors **45**, **46** and **49**. In a closed vial containing the appropriate stannane (0.12 mg) in EtOH (30 μL) were added, in the following order: a 1% acetic acid/ethanol solution (30 μL) (for compound **45** or **49**) or a citrate buffer solution pH 4 (30 μL) (for compound **46**), [^{125}I]NaI (20–50 μL, 55.5–138.8 MBq) and an aqueous solution of chloramine-T monohydrate (15 μL, 0.4 mg/mL for **45**, 0.5 mg/mL for **46** and 0.25 mg/mL for **49**). The resulting solution was vortexed at room temperature for 10 min. The reaction was quenched with an aqueous 0.1 N NaOH solution (20 μL). The mixture was vortexed for 5 min and the vial cap and septum were removed. The reaction mixture was transferred to an Extrelut® column and the vial was rinsed with a solution of H_2O/EtOH (1:1, v/v, 2× 100 μL). After 10 min, the column was eluted with dichloromethane (5× 2 mL). The organic extracts were collected were evaporated under reduced pressure, taken up with methanol (200 μL), and purified by HPLC at a flow rate of 1 mL/min. The fractions containing the product were collected, evaporated to dryness and re-dissolved in dichloromethane (1 mL). An anhydrous 2 N HCl/ether solution (2 mL) was added and the total solution was evaporated to yield the expected [^{125}I]compound. Radiochemical yields based on TLC of exchange reaction mixture and radiochemical purities are given in Table 3.

4.3. Pharmacology

4.3.1. Partition coefficient measurements

The partition coefficient between *n*-octanol and phosphate buffer was determined for each molecule. The measurement was performed by shaking 2 mL of [^{125}I]molecule solution (50 μM in phosphate buffer solution, PBS, pH 7.4) with 2 mL of n-octanol. The activity of each phase was measured. *P* was calculated as the ratio of activities (*n*-octanol/buffer), and its logarithm was determined in order to express lipophilicity.

4.3.2. In vitro binding to melanin

An in vitro experiment was performed to evaluate the binding affinity of new compounds to melanin using synthetic tyrosine-melanin (Sigma) suspended in two different media: H_2O or PBS. The general procedure used was as follows: [^{125}I]compound was added to the melanin suspension (0.5 mg/10 mL). The reaction mixture was incubated at room temperature for 24 h with stirring. After incubation, the tubes were centrifuged at 35,000*g* for 20 min, and aliquots of the supernatants were counted on the gamma counter (Packard, Minaxi γ 5530, Rungis, France).

4.3.3. Cytotoxicity assays

4.3.3.1. Cell culture. Normal human fibroblasts were purchased from Biopredic International (Rennes, France). This frozen culture was obtained from abdominal surgical waste from a 36-year-old female and the cells used in this work were from the 7th to 12th passage of the culture. M4Beu, a human melanoma cell line, was established in the laboratory of Dr. J. F. Doré (INSERM, Unit 218, Lyon, France) from metastatic biopsy specimens and maintained in cell culture for roughly 15 years. Colon adenocarcinoma DLD-1 cells, Jurkat leukaemia cells, and B16F0 murine melanoma cell line cells were purchased from the European Collection of Cell Cultures (ECACC; Salisbury, United Kingdom). Stock cell cultures were maintained as monolayers in 75-cm^2 culture flasks in Eagle's minimum essential medium with Earle's salts (MEM; Gibco–BRL, Invitrogen, Cergy-Pontoise, France) or Dulbecco modified MEM (DMEM, Gibco) for B16F0 supplemented with 10% foetal calf serum (Sigma), and 5 mL of a 100× solution of vitamins (Gibco), 5 mL of 100 mM sodium pyruvate (Gibco), 5 mL of 100× non-essential amino acids (Gibco) and 2 mg of gentamicin base (Gibco). Cells were grown in a humidified 37 °C incubator containing 5% CO_2.

4.3.3.2. Cell survival assays. Two test were used: the resazurin reduction test (RRT) for measuring cellular redox potential, and the Hoechst dye 33342 test for DNA quantization. Cells were plated at a density of 5×10^3 cells per well in 96-well microplates (Nunclon™, Nunc, Roskilde, Denmark) in 100 μL of culture medium and were allowed to adhere for 16 h before treatment with each drug. Stock solution (200×) of each drug was prepared in DMSO (Sigma) and kept at −20 °C until use. Each set of experimental conditions was tested in triplicate. After 48 h of continuous drug exposure, the anti-proliferative effect of each drug was assessed by the RRT and then, cells were frozen and assessed by the Hoechst test as previously described.[57]

RRT. Briefly, RRT was carried out as follows: plates were rinsed with 200 μL PBS (37 °C, Gibco) at 37 °C to which was added 150 μL of a 25 μg/mL (20 μL of a 275 μg/mL for Jurkat cells) solution of resazurin in MEM without phenol red. The plates were incubated for approximately 1 h (6 h for Jurkat cells) at 37 °C in a humidified atmosphere with 5% CO_2 for fluorescence development by living cells. Fluorescence was then measured on the Fluoroskan Ascent FL™ (Labsystems) automated 96-well plate reader using an excitation wavelength of 590 nm and an emission wavelength of 630 nm. In the conditions used here, fluorescence was proportional to the number of living cells in the well. Cell survival percentage is defined as the ratio of fluorescence in drug-treated wells to fluorescence in control wells, after subtraction of blank values. After reading, the plates were emptied and stored at −80 °C for Hoechst dye test.

Hoechst dye 33342 test. Plates were thawed at room temperature for 10 min. A 0.01% (m/v) SDS solution (100 μL) in distilled water was then distributed into each well, and the plates were incubated for 1 h at room temperature and refrozen for 1 h at −80 °C. After thawing, 100 μL of Hoechst dye 3342 solution at 30 μg mL^{-1} in a hypersaline buffer (10 mM Tris–HCl, pH 7.4, 1 mM EDTA and 2 M NaCl) were added to each well. The plates were maintained in dark conditions and incubated in this solution on a plate shaker for 1 h at room temperature. Fluorescence was then measured at 360/460 nm on a microplate fluorescence reader and the IC_{50} was calculated as described above.

4.3.4. In vivo distribution

All experiments were carried out in compliance with the French laws governing animal experimentation. The biodistribution of radioiodinated compounds was studied in C57BL6 male mice bearing the B16F0 murine melanoma.

4.3.4.1. Tumoural model. For transplantation, an aliquot was grown in a monolayer culture to confluence, after which the cells were trypsinised and washed with phosphate buffer saline (PBS) before being resuspended in PBS. Each mouse was subcutaneously administered 0.1 mL (3×10^5 cells) on the left flank. Ten days later, the tumours became palpable, with 98–100% of tumours taken.

4.3.4.2. Quantitative biodistribution on whole body slides. The [^{125}I]molecule was administered intravenously via a tail vein (0.74–0.92 MBq/animal) in 10 animals for each compound. After the injections, two mice were sacrificed by CO_2 inhalation and quickly frozen in liquid nitrogen at different times after administration (1, 3, 6, 24 or 72 h). These frozen animals were cryosectioned using the technique described by Ullberg.[58] Slices of 40 μm were obtained using a Reichert-Jung cryopolycut (Leica Instruments, Rueil Malmaison, France) at −22 °C and dehydrated for 48 h in the cryochamber. Eight slices per mouse and per time-point were selected from a total number of more than 500 slices, for the analyses on distant slices and the section of the organs of interest. The radioactivity contained in the slices was analysed using an AMBIS 4000 detector (Scanalytics, CSPI, San Diego, CA), which is a computer-controlled multi-wire proportional counter validated for evaluation of iodinated agents in mice.[59] Measurements were performed following a 1000-min acquisition time. The quantification of radioactivity in different organs was made after contouring a suitable area on the 2D image of the slice displayed on the computer screen for analysis. Radioactivity per unit area (net cpm/mm^2) was converted into radioactive concentration (kBq/g) and expressed as percentage of injected dose/g of tissue (% ID/g). For comparison of tumour (T) uptake with other tissues, ratios of radioactive concentrations (T/organ) were determined to illustrate the image contrast.

For two animals, urine and faeces were collected until 72 h and counted to determine the cumulative urinary and faecal excretions.

4.3.4.3. Scintigraphic imaging. For each selected compound, the in vivo kinetic profile was tracked in two mice by repeated planar scintigraphic imaging (3 MBq, acquisition time: 10 min) using a gamma camera dedicated for small animals (Biospace, Paris, France). This study made it possible to follow tumoural uptake kinetics in the same animal.

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