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Novel fluorinated acridone derivatives. Part 1: Synthesis and evaluation as potential anticancer agents

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ABSTRACT

We report on the synthesis of a novel series of fluorinated acridones from 5-trifluoromethyl-1,3-cyclohexanedione. The cytotoxic activities of the compounds were studied in several cancer cells. Compounds **9a**, **9c**, **9e**, **9f**, and **9h** exhibited significant anticancer activities in selected cell lines. Compound **9c** is the most active showing GI_{50} that ranged in values from 0.13 to 26 μM, covering a wide range of cancer cell lines.

Cancer, the uncontrolled, rapid, and pathological proliferation of abnormal cells, is the leading cause of human death, after cardiovascular diseases.[1] Over 1 million cases of cancer occur in the United States annually and cancer-related deaths are estimated to reach 12 million world-wide, by the year 2015. Despite considerable progress in the understanding of its biology and pharmacology, cancer remains a serious health problem. Although there has been increasing sophistication of conventional therapeutic strategies, such as surgery, radiotherapy, and chemotherapy, such approaches are capable of remediating approximately half of cancer patients, while over 40% of patients are still likely to die from the disease.[1]

Therefore, the search for potent, safe, and selective anticancer compounds is a crucial aspect of modern cancer research. Natural products are a rich source of new medicinal leads and, therefore, the preparation of natural product-based libraries of compounds is an important area of research in modern drug discovery.[2] As structural building block in many natural products, the acridine or acridone ring is one of the most commonly encountered heterocycles in medicinal chemistry. It is present in many biologically important molecules, such as amsacrine (**1**) (cytotoxic and antiviral agents),[3a,b] botiacrine (**2**) (antiparkinsonian drug),[3c,d] clomacran (**3**) (tranquilizer),[3e,f] monometacrine (antidepressant),[3g,h] acridine carboxamide (DACA) (**4**) (antitumor),[3i–k] and in natural products such as plakinidine **A** (**5a**) and **B** (**5b**) (anthelmintic),[3l] dercitin (**6**) (anticancer),[3m] glyfoline (**7**) (anticancer),[3n] and acronycine (**8**) (antitumor)[3o] (Fig. 1).

In addition, acridine- and acridone-based derivatives have been mostly studied as anticancer agents,[3p,q] which are believed to express their activity through binding or intercalation of DNA.[3r] The planar area of the tricyclic acridine and acridone nuclei aid in targeting DNA by intercalating between nucleotide base pairs in the helix and the positive charge. Examples that are in clinical trials include amsacrine,[4] anilino acridine derivative asulacrine (or CI-921),[5] and *N*-[(2-dimethyl amino)-ethyl] acridine-4-carboxamide **4** (DACA).[6] DACA (**4**) entered phase I clinical trial on the basis of its mixed inhibition of both DNA topoisomerase-I and topoisomerase-II, and excellent activity against solid tumors.[3i–k]

Other derivatives that have shown interesting antitumor properties are acridone-4-carboxamide,[7] and the bis-functionalized acridone/acridine-4-carboxamides.[8] Although, good correlation between high affinity for DNA and cytotoxicity was observed, the intercalating property was found not to be sufficient for antitumor activities. It was therefore proposed that the antitumor properties of acridines and acridones were not only due to their mode of binding to DNA, but also due to their specific interaction with other nuclear receptors.[9]

It has been shown that the introduction of fluorine(s), difluoromethyl, or trifluoromethyl group to bioactive molecules often leads to improved pharmacological properties,[10–12] due to increased membrane permeability, enhanced hydrophobic bind-

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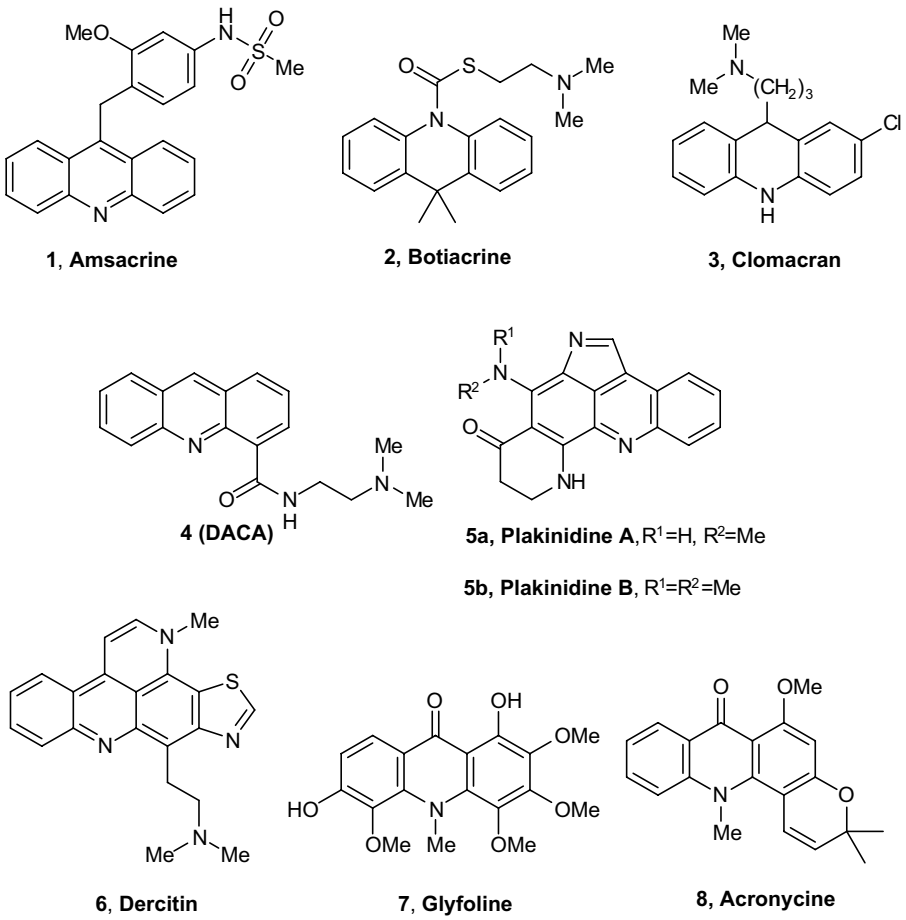


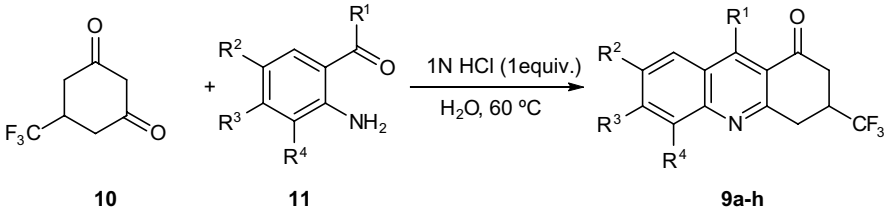
Figure 1. Chemical structures of biologically important acridone- and acridine-based derivatives.

ing, stability against metabolic oxidation, etc. Fluorine, most especially trifluoromethyl can have significant effects on the binding affinity in protein–ligand complexes. This effect can be direct by interaction of the fluorine with the protein, or it can be indirect by modulation of the polarity of other groups of the ligand that interact with the protein. Frequently, it is found that a fluorine substituent leads to a slight enhancement of the binding affinity due to an increased lipophilicity of the molecule that results in an increased affinity for the protein.[13] One typical example is the increased cytotoxicity of taxoids where the phenyl group of the isoserine side chain in docetaxel is replaced by a trifluoromethyl group (CF_3-Ac-docetaxel) resulting in a more than 10-fold increase in antitumor activity. Studies also demonstrated that fluorine substitution has a strong effect on the conformational equilibrium of the side chain in hydrophobic medium.[14]

In continuation of our search for biologically active fluorine-containing compounds, we report in this letter the synthesis and preliminary anticancer activity of a series of new fluorinated acridone derivatives.

The preparation of fluorinated acridone derivatives (**9a–h**) is outlined in Scheme 1.

Recently, we synthesized compound **10**, as a useful intermediate in organic synthesis, as a synthon for the design of biologically active compounds, heterocyclic compounds, and as an efficient building block.[15] The series were prepared using the Friedlander reaction[16] in the presence of a Bronsted acid and water. Typically, a mixture of 5-(trifluoromethyl)cyclohexane-1,3-dione (**10**) and 2-aminoacetophenone or benzophenone (**11**) in 1 N hydrochloric acid was stirred at 60 °C for the designated time (Table 1) as required for the completion of the reaction.[17] The progress of the reaction was monitored by thin layer chromatography (TLC). The cooled reaction mixture was neutralized with aqueous sodium hydroxide (10%), wherein precipitates separated and were filtered to obtain pure crystalline solids.[17] Under the above conditions the reaction proceeded smoothly with almost quantitative yields



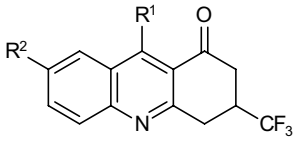
Scheme 1. Synthesis of fluorinated acridone derivatives **9a–h**.

Table 1
Reaction of **10** with benzophenone derivative **11** affording trifluoromethylated acridone **9a–h**

Entry	Compounds	R^1	R^2	R^3	R^4	Time (h)	Yield (%)[a]
1	**9a**	2-Cl-Ph	Cl	H	H	0.5	93
2	**9b**	Ph	H	H	H	0.5	98
3	**9c**	H	Br	H	Br	0.5	90
4	**9d**	Me	O-CH_2-O		H	0.5	94
5	**9e**	Ph	Cl	H	H	0.5	96
6	**9f**	2-F-Ph	Br	H	H	1.0	80
7	**9g**	Ph	NO_2	H	H	2.0	75
8	**9h**	Me	Cl	H	H	0.5	98

[a] Yields refer to pure products.

Table 2
IC_{50} (μM) values on different human cancer cell lines



Compounds	R^1	R^2	Cell lines (IC_{50} ± SEM, μM)			
			BT-549	BT-20	SW-480	JURKAT
9a	2-Cl-Ph	Cl	39.3 ± 0.5	42.1 ± 1.1	21.3 ± 1.2	87.6 ± 0.7
9e	Ph	Cl	16.1 ± 0.3	22.1 ± 1.7	28.4 ± 0.8	12.3 ± 1.6
9f	2-F-Ph	Br	17.4 ± 1.2	23.3 ± 0.6	15.5 ± 1.1	11.6 ± 1.3
9h	Me	Cl	12.1 ± 0.4	6.7 ± 2.5	15.3 ± 1.5	5.1 ± 0.6

IC_{50} (μM) values obtained from Alamarblue™ assays with tested cancer cell lines; means ± SD obtained from three independent experiments performed in triplicate.[18]

Table 3
Percent inhibition of growth at 10 μM of compounds **9a–h** on different human cancer cell lines

Cell line	Compound						
	9a	**9b**	**9c**	**9d**	**9e**	**9f**	**9h**
Breast cancer							
MCF7	<20	<20	41	<20	<20	<20	95
T-47D	<20	<20	66	<20	<20	<20	43
Lung cancer							
HOP-92	38	70	57	<20	37	48	58
A549/ATCC	50	<20	—	67	80	<20	<20
Colon cancer							
HCT-116	38	70	64	<20	25	35	<20
HT-29	<20	<20	77	<20	<20	<20	43
Leukemia							
MOLT-4	46	<20	31	<20	—	<20	95
HL-60 (TB)	<20	45	58	<20	—	29	<20
CCRF-CEM	60	<20	—	<20	<20	28	—
Renal cancer							
CAK1-1	<20	<20	44	<20	66	45	<20
786-0	<20	<20	54	<20	<20	62	<20
Melanoma							
UACC-257	<20	77	39	<20	40	<20	<20
SK-MEL-28	<20	<20	40	<20	<20	<20	<20
CNS cancer							
SF-295	<20	<20	61	32	48	<20	<20
SNB-75	<20	<20	76	25	<20	<20	<20

(Table 1). It is worth mentioning that under basic conditions (NaOH or sodium methoxide), the target compounds (**9a–h**) were obtained in much lower yields, requiring extensive purification by column chromatography.

We first examined the antiproliferative activity of **9a**, **9e**, **9f**, and **9h** in a preliminary screen comprising a panel of human colon (SW480), breast (BT-20 and BT-549), and leukemia (JURKAT) cancer cell lines in 72 h drug exposure Alamarblue™ assays (Table 2).[18] The dose of the compound that inhibited 50% cell proliferation (IC_{50}) was calculated using the data generated from the assay.

Table 4
In vitro anticancer activity of compound **9c** on 60 human cancer cell lines

Cell line	GI_{50} (μM)
Leukemia	
CCRF-CEM	21.9
HL-60 (TB)	2.94
K-562	6.23
MOLT-4	20.8
RPMI-8226	4.32
Non-small-cell lung cancer	
A549/ATCC	4.65
EKVX	3.54
HOP-62	4.52
HOP-92	11.2
NCI-H226	10.7
NCI-H23	3.14
NCI-H322M	6.26
NCI-H460	3.10
NCI-H522	11.1
Colon cancer	
COLO 205	3.23
HCC-2998	2.60
HCT-116	4.03
HCT-15	3.35
HT29	4.42
KM12	2.02
SW-620	15.7
CNS cancer	
SF-268	4.35
SF-295	2.96
SF-539	2.85
SNB-19	11.3
SNB-75	1.46
U251	11.9
Melanoma	
LOX IMVI	19.0
MALME-3M	7.15
M14	5.47
SK-MEL-28	8.98
SK-MEL-5	5.03
UACC-257	16.1
UACC-62	9.87
Ovarian cancer	
IGROV1	1.29
OVCAR-3	1.79
OVCAR-4	6.49
OVCAR-5	1.91
OVCAR-8	26.0
SK-OV-3	7.55
Renal cancer	
786-0	8.12
A498	2.69
ACHN	17.5
CAKI-1	5.03
SN12C	8.82
TK-10	7.89
UO-31	3.86
Prostate cancer	
PC-3	5.75
DU-145	4.13
Breast cancer	
MCF7	0.13
NCI/ADR-RES	2.55
MDA-MB-231/ATCC	5.89
HS 578T	16.3
MDA-MB-435	1.56
BT-549	4.63
T-47D	3.55
MDA-MB-468	5.11

The most sensitive cell line was JURKAT, which gave IC_{50} values range of 5.1–12.3 μM; the least sensitive cell line was BT-20 (IC_{50} values 23.3–42.1 μM) except compound **9h** with IC_{50} values of 6.7 μM.

Because **9a**, **9e**, **9f**, and **9h** exhibited inhibitory activity on the panel of human cancer cell lines, the eight fluorinated acridone derivatives **9a–h** were selected as model for further screening. The synthesized compounds **9a–h** were then submitted to the National Cancer Institute (NCI, Bethesda, MD) for the standard 60 cancer cell line screen. Selected compounds were tested initially at a single dose in the cell panel.[19] Only compounds which satisfy pre-determined threshold inhibition criteria progressed to evaluation in the same full panel using five different concentrations. The concentration causing 50% cell growth inhibition (GI_{50}) compared with the control was calculated.

Results for test compounds are reported as percentage inhibition of the treated cells at single dose of 10 μM in comparison with that of the untreated control cells (Table 3).

It was found that among the series of the eight fluorinated acridone derivatives only compound **9c** exhibited in vitro cytotoxic activity at 10 μM in greater than 90% of the 60 cell lines. As a result, **9c** was selected for a 5-dose repeat screening. The result for the 5-dose testing is shown in Table 4.

This compound shows good anticancer potency in a wide spectrum of cell lines causing 50% cell growth inhibition (GI_{50}), and the values range from 0.13 to 26.0 μM as shown in Table 4.

Among the eight fluorinated acridone derivatives **9a–h**, those containing aryl-substituted analogs (R^1 = Ph) overall showed weaker cell growth inhibition compared with the unsubstituted phenyl (R^1 = H) or methyl-substituted phenyl (R^1 = Me) (Tables 2 and 3). It appears that the substitution at R^1 of the acridone ring is essential for activity, since less bulky groups gave better activity. Compound **9c** (R^1 = H), is the most active, with GI_{50} value of 0.13 μM (Table 4), in breast cancer cell line. Compound **9h** (R^1 = Me) also showed moderate activity, with IC_{50} value of 5.1 and 6.7 μM in leukemia cell line (JURKAT) and breast cancer cell line (BT-20), respectively (Table 2). The introduction of phenyl group at R^1 led to a decrease in activity (Tables 2 and 3). However, compound **9e** and **9f** showed significant growth inhibition of JURKAT with IC_{50} values of 11.6 and 12.3 μM, respectively. Compounds **9e** and **9f** also inhibited BT-549 with IC_{50} values of 16.1 and 17.4 μM, respectively (Table 2).

A new series of trifluoromethylated acridone derivatives have been synthesized and characterized.

The preliminary anticancer studies showed that **9a**, **9c**, **9e**, **9f**, and **9h** represent novel leads for further development. Compound **9c** was the most active among the series and will be subjected to in-depth structure–activity relationship (SAR). The mechanism of action of these novel leads will be the subject of future studies, aimed at identifying highly potent, safe, and selective agents for the treatment of cancer.

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17. *Experimental and spectral data for title compounds.*
Typical procedure for the synthesis of fluorinated acridone **9**:To a mixture of substituted 2-aminoarylketone or 2-aminoarylaldehyde **11** (1.0 mmol) and 5-trifluoromethyl-cyclohexanedione **10** (1 mmol) (1.2 mmol), 1 mL of 1 N HCl aqueous solution was added. The reaction mixture was stirred at 60–75 °C for a designated time. After completion of the reaction (monitored by TLC), the resulting suspension was neutralized with 1 mL of 1 N NaOH. The solid was filtrated, washed with water (3× 6 mL), air-dried to give the product as white or slightly yellow powder. The solid product was further purified by recrystallization with aqueous ethanol when necessary.
7-Chloro-9-(2-chlorophenyl)-3-(trifluoromethyl)-3,4-dihydroacridin-1(2H)-one **9a**:Yellow solid, mp = 182–185 °C. IR (nujol) 2930, 1605, 1495, 1156, 760 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.36–2.47 (dd, 2H), 2.87–2.96 (dd, 2H), 3.06 (m, 1H), 7.4–7.77 (m, 4H), 7.83 (s, 1H), 7.99–8.20 (d, 2H). EIMS *m/z*; 305 (5%), 374 (100%), 376 (50%), 375(25%), 377 (12.6%), 410 (2%) (M^+).

9-Phenyl-3-(trifluoromethyl)-3,4-dihydroacridin-1(2H)-one **9b**:Yellow solid, mp = 203–206 °C. IR (nujol) 2930, 1612, 1491, 1147 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.35–2.48 (dd, 2H), 2.85–2.97 (dd, 2H), 3.02 (m, 1H), 7.21 (d, 2H), 7.41–7.55 (m, 3H), 7.69–7.98 (m, 2H), 8.09–8.15 (d, 2H). EIMS *m*/*z*; 51 (12.5%), 217 (15%), 247 (14%), 313 (50%), 340 (100%), 341 (75%) (M^+). *5,7-Dibromo-3-(trifluoromethyl)-3,4-dihydroacridin-1(2H)-one* **9c**:Yellow solid, mp = 140–143 °C. IR (nujol) 2925, 1600, 1465, 964, 787 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.4–2.49 (dd, 2H), 2.88–2.93 (dd, 2H), 3.22 (m, 1H), 8.32 (d, 1H), 8.59 (d, 1H), 8.91 (s, 1H). EIMS *m*/*z*; 69 (13%), 139 (12%), 220 (12.5%), 2993 (15%), 396 (50%), 397 (25%) 423 (100%) (M^+), 425 (60%).

10-Methyl-7-(trifluoromethyl)-7,8-dihydro-6H-[1,3]dioxolo[4,5-b]acridin-9-one **9d**:Yellow solid, mp = 209–212 °C. IR (nujol) 2925, 1600, 1465, 964, 787 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.39 (s, 3H), 2.4–2.49 (dd, 2H), 2.81–2.97 (dd, 2H), 3.23 (m, 1H), 5.97 (s, 2H), 7.29 (s, 1H), 7.41 (s, 1H). EIMS *m*/*z*; 169 (13%), 227 (25%), 295 (80%), 323 (100%), (M^+).

7-Chloro-9-phenyl-3-(trifluoromethyl)-3,4-dihydroacridin-1(2H)-one **9e**:Yellow solid, mp = 151–153 °C. IR (*nujol*) 2931, 1605, 1491, 1155, 764 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.36 - 2.47 (dd, 2H), 2.87-2.99 (dd, 2H), 3.06 (m, 1H), 7.18-7.55 (m, 5H), 7.83 (s, 1H), 7.99 - 8.21 (d, 2H). EIMS *m*/*z*; 305 (5%), 375 (100%) (M^+).

7-Bromo-9-(2-fluoro-phenyl)-3-(trifluoromethyl)-3,4-dihydroacridin-1(2H)-one **9f:** Yellow solid, mp = 134–136 °C. IR (nujol) 2941, 1609, 1487, 1149, 759 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.36–2.49 (dd, 2H), 2.83–2.97 (dd, 2H), 3.01 (m, 1H), 7.31–7.53 (m, 4H), 7.87 (s, 1H), 8.19–8.23 (d, 2H). EIMS *m*/*z*; 235 (25%), 331 (15%), 359 (12.5%), 411(30%), 439 (100%) (M^+ +1).

7-Nitro-9-phenyl-3-(trifluoromethyl)-3,4-dihydroacridin-1(2H)-one **9g**: Yellow solid. mp = 194–198 °C. IR (*nujol*) 2941, 1609, 1487, 1149, 759 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.36–2.48 (dd, 2H), 2.83–2.97 (dd, 2H), 3.01 (m, 1H), 7.21–7.57 (m, 5H), 7.71- 8.49 (d, 2H), 9.35 (s, 1H). EIMS *m*/*z*; 235 (25%), 331 (15%), 359 (12.5%), 411(30%), 439 (100%) (M^++1).

7-Chloro-9-methyl-3-(trifluoromethyl)-3,4-dihydroacridin-1(2H)-one **9h**: Yellow solid, mp = 122–125 °C. IR (*nujol*) 1679, 1560 cm^{-1}; ^1H NMR (CDCl3) δ 2.39 (s, 3H), 2.4–2.49 (dd, 2H), 2.81–2.97 (dd, 2H), 3.19 (m, 1H), 7.93 (d, 1H), 8.03 (d, 1H), 8.33 (s, 1H). EIMS *m*/*z*; 51 (12.5%), 154 (20%), 217 (23%), 285 (100%), 313 (95%) (M^+).

18. *Materials and methods:* Human colon (SW480), breast (BT-20 and BT-549), and leukemia (JURKAT) cancer cell lines were obtained from the American Type Culture Collection (Manassas, VA). Cell lines were maintained in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 0.15% sodium bicarbonate, 0.011% sodium pyruvate, 0.24% Hepes and 10 ml/L of antibiotic solution. The cells were grown in 150 cm^2 culture plates in an air/CO_2 (95:5) atmosphere at 37 °C and passaged approximately every 3 days. All four cancer cell lines (1×10^4 cells per well) were plated using RPMI 1640 medium containing FBS in 96-well plates and left to attach for 24 h. Cells were then treated with either vehicle (DMSO) or the indicated concentrations of different compounds. Cells were exposed for a total of 72 h to test compounds and vehicle controls. Alamarblue™ reagent dye was added to wells in a 1:10 following exposure period and incubated at 37 °C overnight. The plates were then analyzed using a microtiter plate reader at dual wavelengths (560 nm λ excitation, 590 nm λ emission). Each experiment was done in triplicate and results are expressed as means ± SE for each determination. The IC_{50} values were calculated using linear regression method and are expressed in micromolar (μM).
19. Boyd, M. R.; Paul, K. D. *Drug Dev. Res.* **1995**, *34*, 91.