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European Journal of Medicinal Chemistry 39 (2004) 1029–1038
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
www.elsevier.com/locate/ejmech

Original article

Synthesis of 9-acridinyl sulfur derivatives: sulfides, sulfoxides and sulfones. Comparison of their activity on tumour cells

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Received 30 January 2004; received in revised form 14 May 2004; accepted 1 June 2004

Available online 27 October 2004

Abstract

The synthesis of several acridine thioethers is described. These compounds were oxidized to give new sulfoxides and sulfones. Among 23 compounds prepared, 19 were tested in vitro against the human cancer cell lines panel of NCI screening. Activity is increased 5–10 times from sulfides to sulfoxides. Among substituted groups in the side chain, sulfur mustard, epoxy sulfide and sulfoxide displayed the most interesting activity.

Keywords: Acridine sulfides; Sulfoxides; Sulfones; Anticancer activity; DNA interaction

1. Introduction

Antitumour anthracyclines, aminoanthraquinones and actinomycins, ellipticine and amsacrine (2′-methoxy–4′-methylsulfamido-9-anilino acridine) are known to intercalate into DNA. Intercalation involves the stabilization of ternary complexes between ligand, DNA and topoisomerases [1]. Other derivatives such as busulfan which is a sulfone act as alkylating agents. Hence bifunctionnalized derivatives were prepared and tested. So acridine compounds linked through a 9-aminoalkyl chain to a sulfur mustard moiety have been examined and found to be active on Ehrlich ascites tumour [2].

In our continuing interest in acridine derivatives, acridine sulfides, sulfoxides and sulfones were synthesized. In previous works, some of these compounds were tested for purity determination [3], activity on multiplication of herpes simplex virus type 2 [4] and as chemosensitizers [5]. Permeation properties were also investigated [6].

The synthetic procedures and antiproliferative activity against human cancer cells are now reported.

The activity of 19 compounds was evaluated within the frame of the National Cancer Institute screening Program on approximately 60 different cancer cell lines and some structure–activity relationships are discussed.

2. Chemistry

The sulfides listed in Fig. 1, were synthesized by alkylation of the corresponding 9-thioacridinones [7,8]. Reaction conditions differ according to the haloderivative used:

- alkylation was performed under PTC conditions with TEBAC as catalyst [9] for **1**, **3**, **5**, **13** whilst **19** and **20** were prepared without catalyst,
- bromoepoxypropane reacted in DMF in the presence of potassium carbonate to lead to **6**, **7**, **8**

Abbreviations: PTC, phase transfer catalysis; TEBAC, triethylbenzylammonium chloride; MTD, maximum tolerated dose; MTT, formazan dye.

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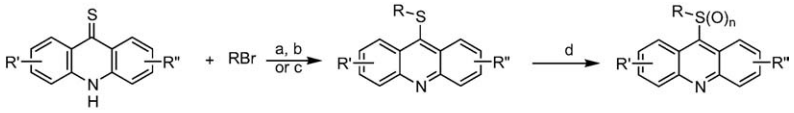
doi:10.1016/j.ejmech.2004.06.015



R = C_2H_4Cl	R' = R" = H	1	n = 1	2
	R' = R" = 2,7-$(OCH_3)_2$	3	n = 1	4
R = C_4H_8Cl	R' = R" = H	5		
R = CH_2–epoxide	R' = R"= H	6		
	R' = 3-Cl	7		
	R' = R" = 2,7-$(OCH_3)_2$	8		
R = $CH_2C_6H_4NO_2$	R' = R" = H	9		
	R' = R" = 2,7-$(OCH_3)_2$	10	n = 1	11
			n = 2	12
R = C_2H_5	R' = R" =2,7-($OCH_3)_2$	13	n = 1	14
			n = 2	15
R = C_2H_4OH	R' = R" = 2,7-$(OCH_3)_2$	16		
R = $C_2H_4N(C_2H_5)_2$	R' = R" = 2,7-$(OCH_3)_2$	17	n = 1	18
R = $CH_2C_6H_5$	R' = 3-Cl	19		
	R' = R" = 2,7-($OCH_3)_2$	20	n = 1	21
			n = 2	22
R = CH=CH_2	R' =R"= 2,7-($OCH_3)_2$		n = 2	23

a) alkl halide, TEBAC, toluene , 110°C; b) alkyl halide, DMF, K_2CO_3; c) alkyl halide, butanone, NaOH, 6M
d) H_2O_2, $(NH_4)_6$ Mo_7 O_{24}. 4 H_2O, THF, buffer pH = 6.8

Fig. 1. Synthetic pathways in of case of thioethers, sulfoxides and sulfones **1–23**.

- alkylation was achieved in butanone and sodium hydroxide solution in case of **17** (Fig. 1).

Several methods for oxidizing sulfides are known [10]. In the case of the thioacridine derivatives, the major problem is not overoxidation of sulfoxide to sulfone but to prevent hydrolysis of the expected compounds leading to acridinone derivatives. It has been shown with 4-pyridine sulfur derivatives that lability of the S–C bond increases from S to SO and SO_2 compounds [11]. Various oxidants in different conditions were tested. Oxone (potassium monopersulfate triple salt) and 3-chloroperbenzoic acid (mCPBA) led to acridinone, oxone tetrabutylammonium salt (oxone TBA) in basic medium, sodium perborate under PTC conditions left the starting thioether unchanged. Finally, a mild reaction with H_2O_2 in tetrahydrofuran using a buffer solution (pH 6.8) and ammonium molybdate as catalyst [12] was successfully applied. However, formation of acridinone cannot be totally avoided, except for the 2,7-dimethoxy derivatives. Depending on the H_2O_2 amount and reaction time, sulfoxides can be isolated while sulfones are isolated using more drastic conditions. The reaction was followed by TLC. Sulfides and sulfones have the same polarity, sulfoxides being more polar. Elimination occurs during oxidation of thioether **3** leading to the ethylenic sulfone **23**. Oxidation of **1** gave sulfoxide **2** with low yield depending on the amount of oxidizer or reaction time. Unreacted thioether was recovered as well as the sulfoxide or acridinone formed. In the latter case no elimination product was detected.

Direct oxidation of **17** did not afford the corresponding sulfoxide or sulfone but elimination process led to the ethylenic sulfone **23**. Intermediate could possibly be an amine oxide which undergoes a Cope elimination [13]. Hence, the corresponding sulfoxide **18** was prepared by amination of the 2′-chloroethyl sulfoxide **4**.

3. Results and discussion

Effects of changes:

- in the side chain,
- in the oxidation state of the sulfur atom,
- in the substituents on the acridinic ring were investigated with the aim to get an insight into the mode of action of compounds. As a result, the GI_{50}, TGI, LC_{50} parameters as defined in the experimental part, are listed in Table 1. To be concise, we present in Table 2 only significant results restricted to a few lines for the most active compounds. In this table, the MG-MID (mean graph mid-point) value

Table 1
Biological [a] and physicochemical [b] data

Compound	GI_{50}	TGI	LC_{50}	log D	p*K*a
1	11.5/1.8	43/3.3	77/5.9	4.64	5.23
2	66/8.3	>100/35	>100	2.84	5.23
3	5.1/0.26	43/0.97	81/7.7 (4) [c]	4.79	5.91
4	5.5/1.5	19.4/2.8	47/5.3 (10) [c]	2.99	5.91
7	2.4/1.05	7.4/2.6	33/5.5 (11) [c]	3.99	4.85
8	15.8/5.7	39/19	70/46	3.50	5.98
9	5.9/1.48	21/2.8	52/5.3 (2) [c]	5.48	5.26
10	72/17	>100/69	>100	5.67	5.94
11	6.4/1.6	21.8/3	56/5.6	3.86	5.94
12	95/39	>100	>100	3.71	2.88
13	12.5/2.6 (3) [c]	53/23	91/53	2.60	6.09
14	6.4/1.7	25/3	56/5.6 (8) [c]	2.79	6.09
15	97/26	>100	>100	2.65	2.76
16	26/2	77/12	95/42	3.30	5.99
17	7.7/1.6	41/3.3	93/50	2.94	5.69, 9.29
18	7.2/0.18	24/0.34	57/0.66	1.15	5.69, 9.29
20	93/15	>100	>100	5.73	6.16
21	8.5/1.35	30/2.6	66/5.1 (2) [c]	3.91	6.16
23	6.3/1.8	39/3.8	87/13.8	2.58	5.60

[a] Cytostatic and cytotoxic mean concentration values (MG-MID) on 60 human tumour cell lines/best activity value in μM.
[b] log D, p*K*a were calculated with PALLAS 2.0 [40].
[c] Number of cell lines.

Table 2
Selected cytotoxicity data (LC_{50} in μM)

	Compounds	1	3	4	7	9	11	14	18	22
Cell lines										
leukemia	HL-60				6.4					
	K562				6.6					
NSCL	H522	6.9		5.5		5.5	5.5	5.5	0.66	5.1
Colon cancer	COLO-205				5.6					
	HCT-116			6.6	5.5		5.9			
	HT-29			6.1	6.3	5.5	7.0	9.1		
Melanoma	LOX IMVI		9.5							
	MALME-3M			6.3		8.3			3.9	
	SK MEL5				5.5					
Ovar. cancer	IGROV1			6.0		6.3	9.5	7.0		
	OVCAR-3				5.7					
Renal cancer	786-0			6.7	6.0	6.3	6.1		8.3	6.6
	ACHN				6.4					
	CAKI-1	5.8		5.7	5.7			5.5		
	SN12C			7.6	7.4			6.6		
	TK-10			6.1	5.6		5.9	7.0		
Prost. cancer	PC3				5.9					
	DU145			9.1	5.6	5.5	6.0	9.5	7.6	
Breast cancer	MDA-N				7.6					

which is the average sensitivity of all tested cell lines and the best activity quoted in a LC_{50} value form are listed.

The epoxy ether **7** shows the best efficacy on 41 strains within the approximately 60 lines studied, while thioether **3** does the same with only seven strains and **18** with two strains. As a result one can select nine compounds with a GI_{50} in the range 0.17–5.1 μM and LC_{50} 0.66–7.2 μM i.e. **3**, **4**, **7**, **9** [14], **11**, **14**, **17**, **18**, **23**. Sulfones **12** and **15** and benzyl thioethers **10** and **20** are inactive compounds. The only active sulfone is the vinyl sulfone **23**.

Six sulfoxides have been tested. When compared to the corresponding sulfides, 4 compounds display better cytotoxicity according to the sequence **14** > **13** , **18** > **17**, **11** > **10**, **21** > **20**. The 2′-chloroethylthio ether **3** is better than sulfoxide **4** but in this case it can be assumed that like the nitrogen mustards which give an aziridinium ion, the active species is in this case a thiiranium ion. Thus, it can be inferred that the activity of sulfoxides is better than that of the corresponding sulfides, except for *R* = 2′-chloroethyl (Fig. 2).

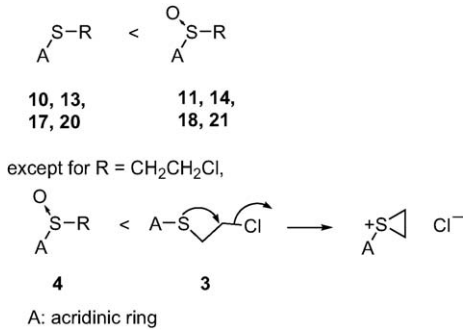


Fig. 2. Compared activity of sulfides and sulfoxides.

The lack of activity of **2** is questionable owing to the instability of this compound towards hydrolysis that easily leads to the corresponding acridinone.

Keeping the acridinic moiety identical with a 2,7-dimethoxy substitution, six thioethers are compared and classified in order of increasing activity, i.e. benzyl (**20**), oxiranylpropyl (**8**) hydroxyethyl (**16**), ethyl (**13**), diethylaminoethyl (**17**) and 2′-chloroethyl (**3**), 9-thio substituted derivatives. The last two acting as a positively charged moiety (ionized nitrogen and thiiranium ion). It can be noted that a 3-chloro substituent in acridine ring **7**, confers a better activity than a 2,7-dimethoxy one in **8**. The presence of chlorine atom would prevent enzymatic destruction [15] but also protects the side chain from hydrolysis which is facilitated by electron deficiency. As dimethoxy substituents are also expected to exhibit similar electronic stabilization, lower activity of **8** is probably due to a detrimental steric hindrance effect.

Intercalation of 9-amino acridinic derivatives into DNA has been studied previously [16]. When a ligand possessing a chromophore binds to DNA, the UV spectrum is altered as a consequence of the change in its environment. Moreover, the long wavelength absorption band of ligands which intercalate between the base-pairs of DNA, undergoes bathochromic and hypochromic changes. In addition, the chains of the DNA duplex melt apart when hydrogen bonds between the base-pairs break on increasing temperature or by titration with acid or alkali. Melting can be monitored conveniently by an increase in absorbance (hyperchromic effect) that results from the disruption of base stacking. The mid-point melting temperature Tm is a characteristic of DNA stability. Measurement of increase in the thermal denaturation (ΔTm) of DNA or a homopolymer as a result of a ligand: DNA complex formation, compared to that of DNA alone measured in the same experiment, provides a value which reflects the affinity of the compound for DNA [17].

Table 3
DNA binding affinity data

Compound	K binding affinity	Number of binding sites	ΔTm (°C)
1	7.91×10^6 M^{-1}	0.15	2.0
2	NI		
3	8.16×10^6 M^{-1}	0.19	2.0
4	NI		
5	NI		
7	NI		
8	NI		
16	0.76×10^6 M^{-1}	0.23	1.2

NI: no intercalation.

Binding to calf thymus DNA has been previously reported [18]. Intercalation occurred only for compounds bearing a protonatable nitrogen in the side chain or in the ring. For some of our compounds, intercalation occurred for the structurally related **1** and **3** derivatives which undergo, like nitrogen mustard, an assisted chlorine elimination leading to a reactive positively charged intermediate (Table 3). It can be noted that sulfoxides **2** and **4**, as well as the 4′-chlorobutyl thio derivative **5** which cannot undergo this reaction, do not intercalate. Alcohol **16** weakly intercalate, possibly stabilized by hydrogen bonding with phosphate groups on the DNA backbone. From binding affinity data of 2′-dimethylaminoethyl-9-thioacridine [18] (Fig. 2, A = acridine, $R = (CH_2)_2N(CH_3)_2$, $k = 0.935 \times 10^6$ M^{-1}, $n = 0.2$, Δ Tm = 1.5), analogous data can be expected for amine derivative **17** as well **18**.

Using two strains, one parental and one deficient in RAD 52 and RAD 6 and so showing hypersensitivity to DNA damaging agents, definite DNA-damaging activity was recorded for four products **1**, **4**, **7**, **14**, as revealed by a ratio of IC_{50} values which is ≥7 for the two strains (Table 4). Such DNA-damaging activity was confirmed by Comet assay using human 549 cells since a tail moment (TM) value higher than 1.3 (value corresponding to solvent control) was obtained with the four compounds tested (Table 4). Interestingly, the IC_{50}s evaluating cytotoxicities of these compounds versus A549 cells, were close to the concentrations which demonstrated DNA-damaging activity with the Comet assay (10 μM). These data strongly suggest that DNA-damaging activity originated in cytotoxicity of compounds. However, using two strains expressing different levels of topoisomerase II, a very weak difference in cytotoxicity was identified for these four compounds suggesting that topoisomerase II

Table 4
Pharmacological profile of compounds **1, 4, 7** and **14** (IC_{50} in μM)

Compound	DNA-damaging activity in yeast			DNA-damaging activity in A549 cells (TM)	Cytotoxicity A549 cells (IC_{50})	Involvement of Top2 in cytotoxicity		
	wt (IC_{50})	Y195 (IC_{50})	Ratio wt/Y195			Low Top2 (IC_{50})	High Top2 (IC_{50})	Ratio lTop2/hTop2
1	>100	1.1	>100	2.6	10	1.7	1.0	1.7
4	6.7	0.22	30	1.7	7	11.6	7.8	1.5
7	3.2	0.35	9	4.6	7	4.0	3.1	1.3
14	95	14	7	2.9	10	67	34	2.0

was involved only in a minor part of cytotoxicity (Table 4). Thus, the mode of action of these compounds differs from that of classical topoisomerase II-poisons such as etoposide.

Thioepoxy derivative **7** has been selected by the NCI for further preliminary testing in the in vivo Hollow Fiber Assay. Twelve selected human tumour cell lines encased in hollow fibers are implanted into aythmic nude mice, in two intraperitoneal (IP) and subcutaneous (SC) sites. Two compound doses are used, giving 48 test combinations (12 cell lines × 2 sites × 2 doses). Six to eight days after administration of the test compound to the mice, fibers are collected, cells removed and growth inhibition is measured using MTT. A value of 2 is assigned for each compound dose which results in a 50% or greater reduction in the viable cell mass. Compound **7** displayed an MTD of 400 and scored a 12 (8 IP + 4 SC) in the assay. One of the cell lines H522, was killed in the experiment. The assay was repeated using the i.v. route of administration in the hope of improving activity. In this case the MTD was also 400 and the hollow fiber score was 8 (2 IP + 6 SC). Combined IP + SC score ≥ 20 and SC score ≥ 8 are the criteria retained by the NCI for further in vivo testing.

According to the literature, the biological activity of sulfoxides can be attributed to their ability to be reduced to sulfides or oxidized to sulfones. The center of a solid tumour cells is usually deprived of oxygen and is equivalent to a low oxygen tension (hypoxia) area resistant to radiotherapy and chemotherapy. The use of bioreductive agents which are selectively toxic to hypoxic cells upon enzymatic reduction has been reported [19,20]. Sulindac sulfone has cancer chemopreventive activity [21] and inhibition of angiogenesis with sulindac or its sulfone metabolite may also contribute to their antineoplastic properties [22].

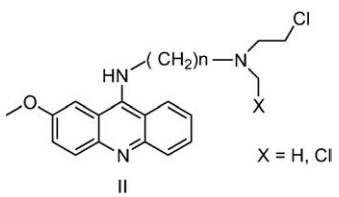
It can be concluded, from a comparison of sulfides and sulfoxides that sulfoxides are not sulfide prodrugs as their activity is greater in the series under evaluation. Nevertheless, sulfoxides can be involved in a redox reaction. The corresponding sulfones are by far less active but they display a low p*K*a that may be responsible for the lack of activity.

The leaving group properties of the sulfoxides have also been related to their biological activity [23]. Furthermore, the antitumour activity of complexes of platinum containing substituted sulfoxides was reported and it is suggested that these complexes may act by binding to DNA with subsequent loss of the sulfoxide ligand [24]. Brefeldin A (BFA) possesses biological activity and particularly antitumour activity by inducing apoptosis in cancer cells although clinical use was precluded due to undesirable pharmacokinetic properties. The sulfoxide analogues present activities comparable to that of BFA and one was shown to be more active than BFA. The sulfide derivatives were much less active that the sulfoxide ones. This fact was related to the ability of the sulfoxide and not the sulfide to undergo elimination that regenerates BFA [25]. It is not probable that acridinic sulfoxides act as leaving groups owing to the fact that compounds bearing 2,7-dimethoxy substituents—which confer a great stability towards ring attack at the C-9 position—display good activity. On the contrary, it seems that stability towards hydrolysis or enzymatic action is favourable to the activity.

Finally, epoxide, sulfoxide, sulfide, amine, alcohol or nitro groups found in the side chains branched on the acridine ring are hydrogen bonding acceptors (HBA) and therefore can interact with hydrogen bond donor groups. The interaction site of the sulfoxide group with enzymes like *Escherichia coli* thymidylate synthase (TS) was deduced from crystallographic studies [26]. Selective binding of the sulfoxide group occurs with positively charged lysine and negatively charged glutamate. The sulfoxides studied here, which display greater activity than sulfides are also better HBA groups (pK_{HB} 2.6 versus 0.3). Besides sulfoxide, amine, oxirane, alcohol, nitro and sulfide are in decreasing order strength from approximately 2.6, 2, 0.97, 0.8, 0.4 to 0.3 according to the pK_{HB} scale (logarithm of the formation constant of 4-fluorophenol: base complexes in CCl_4) [27]. But for other groups than the sulfoxide one, additional interactions would account for activity. Thus, the tertiary amino group can give an ionic bond that is stronger than a hydrogen bonding and the epoxy sulfide **7** (as well as ethenosulfone **23**) can undergo nucleophilic addition. Nevertheless an epoxy group does not seem sufficient as **8** is 5–10 times less active than **7**, possibly due to the detrimental presence of bulky methoxy groups which may prevent interactions. Still with respect to the biological activity, the presence of an oxirane is evidence that the epoxy moiety as well as the bromine atom significantly increases the antiproliferative activity in bromo and epoxyequinols derived from oestrone [28]. Moreover, the DNA cross-linking activity of symmetrical dimers of the azinomycins is based upon the epoxide domain [29].

The DNA interaction of mono and difunctional 9-aminoacridine nitrogen mustards II (Fig. 3), quinacrine mustard analogues has been studied [30] and cytotoxicity to human colon carcinoma HT-29 cells measured. The concentration required to inhibit colony formation by 50% (IC_{50}) equal 0.25 μM for the mono derivative (X = H) and 0.01 μM for the difunctional derivative (X = Cl) when n = 4.

The GI_{50} values for **7** and **9** are 1.65 μM, for **4** and **11**, 1.8 μM, for **17**, 2.6 μM, for **3**, 2.75 μM and for **1**, 5 μM. Derivatives **7**, **9** as well as **4** and **11** show a comparable activity with nitrogen mustard II with n = 2 (IC_{50} = 1.10 μM). A comparison between quinacrine which is a 9-amino acridine (IC_{50} = 55 μM), **17** which is a 9-thioacridine (IC_{50} = 2.6 μM) and **18** which is the corresponding sulfoxide (IC_{50} = 3.8 μM), shows that replacement of the nine nitrogen atom by sulfur atom and sulfoxide group seems to be beneficial to the activity.



II

Fig. 3. Quinacrine mustard analogues.

4. Conclusion

9-Acridinyl sulfur derivatives with various functions in the side chain have been synthesized as sulfides, sulfoxides and sulfones. Some of these compounds display antiproliferative activity against tumour cells. The presence of a positive charge moiety in sulfur mustards or protonatable amines, that of epoxy or sulfoxide group can account for the biological activity which is related to DNA interactions by intercalation as well as by damaging activity.

5. Experimental protocols

5.1. Chemistry

Liquid chromatography was performed on silica gel 60 (230–400 Mesh) and TLC on silica gel 60 F_{254}. Melting points were determined on a Büchi apparatus and are given uncorrected. ^{1}H and ^{13}C NMR spectra were performed on Brüker spectrometer (200 MHz) with TMS as internal reference; chemical shifts δ are given in the parts per million scale with *J* value in Hertz. Mass spectra were obtained on a Varian MAT 311 mass spectrometer. Elemental analysis are within ±0.4% of the theoretical value.

*5.1.1. 9-(2′-Chloroethylthio)-acridine (**1**)*

Method A used for the preparation of **1**, **3**, **5** (the appropriate halide is in this case 4-chloro bromobutane), **13** (with excess ethyl bromide), **19** and **20** (benzyl chloride or TEBAC alone is used).

Thioacridinone (2.1 g, 10 mmol), toluene (100 ml), TEBAC (1 g), excess bromochloroethane (3 g), KOH, 5 M (40 ml) were heated 1 h under reflux. After cooling, the organic phase was separated, the aqueous phase extracted with toluene and the organic phases washed to neutrality and dried over $MgSO_4$. The crude product was purified by liquid chromatography on silica gel (ether).

Yield: 75%. Mp: 98 °C. ^{1}H NMR ($CDCl_3$): 8.44 (d, d, *J* = 8.7 Hz, *J* = 0.5 Hz, 2H); 8.04 (d, *J* = 8.7 Hz, 2H), 7.54 (d, d, d, *J* = 8.7 Hz, *J* = 6.6 Hz, *J* = 1.4 Hz, 2H), 7.34 (d, d, d, *J* = 8.6 Hz, *J* = 6.6 Hz, 1.2 Hz, 2H), 3.15 (t, *J* = 7.3 Hz, 2H), 2.97 (t, *J* = 7.3 Hz, 2H). ^{13}C NMR ($CDCl_3$): 148.77 (s); 140.58 (s); 130.37 (d); 130.26 (d); 129.04 (s); 127.09 (d); 126.38 (d); 42.71 (t); 38.84 (t).

*5.1.2. 9-(2′-Chloroethylsulfinyl)-acridine (**2**)*

Method B: oxidation of sulfides: sulfide (3 mmol), THF (20 ml), buffer solution (20 ml) (from KH_2PO_4, 0.066 M, 53.4 ml and Na_2HPO_4, 2 H_2O, 0.066 M, 46.6 ml), 0.5 g $(NH_4)_6$ Mo_7 O_{24}. 4 H_2O, H_2O_2, 30% (2 ml) were stirred at room temperature. The reaction was followed by T.L.C (ether) and H_2O_2 added if necessary as well buffer solution to maintain the neutrality. Solvents are eliminated under vacuum. The product was extracted with CH_2Cl_2 dried over $MgSO_4$. The solvent was eliminated and the crude product purified by chromatography (ether/chloroform, 10/1, then methanol 5–10%).

Yield: 22%. Mp: 119–120 °C ^{1}H NMR ($CDCl_3$): 8.94 (br.s, 2H), 8.26 (d, *J* = 8.7 Hz, 2H), 7.82 (d, d, d, *J* = 8.7 Hz, *J* = 6.6 Hz, *J* = 1.2 Hz, 2H), 7.63 (d, d, d, *J* = 8.7 Hz, *J* = 6.6 Hz, *J* = 1.2 Hz, 2H), 4.13 (d, d, d, *J* = 11.4 Hz, *J* = 9.1 Hz, *J* = 5 Hz, 1H), 4.0 (d, t, *J* = 13 Hz, *J* = 5.0 Hz, 1H), 3.82 (d, d, d, *J* = 11.4 Hz, *J* = 5.4 Hz, *J* = 5 Hz, 1H), 3.36 (d, d, d, *J* = 13 Hz, *J* = 9.1 Hz, *J* = 5.4 Hz, 1H). ^{13}C NMR ($CDCl_3$): 148.32(s); 141.78 (s); 130.74 (d); 130.45 (d); 127.73 (d); 123.92 (s); 122.18 (d); 56.86 (t); 37.54 (t).

*5.1.3. 2,7-Dimethoxy-9-(2′-chloroethylthio)-acridine (**3**)*

Method A. Yield: 60%. Mp: 157–158 °C. ^{1}H NMR: 8.08 (d, *J* = 9.6 Hz, 2H), 7.91 (d, *J* = 2.7 Hz, 2H), 7.41 (d, d, *J* = 9.6 Hz, *J* = 2.7 Hz, 2H), 4.03 (s, 6H), 3.41 (t, *J* = 7 Hz, 2H), 3.22 (t, *J* = 7.0 Hz, 2H). ^{13}C NMR ($CDCl_3$): 158.63 (s); 144.19 (s); 133.83 (s); 132.07 (d); 130.76 (s); 124.09 (d); 101.78 (d); 55.76 (q); 43.06 (t); 38.11 (t).

*5.1.4. 2,7-Dimethoxy-9-(2′-chloroethylsulfinyl)-acridine (**4**)*

Method B. (From 450 mg of sulfide **3**, after chromatography of the crude product 100 mg of **4** and 200 mg of **23** was recovered).

Mp: 162–163 °C. ^{1}H NMR ($CDCl_3$): 8.10 (shoulder) and 8.09 (d, *J* = 9.5 Hz) (3.1 H), 7.41 (d, d, *J* = 9.5 Hz, *J* = 2.6 Hz, 2H), 4.09 (d, d, d, *J* = 11.4 Hz, *J* = 9.1 Hz, *J* = 4.3 Hz, 1H), 3.96 (s, 6H + m. 1H), 3.76 (d, d, d, *J* = 11.4 Hz, *J* = 5.3 Hz, *J* = 4.8 Hz, 1H), 3.45 (d, d, d, *J* = 13.0 Hz, *J* = 9.0 Hz, *J* = 4.8 Hz, 1H). ^{13}C NMR ($CDCl_3$): 158.58 (s); 143.62 (s); 134.36 (s), 132.20 (d); 125.60 (s); 124.34 (d); 97.64 (d); 56.08 (t); 55.70 (q); 37.87 (t).

*5.1.4.1. 2,7-Dimethoxy-9-(ethenosulfonyl)-acridine (**23**).*

Mp: 180–182 °C. ^{1}H NMR ($CDCl_3$): 8.45 (d, *J* = 2.6 Hz, 2H), 8.10 (d, *J* = 9.4 Hz, 2H), 7.41 (d, d, *J* = 9.4 Hz, *J* = 2.6 Hz, 2H), 6.90 (d, d, *J* = 16.5 Hz, *J* = 9.8 Hz, 1H), 6.59 (d, *J* = 16.5 Hz, 1H), 6.07 (d, *J* = 9.8 Hz, 1H), 4.00 (s, 6H). ^{13}C NMR ($CDCl_3$): 159.65 (s); 144.43 (s); 138.47 (d); 132.47 (d); 130.50 (s); 126.79 (t); 125.13 (s); 123.78 d); 100.31 (d); 55.67 (q).

*5.1.5. 9-(4′-Chlorobutylthio)-acridine (**5**)*

Method A. Yield: 83%. Mp: 84–85 °C. ^{1}H NMR: 8.65 (d, *J* = 8.7 Hz, 2H), 8.14 (d, *J* = 8.6 Hz, 2H), 7.68 (m, 2H), 7.49 (m, 2H), 3.28 (t, *J* = 6.3 Hz, 2H), 2.83 (t, *J* = 7.0 Hz, 2H), 1.72 (m, 2H), 1.48 (t, *J* = 7.0 Hz, 2H). ^{13}C NMR ($CDCl_3$): 148.60 (s); 142.52 (s); 130.15 (d); 130.01 (d); 128.88 (s); 126.54 (d); 44.07 (t); 36.72 (t); 31.21 (t); 27.22 (t).

*5.1.6. 9-(2′,3′-Epoxypropylthio)-acridine (**6**)*

Method C for **6**, **7** and **8**. Thioacridinone (1 g, 5 mmol), DMF (10 ml), 2,3-epoxy bromopropane (1 g, 7.5 mmol), K_2CO_3 (1 g, 7.5 mmol) were heated at 110–110 °C during 15 min. DMF was eliminated under vacuum and the oil extracted with toluene, washed and dried over $MgSO_4$. The product was chromatographed on silica gel (ether).

Yield: 46%. Mp: 79–80 °C. ^{1}H NMR ($CDCl_3$): 8.78 (d, J = 8.7 Hz, 2H), 8.23 (d, J = 8.7 Hz, 2H), 7.78 (m, 2H), 7.61 (m, 2H), 3.13 (m, 1H), 2.93 (m, 2H), 2.45 (m, 1H), 2.10 (m, 1H). ^{13}C NMR ($CDCl_3$): 148.66 (s), 141.20 (s), 130.26 (d), 130.12 (d), 128.91 (s), 126.85 (d), 126.42 (d), 50.90 (d), 47.22 (t), 39.34 (t).

*5.1.7. 3-Chloro-9-(2′,3′-epoxypropylthio)-acridine (**7**)*

Method C. Yield: 38%. Mp: 99–100 °C. ^{1}H NMR ($CDCl_3$): 8.71 (br.d, J = 8.6 Hz, J = 1.2 Hz) and 8.70 (d, J = 9.3 Hz) (2H), 8.16 (d, J = 8.6 Hz) and 8.17 (d, J = 2.0 Hz) (2H), 7.78 (d, d, d, J = 8.6 Hz, J = 6.8 Hz, J = 1.2 Hz, 1H), 7.60 (d, d, d, J = 8.6 Hz, J = 6.8 Hz, J = 1.2 Hz, 1H), 7.50 (d, d, J = 9.3 Hz, J = 2 Hz, 1H), 3.06 (d, d, J = 15.0 Hz, J = 7.7 Hz) and 2.93 (d, d, J = 15.0 Hz, J = 5.3 Hz) and 2.90 (m) (3H), 2.47 (d, d, J = 4.7 Hz, J = 3.7 Hz, 1H), 2.11 (d, d, J = 4.7 Hz, J = 2.2 Hz, 1H). ^{13}C NMR ($CDCl_3$): 149.27 (s); 148.51 (s); 141.88 (s); 136.40 (s); 130.80 (d); 130.31 (d); 128.84 (s); 128.63 (d); 128.17 (d); 128.07 (d); 127.42 (s); 127.25 (d); 126.52 (d); 50.93 (d); 47.29 (t); 39.60 (t).

*5.1.8. 2,7-Dimethoxy-9-(2′,3′-epoxypropylthio)-acridine (**8**)*

Method C. Yield: 32%. Mp: 128–130 °C. ^{1}H NMR ($CDCl_3$): 8.06 (d, J = 9.3 Hz, 2H), 7.93 (d, J = 2.7 Hz, 2H), 7.39 (d, J = 9.3 Hz, J = 2.7 Hz, 2H), 4.01 (s, 6H), 2.95 (m, 3 H), 2.50 (d, d, J = 4.7 Hz, J = 3.7 Hz, 1H), 2.17 (d, d, J = 4.7 Hz, J = 2.2 Hz, 1H). ^{13}C NMR ($CDCl_3$): 158.37 (s), 144.0 (s), 134.40 (s), 131.85 (d), 130.50 (s), 123.90 (d), 101.77 (d), 55.60 (q), 50.87 (d), 47.61 (t), 38.23 (t).

*5.1.9. 2,7-Dimethoxy-9-(4′-nitrobenzylthio)-acridine (**10**)*

2,7-Dimethoxy-thioacridinone (2.7 g, 10 mmol), 4-nitrobenzylbromide (2.2 g, 10 mmol), toluene (100 ml), KOH, 5 M (100 ml) were heated under reflux during 18 h. After cooling, the organic phase was separated, the aqueous phase extracted with toluene and the organic phases dried over $CaSO_4$.

Yield: 75%. Mp: 202–204 °C. ^{1}H NMR ($CDCl_3$): 8.06 (d, J = 9.3 Hz, 2H), 7.85 (d, J = 8.5 Hz, 2H), 7.68 (d, J = 2.6 Hz, 2H), 7.36 (d, d, J = 9.3 Hz, J = 2.6 Hz, 2H), 6.96 (d, J = 8.6 Hz, 2H), 4.07 (s, 2H), 3.91 (s, 6H). ^{13}C NMR ($CDCl_3$): 158.29 (s); 146.72 (s); 145.30 (s); 143.93 (s); 133.41 (s); 131.82 (d); 130.40 (s); 129.30 (d); 123.80 (d); 123.27 (d); 101. 36 (d), 55.42 (q), 39.35 (t).

*5.1.10. 2,7-Dimethoxy-9-(4′-nitrobenzylsulfinyl)-acridine (**11**)*

Oxidation of **10** using method B gave after 6 h a mixture of starting sulfide and sulfoxide and 8% sulfone. Yield: 37%. Mp: 192 °C. ^{1}H NMR ($CDCl_3$): 8.09 (d, J = 9.5 Hz) and 7.92 (d, J = 8.6 Hz + shoulder, 4.6H), 7.38 (d, d, J = 9.5 Hz, J = 2.6 Hz, 2H), 7.03 (d, J = 8.6 Hz, 2H), 4.62 (s, 2H), 3.89 (s, 6H). ^{13}C NMR ($CDCl_3$): 158.29 (s), 147.79 (s), 143.47 (s), 136.73 (s), 133.49 (s), 132.09 (d), 131.14 (d), 124.23 (s), 123.38 (d), 97.61 (d), 58.37 (t), 55.61 (q).

*5.1.11. 2,7-Dimethoxy-9-(4′-nitrobenzylsulfonyl)-acridine (**12**)*

Method B starting from sulfoxide **11**. Yield: 58%. Mp: dec.>195 °C. ^{1}H NMR ($CDCl_3$): 8.35 (d, J = 2.6 Hz, 2H), 8.13 (d, J = 9.6 Hz, 2H), 7.95 (d, J = 8.8 Hz, 2H), 7.45 (d, d, J = 9.6 Hz, J = 2.6 Hz, 2H), 7.12 (d, J = 8.8 Hz, 2H), 4.67 (s, 2H), 3.96 (s, 6H). ^{13}C NMR ($CDCl_3$): 160.14 (s), 144.28 (s), 134.46 (s), 132.70 (d), 131.80 (d), 129.15 (s), 125.79 (s), 123.83 (s), 123.83 (d), 123.42 (d), 99.91 (d), 61.84 (t), 55.71 (q).

*5.1.12. 2,7-Dimethoxy-9-(ethylthio)-acridine (**13**)*

Method A. Yield: 72%. Mp: 129.5–130.5 °C. ^{1}H NMR ($CDCl_3$): 8.12 (d, J = 9.3 Hz, 2H), 8.0 (d, J = 2.7 Hz, 2H), 7.44 (d, d, J = 9.3 Hz, J = 2.7 Hz, 2H), 4.06 (s, 6H), 2.98 (q, J = 7.3 Hz, 2H), 1.19 (t, J = 7.3 Hz, 3H), ^{13}C NMR ($CDCl_3$): 158.54 (s); 144.55 (s); 136.53 (s); 132.24 (d); 131.15 (s); 124.28 (d); 102.66 (d); 56.05 (q); 30.87 (t); 16.00 (q).

*5.1.13. 2,7-Dimethoxy-9-(ethylsulfinyl)-acridine (**14**)*

Method B. Yield: 56%. Mp: 148–149 °C. ^{1}H NMR ($CDCl_3$): from 8.25 to 7.95 (shoulder) + 8.06 (d, J = 9.4 Hz) (4H), 7.38 (d, d, J = 9.4 Hz, 2H), J = 2.6 Hz, 2H), 3.95 (s, 6H), 3.52 (d, q, J = 13 Hz, J = 7.5 Hz, 1H), 3.29 (d, q, J = 13 Hz, J = 7.5 Hz, 1H), 1.27 (t, J = 7.5 Hz, 3H). ^{13}C NMR ($CDCl_3$): 158.11 (s), 143.52 (s); 135.43 (s); 131.91 (d); 125.69 (s); 123.99 (d); 99.15 (d); 55.52 (q); 46.88 (t); 8.25 (q). Mass spectrum: *m/z*: 316 (100%), 286, 239,195,155.

*5.1.14. 2,7-Dimethoxy-9-(ethylsulfonyl)-acridine (**15**)*

Method B. Yield: 16%. Mp: 212–213 °C. ^{1}H NMR ($CDCl_3$): 8.54 (d, J = 2.6 Hz, 2H), 8.09 (d, J = 9.4 Hz, 2H), 7.40 (d, d, J = 9.4 Hz, J = 2.6 Hz, 2H), 4.00 (s, 6H); 3.42 (q, J = 7.4 Hz, 2H), 1.25 (t, J = 7.4 Hz, 3H). ^{13}C NMR ($CDCl_3$): 159.60 (s); 144.32 (s); 132.35 (d); 130.46 (s); 125.62 (s); 123.74 (d); 100.19 (d); 55. 64 (q); 50.86 (t); 7.09 (q).

*5.1.15. 2,7-dimethoxy-9-(2′-hydroxyethylthio)-acridine (**16**)*

2,7-Dimethoxy-9-thioacridinone (2.7 g, 10 mmol), bromethanol (2.5 g, 20 mmol), triethylamine (2 g, 20 mmol), DMF (30 ml) were heated at 110 °C during 22 h. After cooling, KOH, 2.5 M (100 ml) was added and stirred. The precipitate was filtered, dried and purified by chromatography on silica gel (ether).

Yield: 64%. Mp: 168–170 °C. ^{1}H NMR (DMSO-d_6): 7.95 (d, J = 9.3 Hz) and 7.91 (d, J = 2.7 Hz) (4H), 7.35 (d, d, J = 9.3 Hz, J = 2.7 Hz, 2H), 4.85 (t, J = 4.9 Hz, 1H), 3.97 (s, 6H), 3.44 (m, 2H), 2.98 (t, J = 6.5 Hz, 2H). ^{13}C NMR (DMSO-d_6): 157.66 (s), 143.33 (s), 135.46 (s), 131.30 (d), 130.04 (s), 123.43 (d), 101.86 (d), 60.11 (t), 55.24 (q), 38.25 (t).

*5.1.16. 2,7-Dimethoxy-9-(2′-diethylaminoethylthio)-acridine (**17**)*

2,7-Dimethoxy-thioacridinone (2 g, 7.4 mmol), 2-diethylaminochloroethane, hydrochloride (1.8 g, 10 mmol), buta-

none (30 ml), NaOH, 6 M (25 ml) were heated at 110 °C during 15 min. After cooling, water was added and the product extracted with CH_2Cl_2 and dried over $CaSO_4$. The solvent is eliminated under vacuum. The solid was rinsed with acetone and ether and crystallized from ethanol 95%.

Yield: 73%. Mp: 70–70.8 °C. ^{1}H NMR ($CDCl_3$): 8.19 (d, J = 9.4 Hz, 2H), 7.98 (d, J = 2.7 Hz, 2H), 7.41 (d, d, J = 9.4 Hz, J = 2.7 Hz, 2H), 4.04 (s, 6H), 3.01 (m, 2H), 2.61 (m, 2H), 2.43 (q, J = 7.1 Hz, 4H), 0.87 (t, J = 7.1 Hz, 6H). ^{13}C NMR ($CDCl_3$): 158.36 (s); 144.28 (s); 136.53 (s); 132.04 (d); 130.68 (s); 124.07 (d), 102.31 (d); 55.81 (q); 53.11 (t); 47.04 (t); 34.02 (t); 11.91 (q).

*5.1.17. 2,7-Dimethoxy-9-(2′-diethylaminoethylsulfinyl)-acridine (**18**)*

Thioether **3** (0.5 g, 1.7 mmol), DMF (25 ml), KI (0.7 g, 4.3 mmol), K_2CO_3 (0.9 g, 6.5 mmol), diethylamine (0.6 g, 8 mmol) were heated at 100 °C during 3 days or 120 °C during 19 h. After cooling, 10% KOH was added, the precipitate filtered and dried. The pure product was obtained after chromatography on silica gel (ether–ether 5% MeOH).

Yield: 35%. Mp: 234–236 °C (HCl). ^{1}H NMR ($CDCl_3$): 8.5–7.5 (shoulder), 8.06 (d, J = 9.4 Hz) (>2H), 7.37 (d, d, J = 9.4 Hz, J = 2.7 Hz, 2H), 3.93 (s, 6H), 3.70 (m, 1H), 3.30–3.0 (m, 2H), 2.79 (m, 1H), 2.52 (br.q, J = 7.2 Hz, 4H), 0.98 (t, J = 7.2 Hz, 6H). ^{13}C NMR ($CDCl_3$): 158.34 (s); 143.85 (s); 136.67 (s); 132.17 (d); 125.78 (s); 124.34 (d); 98.41 (d); 55.75 (q); 52.11 (t); 46.96 (t); 46.72 (t); 11.66 (q).

*5.1.18. 3-Chloro-9-benzylthio-acridine (**19**)*

Method A: the crude product was purified by chromatography (petroleum ether/ether 5/5).

Yield: 65%. Mp: 118–120 °C. ^{1}H NMR ($CDCl_3$): 8.65 (d, d, J = 8.8 Hz, J = 0.4 Hz, 1H), 8.55 (d, J = 9.3 Hz, 1H), 8.18 (m, 2H), 7.78 (t, d, J = 6.6 Hz, J = 1.2 Hz, 1H), 7.55 (t, d, J = 6.6 Hz, J = 1.0 Hz, 1H), 7.42 (d, d, J = 9.3 Hz, J = 2.0 Hz, 1H), 7.05 (m, 3H), 6.87 (m, 2H), 4.05 (s, 2H). ^{13}C NMR ($CDCl_3$): 149.17 (s); 148.44 (s); 142.34 (s); 136.83 (s); 136.14 (s); 130.58 (d); 129.97 (d); 128.92 (s); 128.53 (d); 128.25 (d); 127.57 (d); 127.26 (d); 126.76 (d); 126.66 (d), 41.82 (t).

*5.1.19. 2,7-Dimethoxy-9-benzylthio-acridine (**20**)*

Method A: the crude product was purified by chromatography (petroleum ether/ether 5/5).

Yield: 80%. Mp: 145–146 °C. ^{1}H NMR ($CDCl_3$): 8.05 (d, J = 9.3 Hz, 2H) 7.75 (d, J = 2.7 Hz, 2H), 7.35 (d, d, J = 9.3 Hz, J = 2.7 Hz, 2H), 7.05 (m, 3H), 6.87 (m, 2H), 3.99 (s, 2H), 3.90 (s, 6H). ^{13}C NMR ($CDCl_3$): 158.15 (s); 144.16 (s); 137.89 (s); 135.29 (s); 131.70 (d); 130.75 (s); 128.7 (d); 128.32 (d); 127.23 (d); 123.88 (d); 102.02 (d); 55.54 (q); 40.61 (t).

*5.1.20. 2,7-Dimethoxy-9-(benzylsulfinyl)-acridine (**21**)*

Method B from **20**. Yield: 36%. Mp: 182–184 °C. ^{1}H NMR ($CDCl_3$): 8.60 (shoulder) and 8.08 (d, J = 9.3 Hz) (2.9 H), 7.38 (d, J = 9.3 Hz, 2H), 7.19–6.97 (m, 4H), 6.83 (d, J = 7.1 Hz, 2H), 4.70 (d, J_{AB} = 12.1 Hz, 1H), 4.63 (d, J_{AB} = 12.1 Hz, 1H), 3.89 (s, 6H). ^{13}C NMR ($CDCl_3$): 158.07 (s); 144 (m); 134.63 (s); 131.83 (d); 130.21 (d); 129.24 (s); 128.47 (d); 128.43 (d); 126.2 (s); 124.13 (d); 98.09 (m); 59.28 (t); 55.57 (q). Mass spectrum: *m/z*: 377 (15%), 361 (23%), 329 (18%), 286 (60%), 270 (100%).

*5.1.21. 2,7-Dimethoxy-9-(benzylsulfonyl)-acridine (**22**)*

Method B from **20**. Yield: 43%. Mp: 292–293 °C. ^{1}H NMR ($CDCl_3$): 8.29 (d, J = 2.6 Hz, 2H), 8.08 (d, J = 9.4 Hz, 2H), 7.38 (d, d, J = 9.4 Hz, J = 2.6 Hz, 2H), 7.16 (m, 1H), 7.05 (m, 2H), 6.88 (br.d, J = 7.2 Hz, 2H), 4.60 (s, 2H), 3.92 (s, 6H). ^{13}C NMR ($CDCl_3$): 159.56 (s); 144.26 (s); 132.23 (d); 130.93 (d); 130.05 (s); 128.89 (d), 128.40 (d); 127.28 (s); 126.02 (s); 123.74 (d); 100.28 (d); 62.69 (t); 55.62 (q).

5.2. Biology

5.2.1. Cytotoxicity testing

Cytostatic and cytotoxic properties of compounds were determined by use of in vitro panel of over 60 different human cancer cell lines in standard procedures at the NCI [31,32]. Results were expressed in terms of standard calculated response parameters: GI_{50} (drug concentration causing a 50% reduction in the cell increase in the test wells relative to the control wells (i.e. 50% growth inhibition), TGI (drug concentration resulting in total growth inhibition) and LC_{50} (drug concentration causing 50% reduction in cells present in the test wells at the end of drug treatment relative to that at the start (i.e., 50% cell kill). Sensitivity data from anticancer drug screens with the NCI tumor cell line panel are routinely displayed as “mean graphs” (MG) [33,34]. For a given drug, MG-MID indicates the average sensitivity of all tested cell lines, the second figure in Table 1 is the concentration corresponding to the best activity displayed by the agent.

5.2.2. DNA interaction: UV spectroscopic studies

5.2.2.1. Materials. Highly polymerised type 1 calf thymus DNA sodium salt was from the Sigma Chemical Corporation.

5.2.2.2. Methods. Stock solutions of DNA and acridine derivatives were prepared as follows: calf thymus DNA (10 mg) was suspended in 0.03 M tris(hydroxymethyl) aminomethane hydrochloride buffer containing 0.018 M NaCl adjusted to pH 7.0 (10 ml) and the solution was kept at 4 °C for at least 3 days before use. The stock solution was then diluted to the working concentration just before use. Derivatives were dissolved in DMF or DMSO at 10^{-3} M concentration also just before use. A series of solutions of compounds (5×10^{-5} M) was investigated from a spectral point of view in the presence of increasing amounts of CT DNA ($2.0–40.0 \times 10^{-5}$ M). Spectra were recorded on a Cary Varian IE spectrophotometer in 0.03 M tris buffer containing 0.018 M NaCl at pH

7.00. Bathochromic shift (if any) was determined and binding affinity constant (k) and number of binding sites/mole macromolecule were calculated from the hypochromic shift recorded at the λ_{max} of each compound using non-linear regression graph fitting software [35]. Concentrations of DNA solutions were determined spectrophotometrically in terms of nucleotide phosphate and calculated from extinction coefficient at 260 nm of 6600 M^{-1} cm^{-1} for calf thymus DNA [36].

Measurement of thermal denaturation profiles of drug: DNA complexes were made for drug: DNA ratios of 1:10 and were recorded on a Cary Varian Model IE spectrophotometer using a Cary temperature controller connected to a Cary 1/3 multicell block. Solutions were allowed to equilibrate for 20 min, then temperature was increased at a rate of 0.5 °C/min. A cell containing the DNA solution alone was always measured along side cells containing drug/DNA mixtures to act as internal standard. Blank cells contained tris buffer solution in all measurements and all cells were stoppered with teflon caps, after debubbling and space was allowed for the expansion of solutions [37]. The mid-point of the thermal denaturation profile of solutions (Tm) was determined by calculating the average absorbance using the instrument thermal application software [17].

5.2.3. Determination of DNA damaging activity using yeast strains

5.2.3.1. Yeast strains. The wild type yeast strain used as control was JN362a (*MATα ura3-52 leu 2 trp1 his7 ade1-2 ISE-2*) and the Rad52-/Rad6- double deficient yeast strains was the Y195 strain which correspond to JN362a strain converted to *rad52*::LEU2 *rad6*::URA3. Both strains were provided by Dr. J. Nitiss (St Jude Children's Research Hospital, Memphis TN, USA).

5.2.3.2. Yeast growth inhibition. Yeasts in an exponentially growing phase in selective liquid media at 30 °C were adjusted to 107 cells/ml. Thereafter, serial 10-fold dilutions of each culture were performed in sterile water, and 2 μl aliquots of each dilution were seeded onto Petri dishes containing selective agar medium including the test compound at various concentrations or solvent alone, i.e. 1% dimethyl sulfoxide, that proved to be non-deleterious to yeast growth. Plates were incubated at 30 °C for 3 days to allow growth of the yeast. The surface of all the plates was then digitally processed using Geldoc 1000 gel documentation system (Bio-Rad, Hercules, CA, USA), and the density of yeast growth for each inoculum was quantitated with the associated Molecular Analyst software provided (Bio-Rad).

For each yeast strain, inhibition of growth by given concentration of drug was measured as a percentage of growth density on the control plate solvent alone. The GraphPad Prism software was used to calculate and draw non-linear regression curves of cytotoxicity, using the "sigmoidal dose–response (variable slope)" mode. Top plateau corresponds to yeast growth unaffected by presence of the test compound, and bottom plateau to the total inhibition of growth. Drug concentrations that reduced the density of growth by 50% (IC_{50} values) were calculated. Furthermore, differential sensitivities observed between two yeast transformants challenged by any test compound were assessed in terms of the ratio of the two respective IC_{50} values. A series of independent experiments indicated that this ratio had a variability of the yeast. Each test compound was evaluated at least twice in independent experiments.

5.2.4. Determination of topoisomerase II-independent cytotoxicity using yeast strains

5.2.4.1. Yeast strains. This assay was performed with two yeast strains, Y136 which express a low level of human topoisomerase II and Y141 which overexpress human topoisomerase II, as detailed previously [38].

5.2.4.2. Yeast growth inhibition. This evaluation was similar to that of determination of DNA damaging activity and was previously described in details [38].

5.2.5. Determination of DNA damaging activity using mammalian cells

DNA damaging activity in human A549 non-small cell lung cancer cells was evaluated using the Comet assay as described previously [39]. DNA damage was detected in A549 cells after 1 h incubation in the presence of 10 μM of test compound. For each comet, the "tail moment" (TM), defined as the product of the percentage of DNA in the tail multiplied by the tail length was calculated using the Komet software (Kinetic Imaging, UK). For each experimental point, 25 comets were analysed. Finally, from each experimental point the mean tail moment was calculated, reflecting the quantity of DNA-strand breaks.

5.2.6. A549 cell growth inhibition

A549 tumour cells were cultivated in RPMI 1640 medium without phenol red (Seromed) containing 5% foetal bovine serum (100 μl/well, 1.25×10^4 cell/ml) in 96 well plates. After a 24-h incubation at 37 °C in a 5% CO_2 incubator, the medium was removed and replaced by medium containing the test agent, after which the plates were incubated for a further 48 h. Cell survival was then evaluated by measurements of luminescence after ATP release into the medium using mammalian cell lysis and luciferine/luciferase solutions from ATP-lite-M™ kit as recommended by the manufacturer (Packard, Rungis, France). Each condition tested was evaluated at least three times in three independent experiments in sextuplet.

Acknowledgements

We are thankful to the National Cancer Institute for the in vitro and in vivo testing. We are indebted to Dr. J.L. Nitiss (St

Jude Children's Hospital, Memphis, USA) for providing yeast strains and plasmids. We would like to thank Yoann Menon, Laurence Lacastaigneratte and Vanessa Offrete for their technical contributions to the experiments.

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