REACTION OF PROFLAVINE DERIVATIVES WITH FORMALDEHYDE: ACCESS TO MONO- AND BIS-(3,4-DIHYDRO[1,3]OXAZINO)ACRIDINES OF PHARMACOLOGICAL INTEREST

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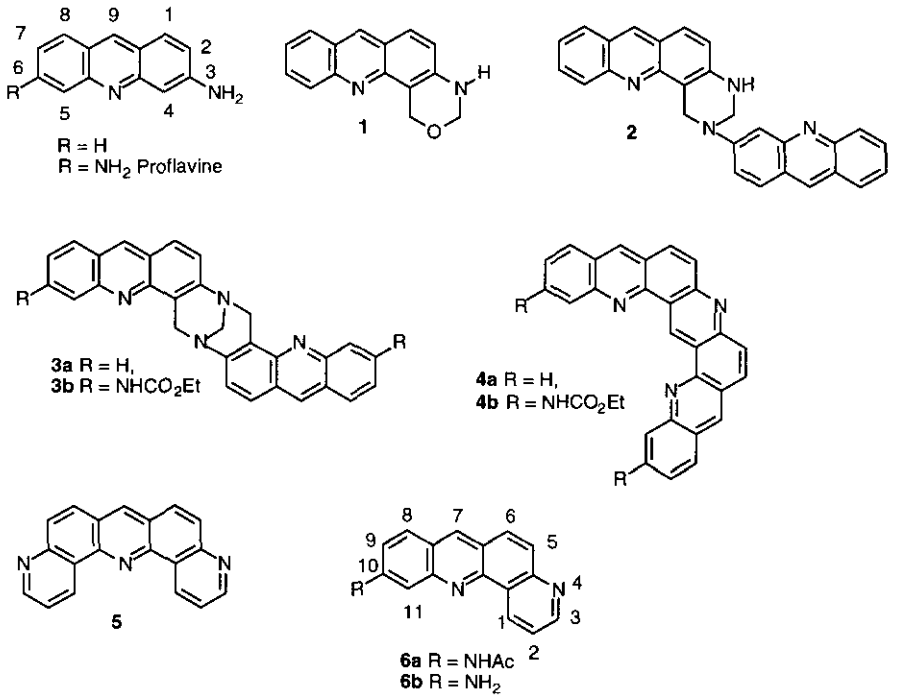
Abstract - Proflavine derivatives react with formaldehyde in acidic media to give 3,4-dihydro[1,3]oxazino condensed compounds. Depending on the nature of the solvents and the substituents attached to the exocyclic nitrogens, mono and bis reaction can occur.

INTRODUCTION.

Acridine derivatives are known to interact with nucleic acids[1] and show a large variety of biological properties (antimalarial, anticancer...).[2] In the course of a project devoted to search for new anticancer drugs, we introduced various functional groups to the acridine ring.[3] We examined in particular the reactivity of aminoacridines in electrophilic substitution reactions.[4-6] A preliminary ^{1}H NMR study had shown that H/D exchange in acidic medium occurs regioselectively at 4 position of 3-aminoacridine and at 4 and 5 positions of proflavine (3,6-diaminoacridine), 2 and 7 positions appearing totally unreactive in the same conditions.[4] We took advantage of this observation to introduce substituents at these positions (C-4 and C-5). Reaction of formaldehyde with 3-aminoacridine in acidic media was studied in detail.[5] The

reaction appeared complex, leading to four different types of compounds through competitive pathways. The dihydrooxazino (**1**) and tetrahydropyrimidino (**2**) derivatives along with the Tröger's Base analogs (**3a**) and acridino[3,4-*j*]benzo[*b*][1,7]phenanthroline (**4a**) were formed (Scheme 1). The reaction is strongly dependent on the stoichiometry in formaldehyde and on the nature of the acid, and compounds (**2**, **3a** and **4a**) could be prepared selectively in yields close to 80 % depending on the conditions used. The dihydrooxazino derivative (**1**) on the other hand was produced in low yields in all conditions, but was prepared in good yield by an independant route involving treatment of the 3-ethoxycarbonylaminoacridine by excess formaldehyde in methanesulfonic acid followed by hydrolysis of the carbamate function.[5]



Scheme 1: Products (**1**~**6**) resulting from electrophilic substitution on 3-aminoacridine and 3,6-diaminoacridine (proflavine)

Substitution on the proflavine ring is more complex due to the presence of two symmetrical amino substituents. For example, Skraup condensation (glycerol in sulfuric acid) has been reported to lead to the bis-pyridoacridine derivative (**5**) through reaction at both C-4 and C-5 sites.[7] Controlled reaction at one site required protection of one of the amino functions. The acetamido groups proved convenient to direct the heterocyclisation of a monoacetylated proflavine into 10-acetamidobenzo[*b*][1,7]phenanthroline (**6**) by treatment with acrolein diethylacetal in refuxing acetic acid.[6] Monoprotected proflavine such as the mono-

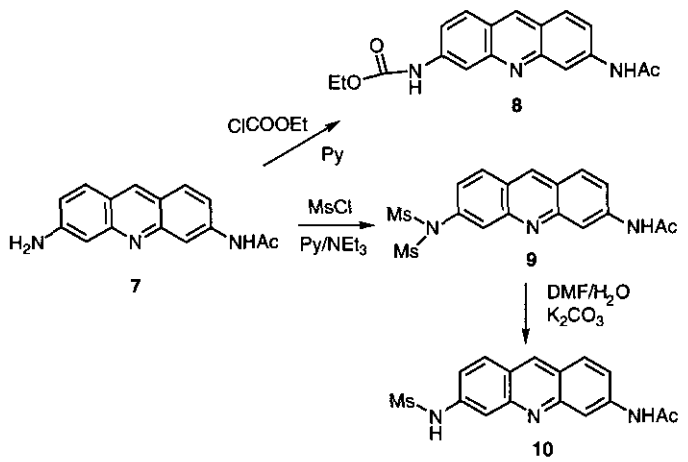
ethoxycarbonylproflavine, yielded the Tröger's Base analogs (**3b**) (75 %) when treated with 1.5 equivalent of formaldehyde in trifluoroacetic acid,[8] or was converted into the fused heptacyclic compound (**4b**) by action of 0.5 equivalent of formaldehyde in 12N HCl (**4b** was used as an intermediate to prepare the first heterocyclic Kekulene analog).[9] The two latter reactions involving formaldehyde as a reagent did not furnish any oxazine derivative. The tetracyclic derivative (**6b**) that possesses the unsubstituted C-11 atom *ortho* to the amino group reacted equally with formaldehyde in acid medium to give C-11 substitued derivatives.[10]

A large screening program involving both *in vitro* and *in vivo* experiments indicated that in the two series, aminoacridine and aminobenzo[*b*]phenanthroline, the 1,3-dihydrooxazino derivatives were quite promising.[3,10] It thus appeared interesting to extend the series to the bifunctional proflavine itself and prepare the corresponding mono- and bisdihydro[1,3]oxazinoacridines. We describe in this paper the synthesis of these compounds and show that the reactivity of proflavine can be controlled by both the nature of the substituents introduced on the exocyclic amines and by the strength of the acid used for the electrophilic substitution. Preliminary results of cytotoxicity are also presented.

RESULTS AND DISCUSSION.

To control the reactivity of the acridine nucleus, the two amines of proflavine were selectively substituted by groups inducing different electronic effects on the ring. Monoacetylproflavine efficiently prepared in good yields (80 %)[6] was used as starting material. The acetylamino group is stable in mild acidic conditions (acetic acid) but slowly hydrolyzes in stronger acids (6N HCl or methanesulfonic acid). We have previously observed that the ethoxycarbonylamino group is stable in acids, and depending on the reaction conditions it can be used to prepare dihydrooxazino derivatives in high yields.[5] Methanesulfonylamino groups were also chosen as they are stable in strong acidic conditions and their high electron-withdrawing properties can be used to modulate substitution on the carbon located on the *ortho* position.

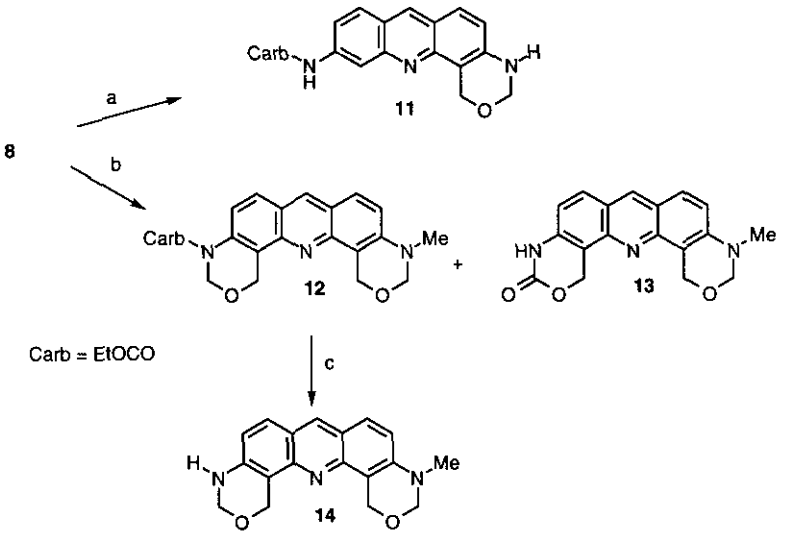
Reaction of monoacetylproflavine (**7**) with ethyl chloroformate in pyridine gave *N*-acetyl-*N'*-ethoxycarbonylproflavine (**8**) in good yield (87 %). A similar reaction performed with methanesulfonyl chloride directly afforded *N,N*-bismethanesulfonyl-*N'*-acetyl derivative (**9**) with 86 % yield. The *N*-methanesulfonyl-*N'*-acetyl compound (**10**) was obtained in 98 % yield by alkaline hydrolysis of **9**.



Scheme 2: Formation of protected proflavines (**8~10**)

The three substituted derivatives (**8~10**) were then reacted with a large excess of formaldehyde in HCl or methanesulfonic acid, as described in the following section (1)-(3).

(1) Reaction of carbamate derivative (**8**).



a) aq. HCHO, 12N HCl, 45°C; b) aq. HCHO, MsOH, rt, 28 h; c) THF/EtOH, $NaOH/H_2O$, 80°C, 3 h

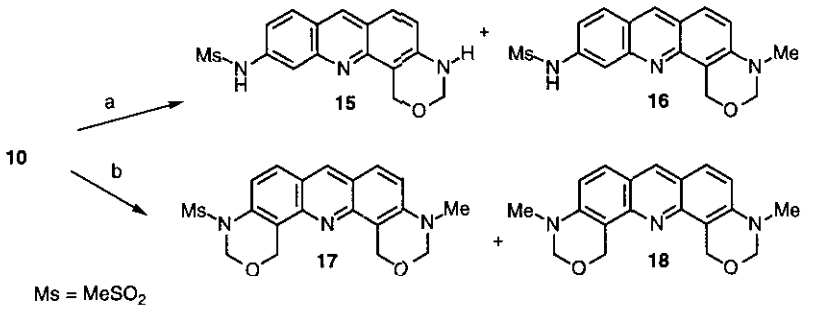
Scheme 3: Reactivity of **8** with formaldehyde

In 12N HCl, one major compound (**11**) could be isolated in 58 % yield by column chromatography. It corresponds to a monooxazine derivative as confirmed by ^{1}H NMR. The nitrogen of the oxazine ring is not substituted.

In methanesulfonic acid, two compounds were obtained, the bisdihydrooxazine derivative (**12**) in 43 % yield and the minor product (**13**) (8 %). The ethoxycarbonyl group of **12** could be easily removed by alkaline treatment, giving **14** in 61 % yield.

(2) Reaction of methanesulfonamide derivative (**10**).

In 6 N HCl, two mono-dihydrooxazino compounds (**15**) and (**16**) were isolated in 77 and 7 % yields, respectively.

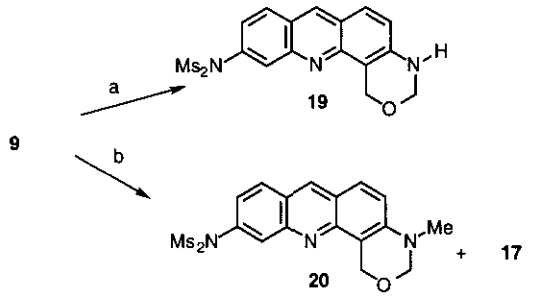


a) $(HCHO)_n$, 6N HCl, 80°C; b) $(HCHO)_n$, MsOH, rt, 7 days

Scheme 4: Reactivity of **10** with formaldehyde

In methanesulfonic acid, compound (**17**) retaining the methanesulfonyl group and the minor compound (**18**) carrying two methyl groups were obtained in 60 and 12 % yields, respectively.

(3) Reaction of the *N,N*-bis(methanesulfonyl)amino compound (**9**).



a) $(HCHO)_n$, 6N HCl, 100°C, 30 h; b) $(HCHO)_n$, MsOH, rt, 2 weeks

Scheme 5: Reactivity of **9** with formaldehyde

In 6 N HCl, product (**19**) was obtained in good yield (87 %).

In methanesulfonic acid, the reaction monitored by HPLC is very slow. After 2 weeks, the mixture was chromatographed, and two products were identified. Compound (**20**) was isolated in 30 % yield and compound (**17**) in 10 % yield.

Starting proflavine	Reaction conditions	Products (yield)
8	12N HCl (45°C, 4 h)	**11** (58)
8	$MeSO_3H$ (rt, 24 h)	**12** (43), **13** (8)
9	6N HCl (100°C, 30 h)	**19** (83)
10	6N HCl (80°C, 3 h)	**15** (77), **16** (7)
9	$MeSO_3H$ (rt, 15 d)	**20** (30), **17** (10)
10	$MeSO_3H$ (rt, 7 d)	**17** (60), **18** (12)

Table 1: Reaction products obtained from substituted proflavines reacting with formaldehyde in various acidic conditions. Yields are given in parentheses.

For the three derivatives (**8~10**) examined, the electrophilic substitution proceeds at the position *ortho* to the acetamido function. In 6N or 12 N HCl, the dihydrooxazine ring thus formed is unsubstituted while in methanesulfonic acid, the dihydrooxazine ring is *N*-methylated. This *N*-methylation with formaldehyde in methanesulfonic acid occuring in the absence of a reducing agent is similar to the Eschweiler-Clark reaction which is usually performed in formic acid.[11] The presence and nature of the amino substituents has a major effect on the formation of a second dihydrooxazine ring. The positions *ortho* to the ethoxycarbonylamino or methanesulfonylamino groups are not reactive in 6N or 12N HCl, the electron-withdrawing effect of the substituent is sufficient to deactivate this position. In these cases, the substituent acts as a protecting group. Meanwhile, using a stronger acid such as methanesulfonic acid, the electrophilic reaction occurs, leading to the corresponding bis-dihydrooxazino compound. The presence of two electron-withdrawing groups in the bis(methanesulfonyl)amino compound (**9**), totally prevents the electrophilic substitution at the *ortho* position, leading to the mono-dihydrooxazino compounds (**19** and **20**) in 6N HCl and methanesulfonic acid, respectively. In the latter solvent the reaction is slow and partial deprotection of one methanesulfonyl group can explain the formation of the bis-dihydrooxazine (**17**). Finally, the ethoxycarbonyl group appears

to be more versatile. It can be hydrolyzed under mild alkaline conditions that do not affect the dihydrooxazine rings (Scheme 3).

CONCLUSION

We have prepared a series of mono- and bis-dihydrooxazino condensed acridines. Common amino protecting groups such as ethoxycarbonyl or methanesulfonyl groups appeared stable in the acidic conditions used for the electrophilic substitution with formaldehyde. They control the regioselectivity of the reaction as it was possible to direct the reaction to a mono or bis substitution by changing the acid. The cytotoxicity of compounds (**14**, **17**, **19** and **20**) has been determined *in vitro* on L1210 cell lines. The lowest IC_{50} values were found for **14** and **19** (1.2 and 10 μM, respectively). These results confirm that functionalization of proflavine with one or two dihydrooxazine rings constitutes a new approach to active compounds.

EXPERIMENTAL

^{1}H NMR were recorded on Bruker AM200 and AM300 spectrometers. Spectra were referenced to the residual proton solvent peaks. Mass spectra were recorded on Delsi Nermag R10-10 instrument. For all products, the purity was ascertained by ^{1}H NMR and HPLC analyses. In several cases, correct elemental analysis could not be obtained due to the polar character of the compounds. All melting points are uncorrected.

3-Acetylamino-6-ethoxycarbonylaminoacridine (8). Ethyl chloroformate (70 mL, 730 mmol) was added to monoacetylproflavine (**7**) (5 g, 20.1 mmol) dissolved in pyridine (300 mL) and the mixture was stirred for 30 min at rt. The solution was filtered and the solvent was evaporated to dryness under reduced pressure. The residue was triturated in diisopropyl ether (200 mL), the solid thus obtained was filtered washed with diisopropyl ether and dried. Compound (**8**) was obtained as the hydrochloride with 87 % yield (6.35 g). Yellow powder (MeOH), mp 255°C; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm) 15.81 (1H, s, NH); 11.41 (1H, s, NH); 10.81 (1H, s, NH); 9.42 (1H, s, H-9); 8.75 (1H, s, H-4); 8.45 (1H, s, H-5);

8.23 (1H, d, J = 9.1 Hz, H-1); 8.22 (1H, d, J = 9.2 Hz, H-8); 7.82 (1H, d, J = 9.1 Hz, H-2); 7.68 (1H, d, J = 9.2 Hz, H-7); 4.23 (2H, q, J = 7.1 Hz, CH_2); 2.23 (3H, s, $COCH_3$), 1.31 (3H, t, J = 7.1 Hz, CH_3); MS (CI, NH_3, C_4H_{10}) m/z = 324 (M^+), 278 (M^+- C_2H_5OH), 235 (M^+- C_2H_5OH - $COCH_3$); UV (EtOH) λmax (ε) 384 (17 700), 273 (71 700), 252 (36 100) nm; *Anal.* Calcd for $C_{18}H_{18}N_3O_3$, HCl, H_2O: C, 57.07; H, 5.59; N, 11.09. Found: C, 57.22; H, 5.34; N, 11.12.

3-Acetylamino-6-(*N,N*-dimethanesulfonyl)aminoacridine (9). Methanesulfonyl chloride (4 mL, 51 mmol) dissolved in pyridine (10 mL) was added to a solution of monoacetylproflavine (**7**) (2.02 g, 8 mmol) in pyridine (200 mL) cooled at 0°C. A precipitate deposited, and it was gradually dissolved by addition of triethylamine (15 mL). After stirring for 20 h at rt, the solvent was evaporated to dryness under reduced pressure, and the residue was stirred overnight in chloroform. After filtration of the insoluble substance, the filtrate was concentrated. Compound (**9**) was purified by column chromatography (silica gel, elution ethyl acetate) and obtained with 86 % yield (2.8 g). Yellow powder (ethyl acetate), mp 240°C (decomp); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 10.42 (1H, s, NH); 9.04 (1H, s, H-9); 8.59 (1H, d, J = 2 Hz, H-4); 8.27 (1H, d, J = 2.2 Hz, H-5); 8.19 (1H, d, J = 9.2 Hz, H-8); 8.12 (1H, d, J = 9.2 Hz, H-1); 7.54 (1H, dd, J = 9.1 and 2.2 Hz, H-7); 7.69 (1H, dd, J = 9.2 and 2 Hz, H-2); 3.60 (6H, s, $2SO_2CH_3$); 2.12 (3H, s, $COCH_3$); MS (EI) m/z = 407 (M^+), 328 (M^+- SO_2CH_3); UV (EtOH) λmax (ε) 358 (8 500), 272, (75 800), 237 (32 500) nm; *Anal.* Calcd for $C_{17}H_{17}N_3O_5S_2$: C, 50.11; H, 4.21; N, 10.31. Found: C, 50.17; H, 4.26; N, 10.46.

3-Acetylamino-6-methanesulfonylaminoacridine (10). A solution of **9** (1 g, 2.4 mmol) in DMF-H_2O (6/1, v/v, 40 mL) mixture was heated for 45 min in the presence of potassium carbonate (2 g, 14.5 mmol). The solvent was then removed under reduced pressure and the resulting solid was stirred in acetone (50 mL) overnight at 0°C. The suspension was filtered to remove the salts and the filtrate was concentrated. Precipitation of monomesyl derivative (**10**) was obtained by adding ether slowly to the concentrated filtrate. It was isolated in 98 % yield (0.79 g). Yellow powder (MeOH), mp 260°C; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm) 10.41 (1H, s, NHAc); 8.81 (1H, s, H-9); 8.45 (1H, d, J = 2 Hz, H-4); 8.01 (2H, d, J = 9.15 Hz, H-1 and H-8); 7.72 (1H, s, H-5); 7.35 (1H, dd, J = 9.1 and 2 Hz, H-7); 7.60 (1H, dd, J = 9.1 and 2

Hz, H-2); 3.11 (3H, s, SO_2CH_3); 2.11 (3H, s, $COCH_3$); MS (EI) m/z = 329 (M^+), (M^+- $COCH_2$), 250 (M^+- SO_2CH_3); UV (EtOH) λmax (ε) 466 (3 500), 281 (17 150), 271 (68 200) nm. *Anal.* Calcd for $C_{16}H_{15}N_3O_3S$: C, 58.35; H, 4.59; N, 12.76. Found: C, 58.46; H, 4.81; N, 12.42.

Reaction of the ethoxycarbonyl derivative (8) with formaldehyde in 12N HCl. Formation of 10-ethoxycarbonylamino-3,4-dihydro-1*H*-[1,3]oxazino[4,5-*c*]acridine (11).

Compound (**8**) (0.5 g, 1.39 mmol) was dissolved in 12N HCl (100 mL) containing 37 % aqueous formaldehyde (4 mL, 53 mmol). The mixture was stirred at 45 °C for 4 h and then cooled to 0 °C. The precipitate thus formed was filtered and then stirred in a water/ethyl acetate/28 % NH_4OH mixture (85 mL, 10/70/5). The aqueous layer was separated, extracted twice with ethyl acetate. The organic layers were collected, dried on magnesium sulfate and then evaporated to dryness. The residue was dissolved in chloroform and the desired dihydrooxazine (**11**) was separated by column chromatography (silica gel, elution chloroform/methanol/28 % NH_4OH mixture (97.5/2.25/0.25, v/v/v)). It was obtained with a 58 % yield (0.26 g). Yellow powder (MeOH), mp 175°C; ^1H NMR (200 MHz, DMSO-d_6) δ (ppm) 10.03 (1H, s, NH); 8.84 (1H, s, H-7); 8.16 (1H, s, H-11); 7.91-6.99 (4H, m, H-5, H-6, H-8 and H-9); 7.01 (1H, br s, NH); 5.24 (2H, s, $ArCH_2O$); 4.79 (2H, s, OCH_2N); 4.20 (2H, q, J = 7 Hz, CH_2); 1.29 (3H, t, J = 7 Hz, CH_3); MS (CI, ammonia, isobutane) m/z = 324 $(M + 1)^+$; UV (EtOH) λmax (ε) 382 (12 700), 290 (40 600), 271 (53 700), 255 (42 600) nm; *Anal.* Calcd for $C_{18}H_{17}N_3O_3$: C, 66.86; H, 5.30; N, 13.00. Found: C, 67.22; H, 5.39; N, 12.94.

Reaction of compound (8) with formaldehyde in methanesulfonic acid. A mixture of **8** (4.8 g, 13.4 mmol) and 37 % aqueous formaldehyde (10 mL, 133 mmol) in methanesulfonic acid (50 mL) was stirred for 7 h at 45°C and then for 21 h at rt. The mixture was added dropwise to a ice/28 % NH_4OH/ethyl acetate mixture (100/100/600, 800 mL). The aqueous phase was separated and extracted twice with ethyl acetate. The organic layers were collected, dried on magnesium sulfate and evaporated to dryness. The residue was triturated in chloroform (25 mL). The insoluble part was filtered, washed with chloroform and dried. Compound (**13**) was thus isolated with 8 % yield (0.34 g). The filtrate was chromatographed on silica gel (elution with a solvent gradient, chloroform/methanol/28 % NH_4OH from 97.5/2.25/0.25 to

91.75/8/0.25 mixture). Fractions containing compound (**12**) were collected and evaporated to dryness. **12** was crystallized from hot ethyl acetate in 43 % yield (2.16 g).

10-Ethoxycarbonylamino-3,4,10,11-tetrahydro-4-methyl-1*H*,13*H*-bis[1,3]oxazino[4,5-*c*:5',4'-*h*]acridine (12). Yellow powder (ethyl acetate), mp 180°C; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm) 8.79 (1H, s, H-7); 7.92-7.39 (4H, m, H-5, H-6, H-8 and H-9); 5.39 (2H, s, $ArCH_2O$); 5.27 (4H, s, $ArCH_2O$ and OCH_2N); 4.76 (2H, s, OCH_2N); 4.26 (2H, q, J = 7 Hz, CH_2); 3.07 (3H, s, NCH_3); 1.30 (3H, t, J = 7 Hz, CH_3); MS (CI, NH_3, isobutane) m/z = 380 $(M + 1)^+$; UV (EtOH) λmax (ε) 375 (10 500), 275 (54 700), 257 (38 400) nm; *Anal.* Calcd for $C_{21}H_{21}N_3O_4$: C, 66.48; H,5.58; N,11.08. Found: C,66.77; H,5.60; N,11.12.

3,4-Dihydro-4-methyl-1*H*,13*H*-bis[1,3]oxazino[4,5-*c*:5',4'-*h*]acridin-11(10*H*)-one (13). Yellow powder (MeOH), mp 218°C; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm) 10.44 (1H, s, N-H); 8.74 (1H, s, H-7); 7.95-7.09 (4H, m, H-5, H-6, H-8 and H-9); 5.84 (2H, s, $ArCH_2OCO$); 5.25 (2H, s, $ArCH_2O$); 4.76 (2H, s, OCH_2N); 3.06 (3H, s, NCH_3); MS (FAB(+), glycerol) m/z = 322 $(M + 1)^+$; UV (EtOH) λmax (ε) 388 (12 500), 297 (29600), 275 (41 300), 257 (42 300) nm; *Anal.* Calcd for $C_{18}H_{15}N_3O_3$ + 0.75 H_2O: C, 64.57; H,4.97; N,12.55. Found: C,64.44; H,4.82; N,12.12.

Hydrolysis of 12: formation of 3,4,10,11-tetrahydro-4-methyl-1*H*,13*H*-bis[1,3]-oxazino[4,5-*c*:5',4'-*h*]acridine (14).

Carbamate protected bis-oxazine (**12**) (2 g, 5.3 mmol) was refluxed for 5 h in a THF/ethanol/water mixture (44/22/1.5, 23.5 mL) in the presence of 30 % NaOH (0.9 mL). The mixture was then cooled to rt. Insoluble material was filtered off and the solvent was evaporated to dryness. The residue thus obtained was solubilized in hot chloroform/THF mixture (10/1, 11 mL) and chromatographed on silica gel pre-treated with chloroform/methanol/28 % NH_4OH (97.5/2.25/0.25, v/v/v) and eluted with chloroform. Compound (**14**) was obtained with 61 % yield (0.99 g). Yellow powder (MeOH), mp 178°C; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm) 8.57 (1H, s, H-7); 7.80-6.93 (4H, m, H-5, H-6, H-8 and H-9); 6.95 (1H, s, NH); 5.24 (2H, s, $ArCH_2O$); 5.18 (2H, s, $ArCH_2O$); 4.78 (2H, s, OCH_2N); 4.72 (2H, s, OCH_2N); 3.04 (3H, s, NCH_3); MS (CI, NH_3, isobutane) m/z = 308 $(M + 1)^+$; UV (EtOH) λmax (ε) 406 (10 300),

298 (22 800), 274 (42 900) nm; *Anal.* Calcd for $C_{18}H_{17}N_3O_2$: C, 70.34; H,5.57; N,13.67. Found: C,70.11; H,5.59; N,13.72.

Reactions of methanesulfonyl derivatives (9) and (10) with formaldehyde in 6N HCl. General procedure.

A solution of compound (**9** or **10**, 0.3 mmol) and paraformaldehyde (0.83 g, 2.8 mmol) in 6N HCl (20 mL) was stirred at 80°C. Disappearence of starting material was monitored by HPLC. When all the starting material has disappeared, the reaction mixture was slowly poured into 10 % aqueous NH_4OH (200 mL) and extracted three times with dichloromethane. The organic layers were collected, dried on sodium sulfate, concentrated and chromatographed on silica gel (elution with dichloromethane/ethyl acetate gradient). The yields obtained for the different products are collected in Table 1

3,4-Dihydro-10-methanesulfonylamino-1*H*-[1,3]oxazino[4,5-*c*]acridine (15). Yellow powder (MeOH), mp 160-162°C; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm) 10.26 (1H, s, N-H); 8.69 (1H, s, H-7); 7.97 (1H, d, J = 8.9 Hz, H-6 or H-8); 7.77 (1H, d, J = 8.9 Hz, H-8 or H-6); 7.69 (1H, d, J = 1.9 Hz, H-11); 7.29 (1H, dd, J = 8.9 and 1.9 Hz, H-9); 7.08 (1H, s, N-H); 7.00 (1H, d, J = 8.9 Hz, H-5); 5.23 (2H, s, $ArCH_2O$); 4.78 (2H, s, OCH_2N); 3.13 (3H, s, CH_3); MS (FAB(+), glycerol/DMSO) m/z = 330 $(M+1)^+$; *Anal.* Calcd for $C_{16}H_{15}N_3O_3S$: C, 58.35; H, 4.59; N, 12.76. Found: C, 58.80; H, 4.63; N, 12.37.

3,4-Dihydro-10-methanesulfonylamino-4-methyl-1*H*-[1,3]oxazino[4,5-*c*]acridine (16). Yellow powder (MeOH), mp 135-137°C; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm) 10.30 (1H, s, N-H); 8.77 (1H, s, H-7); 8.00 (1H, d, J = 9 Hz, H-8); 7.9 (1H, d, J = 9.3 Hz, H-6); 7.71 (1H, s, H-11); 7.35 (1H, d, J = 9.3 Hz, H-5); 7.32 (1H, d, J = 9 Hz, H-9); 5.29 (2H, s, $ArCH_2O$); 4.75 (2H, s, OCH_2N); 3.14 (3H, s, CH_3); 3.06 (3H, s, CH_3); MS (FAB(+), glycerol/DMSO) m/z = 344 $(M+1)^+$; *Anal.* Calcd for $C_{17}H_{17}N_3O_3S$ + 0.15 H_2O: C, 58.99; H, 5.04; N, 12.14. Found: C, 58.81; H, 4.72; N, 11.93.

3,4-Dihydro-10-(*N,N*-bismethanesulfonylamino)-1*H*-[1,3]oxazino[4,5-*c*]acridine (19). Yellow powder (ethyl acetate), mp 210-215°C; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm) 8.69 (1H, s, H-7); 8.20 (1H, d, J = 2.1 Hz, H-11); 8.00 (1H, d, J = 8.8 Hz, H-8); 7.70 (1H, d, J = 9.01 Hz, H-6); 7.33 (1H, dd, J = 8.8 and 2.1 Hz, H-9); 7.00 (1H, d, J = 9.01 Hz, H-5); 5.40 (2H, s, $ArCH_2O$); 4.90 (2H, d,

J = 5 Hz, OCH_2N); 4.40 (1H, s, N-H); 3.50 (6H, s, CH_3); MS (CI, NH_3, isobutane) m/z = 408 $(M)^+$; UV (EtOH) λmax (ε) 435 (5 000), 356 (4 950), 338 (4 0 40), 283 (47 100), 244 (37 000) nm; *Anal.* Calcd for $C_{17}H_{17}N_3O_5S_2$: C, 50.11; H, 4.20; N, 10.31. Found: C, 50.52; H, 4.24; N, 10.0.

Reactions of 9 and 10 with formaldehyde in methanesulfonic acid. General procedure. A mixture of compound (**9**) or (**10**) (1.5 mmol) and paraformaldehyde (0.45 g, 15 mmol)) in methanesulfonic acid (15 mL) was stirred at rt for several days. The reaction was monitored by HPLC. When all starting material has disappeared, the mixture was poured dropwise into water/28 % NH_4OH/dichloromethane mixture. The aqueous phase was separated and extracted twice with dichloromethane. The organic layers were collected, dried on sodium sulfate, concentrated and chromatographed on silica gel (elution with a dichloromethane/ethyl acetate gradient). Yields in reaction products are given in Table 1.

3,4,10,11-Tetrahydro-10-methanesulfonyl-4-methyl-1*H*,13*H*-bis[1,3]oxazino[4,5-*c*:5',4'-*h*]acridine (17). Yellow powder (MeOH), mp 270-275°C; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm) 8.85 (1H, s, H-7); 7.96 (1H, d, J = 9.2 Hz, H-6 or H-8); 7.95 (1H, d, J = 9.2 Hz, H-8 or H-6); 7.59 (1H, d, J = 9.2 Hz, H-5 or H-9); 7.42 (1H, d, J = 9.2 Hz, H-9 or H-5); 5.46 (2H, s, CH_2); 5.28 (2H, s, CH_2); 5.24 (2H, s, CH_2); 4.77 (2H, s, CH_2); 3.07 (3H, s, SO_2CH_3); 3.03 (3H, s, N-CH_3); MS (CI, NH_3, isobutane) m/z = 386 $(M + 1)^+$; UV (EtOH) λmax (ε) 425 (4 160), 368 (5 600), 282 (29 400), 251 (25 600) nm; *Anal.* Calcd for $C_{19}H_{19}N_3O_4S$: C, 59.20; H,4.96; N,10.90. Found: C,59.38; H,4.90; N,10.83.

3,4,10,11-Tetrahydro-4,10-dimethyl-1*H*,13*H*-bis[1,3]oxazino[4,5-*c*:5',4'-*h*]acridine (18). Yellow powder (MeOH), mp 178°C; ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 8.36 (1H, s, H-7); 7.69 (2H, m, H-6 and H-8); 7.11 (2H, m, H-5 and H-9); 5.40 (4H, s, CH_2); 4.74 (4H, s, CH_2); 3.09 (6H, s, N-CH_3); MS (CI, NH_3, isobutane) m/z = 322 $(M + 1)^+$; UV (EtOH) λmax (ε) 409 (10 260), 300 (22 900), 276 (44 300) nm; *Anal.* Calcd for $C_{19}H_{19}N_3O_2$: C, 71.01; H,5.96; N,13.07. Found: C,71.20; H,5.88; N,12.98.

3,4-Dihydro-10-(*N,N*-bismethanesulfonylamino)-4-methyl-1*H*-[1,3]oxazino[4,5-*c*]-acridine (20). Yellow powder (ethyla acetate), mp > 215 °C (decomp); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm) 8.96 (1H, s, H-7); 8.16 (1H, d, J = 2 Hz, H-11); 8.14 (1H, d, J = 8.8 Hz, H-8); 7.8 (1H, d, J = 9.1 Hz, H-6); 7.52 (1H, dd, J = 8.8 and 2 Hz, H-9); 7.49 (1H, d, J = 9.1 Hz, H-5); 5.34 (2H, s, $ArCH_2O$); 4.79 (2H, s, OCH_2N); 3.62 (6H, s, SO_2CH_3); 3.09 (3H, s, N-CH_3); MS (CI, NH_3, isobutane) m/z = 422 $(M + 1)^+$; UV (EtOH) λmax (ε) 435 (3 250), 359 (3 700), 342 (3 250), 286 (62 200), 245 (26 800) nm; *Anal.* Calcd for $C_{18}H_{19}N_3O_5S_2$ + 0.5 H_2O: C, 50.22; H,4.68; N,9.76. Found: C, 50.52; H, 4.24; N, 9.59.

REFERENCES

1. L. S. Lerman, *J. Mol. Biol.*, 1961, **3**, 18; S. F. Kitchen, Y. H. Wang, A. L. BauMStark, W. D. Wilson, and D. W. Boykin, *J. Med. Chem.*, 1985, **28**, 940; J. M. Crenshaw, D. E. Graves, and W. A. Denny, *Biochemistry*, 1995, **34**, 13682.
2. For a recent review article, see: L. R. Ferguson and W. A. Denny, *Mutation Res.*, 1991, **258**, 123; A. Albert, "The Acridines. Their Preparation Physical, Chemical and Biological Properties and Uses", Edward Arnold publishers, London, 1966.
3. A. Duflos, D. C. H. Bigg, J. Lhomme, N. Fixler, C. Etievant, and M. Demeunynck, Pierre Fabre Médicament Patent FR 2,712,290 [*Chem. Abstr.*, 1995, **123**, 313985p]; J. Lhomme, D. C. H. Bigg, A. Duflos, B. Bonnaud, M. Demeunynck, C. Etievant, and R. Kiss, Pierre Fabre Médicament Patent PCT Int. App. WO 94 25,439 [*Chem. Abstr.*, 1995, **122**, 239555j]; A. Duflos, D. C. H. Bigg, J. Lhomme,B. Bonnaud, and C. Etievant, Pierre Fabre Médicament Patent FR 2,716,454 [*Chem. Abstr.*, 1996, **124**, 117108m]
4. J. Fahy, Thèse d'Université, Lille, 1982.
5. A. Tatibouët, N. Fixler, M. Demeunynck, and J. Lhomme, *Tetrahedron*, 1997, **53**, 2891
6. A. Wardani and J. Lhomme, *Tetrahedron Lett.*, 1993, **34**, 6411
7. M. Dufour, N. P. Buu-Hoï, and P. Jacquignon, *J. Chem. Soc. (C)*, 1967, 1415
8. A. Tatibouët, Thèse d'Université, Grenoble, 1996
9. A. Tatibouët, R. Hancock, M. Demeunynck and J. Lhomme, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 1190.
10. A. Wardani, J. Lhomme, D. C. H. Bigg, and H. Salez, Pierre Fabre Médicamant Patent PCT Int App WO 91 07,403 [*Chem. Abstr.*, 1991, **115**, 207973h]
11. M. L. Moore, "Organic Reactions", Vol.5, ed. by R. Adams, W. E. Bachmann, A. H. Blatt, L. F. Fieser, J. R. Johnson, and H. R. Snyder , John Wiley and Sons, London, 1949, p 301.

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