Inhibitors of Platelet Aggregation. 2. 9-{[(Dialkylamino)alkyl]thio}-3-(dimethylamino)acridines and Related Acridine Derivatives[1]

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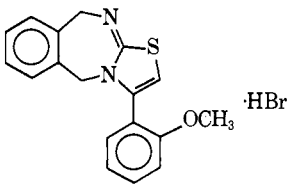
A series of 3,6-bis(dimethylamino)-9-{[(dialkylamino)alkyl]thio}acridines (III) was synthesized in 10–94% yield by the condensation of 3,6-bis(dimethylamino)-9-acridanthione (II) with the appropriate (dialkylamino)-alkyl halide in DMF. 3,6-Bis(diethylamino)-9-{[3-(diethylamino)propyl]thio}acridine (XIII) was prepared similarly. 2- and 3-(dimethylamino)-9-{[3-(dimethylamino)propyl]thio}acridine (XIVa, XVa) and 3-(dimethylamino)-9-[(1-methyl-4-piperidyl)thio]acridine (XVc) were obtained in 51–79% yield from 9-chloro-2- and -3-(dimethylamino)acridine and the appropriate aminothiol in PhOH. Fourteen 3,6-bis(dimethylamino)-9-{[(dialkylamino)alkyl]thio}acridines, XVa, and XVc caused 54–100% inhibition of ADP-induced platelet aggregation *in vitro* at concns of 10^{-5} *M*. Eleven compds also produced >50% inhibition of platelet aggregation in plasma from rabbits given single iv 3–12.5 mg/kg doses. 3,6-Bis(dimethylamino)-9-{[2-(1-pyrrolidinyl)ethyl]-thio}acridine (**6**) caused a significant increase in both primary and secondary bleeding time from a micropuncture wound in the mouse mesentery 4 and 24 hr after a single iv 10 mg/kg dose.

The key role of adenosine diphosphate (ADP) in platelet aggregation and thrombosis[2-6] suggests that compounds active against ADP-induced platelet aggregation may be useful for the prevention and treatment of thrombosis and embolism. In a previous communication from these laboratories it was disclosed that certain 5,10-dihydro-3-(phenyl, thienyl, and furyl)thiazolo[3,2-*b*][2,4]benzodiazepines, exemplified by 5,10-dihydro-3-(*o*-methoxyphenyl)thiazolo-[3,2-*b*][2,4]benzodiazepine·HBr (I), inhibited ADP-induced platelet aggregation *in vitro* and in plasma from rabbits that had been treated with these substances. Moreover, I produced a significant increase in bleeding time from a micropuncture wound in the mouse mesentery.



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We now report the synthesis and biological properties of another novel class of platelet aggregation inhibitors, namely certain 9-{[(dialkylamino)alkyl]thio}-3-(dimethylamino)acridines (III) and related acridine derivatives.

Chemistry.—A series of 3,6-bis(dimethylamino)-9-{[(dialkylamino)alkyl]thio}acridines (III) (**1–19**, Table I) was prepared in 10–94% yield by the condensation of 3,6-bis(dimethylamino)-9-acridanthione (II)[7] with the appropriate dialkylaminoalkyl halide (Scheme I). Optimum yields were obtained when the reaction was carried out at 75–80° in DMF in the presence of 2 equiv of K_2CO_3 (procedure I). However, when II was allowed to react with 2-(2-chloroethyl)-1-methylpyrrolidine[8] under these conditions, tlc (silica–Et_3N–EtOAc) showed that 2 products having a similar R_f were produced. Based on previous observations[8] it was presumed that one component was the normal reaction product, 3,6-bis(dimethylamino)-9-{[2-(1-methyl-2-pyrrolidinyl)ethyl]thio}acridine (**11**), while the other was 3,6-bis(dimethylamino)-9-[(1-methylazepin-4-yl)thio]acridine (IV) which might be expected to be formed by ring enlargement.[8] Alternatively, when 2-(2-chloroethyl)-1-methylpyrrolidine·HCl and II were fused at 135° for 10 min (procedure III), a product containing only one of these components was isolated. Structure **11** was assigned to this material on the basis of the nmr curve which exhibited a partially obscured triplet at δ 2.9, due presumably to CH_2 adjacent to S. In one instance where the acridinethione (II) was condensed with a dialkylaminoalkyl halide in EtOH in the presence of NaOMe (procedure II), 3,6-bis(dimethylamino)-9-ethoxyacridine (V) was isolated as a by-product from the EtOH recrystallization liquors.

The striking effects of the 3,6-bis(dimethylamino)-9-{[(dialkylamino)alkyl]thio}acridines (III) as inhibitors of ADP-induced platelet aggregation *in vitro* (Table I) prompted the synthesis of various other substituted 3,6-bis(dimethylamino)acridine derivatives for evaluation as potential antithrombotic agents. 3,6-Bis(dimethylamino)-9-{[6-(dimethylamino)-2-methyl-4-quinolyl]thio}acridine (VI) was isolated in 8% yield from the condensation of II with 4-chloro-6-(dimethylamino)quinaldine in DMF in the presence of K_2CO_3, while 9-{[3-(diethylmethylammonio)propyl]thio}-3,6-bis(dimethylamino)-10-methylacridinium diiodide (VII) was obtained (60%) by quaternization

(1) Previous paper, E. F. Elslager, J. R. McLean, S. C. Perricone, D. Potoczak, H. Veloso, D. F. Worth, and R. H. Wheelock, *J. Med. Chem.*, **14**, 397 (1971).

(2) J. C. F. Poole and J. E. French, *J. Atheroscler. Res.*, **1**, 251 (1961).

(3) A. J. Honour and R. W. Ross Russell, *Brit. J. Exp. Pathol.*, **43**, 350 (1962).

(4) G. V. R. Born and M. J. Cross, *J. Physiol.*, **168**, 178 (1963).

(5) J. F. Mustard, *Exp. Mol. Pathol.*, **7**, 366 (1967).

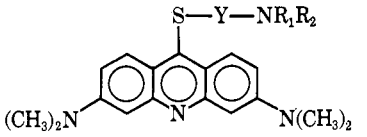
(6) M. G. Davey and E. F. Lüscher, *Sem. Hematol.*, **5**, 5 (1968).

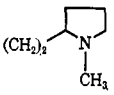
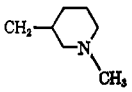
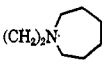
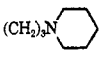
(7) E. F. Elslager, *J. Org. Chem.*, **27**, 4346 (1962).

(8) A. Ebnöther and E. Jucker, *Helv. Chim. Acta.*, **47**, 745 (1964).

TABLE I

3,6-BIS(DIMETHYLAMINO)-9-{[(DIALKYLAMINO)ALKYL]THIO}ACRIDINES



No.	Y-NR_1R_2	Mp, °C	Yield purified, %	Purifcn solvent	Procedure	Formula	Analyses	Inhibition of platelet aggregation *in vitro* Concn, $M \times 10^{-5}$	% inhibition
1	$(CH_2)_2N(CH_3)C_2H_5$	111–113	46	Et_2O–petr ether	I	$C_{22}H_{30}N_4S \cdot 0.66H_2O$	C, H, N, S, H_2O	1	75
2	$(CH_2)_5N(CH_3)_2$	180–183	19	EtOH	II	$C_{22}H_{30}N_4S \cdot 2C_7H_6O_3$[a]	C, H; N[b]	10	100
3	CH_2-α-pyridyl	166–167.5	62	MeCN	I	$C_{23}H_{24}N_4S$	C, H; N[c]	1	72
								1	63
								0.1	44
4	CH_2-β-pyridyl	188–189	66	MeCN	I	$C_{23}H_{24}N_4S$	C, H, N	1	64
								0.1	6
5	CH_2-γ-pyridyl	192–195	32	MeCN	I	$C_{23}H_{24}N_4S$	C, H, N, S	1	78
								0.1	10
6	$(CH_2)_2$-*N*-pyrrolidyl	165–166	70	MeCN	I	$C_{23}H_{30}N_4S$	C, H, N	1	67
								0.1	35
7	$(CH_2)_2$-*N*-morpholinyl	170–171	53	MeCN	I	$C_{23}H_{30}N_4OS$	C, H, N	1	75
								0.1	31
8	$(CH_2)_2N(C_2H_5)_2$	86–88	87	*n*-Hexane	I	$C_{23}H_{32}N_4S \cdot H_2O$	C, H, N, S	1	76
9	$CH_2CH(CH_3)CH_2N(CH_3)_2$	110–111	53	*n*-Heptane	I	$C_{23}H_{32}N_4S$	C, H, N	1	78
								0.1	32
10	$(CH_2)_2$-*N*-piperidyl	135–137	74	Et_2O	I	$C_{24}H_{32}N_4S$	C, H, N, S	1	43
11		70–72	45	EtOAc	III	$C_{24}H_{32}N_4S \cdot 1.5H_2O$	C, H, N, H_2O	10	100
								1	76
								0.1	56
12		98–100	35	Cyclohexane	I	$C_{24}H_{32}N_4S \cdot 0.5H_2O$	C, H, N, H_2O	1	77
13		128–130	61	*n*-Heptane	I	$C_{25}H_{34}N_4S$	C, H, N	1	94
								0.1	23
14		93–95	94	Et_2O	I	$C_{25}H_{34}N_4S$	C, H, N, S	1	54
15	$(CH_2)_2N[CH(CH_3)_2]_2$	111–112	61	*n*-Heptane	I	$C_{25}H_{36}N_4S$	C, H, N	1	100
								0.1	35
16	$CH_2C(CH_3)_2CH_2N(C_2H_5)_2$	90–91	52	MeCN	I	$C_{26}H_{38}N_4S$	C, H, N	1	47
17	$(CH_2)_5N(C_2H_5)_2$	161–163	10	*i*-PrOH	I	$C_{26}H_{38}N_4S \cdot 2C_7H_6O_3 \cdot 0.5H_2O$[a]	C, H, N; H_2O[d]	2	68
18	$(CH_2)_2N(CH_3)CH_2C_6H_5$	108–109	50	*n*-Heptane	I	$C_{27}H_{32}N_4S$	C, H, N	1	22
19	$(CH_2)_2N(CH_3)CH_2C_6H_5$	82–84	46	*n*-Heptane	I	$C_{28}H_{34}N_4S$	C, H, N	1	28
	5,10-Dihydro-3-(*o*-methoxyphenyl)thiazolo[3,2-*b*][2,4]benzodiazepine·HBr (I)							5	100, 80, 73
								1	44, 43, 39
	Methapyrilene·HCl							100	42
								50	25
								10	17
	Adenosine							10	61
								1	25
	N^{α}-(*p*-Tolylsulfonyl)-L-arginine ethyl ester·HCl; TAME·HCl							500	80
								50	53

[a] $C_7H_6O_3$ represents salicylic acid. [b] N: calcd, 8.50; found, 8.91. [c] N: calcd, 14.42; found, 14.01. [d] H_2O: calcd, 1.24; found, 1.77.

of 9-{[3-(diethylamino)propyl]thio}-3,6-bis(dimethylamino)acridine[7] with MeI. Two 9-amino-3,6-bis(dimethylamino)acridine derivatives were also prepared. The reaction of 3,6-bis(dimethylamino)-9-(methylthio)acridine (IX) with 2-[(3-aminopropyl)thio]ethanol in PhOH afforded 2-[(3-{[3,6-bis(dimethylamino)-9-acridinyl]amino}propyl)thio]ethanol (VIII) (87%), while 9-{3-[(diethylamino)methyl]-*p*-anisidino}-3,6-bis(dimethylamino)acridine (X) was obtained in 44% yield by the fusion of N^{α},N^{α}-diethyl-6-methoxytoluene-α,3-diamine[9] and IX at 180°.

To enable an assessment of the effects of larger alkylamino substituents at positions 3 and 6 on platelet aggregation, 3,6-bis(diethylamino)-9-{[3-(diethylamino)propyl]thio}acridine·2HCl (XIII) was synthesized as outlined in Scheme II. 3,6-Bis(diethylamino)acridine·HCl (XI) was prepared from *N*,*N*-diethyl-*m*-phenylenediamine and HCO_2H in glycerol utilizing the experimental conditions recommended by Albert[10] for symmetrical syntheses. The yield of pure product was 17%. This substance was reported earlier by Browning, *et al.*,[11] but was not adequately characterized. Fusion of the free base of XI with S at 240–250° afforded 3,6-bis(diethylamino)-9-acridanthione (XII), which was allowed to react with 3-diethylaminopropyl chloride (procedure I) to give XIII (12%).

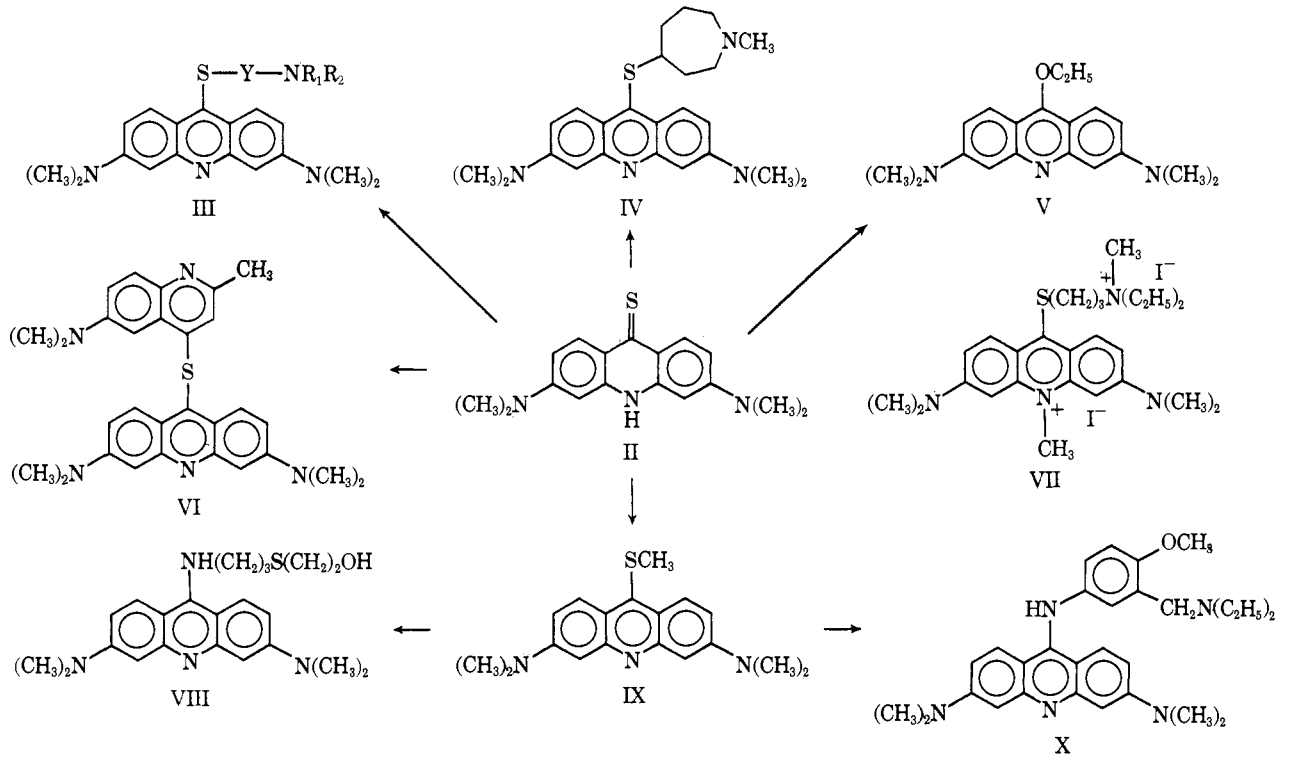
Several mono(dimethylamino)acridine derivatives were also prepared. The condensation of 9-chloro-2-

(9) E. F. Elslager, E. H. Gold, F. H. Tendick, L. M. Werbel, and D. F. Worth, *J. Heterocycl. Chem.*, **1**, 6 (1964).

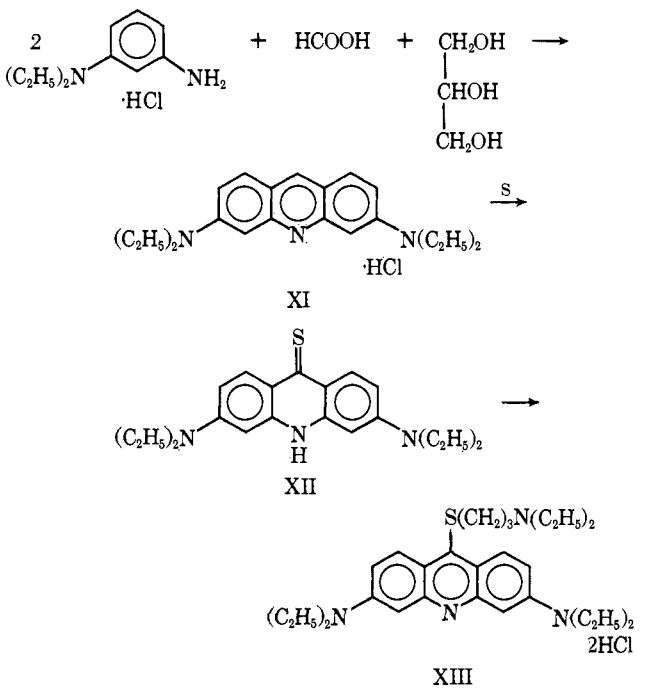
(10) A. Albert, "The Acridines," 2nd ed, Edward Arnold Ltd., London, 1966, p 105.

(11) C. H. Browning, J. B. Cohen, R. Gaunt, and R. Gulbransen, *Proc. Roy. Soc., Ser. B*, **93**, 329 (1922).

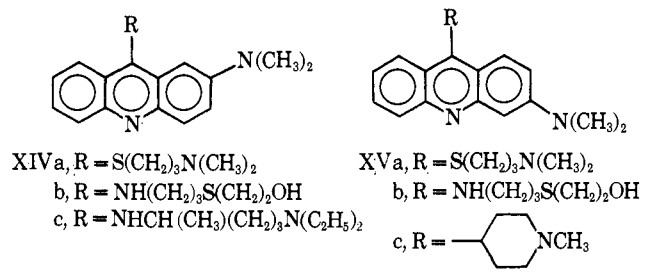
SCHEME I



SCHEME II



dimethylaminoacridine[12] with 3-dimethylamino-1-propanethiol·HCl or 2-[(3-aminopropyl)thio]ethanol in PhOH or with N^1,N^1-diethyl-1,4-pentanediamine in a melt afforded 2-(dimethylamino)-9-{[3-(dimethylamino)propyl]thio}acridine (XIVa) (51%), 2-[(3-{[2-(dimethylamino)-9-acridinyl]amino}propyl)thio]ethanol (XIVb) (84%), and 9-{[4-(diethylamino)-1-methylbutyl]amino}-2-(dimethylamino)acridine (XIVc) (50%). Similarly, 3-(dimethylamino)-9-{[3-(dimethylamino)propyl]thio}acridine (XVa), 2-[(3-{[3-(dimethylamino)-9-acridinyl]amino}propyl)thio]ethanol (XVb), and 3-(dimethylamino)-9-[(1-methyl-4-piperidyl)thio]acridine (XVc) were obtained in 55–79% yield by heating 9-chloro-3-(dimethylamino)-acridine[12] with 3-(dimethylamino)-1-propanethiol, 2-[(3-aminopropyl)thio]ethanol, or 1-methyl-4-piperidinethiol[13] in PhOH.



XIVa, R = $S(CH_2)_3N(CH_2)_2$
b, R = $NH(CH_2)_3S(CH_2)_2OH$
c, R = $NHCH(CH_3)(CH_2)_3N(C_2H_5)_2$

XVa, R = $S(CH_2)_3N(CH_3)_2$
b, R = $NH(CH_2)_3S(CH_2)_2OH$
c, R =

Biology.—The 3,6-bis(dimethylamino)-9-{[(dialkylamino)alkyl]thio}acridines (III) (**1–19**, Table I) and other dialkylaminoacridine derivatives (V–XIII, XIVa–c, XVa–c) (Table II) were tested as inhibitors of ADP-induced platelet aggregation *in vitro* utilizing a modification[1] of the method of Born and Cross.[4] Briefly, when ADP is added to rabbit platelet-rich plasma (PRP) and the PRP is gently agitated, the individual platelets aggregate, or stick together, to form clumps. Each clump contains a large number of platelets. The consequent decrease in the number of particles in suspension causes a decrease in the optical density of the PRP. Compounds that inhibit platelet aggregation minimize or prevent this decrease in the optical density. Colorimetric measurements afford a quantitative measure of the amount of the platelets.[1,4]

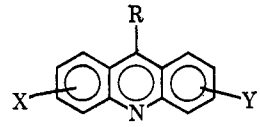
Potent *in vitro* activity is widespread within the

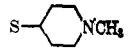
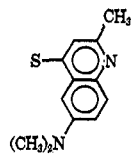
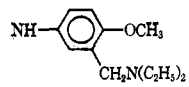
(12) A. Ledochowski and B. Kozinska, *Roczniki Chem.*, **39**, 357 (1965); *Chem. Abstr.*, **63**, 16302*h* (1965).

(13) H. Berrera and R. E. Lyle, *J. Org. Chem.*, **27**, 641 (1962).

TABLE II

EFFECTS OF OTHER (DIALKYLAMINO)ACRIDINES ON THE INHIBITION OF PLATELET AGGREGATION *in Vitro*



Compd	X, Y	R	Formula	Inhibition of platelet aggregation *in vitro* Concn $M \times 10^{-5}$[a]	% inhibition
Acridine orange	3,6-$N(CH_3)_2$	H	$C_{17}H_{19}N_3$	1	31
IX	3,6-$N(CH_3)_2$	SCH_3	$C_{13}H_{22}N_3S$	<20	30
V	3,6-$N(CH_3)_2$	OC_2H_5	$C_{19}H_{23}N_3O$	1	36
XIVa	2-$N(CH_3)_2$	$S(CH_2)_3N(CH_3)_2$	$C_{20}H_{25}N_3S$	1	37
XVa	3-$N(CH_3)_2$	$S(CH_2)_3N(CH_3)_2$	$C_{20}H_{25}N_3S \cdot 2HCl \cdot 3H_2O$	1	95
				0.1	35
XIVb	2-$N(CH_3)_2$	$NH(CH_2)_3S(CH_2)_2OH$	$C_{20}H_{25}N_3OS \cdot HCl$	<10	23
XVb	3-$N(CH_3)_2$	$NH(CH_2)_3S(CH_2)_2OH$	$C_{20}H_{25}N_3OS$	1	13
XVc	3-$N(CH_3)_2$		$C_{21}H_{25}N_3S$	50	100
				10	100
				1	90
XI	3,6-$N(C_2H_5)_2$	H	$C_{21}H_{27}N_3 \cdot HCl$	5	15
XII	3,6-$N(C_2H_5)_2$	SH	$C_{21}H_{27}N_3S$	<50	14
VIII	3,6-$N(CH_3)_2$	$NH(CH_2)_3S(CH_2)_2OH$	$C_{22}H_{30}N_4OS$	10	10
				1	0
XIVc	2-$N(CH_3)_2$	$NHCH(CH_3)(CH_2)_3N(C_2H_5)_2$	$C_{24}H_{34}N_4 \cdot 2C_7H_6O_3$	1	61
VII	3,6-$N(CH_3)_2$, 10-$CH_3{}^+I^-$	$S(CH_2)_3N^+(C_2H_5)_2(CH_3)I^-$	$C_{26}H_{40}N_4SI_2$	50	42
				5	17
XIII	3,6-$N(C_2H_5)_2$	$S(CH_2)_3N(C_2H_5)_2$	$C_{23}H_{42}N_4S \cdot 2HCl \cdot 1.5H_2O$	10	96
				1	40
				0.1	27
VI	3,6-$N(CH_3)_2$		$C_{29}H_{31}N_5S$	<10	11
X	3,6-$N(CH_3)_2$		$C_{29}H_{37}N_5O$	50	74
				10	91
				1	45

[a] Concns given as less than (<) indicate insol compds. These were tested as filtrates of suspensions of the indicated concns.

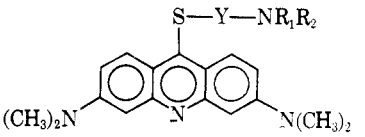
3,6-bis(dimethylamino)-9-{[(dialkylamino)alkyl]thio}-acridine series and 14 compounds (**1–9** and **11–15**) produced 54–100% inhibition of platelet aggregation at a concn of 10^{-5} *M* (Table I). Among congeners (Table II) of these substances, strong *in vitro* effects (37–95% inhibition at 10^{-5} *M*) were retained in other 9-{[(dialkylamino)alkyl]thio}acridines with 3,6-bisdiethylamino (XIII), 2-dimethylamino (XIVa), or 3-dimethylamino (XVa,c) substituents, although quaternization (VII) markedly reduced activity. When S was replaced by N to form a basic side chain (X, XIVc), strong antithrombotic effects (45–61% inhibition at 10^{-5} *M*) were retained. However, activity was usually diminished when the (dialkylaminoalkyl)thio side chain at position 9 was replaced by other substituents which lacked a basic distal function, including H (acridine orange, XI), SH (XII), MeS (IX), and [(aminopropyl)thio]ethanol (VIII, XIVb, XVb).

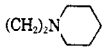
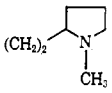
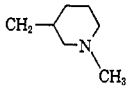
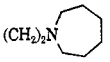
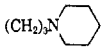
Many of the 3,6-bis(dimethylamino)-9-{[(dialkylamino)alkyl]thio}acridines (Table III) and other (dialkylamino)acridine derivatives (Table IV) also inhibited platelet aggregation in PRP taken from rabbits that had received a single iv dose of the drug prior to blood sampling. As in previous work,[1] female rabbits (New Zealand strain) were anesthetized and a jugular vein and a carotid artery were cannulated for drug administration and blood sampling, respectively. The drug was added to saline and injected during a 5-min period. Blood samples were drawn prior to and at 30- and 60-min intervals posttreatment. Each animal was dosed only once and was sacrificed at the termination of the test. Platelet-rich plasma (PRP) was prepared[1] and an aliquot was added to a tube contg $(HOCH_2)_3CNH_2$ and NaCl, pH 7.0. The mixt was stirred in a Bryston platelet aggregometer with continuous recording of the optical density. An aliquot of a soln of 2.5 or 5.0 μg/ml of ADP in saline was added, and the decrease in optical density was measured. The effect of the acridine on platelet aggregation by ADP was detd by comparing the values obtained with the pre- and posttreatment samples of PRP.

In the above *in vitro–in vivo* test, the 3,6-bis(dimethylamino)-9-{[(dialkylamino)alkyl]thio}acridines (III) (Table III) once again represented the most active group of acridine compds studied (Tables III, IV). Compds **1**, **2**, **6**, **8**, and **10—15** were the most promising. In the presence of 0.25 μg of ADP/ml, drug doses

TABLE III

INHIBITION OF PLATELET AGGREGATION IN PLASMA FROM RABBITS TREATED WITH 3,6-BIS(DIMETHYLAMINO)-9-{[(DIALKYLAMINO)ALKYL]THIO}ACRIDINES



No.	—Y—NR1R2	Single iv dose, mg of base/kg	% inhibition[a] of ADP-induced platelet aggregation at final concns: 0.25 μg of ADP/ml: No. of rabbits tested	0.25 μg of ADP/ml: Posttreatment periods, min 30	0.25 μg of ADP/ml: Posttreatment periods, min 60	0.5 μg of ADP/ml: No. of rabbits tested	0.5 μg of ADP/ml: Posttreatment periods, min 30	0.5 μg of ADP/ml: Posttreatment periods, min 60
1	$(CH_2)_2N(CH_3)C_2H_5$	12.5	2	96 (92–100)	77 (54–100)			
		6.0	4	92 (75–100)	48 (0–83)	3	73 (64–80)	47 (30–60)
		3.0	2	26 (4–48)	0 (0–0)	2	19 (6–33)	0 (0–0)
2	$(CH_2)_3N(CH_3)_2$	12.5	2	100 (100–100)	68 (53–83)			
		6.0	1	75	36	2	51 (44–58)	25 (18–33)
3	CH_2-α-pyridyl	6.0	1	0	24	3	19 (8–31)	0 (0–0)
4	CH_2-β-pyridyl	6.0				2	25 (0–50)	3 (0–7)
5	CH_2-γ-pyridyl	12.5				2	95 (90–100)	
		6.0				1	0	12
6	$(CH_2)_2$-*N*-pyrrolidyl	12.5	5	99 (95–100)	81 (59–100)			
		6.0				2	54 (32–77)	40 (38–43)
		3.0	2	36 (32–40)	14 (0–28)	2	37 (37–38)	12 (10–15)
7	$(CH_2)_2$-*N*-morpholinyl	6.0				4	23 (0–67)	0 (0–0)
8	$(CH_2)_2N(C_2H_5)_2$	12.5	3	61 (17–100)	60 (52–70)			
		6.0				2	73 (54–93)	
		3.0				2	19 (17–22)	11 (0–22)
9	$CH_2CH(CH_3)CH_2N(CH_3)_2$	6.0				3	25 (18–37)	9 (0–25)
10		12.5	3	98 (93–100)	32 (13–83)	1	85	50
		6.0				2	62 (35–89)	72 (45–100)
11		12.5	2	100 (100–100)	70 (57–83)	1	53	41
		6.0	3	68 (40–100)	71 (38–100)	1	65	59
12		12.5	1	100	100	1	24	6
13		12.5	3	100 (100–100)	53 (42–65)	2	56 (51–62)	20 (18–21)
14		12.5	2	99 (98–100)				
		6.0	1	55	57	3	34 (17–56)	27 (14–41)
		3.0				1	0	5
15	$(CH_2)_2N[CH(CH_3)_2]_2$	12.5	3	84 (53–100)	68 (60–79)			
		6.0				2	54 (48–60)	37 (19–55)
		3.0				2	42 (32–53)	16 (13–19)
16	$CH_2C(CH_3)_2CH_2N(C_2H_5)_2$	6.0				3	34 (0–54)	9 (0–27)
17	$(CH_2)_5N(C_2H_5)_2$	6.0	2	28 (21–36)				
18	$(CH_2)_2N(CH_3)CH_2C_6H_5$	6.0				3	7 (0–19)	12 (4–26)
19	$(CH_2)_3N(CH_3)CH_2C_6H_5$	6.0				3	29 (15–37)	33 (8–58)

[a] Average and (range) of values.

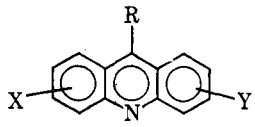
of 6 or 12.5 mg/kg caused an inhibition of platelet aggregation averaging from 55 to 100% and 32 to 100% at posttreatment periods of 30 and 60 min, respectively. At the higher concn of ADP (0.5 μg/ml), the inhibition produced by these substances ranged from an average of 0–95% at 30 min to 0–59% at 60 min. It is noteworthy that the degree of inhibition produced by a given dose of the 3,6-bis(dimethylamino)-9-thioacridines was almost invariably higher at the lower concentration of ADP, suggesting a competition between ADP and the bis(dimethylamino)acridines at some point in the sequence of events leading to platelet aggregation.

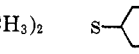
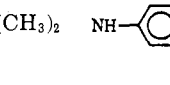
Increases in bleeding time are observed with drugs that interfere with platelet function and/or the coagulation mechanism. In a recent report Herrmann, *et al.*,[14] described a new technique for studying platelet aggregation which depends on the formation of a hemostatic platelet plug following a micropuncture wound in a small vein of the mouse mesentery. The effects of 3,6-bis(dimethylamino)-9-{[2-(1-pyrrolidinyl)ethyl]-thio}acridine (**6**) on mouse mesentery puncture bleeding time were studied utilizing a modification[1] of the above technique. An increase in both the primary and secondary bleeding time was observed 4 and 24 hr after single intravenous 10 mg/kg doses of **6** and at 4 hr

(14) R. G. Herrmann, J. D. Frank, and D. L. Marlett, *Proc. Soc. Exp. Biol. Med.*, **128**, 960 (1968).

TABLE IV

INHIBITION OF PLATELET AGGREGATION IN PLASMA FROM RABBITS TREATED WITH OTHER (DIALKYLAMINO)ACRIDINE DERIVATIVES



				% inhibition[a] of ADP-induced platelet aggregation at final concns:					
				0.25 μg of ADP/ml			0.5 μg of ADP/ml		
No.	X, Y	R	Single intravenous dose, mg of base/kg	No. of rabbits tested	Posttreatment periods, min 30	60	No. of rabbits tested	Posttreatment periods, min 30	60
XIVa	2-N(CH$_3$)$_2$	S(CH$_2$)$_3$N(CH$_3$)$_2$	6.0				1	0	0
XIVb	2-N(CH$_3$)$_2$	NH(CH$_2$)$_3$S(CH$_2$)$_2$OH	6.0	1	21	11	1	6	0
XVb	3-N(CH$_3$)$_2$	NH(CH$_2$)$_3$S(CH$_2$)$_2$OH	6.0				1	0	0
XVc	3-N(CH$_3$)$_2$		6.0	3	Toxic 1/3				
			3.0	3	21 (0–31)	13 (0–38)	3	43 (10–71)	36 (18–71)
VIII	3,6-N(CH$_3$)$_2$	NH(CH$_2$)$_3$S(CH$_2$)$_2$OH	25.0				1	26	4
XIVc	2-N(CH$_3$)$_2$	NHCH(CH$_3$)(CH$_2$)$_3$N(C$_2$H$_5$)$_2$	12.5	1	Toxic 1/1				
			6.0	1	Toxic 1/1				
			3.0	2	Toxic 1/2				
XIII	3,6-N(C$_2$H$_5$)$_2$	S(CH$_2$)$_3$N(C$_2$H$_5$)$_2$	6.0	2	Toxic 2/2				
			3.0	6	55 (31–70)	27 (0–60)			
			1.5	3	44 (28–75)	6 (0–19)			
X	3,6-N(CH$_3$)$_2$		6.0	2	Toxic 1/2				

[a] Average and (range) of values.

TABLE V

EFFECTS OF 3,6-BIS(DIMETHYLAMINO)-9-{[2-(1-PYRROLIDINYL)ETHYL]THIO}ACRIDINE (**6**) ON MOUSE MESENTERY BLEEDING TIME

Single dose, saline or drug, mg of base/kg	Route	Posttreatment period, hr	Primary bleeding[a] No. of mice tested	Primary bleeding[a] Average bleeding time, sec	Secondary bleeding[a] No. of mice tested	Secondary bleeding[a] Average bleeding time, sec	Secondary bleeding[a] No. of mice with secondary bleeding
Saline	Iv	4	10	30 ± 5	10	30 ± 14	5
10	Iv	4	10	93 ± 33[b]	8	47 ± 20	4
Saline	Iv	24	32	48 ± 13	30	16 ± 5	10
10	Iv	24	31	66 ± 14	28	33 ± 8[b]	18
Saline	Oral	4	20	47 ± 14	19	12 ± 3	8
20	Oral	4	18	57 ± 14	18	35 ± 11	10

[a] Average of values ± standard error of the mean. [b] Significant difference from control $-P < 0.05$, Student's t test calculated using the square root of the experimental values.

after single oral doses of 20 mg/kg (Table V). The effects of **6** on primary bleeding at 4 hr and on secondary bleeding at 24 hr were statistically significant ($p = < 0.05$) (Table V).

Experimental Section[15,16]

3,6-Bis(dimethylamino)-9-{[(dialkylamino)alkyl]thio}acridines (1–19, Table I). Procedure I.—To a suspension of 6.0 g (0.02 mole) of 3,6-bis(dimethylamino)-9-acridanthione (II),[7] 5.9 g (0.042 mole) of anhyd K_2CO_3, and 100 ml of DMF was added 4.0 g (0.02 mole) of 1-(3-chloropropyl)piperidine·HCl in 25 ml of DMF, and the mixt was gradually heated to 75°. The mixt was stirred and heated at 75–80° for 3 hr, cooled, and poured into ice–H_2O. The crude base was collected, washed with H_2O, and dissolved in 0.5 N HCl. The soln of the HCl salt was filtered, and the filtrate was made alk with 25% aq NaOH. The base was extd with Et_2O, the combined Et_2O extracts were dried (K_2CO_3), and the Et_2O was removed *in vacuo*. The residue was crystd from Et_2O to give 8.0 g (94%) of 3,6-bis(dimethylamino)-9-[(3-piperidinopropyl)thio]acridine (**14**) as orange crystals, mp 93–95°.

Procedure II.—To a soln of 3.6 g (0.064 mole) of $NaOCH_3$ (95%) in 100 ml of EtOH was added 9.6 g (0.032 mole) of 3,6-bis(dimethylamino)-9-acridanthione (II),[7] and the mixt was heated under reflux for 1 hr. A soln of 5.1 g (0.032 mole) of 3-dimethylaminopropyl chloride in 100 ml of EtOH was then added over 10 min, and the mixt was stirred and boiled under reflux for 18 hr. The mixt was filtered hot, and the filtrate was dild with 300 ml of H_2O. The crude product was extd with Et_2O, the combined Et_2O extracts were dried (K_2CO_3), and the Et_2O was removed *in vacuo*. The residue (6.0 g) was dissolved in 150 ml of Et_2O and to it was added a soln of 5.8 g (0.042 mole) of salicylic acid in 150 ml of Et_2O. The Et_2O was decanted, and the red gum was triturated successively with Et_2O and *i*-PrOH. The crude salt (5.4 g) was crystd from EtOH to give 4.0 g (19%) of 3,6-bis(dimethylamino)-9-{[3-(dimethylamino)propyl]thio}-acridine disalicylate (**2**) as brick red crystals, mp 180–183°.

In one instance in which the above procedure was used, a yellow salicylic acid salt of an unknown by-product was isolated from EtOH recrystallization liquors. This material was converted to the base, recrystd from *i*-PrOH and dried at 80° *in vacuo* for 24 hr to give a yellow-orange cryst solid, mp 162–168°. This compd analyzed correctly for 3,6-bis(dimethylamino)-9-ethoxyacridine (V). *Anal.* ($C_{19}H_{23}N_3O$) C, H, N.

Procedure III.—3,6-Bis(dimethylamino)-9-acridanthione (**II**)[7] (6.0 g, 0.02 mole) and 2-(2-chloroethyl)-1-methylpyrrolidine·HCl (Aldrich) (6.0 g, 0.033 mole) were ground together intimately and fused at 135° for 10 min at which time the melt solidified. The mixt was cooled, and the product was dissolved in 0.5 N HCl and filtered. The filtrate was neutralized with 50% aq NaOH, and the product was extd with $CHCl_3$. The combined $CHCl_3$ exts were washed with H_2O and dried (K_2CO_3), and the $CHCl_3$ was removed *in vacuo*. The residue was crystd successively from Me_2CO–H_2O and EtOAc and dried *in vacuo* at room temp

(15) Melting points (corrected) were taken on a Thomas-Hoover capillary melting point apparatus.

(16) Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values.

for 72 hr to give 4.1 g (45%) of 3,6-bis(dimethylamino)-9-{[2-(1-methyl-2-pyrrolidinyl)ethyl]thio}acridine sesquihydrate (11) as orange-red crystals, mp 70–72°. Two additional recrystns from EtOAc gave an anhyd sample melting at 99–102°. The nmr curve ($CDCl_3$) from this material exhibited a partially obscured triplet at δ 2.9 which was assigned to the CH_2 adjacent to S.

3,6-Bis(dimethylamino)-9-{[6-(dimethylamino)-2-methyl-4-quinolyl]thio}acridine (VI).—3,6-Bis(dimethylamino)-9-acridanthione (II)[7] (15.0 g, 0.05 mole), 4-chloro-6-(dimethylamino)-quinaldine (Eastman) (11.1 g, 0.05 mole), and K_2CO_3 (14.6 g) were heated in DMF at 70–80° for 3 hr and the crude product was processed according to procedure I utilizing $CHCl_3$ as the extn solvent. The product (2.0 g, 8%) was obtained as red crystals from MeCN, mp 285–287°. *Anal.* ($C_{29}H_{31}N_5S$) C, H, S; N: calcd, 14.54; found, 14.02.

9-{[3-(Diethylmethylammonio)propyl]thio}-3,6-bis(dimethylamino)-10-methylacridinium Diiodide (VII).—To 1.0 g (0.0024 mole) of 9-{[3-(diethylamino)propyl]thio}-3,6-bis(dimethylamino)acridine[7] was added 10 ml of MeI, and the mixt was stirred at room temp for 15 min. EtOH (50 ml) was added, and the mixt was heated on a steam bath for 20 min and chilled. The product was collected, recrystd from MeOH–*i*-PrOH, and dried *in vacuo* at 50° for 24 hr to give 1.0 g (60%) of red-brown crystals, mp 248° dec. *Anal.* ($C_{26}H_{40}N_4SI_2$) C, H, N.

2-[(3-{[3,6-Bis(dimethylamino)-9-acridinyl]amino}propyl)thio]ethanol (VIII).—A soln of 10.0 g (0.032 mole) of 3,6-bis-dimethylamino)-9-(methylthio)acridine (IX),[7] 10.8 g (0.080 mole) of 2-[(3-aminopropyl)thio]ethanol, and 50 g of PhOH was stirred and heated on a steam bath for 2.5 hr. The crude HCl salt was converted to the base and crystd from MeCN to give 10.0 g (87%) of yellow-brown crystals, mp 168–170°. *Anal.* ($C_{22}H_{30}N_4OS$) C, H, N, S.

9-{3-[(Diethylamino)methyl]-*p*-anisidino}-3,6-bis(dimethylamino)acridine (X).—N^{α},N^{α}-Diethyl-6-methoxytoluene-α,3-diamine·2HCl[9] (5.6 g, 0.02 mole) was converted to the free base and to it was added 5.0 g (0.016 mole) of 3,6-bis(dimethylamino)-9-(methylthio)acridine (IX).[7] The mixt was stirred and heated gradually to 180° over 1 hr and this temp was maintained for an addl 0.5 hr. The mixt was cooled, and the residue was crystd from MeCN to give 3.3 g (44%) of gold crystals, mp 203–205°. *Anal.* ($C_{29}H_{37}N_5O$) C, H, N.

3,6-Bis(diethylamino)acridine·HCl (XI).—A mixt of *N,N*-diethyl-*m*-phenylenediamine·2HCl (Eastman) (71.2 g, 0.3 mole), 90% HCO_2H (7.8 g, 0.15 mole), 216 g of glycerol, and 6 drops of concd HCl was stirred and heated gradually to 155°. This temp was maintained for 0.5 hr. The reaction mixt was cooled to 100°, 900 ml of H_2O was added, and the mixt was neutralized with 1 *N* NaOH. The product was extd with $CHCl_3$, the extracts were dried (K_2CO_3), and the $CHCl_3$ was removed *in vacuo*. The residue was crystd from MeOH–EtOAc to give 18.0 g, (17%) of the product as orange-red crystals, mp 270–272°. *Anal.* ($C_{21}H_{27}N_3 \cdot HCl$) C, H, N, Cl^-.

3,6-Bis(diethylamino)-9-acridanthione (XII).—3,6-Bis(diethylamino)acridine·HCl (18.0 g, 0.05 mole) was dissolved in hot H_2O and poured into an excess of 0.05 *N* NaOH with vigorous stirring. The free base was extd with $CHCl_3$ and the $CHCl_3$ was removed *in vacuo*. The residue was mixed intimately in a round-bottom flask with 1.6 g of resublimed S and the flask was placed in a preheated oil bath at 200°. The bath temp was raised to 240–250° and maintained at this temp for 3 hr. The flask was allowed to cool to room temp, and the clinker-like residue (13.5 g) was pulverized and used directly as an intermediate for the prepn of 3,6-bis(diethylamino)-9-{[3-(diethylamino)propyl]thio}acridine (XIII). For anal. a small sample was crystd from DMF–MeOH to give orange-brown crystals, mp 307°. *Anal.* ($C_{21}H_{27}N_3S$) C, H, N, S.

3,6-Bis(diethylamino)-9-{[3-(diethylamino)propyl]thio}acridine·2HCl Sesquihydrate (XIII).—3,6-Bis(diethylamino)-9-acridanthione (XII) (9.5 g, 0.027 mole) and 3-(diethylamino)propyl chloride·HCl (5.0 g, 0.027 mole) were allowed to react according to procedure I. The crude base was dissolved in C_6H_6 and chromatographed on alumina (1.4 kg), eluting successively with C_6H_6, C_6H_6–2.5% EtOAc, and C_6H_6–0.5% EtOAc. The product, 1.8 g (12%), was obtained as a hydrated HCl salt, red crystals, mp 170–180° dec. *Anal.* ($C_{28}H_{42}N_4S \cdot 2HCl \cdot 1.5H_2O$) C, H, N; Cl^-: calcd, 12.51; found, 12.93.

2-(Dimethylamino)-9-{[3-(dimethylamino)propyl]thio}acridine (XIVa).—A mixt of 6.0 g (0.023 mole) of 9-chloro-2-(dimethylamino)acridine,[12] 4.5 g (0.023 mole) of 80% 3-(dimethylamino)-1-1-propanethiol·HCl (Evans), and 20 g of PhOH was stirred and heated on a steam bath for 3 hr. Upon cooling, the mixt was poured into 800 ml of Me_2CO with vigorous stirring, and the mixt was boiled for 5 min and cooled. The crude HCl salt was collected and dissolved in H_2O, and the H_2O soln was made strongly alk with 50% aq NaOH. The red base that sepd was extd with Et_2O, the combined exts were dried (K_2CO_3), and the Et_2O was removed finishing *in vacuo*. The residue crystd upon trituration with heptane. Recrystn from heptane afforded 4.0 g (51%) of tiny red needles, mp 59–60°. *Anal.* ($C_{20}H_{25}N_3S$) C, H, N.

2-[(3-{[2-(Dimethylamino)-9-acridinyl]amino}propyl)thio]-ethanol·HCl (XIVb).—9-Chloro-2-(dimethylamino)acridine[12] (3.5 g, 0.014 mole), 2-[(3-aminopropyl)thio]ethanol (1.9 g, 0.014 mole), and PhOH (35 g) were stirred and heated on a steam bath for 2 hr, and the cooled reaction mixt was poured into 1.5 l. of 1:1 Me_2CO–Et_2O containing 5 drops of concd HCl. The cryst product that sepd was collected and recrystd from MeOH to give 4.5 g (84%) of red-brown crystals, mp 168–170°. *Anal.* ($C_{20}H_{25}N_3OS \cdot HCl$) C, H, N, Cl^-, S.

9-{[4-(Diethylamino)-1-methylbutyl]amino}-2-(dimethylamino)acridine Disalicylate (XIVc).—9-Chloro-2-(dimethylamino)acridine[12] (7.0 g, 0.027 mole) and N^1,N^1-diethyl-1,4-pentanediamine (100 ml) were heated under reflux for 4 hr, and the reaction mixt was poured into 2 l. of cold H_2O. The sticky base was sepd by decantation and dissolved in Et_2O, and the Et_2O extract was dried (K_2CO_3). Treatment with an excess of salicylic acid in Et_2O afforded 9.0 g (50%) of brilliant red crystals, mp 176–178°. *Anal.* C, H, N.

3-(Dimethylamino)-9-{[3-(dimethylamino)propyl]thio}acridine·2HCl·3H₂O (XVa).—9-Chloro-3-(dimethylamino)acridine[12] (8.0 g, 0.03 mole) and 80% 3-(dimethylamino)-1-propanethiol·HCl (Evans) (6.1 g, 0.03 mole) were heated with 25 g of PhOH on a steam bath for 3 hr and the mixt was worked up according to the procedure for XIVa. HCl was bubbled into the dried Et_2O extract, and the crude HCl salt was crystd from MeCN to give 11.0 g (79%) of red-brown crystals, mp 87–88°. *Anal.* ($C_{20}H_{25}N_3S \cdot 2HCl \cdot 3H_2O$) C, H, N, Cl^-, S.

2-[(3-{[3-(Dimethylamino)-9-acridinyl]amino}propyl)thio]-ethanol (XVb).—Utilizing the reaction conditions for XIVb 3.5 g (0.014 mole) of 9-chloro-3-(dimethylamino)acridine[12] and 1.9 g (0.014 mole) of 2-[(3-aminopropyl)thio]ethanol were allowed to react in 35 g of PhOH. The crude HCl salt was converted to the base which was crystd from MeCN to give 3.5 g (72%) of yellow crystals, mp 124–126°. *Anal.* ($C_{20}H_{25}N_3OS$) C, H, N.

3-(Dimethylamino)-9-[(1-methyl-4-piperidyl)thio]acridine (XVc).—9-Chloro-3-(dimethylamino)acridine[12] (2.0 g, 0.008 mole) was condensed with 1-methyl-4-piperidinethiol[13] (1.0 g, 0.008 mole) in 20 g of PhOH, and the reaction mixt was poured into 800 ml of Et_2O contg 5 ml of 22% HCl in *i*-PrOH. The crude HCl salt was worked up according to the procedure for XIVa to give 1.5 g (55%) of product as orange needles from MeCN, mp 182–183°. *Anal.* ($C_{21}H_{25}N_3S$) C, H, N.

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