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Note

Synthesis, anti-inflammatory and analgesic activity evaluation of some 2-(9-acridinyl-amino)pyridines and 2-(9-acridinylamino/imino)thiazolines†

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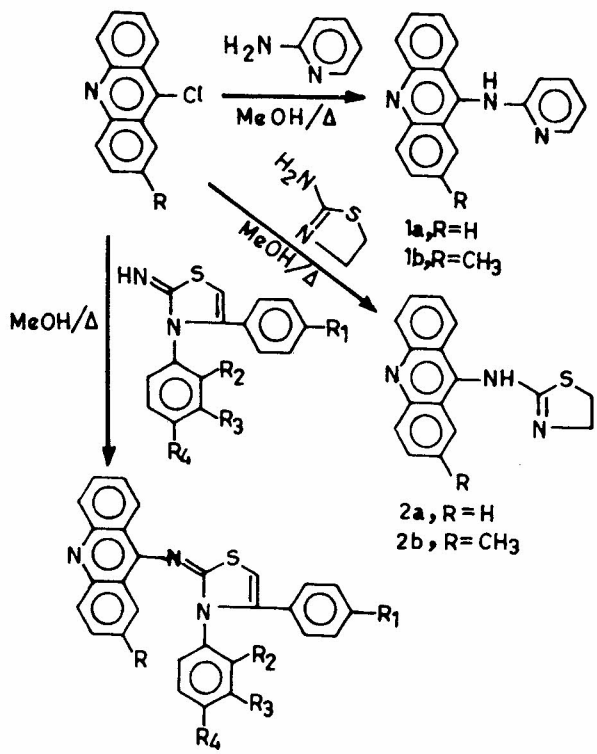
A number of 2-(9-acridinylamino)pyridines **1** and 2-(9-acridinylamino/imino)thiazolines **2** and **3** have been synthesized by the condensation of 9-chloro-acridines with 2-aminopyridine, 2-amino-2-thiazoline and 3, 4-diaryl-2-imino-4-thiazoline, respectively. The resulting products have been evaluated for their anti-inflammatory and analgesic activities. Compound **3j** shows good analgesic activity.

Acridine based antimalarial and antibacterial compounds (mepacrine, azacrine, proflavine and aminacrine) are known[1]. 9-[(Dimethylaminopropyl)amino]-1-nitroacridine has been quite extensively used as an antitumor drug in Poland. Amsacrine has a wide-spread clinical use as an antitumor agent[3,4]. Denny and co-workers have been deeply involved in the synthesis and antitumor evaluation of acridine derivatives[5-8]. Acridine derivatives exhibiting fungicidal[9-11], antimicrobial[12,13] antiparasitic[14] and anti-inflammatory[15] activities are reported in literature. In continuation of our efforts in the search of potential anti-inflammatory compounds[16-21], we wish to report here the synthesis and anti-inflammatory and analgesic activity screening of a number of acridine derivatives (**1-3**).

The required 9-chloroacridines were synthesized by the condensation of N-arylanthranilic acids[22] with phosphorus oxychloride[23]. Condensation of 9-chloroacridine and 9-chloro-2-methylacridine with 2-aminopyridine in methanol under reflux for 8 hr followed by purification by crystallization and chromatography over silica gel gave



	R	R_1	R_2	R_3	R_4
3a	H	H	H	H	H
3b	H	H	CH_3	CH_3	H
3c	H	H	H	H	CH_3
3d	CH_3	H	H	H	H
3e	OCH_3	H	H	H	H
3f	H	OCH_3	H	H	H
3g	H	H	H	H	OCH_3
3h	H	H	H	H	Cl
3i	H	H	OCH_3	H	H
3j	CH_3	H	CH_3	CH_3	H
3k	CH_3	H	H	H	Cl
3l	CH_3	H	H	H	CH_3
3m	CH_3	H	H	H	OCH_3

Scheme I

the corresponding acridinylaminopyridines **1a** and **1b** (Scheme I).

Condensation of 9-chloroacridine and 9-chloro-2-methylacridine with 2-amino-2-thiazoline in methanol under reflux for 16 hr followed by purification by column chromatography over silica gel

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gave the corresponding acridinylamino-2-thiazolines **2a** and **2b** (Scheme I). For structures **2a** and **2b**, the molecular ion peaks were obtained at m/z 219.08292 and 293.09880 (calculated values for $C_{16}H_{13}N_3S$ and $C_{17}H_{15}N_3S$ are m/z 279.08301 and 293.09866, respectively).

3, 4-Diaryl-2-imino-4-thiazolines, prepared by the condensation of phenacyl thiocyanate and amine hydrochlorides as reported in literature[24], on condensation with 9-chloroacridines in methanol under reflux for 10 hr followed by purification by chromatography over silica gel gave the acridinylimino-4-thiazolines **3a-3m** (Scheme I). The structures of all the synthesized compounds **1-3** were supported by their ^{1}H NMR, HRMS and IR spectra (cf. Table I).

All the compounds synthesized were evaluated for their anti-inflammatory and analgesic activities. Compounds **3c, 3d, 3e** and **3f** showed weak analgesic activity and compounds **1b, 2b** and **3e** showed weak anti-inflammatory activity. However, compound **3j** showed a good analgesic activity.

Experimental Section

Melting points were determined on a JSGW apparatus and are uncorrected. Only principal sharply defined IR peaks are reported. ^{1}H NMR spectra were recorded in DMSO-d_6 solutions (5-15%, w/v) with TMS as internal standard. MS peaks were measured by comparison with that of perfluorotributylamine at a resolving power of 15,000. TLC was performed over silica gel G (Merck) coated plates and spots were visualized by iodine vapours or by irradiation with UV light of 254 nm wavelength. Silica gel (60-120 mesh) was used for column chromatography. Elemental analyses (C, H, N) were within ±0.4% of theoretical values.

Condensation of 2-aminopyridine with 9-chloroacridine:Formation of 1a. 9-Chloroacridine (426 mg, 2mmoles) was dissolved in methanol (30 mL) and to it was added 2-aminopyridine (190 mg, 2mmoles). The reaction mixture was heated under reflux for 8 hr, and then solvent removed under reduced pressure. The solid residue

Table I—Physical and spectral data of acridine derivatives **1-3**

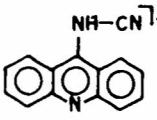
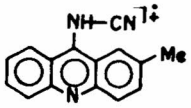
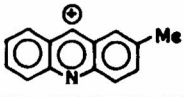
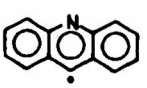
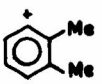
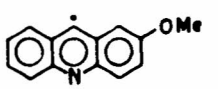
Compd	m.p. °C (solvent of crystallization/elution)*	Yield (%)†	Spectral data
1a	180 (w)	30	^{1}H NMR : δ7.0 (t, 2H, Ar), 7.3 (m, 2H, Ar), 8.0(m, 7H, Ar), 8.2(m, 1H, Ar), 13.2(s, 1H, −NH−, exch.). HRMS (m/z; rel. int.):271. 10915 (M^+; 100.00) (Calcd for $C_{18}H_{13}N_3$:271.11096), 270.10216 ($M^+ - H$, 98.83), 178.06433 ($M^+ - C_5H_5N_2$; 3.51), 78.03472 (C_5H_4N; 3.99).
1b	220 (w − x; 8:2)	36	^{1}H NMR:δ 2.40 (s, 3H, $-CH_3$), 7.2 (t, 2H, Ar). 7.5(m, 5H, Ar), 7.7(t, 2H, Ar), 8.0(s, 1H, Ar), 8.2(d, 1H, Ar), 11.6(s, 1H, −NH−, exch). HRMS (m/z; rel. int.): 285.12545 (M^+; 100.00) (Calcd. for $C_{19}H_{15}N_3$:285.12659), 284.11827($M^+ - H$; 73.99), 270.10375 ($M^+ - CH_3$; 21.51), 207.09119($M^+ - C_5H_4N$; 8.95), 78.03418 (C_5H_4N; 9.60).
2a	192-95 (y − w; 2:8)	30	^{1}H NMR: δ3.3(t, 2H, $-CH_2-$), 3.65(t, 2H, $-CH_2-$). 7.5(m, 2H, Ar), 7.75(m, 2H, Ar), 8.0(d, 4H, Ar), 8.2(s, 1H, −NH−, exch). HRMS(m/z; rel. int.): 279.08292 (M^+:100.00)(Calcd for $C_{16}H_{13}N_3S$: 279.08301), 278.07528($M^+ - H$;  ; 34.74), 251.05145. ($M^+ - CH_2=CH_2$; 4.15), 219.07983(; 34.98).
2b	225 (z − w; 5:5)	52	^{1}H NMR : δ 3.30 (t, 2H, $-CH_2-$), 3.65 (t, 2H, $-CH_2-$), 7.45 (m, 1H, Ar), 7.60(dd, 1H, Ar), 7.7 (m, 1H, Ar), 7.80(s, 1H, Ar) 8.00(m, 3H, Ar), 8.15(bs, 1H, −NH− exch.) signal due to methyl group is merged with DMSO signal at 2.50. HRMS (m/z; rel. int.) : 293.09880 (M^+; 100.00) (Calcd. for $C_{17}H_{15}N_3S$:293.09866, 292.09135 ($M^+ - H$; 31.49), 265.06678 ($M^+ - CH_2=CH_2$; 5.98), 233.09578( ; 30.51), 192.08009  ; 11.36).

Table I—Physical and spectral data of acridine derivatives **1-3**

Compd	m.p. °C (solvent of crystallization/elution)*	Yield (%)†	Spectral data
3a	185-90 (w)	40	^{1}H NMR : δ6.5 (s, 1H, > C = CH −), 7.25(s, 5H, Ar), 7.50(m, 5H, Ar), 7.75(m, 4H, Ar), 8.10(d, 4H, Ar). HRMS (m/z; rel. int.) : 429.12841 (M^+, 21.37) (Calcd. for $C_{28}H_{19}N_3S$:429.12997.
3b	240 (w − x; 8:2)	25	^{1}H NMR : δ2.3 (s, 3H, $-CH_3$), 2.40 (s, 3H, $-CH_3$), 6.1(s, 1H, > C = CH −), 7.3(m, 8H, Ar), 7.5(m, 2H, Ar). 7.7(m, 2H, Ar), 8.1(d, 2H, Ar), 8.2(d, 2H, Ar). HRMS (m/z; rel. int.) : 457.16133(M^+; 100.00)(Calcd. for $C_{30}H_{23}N_3S$: 457.16125), 456.15364 ($M^+ - H$; 12.64). 442.13799($M^+ - CH_3$; 7.63). 380.12305 ($M^+ - C_6H_5$; 6.51). 279.09501(M^+ −  ; 5.53), 105.07093  ; 27.25), 77.03854 ($C_6H_5^+$; 29.22)
3c	195 (z − w; 2:8)	25	^{1}H NMR : δ 2.30 (s, 3H, CH_3), 6.5(s, 1H, > C = CH), 7.2(d, 7H, Ar), 7.5(m, 5H, Ar), 7.75(m, 2H, Ar), 8.10(m, 3H, Ar). HRMS (m/z; rel. int.) : 443.14563(M^+; 100.00)(Calcd. for $C_{29}H_{21}N_3S$:443.14563), 442.13783 ($M^+ - H$; 15.21), 91.05477(C_7H_7; 32.52), 77.03843($C_6H_5^+$; 3.76).
3d	175 (x − w; 2:8)	60	^{1}H NMR : δ2.55 (s, 3H, $-CH_3$), 6.50(s, 1H, > C = CH −), 7.2(m, 5H, Ar), 7.30(m, 1H, Ar), 7.45(m, 3H, Ar), 7.60(m, 3H, Ar), 7.70(m, 2H, Ar), 8.0(m, 3H, Ar) HRMS (m/z; rel. int.) :443.14485(M^+; 100.00)(Calcd for $C_{29}H_{21}N_3S$:443.14563), 251.06239($C_{15}H_{11}N_2S$; 4.02).
3e	130 (w-x; 8:2)	11	^{1}H NMR : δ3.80 (s, 3H, $-OCH_3$) 6.9-7.7(m, 17H, Ar + 1H, > C = CH −). HRMS (m/z; rel. int.) : 459.14076(M^+; 46.38)(Calcd. for $C_{29}H_{21}N_3SO$:459.14053), 444.11733 ($M^+ - CH_3$; 11.34), 251.06468(M^+ −  ; 100.00), 77.03921 ($C_6H_5^+$; 44.20)
3f	130 (w-x; 8:2)	16	^{1}H NMR: δ 3.69(s, 3H, $-OCH_3$), 6.40(s, 1H, > C = CH −), 6.83(d, 2H, Ar), 7.16(d, 2H, Ar), 7.48(m, 5H, Ar), 7.61(d, 2H, Ar), 7.79(m, 2H, Ar), 8.05(d, 3H, Ar), 8.20(d, 1H, Ar). IR(KBr)cm^{-1}: 1606(> C = N −), 1551 and 1506 (Ar).
3g	100 (w − x); 5:5)	16	^{1}H NMR : δ 3.73 (s, 3H, $-OCH_3$), 6.47(s, 1H, > C = CH −), 7.02(d, 2H, Ar), 7.29(m, 4H, Ar), 7.54(m, 4H, Ar), 7.79(m, 3H, Ar), 8.05(d, 3H, Ar), 8.21(d, 1H, Ar). IR(KBr) cm^{-1}; 1599 (> C = N −), 1551 and 1508 (Ar).
3h	112 (w − x; 8:2)	24	^{1}H NMR : δ6.5 (s, 1H, > C = CH −), 7.30 (m, 8H, Ar), 7.52(m, 4H, Ar), 7.70(m, 3H, Ar), 8.05(d, 2H, Ar). IR(KBr) cm^{-1}: 1615 (> C = N −), 1586 and 1551 (Ar).
3i	181 (z − w; 8:2)	16	^{1}H NMR : δ 3.90 (s. 3H, $-OCH_3$), 6.42(s, 1H, > C = CH −), 7.20(m, 9H, Ar), 7.50(m, 3H, Ar), 7.78(t, 2H, Ar), 8.03(d, 3H, Ar). IR(KBr)cm^{-1}: 1604 (> C = N −), 1552 and 1500 (Ar).
3j	95 (w − x; 8:2)	25	^{1}H NMR : δ2.24(s, 3H, $-CH_3$), 2.30(s, 3H, $-CH_3$), 2.39(s, 3H, $-CH_3$), 6.49(s, 1H, > C = CH −), 7.25(m, 8H, Ar), 7.46-7.72(m, 5H, Ar), 7.96(m, 2H, Ar). IR(KBr) cm^{-1}: 1614 (> C = N −), and 1550 (Ar).
3k	120 (w − x; 8:2)	30	^{1}H NMR : δ 6.48 (s, 1H, > C = CH −), 7.27-7.79 (m, 14H, Ar), 8.0(m, 2H, Ar), signal due to $-CH_3$ is merged with DMSO-d_6 signal. IR(KBr) cm^{-1} : 1614 (> C = N −), 1586 and 1549 (Ar).
3l	110 (w-x; 8:2)	28	^{1}H NMR :δ2.29 (s, 3H, $-CH_3$), 2.40(s, 3H, $-CH_3$), 6.46(s, 1H, > C = CH −), 7.27-7.76(m, 14H, Ar), 8.01 (m, 2H, Ar). IR(KBr) cm^{-1} : 1618 (> C = N −), 1549 and 1509 (Ar).
3m	125 (w − x; 5:5)	22	^{1}H NMR : δ 3.7(s, 3H, $-OCH_3$), 6.43(s, 1H, > C = CH −), 7.0(d, 2H, Ar), 7.26(s, 5H, Ar), 7.51(m, 6H, Ar), 8.0(q, 3H, Ar). signal due to $-CH_3$ group is burried under the signal of DMSO-d_6 at δ 2.49. IR(KBr) cm^{-1} : 1618 (> C = N −), 1581 and 1550 (Ar)

* w = Chloroform; x = ethyl acetate; y = hexane; z = pet. ether.

† Yields of **3a-3m** were calculated on the basis of the amount of thiazoline used during condensation.

was stirred with sodium carbonate solution (10% 10 mL) for half an hour and then filtered, washed with water and air dried. The crude product so obtained was crystallized from chloroform to give **1a** as an orange yellow solid, yield 130 mg (30%), m.p. 180°; ^{1}H NMR and HRMS (cf. Table I).

Similarly, the condensation of 9-chloro-2-methylacridine with 2-aminopyridine followed by purification by column chromatography gave compound **1b**; yield, m.p., solvent of elution and spectral data of **1b** are reported in Table I.

Condensation of 2-amino-2-thiazoline with 9-chloroacridine:Formation of 2a. 9-Chloroacridine (426 mg, 2mmoles) was dissolved in methanol (50 mL) and to it was added 2-amino-2-thiazoline (306 mg, 3 mmoles). The reaction mixture was heated under reflux for 16 hr. The crystalline solid separated out was filtered. The filtrate was evaporated and the residue left was subjected to column chromatography over silica gel. Elution which was hexane-chloroform (2:8) gave **2a** as an orange compound which was crystallized from methanol, yield 165 mg (30%), m.p. 192-5°; ^{1}H NMR and HRMS (cf. Table I).

Similarly 9-chloro-2-methylacridine was condensed with 2-amino-2-thiazoline to give **2b**; yield, m.p., solvent of elution and spectral data of **2b** are reported in Table I.

General procedure for condensation of 3, 4-diaryl-2-imino-4-thiazoline with 9-chloroacridines:Formation of 3a-m. 9-Chloroacridine (426 mg, 2 mmoles was dissolved in methanol (50 mL) and to it was added 2-imino-3, 4-diphenyl-4-thiazoline (504 mg, 2 mmoles). The reaction mixture was heated under reflux for 10 hr and then solvent removed under reduced pressure. The solid residue was dissolved in benzene (50 mL) and insoluble material filtered, washed with benzene and air dried. This solid was found to be 2-imino-3, 4-diphenyl-4-thiazoline hydrochloride (250 mg). The benzene solution was concentrated and subjected to column chromatography over silica gel. Elution with chloroform gave **3a,** as a yellow coloured solid, yield 180 mg (40%), m.p. 185-90°; ^{1}H NMR and HRMS (cf. Table I).

Similarly, compounds **3b-m** were prepared. Their yields, m.ps solvents of elution and spectral data are reported in Table I.

Anti-inflammatory activity testing[25]. The testing of anti-inflammatory activity was carried out using the carrageenin-induced paw oedema method in albino rats. Oedema in one of the hind paws was induced by injection of 0.1 mL of 1% carrageenin solution into planter aponeurosis. The volume of the paw was measured plethysmographically immediately after and 3 hr after the injection of carrageenin. The difference in volume gave the amount of oedema developed. Percent inhibition of the oedema between the control group and the compound (100 mg/kg p.o.) treated group was calculated and compared with the group receiving standard drug phenylbutazone (30 mg/kg p.o.). At a dose of 100 mg/kg p.o., none of the compounds showed any significant anti-inflammatory activity.

Analgesic activity testing[26]. Analgesic activity was evaluated in mice using the phenylquinone writhing assay method[26]. Female Swiss mice (15-20 g), bred in the Animal House of CDRI, Lucknow and maintained under standard laboratory conditions, were used in the study. Mice were injected 0.2 mL of 0.02% aqueous solution of phenylquinone (2-phenyl-1, 4-benzoquinone) and observed for writhing for 20 min. Number of writhes, produced by each mouse, was counted during this period. A minimum of 10 writhes, produced by a mouse, was considered positive and used in the analgesic testing on the following day.

The mice, consisting of 5 in each group and showing significant writhing, were given subcutaneously or orally 50 or 100 mg/kg dose of test compounds, 15 min prior to phenylquinone challange. Writhing was again recorded for each mouse in a group and percentage protection was calculated using the following formula:

$$\% \text{ Protection} = 100 - \left(\frac{\text{No. of writhing in treated}}{\text{No. of writhing in untreated}} \times 100 \right).$$

This was taken as percent analgesic response and was averaged in each group of mice. Percent of animals, exhibiting analgesia was determined with each dose.

Compound **3j** exhibited significant analgesic activity.

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