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Synthesis of certain tetrahydroacridine derivatives of anticipated medicinal value

Omar A. Fathhalla · Mosaad S. Mohamed · Madeha A. Farag · Rehab Sayed Ahmed Ismail

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Abstract This study aims at the synthesis and evaluation of the chemotherapeutic activity of a number of 9-substituted tetrahydroacridine derivatives. The starting material, acridine hydrazide, could be prepared through the interaction between cyclohexanone and anthranilic acid, then chlorination of the product, then condensation of the last compound with hydrazine hydrate. The structures of the new compounds were established by IR, ^{1}H NMR, MS spectra, and elemental analysis in certain cases. Antitumor activities as a trial to obtain more effective and less toxic agents were evaluated. The antitumor activity results indicated that the selected tetrahydroacridine derivatives showed antitumor activity against the liver cancer (HEPG2) tumor cell line tested, but with varying intensities in comparison to the known anticancer drugs, 5-fluorouracil and doxorubicin. It was found that compound **VIb** was the most active and induced a marked growth inhibition (0.694 μg/ml concentration) in a dose-dependent manner against liver cancer (HEPG2), while compound **XVIIIa** was second in regards to the growth inhibition activity (2.97 μg/ml concentration).

Keywords 9-Substituted tetrahydroacridine derivatives · The antitumor activity against the liver cancer (HEPG2) tumor cell line

O. A. Fathhalla (✉) · R. S. A. Ismail
Medicinal Chemistry Department, National Research Centre, Dokki, Cairo, Egypt
e-mail: omarfathalla@yahoo.com

M. S. Mohamed · R. S. A. Ismail
Organic Chemistry Department, Faculty of Pharmacy, Helwan University, Cairo, Egypt

M. A. Farag · R. S. A. Ismail
Pharmaceutical Chemistry Department, Faculty of Pharmacy, Helwan University, Cairo, Egypt

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Introduction

Acridine and 1,2,3,4-tetrahydroacridine derivatives, well known as DNA intercalates, have been widely studied from a variety of viewpoints, such as synthesis [1, 2], physicochemical properties [3, 4], structural requirements [5], and biological activities [6, 7]. Due to their polycyclic planar structure, acridine and its derivatives intercalate within DNA and RNA by forming hydrogen bonds and stacking between base pairs, resulting in DNA cross links and strand breaks [8]. A variety of natural and synthetic acridine derivatives have also been tested for antimalarial [9], antiinflammatory [10, 11], and analgesic [12] activities, and some of them have been approved for chemotherapy. The 1,2,4-triazole nucleus has been incorporated into a wide variety of therapeutically important agents. Ribavirin (antiviral), letrozole, and anastrozole (antitumor) are some examples of drugs containing the 1,2,4-triazole moiety [13–17]. 1,3,4-Thiadiazole derivatives are another important class of heterocycles due to their biological activities. The only commercially available 1,3,4-thiadiazole drugs are desaglybuzole, acetazolamide, and furidiazine [18–20]. Moreover, 1,3-thiazoles recently found application in drug development for the treatment of hypertension [21], schizophrenia [22], HIV infections [23], and as new inhibitors of bacterial DNA gyrase B [24]. Furthermore, 1,3-thiazine derivatives have antibacterial [25] and cannabinoid receptor agonist [26] activity.

In view of the above-mentioned findings and in continuation of our efforts to identify new candidates that may be of value in designing new and potent antimicrobial agents [27–29], we decided, in the present work, to synthesize new 1,3-thiazole, 1,3,4-thiadiazole, 1,2,4-triazole, and 1,3-thiazine derivatives bearing acridine and 1,2,3,4-tetrahydroacridine moieties in order to investigate their antimicrobial activities.

Materials and methods

All melting points were uncorrected and measured using Electro-thermal IA 9100 apparatus (Shimadzu, Japan). Microanalytical data were obtained by Vario El-Mentar apparatus (Shimadzu, Japan), at the National Research Centre (NRC), Cairo, Egypt. The found values were within ±0.4 % of the theoretical values. IR spectra (KBr) were recorded on a Perkin-Elmer 1650 spectrophotometer, at the NRC. ^{1}H NMR and ^{13}C NMR spectra were determined on a Varian Mercury 300 MHz spectrometer (Varian, UK) and the chemical shifts were expressed in δ ppm relative to TMS as an internal reference (Faculty of Science, Cairo University, Egypt). Mass spectra were recorded at 70 eV using an EI Ms-QP 1000 EX instrument (Shimadzu, Japan), at the NRC. Follow up of the reactions and checking the purity of the compounds were was done by TLC on silica gel-protected glass plates and the spots were detected by exposure to a UV lamp at $\lambda = 254$.

Method for preparation of 1,2,3,4-tetrahydroacridine (I)

A mixture of cyclohexanone (10 ml) and anthranilic acid (10 g) was placed in a flask provided by a still head and a condenser in a sand bath held at 120 °C for half

an hour. The still head was then lagged and the temperature of the sand bath raised in half an hour to 160 °C, when vigorous boiling set in.

In the next half hour, the temperature was raised to 230 °C, when the content of the flask swelled and solidified. Heating was continued for a further half an hour at 230 °C. Then, the flask was cooled and the contents were grounded with benzene 75 ml. The suspension was refluxed for half an hour, filtered whilst hot, and the residue washed with more benzene to remove the excess aniline and cyclohexanone, washed with petroleum ether, filtered, and dried.

Method for preparation of 9-chloro-1,2,3,4-tetrahydroacridine (II)

A mixture of tetrahydroacridine (19.8 g, 0.1 mol) and 17 ml phosphoryl chloride was heated in a sand bath at 125–130 °C for an hour. The reaction mixture was then cooled, mixed thoroughly with benzene (50 ml), and poured onto 5 N sodium hydroxide (150 ml) using a mechanical stirrer.

After 10 min, it was confirmed that the liquid was still alkaline, and the aqueous layer was extracted successively three times with benzene (each with 20 ml). The combined benzene extracts were dried over sodium sulfate, filtered, dried, and crystallized from acetone.

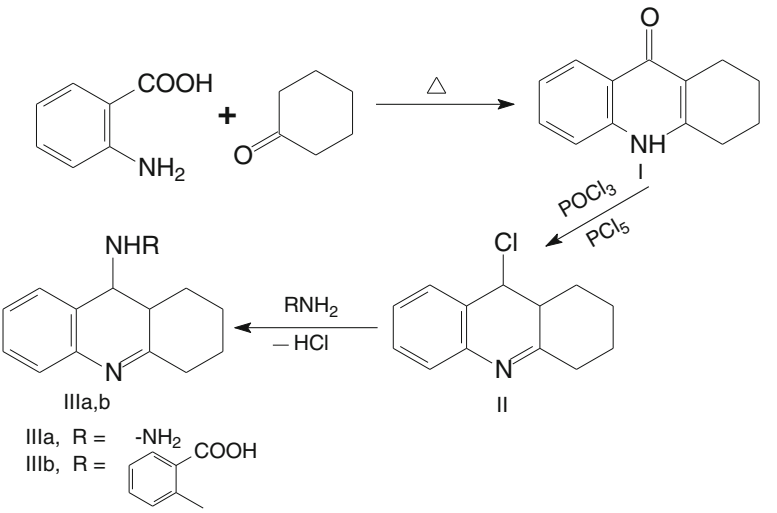
Method for preparation of 9-substituted amino-1,2,3,4-tetrahydroacridine (IIIa, b)

9-hydrazide-1,2,3,4-tetrahydroacridine (IIIa)

A mixture of **II** (21 g) and (21 ml) hydrazine hydrate was dissolved in (70 ml) n-butanol as the solvent, and then the reaction mixture was refluxed for 24 h. The formed precipitate after cooling was filtered off, dried with air suction, and then crystallized from methanol to give 9-hyrazino-1,2,3,4-tetrahydroacridine HCl. Pale yellow powder with m.p. 273–275 °C and yield 51 %. IR spectrum (K Br, cm^{-1}): 3344 (NH), 3335, 3065 (C–H aromatic); ^{1}H NMR spectrum (DMSO-d6, ppm): 1.85 (mm, 4H, $2CH_2$), 2.30 (t, 2H, CH_2), 3.3 (t, 2H, CH_2), 7.2–8.7 (m, 4H, C–H aromatic), 5.8, 9.1, 11.1 (s, 3H, NH, NH_2 exchangeable with D_2O); ^{13}C NMR (DMSO *d6*): δ 20.6, 22.7, 22.8, 31.4, 115.6, 119.5, 119.9, 124.4, 128.2, 128.3, 128.4, 145.4, 147.6, 164.2. Analysis for $C_{13}H_{15}N_3$: required: C, 73.21; H, 7.09; N, 19.70; found: C, 73.19; H, 7.07; N, 19.68.

2-(1,2,3,4-tetrahydroacridin-9-ylamino)benzoic acid (IIIb)

A mixture of **II** (1 g) and anthranilic acid (0.7 g) in 20 ml absolute methanol with 1 ml pyridine was refluxed for 24 h. The produced precipitate was filtered off, dried under air suction, and then crystallized from ethanol. Pale yellow powder with m.p. 125–120 °C and yield 51 %. IR spectrum (K Br, cm^{-1}): 3697 (OH), 3444 (NH), 3335, 3065 (C–H aromatic), 1682 (C=O); ^{1}H NMR spectrum (DMSO-d6, ppm): 1.8 (mm, 4H, $2CH_2$), 2.5 (t, 2H, CH_2), 3.08 (t, 2H, CH_2), 6.1–7.9 (m, 8H, C–H aromatic), 9.3 (s, 1H, NH exchangeable with D_2O), 9.85 (b, 1H, OH exchangeable with D_2O); ^{13}C NMR (DMSO *d6*): δ 20.4, 22.6, 22.7, 31.4, 110.3, 115.6, 116.2,



Scheme 1 Structure of compounds **I–III**

118.7, 119.5, 119.9, 124.4, 128.2, 128.3, 131.1, 134.8, 142.6, 145.4, 146.8, 164.2, 169.4. Analysis for $C_{20}H_{18}N_2O_2$: required: C, 75.45; H, 5.70; N, 8.80; found: C, 75.45; H, 5.70; N, 8.80 (Scheme 1).

General method for preparation of 9-arylidenylhydrazon-1,2,3,4-tetrahydroacridine (IVa–c)

A mixture of **IIIa** (2.13 g, 0.1 mol) and the appropriate aldehyde (0.1 mol), namely, 4-methylbenzaldehyde, 4-methoxybenzaldehyde, and 3,5-dimethoxybenzaldehyde, in absolute ethanol (15 ml) was refluxed for 18–24 h. The solvent was evaporated till it had halved and the solid product after cooling was filtered off, dried with air suction, and then crystallized from ethanol to give the compounds **IVa–c**.

(E)-9-(2-(4-methylbenzylidene)hydrazinyl)-1,2,3,4-tetrahydroacridine (IVa)

Crystallized from ethanol to give pale brown powder with m.p. 160–165 °C and yield 64 %. IR spectrum (KBr, cm^{-1}): 3291 (NH), 3172 (CH, st, aromatic), 1634 (C=N); ^{1}H NMR spectrum (DMSO-d6, ppm): 1.85 (4H, m, $2CH_2$), 2.30 (2H, t, CH_2), 2.5 (3H, s, C–CH_3), 3.3 (2H, t, CH_2), 7.4 (s, H, CH=N), 7.2–8.7 (m, 8H, C–H aromatic), 11.3 (s, H, NH exchangeable with D_2O). Analysis for $C_{21}H_{21}N_3$ (315): required: C, 79.97; H, 6.71; N, 13.32; found: C, 79.95; H, 6.69; N, 13.30.

(E)-9-(2-(4-methoxybenzylidene)hydrazinyl)-1,2,3,4-tetrahydroacridine (IVb)

Crystallized from ethanol to give yellowish brown powder with m.p. 240–243 °C and yield 52 %. IR spectrum (K Br, cm^{-1}): 3213 (NH), 3117 (CH, st, aromatic),

1636 (C=N), 1246–1052 (C–O of phenyl alkyl ether); ^{1}H NMR spectrum (DMSO-d6, ppm): 1.80 (4H, m, $2CH_2$), 2.36 (2H, t, CH_2), 3.3 (2H, t, CH_2), 3.8 (3H, s, OCH_3), 7.7 (H, s, CH=N), 7.4–8.9 (8H, m, C–H aromatic), 11.1 (H, s, NH exchangeable with D_2O). Analysis for $C_{21}H_{21}N_3O$ (331): required: C, 76.11; H, 6.39; N, 12.68; found: C, 76.13; H, 6.49; N, 12.88.

(E)-9-(2-(3,5-dimethoxybenzylidene)hydrazinyl)-1,2,3,4-tetrahydroacridine (IVc)

Crystallized from ethanol to give yellow powder with m.p. 165–166 °C and yield 41 %. IR spectrum (K Br, cm^{-1}): 3194 (NH), 3123 (CH, st, aromatic), 1263–1059 (C–O of phenyl alkyl ether); ^{1}H NMR spectrum (DMSO-d6, ppm): 1.8 (4H, m, $2CH_2$), 2.4 (2H, t, CH_2), 3.25 (2H, t, CH_2), 3.81, 3.83 (6H, s, $2OCH_3$), 7.9 (H, s, CH=N), 6.6–7.8 (7H, m, C–H aromatic), 11.4 (H, s, NH exchangeable with D_2O). Analysis for $C_{22}H_{23}N_3O_2$ (436): required: C, 73.11; H, 6.41; N, 11.63; found: C, 73.01; H, 6.51; N, 11.33.

Method for preparation of 1,2,3,4-tetrahydroacridin-9-yl(N-(1-hydrazinovinyl)cyclohexanamine) (V)

A mixture of **IIIa** (2.1 g, 0.1 mol) and cyclohexyl isothiocyanate (1.28 g, 0.1 mol) in least amounts in ethyl alcohol (10 ml) was refluxed for 10 h, the solvent was evaporated, and the precipitate was successively washed with ether and chloroform and crystallized from ethanol to give product **V** with m.p. 230–235 °C and yield 100 %. IR spectrum (K Br, cm^{-1}): 3370 (NH), 1633 and 1448.28 (C=C, st, aromatic); ^{1}H NMR spectrum (DMSO-d6, ppm): 1.1–2 (10H, m, 5 CH_2), 1.8 (4H, m, $2CH_2$), 2.80 (2H, t, CH_2), 3.3 (2H, t, CH_2), 7.4–8.7 (4H, m, C–H aromatic, CH–NH of cyclohexane), 4.3, 9.4, 10.4 (3H, s, 3NH exchangeable with D_2O). Analysis for $C_{20}H_{25}N_3S$ (339.1): required: C, 70.76; H, 7.42; N, 12.38; found: C, 70.75; H, 7.44; N, 12.44.

General method for preparation of 1-(1,2,3,4-tetrahydroacridin-9-yl)-3-methyl-5-substituted pyrazole (VIa, b)

A mixture of **IIIa** (2.1 g, 0.1 mol) and the appropriate 1,3-dicarbonyl compound (ethyl acetoacetate, acetylacetone) in absolute ethanol (30 ml) was heated in a water bath for 7 h. The reaction mixture was allowed to cool, and the solid formed was filtered off, dried, and then crystallized from ethanol to afford **VIa**, **b**.

3-methyl-1-(1,2,3,4-tetrahydroacridin-9-yl)-1H-pyrazol-5-ol (VIa)

Crystallized from ethanol, m.p. 216–218 °C and yield 22 %. IR spectrum (K Br, cm^{-1}): 3325 (OH), 2957 (C–H of aliphatic), 1675 (C=O of pyrazolone), 1633 and 1448.28 (C=C, st, aromatic); ^{1}H NMR spectrum (DMSO-d6, ppm): 1.8 (mm, 4H, $2CH_2$), 2.6 (s, 3H, CH_3), 2.80 (t, 2H, CH_2), 3.3 (t, 2H, CH_2), 7.2–8.2 (m, 4H, C–H aromatic, 1H, –C=H of pyrazolone), 10.1 (s, H, OH exchangeable with D_2O); ^{13}C NMR (DMSO *d6*): δ 18.0, 20.6, 22.6, 22.7, 31.4, 91.4, 114.5, 122.4, 125.7, 127.2,

127.9, 128.6, 141.8, 144.8, 161.0, 164.5. Analysis for $C_{17}H_{17}N_3O$ (279): required: C, 73.36; H, 5.79; N, 15.10; found: C, 73.55; H, 5.21; N, 15.37.

9-(3,5-dimethyl-1H-pyrazol-1-yl)-1,2,3,4-tetrahydroacridine (VIb)

Crystallized from ethanol to give product of type **VIb** with m.p. 140–142 °C and yield 11 %. ^{1}H NMR spectrum (DMSO-d6, ppm): 1.8 (mm, 4H, $2CH_2$), 2.4, 2.6 (s, s, 6H, $2CH_3$), 2.80 (t, 2H, CH_2), 3.3 (t, 2H, CH_2), 7.2–8.2 (m, 4H, C–H aromatic, 1H, –C=H of pyrazole); ^{13}C NMR (DMSO *d6*): δ 22.8, 22.8, 23.1, 31.4, 11.1, 18.0, 105.0, 114.5, 122.4, 125.7, 127.2, 127.9, 128.6, 144.3, 144.8, 145.4, 164.5. Analysis for $C_{18}H_{19}N_3$ (277): required: C, 77.66; H, 7.24; N, 15.09; found: C, 77.28; H, 7.48; N, 15.14.

General method for preparation of compounds VIIa–d

A mixture of 9-hydrazino-1,2,3,4-tetrahydroacridine (2.1 g, 0.1 mol) and the appropriate trisubstituted vinylcyanide (0.1 mol) in absolute methanol (50 ml) containing triethylamine (1.5–2 ml) (except **VIIb**) was heated under reflux for 24 h. The solvent was evaporated and the precipitate formed was filtered off, dried, and crystallized from ethanol to give the compounds **VIIa–d**.

5-amino-3-(methylthio)-1-(1,2,3,4-tetrahydroacridin-9-yl)-1H-pyrazole-4-carbonitrile (VIIa)

Crystallized from ethanol to give brown powder with m.p. 230–232 °C and yield 60 %. IR spectrum (K Br, cm^{-1}): 3343, 3186 (NH_2), 2940 (C–H aliphatic) and 2216 (C≡N); ^{1}H NMR spectrum (DMSO-d6, ppm): 1.8 (mm, 4H, $2CH_2$), 2.4 (s, 3H, –S–CH_3), 2.50 (t, 2H, CH_2), 3.3 (t, 2H, CH_2), 5.9 (b, 2H, NH_2 exchangeable with D_2O), 6.8–8.1 (m, 4H, C–H aromatic). Analysis for $C_{18}H_{17}N_5S$ (335): required: C, 64.45; H, 5.11; N, 20.88; found: C, 64.55; H, 5.21; N, 20.68.

5-amino-1-(1,2,3,4-tetrahydroacridin-9-yl)-1H-pyrazole-4-carbonitrile (VIIb)

Crystallized from ethanol to give brown powder with m.p. 260–265 °C and yield 57 %. IR spectrum (K Br, cm^{-1}): 3385 (NH_2), 2209 (C≡N), 1632 (C=N); ^{1}H NMR spectrum (DMSO-d6, ppm): 1.8 (mm, 4H, $2CH_2$), 2.50 (t, 2H, CH_2), 3.3 (t, 2H, CH_2), 3.8 (b, 2H, NH_2 exchangeable with D_2O), 6.78.04 (m, 5H, C–H aromatic). Analysis for $C_{17}H_{15}N_5$ (289): required: C, 70.57; H, 5.23; N, 24.20; found: C, 70.58; H, 5.13; N, 24.22.

Methyl 5-amino-3-(1,5-dimethyl-3-oxo-2-phenylpyrazolidin-4-ylamino)-1-(1,2,3,4-tetrahydroacridin-9-yl)-1H-pyrazole-4-carboxylate (VIIc)

Crystallized from ethanol to give yellowish brown powder with m.p. 270–275 °C and yield 19 %. IR spectrum (K Br, cm^{-1}): 3549 (NH_2), 3155, 3099 (C–H of aromatic), 2936 (C–H of CH_3), 1643 (C=O of amide), 1700 (C=O of $COOCH_3$); ^{1}H NMR spectrum (DMSO-d6, ppm): 2.1 (mm, 4H, $2CH_2$), 2.25 (t, 2H, CH_2), 2.6 (s, 3H, C–CH_3), 3.25 (t, 2H, CH_2), 3.49 (s, 3H, N–CH_3), 3.85 (s, 3H, OCH_3), 7.3–7.5

(m, 9H, C–H aromatic), 4.38 (b, 2H, NH_2 exchangeable with D_2O), 9.7 (s, 1H, NH exchangeable with D_2O). Analysis for $C_{29}H_{29}N_7O_3$ (523): required: C, 66.20; H, 5.58; N, 18.80; found: C, 66.18; H, 5.56; N, 18.78.

5-amino-3-(1,5-dimethyl-3-oxo-2-phenylpyrazolidin-4-ylamino)-1-(1,2,3,4-tetrahydroacridin-9-yl)-1H-pyrazole-4-carbonitrile (VIId)

Crystallized from ethanol to give pale brown powder with m.p. 255–257 °C and yield 19 %. IR spectrum (K Br, cm^{-1}): 3424 (NH_2), 3058, (C–H aromatic), 2924 (C–H of CH_3), 2204 (C ≡ N), 1657 (C=O of amide). Analysis for $C_{28}H_{26}N_8O$ (490): required: C, 68.10; H, 5.44; N, 22.84; found: C, 68.08; H, 5.42; N, 33.82.

General method for preparation of 9-(2-{[(4-substituted heterocyclic sulfonyl chloride)-thio]peroxy}hydrazino)-1,2,3,4-tetrahydroacridine (VIIIa–d)

A mixture of **IIIa** (2.13 g, 0.1 mol) and the appropriate sulfonyl chloride [containing a catalytic amount of pyridine (2 ml) in **VIId**] was dissolved in 30 ml absolute methanol and then refluxed for 18–24 h. The solvent was evaporated, and the solid product obtained was filtered off, dried on air suction, and then crystallized from ethanol.

N′-(1,2,3,4-tetrahydroacridin-9-yl)benzenesulfonohydrazide (VIIIa)

Pale yellowish brown powder with m.p. 293–290 °C and yield 45 %. IR spectrum (K Br, cm^{-1}): 3545 (N–H), 3060 (C–H aromatic), 1212–1175 (SO_2). Analysis for $C_{19}H_{19}N_3O_2S$ (353): required: C, 64.57; H, 5.42; N, 11.89; found: C, 64.57; H, 5.32; N, 11.99.

4-methyl-N′-(1,2,3,4-tetrahydroacridin-9-yl)benzenesulfonohydrazide (VIIIb)

Brown powder with m.p. 270–272 °C and yield 55 %. IR spectrum (K Br, cm^{-1}): 3431 (N–H), 3064 (C–H aromatic), 1255–1174 (SO_2); ^{1}H NMR spectrum (DMSO-d6, ppm): 1.8 (mm, 4H, $2CH_2$), 2.50 (t, 2H, CH_2), 3.3 (t, 2H, CH_2), 2.9 (s, 3H, CH_3) 7.4–7.9 (m, 8H, C–H aromatic), 9.4, 9.7 (s, 2H, 2 NH, exchangeable with D_2O). Analysis for $C_{20}H_{21}N_3O_2S$ (367): required: C, 65.37; H, 5.76; N, 11.44; found: C, 65.33; H, 5.66; N, 11.45.

4-bromo-N′-(1,2,3,4-tetrahydroacridin-9-yl)benzenesulfonohydrazide (VIIIc)

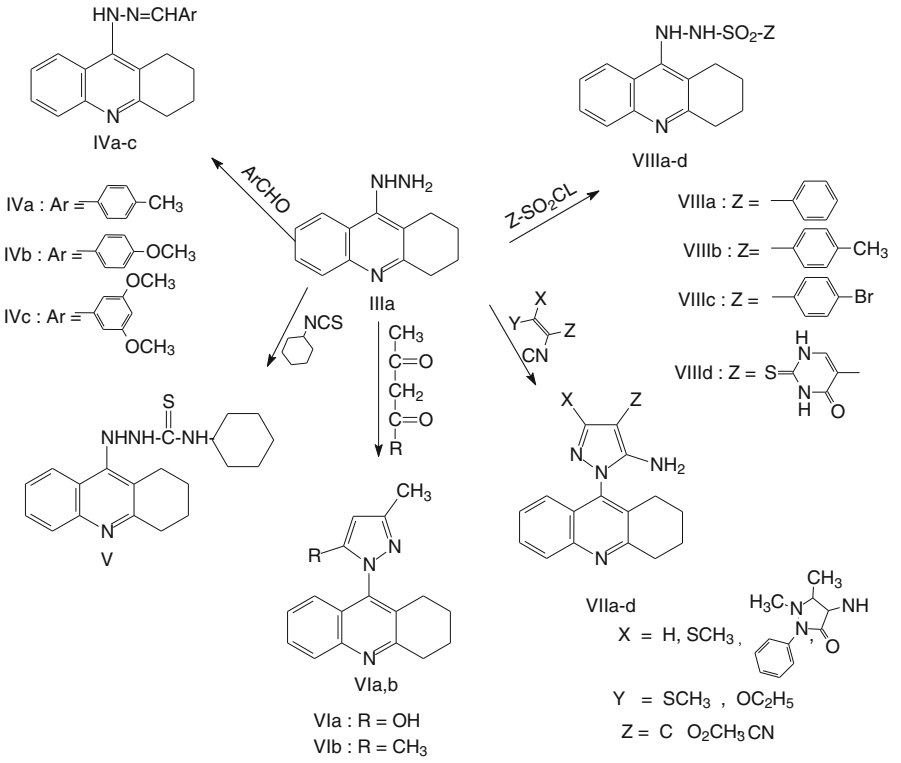
Pale brown powder with m.p. 220–222 °C and yield 51 %. IR spectrum (K Br, cm^{-1}): 3431 (N–H), 3064 (C–H aromatic), 1255–1174 (SO2); ^{1}H NMR spectrum (DMSO-d6, ppm): 1.8 (mm, 4H, $2CH_2$), 2.50 (t, 2H, CH_2), 3.3 (t, 2H, CH_2), 7.4–7.8 (m, 8H, C–H aromatic). 9.4, 9.7 (s, 2H, 2 NH, exchangeable with D_2O); ^{13}C NMR (DMSO *d6*): δ 20.5, 22.8, 22.9, 31.4, 115.6, 119.5, 119.9, 124.4, 126.3, 128.2, 128.3, 129.5, 129.5, 132.0, 132.0, 138.7, 145.4, 147.6, 164.2. Analysis for $C_{19}H_{18}BrN_3O_2S$ (431): required: C, 52.79; H, 4.20; N, 9.82; found: C, 52.78; H, 4.10; N, 9.72.

4-oxo-N′-(1,2,3,4-tetrahydroacridin-9-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-sulfonohydrazide (VIIId)

Pale yellowish powder with m.p. 250–252 °C and yield 40 %. IR spectrum (K Br, cm^{-1}): 3446, 3292 (NH), 3173, 3109 (C–H aromatic), 1719 (C=O), 1632 (C=N), 1216, 1171 (SO_2); ^{1}H NMR spectrum (DMSO-d6, ppm): 1.8 (mm, 4H, $2CH_2$), 2.50 (t, 2H, CH_2), 2.9 (t, 2H, CH_2), 7.3–8.9 (m, 5H, C–H aromatic), 5.6, 11.16, 11.29, 11.47 (m, 4 NH exchangeable with D_2O); ^{13}C NMR (DMSO *d6*): δ 20.6, 22.8, 22.9, 31.4, 103, 115.6, 119.5, 119.9, 124.4, 128.2, 128.3, 145.4, 1467.6, 160, 154.2, 178.1. Analysis for $C_{17}H_{17}N_5O_3S_2$ (403): required: C, 50.61; H, 4.25; N, 17.36; found: C, 50.51; H, 4.15; N, 17.26 (Scheme 2).

General method for preparation of 3-(1,2,3,4-tetrahydroacridine)-2(substituted)-1,3-thiazolidin-4-one (IXa–c)

A mixture of **IVa–c** (0.1 mol) and thioglycolic acid (0.092 g, 0.1 mol) in dry benzene (15 ml) was heated in a water bath for 6–10 h. The volatile solvent was evaporated and the reaction mixture was neutralized with cold dilute sodium bicarbonate solution. The solid was filtered off, dried under air suction, and then crystallized from ethanol to give the compounds **IXa–c**.



Scheme 2 Structure of compounds **IV–VIII**

3-(1,2,3,4-tetrahydroacridin-9-ylamino)-2-p-tolylthiazolidin-4-one (IXa)

Crystallized from ethanol to give brown powder with m.p. 210–215 °C and yield 41 %. IR spectrum (K Br, cm^{-1}): 3237 (NH), 2936 (C–H, st, aliphatic), 1713 (C=O); ^{1}H NMR spectrum (DMSO-d6, ppm): 1.85 (mm, 4H, $2CH_2$), 2.30 (t, 2H, CH_2), 2.5 (s, 3H, CH_3), 3.3 (t, 2H, CH_2), 4.6 (m, 2H, CH_2), 4.8 (s, H, CH–N), 7.2–8.2 (m, 8H, C–H aromatic), 11.88 (s, H, NH exchangeable with D_2O). Analysis for $C_{23}H_{23}N_3OS$ (391): required: C, 70.92; H, 5.95; N, 10.79; found: C, 70.72; H, 5.55; N, 10.69.

2-(4-methoxyphenyl)-3-(1,2,3,4-tetrahydroacridin-9-ylamino)thiazolidin-4-one (IXb)

Crystallized from ethanol to give pale brown powder with m.p. 240–243 °C and yield 52 %. IR spectrum (K Br, cm^{-1}): 3063.37 (NH), 1745 (C=O), 1602 and 1475 (C=C, st, aromatic); ^{1}H NMR spectrum (DMSO-d6, ppm): 1.80 (4H, m, $2CH_2$), 2.36 (2H, t, CH_2), 3.3 (2H, t, CH_2), 3.8 (3H, s, OCH_3), 4.6 (2H, m, CH_2), 4.9 (H, s, CH–N), 7.3–8.7 (8H, m, C–H aromatic), 11.3 (s, H, NH exchangeable with D_2O). Analysis for $C_{23}H_{23}N_3O_2S$ (405): required: C, 68.12; H, 5.72; N, 10.36; found: C, 68.22; H, 5.22; N, 10.26.

2-(3,5-dimethoxyphenyl)-3-(1,2,3,4-tetrahydroacridin-9-ylamino)thiazolidin-4-one (IXc)

Crystallized from ethanol to give pale yellow powder with m.p. 165–166 °C and yield 41 %. IR spectrum (K Br, cm^{-1}): 3418 (NH), 1711 (C=O), 2916 (C–H, st, aromatic), 1455.99 and 1634 (C=C aromatic); ^{1}H NMR spectrum (DMSO-d6, ppm): 1.89 (4H, m, $2CH_2$), 2.5 (2H, t, CH_2), 3.37 (2H, t, CH_2), 3.81, 3.83 (6H, s, $2OCH_3$), 4.5 (2H, m, CH_2), 4.9 (H, s, CH–N), 6.6–8.2 (7H, m, C–H aromatic), 9.3 (H, s, CH–N), 11.4 (H, s, NH exchangeable with D_2O); ^{13}C NMR (DMSO *d6*): δ 14.6, 20.4, 22.6, 22.7, 31.4, 36.0, 55.8, 55.9, 58.0. 98.8, 105.1, 105.2, 115.6, 119.5, 119.9, 124.4, 128.2, 128.3, 141.2, 145.4, 161.6, 161.7, 164.2, 168.8. Analysis for $C_{24}H_{25}N_3O_3S$ (436): required: C, 66.19; H, 5.79; N, 9.65; found: C, 66.29; H, 5.69; N, 9.45.

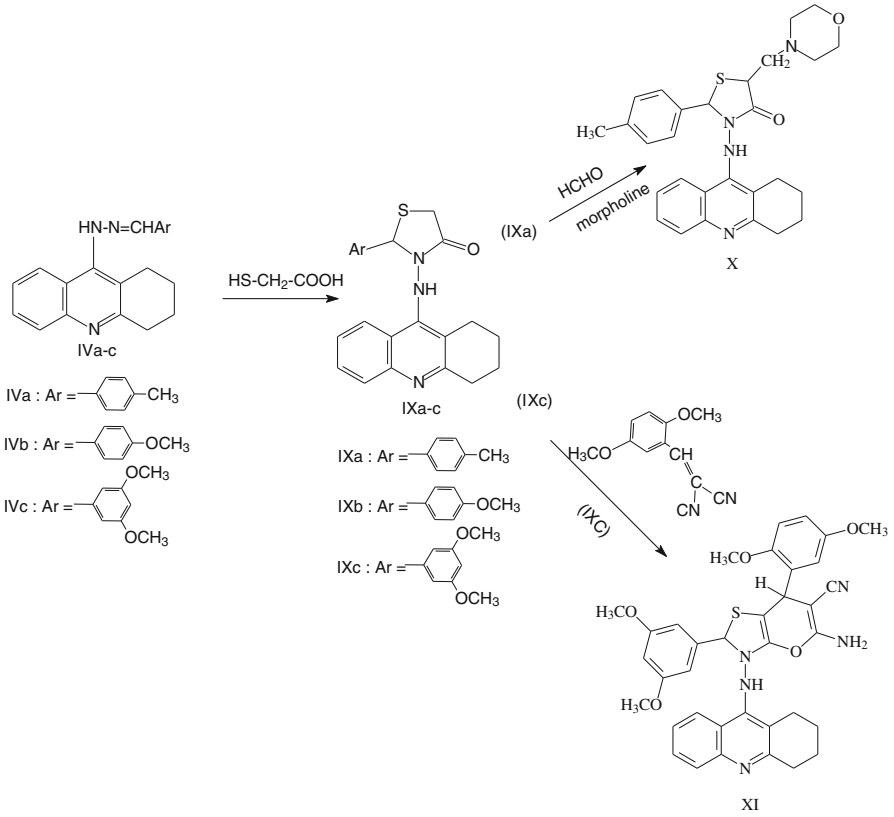
Method for preparation of 3-[1,2,3,4-tetrahydroacridin-9-ylimino]-2-(4-methylphenyl)-5-(morpholin-4-ylmethyl)-1,3-thiazolidin-4-one (X)

A mixture of paraformaldehyde (0.5 g, 0.005 mol) and 15 ml of morpholine in 25 ml absolute ethanol was refluxed for half an hour till complete solubility of the paraformaldehyde. Compound **IXa** (2.5 g, 0.02 mol) in 30 ml of ethanol was warmed and then added to the reaction mixture. The whole mixture was refluxed for 12 h and left at room temperature for 3 days. Then, the volatile material was evaporated. The dry residue was extracted with chloroform and crystallized from methanol to give compound **X** with m.p. 245–147 °C and yield 65 %. IR spectrum (K Br, cm^{-1}): 3373 (NH), 1749 (C=O), 2927 (C–H, st, aromatic); ^{1}H NMR spectrum (DMSO-d6, ppm): 1.85 (4H, m, $2CH_2$), 2.30 (2H, t, CH_2), 2.4 (4H, m, CH_2–N–CH_2), 2.5 (3H, s, C–CH_3), 3.3 (2H, t, CH_2), 3.7 (4H, m, CH_2–O–CH_2), 4.6 (2H, d, CH_2), 7.2–8.6 (8H, m, C–H aromatic, 1H, C–H, of thiazole) 9.2 (H, s, NH,

exchangeable with D_2O). Analysis for $C_{28}H_{32}N_4O_2S$ (488.65): required: C, 68.82; H, 6.60; N, 11.47; found: C, 68.80; H, 6.58; N, 11.45.

Method for preparation of 2-(3,5-dimethoxybenzyl)-3-(1,2,3,4-tetrahydroacridin-9-amino)-7-(2,5-dimethoxybenzyl)-5-amino-6-cyano-3H-thienol (XI)

To a mixture of **IXc** (1.5 g, 0.1 mol) and 2,5-dimethoxybenzylidene malononitrile (0.7 g, 0.1 mol) in 30 ml methanol was added three drops of pyridine to turn it alkaline. Then, it was refluxed for 24 h. After cooling, the formed solid was filtered off, dried under air suction, and then crystallized from ethanol to give product **XI** with m.p. 295–297 °C and yield 55 %. IR spectrum (K Br, cm^{-1}): 3424, 3123 (NH_2), 2293 (C–H st, aromatic), 2183.8 (CN); ^{1}H NMR spectrum (DMSO-d6, ppm): 1.85 (4H, m, $2CH_2$), 2.30 (2H, t, CH_2), 2.5 (3H, s, C–CH_3), 3.3 (2H, t, CH_2), 3.6, 3.7, 3.8, 3.9 (12H, s, $4OCH_3$), 7.1–8.9 (10H, m, C–H aromatic, C–H of pyrane, C–H of thiazole), 5.2–6.1, 10.6 (3H, b, NH, NH_2 exchangeable with D_2O). Analysis for $C_{36}H_{35}N_5O_5S$ (649): required: C, 66.55; H, 5.43; N, 10.78; found: C, 67.41; H, 6.38; N, 10.08 (Scheme 3).



Scheme 3 Structure of compounds **IX–XI**

General method for preparation 1-(1,2,3,4-tetrahydroacridin-9-yl)-3-methylthio-4-cyano5-arylidiniminopyrazole (XIIa–c)

A mixture of **VIIa** (3.3 g, 1 mol) and the appropriate aldehyde (0.1 mol), namely, 4-methoxybenzaldehyde, 4-chlorobenzaldehyde, and 4-nitrobenzaldehyde, in 50 ml absolute ethanol was refluxed for 18–24 h. After cooling, the produced precipitate was filtered off, dried under air suction, and then crystallized from the proper solvent to give compounds **XIIa–c**.

(E)-5-(4-methoxybenzylideneamino)-3-(methylthio)-1-(1,2,3,4-tetrahydroacridin-9-yl)-1H-pyrazole-4-carbonitrile (XIIa)

Crystallized from proper solvent to give yellowish brown powder with m.p. 200–207 °C and yield 58 %. IR spectrum (K Br, cm^{-1}): 3121.22, 3066.26 (C–H aromatic), 2214 (CN), 1633.41 (C=N); ^{1}H NMR spectrum (DMSO-d6, ppm): 1.85 (mm, 4H, $2CH_2$), 2.4 (t, 2H, CH_2), 2.5 (s, 3H, S–CH_3), 3.5 (t, 2H, CH_2), 3.8 (s, 3H, OCH_3), 8.1 (s, H, CH=N), 7.2–7.9 (m, 8H, C–H aromatic). Analysis for $C_{26}H_{23}N_5OS$ (453): required: C, 68.85; H, 5.11; N, 15.44; found: C, 68.95; H, 5.21; N, 15.74.

(E)-5-(4-chlorobenzylideneamino)-3-(methylthio)-1-(1,2,3,4-tetrahydroacridin-9-yl)-1H-pyrazole-4-carbonitrile (XIIb)

Crystallized from proper solvent to give brown powder with m.p. 270–275 °C and yield 54 %. IR spectrum (K Br, cm^{-1}): 3346, 3005 (C–H aromatic), 2212.9 (CN), 1653 (C=N), 1107 (C–Cl); ^{1}H NMR spectrum (DMSO-d6, ppm): 1.82 (mm, 4H, $2CH_2$), 2.6 (t, 2H, CH_2), 2.8 (s, 3H, S–CH_3), 3.8 (t, 2H, CH_2), 8.3 (s, H, CH = N), 7.4–9.1 (m, 8H, C–H aromatic). Analysis for $C_{25}H_{20}ClN_5S$ (457): required: C, 65.57; H, 4.40; N, 15.29; found: C, 65.55; H, 4.38; N, 15.27.

(E)-3-(methylthio)-5-(4-nitrobenzylideneamino)-1-(1,2,3,4-tetrahydroacridin-9-yl)-1H-pyrazole-4-carbonitrile (XIIc)

Pale brown powder with m.p. 250–255 °C and yield 52 %. IR spectrum (K Br, cm^{-1}): 3239, 3080 (C–H aromatic), 2214 (CN), 1632 (C=N), 1330 (NO_2, st, symmetric); ^{1}H NMR spectrum (DMSO-d6, ppm): 1.85 (4H, m, $2CH_2$), 2.5 (2H, t, CH_2), 2.6 (3H, s, S–CH_3), 3.3 (2H, t, CH_2), 7.9 (H, s, CH = N), 7.7–8.7 (8H, m, C–H aromatic). Analysis for $C_{25}H_{20}N_6O_2S$ (468): required: C, 64.09; H, 4.30; N, 17.94; found: C, 64.19; H, 4.40; N, 17.74.

General method for preparation of (±)1-(1,2,3,4-tetrahydroacridin-9-yl)-3-methylthio-4-cyano-5-(2-aryl-1,3-thiazolidine-4-oxo-3-yl)pyrazole (XIIIa–c)

A mixture of **XIIa–c** (0.1 mol) and thioglycolic acid (0.092 g, 0.1 mol) in dry benzene (15 ml) was heating in a water bath for 6–10 h. The volatile solvent was evaporated and the reaction mixture was neutralized with cold dilute sodium

bicarbonate solution. The solid obtained was filtered off, dried under air suction, and then crystallized from ethanol.

5-(4-(4-methoxyphenyl)-2-oxothiazolidin-3-yl)-3-(methylthio)-1-(1,2,3,4-tetrahydroacridin-9-yl)-1H-pyrazole-4-carbonitrile (XIIIa)

Brown powder with m.p. 285–287 °C and yield 47 %. IR spectrum (K Br, cm^{-1}): 3181 (C–H aromatic), 2214 (C≡N), 1644 (C=O); ^{1}H NMR spectrum (DMSO-d6, ppm): 1.85 (mm, 4H, $2CH_2$), 2.4 (t, 2H, CH_2), 2.5 (s, 3H, S–CH3), 3.5 (t, 2H, CH_2), 3.8 (s, 3H, OCH_3), 4.0 (s, H, CH–N), 4.9 (m, 2H, CH_2), 7.2–7.9 (m, 8H, C–H aromatic). Analysis for $C_{28}H_{25}N_5O_2S_2$ (527): required: C, 63.74; H, 4.78; N, 13.27; found: C, 63.64; H, 4.58; N, 13.07.

5-(4-(4-chlorophenyl)-2-oxothiazolidin-3-yl)-3-(methylthio)-1-(1,2,3,4-tetrahydroacridin-9-yl)-1H-pyrazole-4-carbonitrile (XIIIb)

Pale brown powder with m.p. 270–276 °C and yield 51 %. IR spectrum (K Br, cm^{-1}): 3030 (C–H aromatic), 2200 (C≡N), 1654 (C=O); ^{1}H NMR spectrum (DMSO-d6, ppm): 1.82 (mm, 4H, $2CH_2$), 2.6 (t, 2H, CH_2), 2.8 (s, 3H, S–CH_3), 3.8 (t, 2H, CH_2), 4.3 (s, H, CH–N), 4.6 (m, 2H, CH_2), 7.4–9.1 (m, 8H, C–H aromatic). Analysis for $C_{27}H_{22}ClN_5OS_2$ (532): required: C, 60.95; H, 4.17; N, 13.16; found: C, 60.93; H, 4.15; N, 13.14.

3-(methylthio)-5-(4-(4-nitrophenyl)-2-oxothiazolidin-3-yl)-1-(1,2,3,4-tetrahydroacridin-9-yl)-1H-pyrazole-4-carbonitrile (XIIIc)

Brown crystals with m.p. 294–295 °C and yield 24 %. IR spectrum (K Br, cm^{-1}): 3169 (C–H aromatic), 2220 (C≡N), 1646 (C=O); ^{1}H NMR spectrum (DMSO-d6, ppm): 1.88 (mm, 4H, $2CH_2$), 2.5 (t, 2H, CH_2), 2.6 (s, 3H, S–CH_3), 3.2 (t, 2H, CH_2), 4.2 (s, H, CH–N), 4.8 (m, 2H, CH_2), 7.1–8.2 (m, 8H, C–H aromatic). Analysis for $C_{27}H_{22}N_6O_3S_2$ (542): required: C, 59.76; H, 4.09; N, 15.49; found: C, 59.56; H, 4.19; N, 15.29.

Method for preparation of 3-(methylthio)-1-(1,2,3,4-tetrahydroacridin-9-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (XIV)

A mixture of **VIIa** (3.3 g 1 mol) and 33 ml formic acid was refluxed for 24 h. Then ice water was poured into the mixture, its pH adjusted to 6 by using NaOH, and the produced precipitate was filtered off, dried under air suction, and then crystallized from ethanol.

Yellowish brown powder with m.p. 278–280 °C and yield 80 %. IR spectrum (K Br, cm^{-1}): 3395 (NH), 2936 (C–H aliphatic), 2813 (C–H of CH3), 1650 (C=O); ^{1}H NMR spectrum (DMSO-d6, ppm): 1.9 (mm, 4H, $2CH_2$), 2.4 (s, 3H, –S–CH_3), 2.50 (t, 2H, CH_2), 3.1 (t, 2H, CH2), 6.3 (s, 1H, C–H of pyrimidine), 6.8–8.1 (m, 4H, C–H aromatic), 5.2 (s, 1H, NH, exchangeable with D_2O). Analysis for $C_{19}H_{17}N_5OS$ (363): required: C, 62.79; H, 4.71; N, 19.27; found: C, 62.59; H, 4.71; N, 19.27.

Method for preparation of 9-[4-chloro-3-(methylthio)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-1-yl]-1,2,3,4-tetrahydroacridine (XV)

A mixture of **XIV** (3.6 g, 1 mol) and 20 ml phosphoryloxychloride was heated in a sand bath at 80 °C for 7 h. After cooling, ice water was poured into the mixture with stirring and it was left to stand for 5 h to precipitate. The produced precipitate was filtered off, dried under suction, and then crystallized from ethanol.

Brown powder with m.p. 270–275 °C and yield 55 %. IR spectrum (K Br, cm^{-1}): 3076 (C–H aromatic), 2936 (C–H of CH3); ^{1}H NMR spectrum (DMSO-d6, ppm): 1.8 (mm, 4H, $2CH_2$), 2.4 (s, 3H, –S–CH_3), 2.50 (t, 2H, CH_2), 3.2 (t, 2H, CH_2), 7.8 (s, 1H, C–H of pyrimidine), 6.4–8.2 (m, 4H, C–H aromatic). Analysis for $C_{19}H_{16}ClN_5S$ (381.5): required: C, 59.45; H, 4.73; N, 18.24; found: C, 59.55; H, 4.53; N, 18.14.

Method for preparation of 9-[9-(methylthio)-7H-pyrazolo[4,3-e]tetrazolo[1,5-c]pyrimidin-7-yl]-1,2,3,4-tetrahydroacridine (XVI)

A mixture of **XV** (3.6 g, 1 mol) and sodium azide (0.7 g, 0.1 mol) in 15 ml glacial acetic acid was refluxed for 24 h. After cooling, the produced precipitate was filtered off, dried under suction, and then crystallized from ethanol.

Dark brown powder with m.p. 255–257 °C and yield 15 %. IR spectrum (K Br, cm^{-1}): 3078 (C–H aromatic), 2985 (C–H of CH3), 1624 (C=N); ^{1}H NMR spectrum (DMSO-d6, ppm): 1.8 (mm, 4H, $2CH_2$), 2.4 (s, 3H, –S–CH_3), 2.50 (t, 2H, CH_2), 3.2 (t, 2H, CH_2), 8.1 (s, 1H, C–H of pyrimidine), 7.4–8.4 (m, 4H, C–H aromatic). Analysis for $C_{19}H_{16}N_8S$ (388): required: C, 58.75; H, 4.15; N, 28.85; found: C, 58.55; H, 4.25; N, 28.65.

Method for preparation of 1,5-dimethyl-4-{[3-(methylthio)-1(1,2,3,4-tetrahydroacridine)-pyrazolo[3,4-d]pyrimidin-4-yl]amino}-2-phenyl-1,2-dihydro-3H-pyrazol-3-one (XVII)

A mixture of **XV** (3.6 g, 1 mol) and 4-aminoantipyrine (2.8 g) with 0.5 ml of pyridine in 20 ml absolute methanol was refluxed for 24 h. The produced precipitate was filtered off, dried under suction, and then crystallized from ethanol.

Yellow crystal with m.p. 295–297 °C and yield 79 %. ^{1}H NMR spectrum (DMSO-d6, ppm): 1.8 (mm, 4H, $2CH_2$), 2.4 (s, 3H, C–CH_3), 2.45 (s, 3H, –S–CH_3), 2.50 (t, 2H, CH_2), 3.2 (t, 2H, CH_2), 3.7 (s, 3H, N–CH_3), 7.4–8.4 (m, 9H, C–H aromatic, s, 1H of pyrimidine), 10.5 (s, 1H, NH exchangeable with D_2O). Analysis for $C_{30}H_{28}N_8OS$ (548): required: C, 65.43; H, 5.49; N, 20.35; found: C, 65.67; H, 5.14; N, 20.42.

General method for preparation of compounds XVIIIa, b)

A mixture of **XV** (0.1 mol) and anthranilic acid (1 g, 0.1 mol) in n-butanol (20 ml) in case of compound **XVIIIa** and in methanol (20 ml) with 2 ml of pyridine in case

of compound **XVIIIa** was refluxed for 24 h. The produced precipitate was filtered off, dried, and crystallized from methanol.

8-[(1,2,3,4-tetrahydroacridin-9-yl)-1-methylsulfanyl-3H-2,3,4,5a,11-pentaaza-cyclopena[a]anthracen-6-one (XVIIIa)

Pale brown powder with m.p. 290–292 °C and yield 41 %. IR spectrum (K Br, cm^{-1}): 3407 (C–H aromatic), 2935 (C–H of CH_3), 1720 (C=O), 1632 (C=N); ^1H NMR spectrum (DMSO-d6, ppm): 1.8 (mm, 4H, $2CH_2$), 2.4 (s, 3H, –S–CH_3), 2.50 (t, 2H, CH_2), 3.2 (t, 2H, CH_2), 7.6–8.2 (m, 8H, C–H aromatic), 8.9 (s, 1H, CH of pyrimidine); ^{13}C NMR (DMSO *d6*): δ 20.5, 22.8, 22.9, 23.2, 31.4, 105, 114.5, 120.9, 122.4, 122.5, 125.7, 128.6, 128.8, 133, 144.8, 145.4, 147.1, 148.9, 163, 164.5, 170.6. Analysis for $C_{24}H_{20}N_6$ (390): required: C, 73.83; H, 4.65; N, 21.52; found: C, 73.73; H, 4.55; N, 21.32.

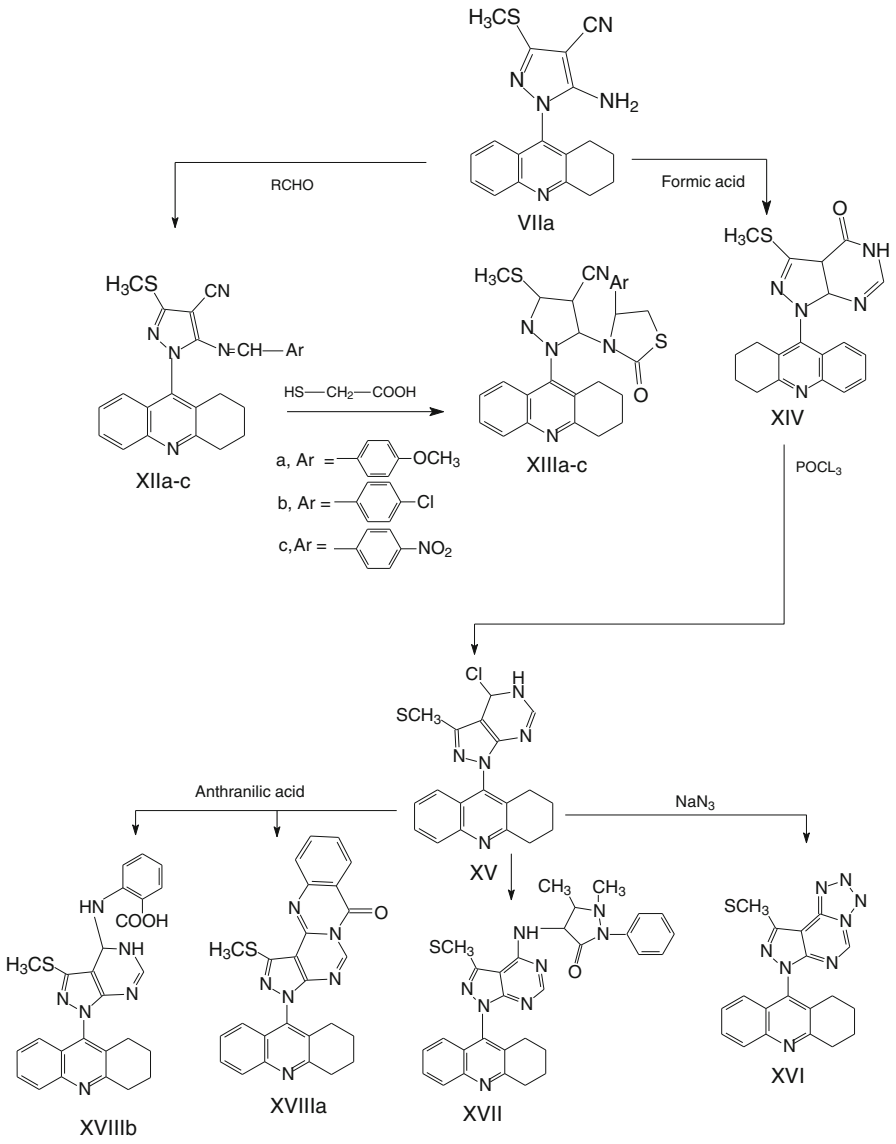
2-(3-(methylthio)-1-(1,2,3,4-tetrahydroacridin-9-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-ylamino)benzoic acid (XVIIIb)

Dark pale brown powder with m.p. 274–272 °C and yield 39 %. IR spectrum (K Br, cm^{-1}): 3739 (O–H), 3390.24 (C–H aromatic), 1720 (C=O), 1632.45 (C=N); ^1H NMR spectrum (DMSO-d6, ppm): 1.8 (mm, 4H, $2CH_2$), 2.0 (s, 1H, NH exchangeable with D_2O), 2.4 (s, 3H, –S–CH_3), 2.50 (t, 2H, CH_2), 3.2 (t, 2H, CH_2), 7.5–8.1 (m, 8H, C–H aromatic), 8.4 (s, 1H, CH of pyrimidine), 10.5 (s, 1H, OH exchangeable with D_2O); ^{13}C NMR (DMSO *d6*): δ 14.6, 20.6, 22.8, 22.9, 31.4, 105, 114.5, 116.4, 122.2, 122.4, 125.7, 127.2, 127.3, 127.9, 128.6, 131.6, 133, 135.3, 144.5, 144.8, 145.4, 148.9, 149.9, 159.0, 159.9, 164.5, 169.4. Analysis for $C_{26}H_{22}N_6O_2S$ (482): required: C, 64.61; H, 4.60; N, 17.42; found: C, 64.51; H, 4.20; N, 17.22 (Scheme 4).

Results and discussion

The synthesis of 10H-1,2,3,4-tetrahydroacridin-9-one I and, particularly, the unsubstituted derivative (R = H) has been carried out by two methods (Fig. 1). Method A (isolating or not the intermediate Schiff base) was discovered by Tiedtke [30] and improved by Reed [31] and Albert [32]. The yields are 40 % (R = H) and 77 % (R = 2−Me) [31–35]. Method B (isolating or not the anil intermediate) was discovered by Sen and Basu [36, 37] and improved by Hughes and Lions [38].

The yield on I (R = H) varies between 20 and 90 % [36], but one must consider that acridones are high melting, very insoluble compounds and difficult to purify. 9-Chlorotetrahydroacridines have been prepared by the reaction of the corresponding 10H tetrahydroacridin-9-ones with phosphorus oxychloride [31–33]. It has been reported [39] that four 9-substituted amino-1,2,3,4-tetrahydroacridines have the general formula of compounds **IIIa, b**, which have been prepared via transformation of the respective 9-chlorotetrahydroacridines. The nucleophilic substitution of the chlorine atom in compound **II** is difficult.



Scheme 4 Structure of compounds **XII–XVIII**

Compounds **IVa–c** were prepared in a good yield by the condensation of **IIIa** with the appropriate aldehyde in absolute ethanol. The precipitate was dried and then crystallized from ethanol to give the title compounds, whose chemical structures were confirmed by microanalytical and spectral data.

Compound **V** was prepared by the condensation of **IIIa** and cyclohexyl isothiocyanate in the least amount in ethyl alcohol. Compounds **VIa, b** were prepared by the condensation of **IIIa** and the appropriate 1,3-dicarbonyl compound (ethylacetoacetate, acetyl acetone) in absolute ethanol to give a good yield. Compounds **VIIa–d**

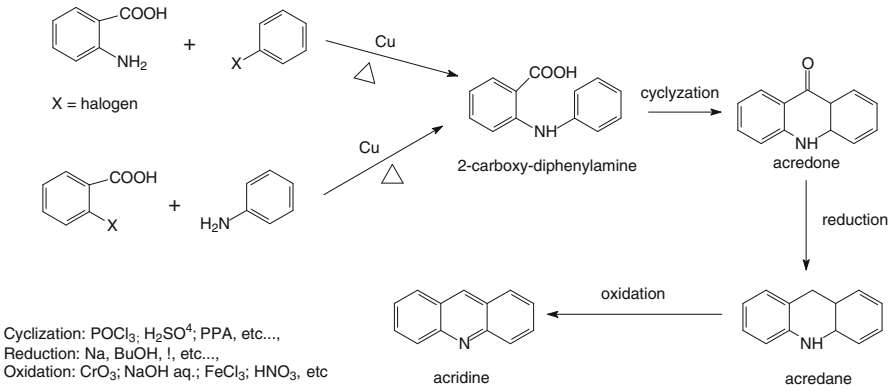


Fig. 1

were prepared by the condensation of **IIIa** and the appropriate trisubstituted vinylcyanide to give the title compounds **VIIa–d**. Compounds **VIIIa–d** were prepared by refluxing **IIIa** and the appropriate heterocyclic sulfonyl chloride (with pyridine in **VIIId**) in absolute methanol to give the corresponding compound. The series of compounds **IXa–c** was prepared by the condensation of **IVa–c** with thioglycolic acid in dry benzene and then neutralizing with sodium bicarbonate solution. The condensation of paraformaldehyde and morpholine in absolute ethanol was progressed till complete solubility of paraformaldehyde and then compound **IXa** was added, which was dissolved in ethanol after heating the reaction mixture and then refluxed to give compound **X**. The compound **IXc** was condensed with 2,5-dimethoxybenzylidene malononitrile in methanol and pyridine to give product **XI**. Compounds **XIIa–c** were prepared by the condensation of **VIIa** and the appropriate aldehyde in absolute ethanol to give the compounds **XIIa–c**. Compounds **XIIIa–c** were achieved by the mixture of **XIIa–c** and thioglycolic acid in dry benzene to give **XIIIa–c**. Compound **XIV** was prepared by the condensation of **VIIa** with formic acid (3.3 ml). The pH was adjusted to 6 by using dilute NaOH to give compound **XIV**. Compound **XV** was prepared by the condensation of **XIV** and phosphoryloxy chloride, which was then poured into ice water with stirring to give compound **XV**. Compound **XVI** was prepared by the mixture of **XV** and sodium azide in glacial acetic acid, which was refluxed to give compound **XVI**. Compound **XVII** was prepared by the condensation of **XV** and 4-aminoantipyrine with pyridine in absolute methanol. Compound **XVIIIa** was prepared by the condensation of **XV** and anthranilic acid in n-butanol. Compound **XVIIIb** was prepared by the condensation of **XV** and anthranilic acid with pyridine in methanol and crystallized from ethanol.

Pharmacology

Antitumor activity

In vitro tests were performed for the new compounds' cytotoxic effects [1–4].

Experiment

Measurement of potential cytotoxicity by SRB assay

The eight tetrahydroacridine derivatives (compounds **I–VIII**) were subjected to a screening system for the evaluation of their antitumor activity against the liver cancer (HEPG2) tumor cell line in comparison to the known anticancer drugs, 5-fluorouracil and doxorubicin.

The potential cytotoxicity of the selected tetrahydroacridine derivatives was tested using the method of Skehan et al. [40] as follows:

1. Cells were plated in 96-well plates (10^4 cells/well) for 24 h before treatment with the compound(s) to allow attachment of cells to the walls of the plate.
2. Different concentrations of the compound under test (0, 1, 2.5, 5, 10 μg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose.
3. Monolayer cells were incubated with the compound(s) for 48 h at 37 °C and in an atmosphere of 5 % CO_2.
4. After 48 h, the cells were fixed, washed, and stained with sulforhodamine B stain.
5. Excess stain was washed with acetic acid and attached stain was recovered with Tris-EDTA buffer.
6. Color intensity was measured using an ELISA reader.
7. The relation between surviving fraction and drug concentration is plotted in order to obtain the survival curve of each tumor cell line treatment with the specified compound.

Biochemical analysis

The biochemical effects of the selected tetrahydroacridine derivatives (eight compounds) on some enzymes such as aspartate and alanine aminotransferases (AST and ALT), alkaline phosphatase (ALP), total protein content, albumin, globulins, creatinine, total lipids, cholesterol, triglycerides, and bilirubin in the serum of mice were evaluated in comparison to 5-fluorouracil and doxorubicin.

Different biochemical parameters were realized in control and treated mice groups as follows:

1. Estimation of ALT, AST, and ALP activity levels were done using a blood autoanalyzer (Olympus AU400, Japan)
2. Total proteins, albumin, globulins, and creatinine in the serum of mice were estimated according to the technique described by Faddah and Soliman [41]
3. Total lipids, cholesterol, triglycerides, and bilirubin in the serum of mice were estimated according to the technique described by Soliman and Faddah [42]

Statistical analysis of the results was performed using Chi-square values (SPSS software package).

Table 1 Effect of some selected tetrahydroacridine derivatives on the hepatic carcinoma cell line HEPG2

Compounds	IC_{50}
Doxorubicin (Dox)	3.56 μg/ml
5-Fluorouracil (5-FU)	5 μg/ml
VIIa	18 μg/ml
VIIIc	19.1 μg/ml
IVa	24.6 μg/ml
VIb	0.694 μg/ml
XVIIIa	2.97 μg/ml
VIIId	14.9 μg/ml
VIIb	21.1 μg/ml
IIIb	18.5 μg/ml

Results

Many tetrahydroacridine derivatives have been synthesized in order to evaluate their antitumor activities as a trial to obtain more effective and less toxic agents. The antitumor activity results indicated that the selected tetrahydroacridine derivatives showed antitumor activity against the liver cancer (HEPG2) tumor cell line tested, but with varying intensities in comparison to the known anticancer drugs 5-fluorouracil and doxorubicin.

Table 1 shows that all compounds exhibited a moderate growth inhibition activity on the tested tumor panel cell line in 1–10 μg/ml concentrations in comparison to the known anticancer drugs 5-fluorouracil and doxorubicin.

It was found that compound **VIb** was the most active and induced a marked growth inhibition (0.694 μg/ml concentration) in a dose-dependent manner against liver cancer (HEPG2), while compound **XVIIIa** was second in regards to the growth inhibition activity (2.97 μg/ml concentration). The rest of the compounds, **VIIa**,**VIIIc**, **IVa**, **VIIId**, **VIIb**, and **IIIb**, exhibited less inhibition activities, with concentrations of 18, 19.1, 24.6, 14.9, 21.1, and 18.5 μg/ml.

It is noticed from the results that the novel derivatives induced a significant growth inhibition towards the liver cancer (HEPG2) cell line in comparison to 5-fluorouracil and doxorubicin after treatment with IC_{50} values (ranging from 0.694 to 2.97 μg/ml concentrations) for compounds **VIb** and **XVIIIa**, while the IC_{50} values for 5-fluorouracil was 5 μg/ml concentration and doxorubicin was 3.56 μg/ml concentration.

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