SYNTHESIS AND BIOLOGICAL ACTIVITY OF SOME BASIC-SUBSTITUTED 4,9-DIHYDRO-3-METHYL-4-OXO-1*H*(2*H*)-PYRAZOLO[3,4-*b*]QUINOLINES

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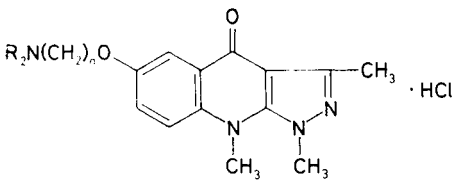
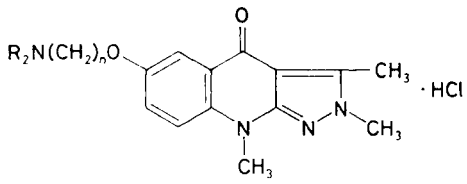
Compounds *Ia*, *Ib* were obtained by an alkylation of 4,9-dihydro-6-hydroxy-1,3,9-trimethyl-4-oxo-1*H*-pyrazolo[3,4-*b*]quinoline (*VIIb*) with the respective dialkylaminoalkyl chloride. The same alkylation of 4,9-dihydro-6-hydroxy-2,3,9-trimethyl-4-oxo-2*H*-pyrazolo[3,4-*b*]quinoline (*VIIIb*) yielded compounds *IIa* and *IIb*. Similar alkylation of 4,9-dihydro-3,9-dimethyl-4-oxo-1*H*-pyrazolo[3,4-*b*]quinoline (*IXa*) and its 6-methoxy derivative (*IXb*) afforded *IIIa*—*IIId*. Compound *IV* was prepared from 4-chloro-3-methyl-1*H*-pyrazolo[3,4-*b*]quinoline (*Xa*) via its 1-(3-dimethylaminopropyl)derivative (*Xb*). Compounds *VIa*, *VIb* were prepared from 4,9-dihydro-6-hydroxy-3,9-dimethyl-4-oxo-1*H*-pyrazolo[3,4-*b*]quinoline (*IXc*) and the respective dialkylaminoalkyl chloride. The compounds prepared were tested for antiviral activity in vivo in mice against influenza virus A2-Hongkong and encephalomyocarditis virus.

Some pyrazolo[3,4-*b*]quinolines have similar biological effects as their acridine analogues[1-3]. Antiviral activity of various dialkylaminoalkoxy derivatives of 10-methyl-9(10*H*)-acridanons[4] has incited us to prepare similar compounds *Ia*, *Ib*, *IIa*, *IIb*, *IIIa*—*IIId*, and *IV*. There are a lot of bis-basic-substituted tricyclic synthetic interferon inducers of a general formula *V* (ref.[1]). Antiviral activity of 3,6-bis(aminoalkoxy)acridines is also known[5]. These facts inspired us to prepare compounds *VIa* and *VIb*.

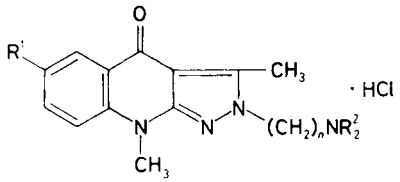
Compounds *Ia* and *Ib* were prepared by alkylation of 4,9-dihydro-6-hydroxy-1,3,9-trimethyl-4-oxo-1*H*-pyrazolo[3,4-*b*]quinoline (*VIIb*) with 2-diethylaminoethyl chloride and 3-dimethylaminopropyl chloride, respectively. Similarly, alkylation of 4,9-dihydro-6-hydroxy-2,3,9-trimethyl-4-oxo-2*H*-pyrazolo[3,4-*b*]quinoline (*VIIIb*) yielded compounds *IIa* and *IIb*. Compounds *VIIb* and *VIIIb* were prepared from 6-methoxy derivatives *VIIa* and *VIIIa*, respectively[6]. The fact, that alkylation of 4,9-dihydro-3,9-dimethyl-4-oxo-1*H*-pyrazolo[3,4-*b*]quinolnie (*IXa*) affords only 2-alkyl derivatives[2,7], was used for the preparation of *IIIa* and *IIIb*. Similarly alkylation of *IXb* yielded *IIIc* and *IIId*. Alkylation of 4-chloro-3-methyl-1*H*-pyrazolo[3,4-*b*]quinoline (*Xa*) with 3-dimethylaminopropyl chloride in dimethyl sulfoxide in the presence of powdered potassium hydroxide at room temperature yielded 4-chloro-3-methyl-1-(3-dimethylaminopropyl)-1*H*-pyrazolo[3,4-*b*]quinoline (*Xb*),



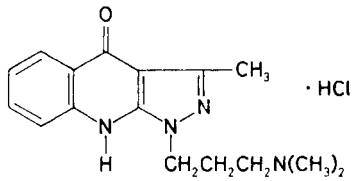
Ia, R = C_2H_5; *n* = 2
Ib, R = CH_3; *n* = 3



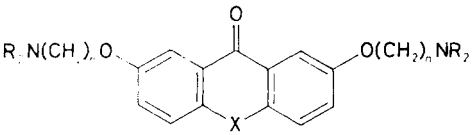
IIa, R = C_2H_5; *n* = 2
IIb, R = CH_3; *n* = 3



IIIa, R^1 = H; R^2 = C_2H_5; *n* = 2
IIIb, R^1 = H; R^2 = CH_3; *n* = 3
IIIc, R^1 = CH_3O; R^2 = C_2H_5; *n* = 2
IIId, R^1 = CH_3O; R^2 = CH_3; *n* = 3

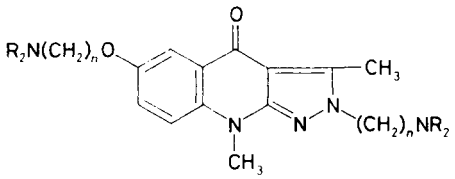


IV



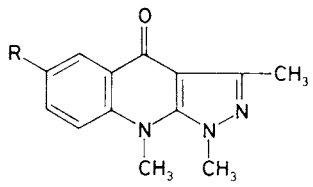
V

X = O, S, CO, bond; R = C_1–C_4 alkyl; *n* = 1–4

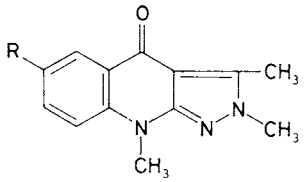


VIa, R = C_2H_5; *n* = 2
VIb, R = CH_3; *n* = 3

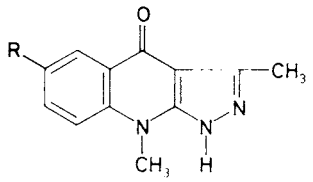
which was converted into *IV* by boiling in dilute hydrochloric acid. Alkylation of 4,9-dihydro-6-hydroxy-3,9-dimethyl-4-oxo-1*H*-pyrazolo[3,4-*b*]quinoline (*IXc*) with two molar equivalents of 2-diethylaminoethyl chloride and 3-dimethylaminopropyl chloride yielded *VIa* and *VIb*, respectively. Compound *IXc* was prepared by demethylation of its 6-methoxy derivative *IXb* with hydrobromic acid[6]. 4,9-Dihydro-6-methoxy-3,9-dimethyl-4-oxo-1*H*-pyrazolo[3,4-*b*]quinoline (*IXb*) was obtained by reaction of 4-(4-methoxyphenyl)-4-methyl-thiosemicarbazide (*XI*) with ethyl 2-chloroacetoacetate, followed by cyclization of formed 3-(N-methyl-4-methoxyanilino)-5-methyl-1*H*-pyrazole-4-carboxylic acid (*XII*) by the action of polyphosphoric acid. Compound *XII* is formed from *XI* by extrusion of sulfur[8] from an unstable 1,3,4-thiadiazine intermediate. The starting 4-(4-methoxyphenyl)-4-methyl-



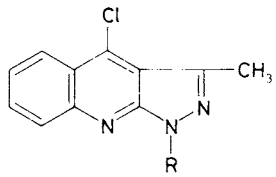
VII a, R = CH_3O
VII b, R = OH



VIII a, R = OCH_3
VIII b, R = OH

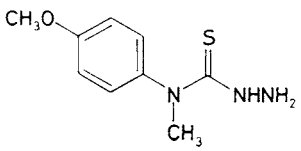


IX a, R = H
IX b, R = OCH_3
IX c, R = OH

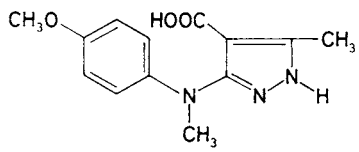


X a, R = H
X b, R = $(CH_2)_3N(CH_3)_2$. HCl

-thiosemicarbazide (*XI*) was obtained from N-methyl-4-methoxyaniline via 2-[N-(4--methoxyphenyl)-N-methyl-thiocarbamoylthio]acetic acid. Similar preparation of 4,9-dihydro-3,9-dimethyl-4-oxo-1*H*-pyrazolo[3,4-*b*]quinoline (*IXa*) has been reported[9].



XI



XII

Compounds *Ia*, *Ib*, *IIa*, *IIb*, *IIIa*–*IIId*, *IV*, *VIa*, and *VIb* were tested in vivo in mice for their efficacy against influenza virus A2-Hongkong and encephalomyocarditis virus after p.o. and s.c. administration. The tests were carried out as described before[7] at the Virological Department of the Institute (Head: Dr. F. Šmejkal). None of the compounds tested exhibited any significant antiviral effect.

In the antitumour tests compounds *VIa* and *VIb* were administered p.o. or s.c. to animals with experimental tumours according to the usual screening scheme[10,11]. In these tests the selected compounds exhibited no marked antitumour effect against ascitic sarcoma (Sa 37) and Ehrlich tumour (STE). Compound *VIb* extended the survival with Yoshida ascitic tumour by 62% in a dose of 25 mg/kg p.o., by 63%

in a dose of 50 mg/kg p.o., by 27% in a dose of 25 mg/kg s.c. and 96% in a dose of 50 mg/kg s.c.

All compounds prepared were tested for their antimicrobial activity in vitro against *Streptococcus β-haemolyticus*, *Streptococcus faecalis*, *Staphylococcus pyogenes aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris*, *Saccharomyces pasterianus*, *Trichophyton mentagrophytes*, *Candida albicans*, and *Aspergillus niger*. The studied compounds had no or relatively small inhibitory effect (minimum inhibitory concentrations higher or equal 100 mg/l).

EXPERIMENTAL

The melting points were determined on an apparatus Mettler FP5 and are not corrected. The UV spectra were taken with a spectrophotometer Perkin–Elmer 550 S in ethanol, molar absorption coefficients are given in $m^2\ mol^{-1}$ (Table I).

General Procedure for Preparation of *Ia*, *Ib*, *IIa*, *IIb*, *IIIa*—*IIId*

Sodium hydride (80% dispersion in mineral oil, 0·9 g, 30 mmol) was added to a stirred suspension of the respective starting compound (Table II) (12 mmol) in N,N-dimethylformamide (50 ml) and the mixture was stirred under nitrogen for 1 h. Then the appropriate dialkylaminoalkyl chloride hydrochloride (14 mmol) was added and the stirring was continued for additional 4 h, the mixture was left to stand overnight and taken to dryness. The residue was boiled with ethanol,

TABLE I
UV spectra (molar absorption coefficients in $m^2\ mol^{-1}$)

Compound	λ_{max}, nm (log ε)
Ia	241 (3·63), 290 (2·69), 355 (2·80), 367 (2·79)
Ib	242 (3·61), 290 (2·69), 355 (2·79), 367 (2·79)
IIa	240 (3·47), 293 (2·99), 405 (2·90)
IIb	240 (3·64), 292 (2·94), 406 (2·89)
IIIa	240 (3·62), 284 (2·87), 373 (2·82)
IIIb	241 (3·63), 284 (2·85), 374 (2·81)
IIIc	239 (3·63), 292 (2·96), 404 (2·86)
IIId	240 (3·70), 293 (3·03), 405 (2·90)
IV	240 (3·64), 275 (2·75), 336 (2·80)
VIa[a]	240 (3·73), 294 (3·07)
VIa[b]	239 (3·66), 293 (2·99)
VIb[a]	240 (3·73), 293 (3·07)
VIb[c]	240 (3·69), 293 (3·02)

[a] Base; [b] dihydrochloride monohydrate; [c] dihydrochloride dihydrate.

TABLE II

Preparation of compounds *Ia*, *Ib*, *IIa*, *IIb*, *IIIa*–*IIId*

Product	Starting compound	Yield (%)	M.p., °C solvent	Formula (mol. weight)	Calculated/found			
					% C	% H	% Cl	% N
Ia	*VIIb*	68	209·8–211·1 2-propanol	$C_{19}H_{26}N_4O_2.HCl$ (378·9)	60·23 59·74	7·18 7·08	9·36 9·58	14·79 14·77
Ib	*VIIb*	80	251–255[a] 2-propanol	$C_{18}H_{24}N_4O_2.HCl$ (364·9)	59·25 59·48	6·91 7·03	9·72 9·91	15·35 15·20
IIa	*VIIIb*	71	241·7–242·6 2-propanol	$C_{19}H_{26}N_4O_2.HCl$ (378·9)	60·23 59·96	7·18 7·20	9·36 9·24	14·79 14·69
IIb	*VIIIb*	60	209·3–213·4 ethanol	$C_{18}H_{24}N_4O_2.HCl$ (364·9)	59·25 59·19	6·91 7·16	9·72 9·92	15·35 15·47
IIIa	*IXa*	66	212·3–218·7 ethanol	$C_{18}H_{24}N_4O.HCl$ (348·9)	61·97 61·66	7·22 7·47	10·16 10·48	16·06 15·61
IIIb	*IXa*	57	200·1–202·1 ethanol	$C_{17}H_{22}N_4O.HCl$ (334·9)	60·98 61·03	6·92 6·76	10·59 10·98	16·73 16·43
IIIc	*IXb*	74	229·5–222·2 2-propanol	$C_{19}H_{26}N_4O_2.HCl$ (378·9)	60·23 60·16	7·18 7·28	9·36 9·11	14·79 15·02
IIId	*IXb*	52	215·7–216·6 2-propanol	$C_{18}H_{24}N_4O_2.HCl$ (364·9)	59·25 58·88	6·91 6·97	9·72 10·10	15·35 15·19

[a] Decomposition.

the insoluble portion was filtered off. The filtrate was acidified with 30% ethanolic solution of hydrogen chloride, and diethyl ether was added. The precipitate was filtered off and crystallized from a suitable solvent (Table II).

4-Chloro-1-(3-dimethylaminopropyl)-3-methyl-1*H*-pyrazolo-[3,4-*b*]quinoline Hydrochloride (*Xb*)

Powdered potassium hydroxide (1·7 g; 30 mmol) was added to a suspension of *Xa* (2·2 g; 10 mmol) in dimethyl sulfoxide (25 ml) and the mixture was stirred at room temperature for 1 h. 3-Dimethylaminopropyl chloride hydrochloride (1·7 g; 11 mmol) was added and the mixture was stirred at room temperature for 8 h and then left to stand overnight. The mixture was poured into water (50 ml), the mixture was extracted with diethyl ether, the extract was washed with water and dried with magnesium sulfate. The filtrate was taken to dryness, the residue was dissolved in ethanol and acidified with 30% ethanolic solution of hydrogen chloride. Separated crystals were filtered off, yield 1·8 g (53%), m.p. 239·3–241·4°C. Ref. 12 reports m.p. 240–242°C (2-propanol). For $C_{16}H_{19}ClN_4.HCl$ (339·3) calculated: 56·64% C, 5·94% H, 20·90% Cl, 16·51% N; found: 56·72% C, 5·87% H, 21·12% Cl, 16·71% N.

4,9-Dihydro-1-(3-dimethylaminopropyl)-3-methyl-4-oxo-1*H*-pyrazolo[3,4-*b*]quinoline Hydrochloride (*IV*)

A mixture of *Xb* (1·1 g; 32 mmol) and 2M hydrochloric acid (7·5 ml) was refluxed for 1 h and then taken to dryness. The residue was crystallized from ethanol; yield 1·0 g (96%), m.p. 247·0 to 248·9°C. For $C_{16}H_{20}N_4O.HCl$ (320·8) calculated: 59·90% C, 6·60% H, 11·05% Cl, 17·46% N; found: 59·79% C, 6·54% H, 11·02% Cl, 17·26% N.

2-[N-(4-Methoxyphenyl)-N-methyl-thiocarbamoylthio]acetic Acid

Carbon disulfide (76 g; 1 mol) was added to a stirred solution of 4-methoxy-N-methylaniline (137 g; 1 mol) in 350 ml of ethanol and 160 ml of concentrated aqueous ammonia and the mixture was stirred at room temperature for 5 h. A solution obtained by neutralization of chloroacetic acid (95 g; 1 mol) in 200 ml of water with powdered sodium carbonate (53 g; 0·5 mol) was then added to the reaction mixture and stirring at room temperature was continued for 8 h. After acidification with concentrated hydrochloric acid and cooling the separated product was filtered off and washed with water; yield 213 g (89%), m.p. 174·1–176·2°C. Analytical sample was obtained by crystallization from ethanol, m.p. 175·2–176·2°C. For $C_{11}H_{13}NO_3S_2$ (271·4) calculated: 48·69% C, 4·83% H, 5·16% N, 23·63% S; found: 49·04% C, 5·02% H, 5·06% N, 23·54% S.

4-(4-Methoxyphenyl)-4-methyl-thiosemicarbazide (*XI*)

100% Hydrazine hydrate (50 g; 1 mmol) was added to a mixture of 2-[N-(4-methoxyphenyl)-N-methyl-thiocarbamoylthio]acetic acid (120 g; 0·5 mol), 150 ml of ethanol and 100 ml of concentrated aqueous ammonia. The mixture was stirred at room temperature for 4 days. The separated crystals were collected on a filter (74 g), the filtrate was after adding additional 100% hydrazine hydrate (10 g; 0·2 mol) left standing for a fornight and another crop of crystals (10·5 g) was obtained. The combined portions were crystallized from ethanol; yield 71·3 g (67%), m.p. 118·5–120·2°C. For $C_9H_{13}N_3OS$ (211·3) calculated: 51·16% C, 6·20% H, 19·89% N, 15·18% S; found: 50·87% C, 6·28% H, 19·96% N, 15·08% S.

4,9-Dihydro-6-methoxy-3,9-dimethyl-4-oxo-1H-pyrazolo[3,4-*b*]quinoline (*IXb*)

Ethyl 2-chloroacetoacetate (8·5 g; 52 mmol) was added to a stirred suspension of *XI* (10·5 g; 50 mmol) in 250 ml of ethanol and the mixture was stirred for 2 h at room temperature, then refluxed for 30 minutes. The insoluble sulfur was removed by filtration of the hot mixture, the filtrate was taken to dryness and the residue was mixed with polyphosphoric acid (100 g) containing 85% of phosphorus pentoxide. The mixture was stirred at 90°C for 2 h, poured into water (1 l) and briefly boiled with activated carbon (2 g). The filtrate was cooled down and left to stand overnight in a refrigerator. The separated product was filtered off and crystallized from ethanol; yield 7·0 g (58%), m.p. 319–323°C. For $C_{13}H_{13}N_3O_2$ (243·3) calculated: 64·19% C, 5·39% H, 17·27% N; found: 63·81% C, 5·72% H, 17·13% N. UV spectrum: λ_{max}, nm (log ε): 239 (3·70), 288 (2·88), 378 (2·79), 397 (2·80); λ_{infl}, nm: 277, 356

4,9-Dihydro-6-hydroxy-3,9-dimethyl-4-oxo-1*H*-pyrazolo[3,4-*b*]quinoline Monohydrate (*IXc*)

A mixture of *IXb* (24·3 g; 0·1 mol) and 48% hydrobromic acid (400 ml) was refluxed for 16 h poured into water (1·5 l) and cooled. The separated product was filtered off, crystallized from 2-methoxyethanol; yield 21·2 g (86%), m.p. 344–348°C. For $C_{12}H_{11}N_3O_2.H_2O$ (247·3) calculated: 58·29% C, 5·30% H, 16·99% N; found: 58·11% C, 5·41% H, 17·11% N. UV spectrum: λ_{max}, nm (log ε): 237 (3·67), 287 (2·87), 377 (2·74), 402 (2·75).

4,9-Dihydro-6-(2-diethylaminoethoxy)-2-(2-diethylaminoethyl)-3,9-dimethyl-4-oxo-2*H*-pyrazolo[3,4-*b*]quinoline (*VIa*)

Sodium hydride (80% dispersion in mineral oil, 9·0 g; 0·3 mol) was added to a stirred suspension of thoroughly dried (130°C, 0·4 kPa) of *IXc* (11·5 g; 50 mmol) in N,N-dimethylformamide (250 ml) and the mixture was stirred under nitrogen for 2 h. 2-Diethylaminoethyl chloride hydrochloride (18·9 g; 110 mmol) was added and the stirring at room temperature was continued for 24 h. The mixture was taken to dryness, the residue as crystallized from hexane; yield 17·1 g (80%), m.p. 70·8–72·3°C. For $C_{24}H_{37}N_5O_2$ (427·6) calculated: 67·42% C, 8·72% H, 16·38% N; found: 67·12% C, 8·86% H, 16·28% N. From 5·3 g of the base 5·9 g (82%) of dihydrochloride monohydrate was obtained by usual way, m.p. 219·8–222·9°C (decomp.). For $C_{24}H_{37}N_5O_2.2\,HCl.H_2O$ (518·5) calculated: 55·59% C, 7·97% H, 13·67% Cl, 13·51% N; found: 55·30% C, 7·97% H, 13·86% Cl, 13·59% N.

4,9-Dihydro-6-(3-dimethylaminopropoxy)-2-(3-dimethylaminopropyl)-3,9-dimethyl-4-oxo-2*H*-pyrazolo[3,4-*b*]quinoline (*VIb*)

This compound was obtained from *IXc* and 3-dimethylaminopropyl chloride hydrochloride according to the procedure described for the preparation of *VIa*; yield 64%, m.p. 90·2–91·4°C (hexane). For $C_{22}H_{33}N_5O_2$ (399·5) calculated: 66·14% C, 8·33% H, 17·53% N; found: 66·16% C, 8·41% H, 17·77% N. From 5·8 g of this base 5·1 g of dihydrochloride dihydrate (69%) was obtained, m.p. 226·1–235·5°C (decomp.). For $C_{22}H_{33}N_5O_2.2\,HCl.2\,H_2O$ (508·5) calculated: 51·97% C, 7·73% H, 13·94% Cl, 13·77% N; found: 51·51% C, 7·26% H, 14·17% Cl, 13·54% N.

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