Synthesis and Biological Activity of Some 2-Substituted Quinazolin-4-ones

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The nonclassical antifolates have been prepared by nucleophilic substitution of bromine in 2-bromomethyl-3*H*-quinazolin-4-one by nitrogen and oxygen nucleophiles. IR and ^{1}H NMR spectra, ^{13}C NMR data of selected compounds, basic antibacterial and cytotoxic activities are presented.

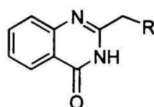
Quinazoline derivatives are used in medicine and agriculture because of their wide-range biological properties. As documented in the literature, many derivatives act as anticancer active agents and antimetabolites from the group of analogues of folic acid. They are antifolate thymidylate synthase (TS) inhibitors [1].

The present research is interested in an alternative class of nonclassical lipophilic TS inhibitors, which are not substrates for folylpolyglutamate synthetase, but retain their cytotoxic activity [2, 3].

In view of the above-mentioned facts, our interest was focused on the utilization of the 2-bromomethyl-3*H*-quinazolin-4-one (*II*) for the synthesis of potentially cytotoxic nonclassical antifolates. The target compounds were prepared by nucleophilic substitution of bromine in the bromomethyl group of *II* by nitrogen and oxygen nucleophiles. Characterization of prepared compounds is given in Table 1. Spectral data are presented in Tables 2 and 3. For selected derivatives the spectral data were supplemented by ^{13}C NMR analyses.

The study of biological properties showed certain antibacterial and cytotoxic activity of some of prepared derivatives. The widest antibacterial effect has been manifested by the derivative *II*, which was effective against *S. aureus*, *B. subtilis*, and *E. coli*. The IC_{50} value for *P. aeruginosa* was 2.94 times lower than the corresponding value for ampicillin. The concentration 100 μg cm^{-3} of this derivative exerted a bacteriostatic effect on *S. aureus*, *B. subtilis*, and *E. coli*.

From the results of cytotoxic study it is evident that the highest cytotoxic activities expressed by IC_{50} values were shown by compounds *I* (< 0.125 μg cm^{-3}), *II* (< 0.125 μg cm^{-3}), *IIIj* (0.068 μg cm^{-3}), and *IIIi* (1.62 μg cm^{-3}). Certain cytotoxic efficacy was exhibited by compounds *IIIk* (4.24 μg cm^{-3}), *IIIg* (8.31 μg cm^{-3}), and *IIIb* (13.77 μg cm^{-3}). The weakest activity was found with derivatives *IIIe* (>100 μg cm^{-3}) and *IIId* (>100 μg cm^{-3}).



I—III

I R = H
II R = Br
IIIa R = 1-morpholinyl
IIIb R = 1-piperidinyl
IIIc R = methoxy
IIId R = ethoxy
IIIe R = isopropoxy
IIIf R = butoxy
IIIg R = phenoxy
IIIh R = 4-nitrophenoxy
IIIi R = 2-nitrophenoxy
IIIj R = *N*,*N*-dimethylamino
IIIk R = *N*-(2-hydroxyethyl)amino
IIIl R = *N*-(methoxycarbonylmethyl)amino
IIIm R = *N*-(1,3-dicarboxypropyl)amino

Table 1. Characterization of the Prepared Compounds *I*—*III*

Compound	Formula M_r	w_i(calc.)/% w_i(found)/% C	H	N	Yield %	M.p. °C
I	$C_9H_8N_2O$	67.49	5.03	17.49	74	243—245
	160.17	67.33	4.98	17.30		
II	$C_9H_7BrN_2O$	45.22	2.95	11.72	65	235—240
	239.07	45.03	2.90	11.65		
IIIa	$C_{13}H_{15}N_3O_2$	63.66	6.16	17.13	78	192—195
	245.28	63.44	6.08	17.02		
IIIb	$C_{14}H_{17}N_3O$	69.11	7.04	17.27	28	174—175
	243.31	68.92	7.00	17.15		
IIIc	$C_{10}H_{10}N_2O_2$	63.15	5.30	14.73	88	218—223
	190.20	62.97	5.21	14.56		
IIId	$C_{11}H_{12}N_2O_2$	64.69	5.92	13.72	60	180—184
	204.23	64.49	5.87	13.52		
IIIe	$C_{12}H_{14}N_2O_2$	66.04	6.47	12.84	33	260—264
	218.25	65.89	6.33	12.67		
IIIf	$C_{13}H_{16}N_2O_2$	67.22	6.94	12.06	46	182—185
	232.28	67.03	6.89	11.96		
IIIg	$C_{15}H_{12}N_2O_2$	71.42	4.79	11.10	85	241—243
	252.27	71.35	4.63	10.87		
IIIh	$C_{15}H_{11}N_3O_4$	60.61	3.73	14.14	50	142—146
	297.27	60.55	3.69	13.88		
IIIi	$C_{15}H_{11}N_3O_4$	60.61	3.73	14.14	43	178—181
	297.27	60.53	3.68	13.87		
IIIj	$C_{11}H_{13}N_3O$	65.01	6.45	20.67	90	163—166
	203.24	64.85	6.36	20.42		
IIIk	$C_{11}H_{13}N_3O_2$	60.26	5.98	19.70	41	221—225
	219.24	59.97	5.73	19.50		
IIIl	$C_{12}H_{13}N_3O_3$	58.29	5.30	16.99	52	193—196
	247.25	58.03	5.21	16.77		
IIIm	$C_{14}H_{15}N_3O_5$	55.08	4.95	13.76	49	215—219
	305.29	54.89	4.86	13.53		

The comparison of the quinazolinones structure and their cytotoxic effect showed that the most active derivatives were substituted in the pyrimidine ring of quinazolinone skeleton by methyl, bromomethyl, and dimethylaminomethyl groups. Substitution of bromine by ethoxy, isopropoxy or nitrophenoxy group caused a considerable decrease of activity.

The aforementioned results show (Table 4) that derivatives *I*, *II*, *IIIi*, and *IIIj* can be included among the potential anticancer drugs.

EXPERIMENTAL

IR spectra were recorded on a Philips PU 9800 FTIR instrument using the KBr technique. ^{1}H NMR spectra were taken on a Tesla BS 587A spectrometer (80 MHz) and ^{13}C NMR spectra on a Varian VXR-300 spectrometer in hexadeuterodimethyl sulfoxide using tetramethylsilane as internal standard.

The starting compounds were prepared according to the literature: 2-methyl-3*H*-quinazolin-4-one [4] and 2-bromomethyl-3*H*-quinazolin-4-one [5].

The antibacterial activity of prepared quinazoline derivatives was evaluated using the G$^+$ bacteria

Table 2. IR Spectral Data of Compounds *I*—*III*

Compound	$\tilde{\nu}$/cm^{-1} ν(C=O)	ν(C=N)	ν(NH)
I	1686	1617	2980
II	1682	1608	3021
IIIa	1674	1607	3088
IIIb	1678	1611	2938
IIIc	1667	1607	3000
IIId	1707	1603	3023
IIIe	1705	1597	3027
IIIf	1713	1603	3021
IIIg	1716	1601	3040
IIIh	1726	1593	2979
		1514*	1338**
IIIi	1684	1606	3424
		1475*	1344**
IIIj	1677	1610	3337
IIIk	1672	1612	3043
			3427***
IIIl	1709	1599	3024
	1662		
IIIm	1682	1608	3017
	1647		

*ν_{as}(NO$_2$), **ν_s(NO$_2$), ***ν(OH).

Table 3. ^{1}H NMR Data of Compounds *I—III*

Compound	δ_i
I	2.30 (s, 3H, CH_3), 7.28—8.09 (m, 4H, H_{arom}), 11.95 (bs, 1H, NH)
II	4.35 (s, 2H, CH_2), 7.39—8.11 (m, 4H, H_{arom}), 12.44 (bs, 1H, NH)
IIIa	3.39 (s, 2H, CH_2), 2.95—3.55 (m, 8H, Mo), 7.33—8.11 (m, 4H, H_{arom}), 11.63 (bs, 1H, NH)
IIIb	1.39—2.40 (m, 10H, Pi), 3.34 (s, 2H, CH_2), 7.34—8.10 (m, 4H, H_{arom})
IIIc	3.28 (s, 3H, CH_3), 4.13 (s, 2H, CH_2), 7.02—7.97 (m, 4H, H_{arom})
IIId	1.15 (t, 3H, CH_3), 3.59 (q, 2H, OCH_2), 4.43 (s, 2H, CH_2O), 7.46—8.15 (m, 4H, H_{arom})
IIIe	1.10 (d, 3H, CH_3); 1.17 (d, 3H, CH_3), 2.67 (m, 1H, CH), 4.52 (s, 2H, CH_2), 7.52—8.16 (m, 4H, H_{arom})
IIIf	1.16 (t, 3H, CH_3), 1.27—1.60 (m, 4H, 2 × CH_2), 3.52 (t, 2H, OCH_2), 4.30 (s, 2H, CH_2O), 7.48—8.14 (m, 4H, H_{arom})
IIIg	5.14 (s, 2H, CH_2), 7.12—8.32 (m, 9H, H_{arom})
IIIh	5.32 (s, 2H, CH_2), 6.88—8.23 (m, 8H, H_{arom})
IIIi	5.27 (s, 2H, CH_2), 7.11—8.10 (m, 8H, H_{arom})
IIIj	2.46; 2.50 (2s, 6H, 2 × CH_3), 3.69 (s, 2H, CH_2), 7.32—8.12 (m, 4H, H_{arom})
IIIk	2.55 (t, 2H, NCH_2), 3.30 (t, 2H, CH_2O), 3.62 (s, 2H, CH_2N), 7.29—8.07 (m, 4H, H_{arom})
IIIl	2.30 (s, 2H, NCH_2), 3.68 (s, 2H, CH_2N), 3.75 (s, 3H, OCH_3), 7.30—8.02 (m, 4H, H_{arom}), 9.09 (bs, 1H, NH)
IIIm	2.07 (m, 2H, CH—$\underline{CH_2}$), 2.25 (m, 2H, CH_2—CO), 3.92 (s, 2H, CH_2N), 4.34 (t, 1H, CH—CO),7.51—8.11 (m, 4H, H_{aro}

Table 4. Biological Activity of the Compounds *I—III*^{b}

Compound	$\rho/(\mu g\ cm^{-3})$									
	S. aureus		*B. subtilis*		*P. aeruginosa*		*E. coli*		HeLa	
	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC
I	>500	>500	>500	>500	>500	>500	>500	>500	< 0.125	0.125
II	15.38	100^c	28.6	100^c	170.0	>500	24.22	100^c	< 0.125	0.125
IIIa	>500	>500	>500	>500	>500	>500	>500	>500	38.74	> 100
IIIb	>500	>500	>500	>500	>500	>500	>500	>500	13.77	> 100
IIIc	>500	>500	>500	>500	>500	>500	>500	>500	61.23	87.60
IIId	>500	>500	>500	>500	>500	>500	>500	>500	> 100	≫ 100
IIIe	>500	>500	>500	>500	>500	>500	>500	>500	> 100	≫ 100
IIIf	>500	>500	>500	>500	>500	>500	>500	>500	75.69	> 100
IIIg	>500	>500	>500	>500	>500	>500	>500	>500	8.31	18.90
IIIh	>500	>500	>500	>500	>500	>500	>500	>500	100	≫ 100
IIIi	445.7	>500	>500	>500	>500	>500	>500	>500	1.62	59.80
IIIj	>500	>500	>500	>500	>500	>500	>500	>500	0.068	12.50
IIIk	>500	>500	>500	>500	>500	>500	>500	>500	4.24	25.00
IIIl	>500	>500	>500	>500	>500	>500	>500	>500	52.99	> 100
IIIm	>500	>500	>500	>500	>500	>500	>500	> 500	69.72	> 100
AMP	0.015	0.04^a	0.7	10^a	500	>500	0.28	1^a	–	

a) Concentration inducing a bacteriocide effect (MBC), *b*) concentration inducing a bacteriostatic effect, AMP – ampicillin.

Staphylococcus aureus and *Bacillus subtilis*; G^- bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. Concentrations 500 μg dm^{-3}, 100 μg dm^{-3}, 10 μg dm^{-3}, 1 μg dm^{-3}, and 0.1 μg dm^{-3} of the tested compounds were used. Chromatographically pure derivatives were dissolved in dimethyl sulfoxide; its final content never exceeded 1.0 vol. % in either control or treated samples. The antibacterial efficacy of the compounds was assayed by a microdilution method in 96-well microtitration plates [6]. To compare the antibacterial activity, ampicillin at concentrations 500 μg dm^{-3}, 100 μg dm^{-3}, 10 μg dm^{-3}, 1 μg dm^{-3}, and 0.1 μg dm^{-3} was used as standard. The antibacterial effect was characterized by IC_{50} values, *i.e.* the minimal concentration of a substance which inhibits bacterial growth by 50 % relative to the control, and MIC values, *i.e.* the minimal concentration of a substance which completely inhibits the bacterial growth. MIC experiments on subcultures dishes were used to assess the minimum bactericidal concentration (MBC) values. Subcultures were prepared separately in Petri dishes containing Müller—Hinton agar and incubated at 37 °C for 48 h. The MBC value was taken as the lowest concentration which showed no visible growth of bacterial colonies in the subculture dishes [7].

The cytotoxic activity of the prepared derivatives was studied on the transformed tumour cell line HeLa. A three-day culture of HeLa cells was trypsinized and was used to prepare a suspension with density 3.5 × 10^4 cells cm^{-3} [8]. The experiments were carried out

in Leighton flasks into which 2 cm^3 of the suspension were pipetted. After 24 h of static culturing at 37 °C, the substances, previously dissolved in dimethyl sulfoxide, were gradually added in seven different concentrations (in the range of 0.52—489.7 μmol dm^{-3}), 0.020 cm^3 of each per culturing flask. First, the effect of the substances on cell morphology was microscopically evaluated after 48 h of incubation at 37 °C [9]. Then, the intensity of growth of the cells was evaluated by the Lowry method stating the content of total cell protein [10]. The cytotoxic activity of the derivatives was stated from inhibitory concentrations IC_{50} which were read out from the toxicity curves.

Preparation of 2-X-Methyl-3*H*-quinazolin-4-ones

a) Reactions of *II* with Secondary Amines and Amino Alcohols Yielding *IIIa, IIIb, IIIj, IIIk*

A mixture of 2-bromomethyl-3*H*-quinazolin-4-one (1 g; 0.004 mol) and *N*-nucleophile (10—15 cm^3) was stirred at room temperature for 24 h, then poured into water (20 cm^3) and extracted with ether. The ethereal solution was dried and evaporated. The residue was crystallized from ethanol.

b) Reactions of *II* with the Amino Acids Yielding *IIIl, IIIm*

A mixture of 2-bromomethyl-3*H*-quinazolin-4-one (0.5 g; 0.002 mol), corresponding amino acid (0.0035 mol), and absolute DMF (30 cm^3) was refluxed for 6 h, cooled and poured into ice-water. After standing for 12 h, water was removed *in vacuo* and the residue was crystallized from acetone or ether.

c) Reactions of *II* with Alcohols Yielding *IIIc—IIIf*

To a solution of sodium alkoxide prepared from Na (0.12 g) and the corresponding absolute alcohol (25 cm^3), 2-bromomethyl-3*H*-quinazolin-4-one (1.2 g; 0.0048 mol) was added and the reaction mixture was heated over a steam bath for 1 h, cooled, shaken with 10 % HCl, and filtered. The residue was washed with H_2O and crystallized from DMF—H_2O.

d) Reactions of *II* with Phenolates Yielding *IIIg—IIIi*

A mixture of *II* (1.2 g; 0.0048 mol) and freshly prepared sodium phenolate (0.15 mol) in 20 cm^3 of absolute ethanol was heated over the steam bath for 1 h. The next procedures were the same as in the previous case with alcohols.

^{13}C NMR Data of Compounds *II, IIId, IIIj,* and *IIIk*

2-Bromomethyl-3*H*-quinazolin-4-one (*II*)

^{13}C NMR spectrum, δ: 33.49 (CH_2), 121.89, 126.06, 127.37, 127.53, 134.50, 145.51 (benzene ring), 158.69 (C=N), 164.73 (C=O).

2-Ethoxymethyl-3*H*-quinazolin-4-one (*IIId*)

^{13}C NMR spectrum, δ: 14.65 (CH_3), 66.49 ($\underline{CH_2}$—CH_3), 67.70 (CH_2—O), 120.60, 123.54, 126.15, 127.58, 135.08, 143.24 (benzene ring), 156.79 (C=N), 160.22 (C=O).

2-(*N,N*-Dimethylamino)methyl-3*H*-quinazolin-4-one (*IIIj*)

^{13}C NMR spectrum, δ: 46.88 (2 × CH_3), 56.40 (CH_2), 120.11, 125.55, 126.29, 126.89, 134.00, 144.50 (benzene ring), 159.01 (C=N), 164.19 (C=O).

2-[*N*-(2'-Hydroxyethyl)amino]methyl-3*H*-quinazolin-4-one (*IIIk*)

^{13}C NMR spectrum, δ: 49.43 (NH—CH_2—CH_2), 51.60 ((HN)N=C—CH_2—NH), 61.55 (CH_2—OH), 120.20, 125.75, 126.19, 126.82, 134.03, 144.38 (benzene ring), 156.39 (C=N), 164.20 (C=O).

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