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Heterocondensed Quinazolones: Synthesis and Protein-Tyrosine Kinase Inhibitory Activity of 3,4-Dihydro-1*H*,6*H*-[1,4]oxazino-[3,4-*b*]quinazolin-6-one Derivatives

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Abstract—1-Benzylidene (**2–14**) and 1-phenylhydrazono derivatives (**15–29**) of 3,4-dihydro-1*H*,6*H*-[1,4]oxazino[3,4-*b*]quinazolin-6-one (**1**) were obtained from the condensation reactions of **1** with a series of aromatic aldehydes and by direct diazonium coupling with aryl-diazonium chlorides. The substances were tested for their ability to inhibit the tyrosine kinase activity of SW-620 (human colon carcinoma) cells. Compounds **8**, **10**, **12**, and **13** showed remarkable inhibitory activity. Copyright © 1996 Elsevier Science Ltd

Introduction

Throughout the last decade, tyrosine kinase inhibition has represented a new concept in antitumor drug research, and many inhibitors of tyrosine kinases have been discovered both as natural products and as the result of rational synthetic chemistry.[1] Arylidene heterocyclics have been reported to inhibit tyrosine phosphorylation in vitro and angiogenesis in vivo.[2] 4-Anilino-quinazolines were referred to as specific inhibitors of epidermal growth factor (EGF) receptor tyrosine kinase in vitro.[3] The most active compound, 4-(3′-bromo-)-anilino-6,7-dimethoxy-quinazoline, has an inhibition constant of 5 pM.[4]

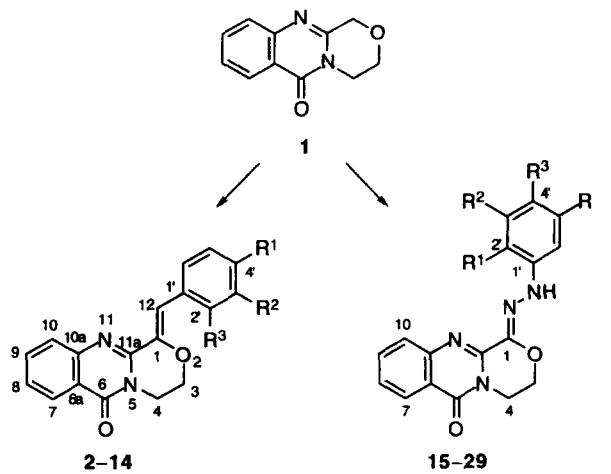
Many protein tyrosine kinase (PTK) inhibitors have been designed as tyrosine analogues, such as the tyrphostins,[5] cinnamamides,[6] styrene derivatives,[7] or phenylhydrazones.[8] Numerous synthetic analogues were systematically developed from naturally occurring inhibitors.[9]

3,4-Dihydro-1*H*,6*H*-[1,4]oxazino[3,4-*b*]quinazolin-6-one (**1**) is known to be the partial structure of some toxic fungus metabolites,[10] and can be variously substituted at position-1, taking into consideration the above mentioned facts. In the present paper, we report the synthesis and biological test of 1-benzylidene- and 1-phenylhydrazono derivatives of **1** (Scheme 1).

Key words: Synthesis, 1-benzylidene-3,4-dihydro-1*H*,6*H*-[1,4]oxazino-[3,4-*b*]quinazolin-6-ones, 1-phenylhydrazono-3,4-dihydro-1*H*,6*H*-[1,4]-oxazino-[3,4-*b*]quinazolin-6-ones, protein kinase inhibitory activity, SW-620 human colon carcinoma cells.

Synthesis

Dihydro-1*H*,6*H*-[1,4]oxazino[3,4-*b*]quinazolin-6-one (**1**) was prepared from 2-chloro-methyl-3,1-benzoxazin-4-one by condensation with ethanolamine using our original method.[10] Compound **1**, having considerable active methylene reactivity,[10] readily condenses with aromatic aldehydes and takes part in diazonium-



Scheme 1.

Table 1. Physical and analytical data of 1-benzylidene-3,4-dihydro-1*H*,6*H*-[1,4]oxazino[3,4-*b*]quinazolin-6-ones (**2–14**)

Compd	Substituents			Mp (°C)	Yield (%)	Molecular formula	Analyses calcd/found (%)			
	R^1	R^2	R^3				C	H	N	Hlg
							74.47	4.86	9.65	
2	H	H	H	157–158	86	$C_{18}H_{14}N_2O_2$	74.16	4.93	9.80	
							66.57	4.03	8.63	10.92
3	H	H	Cl	182–184	46	$C_{18}H_{13}ClN_2O_2$	66.46	3.98	8.58	10.73
							71.24	5.03	8.74	
4	H	H	OMe	161–165	37	$C_{19}H_{16}N_2O_3$	71.11	4.93	8.67	
							70.58	4.61	9.15	
5	H	H	OH	187–192	80	$C_{18}H_{14}N_2O_3$	70.16	4.70	9.27	
							70.58	4.61	9.15	
6	H	OH	H	208–214	34	$C_{18}H_{14}N_2O_3$	70.63	4.65	9.09	
							66.57	4.03	8.63	10.92
7	Cl	H	H	171–174	48	$C_{18}H_{13}ClN_2O_2$	66.41	4.06	8.59	10.98
							70.58	4.61	9.15	
8	OH	H	H	247–249	74	$C_{18}H_{14}N_2O_3$	70.44	4.47	9.09	
							71.24	5.03	8.74	
9	OMe	H	H	157–159	63	$C_{19}H_{16}N_2O_3$	71.30	5.08	8.71	
							72.05	5.74	12.60	
10	$N(Me)_2$	H	H	156–159	56	$C_{20}H_{19}N_3O_2$	72.15	5.80	12.68	
							64.48	3.91	12.53	
11	NO_2	H	H	257–260	77	$C_{18}H_{13}N_3O_4$	64.12	3.82	12.60	
							67.08	4.38	8.69	
12	OH	OH	H	210–213	88	$C_{18}H_{14}N_2O_4$	67.10	4.41	8.63	
							67.68	4.79	8.33	
13	OH	OMe	H	131–135	82	$C_{19}H_{16}N_2O_4$	67.70	4.66	8.29	
							60.19	3.37	7.80	19.74
14	Cl	Cl	H	195–199	78	$C_{18}H_{12}Cl_2N_2O_3$	60.23	3.42	7.73	19.74

Table 2. Physical and analytical data of 1-phenylhydrazono-3,4-dihydro-1*H*,6*H*-[1,4]oxazino[3,4-*b*]quinazolin-6-ones (**15–19**)

Compd	Substituents				Mp (°C)	Yield (%)	Molecular formula	Analyses calcd/found (%)			
	R^1	R^2	R^3	R^4				C	H	N	Hlg
								66.66	4.61	18.29	
15	H	H	H	H	212–214	69	$C_{17}H_{14}N_4O_2$	66.47	4.47	18.54	
								62.96	4.04	17.28	
16	F	H	H	H	208–212	38	$C_{17}H_{13}FN_4O_2$	62.83	3.99	17.23	
								59.92	3.85	16.44	10.40
17	Cl	H	H	H	192–195	61	$C_{17}H_{13}ClN_4O_2$	59.86	3.79	16.29	10.31
								53.00	3.40	14.54	20.74
18	Br	H	H	H	183–185	38	$C_{17}H_{13}BrN_4O_2$	53.03	3.38	14.58	20.69
								47.24	3.03	12.96	29.36
19	I	H	H	H	189–192	46	$C_{17}H_{13}IN_4O_2$	47.08	2.98	12.87	29.06
								64.28	4.79	16.66	
20	OMe	H	H	H	169–173	27	$C_{18}H_{16}N_4O_3$	64.23	4.72	16.53	
								47.24	3.03	12.96	29.36
21	H	I	H	H	134–137	19	$C_{17}H_{13}IN_4O_2$	47.18	2.96	12.88	29.29
								53.00	3.40	14.54	20.74
22	H	H	Br	H	156–160	53	$C_{17}H_{13}BrN_4O_2$	52.78	3.37	14.48	20.63
								61.71	4.03	15.99	
23	H	H	COOH	H	217–221	43	$C_{18}H_{14}N_4O_4$	61.78	3.97	15.90	
								63.49	4.79	14.81	
24	H	H	COOEt	H	225–228	38	$C_{20}H_{18}N_4O_4$	63.38	4.71	14.73	
								65.25	3.95	21.14	
25	H	H	CN	H	305–314	61	$C_{18}H_{13}N_5O_2$	65.28	3.90	21.10	
								57.76	3.50	14.97	
26	H	H	CF_3	H	208–212	31	$C_{18}H_{13}F_3N_4O_2$	57.81	3.43	15.05	
								65.51	4.63	16.08	
27	H	H	$COCH_3$	H	210–212	23	$C_{19}H_{16}N_4O_3$	65.54	4.60	16.03	
								59.71	4.30	13.26	
28	H	COOMe	H	COOMe	250–253	14	$C_{21}H_{18}N_4O_6$	59.80	4.24	13.10	
								60.94	4.26	15.79	9.99
29	Me	H	H	Cl	199–203	24	$C_{18}H_{15}ClN_4O_2$	60.79	4.37	15.63	9.87

coupling reactions. Condensations with aldehydes were performed practically by fusion of **1** with 20% excess of the corresponding aldehyde without catalysator under continuous nitrogen stream, to avoid oxidation side reactions. The melting point of **1** (~160 °C) was found to be the optimal temperature for the reaction.

Diazonium-coupling reactions with the corresponding diazonium chlorides[11] were carried out in ice-cooled acetic acid solutions of **1** in the presence of a two molar excess of sodium acetate. The synthetic and analytical data of the synthetized compounds are listed in Tables 1 and 2.

Compounds have characteristic UV spectra with an absorption maximum in the visible region (Table 3). The ^{1}H NMR data of selected compounds showed that only the *E* isomers are present in DMSO solutions (Table 4).

Biological Testing

The tyrosine kinase inhibitory activities of the synthesized compounds (Tables 5 and 6) were tested using a cell homogenate of SW 620 human colon carcinoma cell line as enzyme preparation and a well known synthetic peptide substrate originating from the autophosphorylation site of *src* oncoprotein pp60*src*, named E11G1.[12] Four 1-benzylidene-oxazino-quinazolone derivatives had significant tyrosine kinase inhibitory activity, while none of the 1-phenylhydrazono-oxazino-quinazolones had any activity. The 4′-hydroxy- (**8**), 3′,4′-dihydroxy- (**12**), 4′-hydroxy-3′-methoxy- (**13**), and 4′-dimethylamino (**10**) substituents on 1-benzylidene-oxazino-quinazolone (**2**) gave structures with relatively good PTK inhibitory activity (IC_{50} = 70–100 μM) and these structures may serve as the basis for further developments. The above molecules are all new molecules, and while some substituted quinazoline structures were found to be very good tyrosine kinase inhibitors,[4] it is noticeable that benzylidene derivatives show activity, but hydrazono derivatives are inactive.

Experimental

Melting points are uncorrected. The UV spectra were recorded on a Zeiss M42 spectrophotometer. All NMR measurements were taken at rt (298 K) using DMSO-d_6 as the solvent and TMS as the internal reference. Assignments are based on 1-D proton and carbon spectra and DEPT and series of selective INEPT spectra as well as one bond proton–carbon shift correlations were established using 2-D shift correlated spectra. 2- or 3-bond range shift correlations were determined by selective INEPT measurements. All spectra were measured with a Bruker AM250 spectrometer. 16 K datapoints were used in the 1-D measurements. The standard Bruker DEPT.AU and

Table 3. UV spectroscopic data of 1-benzylidene-3,4-dihydro-1*H*,6*H*-[1,4]oxazino-[3,4-*b*]quinazolin-6-ones (**2–14**) and 1-phenylhydrazono-3,4-dihydro-1*H*,6*H*-[1,4]oxazino[3,4-*b*]quinazolin-6-ones (**15–29**), in 96% ethanol

Compd	λ	(ε)								
2	356i	(24,700)	346	(25,600)	254i	(7980)	228	(16,500)		
3	346	(25,460)	218	(26,110)						
4	362	(21,280)	228	(24,190)						
5	368	(10,090)	268	(9170)	226	(27,310)				
6	358	(22,710)	224	(24,290)						
7	348	(28,770)	256	(11,290)	228	(22,050)	214	(22,050)		
8	368	(18,840)	214i	(15,220)						
9	366	(22,010)	214	(11,210)						
10	348	(24,720)	228	(19,370)	250	(10,190)	214	(26,610)		
11	378	(18,260)	300	(7720)	218	(17,340)				
12	376	(25,550)	263	(5160)	224	(21,890)				
13	374	(28,620)	268	(23,230)	275i	(19,000)	226	(54,720)		
14	348	(19,100)	228	(12,590)						
15	390	(19,700)	301i	(6960)	294	(7430)	246i	(15,080)	228	(20,700)
16	376	(16,780)	292	(5940)	230	(16,880)				
17	374	(12,240)	296	(4820)	230	(11,200)				
18	374	(10,110)	298	(4920)	228	(13,320)				
19	378	(23,700)	304	(8880)	218	(30,260)				
20	390	(10,890)	288	(5770)	226	(29,780)				
21	386	(16,800)	304	(5400)	254	(11,000)	220	(22,470)		
22	392	(24,480)	306	(18,490)	228	(37,170)				
23	392	(12,800)	286	(6470)	226	(8040)				
24	392	(16,010)	288	(8320)	228	(9770)				
25	388	(9830)	280	(5850)	226	(7470)				
26	380	(30,100)	294	(8690)	258	(15,420)	224	(19,840)		
27	398	(49,620)	300	(19,880)	228	(35,380)				
28	384	(5640)	360	(5330)	227	(9090)				
29	380	(6960)	294	(2370)	248	(3480)				

Table 4. ^{13}C and (^1H) chemical shifts of compounds **3**, **6**, **9**, **12**, **17**, **18**, **21**, **23**, and **25**

Compd	1	3	4	6	6a	7	8	9	10	10a	11a	12	1′	2′	3′	4′	5′	6′	R
		63.9	40.3			126.7	134.3	126.4	127.7			108.0			129.3	128.4	126.3	130.7	
3	144.4	(4.41)	(4.33)	160.6	119.8	(8.25)	(7.70)	(7.40)	(7.72)	147.2	145.2	(7.26)	134.0	132.2	(7.70)	(7.20)	(7.20)	(8.16)	(2′)Cl
		63.6	40.3			125.8	134.5	126.2	127.2			112.1		131.6	115.6		115.6	131.6	
6	141.7	(4.43)	(4.21)	160.1	119.6	(8.12)	(7.70)	(7.47)	(7.70)	147.4	146.3	(7.17)	132.2	(7.66)	(6.82)	157.5	(6.82)	(7.66)	(4′)OH
		63.4	40.5			126.4	134.2	126.1	127.2			112.8		131.4	113.6		113.6	131.4	(4′)MeO
9	141.7	(4.35)	(4.27)	160.7	119.5	(8.20)	(7.67)	(7.38)	(7.67)	147.4	145.8	(7.26)	119.5	(7.75)	(6.88)	159.1	(6.88)	(7.75)	55 (3.81)
		63.6	40.5			126.2	134.6	127.2	128.9			112.5		117.0			115.8	122.6	(3′,4′)OH
12	141.6	(4.43)	(4.23)	160.2	119.5	(8.12)	(7.66)	(7.37)	(7.80)	147.4	146.4	(7.39)	129.0	(7.10)	146.0	145.1	(7.10)	(6.79)	
		64.6	40.1			126.4	134.5	127.7	128.1						127.2	126.4	120.8	114.6	
17	136.8	(4.57)	(4.40)	160.6	120.1	(8.18)	(7.82)	(7.42)	(7.80)	147.2	141.2	—	139.2	117.8	(7.25)	(6.82)	(7.80)	(7.25)	(2′)Cl
		65.1	40.3			126.4	135.2	127.6	129.0						132.5	126.7	121.8	114.7	
18	137.5	(4.62)	(4.43)	159.3	120.1	(8.28)	(7.86)	(7.42)	(7.80)	145.7	142.4	—	140.5	107.4	(7.51)	(6.78)	(7.30)	(7.42)	(2′)Br
		64.3	40.2			126.4	135.3	127.6	128.7					121.5		129.2	127.5	113.1	
21	135.0	(4.62)	(4.36)	159.2	119.5	(8.18)	(7.90)	(7.58)	(7.90)	145.7	143.1	—	144.6	(7.75)	95.4	(7.38)	(7.05)	(7.18)	(3′)I
		64.5	39.3			126.2	134.7	127.1	127.8					112.3	130.8		130.8	112.3	(4′)COOH
23	137.4	(4.63)	(4.32)	159.8	120.3	(8.15)	(7.80)	(7.53)	(7.85)	147.4	142.3	—	148.8	(7.34)	(7.84)	120.3	(7.84)	(7.34)	165.8
		64.7	39.6			126.3	134.8	127.3	127.9					113.2	133.6		133.6	113.2	(4′)CN
25	137.9	(4.65)	(4.32)	159.8	120.1	(8.16)	(7.85)	(7.55)	(7.80)	147.4	142.2	—	148.1	(7.38)	(7.65)	100.2	(7.65)	(7.38)	120.4

Table 5. PTK inhibitory activity of compounds **2–14**[a]

Compd	Inhibition (%)	Compd	Inhibition (%)
2	<10	**9**	<10
3	<10	**10**	83
4	<10	**11**	<10
5	<10	**12**	100
6	12	**13**	87
7	<10	**14**	<10
8	100		

[a]Conditions: temp, 30 °C; concd, 100 μM; solvent, DMSO.

XHCORR.AU microprograms were used for the DEPT and the heteronuclear (proton–carbon) shift correlated measurements, respectively. Selective INEPT spectra were taken using 50 Hz selective proton pulses via the decoupler channel.

1-Benzylidene-3,4-dihydro-1*H*,6*H*-[1,4]oxazino[3,4-*b*]-quinazolin-6-ones (2–4). 1.01 g (0.005 mol) of 3,4-dihydro-1*H*,6*H*-[1,4]oxazino[3,4-*b*]quinazolin-6-one (**1**) and 0.006 mol of the appropriate benzaldehyde were fused on an oil bath at 160 °C under continuous stirring. After removing the water formed in the reaction, the mixture was dissolved in ethanol and clarified by charcoal. The solvent was evapd in vacuo and the crystalline product was washed with ethanol and dried. The resulted compounds **2–14** are listed in Table 1.

1-Phenylhydrazono-3,4-dihydro-1*H*,6*H*-[1,4]oxazino[3,4-*b*]quinazolin-6-ones (15–29). To an ice-cooled solution of the appropriate phenyldiazonium chloride, prepared from the corresponding aniline (0.005 mol) in 1:1 diluted aqueous hydrochloric acid (5 mL) a solution of sodium acetate (8.2 g) in acetic acid (15 mL), then 3,4-dihydro-1*H*,6*H*-[1,4]oxazino[3,4-*b*]quinazolin-6-one (**1**) (1.01 g, 0.005 mol) in acetic acid (5 mL), was gradually added at 0 °C and the reaction mixture was kept at this temperature for 3 h. Then the reaction mixture was allowed to stand overnight at rt. The reaction mixture was diluted with water and the precipitated crystals filtered off, washed with water,

Table 6. PTK inhibitory activity of compounds **15–29**[a]

Compd	Inhibition (%)	Compd	Inhibition (%)
15	<10	**23**	<10
16	<10	**24**	<10
17	13	**25**	<10
18	<10	**26**	<10
19	<10	**27**	<10
20	<10	**28**	<10
21	<10	**29**	<10
22	<10		

[a]Conditions: temp, 30 °C; concd, 100 μM; solvent, DMSO.

and recrystallized from ethanol to give compounds **15–29** (Table 2).

Tyrosine kinase assay. SW 620 human colon tumor cells were homogenized for 1 min by a Teflon homogenizer in 5 vol of buffer (50 mM Tris–HCl, pH 7.8, 50 mM $MgCl_2$, 10 μM $NaVO_3$, 1 mM EDTA, 50 μg/mL Aprotinin). After removing the medium, the tumor cells were centrifuged (1000*g*, 10 min) and then resuspended in 5 vol of the same buffer. The cells were homogenized 30 times by a Dounce homogenizer.

Tyrosine kinase activity was measured as previously described.[13] Compounds tested for tyrosine kinase inhibition were dissolved in DMSO (100 μM). The reaction volume of 250 μL contained 50 mM Tris–HCl, pH 7.8, 50 mM $MgCl_2$, 10 μM $NaVO_3$, 0.1% Nonidet P-40, 20 μm ATP, 2–5 μCi γ-[^{32}P]ATP (Izinta, Budapest, Hungary), 1 mM of synthetic peptide substrate ($E_{11}G_1$: Glu-Asp-Ala-Glu-Tyr-Ala-Ala-Arg-Arg-Arg-Gly), and 60 μL of cell homogenate (2 15 μg protein content). The assay was initiated by the addition of γ-[^{32}P]ATP. After incubation for 10 min at 30 °C, the reaction was stopped by the addition of 150 μL 10% trichloroacetic acid and subsequently, 10 μL of bovine serum albumin (20 mg/mL) was added. The precipitated protein was removed by centrifugation (3200*g*, 25 min) and two 50 μL aliquots of the supernatant were spotted on phosphocellulose paper (Whatman P81). The phosphocellulose papersquares were washed 6 times in 0.5% phosphoric acid and once in acetone. The dried papers were counted for radioactivity in 5 mL of scintilation fluid. For each sample, an appropriate reaction mixture containing no peptide was run as a control. The results are expressed in pmol ^{32}P/mg protein/min.

The biological potency of the analogues was characterized by their ability to inhibit the tyrosine kinase activity of the tumor cells, measured by the incorporation of ^{32}P into the specific peptide substrate.

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