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AN EFFICIENT AND EXPEDITIOUS SYNTHESIS OF A NOVEL 5*H*-NAPHTH[1',2':5,6][1,4]OXAZINO[2,3-*b*]QUINOXALIN-5-ONE AND ITS UNIQUE INHIBITORY ACTIVITY AGAINST A PANEL OF HUMAN CANCER CELL LINES‡

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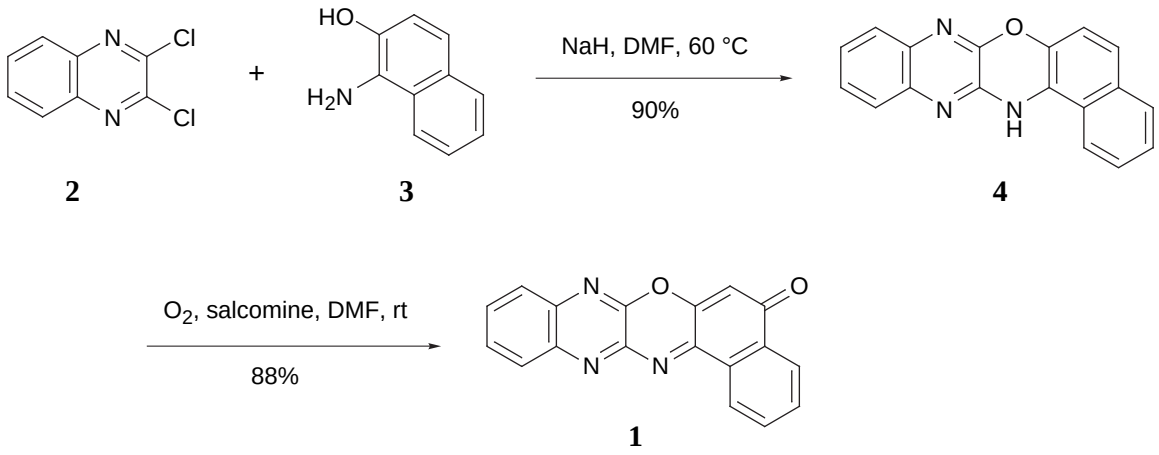
Abstract - The title compound (**1**) was efficiently synthesized in two steps starting from 2,3-dichloroquinoxaline (**2**) and 1-amino-2-naphthol (**3**); the method involves cyclization of **2** with **3** and subsequent salcomine oxidation. The compound (**1**) showed unique inhibitory activity against a panel of human cancer cell lines.

Quinoxaline derivatives are known to possess interesting biological properties which show antibacterial, fungicidal, insecticidal, anthelmic, and cytotoxic activities.[1] Of particular note is the recent report that some quinoxaline derivatives inhibit selectively the platelet-derived growth factor (PDGF) receptor kinase and PDGF-dependent DNA synthesis in cells.[2] On the other hand, a quinonimine system is quite of interest because this system constitutes the structure of several antitumor antibiotics such as actionmycin D,[3] questiomycin A,[4] and glucosylquestiomycin;[5] this quinonimine system is recognized to play an important role in promoting pronounced antitumor activity. As part of our work in a search for new antitumor substances possessing a novel mode of action, we became very much interested in the previously unknown quinoxaline-fused quinonimine ring system because this type of hybrid compound was expected to display a novel characteristic profile as an antitumor agent. In this paper, we describe the efficient and expeditious synthesis of a novel 5*H*-naphth[1',2':5,6][1,4]oxazino[2,3-*b*]quinoxalin-5-one (**1**) and its unique inhibitory activity against a panel of human cancer cell lines.

‡ This paper is dedicated to Professor Shô Ito of Bunri University of Tokushima on the occasion of his 77th birthday.

As shown in Scheme 1, the synthesis commenced with cyclization of commercially available 2,3-dichloroquinoxaline (**2**) and 1-amino-2-naphthol (**3**), respectively. The base-promoted cyclization of **2** with 2-aminophenol leading to the quinoxalinobenzoxazine ring system has been reported [3.0 M KOH (2.3 equiv), *N*,*N*-dimethylformamide (DMF), reflux, 3 h, 90%];[6] therefore, we initially applied the same reaction conditions to our substrates (**2**) and (**3**). However, the reaction was not clean, and the isolated yield of the desired naphthoxazinoquinoxaline (**4**) was very low (~20%). After several experiments, the cyclization was found to proceed effectively by treating a mixture of **2** and **3** with sodium hydride (3.5 equiv) in DMF at 60°C for 30 min, leading to the formation of **4**[7] in 90% yield. The subsequent crucial formation of the quinonimine system[8] was best achieved by reaction of **4** with oxygen in the presence of salcomine[9] [*N*,*N*'-bis(salicylidene)ethylenediiminocobalt(II)] (0.2 equiv) in DMF at room temperature, providing the targeted compound (**1**)[10] in 88% yield. The explored synthetic method seems to be applicable to a large scale preparation of **1** due to operational simplicity and excellent overall yield.

Scheme 1. Synthesis of 5*H*-naphth[1',2':5,6][1,4]oxazino[2,3-*b*]quinoxalin-5-one (**1**)



2 **3** **4** **1**

With compound (**1**) in hand, its cytotoxic activities were assessed by the use of a disease-oriented panel of 39 human cancer cell lines (HCC panel) in the Japanese Foundation for Cancer Research.[11] The number of cell lines and their origin (organs) are as follows: 5 breast, 6 central nervous system, 5 colon, 7 lung, 1 melanoma, 5 ovary, 2 kidney, 6 stomach, and 2 prostate cancers. Dose-response curves were measured at five different concentrations (10^{-4}–10^{-8} M, one log intervals) of **1**, and the concentration causing 50% cell growth inhibition compared with the control (GI_{50}) was calculated. The results are presented in Table 1 as log GI_{50} values. These results clearly disclosed that **1** exhibited appreciable cytotoxic activities against the 39 cell lines in which the DMS273 (lung cancer) cell was especially susceptible to inhibition by **1** (logGI_{50}=-6.76). The delta value [the difference in log GI_{50} value of the most sensitive cell and MG-MID value (mean value of log GI_{50} over all cell lines tested)] and the range value (the difference in log GI_{50} values of the most sensitive cell and the least sensitive cell) of **1** were

Table 1. *in vitro* cytotoxic activity of compound (**1**) against a panel of 39 human cancer cell lines.

Origin of cancer	Cell line	Log GI_{50} (M)[a]	Origin of cancer	Cell line	Log GI_{50} (M)[a]
Breast	HBC-4	-4.64	Lung	DMS114	-5.53
	BSY-1	-5.34			
	HBC-5	-4.59	Melanoma	LOX-IMVI	-5.07
	MCF-7	-4.84			
	MDA-MB-231	-5.52	Ovary	OVCAR-3	-5.52
				OVCAR-4	-5.65
Central nervous system	U-251	-4.79		OVCAR-5	-4.81
	SF-268	-5.15		OVCAR-8	-4.77
	SF-295	-4.72		SK-OV-3	-4.76
	SF-539	-4.91			
	SNB-75	-4.94	Kidney	RXF-631L	-4.70
	SNB-78	-5.28		ACHN	-4.80
Colon	HCC2998	-5.26	Stomach	St-4	-4.91
	KM-12	-4.84		MKN1	-5.38
	HT-29	-4.57[b]		MKN7	-5.51
	HCT-15	-5.21		MKN28	-5.29
	HCT-116	-5.42		MKN45	-5.06
				MKN74	-5.01
Lung	NCI-H23	-5.13			
	NCI-H226	-4.90	Prostate	DU-145	-4.63
	NCI-H522	-5.61		PC-3	-4.82
	NCI-H460	-4.80			
	A549	-4.61	MG-MID[d]		-5.08
	DMS273	-6.76[c]	Delta[e]		1.68
			Range[f]		2.18

a) Log concentration of compound (**1**) for inhibition of cell growth at 50% compared to control.
b) The least sensitive cell.
c) The most sensitive cell.
d) Mean value of log GI_{50} over all cell lines tested.
e) The difference in log GI_{50} value of the most sensitive cell and MG-MID value.
f) The difference in log GI_{50} value of the most sensitive cell and the least sensitive cell.

1.68 and 2.18, respectively (effective value: delta$\geqq$0.5 as well as range$\geqq$1.0), disclosing that this compound showed pronounced selective cytotoxic activity. Furthermore, evaluation of the pattern of differential cytotoxicity using the COMPARE Program[11] demonstrated that the mode of action for **1** was not correlated with that shown by any other anticancer drug developed to date. This suggests that compound (**1**) might be a useful lead compound for anticancer agents with a novel mode of action.

In summary, we have succeeded in synthesizing a novel 5*H*-naphth[1',2':5,6][1,4]oxazino[2,3-*b*]quinoxalin-5-one (**1**) *via* a two-step sequence of reactions which involves cyclization of 2,3-dichloroquinoxaline (**2**) with 1-amino-2-naphthol (**3**) and the subsequent quinonimine formation by salcomine oxidation. Since compound (**1**) proved to exhibit unique inhibitory activity against a panel of human

cancer cell lines, this compound appears to be a good starting point for development of novel types of anticancer drugs. Further studies including *in vivo* experiments, synthesis of analogues, and mode of action are in progress.

REFERENCES AND NOTES

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7. Compound (**4**): pale yellow powder; mp 279-281 °C; IR (KBr): 3196 (w), 3055 (w), 2926 (w), 2854 (w), 1628 (w), 1608 (m), 1587 (s), 1574 (m), 1543 (m), 1520 (w), 1481 (s), 1456 (s), 1406 (s), 1338 (w), 1304 (s), 1284 (w), 1269 (m), 1201 (m), 1143 (w), 1120 (w), 1087 (w), 1001 (w), 979 (w), 945 (w), 927 (w), 866 (w), 794 (m), 763 (s), 736 (m), 652 (w), 601 (m), 557 (w), 538 (m), 499 (w); ^{1}H-NMR (500 MHz, $CDCl_3$)δ: 7.05 (1H, s, NH), 7.20 (1H, d, J=8.6 Hz), 7.38 (1H, t, J=7.2 Hz), 7.42-7.49 (3H, m), 7.51 (1H, d, J=7.2 Hz), 7.57 (1H, t, J=7.2 Hz), 7.62 (1H, d, J=8.0 Hz), 7.70 (1H, d, J=8.6 Hz), 7.80 (1H, d, J=8.0 Hz); EIMS (m/z): 285 (M^+), 142, 129, 111, 97, 85, 83, 71, 69, 57, 55, 43, 41; Anal. Calcd for $C_{18}H_{11}N_3O$: C, 75.78; H, 3.89; N, 14.73. Found: C, 75.62; H, 3.98; N, 14.46.
8. The synthesis of quinonimine derivatives using a combination of iodosylbenzene (PhIO) and vanadyl acetylacetonate [$VO(acac)_2$] has been reported, see, R. Barret and M. Daudon, *Synth. Comm*., 1990, **20**. 1543.
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10. Compound (**1**): pale orange needles; mp 288-290 °C; IR (KBr): 3036 (w), 1641 (s), 1595 (m), 1568 (s), 1496 (w), 1464 (w), 1400 (w), 1379 (w), 1361 (w), 1332 (m), 1309 (s), 1277 (w), 1244 (s), 1213 (m), 1170 (m), 1141 (w), 1126 (w), 1111 (w), 1082 (w), 1005 (w), 902 (w), 817 (w), 785 (m), 765 (s), 711 (m), 671 (w), 625 (w), 607 (w), 520 (w), 495 (w); ^{1}H-NMR (500 MHz, $CDCl_3$)δ : 6.68 (1H, s), 7.79-7.90 (4H, m), 8.05 (1H, dd, J=1.0, 8.3 Hz), 8.26 (1H, dd, J=1.0, 8.3 Hz), 8.28-8.32 (1H, m), 8.88-8.92 (1H, m); EIMS (m/z): 299 (M^+), 284, 227, 163, 113, 101, 75; Anal. Calcd for $C_{18}H_9N_3O_2$: C, 72.24; H, 3.03; N, 14.04. Found: C, 72.05; H, 2.78; N, 13.96.
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