http://dx.doi.org/10.5012/bkcs.2012.33.2.647

Synthesis of 1,6-Disubstituted 4,5,6,7-Tetrahydropyrazolo[3,4-*c*]pyridin-7-one Derivatives and Evaluation of Their Anticancer Activity

Vani Nelamane Devegowda,[†,‡] Seon Hee Seo,[†] Ae Nim Pae,[†,‡] Ghilsoo Nam,[†,‡,*] and Kyung Il Choi[†,‡,*]

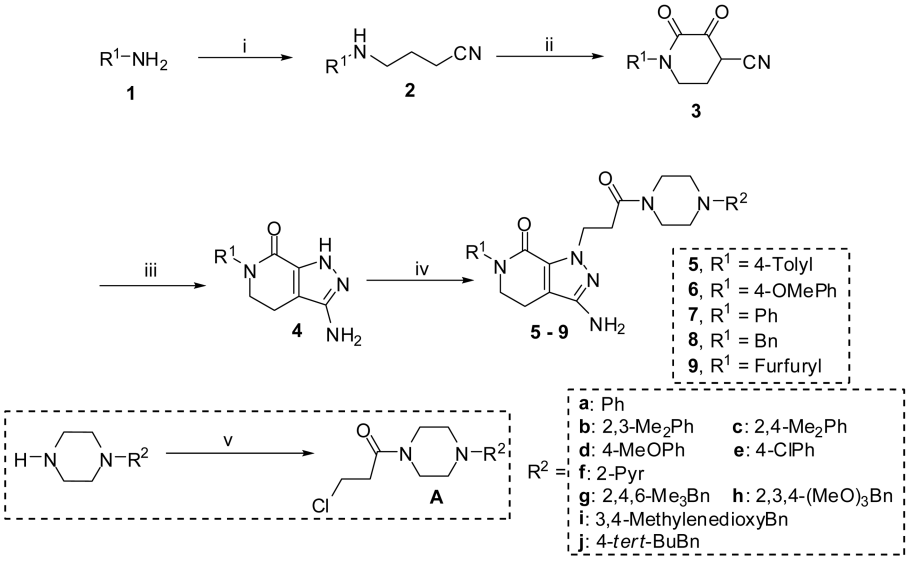
[†]*Life/Health Division, Korea Institute of Science and Technology, Seoul 136-791, Korea.* [*]*E-mail: kichoi@kist.re.kr*
[‡]*School of Science, University of Science and Technology, Daejeon 305-333, Korea.* [*]*E-mail: gsnam@kist.re.kr*
Received August 23, 2011, Accepted October 24, 2011

Promising anticancer compounds of the type 1,6-disubstituted 4,5,6,7-tetrahydropyrazolo[3,4-*c*]pyridin-7-ones were identified. The target compounds were readily synthesized in a large scale *via* a sequence of reactions starting from the commercially available primary amines. Their *in vitro* anti-proliferative activity has been evaluated on prostate (DU-145), colon (HT-29 and HCT-116) and melanoma (A375P) human cancer cell lines. The relationships between the structure and the anticancer activity, covering all tested cancer cell lines, revealed that the compound **5c** with 2,4-dimethylphenyl substituent at R^2 was the most potent with the IC_{50} values in the range as low as 0.16 to 0.40 μM.

Key Words : Tetrahydropyrazolopyridinone, 3-Aminopyrazole, Anti-proliferative activity, Anticancer drugs

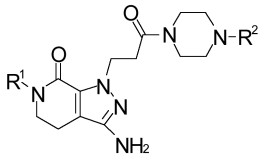
The fundamental research for the development of new drugs against cancer stands first among the priorities of life science research worldwide.[1] The systematic investigation in our research group towards the development of anticancer drugs resulted in designing new molecules based on the bicyclic tetrahydropyrazolopyridinone skeleton. The bicyclic tetrahydropyrazolopyridinones have many interesting pharmacological properties due to their analogy with purines. The core 4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridin-7-one has been reported as an inhibitor of blood coagulation factor Xa.[2]

Among the wide variety of synthetic compounds recognized as potential anticancer drugs, the molecules based on the 3-aminopyrazole moiety have attracted a great interest. The 3-aminopyrazole, a well known adenine mimetic pharmacophore, was found in inhibitors of several classes of kinases such as Aurora,[3] CDK-2[4a] and MAP[5] kinases. These kinases have great importance as the emerging targets in oncology drug discoveries. Based on the previously reported research on the potent anticancer activity of 3-aminopyrazoles, we have incorporated tetrahydropyridinone ring in our target molecules. Herein we report the synthesis and anticancer activity of novel 1,6-disubstituted 4,5,6,7-tetrahydropyrazolo[3,4-*c*]pyridin-7-one derivatives. SAR studies were done using biologically evaluated disubstituted tetrahydropyrazolopyridinone analogues with the aim to find



Scheme 1. General synthetic route of 1,6-disubstituted 4,5,6,7-tetrahydropyrazolo[3,4-*c*]pyridin-7-one analogues. Reagents and conditions: (i) K_2CO_3, 4-bromobutyronitrile, CH_3CN, reflux, 2 days, 80-95%; (ii) NaOEt, diethyloxalate, EtOH, reflux, overnight, 50-75%; (iii) hydrazinemonohydrate, glacial AcOH, EtOH, 65 °C, overnight, 95-100%; (iv) K_2CO_3, **A**, CH_3CN, 80 °C, overnight, 60-95%; (v) chloropropionyl chloride, CH_2Cl_2, rt, 1 h, 60-90%.

Table 1. Structures and Cancer Cell Cytotoxicities of 1,6-Disubstituted 4,5,6,7-tetrahydropyrazolo[3,4-c]pyridin-7-one analogues



Compounds	Substituents		Anti-proliferative activity (% Growth Inhibition at 100, 10 and 1 μM concentration)[a]											
			DU-145			HT-29			HCT-116			A375P		
	R^1	R^2	100 μM	10 μM	1 μM	100 μM	10 μM	1 μM	100 μM	10 μM	1 μM	100 μM	10 μM	1 μM
5a	4-Tolyl	Ph	22	NA[b]	NA	38	4	NA	10	NA	NA	38	7	6
5b	4-Tolyl	2,3-Me_2Ph	75	NA	NA	95	NA	NA	72	5	NA	86	6	4
5c	4-Tolyl	2,4-Me_2Ph	91	86	83	94	91	91	93	86	74	90	78	75
5d	4-Tolyl	4-OMePh	38	15	NA	32	8	7	33	6	NA	44	14	12
5e	4-Tolyl	4-ClPh	80	24	5	91	28	18	92	30	10	95	48	19
5f	4-Tolyl	2-Pyr	ND	ND	ND	ND	ND	ND	25	6	5	38	5	NA
5g	4-Tolyl	2,4,6-Me_3Bn	96	25	9	97	19	12	97	22	NA	98	50	6
5h	4-Tolyl	2,3,4-$(MeO)_3$Bn	52	10	4	45	10	NA	42	NA	NA	43	14	10
5i	4-Tolyl	3,4-methylen dioxyBn	45	12	8	35	6	NA	27	7	6	32	11	3
5j	4-Tolyl	4-*tert*-BuBn	96	49	21	97	47	21	97	54	7	98	61	28
Doxorubicin			88	89	82	89	88	83	93	92	88	96	95	90

[a]Percentage growth inhibition caused by compounds and standard doxorubicin drug against human cancer cell lines at 100, 10 and 1 μM concentration. [b]Inactive. [c]Not determined.

better anticancer molecules.

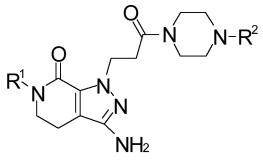
The route adopted for the synthesis of 1,6-disubstituted 4,5,6,7-tetrahydropyrazolo[3,4-*c*]pyridin-7-one derivatives is described in Scheme 1. The key intermediate 3-amino-6-*N*-substituted tetrahydropyrazolopyridinone **4** was synthesized in facile three steps by employing the approach reported by Blatter and Irvington.[6] with modifications to get better yield. The first step was the synthesis of *N*-substituted aminobutyronitrile **2** by the N-alkylation of primary amine **1** with 4-bromobutyronitrile using potassium carbonate as a base.[7] Then the cyclization was accomplished by refluxing **2** with diethyl oxalate and sodium ethoxide in ethanol to give 6-*N*-substituted 2,3-dioxopiperidin-4-carbonitrile **3**,[6] which upon treatment with hydrazine monohydrate in the presence of glacial acetic acid provided the required key intermediate **4** in a quantitative yield.[4b] Preparation of the target compounds **5-9** were achieved by reacting the intermediate **4** with the halide **A** using potassium carbonate as a base; the latter was obtained by reacting 4-substituted piperazine with chloropropionyl chloride.[8] The IR, ^{1}H and ^{13}C NMR and Mass spectral data of all the newly synthesized compounds were consistent with the assigned structure.[9]

The *in vitro* anti-proliferative activity of all the prepared 1,6-disubstituted tetrahydropyrazolopyridinones was evaluated according to the previously reported standard procedure by our research group[10] on four different human cancer cell lines: prostate (DU-145), colon (HT-29 and HCT-116) and melanoma (A375P).

At first, we synthesized compounds **5a-j** having 4-tolyl at R^1 and various substituents at R^2 position to provide some structure activity relationship data to guide the design of additional 1,6-disubstituted tetrahydropyrazolopyridinone derivatives (Table 1). Of them, compounds **5b**, **5c**, **5e**, **5g** and **5j** showed comparable activity to the reference Doxorubicin at 100 mM concentration. At lower concentrations, 10 μM and 1 μM, however, the compound **5c** having 2,4-dimethylphenyl group at R^2 exhibited far superior potency compared to the rest four compounds. It inhibited the growth of the cancer cells in the range of 74-94% even at 1 μM concentration. Overall, bulky R^2 groups seemed to be good for activity, and the electronic effect of the substituents of the phenyl ring of R^2 seemed not to be a determining factor. Interestingly the pattern of methyl substitution in the dimethylphenyl substituent (R^2) was crucial: the activity of the compound **5b** increased drastically by altering the position of a methyl on the phenyl group from 3 to 4-position to give the compound **5c**. The compound **5b** was almost inactive against the tested four cell lines at 10 μM and 1 μM concentrations, while the compound **5c** inhibited the cell growth significantly at the same concentrations. The compound **5j** also showed considerable inhibition at 10 μM concentration and was subjected to IC_{50} measurement.

Based on the above results, R^1 was varied while R^2 was fixed as 2,4-dimethylphenyl group and the *in vitro* anti-proliferative activities of the resulting compounds were evaluated (Table 2). Variation of R^1 in **5c** from 4-tolyl to 4-methoxyphenyl, phenyl and benzyl yielded compounds **6**, **7** and **8**, respectively, with significant inhibitory potential of 72-97% at 100 μM concentration on tested cancer cell lines. Except the compound **8**, however, they showed far inferior anti-proliferative activity to the compound **5c** at lower concentrations. The compound **8** exhibited notable inhibition of 69% and 23% on A375P cancer cell line at 10 μM and 1 μM

Table 2. Inhibitory Effects of Selected Tetrahydropyrazolopyridinone Analogues



Compounds	Substituents		Anti-proliferative activity (% Growth Inhibition at 100, 10 and 1 μM concentration)[a]											
			DU-145			HT-29			HCT-116			A375P		
	R^1	R^2	100 μM	10 μM	1 μM	100 μM	10 μM	1 μM	100 μM	10 μM	1 μM	100 μM	10 μM	1 μM
5c	4-Tolyl	2,4-Me_2Ph	91	86	83	94	91	91	93	86	74	90	78	75
6	4-OMePh	2,4-Me_2Ph	86	13	NA[b]	91	20	11	97	4	NA	97	9	1
7	Ph	2,4-Me_2Ph	72	18	8	74	8	3	79	NA	NA	89	28	13
8	Bn	2,4-Me_2Ph	94	24	2.6	94	21	NA	95	32	NA	97	69	23
9	Furfuryl	2,4-Me_2Ph	ND[c]	ND	ND	ND	ND	ND	64	25	NA	67	34	10
Doxorubicin			88	89	82	89	88	83	93	92	88	96	95	90

[a]Percentage growth inhibition caused by compounds and standard doxorubicin drug against human cancer cell lines at 100, 10 and 1 μM concentration. [b]Inactive. [c]Not determined.

Table 3. *In vitro* anti-proliferative activity of the selected derivatives against human cancer cell lines, IC_{50} (μM)

Compounds	IC_{50}[a] (μM)			
	DU-145	HT-29	HCT-116	A375P
5c	0.40 ± 0.02	0.16 ± 0.01	0.35 ± 0.01	0.34 ± 0.03
5j	11.36 ± 0.71	8.11 ± 1.82	3.55 ± 0.80	6.51 ± 1.87
8	20.98 ± 3.27	17.76 ± 3.31	8.07 ± 1.10	6.55 ± 3.19
Doxorubicin	0.10 ± 0.02	0.11 ± 0.09	0.09 ± 0.05	0.08 ± 0.03

[a]IC_{50}: Drug concentration required to inhibit 50% of cancer cell proliferation. Each experiment was repeated three times in duplicate.

concentrations, respectively. Significant loss in activity was observed upon changing the R^1 to furfuryl group. The compound **9** though having 2,4-dimethylphenyl at R^2 showed only modest anticancer activity of 64% and 67% at 100 μM on HCT-116 and A375P cancer cell lines, respectively.

The IC_{50} values for the compounds **5c**, **5j** and **8,** which exhibited significant inhibition at 10 μM concentrations, are summarized in Table 3. All these compounds decreased cell viability as determined by colorimetric MTT assay, with the IC_{50} values ranging from 0.15 to 20 μM. The compound **5c** was the most potent among the prepared compounds of the library on all tested cancer cell lines with the IC_{50} values of 0.40 on DU-145, 0.16 on HT-29, 0.35 on HCT-116, and 0.34 μM on A375P cancer cell lines, though not superior to the reference. The compounds **5j** and **8** showed moderate values of IC_{50} ranging 3.55-20.98 for the cancer cell lines.

In the present study, we have discovered and explored the structure-activity relationships of 1,6-disubstituted tetrahydropyrazolopyridinones by modifying the R^1 and R^2 substituents. The R^2 group played a key role in enhancing the activity, and 2,4-dimethylphenyl and 4-*tert*-butylbenzyl groups were the most preferred choices in getting potent analogues. Through SAR studies, the compound **5c** was identified, which has submicromolar potency on all the tested cancer cell lines and is significantly more potent than the rest of the prepared compounds. Further modification and evaluation of the compound **5c** is in progress. Studies on the mode of action of these compounds are also under way.

Acknowledgments. Financial support was provided by the Seoul R&BD Program (2G08020), Basic Science Research Program (2010-0021233) through the National Research Foundation of Korea (NRF) funded by the Ministry of Education. The author Ms. Vani N. D. wishes to thank the Korean government MOEHRD for the doctoral program grant (KRF-2007-211-C00028).

References and Notes

1. Gibbs, J. B. *Science* **2000**, *287*, 1969.
2. Pinto, D. J. P.; Orwat, M. J.; Quan, M. L.; Han, Q.; Galemmo, R. A., Jr.; Amparo, E.; Wells, B.; Ellis, C.; He, M. Y.; Alexander, R. S.; Rossi, K. A.; Smallwood, A.; Wong, P. C.; Luettgen, J. M.; Rendina, A. R.; Knabb, R. M.; Mersinger, L.; Kettner, C.; Bai, S.; He, K.; Wexler, R. R.; Lam, P. Y. S. *Bioorg, Med. Chem. Lett.* **2006**, *16*, 4141.
3. Fancelli, D.; Berta, D.; Bindi, S.; Cameron, A.; Cappella, P.; Carpinelli, P.; Catana, C.; Forte, B.; Giordano, P.; Giorgini, M. L.; Mantegani, S.; Marsiglio, A.; Meroni, M.; Moll, J.; Pittalà, V.; Roletto, F.; Severino, D.; Soncini, C.; Storici, P.; Tonani, R.; Varasi, M.; Vulpetti, A.;Vianello, P. *J. Med. Chem.* **2005**, *48*, 3080.
4. (a) Pevarello, P.; Brasca, M. G.; Amici, R.; Orsini, P.; Traquandi, G.; Corti, L.; Piutti, C.; Sansonna, P.; Villa, M.; Pierce, B. S.; Pulici, M.; Giordano, P.; Martina, K.; Fritzen, E. L.; Nugent, R. A.; Casale, E.; Cameron, A.; Ciomei, M.; Roletto, F.; Isacchi, A.; Fogliatto, G.; Pesenti, E.; Pastori, W.; Marsiglio, A.; Leach, K. L.; Clare, P. M.; Fiorentini, F.; Varasi, M.; Vulpetti, A.; Warpehoski, M. A. *J. Med. Chem.* **2004**, *47*, 3367. (b) Brasca, M. G.; Albanese, C.; Amici, R.; Ballinari, D.; Corti, L.; Croci, V.; Fancelli, D.; Fiorentini, F.; Nesi, M.; Orsini, P.; Orzi, F.; Pastori, W.; Perrone, E.; Pesenti, E.; Pevarello, P.; Riccardi-Sirtori, F.; Roletto, F.; Roussel, P.; Varasi, M.; Vulpetti, A.; Mercurio, C. *Chem. Med. Chem.* **2007**, *2*, 841.
5. Regan, J.; Capolino, A.; Cirillo, P. F.; Gilmore, T.; Graham, A. G.; Hickey, E.; Kroe, R. R.; Madwed, J.; Moriak, M.; Nelson, R.; Pargellis, C. A.; Swinamer, A.; Torcellini, C.; Tsang, M.; Moss, N.

J. Med. Chem. **2003**, *46*, 4676.
6. Blatter, H. M.; Irvington, N. J. US 3423414, 1969.
7. Gardner, R. A.; Belting, M.; Svensson, K.; Phanstiel Iv, O. *J. Med. Chem.* **2007**, *50*, 308.
8. Larsen, S. D.; Dipaolo, B. A. *Org. Lett.* **2001**, *3*, 3341.
9. Data for selected compounds: *3-amino-1-[3-{4-(2,4-dimethylphenyl)piperazin-1-yl}propanoyl]-6-N-(p-tolyl)-4,5,6,7-tetrahydropyrazolo[3,4-c]pyridin-7-one* **5c**: Light yellow amorphous solid (1.24 g, 86.1%); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.21-7.15 (m, 4H), 7.01 (s, 1H), 6.96 (d, J = 8.2 Hz, 1H), 6.84 (d, J = 8.0 Hz, 1H), 4.56 (s, 2H, NH_2), 4.37 (t, J = 4.7 Hz, 2H), 3.89 (t, J = 6.4 Hz, 2H), 3.70 (br s, 2H), 3.55 (br s, 2H), 3.00 (t, J = 4.8 Hz, 2H), 2.79 (br s, 4H), 2.70 (t, J = 6.4 Hz, 2H), 2.33 (s, 3H), 2.27 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.6, 161.6, 148.2, 142.2, 142.0, 140.4, 135.8, 133.3, 132.5, 131.9, 129.5, 127.1, 125.5, 119.0, 102.1, 51.9, 51.9, 51.7, 45.9, 42.9, 42.4, 33.4, 21.0, 20.7, 19.5, 17.6; MS (ES+): *m/z* calcd for $C_{28}H_{34}N_6O_2$: 486.61; found 487.93 $[M+H]^+$
3-amino-1-[3-{4-(4-tert-butylbenzyl)piperazin-1-yl}propanoyl]-6-N-(p-tolyl)-4,5,6,7-tetrahydropyrazolo[3,4-c]pyridin-7-one **5j**: Light yellow amorphous solid (150.6 mg, 60.8%); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.33 (d, J = 8.2 Hz, 2H), 7.22-7.14 (m, 6H), 4.54 (s, 2H, NH_2), 4.32 (t, J = 4.8 Hz, 2H), 3.89 (t, J = 6.4 Hz, 2H), 3.56 (br s, 2H), 3.45 (s, 2H), 3.40 (br s, 2H), 2.92 (t, J = 4.8 Hz, 2H), 2.69 (t, J = 6.4 Hz, 2H), 2.37 (br s, 4H), 2.32 (s, 3H), 1.31 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.4, 161.6, 150.2, 142.2, 142.0, 140.4, 135.8, 134.3, 129.5, 128.8, 125.5, 125.2, 102.0, 62.4, 52.7, 52.5, 51.9, 45.3, 42.8, 41.8, 34.5, 33.4, 31.4, 21.0, 19.5; MS (ES+): *m/z* calcd for $C_{31}H_{40}N_6O_2$: 528.69; found 529.95 $[M+H]^+$
3-amino-1-[3-{4-(2,4-dimethylphenyl)piperazin-1-yl}propanoyl]-6-N-benzyl-4,5,6,7-tetrahydropyrazolo[3,4-c]pyridin-7-one **8**: White amorphous solid (170.2 mg, 70.3%); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.32-7.27 (m, 5H), 7.02 (s, 1H), 6.95 (br s, 1H), 6.85 (d, J = 8.0 Hz, 1H), 4.75 (s, 2H), 4.45 (s, 2H, NH_2), 4.36 (t, J = 5.0 Hz, 2H), 3.69 (t, J = 4.5 Hz, 2H), 3.56 (t, J = 4.7 Hz, 2H), 3.43 (t, J = 6.6 Hz, 2H), 3.01 (t, J = 4.9 Hz, 2H), 2.84-2.77 (m, 4H), 2.55 (t, J = 6.6 Hz, 2H), 2.27 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.6, 162.1, 148.2, 141.9, 141.8, 137.7, 133.3, 132.6, 131.9, 128.5, 128.2, 128.1, 127.3, 127.1, 119.0, 101.5, 51.9, 51.7, 49.4, 47.3, 45.9, 42.7, 42.4, 33.4, 20.7, 18.9, 17.6; MS (ES+): *m/z* calcd for $C_{28}H_{34}N_6O_2$: 486.61; found 487.93 $[M+H]^+$.
10. Devegowda, V. N.; Kim, J. H.; Han, K.-C.; Yang, E. G.; Choo, H.; Pae, A. N.; Nam, G.; Choi, K. I. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1630.