STUDIES ON 1,2,4-BENZOTHIADIAZINE 1,1-DIOXIDES VII [1] AND QUINAZOLINONES IV [2]: SYNTHESIS OF NOVEL BUILT-IN HYDROXYGUANIDINE TRICYCLES AS POTENTIAL ANTICANCER AGENTS

Ji-Wang Chern*, Yen-Chywan Liaw+, Chien-Shu Chen, Jiann-Gwo Rong, Chien-Lin Huang, Chao-Han Chan, and Andrew H.-J. Wang+ #

Institute of Pharmacy and Medical Laboratories, National Defense Medical Center, P. O. Box 90048-512, Taipei, Taiwan, Republic of China (100) and +Laboratory of Structural Biology, Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan, ROC.

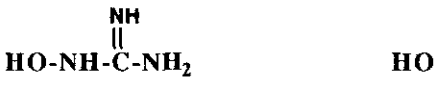
This manuscript is dedicated to Prof. Leroy B. Townsend on the occasion of his 60th birthday.

Abstract- Two representative built-in hydroxyguanidine tricycles containing 1,2,4-benzothiadiazine 1,1-dioxides (3) and quinazolinones (4) were prepared by reductive cyclization of 1-(2-nitrophenylsulfonyl)-2-benzylthio-2-imidazoline (9a), 1-(2-nitrophenylsulfonyl)-2-benzylthio-1,4,5,6-tetrahydropyrimidine (9b), 1-(2-nitrobenzoyl)-2-benzylthio-2-imidazolidine (10a) and 1-(2-nitrobenzoyl)-2-benzylthio-1,4,5,6-tetrahydropyrimidine hydrobromide respectively (10b) with zinc dust in acetic acid under ice-cooling. 2,10-Dihydro-10-hydroxy-3*H*-imidazo[1,2-b][1,2,4]benzothiadiazine 5,5-dioxide (3a) and 2,3,4,11-tetrahydro-11-hydroxypyrimido[1,2-b][1,2,4]benzothiadiazine 6,6-dioxide (3b) were found to be active against solid tumor cell lines such as KB, Colo 205, HeLa, and Hepa-2.

Hydroxyguanidine (1), hydroxyurea (2) and their derivatives constitute a class of anticancer and antiviral agents.[3] It has been reported that these agents inactivate ribonucleotide reductase which is an essential enzyme for the DNA synthesis and cell replication and is considered as an important target for the

Permanent address: Department of Physiology & Biophysics, University of Illinois at Urbana, Champaigan, Urbana, IL 61801, USA.

development of chemotherapeutic agent *via* an inhibition of DNA synthesis.[4] However, due to the hydrophilicity and low molecular weight of these types of compounds, the disadvantage of these compounds such as a short half-life, rapid metabolic transformation to inactive forms, and myelosuppression has limited their application in the clinic.[5] Hydrophobicity is known to play an important role in binding a substrate or inhibitor to the active site of enzyme or receptor and such a hydrophobic region adjacent to the active site of enzyme or receptor has been observed on dihydrofolate reductase,[6] guanase,[7] thymidine phosphorylase,[8] purine nucleoside phosphorylase[9] and α_1-adrenergic receptors.[10] After our careful examinations, we suggested that tricycles with built-in hydroxyguanidine such as 2,10-dihydro-10-hydroxy-3*H*-imidazo[1,2-b][1,2,4]benzothiadiazine 5,5-dioxide (3a), 2,3,4,11-tetrahydro-11-hydroxypyrimido[1,2-b][1,2,4]benzothiadiazine 6,6-dioxide (3b), 2,10-dihydro-10-hydroxyimidazo[2,1-b]quinazolin-5(3*H*)-one (4a) and 2,3,4,11-tetrahydro-11-hydroxy-5*H*-pyrimido[2,1-b]quinazolin-6-one (4b) appear to be more stable and hydrophobic while possessing a rigid structural feature is essential to elicit the biological activities of hydroxyguanidine. This paper describes on the synthesis of this type of compounds and their antitumor activities.



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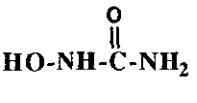

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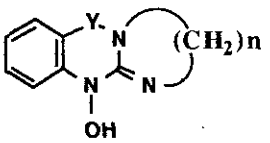


3a, n=2, Y= SO_2
3b, n=3, Y= SO_2
4a, n=2, Y= C=O
4b, n=3, Y= C=O

RESULTS AND DISCUSSION

It is well known that nitroarenes can be converted into their corresponding amines with many reducing agents *via* an intermediary formation of hydroxylamine and the reduction can be stopped at the stage of the hydroxylamines.[11] Accordingly, we reasoned that the preparation of 3a,b and 4a,b could be achieved by careful reduction of nitroarene (9a,b) and (10a,b) with zinc followed by a simultaneous nucleophilic displacement of alkylthio group in the juxtaposition for the intramolecular cyclization with the resulting hydroxylamino group.

The synthetic route for the compounds (3) and (4) is outlined in Scheme. 2-Benzylthio-2-imidazoline hydrobromide (6a), prepared by the benzylation of 2-mercapto-2-imidazoline (5a), was treated with 2-nitrobenzenesulfonyl chloride (7) to give 1-(2-nitrophenylsulfonyl)-2-benzylthio-2-imidazoline

(9a), which was then treated with zinc in acetic acid at 0 °C for 30 min. After the mixture was stirred at room temperature for a further 60 min, the crude product was isolated and was found to be complexed with one equivalent of zinc on the basis of elemental analysis. The product was then dissolved in hot ethanol containing 0.1N EDTA. After cooling to room temperature, a nice crystal of 2,10-dihydro-10-hydroxy-3*H*-imidazo[1,2-b][1,2,4]benzothiadiazine 5,5-dioxide (3a) was obtained in 79% yield. Meanwhile, 2,3-dihydro-1*H*-imidazo[1,2-b][1,2,4]benzothiadiazine 5,5-dioxide (11a) was isolated in 6.8% yield from the filtrate.

At the outset, we reasoned that the products of this reaction could be not only 3a and 11a, but also be a product of *N*-hydroxy nucleophilic displacement of alkylthio group such as 13. To confirm the structure of the major product (3a), following experiments were carried out. However, when the reaction mixture of 9a after reduction with zinc was allowed to stir at room temperature for further 2 days, the deoxygenated product (11a) was obtained exclusively; the major product (3a) was reduced by triphenylphosphine[12] to afford 11a (followed by tlc). Accordingly, alternative structure 13 for the major product was rejected. The mass spectra of the product (3a) exhibited a molecular ion peak at 239 (M^+) together with a fragment ion peak at 223 (M^+-16), whereas the compound (11a) showed the molecular ion peak at 223 (M^+) indicating a loss of oxygen from 3a. On the basis of the fragment ion peak at M^+-16, it initially appeared to

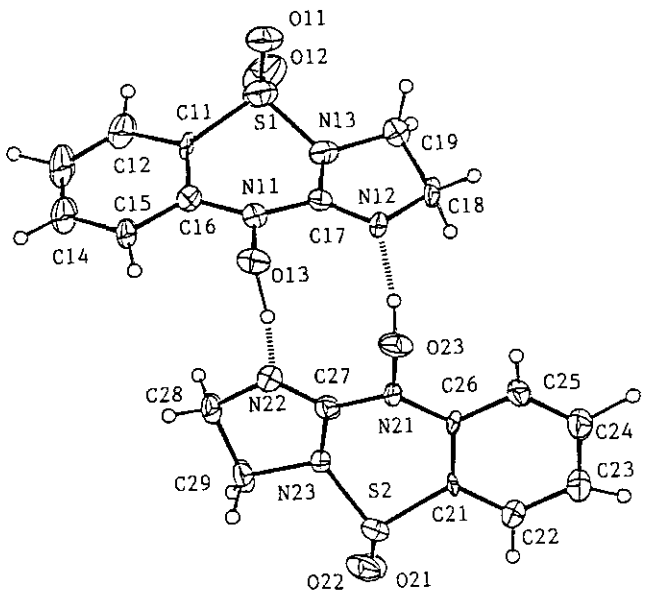
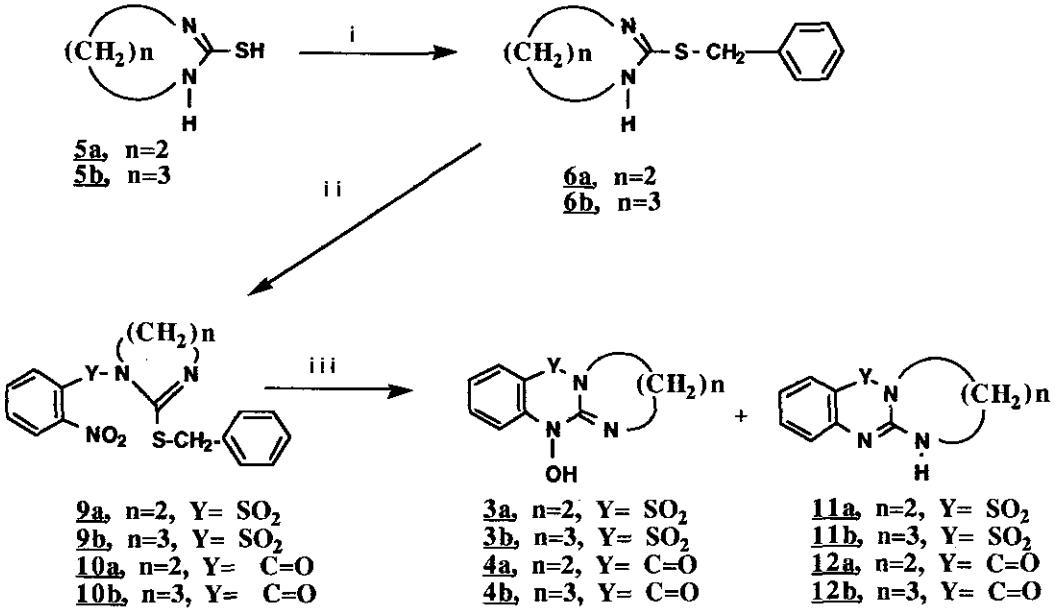


Figure 1. Thermal ellipsoid plot of the molecule (3a) showing the atom numbering and the intermolecular H-Bond within dimer.

be the *N*-oxide (14). However, the ^{13}C nmr spectrum obtained for 11a showed the chemical shift for the C-9a at 146.4 ppm which is in agreement with the values reported by Jackobsen and Treppendahl,[13] indicative of a double bond existing between N-10 and C-10a. Meanwhile, the ^{13}C nmr spectrum of 3a showed the C-9a chemical shift at 138.4 ppm which illustrates a hydroxy group at N-10 position with a

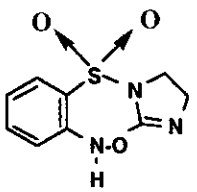
double bond located between C-10a and N-1 is in agreement with previous data.[14] This would lend some support to the fact that the major product obtained by a reductive cyclization of 9a with zinc in acetic acid at low temperature would exist as built-in hydroxyguanidine (3a) instead of the alternative structure (14). To confirm the configuration of 3a, an X-ray structure analysis of 3a was performed. As expected, the thermal ellipsoid plot shows a hydroxy group located on N-10 position. The hydroxy group at N-10 position donates a strong intermolecular hydrogen bond to N-1 of 3a and two molecules form a dimer by two intermolecular hydrogen bonds between N-OH and N-1 position. Similarly, treatment of 1,4,5,6-tetrahydro-2-pyrimidinethiol (5b) with benzyl bromide gave 2-benzylthio-1,4,5,6-tetrahydro-



i. benzyl bromide, CH_3OH, reflux; ii. 2-nitrobenzenlsulfonyl chloride (7) or 2-nitrobenzoyl chloride (8), CH_2Cl_2, TEA, room temperature; iii. Zn, HOAc, 0^0C

pyrimidine hydrobromide (6b) which was then reacted with 7 to furnish 1-(2-nitrophenylsulfonyl)-2-benzylthio-1,4,5,6-tetrahydropyrimidine (9b). Reduction of 9b under the condition analogous to the case of 9a afforded 2,3,4,11-tetrahydro-11-hydroxypyrimido[1,2-b][1,2,4]benzothiadiazine 6,6-dioxide (3b) in 30% yield and 1,2,3,4-tetrahydropyrimido[1,2-b]-[1,2,4]benzothiadiazine 6,6-dioxide (11b) in 10% yield. A prolonged reaction time (for 2 days) caused the exclusive formation of 11b. The ^{13}C nmr spectra for the products (3b) and (11b) showed the chemical shift for the C-10a at 138.50 and 145.02 ppm, respectively, which are in agreement with their structures. Although arylhydroxyamines are traditionally made by careful reduction of nitroarenes, the synthesis of 3a,b is probably due to either the inductive effects of the sulfonyl group which stabilize the hydroxylamine intermediates during the reduction or the propensity of the hydroxylamine group and alkylthiol moiety. Friary *et al.*[15] have reported the reduction of 1-(2-nitrophenylsulfonyl)-2-methylthio-2-imidazolidine (15) with $SnCl_2$ in hydrochloric acid or acetic acid caused the intramolecular cyclization by an elimination of methanethiol to

give the compound (11a). Analogous results were obtained in the reduction of 4-[(4,5-dihydro-2-methylthio-1*H*-imidazol-1-yl)carbonyl]-1-methyl-5-nitro-1*H*-pyrazole (16) with zinc in acetic acid under ice-cooling leading to the formation of the tricyclic 1,6,7,8-tetrahydro-1-methyl-4*H*-imidazo[1,2-a]pyrazolo[3,4-d]pyrimidin-4-one (17) instead of built-in hydroxyguanidine tricycle.[16] In contrast to these results, careful reduction of 15 with zinc in acetic acid under the mild condition resulted in the formation of 3a in 77% yield, together with 11a (14%).



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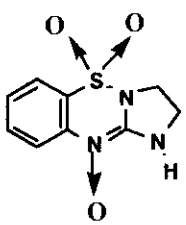

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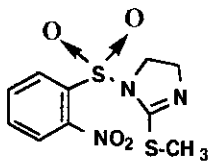

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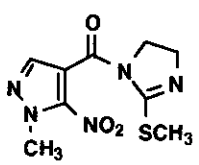
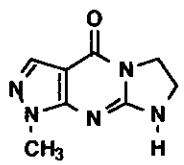


16

Zn, AcOH



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In the field of medicinal chemistry, a carboxamide group is considered as a bioisostere of the sulfonamide moiety.[17] From this point of view, the quinazoline derivatives (4a,b) were prepared by the synthetic method similar to that for 3a,b. Treatment of 2-nitrobenzoyl chloride (8) with 6a and 6b afforded 1-(2-nitrobenzoyl)-2-benzylthioimidazolidine (10a) and 1-(2-nitrobenzoyl)-2-benzylthio-1,4,5,6-tetrahydropyrimidine hydrobromide (10b) in good yield. The reduction of 10a and 10b with zinc dust in acetic acid in ice-cooling furnished 4a and 4b instead of 2,3-dihydroimidazo[2,1-b]quinazolin-5(1*H*)-one (12a) and 1,2,3,4-tetrahydro-6*H*-pyrimido[2,1-b]quinazolin-6-one (12b). To confirm the structures of these two isolated products (4a) and (4b), 12a [18] and 12b [19] were prepared in a good yield by a treatment of isatoic anhydride with 6a and 6b respectively in the presence of potassium carbonate in DMF at 100 °C. The structural determination of compounds (4a) and (4b) was mainly based upon the the elemental analysis and mass spectral data. The mass spectrum of 4a and 4b illustrated that in addition to the molecular ion peak, a molecular fragment ion peak appeared at M^+-16, indicative of the presence of a built-in hydroxyguanidine moiety in the molecule as well.

Cytotoxic evaluation of the compounds (3a,b) by the MTT method[20] showed good activity against cancer cell lines including KB, Colo 205, HeLa and Hep-2 with LD_{50} down to 2.0 μg/ml (Table 1). Interestingly, the compounds (4a,b) did not exhibit a significant cytotoxic activity against above cell lines, though the structural skeletons of 1,2,4-benzothiadiazine 1,1-dioxide and quinazolinone are bioisosteric to each

other.[17] This indicates that the sulfonamide moiety in the tricycles might be essential to the biological activity. A recent synthetic studies on the structural modification of certain hypoglycemic agents led to a discovery of a new class of sulfonylurea as anticancer agents.[21] The mechanism of action of this type of compounds is different from the current anticancer therapeutic agents. The agent primarily accumulates in mitochondria instead of blocking the DNA synthesis and especially for the treatment of solid tumors.[22] More interestingly, the built-in hydroxyguanidine tricycles described herein are potent against solid tumors as well. The initial results indicated that these compounds did not inhibit protein synthesis.

Table 1. Cytotoxic Activity of compound 3a and 3b by the MTT method

Compounds	LD_{50} (μg/ml)			
	KB	Colo 205	Hela	Hep-2
3a	5.6	2.1	2.0	11.1
3b	6.8	3.0	3.2	12.3

Table 2. Atomic coordinates and isotropic thermal parameters of 3a (Biso).E.S.Ds. refer to the last digit printed.

	x	y	z	Biso*
S1	0.67106(4)	0.39555(8)	0.0932(1)	3.40(7)
O11	0.68148(10)	0.32617(24)	0.2165(3)	4.7(2)
O12	0.66164(10)	0.33402(25)	-0.0370(3)	4.6(2)
O13	0.68242(9)	0.70488(22)	0.3621(3)	3.9(2)
N11	0.67697(11)	0.6244(3)	0.2482(4)	2.9(2)
N12	0.61996(11)	0.5977(3)	0.3278(3)	3.2(2)
N13	0.63907(10)	0.4841(3)	0.1397(3)	2.7(2)
C11	0.70199(13)	0.5028(3)	0.0588(4)	2.5(3)
C12	0.72727(13)	0.4813(4)	-0.0407(5)	3.7(3)
C13	0.75499(15)	0.5563(4)	-0.0526(5)	4.7(3)
C14	0.75626(16)	0.6568(4)	0.0392(6)	4.6(3)
C15	0.73111(14)	0.6799(4)	0.1390(5)	3.8(3)
C16	0.70298(14)	0.6043(3)	0.1514(5)	2.6(3)
C17	0.64555(13)	0.5736(3)	0.2459(4)	2.6(3)
C18	0.59091(14)	0.5258(4)	0.2764(5)	3.6(3)
C19	0.60662(14)	0.4288(4)	0.1809(5)	4.0(3)
S2	0.56909(4)	1.06668(10)	0.2285(1)	4.52(8)
O21	0.55595(11)	1.10298(33)	0.0908(4)	6.6(2)
O22	0.57404(11)	1.15726(26)	0.3369(4)	5.6(2)
O23	0.61227(9)	0.78306(22)	0.4935(3)	3.9(2)
N21	0.59759(12)	0.8609(3)	0.3937(4)	3.4(2)
N22	0.65119(11)	0.8995(3)	0.2878(4)	3.0(2)
N23	0.60511(11)	0.9949(3)	0.1957(4)	3.5(2)
C21	0.54583(13)	0.9503(4)	0.3032(5)	3.1(3)
C22	0.51049(15)	0.9486(5)	0.2895(6)	5.2(4)
C23	0.49115(17)	0.8640(6)	0.3610(7)	6.9(5)
C24	0.50735(16)	0.7822(5)	0.4497(6)	5.5(4)
C25	0.54225(14)	0.7790(4)	0.4628(5)	3.7(3)
C26	0.56231(15)	0.8631(4)	0.3890(5)	3.5(3)
C27	0.61934(14)	0.9148(3)	0.2985(4)	2.9(3)
C28	0.66351(14)	0.9765(4)	0.1686(5)	3.3(3)
C29	0.63385(15)	1.0615(4)	0.1355(5)	3.9(3)

*Biso is defined as one third of the trace of the orthogonalised Bij tensor

Table 3. Bond lengths (Å) of 3a

S(1)-O(11)	1.428(3)	S(2)-O(21)	1.419(4)
S(1)-O(12)	1.423(3)	S(2)-O(22)	1.431(3)
S(1)-N(13)	1.657(4)	S(2)-N(23)	1.655(4)
S(1)-C(11)	1.738(4)	S(2)-C(21)	1.733(5)
O(13)-N(11)	1.394(4)	O(23)-N(21)	1.388(5)
N(11)-C(16)	1.370(6)	N(21)-C(26)	1.387(7)
N(11)-C(17)	1.359(6)	N(21)-C(27)	1.360(6)
N(12)-C(17)	1.282(6)	N(22)-C(27)	1.267(7)
N(12)-C(18)	1.474(6)	N(22)-C(28)	1.472(5)
N(12)-H(4)	1.39 (5)	N(23)-C(27)	1.415(5)
N(13)-C(17)	1.418(5)	N(23)-C(29)	1.461(6)
N(13)-C(19)	1.467(6)	C(21)-C(22)	1.394(8)
C(11)-C(12)	1.368(7)	C(21)-C(26)	1.411(7)
C(11)-C(16)	1.419(5)	C(22)-C(23)	1.379(9)
C(12)-C(13)	1.380(7)	C(23)-C(24)	1.380(9)
C(13)-C(14)	1.406(7)	C(24)-C(25)	1.377(8)
C(14)-C(15)	1.369(8)	C(25)-C(26)	1.402(7)
C(15)-C(16)	1.397(7)	C(28)-C(29)	1.535(7)
C(18)-C(19)	1.525(7)		

Table 4. Bond Angle(°) of 3a

O(11)-S(1)-O(12)	117.93(18)	O(21)-S(2)-N(23)	106.86(21)
O(11)-S(1)-N(13)	109.98(19)	O(21)-S(2)-C(21)	111.9(3)
O(11)-S(1)-C(11)	108.59(22)	O(22)-S(2)-N(23)	110.77(23)
O(12)-S(1)-N(13)	107.91(21)	O(22)-S(2)-C(21)	109.55(21)
O(12)-S(1)-C(11)	111.53(20)	N(23)-S(2)-C(21)	98.95(21)
N(13)-S(1)-C(11)	99.30(19)	O(23)-N(21)-C(26)	116.5(3)
O(13)-N(11)-C(16)	118.2(4)	O(23)-N(21)-C(27)	116.0(4)
O(13)-N(11)-C(17)	115.0(3)	C(26)-N(21)-C(27)	126.9(4)
C(16)-N(11)-C(17)	126.8(4)	C(27)-N(22)-C(28)	107.6(4)
C(17)-N(12)-C(18)	107.8(3)	S(2)-N(23)-C(27)	121.8(3)
S(2)-N(23)-C(29)	118.7(3)	C(27)-N(23)-C(29)	105.6(4)
S(1)-N(13)-C(17)	117.6(3)	S(2)-C(21)-C(22)	120.0(4)
S(1)-N(13)-C(19)	118.1(3)	S(2)-C(21)-C(26)	119.9(4)
C(17)-N(13)-C(19)	106.3(3)	C(22)-C(21)-C(26)	119.8(4)
S(1)-C(11)-C(12)	120.4(3)	C(21)-C(22)-C(23)	121.0(5)
S(1)-C(11)-C(16)	117.9(3)	C(12)-C(11)-C(16)	121.1(4)
C(11)-C(12)-C(13)	121.2(4)	C(22)-C(23)-C(24)	118.7(6)
C(12)-C(13)-C(14)	118.0(5)	C(23)-C(24)-C(25)	121.9(5)
C(13)-C(14)-C(15)	121.6(5)	C(24)-C(25)-C(26)	120.0(5)
C(14)-C(15)-C(16)	120.7(4)	N(21)-C(26)-C(21)	119.2(4)
N(21)-C(26)-C(25)	122.4(4)	C(21)-C(26)-C(25)	118.4(5)
N(11)-C(16)-C(11)	119.7(4)	N(21)-C(27)-N(22)	127.5(4)
N(11)-C(16)-C(15)	122.9(4)	N(21)-C(27)-N(23)	117.3(4)
C(11)-C(16)-C(15)	117.4(4)	N(22)-C(27)-N(23)	115.2(4)
N(11)-C(17)-N(12)	127.9(4)	N(22)-C(28)-C(29)	105.1(4)
N(11)-C(17)-N(13)	118.0(4)	N(12)-C(17)-N(13)	114.0(4)
N(12)-C(18)-C(19)	105.0(4)	N(23)-C(29)-C(28)	101.3(3)
N(13)-C(19)-C(18)	101.3(3)	O(21)-S(2)-O(22)	117.26(22)

EXPERIMENTAL

General methods: Melting points were obtained on an Electrothermal apparatus and are uncorrected. Uv spectra were recorded on a Beckman UA 50 spectrophotometer. ^1H and ^{13}C nuclear magnetic resonance spectra were recorded on a Jeol FX-100 or Jeol JNM-EX400 spectrometer from National Taiwan Normal University or on a Bruker Model AM 300 spectrometer from National Taiwan University, Taipei, and are reported in parts per million with DMSO-d_6 as internal standard on a δ scale. EI mass spectra were recorded on Jeol JMS-D100 mass spectrometer from National Taiwan University. Elemental analysis was carried out either on a Heraeus Elemental Analyzer in Cheng-Kong University, Tainan, or on a Perkin-Elmer 240 Elemental Analyzer in National Taiwan University, Taipei. X-Ray structure determination: the x-ray diffraction data were collected at 21°C with a Rigaku AFC5R(RU-300) rotating anode X-ray diffractometer by using the $\omega-2\theta$ scan mode with graphite-monochromated CuKα(λ = 1.5418Å) radiation. 2036 Reflection intensities up to $2\theta=120^{\circ}$ were mearsured. Cell parameters, a=39.286(7), b=11.2159(7), c=9.1284(22)Å, were determined by least squares refinement, the setting angles of 24 accurately centered reflections ($30^{\circ}<2\theta<50^{\circ}$) being used. Throughout data collection the intensities of three standard reflections ([3,5,-2],[2,-7,-2,[3,6,-1]) were monitored every 2 h and this indicated no significant crystal decomposition. The intensity data were corrected for Lorentz and polarization effects and for absorption by a procedure based on azimuthal ψ-scan[23] 1567 reflections with $F > 3\sigma(F)$ were used for structure solution and refinement. The structure was solved in the orthorhombic space group P bca by the direct methods using the program SHELXS-86.[24] Full-matrix least squares refinement was carried out on positional and anisotropic thermal parameters of all non-hydrogen atoms by using the NRCVAX package.[25] The function minimized was $\Sigma w(|Fo|-|Fc|)$, where $w = [\sigma^2(F_o)+0.0001(F_o)^2]^{-1}$. Positions of the hydrogen atoms were all located in difference Fourier map and also included in the refinement with isotropic temperature factors. In the last stage least-squares calculation, goodness of fit = 2.47, $(\Delta\rho)_{max}$ = 0.17 e Å-3, $(\Delta/\sigma)_{max}$=0.052, final R values were R = 0.042, R_w = 0.051. Final atom coordinates, bond lengths and bond angles are listed in Tables 2-4. Tabulations of hydrogen atomic coordinates, anisotropic thermal parameters, structure factors are available from the author Y.-C. L.

2-Benzylthio-2-imidazoline Hydrobromide (6a)

To a mixture of 2-mercaptoimidazoline (10 g, 98 mmol) in methanol (200 ml) was added benzyl bromide (23 ml, 100 mmol). The mixture was allowed to reflux. After 1 h, the mixture was concentrated *in vacuo* to an oily residue. The residue was dissolved in methanol (10 ml) and then ether (206 ml) was added to the solution to afford 6a (26.2 g, 98%). An analytical sample was recrystallized from acetone, mp 172-175 °C (decomp.). ^1H nmr (100 MHz, DMSO-d_6): δ 3.86 (s, 4H, CH_2), 4.59 (s, 2H, CH_2), 7.31-7.41 (m, 5H, Ar-H), 10.27 (br s, NH, HBr); ms: m/z 192 (M^+), Anal. Calcd for $C_{10}H_{13}N_2SBr$: C, 43.95; H, 4.79; N, 10.25. Found: C, 44.01; H, 4.81; N, 10.13.

1-(2-Nitrophenylsulfonyl)-2-benzylthio-2-imidazolidine (9a)

To a mixture of 6a (6.2 g, 22.7 mmol) and triethylamine (8 ml, 57 mmol) in dichloromethame (150 ml) was added dropwise 2-nitrobenzenesulfonyl chloride (5.0 g, 22.6 mmol) under ice-cooling. The mixture was allowed to stir at room temperature for 1 h and then was evaporated *in vacuo* to afford a white solid. To

the residue was added methanol (25 ml) and water (50 ml). The white solid was then collected by filtration and recrystallized from methanol to give 9a (8.2 g, 96%), mp 112 °C (decomp.). ^{1}H nmr (100 MHz, DMSO-d_6): δ 3.91 (s, 4H, CH_2), 4.26 (s, 2H, CH_2), 7.29 (s, 5H, Ar-H), 7.90-8.04 (m, 4H, Ar-H); ^{13}C nmr (25 MHz, DMSO-d_6): δ 35.80, 45.53, 49.45, 53.32, 124.69, 127.09, 128.14, 128.73, 129.08, 129.84, 132.77, 135.41, 136.41, 147.54, 153.63; ms: m/z, 377 (M^+); Anal. Calcd for $C_{16}H_{15}N_3O_4S_2$: C, 50.92; H, 4.00; N, 11.10. Found: C, 50.72; H, 3.83; N, 10.97.

2,10-Dihydro-10-hydroxy-3*H*-imidazo[1,2-*b*][1,2,4]benzothiadiazine 5,5-Dioxide (3a)

The compound (9a) (3.0 g, 7.96 mmol) was dissolved in acetic acid (80 ml) and treated with zinc dust (2.5 g, 38 mmol) in an ice bath for 30 min. The mixture was subsequently allowed to stir at room temperature for further 30 min and then was evaporated *in vacuo* to an oily residue. To the residue was added ethanol (15 ml) and the mixture was evaporated to dryness *in vacuo* at 50 °C to remove excess amount of acetic acid. The resulting white solid was then collected and the crude product was dissolved in ethanol (20 ml) and then treated with 0.1 N EDTA (5 ml) to furnish needle crystals of 3a (1.51 g, 79%), and 2,3-dihydro-1*H*-imidazo[1,2-b][1,2,4]benzo-thiadiazine 5,5-dioxide (11a, 0.12 g, 6.8%) was isolated from the filtrate. However, conducting reaction for 2 days resulted in exclusive formation of 11a (1.59 g, 83%). Compound 3a: mp 195 °C (ethanol); uv λ_{max} nm (ε x 10^4): (MeOH) 275 (1.3); (pH 1) 252 (1.4); (pH 13) 296 (1.3); ^{1}H nmr (300 MHz, DMSO-d_6): δ 3.85 (m, 2H, CH_2), 3.97 (m, 2H, CH_2), 7.25 (t, *J*=7.7 Hz, 1H, Ar-H), 7.47 (d, *J*=8.6 Hz, 1H, Ar-H), 7.75 (m, 1H, Ar-H), 7.83 (d, *J*=8.6 Hz, 1H, Ar-H); ^{13}C nmr (75 MHz, DMSO-d_6): δ 44.01, 51.11, 113.41, 120.70, 122.28, 122.72, 135.16, 138.53, 149.59; Ms: m/z 239 (M^+), 223(M^+-16). Anal. Calcd for $C_9H_9N_3O_3S$: C, 45.18; H, 3.79; N, 17.56. Found: C, 45.18; H, 3.81; N, 17.48. Compound 11a: mp 267 °C (ethanol); ms: m/z 223 (M^+), 166, 158; ^{1}H nmr (300 MHz, DMSO-d_6): δ 3.57 (t, *J*=7.9 Hz, 2H, CH_2), 4.03 (t, *J*=7.9 Hz, 2H, CH_2), 7.17 (t, *J*=8.2 Hz, 2H, Ar-H), 7.57 (t, *J*=7.8 Hz, 1H, Ar-H), 7.78 (d, *J*=7.8 Hz, 1H, Ar-H), 8.23 (s, 1H, NH); ^{13}C nmr (75 MHz, DMSO-d_6): δ 39.55, 41.78, 122.46, 122.87, 123.34, 125.51, 134.94, 146.37, 155.10. Anal. Calcd for $C_9H_9N_3O_2S$: C, 48.42; H, 4.06; N, 18.82. Found: C, 48.78; H, 4.03; N, 18.95.

2-Benzylthio-1,4,5,6-tetrahydropyrimidine Hydrobromide (6b)

The compound (6b) was prepared in 97% yield according to a procedure similar to that of 6a. An analytical sample was recrystallized from acetone, mp 152 °C. ^{1}H nmr (100 MHz, DMSO-d_6): δ 1.78 (t, *J*=5.6 Hz, 2H, CH_2), 3.34 (t, *J*=5.8 Hz, 4H, 2 CH_2), 4.58 (s, 2H, CH_2), 7.34 (s, 5H, Ar-H), 9.98 (br s, 2H, NH+HBr); Ms: m/z, 206 (M^+). Anal. Calcd for $C_{11}H_{15}N_2BrS$: C, 45.99; H, 5.26; N, 9.75. Found: C, 46.16; H, 5.29; N, 9.75.

1-(2-Nitrophenylsulfonyl)-2-benzylthio-1,4,5,6-tetrahydropyrimidine (9b)

The compound (9b) was prepared in 97% yield according to a procedure similar to that of 9a. An analytical sample was prepared by recrystallization from ethanol. mp 85 °C (decomp.). ^{1}H nmr (100 MHz, DMSO-d_6): δ 1.83 (m, 2H, CH_2), 3.48 (m, 2H, CH_2), 3.54 (m, 2H, CH_2), 4.05 (s, 2H, CH_2), 7.20 (s, 5H, Ar-H), 7.81-8.00 (m, 4H, Ar-H); ^{13}C nmr (25 MHz, DMSO-d_6): δ 22.50, 35.39, 46.05, 46.35,

124.69, 126.70, 127.97, 128.55, 129.79, 131.66, 132.60, 135.00, 136.81, 145.25, 146.96; ms: m/z 391 (M^+). Anal. Calcd for $C_{17}H_{17}N_3O_4S_2$: C, 52.16; H, 4.38; N, 10.74. Found: C, 52.14; H, 4.42; N, 10.93.

2,3,4,11-Tetrahydro-11-hydroxypyrimido[1,2-*b*][1,2,4]benzothiadiazine 6,6-Dioxide (3b)

Reduction of 9b under the conditions similar to the case of 3a afforded 3b and 1,2,3,4-tetrahydropyrimido[1,2-b][1,2,4]benzothiadiazine 6,6-dioxide (11b) in 30% and 10% yields, respectively. However, it afforded exclusively 11b in 62% yield when the reaction was run for 2 days. Compound 3b: mp 180-182 °C (ethanol); uv λ_{max} nm($\varepsilon \times 10^4$): (MeOH) 278 (1.6); (pH 1) 263 (1.2); ^{1}H nmr (300 MHz, DMSO-d_6): δ 1.83-1.97 (m, 2H, CH_2), 3.43 (t, *J*=5.6 Hz, 2H, CH_2), 3.79 (t, *J*=5.6 Hz, 2H, CH_2), 7.22 (t, *J*=7.5 Hz, 1H, Ar-H), 7.56 (d, *J*=8.4 Hz, 1H, Ar-H), 7.67-7.85 (m, 2H, Ar-H).; ^{13}C nmr (75 MHz, DMSO-d_6): δ 21.36, 39.88, 41.84, 114.40, 121.32, 121.85, 134.59, 138.50, 141.46.; Ms: m/z 253 (M^+), 237 (M^+ -16), 172; Anal. Calcd. for $C_{10}H_{11}N_3O_3S$: C, 47.42; H, 4.38; N,16.59. Found: C, 47.50; H, 4.24; N, 16.54. Compound 11b: mp 245-248 °C (ethanol); ^{1}H nmr (300 MHz, DMSO-d_6): δ 1.95 (m, 2H, CH_2), 3.28 (t, *J*=5.8 Hz, 2H, CH_2), 3.80 (t, *J*=5.5 Hz, 2H, CH_2), 7.07 (t, *J*=7.9 Hz, 2H, Ar-H), 7.52 (t, *J*=7.7 Hz, 1H, Ar-H), 7.64 (d, *J*=7.7 Hz, 1H, Ar-H), 8.05 (br s, 1H, NH, D_2O exchangeable); ^{13}C nmr (75 MHz, DMSO-d_6): δ 21.64, 38.95, 41.95, 120.78, 120.97, 122.86, 124.11, 133.79, 145.02, 148.83; ms: m/z 237 (M^+), 209, 172, 155; Anal. Calcd for $C_{10}H_{11}N_3O_2S$: C, 50.62; H, 4.67; N, 17.71. Found: C, 50.41; H, 4.36; N, 17.53.

1-(2-Nitrobenzoyl)-2-benzylthio-2-imidazoline (10a)

To a solution of 6a (6 g, 22 mmol) in dichloromethane (150 ml) contained triethylamine (7 ml, 50 mmol) was added 2-nitrobenzoyl chloride (4 g, 22 mmol) and the mixture was allowed to stir at room temperature for 2 h. The mixture was then concentrated *in vacuo* to soild residue which was poured into a solution of ethanol (25 ml) and water (50 ml). The resulting white solid was collected by filtration and was recrystallized from ethanol to afford 10a (7.2 g, 96%). mp 131 °C ;^{1}H nmr (300 MHz, DMSO-d_6): δ 3.63 (br s, 2H, CH_2), 3.86 (t, *J*=8.4 Hz, 2H, CH_2), 4.23 (s, 2H, CH_2), 7.32-7.41 (m, 5H, Ar-H), 7.76 (t, *J*=7.5 Hz, 2H, Ar-H), 7.90 (t, *J*=7.2 Hz, 1H, Ar-H), 8.24 (d, *J*=8.4 Hz, 1H, Ar-H); ms: m/z 341 (M^+). Anal. Calcd for $C_{17}H_{15}N_3O_3S \cdot H_2O$: C, 59.81; H, 4.43; N, 12.31. Found: C, 59.73; H, 4.49; N, 12.29.

2,10-Dihydro-10-hydroxyimidazo[2,1-*b*]quinazolin-5(3*H*)-one (4a)

To a mixture of 10a (3 g, 8.8 mmol) in acetic acid (60 ml) in an ice bath was added zinc dust (2 g, 31 mmol) and the mixture was allowed to stir for 30 min. The temperature was then raised to room temperature and the mixture was stirred for further 30 min. The zinc dust was filtered and the filtrate was evaporated *in vacuo* to oily residue. To the residue was added acetone (5 ml) and ether (25 ml) to give crude solid (1.71 g, 96%) which was subjected to column chromatography (silica gel, 1.5 x 21 cm, chloroform/methanol=8/2) to isolate 4a (0.74 g, 42%). mp 230 °C; uv λ_{max} nm($\varepsilon \times 10^4$): (MeOH) 278 (1.0); (pH 13) 293 (1.1); ^{1}H nmr (400 MHz, DMSO-d_6): δ 3.77 (m, 2H, CH_2), 4.05 (m, 2H, CH_2), 4.33 (br s, 1H, OH), 7.17 (t, *J*=7.8 Hz, 1H, Ar-H), 7.45 (d, *J*=8.3 Hz, 1H, Ar-H), 7.67 (t,

J=7.8 Hz, 1H, Ar-H), 7.84 (d, *J*=7.8 Hz, 1H, Ar-H); ^{13}C nmr (75 MHz, DMSO-d_6): δ 42.4 (t) , 44.7 (t) , 113.3 (d), 115.7 (s), 125.8 (d), 127.3 (d), 136.0 (d), 139.4 (s), 151.0 (s), 156.9 (s) ; ms: m/z, 203 (M^+, 85%), 187 (M^+-16, 100%), 159. Anal. Calcd for $C_{10}H_9N_3O_2 \cdot H_2O$: C, 54.30; H, 5.01; N, 18.99. Found: C, 54.03; H, 4.95; N, 18.74.

1-(2-Nitrobenzoyl)-2-benzylthio-1,4,5,6-tetrahydropyrimidine (10b)

Compound (10b) was prepared in 97% yield using a procedure similar to that which afforded 10a. An analytical sample was prepared by recrystallization from ethanol. mp 100 °C (decomp.); ^{1}H nmr (400 MHz, DMSO-d_6): δ 1.82 (s, 2H, CH_2), 2.50 (t, 2H, CH_2), 3.56 (m, 2H, CH_2), 4.02 (s, 2H, CH_2), 7.23 (s, 5H, Ar-H), 7.60 (d, *J*=7.3 Hz, 1H, Ar-H), 7.71 (t, *J*=6.8 Hz, 1H, Ar-H), 7.75 (d, *J*=1.7 Hz, 1H, Ar-H), 7.83 (t, *J*=6.8 Hz, 1H, Ar-H), 8.21 (d, *J*=7.3 Hz, 1H, Ar-H); ^{13}C nmr (100.4 MHz, DMSO-d_6): δ 22.50, 35.34, 44.80, 46.41, 124.72, 126.78, 127.91, 128.14, 128.89, 131.00, 131.77, 134.89, 137.22, 144.90, 149.47, 166.13; ms: m/z 355 (M^+). Anal. Calcd for $C_{18}H_{17}N_3O_3S$: C, 60.83; H, 4.82; N, 11.82. Found: C, 60.83; H, 4.90; N, 11.87.

2,3,4,11-Tetrahydro-11-hydroxy-6*H*-pyrimido[2,1-*b*]quinazolin-6-one (4b)

To a solution of 10b (3.0 g, 8.44 mmol) in acetic acid (40 ml) in an ice bath was added zinc dust (2.0 g, 31 mmol) and allowed to stir for 20 min. The mixture was stirred at room temperature (25 °C) for additional 30 min. The mixture was then filtered by filtration to remove zinc dust and the filtrate was evaporated *in vacuo* to oily residue. To the oily residue was added ethanol (20 ml) and then evaporated again *in vacuo* repeating for several times to remove acetic acid. Finally, the oily residue was dissolved in ethanol (20 ml) and cooled at -20 °C. The solid was then collected by filtration to obtain 1.32 g (72%) of crude product. This crude product was recrystallized from a mixture of ethanol (15 ml) and water (15 ml) in the presence of 0.1N EDTA (3 ml) to obtain 4b (0.51 g, 28%). mp 240 °C. uv λ_{max} nm (ε x 10^4): (MeOH) 285 (1.4); (pH 13) 291 (1.1); ^{1}H nmr (400 MHz, DMSO-d_6): δ 1.95 (m, 2H, CH_2) , 3.41 (t, *J*=5.90 Hz, 2H, CH_2), 3.96 (t, *J*=5.90 Hz, 2H, CH_2), 7.23 (t, *J*=7.9 Hz, 1H, Ar-H), 7.74 (t, *J*=8.5 Hz, 1H, Ar-H), 7.87 (d, *J*=8.4 Hz, 1H, Ar-H), 7.96 (d, *J*=7.8 Hz, 1H, Ar-H), 8.89 (br s, 1H, N-OH, D_2O exchangeable); ^{13}C nmr (100 MHz, DMSO-d_6) : δ 19.1(t), 38.2 (t), 113.5 (s), 115.1 (d), 122.8 (d), 126.7 (d), 134.9 (d), 141.7 (s), 144.3 (s), 156.7.(s); ms: m/z 217 (M^+), 201 (M^+-16, 80%), 200 (M^+-17, 100%). Anal. Calcd for $C_{11}H_{11}N_3O_2$: C, 60.82; H, 5.10; N, 19.34. Found: C, 60.87; H, 5.06; N, 19.19.

2,3-Dihydroimidazo[2,1-*b*]quinazolin-5(1*H*)-one (12a)

The compound (12a) was prepared according to a known procedure approach.[19] mp 265-266 °C(ethanol) [lit.,[18] 266-268 °C]. Ms: m/z 187 (M^+); ^{1}H nmr (300 MHz, DMSO-d_6): δ 3.61 (t, *J*=8.0 Hz, 2H, CH_2), 4.09 (t, *J*=8.0 Hz, 2H, CH_2), 7.09 (t, *J*=7.8 Hz, 1H, Ar-H), 7.20 (d, *J*=8.2 Hz, 1H, Ar-H), 7.50 (m, 1H, Ar-H), 7.76 (s, 1H, NH), 7.88 (dd, *J*=1.3 Hz, *J*=7.8 Hz, 1H, Ar-H); ^{13}C nmr (100 MHz, DMSO-d_6): δ 46.47, 121.49, 125.86, 128.55, 130.01, 138.13, 155.45, 158.91, 164.48.

1,2,3,4-Tetrahydro-6*H*-pyrimido[2,1-*b*]quinazolin-6-one (12b)

The compound (12b) was prepared according to a known procedure approach.[19] mp 227-228 °C (ethanol) [lit.,[19] 227-229 °C]. ^{1}H nmr (300 MHz, DMSO-d_6): δ, 3.27 (t, *J*=5.7 Hz, 2H, CH_2), 3.38 (m, 2H, CH_2), 3.90 (t, *J*=5.9 Hz, 2H, CH_2), 6.99 (t, J=8.0 Hz, 1H, Ar-H), 7.10 (d, *J*=8.0 Hz, 1H, Ar-H), 7.48 (m, 1H, Ar-H), 7.67 (s, 1H, NH), 7.83 (d, *J*=6.4 Hz, 1H, Ar-H); ^{13}C nmr (100 MHz, DMSO-d_6): δ 19. 86, 38.37, 115.80, 120.53, 123.35, 126.18, 133.90, 149.80, 150.11, 161.39; ms: m/z 201 (M^+).

ACKNOWLEDGEMENT

The present work was supported by research grants (No. NSC 79-0412-B-016-87 & NSC 80-0412-B-016-75 to JWC and NSC 80-0412-B-016-143R to CHC) from National Science Council of the Republic of China.

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20. *In vitro* anticancer assay by the MTT method: Cells were taken from exponential phase cultures and were allowed to grow in a plate containing 96 wells using RPMI-1640 medium supplemented with 5% fetal bovine serum, 1mM glutamine and antibiotics penicillin and streptomycin at 37 °C in a CO_2 incubator. Cell suspensions were trypsinized and disaggregated. 3 x 10^3 Cells were inoculated into each well in 0.18 ml of medium, to which 0.02 ml of drug was added. After 4 days of culture, 0.1 mg of 3-4,5-dimethylthiazol-2,5-diphenyltetrazolium bromide was added to each well and the plate incubated for 4 h. The medium was then removed and 0.2 ml DMSO added to each well and the plates agitated for 10 min. The optical density of each well was measured at 545 nm test wavelength with a 690 nm reference wavelength using a Titertek Multiskan plate reader. Absorbance levelers from drug tested cells were corrected against untreated control values. The results were described in Table 1.

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Received, 9th November, 1992