# SYNTHESIS AND BIOLOGICAL EVALUATION OF 3-HYDROXYMETHYLPYRIMIDO[1,6-*c*][1,3]OXAZINE DERIVATIVES

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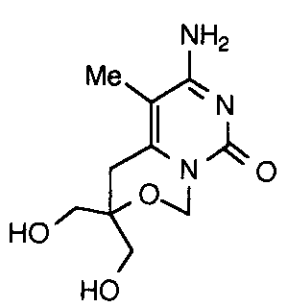
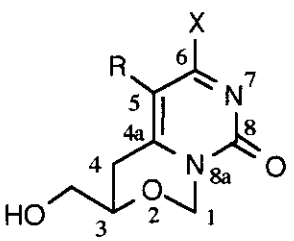
**Abstract**-A number of 5-substituted 3-hydroxymethylpyrimido[1,6-*c*][1,3]-oxazine derivatives were synthesized and evaluated for their biological activity. Compound (**10**) showed slight activity ($GI_{50}$ = 2.7 μM) against MDA-MB-231/ATTC Breast Cancer cell line.

Cyclonucleosides containing a bond from a ribose carbon to a purine or pyrimidine carbon have been known for over 30 years[1] and have served as probes[2-5] to study the relationship between substrate-inhibitor conformation and the specificities of the enzymes of nucleic acid biosynthesis. The class of acyclic nucleoside analogues, especially acyclic purine nucleoside analogues, comprises many compounds that possess significant and therapeutically useful antiherpesvirus activity.[6] Two of the most potent and selective antiviral representatives of this class are acyclovir[7-9] and ganciclovir[10]. Both compounds are acyclic guanine nucleosides. In constrast to these observations, acyclic pyrimidine nucleosides show




**I**




X = OH, $NH_2$
R = H, Cl, Br, I

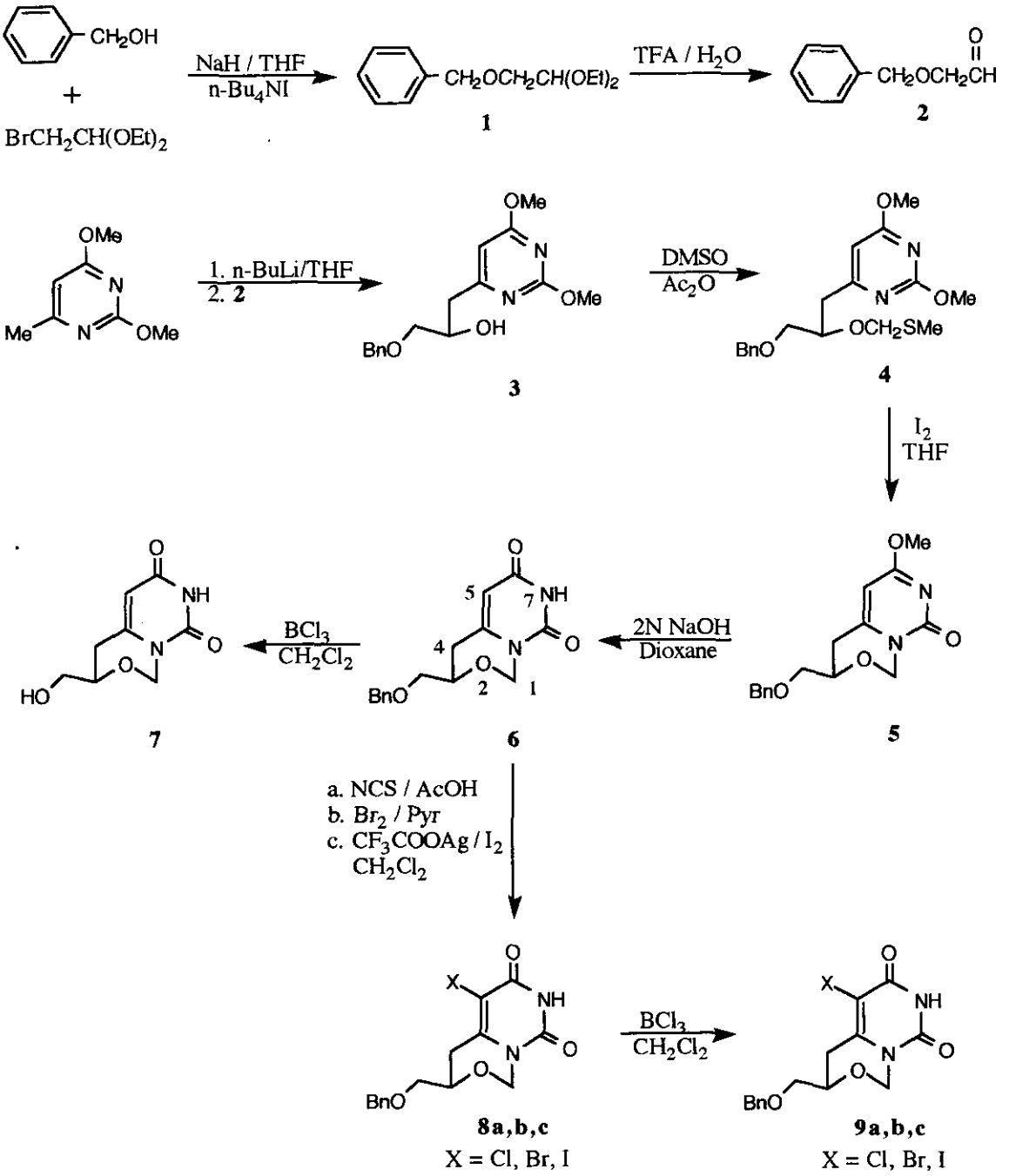
**II**

relatively low or even no significant biological activity. We have recently reported a series of anti-conformationally constrained carbon-bridged pyrimidine acyclic nucleosides and found 6-amino-3,3-bis(hydroxymethyl)-5-methyl-1*H*,2*H*,4*H*-pyrimido[1,6-*c*][1,3]oxazine-8-one (**I**) showed slight activity against HIV[11] and was not cytotoxic to a human T-cell line (CEM-SS cells) at 100 μM. This result prompted us to synthesize more conformationally restricted acyclic pyrimidine nucleosides (**II**) which contain a monohydroxymethyl group other than bishydroxymethyl group existed in compound (**I**). In this article we wish to report on the synthesis and biological activity of these newly designed anti restricted acyclic pyrimidine nucleosides (**II**).

## RESULTS AND DISCUSSION

Target compounds for the present study were prepared by the route designed through a combination and modification of several reported synthetic steps.[11-13] To prepare the pyrimidine intermediate (**3**), the C-6 side chain of pyrimidine was linked *via* an addition reaction of the lithiated 2,4-dimethoxy-6-methylpyrimidine with benzyloxyacetaldehyde (**2**). The 2,4-dimethoxy-6-methylpyrimidine was synthesized from 6-methyluracil by a known methods.[12] To prepare the acyclic sugar moiety, benzyloxyacetaldehyde (**2**), a condensation of benzyl alcohol with bromoacetaldehyde diethylacetal, followed by a deprotection of acetal group in acidic condition were accomplished. Treatment of 2,4-dimethoxy-6-methylpyrimidine with n-butyllithium in dry tetrahydrofuran at -70 °C gave the lithio derivative, which was reacted with **2** at -70 °C to afford **3** in 58% yield. To introduce a C-1 carbon of the acyclic moiety, the conversion of the hydroxy group of **3** to the corresponding methylthiomethyl ether derivative (**4**) was performed. This was accomplished by treating **3** with a mixture of acetic anhydride and anhydrous dimethyl sulfoxide at room temperature for 36 h. Ring closure of **4** was accomplished with iodine in dry tetrahydrofuran at room temperature to give **5**. Treatment of **5** with 2 N sodium hydroxide in dioxane at reflux overnight to give **6**. Debenzylation of **6** was achieved by using boron trichloride in dichloromethane at -70 °C to produce **7** (Scheme I). Halogenation of **6** with *N*-chlorosuccinimide in the presence of acetic acid, bromine water in pyridine, and iodine, silver trifluoroacetate in dichloromethane, respectively, afforded the corresponding 5-halo derivatives (**8a-c**). Deblocking the benzyl group of the 5-halo derivatives (**8a-c**) with boron trichloride in dichloromethane at -55 °C furnished **9a-c**. The cytosine derivative (**11**) (Scheme II) was obtained by a two-step conversion starting with a displacement of the 6-methoxy group of **5** using saturated methanolic ammonia at room

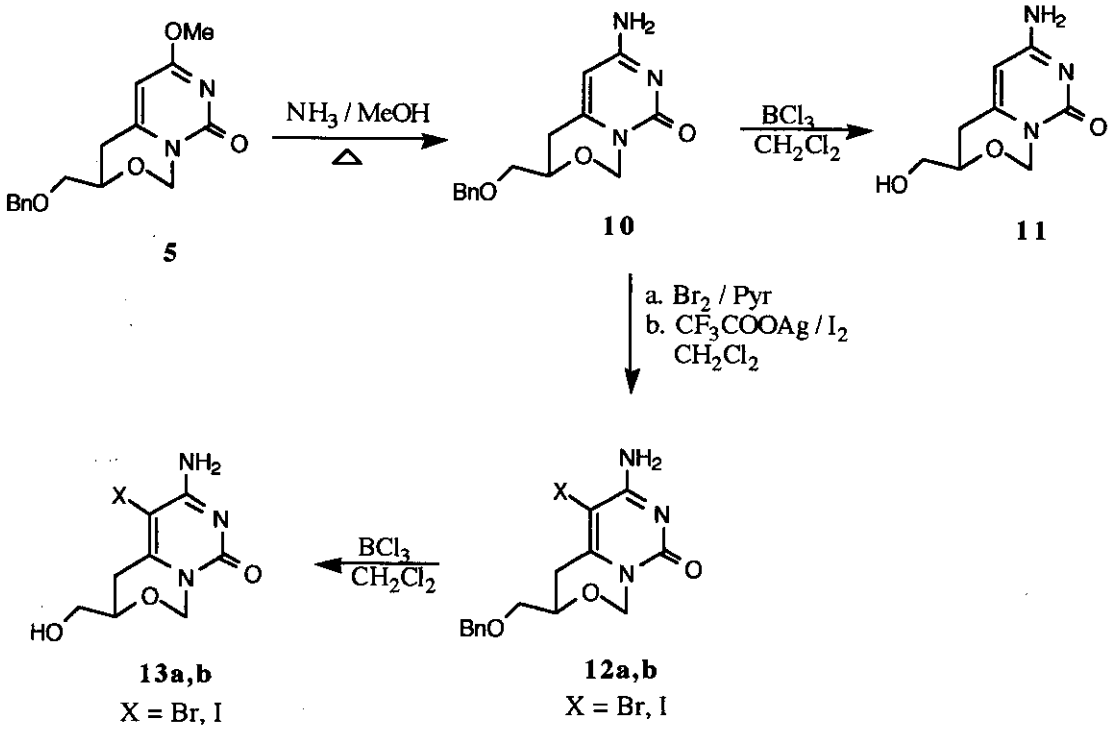
**Scheme I**




temperature, followed by a debenzylation with boron trichloride. The 5-bromo and 5-iodo cytosine derivatives were produced by a two-step reaction starting with halogenation of **10** with bromine water in

pyridine, and iodine, silver trifluoroacetate in dichloromethane, respectively, followed by a debenzylation with boron trichloride.

**Scheme II**




BILOGICAL STUDIES

Compounds (**6**, **7**, **8a**, **9a**, **10**, **11**, **12a**), and (**12b**) were selected by National Cancer Institute (NCI), USA and screened for their in vitro antiviral or antitumor activity[14,15] against human immunodeficiency virus (HIV) or 60 human tumor cell lines derived from nine clinically isolated cancer types (leukemia, lung, colon, brain, melanoma, ovarian, renal, prostate, breast). None of the tested compounds were active against HIV at the highest concentration tested (100 μM). Of the compounds tested, **6**, **10**, **12a**, and **13a** were found slightly active on two human tumor cell lines (UO-31, and MDA-MB-231/ATTC). Compounds (**10**) and (**13a**) were able to inhibit the growth of MDA-MB-231/ATTC breast cancer cells at $GI_{50}$ concentration of 2.7 and 22 μM, respectively (Table 2). Compounds (**6**) and (**12a**) were also able

to inhibit the growth of renal cancer (UO-31) at $GI_{50}$ concentration of 54 and 65 μM, respectively. In this study, a novel series of 3-hydoxymethylpyrimido[1,6-*c*][1,3]oxazine derivatives were synthesized. Two of reasons for the lack of anti-HIV activity in these compound might be attributed to the susceptibility toward phosphorylation by cellular kinases to their triphosphate or the improper spatial position of the 5'-*hydroxy group mimicry*. However, compound (**10**) and (**13a**) showed slight activity against MDA-MB-231/ATTC Breast Cancer cell line and **6** and **12a** exhibited a marginal inhibition on Renal Cancer UO-31 cell line.

Table 1. Results of Antitumor Evaluations of 5-Substituted 3-hydroxymethyl-pyrimido[1,6-*c*][1,3]oxazine derivatives in the NCI in *vitro* Primary Screen

| | $GI_{50}$(μM) | | | |
|---|---|---|---|---|
| Compound<br>Cancer Cell Line | 6 | 10 | 12a | 13a |
| Reanal Cancer<br>(UO-31) | 54 | >100 | 65 | >100 |
| Breast Cancer<br>(MDA-MB-231/ATTC) | >100 | 2.7 | >100 | 22 |

All Compounds were tested in quadruplicate at five different concentrations ($10^{-4}$, $10^{-5}$, $10^{-6}$, $10^{-7}$, $10^{-8}$ M). Calculations were based upon the averaged values from all available tests for each compounds; standard errors averaged less than 10-15% of the respective means.

## EXPERIMENTAL SECTION

**General**. Melting Points were taken on a BUCHI 530 apparatus and are uncorrected. The silica gel used for chromatography was silica gel 60 70-230 mesh (E. Merck, Darmstadt, West Germany), tlc was performed on prescored DC-Alufolien Kieselgel $60F_{254}$ (E. Merk, Darmstart). Compounds were visualized by illuminating under UV light (254 nm). Evaporations were carried out at < 50 °C using a rotary evaporator at reduced pressure (water aspirator). Solvent ratios reported are v/v ratios. $^1$H Nmr spectra were obtained at Varian 300 nmr spectrometer. Where necessary, deuterium exchange, and

homonuclear decoupling experiments were used to obtained proton shift assignments. Ir spectra were recorded on a Perkin-Elmer 938G spectrophotometer. Uv spectra were obtained on a Shimadzu UV-160 spectrometer. Analytical samples were dried under reduced pressure at 78 °C in the presence of $P_2O_5$ for at least 12 h unless otherwise specified. Elemental analyses were obtained from Perkin-Elmer 2400.

**Benzyloxyacetalehyde diethylacetal (1).** To a suspension of sodium hydride (5.76 g, 120 mmol) in dry tetrahydrofuran (40 ml) was added benzyl alcohol (2.16 g, 20 mmol). The mixture was stirred at room temperature for 4 h and then tetrabutylammonium iodide (0.37 g, 1 mmol) and bromoacetaldehyde diethylacetal (3.94 g, 20 mmol) were added at 0 °C. The mixture was heated under reflux for 12 h and then concentrated under reduced pressure. Dichloromethane (60 ml) was added to the residue. The mixture was passaged through celite. The filtrate was washed with water several times, dried under anhydrous sodium sulfate, and concentrated. The residue was chromatographed on silica gel eluting with n-hexane/ehtyl acetate (9/1). The fractions (Rf = 0.28) were collected and concentrated under reduced pressure to give the oily product: 2.93 g (65%). $^1$H Nmr ($CDCl_3$): $\delta$ 1.22 (t, J = 7.1 Hz, 6H, $CH_3$), 3.54 (m, 4H, $CH_2$), 3.70 (m, 2H, $OCH_2$), 4.59 (S, 2H, $CH_2Ph$), 4.67 (t, J = 5.3 Hz, 1H, t-H), 7.34 (m, 5H, Ph); $^{13}$C Nmr ( DMSO-$d_6$): $\delta$ 15.9, 62.8, 7 1.1, 74.0, 101.7, 127.6, 128.2, 128.9, 138.64.

**Benzyloxyacetaldehyde (2)** A mixture of **1** (5.91 g, 30 mmol), trifluoroacetic acid (10 ml, 130 mmol), water (10 ml), and chloroform (10 ml) was stirred at room temperature for 1 h. The mixture was extracted with chloroform, and washed several times with water. The chloroform layer was separated, dried with anhydrous sodium sulfate, and filtered. The filtrate was concentrated under reduced pressure. The residue was chromatographed on silica gel eluting with n-hexane/ethyl acetate (5/1). The fractions (Rf = 0.53) were collected and concentrated under reduced pressure to give oily **2** (1.95 g, 44%). $^1$H Nmr ($CDCl_3$): $\delta$ 4.11 (s, 2H, $PhCH_2$), 4.64 (m, 2H, $OCH_2$), 7.37 (m, 5H, Ph), 9.73 (s, 1H, CHO); $^{13}$C Nmr (DMSO-$d_6$): $\delta$ 74.2, 75.8, 128.4, 128.6, 128.9, 129.3, 201.0. Ms (*m/z*): 149 ($M^+$). Anal. Calcd for $C_9H_{10}O_2$: C, 71.98; H, 6.71. Found: C, 71.64; H, 6.96.

**6-[2-[1-(Benzyloxy)-2-hydroxy]ethyl]methyl-2,4-dimethoxypyrimidine (3)** n-Butyllithium (1.5 M, 3.48 ml, 6 mmol) was added dropwise to a solution of 2,4-dimethoxy-6-methylpyrimidine (0.77 g , 5 mmol) in THF (10 ml) at -70 °C. The temperature was raised to -55 °C and the solution was stirred for 30 min. Compound (**2**) (0.82 g, 5.46 mmol) in THF (2.0 ml) was added dropwise to the solution and the stirring was continued for 2.5 h. The solution was neutralized with acetic acid to pH 7, then the temperature was raised to room temperature, and the solvent was removed. The residue was partitioned

between ethyl acetate and water. The organic layer was separated, dried over $Na_2SO_4$, and then the solvent was removed. The residue was chromatographed on silica gel (100 g, 7 x 12 cm column) and eluted with $CH_2Cl_2$ / MeOH = 9 / 1. The desired fractions were collected and concentrated *in vacuo* to give oily **3** (0.811 g, 58%). Rf: 0.23 (n-hexane / ethyl acetate = 9 / 1). $^1$H Nmr (DMSO-$d_6$): $\delta$ 2.59, 2.75 (m, 1H each, $CH_2$), 3.37 (m, 2H, $OCH_2$), 3.85 (s, 6H, $OCH_3$), 4.08 (m, 1H, t-H), 4.49 (s, 2H, $PhCH_2$), 4.88 (d, J = 5.6 Hz, 1H, OH, $D_2O$ exchangeable), 6.41 (s, 1H, H-5), 7.32 (m, 5H, Ph). Ms (*m/z*): 305 $(M+H)^+$. Anal. Calcd for $C_{16}H_{20}N_2O_4$: C, 63.14; H, 6.62; N, 9.20. Found: C, 63.86; H, 6.62; N, 9.57.

**6-[[2-[1-(Benzyloxy)-2-[(methylthiomethyloxy)ethyl]methyl-2,4-dimethoxypyrimidine (4)** Acetic anhydride (4 ml, 42 mmol) was added to a mixture of **3** (0.811 g, 2.8 mmol) in dry DMSO (4 ml). The solution was stirred at room temperature for 36 h. The solution was extracted with $CHCl_3$ and washed with brine and water. The extracted residue was dried over anhydrous $MgSO_4$, and concentrated to an oily residue. This residue was applied to a silica gel column which was eluted with n-hexane / AcOEt (6 / 1). The desired fractions were collected and concentrated *in vacuo* to give oily **4** (4.5 g, 44%). Rf: 0.20 (n-hexane / AcOEt = 5:1). $^1$H Nmr (DMSO-$d_6$): $\delta$ 1.85 (s, 3H, $SCH_3$), 2.79 (d, J = 7.0 Hz, 2H, $CH_2$), 3.55 (m, 2H, $OCH_2$), 3.86 (s, 6H, $OCH_3$), 4.32 (m, 1H, t-H), 4.49 (s, 2H, $PhCH_2$), 4.65 (s, 2H, $CH_2S$), 6.45 (s, 1H, H-5), 7.32 (m, 5H, Ph). Ms (*m/z*): 365 $(M+H)^+$. Anal. Calcd for $C_{18}H_{24}N_2O_4S$: C, 59.32; H, 6.64; N, 7.69. Found: C, 60.06; H, 6.94; N, 7.77.

**3-Benzyloxymethyl-6-methoxy-1*H*, 3*H*, 4*H*-pyrimido[1,6-*c*][1,3]oxazine (5)** Iodine (4.8 g, 38 mmol) was added into a mixture of **4** (18.6 g , 63.2 mmol) in dry THF (200 ml). The solution was stirred under argon at room temperature for 72 h. A 5% aq. sodium sulfite solution was added until the brown color of the mixture dissapeared and the resulting solution was extracted with $CH_2Cl_2$ . The combined extracts were washed with brine and water and then dried over $MgSO_4$. The $MgSO_4$ was removed by filtration and the solvent was evaporated. The resulting residue was chromatographed on silica gel and eluted with $CH_2Cl_2$ / AcOEt (1 / 1). The fractions (Rf: 0.34) were collected and concentrated *in vacuo* to give oily **5** (4.46 g, 23%). $^1$H Nmr ( DMSO-$d_6$): $\delta$ 2.87 (m, 2H, H-4), 3.56 (d, J = 4.6 Hz, 2H, $OCH_2$), 3.81 (s, 3H, $OCH_3$), 4.14 (m, 1H, t-H), 4.52 (s, 2H, $PhCH_2$), 5.07, 5.45 (d, J = 9.9 Hz, 1H each, H-1), 5.94 (s, 1H, H-5), 7.33 (m, 5H, Ph). Ms (*m/z*): 302 ($M^+$). Anal. Calcd for $C_{16}H_{18}N_2O_4$: C, 63.57; H, 6.00; N, 9.27. Found: C, 63.17; H, 5.97; N, 9.48.

**3-Benzyloxymethyl-1*H*, 3*H*, 4*H*, 7*H*-pyrimido[1,6-*c*][1,3]oxazine-6,8-dione (6)** A

solution of **5** (1.5 g, 5 mmol) in 2 N NaOH / dioxane (1 / 1, 40 ml, 40 mmol) was vigorously stirred under reflux for 14 h. The organic layer (upper layer) was neutralized by Dowex-X 2 ( $H^+$ form) to pH 7. The resin was removed by filtration and the filtrate was concentrated. The resulting residue was chromatographed on silica gel and eluted with $CH_2Cl_2$ / MeOH (9 / 1). The fractions were collected and concentrated *in vacuo* to give **6** (0.46 g, 32%). mp 165-166 °C (methanol). Rf = 0.17 ($CH_2Cl_2$ / EtOAc = 1 / 1). $^1$H Nmr (DMSO-$d_6$): $\delta$ 2.82 (m, 2H, H-4), 3.53 (d, J = 4.4 Hz, 2H, $OCH_2$), 4.13 (m, 1H, t-H), 4.52 (s, 2H, $PhCH_2$), 5.05, 5.36 (d, J = 9.3 Hz, 1H each, H-1), 5.52 (s, 1H, H-5), 7.34 (m, 5H, Ph), 11.26 (s, 1H, NH $D_2O$ exchangeable). $^{13}$C Nmr (DMSO-$d_6$): $\delta$ 28.1, 71.6, 72.1, 72.3, 72.8, 99.8, 127.9, 128.6, 138.5, 150.2, 151.7, 163.1. Ms (*m/z*): 288 ($M^+$). Anal. Calcd for $C_{15}H_{17}N_2O_4$: C, 62.27; H, 5.92; N, 9.68. Found: C, 62.61; H, 5.81; N, 9.34.

**3-Hydroxymethyl-1*H*, 3*H*, 4*H*, 7*H*-pyrimido[1,6-*c*][1,3]oxazine-6,8-dione (7)** A mixture of **6** (347 mg, 1.2 mmol) in $CH_2Cl_2$ (10 ml) was cooled to -78 °C. Boron trichloride (1 M in $CH_2Cl_2$, 1.8 ml, 1.8 mmol) was added via syringe and under argon gas. The mixture was stirred at -78 °C for 3 h then the temperature was raised to -40 °C. A solution of MeOH / $CH_2Cl_2$ (1 : 1, 15 ml) was added, and the cooling bath was removed. The solution was neutralized with methanolic ammonia. After filtration, the solvent was removed under reduced pressure. The residue was dissolved in a small amount of MeOH and then passed through a filter. The filtrate was concentrated to give **7** (150 mg, 63%). Pure **7** was obtained after crystalization with methanol. mp 203-204 °C. Rf = 0.32 ($CH_2Cl_2$ / MeOH = 5 / 1). $^1$H Nmr (DMSO-$d_6$): $\delta$ 2.77 (m, 2H, H-4), 3.45 (m, 2H, $CH_2O$), 3.91 (m, 1H, t-H), 5.00 (t, J = 5.4 Hz, 1H, OH $D_2O$ exchangeable), 5.04, 5.35 (d, J = 9.3 Hz, 1H each, H-1), 5.52 (s, 1H, H-5), 11.25 (s, 1H, NH, $D_2O$ exchangeable). $^{13}$C Nmr (DMSO-$d_6$): $\delta$ 28.0, 63.3, 72.2, 73.9, 99.8, 150.3, 152.1, 163.1. Ms (*m/z*): 198 ($M^+$). Anal. Calcd for $C_8H_{10}N_2O_4$: C, 48.49; H, 5.09; N, 14.14. Found: C, 48.82; H, 5.12; N, 13.99.

**3-Benzyloxymethyl-5-chloro-1*H*, 3*H*, 4*H*, 7*H*-pyrimido[1,6-*c*][1,3]oxazine-6,8-dione (8a)** A mixture of compound (**6**) (289 mg, 1 mmol) and *N*-chlorosuccinimide (200 mg, 1.5 mmol) in glacial acetic acid (7 ml) was heated at 55 °C with stirring for 4 h. The solution was evaporated in *vacuo* to dryness. The residue was coevaporated with methanol (3 x 10 ml) and was added methanolic ammonia and stirred overnight. The solvent was removed and crystalization with methanol to give **8a**, 73 mg. The filtrate was evaporated and the residue was chromatographed with $CHCl_3$ / MeOH (9 / 1). The fractions were collected and concentrated *in vacuo* to give more 143 mg of **8a** (total yield: 45%). mp 133-134 °C

(methanol). Rf: 0.44 ($CHCl_3$/MeOH, 9/1). $^1$H Nmr(DMSO-$d_6$): $\delta$ 2.73, 2.95 (m, 1H each, H-4), 3.62 (m, 2H, $CH_2O$), 4.13 (m, 1H, t-H), 4.53 (s, 2H, $PhCH_2$), 4.95, 5.48 (d, J = 9.3 Hz, 1H each, H-1), 7.34 (m, 5H, Ph), 11.86 (s, 1H, NH, $D_2O$ exchangeable). $^{13}$C Nmr (DMSO-$d_6$): $\delta$ 29.90, 71.44, 72.45, 72.80, 74.29, 105.50, 127.92, 128.66, 138.44, 148.09, 148.97, 158.87, . Ms (*m/z*): 322 ($M^+$). Anal. Calcd for $C_{15}H_{15}N_2O_4Cl$: C, 55.82; H, 4.68; N, 8.68. Found: C, 55.57; H, 4.84; N, 8.79.

**3-Benzyloxymethyl-5-bromo-1*H*, 3*H*, 4*H*, 7*H*-pyrimido[1,6-*c*][1,3]oxazine-6,8-dione (8b)** Bromine water (10 ml) was added to a solution of **6** (289 mg, 1 mmol) in pyridine (50 ml) and the mixture was stirred at room temperature for 8 h. The solvent was removed under reduced pressure. The solid was crystallized from methanol to give **8b** (300 mg, 48%). mp 155-156 °C. Rf: 0.35 ($CH_2Cl_2$ / AcOEt, 4/1). $^1$H Nmr (DMSO-$d_6$): $\delta$ 2.72, 2.93 (m, 1H each, H-4), 3.60 (d, j = 4.9 Hz, 2H, $CH_2O$), 4.13 (m, 1H, t-H), 4.53 (s, 2H, $PhCH_2$), 4.95, 5.47 (d, J = 9.1 Hz, 1H each, H-1), 7.33 (m, 5H, Ph), 11.83 (s, 1H, NH, $D_2O$ exchangeable). $^{13}$C Nmr (DMSO-$d_6$): $\delta$ 29.57, 71.40, 72.70, 72.80, 74.36, 96.44, 106.60, 127.92, 128.67, 128.85, 138.44, 149.22, 149.60, 159.07. Ms (*m/z*): 367 ($M^+$). Anal. Calcd for $C_{15}H_{15}N_2O_4Br$: C, 49.06; H, 4.12; N, 7.63. Found: C, 49.22; H, 4.17; N, 7.38.

**3-Benzyloxymethyl-5-iodo-1*H*, 3*H*, 4*H*, 7*H*-pyrimido[1,6-*c*][1,3]oxazine-6,8-dione (8c)** To a stirred mixture of **6** (289 mg, 1 mmol) and silver trifluoroacetate (221 mg, 1 mmol) in dichloromethane (13 ml) was added dropwise a solution of iodine (170 mg, 1.33 mmol) in 5 ml of dichloromethane at 0 °C (ice bath). The mixture was stirred at room temperature for 2 h. The suspension was filtered and the solvent of filtrate was removed under reduced pressure. The residue was chromatographed with $CH_2Cl_2$ / AcOEt (2 / 1). The fractions were collected and concentrated *in vacuo* to give **8c** (169 mg, 41%). mp 164-165 °C (methanol). Rf: 0.32 ($CH_2Cl_2$/AcOEt, 2/1). $^1$H Nmr (DMSO-$d_6$): $\delta$ 2.73, 2.90 (m, 1H each, H-4), 3.60 (d, J = 4.1 Hz, 2H, $CH_2O$), 4.12 (m, 1H, t-H), 4.53 (s, 2H, $PhCH_2$), 4.95, 5.44 (d, J = 9.4 Hz, 1H each, H-1), 7.34 (m, 5H, Ph), 11.72 (s, 1H, NH, $D_2O$ exchangeable). $^{13}$C Nmr (DMSO-$d_6$): $\delta$ 34.19, 71.38, 72.80, 73.27, 74.33, 106.18, 127.93, 128.00, 128.67, 138.45, 149.66, 152.21, 160.58. Ms (*m/z*): 414 ($M^+$). Anal. Calcd for $C_{15}H_{15}N_2O_4I$: C, 43.50; H, 3.65; N, 6.76. Found: C, 43.60; H, 3.65; N, 6.42.

**3-Hydroxymethyl-5-cholo-1*H*, 3*H*, 4*H*, 7*H*-pyrimido[1,6-*c*][1,3]oxazine-6,8-dione (9a)** A mixture of **8a** (103 mg, 0.9 mmol) in dichlomethane (15 ml) was cooled in an ice-bath. Boron trichloride (1 M in $CH_2Cl_2$, 1.4 ml, 1.4 mmol) was added via syringe and under nitrogen gas. The mixture was stirred in an ice-bath for 3 h. A solvent mixture of methanol / methylene chloride (1 / 1, 10

ml) was added and the cooling bath was removed. The solution was neutralized with saturated methanolic ammonia and then evaporated under reduced pressure. Methanol (40 ml) was added to the residue. The precipitate was collected after filtration and crystallized with methanol to give **9a** (35 mg, 16%). mp 181-182 °C. Rf: 0.15 ($CHCl_3$/MeOH, 9/1). $^1$H Nmr (DMSO-$d_6$): $\delta$ 2.72, 2.89 (m, 1H each, H-4), 3.53 (m, 2H, $CH_2O$), 3.91 (m, 1H, t-H), 4.94, 5.47 (d, J = 9.0 Hz, 1H each, H-1), 5.04 (t, J = 5.4 Hz, 1H, OH), 11.82 (s, 1H, NH, $D_2O$ exchangeable). $^{13}$C Nmr (DMSO-$d_6$): $\delta$ 26.96, 63.08, 74.18, 74.36, 106.61, 148.41, 149.00, 158.89. Ms (*m/z*): 233 ($M^+$). Anal. Calcd for $C_8H_9N_2O_4Cl$: C, 41.30; H, 3.90; N, 12.05. Found: C, 41.00; H, 4.00; N, 11.94.

**3-Hhydroxymethyl-5-bromo-1*H*, 3*H*, 4*H*, 7*H*-pyrimido[1,6-*c*][1,3]oxazine-6,8-dione (9b) and 3-hydroxymethyl-5-iodo-1*H*, 3*H*, 4*H*, 7*H*-pyrimido[1,6-*c*][1,3]oxazine-6,8-dione (9c)** Compound (**9b**) and (**9c**) were prepared in a maner similar to the preparation of **9a**.

**9b**. Reagents: **8b** (160 mg, 0.43 mmol), boron trichloride (1M, 0.64 ml, 0.64 mmol). Yield (98 mg, 83%). mp 188-189 °C (methanol). Rf: 0.17 ($CHCl_3$/MeOH, 9/1). $^1$H Nmr (DMSO-$d_6$): $\delta$ 2.65, 2.87 (m, 1H each, H-4), 3.51 (m, 2H, $CH_2O$), 3.91 (m, 1H, t-H), 4.94, 5.46 (d, J = 9.2 Hz, 1H each, H-1), 5.04 (t, J = 5.4 Hz, 1H, OH, $D_2O$ exchangeable), 11.81 (s, 1H, NH, $D_2O$ exchangeable). $^{13}$C Nmr (DMSO-$d_6$): $\delta$ 29.58, 63.06, 74.43, 74.49, 96.43, 149.27, 149.93, 159.10. Ms (*m/z*): 277 ($M^+$). Anal. Calcd for $C_8H_9N_2O_4Br$: C, 34.68; H, 3.27; N, 10.11. Found: C, 34.96; H, 3.00; N, 10.46.

**9c**. Reagents: **8c** (104 mg, 0.25 mmol), boron trichloride (1M, 0.38 ml, 0.38 mmol). Yield (57 mg, 83%). mp 177-179 °C (methanol). Rf: 0.18 ($CHCl_3$/MeOH, 9/1). $^1$H Nmr (DMSO-$d_6$): $\delta$ 2.66, 2.85 (m, 1H each, H-4), 3.53 (m, 2H, $CH_2O$), 3.90 (m, 1H, t-H), 4.95, 5.43 (d, J = 9.0 Hz, 1H each, H-1), 5.07 (t, J = 5.4 Hz, 1H, OH, $D_2O$ exchangeable), 11.82 (s, 1H, NH, $D_2O$ exchangeable). $^{13}$C Nmr (DMSO-$d_6$): $\delta$ 34.22, 63.02, 74.50, 75.02, 128.28, 149.70, 152.54, 160.60. Ms (*m/z*): 324 ($M^+$). Anal. Calcd for $C_8H_9N_2O_4Br$: C, 29.65; H, 2.80; N, 8.64. Found: C, 29.37; H, 3.03; N, 8.49.

**6-Amino-3-benzyloxymethyl-1*H*, 3*H*, 4*H*-pyrimido[1,6-*c*][1,3]oxazine (10)** A mixture of **5** (1 g, 3.2 mmol) in saturated methanolic ammonia (70 ml) was heated at 120 °C for 14 h in a sealed tube. The solution was cooled to 0 °C and the precipitate was collected by filtration and crystallized with methanol to obtain **10** (661 mg, 71%). mp 248-249 °C (methanol). Rf: 0.18 ($CHCl_3$/MeOH, 9/1). $^1$H Nmr (DMSO-$d_6$): $\delta$ 2.75 (m, 2H, H-4), 3.53 (m, 2H, $CH_2O$), 4.08 (m, 1H, t-H), 4.53 (s, 2H, $PhCH_2$), 4.96, 5.36 (d, J = 8.2 Hz, 1H each, H-1), 5.55 (s, 1H, H-5), 6.96, 7.04 (s, 1H each, $NH_2$, $D_2O$ exchangeable), 7.34 (m, 5H, Ph). $^{13}$C Nmr (DMSO-$d_6$): $\delta$ 28.18, 71.17, 72.73, 73.42, 73.48,

92.16, 127.89, 127.93, 138.50, 151.68, 155.17, 165.65. Ms (*m/z*): 287 ($M^+$). Anal. Calcd for $C_{15}H_{17}N_3O_3$: C, 62.70; H, 5.96; N, 14.62. Found: C, 62.67; H, 5.87; N, 14.57.

**6-Amino-3-hydroxymethyl-1*H*, 3*H*, 4*H*-pyrimido[1,6-*c*][1,3]oxazine (11)** Compound (**11**) was prepared in a maner similar to the preparation of **9a**. Reagents: **10** (287 mg, 1 mmol), boron trichloride (1M, 1.5 ml, 1.5 mmol). Yield (126 mg, 64%). mp 267-268 °C (methanol). Rf: 0.13 ($CHCl_3$/MeOH, 4/1). $^1$H Nmr (DMSO-$d_6$): $\delta$ 2.82 (m, 2H, H-4), 3.48 (m, 2H, $CH_2O$), 3.78 (brs, 1H, OH, $D_2O$ exchangeable), 3.94 (m, 1H, t-H), 5.08, 5.45 (d, J = 9.4 Hz, 1H each, H-1), 5.60 (s, 1H, H-5), 8.56, 9.69 (s, 1H each, $NH_2$, $D_2O$ exchangeable). $^{13}$C Nmr (DMSO-$d_6$): $\delta$ 28.41, 62.99, 73.57, 73.72, 92.12, 146.67, 157.68, 158.74. Ms (*m/z*): 197 ($M^+$). Anal. Calcd for $C_8H_{11}N_3O_3$: C, 48.73; H, 5.62; N, 21.31. Found: C, 48.65; H, 5.44; N, 21.01.

**6-Amino-3-benzyloxymethyl-5-bromo-1*H*, 3*H*, 4*H*-pyrimido[1,6-*c*][1,3]oxazine (12a)** Compound (**12a**) was prepared in a maner similar to the preparation of **8b**. Reagents: **10** (350 mg, 1.28 mmol) in pyridine (60 ml), Bromine water (12 ml). Yield (190 mg, 50%). mp 188-189 °C (methanol). Rf: 0.49 ($CHCl_3$/MeOH, 9/1). $^1$H Nmr (DMSO-$d_6$): $\delta$ 2.76 (m, 2H, H-4), 3.61 (m, 2H, $CH_2O$), 4.10 (m, 1H, t-H), 4.53 (s, 2H, $PhCH_2$), 4.88, 5.45 (d, J = 9.3 Hz, 1H each, H-1), 6.93, 7.69 (s, 1H each, $NH_2$, $D_2O$ exchangeable), 7.34 (m, 5H, Ph). $^{13}$C Nmr (DMSO-$d_6$): $\delta$ 29.52, 71.45, 72.33, 72.79, 75.11, 87.20, 127.92, 127.96, 128.16, 128.68, 138.47, 150.54, 153.43, 162.00. Ms (*m/z*): 366. ($M^+$). Anal. Calcd for $C_{15}H_{16}N_3O_3Br$: C, 49.20; H, 4.40; N, 11.47. Found: C, 48.94; H, 4.32; N, 11.21.

**6-Amino-3-benzyloxymethyl-5-iodo-1*H*, 3*H*, 4*H*-pyrimido[1,6-*c*][1,3]oxazine (12b)** Compound (**12b**) was prepared in a maner similar to the preparation of **8c**. Reagents: **10** (287 mg, 1mmol) and silver trifluoroacetate (221 mg, 1 mmol) in methylene chloride (15 ml), iodine (170 mg, 1.33 mmol) in methylene chloride (5 ml). Yield (285 mg, 69%). mp 165-166 °C (methanol). Rf: 0.44 ($CHCl_3$/MeOH, 9/1). $^1$H Nmr (DMSO-$d_6$): $\delta$ 2.66, 2.84 (m, 1H each, H-4), 3.59 (m, 2H, $CH_2O$), 4.09(m, 1H, t-H), 4.54 (s, 2H, $PhCH_2$), 4.88, 5.43 (d, J = 9.4 Hz, 1H each, H-1), 6.55, 7.68 (s, 1H each, $NH_2$, $D_2O$ exchangeable), 7.35 (m, 5H, Ph). $^{13}$C Nmr (DMSO-$d_6$): $\delta$ 34.28, 71.41, 72.77, 72.98, 75.13, 127.92, 127.98, 128.68, 138.50, 153.28, 154.05, 164.17 Ms (*m/z*): 413 ($M^+$). Anal. Calcd for $C_{15}H_{16}N_3O_3I$: C, 43.60; H, 3.90; N, 10.17. Found: C, 43.79; H, 3.88; N, 9.96.

**6-Amino-3-hydroxymethyl-5-bromo-1*H*, 3*H*, 4*H*-pyrimido[1,6-*c*][1,3]oxazine (13a) and**

**6-Amino-3-hydroxymethyl-5-iodo-1*H*, 3*H*, 4*H*-pyrimido[1,6-*c*][1,3]oxazine (13b)**

Compound (**13a**) and (**13b**) were prepared in a maner similar to the preparation of **9a**.

**13a**. Reagents: **12a** (200 mg, 0.55 mmol) in methylene chloride (15 ml), boron trichloride (1 M, 0.82 ml, 0.82 mmol). Yield (132 mg, 87%). $^1$H Nmr (DMSO-d$_6$): $\delta$ 2.62, 2.80 (m, 1H each, H-4), 3.53 (m, 2H, $CH_2O$), 3.88 (m, 1H, t-H), 4.87, 5.44 (d, J = 9.2 Hz, 1H each, H-1), 5.04 (t, J = 5.6 Hz, 1H, OH, $D_2O$ exchangeable), 6.91, 7.67 (s, 1H each, $NH_2$, $D_2O$ exchangeable). $^{13}$C Nmr (DMSO-d$_6$): $\delta$ 29.48, 63.07, 74.06, 75.21, 87.24, 150.84, 153.50, 162.00 Ms (*m/z*): 276 ($M^+$). Anal. Calcd for $C_8H_{10}N_3O_3Br$: C, 34.80; H, 3.65; N, 15.22. Found: C, 35.09; H, 3.57; N, 14.94.

**13b**. Reagents: **12b** (200 mg, 0.48 mmol) in methylene chloride (15 ml), boron trichloride (1 M, 0.75 ml, 0.75 mmol). Yield (132 mg, 87%). $^1$H Nmr (DMSO-d$_6$): $\delta$ 2.80, 2.88 (m, 1H each, H-4), 3.57 (m, 2H, $CH_2O$), 3.88 (m, 2H, t-H, OH, $D_2O$ exchangeable), 4.98, 5.51 (d, J = 9.2 Hz, 1H each, H-1), 8.49, 9.04 (s, 1H each, $NH_2$, $D_2O$ exchangeable). $^{13}$C Nmr (DMSO-d$_6$): $\delta$ 34.28, 62.07, 71.41, 72.77, 72.98, 75.13, 127.92, 127.98, 128.68, 138.50, 153.28, 154.05, 164.17. Ms (*m/z*): 323 ($M^+$). Anal. Calcd for $C_8H_{10}N_3O_3I$: C, 29.74; H, 3.12; N, 13.01. Found: C, 29.36; H, 3.01; N, 12.94.

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