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Synthesis and Flow Cytometric Evaluation of Novel 1,2,3,4-Tetrahydroisoquinoline Conformationally Constrained Analogues of Nitrobenzylmercaptopurine Riboside (NBMPR) Designed for Probing Its Conformation When Bound to the *es* Nucleoside Transporter

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Novel regioisomers of conformationally constrained analogues of the potent *es* nucleoside transporter ligand, nitrobenzylmercaptopurine riboside (NBMPR), designed for probing its bound (bioactive) conformation, were synthesized and evaluated as *es* transporter ligands by flow cytometry. Purine 6-position 5, 6, 7, or 8-nitro-1,2,3,4-tetrahydroisoquinolylpurine ribosides, in which the nitrobenzyl moiety in NBMPR has been locked into the nitro-1,2,3,4-tetrahydroisoquinoline system, were synthesized by reaction of the appropriate nitro-1,2,3,4-tetrahydroisoquinoline with 6-chloropurine riboside. Flow cytometry was performed using 5-(SAENTA)-*X8*-fluorescein as the competitive ligand. A high degree of variation in the *es* transporter binding capacity of the target compounds was observed, with the K_i values ranging from 0.45 nM for the most tightly bound compound (**4**) to 300 nM for the least tightly bound compound (**5**). The K_i of NBMPR was 0.70 nM, a little higher than that of compound **4**. Compound **4** is the isomer that has the nitro group in the best orientation at the *es* transporter binding site compared to the other three compounds, **2**, **3**, and **5**.

Introduction

Nucleoside transporters are integral membrane glycoproteins, which are responsible for cellular trafficking of physiological nucleosides and their synthetic analogues.[1,2] They are grouped into two categories: (i) equilibrative transporters (most widely distributed) and (ii) concentrative (sodium ion-coupled) transporters (limited to absorptive and secretory tissues and some cultured cell lines). Two equilibrative transporters with similar broad substrate specificities have been identified and designated as *es* (*e*quilibrative *s*ensitive) and *ei* (*e*quilibrative *i*nsensitive) on the basis of their sensitivity or insensitivity to inhibition by *S*[6]-(4-nitrobenzyl)-mercaptopurine riboside (NBMPR, **1**), respectively. Nucleoside transport inhibitors (NTIs) have been shown to have potential therapeutic applications in heart disease and stroke as cardioprotective and neuroprotective agents, respectively,[3−7] in inflammatory disease,[8] and in antimetabolite chemotherapy as biological response modifiers.[9] The *es* transporter is by far the most important nucleoside transporter of most mammalian tissues, especially heart tissue,[10,11] and appears to be the most relevant NT target for therapeutic exploitation. Several chemical classes have been shown to inhibit the *es* nucleoside transporter,[12] the most selective of which are NBMPR and analogues, which inhibit it at low nanomolar concentrations.[1] The therapeutic application of the available *es* transporter inhibitors has been severely hampered by their poor pharmacological profiles having to do with toxicity, lack of in vivo efficacy, and/or lack of selectivity.[12] There is therefore a need for novel inhibitors.

In the absence of a 3D structure of any nucleoside transporter and for that matter its complex with inhibitors, knowledge of the bioactive conformation of the most potent and selective inhibitors will be useful for rational design of new inhibitors.[13] We have therefore embarked on using the conformationally constrained analogue strategy[14] to probe the bioactive conformation of NBMPR, the prototype *es* transporter inhibitor (K_d 0.1−1.0 nM), which is at present unknown. Current structure−activity relationships of *es* transporter inhibitors[12] indicate that for the NBMPR series, the nitrobenzyl moiety (shown in bold in compounds **1**−**5**) confers the high-affinity ligand binding capacity. This moiety is very flexible, with its conformational space involving torsions about the three bonds, *a*, *b*, and *c* (shown in compounds **1**−**5**), making that portion of the molecule the most conformationally variant. In our design, we envisioned that replacement of the purine 6-position substituent of NBMPR (**1**) with nitro-1,2,3,4-tetrahydroisoquinolines (**2**−**5**) will provide analogues in which the conformational flexibility of the nitrobenzyl group is severely restrained. Two of the rotatable bonds (*b* and *c*) in NBMPR have been fixed in the novel tetrahydroisoquinoline analogues (**2**−**5**). This constrains the high-affinity-conferring nitrobenzyl group of NBMPR into a 1,2,3,4-tetrahydroisoquinoline ring system. The most suitable position of the nitro substituent was explored by varying its position on the aromatic portion of the tetrahydroisoquinoline ring as shown in compounds **2**−**5**, where the nitro substituent is in the 5-, 6-, 7- or

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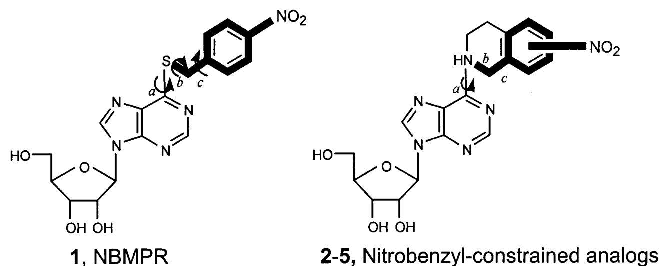
8-position of the 1,2,3,4-tetrahydrisoquinoline ring, respectively. In these tetrahydroisoquinoline analogues, the sulfur atom in NBMPR has of necessity been replaced by an NH. It is known that this replacement does not significantly decrease affinity.[12]

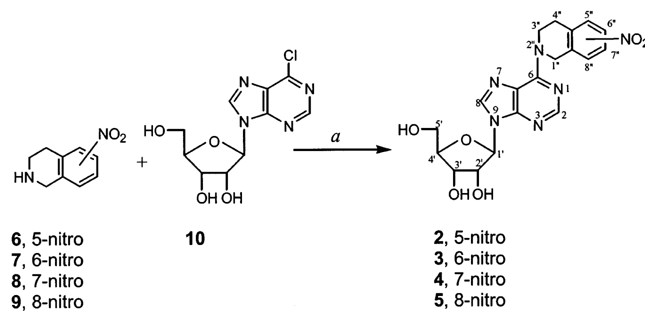


Chemistry

All four new aromatic nitro isomers of 6-position nitro-1,2,3,4-tetrahydoisoquinolinylpurine ribosides NBMPR analogues were synthesized. Several literature methods were employed to synthesize the required nitro-1,2,3,4-tetrahydoisoquinoline (compounds **6**–**9**), which were subsequently reacted with the commercially available 6-chloropurine riboside (**10**), according to reaction Scheme 1, to obtain the target compounds. There are few methods available for the synthesis of tetrahydroisoquinolines containing electron-withdrawing groups on the aromatic ring, which can be problematic.[15] The commercially available 5-nitroisoquinoline (**11**) was reduced using sodium borohydride in ethanol to afford the 5-nitro-1,2,3,4-tetrahydroisoquinoline intermediate (compound **6**) in good yield (90%) according to Scheme 2a.[16] The 7-nitro-1,2,3,4-tetrahydroisoquinoline intermediate (compound **8**) was prepared in 50% yield by nitration of the commercially available 1,2,3,4-tetrahydroisoquinoline using KNO_3 in concentrated sulfuric acid according to the method of Ajao and Bird[17] (see Scheme 2b). The 6- and 8-nitro-1,2,3,4-tetrahydroisoquinoline intermediates **7** and **9** were more difficult to prepare, but were obtained following reaction Scheme 3 according to the report by Tercel et al.[18]

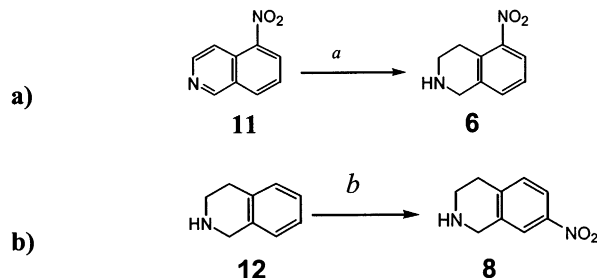
The 7-nitro-1,2,3,4-tetrahydroisoquinoline intermediate (**8**) used to synthesize compound **4** (see Scheme 2) was protected by acetylation to give compound **13**. The NO_2 group of **13** was reduced to an NH_2 group to obtain compound **14**. The NH_2 group of **14** was subsequently protected, and the resulting compound **15** was nitrated to afford the nitro compounds **16** and **17**. Interestingly, when the amount of KNO_3 in the reaction was increased to improve the yields of compounds **16** and **17**, the major product obtained was the dinitro compound, **22** (50%). Compounds **16** and **17** were deprotected to give the free NH_2 substituted compounds **18** and **19**. It must be pointed out that the deprotected compounds **18** and **19** were also obtained as minor products during the nitration of compound **15**. This has not previously been noted. Compounds **18** and **19** were subjected to diazotization using $NaNO_2$ and HCl and then treatment with H_3PO_2 to replace the NH_2 by H, to obtain compounds **20** and **21**. Compounds **20** and **21** were deprotected to obtain the required intermediates **7** and **9**. Compound **23**, which is the analogue of compounds **2**–**5** without the nitro substituent, was also synthesized and tested for comparison. Its synthesis was achieved by reacting compound **12** with compound **10** according to reaction Scheme 1.

Scheme 1[a]



[a] Reaction condition: (a) $CaCO_3$/ethanol.

Scheme 2[a]



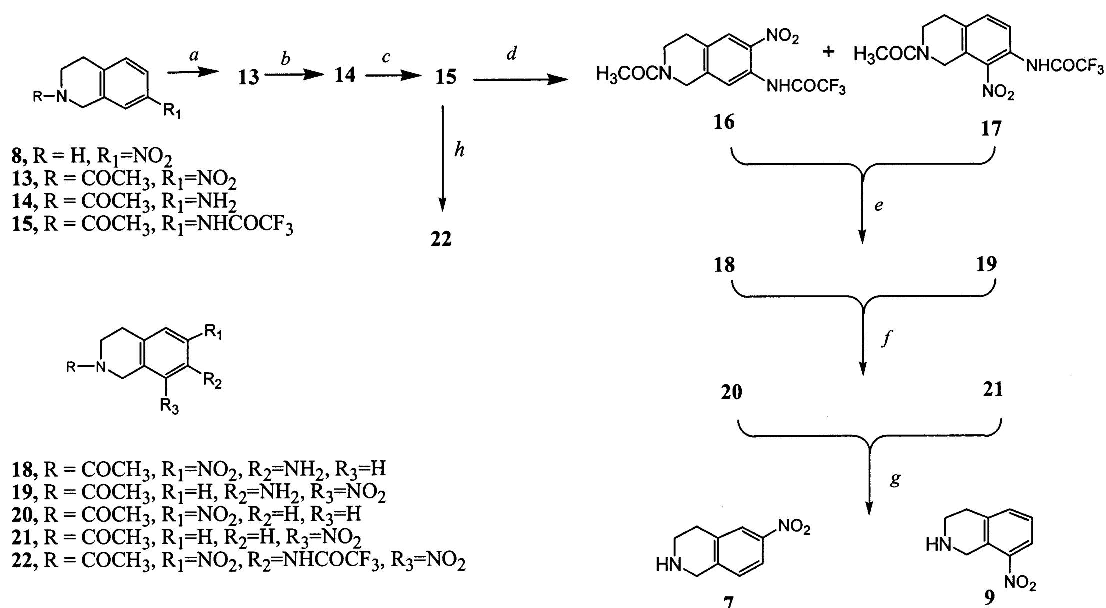
[a] Reaction conditions: (a) $NaBH_4$/EtOH, 0 °C, (b) KNO_3/concentrated H_2SO_4, 5 °C.

In attempts to find better routes for preparing the intermediates **7** and **9** because of their low yields, we tried to prepare compound **9** by the reaction route shown below (Scheme 4). However, the diazotization and replacement of the NH_2 group in compound **27** to obtain 8-nitroisoquinoline (compound **28**), which would have been subsequently reduced to give the desired compound **9**, was not successful. Instead, we obtained 5-hydroxyisoquinoline (compound **29**), the NMR data of which compared well with literature values.[19] To our knowledge, this is the first report of this kind of a result from a diazotization in which an NH_2 and a NO_2 group have both been replaced in one pot. Another unexpected result of the reaction is that the diazonium group was replaced by an OH group instead of a hydrogen atom. One would have expected a replacement with OH had the reaction been carried out in the presence of aqueous HCl, but not after treatment with H_3PO_2.

SAR

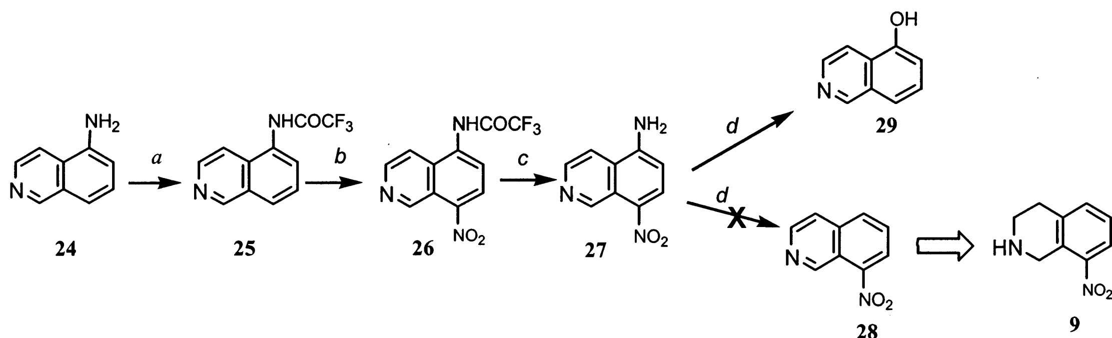
The target constrained NBMPR analogues (**2**–**5**) were tested along with 6-(1,2,3,4-tetrahydroisoquinolinyl)-purine riboside (**23**) as *es* transporter binding ligands by a facile competitive binding flow cytometric assay using the K562 chronic myelogenous leukemia cell line. The high-affinity *es* transporter fluorescent ligand, 5-(SAENTA)-*X8*-fluorescein,[20] was used as the competitive ligand to be displaced by the test compounds. Flow cytometry has several advantages over the conventional radioligand binding assays, in that it eliminates radiation hazards and disposal problems and allows the use of much fewer cells, as few as 5000 cells compared to 2 million cells per sample for comparable radioligand assays. As depicted in Figure 1, these novel constrained

Scheme 3[a]



[a] Reaction conditions: (a) $(Ac)_2O/Et_3N$, CH_2Cl_2; (b) $NH_2NH_2 \cdot xH_2O/FeCl_3 \cdot 6H_2O$/activated carbon/MeOH, reflux; (c) TFAA/TFA/CH_2Cl_2; (d) KNO_3 (1.2 equiv)/concentrated H_2SO_4, 4 °C; (e) HCl/MeOH, reflux; (f) (i) $NaNO_2$/HCl/0 °C, (ii) H_3PO_2; (g) concentrated HCl/MeOH, reflux; (h) KNO_3 (2 equiv)/concentrated H_2SO_4, 5 °C.

Scheme 4[a]



[a] Reaction conditions: (a) TFAA/TFA/CH_2Cl_2/15 min; (b) KNO_3/concentrated H_2SO_4/0 °C,1 h; (c) concentrated HCl/MeOH/reflux/50 min; (d) (i) $NaNO_2$/HCl/0 °C/2 h, (ii) H_3PO_2/4 °C/10 h.

Table 1. Flow Cytometrically-Determined K_i Values

compound	K_i (nM)
1 (NBMPR)	0.70
2	250
3	15
4	0.45
5	300
23	150

analogues of NBMPR exhibited a wide range of binding affinities at the *es* transporter as measured by their ability to displace the *es* nucleoside transporter-specific ligand,[20] shown by the K_i values in Table 1. They range from a subnanomolar concentration for compound **4** (K_i 0.45 nM) to high nanomolar concentrations for compounds **2** and **5**, at least a 500-fold difference. The results provide some surprises based on the known SAR of NBMPR analogues. First, one of the new compounds (**4**) is as good or even possibly better than the prototype *es* transporter inhibitor NBMPR. This is interesting because, generally, when the sulfur at the 6-position in NBMPR is replaced by NH as is present in the new compounds, *es* transporter binding decreases.[12] The comparable affinities of compound **4** (K_i 0.45 nM) and

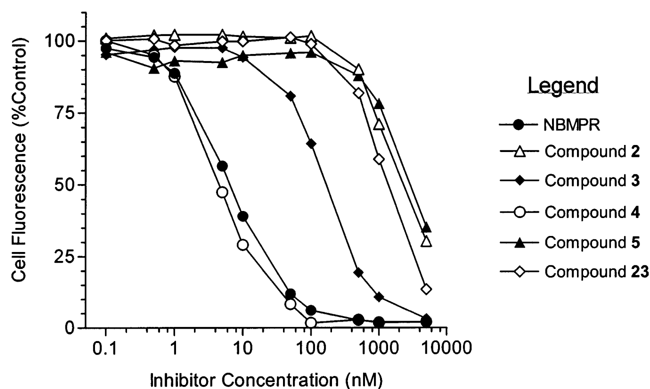


Figure 1. Equilibrium displacement of SAENTA-fluorescein ligand by new tetrahydroisoquinoline constrained NBMPR analogues in K562 cells. Cells were incubated with 25 nM SAENTA fluorescein in the presence or absence of inhibitor for 30 min at room temperature and analyzed by flow cytometry (FACSCalibur[R]). Data were collected on 5000 cells per sample, and mean channel numbers were used as a measure of ligand fluorescence output.

NBMPR (K_i 0.70 nM) indicate that conformational changes at this location in the molecules can make a

significant difference in binding to the *es* transporter. Second, we had thought that compound **3** would probably be the most active of the new analogues since it has the nitro substituent on the tetrahydroisoquinoline ring in an equivalent position to the *p*-nitro substituent in NBMPR. This is because the known SAR suggests that para substitution of the nitro group (as in NBMPR) is the best in terms of *es* transporter binding, followed by meta substitution, which in turn is much better than ortho substitution. As it turned out, the corresponding para compound **3**, is tightly bound (K_i 15 nM), but much less so than the compound corresponding to the meta nitro substitution, compound **4** (K_i 0.45 nM). The compounds corresponding to ortho position substitution, compounds **2** (K_i 250 nM) and **5** (K_i 300 nM) behaved as expected, exhibiting activities comparable to the unsubstituted tetrahydroisoquinoline analogue compound **23** (K_i 150 nM). It appears that compound **3** most captures the bioactive conformation of the nitrobenzyl group of NBMPR and should serve as the best template for rational design of *es* transporter inhibitors among these novel NBMPR analogues. These results offer insights into the bioactive conformation of NBMPR at the 6-position substituent, which is responsible for high affinity binding to the *es* transporter.[21] The solid state conformation of NBMPR has been determined by X-ray diffraction[22] and a solution conformation has also been determined by NMR.[23] The X-ray structure reveals a syn orientation of the purine system about the glycosidic bond, whereas the solution conformation reveals the preponderance of a high-anti orientation of the purine system about the glycosidic linkage. Conformational space analysis of the novel conformationally constrained analogues reported here will shed light on the bioactive conformation of NBMPR and provide insights for further *es* transporter inhibitor design.

The high *es* transporter binding affinity of compound **4**, which is at least equivalent in potency to NBMPR, is of interest since this compound will be less likely to lose the nitrobenzyl moiety, and should overcome a major disadvantage of NBMPR, which has to do with the in vivo lability of the nitrobenzyl moiety. The nitrobenzyl group of NBMPR is lost in vivo to form the immunosuppressive product mercaptopurine riboside,[24] with a dramatically reduced *es* transporter affinity (up to a 1000-fold decrease). This lability of the nitrobenzyl group has actually allowed NBMPR to be used as an effective photolabeling agent of the *es* nucleoside transporter.[25-27] This has hampered the clinical applications of NBMPR. Therefore preclinical development of the novel compounds **3** and **4**, which lock the nitrobenzyl moiety in place, and are likely to prolong in vivo activity compared to NBMPR, is warranted. Compounds **3** and **4** are worth further investigation as novel nucleoside transport inhibitors with potential for clinical and research applications.

Experimental Section

Chemistry. Thin-layer chromatography (TLC) was conducted on silica gel F_{254} plates (Analtech). Compounds were visualized by UV light or 5% H_2SO_4 in EtOH spraying reagent. ^1H,^{13}C spectra were recorded on Bruker ARX (300 MHz) instruments, using $CDCl_3$, CD_3OD, $(CD_3)_2SO$, or CD_3COCD_3 as solvents and tetramethylsilane (TMS) as internal standard. Flash column chromatography was performed on Fisher silica gel (170−400 mesh). Melting points were determined using a Fisher-Johns Melting Point Apparatus and are reported uncorrected. Mass spectra were obtained on a Bruker-HP Esquire-LC mass spectrometer, and IR spectra in KBr with a Perkin-Elmer (System 2000 FT-IR) spectrometer. All solvents and reagents were bought from Aldrich and used without further purification.

General Method for the Preparation of Compounds 2, 3, 4, 5, 23. A mixture of 6-chloropurine riboside (**10**, 100 mg, 0.35 mmol), (mono-NO_2)-1,2,3,4-tetrahydroisoquinoline (0.88 mmol), and calcium carbonate (70 mg, 0.70 mmol) in EtOH (5 mL) was stirred under refluxing for 15 h. The reaction mixture was filtered, and the filtrate was evaporated in vacuo at 40 °C. The residue was purified by flash silica gel chromatography followed by recrystallization from MeOH.

6-{[Mono- (5, 6, 7, or 8)-NO_2-] 1,2,3,4-tetrahydroisoquino-2-yl}purine Riboside (Compounds 2, 3, 4, 5, 23). Compound 2: yield 65%; mp 159−160 °C; IR: 3438, 1604, 1528, 1352 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.15 (1H, s, H-2), 7.86 (1H, d, J = 7.5 Hz, H-6″), 7.84 (1H, s, H-8), 7.49 (1H, d, J = 7.5 Hz, H-8″), 7.36 (1H, t, J = 7.8, 8.1 Hz, H-7″), 6.53 (1H, br d, J = 9.9 Hz, OH-2′), 5.81 (1H, d, J = 7.5 Hz, H-1′), 5.36 (1H, br s, OH-3′), 5.07 (1H, q, J = 6.9, 5.1 Hz, H-2′), 4.53−4.45 (3H, br m, OH-5′, H-3′, 1″A), 4.35 (1H, s, H-1″B), 3.94 (1H, d, J = 12.9 Hz, H-4′), 3.76 (2H, br m, H-5′), 3.28 (2H, t, J = 6.0 Hz, H3″ or H4″). Anal. ($C_{19}H_{20}N_6O_6$) C, H, N.

Compound 3: yield 66%; mp 127.5−129.5 °C; MS (ESI) m/z 451 (M + Na)$^+$; IR: 3437, 1593, 1523, 1350 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 8.30 (1H, s, H-2), 8.29 (1H, s, H-8), 8.10 (2H, m, H-5″, 7″), 7.50 (1H, d, J = 8.4 Hz, H-8″), 5.98 (1H, d, J = 6.3 Hz, H-1′), 5.50 (2H, br s, OH-2′, 3′), 4.74 (1H,t, J = 6.0 Hz, OH-5′), 4.58 (3H, br s, H-2′, 3′, 1″A), 4.32 (1H, q, J = 5.1, 2.7 Hz, H-4′), 4.17 (1H,d, J = 2.4 Hz, H-1″B) 3.89 (1H, q, J = 12.6, 2.4 Hz, H-5′A), 3.74 (1H, q, J = 9.9, 2.4 Hz, H-5′B), 3.15 (2H, t, J = 6.0 Hz, H3″ or H4″). Anal. ($C_{19}H_{20}N_6O_6 \cdot H_2O$): C, H, N.

Compound 4: yield 61%; mp 172−173 °C; IR: 3422, 1597, 1531, 1346 cm^{-1};^1H NMR (300 MHz, $CDCl_3$) δ 8.18 (1H, s, H-2), 8.08 (1H, s, H-8″), 8.02 (1H, d, J = 8.4 Hz, H-6″), 7.81 (1H, s, H-8), 7.29 (1H, d, J = 8.4 Hz, H-5″), 6.46 (1H, br d, J = 10.2 Hz, OH-2′), 5.81 (1H, d, J = 7.5 Hz, H-1′), 5.46 (1H, br s, OH-3′), 5.07 (1H, q, J = 6.9, 5.1 Hz, H-2′), 4.60−4.47 (3H, br m, OH-5′, H-3′, 1″A), 4.35 (1H, s, H-1″B), 3.94 (1H, d, J = 12.3 Hz, H-4′), 3.76 (1H, br t, J = 12.3, 10.5 Hz, H-5′A), 3.61 (1H, br d, J = 3.9 Hz, H-5′B), 3.10 (2H, t, J = 6.0 Hz, H3″ or H4″). Anal. ($C_{19}H_{20}N_6O_6$) C, H, N.

Compound 5: yield 74%; mp 148−149 °C; MS (ESI) m/z 451 (M + Na)$^+$; IR: 3370, 1590, 1530, 1343 cm^{-1}; ^1H NMR (300 MHz, CD_3COCD_3) δ 8.26 (1H, s, H-2), 8.25 (1H, s, H-8), 7.94 (1H, d, J = 7.5 Hz, H-7″), 7.60 (1H, d, J = 7.8 Hz, H-5″), 7.47 (1H, t, J = 7.8, 7.8 Hz, H-6″), 5.95 (1H, d, J = 6.6 Hz, H-1′), 5.78 (1H, br s, OH-2′), 5.63 (1H, d, J = 9.9 Hz, OH-3′), 4.90 (1H, m, H-2′), 4.64 (2H, br m, OH-5′, H-3′), 4.37 (2H, q, J = 6.0, 3.9 Hz, H-4′, 1″A), 4.17 (1H, s, H-1″B), 3.84 (1H, m, H-5′A), 3.71 (1H, m, H-5′B), 3.21 (2H, t, J = 6.0 Hz, H3″ or H4″). Anal. ($C_{19}H_{20}N_6O_6$) C, H, N.

Compound 23: yield 95%; mp 157.5−159 °C; IR: 3413, 1600 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 8.45 (1H, s, H-2), 8.29 (1H, s, H-8), 7.20 (4H, m, H-5″, 6″, 7″, 8″), 5.92 (1H, d, J = 6.0 Hz, H-1′), 6.47−5.18 (4H, br m, OH-2′, 5′, 3′,H-2′), 4.57 (2H, br m, H-3′, 1″A), 4.14 (1H, q, J = 8.1, 5.1 Hz, H-4′), 3.96 (1H, s, H-1″B), 3.67 (1H, m, H-5′A), 3.56 (1H, m, H-5′B), 3.93 (2H, t, J = 6.0 Hz, H3″ or H4″).

5-Nitro-1, 2,3,4-tetrahydroisoquinoline (6). To a solution of **11** (500 mg, 2.85 mmol) in acetic acid (50 mL) was added small portions of $NaBH_4$ until TLC examination of the reaction mixture indicated the absence of the starting material. The solution was then poured onto ice, basified with $NH_3 \cdot H_2O$, and then extracted three with CH_2Cl_2. The organic layer was washed once with water, dried over sodium sulfate, and evaporated in vacuo. The residue was purified by silica gel chromatography to give compound **6** (450 mg, yield 90%); ^1H NMR (300 MHz, $CDCl_3$) δ 7.80 (1H, t, J = 4.8, 4.5 Hz, H-7), 7.28 (2H, d, J = 5.7 Hz, H-6,8), 4.11 (2H, s, $ArCH_2N$), 3.17

(2H, t, J = 5.7 Hz, $ArCH_2CH_2N$), 3.10 (2H, t, J = 5.7 Hz, $ArCH_2CH_2N$).

7-Nitro-1, 2,3,4-tetrahydroisoquinline (8). An ice-cold solution of compound **12** (10.8 g, 80 mmol) in concentrated sulfuric acid (40 mL) was treated with potassium nitrate (8.8 g, 87 mmol) in small portions, keeping the temperature below 5 °C. The reaction was left overnight at room temperature and poured onto ice. The resulting solution was basified with $NH_3 \cdot H_2O$, extracted with CH_2Cl_2. The extract concentrated to dryness in vacuo. The crude product obtained was converted to the hydrochloride salt and crystallized from methanol to give 8.5 g of the hydrochloride (yield 50%), which was basified to give compound **8**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98 (1H, d, J = 8.1 Hz, H-6), 7.91 (1H, s, H-8), 7.24 (1H, d, J = 8.4 Hz, H-5), 4.10 (2H, s, H-1), 3.17 (2H, t, J = 6.0 Hz, H-3), 2.89 (2H, t, J = 6.0 Hz, H-4).

***N*-Acetyl-7-nitro-1, 2,3,4-tetrahydroisoquinline (13).** A mixture of compound **8** (5.4 g, 26 mmol), Et_3N (9.8 mL, 70 mmol), and acetic anhydride (5.0 mL, 51 mmol) in CH_2Cl_2 (70 mL) was stirred at reflux temperature for 1 h. The reaction mixture was cooled and poured onto ice. The resulting solution was extracted with CH_2Cl_2. The organic layer was dried over sodium sulfate and evaporated in vacuo. The residue was subjected to by flash silica gel chromatography to obtain 5.3 g of compound **13** (94% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05−8.03 (2H, m, H-6,8), 7.43−7.30 (1H, m, H-5), 4.83 (1.2H, s, $ArCH_2N$), 4.72 (0.8H, s, $ArCH_2N$), 3.87 (0.8H, t, J = 5.7 Hz, $ArCH_2CH_2N$), 3.74 (1.2H, t, J = 5.7 Hz, $ArCH_2CH_2N$), 3.02 (1.2H, t, J = 5.7 Hz, $ArCH_2CH_2N$), 2.95 (0.8H, t, J = 5.7 Hz, $ArCH_2CH_2N$), 2.21 (1.2H, s, $COCH_3$), 2.20 (1.8H, s, $COCH_3$).

***N*-Acetyl-7-amino-1, 2,3,4-tetrahydroisoquinoline (14).** A mixture of compound **13** (5.1 g, 23 mmol), activated carbon (4.2 g), ferric chloride hexahydrate (2.1 g, 7.6 mmol), and methanol (140 mL) was stirred under reflux for 20 min. To the boiling mixture was added hydrazine hydrate (8.5 g, 265 mmol) dropwise, the mixture was refluxed for an additional 4 h, cooled, and filtered, and the residue was washed with methanol. The filtrate was concentrated in vacuo and the residue recrystallized from EtOAc to give compound **14** (3.8 g, 85% yield). ^{1}H NMR [300 MHz, $(CD_3)_2SO$], a 3:2 mixture of amide conformers doubling most signals, δ 6.80 (1H, d, J = 8.1 Hz, H-5), 6.40 (1H, d, J = 7.8 Hz, H-6), 6.33 (1H, s, H-7), 4.89 (2H, br s, NH_2), 4.46 (0.8H, s, $ArCH_2N$), 4.41 (1.2H, s, $ArCH_2N$), 3.57 (2H, t, J = 5.7 Hz, $ArCH_2CH_2N$), 2.66 (1.2H, t, J = 6.0 Hz, $ArCH_2CH_2N$), 2.55 (0.8H, t, J = 6.0 Hz, $ArCH_2CH_2N$), 2.06 (1.2H, s, $COCH_3$), 2.05 (1.8H, s $COCH_3$).

***N*-Acetyl-7- trifluoroacetamido-1, 2,3,4-tetrahydroisoquinoline (15).** A solution of compound **14** (3.0 g, 17 mmol), trifluoroacetic acid (2 mL, 28 mmol), and trifluoroacetic anhydride (4 mL, 28 mmol) in CH_2Cl_2 was refluxed for 15 min and cooled, and the mixture was poured onto ice and extracted with CH_2Cl_2. The extract was dried over sodium sulfate and concentrated in vacuo. The crude product was chromatographed over silica gel to give 4.5 g of compound **15** (94% yield). $_1$H NMR (300 MHz, $(CD_3)_2SO$), a 3:2 mixture of amide conformers doubling most signals, δ 11.24 (0.8H, s, NH), 11.21 (1.2H, s, NH), 7.55−7.40 (2H, m, H5,8), 7.22 (0.6H,s, H-6), 7.20 (0.4H, s, H-6), 4.64 (0.8H, s, $ArCH_2N$), 4.57 (1.2H, s, $ArCH_2N$), 3.65 (2H, t, J = 6.3 Hz, $ArCH_2CH_2N$), 2.84 (1.2H, t, J = 6.0 Hz, $ArCH_2CH_2N$), 2.73 (0.8H, t, J = 6.0 Hz, $ArCH_2CH_2N$), 2.06 (1.2H, s, $COCH_3$), 2.05 (1.8H,s $COCH_3$).

***N*-Acetyl-7-trifluoroacetamido-6 (8)-nitro-1, 2,3,4-tetrahydroisoquinoline (16, 17) and *N*-Acetyl-7-amino-6 (8)-nitro-1, 2,3,4-tetrahydroisoquinoline (18, 19).** Compound **15** (4.5 g, 16 mol) was dissolved in 30 mL of ice-cold concentrated H_2SO_4. To this solution was added powdered potassium nitrate (2 g, 20 mmol) portionwise, keeping the temperature around 4 °C. The mixture was stirred at 0 °C overnight and then poured onto ice. The resulting solution was basified with ammonium hydroxide and extracted with CH_2Cl_2. The organic layer was dried over sodium sulfate and concentrated to dryness. The product was a mixture of compounds **16** and **17** (as determined by ^{1}H NMR). Hydrolysis of compounds **16** and **17** was achieved by refluxing in MeOH (60 mL) and concentrated HCl (15 mL) for 50 min. The reaction mixture was concentrated in vacuo to get rid of MeOH, basified with ammonium hydroxide, and then extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated. The crude product was chromatographed over silica gel to give 1.1 g of compound **18** and 0.5 g of compound **19**, 30 and 15% yields, respectively. Compound **16**: MS (ESI) m/z 354 $(M + Na)^+$, m/z 330 $(M - H)^-$; ^{1}H NMR (300 MHz, $CDCl_3$), a 3:2 mixture of amide conformers doubling most signals, δ 11.38 (0.4H, s, NH), 11.32 (0.6H, s, NH), 8.56 (1H, s, H-5), 8.15 (0.4H, s, H-8), 8.13 (0.6H, s, H-8), 4.86 (1.2H, s, $ArCH_2N$), 4.74 (0.8H, s, $ArCH_2N$), 3.88 (0.8H, t, J = 6.0 Hz, $ArCH_2CH_2N$), 3.74 (1.2H, t, J = 6.0 Hz, $ArCH_2CH_2N$), 2.99 (1.2H, t, J = 6.0 Hz, $ArCH_2CH_2N$), 2.93 (0.8H, t, J = 6.0 Hz, $ArCH_2CH_2N$), 2.21 (3H, s, $COCH_3$). Compound **17**: MS (ESI) m/z 354 $(M + Na)^+$, m/z 330 $(M - H)^-$;^{1}H NMR (300 MHz, $CDCl_3$), a 3:2 mixture of amide conformers doubling most signals, δ 9.64 (1H, br s, NH), 8.21 (0.4H, d, J = 8.7 Hz, H-5), 8.18 (0.6H, d, J = 8.7 Hz, H-5), 7.46 (0.4H, d, J = 8.7 Hz, H-6), 7.43 (0.6H, d, J = 8.7 Hz, H-6), 4.89 (1.2H, s, $ArCH_2N$), 4.75 (0.8H, s, $ArCH_2N$), 3.86 (0.8H, t, J = 6.0 Hz, $ArCH_2CH_2N$), 3.78 (1.2H, t, J = 6.0 Hz, $ArCH_2CH_2N$), 3.01 (1.2H, t, J = 6.0 Hz, $ArCH_2CH_2N$), 2.96 (0.8H, t, J = 6.0 Hz, $ArCH_2CH_2N$), 2.06 (3H, s, $COCH_3$). Compound **18**: MS (ESI) m/z 258 $(M + Na)^+$; ^{1}H NMR (300 MHz, $CDCl_3$), a 2:1 mixture of amide conformers doubling most signals, δ 7.94 (1H, s, H-5), 6.60 (1H, s, H-8), 5.96 (2H, br s, NH_2), 4.68 (1.3H, s, $ArCH_2N$), 4.56 (0.7H, s, $ArCH_2N$), 3.80 (0.7H, t, J = 6.0 Hz, $ArCH_2CH_2N$), 3.67 (1.3H, t, J = 6.0 Hz, $ArCH_2CH_2N$), 2.85 (1.3H, t, J = 6.0 Hz, $ArCH_2CH_2N$), 2.79 (0.7H, t, J = 6.0 Hz, $ArCH_2CH_2N$), 2.18 (3H, s, $COCH_3$); ^{13}C NMR δ 169.9 and 169.7 ($COCH_3$), 143.6 and 143.2, 142.5 and 141.5, 131.5 and 131.3, 123.4 and 123.5 (C-6, 7,9,10), 126.1 and 125.5, 116.1 and 115.5 (C-5,8), 48.1, 44.4, 44.1, 39.9 (C-1,3), 28.5 and 27.5 (C-4), 22.1 and 21.8 ($COCH_3$). Compound **19**: MS (ESI) m/z 258 $(M + Na)^+$, m/z 234 $(M - H)^-$; ^{1}H NMR (300 MHz, $CDCl_3$), a 3:2 mixture of amide conformers doubling most signals, δ 7.10 (0.6H, d, J = 8.4 Hz, H-5), 7.05 (0.4H, d, J = 8.7 Hz, H-5), 6.73 (0.6H, d, J = 8.4 Hz, H-6), 6.68 (0.4H, d, J = 8.4 Hz, H-6), 5.52 (1.2H, s, NH2), 5.34 (0.8H, s, NH_2), 4.93 (0.8H, s, $ArCH_2N$), 4.81 (1.2H, s, $ArCH_2N$), 3.79 (1.2H, t, J = 6.0 Hz, $ArCH_2CH_2N$), 3.68 (0.8H, t, J = 6.0 Hz, $ArCH_2CH_2N$), 2.83 (0.8H, t, J = 6.0 Hz, $ArCH_2CH_2N$), 2.77 (1.2H, t, J = 6.0 Hz, $ArCH_2CH_2N$), 2.16 (3H, s, $COCH_3$). ^{13}C NMR δ 169.5 and 169.2 ($COCH_3$), 142.9 and 142.4, 131.0 and 130.1, 125.7, 124.3 (C-7, 8,9,10), 135.4 and 134.5, 117.7 and 117.2 (C-5, 6), 47.3, 43.7, 42.9, 39.1 (C-1, 3), 29.0 and 28.1 (C-4), 22.1 and 21.6 ($COCH_3$).

***N*-Acetyl-6 (8)-nitro-1, 2,3,4-tetrahydroisoquinoline (20, 21).** To a stirred solution of compound **18** (**19**) (290 mg, 1.23 mmol) in HCl (6 M, 4 mL) was added dropwise a solution of $NaNO_2$ (110 mg, 1.6 mmol) in water (0.5 mL) at 0 °C. After the mixture was stirred at 0 °C for 2 h, hypophosphorous acid (50% aqueous solution, 1.4 mL) was added dropwise, and the mixture was stirred at 40 °C for 10 h. The reaction mixture was then poured into water and extracted with methylene chloride. The organic layer was dried over $NaSO_4$ and evaporated. The crude product was purified by silica gel chromatography to afford compound **20** (**21**). Compound **20** (176 mg, 65%): MS (ESI) m/z 243 $(M + Na)^+$, m/z 219 $(M - H)^-$; ^{1}H NMR (300 MHz, $CDCl_3$), a 2:1 mixture of amide conformers doubling most signals, δ 8.08−8.03 (2H, m, H-5, 7), 7.31 (1H, d, J = 8.7 Hz, H-8), 4.83 (1.3H, s, $ArCH_2N$), 4.72 (0.7H, s, $ArCH_2N$), 3.87 (0.7H, t, J = 6.0 Hz, $ArCH_2CH_2N$), 3.74 (1.3H, t, J = 6.0 Hz, $ArCH_2CH_2N$), 3.02 (1.3H, t, J = 5.7 Hz, $ArCH_2CH_2N$), 2.96 (0.7H, t, J = 5.7 Hz, $ArCH_2CH_2N$), 2.21 (3H, s, $COCH_3$). Compound **21** (233 mg, 86%): MS (ESI) m/z 243 $(M + Na)^{+;}$ ^{1}H NMR (300 MHz, $CDCl_3$), a 3:2 mixture of amide conformers doubling most signals, δ 7.97 (0.6H, d, J = 10.2 Hz, H-7), 7.94 (0.4H, d, J = 10.2 Hz, H-7), 7.49−7.32 (2H, m, H-6, 5), 5.09 (0.8H, s, $ArCH_2N$), 4.99 (1.2H, s, $ArCH_2N$), 3.86 (1.2H, t, J = 6.0 Hz, $ArCH_2CH_2N$), 3.75 (0.8H, t, J = 6.0 Hz, $ArCH_2CH_2N$), 3.03 (0.8H, t, J = 6.0 Hz, $ArCH_2CH_2N$), 2.98 (1.2H, t, J = 6.0 Hz, $ArCH_2CH_2N$), 2.21 (1.8H, s, $COCH_3$), 2.19 (1.2H, s, $COCH_3$).

6(8)-Nitro-1, 2,3,4-tetrahydroisoquinoline (7 and 9). Hydrolysis of **20** (**21**) (190 mg, 0.86 mmol) in MeOH (6 mL) and concentrated hydrochloride acid (3 mL) at refluxing temperature for 10 h afforded 6(and 8)-nitro-1,2,3,4-tetrahydroisoquinolines (100 mg, 66%), which were separated by flash silica gel chromatography. 6-NO_2-1,2,3,4-tetrahydroisoquinoline (**20**): MS (ESI) *m*/*z* 179 (M + H)$^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.99−7.96 (2H, m, H-5, 7), 7.16 (1H, d, J = 9.0 Hz, H-8), 4.10 (2H,s, ArCH_2N), 3.17 (2H, t, J = 6.0 Hz, ArCH_2-CH_2N), 2.90 (2H, t, J = 6.0 Hz, ArCH_2CH_2N). 8-NO_2-1,2,3,4-tetrahydroisoquinoline (**21**): MS (ESI) *m*/*z* 179 (M + H)$^+$; ^{1}H NMR (300 MHz, CD_3OD) δ 7.80 (1H, d, J = 8.1 Hz, H-7), 7.44 (1H, d, J = 7.5 Hz, H-5), 7.33 (1H, t, J = 8.1, 7.8 Hz, H-6), 4.16 (2H, s, ArCH_2N), 3.07 (2H, t, J = 6.0 Hz, ArCH_2CH_2N), 2.92 (2H, t, J = 6.0 Hz, ArCH_2CH_2N).

***N*-Acetyl-6,8-dinitro-7-trifluoroacetamido-1,2,3,4-tetrahydroisoquinoline (22).** Compound **15** (426 mg, 1.49 mmol) was dissolved in 5 mL was ice-cold concentrated H_2SO_4. To this solution was added powdered potassium nitrate (301.3 mg, 2.98 mmol) portionwise, keeping the temperature under 5 °C. The mixture was stirred at 4 °C for a further 4 h and then poured onto ice. The resulting solution was basified with ammonium hydroxide and extracted with CH_2Cl_2. The organic layer was dried over sodium sulfate and concentrated to dryness. The crude product was chromatographed over silica gel to give 280 mg of *N*-acetyl-6,8-dinitro-7-trifluoroacetamido-1,2,3,4-tetrahydroisoquinoline (50%). MS (ESI) *m*/*z* 399 (M + Na)$^+$, *m*/*z* 375 (M − H)$^-$; IR: 3448, 1640, 1647, 1270, 1171 cm^{-1}; 1H NMR (300 MHz, DMSO-d_6), a 3:1 mixture of amide conformers doubling some signals, δ 7.74 (1H, s, H-5), 4.48 (2H, s, ArCH_2N), 3.70 (2H, t, J = 5.7 Hz, ArCH_2CH_2N), 2.90 (1.5H, t, J = 5.7 Hz, ArCH_2CH_2N), 2.78 (0.5H, t, J = 5.7 Hz, ArCH_2CH_2N), 2.07 (2.3H, s, $COCH_3$), 2.07 (0.7H, s, $COCH_3$).

5- Trifluoroacetamidoisoquinoline (25). A solution of **24** (291.3 mg, 2 mmol), trifluoroacetic acid (0.7 mL, 9.8 mmol) and trifluoroacetic anhydride (1.4 mL, 9.8 mmol) in CH_2Cl_2 (6 mL) was refluxed for 15 min. The reaction mixture was cooled, poured onto ice, and neutralized with ammonium hydroxide to obtain a light yellow solid, which was filtered and dried to afford compound **25** (432.3 mg, 90% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.37 (1H, s, H-1), 8.69 (1H, d, J = 6.0 Hz, H-3), 8.22 (1H, d, J = 8.4 Hz, H-8), 8.00 (1H, d, J = 6.0 Hz, H-4), 7.72 (1H, t, J = 8.1, 8.4 Hz, H-7), 7.61 (1H, d, J = 8.1 Hz, H-6).

5-Trifluoroacetamido-8-nitroisoquinoline (26). Compound **25** (300 mg, 1.3 mmol) was dissolved in 3.5 mL of ice-cold concentrated H_2SO_4. To this solution was added powdered potassium nitrate (200 mg, 2.0 mmol) portionwise, keeping the temperature around 0 °C. The mixture was stirred at 3 °C for further 1 h and then poured onto ice. The resulting solution was basified with ammonium hydroxide and extracted with CH_2Cl_2. The organic layer was dried over sodium sulfate and concentrated to dryness. The crude product was chromatographed over silica gel to give 214 mg of compound **26** (60%). MS (ESI) *m*/*z* 286 (M + H)$^+$; IR: 3422, 1740, 1562, 1523, 1312, 1340 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.96 (1H, s, NH), 9.86 (1H, s, H-1), 8.80 (1H, d, J = 6.0 Hz, H-3), 8.55 (1H, d, J = 8.1 Hz, H-7), 8.10 (1H, d, J = 8.4 Hz, H-6), 8.00 (1H, d, J = 5.7 Hz, H-4).

5-Amino-8-nitroisoquinoline (27). Compound **26** (100 mg, 0.35 mmol) was hydrolyzed in MeOH (2.5 mL) and concentrated HCl (0.6 mL) at refluxing temperature for 50 min. The reaction mixture was evaporated in vacuo, basified with ammonium hydroxide, and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated; the crude product was chromatographed over silica gel to give 57 mg of compound **27** (85%). MS (ESI) *m*/*z* 190 (M + H)$^+$; IR: 3328, 1650, 1558, 1276 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.16 (1H, s, H-1), 8.60 (1H, d, J = 5.7 Hz, H-3), 8.45 (1H, d, J = 8.7 Hz, H-7), 8.17 (1H, d, J = 5.7 Hz, H-4), 7.82 (2H, s, NH_2), 6.83 (1H, d, J = 9.0 Hz, H-6).

5-Hydroxyisoquinoline (29). To a stirred solution of compound **27** (40 mg, 0.21 mmol) in HCl (6 N, 1.4 mL) was added dropwise a solution of $NaNO_2$ (20 mg, 0.29 mmol) in water (0.1 mL) at 0 °C. After the mixture was stirred at 0 °C for 2 h, hypophosphorous acid (50% aqueous solution, 0.3 mL) was added dropwise, and the mixture was stirred at 4 °C for 10 h. The reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was dried over $NaSO_4$ and evaporated. The crude product was chromatographed over silica gel to obtain compound **29** (15 mg, 49%); MS (ESI) *m*/*z* 146 (M + H)$^+$; ^{1}H NMR (300 MHz, DMSO-d_6), δ 10.69 (1H, s, OH), 9.45 (1H, s, ArCH_2N), 8.44 (1H, d, J = 9.0 Hz, H-3), 7.70 (1H, d, J = 5.7 Hz, H-4), 7.56 (1H, t, J = 7.8, 7.8 Hz, H-7), 7.34 (1H, d, J = 8.1 Hz, H-8). 6.97 (1H, d, J = 7.8 Hz, H-6). Literature:[19] ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.22 (1H, s, ArCH_2N), 8.51 (1H, d, J = 5.9 Hz, H-3), 8.05 (1H, d, J = 5.9 Hz, H-4), 7.46 (2H, m, H-7 and H-8), 7.07 (1H, d, J = 7.4 Hz, H-6).

Biological Testing. The compounds were tested to determine their *es* nucleoside transporter binding ability by a flow cytometric assay.[20] Human leukemia K562 cells growing in RPMI 1640 medium were washed once and resuspended at 1.6×10^6 cells/mL in phosphate-buffered saline at pH 7.4, and incubated with 5-(SAENTA)-*X8*-fluorescein (25 nM) in the presence or absence of varying concentrations of test compounds at room temperature for 45 min. Flow cytometric measurements of cell-associated fluorescence were then performed with a FACSCalibur (Becton Dickinson, San Jose, CA) equipped with a 15 mW-argon laser (Molecular Resources Flow Cytometry Facility, University of Tennessee Health Sciences Center). In each assay, 5000 cells were analyzed from suspensions of 4×10^5 cells/mL. The units of fluorescence were arbitrary channel numbers. Percentage (%) of control (i.e., *es* transporter-specific fluorescence in the presence of SAENTA-fluor without test compounds) was calculated for each sample by the equation below (eq 1).

$$\%\ \text{control} = \frac{(\text{SF}_s) \times 100}{(\text{SF}_f)} \quad (1)$$

where SF_s is the *es* transporter-specific fluorescence of test samples, and SF_f is the *es* transporter-specific fluorescence of the SAENTA-fluor ligand standard in mean channel numbers.

The results were fed into the PRISM program (GraphPad, San Diego, CA) to derive concentration-dependent curves, as shown in Figure 1. From these curves, the IC_{50} values were obtained and used to calculate inhibition constants (K_i) values from eq 2

$$K_i = \text{IC}_{50}/(1 + [\text{L}]/K_\text{L}) \quad (2)$$

where [L] and K_L are the concentration and the K_d value of the SAENTA-fluor, respectively.

The K_i values were used to compare the abilities of the new compounds to displace the *es* transporter-specific ligand (5-(SAENTA)-*X8*-fluorescein,[20] and for that matter their affinity for the *es* transporter.

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