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Synthesis, Flow Cytometric Evaluation and Identification of Highly Potent Dipyridamole Analogs as Equilibrative Nucleoside Transporter 1 (ENT1) Inhibitors[a]

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Abstract

Dipyridamole (Persantine) is a clinically used vasodilator with equilibrative nucleoside transporters 1, and 2 (ENT1 and ENT2) inhibitory activity albeit less potent than the prototype ENT1 inhibitor nitrobenzylmercaptopurine riboside (NBMPR). Dipyridamole is a good candidate for further exploration because it is a non-nucleoside and has a proven record of safe use in humans. A series of dipyridamole analogs were synthesized with systematic modification, and evaluated as ENT1 inhibitors by flow cytometry. Compounds with much higher potency were identified, the best being 2,6-bis(diethanolamino)-4,8-diheptamethyleneimino-pyrimido[5,4-*d*]pyrimidine **(13)**, with a K_i of 0.49 nM, compared to a K_i of 308 nM for dipyridamole. Compound **13** is similar in potency to the prototype potent ENT1 inhibitor NBMPR (0.43 nM). For the first time, a dipyridamole analog has been identified that is equipotent with NBMPR. The SAR indicated that diethanolamine substituted analogs were more active than monoethanolamine compounds. Also, free hydroxyl groups are not essential for activity.

Keywords

Equilibrative nucleoside transporter ENT1 Inhibitors; Dipyridamole Analogs; NBMPR

Introduction

Nucleoside transporters are specialized integral membrane glycoproteins known to mediate the cellular influx or efflux of physiological nucleosides or nucleobases, as well as many synthetic analogs.[1-2] Currently, nucleoside transporters have been classified into two families: (i) the equilibrative nucleoside transporter family (ENTs), and (ii) the concentrative nucleoside transporter family (CNTs).[3-4] The equilibrative family facilitates the transport of nucleosides or nucleobases down their concentration gradients; in contrast, the concentrative family transports nucleosides against their concentration gradients by coupling with a sodium ion gradient. Nucleoside transporter inhibitors have potential therapeutic applications in ischemic

[a]Abbreviations: Cbz, carbobenzoxy; CNTs, concentrative nucleoside transporters; DMSO, dimethylsulfoxide; ENT1, Equilibrative Nucleoside Transporter 1; ENT2, Equilibrative Nucleoside Transporter 2; ENT3, Equilibrative Nucleoside Transporter 3; ENT4, Equilibrative Nucleoside Transporter 4; ESI, electrospray ionization; LC, liquid chromatography; MS, mass spectrometry; NBMPR, nitrobenzylmercaptopurine riboside; NMR, nuclear magnetic resonance; NTIs, nucleoside transporter inhibitors; SAR, structure-activity relationship; CDKs, cyclin dependent kinases; TCPP, 2,4,6,8-tetrachloropyrimido[5,4-*d*]pyrimidine; TLC, thin-layer chromatography; TMS, tetramethylsilane;

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heart disease and stroke[5-10], in inflammatory disease,[11] and as biological response modifiers in antimetabolite chemotherapy.[12] A comprehensive summary of nucleoside transport inhibitors as potential therapeutic agents has been published.[13]

Equilibrative nucleoside transporters were the first to be identified because of their broad tissue distribution. They were initially subdivided into *es* (equilibrative sensitive) or ENT1, and *ei* (equilibrative insensitive) or ENT2 according to their sensitivities to inhibition by nanomolar concentrations of 4-nitrobenzylmercaptopurine ribonucleoside (NBMPR). Four subtypes of ENTs (ENT1, ENT2, ENT3 and ENT4) have now been identified and cloned.[3] The ENT1 transporter is the most widely distributed nucleoside transporter with the highest abundance in most tissues studied.[14-15] This makes it the most relevant NT target for therapeutic exploration. Several chemical classes have been shown to inhibit ENT1.[13] Among them, three classes are most significant (Figure 1). These are purine nucleoside analogs of which NBMPR is the prototype, pyrimidopyrimidine analogs such as the antithrombotic and vasodilating agent dipyridamole, and flazine calcium channel blockers represented by lidoflazine.

NBMPR is a more potent ENT1 inhibitor (e.g. K_i of 0.7 nM)[16] than dipyridamole (e.g. K_i of 8.8)[17]. Draflazine, a lidoflazine analog, also exhibits high ENT1 inhibitory activity (IC_{50} = 0.28-10 nM).[18] However, NBMPR and the flazine compounds like draflazine are poor candidates for further exploration. NBMPR has immunosuppressive and mutagenic activities deriving from its 6-mercaptopurine metabolite.[19-21] The flazines are nosnspecific, having calcium channel antagonist activity that is thought to contribute significantly to their cardioprotective effects.[22-24] As a potent ENT1 inhibitor, dipyridamole has broad pharmacological effects. It is an effective coronary vasodilator (used as an antianginal drug) through the increasing of extracellular adenosine concentration stemming from its ENT inhibitory activity.[5,25,26] Dipyridamole also has antiplatelet effects attributed to phosphodiesterase inhibition.[5] Co-administration of ENT1 inhibitors such as dipyridamole and antimetabolites such as 5-fluorouracil, has been shown to result in synergism and might improve the therapeutic index of antimetabolites, where target cells have a higher ENT1 expression than normal cells.[27,28] Synergism results not only from inhibition of nucleoside salvage, but also from increasing the intracellular concentration of 5-fluorodeoxyuridine caused by blockade of its efflux by dipyridamole. Thus, the intracellular level of the active product, 5-fluorodeoxyuridine monophosphate, increases, resulting in higher therapeutic efficacy.[29-30]

Besides mammalian tissues, nucleoside transporters are also found in parasites such as *Plasmodium falciparum*, the malarial parasite.[31,32] Parasites rely on salvage pathways to meet their purine and purine nucleoside needs since they do not have *de novo* purine biosynthetic pathways.[33] Nucleoside transporters of parasites have limited homologies with the human ENT1, and have been shown to be inhibited by dipyridamole but not NBMPR or lidoflazine.[34] Some parasites like *Toxoplasma gondii* can even transport NBMPR.[35] A study of the antimalarial activity of dipyridamole showed that it was effective against all of the erythrocytic stages such as rings, trophozoites and schizonts; it had an IC_{50} of 30 nM by itself, and lowered the IC_{50} of chloroquine from 97.0 nM to 13.7 nM at a concentration of 0.1 nM.[36]

In light of these positive attributes of dipyridamole, we selected it as a candidate for further structure-activity relationship (SAR) exploration for ENT1 transporter inhibitory activity. Many dipyridamole analogs have been reported, and evaluated for their effects as antiplatelet and cardioprotective agents.[37-41] Some dipyridamole analogs have also been synthesized and evaluated for their inhibitory activities against cyclin dependent kinases (CDKs), with negative results.[42] A more recent publication disclosed the synthesis and biological evaluation of a series of dipyprdamole analogs for their ENT1 inhibitory activities, and some of them showed only slightly higher activities than dipyridamole.[43] In this paper, a series of dipyridamole

analogues were synthesized for a more systematic and comprehensive evaluation of ENT1 SAR. Some of the compounds showed comparative activity to NBMPR, which is a much more potent ENT1 inhibitor than dipyridamole.

Chemistry

For the synthesis of these dipyridamole analogs, commercially available starting materials, 2,4,6,8-tetrachloropyrimido[5,4-*d*]pyrimidine (TCPP) and dipyridamole, were used based on the structures of individual final products. For the preparation of the major dipyridamole analogs (compounds **1**-**8**, **11**-**71**, and **73**) (Scheme 1), an excess of the appropriate amine (about 4-fold excess) was reacted with TCPP in anhydrous THF. The resulting 2,6-dichloro intermediates were individually reacted with diethanolamine, ethanolamine or morpholine at 150 °C in DMSO as solvent to obtain the target products. For the preparation of compounds **9** and **10** (Scheme 1), the appropriate Grignard reagents were used for the first step, followed by reaction with diethanolamine in the second step.

For the preparation of compounds **74**-**79** (Scheme 2), dipyridamole was used as starting material. Dipyridamole was acylated or alkylated[44] to afford the desired products. Compound **78** was a dialkylated product, instead of the intended tetra-alkylated product. It appears that the introduction of the first isopropyl group at each side of dipyridamole prevented the introduction of a second isopropyl group on the remaining hydroxyl groups under the reaction conditions. This could be possibly due to steric hindrance. In total, 79 dipyridamole analogs with diverse substituents were synthesized in this study. The core pyrimido[5,4-*d*]pyrimidine system and the symmetrical feature in dipyridamole was maintained, with the exception of compound **8**, which had two different substituents at the at the 4- and 8-positions. Compound **8** was planned to be symmetrical, but the conditions in the second reaction step caused a loss of one Cbz group to produce the unsymmetrical compound.

Biological Studies

The compounds and positive controls, dipyridamole, NBMPR and lidoflazine were subjected to a flow cytometric assay with SAENTA-fluorescein (Figure 2) as the fluorescent probe.[44] 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 less amount of cells, as few as 5000 cells compared to 2 million cells per sample for comparable radioligand assays. SAENTA-fluorescein is a NBMPR analog, and it was used successfully used in several studies to determine the ENT1 inhibitory activities of NBMPR analogs.[16,17] Studies with radiolabeled ligands have shown that NBMPR, dipyridamole and lidoflazine displace each other at the binding sites on the ENT1 transporter.[45-47] Thus, we expected the new compounds would similarly displace SAENTA-fluorescein from the NBMPR binding site on the ENT1 transporter.

Dipyridamole itself is a fluorescent molecule (Exλ_{max} = 280 nm, Em λ_{max} = 490 nm),[48] but at the experimental wavelengths sets for SAENTA-fluorescein (Ex λ = 488 nm, Em λ = 533 nm), dipyridamole and its analogs, with the exception of compounds **9** and **10**, had insignificant absorbance and emission, which did not interfere with the detection of bound SAENTA-fluorescein. Human erythroleukemia K562 cells were used as the ENT1 transporter source for the binding experiments. This cell line expresses high levels of ENT1 protein, with very limited fraction of other nucleoside transporters,[49] and has been used widely for assessing ENT1 binding affinity of compounds.[50-53] Compounds were first screened at 10 μM, and those compounds that showed good inhibitory activities (% Inhibition > 40 %) were further tested at 10 concentration levels to generate dose-dependent curves from which the IC_{50} values were derived and used to calculate the corresponding K_i values. The inhibitory activities of the highly fluorescent dipyridamole analogs like **9** and **10** could not be determined by this method.

Structure–Activity Relationships

All dipyridamole analogs had the core structure of 2,4,6,8-tetra-substituted-pyrimido[5,4-*d*] pyrimidine. They maintained the symmetric feature as in the case of dipyridamole, with the exception of compound **8**, which had two different substituents at the 4-, and 8-positions of the core pyrimidopyrimidine structure. The ENT1 inhibitory activities are summarized in Tables 1-4. In all tables, the activities of one negative control (DMSO) and three positive controls, NBMPR, lidoflazine and dipyridamole, are listed for comparison.

Compounds listed in Table 1 are dipyridamole analogs with ring structures at the 4- and 8-positions of the pyrimidopyrimidine template; compounds listed in Table 2 are analogs with open-chain tertiary amines at the pyrimidopyrimidine 4- and 8-positions. Compounds listed in Table 3 have primary or secondary amine substituents at the 4- and 8-positions of the core structure. Compounds listed in Table 4 are derivatives of dipyridamole. In this study, NBMPR had a K_i of 0.43 nM, dipyridamole a K_i of 8.18 nM and lidoflazine a K_i of 279.9 nM, which are in agreement with the literature.

For substituents at the 4- and 8-positions of the pyrimido[5,4-*d*]pyrimidine, nitrogen-containing monocyclic ring structures usually gave analogs with good inhibitory activities, as in the case of compounds **2**, **4**, **11**, **13** and **15**. Increasing ring size from 5 (compound **2**) to 8 (compound **13**), increased inhibitory activity accordingly, with K_i values going from 99.7 nM to 0.49 nM, about 200-fold increase in inhibitory activity. Compound **13** was the most active analog in the series with comparable activity to one of best ENT1 nucleoside analog inhibitors, NBMPR (K_i of 0.43 nM). Compared to dipyridamole (K_i = 8.18 nM), compound **13** is 16 times more potent. A ring size of eight was optimal since a further increase in ring size to nine, decreased activity as can be seen with compound **15**, which had a K_i of 0.77 nM. The effect of ring size could be due to an increased hydrophobic effect since the piperidine ring in dipyridamole (K_i = 8.18 nM) provided higher inhibitory activity than the morpholino or piperazine rings in compounds **5** (K_i = 6,956 nM) and **6** (practically inactive), respectively. The binding pocket at the 4- and 8-positions also has limits on the ring size it can accommodate. Further, not only does the ring size matter, but also the ring flexibility is important, with flexible rings affording higher activity than rigid ring systems. This is evident in comparing the activities of compound **15** (K_i = 13.6 nM) and compound **17** (K_i = 3,416 nM). Compounds with N-(bis-hydroxyethyl) substituents at the 2- and 6-positions (Type A in Tables 1-4) were much more potent than the corresponding N-(monohydroxyethyl) substituted analogs (Type B in Tables 1-4).

The open chain analogs (compound **21**-**38**) were less active than the cyclic counterparts. Compounds with carbon chain length from 1 to 4 (compounds **21**, **23**, **25** and **27**) exhibited low inhibitory activities. Increasing the chain length (compound **31**), or branching it (compounds **29** and **33**) led to a decrease in activity. Compound **35** has polar oxygen atoms in the side chain, which also resulted in low activity. Compound **37** has dibenzylamino groups at the 4- and 8-positions and was inactive. In this set also, the N-(monohydroxyethyl) substituted analogs (compounds **22**, **24**, **26**, **28**, **30**, **32**, **34**, **36**, and **38**) were less active than the N-(bis-hydroxyethyl) counterparts.

The analogues which contained a primary or secondary amine (compounds **39**-**71**) at the 4- and 8-positions had lower inhibitory activities relative to dipyridamole. The most active compounds in the group, **52**, **58** and **64** were only about half as active as dipyridamole. These are analogs with *tert*-butylamino, *iso*-pentylamino and cyclopentylamnio groups at the 4- and 8-positions. Again, analogs with N-(monohydroxyethyl) substitution were less active than the N-(bis-hydroxyethyl) counterparts.

Compounds **72**-**79** are 2- and 6-substituted dipyridamole analogs. The presence of a 2′-hydroxyethoxy group at the 2- and 6-positions (compound **72**) resulted in a steep drop in activity, compared to dipyridamole. However, compound **72** exhibited higher activity than compound **1**, the N-(monohydroxyethyl) counterpart of dipyridamole. This indicates that a hydrogen atom on the 2- and 6-position nitrogen is unfavorable for potent activity. Compound **73** has the diethanolamino groups at the 2- and 6-postions locked into morpholino rings; and this modification caused a loss of activity. Esterification of dipyridamole (compound **74** and **75**) maintained relatively good activity compared to dipyridamole, which indicates that free hydroxyl groups are not necessary for activity. Esterification introduces additional oxygen atoms, which might participate in additional hydrogen-bonding that probably compensates for the loss of activity caused by an increase in lipophilicity. In contrast, ether type lipophilic modification at same positions caused a decrease in activity as in the case compounds **76** to **78**. Interestingly, compound **79**, which has one free hydroxyl group at the 2- and 6-positions, exhibited a higher potency than dipyridamole. The reasons for the higher potency of **79** relative to dipyridamole are not apparent. Some compounds, namely **7**, **25**, and **27**, had a % inhibition above 40 %, but no IC_{50}s, could be determined due to low solubility.

These dipyridamole analogs had modifications at two important regions with regard to ENT1 inhibitory activity (see Figure 3). Region 1 should be lipophilic to obtain the highest ENT1 inhibitory activities, with single nitrogen-containing flexible rings being preferred to carbocyclic, morpholine, piperazine or rigid multicyclic ring systems. For the nitrogen-containing flexible rings, an 8-membered ring is optimal. Region 2 should be hydrophilic region with diethanolamino group providing optimal activity, although it is not essential; small lipophilic modifications over the hydroxyl groups are well tolerated.

Conclusion

In this study, a substantial number of dipyridamole analogs were synthesized and explored for their inhibitory activity against ENT1 transporter using a flow cytometric method. Compounds with much higher activity than dipyridamole were identified for the first time, with the best, compound **13**, being 16 times more active than dipyridamole, and having comparative activity to the potent ENT1 standard inhibitor NBMPR. The study has also revealed important structural determinants for ENT1 inhibitory activity in this series, among which are the requirements for a lipophilic medium to large size nitrogen containing lipophilic rings at the 4- and 8-positions, and hydrophilic, hydrogen-bond acceptor substituents at the 2- and 6-positions. The newly identified higher potency dipyridamole analog, compound **13**, may facilitate the therapeutic exploitation of the ENT1 inhibitory activity of dipyridamole and related compounds.

Experimental Section

Chemistry

Thin-layer chromatography (TLC) was conducted on silica gel plates (Analtech). Compounds were visualized by UV light (254 and 365 nm). 1D NMR spectra were recorded on a Varian Inova 500 MHz NMR instruments by using $CDCl_3$ or $(CD_3)_2SO$ as solvents and tetramethylsilane (TMS) as an 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 were reported uncorrected. Mass spectra were obtained on a Bruker-HP ESQUIRE Ion Trap LC/MS(n) system. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA. All solvents and reagents were purchased from Aldrich or other major chemical companies, and used without further purification. All reactions were carried under argon gas.

2,6-Bis(diethanolamino)-4,8-disubstituted-pyrimido[5,4-*d*]pyrimidine, or 2,6-diethanolamino-4,8-disubstituted-pyrimido[5,4-*d*]pyrimidine. General procedure I

To a solution of 2,4,6,8-tetra-chloro-pyrimido[5,4-*d*]pyrimidine (TCPP) (0.27 g, 1 mmole) in anhydrous THF (10 ml), appropriate amine (4.2 mmole) was added in this first step. The reaction was stirred on an ice-water bath for 20 min, and then water (100 ml) was added to precipitate the reaction intermediate. After drying over P_2O_5, the intermediate was dissolved in DMSO (3 ml), and an appropriate amine (diethanolamine, ethanolamine or morpholine) (3 ml) was added and the reaction was heated at 150 °C for 6 hours with stirring. Then, the product was purified by flash silica gel chromatography.

2,6-Bis(diethanolamino)-4,8-disubstituted-pyrimido[5,4-*d*]pyrimidine. General procedure II

To a solution of 2,4,6,8-tetra-chloro-pyrimido[5,4-*d*]pyrimidine (TCPP) (0.27 g, 1 mmole) in anhydrous THF (10 ml), appropriate Grignard reagent (2.1 mmole) was added at this first step. The reaction was stirred in ice-water bath for 20min, and then water (100 ml) was added to precipitate the reaction intermediate. After drying over P_2O_5, the intermediate was dissolved in DMSO (3 ml), and diethanolamine (3 ml) was added; and the reaction was heated at 150 ° C for 6 hours with stirring. Then, the product was purified by flash silica gel chromatography.

2,6-Bis(dialkoxylethylamino)-4,8-disubstituted-pyrimido[5,4-*d*]pyrimidine. General procedure III

NaH (60% in mineral oil, 0.28 g, 7 mmole) was added to a solution of dipyridamole (0.35 g, 0.69 mmole) in anhydrous DMF (10 ml), and the reaction was stirred at room temperature for 2 hours; and then appropriate alkyl halide (32 mmole) was added, and the reaction was stirred for overnight. The reaction mixture was participated between CH_2Cl_2 (60 ml) and H_2O (50 ml), and the organic layer was separated, the left aqueous solution was extracted with CH_2Cl_2 (20 ml × 2), and all organic solutions were incorporated and dried over anhydrous Na_2SO_4. Then the CH_2Cl_2 was removed under reduced pressure, and the residue was subjected to flash silica gel chromatography for purification of the product.

2,6-Diethanolamino-4,8-dipiperidino-pyrimido[5,4-*d*]pyrimidine (1)

Compound **1** was prepared by general procedure I with piperidine (0.41 ml, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. The product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=16/1) to give a yellow powdery solid (162 mg, 39%). Mp: 152-153 °C; MS (ESI) *m/z* 417 $(M + H)^+$, 439 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 6.016 (t, 2H, 2 × NH, disappeared after D_2O, J = 5.5 Hz), 4.606 (t, 2H, 2 × OH, disappeared after D_2O, J = 5.5 Hz), 4.057 (br s, 8H, 2 × $N(CH_2CH_2)_2CH_2$), 3.513 (q, 4H, 2 × $NHCH_2CH_2OH$, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.269 (q, 4H, 2 × $NHCH_2CH_2OH$, J_1 = 5.5 Hz, J_2 = 6 Hz), 1.641 (br d, 4H, 2 × $N(CH_2CH_2)_2CH_2$, J = 4.5 Hz), 1.592 (br d, 8H, 2 × $N(CH_2CH_2)_2CH_2$, J = 4.5 Hz); Anal. ($C_{20}H_{32}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-dipyrrolidinyl-pyrimido[5,4-*d*]pyrimidine (2)

Compound 2 was prepared by general procedure I with pyrroline (0.35 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=15/1) to give a yellow power solid (252 mg, 53%). Mp: 212-213 °C; MS (ESI) *m/z* 477 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 4.688 (m, 4H, 4 × OH, disappeared after D_2O exchange), 4.119 (br s, 8H, 2 × $N(CH_2CH_2)_2$), 3.592 (br s, 16H, 2 × $N(CH_2CH_2OH)_2$), 1.877 (br s, 8H, 2 × $N(CH_2CH_2)_2$); Anal. Calcd ($C_{22}H_{36}N_8O_4$): C, H, N.

2,6-Diethanolamino-4,8-dipyrrolidinyl-pyrimido[5,4-*d*]pyrimidine (3)

Compound 3 was prepared by general procedure I with pyrroline (0.35 ml, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=15/1) to give a yellow power solid (176 mg, 45%). Mp: 219-220 °C; MS (ESI) *m/z* 389 $(M + H)^+$, 411 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 5.774 (t, 2H, 2 × NH, disappeared after D_2O), 4.591 (t, 2H, 2 × OH, disappeared after D_2O exchange), 4.006 (br s, 8H, 2 × $N(CH_2CH_2)_2$), 3.505 (q, 4H, 2 × $NHCH_2CH_2OH$, J = 6 Hz), 3.292 (q, 4H, 2 × $NHCH_2CH_2OH$, J_1 = 6 Hz), 1.863 (br s, 8H, 2 × $N(CH_2CH_2)_2$); Anal. ($C_{18}H_{28}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-dimorpholino-pyrimido[5,4-*d*]pyrimidine (4)

Compound 4 was prepared by general procedure I with morpholine (0.37 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=16/1) to give a yellow power solid (274 mg, 54%). Mp: 205-206 °C; MS (ESI) *m/z* 509 $(M + H)^+$, 531 $(M + Na)^+$; ^{1} H NMR (DMSO-d_6) δ 4.689 (t, 4H, 4 × OH, disappeared after D_2O), 4.121 (br s, 8H, 2 × $N(CH_2CH_2)_2O$), 3.715 (t, 8H, 2 × $N(CH_2CH_2)_2O$), 3.573 (br s, 16H, 2 × $N(CH_2CH_2OH)_2$); Anal. ($C_{22}H_{36}N_8O_6 \cdot 0.5\ H_2O$) C, H, N.

2,6-Diethanolamino-4,8-dimorpholino-pyrimido[5,4-*d*]pyrimidine (5)

Compound 5 was prepared by general procedure I with morpholine (0.37 ml, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=15/1) to give a yellow power solid (211 mg, 50%). Mp: 203-204 °C; MS (ESI) *m/z* 421 $(M + H)^+$, 443 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 6.186 (t, 2H, 2 × NH, disappeared after D_2O), 4.619 (t, 2H, 2 × OH, disappeared after D_2O), 4.128 (br s, 8H, 2 × $N(CH_2CH_2)_2O$), 3.708 (t, 8H, 2 × $N(CH_2CH_2)_2O$), 3.504 (q, 4H, 2 × $NHCH_2CH_2OH$), 3.254 (q, 4H, 2 × $NHCH_2CH_2OH$); Anal. ($C_{18}H_{18}N_8O_4 \cdot 0.5\ H_2O$) C, H, N.

2,6-Bis(diethanolamino)-4,8-di-(N-methyl-piperazino)-pyrimido[5,4-*d*]pyrimidine (6)

Compound 6 was prepared by general procedure I with 1-methylpiperazine (0.47 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=1/1) to give a yellow power solid (273 mg, 51%). Mp: 199-200 °C; MS (ESI) *m/z* 535 $(M + H)^+$, 557 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 4.719 (t, 4H, 4 × OH, disappeared after D_2O), 4.122 (br s, 8H, 2 × N $(CH_2CH_2)_2NCH_3$), 3.591 (br s, 16H, 2 × $N(CH_2CH_2OH)_2$), 2.434 (t, 8H, 2 × N $(CH_2CH_2)_2NCH_3$), 2.219 (s, 6H, 2 × CH_3); Anal. ($C_{24}H_{42}N_{10}O_4$) C, H, N.

2,6-Bis(diethanolamino)-4,8-di-(N-BOC-piperazino)-pyrimido[5,4-*d*]pyrimidine (7)

Compound 7 was prepared by general procedure I with N-BOC-piperazine (0.78 g, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=5/1) to give a yellow power solid (304 mg, 43%). Mp: 223-224 °C; MS (ESI) *m/z* 707 $(M + H)^+$, 729 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 4.749 (br t, 4H, 4 × OH, disappeared after D_2O), 4.121 (br s, 8H, 2 × $N(CH_2CH_2)_2$N-BOC), 3.611 (s, 16H, 2 × $N(CH_2CH_2OH)_2$), 3.489 (br s, 8H, 2 × $N(CH_2CH_2)_2$N-BOC), 1.465 (s, 18H, 6 × CH_3). Anal. ($C_{32}H_{54}N_{10}O_8$) C, H, N.

2,6-Bis(diethanolamino)-4-piperazino-8-(N-Cbz-piperazino)-pyrimido[5,4-*d*]pyrimidine (8)

Compound 8 was prepared by general procedure I with benyl piperazine-1-carboxylate (0.93 g, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=1.5/1) to give a yellow power

solid (77 mg, 12%). Mp: 133-134 °C; MS (ESI) *m/z* 641 $(M + H)^+$, 663 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 7.388 (d, 3H, Ar-H-3, Ar-H-4, Ar-H-5), 7.335 (m, 2H, Ar-H-2, Ar-H-6), 5.121 (s, 2H, PhCH_2), 4.699 (t, 4H, 4 × OH, disappeared after D_2O), 4.119 (br s, 4H, N(CH_2CH_2)$_2$NH), 4.031 (br s, 4H, N(CH_2CH_2)$_2$NCbz), 3.570 (br s, 21H, 2 × N(CH_2CH_2OH)$_2$, N(CH_2CH_2)$_2$N*H*), 2.789 (br s, 4H, N(CH_2CH_2)$_2$NCbz); Anal. ($C_{30}H_{44}N_{10}O_6$) C, H, N.

2,6-Bis(diethanolamino)-4,8-dicyclohexyl-pyrimido[5,4-*d*]pyrimidine (9)

Compound 9 was prepared by general procedure II with cyclehexylmagnesium chloride solution (2.0 M in diethyl ether, 1.05 ml, 2.1 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=24/1) to give a yellow power solid (226 mg, 45%). Mp: 226-228 °C; MS (ESI) *m/z* 503 $(M + H)^+$, 525 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 4.782 (t, 4H, 4 × OH, disappeared after D_2O), 3.717 (br s, 8H, 2 × N(CH_2CH_2OH)$_2$), 3.675 (br s, 8H, 2 × N(CH_2CH_2OH)$_2$), 3.602 (m, 2H, 2 × C*H*(CH_2CH_2)$_2$$CH_2$), 1.892 − 1.815 (m, 8H, 2 × CH(CH_2CH_2)$_2$$CH_2$), 1.753 (d, 2H, 2 × CH($CH_2CH_2$)$_2CH_A$$H_B$), 1.589 − 1.397 (m, 8H, 2 × CH(CH_2CH_2)$_2$$CH_2$), 1.283 (m, 2H, 2 × CH($CH_2CH_2$)$_2$$CH_A$$H_B$); Anal. ($C_{26}H_{42}N_6O_4$) C, H, N.

2,6-Bis(diethanolamino)-4,8-diphenyl-pyrimido[5,4-*d*]pyrimidine (10)

Compound 10 was prepared by general procedure II with phenylmagnesium chloride solution (2.0 M in tetrahydrofuran, 1.05 ml, 2.1 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=9/1) to give a red power solid (29 mg, 5.9%). Mp: 208-209 °C; MS (ESI) *m/z* 491 $(M + H)^+$, 513 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 8.470 (m, 4H, 2 × Ar-H-2, 2 × Ar-H-6), 7.557 (m, 6H, 2 × Ar-H-3, 2 × Ar-H-4, 2 × Ar-H-5), 4.805 (t, 4H, 4 × OH, disappeared after D_2O, *J* = 5Hz), 3.793 (br s, 8H, 2 × N(CH_2CH_2OH)$_2$), 3.708 (t, 8H, 2 × N(CH_2CH_2OH)$_2$, *J* = 5Hz); Anal. ($C_{26}H_{30}N_6O_4$) C, H, N.

2,6-Bis(diethanolamino)-4,8-dihexamethyleneimino-pyrimido[5,4-*d*]pyrimidine (11)

Compound 11 was prepared by general procedure I with hexamethyleneimine (0.48 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (Hexane/Acetone=2/1) to give a yellow power solid (213 mg, 40%). Mp: 212-213 °C; MS (ESI) *m/z* 533 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 4.677 (br t, 4H, 4 × OH, disappeared after D_2O), 4.129 (br, s, 8H, 2 × N($CH_2CH_2CH_2$)$_2$), 3.572 (s, 16H, 2 × N(CH_2CH_2OH)$_2$), 1.775 (br s, 8H, 2 × N($CH_2CH_2CH_2$)$_2$), 1.511 (br s, 8H, 2 × N($CH_2CH_2CH_2$)$_2$); Anal. ($C_{26}H_{44}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-dihexamethyleneimino-pyrimido[5,4-*d*]pyrimidine (12)

Compound 12 was prepared by general procedure I with hexamethyleneimine (0.48 ml, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=20/1) to give a yellow power solid (106 mg, 24%). Mp: 166-167 °C; MS (ESI) *m/z* 445 $(M + H)^+$, 467 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 5.787 (q, 2H, 2 × NH, disappeared after D_2O, *J* = 5.5 Hz), 4.592 (t, 2H, 2 × OH, disappeared after D_2O, *J* = 5.5 Hz), 4.112 (br s, 8H, 2 × N($CH_2CH_2CH_2$)$_2$), 3.514 (q, 4H, 2 × NHCH$_2$CH_2OH, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.246 (t, 4H, 2 × NHCH_2CH$_2$OH, J_1 = 5.5 Hz, J_2 = 6 Hz), 1.776 (br s, 8H, 2 × N($CH_2CH_2CH_2$)$_2$), 1.500 (br s, 8H, 2 × N($CH_2CH_2CH_2$)$_2$); Anal. ($C_{22}H_{36}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-diheptamethyleneimino-pyrimido[5,4-*d*]pyrimidine (13)

Compound 13 was prepared by general procedure I with heptamethyleneimine (0.53 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was

purified by flash silica gel chromatography (Hexane/Acetone=3/1) to give a yellow power solid (219 mg, 39%). Mp: 204-205 °C; MS (ESI) *m/z* 561 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 4.681 (t, 4H, 4 × OH, disappeared after D_2O), 4.091 (br, s, 8H, 2 × $N(CH_2CH_2CH_2)_2CH_2$), 3.576 (br s, 16H, 2 × $N(CH_2CH_2OH)_2$), 1.782 (br s, 8H, 2 × $N(CH_2CH_2CH_2)_2CH_2$), 1.541 (br s, 8H, 2 × $N(CH_2CH_2CH_2)_2CH_2$), 1.479 (br s, 4H, 2 × $N(CH_2CH_2CH_2)_2CH_2$); Anal. ($C_{28}H_{48}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-diheptamethyleneimino-pyrimido[5,4-*d*]pyrimidine (14)

Compound 14 was prepared by general procedure I with heptamethyleneimine (0.53 ml, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (Hexane/Acetone=2/1) to give a yellow power solid (246 mg, 43%). Mp: 150-151 °C; MS (ESI) *m/z* 573 $(M + H)^+$, 495 $(M + Na)^+$, 511 $(M + K)^+$; ^{1}H NMR (DMSO-d_6) δ 5.751 (t, 2H, 2 × NH, disappeared after D_2O, J = 6 Hz), 4.592 (t, 2H, 2 × OH, disappeared after D_2O, J = 5.5 Hz), 4.077 (br, s, 8H, 2 × N $(CH_2CH_2CH_2)_2CH_2$), 3.508 (q, 4H, 2 × $NHCH_2CH_2OH$, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.256 (q, 4H, 2 × $NHCH_2CH_2OH$, J_1 = 6 Hz, J_2 = 6 Hz), 1.786 (br s, 8H, 2 × $N(CH_2CH_2CH_2)_2CH_2$), 1.527 (br s, 8H, 2 × $N(CH_2CH_2CH_2)_2CH_2$), 1.468 (br s, 4H, 2 × $N(CH_2CH_2CH_2)_2CH_2$); Anal. ($C_{24}H_{40}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-di-octomethyleneimino-pyrimido[5,4-*d*]pyrimidine (15)

Compound 15 was prepared by general procedure I with octomethyleneimine (0.54 g, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (Hexane/Acetone=2.5/1) to give a yellow power solid (65 mg, 11%). Mp: 213-214 °C; MS (ESI) *m/z* 589 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 4.686 (t, 4H, 4 × OH, disappeared after D_2O), 4.055 (br, s, 8H, 2 × $N(CH_2CH_2CH_2CH_2)_2$), 3.602 (m, 16H, 2 × $N(CH_2CH_2OH)_2$), 1.811 (s, 8H, 2 × $N(CH_2CH_2CH_2CH_2)_2$), 1.648 (s, 8H, 2 × $N(CH_2CH_2CH_2CH_2)_2$), 1.451 (s, 8H, 2 × $N(CH_2CH_2CH_2CH_2)_2$); Anal. Calcd ($C_{30}H_{52}N_8O_4$): C 61.20, H 8.90, N 19.03; Found: C 60.73, H 8.84, N 18.87.

2,6-Diethanolamino-4,8-di-octomethyleneimino-pyrimido[5,4-*d*]pyrimidine (16)

Compound 16 was prepared by general procedure I with octomethyleneimine (0.54 g, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (Hexane/Acetone=2.5/1) to give a yellow power solid (60 mg, 12%). Mp: 167 °C; MS (ESI) *m/z* 501 $(M + H)^+$, 523 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 5.746 (t, 2H, 2 × NH, disappeared after D_2O, J = 6 Hz), 4.601 (t, 2H, 2 × OH, disappeared after D_2O, J = 5.5 Hz), 4.039 (br, s, 8H, 2 × $N(CH_2CH_2CH_2CH_2)_2$), 3.514 (q, 4H, 2 × $NHCH_2CH_2OH$, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.284 (q, 4H, 2 × $NHCH_2CH_2OH$, J_1 = 6 Hz, J_2 = 5.5 Hz), 1.822 (br s, 8H, 2 × $N(CH_2CH_2CH_2CH_2)_2$), 1.645 (br s, 8H, 2 × N $(CH_2CH_2CH_2CH_2)_2$), 1.440 (s, 8H, 2 × $N(CH_2CH_2CH_2CH_2)_2$); Anal. ($C_{26}H_{44}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-dinontropano-pyrimido[5,4-*d*]pyrimidine (17)

Compound 17 was prepared by general procedure I with nontropane[54] (0.47 g, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=10/1) to give a yellow power solid (250 mg, 45%). Mp: 244-245 °C; MS (ESI) *m/z* 557 $(M + H)^+$, 579 $(M + Na)^+$; ^{1}H NMR[56] (DMSO-d_6) δ 6.203 (br s, 2H), 4.908 (br s, 2H), 4.695 (t, 4H, 4 × OH, disappeared after D_2O), 3.581 (br s, 16H, 2 × $N(CH_2CH_2OH)_2$), 1.942-1.455 (series of br s, 20H); Anal. ($C_{28}H_{44}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-dinontropanopyrimido[5,4-*d*]pyrimidine (18)

Compound 18 was prepared by general procedure I with nontropane[54] (0.47 g, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 16/1) to give a yellow power solid compound 7 (128 mg, 27%). Mp: 254-255 °C; MS (ESI) *m/z* 469 $(M + H)^+$, 491 $(M + Na)^+$; ^{1}H NMR[57] (DMSO-d_6) δ 6.319 (br s, 2H), 5.901 (t, 2H, 2 × N*H*CH_2CH_2OH, disappeared after D_2O, J = 5.5 Hz), 4.909 (br s, 2H), 4.584 (t, 2H, 2 × OH, disappeared after D_2O, J = 5.5 Hz), 3.515 (q, 4H, 2 × $NHCH_2CH_2OH$, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.243 (d, 4H, 2 × $NHCH_2CH_2OH$, J = 5.5 Hz), 1.938-1.430 (series of br s, 20H); Anal. ($C_{24}H_{36}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-di-(4-azatricyclo[4.3.1.1^{3,8}]undecane)-pyrimido[5,4-*d*] pyrimidine (19)

Compound 19 was prepared by general procedure I with 4-azatricyclo[4.3.1.1^{3,8}] undecane[55] (0.64 g, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=20/1) to give a yellow power solid (262 mg, 41%). Mp: 252-253 °C; MS (ESI) *m/z* 637 $(M + H)^+$, 659 $(M + Na)^+$; ^{1}H NMR[57] (DMSO-d_6) δ 5.765 (br s, 2H), 4.680 (t, 4H, 4 × OH, disappeared after D_2O), 3.890 (br s, 4H), 3.589 (br s, 16H, 2 × $N(CH_2CH_2OH)_2$), 2.299 (br s, 2H), 1.959 (t, 4H), 1.929 (br s, 8H), 1.759-1.733 (br d, 4H), 1.604-1.516 (m, 8H); Anal. ($C_{34}H_{52}N_8O_4 \cdot 0.5\ H_2O$) C, H, N.

2,6-Diethanolamino-4,8-di-(4-azatricyclo[4.3.1.13,8]undecane)-pyrimido[5,4-*d*]pyrimidine (20)

Compound 20 was prepared by general procedure I with 4-azatricyclo[4.3.1.1^{3,8}] undecane[55] (0.64 g, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (Hexane/Acetone=2.5/1) to give a yellow power solid (198 mg, 36%). Mp: 194-196 °C; MS (ESI) *m/z* 549 $(M + H)^+$, 571 $(M + Na)^+$; ^{1}H NMR[55] (DMSO-d_6) δ 5.746 (t, 2H, 2 × NH, disappeared after D_2O, J = 6 Hz), 5.720 (br s, 2H), 4.580 (t, 2H, 2 × OH, disappeared after D_2O, J = 5.5 Hz), 3.834 (br s, 4H), 3.505 (q, 4H, 2 × $NHCH_2CH_2OH$, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.289 (q, 4H, 2 × $NHCH_2CH_2OH$, J_1 = 6 Hz, J_2 = 6 Hz), 2.287 (br s, 2H), 1.951-1.926 (m, 12H), 1.793-1.767 (br d, 4H), 1.605-1.510 (m, 8H); Anal. ($C_{30}H_{44}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-di-(dimethylamino)-pyrimido[5,4-*d*]pyrimidine (21)

Compound 21 was prepared by general procedure I with dimethylamine solution (2.0 M in tetrahydrofuran, 2.1 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/ MeOH=12/1) to give a yellow power solid (174 mg, 41%). Mp: 207-208 °C; MS (ESI) *m/z* 425 $(M + H)^+$, 447 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 4.704 (t, 4H, 4 × OH, disappeared after D_2O, J = 5.5Hz), 3.597 (m, 16H, 2 × $N(CH_2CH_2OH)_2$, J = 5.5Hz), 3.409 (br s, 12H, 4 × CH_3); Anal. ($C_{18}H_{32}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-di-(dimethylamino)-pyrimido[5,4-*d*]pyrimidine (22)

Compound 22 was prepared by general procedure I with dimethylamine solution (2.0 M in tetrahydrofuran, 2.1 ml, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=9/1) to give a yellow power solid (40 mg, 12%). Mp: 159-161 °C; MS (ESI) *m/z* 337 $(M + H)^+$, 359 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 5.954 (br s, 2H, 2 × NH, disappeared after D_2O), 4.634 (t, 2H, 2 × OH, disappeared after D_2O), 3.509 (q, 4H, 2 × $NHCH_2CH_2OH$), 3.371 (br s, 12H, 4 × CH_3), 3.284 (t, 4H, 2 × $NHCH_2CH_2OH$); Anal. ($C_{14}H_{24}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-di-(diethylamino)-pyrimido[5,4-*d*]pyrimidine (23)

Compound 23 was prepared by general procedure I with diethylamine (0.44 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=10/1) to give a yellow power solid (187 mg, 39%). Mp: 165-166 °C; MS (ESI) *m/z* 481 $(M + H)^+$, 503 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 4.692 (t, 4H, 4 × OH, disappeared after D_2O), 3.914 (br s, 8H, 4 × CH_2CH$_3$), 3.589 (br s, 16H, 2 × N(CH_2CH_2OH)$_2$), 1.205 (t, 12H, 4 × CH$_2$CH_3); Anal. ($C_{22}H_{40}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-di-(diethylamino)-pyrimido[5,4-*d*]pyrimidine (24)

Compound 24 was prepared by general procedure I with diethylamine (0.44 ml, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=16/1) to give a yellow power solid (122 mg, 31%). Mp: 127 °C; MS (ESI) *m/z* 393 $(M + H)^+$, 415 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 5.787 (t, 2H, 2 × NH, disappeared after D_2O, J = 5.5 Hz), 4.599 (t, 2H, 2 × OH, disappeared after D_2O, J = 6 Hz), 3.902 (br s, 8H, 4 × CH_2CH$_3$), 3.506 (q, 4H, 2 × NHCH$_2$CH_2OH, J_1 = 6 Hz, J_2 = 6 Hz), 3.265 (q, 4H, 2 × NHCH_2CH$_2$OH, J_1 = 6 Hz, J_2 = 5.5 Hz), 1.198 (t, 12H, 4 × CH$_2$CH_3); Anal. ($C_{18}H_{32}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-di-(dipropylamino)-pyrimido[5,4-*d*]pyrimidine (25)

Compound 25 was prepared by general procedure I with dipropylamine (0.58 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=18/1) to give a yellow power solid (81 mg, 15%). Mp: 150-151 °C; MS (ESI) *m/z* 537 $(M + H)^+$, 559 $(M + Na)^+$, 575 $(M + K)^+$; ^{1}H NMR (DMSO-d_6) δ 4.707 (br s, 4H, 4 × OH, disappeared after D_2O), 3.846 (br s, 8H, 4 × CH_2CH$_2$CH$_3$), 3.586 (br s, 16H, 2 × N(CH_2CH_2OH)$_2$), 1.637 (q, 8H, 4 × CH$_2$CH_2CH$_3$, J = 7.5 Hz), 0.878 (t, 12H, 4 × CH$_2$CH$_2$CH_3, J = 7.5 Hz); Anal. ($C_{26}H_{48}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-di-(dipropylamino)-pyrimido[5,4-*d*]pyrimidine (26)

Compound 26 was prepared by general procedure I with dipropylamine (0.58 ml, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=12/1) to give a yellow power solid (85 mg, 19%). Mp: 144-145 °C; MS (ESI) *m/z* 449 $(M + H)^+$, 471 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 5.723 (t, 2H, 2 × NH, disappeared after D_2O, J = 6 Hz), 4.618 (t, 2H, 2 × OH, disappeared after D_2O, J = 5.5 Hz), 3.837 (br s, 8H, 4 × CH_2CH$_2$CH$_3$), 3.518 (q, 4H, 2 × NHCH$_2$CH_2OH, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.288 (q, 4H, 2 × NHCH_2CH$_2$OH, J_1 = 6 Hz, J_2 = 6 Hz), 1.644 (m, 8H, 4 × CH$_2$CH_2CH$_3$, J = 7.5 Hz), 0.876 (t, 12H, 4 × CH$_2$CH$_2$CH_3, J = 7.5 Hz); Anal. ($C_{22}H_{40}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-di-(dibutylamino)-pyrimido[5,4-*d*]pyrimidine (27)

Compound 27 was prepared by general procedure I with dibutylamine (0.71 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 27/1) to give a yellow power solid (157 mg, 27%). Mp: 126-127 °C; MS (ESI) *m/z* 593 $(M + H)^+$, 615 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 4.705 (t, 4H, 4 × OH, disappeared after D_2O, J = 5 Hz), 3.876 (br s, 8H, 4 × CH_2CH$_2$CH$_2$CH$_3$), 3.581 (br s, 16H, 2 × N(CH_2CH_2OH)$_2$), 1.591 (m, 8H, 4 × CH$_2$CH_2CH$_2$CH$_3$, J = 8 Hz), 1.308 (m, 8H, 4 × CH$_2$CH$_2$CH_2CH$_3$, J_1 = 8 Hz, J_2 = 7.5 Hz), 0.903 (t, 12H, 4 × CH$_3$, J = 7.5 Hz); Anal. ($C_{30}H_{56}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-di-(dibutylamino)-pyrimido[5,4-*d*]pyrimidine (28)

Compound 28 was prepared by general procedure I with dibutylamine (0.71 ml, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (Hexane/Acetone = 5/1) to give a yellow power solid (124 mg, 22%). Mp: 129-130 °C; MS (ESI) *m/z* 505 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 5.687 (t, 2H, 2 × NH, disappeared after D_2O, J = 5.5 Hz), 4.615 (t, 2H, 2 × OH, disappeared after D_2O, J = 5.5 Hz), 3.870 (br s, 8H, 4 × CH_2CH$_2$CH$_2$CH$_3$), 3.505 (q, 4H, 2 × NHCH$_2$CH_2OH, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.267 (q, 4H, 2 × NHCH_2CH$_2$OH, J_1 = 6 Hz, J_2 = 6 Hz), 1.596 (m, 8H, 4 × CH$_2$CH_2CH$_2$CH$_3$, J = 7.5 Hz), 1.308 (m, 8H, 4 × CH$_2$CH$_2$CH_2CH$_3$, J = 7.5 Hz), 0.907 (t, 12H, 4 × CH$_3$, J = 7.5 Hz); Anal. ($C_{26}H_{48}N_8O_2 \cdot 0.5\ H_2O$) C, H, N.

2,6-Bis(diethanolamino)-4,8-di-(diisobutylamino)-pyrimido[5,4-*d*]pyrimidine (29)

Compound 29 was prepared by general procedure I with diisobutylamine (0.73 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=14/1) to give a yellow power solid (59 mg, 10%). Mp: 169-171°C; MS (ESI) *m/z* 593 $(M + H)^+$, 615 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 4.669 (br s, 4H, 4 × OH, disappeared after D_2O), 3.808 (br s, 8H, 4 × CH_2CH(CH$_3$)$_2$), 3.528 (br s, 16H, 2 × N(CH_2CH_2OH)$_2$), 1.890 (br s, 4H, 4 × CH$_2$CH(CH$_3$)$_2$), 0.752 (br s, 24H, 4 × CH$_2$CH(CH_3)$_2$); Anal. ($C_{30}H_{56}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-di-(diisobutylamino)-pyrimido[5,4-*d*]pyrimidine (30)

Compound 30 was prepared by general procedure I with diisobutylamine (0.73 ml, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=18/1) to give a yellow power solid (212 mg, 42%). Mp: 154°C; MS (ESI) *m/z* 505 $(M + H)^+$, 527 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 5.821 (t, 2H, 2 × NH, disappeared after D_2O, J = 5.5 Hz), 4.634 (t, 2H, 2 × OH, disappeared after D_2O, J = 5.5 Hz), 3.881 (br s, 8H, 4 × CH_2CH(CH$_3$)$_2$), 3.532 (q, 4H, 2 × NHCH$_2$CH_2OH, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.276 (q, 4H, 2 × NHCH_2CH$_2$OH, J_1 = 6 Hz, J_2 = 5.5 Hz), 1.983 (m, 4H, 4 × CH$_2$CH(CH$_3$)$_2$, J = 6.5 Hz), 0.840 (d, 24H, 4 × CH$_2$CH(CH_3)$_2$, J = 6.5 Hz); Anal. ($C_{26}H_{48}N_8O_2 \cdot 0.5\ H_2O$) C, H, N.

2,6-Bis(diethanolamino)-4,8-di-(dipentylamino)-pyrimido[5,4-*d*]pyrimidine (31)

Compound 31 was prepared by general procedure I with dipentylamine (0.85 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 20/1) to give a yellow power solid (145 mg, 22%). Mp: 130-131 °C; MS (ESI) *m/z* 649 $(M + H)^+$, 671 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 4.706 (t, 4H, 4 × OH, disappeared after D_2O), 3.865 (br s, 8H, 4 × CH_2(CH$_2$)$_3$CH$_3$), 3.578 (q, 16H, 2 × N(CH_2CH_2OH)$_2$), 1.604 (m, 8H, 4 × CH$_2$CH_2(CH$_2$)$_2$CH$_3$), 1.332 - 1.229 (m, 16H, 4 × CH$_2$CH$_2$(CH_2)$_2$CH$_3$), 0.869 (t, 12H, 4 × CH$_3$); Anal. ($C_{34}H_{64}N_8O_4 \cdot 0.5\ H_2O$) C, H, N.

2,6-Diethanolamino-4,8-di-(dipentylamino)-pyrimido[5,4-*d*]pyrimidine (32)

Compound 32 was prepared by general procedure I with dipentylamine (0.85 ml, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (Hexane/Acetone = 15/1) to give a yellow power solid (26 mg, 4.6%). Mp: 128-129 °C; MS (ESI) *m/z* 561 $(M + H)^+$, 583 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 5.659 (t, 2H, 2 × NH, disappeared after D_2O, J = 5.5 Hz), 4.619 (t, 2H, 2 × OH, disappeared after D_2O, J = 5.5 Hz), 3.860 (br s, 8H, 4 × CH_2(CH$_2$)$_3$CH$_3$), 3.504 (q, 4H, 2 × NHCH$_2$CH_2OH, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.269 (q, 4H, 2 × NHCH_2CH$_2$OH, J_1 = 6 Hz, J_2 = 5.5 Hz), 1.609 (m, 8H, 4 × CH$_2$CH_2(CH$_2$)$_2$CH$_3$, J_1 = 7.5 Hz, J_2 = 7 Hz), 1.255 (m, 16H, 4 × CH$_2$CH$_2$(CH_2)$_2$CH$_3$), 0.871 (t, 12H, 4 × CH$_3$, J = 7 Hz); Anal. ($C_{30}H_{56}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-di-(diisopentylamino)-pyrimido[5,4-*d*]pyrimidine (33)

Compound 33 was prepared by general procedure I with diisopentylamine (0.86 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (Hexane/Acetone = 7/1) to give a yellow power solid (156 mg, 24%). Mp: 129°C; MS (ESI) *m/z* 671 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 4.705 (t, 4H, 4 × OH, disappeared after D_2O, J = 5.5 Hz), 3.864 (br s, 8H, 4 × CH_2CH$_2$CH(CH$_3$)$_2$), 3.578 (br s, 16H, 2 × N(CH_2CH_2OH)$_2$), 1.603 (m, 8H, 4 × CH$_2$CH_2CH(CH$_3$)$_2$), 1.301 (m, 16H, 4 × CH$_2$CH$_2$CH(CH$_3$)$_2$, 4 × CH$_3$), 0.868 (t, 12H, 4 × CH$_3$, J = 7 Hz); Anal. ($C_{34}H_{64}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-di-(diisopentylamino)-pyrimido[5,4-*d*]pyrimidine (34)

Compound 34 was prepared by general procedure I with diisopentylamine (0.86 ml, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (Hexane/Acetone = 15/1) to give a yellow power solid (125 mg, 22%). Mp: 97-98°C; MS (ESI) *m/z* 561 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 5.727 (m, 2H, 2 × NH, disappeared after D_2O, J = 5.5 Hz), 4.621 (m, 2H, 2 × OH, disappeared after D_2O, J = 5.5 Hz), 3.858 (br d, 8H, 4 × CH_2CH$_2$CH(CH$_3$)$_2$), 3.510 (q, 4H, 2 × NHCH$_2$CH_2OH, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.269 (q, 4H, 2 × NHCH_2CH$_2$OH, J_1 = 5.5 Hz, J_2 = 6 Hz), 1.841 - 1.335 (m, 8H, 4 × CH$_2$CH_2CH(CH$_3$)$_2$), 1.321 - 1.058 (m, 12H, 4 × CH$_3$), 0.884 - 0.778 (m, 16H, 4 × CH$_2$CH$_2$CH(CH$_3$)$_2$), 4 × CH$_3$); Anal. ($C_{30}H_{56}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-di-(bis(2-methoxyethyl)amino)-pyrimido[5,4-*d*]pyrimidine (35)

Compound 35 was prepared by general procedure I with bis(2-methoxyethyl)amine (0.65 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 10/1) to give a yellow power solid (260 mg, 43%). Mp: 104-105 °C; MS (ESI) *m/z* 601 $(M + H)^+$, 623 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 4.685 (br s, 4H, 4 × OH, disappeared after D_2O), 4.145 (br s, 8H, 4 × CH_2CH$_2$OCH$_3$), 3.594 (t, 8H, 4 × CH$_2$CH_2OCH$_3$), 3.557 (br s, 16H, 2 × N(CH_2CH_2OH)$_2$), 3.260 (s, 12H, 4 × CH$_3$); Anal. ($C_{26}H_{48}N_8O_8$) C, H, N.

2,6-Diethanolamino-4,8-di-(bis(2-methoxyethyl)amino)-pyrimido[5,4-*d*]pyrimidine (36)

Compound 36 was prepared by general procedure I with bis(2-methoxyethyl)amine (0.65 ml, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 14/1) to give a yellow power solid (100 mg, 20%). Mp: 68-69 °C; MS (ESI) *m/z* 513 $(M + H)^+$, 535 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 5.905 (t, 2H, 2 × NHCH$_2$CH$_2$OH, disappeared after D_2O), 4.598 (t, 2H, 2 × NHCH$_2$CH$_2$OH, disappeared after D_2O, J = 5 Hz), 4.125 (br s, 8H, 4 × CH_2CH$_2$OCH$_3$), 3.595 (t, 8H, 4 × CH$_2$CH_2OCH$_3$), 3.496 (q, 4H, 2 × NHCH$_2$CH_2OH, J = 5 Hz), 3.257 (s, 12H, 4 × CH$_3$), 3.235 (br s, 4H, 2 × NHCH_2CH$_2$OH); Anal. ($C_{22}H_{40}N_8O_6$) C, H, N.

2,6-Bis(diethanolamino)-4,8-bis(dibenzylamino)-pyrimido[5,4-*d*]pyrimidine (37)

Compound 37 was prepared by general procedure I with dibenzylamine (0.83g, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 16/1) to give a yellow power solid compound 9 (332 mg, 46%). Mp: 199 °C; MS (ESI) *m/z* 729 $(M + H)^+$, 751 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 7.324 (t, 8H, 4 × Ar-H-3, 4 × Ar-H-5, J_1 = 7.5 Hz, J_2 = 7 Hz), 7.261 - 7.224 (m, 12H, 4 × Ar-H-2, 4 × Ar-H-6, 4 × Ar-H-4), 5.317 (br s, 8H, 4 × CH_2Ph), 4.564 (t, 4H, 4 × OH, disappeared after D_2O, J = 5 Hz), 3.259 (br d, 16H, 2 × N(CH_2CH_2OH)$_2$); Anal. ($C_{42}H_{48}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-bis(dibenzylamino)-pyrimido[5,4-*d*]pyrimidine (38)

Compound 38 was prepared by general procedure I with dibenzylamine (0.83g, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 20/1) to give a yellow power solid compound 8 (125 mg, 20%). Mp: 215-216 °C; MS (ESI) *m/z* 641 $(M + H)^+$, 663 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 7.327 (t, 8H, 4 × Ar-H-3, 4 × Ar-H-5, J_1 = 7 Hz, J_2 = 7.5 Hz), 7.289 (d, 8H, 4 × Ar-H-2, 4 × Ar-H-6, J = 7 Hz), 7.247 (t, 4H, 4 × Ar-H-4, J_1 = 7.5 Hz, J_2 = 7 Hz), 5.970 (t, 2H, 2 × N*H*CH_2CH_2OH, disappeared after D_2O, J = 5.5 Hz), 4.279 (br s, 8H, 4 × C*H*$_2$Ph), 4.419 (t, 2H, 2 × $NHCH_2CH_2O$*H*, disappeared after D_2O, J = 5.5 Hz), 3.243 (q, 4H, 2 × $NHCH_2C$*H*$_2$OH, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.927(br s, 4H, 2 × NHC*H*$_2CH_2OH$); Anal. ($C_{38}H_{40}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-diamino-Pyrimido[5,4-*d*]pyrimidine (39)

Compound 39 was prepared by general procedure I with ammonia solution (7N in methanol, 0.6 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=7/1) to give a yellow power solid (63 mg, 17%). Mp: 225-226°C; MS (ESI) *m/z* 369 $(M + H)^+$, 391 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 7.194 (br s, 2H, 2 × N*H*$_A$H$_B$, disappeared after D_2O), 6.663 (br s, 2H, 2 × NH$_A$*H*$_B$, disappeared after D_2O), 4.675 (t, 4H, 4 × OH, disappeared after D_2O, J = 4.5 Hz), 3.625 (t, 8H, 2 × N(C*H*$_2CH_2OH)_2$, J = 5 Hz), 3.584 (t, 8H, 2 × $N(CH_2C$*H*$_2OH)_2$, J_1 = 4.5 Hz, J_2 = 5 Hz); Anal. Calcd ($C_{14}H_{24}N_8O_4$) C 45.64, H 6.57, N 30.42; Found: C 45.29, H 6.73, N 29.58.

2,6-Bis(diethanolamino)-4,8-dimethylamino-pyrimido[5,4-*d*]pyrimidine (40)

Compound 40 was prepared by general procedure I with methylamine solution (2M in tetrahydrofuran, 2.1 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=7/1) to give a yellow power solid (190 mg, 48%). Mp: 213-214°C; MS (ESI) *m/z* 397 $(M + H)^+$, 419 $(M + Na)^+$, 435 $(M + K)^+$; ^{1}H NMR (DMSO-d_6) δ 7.154 (q, 2H, 2 × N*H*CH_3, disappeared after D_2O, J = 4.5 Hz), 4.675 (br s, 4H, 4 × OH, disappeared after D_2O), 3.676 (t, 8H, 2 × N ($CH_2CH_2OH)_2$), 3.619 (t, 8H, 2 × $N(CH_2CH_2OH)_2$), 2.949 (d, 6H, 2 × NHC*H*$_3$, J = 4.5 Hz); Anal. ($C_{16}H_{28}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-dimethylamino-pyrimido[5,4-*d*]pyrimidine (41)

Compound 41 was prepared by general procedure I with methylamine solution (2M in tetrahydrofuran, 2.1 ml, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=15/1) to give a yellow power solid (176 mg, 57%). Mp: 212°C; MS (ESI) *m/z* 309 $(M + H)^+$, 331 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 7.120 (q, 2H, 2 × N*H*CH_3, disappeared after D_2O, J = 5 Hz), 5.947 (t, 2H, 2 × N*H*CH_2CH_2OH, disappeared after D_2O, J = 6 Hz), 4.610 (t, 2H, 2 × $NHCH_2CH_2O$*H*, disappeared after D_2O, J = 5.5 Hz), 3.530 (q, 4H, 2 × $NHCH_2C$*H*$_2$OH, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.402 (q, 4H, 2 × NHC*H*$_2CH_2OH$, J_1 = 6 Hz, J_2 = 6 Hz), 2.920 (d, 6H, 2 × NHC*H*$_3$, J = 5 Hz); Anal. ($C_{12}H_{20}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-diethylamino-pyrimido[5,4-*d*]pyrimidine (42)

Compound 42 was prepared by general procedure I with ethylamine solution (2M in tetrahydrofuran, 2.1 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/ MeOH=10/1) to give a yellow power solid (174 mg, 41%). Mp: 188-189°C; MS (ESI) *m/z* 425 $(M + H)^+$, 447 $(M + Na)^+$, 463 $(M + K)^+$; ^{1}H NMR (DMSO-d_6) δ 7.151 (br s, 2H, 2 × N*H*CH_2CH_3, disappeared after D_2O), 4.690 (s, 4H, 4 × OH, disappeared after D_2O), 3.668 (br

s, 8H, 2 × N(C*H*$_2$CH$_2$OH)$_2$), 3.622 (br s, 8H, 2 × N(CH$_2$C*H*$_2$OH)$_2$), 3.469 (br s, 4H, 2 × C*H*$_2$CH$_3$), 1.190 (t, 6H, 2 × CH$_2$C*H*$_3$); Anal. (C$_{18}$H$_{32}$N$_8$O$_4$) C, H, N.

2,6-Diethanolamino-4,8-diethylamino-pyrimido[5,4-*d*]pyrimidine (43)

Compound 43 was prepared by general procedure I with ethylamine solution (2M in tetrahydrofuran, 2.1 ml, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (CH$_2$Cl$_2$/MeOH=24/1) to give a yellow power solid (205 mg, 61%). Mp: 175-176°C; MS (ESI) *m/z* 337 (M + H)$^+$, 357 (M + Na)$^+$, 375 (M + K)$^+$; ^{1}H NMR (DMSO-*d*$_6$) δ 7.057 (br s, 2H, 2 × N*H*CH$_2$CH$_3$, disappeared after D$_2$O), 5.941 (t, 2H, 2 × N*H*CH$_2$CH$_2$OH, disappeared after D$_2$O, *J* = 6 Hz), 4.621 (t, 2H, 2 × NHCH$_2$CH$_2$O*H*, disappeared after D$_2$O, *J* = 5.5 Hz), 3.532 (q, 4H, 2 × NHCH$_2$C*H*$_2$OH, *J* = 5.5 Hz), 3.442 (m, 4H, 2 × C*H*$_2$CH$_3$, *J* = 6 Hz), 3.392 (q, 4H, 2 × NHC*H*$_2$CH$_2$OH, *J* = 6 Hz), 1.174 (t, 6H, 2 × CH$_2$C*H*$_3$, *J* = 6 Hz); Anal. (C$_{14}$H$_{24}$N$_8$O$_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-dipropylamino-pyrimido[5,4-*d*]pyrimidine (44)

Compound 44 was prepared by general procedure I with propylamine (0.35 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH$_2$Cl$_2$/MeOH=8/1) to give a yellow power solid (217 mg, 48%). Mp: 145-146°C; MS (ESI) *m/z* 453 (M + H)$^+$, 475 (M + Na)$^+$; ^{1}H NMR (DMSO-d6) δ 7.154 (t, 2H, 2 × N*H*CH$_2$CH$_2$CH$_3$, disappeared after D$_2$O), 4.701 (t, 4H, 4 × OH, disappeared after D$_2$O), 3.670 (br d, 8H, 2 × N(C*H*$_2$CH$_2$OH)$_2$), 3.631 (t, 8H, 2 × N(CH$_2$C*H*$_2$OH)$_2$), 3.400 (m, 4H, 2 × C*H*$_2$CH$_2$CH$_3$), 1.607 (m, 4H, 2 × CH$_2$C*H*$_2$CH$_3$, *J* = 7.5 Hz), 0.910 (t, 6H, 2 × CH$_2$CH$_2$C*H*$_3$, *J* = 7.5 Hz); Anal. (C$_{20}$H$_{36}$N$_8$O$_4$) C, H, N.

2,6-Diethanolamino-4,8-dipropylamino-pyrimido[5,4-*d*]pyrimidine (45)

Compound 45 was prepared by general procedure I with propylamine (0.35 ml, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (CH$_2$Cl$_2$/MeOH=10/1) to give a yellow power solid (196 mg, 54%). Mp: 147-148°C; MS (ESI) *m/z* 365 (M + H)$^+$, 387 (M + Na)$^+$; ^{1}H NMR (DMSO-*d*$_6$) δ 7.055 (br s, 2H, 2 × N*H*CH$_2$CH$_2$CH$_3$, disappeared after D$_2$O), 5.957 (br s, 2H, 2 × N*H*CH$_2$CH$_2$OH, disappeared after D$_2$O), 4.636 (br s, 2H, 2 × NHCH$_2$CH$_2$O*H*, disappeared after D$_2$O), 3.544 (br s, 4H, 2 × NHCH$_2$C*H*$_2$OH), 3.376 (br s, 8H, 2 × NHC*H*$_2$CH$_2$OH, 2 × C*H*$_2$CH$_2$CH$_3$), 1.591 (m, 4H, 2 × CH$_2$C*H*$_2$CH$_3$, *J* = 7.5 Hz), 0.901 (t, 6H, 2 × CH$_2$CH$_2$C*H*$_3$, *J* = 7.5 Hz); Anal. (C$_{16}$H$_{28}$N$_8$O$_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-diisopropylamino-pyrimido[5,4-*d*]pyrimidine (46)

Compound 46 was prepared by general procedure I with isopropylamine (0.36 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH$_2$Cl$_2$/MeOH=8/1) to give a yellow power solid (201 mg, 44%). Mp: 188-190°C; MS (ESI) *m/z* 453 (M + H)$^+$, 475 (M + Na)$^+$; ^{1}H NMR (DMSO-*d*$_6$) δ 6.547 (d, 2H, 2 × N*H*CH(CH$_3$)$_2$, disappeared after D$_2$O), 4.706 (br s, 4H, 4 × OH, disappeared after D$_2$O), 4.218 (m, 2H, 2 × NHC*H*(CH$_3$)$_2$), 3.662 (t, 8H, 2 × N(C*H*$_2$CH$_2$OH)$_2$), 3.620 (br s, 8H, 2 × N(CH$_2$C*H*$_2$OH)$_2$), 1.264 (d, 12H, 2 × NHCH(C*H*$_3$)$_2$); Anal. (C$_{20}$H$_{36}$N$_8$O$_4$) C, H, N.

2,6-Diethanolamino-4,8-diisopropylamino-pyrimido[5,4-*d*]pyrimidine (47)

Compound 47 was prepared by general procedure I with isopropylamine (0.36 ml, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (CH$_2$Cl$_2$/MeOH=14/1) to give a yellow power solid (165 mg, 45%). Mp: 167°C; MS (ESI) *m/z* 365 (M + H)$^+$, 387 (M + Na)$^+$, 403 (M + K)$^+$; ^{1}H NMR

(DMSO-d_6) δ 6.590 (d, 2H, 2 × N*H*CH(CH_3)$_2$, disappeared after D_2O), 6.008 (t, 2H, 2 × N*H*CH_2CH_2OH, disappeared after D_2O, J = 6 Hz), 4.646 (br s, 2H, 2 × $NHCH_2CH_2O$*H*, disappeared after D_2O), 4.241 (m, 2H, 2 × NHC*H*(CH_3)$_2$, J = 6.5 Hz), 3.535 (br s, 4H, 2 × $NHCH_2C$*H*$_2$OH), 3.382 (m, 4H, 2 × NHC*H*$_2CH_2OH$, J = 6 Hz), 1.235 (d, 12H, 2 × NHCH(C*H*$_3$)$_2$ J = 6.5 Hz); Anal. ($C_{16}H_{28}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-dibutylamino-pyrimido[5,4-*d*]pyrimidine (48)

Compound 48 was prepared by general procedure I with butylamine (0.42 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=10/1) to give a yellow power solid (82 mg, 17%). Mp: 117-118°C; MS (ESI) *m/z* 481 $(M + H)^+$, 503 $(M + Na)^+$, 519 $(M + K)^+$; ^{1}H NMR (DMSO-d_6) δ 7.135 (br s, 2H, 2 × N*H*$CH_2CH_2CH_2CH_3$, disappeared after D_2O), 4.693 (br s, 4H, 4 × OH, disappeared after D_2O), 3.658 (br s, 8H, 2 × N(CH_2*H*$_2CH_2OH)_2$), 3.617 (br s, 8H, 2 × N(CH_2CH_2*H*$_2OH)_2$), 3.431 (br s, 4H, 2 × NHC*H*$_2CH_2CH_2CH_3$), 1.580 (m, 4H, 2 × $NHCH_2$C*H*$_2CH_2CH_3$, J = 7 Hz), 1.350 (m, 4H, 2 × $NHCH_2CH_2$C*H*$_2CH_3$, J_1 = 7 Hz, J_2 = 7.5 Hz), 0.919 (t, 6H, 2 × $NHCH_2CH_2CH_2$C*H*$_3$, J = 7.5 Hz); Anal. Calcd ($C_{22}H_{40}N_8O_4 \cdot 0.5$ H_2O): C 53.86, H 8.63, N 22.84; Found: C 54.32, H 8.34, N 22.72.

2,6-Diethanolamino-4,8-dibutylamino-pyrimido[5,4-*d*]pyrimidine (49)

Compound 49 was prepared by general procedure I with butylamine (0.42 ml, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=14/1) to give a yellow power solid (70 mg, 18%). Mp: 127-128°C; MS (ESI) *m/z* 393 $(M + H)^+$, 415 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 7.018 (br s, 2H, 2 × N*H*$CH_2CH_2CH_2CH_3$, disappeared after D_2O), 5.938 (t, 2H, 2 × N*H*CH_2CH_2OH, disappeared after D_2O, J = 5.5 Hz), 4.618 (br s, 2H, 2 × $NHCH_2CH_2O$*H*, disappeared after D_2O), 3.529 (m, 4H, 2 × $NHCH_2$C*H*$_2$OH), 3.404 (m, 4H, 2 × NHC*H*$_2CH_2CH_2CH_3$, J = 7 Hz), 3.380 (m, 4H, 2 × NHC*H*$_2CH_2$OH, J = 5.5 Hz), 1.556 (m, 4H, 2 × $NHCH_2$C*H*$_2CH_2CH_3$, J_1 = 7 Hz, J_2 = 7.5 Hz), 1.345 (m, 4H, 2 × $NHCH_2CH_2$C*H*$_2CH_3$, J_1 = 7.5 Hz, J_2 = 7 Hz), 0.921 (t, 6H, 2 × $NHCH_2CH_2CH_2$C*H*$_3$, J = 7 Hz); Anal. ($C_{18}H_{32}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-diisobutylamino-pyrimido[5,4-*d*]pyrimidine (50)

Compound 50 was prepared by general procedure I with isobutylamine (0.36 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=9/1) to give a yellow power solid (58 mg, 12%). Mp: 162-163°C; MS (ESI) *m/z* 481 $(M + H)^+$, 503 $(M + Na)^+$, 519 $(M + K)^+$; ^{1}H NMR (DMSO-d_6) δ 7.145 (br s, 2H, 2 × N*H*CH_2CH(CH_3)$_2$, disappeared after D_2O), 4.703 (br s, 4H, 4 × OH, disappeared after D_2O), 3.649 (d, 8H, 2 × N(CH_2*H*$_2CH_2OH)_2$), 3.619 (d, 8H, 2 × N(CH_2CH_2*H*$_2OH)_2$), 3.278 (t, 4H, 2 × NHC*H*$_2$CH(CH_3)$_2$, J = 6.5 Hz), 1.957 (m, 2H, 2 × $NHCH_2$C*H*(CH_3)$_2$, J_1 = 6.5 Hz, J_2 = 7 Hz), 0.917 (d, 12H, 2 × $NHCH_2$CH(CH_3)$_2$, J = 7 Hz); Anal. ($C_{22}H_{40}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-diisobutylamino-pyrimido[5,4-*d*]pyrimidine (51)

Compound 51 was prepared by general procedure I with isobutylamine (0.36 ml, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=12/1) to give a yellow power solid (78 mg, 20%). Mp: 141-142°C; MS (ESI) *m/z* 393 $(M + H)^+$, 415 $(M + Na)^+$, 431 $(M + K)^+$; ^{1}H NMR (DMSO-d_6) δ 7.019 (t, 2H, 2 × N*H*CH_2CH(CH_3)$_2$, disappeared after D_2O), 5.980 (t, 2H, 2 × N*H*CH_2CH_2OH, disappeared after D_2O, J = 5.5 Hz), 4.631 (t, 2H, 2 × $NHCH_2CH_2O$*H*, disappeared after D_2O, J = 5.5 Hz), 3.537 (q, 4H, 2 × $NHCH_2$C*H*$_2$OH, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.377 (q, 4H, 2 × NHC*H*$_2CH_2$OH, J_1 = 5.5 Hz, J_2 = 6 Hz), 3.255 (t, 4H, 2 × NHC*H*$_2$CH

$(CH_3)_2$, J = 6.5 Hz), 1.954 (m, 2H, 2 × $NHCH_2CH(CH_3)_2$, J_1 = 6.5 Hz, J_2 = 7 Hz), 0.914 (d, 12H, 2 × $NHCH_2CH(CH_3)_2$, J = 7 Hz); Anal. Calcd ($C_{18}H_{32}N_8O_2$): C 55.08, H 8.22, N 28.55; Found; C 54.46, H 8.15, N 28.22.

2,6-Bis(diethanolamino)-4,8-di(*tert*-butylamino)-pyrimido[5,4-*d*]pyrimidine (52)

Compound 52 was prepared by general procedure I with ***tert***-butylamine (0.44 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=6/1) to give a yellow power solid (173 mg, 36%). Mp: 244-245°C; MS (ESI) *m/z* 481 $(M + H)^+$, 503 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 6.475 (s, 2H, 2 × $NHC(CH_3)_3$, disappeared after D_2O), 4.748 (t, 4H, 4 × OH, disappeared after D_2O), 3.643 (br s, 16H, 2 × $N(CH_2CH_2OH)_2$), 1.474 (s, 18H, 2 × $NHC(CH_3)_3$); Anal. ($C_{22}H_{40}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-di(*tert*-butylamino)-pyrimido[5,4-*d*]pyrimidine (53)

Compound 53 was prepared by general procedure I with ***tert***-butylamine (0.44 ml, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=9/1) to give a yellow power solid (190 mg, 48%). Mp: 154°C; MS (ESI) *m/z* 393 $(M + H)^+$, 415 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 6.456 (s, 2H, 2 × $NHC(CH_3)_3$, disappeared after D_2O), 6.135 (t, 2H, 2 × $NHCH_2CH_2OH$, disappeared after D_2O, J = 6 Hz), 4.652 (br s, 2H, 2 × $NHCH_2CH_2OH$, disappeared after D_2O), 3.536 (q, 4H, 2 × $NHCH_2CH_2OH$, J = 5.5 Hz), 3.338 (q, 4H, 2 × $NHCH_2CH_2OH$, J_1 = 5.5 Hz, J_2 = 6 Hz), 1.477 (s, 18H, 2 × $NHC(CH_3)_3$); Anal. ($C_{18}H_{32}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-diamylamino-pyrimido[5,4-*d*]pyrimidine (54)

Compound 54 was prepared by general procedure I with amylamine (0.49 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=12/1) to give a yellow power solid (190 mg, 37%). Mp: 128°C; MS (ESI) *m/z* 509 $(M + H)^+$, 531 $(M + Na)^+$, 547 $(M + K)^+$; ^{1}H NMR (DMSO-d_6) δ 7.139 (br s, 2H, 2 × $NHCH_2CH_2(CH_2)_2CH_3$, disappeared after D_2O), 4.690 (br s, 4H, 4 × OH, disappeared after D_2O), 3.662 (br s, 8H, 2 × $N(CH_2CH_2OH)_2$), 3.624 (d, 8H, 2 × $N(CH_2CH_2OH)_2$), 3.423 (br s, 4H, 2 × $NHCH_2CH_2(CH_2)_2CH_3$), 1.598 (m, 4H, 2 × $NHCH_2CH_2(CH_2)_2CH_3$), 1.320 (m, 8H, 2 × $NHCH_2CH_2(CH_2)_2CH_3$), 0.882 (t, 6H, 2 × $NHCH_2CH_2(CH_2)_2CH_3$); Anal. ($C_{24}H_{44}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-diamylamino-pyrimido[5,4-*d*]pyrimidine (55)

Compound 55 was prepared by general procedure I with amylamine (0.49 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=17/1) to give a yellow power solid (155 mg, 37%). Mp: 147-148°C; MS (ESI) *m/z* 421 $(M + H)^+$, 443 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 7.032 (t, 2H, 2 × $NHCH_2CH_2(CH_2)_2CH_3$, disappeared after D_2O), 5.931 (t, 2H, 2 × $NHCH_2CH_2OH$, disappeared after D_2O), 4.620 (t, 2H, 2 × $NHCH_2CH_2OH$, disappeared after D_2O), 3.530 (q, 4H, 2 × $NHCH_2CH_2OH$), 3.382 (m, 8H, 2 × $NHCH_2CH_2(CH_2)_2CH_3$, 2 × $NHCH_2CH_2OH$), 1.582 (m, 4H, 2 × $NHCH_2CH_2(CH_2)_2CH_3$), 1.309 (m, 8H, 2 × $NHCH_2CH_2(CH_2)_2CH_3$), 0.882 (t, 6H, 2 × $NHCH_2CH_2(CH_2)_2CH_3$); Anal. ($C_{20}H_{36}N_8O_2 \cdot 0.5\ H_2O$) C, H, N.

2,6-Bis(diethanolamino)-4,8-diisopentylamino-pyrimido[5,4-*d*]pyrimidine (56)

Compound 56 was prepared by general procedure I with isopentylamine (0.49 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=22/1) to give a yellow power solid (346 mg, 68%). Mp: 124-125°C; MS (ESI) *m/z* 509 $(M + H)^+$, 531 $(M + Na)^+$, 547 $(M + K)^+$; ^{1}H

NMR (DMSO-d_6) δ 7.131 (t, 2H, 2 × N*H*$CH_2CH_2CH(CH_3)_2$, disappeared after D_2O), 4.679 (t, 4H, 4 × OH, disappeared after D_2O), 3.666 (t, 8H, 2 × N(C*H*$_2CH_2OH)_2$), 3.621 (d, 8H, 2 × N(CH_2C*H*$_2OH)_2$), 3.454 (q, 4H, 2 × NHC*H*$_2CH_2CH(CH_3)_2$), 1.621 (m, 2H, 2 × NHCH_2CH_2C*H*$(CH_3)_2$), 1.505 (m, 4H, 2 × NHCH_2C*H*$_2$CH$(CH_3)_2$), 0.936 (d, 12H, 2 × NHCH_2CH_2CH(C*H*$_3)_2$); Anal. ($C_{24}H_{44}N_8O_4 \cdot 0.5\ H_2O$) C, H, N.

2,6-Diethanolamino-4,8-diisopentylamino-pyrimido[5,4-*d*]pyrimidine (57)

Compound 57 was prepared by general procedure I with isopentylamine (0.49 ml, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=30/1) to give a yellow power solid (235 mg, 56%). Mp: 132-133°C; MS (ESI) *m/z* 421 $(M + H)^+$, 443 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 6.999 (br s, 2H, 2 × N*H*$CH_2CH_2CH(CH_3)_2$, disappeared after D_2O), 5922 (t, 2H, 2 × N*H*CH_2CH_2OH, disappeared after D_2O), 4.621 (br s, 2H, 2 × NHCH_2CH_2O*H*, disappeared after D_2O), 3.538 (q, 4H, 2 × NHCH_2C*H*$_2$OH), 3.422 (q, 4H, 2 × NHC*H*$_2CH_2OH$), 3.384 (m, 4H, 2 × NHC*H*$_2CH_2CH(CH_3)_2$), 1.614 (m, 2H, 2 × NHCH_2CH_2C*H*$(CH_3)_2$), 1.490 (m, 4H, 2 × NHCH_2C*H*$_2$CH$(CH_3)_2$), 0.931 (d, 12H, 2 × NHCH_2CH_2CH(C*H*$_3)_2$); Anal. ($C_{20}H_{36}N_8O_2 \cdot H_2O$) C, H, N.

2,6-Bis(diethanolamino)-4,8-di(*tert*-amylamino)-pyrimido[5,4-*d*]pyrimidine (58)

Compound 58 was prepared by general procedure I with *tert*-amylamine (0.49 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=14/1) to give a yellow power solid (245 mg, 48%). Mp: 212-213°C; MS (ESI) *m/z* 509 $(M + H)^+$, 531 $(M + Na)^+$, 547 $(M + K)^+$; ^{1}H NMR (DMSO-d_6) δ 6.432 (s, 2H, 2 × N*H*C$(CH_3)_2CH_2CH_3$, disappeared after D_2O), 4.757 (d, 4H, 4 × OH, disappeared after D_2O), 3.637 (s, 16H, 2 × N(C*H*$_2$C*H*$_2$OH)$_2$), 1.841 (q, 4H, 2 × NHC$(CH_3)_2$C*H*$_2CH_3$, *J* = 7.5 Hz), 1.415 (s, 12H, 2 × NHC(C*H*$_3)_2CH_2CH_3$), 0.833 (t, 6H, 2 × NHC$(CH_3)_2CH_2$C*H*$_3$, *J* = 7.5 Hz); Anal. ($C_{24}H_{44}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-di(*tert*-amylamino)-pyrimido[5,4-*d*]pyrimidine (59)

Compound 59 was prepared by general procedure I with *tert*-amylamine (0.49 ml, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=18/1) to give a yellow power solid (196 mg, 47%). Mp: 167-168°C; MS (ESI) *m/z* 421 $(M + H)^+$, 443 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 6.391 (s, 2H, 2 × N*H*C$(CH_3)_2CH_2CH_3$, disappeared after D_2O), 6.143 (t, 2H, 2 × N*H*CH_2CH_2OH, disappeared after D_2O, *J* = 6 Hz), 4.653 (br s, 2H, 2 × NHCH_2CH_2O*H*, disappeared after D_2O), 3.552 (q, 4H, 2 × NHCH_2C*H*$_2$OH, *J* = 5.5 Hz), 3.328 (q, 4H, 2 × NHC*H*$_2CH_2OH$, J_1 = 5.5 Hz, J_2 = 6 Hz), 1.881 (q, 4H, 2 × NHC$(CH_3)_2$C*H*$_2CH_3$, *J* = 7.5 Hz), 1.414 (s, 12H, 2 × NHC(C*H*$_3)_2CH_2CH_3$), 0.814 (t, 6H, 2 × NHC$(CH_3)_2CH_2$C*H*$_3$, *J* = 7.5 Hz); Anal. Calcd ($C_{20}H_{36}N_8O_2 \cdot 0.5\ H_2O$): C 55.92, H 8.68, N 26.09; Found: C 56.02, H 8.67, N 25.62.

2,6-Bis(diethanolamino)-4,8-dicyclopropylaminopyrimido[5,4-*d*]pyrimidine (60)

Compound 60 was prepared by general procedure I with cyclopropylamine (0.31 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 14/1) to give a yellow power solid (220 mg, 49%). Mp: 225 °C; MS (ESI) *m/z* 449 $(M + H)^+$, 471 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 7.060 (d, 2H, 2 × N*H*C$(CH_2)_2$, disappeared after D_2O, *J* = 3.5 Hz), 4.688 (s, 4H, 4 × OH, disappeared after D_2O), 3.681 (t, 8H, 2 × N(C*H*$_2CH_2OH)_2$), 3.627 (d, 8H, 2 × N(CH_2C*H*$_2OH)_2$), 2.796 (m, 2H, 2 × NHC*H*$(CH_2)_2$), 0.783 (m, 4H, 2 × NHCH$(CH_2)_2$-$H_{2a,3a}$), 0.622 (m, 4H, 2 × NHCH$(CH_2)_2$-$H_{2e,3e}$); Anal. ($C_{20}H_{32}N_8O_4 \cdot 0.25\ H_2O$) C, H, N.

2,6-Diethanolamino-4,8-dicyclopropylaminopyrimido[5,4-*d*]pyrimidine (61)

Compound 61 was prepared by general procedure I with cyclopropylamine (0.31 ml, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 17/1) to give a yellow power solid (156 mg, 43%). Mp: 199 °C; MS (ESI) *m/z* 361 $(M + H)^+$, 383 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 6.983 (d, 2H, 2 × N*H*CH$(CH_2)_2$, disappeared after D_2O, J = 3.5 Hz), 5.003 (t, 2H, 2 × N*H*CH_2CH_2OH, disappeared after D_2O, J = 6 Hz), 4.633 (t, 2H, 2 × $NHCH_2CH_2O$*H*, disappeared after D_2O, J_1 = 5 Hz, J_2 = 5.5 Hz), 3.525 (q, 4H, 2 × $NHCH_2CH_2OH$, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.396 (q, 4H, 2 × NHC*H*$_2CH_2OH$, J = 6 Hz), 2.869 (m, 2H, 2 × NHC*H*$(CH_2)_2$), 0.748 (m, 4H, 2 × NHCH$(CH_2)_2$-$H_{2a,3a}$), 0.633 (m, 4H, 2 × NHCH $(CH_2)_2$-$H_{2e,3e}$); Anal. ($C_{16}H_{24}N_8O_2 \cdot 0.25\ H_2O$) C, H, N.

2,6-Bis(diethanolamino)-4,8-dicyclobutylaminopyrimido[5,4-*d*]pyrimidine (62)

Compound 62 was prepared by general procedure I with cyclobutylamine (0.37 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 16/1) to give a yellow power solid (212 mg, 45%). Mp: 222-223 °C; MS (ESI) *m/z* 477 $(M + H)^+$, 499 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 6.992 (br s, 2H, 2 × N*H*CH$(CH_2)_2CH_2$, disappeared after D_2O), 4.701 (s, 4H, 4 × OH, disappeared after D_2O), 4.467 (br s, 2H, 2 × NHC*H*$(CH_2)_2CH_2$), 3.615 (br d, 16H, 2 × N $(CH_2CH_2OH)_2$, 2.295 (m, 4H, 2 × NHCH$(CH_2)_2CH_2$-$H_{2a,4a}$), 2.196 (m, 4H, 2 × NHCH $(CH_2)_2CH_2$-$H_{2e,4e}$), 1.729 (m, 4H, 2 × NHCH$(CH_2)_2CH_2$); Anal. ($C_{22}H_{36}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-dicyclobutylaminopyrimido[5,4-*d*]pyrimidine (63)

Compound 63 was prepared by general procedure I with cyclobutylamine (0.37 ml, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 19/1) to give a yellow power solid (183 mg, 47%). Mp: 189-190 °C; MS (ESI) *m/z* 389 $(M + H)^+$, 411 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 7.052 (d, 2H, 2 × N*H*CH$(CH_2)_2CH_2$, disappeared after D_2O, J = 8 Hz), 5.971 (t, 2H, 2 × N*H*CH_2CH_2OH, disappeared after D_2O, J = 5.5 Hz), 4.645 (t, 2H, 2 × $NHCH_2CH_2O$*H*, disappeared after D_2O, J_1 = 4.5 Hz, J_2 = 5.5 Hz), 4.555 (m, 2H, 2 × NHC*H*$(CH_2)_2CH_2$, J_1 = 8 Hz, J_2 = 8.5 Hz), 3.533 (q, 4H, 2 × $NHCH_2CH_2OH$, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.405 (q, 4H, 2 × NHC*H*$_2CH_2OH$, J_1 = 5.5 Hz, J_2 = 6 Hz), 2.272 (m, 4H, 2 × NHCH$(CH_2)_2CH_2$-$H_{2a,4a}$), 2.097 (m, 4H, 2 × NHCH$(CH_2)_2CH_2$-$H_{2e,4e}$), 1.675 (m, 4H, 2 × NHCH$(CH_2)_2CH_2$); Anal. Calcd ($C_{18}H_{28}N_8O_2$): C, H, N.

2,6-Bis(diethanolamino)-4,8-dicyclopentylamino-pyrimido[5,4-*d*]pyrimidine (64)

Compound 64 was prepared by general procedure I with cyclopentylamine (0.42 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=10/1) to give a yellow power solid (97 mg, 19%). Mp: 211-212 °C; MS (ESI) *m/z* 505 $(M + H)^+$, 527 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 6.619 (d, 2H, 2 × N*H*CH$(CH_2CH_2)_2$, disappeared after D_2O), 4.704 (br s, 4H, 4 × OH, disappeared after D_2O), 4.296 (m, 2H, 2 × NHC*H*$(CH_2CH_2)_2$), 3.657 (t, 8H, 2 × N $(CH_2CH_2OH)_2$), 3.615 (d, 8H, 2 × N$(CH_2CH_2OH)_2$), 2.016 (m, 4H, 2 × NHCH $(CH_{ax}H_{eq}CH_2)_2$), 1.720 (m, 4H, 2 × NHCH$(CH_2CH_{ax}H_{eq})_2$), 1.587 (m, 8H, 2 × NHCH $(CH_2CH_{ax}H_{eq})_2$, 2 × NHCH$(CH_{ax}H_{eq}CH_2)_2$); Anal. ($C_{24}H_{40}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-dicyclopentylamino-pyrimido[5,4-*d*]pyrimidine (65)

Compound 65 was prepared by general procedure I with cyclopentylamine (0.42 ml, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=18/1) to give a yellow power solid (175 mg, 42%). Mp: 203-204 °C; MS (ESI) *m/z* 417 $(M + H)^+$, 439 $(M + Na)^+$; ^{1}H NMR

(DMSO-d_6) δ 6.678 (d, 2H, 2 × N*H*CH(CH_2CH_2)$_2$, disappeared after D_2O), 6.028 (t, 2H, 2 × N*H*CH_2CH_2OH, disappeared after D_2O, J = 6 Hz), 4.651 (t, 2H, 2 × NHCH$_2$CH$_2$O*H*, disappeared after D_2O, J = 5 Hz), 4.337 (q, 2H, 2 × NHC*H*(CH_2CH_2)$_2$), 3.534 (q, 4H, 2 × NHCH$_2$C*H*$_2$OH, J = 5 Hz), 3.380 (q, 4H, 2 × NHC*H*$_2$CH$_2$OH, J = 6 Hz), 1.998 (m, 4H, 2 × NHCH($CH_{ax}H_{eq}CH_2$)$_2$), 1.708 (m, 4H, 2 × NHCH($CH_2CH_{ax}H_{eq}$)$_2$), 1.559 (m, 8H, 2 × NHCH($CH_2CH_{ax}H_{eq}$)$_2$, 2 × NHCH($CH_{ax}H_{eq}CH_2$)$_2$); Anal. ($C_{20}H_{32}N_8O_2 \cdot 0.5\ H_2O$) C, H, N.

2,6-Bis(diethanolamino)-4,8-dicyclohexylamino-pyrimido[5,4-*d*]pyrimidine (66)

Compound 66 was prepared by general procedure I with cyclohexylamine (0.48 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=12/1) to give a yellow power solid (320 mg, 60%). Mp: 198-199 °C; MS (ESI) *m/z* 533 $(M + H)^+$, 555 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 6.586 (d, 2H, 2 × N*H*CH(CH_2CH_2)$_2$CH$_2$, disappeared after D_2O), 4.708 (t, 4H, 4 × OH, disappeared after D_2O), 3.891 (m, 2H, 2 × NHC*H*(CH_2CH_2)$_2$CH$_2$), 3.637 (m, 16H, 2 × N(C*H*$_2$C*H*$_2$OH)$_2$), 1.916 (m, 4H, 2 × NHCH($CH_{ax}H_{eq}CH_2$)$_2$CH$_2$), 1.729 (m, 4H, 2 × NHCH($CH_2CH_{ax}H_{eq}$)$_2$CH$_2$), 1.593 (m, 2H, 2 × NHCH(CH_2CH_2)$_2$CH$_{ax}H_{eq}$), 1.442-1.330 (m, 8H, 2 × NHCH($CH_2CH_{ax}H_{eq}$)$_2$CH$_2$, 2 × NHCH($CH_{ax}H_{eq}CH_2$)$_2$CH$_2$), 1.248 (m, 2H, 2 × NHCH(CH_2CH_2)$_2$CH$_{ax}H_{eq}$); Anal. ($C_{26}H_{44}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-dicyclohexylamino-pyrimido[5,4-*d*]pyrimidine (67)

Compound 67 was prepared by general procedure I with cyclohexylamine (0.48 ml, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=15/1) to give a yellow power solid (44 mg, 10%). Mp: 163 °C; MS (ESI) *m/z* 445 $(M + H)^+$, 467 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 6.589 (d, 2H, 2 × N*H*CH(CH_2CH_2)$_2$CH$_2$, disappeared after D_2O), 6.033 (t, 2H, 2 × N*H*CH_2CH_2OH, disappeared after D_2O), 4.632 (t, 2H, 2 × NHCH$_2$CH$_2$O*H*, disappeared after D_2O), 3.915 (q, 2H, 2 × NHC*H*(CH_2CH_2)$_2$CH$_2$), 3.525 (q, 4H, 2 × NHCH$_2$C*H*$_2$OH), 3.355 (q, 4H, 2 × NHC*H*$_2$CH$_2$OH), 1.901 (m, 4H, 2 × NHCH($CH_{ax}H_{eq}CH_2$)$_2$CH$_2$), 1.735 (m, 4H, 2 × NHCH($CH_2CH_{ax}H_{eq}$)$_2$CH$_2$), 1.600 (m, 2H, 2 × NHCH(CH_2CH_2)$_2$CH$_{ax}H_{eq}$), 1.399-1.304 (m, 8H, 2 × NHCH($CH_2CH_{ax}H_{eq}$)$_2$CH$_2$, 2 × NHCH($CH_{ax}H_{eq}CH_2$)$_2$CH$_2$), 1.225 (m, 2H, 2 × NHCH(CH_2CH_2)$_2$CH$_{ax}H_{eq}$); Anal. ($C_{22}H_{36}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-diphenylamino-pyrimido[5,4-*d*]pyrimidine (68)

Compound 68 was prepared by general procedure I with aniline (0.39 ml, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH=14/1) to give a yellow power solid (186 mg, 36%). Mp: 209-210 °C; MS (ESI) *m/z* 519 (M - H)-; ^{1}H NMR (DMSO-d_6) δ 8.918 (s, 2H, 2 × N*H*Ar, disappeared after D_2O), 7.916 (d, 4H, 2 × Ar-H-2, 2 × Ar-H-6, J = 8 Hz), 7.404 (t, 4H, 2 × Ar-H-3, 2 × Ar-H-5, J = 8 Hz), 7.105 (t, 2H, 2 × Ar-H-4), 4.753 (t, 4H, 4 × OH, disappeared after D_2O, J = 5.5 Hz), 3.789 (br s, 8H, 2 × N(C*H*$_2$CH$_2$OH)$_2$), 3.689 (q, 8H, 2 × N(CH_2C*H*$_2$OH)$_2$, J = 5.5 Hz); Anal. ($C_{26}H_{32}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-diphenylaminopyrimido[5,4-*d*]pyrimidine (69)

Compound 69 was prepared by general procedure I with aniline (0.39 ml, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 16/1) to give a yellow power solid compound 4 (138 mg, 32%). Mp: 233-234 °C; MS (ESI) *m/z* 433 $(M + H)^+$, *m/z* 455 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 8.937 (s, 2H, 2 × N*H*Ar, disappeared after D_2O), 8.010 (d, 4H, 2 × Ar-H-2, 2 × Ar-H-6, J = 8 Hz), 7.388 (t, 4H, 2 × Ar-H-3, 2 × Ar-H-5, J_1 = 7.5 Hz, J_2 = 8.5 Hz), 7.092 (t, 2H, 2 × Ar-H-4, J_1 = 7.5 Hz, J_2 = 7 Hz), 6.564 (br s, 2H, 2 × N*H*CH_2CH_2OH, disappeared after D_2O), 4.694 (t, 2H, 2 × OH, disappeared after D_2O, J = 5.5 Hz), 3.613 (q, 4H, 2 ×

NHC*H*$_2$CH$_2$OH, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.526 (q, 4H, 2 × NHCH$_2$C*H*$_2$OH, J_1 = 5.5 Hz, J_2 = 6 Hz); Anal. Calcd ($C_{22}H_{24}N_8O_2 \cdot 0.5\ H_2O$): C 59.85, H 5.71, N 25.38; Found; C 60.31, H 5.69, N 25.23.

2,6-Bis(diethanolamino)-4,8-bis(dibenzylamino)-pyrimido[5,4-*d*]pyrimidine (70)

Compound 70 was prepared by general procedure I with dibenzylamine (0.83g, 4.2 mmole) at the first step, and diethanolamine (3 ml, 30 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 16/1) to give a yellow power solid compound 9 (332 mg, 46%). Mp: 199 °C; MS (ESI) *m/z* 729 $(M + H)^+$, 751 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 7.324 (t, 8H, 4 × Ar-H-3, 4 × Ar-H-5, J_1 = 7.5 Hz, J_2 = 7 Hz), 7.261 - 7.224 (m, 12H, 4 × Ar-H-2, 4 × Ar-H-6, 4 × Ar-H-4), 5.317 (br s, 8H, 4 × C*H*$_2$Ph), 4.564 (t, 4H, 4 × OH, disappeared after D_2O, J = 5 Hz), 3.259 (br d, 16H, 2 × N(C*H*$_2$C*H*$_2$OH)$_2$); Anal. ($C_{42}H_{48}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-bis(dibenzylamino)-pyrimido[5,4-*d*]pyrimidine (71)

Compound 71 was prepared by general procedure I with dibenzylamine (0.83g, 4.2 mmole) at the first step, and ethanolamine (3 ml, 50 mmole) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 20/1) to give a yellow power solid compound 8 (125 mg, 20%). Mp: 215-216 °C; MS (ESI) *m/z* 641 $(M + H)^+$, 663 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 7.327 (t, 8H, 4 × Ar-H-3, 4 × Ar-H-5, J_1 = 7 Hz, J_2 = 7.5 Hz), 7.289 (d, 8H, 4 × Ar-H-2, 4 × Ar-H-6, J = 7 Hz), 7.247 (t, 4H, 4 × Ar-H-4, J_1 = 7.5 Hz, J_2 = 7 Hz), 5.970 (t, 2H, 2 × N*H*CH$_2$CH$_2$OH, disappeared after D_2O, J = 5.5 Hz), 4.279 (br s, 8H, 4 × C*H*$_2$Ph), 4.419 (t, 2H, 2 × NHCH$_2$CH$_2$O*H*, disappeared after D_2O, J = 5.5 Hz), 3.243 (q, 4H, 2 × NHCH$_2$C*H*$_2$OH, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.927(br s, 4H, 2 × NHC*H*$_2$CH$_2$OH); Anal. ($C_{38}H_{40}N_8O_2$) C, H, N.

2,6-Di-(2′-hydroxyethoxy)-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (72)

Compound **72** was prepared by a literature procedure.[43]

2,6-Dimorpholino-4,8-dipiperidino-pyrimido[5,4-*d*]pyrimidine (73)

Compound 73 was prepared by general procedure I with piperidine (0.41 ml, 4.2 mmole) at the first step, and morpholine (3 ml, 34 mmole) at the second step. Product was purified by flash silica gel chromatography (Hexane/Acetone=10/1) to give a yellow power solid (173 mg, 37%). Mp: 203-204 °C; MS (ESI) *m/z* 469 $(M + H)^+$, 491 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 4.076 (br s, 8H, 2 × N(C*H*$_2$CH$_2$)$_2$CH$_2$), 3.663 (t, 8H, 2 × N(C*H*$_2$CH$_2$)O, J = 5 Hz), 3.542 (t, 8H, 2 × N(CH$_2$C*H*$_2$)O, J = 5 Hz), 1.664 (br d, 4H, 2 × N(CH$_2$CH$_2$)$_2$C*H*$_2$, J = 4.5 Hz), 1.608 (br d, 8H, 2 × N(CH$_2$C*H*$_2$)$_2$CH$_2$, J = 4.5 Hz); Anal. ($C_{24}H_{36}N_8O_2$) C, H, N.

2,6-Bis[*N,N*-di-(2′-formyloxy)ethylamino]-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (74)

Dipyridamole (0.51 g, 1 mmole) was dissolved in formic acid (10 ml, 0.25 mole); the reaction was stirred at reflux for 6 hours, and then the solvent was evaporated under reduced pressure. The residue was dissolved in methylene chloride (50 ml) and washed with 10% $NaHCO_3$ solution, and then the organic layer was separated and dried by anhydrous Na_2SO_4. After the solvent was removed, The residue was subjected to flash silica gel chromatography (Hexane:Acetone = 6:1) to give g yellow fluorescent power compound 74 (0.567 g, 92%). mp 129-130 °C (lit:25 128-130 °C). MS (ESI) *m/z* 639 $(M + Na)^+$, *m/z* 617 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 8.225 (s, 4H, 4 × CHO), 4.289 (t, 8H, 2 × N(CH$_2$C*H*$_2$OCHO)$_2$, J = 5.5 Hz), 4.052 (m, 8H, 2 × N(C*H*$_2$CH$_2$)$_2$CH$_2$), 3.784 (t, 8H, 2 × N(C*H*$_2$CH$_2$OCHO)$_2$, J = 5.5 Hz), 1.646 (m, 4H, 2 × N(CH$_2$CH$_2$)$_2$C*H*$_2$), 1.599 (m, 8H, 2 × N(CH$_2$C*H*$_2$)$_2$CH$_2$); Anal. ($C_{28}H_{40}N_8O_8$) C, H, N.

2,6-Bis[*N,N*-di-(2′-acetoxy)ethylamino]-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (75)

In an ice-water bath, acetyl chrolide (1.45 ml, 20 mmole) was added to a solution dipyridamole (0.51 g, 1 mmole) and a catalytic amount of DMAP in anhydrous THF (30 ml). The reaction was stirred for 3 hours, and then the solvent was evaporated under reduced pressure. The residue was dissolved in methylene chloride (50 ml) and washed with 10% $NaHCO_3$ solution, and then the organic layer was separated and dried by anhydrous Na_2SO_4. After the solvent was removed, the residue was subjected to flash silica gel chromatography (Hexane:Acetone = 10:1) to give g yellow fluorescent needle-like compound 75 (0.64 g, 95%). mp 121-122 °C (lit:25 123-124 °C). MS (ESI) *m/z* 695 $(M + Na)^+$, *m/z* 673 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 4.191 (t, 8H, 2 × $N(CH_2CH_2OCOCH_3)_2$, J = 5.5 Hz), 4.046 (m, 8H, 2 × $N(CH_2CH_2)_2CH_2$), 3.746 (t, 8H, 2 × $N(CH_2CH_2OCOCH_3)_2$, J = 5.5 Hz), 1.982 (s, 12H, 4 × CH_3), 1.649 (m, 4H, 2 × $N(CH_2CH_2)_2CH_2$), 1.598 (m, 8H, 2 × $N(CH_2CH_2)_2CH_2$); Anal. ($C_{32}H_{48}N_8O_8$) C, H, N.

2,6-Bis[*N,N*-di-(2′-methoxy)ethylamino]-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (76)

Compound 76 was prepared by general procedure III with MeI (2 ml, 32 mmole) as alkyl halide. The residue was subjected to flash silica gel chromatography (Hexane:Acetone = 8:1) to give yellow fluorescent power compound **56** (247 mg, 64%). Mp: 64 – 65 °C. MS (ESI) *m/z* 583 $(M + Na)^+$, 561 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 4.052 (br s, 8H, 2 × $N(CH_2CH_2)_2CH_2$), 3.766 (t, 8H, 2 × $N(CH_2CH_2OCH_3)_2$, J = 6 Hz), 3.574 (t, 8H, 2 × $N(CH_2CH_2OCH_3)_2$, J = 6 Hz), 3.351 (s, 12H, 4 × CH_3), 1.683 (s, 12H, 2 × $N(CH_2CH_2)_2CH_2$); Anal. ($C_{28}H_{48}N_8O_4$) C, H, N.

2,6-Bis[*N,N*-di-(2′-ethoxy)ethylamino]-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (77)

Compound 77 was prepared by general procedure III with ethyl bromide (2.4 ml, 32 mmole) as alkyl halide. The residue was subjected to flash silica gel chromatography (Hexane:Acetone = 12:1) to give yellow fluorescent power compound 57 (520 mg, 84%). Mp: 58-59 °C; MS (ESI) *m/z* 639 $(M + Na)^+$, 617 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 4.049 (br s, 8H, 2 × $N(CH_2CH_2)_2CH_2$), 3.776 (br s, 8H, 2 × $N(CH_2CH_2OCH_2CH_3)_2$), 3.624 (br s, 8H, 2 × $N(CH_2CH_2OCH_2CH_3)_2$), 3.500 (q, 8H, 4 × CH_2CH_3), 1.708 (br s, 12H, 2 × $N(CH_2CH_2)_2CH_2$), 1.194 (t, 12H, 4 × CH_2CH_3); Anal. ($C_{32}H_{56}N_8O_4$) C, H, N.

2,6-Bis[*N,N*-di-(2′-propoxy)ethylamino]-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (78)

Compound **78** was prepared by general procedure III with propyl bromide (2.9 ml, 32 mmole) as alkyl halide. The residue was subjected to flash silica gel chromatography (Hexane:Acetone = 13:1) to give yellow fluorescent power compound 58 (570 mg, 85%). Mp: 32-34 °C; MS (ESI) *m/z* 695 $(M + Na)^+$, 673 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 4.056 (br s, 8H, 2 × $N(CH_2CH_2)_2CH_2$), 3.779(br s, 8H, 2 × $N(CH_2CH_2OCH_2CH_2CH_3)_2$), 3.616 (br s, 8H, 2 × $N(CH_2CH_2OCH_2CH_2CH_3)_2$), 3.399(t, 8H, 4 × $CH_2CH_2CH_3$, J = 6.5 Hz), 1.699 (br s, 12H, 2 × $N(CH_2CH_2)_2CH_2$), 1.578 (m, 8H, 4 × $CH_2CH_2CH_3$, J_1 = 6.5 Hz, J_2 = 7.5 Hz), 0.915 (t, 12H, 4 × $CH_2CH_2CH_3$, J = 7.5 Hz); Anal. ($C_{36}H_{64}N_8O_4$) C, H, N.

2,6-[*N*-(2-hydroxyethyl)-*N*-(2-isopropoxyethyl)-amino]-4,8-di-piperidino-pyrimido[5,4-*d*] pyrimidine (79)

Compound 79 was prepared by general procedure III with isopropyl bromide (3 ml, 32 mmol) as alkyl halide. The residue was subjected to flash silica gel chromatography (Hexane:Acetone = 2:1) to give yellow fluorescent power compound 58 (326 mg, 55%). Mp: 56-58 °C; MS (ESI) *m/z* 611 $(M + Na)^+$, 589 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 4.574 (m, 2H, 2 × OH, disappeared after D_2O), 4.058 (br s, 8H, 2 × $N(CH_2CH_2)_2CH_2$), 3.631 (t, 4H, 2 × CH_2OH), 3.570 (br s, 8H, 2 × $(CH_3)_2CHOCH_2CH_2NCH_2CH_2OH$), 3.525 (m, 6H, 2 × $(CH_3)_2CHOCH_2$), 1.646 (br

s, 4H, 2 × $N(CH_2CH_2)_2CH_2$), 1.597 (br s, 8H, 2 × $N(CH_2CH_2)_2CH_2$), 1.075 (d, 12H, 4 × CH_3); Anal. ($C_{30}H_{52}N_8O_4$) C, H, N.

Flow Cytometric Assays

The compounds were tested to determine their ENT1nucleoside transporter binding ability by a flow cytometric assay.[44] Briefly, human leukemia K562 cells growing in RPMI 1640 medium were washed once, resuspended at 1.6×10^6 cells/ml in phosphate-buffered saline at pH 7.4, and incubated with 5-(SAENTA)-*X8*-fluorescein (30 nM) in the presence or absence of varying concentrations of test compounds at room temperature for 45 mins. 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., ENT1 transporter-specific fluorescence in the presence of SAENTA-fluorescein without test compounds) was calculated for each sample by the equation below (eq 1).

$$\%\text{Inhibition} = 100\% - \frac{(SF_s) \times 100\%}{(SF_f)} \quad \text{(eq 1)}$$

where *SFs* is the ENT1 transporter-specific fluorescence of test samples, and SF_f is the ENT1 transporter-specific fluorescence of the SAENTA-fluorescein ligand standard in mean channel numbers. The results were fed into the PRISM program (GraphPad, San Diego, CA) to derive concentration-dependent curves. From these curves, the IC_{50} values were obtained and used to calculate inhibition constants (*K*i) values from eq 2:

$$K_i = IC_{50}/(1+[L]/K_L) \quad \text{(eq 2)}$$

where [*L*] and K_L are the concentration and the K_d value of the SAENTA-fluorescein, respectively[57]. The K_i values were used to compare the abilities of the new compounds to displace the ENT1 transporter-specific ligand (5-(SAENTA)-*X8*-fluorescein[24] and, for that matter, their affinity for the ENT1 transporter.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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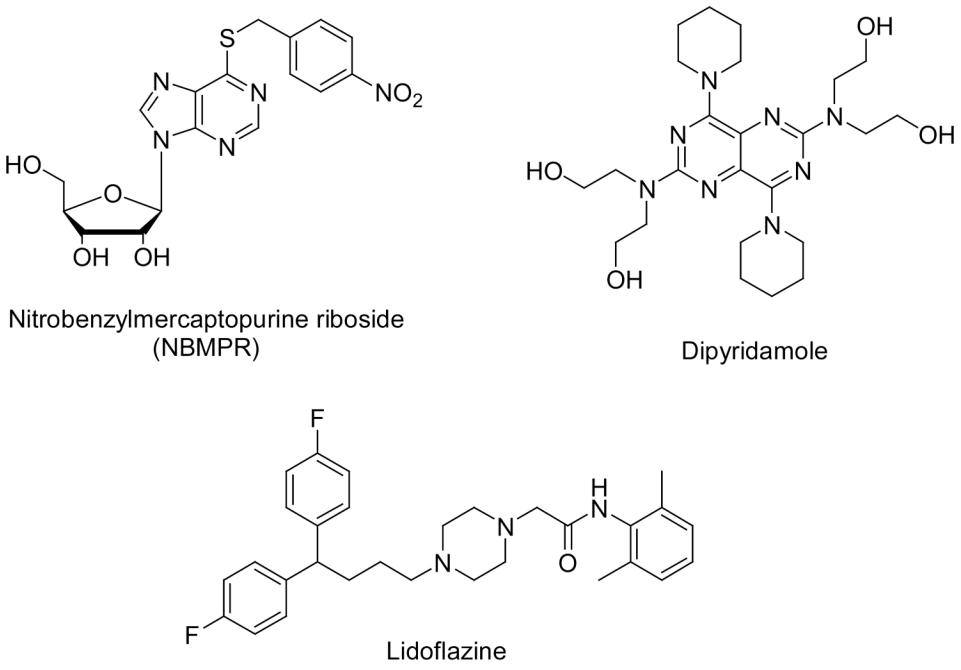
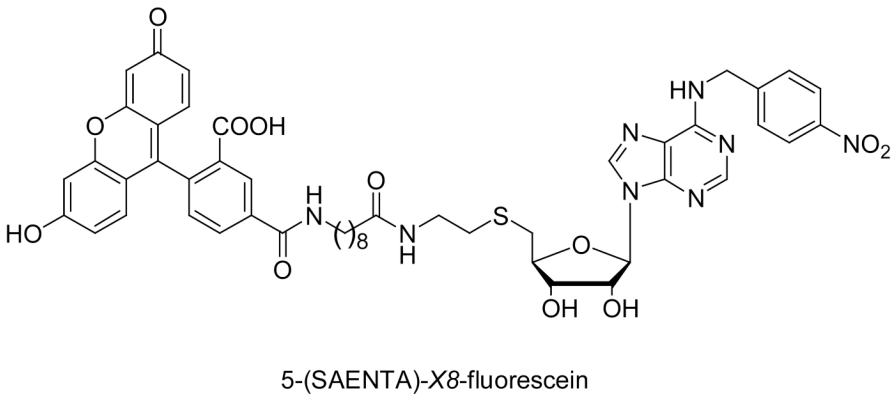


Figure 1.
Representatives of the three main ENT1 inhibitory chemical classes



5-(SAENTA)-*X8*-fluorescein

Figure 2.
Structure of SAENTA-fluorescein

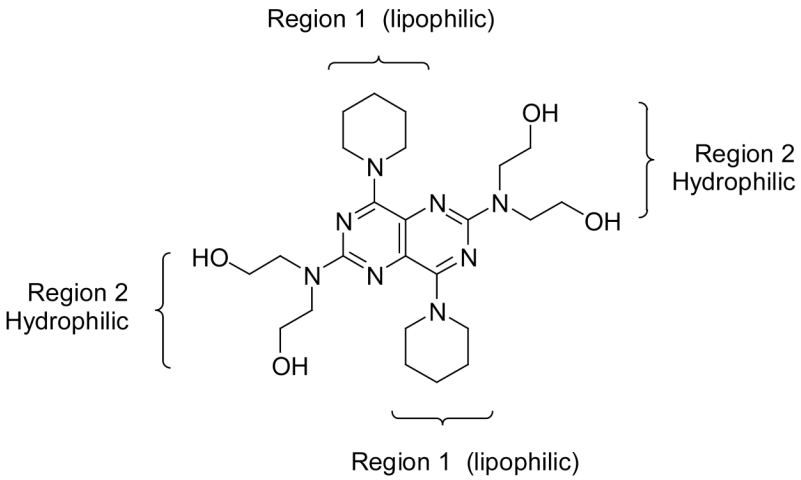
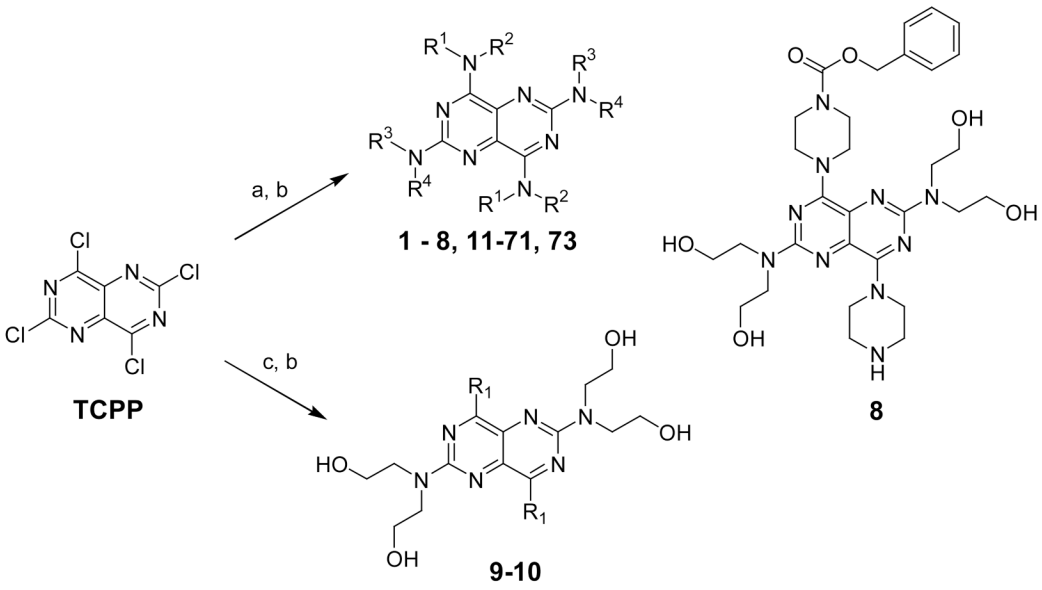
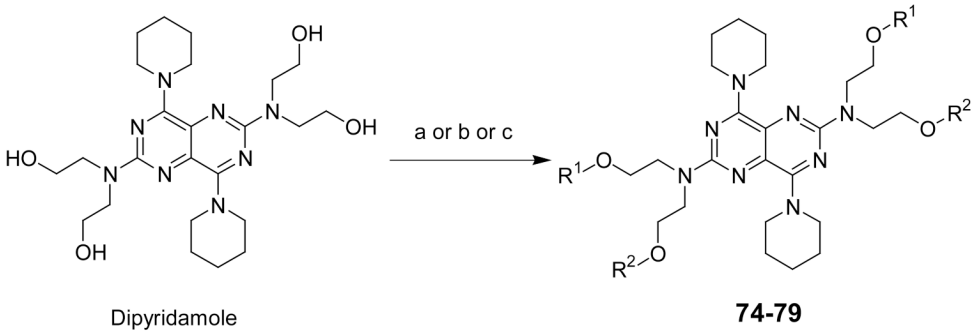


Figure 3.
Representative regions for dipyridamole analogs



Scheme 1a.

[a]Reagents and conditiond: (a) NHR^1R^2, Anhydrous THF, 0 - 5 °C; (b) NHR^3R^4, DMSO, 150 °C; (c) R^1MgCl, Anhydrous THF, 0 - 5 °C.

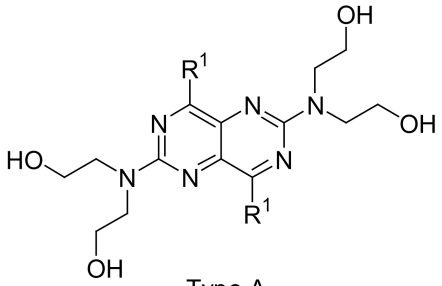


Scheme 2a.

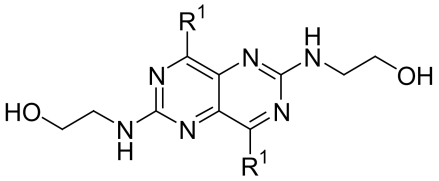
[a]Reagents and conditions: (a) HCOOH, 100 °C (compound **74**); (b) CH_3COCl, DMAP, anhydrous THF, 0-5 °C (compound **75**); (c) NaH, R^1I, anhydrous DMF ($R^2 = R^1$ for **76**-**78**; R^2 = H for **79**).

Table 2

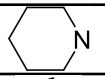
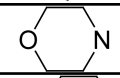
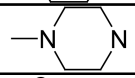
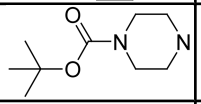
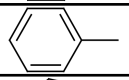
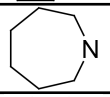
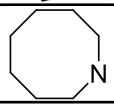
Inhibitory activities of compounds with different ring systems at the 4- and 8-positions



Type A



Type B

Comp.	Type	R^1	ENT1 inhibitory activity in K562 cells determined by flow cytometry		
			%Inhibition at 10μM	IC_{50} (nM)	K_i (nM)
DMSO	-	-	0.0 ± 0.7	N. D.[a]	N. D.
NBMPR	-	-	97.1 ± 0.4	7.6	0.43
Lidoflazine	-	-	90.0 ± 0.2	4954	279.9
Dipyridamole	A		86.7 ± 0.1	144.8	8.18
1	B		53.5 ± 0.1	12,229	690.9
2	A		85.7 ± 0.3	1,764	99.7
3	B		19.3 ± 1.1	ND	ND
4	A		70.4 ± 0.4	6,956	393
5	B		13.5 ± 0.3	ND	ND
6	A		0.9 ± 0.7	ND	ND
7	A		44.6 ± 2.1	ND	ND
8[b]	A	-	68.8 ± 0.0	7,947	449
9	A		N. D.	ND	ND
10	A		N. D.	ND	ND
11	A		94.4 ± 0.2	15.2	0.86
12	B		28.7 ± 0.1	ND	ND
13	A		93.1 ± 0.3	8.67	0.49
14	B		78.6 ± 0.3	375	21.2

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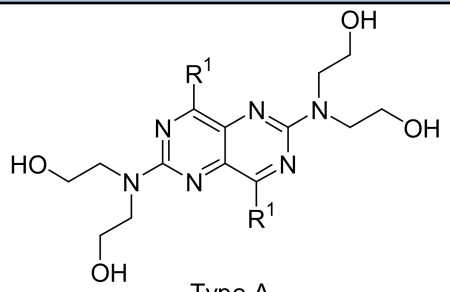
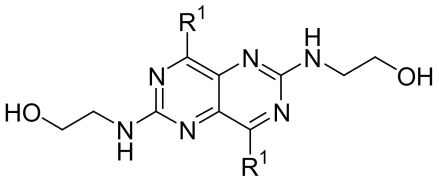
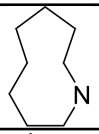
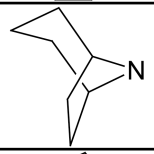
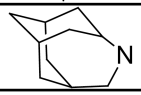


Type A



Type B

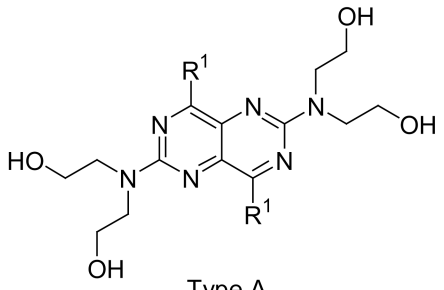
Comp.	Type	R[1]	ENT1 inhibitory activity in K562 cells determined by flow cytometry		
			%Inhibition at 10μM	IC_{50} (nM)	K_i (nM)
15	A		78.0 ± 0.4	13.6	0.77
16	B		69.3 ± 0.3	672	38
17	A		85.0 ± 0.1	3,416	193
18	B		14.9 ± 1.7	ND	ND
19	A		24.7 ± 0.2	ND	ND
20	B		16.2 ± 1.9	ND	ND

[a] ND = Not Determined.

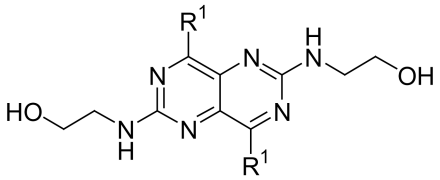
[b] For structure of compound **8**, see Scheme 1.

Table 2

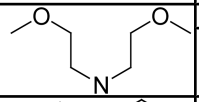
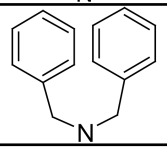
Inhibitory activities of compounds with open chain 4- and 8-position substituents



Type A



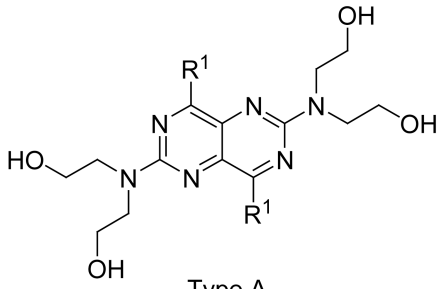
Type B

Comp.	Type	R[1]	ENT1 inhibitory activity in K562 cells determined by flow cytometry		
			%Inhibition at 10μM	IC_{50} (μM)	K_i (nM)
DMSO	-	-	0.0 ± 0.7	ND[a]	ND
NBMPR	-	-	97.1 ± 0.4	7.6	0.43
Lidoflazine	-	-	90.0 ± 0.2	4,954	279.9
DP	A		86.7 ± 0.1	144.8	8.18
21	A	Me_2N	49.7 ± 0.1	3,828	216.7
22	B		9.9 ± 0.4	ND	ND
23	A	Et_2N	57.0 ± 0.7	3,831	216.4
24	B		30.4 ± 0.1	ND	ND
25	A	$(n\text{-Propyl})_2N$	41.6 ± 1.4	ND	ND
26	B		13.0 ± 0.1	ND	ND
27	A	$(n\text{-Butyl})_2N$	53.6 ± 2.9	ND	ND
28	B		3.0 ± 4.3	ND	ND
29	A	$(iso\text{-Butyl})_2N$	19.3 ± 1.8	ND	ND
30	B		6.6 ± 0.1	ND	ND
31	A	$(n\text{-Pentyl})_2N$	7.2 ± 1.3	ND	ND
32	B		-4.6 ± 1.9	ND	ND
33	A	$(iso\text{-Pentyl})_2N$	8.2 ± 0.8	ND	ND
34	B		2.7 ± 1.2	ND	ND
35	A		38.4 ± 0.4	ND	ND
36	B		9.9 ± 0.4	ND	ND
37	A		-65.6 ± 1.8	ND	ND
38	B		-8.0 ± 1.6	ND	ND

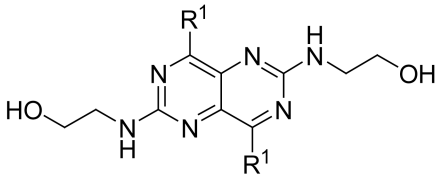
[a] ND = Not Determined.

Table 3

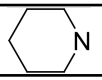
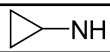
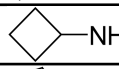
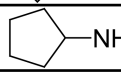
Inhibitory activities of compounds with free hydrogen on the nitrogen of 4- and 8-position substituents

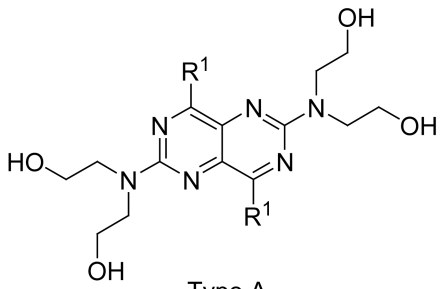


Type A

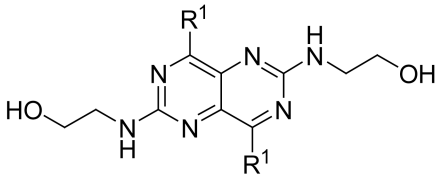


Type B

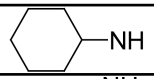
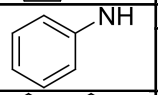
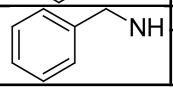
Comp.	Type	R^1	ENT1 inhibitory activity in K562 cells determined by flow cytometry		
			%Inhibition at 10μM	IC_{50} (nM)	K_i (nM)
DMSO	-	-	0.0 ± 0.7	ND[a]	ND
NBMPR	-	-	97.1 ± 0.4	7.6	0.43
Lidoflazine	-	-	90.0 ± 0.2	4954	279.9
DP	A		86.7 ± 0.1	144.8	8.18
39	A	NH_2	6.5 ± 0.1	ND	ND
40	A	MeNH	4.1 ± 0.9	ND	ND
41	B		-1.0 ± 0.1	ND	ND
42	A	EtNH	24.0 ± 1.0	ND	ND
43	B		0.7 ± 0.5	ND	ND
44	A	n-PropylNH	79.5 ± 1.0	5,310	300
45	B		5.7 ± 0.3	ND	ND
46	A	iso-PropylNH	81.7 ± 1.4	3,381	191
47	B		14.1 ± 1.2	ND	ND
48	A	n-ButylNH	87.3 ± 0.1	2,407	136
49	B		61.6 ± 0.1	8,655	489
50	A	iso-ButylNH	92.0 ± 0.2	673	38
51	B		24.9 ± 0.1	ND	ND
52	A	tert-ButylNH	81.2 ± 0.1	297	16.8
53	B		28.0 ± 0.1	ND	ND
54	A	n-PentylNH	56.4 ± 0.4	2,476	139.9
55	B		12.5 ± 1.4	ND	ND
56	A	iso-PentylNH	86.8 ± 0.6	2,136	120.7
57	B		7.56 ± 0.1	ND	ND
58	A	tert-PentylNH	94.4 ± 0.2	260	14.7
59	B		16.1 ± 0.3	ND	ND
60	A		48.7± 0.9	7,554	427.6
61	B		6.2 ± 1.3	ND	ND
62	A		84.1 ± 0.3	1,838	104.1
63	B		23.0 ± 2.6	ND	ND
64	A		90.8 ± 0.2	279.7	15.8
65	B		4.9 ± 0.2	ND	ND



Type A



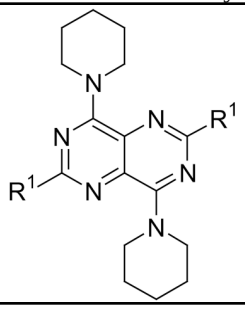
Type B

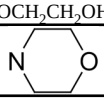
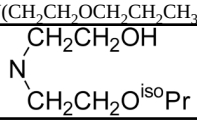
Comp.	Type	R^1	ENT1 inhibitory activity in K562 cells determined by flow cytometry		
			%Inhibition at 10μM	IC_{50} (nM)	K_i (nM)
66	A		80.9 ± 0.1	940	53.1
67	B		1.4 ± 0.3	ND	ND
68	A		5.8 ± 0.5	ND	ND
69	B		6.6 ± 3.9	ND	N. D.
70	A		11.9 ± 1.8	ND	ND
71	B		3.8 ± 0.2	ND	ND

[a] ND = Not Determined.

Table 4

Inhibitory activities of compounds with modification at the hydroxyl groups of dipyridamole



Comp.	R^1	ENT1 inhibitory activity in K562 cells determined by flow cytometry		
		% Inhibition at 10μM	IC_{50} (nM)	K_i (nM)
DMSO	-	0.0 ± 0.7	ND[a]	ND
NBMPR	-	97.1 ± 0.4	7.6	0.43
Lidoflazine	-	90.0 ± 0.2	4954	279.9
DP	$N(CH_2CH_2OH)_2$	86.7 ± 0.1	144.8	8.18
72[b]	OCH_2CH_2OH	60.7 ± 0.7	5,746	325.2
73		13.0 ± 0.4	ND	ND
74	$N(CH_2CH_2OOCH_3)_2$	91.3 ± 0.2	145	8.2
75	$N(CH_2CH_2OOCCH_3)_2$	90.4 ± 0.3	302	17.1
76	$N(CH_2CH_2OCH_3)_2$	66.2 ± 1.1	1,621	91.6
77	$N(CH_2CH_2OCH_2CH_3)_2$	8.4 ± 0.1	ND	ND
78	$N(CH_2CH_2OCH_2CH_2CH_3)_2$	2.3 ± 0.4	ND	ND
79		73.8 ± 1.1	76	4.3

[a] ND = Not Determined.

[b] Prepared by a literature procedure.[40]