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PHOSPHONATE DERIVATIVES OF N^9-BENZYLGUANINE: A NEW CLASS OF POTENT PURINE NUCLEOSIDE PHOSPHORYLASE INHIBITORS

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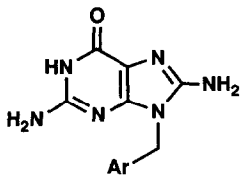
Abstract: New phosphonate derivatives of N^9-benzylguanine (**5a** - **5d**) have been designed and prepared as purine nucleoside phosphorylase inhibitors. Enzyme inhibition studies with PNP from calf spleen or human erythrocytes demonstrate that the trifluorophosphonate **5b**, the fluorovinylphosphonate **5c** and the vinylphosphonate **5d** , with K_i values around 1 nM, are the most potent inhibitors of PNP ever reported.

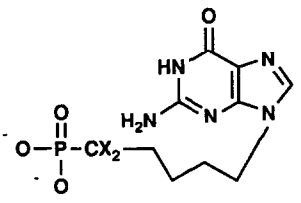
Purine nucleoside phosphorylase (EC.2.4.2.1; PNP), a key enzyme in the purine salvage pathway[1] is believed to be a target for drug design[2] . PNP inhibitors might be useful as immunosuppressive agents as well as in the treatment of T-cell leukemia, gout[3] and some parasitic diseases[4]. In addition PNP inhibitors may protect purine nucleosides used as chemotherapeutic agents such as 2',3'-dideoxyinosine and 6-thiopurine 2'-deoxynucleosides against PNP metabolism[5,6].

PNP catalyzes the reversible phosphorolysis of guanosine and inosine nucleosides (or deoxynucleosides) to their respective free base and ribose-1-phosphate (or deoxyribose-1-phosphate). This reaction proceeds via a ternary complex of enzyme, nucleoside, and orthophosphate[1]. Metabolically stable "multisubstrate" acyclic nucleotide analogues containing a purine and a phosphate-like moiety such as 9-phosphonoalkyl derivatives of hypoxanthine and guanine have been designed and synthesized[7]. The most potent inhibitor of human erythrocytic PNP in this series was 9-(5,5-difluoro-5-phosphonopentyl)guanine **4** and its K_i value was found[7] to be 18 nM. Since PNP is not a rate-limiting enzyme and has a very high activity in humans[2], a more potent inhibitor may be necessary. Other potent PNP inhibitors are 9-aryl substituted analogues of 8-aminoguanosine. In this series, the most potent inhibitors are 8-amino-9-benzylguanine[8] **1**, with a K_i value of 200 nM (four times lower than the K_i of 8-aminoguanosine) and 8-amino-9-(2-thienylmethyl)guanine[9] **2**, with a K_i value of 67 nM.

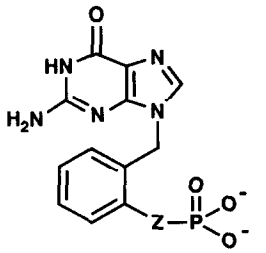
Scheme 1



1: Ar = Ph
2: Ar = 2-thienyl



3: X = H
4: X = F



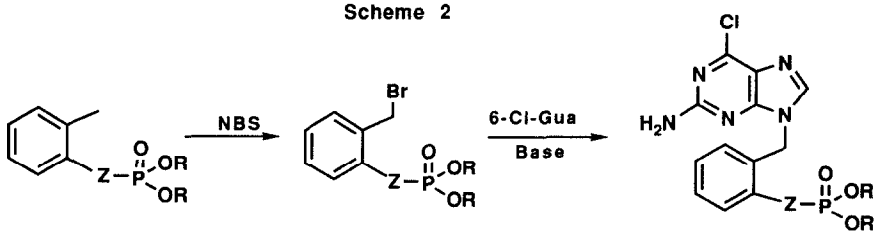
5a: Z = CH_2CF_2 ; **5b**: Z = $CHFCF_2$
5c: Z = CH=CF ; **5d**: Z = CH=CH

Here, we report our results regarding synthesis and inhibitory properties of the phosphonate derivatives of 9-benzylguanine **5a** - **5d**. These compounds were designed as PNP inhibitors to study the possible synergism of a N-9 benzyl substituent of guanine and a phosphonate moiety. Finally, the importance of the phosphate surrogate (fluorinated phosphonates, vinylphosphonate and fluorovinylphosphonate) was also studied and is reported.

The 9-arylphosphonate derivatives of guanine **5a** -**5d** have been obtained from the phosphonate diesters derivatives of 2-amino-6-chloropurine **8a** - **8d** after reaction with excess trimethylsilylbromide[10] in acetonitrile followed by acid aqueous hydrolysis (1 N HCl, 80°C, 15 h).

The phosphonate diester derivatives of 2-amino-6-chloropurines **8a** - **8d** have been prepared by a common strategy from the arylphosphonates **6a** - **6d** (scheme 2).

Scheme 2



6 7 8

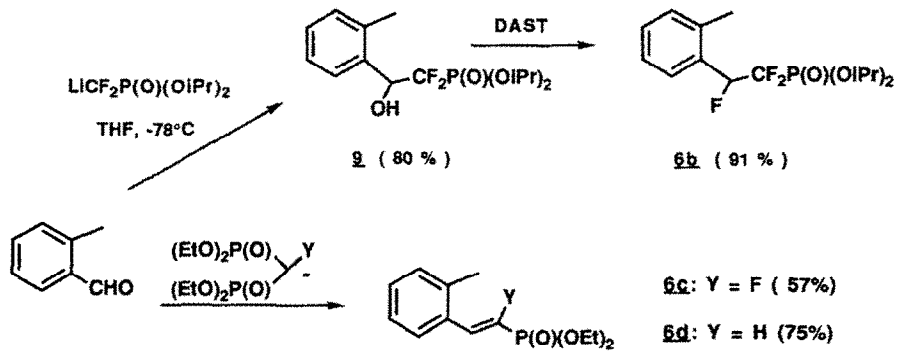
entry	Z	R	Base	Yield (%)
a	o-CH_2CF_2	Et	NaH	42
b	o-$CHFCF_2$	i-Pr	K_2CO_3	65
c	o-CH=CF	Et	K_2CO_3	65
d	o-CH=CH	Et	K_2CO_3	56

The key step of the synthesis involves the condensation of the intermediary benzylbromides **7a** - **7d** with the anion of 2-amino-6-chloropurine in DMF at 20°C to give the expected condensation products **8a** - **8d**. This reaction was found to proceed with a high control (> 90%) of N^9-regioselectivity as determined by ^1H NMR analysis[11]. The benzylbromides **7a** - **7d** have all been obtained by reacting the tolylphosphonates **6a** - **6d** with N-bromosuccinimide in carbon tetrachloride. It is noteworthy that only the depicted (scheme 2) benzylic bromides are formed even in the case of the tolylphosphonates **6a** and **6b** where no benzylic bromination could be detected next to the difluorophosphonate group.

The difluorophosphonate **6a** was obtained in 43% yield by condensing diethylphosphinyldifluoromethyllithium[12] with ortho-α-bromoxylene; the use of the organozinc reagent [$BrZnCF_2P(O)(OEt)_2$] in the presence of copper (I) bromide in DME (conditions described[13] for allylic substitution) gave only 20% yield of the adduct **6a**.

The trifluorophosphonate **6b**, the vinylfluorophosphonate **6c** and the vinylphosphonate **6d** have been prepared from o-tolualdehyde according to scheme 3.

Scheme 3



Condensation of o-tolualdehyde with diisopropylphosphinyl difluoromethyllithium at -78°C in THF followed by acid aqueous hydrolysis at -50°C gave 80% of the alcohol **9** which after reaction with diethylaminosulfurtrifluoride (DAST) was transformed into the expected trifluoroderivative **6b** in 91% yield. The bis-phosphonate anions required to prepare the vinyl phosphonates **6c** or **6d** have been obtained by deprotonation of the corresponding bis-phosphonates with n-butyllithium in THF at -78°C. Tetraethyl fluoromethylene bisphosphonate was prepared by decomposition of diethylphosphinylchlorofluoromethane in the presence of n-butyllithium as previously described[14]. The fluorovinylphosphonate **6c** could also be isolated in 40% yield in a one pot reaction where diethylphosphinylchlorofluoromethane was reacting with 2 equivalents of n-butyllithium before to be condensed with excess o-toluadehyde. Both vinylphosphonates **6c** and **6d** were isolated as the E isomers as demonstrated by ^{1}H NMR analysis (for **6c**: $J_{H\text{-}P}$ = 11 Hz; $J_{H\text{-}F}$ = 40 Hz; for **6d**: $J_{H\text{-}H}$ = 15 Hz; $J_{Ha\text{-}P}$ = 17 Hz; $J_{Hb\text{-}P}$ = 22 Hz).

Compounds **5a** - **5d**[15] were found to be extremely potent inhibitors of PNP from either human erythrocyte or calf spleen. Apparent inhibition constants (K_i) obtained for compounds **5** at pH 7.4, with orthophosphate concentration fixed at 1 mM, are listed in the table.

Table: Inhibition constants for PNP inhibitors.

Compound	PNP			
	human erythrocyte		calf spleen	
	K_i (nM) 1 mM Pi	IC_{50} (nM) 50 mM Pi	K_i (nM) 1 mM Pi	IC_{50} (nM) 50 mM Pi
5a	13 ± 1	300	4 ± 1	200
5b	1.3 ± 0.1	85	0.6 ± 0.1	100
5c	1.8 ± 0.1	77	0.8 ± 0.3	60
5d	3.2 ± 0.3	155	0.8 ± 0.2	85

Determination of K_i values were performed as described previously[7] using commercial sources of PNP (Sigma Chemical Co.). IC_{50} were measured in the presence of 50 μM inosine and 50 mM Pi.

As shown for other phosphonates[7] inhibition decreased with increasing concentrations of orthophosphate. At 50 mM orthophosphate, IC_{50} values are substantially higher than the K_i values measured at 1 mM Pi (see Table 1), suggesting that compounds **5** are multisubstrate analogue inhibitors. In conclusion, compounds **5b**, **5c** and **5d** are the most potent inhibitors of human erythrocyte PNP reported so far. In addition they are about 10 times more potent than the best inhibitors of the calf spleen enzyme designed very recently from cristallographic and modeling methods[16]. More particularly, compounds **5b-d** are about 20 times more potent than 9[2-(phosphonoethyl)phenyl]guanine[16], demonstrating the importance of fluorines or unsaturation in our inhibitors.

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