

Nucleotide analogue, method of synthesis of nucleotide analogue, use of nucleotide analogue, antiviral pro-nucleotide, pharmaceutical composition

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Abstract

The object of the invention are 1) nucleotide analogues, 2) antiviral pro-nucleotides, 3) a use of nucleotide analogues and pharmaceutical composition, 4) a phosphorylating agent for synthesis of nucleotide analogue, and 5) a method of synthesis of nucleotide analogues. More precisely, the invention applies to the new group of nucleotide analogues and their use in partial or complete inhibition of human immunodeficiency virus (HIV).

State of the art

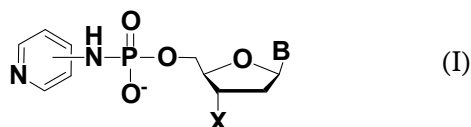
Despite numerous known solutions exploiting nucleoside derivatives as compounds for treating viral diseases, there is still a continuous need for an efficient solution allowing preparation of pharmaceutical compositions comprising water-soluble compounds showing the

same or higher activity as the starting nucleoside and displaying simultaneously low toxicity.

The aim of the current invention is to find efficient compounds being a novel group of nucleotide derivatives, the physicochemical properties and anti-HIV pharmacokinetic parameters of which would be better than

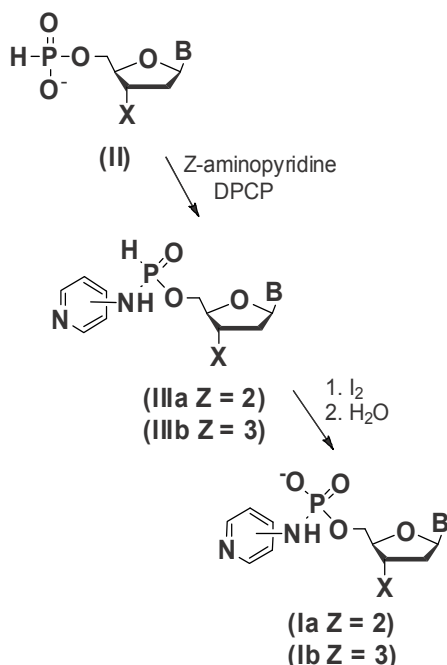
those of the parent nucleoside analogues. The invention applies to compounds without chiral centres at the phosphorus atom that would act as pro-nucleotides and be transformed into the respective nucleosid-5'-yl phosphate.

The subject matter of the invention is a nucleotide analogue of the formula (I)

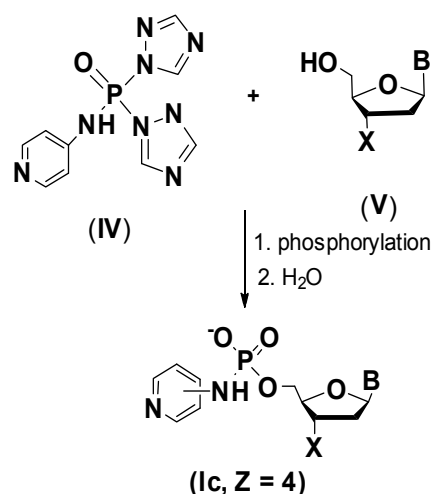


where X stands for N₃ and B stands for thymidine-1-yl, or X stands for H and B stands for uracil-1-yl or adenine-9-yl or hypoxanthin-9-yl as anti-HIV pro-nucleotide.

The next subject of the invention is the way of synthesis and preparation of the nucleotide analogues characterized in that the synthesis of nucleoside phosphor[N-(pyridin-Z-yl)]amidates in which Z indicates the position of the nitrogen atom in the pyridine ring (2 or 3), and the nucleoside analogues is depicted with formula (I) and which synthesis is carried out according to the scheme below.



In synthesis of nucleoside-5'-yl phosphor[N-(pyridin-4-yl)]amidates described with formula (Ic), for phosphorylation of nucleoside analogues V di(1H-1,2,4-triazol-1-yl) phosphor[N-(pyridin-4-yl)]amidate described with formula (IV) was used and the reaction was carried out according to scheme:



and the obtained final nucleotide derivatives are described with formula (Ic), where for (VT) and (ITc): X = N₃, B = thymine; for (VU) and (IUc): X = H, B = uracil; for (VA) and (IAc): X = H, B = adenine; and for (VH) and (IHc): X = H, B = hypoxanthine.

Anti-HIV activity (EC₅₀ and EC₉₀) – parameter EC₅₀ is defined as concentration of compounds in which replication of HIV is inhibited by 50% and parameter EC₉₀ is defined as concentration of compounds in which replication of HIV is inhibited by 90% and were determined in HIV infected CEM-T4 cells (Table 1).

Compounds obtained by Applicants constitute a new group of nucleotide derivatives, which cytotoxicity were lower (or much lower) than nucleosides they derived from (AZT and ddU) and which anti-HIV potencies were comparable or markedly better than the parent nucleosides. They are pro-nucleotides what was proved by the observed anti-HIV activity of ddU derivative. This feature and very good solubility in water, as well as very low cytotoxicity and retained anti-HIV activity make the applicants compounds more valuable as a potential therapeutics against HIV as compare to drugs applied in AIDS therapy so far.

The following basic properties of the examined compounds make them a potential therapeutics:

- very good solubility in aqueous media [phosphoric buffer pH 7.5, cell culture medium (RPMI/FBS 9 : 1 (v/v)],
- comparable or higher anti-HIV activity than the parent nucleosides,
- low cytotoxicity,
- the compounds are pro-nucleotides,
- the compounds are stable during storage, in aqueous buffers of pH = 1 (7 days), i. e. the acidity identical

Table 1. Cytotoxicity (CC) and anti-HIV activity (EC) of selected series of compounds (**Ic**) and nucleosides (**V**) they derived from

Compound	CC ₅₀ [μM]	CC ₉₀ [μM]	EC ₅₀ [μM]	EC ₉₀ [μM]	SI ₅₀
(ITc)	≥190	≥190	0.0011	0.006	≥19000
AZT	60	–	0.0011	0.016	6000
(IUc)	≥1000	≥1000	0.44	>1	≥2273
ddU	≥250*	–	48*	–	>5.2*
(IAc)	≥1000	≥1000	1.7	2.65	≥588
ddA	≥250*	–	2.5*	–	≥100*
(IHc)	≥5300	≥5300	20.00	>20	≥215
ddI	≥100**	–	1.1**	–	≥91**

*Estimated in cells CEM-T4; Baba M. et al. (1987) *Biochem. Biophys. Res. Commun.* 142(1): 128-134;

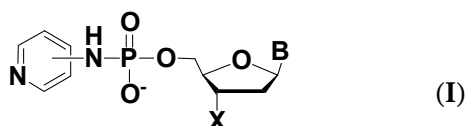
**Imbach J.-L. et al. (1994) *Biochem. Pharmacol.* 48(1): 11-14

as in the stomach, so they are promising candidates for oral delivery,

- the compounds do not contain chiral centres at the phosphorus atom, which may affect their biological activity.

Patent claims

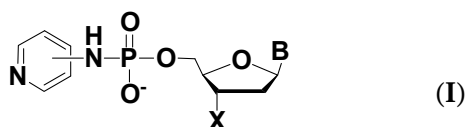
1. Nucleotide analogue described by formula (I)



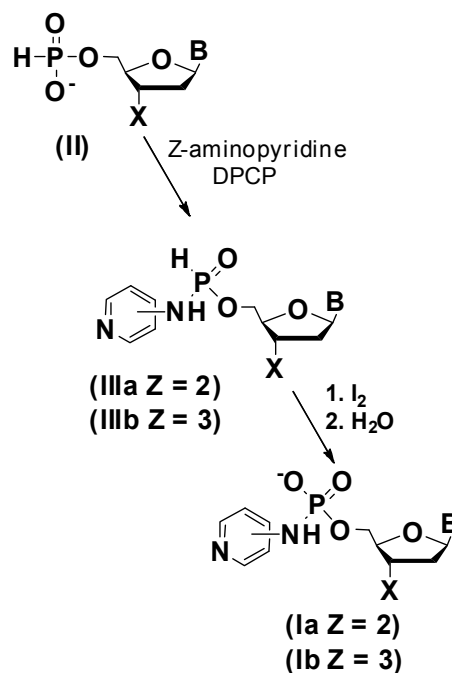
where X is N₃ and B is thymine-1-yl, or X is H and B is uracil-1-yl or adenine-9-yl or hypoxanthine-9-yl.

2. Nucleotide analogue wherein a compound is an anti-HIV pro-nucleotide.

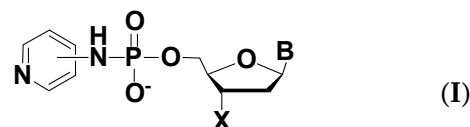
3. A method of synthesis of the nucleotide analogues characterized in that the synthesis of phosphor[N-(pyridin-Z-yl)]amidates of nucleoside analogues described by formula (I), where Z indicates the position of the nitrogen atom in the pyridine ring (2 or 3),



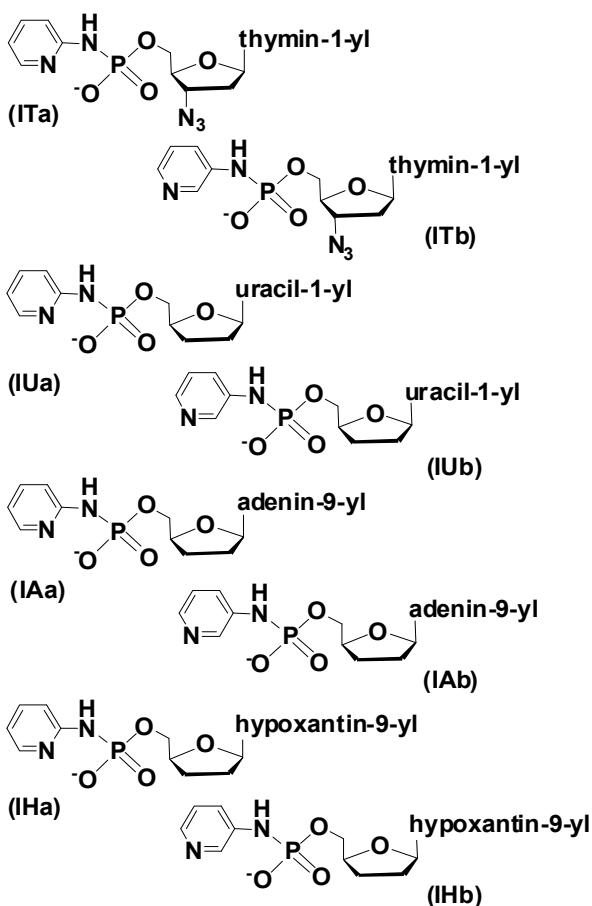
is carried out according to the scheme below:



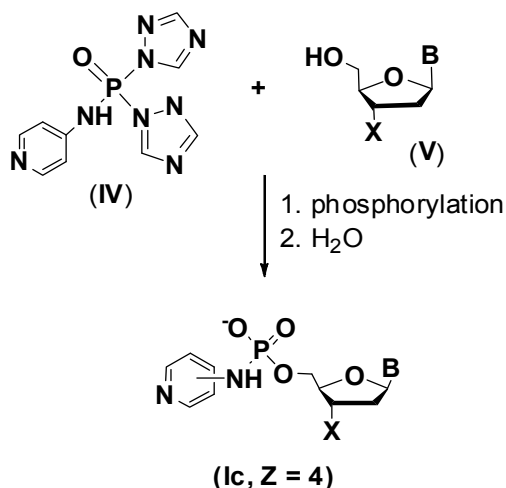
where the compound is described with formula (I)



in which X and B are the same as in the compounds depicted with formulas (**ITa,b**), (**IUa,b**), (**IAa,b**) and (**IHa,b**) presented below:



4. A phosphorylation method characterized in that di(1H-1,2,4-triazol-1-yl)phosphor[N-(pyridin-4-yl)amidate (IV) is used as the phosphorylating agent in the synthesis of nucleoside-5'-yl phosphor[N-(pyridin-4-yl)]amidates described with formula (Ic), in the phosphorylation of nucleoside analogues described with formula (V) and the synthesis is carried out as presented in the following scheme:



and the obtained final nucleotide derivatives are described with formula (Ic), where for (VT) and (ITc): X = N₃, B = thymine; for (VU) and (IUc): X = H, B = uracil; for (VA) and (IAc): X = H, B = adenine; and for (VH) and (IHc): X = H, B = hypoxantine.

5. Nucleotide analogue according to claim 1 to 4 for use as antiviral pronucleotide, preferably derivative of 4-aminopyridine, against HIV virus.

6. Nucleotide analogue according to claim 5, for use in production of antiviral drug, preferentially drug for treatment of HIV infections including AIDS.

7. Antiviral pronucleotide, the nucleotide analogue according to claim 1 to 5.

8. Pharmaceutical composition containing nucleotide analogue according to claim 1 to 5.

9. Pharmaceutical composition according to claim 8, wherein it is antiviral drug, preferentially for treatment of HIV infection and AIDS.

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