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Synthesis of benzo-, pyrido-, thieno- and imidazo-fused *N*-hydroxy-4-oxopyrimidine-2-carboxylic acid derivatives

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ABSTRACT

N-Hydroxy-4-oxoquinazoline-2-carboxamide derivatives (cyclic hydroxamic acids) and related pyrido- and thieno-substituted analogues, as well as a *N*-hydroxyhypoxanthine-2-carboxamide were synthesised for the first time, by means of a four-step sequence that involves a smooth reaction of aminohydroxamates with methyl trimethoxyacetate. Other strategies were unsuccessful.

Raltegravir (Isentress, MK-0518), developed by Merck, is the first HIV-1 integrase inhibitor (INI) approved by the FDA.[1] Raltegravir and its congeners (Fig. 1),[1b,c] elvitegravir (Gilead) and S/GSK candidates have similar pharmacophoric elements and mechanism of action.[1d,e]

The interest of some of us in hydroxamates[2a] and the well-known chelating power of hydroxamic acids[2b] prompted us to propose alternative candidates based on the replacement of the C–OH group of raltegravir by a N–OH group, which would enhance the acidity of the OH proton and would improve the coordination with magnesium ions. Thus, we designed *N*-hydroxypyrimidinone derivatives as shown in Figure 1 (bottom). The challenge was to develop a general approach, as simple as possible, to these unknown molecules.

The strategies analysed by us at the beginning of our project are summarised in Scheme 1. In the first approach (**a**), we envisaged the N-oxidation of N3 of 4-amino-pyrimidines and of N1 of adenine,[3] where the π-electron donating ability of the amino group is crucial for the formation of the *N*-oxide. The subsequent deamination, either enzymatically or via diazotization,[4] should afford the desired *N*-hydroxy pyrimidones (cyclic hydroxamic acids). While 2-cyano derivatives of adenine and adenosine (Scheme 1, top, X = CN)[5] gave the *N*-oxide in acceptable conversions (with a large excess of *m*-CPBA in CH_2Cl_2, as the reagent of choice among eight reagents examined), the corresponding methyl ester (X = COOMe) and amide (X = $CONHCH_2C_6H_4F$ = CONH-4-FBn) did not. In other words, a slight increase in the steric hindrance at position 2 hampered the N-oxidation. Although we achieved the conversion of X = CN into X = COOMe with NaOMe in MeOH at 50 °C, the methoxy group could not be replaced by the 4-fluorobenzylamino group (a substitution that was very easy when the N-oxide substituent was absent). Moreover, whereas deamination of adenine derivatives (to obtain hypoxanthine derivatives) was quite efficient in our hands, either with adenosine deaminase or via nitrosation, the N-oxide derivatives did not react. In short, the route via formation of N-oxides is not feasible.

Approach **b** involved a ring opening–ring closing process by using hydroxylamine (or a protected derivative of it, such as NH_2OBn or NH_2OTr) to attack the oxazinones of Scheme 1. We prepared the corresponding imidazo-oxazinone[6] by the treatment of the *o*-amino carboxylic acid with a large excess of ClCOCOOMe

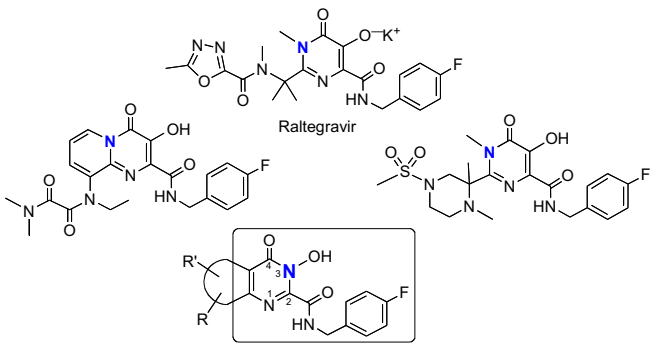

Figure 1. Raltegravir and two examples of congeners. *N*-Hydroxypyrimidin-4-one derivatives proposed as alternative candidates.

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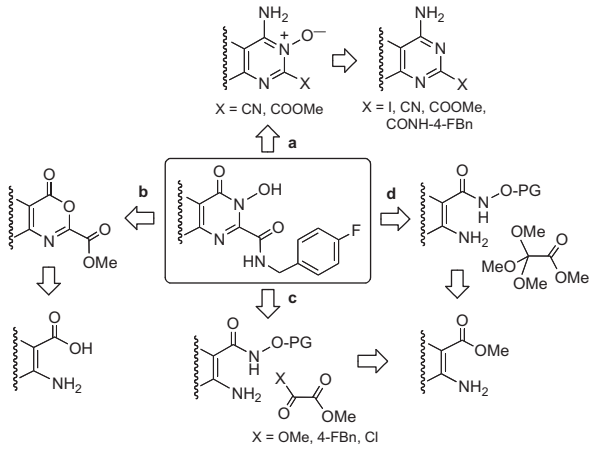
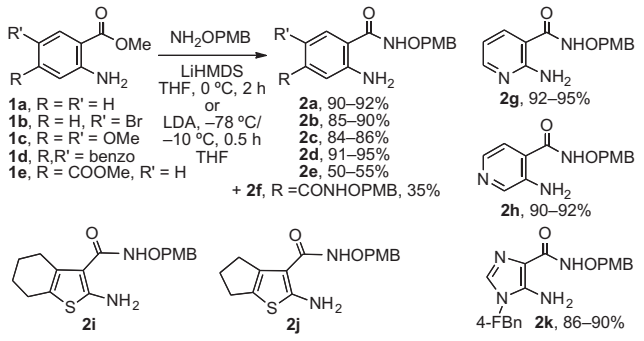


Scheme 1. Retrosynthetic analysis of *N*-hydroxypyrimidin-4-ones.

and DIPEA in CH_3CN. Opening with hydroxylamine derivatives gave mixtures coming from the attack of the hydroxylamines at the diverse electrophilic positions. Moreover, heating the open compounds in Ac_2O gave rise to degradation compounds.

According to disconnection **c**, the hydroxamates (PG = Bn or PG = 4-methoxybenzyl = PMB), prepared from the amino ester, benzylamine or 4-methoxybenzylamine and $LiN(SiMe_3)_2$ (LiHMDS) were heated with dimethyl oxalate and NaOEt,[7] in search of direct cyclisation. Only starting material was recovered. Heating with MeOCO-CONH-4-FBn was also unsuccessful. With ClCOCOOMe and DIPEA or in pyridine, mainly the *O*-acylation occurred. On the other hand, with K_2CO_3 or with DMAP, NHCOCOOMe derivatives were obtained, but their cyclisation to *N*-hydroxypyrimidinones under several dehydrating conditions did not take place.

Among all the approaches that we attempted only that one indicated as **d** in Scheme 1 turned out to be productive. Although the reaction of vicinal amino carboxamides with alkyl orthoformates such as $CH(OR)_3$ is classical[8] and orthoesters such as methyl 2,2,2-trimethoxyacetate[9] have been used with vicinal diamines or aminophenols,[9d,e] there are no reported precedents, according to a SciFinder search, of the reaction of amino hydroxamates with this oxalic acid orthoester. We first examined the conversion of amino ester **1a** (methyl anthranilate) into benzohydroxamate **2a** (Scheme 2). An excess of a strong base such as LiHMDS was required.[10] With a stronger base such as LDA the reaction was even faster.[10] Without the strong base the reaction did not progress, even in refluxing 1,4-dioxane. The more general conditions starting from amino esters **1a–e**, the analogous pyridines **1g** and **1h** and the methyl 5-aminoimidazole-4-carboxylate **1k**[11] are shown in Scheme 2, which gave rise to good-to-excellent yields of hydroxamates of type **2** (in the case of **1e**, a mixture was obtained, but **2e** and **2f** were readily separated by column chromatography). On the other hand, owing to the decomposition of amino esters **1i** and **1j** in strong basic media, we prepared **2i** and **2j** by the standard coupling of the corresponding amino acids with *O*-(4-methoxybenzyl)hydroxylamine (NH_2OPMB) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC).[12]



Scheme 2. Amino hydroxamates **2a–k**.

We then subjected **2a** and $(MeO)_3CCOOMe$ excess to a screening of solvents, acid catalysts, temperatures and reaction times. Most relevant results are shown in Table 1.

Acid catalysis was required, as expected for an orthoester, to achieve the complete conversion of **2a into 3a**. Not only TsOH and Lewis acids were active, in several solvents (see entries 2–10 of Table 1) but also weaker acids such as AcOH could be used (entry 11). With 2 mol % of TsOH in refluxing CH_3CN (entry 12), the cyclisation occurred in 4 h.

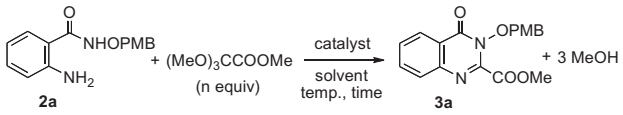
This cyclisation or condensation is general, as it could be applied to 2-aminohydroxamates **2b–f**, to aminopyridines **2g** and **2h** and to thiophene derivatives **2i** and **2j**, as indicated in Table 2, with some adaptations. Afterwards, the ester groups of **3a–j** were transformed into carboxamides **4a–j**,[13] which were subjected to the cleavage of the O–PMB bond with TFA, to give the desired cyclic hydroxamic acids, **5a–j**.

Finally, we obtained *N*-hydroxyhypoxanthine **5k**[14] in three high-yielding steps from **2k** as detailed in Scheme 3.

Samples of the molecules thus prepared (**5a**, **5e**, **5f** and **5k**) were tested as inhibitors of the 3'-processing and strand transfer activities of HIV-1 integrase at a single 10 μM concentration, with regard to raltegravir;[15a] unfortunately, no activity was observed. They also appeared to be inactive for concentrations up to 10 μM in an HIV-1 antiviral, single-round-of-infection assay.[15b]

In summary, we have synthesised new benzo-, pyrido-, thieno- and imidazo-fused *N*-hydroxypyrimidinones (quinazolinone, pyridopyrimidinone, thienopyrimidinone and hypoxanthine ring systems) with carboxyl derivatives at position 2. The optimized sequence involves the reaction of ester groups with NH_2OPMB and a strong base (LDA or LiHMDS) as well as a crucial acid-catalysed condensation of these amino hydroxamates with methyl trimethoxyacetate, which has been carefully investigated to provide

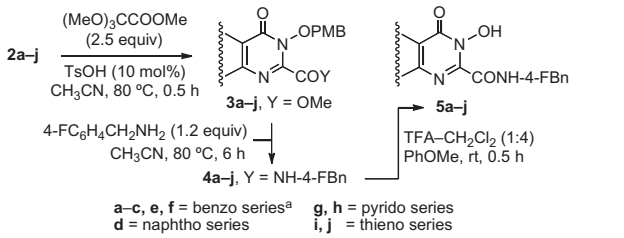
Table 1
Survey of cyclisation conditions[a]



Entry	*n*	Catalyst (mol %)	Solvent	Temp. (°C)	Time (h)	Yield (%)
1	2.5	—	DMF	90	15	⩽1
2	2.0	TsOH (10)[b]	CH_3CN	20	4	95
3	2.5	$BF_3{\cdot}Et_2O$ (10)	CH_3CN	20	4	97
4	2.5	$BF_3{\cdot}Et_2O$ (10)	CH_3CN	80	0.5	96
5	2.5	$Sc(OTf)_3$ (10)[c]	CH_3CN	20	4	96
6	2.5	$Sc(OTf)_3$ (10)	CH_3CN	80	0.5	95[d]
7	2.5	TsOH (10)	CH_3CN	80	0.5	98
8	2.5	TsOH (10)	DMF	90	0.5	98
9	2.5	TsOH (10)	Dioxane	90	0.5	97
10	2.5	TsOH (10)	Toluene	90	3	97
11	2.5	AcOH		90	0.5	98
12	2.0	TsOH (2)	CH_3CN	80	4	98

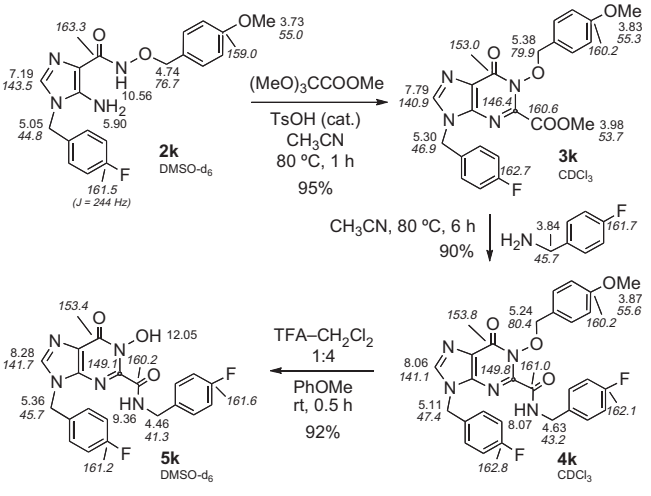
[a] From 1 mmol of **2a** in 10 mL of solvent.
[b] TsOH means commercially available 4-toluenesulfonic acid-hydrate.
[c] Scandium trifluoromethanesulfonate of 99% purity. In trials with 1 mol % of TfOH, under the conditions of entry 5, only a conversion of 10% was noted after 72 h. Thus, the activity of $Sc(OTf)_3$ is not due to the possible content of TfOH as impurity.
[d] The O–PMB bond was cleaved under these conditions: the yield refers to PMB-deprotected **3a**.

Table 2
From amino esters **2a–j** to quinazolinones **5a–f**, pyridopyrimidinones **5g** and **5h** and thienopyrimidinones **5i** and **5j**[a]



Entry	Step 1	Yield (%)	Step 2	Yield[b] (%)	Step 3	Yield (%)
1	**2a** to **3a**	95–98	**3a** to **4a**	91	**4a** to **5a**	95
2	**2b** to **3b**	98	**3b** to **4b**	86	**4b** to **5b**	92
3	**2c** to **3c**	92	**3c** to **4c**	87	**4c** to **5c**	90
4	**2d** to **3d**	85	**3d** to **4d**	85	**4d** to **5d**	90
5	**2e** to **3e**	89	**3e** to **4e**	89	**4e** to **5e**	95
6	**2f** to **3f**	91	**3f** to **4f**	93	**4f** to **5f**[c]	97
7	**2g** to **3g**	90[d]	**3f** to **4g**	85	**4g** to **5g**	87
8	**2h** to **3h**	85[e]	**3h** to **4h**	85	**4h** to **5h**	92
9	**2i** to **3i**	92[f]	**3i** to **4i**	70[g]	**4i** to **5i**	90
10	**2j** to **3j**	90[f]	**3j** to **4j**	75[g]	**4j** to **5j**	92

[a] See Scheme 2 for the substituents (groups/rings).
[b] Similar results in refluxing THF.
[c] The CONHOPMB group at position 4 of the aromatic ring was also cleaved to CONHOH.
[d] 120 mol % of TsOH·H_2O was required in this case, owing to the basicity of the 2-aminopyridine moiety. Refluxing AcOH was not sufficient whereas heating at 140 °C (MW) gave rise to degradation. On the other hand, heating overnight with 10 mol % of BF_3, at 80 °C in a closed vial caused an almost complete disappearance of **2g** (to give in 80% isolated yield the desired **3g**).
[e] This cyclisation was performed in refluxing AcOH.
[f] For 2 h in refluxing CH_3CN.
[g] With 220 mol % of 4-FBnNH_2 and dropwise addition of 200 mol % of LDA, in THF at −78 °C, stirring for 4 h. Heating with 4-FBnNH_2 (as in entries 1–8) gave rise to decarboxylation.



Scheme 3. From **2k** to **5k**. Chemical shifts (δ_H in regular type, δ_C in italics). Assignments confirmed by 2D NMR (HSQC).

excellent yields of the cyclic compounds under very mild conditions. Overall, the protocol reported herein for the first time for the synthesis of *N*-hydroxypyrimidinones **5a–k** (four-steps, most of them in 85–98% yields, up to 76% overall yield) appears to be of wide scope. In short, in the unsuccessful search for new antiviral drugs we have developed the best procedure to date for reaching hitherto unknown pyrimidinone-2-carboxylic acid derivatives, which may enjoy other applications.[16]

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5. We prepared them as follows: conversion of protected guanosines to 2-amino-6-chloropurine derivatives, diazotization to yield 6-chloro-2-iodo analogues, replacement of iodide with cyanide (with CuCN, in the presence of Pd(0)/Xantphos), and amino-dechlorination. We introduced the amino group on C6 with NH_3/1,4-dioxane (without concomitant deacetylation of tri-*O*-acetyladenosines). See: (a) Grunewald, C.; Kwon, T.; Piton, N.; Forster, U.; Wachtveitl, J.; Engels, J. W. *Bioorg. Med. Chem.* **2008**, *16*, 19–26; (b) Ohno, M.; Gao, Z.-G.; Van Rompaey, P.; Tchilibon, S.; Kim, S.-K.; Harris, B. A.; Gross, A. S.; Duong, H. T.; Van Calenbergh, S.; Jacobson, K. A. *Bioorg. Med. Chem.* **2004**, *12*, 2995–3007; (c) Sakamoto, T.; Ohsawa, K. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2323–2326 (this method, reported for aromatic rings, was the most efficient in our hands).
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10. Optimized conditions: 1.5 equiv of $PMBONH_3^+Cl^-$ plus 4.5 equiv of LiHMDS (at 0 °C for 2 h) or 4.1 equiv of LDA (from −78 °C to −10 °C, 0.5 h); yields were similar. We could also prepare 2-aminobenzohydroxamic acid (deprotected **2a**) by treatment of **1a** with a very large excess of NH_2OH/MeOH in a sealed tube, but this procedure failed with the less reactive esters of our series. For direct substitutions of NHOH for OMe, see: (a) Griffith, D.; Krot, K.; Comiskey, J.; Nolan, K. B.; Marmion, C. J. *Dalton Trans.* **2008**, 137–147. and references cited therein; (b) Riva, E.; Gagliardi, S.; Mazzoni, C.; Passarella, D.; Rencurosi, A.; Vigo, D.; Martinelli, M. *J. Org. Chem.* **2009**, *74*, 3540–3543; for the direct substitution of NHOBn for OMe, see: (c) Gissot, A.; Volonterio, A.; Zanda, M. *J. Org. Chem.* **2005**, *70*, 6925–6928; (d) Guzzo, P. R.; Miller, M. J. *J. Org. Chem.* **1994**, *59*, 4862–4867.
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14. *Compound* **5k**: mp 262.5–264.0 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.46 (d, J = 6.1 Hz, 2H), 5.36 (s, 2H), 7.19 (m, 4H), 7.39 (m, 4H), 8.28 (s, 1H), 9.36 (t, J = 6.1 Hz, 1H), 12.05 (s, 1H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 41.3, 45.7, 115.0 (d, J = 21.4 Hz), 115.5 (d, J = 21.5 Hz), 123.5, 129.1 (d, J = 8.2 Hz), 129.7 (d, J = 8.4 Hz), 132.6 (d, J = 3.0 Hz), 134.3 (d, J = 3.0 Hz), 141.7, 144.9, 149.1, 153.4, 160.2, 161.2 (d, J = 242.4 Hz), 161.6 (d, J = 244.0 Hz); HMRS (ESI, m/z), calcd for $C_{20}H_{16}F_2N_5O_3^+$ ($M+H^+$) 412.1216, found 412.1204.
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16. Regarding 2-carboxyl or 2-carbonyl derivatives of pyrimidin-4-ones, 75 patents can be found via SciFinder from 2000 to 2010 aimed at treating diverse diseases.