N,O-Nucleosides from Ene Reaction of (Nitrosocarbonyl)mesitylene with Crotyl Alcohol: Selectivity, Scope, and Limitations

Serena Carosso a
Misal Giuseppe Memeo a
Bruna Bovio a
Elena Valletta b
Beatrice Macchi a
Paolo Quadrelli* a

a Department of Chemistry, University of Pavia, Viale Taramelli 12, 27100 Pavia, Italy
paolo.quadrelli@unipv.it
b Department of System Medicine, University of Rome Tor Vergata, Via Montpellier 1, 00133 Roma, Italy

Received: 09.12.2016
Accepted after revision: 09.01.2017
Published online: 01.02.2017
DOI: 10.1055/s-0036-1588695; Art ID: ss-2016-t0846-op

Abstract The (nitrosocarbonyl)mesitylene intermediate undergoes an ene reaction with crotyl alcohol, affording two regioisomeric adducts in fair yields. The sterically demanding (nitrosocarbonyl)mesitylene slightly shifts the C2/Markovnikov orientation towards a C3/anti-Markovnikov pathway, affording a 5-hydroxyisoxazolidine that serves as a synthon for the preparation of N,O-nucleoside analogues through the Vorbrüggen protocol. The selectivity of the ene reaction is discussed in the light of C=C bond polarization and steric effects. The structures of the N,O-nucleosides are assigned and discussed on the basis of spectroscopic observations and X-ray analysis.

Key words N,O-nucleosides, ene reaction, nitroso carbonyls, crotyl alcohol, Vorbrüggen protocol

The ene reactions of aromatic nitrosocarbonyl intermediates 1 (Scheme 1) with allylic alkoxy olefins were investigated and the synthesis of N,O-nucleoside analogues through the Vorbrüggen protocol was proposed.1 The Chiacchio and Romeo groups recently described the synthesis of truncated phosphonated C-1′-branched N,O-nucleosides as HIV inhibitors or inducers of apoptosis based on 1,3-dipolar cycloadditions of nitrones with vinyl acetate, followed by coupling with silylated nucleobases.2 The same approach was also followed by the research groups of Bortolini and Maiuolo for the synthesis of antiproliferative drugs.3 The 1,3-dipolar cycloaddition synthetic strategy is a valuable method for spacer substitution in classical nucleosides. Furthermore, the introduction of a side chain led to several branched nucleosides which were found to be potential antitumor or antiviral agents,4 and an interesting cytotoxicity and apoptotic activity was also observed.5 Our alternative for the synthesis of N,O-nucleosides relies upon the mild oxidation of aromatic nitrile oxides with tertiary amine N-oxides to generate the nitrosocarbonyl intermediates 1 and the ene reaction with allylic alcohols such as 3-methylbut-2-en-1-ol (Scheme 1).6 3-Methylbut-2-en-1-ol can be regarded as a trisubstituted allylic alkoxy olefin, and the allylic hydrogens on the more congested side of the alkene are exclusively abstracted (the ‘cis effect’), thus resembling singlet oxygen behavior.

Scheme 1 Ene reaction of nitrosocarbonyls 1 with 3-methylbut-2-en-1-ol and synthesis of isoxazolidine nucleosides

The canon (a general rule) for these reactions is that they proceed straightforwardly to the ene adducts in accordance with the prevailing HOMO(olefin)–LUMO(nitrosocarbonyl) interaction, somewhat enforced by the polarization of the C=C bond induced by the slightly electron-withdrawing group CH2OH.7 (Nitrosocarbonyl)benzene 1P (P = phenyl) follows a Markovnikov (M) orientation and preferentially...
abstracts the twix hydrogens over the lone ones. With the more sterically demanding (nitrosocarbonyl)mesitylene (1M; M = mesityl), the Markovnikov directing effect is diminished and twix and lone abstraction are comparable (Scheme 1).8

The anti-Markovnikov (AM) pathway becomes the preferred one when the bulkier anthracene aromatic ring replaces mesitylene in the nitrosocarbonyl structure 1A (A = anthryl).1 The anti-Markovnikov route leads to enol formation and subsequent cyclization to the isoxazolidine isoxd, resembling the structures obtainable through nitrogen additions to vinyl acetate derivatives (Scheme 1). By adapting the Vorbrüggen protocol, a library of N,O-nucleosides NO-N was prepared from commercially available uracils and purines.19

On pursuing our research in nitrosocarbonyl ene reactions, we extended the studies to another allylic alcohol, the disubstituted crotyl alcohol, which was allowed to react in the presence of the mesitylene nitrosocarbonyl 1M. The products of the ene reaction were characterized and the structures were assigned, allowing a closer examination of the selectivity outcome and disclosing new synthetic applications. The diastereomeric isoxazolidines deriving from formal anti-Markovnikov addition to the alcohols are presented as valuable synthons toward the preparation of new N,O-nucleosides N,O-N was prepared from commercially available uracils and purines.19

Addition of a dichloromethane (CH2Cl2) solution of mesitonitrile oxide (MNO) to a stirred solution of N-methylmorpholine N-oxide (NMO, 1.1 equiv) in CH2Cl2 in the presence of an excess (5 equiv) of crotyl alcohol (mixture of cis and trans) afforded, after standing overnight at r.t., the ene adducts 2 and 3 (Scheme 2), which were isolated by chromatographic separation of the reaction mixture in 50% and 23% yield, respectively.

The structures of the isolated ene adducts were determined by the corresponding analytical and spectroscopic data. In the 1H NMR spectrum of the ene adduct 2, the typical olefinic methylene signals are found as doublets at δ = 5.08 and 5.23, while the olefinic methine proton is typically found more deshielded at δ = 5.95 as a multiplet. The N-OH group is found strongly deshielded as a broad signal at δ = 10.40. The other signals are found in the expected ranges for the reported structure; in particular, the OH group resonates at δ = 3.34 as a doublet, also shown in the IR spectrum at 3152 cm⁻¹. Compound 2 is derived from the addition of (nitrosocarbonyl)mesitylene (1M) to the hydroxymethyl substituted carbon atom C2 of the double bond of crotyl alcohol. Structure 2 belongs to the family of compounds obtained in previous work1,9 from 3-methylbut-2-en-1-ol in full accordance with a Markovnikov orientation.8,10 In the present case of crotyl alcohol, it is somewhat inappropriate to consider the carbon atom C2 the site of Markovnikov addition (C2/M addition), because of the 1,2-disubstitution of the C=C bond of the alcohol; however, this is useful and done for the sake of comparison with previous results.1,9

If the addition of the electrophilic (nitrosocarbonyl)mesitylene (1M) occurs at the C3 carbon atom, the product of the ene reaction is represented by the 5-hydroxyisoxazolidine 3; the primary adduct is the enol 4 that evolves into the non-isolable aldehyde 5, which undergoes cyclization to the stable hemiacetal 3 (Scheme 3). This type of 5-hydroxyisoxazolidine is usually prepared by reaction of hydroxymalic acids and α,β-unsaturated aldehydes11 or through the ene protocol of nitrosocarbons and allylic alcohols.1 As in the previous cases,1,9 the origin of isoxazolidine 3 was undoubtedly attributed to the diminishing of the C2/M addition due to the more sterically demanding mesityl group that activates the C3/AM pathway.8,10

The structure of 3 was assigned on the basis of the corresponding analytical and spectroscopic data. All the NMR spectra indicate the presence of an inseparable mixture of diastereoisomers in a 2:1 ratio 2:1 (Schemes 2 and 3). In the case of the major diastereoisomer, the 1H NMR (CDCl3) spectrum indicates the presence of a deshielded proton at δ = 5.89 as a triplet (J = 4 Hz) coupled with two protons at δ = 2.03 and 2.51. The methyl group is found at δ = 1.03 as a doublet (J = 6 Hz), because of the coupling with the methine proton at δ = 3.98 (q, J = 6 Hz). The OH group is confirmed by the corresponding IR spectrum (strong band at 3284 cm⁻¹). The 13C NMR spectrum presents a signal at δ = 98.3 resembling an acetal-type carbon atom. The structure assignment as shown in Scheme 2 was confirmed beyond any reasonable doubt from the X-ray analysis (Figure 1, see also Supporting Information).

The 5-hydroxy-isoxazolidine 3 obtained from the ene reaction of (nitrosocarbonyl)mesitylene (1M) with crotyl alcohol was used as a synthon to prepare N,O-nucleoside analogues by adapting the Vorbrüggen protocol12 as well as...
other procedures for similar compounds.\textsuperscript{5,13} The diastereoisomeric acetyl derivatives 6\textit{a,b} were prepared according to standard procedures (Scheme 4).\textsuperscript{1,14} The acetylated compounds were obtained in nearly quantitative yield, separated by column chromatography and fully characterized. The acetylated compounds 6\textit{a} and 6\textit{b} are diastereoisomers, each one obtained as a racemic mixture of enantiomers, isolated in 45% and 47% yields, respectively. The structures reported in Scheme 4 were assigned on the basis of the corresponding analytical and spectroscopic data and the diastereoisomeric outcome was determined by NOESY experiments. In the \textsuperscript{1}H NMR spectra (CDCl\textsubscript{3}), the presence of the acetyl groups is shown by two singlets at \(\delta = 2.06\) for 6\textit{a} and at \(\delta = 1.90\) for 6\textit{b}, while in the corresponding \textsuperscript{13}C NMR spectra two additional signals corresponding to C=O groups were both detected at \(\delta = 169.0\). The NOESY experiments allowed for the attribution of the reported stereochemistry (NOE correlations in red in Scheme 4). In the stereoisomer 6\textit{a}, the proton at \(\delta = 6.33\) correlates with one of the two methylene protons at \(\delta = 2.25\), while the other one at \(\delta = 2.72\) correlates with the CH–N proton found at \(\delta = 4.65\). In the stereoisomer 6\textit{b}, the proton at \(\delta = 6.18\) correlates with one of the two methylene protons at \(\delta = 2.22\), which displays a second correlation with the CH–N proton found at \(\delta = 4.62\); this relationship is also confirmed by the correlation between the methyl group at \(\delta = 1.43\) with the methylene proton at \(\delta = 2.20\) (Scheme 4).

To prepare the uracil derivatives, the Vorbrüggen protocol can be applied both on a previously silylated heterobase or on a commercial compound in the presence of silylating agents.\textsuperscript{12,15} In the cases at hand, we report the syntheses conducted on the commercial uracil base used in the presence of an \textit{in situ} prepared silylating agent, bis(trimethylsilyl)acetamide (BSA) and trimethylsilyl trifluoromethanesulfonate (TMSOTf) as reaction promoter.\textsuperscript{16}

The acetylated isoxazolidines 6\textit{a} and 6\textit{b} were added under nitrogen atmosphere at r.t. to a solution of uracil (2 equiv) and BSA (2 equiv) and the solutions became clear after boiling in CH\textsubscript{2}Cl\textsubscript{2} for a couple of hours. The mixtures were then ice-cooled at 0 °C and TMSOTf (1 equiv) was added and the reaction mixtures refluxed overnight (Scheme 5). The desired compounds 7\textit{a,b} were obtained as white solids separated by column chromatography and fully characterized. Nucleoside analogues 7\textit{a,b} were isolated in good yields \(37–49\%\) and their structures determined by analytical and spectroscopic data. The reactions were performed on a single diastereoisomer of the starting compound and gave mixtures of both diastereoisomers of the products belonging to the family of uracil derivatives in a nearly 1:1 ratio. Table 1 reports the yields, physicochemical data and the relevant \textsuperscript{1}H NMR spectroscopic data supporting the structural assignments given in Scheme 5.

The \textsuperscript{1}H NMR spectra of uracil derivatives 7\textit{a,b} were typical for this type of compound, with doublets \(J = 8 \text{ Hz}\) at \(\delta = 5.69\) and 7.70 (7\textit{a}) and \(\delta = 5.63\) and 7.65 (7\textit{b}), clearly related
Strategizes operating mechanism of the Vorbrüggen protocol on sub-
scheme 5). These results furnished some insights on the
these nucleoside analogues is that both the diastereois-
antiomers. The most intriguing fact in the synthesis of
out that the two products exist as racemic mixtures of en-
products 7a and 7b can be separated once the reaction ends.

The uracil nucleoside analogues 7a and 7b could be sub-
titted for biological evaluation without any further structural modifications, either for their antiviral behavior or, in other cases, to compare their activities with those of re-
ported carbocyclic and heterocyclic structures.5,13,17,18

To expand the synthetic applications of the Vorbrüggen
protocol on the acetylated isoxazolidines 6a,b and deter-
mine the scope and limitations of the method,19 we inves-
tigated the functionalization with the commercially available
6-chloropurine, being aware of the possible increasing
to the uracil double bond, while both imide NH groups
appeared highly deshielded at δ = 11.33 as singlets. The other
signals are consistent for the isoxazolidine structure. The
configurations of the two diastereoisomers 7a and 7b were
assigned on the basis of NOESY experiments and the cor-
relations are reported in red in Scheme 5. Compound 7a
shows the uracil ring cis-related to the methyl group of the
isoxazolidine ring, while in compound 7b the uracil is
found trans to the methyl group. It must further be pointed
out that the two products exist as racemic mixtures of en-
antiomers. The most intriguing fact in the synthesis of
these nucleoside analogues is that both the diastereois-
omers are obtained from each starting acetyl derivative: 6a
gives a mixture of 7a and 7b; the same happens if the reac-
tion is conducted starting from 6b (see yellow inset in
Scheme 5). These results furnished some insights on the
operating mechanism of the Vorbrüggen protocol on sub-
strates 6 and deserve a discussion (vide infra). For this
reason, for preparative purposes, the reaction can be conduct-
ed simply on a diastereoisomeric mixture of 6a,b, and pro-
Table 1  Yields and Physicochemical and Spectroscopic Data of Compounds 7a,b

<table>
<thead>
<tr>
<th>Compound</th>
<th>mp (°C)a</th>
<th>Yield from 6a (%)</th>
<th>Yield from 6b (%)</th>
<th>IR (cm⁻¹) νmax</th>
<th>νC=O</th>
</tr>
</thead>
<tbody>
<tr>
<td>7a</td>
<td>&gt;210</td>
<td>49</td>
<td>46</td>
<td>3164</td>
<td>1724, 1669</td>
</tr>
<tr>
<td>7b</td>
<td>&gt;210</td>
<td>47</td>
<td>37</td>
<td>3173</td>
<td>1702, 1637</td>
</tr>
</tbody>
</table>

1H NMR (δ, DMSO)

<table>
<thead>
<tr>
<th>Signal</th>
<th>δ H3C</th>
<th>δ CH–N</th>
<th>δ =CH–CO</th>
<th>δ O–CH–Ur</th>
<th>δ N–CH=</th>
<th>δ NH</th>
</tr>
</thead>
<tbody>
<tr>
<td>7a</td>
<td>2.29, 2.93 (m)</td>
<td>4.67 (d)</td>
<td>5.69 (d)</td>
<td>6.19 (t)</td>
<td>7.70 (d)</td>
<td>11.33 (s)</td>
</tr>
<tr>
<td>7b</td>
<td>2.50, 2.95 (m)</td>
<td>4.84 (m)</td>
<td>5.63 (d)</td>
<td>6.27 (d)</td>
<td>7.65 (d)</td>
<td>11.33 (s)</td>
</tr>
</tbody>
</table>

*From ethanol.
complexity of the reaction mixture. In fact, it is well known that the purine rings could give regioisomeric adducts at the N7 and N9 nitrogen atoms; if this is transferred to a substrate existing as a mixture of diastereoisomers, the stereochemical outcome increases its complexity.

The acetylated isoxazolidines $6a, b$ were added under a nitrogen atmosphere at r.t. to a solution of 6-chloropurine (2 equiv) and BSA (2 equiv) and the solutions became clear after boiling in CH$_2$Cl$_2$ for a couple of hours. The mixtures were then ice-cooled at 0 °C and TMSOTf (1 equiv) was added and the reaction mixtures were refluxed overnight. Scheme 6 reports on the structures of the four possible products.

On the basis of previous observations, the reaction was conducted on the mixture of the diastereoisomeric acetylated compounds $6a, b$ to give a mixture of four possible diastereoisomeric products where the purine ring can be linked at the isoxazolidine moiety through the N7 and/or N9 nitrogen atoms. Two compounds $8a, b$ were obtained as white solids separated by column chromatography and characterized. The purine nucleoside analogues $8a, b$ were isolated in moderate yields (33% and 40%, respectively) and their structures were determined with analytical and spectroscopic data through a careful and detailed analysis in particular of the NMR data. Table 2 reports the yields, physicochemical data and the relevant $^1$H NMR spectroscopic data supporting the structural assignments given in Scheme 6.

As reported, quite surprisingly, only two compounds were detected and separated from the reaction mixture. Both contain the 6-chloropurine rings coupled with the isoxazolidine moiety. The major task was the stereochemical assignment, since spectroscopic data alone give only a few suggestions on the relationship between the methyl group of the isoxazolidine ring and the purine ring. A definitive answer came from the X-ray crystal structure analysis of compound $8a$; the structure $8aN7$ was assigned. Combining information from the diffractometric analysis and the chemical shift data from the NMR spectra, it was possible to define the stereochemical outcome of this reaction (Figure 2).

Figure 2  X-ray crystal structure of compound $8aN7$

For compound $8b$, only the spectroscopic data are available to determine the structure. NOESY experiments gave successful answers on the stereochemistry of $8b$, which was confirmed to belong to the trans series of the purine adducts, i.e. the purine ring and the methyl group are trans-related on the isoxazolidine moiety, as shown in Scheme 6, where the NOE correlations are indicated by red arrows. In the NMR spectrum we have also detected a minor component relative to $8b$, not separable from the major; the spectroscopic (NMR) data are reported in Table 2. The signals do not correspond to hindered rotations, since we have verified the matter through variable temperature experiments, and no signal coalescence occurred in the spectra recorded.
in DMSO. Compound 8b is made up of an inseparable mixture of two products, and we have tentatively assigned the reported structures on the basis of NMR observations.

In Table 2 the NMR signals related to compound 8b were separately assigned to the regioisomers 8bN7 (major) and 8bN9 (minor). This was done after comparison of the chemical shifts of the representative protons. These values for the 8bN7 regioisomer are more deshielded than those of the 8bN9 regioisomer, corresponding well with the observed values of solely compound 8aN7. Due to the lack of pure compounds available, these products were not used for biological evaluation.

We investigated the ene reactions of (nitrosocarbonyl)mesitylene, generated through the mild oxidative protocol with NMO, with crotyl alcohol, a 1,2-disubstituted allylic alcohol. The fast oxidation process of nitrile oxide to the nitrosocarbonyl intermediate prevents a possible side reaction, i.e. the addition of the electrophilic nitrile oxide to the reactive 1,2-disubstituted allylic alcohols. In fact, the alcohol employed is prone to undergo 1,3-dipolar cycloaddition, being a slightly reactive dipolarophile. From the reaction mixtures, the presence of adducts between the aromatic nitrile oxides and crotyl alcohol were not observed in isolable amounts, even in the crude mixtures.

(Nitrosocarbonyl)mesitylene (1M) adds to crotyl alcohol affording adducts 2 and 3, which derive, respectively, from the preferred C2/M path or the competitive C3/AM route in the ene reaction (Scheme 7). The results are discussed in terms of two grounds: (a) polarization of the C=C bond and (b) steric effects.

In crotyl alcohol, the methyl and the hydroxymethylene groups are the substituents of the C=C bond and both concur to establish the polarization towards the C2 carbon atom as shown in Scheme 7. The methyl group (C4) is a modest donor, while hydroxymethylene (C1) is slightly electron withdrawing. As a consequence, the electron-rich C2 carbon atom is apt to receive the addition of the electrophilic nitrosocarbonyl intermediate. The subsequent abstraction of the allylic hydrogen on the methyl group leads to the Markovnikov (M) preference (adduct 2) of the ene reaction in accordance with the prevailing HOMOLOMO (olefin)–LUMO (nitrosocarbonyl) interaction. However, the reaction conducted with the sterically demanding (nitrosocarbonyl)mesitylene (1M) does not leave the C3/AM path unpopulated, and this deserves a brief comment. The canon described above is respected.

In previous work, we demonstrated that increasing the bulkiness of an aromatic substituent on a nitrosocarbonyl moiety leads to the AM ene reaction pathway becoming preferred over the M path. The mesitylene ring is located in the middle of this ranking (Scheme 8, cf. Scheme 1) and the regioselectivity is akin to previous results obtained with 3-methylbut-2-en-1-ol. From a mechanistic point of view, we pointed out that, when bulkier substituents replace the phenyl group (Schemes 1 and 8), the M path is raised energetically because of a steric effect and the AM path is favored affording a mixture of regioisomeric compounds. We have recently detailed the selectivity outcome in the ene reaction of (nitrosocarbonyl)mesitylene (1M) with trisubstituted olefins, and the results show the remarkable influence of the nitrosocarbonyl substituent on the selectivities in ene reactions. Model calculations on the reaction of 1M with tetramethylethylene (TME) shed light on the factors involved in the varying selectivities. In these reactions of trimethylethylene, the M path is favored in the case of (nitrosocarbonyl)benzene, while steric hindrance in the approach of 1M compensates somewhat its electronic preference and mixtures of M and AM adducts are formed (Figure 3). This mechanism was found at work also in the case of 3-methylbut-2-en-1-ol and remarkably influences and enforces the selectivity in the reaction of (nitrosocarbonyl)anthracene with 3-methylbut-2-en-1-ol.

As we have demonstrated, in the reactions of (nitrosocarbonyl)benzene with TME, the transition structure (TS) of the addition step shows no special hindrance between the...
addends while in the TS of 1M the mesityl group is twisted out of the nitrosocarbonyl plane as usual, causing unfavorable steric crowding between its ortho methyl and the 'trans' distal TME methyl as depicted in Figure 3. Moving to the case of crotyl alcohol, the evaluation of the steric effect is depicted in Figure 4. Taking into account that crotyl alcohol was used as a mixture of the cis and trans arrangements of the crotyl alcohol, the distorted mesitylene group of the enophile points the ortho methyl towards a simple hydrogen atom. Nevertheless, the crotyl alcohol contributes, although quantitatively less, in orienting the reaction towards the AM path significantly, despite the fact that the majority of the ene products result from the M route. The ene addition according to the C3/AM pathway is not equal to zero and the obtained results suggest the intrinsic relevance of the AM path. It results in enol formation and, although unstable, this intermediate immediately undergoes subsequent cyclization to the highly stable isoxazolidine structure. This can be the unexpected and powerful driving force able to somewhat compensate the electronic bias of the ene reaction and the weakness of steric effects.

5-Hydroxyisoxazolidine 3, resembling structures obtainable through nitrene cycloadditions, is a useful synthon for the preparation of N,O-nucleoside analogues. By adapting the Vorbrüggen protocol,12,19 as well as known procedures for similar compounds,5,13 we have prepared the uracil derivatives 7a,b and the 6-chloropurine derivatives 8a,b in good to excellent yields, even though in this last case the synthesis displays some deficiencies.

The stereochemical outcome of the functionalization reactions with in situ silylated heterobases according to the Vorbrüggen protocol clearly demonstrates that the triflate-promoted replacement of the acetoxy group with the heterobase in isoxazolidine 6 proceeds through the stabilized cation 6' as shown in Scheme 9. Due to the flatness of cat-
ion 6, the addition of the silylated heterobase occurs on both faces of the isoxazolidine ring, producing mixtures of diastereoisomeric products.

These products constitute a new class of nucleoside analogues with different heterobases, easily inserted on the isoxazolidine ring. Their potential in terms of biological activity is totally unexplored, but we believe could be promising, if we compare the new nucleoside structures with those of known compounds, both hetero- and carbocyclic, recently reported in the literature to be interesting adenosine receptor agonists.21

With the exception of compounds 8a, b, both the uracil derivatives 7a, b were sent to the Southern Research Institute (SRI) of Birmingham (AL, USA) within the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) program and tested against the Mycobacterium tuberculosis H37Rv strain. ACF) and tested against the Antimicrobial Acquisition and Coordinating Facility (TAACF) program and tested against the Mycobacterium tuberculosis H37Rv strain. The minimum inhibition concentration (MIC) was 6.03 for all the compounds with %Inh in the range 22–34. Further antiviral, antitumor, and apoptosis tests are currently under evaluation.

All melting points are uncorrected. Elemental analyses were done on an elemental analyzer. 1H and 13C NMR spectra were recorded on a 300 MHz spectrometer (solvents specified). Chemical shifts are expressed in ppm relative to internal tetramethylsilane. IR spectra (Nujol) were recorded on a spectrophotometer. Column chromatography and TLC were carried out on silica gel H60 and GF254, respectively; (Nujol) were recorded on a spectrophotometer. Column chromatography and TLC were carried out on silica gel H60 and GF254, respectively; eluants were cyclohexane–EtOAc, 9:1 to pure EtOAc; when specified, (Nujol) were recorded on a spectrophotometer. Column chromatography was performed with silica gel 60 (60–200 mesh). The minimum inhibition concentration (MIC μg/ml) were found >6.03 for all the compounds with %Inh in the range 22–34. Further antiviral, antitumor, and apoptosis tests are currently under evaluation.

**5-Hydroxy-3-methyl-2-(2,4,6-trimethylbenzoyl)-1,2-isoxazolidine (3)**

Yield: 2.77 g (23%); white crystals (EtOH); mp 134–137 °C.

IR (Nujol): 3284 (OH), 1639 (C=O) cm–1.

1H NMR (300 MHz, CDCl3): δ = 10.40 (br s, 1 H, N-OH), 6.84 (s, 2 H, Ph), 5.95 (ddd, J = 17, 11, 9 Hz, 1 H, =CH), 5.23 (d, J = 11 Hz, 1 H, =CH2), 5.08 (d, J = 17 Hz, 1 H, =CH2), 4.12 (m, 1 H, CH2–OH), 3.98 (t, J = 9 Hz, 1 H, CH–N), 3.34 (d, J = 8 Hz, 1 H, CH2–OH), 2.31 (s, 3 H, CH3), 2.19 (s, 6 H, CH3).

13C NMR (75 MHz, CDCl3): δ = 169.4 (C=O), 139.0, 135.1, 134.4 (Ar–om.), 131.5 (CH=), 130.2, 128.5 (Arom.), 128.1, 118.8 (CH=), 66.2 (CH2), 61.2 (CH–N), 21.0, 19.4, 18.8 (CH3).

Anal. Calcd for C14H19NO3 (249.30): C, 74.89; H, 7.65; N, 5.64. Found: C, 74.65; H, 7.63; N, 5.65.

**5-Acetoxy-3-methyl-2-(2,4,6-trimethylbenzoyl)-1,2-isoxazolidine (6a, b)**

Ac–O (2.2 equiv), DMAP (0.34 equiv), and Et3N (2.2 equiv) were added to a stirred, ice-cooled solution of 3 (1.26 g, 5.06 mmol) in anhydrous CH2Cl2 (150 mL). The reaction mixture was allowed to stir at r.t. for 24 h. After dilution with an equivalent volume of CH2Cl2, the organic phase was washed with a saturated solution of NaHCO3 and dried over anhydrous Na2SO4. After filtration, the solvent was evaporated and an oily residue was obtained, corresponding to a mixture of the two diastereoisomers 6a, b. The acetyl derivatives 6a and 6b were then separated by column chromatography and fully characterized.

**5,6-Dihydroxy-N-(1-hydroxybut-3-en-2-yl)-2,4,6-trimethylbenzamide (2)**

Yield: 6.2 g (50%); white crystals (EtOH); mp 137–140 °C.

IR (Nujol): 3152 (OH), 1619 cm–1.
5-[2,4(1H,3H)-Dioxopirimidin-1-yl]-3-methyl-2-(2,4,6-trimethylbenzoyl)-1,2-isoazolidine (7a,b)

A solution of uracil (0.20 g, 1.78 mmol, 2 equiv) and BSA (2 equiv) in anhydrous CH2Cl2 (20 mL) was refluxed under a N2 atmosphere for 15–20 min until it became clear. It was then cooled to r.t. before a solution of isoxazolidine 6a or 6b (0.26 g, 0.89 mmol) in CH2Cl2 (10 mL) was added dropwise. The mixture was cooled to 0 °C and TMSOTf (0.20 mL, 1 equiv) was added. The reaction mixture was refluxed under stirring overnight and finally quenched with a saturated solution of NaHCO3 (pH 7). The mixture was diluted with an equivalent volume of CH2Cl2 and washed with H2O, before it was finally dried over Na2SO4. From the residue, nucleosides 7a and 7b were isolated after column chromatography (CHCl3 and CHCl3/MeOH) and fully characterized.

Nucleoside 7a

Yield: 0.15 g (49% from 6a); 0.14 g (46% from 6b); white solid (EtOH); mp >210 °C (dec.).

IR (Nujol): 3173 (NH), 1702, 1637 (C=O) cm–1.

13C NMR (75 MHz, DMSO): δ = 164.4, 162.8, 152.3, 148.1 (C=N), 142.6, 138.4, 134.4, 133.4, 133.2, 127.6 (Arom.), 122.1 (CH-O), 118.9, 118.6, 116.5, 116.3, 115.9, 112.2, 111.4, 84.1 (CH-N), 74.7, 74.4, 72.6, 52.6 (CH2), 26.8, 25.3, 19.1, 18.4 (CH3).


Nucleoside 7b

Yield: 0.144 g (47% from 6a), 0.11 g (37% from 6b); white solid (EtOH); mp >210 °C (dec.).

IR (Nujol): 3173 (NH), 1724, 1669 (C=O) cm–1.

1H NMR (300 MHz, DMSO): δ = 11.33 (s, 1 H, NH), 7.70 (d, J = 8 Hz, 1 H, =CH-N), 6.86 (s, 2 H, Ph), 6.19 (t, J = 8 Hz, 1 H, O-CH-N), 5.69 (d, J = 8 Hz, 1 H, CO-CH=), 4.67 (d, J = 7 Hz, 1 H, CH-N), 2.93 and 2.29 (m, 1 H + 1 H, CH2), 2.23 and 2.19 (s, 9 H, CH3), 1.50 (d, J = 6 Hz, 3 H, CH3).


Biological Tests

Primary screen (dose response): determination of a 90% inhibitory concentration (IC90). The initial screen was conducted against Mycobacterium tuberculosis H37Rv (ATCC 27294) in BACTEC 12B medium by using the Microplate Alamar Blue Assay (MABA). The compounds were tested in ten twofold dilutions, typically from 100 μg/mL to 0.19 μg/mL. The IC90 is defined as the concentration effecting a reduction in fluorescence of 90% relative to controls. This value was obtained from the dose–response curve using a curve-fitting program. Any IC90 value of ≥10 μg/mL is considered ‘active’ for antitubercular activity. For further information, consult the URL http://www.taaf.org.

X-ray Crystallography

A summary of the crystal data, data collection, and structures refinement of compounds 3 and 8aN7 are given in Tables S1–S3 of the Supporting Information. The structures are solved by direct methods; non-hydrogen atoms were refined anisotropically, and hydrogen atoms, located from the difference Fourier synthesis, were refined isotropically.2425 CCDC 964611 and 964612 contain the supplementary
crystallographic data for 3 and 8aN7, respectively. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Acknowledgment

Financial support by the University of Pavia, MIUR (PRIN 2011, CUP: B1J121002450001) and Steroid S.p.a. – V.le Spagna, 156, 20093 Cologno Monzese (MI), Italy is gratefully acknowledged. Thanks are also due to the Consorzio Interuniversitario Nazionale Metodologie e Processi Innovativi di Sintesi (CINMPS) for research support. We warmly thank Dr. J. Noah (SRI) for the biological tests.

Supporting Information

Supporting information for this article is available online at http://dx.doi.org/10.1055/s-0036-1588695.

References


Synthesis S. Carosso et al.

Paper

1981


This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.


