

Research Article

#Nitrosocarbonyls 1: Antiviral Activity of *N*-(4-Hydroxycyclohex-2-en-1-yl)quinoline-2-carboxamide against the Influenza A Virus H1N1

Dalya Al-Saad, Misal Giuseppe Memeo, and Paolo Quadrelli

Department of Chemistry, University of Pavia, Viale Taramelli 12, 27100 Pavia, Italy

Correspondence should be addressed to Paolo Quadrelli; paolo.quadrelli@unipv.it

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Influenza virus flu A H1N1 still remains a target for its inhibition with small molecules. Fleeting nitrosocarbonyl intermediates are at work in a short-cut synthesis of carbocyclic nucleoside analogues. The strategy of the synthetic approaches is presented along with the *in vitro* antiviral tests. The nucleoside derivatives were tested for their inhibitory activity against a variety of viruses. Promising antiviral activities were found for specific compounds in the case of flu A H1N1.

“Dedicated to the CINMPIS on the occasion of its 20th anniversary”

1. Introduction

Influenza virus flu A H1N1 still remains a target for its inhibition with small molecules. The outbreak of the human pandemic influenza A (H1N1) caused a considerable public concern. Recent studies focused the attention on the understanding of novel viruses by analyzing the relationship between molecular characteristics and pathogenic properties [1]. Results of this analysis indicated that the human pandemic influenza A (H1N1) virus was a new reassorted virus combining genetic materials from the avian flu (H1N1) virus, classical swine flu (H1N1) virus, human flu (H3N2) virus, and Eurasian swine flu (H1N1) virus. The analysis of the sequences for receptor-binding and cleavage sites of hemagglutinin (HA) allowed for determining that replication could be inhibited by molecules such as oseltamivir (Tamiflu), a diamine derivative of the shikimic acid [2], and zanamivir (Relenza), a derivative of the 3,4-diamino-2-(hydroxymethyl)-3,4-dihydro-2H-pyran-6-carboxylic acid [3–5].

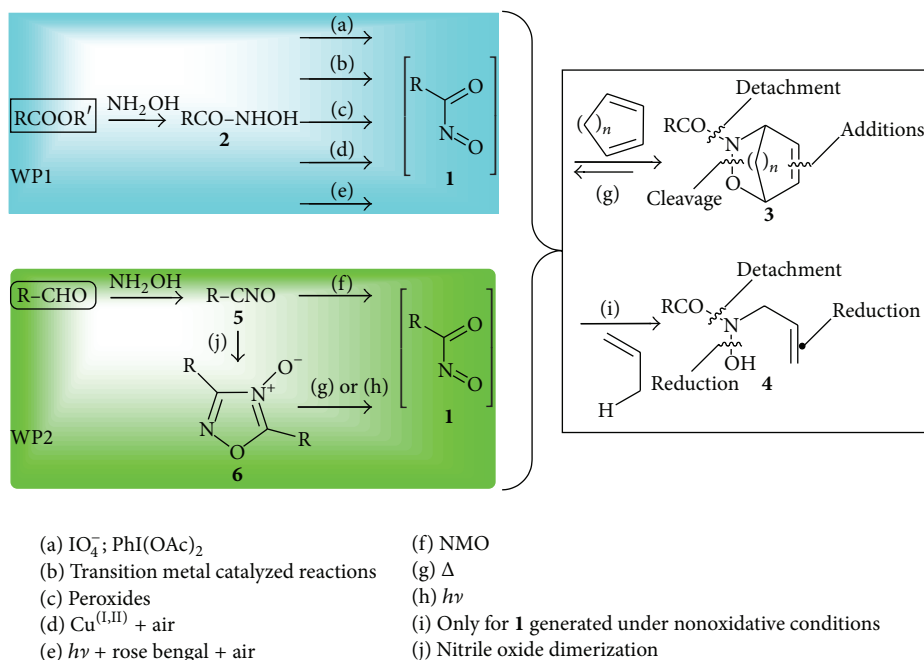
The influenza A virus is an orthomyxovirus, constituted by receptor-binding complexes containing two primary structural proteins: hemagglutinin (HA) and neuraminidase

(NA). HA is the primary protein responsible for binding to receptor sites on the cell membrane, allowing the virion to enter the cell [4–8].

On pursuing our studies and in the search of novel small molecules with potential antiviral activity, we prepared some nucleoside derivatives, found moderately active against human herpes and varicella viruses [9] or strongly active against the human papilloma virus (HPV) [10] as well as influenza A virus H1N1 [11]. The synthetic protocol relied upon the nitrosocarbonyl chemistry.

Nitrosocarbonyls are fleeting intermediates whose generation as well as synthetic applications represents a remarkable piece of organic chemistry [12]. The nitrosocarbonyl intermediates **1** (R-CONO) are highly reactive species discovered at the beginning of the seventies by Kirby and their powerful dienophilic and enophilic activities were reported in 1973 in his seminal paper [13].

Nitrosocarbonyl or acylnitroso intermediates attracted a great deal of attention in the area during the last decades since highly functionalized molecules can be readily achieved [13–16]. The generation of nitrosocarbonyls **1** can be easily performed according to two main synthetic approaches



SCHEME 1: Generation methods of nitrosocarbonyl intermediates and reactivity with dienes and alkenes.

summarized in the two working packages (WP1 and WP2) reported in Scheme 1.

WP1 shows the use of hydroxamic acids **2** as starting compounds whose oxidation is performed either with the more soluble tetraalkylammonium periodate salts or $\text{PhI}(\text{OAc})_2$ (path a) [17]. Other oxidative conditions were developed such as the use of transition metal catalyzed reactions [18–21] and peroxides [22, 23] (paths b and c). Recently, the oxidation with copper salts in the presence of air [24, 25] (path d) or photochemical sensitized oxidation processes [26] (path e) became valuable mild methods for nitrosocarbonyl generation [27].

WP2 shows the use of nitrile oxides **5** for the case at hand; these 1,3-dipoles find in the wide family of the aldehydes the main and most convenient source with the possibility of proposing a variety of substituents. This valuable alternative towards nitrosocarbonyls **1** relies upon the mild oxidation of nitrile oxides **5** with *N*-methylmorpholine-*N*-oxide (path f, NMO) [28] and the clean thermolysis (path g) and photolysis (path h) of 1,2,4-oxadiazole-4-oxides **6**, the most reactive dimer of nitrile oxides **5** [29, 30].

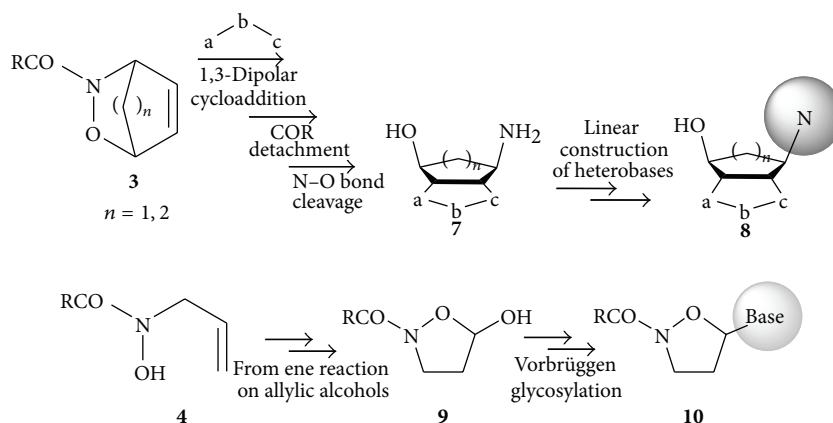
During our studies, we developed the synthesis of a new class of isoxazoline-carbocyclic nucleosides **8** starting from the regioisomeric aminols **7** (Scheme 2) through elaboration of the HDA cycloadducts **3** [31–34]. From the ene adduct of nitrosocarbonyls and the 3-methyl-2-buten-1-ol, the isoxazolidine **9**, the preparation of new *N,O*-nucleoside pyrimidine analogues **10** was achieved through the Vorbrüggen protocol [35, 36]. We wish to report here the application of the WP2 protocol for the rapid and easy synthesis of new promising active nucleoside analogues. This simplified synthetic strategy implies the replacement of traditional heterobases, such as purines or pyrimidines, with selected

and easy available heterocycles containing one nitrogen atom; once prepared, the products are submitted for *in vitro* tests to determine eventual activity and as a consequence further structural modifications. The required heterocyclic aldehydes containing one nitrogen atom are converted into the corresponding nitrile oxides as convenient source for the required nitrosocarbonyl intermediates.

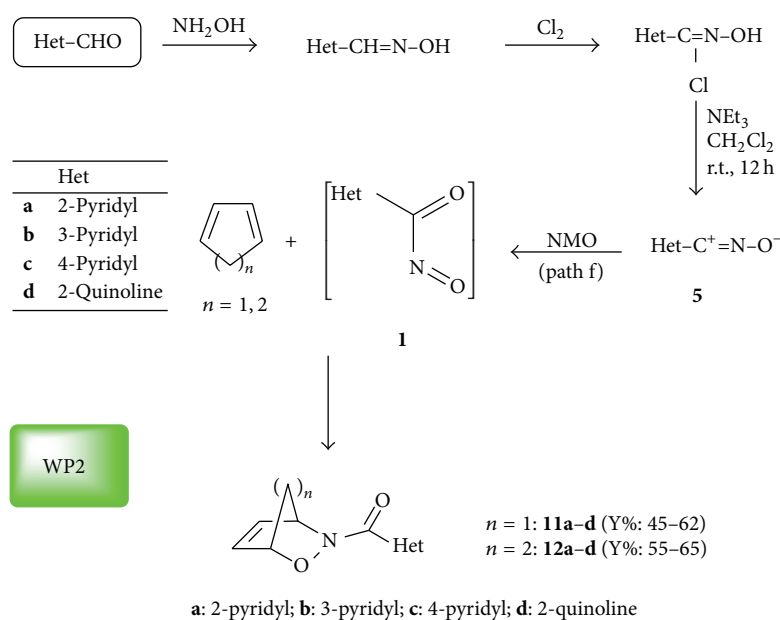
2. Results

2.1. Oxidation of Nitrile Oxides and Cleavage of N–O Bonds. Pyridine and quinoline hydroximoyl chlorides were prepared according to the literature procedures (Scheme 3) [37–39]. The selected aldehydes were converted in high yields into the corresponding oximes following the classical methods [37–39] and from the latter the desired hydroximoyl chlorides were obtained upon chlorination with chlorine gas in chloroform as solvent at -20°C for 1.5 h. The *in situ* generation of the nitrile oxides **5a–d** (Et_3N , 1.2 equiv.) is required in the present cases since none of the nitrile oxides at hand displayed any stability at room temperature, possibly in the solid state, for a long time. The mild oxidation of these 1,3-dipoles with *N*-methylmorpholine *N*-oxide (NMO, 1.3 equiv.) is conducted one-pot in the presence of the required trapping diene (freshly distilled cyclopentadiene or 1,3-cyclohexadiene, 2 equiv.) to afford the nitrosocarbonyl HDA cycloadducts **11a–d** and **12a–d**.

Compounds **11a–d** and **12a–d** were isolated from good to high yields (45–65%) and fully characterized from their analytical and spectroscopic data, except for **11d**, already reported [11]. For the new products, the spectroscopic data are consistent with the reported structures; we wish to point out the most relevant and diagnostic ^1H NMR (CDCl_3)



SCHEME 2: Synthesis of nucleoside analogues from HDA and ene adducts of nitrosocarbonyl intermediates.



SCHEME 3: Application of the WP2 protocol to the synthesis of nitrosocarbonyl HDA cycloadducts **11a-d** and **12a-d** from heteroaromatic nitrile oxides.

signals relative to the 2,3-oxazanorborn-5-ene moieties in the products **11** at hand. The olefinic protons and the deshielded bridge-head protons were found in the range $\delta = 6.37-6.67$ and $\delta = 5.37-6.16$, respectively. Similarly, the 2,3-oxazabicyclo[2.2.2]oct-5-ene moieties of the products **12** are clearly observed in the $^1\text{H NMR}$ (CDCl_3) spectra. The olefinic protons and the deshielded bridge-head protons were found, respectively, in the ranges $\delta = 5.74-6.71$ and $\delta = 4.79-5.55$.

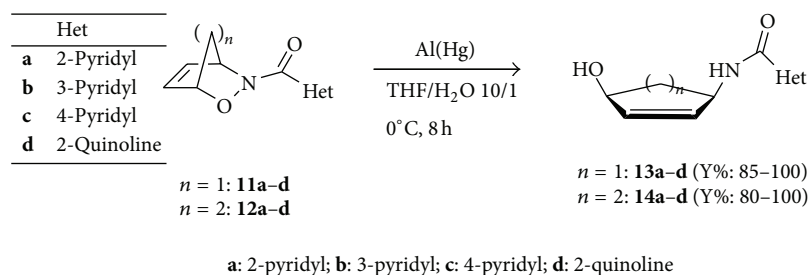
Compounds **11a-d** and **12a-d** were then submitted to the mild reductive cleavage of the N-O bond using the Al(Hg) amalgam in THF/ H_2O as solvents at 0°C [40]. The 4-hydroxycyclopent-2-enyl heterocyclic amides **13a-d** and **14a-d** were obtained in very high yields and the structures were attributed on the basis of their fully consistent analytical and spectroscopic data (Scheme 4).

The most relevant and diagnostic $^1\text{H NMR}$ signals are those relative to the olefinic protons of the cyclopentene and

cyclohexene moieties found at $\delta = 5.94-6.15$ (dd, $J = 5$ Hz in CDCl_3) and at $\delta = 5.66-6.05$ (dd, $J = 10$ Hz in DMSO), respectively. All the other signals are found in the expected range for the given attributions.

Samples of the compounds **13a-d** and **14a-d** were straight submitted for a primary *in vitro* antiviral screening in collaboration with the NIH/NIAID (USA).

2.2. Antiviral Activity. This rapid access to the target molecules allowed for their preparation in good amounts suitable for several biological tests. Compounds **13a-d** and **14a-d** were tested for their inhibitory activity against herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), vaccinia virus (VV), and hepatitis B virus (HBV). The antiviral activity of the above-reported compounds was tested *in vitro* in cell line HFF (strain E-377 for HSV-1, strain MS for HSV-2, and strain Copenhagen for VV) and cell line HepG 2.2.2.15 (strain



SCHEME 4: Reductive cleavage of N–O bonds and synthesis of the 4-hydroxycyclopent-2-enyl heterocyclic amides **13a–d** and **14a–d**.

aywl) for HBV. A test against human papilloma virus (HPV) was conducted on HEK 293 cells (strain HPV-11). Finally, the compounds were tested against respiratory virus influenza A H1N1 cell line MDCK (strain California 07/2009) and neuraminidase (NA) cell line HFF (strain NA).

Both compounds **13a–d** and **14a–d** were found inactive against HSV-1 and -2; EC_{50} and CC_{50} values were >300 with a $SI_{50} = 1$ (drug concentration range 0.096–300 μM ; acyclovir control concentration range 0.032–100 μM). Compounds **13a–d** and **14a–d** also displayed a complete inactivity against the VV; EC_{50} and CC_{50} values were >300 with a $SI_{50} = 1$ (drug concentration range 0.096–300 μM ; cidofovir control concentration range 0.096–300 μM).

The *in vitro* tests against HBV revealed a substantial inactivity of compounds **13a–d** and of compounds **14a, b**, and **d** since the EC_{50} and CC_{50} values were >20 with a $SI_{50} = 1$ (drug concentration range 0.063–20 μM ; 3TC control concentration range 0.00032–0.1 μM). Compound **14c** only gave a different value of $EC_{50} = 9.64$ even though the CC_{50} value was found >100 with a $SI_{50} = 1$, while the cidofovir used as reference data gave $EC_{50} = 180$, $CC_{50} > 200$ with a $SI_{50} > 1$.

The *in vitro* tests against HPV revealed a complete inactivity of compounds **13a–d** and **14a–d** since the EC_{50} and CC_{50} values were >20 with a $SI_{50} = 1$ (drug concentration range 0.063–20 μM ; 3TC control concentration range 0.00032–0.1 μM). The NA tests gave for compounds **13b, c** and **14a–c** comparable results ($CC_{50} > 300$) with those of cidofovir used as control drug (drug assay: neutral red (toxicity); drug concentration range 0.096–300 mM; cidofovir control concentration range 0.096–300 mM).

The derivatives **13a–d** and **14a–d** were also tested against the respiratory influenza A H1N1 virus and Table 1 reports the primary antiviral activities of the tested compounds and control experiments on the specified drug. The majority of the compounds were found to be inactive, but in a single case **14d** (Table 1, entries 15 and 16) the EC_{50} values are just 10 times those of the reference compound while the SI_{50} values require to be increased by the same factor.

3. Discussion and Conclusions

These primary antiviral activity data represent somewhat the prelude to possible developments for more active compounds through modifications of the synthetic pathway,

chosen on the basis of previous results [11]. Indeed the synthesis is suitable for convenient, easy, and straightforward structural modifications. The larger activity shown by those compounds, which bear a naphthyl-like residue, that is, the quinolone ring, could suggest a possible mechanism based on DNA intercalation. Polycyclic aromatic hydrocarbons are known as DNA-intercalating agents and molecular modeling studies on these structures confirm the degree of binding when a polycyclic aromatic residue is linked to an heterocyclic ring [41, 42].

Polyaromatic groups may be active through their ability to establish π - π stacking interactions with themselves as well as DNA intercalators; these mechanisms are in action in different cases depending upon the biological targets [43].

An heterocyclic ring bearing a single heteroatom (the pyridine ring) seems to be somewhat inefficient from the biological point of view even though the nitrogen atom orientation can be easily changed in compound **14** from **a** to **c** (Figure 1).

On the other hand, the quinoline ring possesses the structural features to display this type of action mechanism as shown in many cases in the recent literature [44, 45]. The quinoline is a naphthalene-like heterocycle and assumes different orientations suitable to adapt the structure during the interaction with DNA or active sites of proteins involved into the virus replication (DFT conformational studies at the B3LYP/6-31G(d) [46] level; Figure 2).

Docking studies are pursued to determine the key point concerning the SAR able to orientate positively the synthesis towards a specific target. Moreover, the fragment-based drug design (FBDD) represents a challenging approach in the case at hand; the reported structures seem to fit nicely with the application of computational FBDD for selecting the structural modification to increase the biological activity [47, 48].

The simplified syntheses described rely upon the valuable chemistry of nitrosocarbonyl intermediates and the easy synthetic elaboration of the corresponding hetero-Diels-Alder cycloadducts. The replacement of traditional heterobases, such as purines or pyrimidines, with selected and easy available heterocycles containing one nitrogen atom, although simple or elemental, allows for a viral target oriented production of small molecules able to display some inhibition activity and suitable for further development under structural modification suggested by docking as well as FBDD analyses.

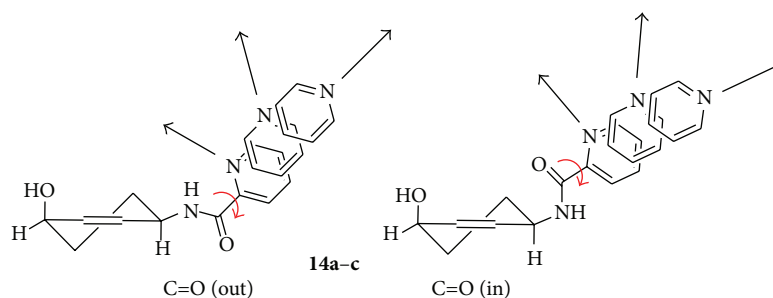


FIGURE 1: Orientations of the pyridine rings in conformers **14a-c**. Red arrows indicate rotation around the C=O-Het bond for all the possible conformers of the pyridine rings.

TABLE 1: Primary antiviral activities of compounds **13a-d** and **14a-d** against influenza A H1N1 virus.

Entry	Compounds	EC ₅₀	CC ₅₀ ^[c]	SI ₅₀
1	13a ^[a]	>100	>100	0
2	13a ^[b]	>100	>100	0
3	13b ^[a]	>100	>100	0
4	13b ^[b]	>100	>100	0
5	13c ^[a]	>100	>100	0
6	13c ^[b]	>100	>100	0
7	13d ^[a]	>100	>100	0
8	13d ^[b]	>100	>100	0
9	14a ^[a]	>100	>100	0
10	14a ^[b]	>100	>100	0
11	14b ^[a]	>100	>100	0
12	14b ^[b]	>100	>100	0
13	14c ^[a]	>100	>100	0
14	14c ^[b]	>100	>100	0
15	14d ^[a]	37	>100	>2.7
16	14d ^[b]	54	74	1.4
17	Ribavirin ^[a]	3.2	>100	>31
18	Ribavirin ^[b]	3.1	42	14

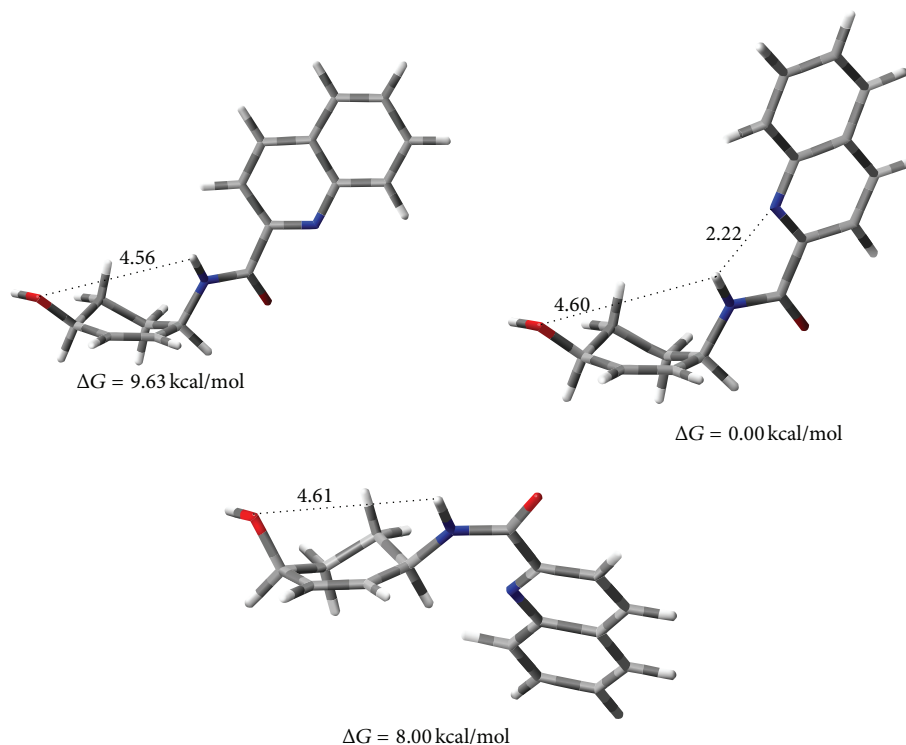
[a] Control assay: visual (cytopathic effect/toxicity). [b] Control assay: neutral red (cytopathic effect/toxicity). [c] Drug control range 0.1–100 $\mu\text{g}/\text{mL}$; ribavirin control concentration range 0.1–100 $\mu\text{g}/\text{mL}$.

4. Experimental Section

4.1. General. All melting points are uncorrected. Elemental analyses were done on a C. Erba 1106 elemental analyzer. IR spectra (Nujol mulls) were recorded on an FT-IR Perkin-Elmer RX-1. ¹H- and ¹³C-NMR spectra were recorded on a Bruker AVANCE 300 in the specified deuterated solvents. Chemical shifts are expressed in ppm from internal tetramethylsilane (δ). Column chromatography and tlc: silica gel 60 (0.063–0.200 mm) (Merck); eluant cyclohexane/ethyl acetate from 9:1 to 5:5. The identification of samples from different experiments was secured by mixed mps and superimposable IR spectra. Compound purification: all the products described were suitably purified; solid compounds were recrystallized from proper solvents and oily compounds were purified through bulb-to-bulb distillation (Kugelrohr distillation, Buchi GKR-51).

4.2. Materials. Cyclopentadiene was freshly distilled from the dimer purchased from Sigma-Aldrich. 1,3-Cyclohexadiene, pyridine-carbaldehyde, 2-quinoline-carbaldehyde, and 4-bromothiazole-2-carbaldehyde were purchased from Sigma-Aldrich. All other reagents and solvents were purchased from Sigma-Aldrich and Alfa-Aesar and used without any further purification. The heteroaromatic hydroximoyl chlorides required for the *in situ* generation of the nitrile oxides **5** were prepared according to the literature reported procedures [26].

4.3. Synthesis of Cycloadducts **11a-d and **12a-d**.** To a dichloromethane (DCM, 50 mL) solution of 1.5 equivalents of NMO and 2.1 equivalents of Et₃N, 2 equivalents of freshly distilled cyclopentadiene were added. The mixture was then cooled down with ice and a DCM (50 mL) solution containing the heteroaromatic hydroximoyl chloride of choice (1 equiv.)

FIGURE 2: Conformers of compound **14d**.

was added dropwise under vigorous stirring at 0°C for a couple of hours. The reaction was left under stirring at room temperature for 48 h. After this period of time, the reaction is quenched by pouring the mixture in brine (100 mL) and the products were extracted from the water phase with DCM (2 × 50 mL). The residue obtained upon evaporation of the solvent was crystallized from proper solvent. Compounds **11a-d** and **12a-d** were obtained in the reported yield and fully characterized.

4.4. N-Pyridin-2-oyl 2,3-Oxazanorborn-5-ene 11a. 1.24 g (59%), oil. IR: $\nu = 1734$ (C=O), 1655 (C=N) cm^{-1} . ^1H NMR (300 MHz, CDCl_3 , 25°C): $\delta = 1.87$ and 2.17 (d, 1H + 1H, $J = 9$ Hz, CH_2), 5.41 (s, 1H, CH-N), 6.16 (s, 1H, CH-O), 6.37 (s, 2H, CH=CH), 7.40 , 7.80 , 7.99 and 8.63 (m, 4H, 2-Py). ^{13}C NMR (75 MHz, CDCl_3 , 25°C): $\delta = 47.9$; 66.3 ; 83.3 ; 124.0 ; 125.3 ; 132.0 ; 133.5 ; 136.5 ; 147.8 ; 151.1 ; 164.7 . $\text{C}_{11}\text{H}_{10}\text{O}_2\text{N}_2$ (202.21): calcd. C, 65.34; H, 4.98; N, 13.85; found C 65.30, H 4.92, N 13.84.

4.5. N-Pyridin-3-oyl 2,3-Oxazanorborn-5-ene 11b. 1.30 g (62%), oil. IR: $\nu = 1701$ (C=O), 1648 (C=N) cm^{-1} . ^1H NMR (300 MHz, CDCl_3 , 25°C): $\delta = 1.91$ and 2.15 (d, 1H + 1H, $J = 9$ Hz, CH_2), 5.37 (s, 2H, CH-N and CH-O), 6.40 and 6.64 (s, 2H, CH=CH), 7.37 , 8.12 , 8.70 and 9.02 (m, 4H, 3-Py). ^{13}C NMR (75 MHz, CDCl_3 , 25°C): $\delta = 48.3$; 63.5 ; 85.0 ; 123.0 ; 129.9 ; 133.2 ; 136.8 ; 149.6 ; 151.6 ; 169.3 ; 174.2 . $\text{C}_{11}\text{H}_{10}\text{O}_2\text{N}_2$ (202.21): calcd. C, 65.34; H, 4.98; N, 13.85; found C 65.35, H 4.91, N 13.83.

4.6. N-Pyridin-4-oyl 2,3-Oxazanorborn-5-ene 11c. 1.07 g (51%), oil. IR: $\nu = 1705$ (C=O), 1652 (C=N) cm^{-1} . ^1H NMR (300 MHz, CDCl_3 , 25°C): $\delta = 2.01$ and 2.19 (d, 1H + 1H, $J = 9$ Hz, CH_2), 5.45 (s, 1H, CH-N), 5.58 (bs, 1H, CH-O), 6.46 and 6.75 (s, 2H, CH=CH), 7.98 and 8.78 (m, 4H, 4-Py). ^{13}C NMR (75 MHz, CDCl_3 , 25°C): $\delta = 48.5$; 65.1 ; 85.6 ; 124.4 ; 130.2 ; 132.4 ; 133.5 ; 136.7 ; 145.3 ; 153.3 ; 160.2 . $\text{C}_{11}\text{H}_{10}\text{O}_2\text{N}_2$ (202.21): calcd. C, 65.34; H, 4.98; N, 13.85; found C 65.30, H 4.92, N 13.82.

4.7. N-Pyridin-2-oyl 2,3-Oxazabicyclo[2.2.2]oct-5-ene 12a. 1.34 g (60%), m.p. 110–113°C from ethanol. IR: $\nu = 1637$ (C=O and C=N) cm^{-1} . ^1H NMR (300 MHz, CDCl_3 , 25°C): $\delta = 1.55$ and 2.35 (d, 2H + 2H, $J = 8$ Hz, CH_2CH_2), 4.97 (bs, 1H, CH-N), 5.55 (s, 1H, CH-O), 6.59 (s, 2H, CH=CH), 7.35 , 7.76 , 7.88 and 8.62 (m, 4H, 2-Py). ^{13}C NMR (75 MHz, CDCl_3 , 25°C): $\delta = 21.7$, 23.3 ; 51.5 ; 71.8 ; 124.8 ; 130.9 ; 131.8 ; 136.8 ; 148.5 ; 152.6 ; 162.2 . $\text{C}_{12}\text{H}_{12}\text{O}_2\text{N}_2$ (216.24): calcd. C, 66.65; H, 5.59; N, 12.96; found C 66.60, H 5.52, N 12.94.

4.8. N-Pyridin-3-oyl 2,3-Oxazabicyclo[2.2.2]oct-5-ene 12b. 1.46 g (65%), m.p. 81–82°C from ethanol. IR: $\nu = 1641$ (C=O and C=N) cm^{-1} . ^1H NMR (300 MHz, CDCl_3 , 25°C): $\delta = 1.56$ and 2.23 (m, 2H + 2H, CH_2CH_2), 4.79 (bs, 1H, CH-N), 5.45 (b, 1H, CH-O), 6.54 and 6.71 (m, 2H, CH=CH), 7.31 , 8.00 , 8.63 and 8.91 (m, 4H, 3-Py). ^{13}C NMR (75 MHz, CDCl_3 , 25°C): $\delta = 20.8$; 23.3 ; 46.8 ; 72.1 ; 122.7 ; 129.8 ; 131.6 ; 133.1 ; 136.4 ; 149.1 ; 151.2 ; 166.0 . $\text{C}_{12}\text{H}_{12}\text{O}_2\text{N}_2$ (216.24): calcd. C, 66.65; H, 5.59; N, 12.96; found C 66.61, H 5.53, N 12.97.

4.9. *N*-Pyridin-4-oyl 2,3-Oxazabicyclo[2.2.2]oct-5-ene **12c**. 1.23 g (55%), m.p. 83–85°C from ethanol. IR: $\nu = 1645$ (C=O and C=N) cm^{-1} . ^1H NMR (300 MHz, CDCl_3 , 25°C): $\delta = 1.54$ and 2.23 (m, 2H + 2H, CH_2CH_2), 4.78 (bs, 1H, CH-N), 5.45 (bs, 1H, CH-O), 6.56 and 6.74 (m, 2H, CH=CH), 7.53 and 8.67 (m, 4H, 4-Py). ^{13}C NMR (75 MHz, CDCl_3 , 25°C): $\delta = 20.7$; 23.3; 46.8; 72.2; 122.6; 131.6; 133.1; 141.3; 149.5; 150.2; 165.6. $\text{C}_{12}\text{H}_{12}\text{O}_2\text{N}_2$ (216.24): calcd. C, 66.65; H, 5.59; N, 12.96; found C 66.62, H 5.58, N 12.96.

4.10. *N*-Quinol-2-oyl 2,3-Oxazabicyclo[2.2.2]oct-5-ene **12d**. 1.60 g (58%), m.p. 148–150°C from ethanol. IR: $\nu = 1652$ (C=O and C=N) cm^{-1} . ^1H NMR (300 MHz, CDCl_3 , 25°C): $\delta = 1.60$ and 2.47 (d, 2H + 2H, $J = 8$ Hz, CH_2CH_2), 5.05 (s, 1H, CH-N), 5.74 (s, 1H, CH-O), 6.62 (s, 2H, CH=CH), 7.63, 7.81, 7.87, 7.99, 8.13 and 8.28 (m, 6H, 2-Qui). ^{13}C NMR (75 MHz, CDCl_3 , 25°C): $\delta = 21.9$, 23.3; 51.7; 71.8; 121.4; 127.6; 127.7; 129.8; 129.9; 131.6; 132.0; 137.0; 146.3; 151.9; 161.9. $\text{C}_{16}\text{H}_{14}\text{O}_2\text{N}_2$ (266.29): calcd. C, 72.16; H, 5.30; N, 10.52; found C 72.12, H 5.32, N 10.54.

4.11. *Reductive Cleavage of N-O Bonds and Synthesis of Compounds 13a-d and 14a-d*. To a stirred solution of the cycloadducts **11a-d** and **12a-d** (1 g) in 200 mL of THF/ H_2O 10/1 under nitrogen and at 0°C, 1 g of Al(Hg) is added portionwise and the reaction is conducted until complete consumption of the starting materials (monitored by TLC). After completion, the solutions were diluted with THF and filtered over celite. The organic phases were then evaporated to dryness to leave the corresponding aminols **13a-d** and **14a-d** in the reported yield and were fully characterized.

4.12. *N*-(4-Hydroxycyclopent-2-enyl)picolinamide **13a**. 0.99 g (98%), oil. IR: $\nu = 3370$ (OH), 1655 (C=O) cm^{-1} . ^1H NMR (300 MHz, CDCl_3 , 25°C): $\delta = 1.79$ (dt, 1H, $J = 14$, 3.5 Hz, HC-H), 1.82 (b, 1H, OH), 2.88 (dt, 1H, $J = 14$, 7.5 Hz, H-CH), 4.86 (m, 2H, CH-N and CH-O), 5.94 and 6.13 (m, 1H + 1H, CH=CH), 7.45, 7.87, 8.20 and 8.56 (m, 4H, 2-Py), 8.55 (bs, 1H, NH). ^{13}C NMR (75 MHz, CDCl_3 , 25°C): $\delta = 41.1$; 54.0; 75.5; 122.1; 126.2; 133.3; 136.8; 137.4; 147.9; 149.7; 163.7. $\text{C}_{11}\text{H}_{12}\text{O}_2\text{N}_2$ (204.23): calcd. C, 64.69; H, 5.92; N, 13.72; found C 64.70, H 5.92, N 13.74.

4.13. *N*-(4-Hydroxycyclopent-2-enyl)nicotinamide **13b**. 0.98 g (97%), m.p. 125–127°C from ethyl acetate. IR: $\nu = 3350$ (OH), 1652 (C=O) cm^{-1} . ^1H NMR (300 MHz, CDCl_3 , 25°C): $\delta = 1.73$ (dt, 1H, $J = 14$, 3 Hz, HC-H), 2.85 (dt, 1H, $J = 14$, 8 Hz, H-CH), 3.30 (b, 2H, OH e NH), 4.83 (d, 1H, $J = 7$ Hz, CH-O), 5.07 (m, 1H, CH-N), 5.96 and 6.09 (d, 1H + 1H, $J = 4$ Hz, CH=CH), 7.48, 8.27, 8.70 and 9.17 (m, 4H, 3-Py). ^{13}C NMR (75 MHz, CDCl_3 , 25°C): $\delta = 40.8$; 53.9; 75.2; 123.8; 130.6; 133.7; 136.5; 136.7; 147.3; 151.0; 164.4. $\text{C}_{11}\text{H}_{12}\text{O}_2\text{N}_2$ (204.23): calcd. C, 64.69; H, 5.92; N, 13.72; found C 64.71, H 5.93, N 13.75.

4.14. *N*-(4-Hydroxycyclopent-2-enyl)isonicotinamide **13c**. 1.00 g (100%), m.p. 170–175°C from ethyl acetate. IR: $\nu = 3326$ (OH), 1640 (C=O) cm^{-1} . ^1H NMR (300 MHz, CDCl_3 , 25°C):

$\delta = 1.71$ (dt, 1H, $J = 14$, 3 Hz, HC-H), 2.34 (s, 1H, OH), 2.85 (dt, 1H, $J = 14$, 8 Hz, H-CH), 4.84 (d, 1H, $J = 7$ Hz, CH-O), 5.00 (m, 1H, CH-N), 5.97 and 6.13 (m, 1H + 1H, $J = 5$ Hz, CH=CH), 6.64 (d, 1H, $J = 7$ Hz, NH), 7.66 and 8.76 (m, 4H, 4-Py). ^{13}C NMR (75 MHz, CDCl_3 , 25°C): $\delta = 41.1$; 54.3; 75.2; 120.9; 133.4; 137.1; 141.6; 150.2; 164.7. $\text{C}_{11}\text{H}_{12}\text{O}_2\text{N}_2$ (204.23): calcd. C, 64.69; H, 5.92; N, 13.72; found C 64.71, H 5.91, N 13.73.

4.15. *N*-(4-Hydroxycyclopent-2-enyl)quinoline-2-carboxamide **13d**. 0.86 g (85%), m.p. 89–90°C from ethyl acetate. IR: $\nu = 3420$ (OH), 3357 (NH), 1667 (C=O) cm^{-1} . ^1H NMR (300 MHz, CDCl_3 , 25°C): $\delta = 1.85$ (dt, 1H, $J = 14$, 4 Hz, HC-H), 2.86 (b, 1H, OH), 2.92 (dt, 1H, $J = 14$, 8 Hz, H-CH), 4.84 (m, 1H, CH-O), 4.95 (m, 1H, CH-N), 5.98 and 6.15 (m, 1H + 1H, CH=CH), 7.63, 7.77, 7.86, 8.09 and 8.29 (m, 6H, 2-Qui), 8.62 (d, 1H, $J = 7$ Hz, NH). ^{13}C NMR (75 MHz, CDCl_3 , 25°C): $\delta = 41.0$; 54.1; 75.4; 118.7; 127.6; 127.9; 129.2; 129.4; 130.2; 133.3; 136.9; 137.6; 146.2; 149.4; 163.9. $\text{C}_{15}\text{H}_{14}\text{N}_2\text{O}_2$ (254.28): calcd. C, 70.85; H, 5.55; N, 11.02; found C 70.81, H 5.51, N 11.03.

4.16. *N*-(4-Hydroxycyclohex-2-enyl)picolinamide **14a**. 0.95 g (94%), oil. IR: $\nu = 3378$ (OH), 1667 (C=O) cm^{-1} . ^1H NMR (300 MHz, DMSO, 25°C): $\delta = 1.62$ and 1.77 (m, 4H, CH_2CH_2), 4.00 (s, 1H, CH-O), 4.43 (m, 1H, CH-N), 4.78 (d, 1H, $J = 6$ Hz, OH), 5.67 and 5.86 (m, 1H + 1H, CH=CH), 7.63, 8.04 and 8.64 (m, 4H, 2-Py), 8.33 (d, 1H, $J = 5$ Hz, NH). ^{13}C NMR (75 MHz, DMSO, 25°C): $\delta = 25.1$; 28.8; 44.1; 63.0; 121.8; 126.7; 128.7; 134.1; 137.9; 148.5; 149.6; 162.9. $\text{C}_{12}\text{H}_{14}\text{O}_2\text{N}_2$ (218.25): calcd. C, 66.04; H, 6.47; N, 12.84; found C 66.01, H 6.42, N 12.84.

4.17. *N*-(4-Hydroxycyclohex-2-enyl)nicotinamide **14b**. 0.93 g (92%), m.p. 95–99°C from ethyl acetate. IR: $\nu = 3286$ (OH), 1661 (C=O) cm^{-1} . ^1H NMR (300 MHz, DMSO, 25°C): $\delta = 1.72$ (m, 4H, CH_2CH_2), 4.03 (s, 1H, CH-O), 4.43 (m, 1H, CH-N), 5.66 and 5.82 (d, 1H + 1H, $J = 9$ Hz, CH=CH), 7.51, 8.25, 8.70 and 9.03 (m, 4H, 3-Py), 8.74 (d, 1H, $J = 8$ Hz, NH). ^{13}C NMR (75 MHz, DMSO, 25°C): $\delta = 24.8$; 28.9; 45.1; 62.7; 123.5; 129.3; 130.0; 133.3; 135.6; 148.3; 151.4; 164.3. $\text{C}_{12}\text{H}_{14}\text{O}_2\text{N}_2$ (218.25): calcd. C, 66.04; H, 6.47; N, 12.84; found C 66.03, H 6.43, N 12.86.

4.18. *N*-(4-Hydroxycyclohex-2-enyl)isonicotinamide **14c**. 1.00 g (100%), m.p. 245–250°C (dec.) from ethyl acetate. IR: $\nu = 3306$ (OH), 1651 (C=O) cm^{-1} . ^1H NMR (300 MHz, DMSO, 25°C): $\delta = 1.71$ (m, 4H, CH_2CH_2), 4.15 (s, 1H, CH-O), 4.41 (m, 1H, CH-N), 5.63 and 5.83 (d, 1H + 1H, $J = 9$ Hz, CH=CH), 7.79 and 8.70 (m, 4H, 4-Py), 8.81 (d, 1H, $J = 8$ Hz, NH). ^{13}C NMR (75 MHz, DMSO, 25°C): $\delta = 24.7$; 28.9; 45.2; 62.7; 121.5; 124.9; 129.1; 133.3; 141.4; 150.0; 164.3. $\text{C}_{12}\text{H}_{14}\text{O}_2\text{N}_2$ (218.25): calcd. C, 66.04; H, 6.47; N, 12.84; found C 66.06, H 6.41, N 12.85.

4.19. *N*-(4-Hydroxycyclohex-2-enyl)quinoline-2-carboxamide **14d**. 0.81 g (80%), m.p. 80–84°C from ethyl acetate. IR: $\nu = 3382$ (OH), 1668 (C=O) cm^{-1} . ^1H NMR (300 MHz, CDCl_3 ,

25°C): δ = 1.99 (m, 4H, CH₂CH₂), 4.31 (s, 1H, CH-O), 4.72 (m, 1H, CH-N), 5.92 and 6.06 (d, 1H + 1H, J = 9 Hz, CH=CH), 7.66, 7.77, 7.79, 8.13 and 8.33 (m, 6H, 2-Qui), 8.23 (d, 1H, J = 8 Hz, NH). ¹³C NMR (75 MHz, CDCl₃, 25°C): δ = 25.3; 29.1; 44.8; 64.5; 118.7; 127.6; 127.9; 129.2; 129.5; 130.1; 130.7; 132.6; 137.5; 146.3; 149.5; 163.7. C₁₆H₁₆O₂N₂ (268.31): calcd. C, 71.62; H, 6.01; N, 10.44; found C 71.66, H 6.01, N 10.45.

4.20. Computational Methods. All calculations were carried out using the Gaussian 09 [46] program package through optimizations in the gas phase at the B3LYP/6-31G(d) level. Vibrational frequencies were computed to verify that the optimized structures were minima.

4.21. Antiviral Assays. The National Institute of Allergy and Infectious Diseases (NIAID) established the AACF under a contract with Southern Research Institute. The NIAID, through the AACF, provides free and confidential services for suppliers, who are interested in submitting compounds to be evaluated for antiviral activity. Tested compounds were delivered in standard DMSO solutions. The methods applied for the different assays can be found at the URL via the internet at <http://niaid-aacf.org/>.

The antiviral tests were conducted on the derivatives **13a-d** and **14a-d** suitably purified; compounds **13b-d** and **14b-d** were recrystallized from proper solvents and compounds **13a** and **14a** were purified through bulb-to-bulb distillation (Kugelrohr distillation).

The following control assays were applied: herpes simplex virus 1 (crystal violet), herpes simplex virus 2 (crystal violet), vaccinia virus (crystal violet), hepatitis B virus (DNA hybridization/Luciferase reporter/CytoTox-1). The antiviral activity of the above-reported compounds was tested *in vitro* in cell line HFF (strain E-377 for HSV-1, strain G for HSV-2, strain Ellen for VZV, and strain Copenhagen for VV), Vero 76 cell line (strain Adames) for PTV, cell line 2.2.15 (strain ayw) for HBV, and cell line Huh-Luc/Neo ET (strain CON-1) for HCV. The following control assays were applied: influenza A H1N1 cell line MDCK (strain California 7/2009; CellTiter-Glo) and neuraminidase (NA) cell line HFF (strain NA; neutral Red). HPV (HEK 293 cells, strain HPV-11).

Control drug reference data: HSV-1: acyclovir, EC₅₀ 3.29; CC₅₀ > 100, SI₅₀ > 30; control assay: crystal violet (cytopathic effect/toxicity). HSV-2: acyclovir, EC₅₀ 3.7; CC₅₀ > 100, SI₅₀ > 27; control assay: crystal violet (cytopathic effect/toxicity). VV: cidofovir, EC₅₀ 6.07; CC₅₀ > 300, SI₅₀ > 4.9; control assay: crystal violet (cytopathic effect/toxicity). HBV: 3TC, EC₅₀ 0.01; CC₅₀ > 0.1, SI₅₀ > 10; control assay: polymerase chain reaction (Virion/CellTiter 96, toxicity). H1N1: ribavirin, EC₅₀ 3.2; CC₅₀ > 100, SI₅₀ > 31; control assay: visual and neutral red. NA: cidofovir, CC₅₀ > 300; control assay: neutral red. HSV-1: cidofovir, EC₅₀ 180; CC₅₀ > 200, SI₅₀ > 1; control assay: crystal violet polymerase chain reaction (DNA/cell proliferation, toxicity).

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

- [1] S. Payungporn, N. Panjaworayan, J. Makkoch, and Y. Poovorawan, "Molecular characteristics of the human pandemic influenza A virus (H1N1)," *Acta Virologica*, vol. 54, no. 3, pp. 155–163, 2010.
- [2] M. Karpf and R. Trussardi, "Efficient access to oseltamivir phosphate (Tamiflu) via the O-trimesylate of shikimic acid ethyl ester," *Angewandte Chemie International Edition*, vol. 48, no. 31, pp. 5760–5762, 2009.
- [3] X.-B. Zhu, M. Wang, S. Wang, and Z.-J. Yao, "Concise synthesis of zanamivir and its C4-thiocarbamido derivatives utilizing a [3+2]-cycloadduct derived from D-glucono- δ -lactone," *Tetrahedron*, vol. 68, no. 8, pp. 2041–2044, 2012.
- [4] J.-J. Shie, J.-M. Fang, P.-T. Lai et al., "A practical synthesis of zanamivir phosphonate congeners with potent anti-influenza activity," *Journal of the American Chemical Society*, vol. 133, no. 44, pp. 17959–17965, 2011.
- [5] J. H. Beigel, "Antiviral compounds in the pipeline to tackle H1N1 influenza infection," *Drugs of the Future*, vol. 35, no. 5, pp. 385–392, 2010.
- [6] K. Subbarao and J. Katz, "Avian influenza viruses infecting humans," *Cellular and Molecular Life Sciences*, vol. 57, no. 12, pp. 1770–1784, 2000.
- [7] Q.-S. Du, H. Wei, R.-B. Huang, and K.-C. Chou, "Progress in structure-based drug design against influenza A virus," *Expert Opinion on Drug Discovery*, vol. 6, pp. 619–631, 2011.
- [8] E. Feng, D. Ye, J. Li et al., "Recent advances in neuraminidase inhibitor development as anti-influenza drugs," *ChemMedChem*, vol. 7, no. 9, pp. 1527–1536, 2012.
- [9] P. Quadrelli, R. Scrocchi, P. Caramella, A. Rescifina, and A. Piperno, "From cyclopentadiene to isoxazoline-carbocyclic nucleosides: a rapid access to biological molecules through nitrosocarbonyl chemistry," *Tetrahedron*, vol. 60, no. 16, pp. 3643–3651, 2004.
- [10] Y. Moggio, L. Legnani, B. Bovio, M. G. Memeo, and P. Quadrelli, "Synthesis of novel anthracene derivatives of isoxazolino-carbocyclic nucleoside analogues," *Tetrahedron*, vol. 68, no. 5, pp. 1384–1392, 2012.
- [11] P. Quadrelli, M. Mella, L. Legnani, and D. Al-Saad, "From cyclopentadiene to isoxazoline-carbocyclic nucleosides; synthesis of highly active inhibitors of influenza A virus H1N1," *European Journal of Organic Chemistry*, vol. 2013, no. 21, pp. 4655–4665, 2013.
- [12] B. S. Bodnar and M. J. Miller, "The nitrosocarbonyl hetero-diels-alder reaction as a useful tool for organic syntheses," *Angewandte Chemie International Edition*, vol. 50, pp. 5630–5647, 2011.
- [13] G. W. Kirby, "Tilden Lecture. Electrophilic C-nitroso-compounds," *Chemical Society Reviews*, vol. 6, no. 1, p. 1, 1977.
- [14] M. D. Surman, M. J. Mulvihill, and M. J. Miller, "Regio- and stereoselective ring openings of 3-Aza-2-oxabicyclo[2.2.1]hept-5-ene systems with copper catalyst-modified grignard reagents:

- application to the synthesis of an inhibitor of 5-lipoxygenase," *The Journal of Organic Chemistry*, vol. 67, no. 12, pp. 4115–4121, 2013.
- [15] C. P. Chow, K. J. Shea, and S. M. Sparks, "Type 2 intramolecular *N*-acylnitroso Diels-Alder reaction: stereoselective synthesis of bridged bicyclic oxazinolactams," *Organic Letters*, vol. 4, pp. 2637–2640, 2002.
- [16] T. Sato, S. Aoyagi, and C. Kibayashi, "Enantioselective total synthesis of (+)-azimine and (+)-carpaine," *Organic Letters*, vol. 5, no. 21, pp. 3839–3842, 2003.
- [17] W. Adam, N. Bottke, O. Krebs, and C. R. Saha-Möller, "Allylic amidation of olefins by ene reaction of acylnitroso compounds generated in situ by oxidation of hydroxamic acids," *European Journal of Organic Chemistry*, vol. 1999, pp. 1963–1965, 1999.
- [18] S. Iwasa, K. Tajima, S. Tsushima, and H. Nishiyama, "A mild oxidation method of hydroxamic acids: efficient trapping of acyl nitroso intermediates," *Tetrahedron Letters*, vol. 42, no. 34, pp. 5897–5899, 2001.
- [19] K. R. Flower, A. P. Lightfoot, H. Wan, and A. Whiting, "The development and application of ruthenium catalysed oxidations of a hydroxamic acid and in situ Diels-Alder trapping of the acyl nitroso derivative," *Journal of the Chemical Society. Perkin Transactions 1*, no. 18, pp. 2058–2064, 2002.
- [20] S. Iwasa, A. Fakhruddin, Y. Tsukamoto, M. Kameyama, and H. Nishiyama, "Iridium(I)-catalyzed hydrogen peroxide oxidation of hydroxamic acids and hetero Diels-Alder reaction of the acyl nitroso intermediates with cyclopentadiene," *Tetrahedron Letters*, vol. 43, no. 35, pp. 6159–6161, 2002.
- [21] M. F. A. Adamo and S. Bruschi, "Generation of acylnitroso dienophiles: a study of metal catalysis," *Journal of Organic Chemistry*, vol. 72, no. 7, pp. 2666–2669, 2007.
- [22] A. Fakhruddin, S. Iwasa, H. Nishiyama, and K. Tsutsumi, "Ene reactions of acyl nitroso intermediates with alkenes and their halocyclization," *Tetrahedron Letters*, vol. 45, no. 51, pp. 9323–9326, 2004.
- [23] D. Atkinson, M. A. Kabeshov, M. Edgar, and A. V. Malkov, "Intramolecular carbonyl nitroso ene reaction catalyzed by iron(III) chloride/hydrogen peroxide as an efficient tool for direct allylic amination," *Advanced Synthesis & Catalysis*, vol. 353, no. 18, pp. 3347–3351, 2011.
- [24] D. Chaiyavej, L. Cleary, A. S. Batsanov, T. B. Marder, K. J. Shea, and A. Whiting, "Copper(II)-catalyzed room temperature aerobic oxidation of hydroxamic acids and hydrazides to acylnitroso and Azo intermediates, and their Diels-Alder trapping," *Organic Letters*, vol. 13, no. 13, pp. 3442–3445, 2011.
- [25] C. P. Frazier, A. Bugarin, J. R. Engelking, and J. Read De Alaniz, "Copper-catalyzed aerobic oxidation of *N*-substituted hydroxylamines: efficient and practical access to nitroso compounds," *Organic Letters*, vol. 14, no. 14, pp. 3620–3623, 2012.
- [26] Y. C. Teo, Y. Pan, and C. H. Tan, "Organic dye-photocatalyzed acylnitroso ene reaction," *ChemCatChem*, vol. 5, no. 1, pp. 235–240, 2013.
- [27] M. G. Memeo and P. Quadrelli, "A new life for nitrosocarbonyls in pericyclic reactions," *Arkivoc*, vol. 2013, no. 1, pp. 418–423, 2013.
- [28] P. Quadrelli, M. Mella, P. Paganoni, and P. Caramella, "Cycloadditions of nitrile oxides to the highly reactive *n*-acyl-2-oxa-3-azanorborn-5-enes afford versatile cycloadducts and a convenient entry to highly functionalized derivatives," *European Journal of Organic Chemistry*, vol. 2000, no. 14, pp. 2613–2620, 2000.
- [29] P. Quadrelli, M. Mella, and P. Caramella, "A photochemical generation of nitrosocarbonyl intermediates," *Tetrahedron Letters*, vol. 40, no. 4, pp. 797–800, 1999.
- [30] P. Quadrelli, G. Campari, M. Mella, and P. Caramella, "A thermal fragmentation of 1,2,4-oxadiazole-4-oxides to nitriles and nitrosocarbonyls," *Tetrahedron Letters*, vol. 41, no. 12, pp. 2019–2022, 2000.
- [31] P. Quadrelli, M. Mella, S. Carosso, B. Bovio, and P. Caramella, "A straightforward synthesis of isoxazoline-based carbocyclic nucleosides from 1,3-cyclohexadiene through nitrosocarbonyl chemistry," *European Journal of Organic Chemistry*, no. 36, pp. 6003–6015, 2007.
- [32] M. Savion, M. G. Memeo, B. Bovio, G. Grazioso, L. Legnani, and P. Quadrelli, "Synthesis and molecular modeling of novel dihydroxycyclopentane-carbonitrile nor-nucleosides by bromonitrile oxide 1,3-dipolar cycloaddition," *Tetrahedron*, vol. 68, no. 7, pp. 1845–1852, 2012.
- [33] P. Quadrelli, M. Mella, S. Carosso, and B. Bovio, "*N*, *O*-nucleosides from ene reactions of nitrosocarbonyl intermediates with the 3-methyl-2-buten-1-ol," *The Journal of Organic Chemistry*, vol. 78, no. 2, pp. 516–526, 2013.
- [34] P. Quadrelli, S. Romano, A. Piccanello, and P. Caramella, "The remarkable *cis* effect in the ene reactions of nitrosocarbonyl intermediates," *Journal of Organic Chemistry*, vol. 74, no. 6, pp. 2301–2310, 2009.
- [35] P. Quadrelli, M. Mella, S. Carosso, and B. Bovio, "*N*, *O*-nucleosides from ene reactions of nitrosocarbonyl intermediates with the 3-methyl-2-buten-1-ol," *Journal of Organic Chemistry*, vol. 78, no. 2, pp. 516–526, 2013.
- [36] C. Grundmann and P. Grünanger, *The Nitrile Oxides*, Springer, Heidelberg, Germany, 1971.
- [37] D. Sümegén, "The preparation of 3,4-diarylfuroxans and 3,5-diaryl-1,2,4-oxadiazol-4-oxides," *Chimica Acta Turcica*, vol. 13, p. 393, 1985.
- [38] S. S. Ghabrial, I. Thomsen, and K. B. G. Torssell, "Synthesis of biheteroaromatic compounds via the isoxazoline route," *Acta Chemica Scandinavica, Series B: Organic Chemistry and Biochemistry*, vol. B41, no. 6, pp. 426–434, 1987.
- [39] K. S. A. Vallin, D. W. Posaric, Z. Hameršak, M. A. Svensson, and A. B. E. Minidis, "Efficient chemoenzymatic dynamic kinetic resolution of 1-heteroaryl ethanols," *The Journal of Organic Chemistry*, vol. 74, no. 24, pp. 9328–9336, 2009.
- [40] G. E. Keck, S. Fleming, D. Nickell, and P. Weider, "Mild and efficient methods for the reductive cleavage of nitrogen-oxygen bonds," *Synthetic Communications*, vol. 9, no. 4, pp. 281–286, 1979.
- [41] A. Rescifina, U. Chiacchio, A. Corsaro, A. Piperno, and R. Romeo, "Isoxazolidinyl polycyclic aromatic hydrocarbons as DNA-intercalating antitumor agents," *European Journal of Medicinal Chemistry*, vol. 46, no. 1, pp. 129–136, 2011.
- [42] A. Rescifina, U. Chiacchio, A. Piperno, and S. Sortino, "Binding of a non-ionic pyrenylisoxazolidine derivative to double-stranded polynucleotides: spectroscopic and molecular modelling studies," *New Journal of Chemistry*, vol. 30, no. 4, pp. 554–561, 2006.
- [43] M. Sekine, Y. Oeda, Y. Iijima, H. Taguchi, A. Ohkubo, and K. Seio, "Synthesis and hybridization properties of 2'-*O*-methylated oligoribonucleotides incorporating 2'-*O*-naphthyluridines," *Organic & Biomolecular Chemistry*, vol. 9, no. 1, pp. 210–218, 2011.

- [44] K. G. Byler, C. Wang, and W. N. Setzer, "Quinoline alkaloids as intercalative topoisomerase inhibitors," *Journal of Molecular Modeling*, vol. 15, no. 12, pp. 1417–1426, 2009.
- [45] W. Li, Z.-W. Zhang, S.-Y. Wang, S. M. Ren, and T. Jiang, "Synthesis and analysis of potential DNA intercalators containing quinoline-glucose hybrids," *Chemical Biology & Drug Design*, vol. 74, p. 80, 2009.
- [46] M. J. Frisch, G. W. Trucks, H. B. Schlegel et al., *Gaussian 09, Revision D.01*, Gaussian Inc, Wallingford, Conn, USA, 2009.
- [47] C. Sheng and W. Zhang, "Fragment informatics and computational fragment-based drug design: an overview and update," *Medicinal Research Reviews*, vol. 33, no. 3, pp. 554–598, 2013.
- [48] A. Kumar, A. Voet, and K. Y. Zhang, "Fragment based drug design: from experimental to computational approaches," *Current Medicinal Chemistry*, vol. 19, no. 30, pp. 5128–5147, 2012.