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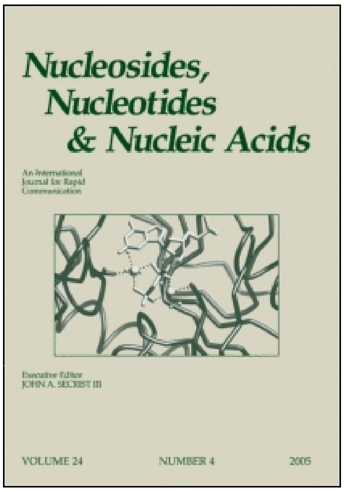

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PHOTOLABELING PROBES OF RIBAVIRIN AND EICAR

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▫ *Ribavirin, the only small molecule available so far for treating hepatitis C virus infection, was recently used in an emergency context to treat patients with severe acute respiratory syndrome in the early stages of the disease. EICAR, one of the most potent congeners of ribavirin, has 10 to 100 times greater antiviral potency than ribavirin. The mechanisms underlying the antiviral effects of ribavirin and EICAR have not yet been definitely elucidated, but they seem to be similar. In order to study the mechanisms responsible for their antiviral effects using a photolabeling approach, we have developed photolabeling probes of ribavirin and EICAR, in which an azido group was introduced into the pseudobases of triazole and imidazole, respectively. The ribavirin photoprobes were obtained by directly coupling the azidotriazole to the protected ribose sugar, while the EICAR probe was prepared by diazotizing AICAR and subsequently substituting with* NaN_3*. All these probes showed a fast, clear-cut photochemical reaction, which suggests that they are promising tools for use in photolabeling studies.*

Keywords Ribavirin, EICAR, Photolabeling, Azole Nucleosides, Photolabeling Probes

INTRODUCTION

Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide, Scheme 1) was the first synthetic nucleoside showing a broad spectrum of antiviral activity against many RNA and DNA viruses.[1] It is used for the clinical treatment of patients infected with Lassa fever virus and respiratory syncytial virus. In association with

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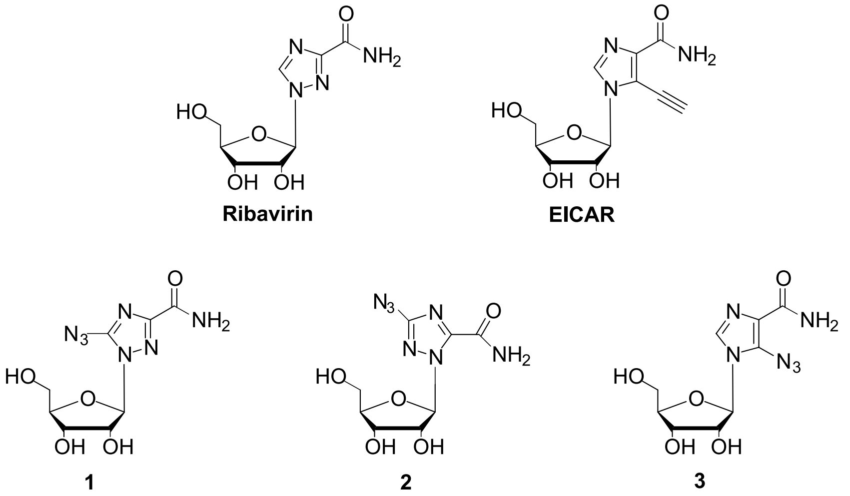

SCHEME 1 Ribavirin, EICAR and the proposed photolabeling probes **1**–**3**.

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interferon-α, it is the only drug available to date for treating patients infected with hepatitis C virus.[2] It was recently used to treat patients with severe acute respiratory syndrome in mainland China, Hong Kong, and Canada, where it was particularly successful when prescribed in the early stages of the disease.[3,4] Since its discovery over 30 years ago, ribavirin has provided a valuable means of treating a broad spectrum of serious viral infections worldwide. Meanwhile, various analogues for ribavirin have been synthesized, in the hope that they will also turn out to be potent antiviral agents. One of the most potent congeners of ribavirin is 5-ethynyl-1-β-D-ribofuranosyl-1*H*-imidazole-4-carboxamide (EICAR, Scheme 1).[5] The antiviral potency of EICAR is approximately 10 to 100 times greater than that of ribavirin.[6–8] In addition to its antiviral activity, EICAR exhibits antitumor activity and inhibits the growth of various tumor cells in vitro.[9,10]

Although ribavirin has been used with success for clinical purposes, its mechanisms of action are still remarkably controversial and many hypotheses have been proposed to explain its modes of action.[11,12] The mechanisms underlying the antiviral effects of EICAR have not yet been definitely elucidated either, but they seem to be similar to those of ribavirin.[6–8] The hypotheses proposed so far for ribavirin include inhibition of the host inosine monophosphate dehydrogenase, inhibition of viral RNA polymerases and viral capping enzymes, lethal mutagenesis of viral RNA genomes, and modulation of the host immune responses.[11,12] Ribavirin is known to be a pleiotropic agent with many complex intrinsic mechanisms that might affect its overall antiviral properties. The antiviral mechanisms of ribavirn and EICAR therefore seem to be highly complex and to involve multiple modes of action. In studies on the molecular mechanisms responsible for the antiviral activity of ribavirin and EICAR, photoaffinity labeling seemed to be a useful approach.

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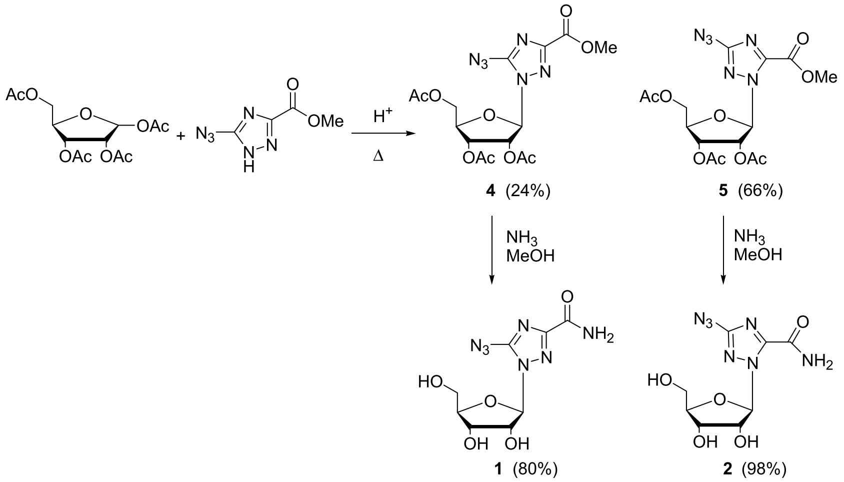
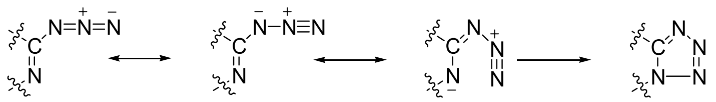
Photoaffinity labeling is an efficient method of studying the interactions between biologically relevant ligands and their target macromolecules.[13,14] This method involves the use of photoactivatable probes which, when exposed to light, will produce highly reactive species such as nitrene or carbene, leading to a process of covalent cross-linkage with the protein at the binding site.[15] This can be used to identify the macromolecular targets that interact with the probes and to map the binding sites of the probes on the targets, and thus to study the modes of action of the ligands. Arylazides are by far the most frequently used photoaffinity probes because they can be easily synthesized, as well as being chemically stable in the dark and highly reactive upon irradiation.[15] With a view to studying the mechanisms underlying the antiviral activity of ribavirin and EICAR using photolabeling procedures, we have synthesized triazoylazides **1** and **2** and imidazoylazide **3** for use as photolabeling probes (Scheme 1).[16–18] Probes **1** and **3** were given the same azole nucleoside structure as ribavirin and EICAR, which is necessary to ensure that the probes remain biologically active, while adding an azido group to the pseudobase triazole or imidazole to make it photochemically reactive. Probe **2** is an isomer of **1** and is therefore expected to have the similar properties as **1**. Here we report on the characterization of these probes.

RESULTS AND DISCUSSION

We attemped several methods of synthesizing probes **1** and **2**.[16,17] The most convenient method of synthesis turned out to be the fusion method using methyl 5-azido-1,2,4-triazole-3-carboxylate and 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose in the presence of bis(*p*-nitrophenyl)phosphate at 120°C (Scheme 2).[17] Under this



SCHEME 2 Synthesis of probes **1** and **2**.



SCHEME 3 Isomerization between an azide and tetraazole functionality.

reaction condition, the azidotriazoles of both the starting material and the products were found to be highly stable, and the total yield of the reaction was as high as 90%. The structures of the two isomers **4** and **5** obtained could be deduced by comparing their NMR spectra with those of the corresponding triazole nucleoside isomers.[17] The isomer ratio for **4** and **5** was around 24/66, in favor of **5**. The reason for this may be explained that the azido group, when occurred in a position adjacent to the nucleosidic bond, may have resulted in steric hindrance in **4**. Therefore, **4** might be less thermodynamically favorable than **5**. Treatment of **4** and **5** in NH_3/MeOH gave the corresponding probes **1** and **2**, respectively.

It has been reported that the azido group attached to the carbon atom of the azomethine bond can readily undergo ring closure and form a tetrazole, resulting in an equilibrium between the azide isomer and the tetrazole isomer (Scheme 3).[19,20] It is necessary for both **1** and **2** to retain the azide structure in order to acquire the relevant photochemical properties. We therefore checked the structures of **1** and **2** by performing infrared spectral analyses and X-ray diffraction as well. Both **1** and **2** showed an intense band at 2161 cm^{-1} in the infrared spectra, resembling that characteristic of azides. Furthermore, the X-ray analyses unambigously confirmed the azide structure of both **1** and **2** (Figure 1).

The synthesis of probe **3** was started with 5-amino-1-β-D-ribofuranosyl-1*H*-imidazole-4-carboxamide (AICAR), followed by diazotization and subsequently substitution with NaN_3 (Scheme 4).[18] In this way, probe **3** was obtained with a yield of around 40%, occasionally reaching 60%.[18] It was previously reported that the diazotization of AICAR with $NaNO_2$ in 6 N HCl at −25°C gave the ring closure product 2-azainosine as the main product.[21] However, we did not succeed in isolating the ring closure product 2-azainosine under the present experimental conditions. The synthesis of **3** developed by us is therefore much shorter and more convenient than that described by Fujii,[22,23] which starts with 5-amino-*N*′-methoxy-1-β-D-ribofuranosyl-1*H*-imidazole-4-carboxamidine, followed by a two-step process of synthesis. In addition, the starting material in the method developed by Fujii is not commercially available and its synthesis from adenosine requires a three-step process, *via* oxidation, methylation, and ring opening.[22,23]

All these probes are stable in the dark and undergo a rapid process of photodecomposition when exposed to light. A photochemical study was carried out with each probe in a buffered solution of 100 mM phosphate at pH 7.4 or in MeOH or EtOH. Figure 2 shows the absorption spectra of probes **1–3** as well as their rate/pattern of photodecomposition in response to irradiation at 260 nm. Irradiation of each compound quickly led to the disappearance of its absorption band, and the

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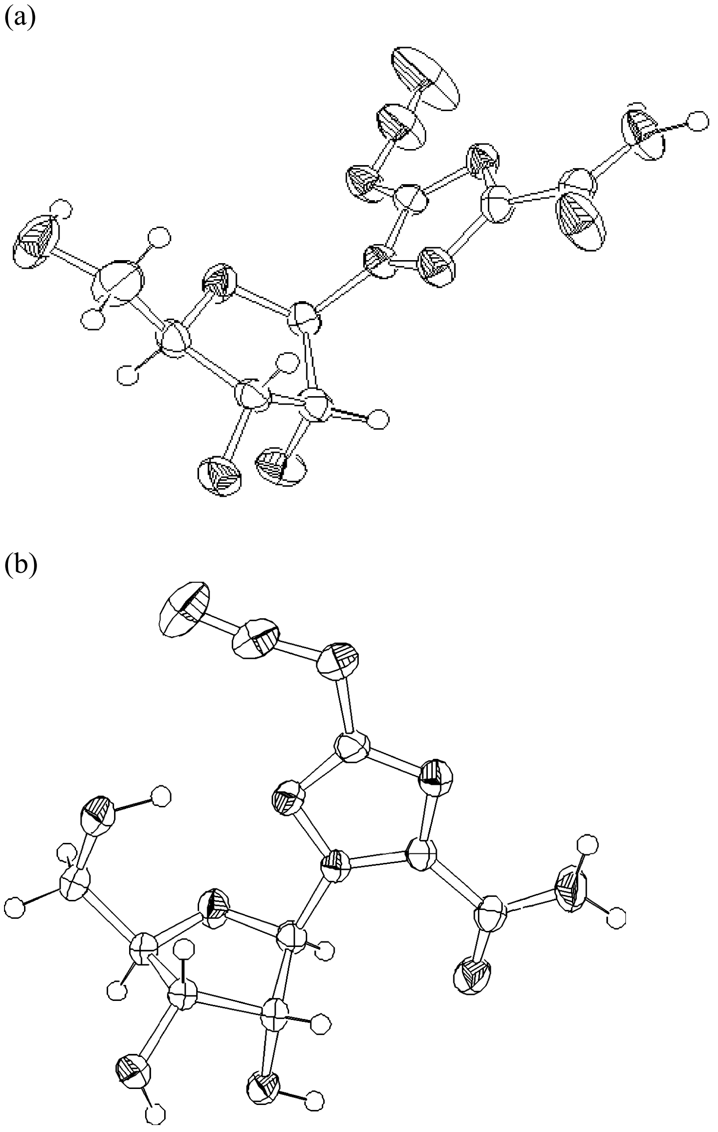
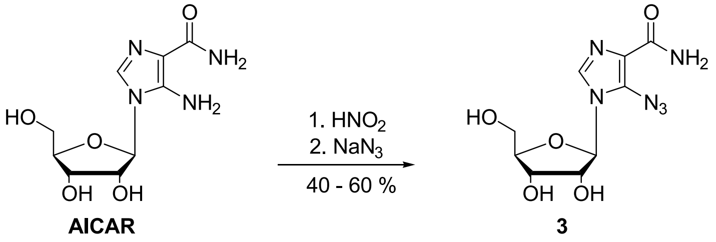


FIGURE 1 X-ray structures of **1** (a) and **2** (b).

isobestic points observed point to the occurrence of a single photodecomposition process. Moreover, HPLC analyses indicated that the photodecomposition process of these probes was a clear-cut reaction, giving a main photoproduct (Figure 3). These results indicate that these probes are promising photolabeling probes for studying the molecular mechanisms underlying the antiviral activity of ribavirin and EICAR. Further investigations are now under way to identify the photoproducts as well as the mechanisms involved in the photoreaction in the case of these probes.



SCHEME 4 Synthesis of probe **3**.

EXPERIMENTAL PART

X-Ray Crystal Structure

Crystals were grown by slowly evaporating a solution of **1** or **2** in H_2O and MeOH, respectively.

1: $C_{32}H_{48}N_{28}O_{22}$, (Mr 1176.98), Monoclinic space group *P21*, $Z = 2$, $a = 14.9540$ (3), $b = 7.50300$ (10), $c = 22.8780$ (6) Å, $\alpha = 90.00$, $\beta = 105.8511$ (7), $\gamma = 90.00$, $V = 2469.30$ (9) $Å^3$, MoK_a radiation, $\lambda = 0.71073$ Å, $0.93° < \theta < 28.80°$, 6673 reflections, $T = 293$ K on a Brucker-Nonius Kappa CCD. The structure was solved using direct methods (SHELXS 97) and refined with SHELXL97 to final $R(F^2 > 4\sigma F^2) = 0.0619$ and $wR = 0.1265$ $[w = 1/[\sigma^2(Fo^2) + (0.0647P)^2 + 1.2442P]$, where $P = (Fo^2 + 2Fc^2)/3]$.

2: $C_8H_{11}N_7O_5$, (Mr 285.24), Orthorhombic space group $P2_12_12_1$, $Z = 4$, $a = 9.8790$ (1), $b = 10.6980$ (2), $c = 11.1820$ (2) Å, $\alpha = 90.00$, $\beta = 90.00$, $\gamma = 90.00$, $V = 1181.78$ (3) $Å^3$, MoK_a radiation, $\lambda = 0.71073$ Å, $1.82 < \theta < 26.34$, 1351 reflections, $T = 293$ K on a Brucker-Nonius Kappa CCD. The structure was solved using direct methods (SHELXS 97) and refined with SHELXL97 to final $R(F^2 > 4\sigma F^2) = 0.0331$ and $wR = 0.1068$ $[w = 1/[\sigma^2(Fo^2) + (0.0802P)^2 + 0.1059P]$, where $P = (Fo^2 + 2Fc^2)/3]$.

Crystallographic data obtained on the structures of **1** and **2** have been deposited in the Cambridge Crystallographic Data Center with deposition Nos. CCDC 251212 and 251213, respectively. Copies of the data can be obtained free of charge on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ UK (Fax: +33 4 91 82 94 91; E-mail: deposit@ccdc.cam.ac.uk).

General Photolysis Procedure

Probes **1–3** were dissolved in 100 mM phosphate buffer at pH 7.4 or in MeOH or in EtOH. The solutions were photolyzed under stirring using a 150 W USHIO Xenon Short Arc Lamp or a 125 W Philipp mercury lamp for 0–30 min at 20°C. The absorption spectra of the irradiated samples were recorded using a CARY UV spectrophotometer (probes **1–2**) or a Perkin Elmer Lambda 35 UV/VIS spectrophotometer (probe **3**). The irradiated sample was withdrawn for HPLC analysis.

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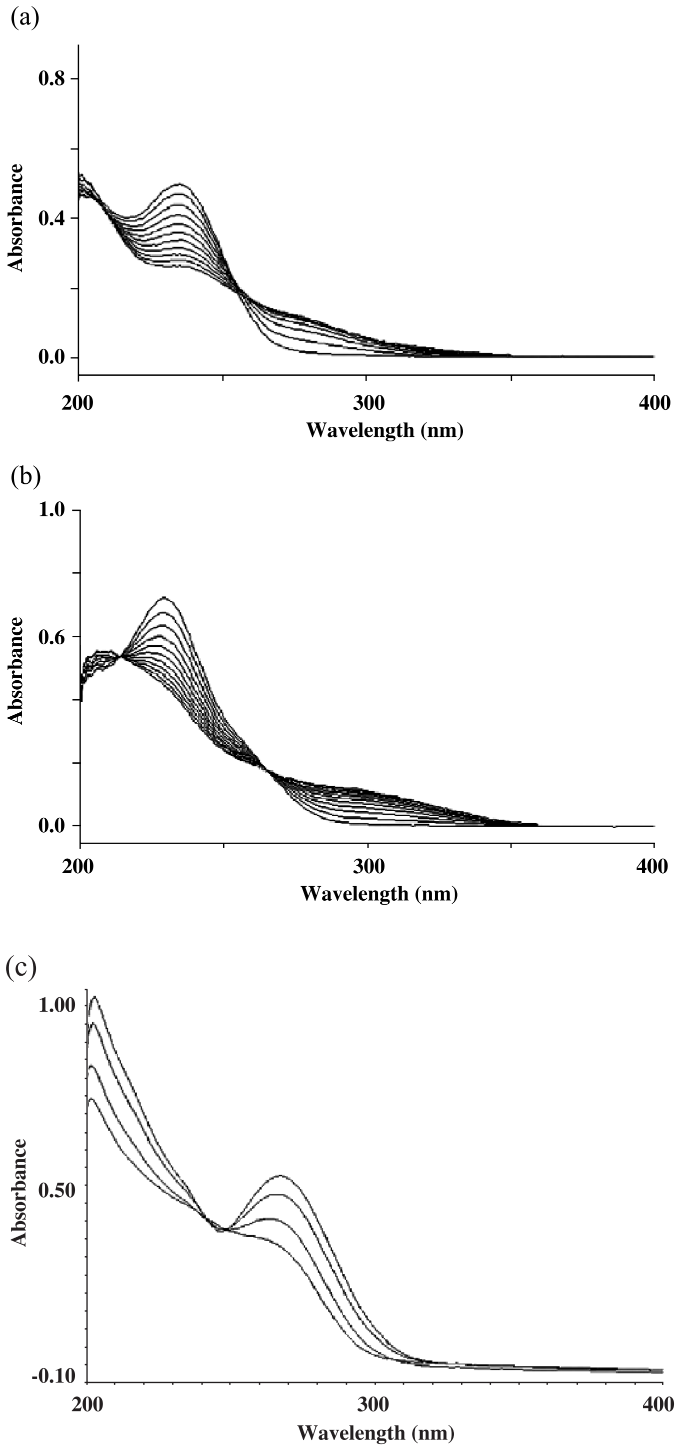

FIGURE 2 Evolution of the absorption spectra during irradiation of **1**–**3**. (a) **1** in 100 mM phosphate, pH 7.4; (b) **2** in EtOH; (c) **3** in 50 mM phosphate, pH 7.4.

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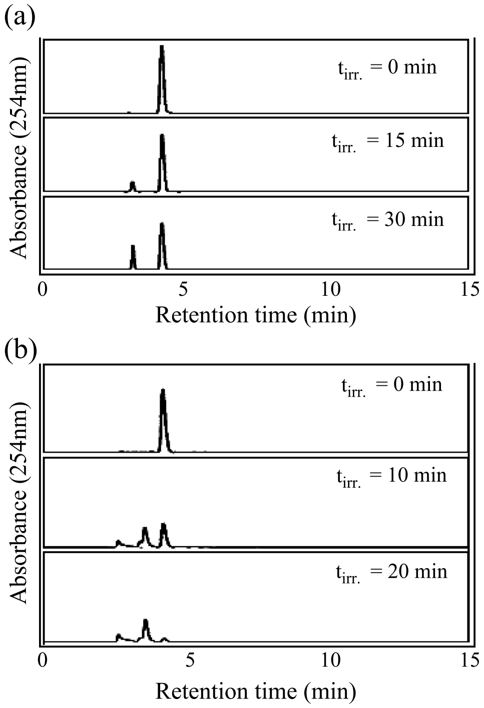

FIGURE 3 HPLC analyses of the photoirradiation of **1** and **3** in phosphate buffer, pH 7.4. (a) **1** in 100 mM phosphate, pH 7.4; (b) **3** in 100 mM phosphate, pH 7.4.

HPLC Analyses

HPLC were performed on a Waters™ 600 pump with a Waters 600 controller and the components were detected using a Waters 2996 photodiode array detector. A C_{18} column (4.6 × 250 mm) was used for HPLC analysis.

CONCLUSION

Two photolabeling probes based on ribavirin, **1** and **2**, and one on EICAR, **3**, were developed with a view to studying the mechanisms responsible for the antiviral effects of ribavirin and EICAR using a photolabeling approach. These probes were synthesized using short, convenient methods, involving the direct coupling of the azidotriazole to the protected ribose sugar in the case of **1** and **2**, and the diazotization of AICAR and the subsequent substitution with NaN_3 in that of **3**. The photodecomposition of these probes was studied in various solvents and was consistently found to be a fast, clear-cut photochemical reaction. These probes therefore constitute promising tools for studying the mechanisms underlying the antiviral effects of ribavirin and EICAR using the photolabeling approach. In addition, both triazoylazides and imidazoylazides may constitute a useful new class of photoprobes that will extend the existing range of photolabeling reagents available.

REFERENCES

1. Sidwell, R.W.; Huffman, J.H.; Khare, G.P.; Allen, L.B.; Witkowski, J.T.; Robins, R.K. Broad-spectrum antiviral activity of virazole: 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide. Science **1972**, *177*, 705–706.
2. De Clercq, E. Stategies in the design of antiviral drugs. Nat. Rev., Drug Discov. **2002**, *1*, 13–25.
3. So, L.K.-Y.; Lau, A.C.W.; Yam, L.Y.C.; Cheung, T.M.T.; Poon, E.; Yung, R.W.H.; Yuen, K.Y. Development of a standard treatment protocol for severe acute respiratory syndrome. Lancet **2003**, *361*(9369), 1615.
4. Koren, G.; King, S.; Knowles, S.; Phillips, E. Ribavirin in the treatment of SARS: a new trick for an old drug? CMAJ **2003**, *168*, 1289–1292.
5. De Clercq, E.; Cools, M.; Balzarini, J.; Snoeck, R.; Andrei, G.; Hosoys, M.; Shigeta, S.; Ueda, T.; Minakawa, N.; Matsuda, A. Antiviral activities of 5-ethyly-1-β-D-ribofuranosylimidazole-4-carboxamide and related compounds. Antimicrob. Agents Chemother. **1991**, *35*(4), 679–684.
6. Minakawa, N.; Matsuda, A. Mechanism-based design of inosine 5′-monophosphate dehydrogenase inhibitors: synthesis and biological activities of 5-ethyly-1-beta-D-ribofuranosylimidazole-4-carboxamide (EICAR) and its derivatives. Curr. Med. Chem. **1999**, *6*, 615.
7. Balzarini, J.; Karlsson, A.; Wang, L.; Bohman, C.; Horska, K.; Votruba, I.; Fridland, A.; Van Aerschot, A.; Herdewijin, P.; De Clercq, E. Eicar (5-ethynyl-1-beta-D-ribofuranosylimidazole-4-carboxamide) a novel potent inhibitor of inosinate dehydrogenase activity and guanylate biosynthesis. J. Biol. Chem. **1993**, *268*, 24591–24598.
8. Jashes, M.; Mlynarz, G.; De Clercq, E.; Sandino, A.M. Inhibitory effects of EICAR on infectious pancreatic necrosis virus replication. Antivir. Res. **2000**, *45*, 9–17.
9. Minakawa, N.; Takeda, T.; Sasaki, T.; Matsuda, A.; Ueda, T. Nucleosides and nucleotides 96: synthesis and antitumor activity of 5-Ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide (EICAR) and its derivatives. J. Med. Chem. **1991**, *34*(2), 778–786.
10. Matsuda, A.; Minakawa, N.; Sasaki, T.; Ueda, T. The design, synthesis and antileukemic activity of 5-alkynyl-1-β-D-ribofuranosylimidazole-4-carboxamide. Chem. Pharm. Bull. **1988**, *36*(7), 2730–2733.
11. Hong, Z.; Cameron, C.E. Pleiotropic mechanisms of ribavirin antiviral activities. Prog. Drug Res. **2002**, *59*, 41–69.
12. Graci, J.D.; Cameron, C.E. Quasispecies, error catastrophe and the antiviral activity of ribavirin. Virology **2002**, *298*, 175–180.
13. Dorman, G.; Prestwich, G.D. Using photolabile ligands in drug discovery and development. Trends Biotech. **2000**, *18*(2), 64–77.
14. Dorman, G. Photoaffinity labeling in biological signal transduction. Top. Curr. Chem. **2000**, *211*, 169–225.
15. Kotzyba-Hibert, F.; Kapfer, I.; Goeldner, M. Recent trends in photoaffinity labeling. Angew. Chem., Int. Ed. **1995**, *34*(12), 1296–1312.
16. Wu, Q.; Zhu, X.; Wan, J.; Qu, F.; Peng, L. Synthesis of a photolabeling probe for the study of antiviral mechanism of ribavirin. Chin. Chem. Lett. **2004**, *15*(8), 907–910.
17. Wu, Q.; Qu, F.; Wan, J.; Zhu, X.; Xia, Y.; Peng, L. Design, synthesis and characterization of photolabeling probes for the study of the mechanisms of the antiviral effects of ribavirin. Helv. Chim. Acta **2004**, *87*, 811–819.
18. Wu, Q.; Wan, J.; Xia, Y.; Zhou, J.; Qu, F.; Peng, L. Synthesis and characterization of potential photolabeling probes for studying the antiviral mechanisms of EICAR. Heterocycles **2004**, *63*(3), 671–679.
19. TI'LER, M. Some aspects of azido-tetrazolo isomerization. Synthesis **1973**, *3*, 123.
20. Lioux, T.; Gosselin, G.; Mathé, C. Azido/tetrazole tautomerism in 2-azidoadenine-β-D-pentofuranonucleoside derivatives. Eur. J. Org. Chem. **2003**, *20*, 3997.
21. Kawana, M.; Ivanovics, G.A.; Rousseau, R.J.; Robins, R.K. Azapurine nucleosides. 3: synthesis of 7-(beta-D-ribofuranosyl)imidazo[4,5-d]-gamma-triazin-4-one (2-azainosine) and related derivatives. J. Med. Chem. **1972**, *15*(8), 841–843.
22. Saito, T.; Asahi, Y.; Nakajima, S.; Fujii, T. Purines. LXIV. Syntheses of 9-methyl-2-azaadenine-1-oxide, its O-methyl derivative, and 1-substituted 5-azidoimidazole-4-carboamides. Chem. Pharm. Bull. **1994**, *42*, 2263–2268.

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23. Saito, T.; Asahi, Y.; Nakajima, S.; Fujii, T. A unique transformation of 6-amino-N-methoxyimidazole-4-carboxamide by diazotization: synthesis of the 5-azido analogue of AICA riboside. Heterocycles **1990**, *30*(1), 329–332.
24. Srivastava, P.C.; Streeter, D.G.; Matthews, T.R.; Allen, L.B.; Sidwell, R.W.; Robins, R.K. Synthesis and antiviral and antimicrobial activity of certain 1-β-D-ribofuranosyl-4,5-disubstituted imidazoles. J. Med. Chem. **1976**, *19*(8), 1020–1026.

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