SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF UNSATURATED PYRIMIDINE CARBOACYCLONUCLEOSIDE ANALOGUES

Jamal KRIM[a1,b], Bouchra SILLAHI[a2], Moha TAOURIRTE[a3], Said EDDARIR[a4] and Joachim W. ENGELS[b,*]

[a] *Laboratoire de Chimie Bioorganique et Macromoléculaire, Faculté des Sciences et Techniques, Guéliz 40 000, Marrakech, Maroc; e-mail:* [1] *krimjamal@gmail.com,* [2] *bouchra.sillahi@gmail.com,* [3] *taourirte@fstgmarrakech.ac.ma,* [4] *eddarir@yahoo.fr*

[b] *Institut für Organische Chemie und Chemische Biologie, J. W. Goethe Universität, Max-von-Laue Str. 7, 60438 Frankfurt am Main, Germany; e-mail: joachim.engels@chemie.uni-frankfurt.de*

Received April 20, 2011
Accepted June 17, 2011
Published online October 30, 2011

Dedicated to Professor Antonín Holý on the occasion of his 75th birthday.

The novel pyrimidine carboacyclonucleoside analogues containing propargylated aryl side chains were synthesized via palladium-catalyzed cross-coupling reactions as a key step. The synthesized compounds were screened for their antibacterial activity against four microorganisms: *Staphylococcus aureus* (CIP 53.154; Gram positive), *Enterococcus hirae* (CIP 58.55; Gram positive), *Pseudomonas aeruginosa* (CIP A22; Gram negative), *Escherichia coli* (CIP 54.8; Gram negative). Some of the prepared products showed promising antibacterial activity against the nosocomial *E. hirae*.

Keywords: *N*-Alkylation; Sonogashira cross-coupling; Thymine; Antibacterial activity; Alkynes; Alkylation; Antibiotics; Nucleobases; Palladium.

The emergence of resistant strains of bacteria to major classes of antimicrobial agents is recognized as a serious health concern[1]. Even though pharmaceutical industries have produced a number of novel antibiotics in the last three decades, resistance to these drugs by microorganisms has increased dramatically. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents[2]. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotics, develop research to better understand the genetic mecha-

doi:10.1135/cccc2011083

nisms of resistance, and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient. Consequently, the search for new chemotherapeutic agents constitutes a real challenge for microbiologists, pharmacologists as well as medicinal chemists.

Nucleoside analogues display a wide range of biological activities, such as antitumor, antiviral and chemotherapeutic[3–5]. Various acyclic and cyclic nucleoside analogues were synthesized and evaluated for antibacterial and antifungal activity[6–11]. Some prepared products showed promising antimicrobial activity. In addition, it has been shown that the presence of conjugated system plays a fundamental role in determining bioactivity, due to its ability to act as a Michael acceptor for the addition of protein functional groups[12,13].

Efforts aimed at synthesizing and isolating new active carboacyclonucleosides now require the development and elaboration of new strategies yielding facile and rapid access to a large variety of compounds. In view of the stimulating results reported for unsaturated acyclonucleosides[14,15] and as a part of our ongoing drug discovery efforts, this study aims to synthesize novel unsaturated carboacyclonucleosides. This paper reports their synthetic routes from the simple propargylated nucleobases by Sonogashira cross-coupling. The cross-coupling reaction proceeds in the presence of catalytic amounts of palladium complexes like $Pd(PPh_3)_2Cl_2$, a catalytic amount of copper(I) iodide, an organic amine base and DMF as solvent at room temperature.

RESULTS AND DISCUSSION

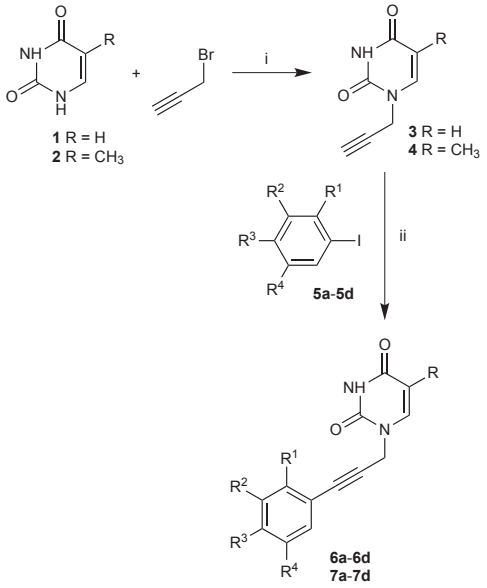
Chemistry

The first step of the synthesis was the preparation of propargylated nucleobases. For this, uracil and thymine were used as starting materials that were treated with propargyl bromide in the presence of K_2CO_3. All reactions were carried out in DMF, as it is an excellent solvent for dissolving both nucleobases[16,17] (Scheme 1) and inorganic salts. All the pyrimidine derivatives were exclusively alkylated at N-1 position **3** and **4** as confirmed by their ^{1}H NMR spectra and comparison with authentic samples[16].

The second step was the introduction of various aryl iodides at the terminal triple bond by Sonogashira reaction. The palladium-catalyzed cross-coupling reaction of aryl iodides with propargyl pyrimidines proceeds efficiently in the presence of catalytic amounts of palladium complexes such

as $Pd(PPh_3)_2Cl_2$, a catalytic amount of copper(I) iodide, an organic amine base and DMF as solvent.

We examined the reaction of various aryl iodides **5a–5d** with the propargyl pyrimidines **3** and **4**. The presence of methoxy groups in the *meta*-position on the aryl iodide seems to be advantageous. Electron donors in *para*-positions, however, are inferior to electron withdrawing groups like NO_2. When *p*-iodoanisole was used as the aryl iodide, the Sonogashira coupling reaction yield decreased to 50 or 60%. The results are summarized in Table I.



SCHEME 1
(i) K_2CO_3, DMF, r.t.; (ii) $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, DMF, r.t.

Antimicrobial Activity

Newly synthesized compounds **6a–6d** and **7a–7d** were tested for antimicrobial activity against the following bacterial strains obtained from the Pasteur Institute Collection: Gram (+): *Staphylococcus aureus* (CIP 53.154), *Enterococcus hirae* (CIP 58.55); Gram (–): *Pseudomonas aeruginosa* (CIP A22), *Escherichia coli* (CIP 54.8).

The compounds were dissolved in 5% DMSO/H_2O and added to the culture medium (nutrient agar for bacteria) immediately before it was emptied into the Petri dishes. The concentrations tested ranging from 27 to

217 μg/ml. Inocula of the bacteria were prepared from 18 h cultures and the suspensions of microorganisms (spot content 2 μl of 108 cells per ml) were inoculated onto the surface of the Petri dishes immediately after their preparation (three replicates). The negative control received the same quantity of the 5% DMSO mixed with the culture medium and bacteria. Suspension of each microorganism was prepared and applied to plates with serially di-

TABLE I
Pd-Catalyzed reaction of aryl iodides **5a–5d** with propargyl pyrimidines

Compounds	R	R^1	R^2	R^3	R^4	Yield, %
6a	H	NO_2	H	H	H	80
6b	H	H	H	NO_2	H	90
6c	H	H	OCH_3	H	OCH_3	92
6d	H	H	H	OCH_3	H	50
7a	CH_3	NO_2	H	H	H	80
7b	CH_3	H	H	NO_2	H	92
7c	CH_3	H	OCH_3	H	OCH_3	96
7d	CH_3	H	H	OCH_3	H	60

TABLE II
Minimum inhibitory concentration (MIC in μg/ml) of medium

Compounds	*Escherichia coli*	*Staphylococcus aureus*	*Enterococcus hirae*	*Pseudomonas aeruginosa*
6a	–	–	27	–
6b	–	–	27	–
6c	54	–	27	–
6d	–	–	108	–
7a	–	–	108	–
7b	–	–	–	–
7c	–	–	54	–
7d	54	–	54	–
Amoxicillin	3.1	0.2	0.5	0.2

luted compounds (DMSO/H_2O) to be tested and incubated (24 h) at 37 °C. After the incubation period the growth was visually evaluated by comparison with those of control plates. The minimum inhibitory concentration (MIC) was taken as the lowest concentration that completely inhibited growth after incubation. The MICs of the standards (Amoxicillin) were also determined in parallel experiments, to control the sensitivity of the bacteria.

No antibacterial activity of **6a–6d** and **7a–7d** was detected against *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains. All these compounds except **7b** showed antibacterial activity (MIC ≈ 27–108 μg/ml) against *Enterococcus hirae*, a strain, known to be present in hospitals (Table II). In addition compounds **6c** and **7d** exhibit some activity against *E. coli* (MIC ≈ 54 μg/ml).

CONCLUSIONS

In summary, the palladium-catalyzed cross-coupling reaction of various aryl iodides with propargyl pyrimidines was achieved by using Sonogashira reactions. Newly synthesized compounds **6a–6d** and **7a–7d** were tested for their antimicrobial activity. No antibacterial activity of **6a–6d** and **7a–7d** was detected against *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains, some of them exhibit a good activity against *Enterococcus hirae* strains (MIC ≈ 27 μg/ml) and *E. coli* (MIC ≈ 54 μg/ml).

EXPERIMENTAL

Melting points were determined in open capillary tubes and are uncorrected. NMR spectra were recorded at 300 MHz (^{1}H, ^{13}C) Bruker (in DMSO-d_6, $CDCl_3$) using TMS as an internal reference. All chemical shifts (δ) are expressed in ppm and coupling constant (*J*) are given in Hz. Mass spectra were obtained using ESI/MS and (FAB^+). DMF was distilled prior to use and stored over 4Å molecular sieves. Precoated Merck Silica Gel 60F-254 plates were used for thin layer chromatography (TLC) and the spots were detected under UV light (254 nm). Column chromatography (CLC) was performed using silica gel (0.063–0.2 mm) Fluka. All reagents used were purchased from Aldrich.

Synthesis of the Monopropargyl Pyrimidines. General Procedure

The mixture of 1 mmol of the pyrimidine base (thymine and uracil), 0.5 mmol of K_2CO_3 and 1 mmol of propargyl bromide in 20 ml of anhydrous DMF was stirred at room temperature for 24 h. After removal of the solvent under reduced pressure, the obtained residue was purified on silica gel column (CH_2Cl_2 and MeOH 99:1).

N-1-Propargyl thymine (**3**): Yield 56%; solid, m.p. 154–156 °C. ^{1}H NMR (DMSO-d_6): 1.75 (s, 3 H, CH_3); 3.37 (t, 1 H, CH, *J* = 2.2); 4.46 (d, 2 H, CH_2N, *J* = 7.8); 7.55 (s, 1 H, H-6); 11.35 (s, 1 H, NH). ^{13}C NMR (DMSO-d_6): 11.87; 36.30; 75.58; 78.61; 109.38; 140.04; 150.33; 164.09. FAB-MS, *m/z*: calculated for $C_8H_8N_2O_2$ $[M + H]^+$ 165.06, found 165.

N-1-Propargyl uracil (**4**): Yield 54%; solid, m.p. 164–166°C. ^{1}H NMR (DMSO-d_6): 3.43 (t, 1 H, CH, J = 2.3); 4.57 (d, 2 H, CH_2N, J = 2.3); 5.7 (d, 1 H, H-5, J = 7.8); 7.75 (d, 1 H, H-6, J = 7.8); 11.44 (s, 1 H, NH). ^{13}C NMR (DMSO-d_6): 36.60; 75.79; 78.42; 101.67; 144.42; 150.35; 163.50. FAB-MS, *m/z*: calculated for $C_7H_6N_2O_2$ $[M + H]^+$ 151.04, found 151.

Sonogashira Cross-Coupling. General Procedure

Aryl iodides **5a–5d** (5 mmol) were dissolved in a mixture of dry DMF (15 ml), dry Et_3N (3 equiv.) and propargyl pyrimidine (2 equiv.). CuI (0.2 equiv.) and Pd $(PPh_3)_2Cl_2$ (0.1 equiv.) were then added and the reaction mixture was stirred at room temperature until complete (typically 5–20 h, checked by TLC). The solvent was removed by rotary evaporation, and the residue was purified on silica gel with dichloromethane and methanol (95:5) to give the desired compound.

1-(3-(2-Nitrophenyl)prop-2-ynyl)uracil (**6a**): Yield 80%; m.p. 199 °C. ^{1}H NMR (DMSO-d_6): 4.38 (d, 2 H, CH_2-N, J = 2.3); 6.13 (d, 1 H, H-5, J = 7.8); 7.65–7.9 (m, 5 H, 4CH and H-6); 11.4 (s, 1 H, NH). FAB-MS, *m/z*: calculated for $C_{13}H_9N_3O_4$ $[M + H]^+$ 272.06, found 272.

1-(3-(4-Nitrophenyl)prop-2-ynyl)uracil (**6b**): Yield 90%; m.p. > 250 °C. ^{1}H NMR (DMSO-d_6): 4.83 (s, 2 H, CH_2-N); 5.65 (d, 1 H, H-5, J = 7.8); 7.75 (d, 2 H, 2CH, J = 8.7); 7.84 (d, 1 H, H-6, J = 7.8); 8.22 (d, 2 H, 2CH, J = 8.7); 11.4 (s, 1 H, NH). FAB-MS, *m/z*: calculated for $C_{13}H_9N_3O_4$ $[M + H]^+$ 272.06, found 272.

1-(3-(3,5-Dimethoxyphenyl)prop-2-ynyl)uracil (**6c**): Yield 92%; m.p. 141 °C. ^{1}H NMR (DMSO-d_6): 4.75 (s, 2 H, CH_2-N); 3.77 (s, 6 H, 2 × OCH_3); 5.65 (d, 1 H, H-5, J = 7.8); 6.53 (s, 1 H, CH); 6.59 (s, 2 H, 2CH); 7.78 (d, 1 H, H-6, J = 7.8); 11.45 (s, 1 H, NH). FAB-MS, *m/z*: calculated for $C_{15}H_{14}N_2O_4$ $[M + H]^+$ 287.10, found 287.

1-(3-(4-Methoxyphenyl)prop-2-ynyl)uracil (**6d**): Yield 50%; m.p. > 250 °C. ^{1}H NMR (DMSO-d_6): 3.76 (s, 3 H, OCH_3); 4.72 (s, 2 H, CH_2-N); 5.65 (d, 1 H, H-5, J = 7.8); 6.9 (d, 2 H, 2CH, J = 9); 7.39 (d, 2 H, 2CH, J = 9); 7.8 (d, 1 H, H-6, J = 7.8); 11.4 (s, 1 H, NH). FAB-MS, *m/z*: calculated for $C_{14}H_{12}N_2O_3$ $[M + H]^+$ 257.08, found 257.

1-(3-(2-Nitrophenyl)prop-2-ynyl)thymine (**7a**): Yield 80%; m.p. 208 °C. ^{1}H NMR (DMSO-d_6): 1.9 (s, 3 H, CH_3); 4.38 (s, 2 H, CH_2-N); 7.58–7.81 (m, 5 H, 4CH and H-6); 11.4 (s, 1 H, NH). FAB-MS, *m/z*: calculated for $C_{14}H_{11}N_3O_4$ $[M + H]^+$ 286.07, found 286.

1-(3-(4-Nitrophenyl)prop-2-ynyl)thymine (**7b**): Yield 92%; m.p. 242 °C. ^{1}H NMR (DMSO-d_6): 1.87 (s, 3 H, CH_3); 4.39 (s, 2 H, CH_2-N); 7.73 (d, 2 H, 2CH, J = 9); 8.02 (s, 1 H, H-6); 8.22 (d, 2 H, 2CH, J = 9); 11.42 (s, 1 H, NH). FAB-MS, *m/z*: calculated for $C_{14}H_{11}N_3O_4$ $[M + H]^+$ 286.07, found 286.

1-(3-(3,5-Dimethoxyphenyl)prop-2-ynyl)thymine (**7c**): Yield 96%; m.p. 128 °C. ^{1}H NMR (DMSO-d_6): 1.78 (s, 3 H, CH_3); 3.74 (s, 6 H, 2 × OCH_3); 4.75 (s, 2 H, CH_2-N); 6.53 (s, 1 H, CH); 6.65 (s, 2 H, 2CH); 7.64 (s, 1 H, H-6); 11.4 (s, 1 H, NH). FAB-MS, *m/z*: calculated for $C_{16}H_{16}N_2O_4$ $[M + H]^+$ 301.11, found 301.

1-(3-(4-Methoxyphenyl)prop-2-ynyl)thymine (**7d**): Yield 60%; m.p. 207 °C. ^{1}H NMR (DMSO-d_6): 1.79 (s, 3 H, CH_3); 3.76 (s, 3 H, OCH_3); 4.70 (s, 2 H, CH_2-N); 6.78 (d, 2 H, 2CH, J = 8.7); 7.21 (d, 2 H, 2CH, J = 8.7); 7.59 (m, 1 H, H-6); 11.4 (s, 1 H, NH). FAB-MS, *m/z*: calculated for $C_{15}H_{14}N_2O_3$ $[M + H]^+$ 271.10, found 271.

We thank Prof. F. Benkhalti and Dr. H. Bouaamama for supporting the biochemical and pharmacological data (experiments).

REFERENCES

1. Amyes S. G. B., Gemmell C. G.: *J. Med. Microbiol.* **1997**, *46*, 436.
2. Cohen M. L.: *Science* **1992**, *257*, 1050.
3. Zhou W., Gumina G., Chong Y., Wang J., Shinazi R. F., Chu C. K.: *J. Med. Chem.* **2004**, *47*, 3399.
4. Robins R. K., Kini G. D. in: *The Chemistry of Antitumor Agents* (D. E. V. Wilman, Ed.), p. 299. Chapman and Hall, New York 1990.
5. MacCoss M., Robins M. J. in: *The Chemistry of Antitumor Agents* (D. E. V. Wilman, Ed.), p. 261. Chapman and Hall, New York 1990.
6. Hassan N. A., Hegab M. I., Rashad A. E., Fahmy A. A., Abdel-Megeid F. M. E.: *Nucleosides, Nucleotides Nucleic Acids* **2007**, *26*, 379.
7. Goudgaon N. M., Vijayalaxmi A.: *Indian J. Pharm. Sci.* **2003**, *65*, 545.
8. Shaban M. A. E., Nasr A. Z., Morgaan A. E. A.: *Pharmazie* **2000**, *55*, 87.
9. Stefańska J. Z., Gralewska R., Starościak B. J., Kazimierczuk Z.: *Pharmazie* **1999**, *54*, 879.
10. Kimura K., Bugg T. D. H.: *Nat. Prod. Rep.* **2003**, *20*, 252.
11. Srivastava R., Bhargava A., Singh R. K.: *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6239.
12. Favier L. S., Maria A. O. M., Wendel G. H., Borkowski E. J., Giordano O. S., Pelzer L., Tonn C. E.: *J. Ethnopharmacol.* **2005**, *100*, 260.
13. Rungeler P., Castro V., Mora G., Goren N., Vichnewski W., Pahl H. L., Merfort I., Schmidt T.: *Bioorg. Med. Chem.* **1999**, *7*, 2343.
14. Lazrek H. B., Redwane N., Taourirte M., Rochdi A., Barascut J. L., Imbach J. L.: *Nucleosides Nucleotides* **1999**, *18*, 673.
15. Rochdi A., Taourirte M., Lazrek H. B., Barascut J. L., Imbach J. L.: *Molecules* **2000**, *5*, 1139.
16. Lazrek H. B., Taourirte M., Oulih T., Barascut J. L., Imbach J. L., Witvrouw M., De Clercq E.: *Nucleosides Nucleotides* **2001**, *20*, 1949.
17. Krim J., Sillahi B., Taourirte M., Rakib E. M., Engels J. W.: *ARKIVOC* **2009**, *xiii*, 142.