Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 1195–1198
Bioorganic & Medicinal Chemistry Letters

Synthesis and antibiotic activity of a small molecules library of 1,2,3-triazole derivatives

Marie Aufort, Jean Herscovici, Pascale Bouhours, Nicole Moreau and Christian Girard*

Unité de Pharmacologie Chimique et Génétique UMR8151 CNRS / U640 INSERM, and Equipe de Biochimie, Laboratoire de Synthèse Organique Sélective et Produits Naturels UMR7573 CNRS, IFR 2769 Chimie Moléculaire de Paris Centre, Ecole Nationale Supérieure de Chimie de Paris, 75005 Paris, France

Received 26 November 2007; revised 28 November 2007; accepted 29 November 2007
Available online 4 December 2007

Abstract—A small library of simple 1,4-disubstituted 1,2,3-triazoles was prepared using a known one-pot procedure starting from organic halides and terminal alkynes. The compounds were then tested for their antibacterial activity against normal and resistant species of *Staphylococcus aureus*.

Drug resistance to antibiotics, especially the multiple one (MDR), is nowadays an alarming and major public health concern worldwide.[1] Many strains of bacteria have developed resistances through the years following the clinical use of the first antibiotics and there is continuous emergence and spreading of antibiotic-resistant bacteria. Several strains causing infectious diseases that seemed to be in control are once again causing deaths each year due to the absence of an appropriate antibiotic.[2] There is thus still a need for seeking new molecules that can be added to the pharmacopeia in order to treat these infections.[3]

Staphylococcus aureus is a bacterium found on the skin and mucosa of one-third of the population. *S. aureus* is very reactive towards the antibiotic pressure and was the first species in which a penicillin resistance was found right after the beginning of its industrial production. *S. aureus* is furthermore the causal agent in 40% of septicemia cases and half infections caused by this bacterium are presenting MDR (penicillin, methicillin, tetracyclin, erythromycin, etc.).[4]

Triazole ring is a potential pharmacophore that has gained in interest over the past few years. Several derivatives have already shown interesting biological activities and a few were found to be efficient in the treatment of several infections.[5] For the 1,2,3 isomer, this new life is mainly due to the discovery of copper (I)-catalyzed versions of the usual thermal conditions for the [3+2] Huisgen's cycloaddition reaction between azides and terminal alkynes.[6]

In this communication, we wish to report the synthesis of a series of 1,2,3-triazoles and the results for their biological activity evaluation on some normal and resistant bacterial strains.

The triazole synthesis was conducted accordingly to previously published one-pot procedures.[6b,7] Advantages of these methods are the in situ generation of copper (I) salts needed for the catalysis from the stable copper (II) sulfate/sodium ascorbate redox system, in situ synthesis of the organic azide from an organic halide and sodium azide, and the isolation of the sole 1,4-disubstituted isomer. Several attempts were made in order to optimize the reaction conditions for our needs: equivalents of NaN_3, number of mol% of $CuSO_4$ and sodium ascorbate, with or without added base (Na_2CO_3) and catalyst (proline) and this in different solvent systems (DMF, dioxane, DMSO, with or without water, degassed or not) at room temperature and higher, for short and long reaction times. The best general conditions were found to be 1.2 equiv NaN_3, 5 mol% $CuSO_4$, 0.1 equiv Na ascorbate, 0.2 equiv Na_2CO_3, 0.2 equiv DL-proline in DMSO/H2O 9/1 at 65 °C until the reaction was complete (Fig. 1).[7a] All compounds were obtained by simple aqueous work-up followed by a purification.

Keywords: Click-chemistry; Huisgen's cycloaddition; Triazole; Antibiotic; MDR; *S. aureus*.
* Corresponding author. Tel.: +33 1 44 27 67 22; fax: +33 1 43 25 79 75; e-mail: Christian-Girard@enscp.fr

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.11.111

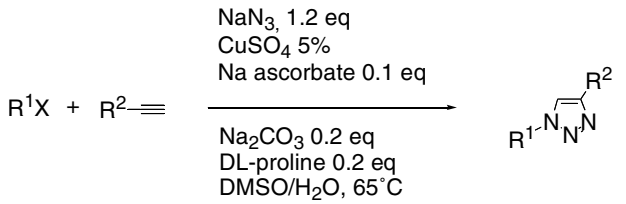

Figure 1. One-pot copper (I)-catalyzed 1,4-disubstituted 1,2,3-triazole synthesis.

The results for the synthesis of thirty derivatives are presented in Table 1. The compounds were isolated in variable yields ranging from poor to good (5–96% yield, 40% average). The reactions were conducted using alkyl, allyl and benzyl bromides as well as with iodoaromatics giving the opportunity to introduce different substitutions on the N1 of the triazole. Reactions of propargylphthalimide (triazoles **1**–**9**) gave highly crystalline compounds with the phthalimidomethyl group on the C4 of the ring, the yields being lower with iodoaryl partners. When using phenyl propargyl ether (**10**–**15**), good yields of the corresponding C4-phenoxymethyl substituted triazoles were obtained in most cases. The same was observed for propiolaldehyde diethyl acetal (**16**–**20**). The yields for the reaction of propiolic acid (**21**–**25**) were fair after, of course, acidification during the work-up procedure. However, reactions of propiolic acid methyl ester (**26**–**28**) only gave poor yields. This was due to the competitive saponification reaction as well as decarboxylation observed in some cases. Finally, unprotected alcohols like the propargyl one can be used (**29**) whereas propargyl amine (**30**) gave N-alkylated products and can only be treated with the azide generated ex situ.

The products were then tested for their biological activity on a reference strain of *S. aureus* (ATCC 25923) and resistant strains 1199B NorA and RN4220 pUL5054 MsrA.[8] Solutions were prepared in DMSO and added to the strains in Mueller–Hinton broth in 96-well plates. The growth and its inhibition were measured by the optical density followed over 24 h. The results of the inhibition on the three strains are presented in Table 2.

For the C4 phthalimidomethyl derivatives (**1**–**9**), some of them showed a moderate inhibition of *S. aureus* strains. The derivative bearing an octyl chain on N1 (**1**) inhibited the growth of MsrA strain at 40%. A dodecyl chain at this position (**2**) gave an inhibition of 40% for NorA strain and an almost equivalent (43%) for MsrA. An allyl (**3**) substituent made the activity drop to lower values. A benzyl (**4**) group gave a triazole with a better activity against the NorA strain (57%). For the compound substituted by a phenyl ring (**5**), the activity on all the strains was low. Introduction of a *para*-nitro (**6**) group on the aromatic ring gave a product more specific for *S. aureus* MsrA (48.1%), while a methoxy (**7**) one lowered the potency. A pyridine nucleus on the N1 gave a compound with a moderate inhibition of the MsrA strain (46%).

Table 1. Synthesis of 1,4-disubstituted-1,2,3-triazoles **1**–**30** using a one-pot method[a]

Triazole	R^1X	R^2	Yield, %[b]	Triazole	R^1X	R^2	Yield, %[b]	Triazole	R^1X	R^2	Yield, %[b]
1	$CH_3(CH_2)_7Br$	$PhthNCH_2$	62	**11**	$PhCH_2Br$	$PhOCH_2$	96	**21**	$H_2C{=}CHCH_2Br$	CO_2H	36[c]
2	$CH_3(CH_2)_{11}Br$	$PhthNCH_2$	5	**12**	PhI	$PhOCH_2$	52	**22**	$PhCH_2Br$	CO_2H	20[c]
3	$H_2C{=}CHCH_2Br$	$PhthNCH_2$	69	**13**	*p*-NO_2PhI	$PhOCH_2$	67	**23**	PhI	CO_2H	53[c]
4	$PhCH_2Br$	$PhthNCH_2$	82	**14**	*p*-MeOPhI	$PhOCH_2$	12	**24**	*p*-MeOPhI	CO_2H	36[c]
5	PhI	$PhthNCH_2$	30	**15**	3-Iodopyridine	$PhOCH_2$	67	**25**	3-Iodopyridine	CO_2H	24[c]
6	*p*-NO_2PhI	$PhthNCH_2$	9	**16**	$H_2C{=}CHCH_2Br$	$CH(OEt)_2$	24	**26**	$H_2C{=}CHCH_2Br$	CO_2Me	9
7	*p*-MeOPhI	$PhthNCH_2$	15	**17**	$PhCH_2Br$	$CH(OEt)_2$	67	**27**	$PhCH_2Br$	CO_2Me	32
8	3-Iodopyridine	$PhthNCH_2$	33	**18**	PhI	$CH(OEt)_2$	65	**28**	3-Iodopyridine	CO_2Me	9
9	2-Iodothiophene	$PhthNCH_2$	6	**19**	*p*-MeOPhI	$CH(OEt)_2$	14	**29**	$PhCH_2Br$	$HOCH_2$	60
10	$H_2C{=}CHCH_2Br$	$PhOCH_2$	70	**20**	3-Iodopyridine	$CH(OEt)_2$	53	**30**	$PhCH_2N_3$	H_2NCH_2	53[d]

[a] See the example for triazole **4** at the end of this article.
[b] Isolated and purified yield. All compounds gave correct IR, mp, ^1H and ^{13}C NMR and LC–MS analyses.
[c] Isolated after acidification of the reaction mixture.
[d] Reaction conducted directly with the azide. The use of the corresponding bromide gave N-alkylated products.

Table 2. Inhibition of the growth of strains of *S. aureus* by triazoles **1**–**30** at 0.1 mg mL^{-1} after 24 h

Triazole	% Inhibition[a] *S. aureus* ATCC 25923	% Inhibition[a] *S. aureus* 1199B NorA	% Inhibition[a] *S. aureus* RN4220 MsrA	% Inhibition[a,b] *S. aureus* 1199B NorA+cip	% Inhibition[a,b] *S. aureus* RN4220 MsrA+ery
1	na	27.4 (±0.4)	40 (±2)	25 (±1)	24 (±9)
2	20 (±4)	40 (±2)	43 (±3)	45 (±6)	65 (±4)
3	na	18 (±4)	33 (±16)	23 (±2)	36 (±11)
4	na	57 (±10)	30 (±4)	36 (±1)	44 (±9)
5	na	17 (±3)	24 (±4)	17 (±3)	41 (±13)
6	22 (±3)	35 (±3)	48.1 (±0.1)	38 (±3)	72 (±4)
7	24 (±3)	15 (±6)	24.1 (±0.7)	18 (±5)	48 (±3)
8	24 (±1)	22 (±1)	46 (±1)	26.6 (±0.1)	71 (±2)
10	16 (±3)	15 (±3)	32 (±2)	20 (±9)	38 (±5)
11	12 (±3)	21 (±6)	32.7 (±0.2)	20 (±8)	43 (±4)
12	31 (±4)	11 (±2)	11 (±2)	26 (±1)	41 (±11)
13	27 (±4)	18 (±3)	34 (±3)	24 (±2)	56 (±19)
14	10 (±4)	11 (±3)	21 (±3)	16(±2)	39 (±7)
15	22 (±3)	22 (±4)	30 (±3)	56 (±5)	58 (±22)
16	17 (±2)	na	6 (±2)	na	40 (±3)
17	32 (±7)	6 (±1)	19 (±11)	8 (±2)	28 (±13)
18	na	na	na	na	na
19	na	11 (±4)	9 (±2)	71 (±10)	25 (±8)
20	na	6.3 (±0.3)	na	na	na
21	na	na	na	6 (±1)	na
22	na	8 (±1)	31 (±2)	9 (±3)	22 (±2)
23	na	18.0 (±0.7)	54 (±2)	15 (±1)	74.9 (±0.8)
24	na	21 (±6)	38 (±1)	24 (±5)	45.8 (±0.6)
25	14 (±5)	23 (±9)	52.6 (±0.1)	24 (±8)	78 (±1)
26	21 (±2)	16 (±2)	44 (±1)	16 (±3)	71 (±1)
27	25 (±7)	15 (±6)	15 (±2)	18 (±7)	33 (±3)
28	16 (±4)	na	11 (±4)	na	78.3 (±0.6)
29	6.1 (±0.3)	6 (±3)	na	na	na

[a] Values are means of two experiments; standard deviation is given in parentheses (na, not active; nm, not measured).
[b] Measured in the presence of ciprofloxacin (cip) at 4 mg L^{-1}, and erythromycin (ery) at 16 mg L^{-1} (antibiotics not active at this concentration).

In the case of the C4 phenyloxymethyl substituted triazoles (**10**–**15**) and the C4 formyl diethyl acetal derivatives (**16**–**20**), all compounds had quite low inhibition on the strains ($\leqslant$30%). In the case of the C4-carboxy bearing triazoles (**21**–**25**), the derivatives with the N1-phenyl (**21**), *p*-methoxyphenyl (**24**) and pyridine (**25**) were moderately active on the MsrA strain, with 54%, 38% and 53% inhibition, respectively. The methoxycarbonyl derivatives **26**–**28** were not as active as their carboxy parents; only the allyl substituted product **26** gave 44% inhibition of growth on *S. aureus* MsrA. The C4-hydroxymethyl compound **29** was not active at all on all tested strains.

The triazole library was then tested on the resistant strains but in the presence of the antibiotic for which the strain has lost its sensitivity, in order to evaluate any possible synergetic effects (Table 2). The tests were conducted using doses of antibiotics below the inhibition concentration: namely ciprofloxacin (cip) at 4 mg L^{-1} for NorA, and erythromycin (ery) at 16 mg L^{-1} for MsrA.

In the case of *S. aureus* 1199B NorA and ciprofloxacin, only a few compounds were found to have an effect. While the triazole **2** is giving an equivalent inhibition value (45% vs 40%), 4-phenoxymethyl-1-(3-pyridyl)triazole (**15**) and 4-(diethoxymethyl)-1-(4-methoxyphenyl)triazole (**19**) gave a two to six time increase of growth inhibition: from 22% to 56% and 11% to 71%, respectively.

For the strain RN4220 MsrA and erythromycin, almost all compounds were found to be more active using this protocol. For the C4-phthalimidomethyl series (**1**–**9**), while the triazole **1** lost its activity and **3** stayed at the same level, all other derivatives were up to two times more potent. An increase of around 50% was observed for the triazoles **2** (43–65%), **4** (30–44%), **6** (48–72%) and **8** (46–71%), while the derivatives **5** and **7** have shown a important rise around 100%, going up from 24% to 41% and 24% to 48%, respectively. In the series of C4-phenoxymethyltriazoles (**10**–**15**), a similar effect was encountered and the inhibitions were increased by 50% for **11** (33–43%) and **13** (34–56%), by two times for **14** (21–39%) and **15** (30–58%), and up to four times for the triazole **12** (11–41%). For the C4-formyl diethylacetal derivatives (**16**–**20**), significant increases of inhibition of three times for **19** (9–25%) and seven times for **16** (6–40%) were observed. In the case of the carboxylic acids (**21**–**25**), the triazoles **23** (54–75%) and **25** (53–78%) were found to be 50% more active. For the methyl ester analogues (**26**–**28**), a similar increase was observed for the compound **26** (44–71%) while it was of two to six times for **27** (15–33%) and **28** (11–78%).

We presented in this communication our results for the synthesis and antibiotic activity measurements of a thirty compounds library of 1,4-disubstituted-1,2,3-triazoles. The triazoles were not very active on a reference (ATCC 25923) and two resistant strains (1199B NorA, RN4220 MsrA) of *S. aureus*.

However, a few products were found to have a moderate activity on the NorA strain in the presence of ciprofloxacin. This phenomenon was even more important while testing the triazoles on the MsrA strain in the presence of erythromycin. In this case, several compounds of the library have shown a good inhibition in the presence of this antibiotic, some of them reaching levels up to 70–80% growth inhibition.

These results seem to demonstrate that 1,2,3-triazoles could be interesting candidates for further antibiological activity testing. Investigations on other members of this class of compounds, as well as on their mode of action, will be reported in due course.

*Typical procedures for triazole synthesis and tests. 1-Benzyl-4-(N-phthalimidomethyl)-1,2,3-triazole (**4**):* In a 10 mL Schlenk tube was placed 185 mg (1 mmol) of *N*-propargylphthalimide in 2 mL of DMSO/water 9:1. Under stirring was introduced successively 119 μL (1 mmol, 1 equiv) of benzyl bromide, 23 mg (0.2 mmol, 0.2 equiv) of D,L-proline, 21 mg (0.2 mmol, 0.2 equiv) de Na_2CO_3, 78 mg de NaN_3 (1.2 mmol, 1.2 equiv), 100 μL of freshly prepared 1 M sodium ascorbate (0.1 mmol, 10 mol%) and 50 μL of 1 M $CuSO_4{\cdot}5H_2O$ (0.05 mmol, 5 mol%). The tube was capped with a septum and the mixture stirred at 65 °C overnight. The mixture was poured into 20 mL of ice-cold water and stirred for 0.5 h. The resulting solid was filtered, washed with water and dried under vacuum. The crude product was purified on a short pad silica gel column using a heptane to ethyl acetate gradient. 1-Benzyl-4-(*N*-phthalimidomethyl)-1,2,3-triazole (**4**) (260 mg, 82%) was isolated as a white solid.

TLC. $R_f = 0.21$ (heptane/AcOEt, 4:6). Mp 179–181 °C. *FTIR.* ν 3110, 3076, 3038, 2849, 1708, 1432, 1402 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 4.97 (s, 2H), 5.49 (s, 2H), 7.25–7.37 (m, 5H), 7.51 (s, 1H), 7.70–7.85 (m, 4H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 33.1, 54.2, 122.7, 123.4, 128.1, 128.7, 129.1, 132.0, 134.1, 134.5, 143.1, 167.6 ppm. *LC–MS.* $R_t = 9.10$ min, m/z 319 $(M+H)^+$.

Inhibition tests. Four isolated colonies of the strain were suspended in Mueller–Hinton broth (1 mL) and incubated at 37 °C for 18 h. This inoculum (100 μL) was diluted in 10 mL of the same medium to be used in inhibition tests. Solutions of the compounds to be tested were prepared in DMSO (10 mg mL^{-1}). The tests were run in 96-well format microplates using a Biomek 2000 (Beckman-Coulter). Two microliters of the compound solution was added to 198 μL of each inoculum (for a final concentration of 0.1 mg mL^{-1} of the molecule). Three blanks were also prepared by adding 2 μL DMSO and 2 μL of Mueller–Hinton medium, both to 198 μL of inoculum, and the third one made from 200 μL of Mueller–Hinton medium alone. Controls were also made using antibiotics known to inhibit the strains' growth: ampicillin for *S. aureus* ATCC 25923 (at 0.25 mg L^{-1}), ciprofloxacin for *S. aureus* 1199B NorA (at 4 and 32 mg L^{-1}) and erythromycin for *S. aureus* RN4220 pUL5054 MsrA (at 16 and 256 mg L^{-1}). The plates were incubated at 37 °C and optical densities read over a 24 h time.

Acknowledgments

This work was made possible by grants from CNRS (7573 and 8151) and INSERM (U640). The authors also wish to thank for their financial support SESAME and CPER programs (Ile-de-France) and Pr. Jean-Marc Paris for helpful discussions.

References and notes

1. (a) Mitscher, L. A.; Pillai, S. P.; Gentry, E. J.; Shankel, D. M. *Med. Res. Rev.* **1999**, *19*, 477; (b) Yoneyama, H.; Katsumata, R. *Biosci. Biotechnol. Biochem.* **2006**, *70*, 1060.
2. Lim, S.-M.; Webb, S. A. R. *Anaesthesia* **2005**, *60*, 887.
3. (c) Fluit, A. C.; van der Bruggen, J. T.; Aarestrup, F. M.; Verhoef, J.; Jansen, W. T. *Clin. Microbiol. Infect.* **2006**, *12*, 410; (d) Livermore, D. M. *Clin. Microbiol. Infect.* **2004**, *10*, 1; (e) Alanis, A. J. *Arch. Med. Res.* **2005**, *36*, 697.
4. (a) Grundmann, H.; Aires-de-Sousa, M.; Boyce, J.; Tiemersma, E. *Lancet* **2006**, *368*, 874; (b) Schito, G. C. *Clin. Microbiol. Infect.* **2006**, *12*, 3; (c) Bal, A. M.; Gould, I. M. *Expert Opin. Pharmacother.* **2005**, *6*, 2257.
5. For a review and some recent examples on 1,2,3- and 1,2,4-triazoles, see: (a) Como, J. A.; Dismukes, W. E. *N. Engl. J. Med.* **1994**, *330*, 2126; (b) Dandia, A.; Singh, R.; Khaturia, S.; Mérienne, C.; Morgant, G.; Loupy, A. *Bioorg. Med. Chem.* **2006**, *14*, 2409; (c) Lin, R.; Connolly, P. J.; Huang, S.; Wetter, S. K.; Lu, Y.; Murray, W. V.; Emanuel, S. L.; Gruninger, R. H. *J. Med. Chem.* **2005**, *48*, 4208; (d) Tehranchian, S.; Akbarzadeh, T.; Fazeli, M. R.; Jamalifar, H.; Shafiee, A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1023; (e) Alam, M. S.; Kajiki, R.; Hanatani, H.; Kong, X.; Ozoe, F.; Matsui, Y.; Matsumara, F.; Ozoe, Y. *J. Agric. Food Chem.* **2006**, *54*, 1361; (f) Wroblewski, A. E.; Glowacka, I. E. *Tetrahedron: Asym.* **2005**, *16*, 4056; (g) Tornøe, C. W.; Sanderson, S. J.; Mottram, J. C.; Coombs, G. H.; Meldal, M. *J. Comb. Chem.* **2004**, *6*, 312.
6. (a) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057; (b) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596; (c) Fazio, F.; Bryan, M. C.; Blixt, O.; Paulson, J. C.; Wong, C.-H. *J. Am. Chem. Soc.* **2002**, *124*, 14397; For reviews, see (d) Kolb, H. C.; Sharpless, K. B. *Drug Discov. Today* **2003**, *8*, 1128; (e) Bock, V. D.; Hiemstra, H.; van Maarseveen, J. H. *Eur. J. Org. Chem.* **2006**, 51; (f) Wu, P.; Fokin, V. V. *Aldrichim. Acta* **2007**, *40*, 7.
7. (a) Feldman, A. K.; Colasson, B.; Fokin, V. V. *Org. Lett.* **2004**, *6*, 3897; (b) Appukkuttan, P.; Dehaen, W.; Fokin, V. V.; Van der Eycken, E. *Org. Lett.* **2004**, *6*, 4223; (c) Kamijo, S.; Jin, T.; Huo, Y.; Yamamoto, Y. *J. Org. Chem.* **2004**, *69*, 2386; (d) Kacprzak, K. *Synlett* **2005**, 943; (e) Yan, Z.-Y.; Zhao, Y.-B.; Fan, M.-J.; Liu, W.-M.; Liang, Y.-M. *Tetrahedron* **2005**, *61*, 9331; (f) Chittaboina, S.; Xie, F.; Wang, Q. *Tetrahedron Lett.* **2005**, *46*, 2331; (g) Zhao, Y.-B.; Yan, Z.-Y.; Liang, Y.-M. *Tetrahedron Lett.* **2006**, *47*, 1545; (h) Beckmann, H. S. G.; Wittmann, V. *Org. Lett.* **2007**, *9*, 1.
8. Kaatz, G. W.; Seo, S. M.; Ruble, C. A. *Antimicrob. Agents Chemother.* **1993**, *37*, 1086; Ross, J. I.; Eady, E. A.; Cove, J. H.; Cunliffe, W. J.; Baumberg, S.; Wootton, J. C. *Mol. Microbiol.* **1990**, *4*, 1207.