Bioorganic & Medicinal Chemistry Letters 21 (2011) 4561–4563

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Antiplasmodial and cytotoxicity evaluation of 3-functionalized 2-azetidinone derivatives

Pardeep Singh [a], Shaveta Sachdeva [a], Raghu Raj [a], Vipan Kumar [a,*], Mohinder P. Mahajan [a], Shereen Nasser [b], Livia Vivas [b], Jiri Gut [c], Phillip J. Rosenthal [c], Tzu-Shean Feng [d], Kelly Chibale [d]

[a] Department of Chemistry, Guru Nanak Dev University, Amritsar 143005, India
[b] London School of Hygiene and Tropical Medicine, Keppel Street, London, UK
[c] Department of Medicine, San Francisco General Hospital, CA 94143, USA
[d] Department of Chemistry and Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Private Bag, Rondebosch 7701, South Africa

ARTICLE INFO

Article history:
Received 8 February 2011
Revised 27 May 2011
Accepted 31 May 2011
Available online 12 June 2011

Keywords:
c-3 Functionalized b-lactams
Triazoles
Click chemistry
Antiplasmodial evaluation

ABSTRACT

3-Azido-, 3-amino- and 3-(1,2,3-triazol-1-yl)-β-lactams were synthesized and evaluated for their antiplasmodial activity against four strains of *Plasmodium falciparum* and KB cells for their cytotoxicity profiles. The presence of a cyclohexyl substituent at N-1 and a phenyl group on the triazole ring markedly improved the activity profiles of triazole-tethered β-lactam exhibiting IC_{50} values of 1.13, 1.21 and 1.00 μM against 3D7, K1 and W2 strains respectively.

Malaria is the most common of the parasitic diseases in tropical and subtropical regions, and it is estimated that about 40% of the world's population lives in malaria endemic areas. With 300–500 million clinical cases and nearly 1 million deaths each year, malaria remains a major issue in health control, especially in developing countries.[1] Quinoline-containing compounds have long been used for the treatment of malaria, and systematic modification has led to a variety of antimalarial drugs with diverse substitutions around the quinoline ring.[2] The emergence of *Plasmodium falciparum* strains resistant to chloroquine, the cheapest and most widely used drug has caused global concern as the alternatives available are expensive and mostly beyond the reach of the affected population. Urgent efforts are therefore necessary to identify new classes of antimalarials[3] and develop them as drugs with varied modes of action to overcome the problem of resistance.[4]

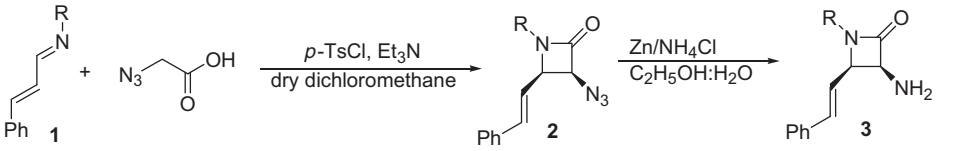
Since the advent of penicillin, β-lactam antibiotics have been the subject of much discussion and investigation; within both the scientific and public sectors.[5] The need for potent and effective β-lactamase antibiotics as well as more effective β-lactamase inhibitors has motivated medicinal chemists to design new functionalized 2-azetidinones, apart from their clinical use as antibacterial agents. These compounds have also been used as synthons in the preparation of various heterocyclic compounds of biological significance.[6] Because of this general trend of β-lactam use, the search for clinically useful β-lactams that are antibiotics or have other medically importance properties will continue. However, to the best of our knowledge, not many reports have appeared in literature concerning the exploration of β-lactams as potential antimalarial agents.[7] In continuation with our pursuits for the synthesis of functionalized monocyclic β-lactam-derived medicinally important scaffolds,[8] we report herein the synthesis and evaluation of 3-azido-, 3-amino- and 3-(1,2,3-triazol-1-yl)-β-lactams and their evaluation against chloroquine sensitive (CQ-S) and chloroquine resistant (CQ-R) strains of *P. falciparum* along with their cytotoxic profiles against KB cells. The styryl group has been introduced at C-4 position of the β-lactam ring because of its well documented potential in enhancing the anticancer profiles against KB cells.[9]

The desired scaffolds were synthesized by Staudinger reaction of appropriately functionalized 1-azadienes with azido-ketene generated in situ from azido-acetic acid in the presence of *p*-toluene sulphonylchloride and triethylamine in dry dichloromethane. The structure to 3-azido-2-azetidinones **2**, thus formed is assigned on the basis of analytical data and spectral evidences (Scheme 1). The *cis*-stereochemistry to the products was assigned on the basis of observed coupling constant $J = 5.4$ Hz between H^1 and H^2.[10]

The 3-azido-2-azetidinone was then reduced by following the zinc-ammonium chloride reduction protocol[11] to result in the isolation of corresponding 3-amino-2-azetidinones which were again

* Corresponding author.
E-mail address: vipan_org@yahoo.com (V. Kumar).

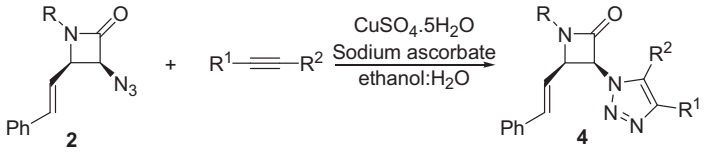
0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.05.119



Entry	R	% yield
2a	C_6H_5	74
2b	p-C_6H_4-CH_3	76
2c	p-C_6H_5Cl	77
2d	C_6H_{11}	73
2e	$C_6H_5CH_2$	75

Entry	R	% yield
3a	p-C_6H_5Cl	81
3b	C_6H_{11}	80
3c	p-C_6H_4-CH_3	83
3d	C_6H_5	79

Scheme 1.



Entry	R	R^1	R^2	% yield
4a	p-C_6H_5-CH_3	CO_2CH_3	CO_2CH_3	88
4b	C_6H_{11}	CO_2CH_3	CO_2CH_3	87
4c	p-C_6H_4-Cl	CO_2CH_3	CO_2CH_3	90
4d	C_6H_5	C_6H_5	H	83
4e	C_6H_{11}	C_6H_5	H	85
4f	p-C_6H_4-Cl	C_6H_5	H	86
4g	p-C_6H_5-CH_3	C_6H_5	H	88

Scheme 2.

characterized on the basis of analytical and spectral data. The 3-azido-2-azetidinone was also explored in click chemistry approaches utilizing azide-alkyne cycloaddition in the presence of $CuSO_4{\cdot}5H_2O$/sodium ascorbate in ethanol–water mixture at room temperature. The reaction resulted in good to excellent yields of triazole-tethered β-lactams which were assigned the structure on the basis of spectral studies and analytical data (Scheme 2).[12]

The synthesized β-lactams **2a–e** to **3a–d** were tested for their antimalarial potential against D10 (Chloroquine sensitive) strain of *P. falciparum*. Although the compounds didn't show any considerable antiplamodial activity when compared to the standard agent Chloroquine, they displayed an interesting behaviour with respect to the substituents on N-1 and C-3 of the lactam ring.

As evident from the activity data, compounds with azido substituent at C-3 (**2a**–**2e**) displayed much better antimalarial profiles as compared to the amine scaffolds (**3a**–**3d**). The activity was further enhanced with the introduction of *N*-alkyl substituent on N-1 with **2d** and **2e** having cyclohexyl and benzyl substituents displayed IC_{50} values of 2.36 and 4.70 μM respectively. The C-3 triazole tethered β-lactams were evaluated against 3D-7 (Chloroquine

Table 1
Antiplasmodial and cytotoxic profiles of the test compounds

Compound	IC_{50}[a] (μM) (D10) (CQ-S)	*c* log *P*[b]	Compound	3D7(CQ-S)[a] IC_{50} (μM)	K1 (CQ-R)[a] IC_{50} (μM)	W2 (CQ-R)[a] IC_{50} (μM)	KB cells (% inhibition)[a] (10 μM)	(100 μM)	IC_{50} (μM)	*c* log *P*[b]
2a	10.35	4.27	**4a**	ND	ND	>20	ND	ND	ND	4.00
2b	5.83	4.77	**4b**	>45.66	>45.66	>20	−7.70	8.07	>228.31	3.45
2c	4.47	5.13	**4c**	25.05	12.27	>20	19.48	56.48	145.29	4.35
2d	2.36	4.24	**4d**	>51.02	>51.02	11.725	10.02	58.26	91.25	5.80
2e	4.70	3.96	**4e**	1.13	1.21	1	76.75	99.70	8.36	5.93
3a	38.06	4.38	**4f**	7.86	7.09	1	16.84	79.30	84.17	6.65
3b	18.59	3.63	**4g**	11.99	3.42	1	14.66	80.79	89.15	6.30
3c	59.85	4.03	CQ	0.002	0.012	0.02366	31.42	96.95	35.95	
3d	29.28	3.53								
CQ (Chloroquine)	0.002		POD (Podophyllotoxin) (0.50 μM)				81.49	84.70	0.0005	

[a] CQ-S: chloroquine sensitive, CQ-R: chloroquine resistant.
[b] Calculated using Chemdraw Ultra 10.0;[14] ND = not determined.

sensitive); K1 (Chloroquine resistant); W2 (Chloroquine resistant) strains for their malarial efficacy and KB cells, a cell line derived from a human carcinoma of the nasopharynx, typically used as an assay for antineoplastic agents, for cytotoxic profiles. KB cells are maintained as monolayers in RPMI1640 + 10% HIFCS. All cultures and assays are conducted at 37 °C under an atmosphere of 5% CO_2/95% air mixture with Podophyllotoxin (POD) as the control drug.[13]

The test compounds showed comparatively much weaker cytotoxic profiles compared to the standard agent Podophyllotoxin while a comparable activity was observed when compared with chloroquine. The results clearly revealed that the compounds showed concentration dependent cytotoxicity with significant increase in growth inhibition as the concentration increases from 10 μM to 100 μM as shown by the compounds **4e**, **4f** and **4g**. The compounds **4d**–**4g** viz phenyl substituted triazoles have shown potentially better cytotoxic profile compared to the carbomethoxy-substituted triazoles (**4a**–**4c**). The enhancement in activity has been observed in case of *N*-cyclohexyl derivative **4e** exhibiting >99% growth inhibition with IC_{50} value of 8.36 μM better than that of CQ (Table 1). A similar trend was observed in anti-malarial evaluation. The compounds **4e**, **4f** and **4g** showed better antimalarial efficacy among the test compounds with **4e** exhibiting an IC_{50} value of 1.13, 1.21 and 1.00 μM against 3D-7, K1 and W2 strains. This further confirms the presence of alkyl substituent preferably cyclohexyl at N-1 and phenyl ring on the triazole being critical for good activity profiles.

In conclusion, the synthesis of 3-azido-, 3-amino- and 3-(1,2,3-triazol-1-yl)-β-lactams and their evaluation for antiplasmodial and cytotoxic activity has been described. The compounds with an *N*-cyclohexyl substituent in the β-lactam ring and a phenyl group on the triazole showed better cytotoxicity as well as antiplasmodial activity. The results described indicate that these compounds could serve as the basis for the development of a new group of non-cytotoxic antiplasmodial agents.

Acknowledgement

Financial assistance from DST New Delhi under Fast Track Young Scientist Scheme (VK) is gratefully acknowledged

Reference and notes

1. Guinovart, C.; Navia, M. M.; Tanner, M.; Alonso, P. L. *Curr. Mol. Med.* **2006**, *6*, 137.
2. (a) Kumar, A.; Katiyar, S. B.; Agarwal, A.; Chauhan, P. M. S. *Curr. Med. Chem.* **2003**, *10*, 1137; *Chemotherapy of Malaria*; Bruce-Chwatt, L. J., Black, R. H., Canfield, C. J., Clyde, D. F., Peters, W., Wernsdorfer, W. H., Eds., Second ed; World Health Organization: Geneva, 1986.
3. (a) Edwards, G.; Biagini, G. A. *Br. J. Clin. Pharmacol.* **2006**, *61*, 690; (b) Mutabingwa, T. K. *Acta Trop.* **2005**, *95*, 305.
4. (a) Vennerstrom, J. L.; Arbe-Barnes, S.; Brun, R.; Charman, S. A.; Chiu, C. K.; Chollet, J.; Dong, Y.; Dorn, A.; Hunziker, D.; Matile, H.; McIntosh, K.; Padmanilayam, M.; Tomas, J. S.; Scheurer, C.; Scorneaux, B.; Tang, Y.; Urwyler, H.; Wittlin, S.; Charman, W. N. *Nature* **2004**, *430*, 900; (b) Haynes, R. K.; Fugmann, B.; Stetter, J.; Rieckmann, K.; Heilmann, H.; Chan, H.; Cheung, M.; Lam, W.; Wong, H.; Croft, S.; Vivas, L.; Rattray, L.; Stewart, L.; Peters, W.; Robinson, B.; Edstein, M.; Kotecka, B.; Kyle, D. E.; Beckermann, B.; Gerisch, M.; Radtke, M.; Schmuck, G.; Steinke, W.; Wollborn, U.; Schmeer, K.; Romer, A. *Angew. Chem., Int. Ed.* **2006**, *45*, 2082.
5. Bose, A. K.; Manhas, M. S.; Banik, B. K.; Sriranjan, V. β-Lactams: Cyclic Amides of Distinction. In *The Amide Linkage: Selected structural Aspects in Chemistry, Biochemistry, and Material Science*; Greenberg, A., Breneman, C. M., Liebman, J. F., Eds.; Wiley Interscience: New York, 2000; p 157.
6. (a) Chen, A.; Nelson, A.; Tanikkul, A. N.; Thomas, E. J. *Tetrahedron Lett.* **2001**, *42*, 1251; (b) Brain, C. T.; Chen, A.; Nelson, A.; Tanikkul, N.; Thomas, E. J. *Tetrahedron Lett.* **2001**, *42*, 1247; (c) Sohma, Y.; Hayashi, Y.; Ito, T.; Matsumoto, H.; Kimura, T.; Kiso, Y. *J. Med. Chem.* **2003**, *46*, 4124; (d) Palomo, C.; Aizpurua, J. M.; Ganboa, I.; Oiarbide, M. *Curr. Med. Chem.* **2004**, *11*, 1837; (e) Ojima, I. *Acc. Chem. Res.* **1995**, *28*, 383; (f) Ojima, I.; Delaloge, F. *Chem. Soc. Rev.* **1997**, *26*, 377; (g) Fisher, J. F.; Meroueh, S. O.; Mobashery, S. *Chem. Rev.* **2005**, *105*, 395; (h) D'hooghe, M.; Dekeukeleire, S.; Leemans, E.; De Kimpe, N. *Pure Appl. Chem.* **2010**, *82*, 1749.
7. Nivsarkar, M.; Thavaselvam, D.; Prasanna, S.; Sharma, M.; Kaushik, M. P. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1371.
8. (a) D'hooghe, M.; Dekeukeleire, S.; Mollet, K.; Lategan, C.; Smith, P. J.; Chibale, K.; De Kimpe, N. *J. Med. Chem.* **2009**, *52*, 4058; (b) Anand, A.; Bhargava, G.; Kumar, V.; Mahajan, M. P. *Tetrahedron Lett.* **2010**, *51*, 2312; (c) Singh, P.; Bhargava, G.; Kumar, V.; Mahajan, M. P. *Tetrahedron Lett.* **2010**, *51*, 4272.
9. Nam, D. H.; Lee, K. Y.; Moon, C. S.; Lee, Y. S. *Eur. J. Med. Chem.* **2010**, *45*, 4288.
10. *3-Azido-1-cyclohexyl-4-styryl-azetidin-2-one* (**2d**). Yellow oil, yield: 72%; IR (KBr) ν_{max}: 2108, 1755, 1514, 1388 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 1.0–1.9 (m, 10H, Cyclohexyl H), 3.46 (m, 1H, Cyclohexyl H) 4.42 (dd, J = 5.4 Hz, 9.0 Hz, 1H, H_2), 4.7 (d, J = 5.4 Hz,1H, H_1), 6.15 (dd, J = 9.0 Hz, 15.9 Hz,1H, H_3), 6.68 (d, J = 15.9 Hz 1H, H_4), 7.26–7.45 (m, 5H, Ar-H), ^{13}C ($CDCl_3$, 75 Hz): 22.3, 27.1, 31.1, 47.3, 53.3, 63.4, 123.3, 126.1, 127.0, 127.1, 128.0, 134.3, 173.3; MS m/z 296 (M^+); Anal. calcd for $C_{17}H_{20}N_4O$: C, 68.89 H,6.80; N, 18.90. Found: C, 68.83; H, 6.72; N, 18.86.
11. Lin, W.; Zhang, X.; He, Z.; Jin, Y.; Gong, L.; Mi, A. *Synth. Commun.* **2002**, *32*, 3279.
12. *General Procedure for the synthesis of triazole-tethered β-lactams* (**4**). To a stirred solution of azide (1 mmol) in ethanol:water (10:1) was added in succession phenyl acetylene (1.1 mmol), copper sulphate (0.055 mmol) and sodium ascorbate (0.143 mmol) at room temperature. On completion, as monitored by tlc, water (15 ml) was added to the reaction mixture and extracted with chloroform (2 × 50). Combined oragnic layers were dried over anhydrous sodium sulphate and concentrated under reduce pressure to result in a crude product which was recrystallized using chloroform: hexane (2:10) mixture.
1-Cyclohexyl-3-(4-phenyl-[1,2,3]triazol-1-yl)-4-styryl-azetidin-2-one (**4e**). White solid, yield 70%; mp 195–197 °C; IR (KBr) ν_{max}: 1755, 1514, 1388 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 1.0–1.9 (m,10H, Cyclohexyl ring), 3.46 (m,1H, Cyclohexyl ring), 5.13 (dd,J = 5.4 Hz, 9.0 Hz, 1H, H_2), 5.80 (dd, J = 9.0 Hz, 15.9 Hz, 1H, H_3), 6.36 (d, J = 5.4 Hz, 1H, H_1), 6.71 (d, J = 15.9 Hz, 1H, H_4), 7.26–7.78 (m, 10H, Ar-H), 7.94 (s, 1H, triazole ring), ^{13}C ($CDCl_3$, 75 Hz): 22.3, 27.1, 31.1, 47.3, 60.4, 67.6, 123.5, 126.4, 127.2, 127.6, 127.9, 128.8, 129.0, 129.4, 131.4, 134.5, 134.8, 136.4, 173.3.; MS m/z 398 (M^+); Anal. calcd for $C_{25}H_{26}N_4O$: C, 75.35 H, 6.58; N, 14.06. Found: C, 75.30; H, 6.52; N, 13.99.
13. (a) Damayanthi, Y.; Lown, J. W. *Curr. Med. Chem.* **1998**, *5*, 205; (b) You, Y. *Curr. Pharm. Des.* **2005**, *11*, 1695; (c) Xu, H.; Lv, M.; Tian, X. *Curr. Med. Chem.* **2009**, *16*, 327.
14. (a) Kasim, N. A.; Whitehouse, M.; Ramachandran, C.; Bermejo, M.; Lennernas, H.; Hussain, A. S.; Junginger, H. E.; Stavchansky, S. A.; Midha, K. K.; Shah, V. P.; Amidon, G. L. *Mol. Pharma.* **2004**, *1*, 85–96; (b) Podunavac-kuzmanovic, S. O.; Cvetkovic, D. D. *Chem. Ind. Chem. Eng. Quart.* **2011**, *17*, 9; (c) Anoaica, P. G.; Anoaica, M. B.; Amzoiu, E.; Giubelan, L.; Tatucu, M. *Farmacia* **2010**, *58*, 515; (d) Uesawa, Y.; Mohri, K. *Yakugaku Zasshi* **2008**, *128*, 117.