Bioorganic & Medicinal Chemistry Letters 22 (2012) 57–61

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis, docking and in vitro antimalarial evaluation of bifunctional hybrids derived from β-lactams and 7-chloroquinoline using click chemistry

Pardeep Singh [a], Parvesh Singh [c], Malkeet Kumar [a], Jiri Gut [d], Philip J. Rosenthal [d], Kewal Kumar [a], Vipan Kumar [a,*], Mohinder P. Mahajan [a,b], Krishna Bisetty [c]

[a] Department of Chemistry, Guru Nanak Dev University, Amritsar 143 005, India
[b] Apeejay Stya Research Foundation, Gurgaon, India
[c] Department of Chemistry, Durban University of Technology, Durban 4000, South Africa
[d] Department of Medicine, University of California, San Francisco, CA, USA

ARTICLE INFO

Article history:
Received 10 September 2011
Revised 16 November 2011
Accepted 19 November 2011
Available online 28 November 2011

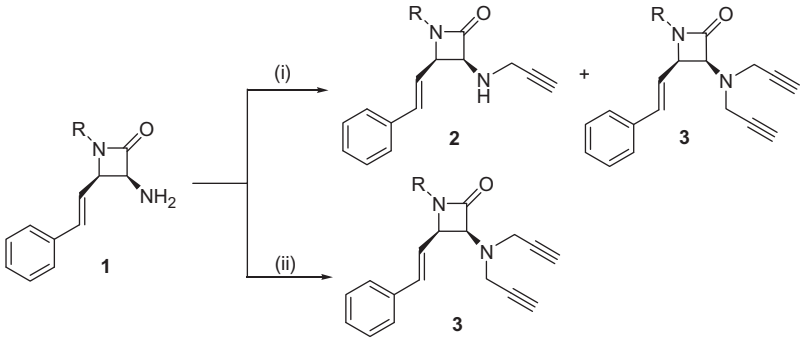
ABSTRACT

1,2,3-Triazole tethered β-lactam and 7-chloroquinoline bifunctional hybrids were synthesized and evaluated as potential antimalarial agents. Activity against cultured *Plasmodium falciparum* was dependent on the N-substituent of the β-lactam ring as well as the presence of bis-triazole at the C-3 position. The observed activity profiles were further substantiated by docking studies via inhibition of *P. falciparum* dihydrofolate reductase (PfDHFR), a potential target for the development of new anti-malarials.

© 2011 Elsevier Ltd. All rights reserved.

Malaria is a tropical parasitic disease and one of the top three killers among communicable diseases.[1] It is a public health problem in more than 90 countries inhabited by about 40% of the world population. Despite decades of research and successful development of combination therapy, malaria remains a devastating disease in part because of resistance of *Plasmodium falciparum* to standard antimalarial drugs including chloroquine.[2] Chloroquine probably acts against *P. falciparum* by inducing heme accumulation in the parasite membrane, consequently disturbing cation homeostasis and resulting in parasite death.[3,4] CQ resistance had largely been attributed to mutations in the PfCRT gene that encodes for the protein believed to mediate efflux of the drug from the digestive vacuole of the parasite,[5] leading to sub-optimal drug concentrations. Resistance is also increasing to multiple other antimalarial drugs, and of particular concern are early signs of resistance to new artemisinin-based therapies.[6]

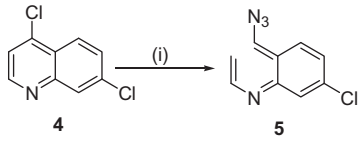
Development of antiplasmodial agents aimed at a single parasite target or specialized process has failed to stem the tide of drug resistance. In this sense, double-drug development and multi-therapeutic strategies, which utilize heterocyclic skeleton of two drugs, are promising.[7–9] These strategies have the potential to overcome resistance mechanisms. Given the unique pharmacological effect of quinoline based antimalarials, we are interested in the design and synthesis of quinoline-containing dual inhibitors or 'double drugs' that will potentially inhibit hemozoin formation, but not be recognized by the proteins involved in chloroquine efflux.[10]



Scheme 1. Reagents and conditions: (i) K_2CO_3 (1.2 mmol), propargyl bromide (1.1 mmol), DMF, rt, 6 h; (ii) K_2CO_3 (2.2 mmol), propargyl bromide (2.5 mmol), DMF, rt, 6 h.

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

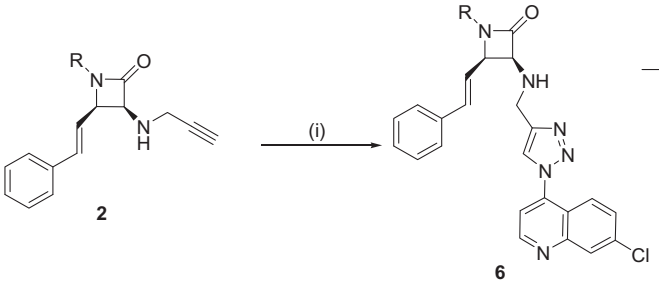
0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.11.082



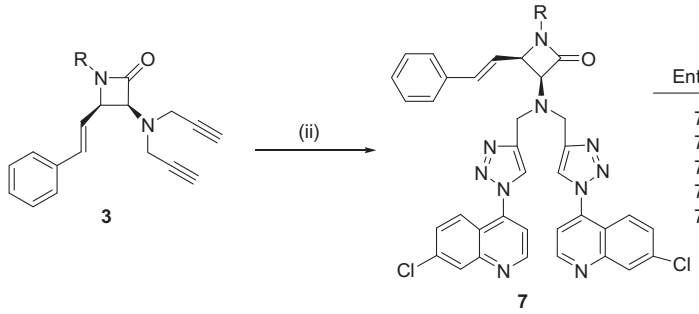
Scheme 2. Reagents and conditions :(i) NaN_3, DMF, 65 °C, 6h.

Further, rationalization of literature have revealed the increased utility of β-lactams as potential antimalarials.[11] Recent reports from our group have described the synthesis and antimalarial/anticancer evaluation of C-3 functionalized β-lactam derivatives with marked dependency of activity on the substituents at N-1 of the β-lactam ring and C-3 of the substituted triazole ring.[12]

In continuation of our pursuit of the synthesis of biologically potent novel functional entities,[13] we present herein the synthesis and evaluation of triazole tethered bifunctional hybrids of 7-chloroquinoline with substituted β-lactams. We envisaged that combining two intrinsically antimalarial moieties, 7-chloroquinoline (hemozoin formation inhibitor) and a β-lactam (potential inhibitor of dihydrofolate reductase)[14] linked via a triazole (structurally related to triazine) would result in the development of potent antimalarials. The styryl group has been introduced at C-4 position of the β-lactam ring because of its well documented



Entry	R	%yield
6a	*p*-C_6H_5-CH_3	84
6b	C_6H_5	82
6c	*p*-C_6H_5-F	85
6d	C_6H_{11}	84
6e	$C_6H_5CH_2$	83



Entry	R	%yield
7a	*p*-C_6H_5-CH_3	85
7b	C_6H_5	87
7c	*p*-C_6H_5-F	85
7d	C_6H_{11}	88
7e	$C_6H_5CH_2$	86

Scheme 3. Reagents and conditions: (i) **5** (1 mmol), $CuSO_4{\cdot}5H_2O$ (0.05 mmol), sodium ascorbate (0.13 mmol), EtOH–H_2O, rt, 8 h; (ii) **5** (2 mmol), sodium ascorbate (0.26 mmol), $CuSO_4{\cdot}5H_2O$ (0.1 mmol), EtOH–H_2O, rt, 8 h.

Table 1
Antimalarial and docking results of the test compounds

Compound	R	W2 (CQ-R)[a] IC_{50} (μM)	*c*log*P*[b]	Wild type		Mutant type	
				Donor/acceptor hydrogen bond	Binding energy[c] (Kcal/mol)	Donor/acceptor hydrogen bond	Binding energy[c] (Kcal/mol)
6a	*p*-C_6H_5–CH_3	>10	7.16	Triazole-N···HO-SER[108]	1.2	——	−5.9
6b	C_6H_5	1.9	6.66	Quinoline-N···HN-GLY[44] Triazole-H···HN-SER[108]	−13.3	NH···O-ASN[108]	−11.0
6c	*p*-C_6H_5–F	>10	7.52	Triazole-N···HO-SER[108]	−4.5	——	20.0
6d	C_6H_{11}	4.3	6.76	NH···O-SER[111]	−17.2	Triazole-N···HO-SER[111]	−15.1
6e	$C_6H_5CH_2$	5.9	6.48	Quinoline-Cl···HO-THR[107]	−16.5	——	−11.2
7a	*p*-C_6H_5–CH_3	1.5	10.32	Triazole-N···HN-SER[22] Triazole-N···HO-SER[22] Triazole-N···HO-SER[22] Lactam-O···HN-ARG[38]	−42.5	Triazole-N···HO-SER[22]	−53.5
7b	C_6H_5	2.1	9.82	Lactam-O···HN-ARG[38]	−39.8	Lactam-O···HN-ARG[38] Triazole-N···HO-THR[36]	−51.2
7c	*p*-C_6H_5–F	1.1	10.68	Triazole-N···HN-SER[22] Triazole-N···HO-SER[22] Lactam-O···HN-ARG[38]	−49.4	Triazole-N···HN-SER[22] Triazole-N···HO-SER[22] Lactam-O···HN-ARG[38] Quinoline-Cl···HN-ASN[595]	−61.3
7d	C_6H_{11}	2.8	9.92	Triazole-N···HN-SER[22] Triazole-N···HO-SER[22]	−50.1	Lactam-O···HN-ARG[38] Triazole-N···HO-SER[22] Triazole-N···HO-SER[22]	−62.4
7e	$C_6H_5CH_2$	2.7	9.64	Quinoline-Cl···HN-THR[36]	−41.6	Quinoline-Cl···HN-THR[36]	−55.1

[a] CQ-R: chloroquine resistant.
[b] Calculated using Chemdraw Ultra 10.0.
[c] Binding energy = energy of complex − energy of ligand − energy of receptor.[19]

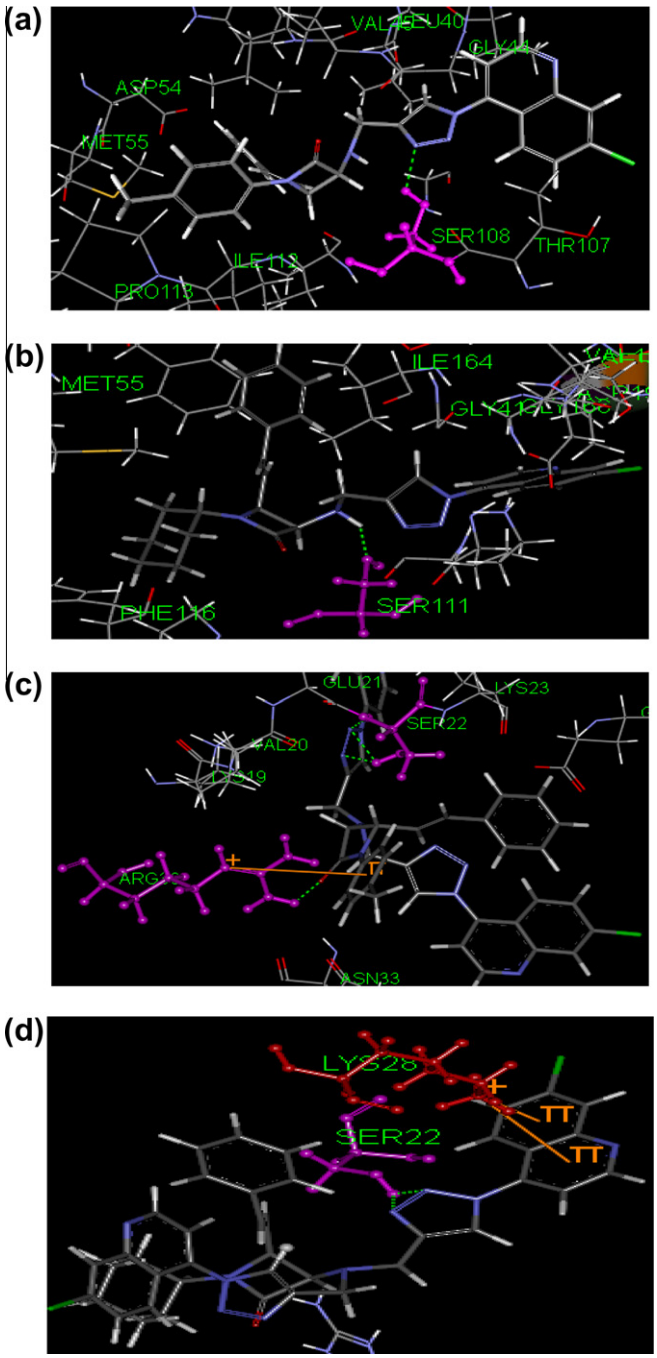


Figure 1. Docked conformations of compound **6a** (a), **6d** (b), **7a** (c) and **7d** (d) showing important amino acid residues of wild-type PfDHFR. All interacting amino acids are presented in ball and stick form, while ligands are shown as sticks. Amino acids exhibiting cation-π and π–π interactions with the ligands are colored in red, while those participating in hydrogen bond formation are colored in pink. Hydrogen bonds are shown as dotted green lines.

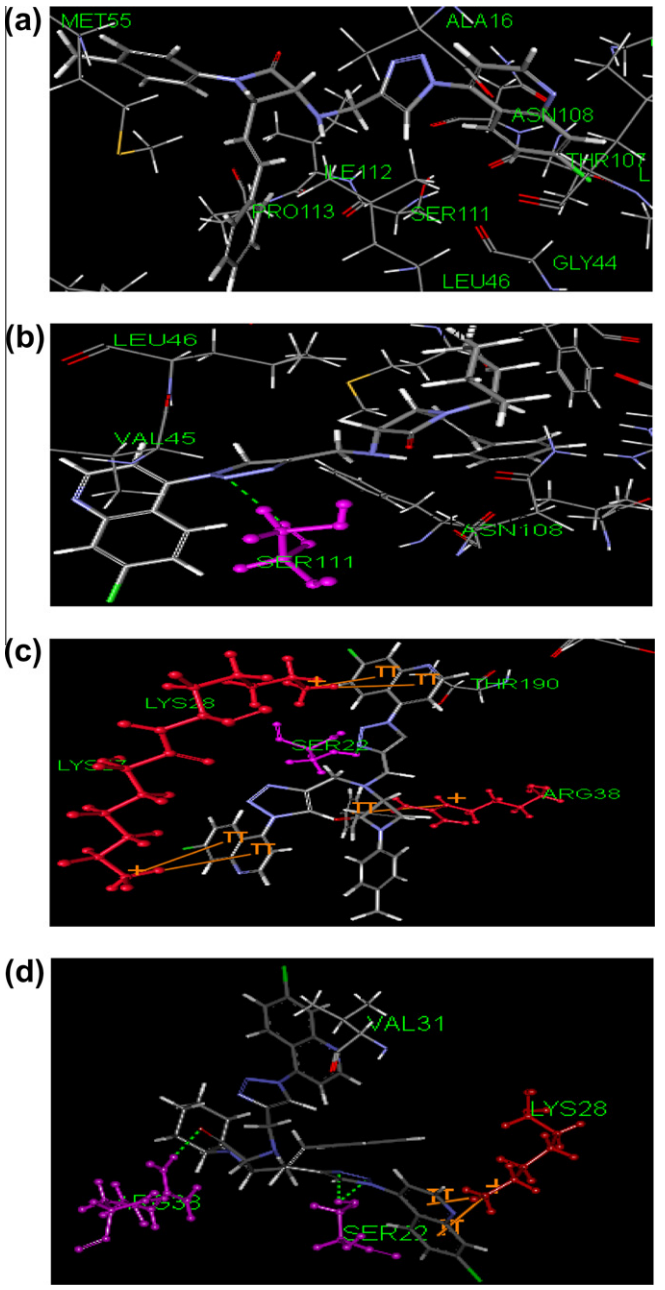


Figure 2. Docked conformations of compound **6a** (a), **6d** (b), **7a** (c) and **7d** (d) showing important amino acid residues of quadruple mutant type PfDHFR. All interacting amino acids are presented in ball and stick form, while ligands are shown as sticks. Amino acids exhibiting cation-π and π-π interactions with the ligands are colored in red, while those participating in hydrogen bond formation are colored in pink. Hydrogen bonds are shown as dotted green lines.

potential in enhancing the anticancer profiles against KB cells.[15] A molecular docking study was conducted to gain insight into the mechanism of action of the novel compounds.

3-Amino-2-azetidinone **1**, required for the synthesis of the desired hybrids was synthesised by a previously reported procedure.[12] The N-alkylation of **1** was carried out using propargyl bromide in anhydrous DMF in the presence of potassium carbonate as a base. Interestingly, use of 1.1 equiv of propargyl bromide resulted in the isolation of mono as well as di-propargylated products in the ratio of 75:25 as evidenced by the ^{1}H NMR analysis of the crude reaction mixture. Both the products were isolated using column chromatography eluting with a mixture of EtOAc–hexane (10:90 for compound **2** and 30:70 for compound **3**). The treatment of **1** with 2.1 equiv of propargyl bromide resulted in the isolation of exclusive di-propargylated product **3** in good yields. The structures of compounds **2** and **3** were assigned on the basis of analytical evidences and spectral data (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.

4-Azido-7-chloroquinoline **5**, another precursor required for the synthesis of target scaffolds was prepared by the method described by de Souza et al.[16] involving the treatment of 4,7-dichloroquinoline

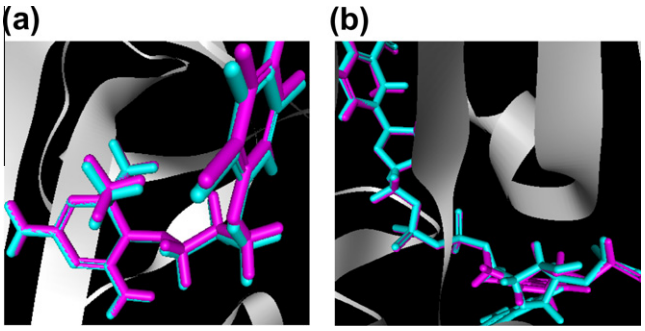


Figure 3. (a) Overlay of the WR99210 conformations obtained from docking (in Pink) and the X-ray complex (in Light Blue) of wild-type PfDHFR. (b) Overlay of the NDP610 conformations obtained from docking (in Pink) and the X-ray structure (in Light Blue) of mutant PfDHFR. Ligands are shown in sticks while proteins are presented in ribbon format.

with 2 equiv of sodium azide in anhydrous DMF at 60 °C for 6 h (Scheme 2).

The desired hybrids **6** and **7** were synthesized using Huisgen's 1,3-dipolar cycloaddition reaction of **2** or **3** with 4-azido-7-chloroquinoline **5** (Scheme 3). The structures of the hybrids were assigned on the basis of analytical and spectral data.[17]

The antimalarial activities of synthesized β-lactam based hybrids **6a–e** and **7a–e** were evaluated against the chloroquine resistant strain of *P. falciparum* using methods as previously described[18] (Table 1). The test compounds were not as active as the control drugs, However, with the exception of **6a** and **6c**, all the test compounds showed a reasonable antimalarial activity with IC_{50}s ranging from 1.1 to 5.9 μM. The presence of mono- and bis-1,2,3-triazole tethered 7-chloroquinolines considerably influence the antimalarial profile with bis-compounds exhibiting better activity than corresponding mono-scaffolds except for **6b** and **7b**. The increase in antimalarial activity in the bis-triazole might be attributed to either solubility enhancing properties of triazole rings or increased heme-binding of 7-chloroquinolines. Further, the presence of a substituent on the nitrogen of the β-lactam ring also influenced activity with the antimalarial potency of *N*-aryl substituted hybrids influenced by the presence of a C-3 triazole ring while this effect was minimal in the case of *N*-alkyl derivatives (compare **6d–e** with **7d–e**), substantiating our previous observation of activity enhancement by an N-alkyl group on β-lactams.

In order to substantiate the observed activity profile and to offer insight into the mechanisms of action of the test compounds, molecular docking studies were performed into the binding pocket of *P. falciparum* dihydrofolate reductase (PfDHFR) considering both the wild type (1J3I.pdb) and a quadruple mutant (N51I, C59R, S108 N, I164L, 3QG2.pdb). The docking simulations were performed using the Ligand Fit[19,20] module of the Discovery Studio (DS, version 2.5, Accelrys Software Inc.). The docked conformations of some compounds (**6a**, **6d**, **7a**, and **7d**) in the active site of wild type and mutant PfDHFR enzymes are diagrammatically represented in Figures 1 and 2, respectively, while the corresponding docking results viz donor–acceptor H bond and binding energy for each compound are summarized in Table 1. The results reveal a significant difference in the predicted binding energies (BEs) of the complexes of mono-triazoles (**6**) and bis-triazoles (**7**) especially in the case of the mutant PfDHFR. For instance, compound **7c**, observed to be the most potent anti-malarial compound (IC_{50} = 1.1), exhibited the lowest BEs for both wild type (−49.4 kcalmol^{-1}) and mutant (−61.3 kcalmol^{-1})-PfDHFR complexes. The greater intermolecular hydrogen bonding and other non-bonded forces (π–π and cation-π interactions) between the amino acid residues of both enzymes, as depicted in Figures 1 and 2, may account for the lower BEs and higher stability of these complexes. The triazole ring of compounds **7** was predicted to be predominantly engaged in hydrogen bonding with key amino acid residues (Ser22, Arg38, Thr36) of both PfDHFR enzymes, suggesting a role in the biological activity of these compounds. Similarly, the absence or lower extent of intermolecular hydrogen bonding and non-bonded interactions in the mono-triazole compounds **6a** and **6c** could be responsible for the higher BEs of their complexes with both enzymes, and these results substantiate a good relationship between the anti-malarial activity and BEs of the complexes. Furthermore, the anti-malarial effect of compounds bearing alkyl substituents on their lactam-nitrogens (**6** and **7**) (Table 1), can also be explained on the basis of the stability of their complexes with the PfDHFR enzymes. For example, all *N*-alkyl compounds, except **7e**, irrespective of the PfDHFR enzymes studied, exhibited lower BEs for their complexes compared to their corresponding *N*-aryl analogues. Finally, the predicted binding conformations of selective inhibitors (WR99210 and NDP 610, for wild type and quadruple mutant PfDHFR, respectively) with a root mean square deviation (all atoms) <1 Å, with respect to their X-ray structures (http://www.pdb.org), clearly validated the proposed binding conformations of the synthesized compounds (Figs. 3a and b).

In conclusion the present Letter describes the synthesis of β-lactam-7-chloroquinoline bifunctional hybrids and their evaluation as antimalarial agents against W2 strain of *P. falciparum*. The observed activity profile were further corroborated via docking simulations performed using the ligand fit module. Further studies in order to improve the activity profiles of the scaffolds are underway in the lab and will soon be communicated.

Acknowledgement

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

References and notes

1. Sachs, J.; Malaney, P. *Nature* **2002**, *415*, 680.
2. Winstanley, P. A.; Ward, S. A.; Snow, R. W. *Microbes Infect.* **2002**, *4*, 157.
3. Biagini, G. A.; O'Neill, P. M.; Nzila, A.; Ward, S. A.; Bray, P. G. *Trends in Parasitol.* **2003**, *19*, 479.
4. Ginsburg, H.; Famin, O.; Zhang, J.; Krugliak, M. *Biochem. Pharmacol.* **1998**, *56*, 1305.
5. Cravo, P.; Culleton, R.; Afonso, A.; Ferreira, I. D.; do Rosario, V. E. *Anti-Infect. Agents Med. Chem.* **2006**, *5*, 63.
6. Dondorp, A. M.; Nosten, F.; Yi, P.; Das, D.; Phyo, A. P.; Tarning, J.; Lwin, K. M.; Ariey, F.; Hanpithakpong, W.; Lee, S. J.; Ringwald, P.; Silamut, K.; Imwong, M.; Chotivanich, K.; Lim, P.; Herdman, T.; An, S. S.; Yeung, S.; Singhasivanon, P.; Day, N. P. J.; Lindegardh, N.; Socheat, D.; White, N. J. *N. Engl. J. Med.* **2009**, *361*, 455.
7. Morphy, R.; Rankovich, Z. *J. Med. Chem.* **2005**, *48*, 6523.
8. Espinoza-Fonseca, L. M. *Bioorg. Med. Chem.* **2006**, *14*, 896.
9. Meunier, B. *Acc. Chem. Res.* **2008**, *41*, 69.
10. Kouznetsov, V. V.; Gomez-Barrio, A. *Eur. J. Med. Chem.* **2009**, *44*, 3091.
11. (a) Nivsarkar, M.; Thavaselvam, D.; Prasanna, S.; Sharma, M.; Kaushik, M. P. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1371; (b) D'hooghe, M.; Dekeukeleire, S.; Mollet, K.; Lategan, C.; Smith, P. J.; Chibale, K.; De Kimpe, N. *J. Med. Chem.* **2009**, *52*, 4058; (c) Avile, E.; Rodriguez, A. *Org. Lett.* **2010**, *12*, 5290.
12. Singh, P.; Sachdeva, S.; Raj, R.; Kumar, V.; Mahajan, M. P.; Nasser, S.; Vivas, L.; Gut, J.; Rosenthal, P. J.; Feng, T. S.; Chibale, K. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4561.
13. (a) Anand, A.; Bhargava, G.; Kumar, V.; Mahajan, M. P. *Tetrahedron Lett.* **2010**, *51*, 2312; (b) Singh, P.; Bhargava, G.; Kumar, V.; Mahajan, M. P. *Tetrahedron Lett.* **2010**, *51*, 4272; (c) Raj, R.; Mehra, V.; Singh, P.; Kumar, V.; Bhargava, G.; Mahajan, M. P.; Handa, S.; Slaughter, L. G. *Eur. J. Org. Chem.* **2011**, *14*, 2697; (d) Singh, P.; Kaur, S.; Kumar, V.; Bedi, P. M. S.; Mahajan, M. P.; Sehar, I.; Pal, H. C.; Saxena, A. K. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3017; (e) Singh, P.; Mehra, V.; Anand, A.; Kumar, V.; Mahajan, M. P. *Tetrahedron Lett.* **2011**, *52*, 5060; (f) Singh, P.; Raj, R.; Kumar, V.; Bedi, P. M. S.; Mahajan, M. P.; Kaur, T.; Saxena, A. K. *Eur. J. Med. Chem.* **2011**, 0000.
14. (a) Walsh, J. J.; Coughlan, D.; Heneghan, N.; Gaynor, C.; Bell, A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3599; (b) Ratelli, G.; Pacchioni, S.; Sirawaraporn, W.; Sirawaraporn, R.; Parenti, M. D.; Ferrari, A. M. *J. Med. Chem.* **2003**, *46*, 2834; (c) Sunduru, N.; Sharma, M.; Srivastava, K.; Rajakumar, S.; Puri, S. K.; Saxena, J. K.; Chauhan, P. M. S. *Bioorg. Med. Chem. Lett.* **2009**, *17*, 6451; (d) Kumar, A.;

Srivastava, K.; Kumar, S. R.; Siddiqi, M. I.; Puri, S. K.; Sexana, J. K.; Chauhan, P. M. S. *Eur. J. Med. Chem.* **2011**, *46*, 676; (e) Kumar, A.; Srivastava, K.; Rajakumar, S.; Puri, S. K.; Chauhan, P. M. S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6530; (f) Gupta, L.; Srivastava, K.; Singh, S.; Puri, S. K.; Chauhan, P. M. S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3306.

15. Nam, D. H.; Lee, K. Y.; Moon, C. S.; Lee, Y. S. *Eur. J. Med. Chem.* **2010**, *45*, 4288.
16. De Souza, M. V. N.; Pais, K. C.; Kaiser, C. R.; Peralta, M. A.; de L Ferreira, M.; Lourenco, M. C. S. *Bioorg. Med. Chem.* **2009**, *17*, 1474.
17. *General procedure and analytical data for β-lactam-chloroquinoline hybrids* (**6** and **7**): To a stirred solution of azide **5** (1 mmol for **2** and 2 mmol for **3**) in ethanol–water (10:1) was added in succession appropirate acetylenic lactam **2** or **3** (1 mmol), copper sulphate (0.055 mmol for **2** and 0.1 mmol for **3**) and sodium ascorbate (0.13 mmol for **2** and 0.26 for **3**) at room temperature. On completion, as monitored by tlc, water (15 ml) was added to the reaction mixture and extracted with chloroform (2 × 50 ml). Combined organic layers were dried over anhydrous sodium sulphate and concentrated under reduced pressure to result in a crude product which was purified by silica gel chromatography.
3-{[1-(7-Chloroquinolin-4-yl)-1*H*-[1,2,3]triazol-4-ylmethyl]-amino}-4-styryl-1-p-tolyl-azetidin-2-one (**6a**): White solid, Yield 84%; mp 157–158 °C; IR (KBr) ν_{max}: 3017, 1747, 1523 cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$): δ 2.29 (s, 3H, $-CH_3$), 4.29 (AB quartet, J = 7.5, 13.7 Hz, 2H, $-CH_2-$), 4.74 (d, J = 5.1 Hz, 1H, H_1), 4.92 (dd, J = 5.1, 8.1 Hz, 1H, H_2), 6.44 (dd, J = 8.1, 15.9 Hz, 1H, H_3), 6.79(d, J = 15.9 Hz, 1H, H_4), 7.09–7.32 (m, 9H, ArH), 7.35 (d, J = 4.2 Hz, 1H, H_5) 7.54 (dd, J = 1.8, 9.0 Hz, 1H, H_8), 8.01 (d, J = 9.0 Hz, 1H, H_9), 8.21 (s, 1H, triazole ring), 8.23 (d, J = 1.8 Hz, 1H, H_7), 8.97 (d, 1H, J = 4.2 Hz, H_6); ^{13}C NMR: ($CDCl_3$, 75 Hz): 20.9, 49.1, 60.1, 61.3, 115.7, 117.2, 120.3, 122.2, 123.9, 124.7, 126.5, 127.4, 127.7, 128.7, 128.9, 129.4, 129.6, 132.4, 134.2, 135.2, 135.7, 136.8, 142.7, 150.1, 151.2, 172.1; MS m/z 522(M^+); Analysis calculated for $C_{30}H_{25}ClN_6O$: C, 69.16 H, 4.84; N, 16.13. Found: C, 69.12; H, 4.79; N, 16.10.
3-{Bis-[1-(7-chloroquinolin-4-yl)-1*H*-[1,2,3]triazol-4-ylmethyl]-amino}-1-cyclohexyl-4-styryl-azetidin-2-one (**7d**): White solid, Yield 88%; mp 133–134 °C; IR (KBr) ν_{max}: 1755, 1512 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.09–1.96 (m, 10H, cyclohexyl ring), 3.56 (m, 1H, cyclohexyl H), 4.28 (AB quartet, J = 13.7, 7.5 Hz, 4H, 2x-CH_2-), 4.41 (dd, J = 5.1, 8.7 Hz, 1H, H_2), 4.6 (d, J = 5.1 Hz, 1H, H_1), 6.32 (dd, J = 8.7, 15.9 Hz, 1H, H_3), 6.74 (d, J = 15.9 Hz, 1H, H_4), 7.12–7.29 (m, 5H, ArH), 7.35 (d, J = 4.4 Hz, 2H, H_8) 7.55 (dd, J = 2.1 Hz, 9.3 Hz, 2H, H_{10}), 8.01 (d, J = 9.3 Hz, 2H, H_9), 8.19 (s, 2H, triazole ring), 8.26 (d, J = 2.1 Hz, 2H, H_7), 9.01 (d, J = 4.4 Hz, 2H, H_6); ^{13}C NMR ($CDCl_3$, 75 Hz): 22.4, 27.3, 30.9, 47.2, 58.3, 59.9, 60.8, 122.2, 124.5, 125.5, 126.6, 128.5, 128.6, 129.4,129.6, 132.4, 134.3, 134.8, 135.4, 136.4, 136.9, 143.2, 150.1, 151.3 173.2.; MS m/z 755(M^+); Analysis calculated for $C_{41}H_{36}Cl_2N_{10}O$: C, 65.16 H, 4.80; N, 18.53. Found: C, 65.31; H, 4.68; N, 18.77.
18. Coteron, J. M.; Catterick, D.; Castro, J.; Chaparro, M. J.; Diaz, B.; Fernandez, E.; Ferrer, S.; Gamo, F. J.; Gordo, M.; Gut, J.; Heras, L. D.; Legac, J.; Marco, M.; Miguel, J.; Munoz, V.; Porras, E.; de la Rosa, J. C.; Ruiz, J. R.; Sandoval, E.; Ventosa, P.; Rosenthal, P. J.; Fiandor, J. M. *J. Med. Chem.* **2010**, *53*, 6129.
19. Venkatachalam, C. M.; Jiang, X.; Oldfield, T.; Waldman *J. Mol. Graphics Modell.* **2003**, *21*, 289.
20. Choi, Y.; Kim, D. W.; Park, H.; Hwang, S.; Jeong, K.; Jung, S. *Bull. Korean Chem. Soc.* **2005**, *26*, 769.