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Exploration of click reaction for the synthesis of modified nucleosides as chitin synthase inhibitors

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ABSTRACT

Click reaction approach toward the synthesis of two sets of novel 1,2,3-triazolyl linked uridine derivatives **19a–19g** and **21a–21g** was achieved by Cu(I)-catalyzed 1,3-dipolar cycloaddition of 5′-azido-5′-deoxy-2′,3′-*O*-(1-methylethylidene)uridine (**17**) with propargylated ether of phenols **18a–18g** and propargylated esters **20a–20g**. Structure of one of the representative compound **19d** was unambiguously confirmed by X-ray crystallography. Chitin synthase inhibition study of all these compounds **19a–19g** and **21a–21g** was carried out to develop antifungal strategy. Compounds **19d, 19e, 19f,** and **21f** were identified as potent chitin synthase inhibitors by comparing with nikkomycin. Compounds **19a, 19b, 19c, 19d, 21a**, and **21b** showed good antifungal activity against human and plant pathogens. Compounds **19a, 19b, 19f, 21c, 21f**, and **21g** were identified as lead chitin synthase inhibitors for further modifications by comparing results of inhibition of growth, % germ tube formation and chitin synthase activity.

Recently Zhou et al. have reported the synthesis of 1,2,3-triazole functionalized thymidines **1**, **2**, and **3** (Fig. 1), which were found to be antiviral.[1] Synthesis of triazole linked coumarin nucleoside conjugates **4** and **5** (Fig. 1) was documented by Kosiova et al.[2] These materials have wide applications as fluorescent probes and signaling units. Solid phase synthesis of 1,2,3-triazolyl uridines **6** (Fig. 1) from 5′-azido uridine and mono substituted and carboxy-substituted alkyne components was reported by Epple et al.[3] The synthesis of 4-substituted triazolyl-nucleosides **7** and **8** (Fig. 1) showing cytostatic activity on CEM tumor cell lines was reported by Akri et al.[4]

The chitin, β-1,4-linked *N*-acetylglucosamine polymer, is the main structural component of the fungal cell wall. As it is absent in plants and mammals, targeting the enzyme chitin synthase involved in the synthesis of chitin could be one of the eco-friendly strategies for the control of plant pathogenic fungi in agriculture.[5] Discovery of polyoxins **9** (Fig. 2) which are peptidyl nucleoside antibiotics, isolated from an actinomycete, *Streptomyces cacaoi* in the sixties by Isono and co-workers[6,7] has opened a new era in the field of development of novel fungicides.[8–10] Naturally occurring antifungal uridine nucleosides such as polyoxins **9**, nikkomycin Z (NZ) **10** (Fig. 2) and uridine di-phosphate (UDP)[11] are known to be chitin synthase inhibitors. The inhibitory activity of these molecules is due to their structural similarity to UDP-*N*-acetyl-D-glucosamine, a substrate for chitin synthase. Therefore most of these compounds possess uridine as a basic constituent with peptide side chain. Modifications for enhanced biological activities at uracil moiety, ribose ring and/or phosphate chain, as well as in the peptide side chain of NZ have been reported in the literature.[12,13]

Phenols and phenyl acetic acids are present in a large number of biologically active compounds. Phenolic ether linkages are present in many naturally occurring bioactive molecules like exophilic acid **11** inhibiting HIV-integrase,[14] methyl-4-[[(2*E*)-3,7-dimethyl-2,6-octadienyl]oxy]-3-hydroxybenzoate **12** (Fig. 3) which is antitumoral, antimicrobial and antioxidative too.[15] Along with these some synthesized compounds like C_3-symmetric triantennary *N*-acetyl-(1–6)-β-D-glucosamine octadecaoligosaccharide derivatives **13** (Fig. 3) containing phenolic ether linkage showed good antitumor activity.[16]

Compounds containing phenyl acetic acid ester linkages such as methyl-2-acetyl-3,5-dihydroxy phenyl acetate **14** possesses antibacterial activity,[17] some comb sugar polymers **15** (Fig. 3) are useful in protein–carbohydrate interaction study.[18]

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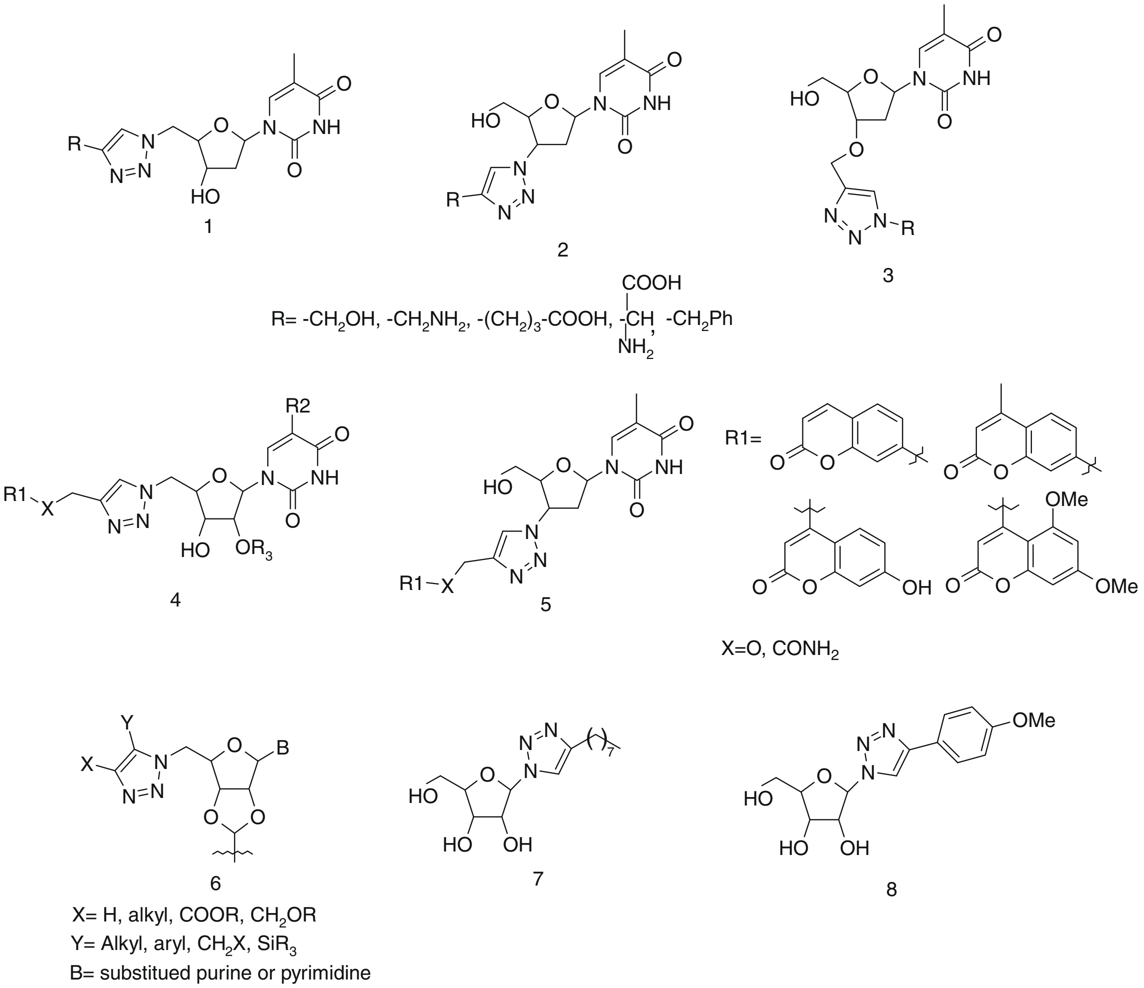


Figure 1. Synthetic nucleoside analogues.

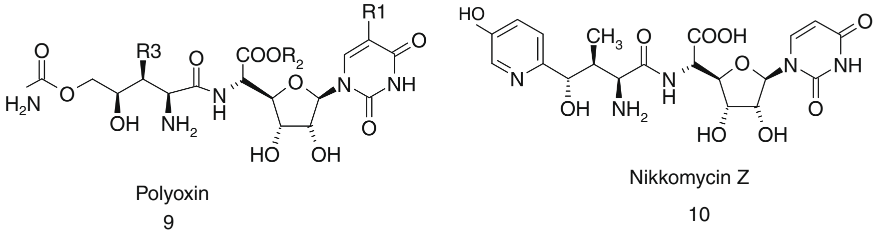


Figure 2. Structure of naturally occurring antifungal uridine nucleosides.

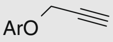
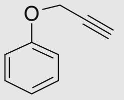
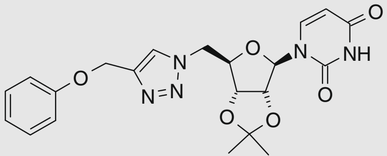
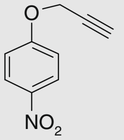
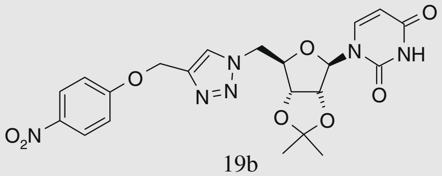
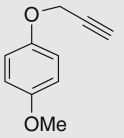
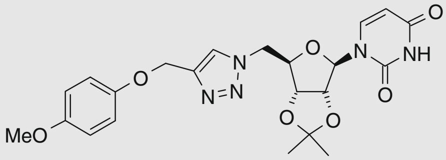
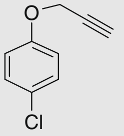
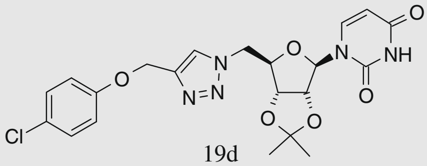
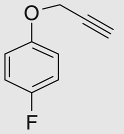
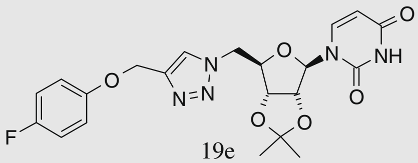
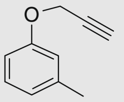
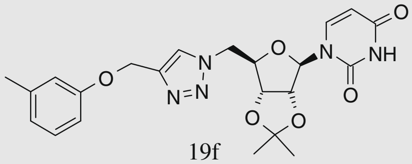
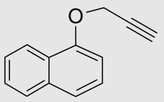
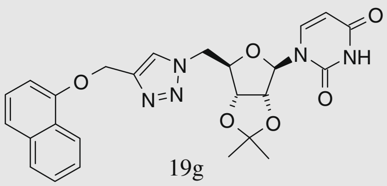
In the present investigations modification in uridine nucleosides with 1,2,3-triazoles substituted at 5′ position of uridine was chosen. The design for this target was based on replacing the peptide bond of nikkomycin **10** with triazole moiety. 1,2,3-Triazole unit may be considered as a surrogate for a peptide bond as these triazoles have atom placement and electronic properties similar to the peptide bond.[19] It was also established that 1,2,3-triazole cores interact strongly with biological targets through hydrogen bonding to nitrogen atoms and their large dipole moments.[20] The construction of 1,2,3-triazole from substituted azide and alkyne derivative in presence of Cu(I) catalyst using click chemistry proved to be powerful tool in a biomedical research, ranging from combinatorial chemistry and target-template in situ chemistry, for lead discovery, to bioconjugation strategies for proteomics and DNA research.[21]

Synthesis of targeted 1,2,3-triazole substituted uridine derivatives **19a**–**19g** and **21a**–**21g** was achieved using Cu(I)-catalyzed Sharpless click chemistry[22] approach from 5′-azidouridine **17** and propargyl derivatives of phenols **18a**–**18g** (Table 1) and propargyl derivatives of acids **20a**–**20g** (Table 2). The addition of Cu(I) catalyst strongly activates terminal acetylenes toward 1,3-dipole in organic azides, exclusively forming the 1,4-disubstituted regio-isomer.

Thus compound **17** was obtained from compound **16** by using reported procedure with a 95% yield (Scheme 1).[23]

Propargyl ethers of phenol were prepared by the reaction of phenols with propargyl bromide and K_2CO_3 in DMF. Propargyl esters were synthesized in excellent yield by heating acids in excess of propargyl alcohol using catalytic amount of *p*-toluenesulfonic acid (*p*-TSA) at 80 °C. These propargylated compounds were then reacted with 5′-azidouridine **17** in the presence of copper sulfate

Table 1
Synthesis of 1,2,3-triazole derivatives of uridine by click reaction between 5′-azidouridine **17** and propargyl ethers **18a–18g**

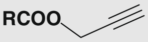
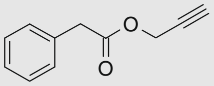
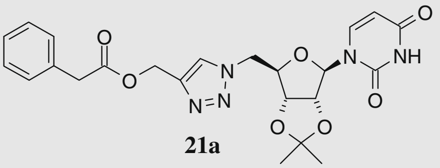
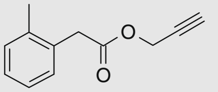
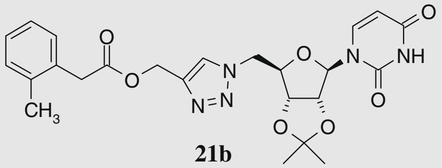
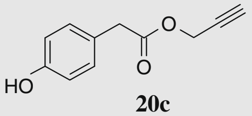
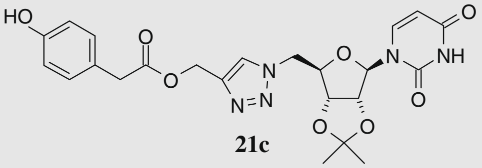
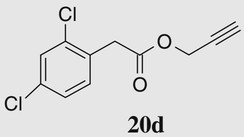
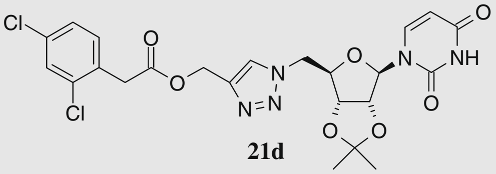
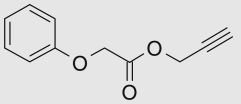
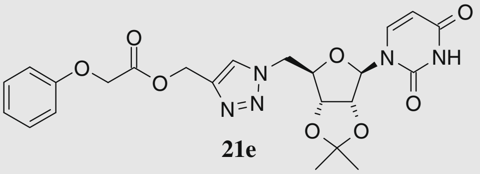
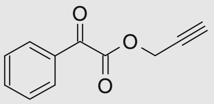
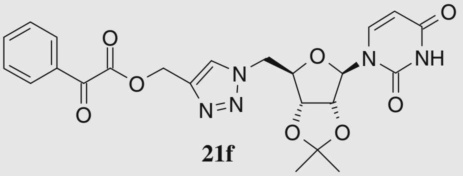
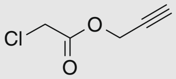
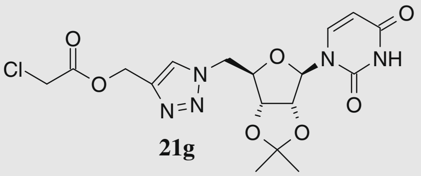
Sr. No		Product	Yield (%)
1	 18a	 19a	56
2	 18b	 19b	69
3	 18c	 19c	71
4	 18d	 19d	65
5	 18e	 19e	70
6	 18f	 19f	73
7	 18g	 19g	83

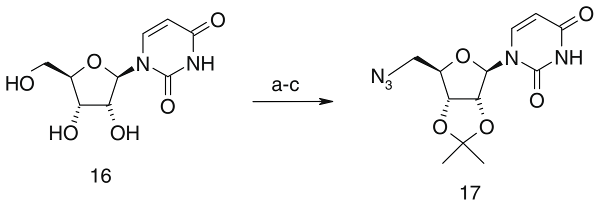
and sodium ascorbate in *t*-butanol/water (8:2) to yield exclusively 1,4-disubstituted-1,2,3-triazoles at 5′ position of uridine (Schemes 2 and 3 and Tables 1 and 2).[33] The structures of final compounds **19a–19g** and **21a–21g** were confirmed by IR, ^{1}H NMR, ^{13}C NMR, MS spectra and elemental analysis. [34–36]

To get single crystal an ethyl acetate solution of nucleoside **19d** was subjected to slow evaporation. The molecular structure of **19d** was unambiguously confirmed (ORTEP diagram, Fig 4). [37]

Screening of all the synthesized compounds was carried out using NCL isolates. The fungal strains *Candida albicans* and *Cryptococcus neoformans* (human pathogens), *Benjaminiella poitrasii* were maintained on YPG (yeast extract, 0.3%; peptone, 0.5%; and glucose, 1%) agar slants, and *Fusarium oxysporum* (plant pathogen) was maintained on PDA (potato, 20%; dextrose, 2%) slants at 28 °C as described earlier.[24,25] MIC (minimum inhibitory concentration for 90% inhibition of growth) and IC$_{50}$ (50% inhibition of

Table 2
Synthesis of 1,2,3-triazole derivatives of uridine by click reaction between 5′-azidouridine **17** and propargyl esters **20a–20g**

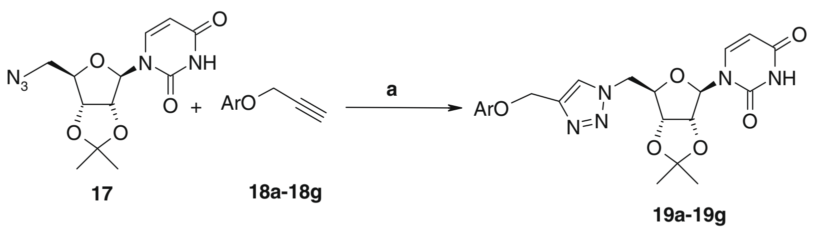
Sr. No.	 RCOO	Product	Yield (%)
1	 **20a**	 **21a**	60
2	 **20b**	 **21b**	74
3	 **20c**	 **21c**	50
4	 **20d**	 **21d**	57
5	 **20e**	 **21e**	55
6	 **20f**	 **21f**	53
7	 **20g**	 **21g**	60



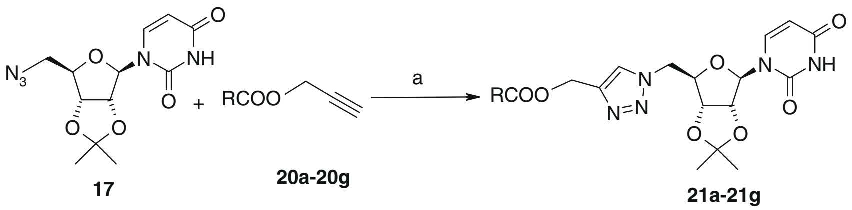
Scheme 1. Reagents and conditions: (a) dimethoxy propane/acetone, *p*-TSA, 28 °C; (b) *p*-TsCl, pyridine, 28 °C; (c) NaN_3, DMF, 60 °C.

growth) of the synthesized compounds were determined according to standard broth microdilution technique as per NCCLS guidelines.[26–28] Compounds were solubilized in DMSO (2.5% v/v), and stock solutions of 1.28 mg/mL were prepared to find out MIC and IC_{50}. Nikkomycin, amphotericin B, and fluconazole (stock solutions of 1.28 mg/mL), known reference antifungal agents were also dissolved in DMSO (2.5% v/v), and were used as a positive control.

As seen in Table 3 synthesized 1,2,3-triazolyl linked uridine compounds **19a–19g** and **21a–21g** were tested for their antifungal activity. It was observed that aryl ether 1,2,3-triazolyl



Scheme 2. Reagents and conditions: (a) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, t-BuOH/H_2O, 28 °C.



Scheme 3. Reagents and conditions: (a) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, t-BuOH/H_2O, 28 °C.

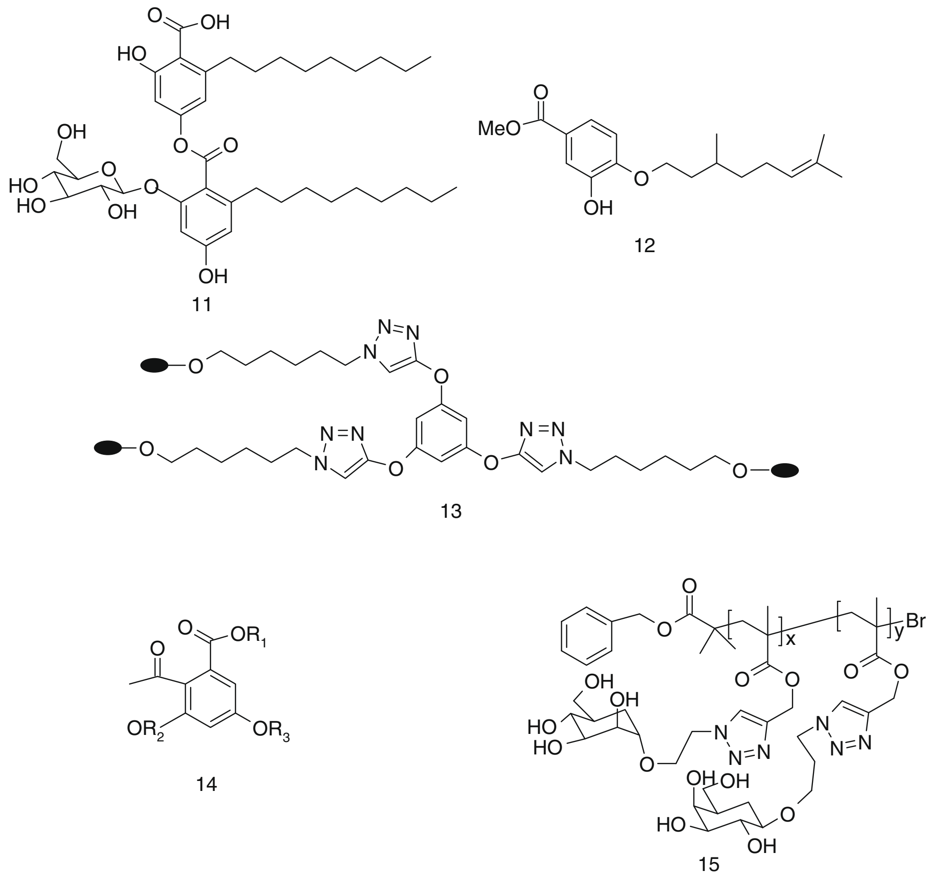


Figure 3. Compounds containing ether and ester linkage.

linked uridine compounds **19a**–**19g** were found to be active against all tested fungal strains with MIC values 8–128 µg/mL (0.003–0.305 µmol). The activity of compounds **19b**–**19d** was found to be higher than that of standard nikkomycin and fluconazole against *C. neoformans* and *C. albicans* with MIC of 32 and 24 µg/mL (0.066 and 0.136 µmol). Compound **19a** found to be most potent with lowest MIC of 8 µg/mL (0.018 µmol) against *C. neoformans* as compared to all standards used.

Aryl ester 1,2,3-triazolyl linked uridine compounds **21a–21g** also found to be active against all tested fungal strains with MIC of values 16–128 µg/mL (0.033–0.257 µmol). Compounds **21a, 21b** showed MIC of 31 and 32 µg/mL (0.064 µmol) against human pathogens *C. neoformans* and *C. albicans*, respectively which was comparable to nikkomycin and fluconazole. Compound **21g** showed MIC of 66 µg/mL (0.149 µmol) against *C. albicans* which was comparable to nikkomycin, while it showed

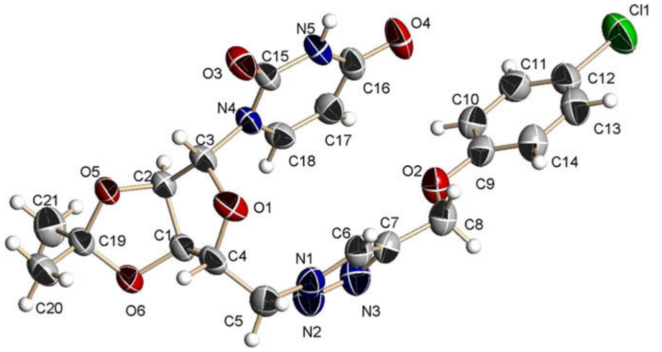


Figure 4. ORTEP diagram of compound **19d**.

Table 3
Antifungal activity detection by using micro broth dilution method

Compound No.	Growth inhibitory concentration in μg/mL					
	F. oxysporum		*C. neoformans*		*C. albicans*	
	MIC[a]	IC_{50}[b]	MIC[a]	IC_{50}[b]	MIC[a]	IC_{50}[b]
19a	>128	128	8	4	>128	68
19b	64	32	32	14	>128	>128
19c	124	70	49	35	24	16
19d	32	16	57	30	32	18
19e	>128	128	128	64	>128	9
19f	64	32	64	8	128	34
19g	64	32	64	32	>128	34
21a	32	16	31	18	32	18
21b	>128	64	64	32	32	18
21c	57	35	>128	128	>128	>128
21d	>128	>128	64	32	>128	32
21e	33	16	>128	128	>128	64
21f	106	55	>128	16	>128	>128
21g	16	8	>128	125	66	32
Nikkomycin	8	6	30	16	64	32
Fluconazole	8	4	32	16	32	14
Amphotericin B	16	8	16	8	2	0.5

Positive control, Amphotericin B, Fluconazole, and Nikkomycin.
Negative control, DMSO, No inhibition.

[a] Minimum inhibitory concentration for 90% inhibition of growth.

[b] 50% Inhibition of growth.

potent inhibition of *F. oxysporum* with MIC value 16 μg/mL (0.036 μmol).

Most of the pathogenic fungi change their morphology reversibly between viz. unicellular yeast or filamentous hypha for survival and proliferation in the host. In other words, it is a change from a nonpathogenic/saprophytic to pathogenic form. Therefore, the compounds which retard this dimorphic transition can have a potential as an antifungal drug. A nonpathogenic dimorphic fungus *B. poitrasii* was used as a model[29] to check the effect of compounds on yeast to hypha transition. Yeast inoculum was grown in YPG (glucose 1%) medium at 37 °C for 24 h and the transition to the hypha form was studied in YP medium at 28 °C. The yeast cells were inoculated in YP broth (with and without compounds) at 28 °C for 6 h and the percentage of cells forming germ tubes was assessed as described earlier.[30] Nikkomycin Z (chitin synthase inhibitor) was used as a positive control in the yeast-hypha transition experiment (Table 4). It was observed that compounds**19b, 19c, 19g, 21a**, and **21f** exhibited >70% of inhibition while **19e, 19f, 21b, 21c, 21g** showed >60% of inhibition at 4 μg/mL. While remaining compounds showed moderate activity as compared to nikkomycin Z.

Chitin synthase activity of *B. poitrasii* cells was estimated with and without compounds (4 μg/mL) using a non-radioactive chitin synthase assay, according to Lucero et al.[31] The assay involved binding of synthesized chitin to WGA-coated surface followed by detection of polymer with horseradish peroxidase-WGA conjugate. Activity of horseradish peroxidase was determined by measuring the absorbance at 430 nm. It was observed that compounds **19d, 19e, 19f**, and **21f** inhibited 80–95% of chitin synthase activity. Compounds **19a, 19b, 19g, 21a, 21c, 21e**, and **21g** exhibited 70–80% inhibition of chitin synthase activity. All other compounds exhibited inhibition in the range of 60–70% except **21b.** (Table 4).

For the screening of antifungal compounds, the inhibition of spore germination or yeast to hypha differentiation assays are more sensitive than the growth inhibition assay.[29] Moreover, the number of pathogens exhibit differentiation (yeast or hypha) for the survival and then proliferation in the host.[29] The comparative evaluation of compounds using in vitro inhibition of chitin synthase activity, inhibition of yeast to hypha transition (measured as% germ tube formation) and inhibition of yeast growth can be used to identify target specificity, hydrophobicity, and toxicity, in general. Results of inhibition of growth, germ tube formation and chitin

Table 4
Comparative analysis of all compounds using whole cell based assay, yeast to hypha transition and chitin synthase inhibition against *Benjaminiella poitrasii*

Group	Compound	% Inhibition		
		Chitin synthase activity[a]	% Germ tube formation[b]	Growth[c]
I	**Nikkomycin**	96.56	>95	45.45
	19d	95.08	58.13	34.65
	19e	84.06	64.70	9.43
	19f	82.14	69.23	34.43
	21f	80.04	72.00	51.52
II	**19a**	77.15	45.09	21.51
	19b	77.78	78.20	17.45
	19c	68.48	71.42	29.30
	19g	76.86	72.72	32.44
	21a	71.00	75.00	31.88
	21c	73.39	61.53	28.21
	21d	67.00	57.14	38.64
	21e	74.00	52.50	56.25
	21g	75.71	60.00	30.21
III	**21b**	36.38	67.21	27.28

Concentration of compounds 4 μg/mL.
Inhibition of chitin synthase activity—Group I, >80%; Group II, 60–70%; Group III, <60%.

[a] Chitin synthase assay. Activity in control 14.86×10^{-2} nmol GlcNAc/mg of protein/min.

[b] Germ tube formation inhibition at 28 °C for 6 h. Control DMSO (2.5%, v/v).

[c] Standard broth microdilution technique, control DMSO (2.5%, v/v).

synthase activity at 4 ppm concentration against *B. poitrasii* were compared (Table 4). With nikkomycin, for instance, inhibition of chitin synthase activity and% germ tube formation were >95% while that of growth was 45% (Table 4). This indicated that nikkomycin uptake by *B. poitrasii* was high enough to show antifungal activity, unlike earlier report of low nikkomycin uptake by *C. albicans.*[32] Based on the in vitro inhibition of chitin synthase activity all the compounds were divided in 3 groups (Table 4). From group I however **19e** did not show growth inhibition comparable to other two parameters which could be attributed to less uptake by the cells. While compounds **19c** and **21a** showed higher inhibition of germ tube formation as compared to chitin synthase activity. These compounds may be acting on some additional targets. The compound **21b** must be acting on some other target since it showed lesser inhibition of chitin synthase activity as compared to other two assays.

Obi et al. reported that in case of synthesized nikkomycin analogues anti-chitin synthase activity was found to be enhanced by introducing β-dimethyl group as β-methyl group of nikkomycin.[12] Furthermore, introduction of hydroxyl group in terminal aryl moiety resulted in significantly enhanced anti-chitin synthase activity. In present investigation introduction of methyl group at *meta* position of benzene ring of compound **19f** showed higher anti-chitin synthase activity. Whereas in compound **21b** decrease in anti-chitin synthase activity was observed, which may be attributed to *ortho* position of methyl group on benzene ring. Unlike reported by Obi et al. in case of compound **21c** introduction of hydroxyl group in benzene ring did not show significant increase in anti-chitin synthase activity.

In conclusion the current endeavor enables a practical, reliable and efficient synthesis of several novel 1,4-disubstituted-1,2,3-triazolyluridine derivatives by 'click chemistry' approach, most of which showed significant antifungal activity. Compound **19a** showed potent antifungal activity as compared to all three standards used against *C. neoformans* with MIC of 8 μg/mL (0.018 μmol). Compounds **19c, 19d, 21a**, and **21b** demonstrated potent antifungal activity in comparison to nikkomycin with MIC value of 24–32 μg/mL (0.048–0.067 μmol) against *C. albicans.* Compounds **19b, 19c, 19g, 21a**, and **21f** have profound suppressive effect on yeast-hypha transition, exhibiting >70% inhibition at concentration 4 μg/mL. Compounds **19d, 19e, 19f**, and **21f** exhibited >80% inhibition of chitin synthase activity comparable to that of nikkomycin at a concentration 4 μg/mL. Comparing the results of all three types of assays, compounds **19a, 19b, 19f, 21c, 21f**, and **21g** can be identified as lead chitin synthase inhibitors for further modifications to increase their antifungal as well as application potential in health care and in agriculture.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.02.019.

References and notes

1. Zhou, L.; Amer, A.; Korn, M.; Burda, R.; Balzarini, J.; Clercq, E. D.; Kern, E. R.; Torrence, P. F. *Antiviral Chem. Chemother.* **2005**, *16*, 375.
2. Kosiova, I.; Kovackova, S.; Kois, P. *Tetrahedron* **2007**, *63*, 312.
3. Epple, R.; Kudirka, R.; Greenberg, W. A. *J. Comb. Chem.* **2003**, *5*, 292.
4. Akri, K. E.; Bougrin, J.; Faraj, A.; Benhida, R. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6656.
5. Patil, R. S.; Deshpande, A. M.; Natu, A. A.; Nahar, P.; Chitnis, M.; Ghormade, V.; Laxman, R. S.; Rokade, S.; Deshpande, M. V. *J. Biol. Control* **2001**, *15*, 157.
6. Isono, K.; Asahi, K.; Suzuki, S. *J. Am. Chem. Soc.* **1969**, *91*, 7490.
7. Isono, K.; Suzuki, S. *Heterocycles* **1979**, *13*, 333.
8. Uramoto, M.; Kobinata, K.; Isono, K.; Higashijima, T.; Isono, K.; Miyazawa, T.; Jenkins, E. E.; McCloskey, J. A. *Tetrahedron Lett.* **1980**, *21*, 3395.
9. Zhang, D.; Miller, M. *J. Curr. Pharm. Des.* **1999**, *5*, 73.
10. Deshpande, M. V. In *Wealth and Sustainable Environment*; Varma, A., Ed.; Malhotra Publishing House: New Delhi, 1998; pp 281–291.
11. Muzzarelli, R. A. A. *Chitin*; Pergamon: Oxford, 1977.
12. Obi, K.; Uda, J. I.; Iwase, K.; Sugimoto, O.; Ebisu, H. *Biorg. Med. Chem. Lett.* **2000**, *10*, 1451.
13. Behr, J-B.; Gourlain, A. H.; Guillerm, G. *Biorg. Med. Chem. Lett.* **2003**, *13*, 1713.
14. Ondeyka, J. G.; Zink, D. L.; Dombrowaski, A. W.; Polishook, J. D.; Felock, P. J.; Hazuda, D. J.; Singh, S. B. *J. Antibiot.* **2003**, *56*, 1018.
15. Baek, S. H.; Oh, H. J.; Lim, J. A.; Chun, H. J.; Lee, H. O.; Ahn, J. W.; Perry, N. B.; Kim, H. M. *Bull. Korean Chem. Soc.* **2004**, *25*, 195.
16. Chen, Q.; Yang, F.; Du, Y. *Carbohydr. Res.* **2005**, *340*, 2476.
17. Varma, G. B.; Fatope, M. O.; Marwah, R. G.; Deadman, M. E.; At-Rawahi, F. K. *Phytochemistry* **2006**, *67*, 1925.
18. Ladmiral, V.; Mantovani, G.; Clarkson, G. J.; Cauet, S.; Irwin, J. L.; Haddleton, D. M. *J. Am. Chem. Soc.* **2006**, *128*, 4823.
19. Bock, V. D.; Perciaccante, R.; Jansen, T. P.; Hirmstra, H.; Van Maarseveen, J. H. *Org. Lett.* **2006**, *5*, 919.
20. Kolb, H. C.; Sharpless, K. B. *Drug Discovery Today* **2003**, *8*, 1128.
21. Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004.
22. Huisgen, R. In *1,3-Dipolar cycloaddition Chemistry*; Padwa, A., Ed.; Wiley: New York, 1984.
23. Sasaki, T.; Minamoto, K.; Suzuki, T.; Sugiura, T. *J. Org. Chem.* **1979**, *44*, 1424.
24. Gholap, A. R.; Toti, K. S.; Shirazi, F.; Kumari, R.; Bhat, M. K.; Deshpande, M. V.; Srinivasan, K. V. *Bioorg. Med. Chem.* **2007**, *15*, 6705.
25. Salunke, D. B.; Hazra, B. G.; Pore, V. S.; Bhat, M. K.; Nahar, P. B.; Deshpande, M. V. *J. Med. Chem.* **2004**, *47*, 1591.
26. National Committee for Clinical Laboratory Standard. Reference Method for Broth Dilution Antifungal susceptibility Testing of Yeast, Approved Standard. Document M27-A; National Committee for Clinical Laboratory Standards: Wayne, PA, USA, 1997.
27. National Committee for Clinical Laboratory Standard. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Conidium Forming Filamentous Fungi: Proposed Standard. Document M38-P; National Committee for Clinical Laboratory Standard: Wayne, PA, USA, 1998.
28. Yamamoto, N.; Fujita, J.; Shinzato, T.; Hig, F.; Tateyama, M.; Tohyama, M.; Nakasone, I.; Yamaneb, N. *Int. J. Antimicrob. Agents* **2006**, *27*, 171.
29. Ghormade, V.; Deshpande, M. V. *Naturwissenschaften* **2000**, *87*, 236.
30. Deshpande, M. V.; Donnell, R. O.; Gooday, G. W. *FEMS Microbiol. Lett.* **1997**, *152*, 327.
31. Lucero, A. H.; Kuranda, J. M.; Bulik, A. D. *Anal. Biochem.* **2002**, *305*, 97.
32. Yarden, J. C.; Gonneau, M.; Sarthou, P.; Le Goffic, F. *J. Bacteriol.* **1984**, *160*, 884.
33. *General experimental protocol for the synthesis of 1,2,3-triazoles **19a–19g** and **21a–21g***: To the stirred solution of **17** (1 mmol) and propargyl ethers **18a–18g** (1 mmol) or propargyl esters **20a–20g** (1 mmol) in 10 mL of tertiary butanol/water (8:2) was added copper sulfate (24 mg, 5 mol %) and sodium ascorbate (40 mg, 10 mol %). Reaction mixture was stirred at 28 °C for 4–12 h. Completion of the reaction was monitored by TLC. Tertiary butanol was removed under reduced pressure, reaction mixture was partitioned between ethyl acetate and water. Organic phase was washed successively with water, brine, and dried over sodium sulfate and concentrated to furnish compounds **19a–19g** or **21a–21g**, yields ranging from 50% to 83%.
34. *Spectral data and elemental analysis for 5′-deoxy-2′,3′-O-(methylethylidene)-5′-{4-[(3-methoxyphenoxy)methyl]-1,2,3-triazol-1-yl}uridine (**19c**)*: ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 9.82$ (s, 1H), 7.61 (s, 1H), 7.05 (d, 1H, $J = 8$ Hz), 6.84 (q, 4H, $J = 8$ Hz), 5.17 (d, 1H, $J = 8$ Hz), 5.50 (s, 1H), 5.12 (s, 2H), 5.03 (dd, 1H, $J = 1.6$ Hz), 4.98 (m, 1H), 4.67 (br s, 2H), 4.48 (m, 1H), 3.74 (s, 3H), 1.52 (s, 3H), 1.32 (s, 3H). ^{13}C ($CDCl_3$, 50 MHz): 174.9, 163.8, 154.2, 152.3, 150.3, 144.3, 143.4, 125.0, 115.8, 114.7, 102.8, 96.1, 86.2, 84.2, 81.8, 62.6, 55.6, 51.8, 27.0, 25.2, 20.7 ppm. MS *m/z*: (MH^+) 472.10; IR ($CHCl_3$) v: 1714, 1693 cm^{-1}. Anal. Calcd for $C_{22}H_{25}N_5O_7$: C, 56.05; H, 5.35; N, 14.85. Found: C, 56.00; H, 5.25; N, 14.55.
35. *Spectral data and elemental analysis for 5′-{4-[(4-hydroxyphenylacetoxy)methyl)]--1,2,3-triazol-1-yl}-5′-deoxy-2′,3′-O-(methylethylidene)uridine (**21c**)*: ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 9.88$. (s, 1H), 7.41 (s, 1H), 7.05 (d, 1H, $J = 8$ Hz), 7.01 (d, 1H, $J = 6$ Hz), 6.73 (d, 2H, $J = 6$ Hz), 5.07 (d, 1H, $J = 8$ Hz), 5.47(s, 1H), 5.20 (s, 2H), 5.04 (dd, 1H, $J = 1.6$ Hz), 4.89 (m, 1H), 4.64 (m, 2H), 4.45 (m, 1H), 3.63 (s, 2H), 1.53 (s, 3H), 1.33 (s, 3H). ^{13}C ($CDCl_3$, 50MHz): 176.2, 168.2, 159.8, 154.2, 147.6, 146.6, 134.2, 129.0, 128.4, 119.3, 118.7, 106.5, 100.0, 90.0, 88.1, 85.7, 61.5, 55.8, 44.0, 30.7, 28.9 ppm. MS *m/z*: (MH^+) 501.03; IR ($CHCl_3$) v: 3338, 1683, 1693 cm^{-1}. Anal. Calcd for $C_{23}H_{25}N_5O_8$: C, 55.31; H, 5.05; N, 14.02. Found: C, 55.00; H, 4.99; N, 14.00.
36. Spectral data and elemental analysis data for the characterization of compounds **19a**, **19b**, **19d–19g** and **21a**, **21b**, **21d–21g** have been incorporated as Supplementary data.
37. *Crystallographic data for 5′-{4-[(4-chlorophenoxy)methyl]-1,2,3-triazol-1-yl}-5′-deoxy-2′,3′-O-(methylethylidene)uridine (**19d**)*: The single-crystal diffraction data were collected on a Bruker AXS Smart Apex CCD diffractometer at 297(2) K. The X-ray generator was operated at 50 kV and 30 mA using graphite-monochromatized (Mo $K_\alpha = 0.71073$ Å) radiation. Data were collected with ω scan width of 0.3° and with four different settings of φ (0°, 90°, 180° and 270°) keeping the sample-to-detector distance fixed at 6.145 cm and the detector position (2θ) fixed at −28°. $C_{21}H_{22}Cl_1N_5O_6$: $M = 475.89$, crystal dimensions $0.62 \times 0.38 \times 0.19$ mm^3, $T = 297(2)$ K,

monoclinic, space group $P2_1$, $a = 11.4808(15)$, $b = 5.6022(7)$, $c = 17.836(2)$ Å, $\beta = 107.144(2)^\circ$; $V = 1096.2(2)$ Å^3; $Z = 2$; $\rho_{\mathrm{calcd}} = 1.442$ g cm^{-3}, μ (Mo K$_\alpha$) = 0.224 mm^{-1}, $F(000) = 496$, $2\theta_{\max} = 52.00^\circ$, 11,494 reflections collected, 4274 unique, 4141 observed ($I > 2\sigma(I)$) reflections, 300 refined parameters, R value 0.0324, $wR_2 = 0.0835$ (all data $R = 0.0334$, $wR_2 = 0.0844$), $S = 1.036$, minimum and maximum transmission 0.8737 and 0.9587; maximum and minimum residual electron densities +0.194 and −0.185 eÅ^{-3}.

All the data were corrected for Lorentzian, polarization and absorption effects using Bruker's SAINT and SADABS programs. SHELX-97 (G. M. Sheldrick, SHELX-97 program for crystal structure solution and refinement, University of Gottingen, Germany, 1997) was used for structure solution and full-matrix least-squares refinement on F^2. All non-hydrogen atoms were refined anisotropically while all hydrogen atoms were refined with appropriate geometric constraints.

Crystallographic data (excluding structure factors) for the structure **19d** in this paper have been deposited with Cambridge crystallographic data center as supplementary publication number CCDC 709791.