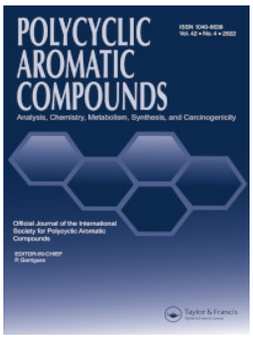


Polycyclic Aromatic Compounds

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/gpol20

Tetrazoloquinoline-1,2,3-Triazole Derivatives as Antimicrobial Agents: Synthesis, Biological Evaluation and Molecular Docking Study

Mubarak H. Shaikh, Dnyaneshwar D. Subhedar, Satish V. Akolkar, Amol A. Nagargoje, Vijay M. Khedkar, Dhiman Sarkar & Bapurao B. Shingate

To cite this article: Mubarak H. Shaikh, Dnyaneshwar D. Subhedar, Satish V. Akolkar, Amol A. Nagargoje, Vijay M. Khedkar, Dhiman Sarkar & Bapurao B. Shingate (2022) Tetrazoloquinoline-1,2,3-Triazole Derivatives as Antimicrobial Agents: Synthesis, Biological Evaluation and Molecular Docking Study, Polycyclic Aromatic Compounds, 42:4, 1920-1941, DOI: 10.1080/10406638.2020.1821229

To link to this article: https://doi.org/10.1080/10406638.2020.1821229

View supplementary material

Published online: 16 Sep 2020.

Submit your article to this journal

Article views: 334

View related articles

View Crossmark data

Citing articles: 7 View citing articles

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=gpol20

POLYCYCLIC AROMATIC COMPOUNDS
2022, VOL. 42, NO. 4, 1920–1941
https://doi.org/10.1080/10406638.2020.1821229

Taylor & Francis
Taylor & Francis Group

Check for updates

Tetrazoloquinoline-1,2,3-Triazole Derivatives as Antimicrobial Agents: Synthesis, Biological Evaluation and Molecular Docking Study

Mubarak H. Shaikh[a,b], Dnyaneshwar D. Subhedar[a], Satish V. Akolkar[a], Amol A. Nagargoje[a,c], Vijay M. Khedkar[d], Dhiman Sarkar[e], and Bapurao B. Shingate[a]

[a]Department of Chemistry, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, India; [b]Department of Chemistry, Radhabai Kale Mahila Mahavidyalaya, Ahmednagar, India; [c]Department of Chemistry, Khopoli Municipal Council College, Khopoli, India; [d]Department of Pharmaceutical Chemistry, School of Pharmacy, Vishwakarma University, Pune, India; [e]Combi-Chem Resource Centre, CSIR-National Chemical Laboratory, Pune, India

ABSTRACT

In search of new active molecules, a small focused library of tetrazoloquinoline-based 1,2,3-triazoles has been efficiently prepared *via* click chemistry approach. Several derivatives were found to be exhibiting promising antimicrobial and antioxidant activity characterized by their lower minimum inhibitory concentration values. All the synthesized compounds exhibited excellent antibacterial activity against Gram negative bacteria *E. coli* and *F. devorans* and antifungal activity against *C. albicans* and *A. niger*. Further, these compounds were tested for their antitubercular activity against dormant *MTB H37Ra* and dormant *M. bovis BCG* using XRMA assay protocol and showed no significant activity. Also, the synthesized compounds were found to have potential antioxidant activity with IC_{50} range = 12.48–50.20 μg/mL. Furthermore, to rationalize the observed biological activity data, the molecular docking study also been carried out against the active site of fungal *C. albicans* enzyme P450 cytochrome lanosterol 14α-demethylase, which revealed a significant correlation between the binding score and biological activity for these compounds. The results of the *in vitro* and *in silico* study suggest that the triazole-incorporated tetrazoloquinolines may possess the ideal structural requirements for further development of novel therapeutic agents.

ARTICLE HISTORY
Received 21 June 2020
Accepted 5 September 2020

KEYWORDS
1,2,3-Triazole; antimicrobial; antioxidant; docking study; ADME prediction

Introduction

In recent years, life threatening systemic fungal infections have become increasingly common, especially in immunocompromised hosts suffering from tuberculosis, cancer, or AIDS and in organ transplant cases. Development of resistance against available antifungal agents (generally azoles) is also an alarming factor. Commonly used azole antifungal agents are fluconazole, itraconazole, miconazole, and voriconazole displayed broad spectrum antifungal activity.[1] Azoles have broad spectrum activities against most yeasts and filamentous fungi and are the drug of choice for antifungal chemotherapy.[2] These antifungal drugs inhibiting CYP51 in the process of biosynthesis of ergosterol through a mechanism in which the heterocyclic nitrogen atom (*N*-4

CONTACT Bapurao B. Shingate bapushingate@gmail.com Department of Chemistry, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad 431 004, India.

Supplemental data for this article is available online at https://doi.org/10.1080/10406638.2020.1821229.

© 2020 Taylor & Francis Group, LLC

of triazole) binds to the heme iron atom.[3] However, increasing use of these antifungal drugs has led to increase in resistance to these drugs.[4–6]

Heterocyclic compounds play an important role in designing new class of structural entities for medicinal applications. Quinoline and their derivatives are pharmacologically important heterocyclic compounds because of their wide existence in alkaloids, therapeutics, and synthetic analogues with interesting biological activities such as antimalarial,[7] analgesic,[8] anticancer,[9] anti-inflammatory,[10] antiviral,[11] antihelmintic,[12] anti-protozoal,[13] cardiovascular,[14] hypoglycemic,[15] and antimicrobial activity.[16]

Triazoles are stable to acidic/basic hydrolysis and also reductive/oxidative conditions, indicative of a high aromatic stabilization. This moiety is relatively resistant to metabolic degradation. Over the past two decades 1,2,3-triazole and its derivatives have attracted continued interest in the medicinal field and are reported to possess a wide range of biological activities such as antifungal,[17] antitubercular,[18] antiallergic,[19]a anti-HIV,[19]a antibacterial,[19]b α-glycosidase inhibitor,[20] antimicrobial,[21] anticoccidiostats,[22] anticonvulsant,[23] antimalarial,[24] antiviral,[25] and antimycobacterial.[26] Triazole has been used to improve the pharmacokinetic properties of the desired drug.[27]

Click chemistry is a newer approach for the synthesis of drug-like molecules that can accelerate the drug discovery process by utilizing a few practical and reliable reactions. Sharpless[28] and Meldal[29] groups have reported the dramatic rate enhancement (up to 10^7 times) and improved regio-selectivity of the Huisgen 1,3-dipolar cycloaddition reaction of an organic azide and terminal acetylene to afford, regio-specifically, the 1,4-disubstituted-1,2,3-triazole in the presence of Cu (I) catalyst. The Cu (I)-catalyzed azide alkyne cycloaddition (CuAAC) reaction has successfully fulfilled the requirement of "click chemistry" as prescribed by Sharpless and within the past few years has become a premier component of synthetic organic chemistry.[30]

Tetrazoles can act as pharmacophore for the carboxylate group, increasing their utility. Angiotensin II blocker often contain tetrazoles, as Losartan and candesartan. Tetrazoles and its derivatives displays various biological activities such as antibacterial,[31]a,b anti-inflammatory,[31]a,b antinociceptive,[31]a,b hypoglycemic,[31]a,b anticancer[31]a,b antifungal,[31]c antiviral,[31]a antitubercular & antimalarial,[31]d and cyclo-oxygenase inhibitors activities.[31]e They are used as catalyst in the synthesis of phosphonates.[31]a

In recent years, a library of quinoline derivatives conjugated with 1,2,3-triazole were synthesized and proved to possess different bioactivity such as antimicrobial activity[32] (Figure 1, **A**), *β*-haematin inhibitor[33] (Figure 1, **B**), antimalarial agent[33] (Figure 1, **C**), antifungal agent[16] (Figure 1, **D**), antimalarial and cytotoxic activity[34] (Figure 1, **E**), DNA binding and photonuclease activity[35] (Figure 1, **F**).

In continuation of our earlier work[16,36] on synthesis and biological properties of heterocyclic moieties and the importance of tetrazoloquinoline-1,2,3-triazole moieties as a single molecular scaffold, herein we would like to report the design and syntheses of new tetrazoloquinoline-linked triazole hybrids and their evaluation for antimicrobial and antioxidant activities. The computational parameters like docking study for antimicrobial activity and ADME prediction of synthesized tetrazoloquinoline-triazole conjugates **5a-i** were also performed.

Results and discussion

Chemistry

We have described a protocol for the syntheses of a series of new derivatives of 8-methoxy-4-((4-(phenoxymethyl)-1*H*-1,2,3-triazol-1-yl)methyl)tetrazolo[1,5-*a*]quinoline **5a-i** as a potential antimicrobial, antioxidant, and antitubercular agents from commercially available starting materials. These compounds were formed by the fusion of substituted (prop-2-yn-1-yloxy)benzenes **4a-h**,

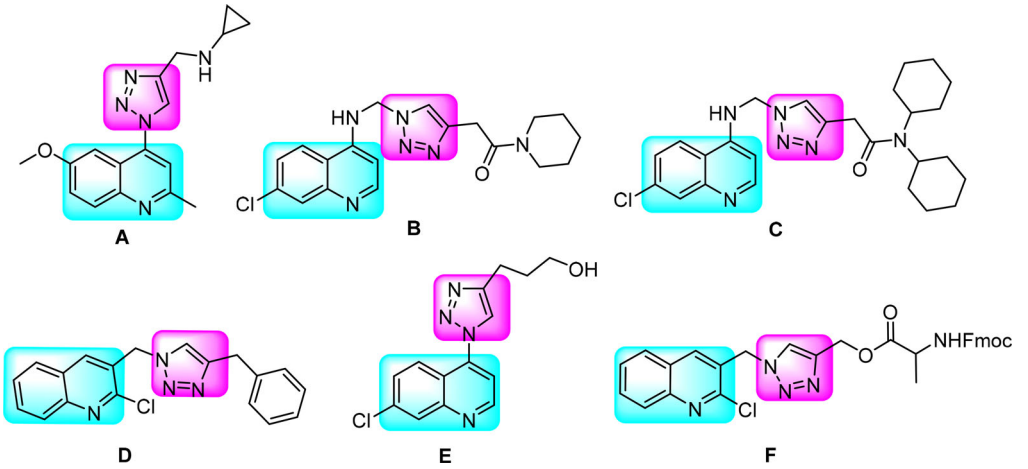
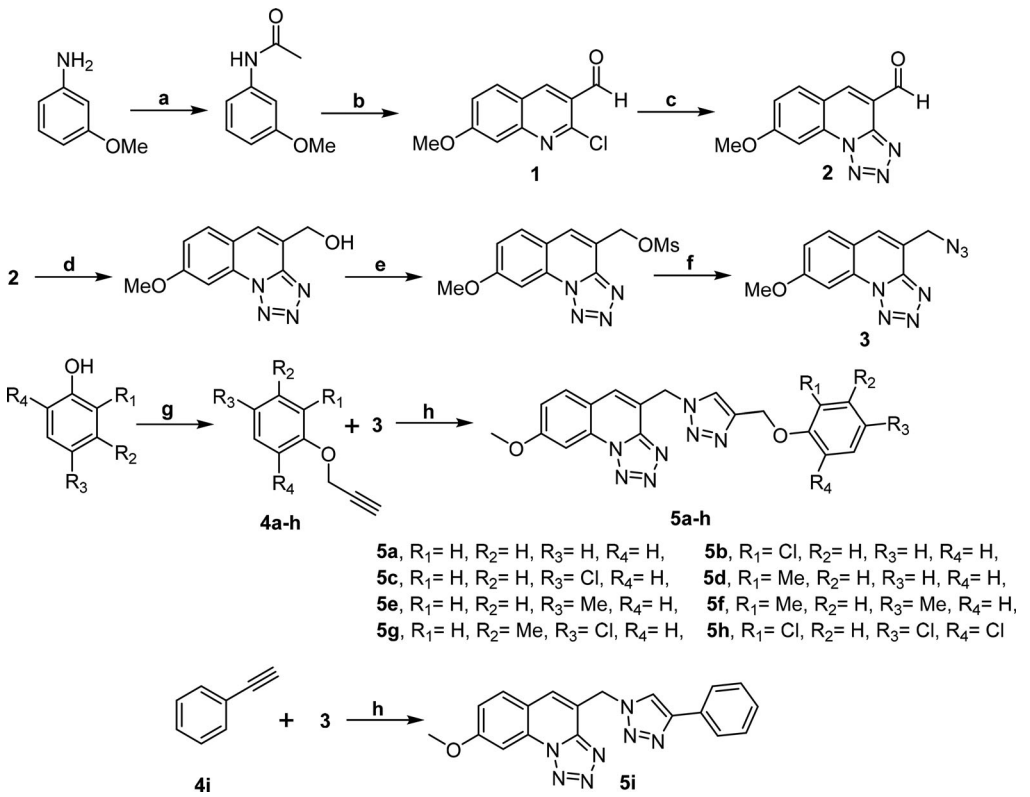


Figure 1. Triazole incorporated quinoline derivatives **A–F**.

phenyl acetylene **4i** and 4-(azidomethyl)-8-methoxytetrazolo[1,5-*a*]quinoline **3** *via* click chemistry approach (Scheme 1). The synthesis of starting material 4-(azidomethyl)-8-methoxytetrazolo[1,5-*a*]quinoline **3** was prepared from 8-methoxytetrazolo[1,5-*a*]quinoline-4-carbaldehyde **2** *via* $NaBH_4$ reduction, mesylation followed by nucleophilic substitution reaction of sodium azide (Scheme 1).

The synthesis of methoxytetrazolo[1,5-*a*]quinoline-4-carbaldehyde **2** was prepared from 3-methoxyaniline (*m*-anisidine) which on acylation followed by Vilsmeier-Haack formylation at 80 °C for 8 hr to generate 2-chloro-7-methoxyquinoline-3-carbaldehyde **1**, which on further reaction with sodium azide in DMF at 80 °C for 6 hr produces tetrazoloquinoline aldehyde **2** in good yield. The commercially available phenols have been alkylated with propargyl bromide in the presence of K_2CO_3 as a base in *N,N*-dimethylformamide (DMF) afforded the corresponding (prop-2-yn-1-yloxy)benzene derivatives **4a-h** in good to excellent yield (Supporting Information). Finally, the Huisgen's CuAAC reaction has been performed on 4-(azidomethyl)-8-methoxytetrazolo[1,5-*a*]quinoline **3** and (prop-2-yn-1-yloxy)benzene derivatives **4a-h** and phenylacetylene **4i** in the presence of $Cu(OAc)_2$ in *t*-BuOH-H_2O (3:1) at room temperature for 16–22 hr gives the corresponding 1,4-disubstituted-1,2,3-triazole based tetrazoloquinoline derivatives **5a-h** and **5i**, respectively, in quantitative isolated yield (86–90%) (Scheme 1). The formation of compounds **3** and **5a-i** were confirmed by ^{1}H NMR, ^{13}C NMR, and HRMS spectral analysis. In the ^{1}H NMR spectrum of the compound **5a**, the two methylene groups attached to nitrogen and oxygen showed singlet at δ 5.16 and 6.09 ppm, respectively. In addition to this, the signal observed at δ 4.06 ppm indicates the -OCH_3 proton present on the quinoline ring. Similarly signal observed at δ 8.01 ppm indicates the proton present on the triazole ring. In the ^{13}C NMR spectrum for compound **5a**, the signals at δ 61.5 and 66.2 ppm indicates the presence of methylene carbon attached to the nitrogen of triazole ring and oxygen to phenyl ring, respectively, and signal at δ 54 ppm indicates the presence of methoxy carbon.

For compound **5a**, the calculated mass for $[M+H]^+$ is 388.1516 and in HRMS, the $[M+H]^+$ peak observed at 388.1513. Furthermore, to expand the series, 1,4-disubstituted-1,2,3-triazole tetrazoloquinoline derivatives **5 b-i** with various substituent has been prepared by the cycloaddition reaction of 4-(azidomethyl)-8-methoxytetrazolo[1,5-*a*]quinoline **3** and alkynes (prop-2-yn-1-yloxy)benzene derivatives **4 b-h** and phenylacetylene **6** (Scheme 1) under similar reaction condition in good to excellent yields.



Scheme 1. Reagents and conditions: (a) acetic acid, 110–120 °C, 6 hr; (b) DMF, $POCl_3$, 0–80 °C, 8 hr, (c) NaN_3, DMF, 80 °C, 6 hr; (d) $NaBH_4$, methanol, 0 °C to rt, 2 hr; (e) MsCl, DCM, 0 °C to rt, 4 hr; (f) NaN_3, DMF, 80 °C, 2 hr; (g) propargyl bromide, K_2CO_3, DMF; (h) $Cu(OAc)_2$ (20 mol%), *t*-BuOH-H_2O (3:1), rt.

Biological evaluation

Antibacterial activity

Minimum inhibitory concentration (MIC) values for bacteria determined according to the two-fold broth micro-dilution method using Muller-Hinton broth in 96-well micro-test plates recommended by National Committee for Clinical Laboratory Standards (NCCLS) guidelines.[37] All the tested tetrazoloquinoline-1,4-disubstituted 1,2,3-triazole based derivatives **5a-i** shows significant antibacterial activity (Table 1). For bacterial strain *S. aureus*, it can be seen that, the compounds **5b**, **5d**, **5e**, **5f**, and **5g** shows excellent inhibitory activity with MIC value 8 µg/mL, which is two-fold more potent than the clinical drug ampicillin and kanamycin (MIC 16 µg/mL) and equivalent to the chloramphenicol (8 µg/mL). However, the compounds **5a**, **5c**, **5h**, and **5i** also possess equivalent antibacterial effect against *S. aureus* with MIC value 16 µg/mL compared to the ampicillin and kanamycin. Compounds **5b**, **5c**, **5d**, **5f**, **5h**, and **5i** with MIC value 16 µg/mL exhibit equivalent antibacterial activity for *M. luteus* compared to the standard drug ampicillin. All the synthesized compounds show considerable activity against *B. cereus*, especially compounds **5e**, **5f**, **5g**, and **5i** with MIC value 4 µg/mL exhibited four-fold more activity compared to the ampicillin and two-fold more potent than kanamycin and chloramphenicol. Compound **5h** shows equivalent activity compared to the standard drug kanamycin and chloramphenicol. Compounds **5b** and **5c** show good activity against *B. cereus* compared to the ampicillin. It can be seen that, all the synthesized tetrazoloquinoline-1,2,3-triazole-based derivatives **5a-i** possess comparable activity against *E. coli* as compared to ampicillin, kanamycin, and chloramphenicol.

Table 1. *In vitro* antimicrobial evaluation of synthesized compounds **3** and **5a-i** MIC values (μg/mL).

	Antibacterial activity											
	Gram + ve bacteria			Gram –ve bacteria			Antifungal activity					
Entry	*SA*	*ML*	*BC*	*EC*	*PF*	*FD*	*CA*	*AN*	*CL*	*PC*	*AF*	*CN*
3	128	128	256	256	256	256	128	128	256	256	256	256
5a	16	32	32	16	16	8	8	8	8	32	32	128
5b	8	16	16	8	8	32	16	16	32	64	16	32
5c	16	16	16	4	8	4	4	8	8	16	16	16
5d	8	16	32	4	128	16	16	32	16	32	16	128
5e	8	32	4	16	16	16	8	8	8	64	16	16
5f	8	16	4	4	16	8	16	8	16	64	32	32
5g	8	32	4	4	4	4	4	8	8	32	8	128
5h	16	16	8	4	4	4	4	8	8	32	128	256
5i	16	16	4	4	128	4	8	16	32	128	256	256
AP	16	16	16	16	16	16	–	–	–	–	–	–
KM	16	8	8	16	16	16	–	–	–	–	–	–
CP	8	8	8	8	8	8	–	–	–	–	–	–
MA	–	–	–	–	–	–	16	16	16	16	16	16
AB	–	–	–	–	–	–	16	8	16	16	8	16
FA	–	–	–	–	–	–	8	8	8	8	8	8

SA, Staphylococcus aureus; ML, Micrococcus luteus; BC, Bacillus cereus; EC, Escherichia coli; PF, Pseudomonas fluorescens; FD, Flavobacterium devorans; AN, Aspergillus niger; PC, Penicillium chrysogenum; CL, Curvularia lunata; CA, Candida albicans; AF, Aspergillus flavus; CN, Cryptococcus neoformans; AP, Ampicillin; KM, Kanamycin; CP, Chloramphenicol; MA, Miconazole; AB; Amphotericin B; FA, Fluconazole.

Compounds **5c**, **5d**, **5f**, **5g**, **5h**, and **5i** with MIC value 4 μg/mL shows four-fold more activity compared to the ampicillin, kanamycin, and two-fold more activity compare to the chloramphenicol against *E. coli*. Compound **5b** with MIC value 8 μg/mL, possesses equivalent activity compared to the standard drug chloramphenicol. Compounds **5g** and **5h** with MIC value 4 μg/mL show four-fold more activity compared to the ampicillin, kanamycin, and two-fold more activity compared to the chloramphenicol against bacterial strain *P. fluorescens*. Compounds **5b** and **5c** shows two-fold more activity compared to the standard ampicillin, kanamycin, and show equivalent activity compared to the chloramphenicol. Compounds **5a**, **5e**, and **5f** show equivalent activity compared to the standard drug ampicillin and chloramphenicol with MIC value 16 μg/mL. Compounds **5c**, **5g**, **5h**, and **5i** with MIC value 4 μg/mL show four-fold more activity compared to the ampicillin, kanamycin, and two-fold more activity compared to the chloramphenicol against bacterial strain *F. devorans*.

Compounds **5a** and **5f** (MIC = 8 μg/mL) show two-fold more activity compared to the standard ampicillin, kanamycin, and shows equivalent activity compared to the chloramphenicol. Compounds **5d** and **5e** show equivalent activity compared to the ampicillin and chloramphenicol with MIC value 16 μg/mL. In general, for Gram positive bacteria, among all the synthesized compounds **3** and **5a-i**, compounds **5e**, **5f**, **5g**, and **5i** show promising antibacterial activity against bacterial strain *B. cereus* and all the synthesized compounds exhibited excellent antibacterial activity against Gram negative bacteria *E. coli* and *F. devorans* compared to the standard drugs.

Antifungal activity

Fungi were subcultured in potato dextrose broth medium. MIC of the synthesized compounds was determined using potato dextrose broth in 96-well micro-test plates recommended by NCCLS guidelines.[37] In case of antifungal activity, all the synthesized 1,4-disubstituted 1,2,3-triazole-based tetrazoloquinoline derivatives **5a-i** show good to moderate activity against *C. albicans*, *A. niger*, *C. lunata*, *P. chrysogenum*, *A. flavus*, and *C. neoformans* strains (Table 1). Compounds **5c**, **5g**, and **5h** with MIC value 4 μg/mL, exhibited four-fold more activity compared to the standard drug miconazole, amphotericin B and two-fold more activity compared to the fluconazole against the fungicidal strain *C. albicans*. Compounds **5a**, **5e**, and **5i** with MIC values 8 μg/mL,

exhibited two-fold more activity compared to the standard drug miconazole, amphotericin B and equivalent potency compared to the standard fluconazole. Compounds **5b**, **5d**, and **5f** with MIC values 16 μg/mL, exhibited equivalent activity compared to the standard drug miconazole and amphotericin B against the fungal strain *C. albicans*. Compounds **5a**, **5c**, **5e**, **5f**, **5g**, and **5h** with MIC values 8 μg/mL, exhibited two-fold more activity compared to the standard drug miconazole, amphotericin B and equivalent activity compared to the fluconazole against the fungicidal strain *A. niger*. Compounds **5b** and **5i** with MIC values 16 μg/mL, exhibited equivalent potency compared to the standard drug miconazole against the fungal strain *A. niger*. Compounds **5a**, **5c**, and **5e** with MIC values 8 μg/mL, exhibited two-fold more activity compared to the standard drug miconazole, amphotericin B and equivalent activity compared to the fluconazole against the fungicidal strain *C. lunata*. Compounds **5d** and **5f** with MIC values 16 μg/mL, exhibited equivalent potency compared to the standard drug miconazole and amphotericin B against the fungal strain *C. lunata*. Compound **5c** with MIC values 16 μg/mL, exhibited equivalent potency compared to the standard drug miconazole and amphotericin B against the fungal strain *P. chrysogenum*. Compound **5g** with MIC values 8 μg/mL, exhibited two-fold more activity compared to the standard drug miconazole and equivalent potency compared to the amphotericin B and fluconazole against the fungal strain *A. flavus*. Compounds **5b**, **5c**, **5d**, and **5e** with MIC value 16 μg/mL exhibited equivalent activity as compared to standard drug miconazole for fungal strain *A. flavus*. Compounds **5c** and **5e** with MIC value of 16 μg/mL, show equivalent antifungal activity against *C. neoformans* as compared to standard drug miconazole and fluconazole. Overall, the starting material 4-(azidomethyl)-8-methoxytetrazolo[1,5-*a*]quinoline **3** exhibited very less antifungal activity but the 1,2,3-triazoles derived from the azide **3** shows excellent antifungal activity compared to the standard antifungal drugs miconazole, amphotericin B and fluconazole.

Antitubercular activity

All the synthesized compounds **5a-i** showed antibacterial activity against both Gram positive and Gram negative bacteria, especially against the Gram negative bacteria. As these compounds have shown significant antibacterial activities, we extended our study for evaluation of antitubercular activity. The newly synthesized 1,4-disubstituted-1,2,3-triazole containing tetrazoloquinoline derivatives **5a-i** were screened for *in vitro* antitubercular activity against *MTB H37Ra* (ATCC 25177) and *M. bovis BCG* (ATCC 35743) in liquid medium. In a preliminary screening (Supporting Information, Table S1), the antimycobacterial activity of these compounds was assessed at concentrations of 30, 10, and 3 μg/mL using an established XTT Reduction Menadione assay (XRMA) anti-tubercular screening protocol[38] using first-line antitubercular drugs rifampicin and isoniazid as reference standards and the MIC and IC_{50} values are presented in Table 2. The MIC, that is, concentration of compounds required to completely inhibit MTB growth, were recorded. The MIC was calculated from a dose response curve. The compounds with more than 90% inhibition of initial primary screening were further assayed for secondary screening, that is, determination of MIC against dormant *MTB H37Ra* and dormant *M. bovis BCG* clinical isolates (drug sensitive and resistant). The tetrazoloquinoline-1,2,3-triazole conjugates **5a-i** (MIC range > 30 μg/mL) were found to be particularly inactive against dormant *MTB H37Ra*, dormant *M. bovis BCG*.

Antioxidant activity

In the present study, antioxidant activity of the synthesized compounds has been assessed *in vitro* by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay[39] and all the synthesized compounds **5a-i** show good to moderate antioxidant activity as compared to the standard drug BHT (Butylated Hydroxy Toluene) (Table 2). Compounds **5b** and **5d** having *chloro*-substituent at *ortho*- and *para*-position of phenyl ring, respectively, shows potent activity (IC_{50} 12.48 and 16.30 μg/mL, respectively) as compared to the standard drug BHT. However, the compound **5g**

Table 2. *In vitro* antitubercular activity against dormant *MTB H37Ra*, dormant *M. bovis BCG* and DPPH radical scavenging activity of compound **3** and **5a-i**.

	MTB H37Ra dormant		*M. bovis* BCG dormant		
Compounds	MIC	IC_{50}	MIC	IC_{50}	DPPH IC_{50} (μg/mL)
3	>30	>30	>30	>30	50.20
5a	>30	>30	>30	>30	29.43
5b	>30	>30	>30	>30	12.48
5c	>30	>30	>30	>30	16.30
5d	>30	>30	>30	>30	40.41
5e	>30	>30	>30	>30	26.78
5f	>30	>30	>30	>30	32.55
5g	>30	>30	>30	>30	15.19
5h	>30	>30	>30	>30	19.20
5i	>30	>30	>30	>30	33.82
RP	0.043 ± 0.15	0.0018 ± 0.009	0.041 ± 0.01	0.0016 ± 0.002	NT
INH	0.075 ± 0.25	0.0025 ± 0.0007	0.045 ± 0.02	0.0023 ± 0.001	NT
BHT	NT	NT	NT	NT	16.47

RP: Rifampicin; INH: Isoniazid; BHT: Butylated hydroxy toluene; NT: Not tested.

(15.19 μg/mL) with *methyl*-group at *meta*- and *chloro*-group at *para*-position of phenyl ring shows excellent antioxidant activity as compared to the BHT. The starting material 4-(azidomethyl)-8-methoxytetrazolo[1,5-*a*]quinoline **3** exhibited very less antioxidant activity as compared to standard drugs.

Computational study

Molecular docking

Molecular docking is now an established approach in drug discovery for predicting the binding mode of a specified compound within the active site of the protein target of interest and the types of thermodynamic interactions governing the protein-inhibitor complexation especially in the absence of available resources to carry out the enzymatic assays. Thus, with the aim of rationalizing the promising antifungal activity portrayed by the title compounds tetrazoloquinoline-1,2,3-triazole derivatives (**5a-i**) and to gain an insight into the molecular basis of their interactions, a molecular docking study was carried out against the fungal sterol 14α-demethylase (CYP51) as the target enzyme. Sterol 14α-demethylase (CYP51) is an ancestral activity of the cytochrome P450 superfamily. It converts lanosterol into 4,4′-dimethyl cholesta-8,14,24-triene-3-β-ol-a step required for ergosterol biosynthesis which is an essential component of the fungal cytoplasmic membrane. Inhibition of CYP51 causes depletion of ergosterol coupled with an accumulation of 14-methyl sterols resulting in impaired cell growth in fungi. This crucial role of CYP51 in fungi makes it an important target for drug design. A perusal of the docking poses obtained for all these tetrazoloquinoline-1,2,3-triazole derivatives revealed that they could snugly fit into the active site of CYP51 with varying degree of binding affinities adopting a very homologous orientation and at co-ordinates very close to that of the native ligand-fluconazole (Figure 2). The resulting enzyme-inhibitor complexation was stabilized through a network of steric and electrostatic interactions with the active site residues.

To gauze the accuracy and reliability of the docking protocol, the co-crystallized ligand (fluconazole) was extracted from the crystal structure and again subjected to dock into the same binding pocket defining the above-mentioned parameters. The docked conformation of fluconazole comparing with the experimental binding mode as in X-ray is shown in Figure 3. The result show that the docking protocol could reproduce the X-ray bound conformation of fluconazole with an RMSD of less than 1.0 Å indicating the reliability of the docking protocol in accurately predicting the binding mode for the title molecules.

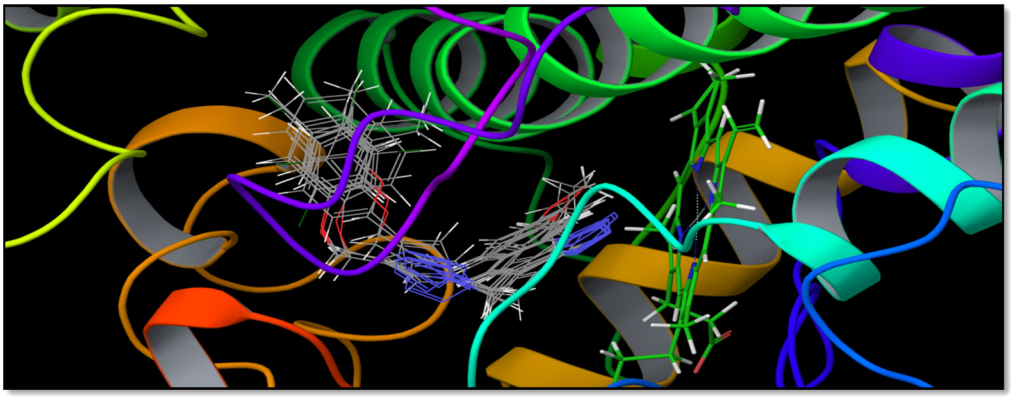


Figure 2. Docking-based binding mode of tetrazoloquinoline-1,2,3-triazole derivatives (**5a-i**) into the active site of fungal sterol 14α-demethylase (CYP51).

The minimum energy (Glide energy) for each of these complexes was observed to be negative ranging from −58.54 kcal/mol to −49.70 kcal/mol while the docking scores ranged from −7.79 to −7.14 with a significant correlation between their docking score and the experimentally observed MIC values—the active compounds possessing higher scores while those with relatively lower activity were also predicted to have lower docking scores. The binding energy for the reference ligand-fluconazole was found to be −52.92 kcal/mol with a docking score of −7.34. The binding energy signifies the energy required for a ligand to cover the entire enzyme surface and its putative interactions with the amino acid residues. A higher negative value for the binding energy (and docking score) signifies a good binding affinity for the ligand toward the target enzyme and vice versa. Furthermore, a detailed analysis of the per-residue interactions between the enzyme and these compounds was carried out to identify the most significantly interacting residues and the type of thermodynamic elements (bonded and non-bonded interactions) governing the binding of these molecules to the target. This analysis is elucidated in the next section for one of the most active compound **5g** while the results for the remaining compounds are summarized in Table 3 and their binding modes are provided in the Supporting Information as Figure 4.

The lowest energy docked conformation of **5 g** into the active site of CYP51 showed that the inhibitor binds at the same co-ordinates as the native ligand with a significantly higher binding affinity resulting in a docking score of −7.79 and a binding energy −58.54 kcal/mol (Figure 4). The higher binding affinity can be explained in terms of the specific bonded and non-bonded per-residue interactions with the residues lining the active site.

The complexation of **5g** with CYP51 is observed to be stabilized through an extensive network of van der Waals interactions with Ala291 (−3.08 kcal/mol), Ala288 (−1.55 kcal/mol), Ala287 (−1.99 kcal/mol), Tyr116 (−3.54 kcal/mol), Phe110 (−1.94 kcal/mol), and Tyr103 (−3.54 kcal/mol) through quinoline-tetrazole backbone while 1,2,3-triazole heterocycle was engaged in similar interactions through Val461 (−3.28 kcal/mol), Met460 (−2.52 kcal/mol), Thr459 (−1.28 kcal/mol), Leu356 (−2.46 kcal/mol), and Met106 (−4.00 kcal/mol) residues in the active site. Furthermore, the substituted aromatic ring connected to 1,2,3-triazole heterocycle also showed favorable van der Waals interactions through His294 (−1.40 kcal/mol), Phe290 (−3.98 kcal/mol), Leu208 (−3.02 kcal/mol), and Glu205 (−1.96 kcal/mol) residues. The compound was also involved in a set of relatively few but significant electrostatic interactions as well through Met106 (−1.50 kcal/mol) and Tyr103 (−1.18 kcal/mol) residues in the active site CYP51. The excellent binding affinity of **5g** toward CYP51 can also be attributed to a very strong van der Waals (−10.62 kcal/mol) as well as electrostatic (−9.425 kcal/mol) interactions with heme moiety in the active site. Furthermore, a prominent π-π stacking interaction was observed through the aromatic ring of quinoline-tetrazole backbone with the Phe110 having a bonding distance of 2.451 Å which as well contributed significantly to the stability of the **5g** in the active site of the

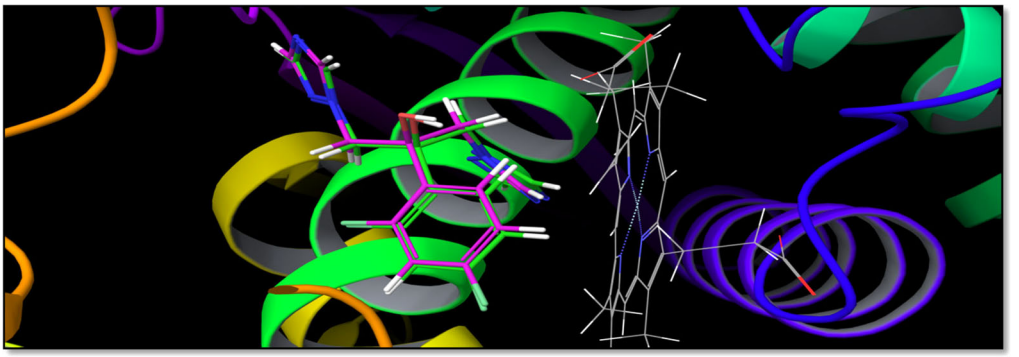


Figure 3. Validation of the molecular docking protocol: super-imposed image of the structures of fluconazole from the crystal structure (orange carbon chain) and the docked conformation (purple carbon chain).

enzyme. Such π-π stacking interactions serve as an "anchor" for channelizing the 3 D orientation of the ligand in its active site and also facilitate the steric and electrostatic interactions thereby contributing to the stability of the enzyme-inhibitor complex.

A similar network of interaction was observed for the other quinoline-tetrazole-1,2,3-triazole derivatives as well but decreasing gradually with their observed antifungal activity. The per-residue interaction analysis revealed that the primary driving forces for mechanical interlocking is the steric complementarity between the ligand and the active site of CYP51 which is evident from the relatively higher number of van der Waals interactions over other components contributing to the binding scores. Interestingly, all the quinoline-tetrazole-1,2,3-triazole derivatives investigated herein were found coordinated to the iron of the heme group present in the active site. This is a very important observation as the native ligand-fluconazole is also coordinated with the metal ion in the active site of CYP51. Thus, these quinoline-tetrazole-1,2,3-triazoles may as well share the same inhibition mechanism as fluconazole making them pertinent starting points for structure-based drug design.

In silico ADME prediction

The success of a drug is determined not only by good efficacy but also by an acceptable ADME (absorption, distribution, metabolism, and excretion) profile. In the present study, we have calculated molecular volume (MV), molecular weight (MW), logarithm of partition coefficient (miLog *P*), number of hydrogen bond acceptors (n-ON), number of hydrogen bonds donors (n-OHNH), topological polar surface area (TPSA), number of rotatable bonds (n-ROTB), and Lipinski's rule of five[40] using Molinspiration online property calculation toolkit.[41] Absorption (% ABS) was calculated by: % ABS = 109 – (0.345 × TPSA).[42] Drug-likeness model score (a collective property of physic-chemical properties, pharmacokinetics, and pharmacodynamics of a compound is represented by a numerical value) was computed by MolSoft software.[43]

A computational study of all the synthesized **5a-i** was performed for prediction of ADME properties and the value obtained is presented in Table 4.

It is observed that, the compounds exhibited a good % ABS (% absorption) ranging from 77.16 to 80.35%. Furthermore, none of the synthesized compounds **5a-i** violated Lipinski's rule of five (miLog $P \leq 5$). A molecule likely to be developed as an orally active drug candidate should not show more than one violation of the following four criteria: miLog *P* (octanol-water partition coefficient) $\leq$ 5, molecular weight $\leq$ 500, number of hydrogen bond acceptors $\leq$ 10 and number of hydrogen bond donors $\leq$ 5.[44] The larger the value of the drug likeness model score, the higher is also probability that the particular molecule will be active. All the tested compounds followed the criteria for orally active drug and therefore, these compounds may have a good potential for eventual development as oral agents.

Table 3. Quantitative estimate of the per-residue interactions for the tetrazoloquinoline-1,2,3-triazole derivative (**5a-i**) with the *fungal sterol 14α-demethylase (CYP51) enzyme*.

			Per-residue interaction energy analysis		
Cpd	Docking score	Binding energy	van der Waals (kcal/mol)	Electrostatic (kcal/mol)	π-π stacking (Å)
5a	−7.40	−53.57	500(−10.79), Val461(−2.92), Met460(−2.41), Thr459(−1.19), Leu356(−2.21), Ala291(−2.31), Phe290(−2.30), Ala288(−1.24), Ala287(−1.59), Leu208(−1.26), Tyr116(−2.45), Phe110(−1.52), Met106(−3.16), Tyr103(−2.92)	500(−7.37), Met106(−1.25), Tyr103(−1.09)	Tyr116(2.07)
5b	−7.1	−50.77	500(−10.72), Val461(−2.41), Met460(−1.78), Thr459(−1.08), Leu356(−2.10), Ala291(−2.03), Phe290(−2.19), Ala288(−1.14), Ala287(−1.11), Leu208(−1.14), Leu127(−1.04), Tyr116(−1.98), Phe110(−1.27), Met106(−2.92), Tyr103(−3.75)	500(−6.75), Tyr116(−1.32), Met106(−1.10), Tyr103(−1.017)	Tyr116(2.06)
5c	−7.78	−57.71	500(−10.85), Val461(−3.18), Met460(−3.11), Thr459(−1.42), Leu356(−2.10), His294(−1.40), Ala291(−2.91), Phe290(−3.51), Ala288(−1.43), Ala287(−1.96), Leu208(−2.96), Glu205(−1.85), Tyr116(−3.55), Phe110(−1.77), Met106(−3.83), Ile105(−2.63), Tyr103(−3.05)	500(−9.67), Met106(−1.43), Tyr103(−1.19)	Phe110(2.39)
5d	−7.14	−49.70	500(−10.52), Val461(−2.50), Met460(−1.71), Thr459(−1.00), Leu356(−2.16), Ala291(−2.04), Phe290(−2.09), Ala288(−1.14), Ala287(−1.05), Leu208(−1.18), Leu127(−1.00), Tyr116(−1.95), Phe110(−1.25), Met106(−2.59), Tyr103(−2.61)	500(−6.76), Tyr116(−1.11), Met106(−1.19)	Tyr116(2.066)
5e	−7.33	−53.50	500(−10.42), Val461(−2.70), Met460(−2.35), Thr459(−1.13), Leu356(−2.27), His294(−1.11), Ala291(−2.21), Phe290(−2.25), Ala288(−1.24), Ala287(−1.57), Leu208(−1.33), Tyr116(−2.25), Phe110(−1.42), Met106(−3.03), Tyr103(−3.14)	500(−7.29), Tyr116(−0.86), Met106(−1.24), Tyr103(−1.09)	Tr116(2.07)
5f	−7.26	−50.74	500(−10.44), Val461(−2.40), Met460(−1.41), Thr459(−1.07), Leu356(−2.17), His294(−1.34), Ala291(−2.18), Phe290(−2.16), Ala288(−1.06), Ala287(−1.05), Leu208(−1.04), Glu205(−1.67), Tyr116(−1.35), Phe110(−1.15), Met106(−2.29), Tyr103(−2.83)	500(−7.05), Tyr116(−1.22), Met106(−1.12)	Tyr116(1.97)
5g	−7.79	−58.54	500(−10.62), Val461(−3.28), Met460(−2.52), Thr459(−1.28), Leu356(−2.46), His294(−1.40), Ala291(−3.08), Phe290(−3.98), Ala288(−1.55), Ala287(−1.99), Leu208(−3.02), Glu205(−1.96), Tyr116(−3.54), Phe110(−1.94), Met106(−4.00), Tyr103(−3.54)	500(−9.42), Met106(−1.5), Tyr103(−1.18)	Phe110(2.45)
5h	−7.72	−57.51	500(−10.87), Val461(−3.13), Met460(−2.29), Thr459(−1.58), Leu356(−2.58), His294(−1.37), Ala291(−2.94), Phe290(−3.29), Ala288(−1.45), Ala287(−1.84), Leu208(−3.40), Glu205(−1.98), Tyr116(−3.42), Phe110(−1.81), Met106(−3.72), Tyr103(−3.01)	500(−9.41), Tyr116(−1.31), Tyr103(−1.13)	Tyr116(2.07)
5i	−7.42	−51.64	500(−10.26), Val461(−2.78), Met460(−2.53), Thr459(−1.14), Leu356(−2.26), Ala291(−2.33), Phe290(−2.29), Ala288(−1.28), Ala287(−1.67), Leu208(−1.49), Tyr116(−2.29), Phe110(−1.40), Met106(−3.01), Tyr103(−2.81)	500(−7.97), Tyr116(−1.18), Tyr103(−1.016)	Tyr103(2.92), Tyr116(2.01)

Conclusion

We have synthesized new 1,4-disubstituted 1,2,3-triazole-based tetrazoloquinoline derivatives *via* click chemistry approach and evaluated for biological activity. The synthesized compound displays promising antibacterial, antifungal, and antioxidant activity as compared to the respective standard drugs and unfortunately does not show antitubercular activity. Compounds **5g** and **5h** displayed significant antibacterial activity as compared to the standard antibacterial drug. Compounds **5c**, **5g**, and **5h** displayed significant antifungal activity as compared to the standard

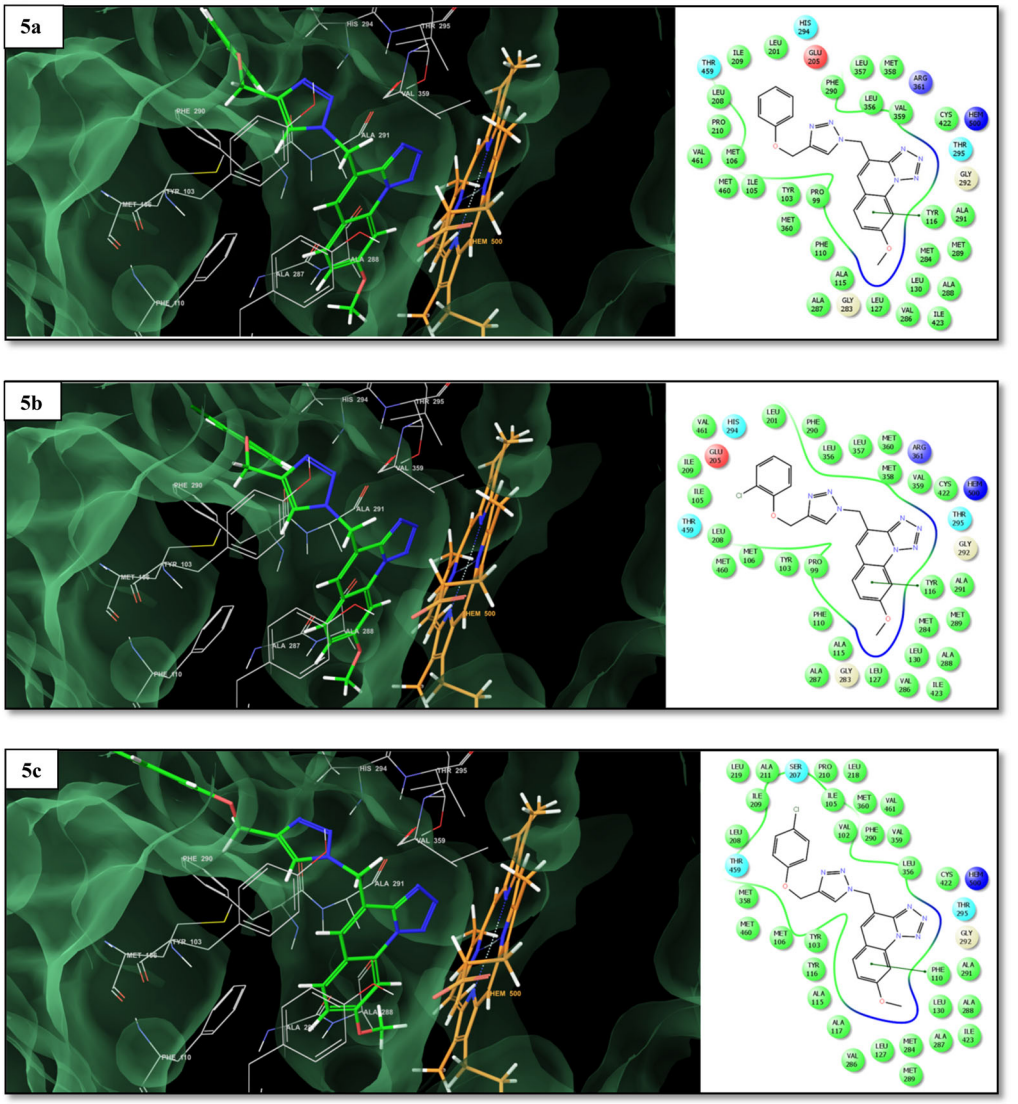


Figure 4. Docking-based binding mode of **5a**, **5b**, **5c**, **5d**, **5e**, **5f**, **5g**, **5h**, and **5i** into the active site of sterol 14α-demethylase (CYP51) (the π-π stacking interaction is represented using the green line).

antifungal drug. Compounds **5b**, **5d**, and **5g** shows potential antioxidant activity when compared with standard BHT. The trend observed in the antifungal activity for these tetrazoloquinoline-1,2,3-triazole derivatives was further rationalized by molecular docking studies with respect to their binding energy toward target enzyme sterol 14α-demethylase (CYP51). The theoretical predictions from molecular docking studies were found to be in agreement with the experimental antifungal data. Furthermore, the quantitative estimation of the per-residue interactions between these tetrazoloquinoline-1,2,3-triazoles and CYP51 enzyme helps to speculate regarding the detailed binding patterns in the cavity and the most significantly interacting the residues as well as the type of thermodynamic interactions governing the binding of these molecules which can provide an ample opportunity for medicinal chemist to design more specific and potent analogues targeting CYP51. Furthermore, analysis of the ADME parameters for synthesized compounds predicted good drug like properties and

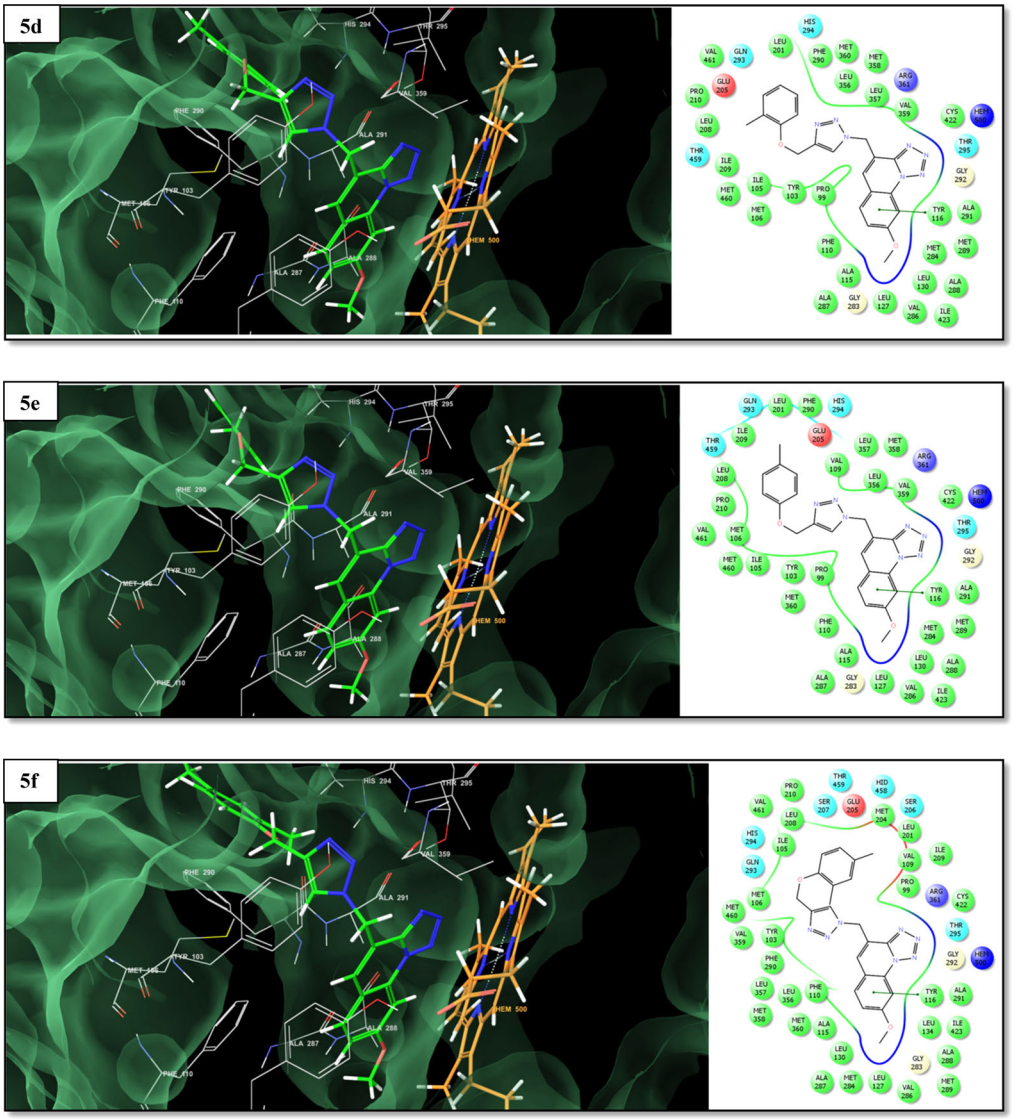


Figure 4. Continued.

can be developed as oral drug candidate. Thus, suggesting that compounds from present series can be further optimized and developed as a lead molecule.

Experimental

General procedure for the synthesis of 1-(prop-2-ynyloxy)benzene or substituted 1-(prop-2-ynyloxy)benzene (4a-h)

To the stirred solution of phenol or substituted phenol (20 mmol) in *N,N*-dimethylformamide (DMF) (20 mL), K_2CO_3 (24 mmol) was added. The reaction mixture was stirred at room temperature for 30 min, which results into the corresponding oxyanion. To this mixture, propargyl bromide (20 mmol) was added and stirred for 2 hr. The progress of the reaction was monitored by TLC using ethyl acetate:hexane as a solvent system. The reaction was quenched by crushed ice.

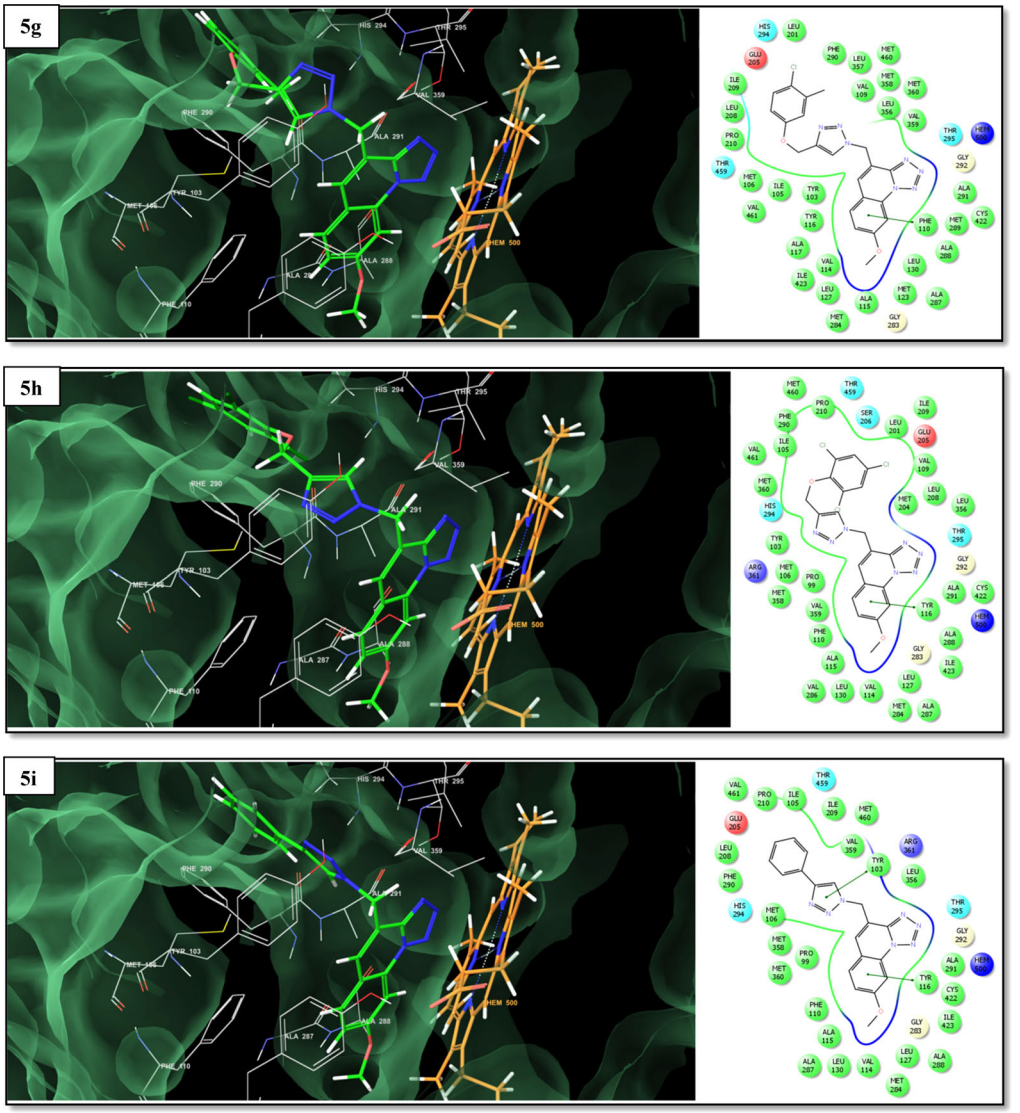


Figure 4. Continued.

In case of solid product, it was filtered and the obtained crude solid product was crystallized using ethanol. The crystallized products were taken for next step. When the products are liquid, it has been extracted in ethyl acetate (20 mL × 3). The combined organic layers were dried over $MgSO_4$. The solvent was removed under a reduced pressure and used for further reaction without purification.

General procedure for the synthesis of 8-methoxy-4-((4-(phenoxymethyl)-1H-1,2,3-triazol-1-yl)methyl)tetrazolo[1,5-a]quinoline 5a-i

To the solution of 1-(prop-2-ynyloxy)benzene and phenyl acetylene **(4a-i)** (0.5 mmol), 4-(azidomethyl)-8-methoxytetrazolo[1,5-*a*]quinoline **3** (0.5 mmol) and copper diacetate ($Cu(OAc)_2$) (20 mole %) in *t*-BuOH-H_2O (3:1, 8 mL) and the resulting mixture was stirred at room temperature for 16–22 hr. The progress of the reaction was monitored by TLC using ethyl acetate:hexane

Table 4. Pharmacokinetic parameters important for good oral bioavailability and its drug likeness model score.

Entry	% ABS	TPSA (A^2)	n-ROTB	MV	MW	miLog *P*	n-ON	n-OHNH	Lipinski violation	Drug-likeness model score
Rule	–	–	–	–	<500	≤5	<10	<5	≤1	-
5a	77.16	92.28	6	332.93	387.40	3.01	9	0	0	−0.24
5b	77.16	92.28	6	346.47	421.85	3.64	9	0	0	0.18
5c	77.16	92.28	6	346.47	421.85	3.69	9	0	0	0.21
5d	77.16	92.28	6	349.49	401.43	3.42	9	0	0	0.14
5e	77.16	92.28	6	349.49	401.43	3.46	9	0	0	−0.24
5f	77.16	92.28	6	366.05	415.46	3.84	9	0	0	−0.10
5g	77.16	92.28	6	363.03	435.88	4.07	9	0	0	0.32
5h	77.16	92.28	6	373.54	490.74	4.90	9	0	0	0.10
5i	80.35	83.04	4	307.14	357.38	2.80	8	0	0	−0.03

% ABS: Percentage absorption, TPSA: Topological polar surface area, n-ROTB: Number of rotatable bonds, MV: Molecular volume, MW: Molecular weight, miLog *P*: Logarithm of partition coefficient of compound between n-octanol and water, n-ON Acceptors: Number of hydrogen bond acceptors, n-OHNH donors: Number of hydrogen bonds donors.

as a solvent system. The reaction mixture was quenched with crushed ice and extracted with ethyl acetate (2 × 25 mL). The organic extracts were washed with brine solution (2 × 25 mL) and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to afford the corresponding crude compounds. The obtained crude compounds were crystallized using ethanol and ethyl acetate.

Synthesis of 2-chloro-7-methoxyquinoline-3-carbaldehyde[45]

To a solution of *N*-(3-methoxyphenyl)acetamide (5 mmol) in dry DMF (15 mmol) at 0–5 °C with stirring $POCl_3$ (60 mmol) was added drop wise and the mixture stirred at 80–90 °C for time 8 hr. After the completion of reaction (checked by TLC), the mixture was poured into crushed ice, stirred for 5 min and the resulting solid filtered, washed well with water and dried. The compounds were purified by recrystallization from ethyl acetate.

Synthesis of 8-methoxytetrazolo[1,5-a]quinoline-4-carbaldehyde

2-Chloro-7-methoxyquinoline-3-carbaldehyde (5 mmol), sodium azide (10 mmol), and DMF (10 mL) were taken in round bottom flask as per reported procedure.[46] The reaction mixture was slowly heated at 80 °C for 2 hr. After the completion of reaction (checked by TLC), the product was filtered and washed with ethanol. The crude product was purified by crystallization in DMF. Melting point is 240–242 °C.

Synthesis of (8-methoxytetrazolo[1,5-a]quinolin-4-yl)methanol

8-methoxytetrazolo[1,5-*a*]quinoline-4-carbaldehyde (1 equiv) were taken in round bottom flask, methanol used as a solvent and allowed reaction mixture for stirring below 0 °C. Then, $NaBH_4$ (3 equiv.) were added slowly with constant stirring and maintaining the temperature below 0 °C for 2 hr. The progress of the reaction was monitored by thin layer chromatography (TLC) using ethyl acetate:hexane as a solvent system. After completion of the reaction as indicated by TLC, the reaction mixture was then poured on crushed ice and extracted in ethylacetate (3 × 10 mL). The combined organic layer was dried over $MgSO_4$. Solvent was removed under reduced pressure and the (8-methoxytetrazolo[1,5-*a*]quinolin-4-yl)methanol were sufficiently pure to use without further work up.

Synthesis of (8-methoxytetrazolo[1,5-a]quinolin-4-yl)methyl methanesulfonate

To a mixture of (2-chloro quinolin-3-yl)methanol 1 (1 equiv) in acetone, triethyl amine (2 equiv) was added at 0 °C. Methane sulfonyl chloride (1.5 equiv) in acetone was added dropwise in 10 min at 0 °C, and stirred for 4 hr. The progress of the reaction was monitored on TLC. After completion of the reaction, reaction mixture was poured on crushed ice. The solid obtained was extracted with ethylacetate (2 × 20 mL) and washed with brine (2 × 20 mL). Thus, organic layer was separated, dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure. The obtained crude product was crystallized using ethanol/ethylacetate obtain pure compound (8-methoxytetrazolo[1,5-*a*]quinolin-4-yl)methyl methanesulfonate.

Synthesis of 4-(azidomethyl)-8-methoxytetrazolo[1,5-a]quinoline (3)

To a solution of (8-methoxytetrazolo[1,5-*a*]quinolin-4-yl)methyl methanesulfonate (1 equiv) in dry DMF, sodium azide (2 equiv) was added and stirred at 80 °C for 2 hr. The progress of the reaction was monitored on TLC. After completion of reaction, reaction mixture was poured on crushed ice. The solid obtained was extracted with EtOAc (2 × 25 mL). The organic extract was washed with water and brine. The solvent was removed under reduced pressure to afford crude product 3, which was purified by crystallization using ethanol/ethylacetate with 92% yield. Mp 133–135 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 4.05 (*s*, 3H, -OMe), 4.95 (*s*, N-CH_2), 7.40–7.46 (*m*, 1H), 7.98–7.99 (*d*, 1H), and 8.15–8.25 (*m*, 2H). HRMS calculated $[M+H]^+$ for $C_{11}H_{10}N_7O$: 256.0941, found: 256.0935.

8-Methoxy-4-((4-(phenoxymethyl)-1H-1,2,3-triazol-1-yl)methyl)tetrazolo[1,5-a]quinoline (5a)

The compound **5a** as a pale pink solid and was obtained *via* 1,3-dipolar cycloaddition reaction between 4-(azidomethyl)-8-methoxytetrazolo[1,5-*a*]quinoline **3** and (prop-2-yn-1-yloxy)benzene **4a** in 17 hr with 90% yield. Mp 150 °C. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 4.06 (*s*, 3H, -OMe), 5.16 (*s*, N-CH_2), 6.09 (*s*, O-CH_2), 6.92–7.06 (*m*, 3H), 7.27–7.35 (*m*, 2H), 7.43–7.47 (*d*, 1H), 8.01 (*s*, 1H), 8.16 (*s*, 1H), 8.20 (*s*, 1H) and 8.44 (*s*, 1H). ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 49.3, 56.8, 61.8, 99, 117, 117.6, 118.6, 125.1, 126, 129.7, 131.8, 131.9, 133.6, 143, 147.3, 157.4 and 162.3. HRMS calculated $[M+H]^+$ for $C_{20}H_{17}N_7O_2$: 388.1516, found: 388.1513.

4-((4-((2-Chlorophenoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-8-methoxytetrazolo[1,5-a]quinoline (5b)

The compound **5b** as a off white solid and was obtained *via* 1,3-dipolar cycloaddition reaction between 4-(azidomethyl)-8-methoxytetrazolo[1,5-*a*]quinoline **3** and 1-chloro-2-(prop-2-yn-1-yloxy)benzene **4b** in 19 hr with 88% yield. Mp 139–140 °C. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 4.03 (*s*, 3H, -OMe), 5.14 (*s*, N-CH_2), 6.06 (*s*, O-CH_2), 7.02–7.06 (*m*, 2H), 7.29–7.45 (*m*, 3H), 7.98–7.99 (*d*, 1H), 8.13 (*s*, 1H), 8.17 (*s*, 1H), and 8.41 (*s*, 1H).

4-((4-((4-Chlorophenoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-8-methoxytetrazolo[1,5-a]quinoline (5c)

The compound **5c** as a off white solid and was obtained *via* 1,3-dipolar cycloaddition reaction between 4-(azidomethyl)-8-methoxytetrazolo[1,5-*a*]quinoline **3** and 1-chloro-4-(prop-2-yn-1-yloxy)benzene **4c** in 20 hr with 87% yield. Mp 180 °C. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 4.06 (*s*, 3H, -OMe), 5.17 (*s*, N-CH_2), 6.09 (*s*, O-CH_2), 7.05–7.09 (*m*, 2H), 7.32–7.37 (*m*, 2H), 7.43–7.48 (*3*, 1H), 8.01–8.02 (*d*, 1H), 8.16 (*s*, 1H), 8.20 (*s*, 1H), and 8.44 (*s*, 1H). ^{13}C NMR (50 MHz,

$CDCl_3$, δ ppm): 48.8, 56.3, 61.3, 98.4, 116.5, 117, 118.1, 124.6, 125.5, 129.2, 131.2, 131.3, 133.1, 142.5, 146.8, 156.8, and 161.8. HRMS calculated $[M+H]^+$ for $C_{20}H_{17}N_7O_2Cl$: 422.1127, found: 422.1123.

8-Methoxy-4-((4-((o-tolyloxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)tetrazolo[1,5-a]quinoline (5d)

The compound **5d** as a pale pink solid and was obtained *via* 1,3-dipolar cycloaddition reaction between 4-(azidomethyl)-8-methoxytetrazolo[1,5-*a*]quinoline **3** and 1-methyl-2-(prop-2-yn-1-yloxy)benzene **4d** in 17 hr with 89% yield. Mp 145 °C.

8-Methoxy-4-((4-((p-tolyloxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)tetrazolo[1,5-a]quinoline (5e)

The compound **5a** as a pale pink solid and was obtained *via* 1,3-dipolar cycloaddition reaction between 4-(azidomethyl)-8-methoxytetrazolo[1,5-*a*]quinoline **3** and 1-methyl-4-(prop-2-yn-1-yloxy)benzene **4e** in 16 hr with 90% yield. Mp 152–153 °C.

4-((4-((2,4-Dimethylphenoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-8-methoxytetrazolo[1,5-a]quinoline (5f)

The compound **5f** as a off white solid and was obtained *via* 1,3-dipolar cycloaddition reaction between 4-(azidomethyl)-8-methoxytetrazolo[1,5-*a*]quinoline **3** and 2,4-dimethyl-1-(prop-2-yn-1-yloxy)benzene **4f** in 18 hr with 90% yield. Mp 135 °C. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 2.06 (*s*, 3H), 2.18 (*s*, 3H), 4.04 (*s*, 3H, -OMe), 5.10 (*s*, N-CH_2), 6.07 (*s*, O-CH_2), 6.93–6.96 (*m*, 3H), 7.39–7.45 (*m*, 1H), 7.99–8.00 (*d*, 1H), 8.13–8.17 (*m*, 2H), and 8.39 (*s*, 1H). HRMS calculated $[M+H]^+$ for $C_{22}H_{22}N_7O_2$: 416.1829, found: 416.1825.

4-((4-((4-Chloro-3-methylphenoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-8-methoxytetrazolo [1,5-a]quinoline (5 g)

The compound **5f** as a pale pink solid and was obtained *via* 1,3-dipolar cycloaddition reaction between 4-(azidomethyl)-8-methoxytetrazolo[1,5-*a*]quinoline **3** and 1-chloro-2-methyl-4-(prop-2-yn-1-yloxy)benzene **4g** in 21 hr with 86% yield. Mp 150 °C. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 2.27 (*s*, 3H), 4.04 (*s*, 3H, -OMe), 5.13 (*s*, N-CH_2), 6.07 (*s*, O-CH_2), 6.85–7.03 (*m*, 2H), 7.26–7.46 (*m*, 2H), 7.99–8.00 (*d*, 1H), 8.14 (*s*, 1H), 8.18 (*s*, 1H), and 8.41 (*s*, 1H). HRMS calculated $[M+H]^+$ for $C_{21}H_{19}N_7O_2Cl$: 436.1283, found: 436.1283.

8-Methoxy-4-((4-((2,4,6-trichlorophenoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)tetrazolo [1,5-a]quinoline (5 h)

The compound **5h** as a off white solid and was obtained *via* 1,3-dipolar cycloaddition reaction between 4-(azidomethyl)-8-methoxytetrazolo[1,5-*a*]quinoline **3** and 1,3,5-trichloro-2-(prop-2-yn-1-yloxy)benzene **4 h** in 22 hr with 86% yield. Mp 143–145 °C.

8-Methoxy-4-((4-phenyl-1H-1,2,3-triazol-1-yl)methyl)tetrazolo[1,5-a]quinoline (5i)

The compound **5i** as a pale pink solid and was obtained *via* 1,3-dipolar cycloaddition reaction between 4-(azidomethyl)-8-methoxytetrazolo[1,5-*a*]quinoline **3** and phenyl acetylene **4i** in 19 hr

with 88% yield. Mp 175–177 °C. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 4.07 (*s*, 3H, -OMe), 6.12 (*s*, N-CH_2), 7.34–7.47 (*m*, 4H), 7.84–7.88 (*m*, 2H), 8.04–8.05 (*d*, 1H), 8.21–8.26 (*m*, 2H), and 8.72 (*s*, 1H). ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 48.9, 56.3, 98.5, 117, 117.5, 118.1, 122.2, 125.2, 127.9, 128.9, 130.8, 131.3, 131.4, 133.1, 146.4, 146.9, and 161.8.

Experimental protocol for biological activity

Antibacterial activity

The antimicrobial susceptibility testing of newly synthesized compounds were performed *in vitro* against bacterial strains *viz*., Gram positive *Staphylococcus aureus* (ATCC No. 29737), *Micrococcus luteus* (ATCC No. 398), *Bacillus cereus* (ATCC No. 6630), and Gram negative *Escherichia coli* (NCIM No. 2256), *Pseudomonas fluorescens* (NCIM No. 2173), and *Flavobacterium devorans* (ATCC No. 10829), respectively, to find out MIC. The MIC (μg/mL) were defined as the lowest concentrations of compound that completely inhibit the growth of each strain. Serial two-fold dilutions of all samples were prepared in triplicate in micro titer plates and inoculated with suitably prepared cell suspension to achieve the required initial concentration. Serial dilutions were prepared for screening. Dimethyl sulfoxide (DMSO) was used as solvent control. Ampicillin, kanamycin, and chloramphenicol were used as a standard antibacterial drug. The concentration range of tested compounds and standard was 256–0.5 µg/mL. The plates were incubated at 37 °C for all micro-organisms; absorbance at 595 nm was recorded to assess the inhibition of cell growth after 24 hr. The compounds which are showing promising antibacterial activity were selected for MIC studies. The MIC was determined by assaying at 256, 128, 64, 32, 16, 8, 4, 2, 1, and 0.5 µg/mL concentrations along with standards at the same concentrations.

Antifungal activity

The antifungal activity was evaluated against different fungal strains such as *Aspergillus niger* (NCIM No. 1196), *Penicillium chrysogenum* (NCIM No. 723), *Curvularia lunata* (NCIM No. 1131), *Candida albicans* (NCIM No. 3471), *Aspergillus flavus* (NCIM No. 539), and *Cryptococcus neoformans* (NCIM No. 3378). Fluconazole, miconazole, and amphotericin B were used as standard drugs for the comparison of antifungal activity. The plates were incubated at 37 °C for all micro-organisms; absorbance at 410 nm was recorded to assess the inhibition of cell growth after 48 hr. The lowest concentration inhibiting growth of the organisms was recorded as the MIC. DMSO was used as a solvent or negative control. In order to clarify any effect of DMSO on the biological screening, separate studies were carried out with solutions alone of DMSO and showed no activity against any microbial strains. The compounds which are showing promising antifungal activity were selected for MIC studies. The MIC was determined by assaying at 256, 128, 64, 32, 16, 8, 4, 2, 1, and 0.5 µg/mL concentrations along with standards at the same concentrations.

Antitubercular activity protocol

Compounds were tested for their *in vitro* effects against *MTB H37Ra* (ATCC 25177) which is susceptible to control drugs (Rifampicin, Isoniazid, Ethambutol, and Pyrazinamide). Compounds were screened for their inhibitory effect on MTB by *in vitro* according to standard XTT Reduction Menadione Assay (XRMA) protocol as described previously.[38] Dimethyl Sulfoxide (DMSO) was used as a solvent or negative control. In order to clarify any effect of DMSO on the biological screening, separate studies were carried out with solutions alone of DMSO and showed no activity against any mycobacteria. Rifampicin and Isoniazide was used as positive control for assay. Primary screening was done against MTB at 30, 10, and 3 µg/mL concentration of

compound. Those compounds were shown more than 90% inhibition at 30 μg/mL which were selected for dose response. The MIC (in μg/mL) was recorded as the lowest concentration/highest dilution of the compounds/control drugs that completely inhibited the growth of MTB cultures.

The *in vitro* effect of compounds against *M. bovis BCG* (ATCC 35743) was done according to standard NR assay protocol as described previously.[38] Briefly in NR assay, take 80 μL of culture from incubated 96 well plate into another 96 well plate, then add 80 μL of 1% sulfanilic acid in 20% of conc. HCl, incubate it for 10 min at room temperature then add 80 μL of 0.1% NEDD solution in D/W. Finally, the optical density of the suspension was measured at 540 nm using micro plate reader. MIC and IC_{50} values were calculated using origin9 software. The % inhibition of bacilli was measured using following formula,

$$\% \text{ Inhibition} = [(\text{Abs of control}) - (\text{Abs of test sample})/(\text{Abs of control}) - (\text{Abs of blank})] \times 100.$$

Control: cell growth in medium without compound, with DMSO
Test: cell growth in presence of compound
Blank: culture medium without cells.

DPPH radical scavenging activity

The hydrogen atom or electron donation ability of the compounds was measured from the bleaching of the purple-colored methanol solution of 1,1-diphenyl-1-picrylhydrazyl (DPPH).[39] The spectrophotometric assay uses the stable radical DPPH as a reagent. One milliliter of various concentrations of the test compounds (5, 10, 25, 50, and 100 μg/mL) in methanol was added to 4 mL of 0.004% (w/v) methanol solution of DPPH. After a 30-min incubation period at room temperature, the absorbance was measured against blank at 517 nm. The percent inhibition (I %) of free radical production from DPPH was calculated by the following equation.

$$\% \text{ of scavenging} = [(\text{A control} - \text{A sample})/\text{A blank}] \times 100$$

Where "A control" is the absorbance of the control reaction (containing all reagents except the test compound) and "A sample" is the absorbance of the test compound. Tests were carried at in triplicate.

Molecular docking

To gain an insight into the binding mode of quinoline-tetrazole-1,2,3-triazole derivatives into the active site of fungal sterol 14α-demethylase (CYP51) enzyme and to increase the understanding of their action as antifungal agents, the molecular docking study was performed using the *Glide* (Grid-Based Ligand Docking with Energetics) program of Schrodinger molecular modeling suite.[47] *Glide* is an interactive molecular graphics program for analyzing the enzyme-inhibitor interactions and identifying potential binding site of the bio-macromolecular targets. The algorithm carries out a systematic search for favorable interactions between the ligand(s) and the target enzyme through a complete search of the conformational, orientation, and positional space of the docked ligand by adopting a funnel type approach and eliminates unwanted conformations using a scoring function followed by energy optimization. With this purpose, the starting coordinates of the sterol 14α-demethylase (CYP51) in complex with its inhibitor-fluconazole were retrieved from the Protein Data Bank (PDB) (www.rcsb.org) (PDB code: 3KHM) and further modified to be used for *Glide* docking. The crystal structure was preprocessed the *Protein Preparation Wizard in Glide* which involved eliminating the crystallographically observed water molecules (as none of them were observed to be conserved), assigning the correct bond orders

followed by addition of missing hydrogen atoms corresponding to pH 7.0 (considering the appropriate ionization states for the acidic as well as basic amino acid residues). Following the assignment of appropriate charge and protonation state, the enzyme-inhibitor complex was subjected to energy minimization (until the average root-mean-square deviation (RMSD) of the non-hydrogen atoms reached 0.3 Å) using Optimized Potentials for Liquid Simulations-2005 (OPLS-2005) force field in order to relieve the steric clashes among the residues due to addition of hydrogen atoms. After ensuring that enzyme-inhibitor complex is in the correct form, the shape and properties of the active site of the enzyme was characterized and setup for the docking study using the *receptor grid generation* panel in *Glide*. With the non-covalently bound native ligand-fluconazole in place, the active site grid was defined by a box of 10X10X10Å dimensions centered on the centroid of fluconazole in the crystal complex which was sufficiently large to explore a bigger surface of the enzyme. The co-crystallized ligand serves as the reference co-ordinate signifying the active site of a ligand with respect to the target.

The 3 D-structures of all the tetrazoloquinoline-1,2,3-triazole derivatives (**3**, **5a-i**) were sketched using the *build* panel within Maestro and were optimized using the *LigPrep* module which involved addition of hydrogens, adjusting realistic bond lengths and angles, correcting the chiralities, ionization states and ring conformations, and generation of tautomers. The partial atomic charges were ascribed for these structures using the OPLS-2005 force-field and finally each of these structures was subjected to energy minimization until energy gradient of 0.001 kcal/mol/Å is reached. The optimized enzyme and ligand structures were then used as input for carrying out docking study utilizing the *extra precision* (XP) Glide scoring function to rank the docking poses and to estimate the binding affinities of these ligands to the target. This scoring function is equipped with force field-based parameters accounting for contributions from van der Waals and coulombic interaction energies along with terms for solvation, repulsive, hydrophobic, hydrogen bonding, and metal-ligand interactions all integrated in an empirical energy functions. The output files in terms of the docking poses of the ligands were visualized and analyzed for the key elements of interaction with the enzyme using the Pose Viewer utility in *Maestro*.

Acknowledgement

Authors are also thankful to the Head, Department of Chemistry, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431 004, India for providing laboratory facility. We also thank Schrodinger Inc. for providing the Demo license of Schrodinger Suite and especially Craig Coel for the valuable technical support that has tremendously helped in this study.

Disclosure statement

There are no conflicts of interest.

Funding

The authors MHS, DDS, and SVA are very much grateful to the Council for Scientific and Industrial Research (CSIR), New Delhi for the award research fellowship.

References

1. D. J. Sheehan, C. A. Hitchcock, and C. M. Sibley, "Current and Emerging Azole Antifungal Agents," *Clinical Microbiology Reviews* 12, no. 1 (1999): 40–79.
2. R. Cha, and J. D. Sobel, "Fluconazole for the Treatment of Candidiasis: 15 Years Experience," *Expert Review of Anti-Infective Therapy* 2, no. 3 (2004): 357–66.

3. N. H. Georgopapadakou and T. J. Walsh, "Antifungal Agents: Chemotherapeutic Targets and Immunologic Strategies," *Antimicrobial Agents and Chemotherapy* 40, no. 2 (1996): 279–91.
4. M. A. Pfaller, S. A. Messer, R. J. Hollis, and R. N. Jones, "In Vitro Activities of Posaconazole (Sch 56592) Compared with Those of Itraconazole and Fluconazole against 3685 Clinical Isolates of Candida Spp. and *Cryptococcus neoformans*," *Antimicrobial Agents and Chemotherapy* 45, no. 10 (2001): 2862–4.
5. L. Jeu, F. J. Piacenti, A. G. Lyakhovetskiy, and H. B. Fung, "Voriconazole," *Clinical Therapeutics* 25, no. 5 (2003): 1321–81.
6. G. I. Lepesheva, N. G. Zaitseva, W. D. Nes, W. Zhou, M. Arase, J. Liu, G. C. Hill, and M. R. Waterman, "CYP51 from *Trypanosoma cruzi*: A Phyla-Specific Residue in the B' Helix Defines Substrate Preferences of Sterol 14Alpha-Demethylase," *The Journal of Biological Chemistry* 281, no. 6 (2006): 3577–85.
7. H. Shiraki, M. P. Kozar, V. Melendez, T. H. Hudson, C. Ohrt, A. J. Magill, and A. J. Lin, "Antimalarial Activity of Novel 5-Aryl-8-Aminoquinoline Derivatives," *Journal of Medicinal Chemistry* 54, no. 1 (2011): 131–42.
8. C. Manera, M. G. Cascio, V. Benetti, M. Allara, T. Tuccinardi, A. Martinelli, G. Saccomanni, E. Vivoli, C. Ghelardini, V. D. Marzo, et al. "New 1,8-Naphthyridine and Quinoline Derivatives as CB2 Selective Agonists," *Bioorganic & Medicinal Chemistry Letters* 17, no. 23 (2007): 6505–10.
9. S. B. Marganakop, R. R. Kamble, T. Taj, and M. Y. Kariduraganvar, "An Efficient One-Pot Cyclization of Quinoline Thiosemicarbazones to Quinolines Derivatized with 1,3,4-Thiadiazole as Anticancer and Anti-Tubercular Agents," *Medicinal Chemistry Research* 21, no. 2 (2012): 185–91.
10. A. M. Gilbert, M. G. Bursavich, S. Lombardi, K. E. Georgiadis, E. Reifenberg, C. Flannery, and E. A. Morris, "*N*-((8-Hydroxy-5-Substituted-Quinolin-7-yl)(Phenyl)Methyl)-2-Phenyloxy/Amino-Acetamide Inhibitors of ADAMTS-5 (Aggrecanase-2)," *Bioorganic & Medicinal Chemistry Letters* 18, no. 24 (2008): 6454–7.
11. S. Chen, R. Chen, M. He, R. Pang, Z. Tan, and M. Yang, "Design, Synthesis, and Biological Evaluation of Novel Quinoline Derivatives as HIV-1 Tat-TAR Interaction Inhibitors," *Bioorganic & Medicinal Chemistry* 17 (2009): 1948–56.
12. S. Rossiter, J.-M. Péron, P. J. Whitfield, and K. Jones, "Synthesis and Anthelmintic Properties of Arylquinolines with Activity against Drug-Resistant Nematodes," *Bioorganic & Medicinal Chemistry Letters* 15, no. 21 (2005): 4806–8.
13. X. Ma, W. Zhou, and R. Brun, "Synthesis, In Vitro Antitrypanosomal and Antibacterial Activity of Phenoxy, Phenylthio or Benzyloxy Substituted Quinolones," *Bioorganic & Medicinal Chemistry Letters* 19, no. 3 (2009): 986–9.
14. T. A. Rano, E. S. McMaster, P. D. Pelton, M. Yang, K. T. Demarest, and G. H. Kuo, "Design and Synthesis of Potent Inhibitors of Cholesteryl Ester Transfer Protein (CETP) Exploiting a 1,2,3,4-Tetrahydroquinoline Platform," *Bioorganic & Medicinal Chemistry Letters* 19, no. 9 (2009): 2456–60.
15. D. Edmont, R. Rocher, C. Plisson, and J. Chenault, "Synthesis and Evaluation of Quinoline Carboxyguanidines as Antidiabetic Agents," *Bioorganic & Medicinal Chemistry Letters* 10, no. 16 (2000): 1831–4.
16. A. H. Kategaonkar, P. V. Shinde, A. H. Kategaonkar, S. K. Pasale, B. B. Shingate, and M. S. Shingare, "Synthesis and Biological Evaluation of New 2-Chloro-3-((4-Phenyl-1*H*-1,2,3-Triazol-1-yl)Methyl)Quinoline Derivatives via Click Chemistry Approach," *European Journal of Medicinal Chemistry* 45, no. 7 (2010): 3142–6.
17. (a) R. C. Venkata, V. D. Mukund, G. T. Santosh, and K. Yadagiri, "Novel 1,2,3 triazole antifungal agents and preparation thereof" (US Patent US 9,981,923B2;(b) K. N. Venugopala, M. A. Khedr, Y. R. Girish, S. Bhandary, D. Chopra, M. A. Morsy, B. E. Aldhubiab, P. K. Deb, M. Attimarad, A. B. Nair, et al., "Crystallography, *In Silico* Studies, and *In Vitro* Antifungal Studies of 2, 4, 5 Trisubstituted 1, 2, 3-Triazole Analogues," *Antibiotics* 9 (2020): 350.
18. K. N. Venugopala, G. B. Dharma Rao, S. Bhandary, M. Pillay, D. Chopra, B. E. Aldhubiab, M. Attimarad, O. I. Alwassil, S. Harsha, and K. Mlisana, "Design, Synthesis, and Characterization of (1-(4-Aryl)-1*H*-1,2,3-Triazol-4-yl)Methyl, Substituted Phenyl-6-Methyl-2-Oxo-1,2,3,4-Tetrahydropyrimidine-5-Carboxylates against *Mycobacterium tuberculosis*," *Drug Design, Development and Therapy* 10 (2016): 2681–90.
19. (a) S. G. Agalave, R. S. Maujan, and V. S. Pore, "Click Chemistry: 1,2,3-Triazoles as Pharmacophores," *Chemistry - An Asian Journal* 6 (2011): 2696–2718 and references cited therein;(b) M. Hussain, T. Qadri, Z. Hussain, A. Saeed, P. A. Channar, S. A. Shehzadi, M. Hassan, F. A. Larik, T. Mahmood, and A. Malik, "Synthesis, Antibacterial Activity and Molecular Docking Study of Vanillin Derived 1,4-Disubstituted 1,2,3-Triazoles as Inhibitors of Bacterial DNA Synthesis," *Heliyon* 5 (2019): e02812.
20. M. R. Senger, L. C. Gomes, S. B. Ferreira, C. R. Kaiser, V. F. Ferreira, and F. P. Silva, "Kinetics Studies on the Inhibition Mechanism of Pancreatic α-Amylase by Glycoconjugated 1*H*-1,2,3-Triazoles: A New Class of Inhibitors with Hypoglycemiant Activity," *ChemBioChem: A European Journal of Chemical Biology* 13, no. 11 (2012): 1584–93.

21. T. El. Malah, H. F. Nour, A. A. E. Satti, B. A. Hemdan, and W. A. El-Sayed, "Design, Synthesis, and Antimicrobial Activities of 1,2,3-Triazole Glycoside Clickamers," *Molecules* 25, no. 4 (2020): 790.
22. M. J. Genin, D. A. Allwine, D. J. Anderson, M. R. Barbachyn, D. Edward Emmert, S. A. Garmon, D. R. Graber, K. C. Grega, J. B. Hester, D. K. Hutchinson, et al. "Substituent Effects on the Antibacterial Activity of Nitrogen-Carbon-Linked (Azolylphenyl)Oxazolidinones with Expanded Activity against the Fastidious Gram-Negative Organisms *Haemophilus influenzae* and *Moraxella catarrhalis*," *Journal of Medicinal Chemistry* 43, no. 5 (2000): 953–70.
23. R. J. Bochis, J. C. Chabala, E. Harris, L. H. Peterson, L. Barash, T. Beattie, J. E. Brown, D. W. Graham, F. S. Waksmunski, M. Tischler, et al. "Benzylated 1,2,3-Triazoles as Anticoccidiostats," *Journal of Medicinal Chemistry* 34, no. 9 (1991): 2843–52.
24. J. L. Kelley, C. S. Koble, R. G. Davis, E. W. Mclean, F. E. Soroko, and B. R. Cooper, "1-(Fluorobenzyl)-4-Amino-1*H*-1,2,3-Triazolo[4,5-c]Pyridines: Synthesis and Anticonvulsant Activity," *Journal of Medicinal Chemistry* 38, no. 20 (1995): 4131–4.
25. R. Raj, P. Singh, P. Singh, J. Gut, P. J. Rosenthal, and V. Kumar, "Azide-Alkyne Cycloaddition en Route to 1*H*-1,2,3-Triazole-Tethered 7-Chloroquinoline-Isatin Chimeras: Synthesis and Antimalarial Evaluation," *European Journal of Medicinal Chemistry* 62 (2013): 590–6.
26. M. H. Shaikh, D. D. Subhedar, M. Arkile, V. M. Khedkar, N. Jadhav, D. Sarkar, and B. B. Shingate, "Synthesis and Bioactivity of Novel Triazole Incorporated Benzothiazinone Derivatives as Antitubercular and Antioxidant Agent," *Bioorganic & Medicinal Chemistry Letters* 26, no. 2 (2016): 561–9.
27. B. L. Wilkinson, H. Long, E. Sim, and A. J. Fairbanks, "Synthesis of Arabino Glycosyl Triazoles as Potential Inhibitors of Mycobacterial Cell Wall Biosynthesis," *Bioorganic & Medicinal Chemistry Letters* 18, no. 23 (2008): 6265–7.
28. V. V. Rostovtsev, L. G. Green, V. V. Fokin, and K. B. Sharpless, "A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective "Ligation" of Azides and Terminal Alkynes," *Angewandte Chemie International Edition* 41, no. 14 (2002): 2596–9.
29. C. W. Tornoe, C. Christensen, and M. Meldal, "Peptidotriazoles on Solid Phase: [1,2,3]-Triazoles by Regiospecific Copper(I)-Catalyzed 1,3-Dipolar Cycloadditions of Terminal Alkynes to Azides," *The Journal of Organic Chemistry* 67, no. 9 (2002): 3057–64.
30. H. C. Kolb, M. G. Finn, and K. B. Sharpless, "Click Chemistry: Diverse Chemical Function from a Few Good Reactions," *Angewandte Chemie International Edition* 40, no. 11 (2001): 2004–21.
31. (a) V. A. Ostrovskii, R. E. Trifonov, and E. A. Popova, "Medicinal Chemistry of Tetrazoles," *Russian Chemical Bulletin* 61 (2012): 768–80 and references cited therein;(b) P. B. Mohite and V. H. Bhaskar, "Potential Pharmacological Activities of Tetrazoles in the New Millennium," *International Journal of PharmTech Research* 3 (2011): 1557–66 and references cited therein;(c) S. Q. Wang, Y. F. Wang, and Z. Xu, "Tetrazole Hybrids and Their Antifungal Activities," *European Journal of Medicinal Chemistry* 170 (2019): 225–34;(d) C. Gao, L. Chang, Z. Xu, X. F. Yan, C. Ding, F. Zhao, X. Wu, and L. S. Feng, "Recent Advances of Tetrazole Derivatives as Potential Anti-Tubercular and Anti-Malarial Agents," *European Journal of Medicinal Chemistry* 163 (2018): 404–12;(e) P. F. Lamie, J. N. Philoppes, A. A. Azouz, and N. M. Safwat, "Novel Tetrazole and Cyanamide Derivatives as Inhibitors of Cyclooxygenase-2 Enzyme: Design, Synthesis, Anti-Inflammatory Evaluation, Ulcerogenic Liability and Docking Study," *Journal of Enzyme Inhibition and Medicinal Chemistry* 32 (2017): 805–20.
32. K. D. Thomas, A. V. Adhikari, and N. S. Shetty, "Design, Synthesis and Antimicrobial Activities of Some New Quinoline Derivatives Carrying 1,2,3-Triazole Moiety," *European Journal of Medicinal Chemistry* 45, no. 9 (2010): 3803–10.
33. M. C. Joshi, K. J. Wicht, D. Taylor, R. Hunter, P. J. Smith, and T. J. Egan, "In Vitro Antimalarial Activity, β-Haematin Inhibition and Structure-Activity Relationships in a Series of Quinoline Triazoles," *European Journal of Medicinal Chemistry* 69 (2013): 338–47.
34. G. R. Pereira, G. C. Brandao, L. M. Arantes, H. A. de Oliveira, Jr., R. C. de Paula, M. F. A. do Nascimento, F. M. dos Santos, R. K. da Rocha, J. C. D. Lopes, and A. B. de. Oliveira, "7-Chloroquinolinotriazoles: Synthesis by the Azide-Alkyne Cycloaddition Click Chemistry, Antimalarial Activity, Cytotoxicity and SAR Studies," *European Journal of Medicinal Chemistry* 73 (2014): 295–309.
35. T. Aravinda, H. S. Bhojya Naik, and H. R. Prakash Naik, "1,2,3-Triazole Fused Quinoline-Peptidomimetics: Studies on Synthesis, DNA Binding and Photonuclease Activity," *International Journal of Peptide Research and Therapeutics* 15, no. 4 (2009): 273–9.
36. (a) D. D. Subhedar, M. H. Shaikh, M. A. Arkile, A. Yeware, D. Sarkar, and B. B. Shingate, "Facile Synthesis of 1,3-Thiazolidin-4-Ones as Antitubercular Agents," *Bioorganic & Medicinal Chemistry Letters* 26 (2016): 1704–8;(b) D. D. Subhedar, M. H. Shaikh, L. Nawale, A. Yeware, D. Sarkar, F. A. K. Khan, J. N. Sangshetti, and B. B. Shingate, "Novel Tetrazoloquinoline-Rhodanine Conjugates: Highly Efficient Synthesis and Biological Evaluation," *Bioorganic Medicinal & Chemistry Letters* 26 (2016): 2278–83;(c) M. H. Shaikh, D. D. Subhedar, F. A. K. Khan, J. N. Sangshetti, and B. B. Shingate, "1,2,3-Triazole Incorporated Coumarin

Derivatives as a Potential Antifungal and Antioxidant Agents," *Chinese Chemical Letters* 27 (2016): 295–301; (d) M. H. Shaikh, D. D. Subhedar, L. Nawale, D. Sarkar, F. A. K. Khan, J. N. Sangshetti, and B. B. Shingate, "1,2,3-Triazole Derivatives as Antitubercular Agents; Synthesis, Biological Evaluation and Molecular Docking Study," *Medicinal Chemistry Communications* 6 (2015): 1104–16.

37. (a) 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, 1997);(b) 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, 1998);(c) National Committee for Clinical Laboratory Standards, "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically Approved Standard", 5th ed. (M7-A5, NCCLS, Villanova, PA, 2000).
38. (a) A. Khan, S. Sarkar, and D. Sarkar, "Bactericidal Activity of 2-Nitroimidazole against the Active Replicating Stage of *Mycobacterium bovis* BCG and *Mycobacterium tuberculosis* with Intracellular Efficacy in THP-1 Macrophages," *International Journal of Antimicrobial Agents* 32 (2008): 40–5;(b) U. Singh, S. Akhtar, A. Mishra, and D. Sarkar, "A Novel Screening Method Based on Menadione Mediated Rapid Reduction of Tetrazolium Salt for Testing of Anti-Mycobacterial Agents," *Journal of Microbiological Methods* 84 (2011): 202–7;(c) S. Sarkar and D. Sarkar, "Potential Use of Nitrate Reductase as a Biomarker for the Identification of Active and Dormant Inhibitors of *Mycobacterium tuberculosis* in a THP-1 Infection Model," *Journal of Biomolecular Screening* 17 (2012): 966–73.
39. M. Burits and F. Bucar, "Antioxidant Activity of *Nigella sativa* Essential Oil," *Phytotherapy Research* 14, no. 5 (2000): 323–8.
40. C. A. Lipinski, L. Lombardo, B. W. Dominy, and P. J. Feeney, "Experimental and Computational Approaches to Estimate Solubility and Permeability in Drug Discovery and Development Settings," *Advanced Drug Delivery Reviews* 46, no. 1–3 (2001): 3–26.
41. Molinspiration Chemoinformatics, Brastislava, Slovak Republic, 2014, http://www.molinspiration.com/cgi-bin/properties.
42. Y. H. Zhao, M. H. Abraham, J. Le, A. Hersey, C. N. Luscombe, G. Beck, B. Sherborne, and I. Cooper, "Rate Limited Steps of Human Oral Absorption and QSAR Studies," *Pharmaceutical Research* 19, no. 10 (2002): 1446–57.
43. "Drug-Likeness and Molecular Property Prediction," http://www.molsoft.com/mprop/.
44. P. Ertl, B. Rohde, and P. Selzer, "Fast Calculation of Molecular Polar Surface Area as a Sum of Fragment-Based Contributions and Its Application to the Prediction of Drug Transport Properties," *Journal of Medicinal Chemistry* 43, no. 20 (2000): 3714–7.
45. R. M. Singh and A. Srivastava, "Vilsmeier-Haack Reagent: A Facile Synthesis of 2-Chloro-3-Formylquinolines from N-Acetamides and Transformation into Different Functionalities," *Indian Journal of Chemistry* 44B (2005): 1868–75.
46. N. K. Ladani, M. P. Patel, and R. G. Patel, "An Efficient Three Component One-Pot Synthesis of Some New Octahydroquinazolinone Derivatives and Investigation of Their Antimicrobial Activities," *Arkivoc* vii (2009): 292–302.
47. (a) Schrodinger Suite 2015-4 QM-Polarized Ligand Docking Protocol; Glide Version 6.9 (New York, NY: Schrodinger, LLC, 2015); Jaguar Version 9.0 (New York, NY: Schrodinger, LLC, 2015); QSite Version 6.9 (New York, NY: Schrodinger, LLC, 2015;(b) R. A. Friesner, R. B. Murphy, M. P. Repasky, L. L. Frye, J. R. Greenwood, T. A. Halgren, P. C. Sanschagrin, and D. T. Mainz, "Extra Precision Glide: Docking and Scoring Incorporating a Model of Hydrophobic Enclosure for Protein-Ligand Complexes," *Journal of Medicinal Chemistry* 49 (2006): 6177–96 and related references cited therein.