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An Expedient Route to Highly Diversified [1,2,3]Triazolo[1,5-a][1,4]Benzodiazepines and their Evaluation for Antimicrobial, Antiproliferative and *In Silico* studies

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An efficient diversity oriented synthesis of [1,2,3]triazolo[1,5-a][1,4]benzodiazepines has been developed by sequential diazotization, azidation and cycloaddition reactions in a one-pot fashion. This strategy allows an easy accessibility of triazole fused [1,4]benzodiazepines in good yields. The main objective of this methodology is to introduce various substituents at all possible positions under mild reaction conditions. All the synthesized compounds were evaluated for their antimicrobial, anticancer and *in silico* activity. Among the tested compounds (**2a–n**), the derivatives **2a, 2b, 2d, 2k, 2g, 2j, 2m** and **2l** have displayed broad spectrum of antibacterial activity. Anticancer activity results revealed that compounds **2a, 2g and 2m** exhibited potent *in vitro* anticancer activity against A549 lung adenocarcinoma cancer cell line. Further, molecular docking studies of all the synthesized compounds were performed to gain a comprehensive understanding of the plausible binding modes and also to compare the theoretical and experimental results of these compounds.

Introduction

Benzodiazepines are privileged heterocyclic structures and their synthesis has been receiving much attention in the field of medicinal and pharmaceutical chemistry owing to their applications as anticonvulsants,[1] anti-inflammatories,[2] HIV inhibitors,[3] farnesyl-transferase inhibitors[4] and receptor ligands in neurodegenerative diseases[5] Benzodiazepine derivatives have also been reported to possess antibacterial, antifungal and antitumor activities.[6] Fused heterocyclic benzodiazepines have attracted considerable attention due to their highly potent anxiolytic[7] or anti-depressant activity.[8] 1,2,3-triazole derived molecules found to have antiprotozoal, antiviral, antileishmanial and anticancer properties.[9]

Furthermore, fused-ring systems consisting of multifarious heterocycles such as 1,4-benzodiazepine and triazole substructures have attracted considerable attention due to their highly potent biological activities. Some of the examples for triazolo benzodiazepines are alprazolam (1), estazolam (2) are used as anxiolytic agents[10] whereas triazolam (3), adinazolam(4) are known as antidepressants[11] and compound (5), derived from the further elaborated [1,2,3]triazolo[1,4]benzodiazepine skeleton, displays activity as protease inhibitors (Fig. 1).[12]

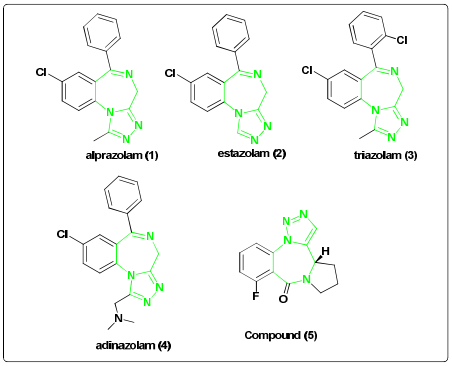
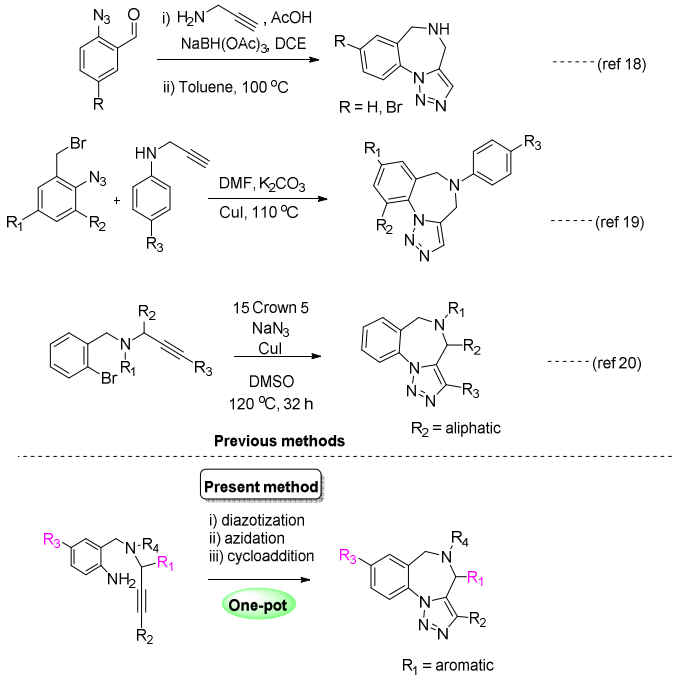


Fig 1 Selected examples of triazolo-[1,4]benzodiazepines

Careful examination of the literature indicates that the majority of syntheses have been developed through inter/intra molecular cycloaddition to generate the triazole ring.[13] The efficiency of the cycloaddition has been successfully demonstrated in both aqueous[14] and organic solvents involving metal catalyzed[15]/metal-free reactions[16] and various thermal conditions. Ideally an intramolecular 1,3-dipolar cycloaddition reaction can be expected to furnish two annulated cyclic rings

in comparison to the intermolecular format. Several groups have reported intramolecular azide-alkyne 1,3-dipolar cycloaddition strategy for the synthesis of triazole linked polyhetrocycles.[17] However most of these strategies predominantly follows multistep format to promote 1,3-dipolar cycloaddition with limited applications.[18]

Numerous reports demonstrate the formation of triazole fused benzodiazepines. Though the efficient methodologies to prepare the diversely functionalized fused 1,4-benzodiazepines remains a challenge to modern synthetic organic chemists.[19] Therefore, new and efficient methods that could be carried out under milder and eco-friendly condition always demand special importance to the field of synthetic organic chemistry. Martin *et al* have synthesized 1,4-benzodiazepines fused with a 1,2,3-triazole ring and diversified them *via* a variety of refunctionalizations.[20] Copper catalyzed tandem Ullmann C–N coupling followed by azide-alkyne cycloaddition approach has been described by Majumdar and co-worker.[21] Eycken group have reported Cu-catalyzed azidation-cyclization reaction for the synthesis of fused triazoles.[22] Although the above mentioned method is efficient, it is limited to the aliphatic substituents at C3- position and it requires metal-catalyst. Our rationale is based on the idea of assembling an aromatic substituent at C3- position of triazolo-1,4-benzodiazepines involving *in situ* generated aryl azide under mild reaction condition (Scheme 1). Prompted by the synthetic interest and inevitable medicinal properties of triazole fused benzodiazepines we report herein a facile route to highly substituted [1,2,3]triazolo[1,5-a][1,4]benzodiazepines under metal-free conditions and the evaluation of the biological activity of resulting compounds.



Scheme 1 Methods for the preparation of triazolo-1,4-benzodiazepines

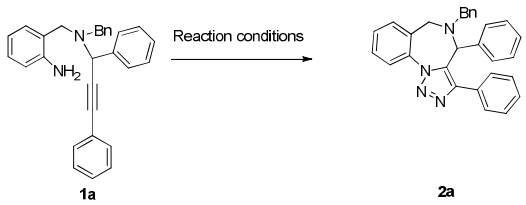
Results and discussion

Chemistry

We have developed many general approaches for preparing the various heterocyclic scaffolds of possible medical relevance by a strategy that involves sequencing multicomponent assembly processes (MCAPs) with subsequent cyclizations.[23] As a continuation of these investigations, we found an expedient route to a compound bearing the triazolo-1,4-benzodiazepine scaffold.

Our synthetic approach started with 2-amino-*N*-benzylpropargylamine **1a** as a model substrate to synthesize fused triazoles **2a** mild reaction condition. This precursor **1a** can be obtained using the sequential A^3-coupling reaction of *N*-benzyl-1-(2-nitrophenyl)methanamine, aromatic aldehyde, alkyne followed by the reduction of $–NO_2$ group (as mentioned in the supporting information). In an exploratory experiment the reaction conditions for the one-pot diazotization, azidation and cycloaddition were investigated.

Table 1 Optimization of reaction conditions



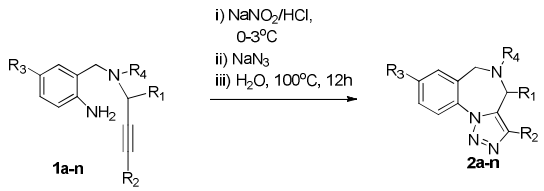
Entry	Solvent	Temp (°C)	Time (h)	Isolated Yield (%)
1	Diethyl ether	0	5	Trace [a]
2	Diethyl ether	0	12	Trace [a]
3	Benzene	60	12	20[b]
4	Toluene	80	12	30[b]
5	Toluene	100	24	45[b]
5	DMSO	120	24	48[c]
6	EtOH	60	12	55[c]
7	H_2O/DMSO	100	12	68[c]
8	H_2O/EtOH	100	12	75[c]
9	H_2O	rt	12	50[c]
10	H_2O	rt	24	62[c]
11	H_2O	80	12	78[c]
12	H_2O	100	12	86[c]
13	H_2O	100	24	85[c]

[a]$NaNO_2$(2eq), 1N HCl, glacial acetic acid (7ml), NaN_3 (6 eq), [b]$NaNO_2$(2eq), 1N HCl, NaN_3 (2 eq), [c] $NaNO_2$(1.4 eq), 2N HCl , NaN_3 (1.4eq)

The investigation on the optimization of reaction parameters included temperature, solvent and reaction time (Table 1). A variety of solvents were explored which included diethyl ether, benzene, toluene, EtOH, DMSO and H_2O. The reaction was

found to proceed well using polar protic solvents (Table 1 entries 6-13) when compared to other solvents (Table 1, entries 1-5). Interestingly H_2O was found to be the best solvent for the formation of fused triazoles (Table 1, entries 7-13). Subsequently the effect of reaction temperature was studied (Table 1, entries 9-12). At room temperature the reaction proceeded slowly and resulted in a lower yield of the product. The increase in temperature increased the rate of the reaction. The scope of reaction time was also studied (Table 1 entries 12 & 13). Thus the best result was obtained when 1.4 eq of $NaNO_2$, 2N of HCl, 1.4 eq of NaN_3 and 2ml of H_2O were used for the *in situ* generation of azide and followed by the cycloaddition reaction to obtain the desired product in a single step.

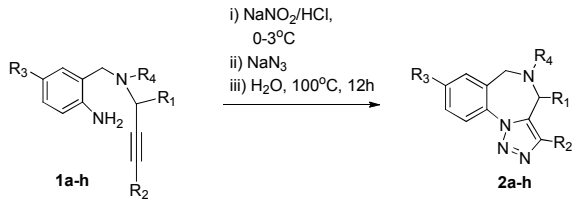
With these optimal conditions in hand, we set out to explore the scope of our method for the preparation and diversification of 2-amino-*N*-benzylpropargylamines (Scheme 2).

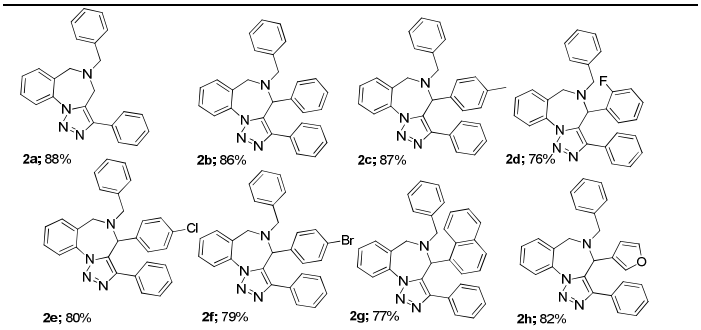


Scheme 2 Preparation of diversified triazolo-1,4-benzodiazepines

We can diversify the final products by varying *N*-benzyl/alkyl-1-(2-nitrophenyl)methanamine, aromatic aldehyde, alkyne used for the A^3-coupling reaction (see supporting information). We utilized a variety of commercial aldehydes such as paraformaldehyde, 1-naphthaldehyde, furan-3-carbaldehyde and other aromatic aldehydes (R_1-CHO) (R_1 = -Ph, *p*-MeC_6H_4, *o*-FC_6H_4, *p*-ClC_6H_4, *p*-BrC_6H_4) (Scheme 3). Aliphatic aldehyde provides higher yield when compared to aromatic and hetero aromatic aldehydes. The halo substituents gave lower yield when compared to the electron donating methyl substituent on the phenyl ring of aldehydes. Next, we examined the substrate scope by varying the alkynes substituents. The reaction is tolerant for both alkyl and aryl substituted terminal alkynes (R_2 = *n*-butyl, *p*-MeC_6H_4, *p*-FC_6H_4). Methyl substituted aromatic alkynes gives higher yield than the other alkynes. The desired compounds are obtained as a single diastereoisomer. At the amine position we have introduced alkyl (n-butyl, n-hexyl) and benzyl substituents. The main advantage of this method is that we can introduce the substituents even at the R_3 position (Scheme 4).

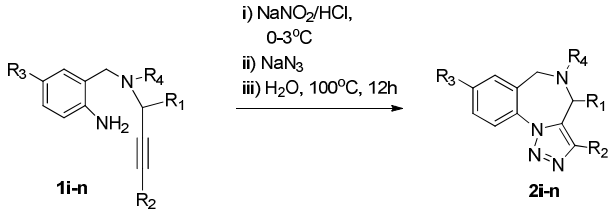
A plausible reaction mechanism for the formation of triazole fused [1,4]benzodiazepines is depicted in Scheme 5. 2-amino-*N*-benzylpropargylamine **1** was subjected to diazotization reaction using sodium nitrite in dilute HCl to generate diazonium salt **A**. Subsequent treatment of *in situ* generated diazonium salt **A** with sodium azide produced the corresponding azido compound **B**. Concurrent intramolecular azide-alkyne cycloaddition of **B** under thermal conditions would lead to the formation of annulated 7- and 5-membered heterocyclic rings as a desired product **2**.

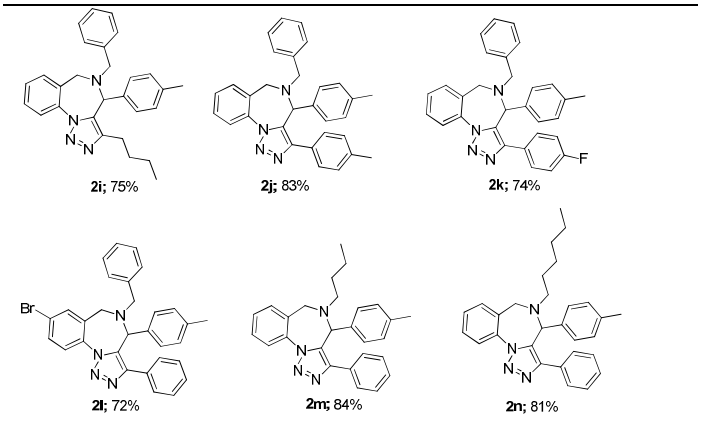




Scheme 3 Scope of aldehydes

The structure of all prepared compounds were elucidated by the ^1H, ^{13}C NMR experiments and further proved by the mass data.

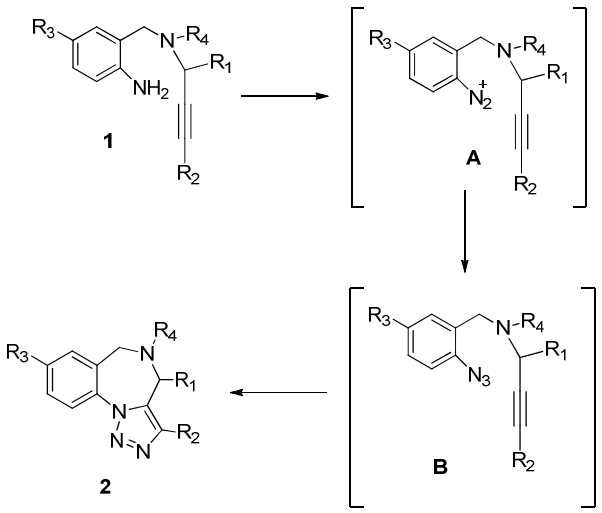




Scheme 4 Exploring the scope of alkynes (**2i-k**) and various substituents at R_3 and R_4 (**2l-n**)

The disappearance of characteristic peak corresponds to $-NH_2$ protons in ^1H NMR spectra and the disappearance of peaks corresponds to acetylenic carbons at the δ value 84.9, 88.8 ppm indicates the formation of cyclized product. The

stereochemistry of the compounds is clearly identified from the single crystal X-ray analysis of compound **2e** (Fig. 2)[24] which further supports the NMR spectroscopy.



Scheme 5 Proposed Mechanism for the formation of **2**:

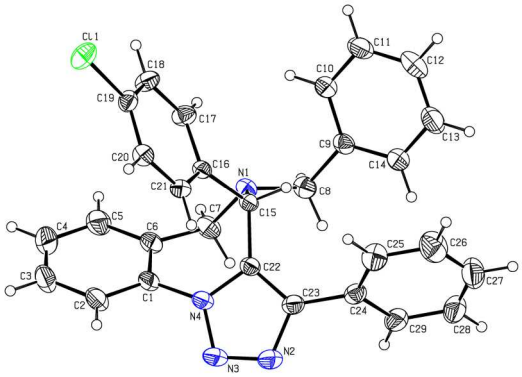


Fig 2 ORTEP of compound **2e**

Pharmacology

Antimicrobial activity

All the synthesized compounds were screened for the antimicrobial activities against eleven bacteria and two fungi using well method. The results revealed that most of the synthesized compounds exhibited good antimicrobial activities against *S. paratyphi B*, *E. aerogens*, *S. epidermidis*, *S. typhimurium*, *K. pneumonia*, *P. aeruginosa* and *S. aureus*. The results are summarized in Table 2 and Fig. 3. Compounds **2a**, **2b**, **2c**, **2d**, **2g**, **2j** and **2m** have shown excellent activities nearly equal to the standard and **2k** is showing better activity than the standard drug against *S. aureus* at 1mg/well. Moreover the compounds **2a**, **2b**, **2d**, **2k**, **2g**, **2j**, **2m** and **2l** showed good antibacterial activity over the others. All tested compounds showed moderate antifungal activity against *C. albican* and *M. pachydermatis*.

The Minimum Inhibitory Concentration (MIC) values of active compounds against bacteria are given in Table 3 and Fig. 4. Significant MIC values were observed against gram positive and gram negative bacteria. The results revealed that the fused triazoles **2a**, **2b**, **2d**, **2j**, **2l** and **2m** have shown good antibacterial activity against tested organisms. Among all tested compounds phenyl ring containing compound **2b** has shown significant MIC values against *P. vulgaris*, *S. flexneri*, 4-methyl substituted aromatic rings containing compound **2j** is potent against *S. aureus* (MRSA), *P. vulgaris* and *S. flexneri*. The naphthyl substituted compound **2g** is active against *S. flexneri* and *P. vulgaris*. The *N*-butyl substituted fused triazole compound **2m** showed significant MIC values against *P. vulgaris* and *S. aureus* (MRSA).

Anticancer activity

Anti-cancer activity studies have been performed for the synthesized compounds **2a**, **2b**, **2g**, **2j**, **2l** and **2m** (Table 4 and Fig. 5). All the tested compounds showed good cytotoxicity activity against cell line, however some of the synthesized compounds showed prominent cytotoxic activity *in vitro* against A549 adenocarcinoma lung cancer cell line. The anticancer activity against A549 cell line was observed at 200 to 50µg/mL concentration. In general alkyl substituents at -C3 and -N4 position shows better activity than the other compounds. Also, naphthyl substituent at –C3 position increases the activity of the product. Interestingly, among all the tested compounds **2a**, **2m** and **2g** showed very good activity with IC_{50} value at 50µg/mL. In particular, among the tested compounds **2a**, **2m** and **2g** showed very high activity 64.3 %, 61.9 % and 75.3 % at 200µg/mL concentrations against A549 lung adenocarcinoma cancer cell line. All concentrations used in the experiment decreased the cell viability significantly ($P<0.05$) in a concentration-dependent manner.

Molecular docking studies

To rationalize the pharmacological results, molecular docking studies were performed using the AutoDock Tools (ADT) version 1.5.6 and AutoDock version 4.2.5.1 docking program[25] on a DNA Gyrase (PDB ID: 2XCS) and Anaplastic Lymphoma Kinase(ALK) receptor (PDB ID: 2XP2)[26] to simulate the interaction of the synthesized compounds with the receptor binding site.

The co-crystallized ligand was extracted from the complex and submitted for one-ligand run calculation in order to verify the reproducibility of the docking calculation. This reproduced top scoring conformation falling within root-mean-square deviation (RMSD) value of 1.63 Å with bound X-ray conformation for 2XP2, suggesting this method is valid enough to be used for docking studies of other compounds.

The same protocol was applied to all the synthesized compounds and docking simulation was performed in the same active site using AutoDock after the validation study. All dockings were taken into 2.5 million energy evaluations were performed for each of the test molecules. Docked ligand

conformations were analyzed in terms of energy, hydrogen bonding, hydrophobic and π-π interaction between ligand and DNA topoisomerase IV receptor. The ligand-receptor interactions were clearly analyzed, and final coordinates of the ligand and receptor were saved. After docking, output was exported to PyMOL software for display of the ligand with the receptor binding site.[27] The free energy of binding (FEB) of all compounds were calculated from the docking scores (Table 5).

Docking studies of synthesized compounds with 2XCS receptor show that all the docked compounds bind efficiently with the receptor and exhibits free energy of binding value from -9.13 to -11.07 kcal/mol. Interestingly, among all the compounds docked, compound **2g** exhibits very high binding with 2XCS receptor and forms two polar interactions with three amino acid namely MET-1121, resulted in the binding energy of -11.07 kcal/mol. As shown in Fig. 6, in the compound **2g**, two nitrogens of triazole ring interact with N-H of MET-1121, forms two polar interactions with the distance of 2.1 and 2.5 Å. In addition, naphthyl moiety exhibits hydrophobic interaction with GLY-1117, ALA-1118, ALA-1119 and ALA-1120 amino acids.

Synthesized compounds efficiently bind with the active site of 2XP2 receptor and exhibits free energy of binding value from -7.70 to -10.07 kcal/mol. All the synthesized compounds interact in the 24 active site amino acids namely ARG-1120, LEU-1122, GLY-1123, VAL-1130, GLU-1132, ALA-1148, LYS-1150, LEU-1196, GLU-1197, LEU-1198, MET-1199, ALA-1200, GLY-1201, GLY-1202, ASP-1203, SER-1206, PHE-1207, GLU-1210, ARG-1253, ASN-1254, CYS-1255, LEU-1256, GLY-1269 and ASP-1270. Among all the compounds docked, compound **2g** exhibits very high binding with 2XP2 receptor and forms two polar interactions with ASP-1203, resulted in the binding energy of -10.07 kcal/mol. As shown in Fig 7, in the compound **2g**, two nitrogens of triazole ring interact with C=O of ASP-1203, forms two polar interactions with the distance of 2.7 and 2.9 Å. Further, naphthyl and phenyl moieties exhibit hydrophobic interaction with LEU-1122, VAL-1130, ALA-1148, LEU-1198 and ALA-1200 amino acids.

Table 2 *In vitro* antimicrobial activity of synthesized compounds

Compounds	Zone of inhibition in mm												
	Gram positive bacteria				Gram negative bacteria							Fungi	
	S. epidermidis	*S. aureus*	*S. aureus (MRSA)*	*M. luteus*	*E. aerogens*	*S. typhimurium*	*K. pneumoniae*	*P. vulgaris*	*S. flexneri*	*S. Paratyphi B*	*P. aeruginosa*	*C. albicans*	*M. pachyderm atis*
2a	17	15	15	18	18	16	14	22	21	15	16	NI	NI
2b	15	12	13	19	16	14	16	23	25	14	15	10	12
2c	12	13	NI	10	10	11	13	10	NI	12	13	NI	NI
2d	12	12	10	14	13	NI	12	10	16	12	14	10	10
2e	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	16	12	12
2f	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
2g	14	13	18	22	20	17	18	23	26	15	NI	NI	NI
2h	14	12	10	10	11	11	10	12	10	10	12	NI	13
2i	12	10	13	15	12	11	14	11	10	13	14	10	10
2j	15	12	23	18	12	22	17	24	23	14	15	NI	13
2k	13	15	12	16	16	13	14	15	12	14	10	12	10
2l	15	13	10	18	20	20	14	17	19	16	12	NI	13
2m	10	12	23	20	18	22	18	25	21	17	18	10	13
2n	NI	NI	13	NI	14	12	NI	NI	NI	10	13	NI	NI
Streptomycin	26	14	30	26	22	24	20	30	30	18	30	NA	NA
Ketoconazole	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	28	26

NA-not applicable NI – no inhibition

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Table 3 MIC (mg/ml) of compounds tested against bacteria

Compounds	Gram positive bacteria				Gram negative bacteria						
	S. epidermidis	*S. aureus*	*S. aureus* (MRSA)	*M. luteus*	*E. aerogens*	*S. typhimurium*	*K. pneumoniae*	*P. vulgaris*	*S. flexneri*	*S. paratyphi B*	*P. aeruginosa*
2a	125	125	125	125	125	125	250	62.5	62.5	125	125
2b	125	250	250	62.5	125	250	125	31.25	31.25	250	125
2d	250	250	500	250	250	NI	250	500	125	250	250
2g	250	250	125	62.5	250	125	125	31.25	31.25	125	125
2j	125	250	31.25	125	250	62.5	125	31.25	31.25	250	31.25
2k	250	125	250	125	125	250	250	125	250	250	500
2l	250	250	250	125	62.5	62.5	250	125	62.5	125	250
2m	500	250	31.25	62.5	125	62.5	125	31.25	62.5	125	62.5
Streptomycin	25	6.25	6.25	6.25	25	6.25	6.25	6.25	6.25	30	25
Ciprofloxacin	6.25	<0.78	<0.78	>100	<0.78	>100	6.25	6.25	<0.78	6.25	6.25

Table 4 Anticancer activity of synthesized compounds against A549 cancer cell line

Conc (µg/mL)	**2a**		**2b**		**2g**		**2j**		**2l**		**2m**	
	%	Mean±S.D	%	Mean±S.D	%	Mean±S.D	%	Mean±S.D	%	Mean±S.D	%	Mean±S.D
50	21.1	1.134±0.004	21.7	1.124±0.004	55.9	0.632±0.004	4.8	1.367±0.005	4.4	1.373±0.006	35.9	0.921±0.0062
100	34.9	0.935±0.002	34.4	0.942±0.003	64.3	0.512±0.007	28.6	1.025±0.008	26.5	1.056±0.006	52.6	0.681±0.0039
200	64.3	0.512±0.006	51.4	0.698±0.009	75.3	0.354±0.008	41.2	0.845±0.004	35.9	0.921±0.004	61.9	0.546±0.0043

Table 5 Binding energy and the interaction of ligands with the DNA Gyrase and ALK receptor

Compound	**Binding energy (kcal/mol)[a]**	
	DNA Gyrase (2XCS)	**ALK (2XP2)**
2a	-10.24	-7.70
2b	-10.66	-8.58
2c	-9.79	-8.89
2d	-10.60	-8.65
2e	-9.93	-8.95
2f	-10.24	-9.07
2g	-11.07	-10.07
2h	-9.84	-8.24
2i	-9.32	-8.17
2j	-10.24	-8.37
2k	-9.75	-8.42
2l	-10.48	-8.94
2m	-9.13	-7.77
2n	-9.48	-8.24
Crystallized ligand	-13.81	-8.40
Standard drug	-7.94[b] -5.85[c]	

[a]Calculated by Autodock; [b]Streptomycin; [c]Ciprofloxacin

Conclusions

In conclusion, an efficient method for the synthesis of highly diversified [1,2,3]triazolo[1,5-a][1,4]benzodiazepine derivatives (**2a–n**) under mild reaction condition were reported in good yields *via* MCR approach and evaluated for their *in vitro* antimicrobial and anticancer studies. Among the series, compounds **2a, 2b, 2d, 2k, 2g, 2j, 2m** and **2l** were found to be potent with respect to standard drugs streptomycin and ciprofloxacin. Anticancer studies revealed that, the compound **2g** possessing naphthyl group showed potent anticancer activity against A549 lung adenocarcinoma cancer cell line. In order to support the *in vitro* antibacterial and anticancer results, the synthesized compounds were docked in to the plausible target enzymes. The binding energies and H-bond interactions with amino acids in active site of target enzyme well supported the *in vitro* results. These compounds [1,2,3]triazolo[1,5-a][1,4]benzodiazepine can be promising therapeutic agents for A549 lung adenocarcinoma cancer cell line.

Experimental

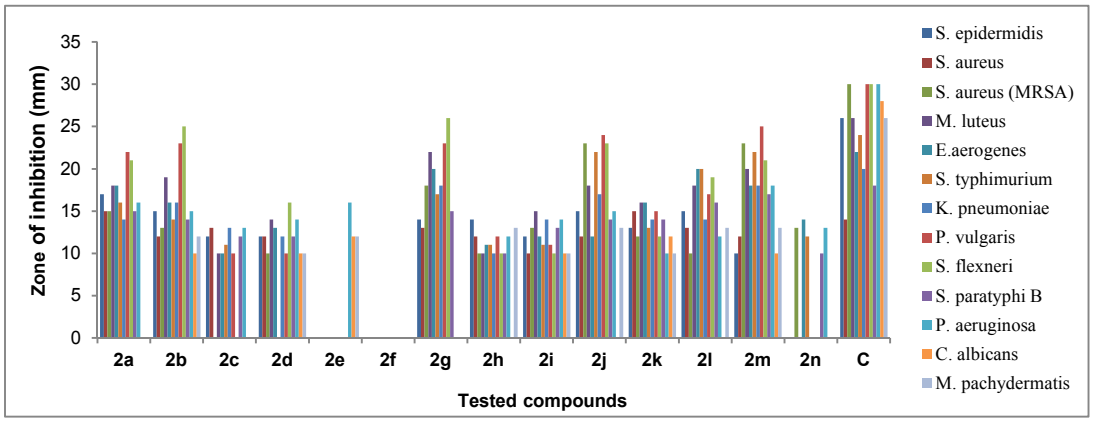
Chemistry

Analytical TLC was performed on precoated aluminium sheets of silica gel 60F254 of 0.2 mm thickness (Merck, Germany). Melting points were determined on Gallenkamp melting point apparatus and are uncorrected. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in $CDCl_3$ solutions with TMS as an internal standard on a Brucker Avance DPX-400 MHz instrument. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, δ = 0.00) as internal standard and expressed in parts per million. The number of protons (n) for a given resonance was indicated as nH. Coupling constants (J) are given in hertz. Spin multiplicities are given as s (singlet), d (doublet), t (triplet) and m (multiplet). Mass spectra were recorded under Mass spectra were recorded using ESI/HRMS at 60000 resolution in Thermoscientific Exactive mass spectrometer and ESI/MS using a Thermo Finnigan LCQ Advantage MAX 6000 ESI mass spectrometer. Elemental analyses were recorded using a Thermo Finnigan FLASH EA1112 CHN analyzer.

Experimental procedure for the synthesis of (2a-n)

To a stirred and cooled (0–3 °C) solution of 2-amino-*N*-benzylpropargylamine (0.90 mmol) in 2 N HCl (8.0 mL) was added $NaNO_2$ (1.26 mmol) in 2mL H_2O dropwise during 35 min and the mixture was allowed to stir for another 30 min at the same temperature. A solution of NaN_3 (1.26 mmol) in 2 mL H_2O was added dropwise during 35 min under ice-cooled condition and the stirring was continued for another 15 min. The reaction mixture was allowed to come to room temperature during about 45 min. It was then heated at 100 °C for 12 h to obtain a desired fused triazole.

5-Benzyl-3-phenyl-5,6-dihydro-4H-benzo[f][1,2,3]triazolo[1,5-a][1,4]diazepine (2a): White solid. Yield: 88%. mp: 154-156 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J= 8.0 Hz, 1H), 7.79 (d, J = 7.6 Hz, 2H), 7.59 (t, J = 7.6 Hz, 1H), 7.51-7.37 (m, 5H), 7.35 (d, J = 4.4 Hz, 4H), 7.33-7.27 (m, 1H), 3.74 (d, J = 6.4 Hz, 4H), 3.65 (s, 2H).^{13}C NMR (100 MHz, $CDCl_3$) δ 145.4, 137.9, 136.9, 131.1, 130.7, 130.2, 129.6, 129.1, 128.9, 128.88, 128.6, 128.3, 127.6, 127.5, 122.8, 60.3, 54.5, 45.1. HRMS (ESI): Mass calculated for $C_{23}H_{21}N_4$ $[M+H]^+$353.1761, found, $[M+H]^+$, 353.1772.



C= Streptomycin for bacteria, Ketoconazole for fungi

Fig 3 Comparison of antimicrobial activity of synthesized compounds and standard drugs

5-Benzyl-3,4-diphenyl-5,6-dihydro-4H-benzo[f][1,2,3]triazolo[1,5-a][1,4]diazepine (2b): White solid. Yield: 86%. mp: 197-199 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, J = 7.6 Hz, 1H), 7.63-7.62 (m, 2H), 7.41-7.40 (m, 3H), 7.26-7.18 (m, 8H),7.05- 6.94 (m, 5H), 5.46 (s, 1H), 3.99 (d, J = 12.8 Hz, 1H), 3.90 (d, J = 13.2 Hz, 1H), 3.71 (d, J = 13.2 Hz, 1H), 3.52 (d, J = 12.8 Hz, 1H).^{13}C NMR (100 MHz, $CDCl_3$) δ 147.4, 140.3, 137.8, 137.2, 133.0, 130.6, 130.3, 130.0, 129.0, 129.0, 128.8, 128.7, 128.5, 128.3, 128.1, 127.8, 127.6, 126.8, 126.7, 122.3, 60.7, 57.2, 54.7. HRMS (ESI): Mass calculated for $C_{29}H_{24}N_4$ $[M+H]^+$ 429.2074, found, $[M+H]^+$,429.2072.

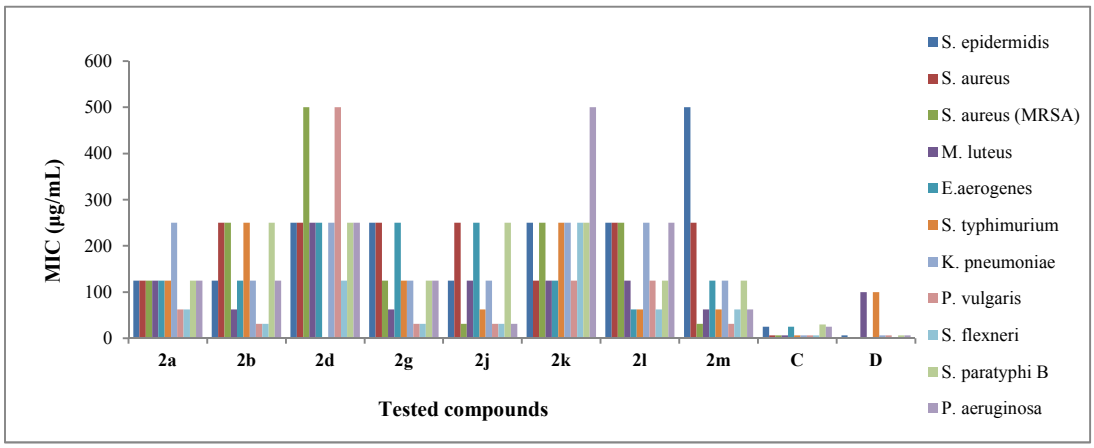
5-Benzyl-3-phenyl-4-(p-tolyl)-5,6-dihydro-4H-benzo[f][1,2,3]triazolo[1,5-a][1,4]diazepine (2c): White solid. Yield: 87%. mp: 149-150 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, J = 8.0 Hz, 1H), 7.57-7.59 (m, 2H), 7.39-7.38 (m, 3H), 7.32-7.27 (m, 1H), 7.17-7.24 (m, 7H), 6.92 (d, J = 8.0 Hz, 2H), 6.81 (d, J = 8.0 Hz, 2H), 5.41 (s, 1H), 3.99 (d, J = 13.2 Hz, 1H), 3.85 (d, J = 13.1 Hz, 1H), 3.68 (d, J= 13.2 Hz, 1H), 3.54 (d, J = 13.2 Hz, 1H), 2.15 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.2, 136.5, 132.5, 130.7, 130.3, 129.0, 128.8, 128.7, 128.6, 128.4, 128.2, 128.1, 127.5, 126.7, 124.9, 122.3, 121.1, 60.4, 57.4, 54.6, 20.9. HRMS (ESI): Mass calculated for $C_{30}H_{27}N_4$ $[M+H]^+$ 443.2230, found, $[M+H]^+$,443.2223.

5-Benzyl-4-(2-fluorophenyl)-3-phenyl-5,6-dihydro-4H-benzo[f][1,2,3]triazolo[1,5-a][1,4]diazepine (2d): White solid. Yield: 76%. mp: 151-153 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, J = 7.6 Hz, 1H), 7.48-7.40 (m, 4H), 7.37 (t, J = 7.4 Hz, 1H), 7.25-7.30 (m, 9H), 6.98 (dd, J = 13.2, 7.2 Hz, 1H), 6.80 (t, J = 7.6 Hz, 1H), 6.70-6.65 (m, 1H), 5.40 (s, 1H), 3.95 (d, J = 13.6 Hz, 1H), 3.78 (q, J = 13.6 Hz, 2H), 3.64 (d, J = 13.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.1, 158.6, 146.6, 137.9, 136.9, 132.5, 130.7, 130.5, 130.0 (d, J = 3.3 Hz), 129.6, 129.2,

129.1 (d, J = 8.3 Hz), 128.8, 128.7, 128.6 (d, J = 4.4 Hz), 128.3, 128.0, 127.6, 126.2 (d, J = 11.7 Hz), 123.3 d, J = 3.4 Hz), 122.6, 115.2, 115.0, 59.0, 54.1, 53.3. MS (ESI) for $C_{29}H_{23}FN_4$ m/z = 447 $[M+H]^+$. Elemental analysis calc for $C_{29}H_{23}FN_4$: C, 78.01; H, 5.19; F, 4.25; N, 12.55 found, C 78.04, H, 5.16; F, 4.23; N, 12.58.

5-Benzyl-4-(4-chlorophenyl)-3-phenyl-5,6-dihydro-4H-benzo[f][1,2,3]triazolo[1,5-a][1,4]diazepine (2e): White solid. Yield: 80%. mp: 181-183 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, J = 8.0 Hz, 1H), 7.61-7.59 (m, 2H), 7.42-7.41 (m, 3H), 7.32-7.28 (m, 1H), 7.27-7.23 (m, 5H), 7.17-7.19 (m, 2H), 6.98-6.93 (m, 4H), 5.38 (s, 1H), 3.94 (dd, J = 26.4, 12.8 Hz, 2H), 3.70 (d, J = 12.8 Hz, 1H), 3.51 (d, J = 12.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.5, 139.0, 137.6, 137.1, 132.6, 132.4, 130.4, 130.3, 129.9, 129.2, 129.0, 128.9, 128.8, 128.5, 128.4, 128.1, 128.0, 127.9, 127.7, 122.3, 60.7, 56.6, 54.8. HRMS (ESI): Mass calculated for $C_{29}H_{24}ClN_4$ $[M+H]$+ 463.1684, found, $[M+H]$+,463.1676.

5-Benzyl-4-(4-bromophenyl)-3-phenyl-5,6-dihydro-4H-benzo[f][1,2,3]triazolo[1,5-a][1,4]diazepine (2f): White solid. Yield: 79%. mp: 178-180 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, J = 7.6 Hz, 1H), 7.60-7.59 (m, 2H), 7.42-7.41 (m, 3H),



C = Streptomycin, D = Ciprofloxacin

Fig 4 Comparison of MIC (mg/ml) values of synthesized compounds and standard drugs.

7.33-7.29 (m, 1H), 7.27-7.23 (m, 5H), 7.17-7.16 (m, 2H), 7.10 (d, J = 8.0 Hz, 2H), 6.90 (d, J = 8.0 Hz, 2H), 5.35 (s, 1H), 3.94 (dd, J = 27.0, 12.8 Hz, 2H), 3.69 (d, J = 12.8 Hz, 1H), 3.51 (d, J = 12.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.5, 139.5, 137.5, 137.1, 132.3, 130.9, 130.4, 130.3, 129.8, 129.3, 129.0, 128.9, 128.8, 128.5, 128.4, 128.3, 128.1, 127.7, 122.3, 120.8, 60.7, 56.6, 54.8. MS (ESI) for $C_{29}H_{23}BrN_4$ m/z = 507 $[M+H]$+. Elemental analysis calc for $C_{29}H_{23}BrN_4$: C, 68.64; H, 4.57; Br, 15.75; N, 11.04 found, C, 68.67; H, 4.55; Br, 15.71; N, 11.06.

5-Benzyl-4-(naphthalen-1-yl)-3-phenyl-5,6-dihydro-4H-benzo[f][1,2,3]triazolo[1,5-a][1,4]diazepine (2g): White solid. Yield: 77%. mp: 64-65 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (d, J = 8.0 Hz, 1H), 7.89 (d, J = 8.0 Hz, 1H), 7.79 (d, J = 7.6 Hz, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.54-7.41 (m, 4H), 7.36-7.32 (m, 3H), 7.27-7.20 (m, 4H), 7.15-7.06 (m, 6H), 5.86 (s, 1H), 4.01 (d, J = 16.8 Hz, 1H), 3.85 (d, J = 16.8 Hz, 1H), 3.79 (d, J = 13.6 Hz, 1H), 3.65 (d, J = 13.2 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.6, 137.0, 134.3, 134.1, 131.0, 130.4, 129.1, 129.0, 128.7, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.5, 127.3, 126.3, 125.8, 125.0, 123.8, 122.6, 58.4, 57.0, 52.6. HRMS (ESI): Mass calculated for $C_{33}H_{27}N_4$ $[M+H]^+$ 479.2230, found, $[M+H]^+$,479.2226.

5-Benzyl-4-(furan-3-yl)-3-phenyl-5,6-dihydro-4H-benzo[f][1,2,3]triazolo[1,5-a][1,4]diazepine (2h): White solid. Yield: 82%. mp: 168-170 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, J = 7.6 Hz, 1H), 7.63-7.61 (m, 2H), 7.43-7.33 (m, 4H), 7.32-7.18 (m, 7H), 7.02 (s, 1H), 6.90 (s, 1H), 5.81 (s, 1H), 5.32 (s, 1H), 3.98 (d, J = 12.8 Hz, 1H), 3.85 (d, J = 13.2 Hz, 1H), 3.68 (d, J = 13.2 Hz, 1H), 3.52 (d, J = 12.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.5, 142.8, 140.8, 137.8, 137.2, 132.2, 130.5, 130.3, 130.1, 129.1, 128.9, 128.8, 128.5, 128.4, 128.1, 127.6, 126.0, 122.2, 109.0, 60.6, 55.0, 51.2. HRMS (ESI): Mass calculated for $C_{27}H_{23}N_4O$ $[M+H]^+$ 419.1866, found, $[M+H]^+$,419.1858.

5-Benzyl-3-butyl-4-(p-tolyl)-5,6-dihydro-4H-benzo[f][1,2,3]triazolo[1,5-a][1,4]diazepine (2i): Yellow oil. Yield: 75%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, J = 7.6 Hz, 1H), 7.48-7.43 (m, 3H), 7.37 (t, J = 7.2 Hz, 3H), 7.31-7.23 (m, 4H), 7.08 (d, J = 7.6 Hz, 2H), 4.65 (s, 1H), 3.78-3.71 (m, 2H), 3.57 (d, J = 14.0 Hz, 1H), 3.51 (d, J = 13.6 Hz, 1H), 2.30 (s, 3H), 2.23-2.16 (m, 1H), 2.00-1.93 (m, 1H), 1.52-1.38 (m, 2H), 1.22-1.15 (m, 2H), 0.82 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.8, 138.6, 137.6, 137.1, 135.8, 132.6, 130.5, 129.1, 128.8, 128.6, 128.5, 128.0, 127.4, 122.6, 60.3, 57.3, 51.7, 32.3, 24.8, 22.6, 21.1, 13.8. HRMS (ESI): Mass calculated for $C_{28}H_{31}N_4$ $[M+H]^+$ 425.2543, found, $[M+H]^+$, 425.2536.

5-Benzyl-3,4-di-p-tolyl-5,6-dihydro-4H-benzo[f][1,2,3]triazolo[1,5-a][1,4]diazepine (2j): Semi solid. Yield: 83%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 8.0 Hz, 1H), 7.53 (d, J = 7.6 Hz, 1H), 7.48 (d, J = 7.6 Hz, 2H), 7.28-7.19 (m, 9H), 6.91 (d, J = 8.0 Hz, 2H), 6.81 (d, J = 7.6 Hz, 2H),

5.42 (s, 1H), 3.98 (d, *J* = 12.8 Hz, 1H), 3.86 (d, *J* = 13.2 Hz, 1H), 3.70 (d, *J* = 13.2 Hz, 1H), 3.52 (d, *J* = 13.2 Hz, 1H), 2.41 (s, 3H), 2.15 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.1, 138.0, 137.3, 136.4, 133.0, 130.2, 130.0, 129.4, 129.0, 128.8, 128.6, 128.5, 128.4, 128.0, 127.8, 127.5, 126.7, 122.2, 60.5, 57.5, 54.6, 21.3, 20.8. MS (ESI) for $C_{31}H_{28}N_4$ *m/z* = 457 $[M+H]^+$. Elemental analysis calc for $C_{31}H_{28}N_4$: C, 81.55; H, 6.18; N, 12.27 found, C, 81.52; H, 6.21; N, 12.25.

5-Benzyl-3-(4-fluorophenyl)-4-(p-tolyl)-5,6-dihydro-4H-benzo[f][1,2,3]triazolo[1,5-a][1,4]diazepine (2k): White solid. Yield: 74%. mp: 144-145 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, *J* = 8.0 Hz, 1H), 7.48 (t, *J* = 6.4 Hz 2H), 7.33 (t, *J* = 7.4 Hz, 1H), 7.23-7.19 (m, 7H), 7.04 (t, *J* = 8.4 Hz, 2H), 6.93 (d, *J* = 7.6 Hz, 2H), 6.83 (d, *J* = 7.6 Hz, 2H), 5.28 (s, 1H), 3.99 (d, *J* = 13.6 Hz, 1H), 3.82 (d, *J* = 13.2 Hz, 1H), 3.66 (d, *J* = 13.2 Hz, 1H), 3.57 (d, *J* = 13.6 Hz, 1H), 2.16 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.6, 146.3, 137.8, 137.1, 136.8, 133.3, 130.3, 130.0, 129.9, 129.8, 128.9, 128.7, 128.6, 128.5, 127.6, 126.8, 122.3, 115.7, 115.5, 59.9, 57.6, 54.3, 20.9. MS (ESI) for $C_{31}H_{28}N_4$ *m/z* = 461 $[M+H]^+$. Elemental analysis calc for $C_{30}H_{25}FN_4$: C, 78.24; H, 5.47; F, 4.13; N, 12.17found C, 78.21; H, 5.51; F, 4.10; N, 12.19.

5-Benzyl-8-bromo-3-phenyl-4-(p-tolyl)-5,6-dihydro-4H-benzo[f][1,2,3]triazolo[1,5-a][1,4]diazepine (2l): White solid. Yield: 72%. mp: 185-186 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.4 Hz, 1H), 7.56 – 7.52 (m, 2H), 7.44 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.39-7.37 (m, 3H), 7.35 (d, *J* = 2.0 Hz, 1H), 7.23-7.21 (m, 3H), 7.19-7.16 (m, 2H), 6.91 (q, *J* = 8.4 Hz, 4H), 5.44 (s, 1H), 3.97 (d, *J*= 14.0 Hz, 1H), 3.82 (d, *J* = 13.2 Hz, 1H), 3.68 (d, *J* = 13.2 Hz, 1H), 3.55 (d, *J* = 13.6 Hz, 1H), 2.20 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.4, 137.5, 137.0, 136.8, 136.2, 133.5, 132.9, 131.8, 131.0, 130.4, 128.9, 128.7, 128.5, 128.3, 128.0, 127.6, 126.8, 123.8, 122.0, 60.0, 57.7, 53.9, 20.9. HRMS (ESI): Mass calculated for $C_{30}H_{26}BrN_4$ $[M+H]^+$ 521.1335, found, $[M+H]^+$, 521.1338.

5-Butyl-3-phenyl-4-(p-tolyl)-5,6-dihydro-4H-benzo[f][1,2,3]triazolo[1,5-a][1,4]diazepine (2m): White solid. Yield: 84%. mp: 120-122 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81-7.79 (m, 1H), 7.69-7.67 (m, 2H), 7.45-7.37 (m, 3H), 7.31-7.27 (m, 2H), 7.25-7.22 (m, 1H), 6.84 (q, *J* = 8.9 Hz, 4H), 5.45 (s, 1H), 3.95 (d, *J* = 13.2 Hz, 1H), 3.56 (d, *J* = 13.2 Hz, 1H), 2.73-2.69 (m, 2H), 2.16 (s, 3H), 1.55-1.48 (m, 2H), 1.40-1.31 (m, 2H), 0.87 (t, *J* = 7.4 Hz, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ 147.0, 137.7, 137.1, 136.4, 133.7, 130.8, 130.3, 130.1, 128.7, 128.7, 128.6, 128.5, 128.3, 128.0, 126.7, 122.1, 58.5, 55.9, 54.5, 30.0, 20.9, 20.3, 13.9. HRMS (ESI): Mass calculated for $C_{27}H_{29}N_4$ $[M+H]^+$409.2387, found, $[M+H]^+$, 409.2394.

5-Hexyl-3-phenyl-4-(p-tolyl)-5,6-dihydro-4H-benzo[f][1,2,3]triazolo[1,5-a][1,4]diazepine (2n): Yellow oil. Yield: 81%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81-7.79 (m, 1H), 7.69-7.66 (m, 2H), 7.45-7.36 (m, 3H), 7.31-7.27 (m, 2H), 7.26-7.22 (m, 1H), 6.84 (q, *J* = 8.9Hz, 4H), 5.45 (s, 1H), 3.94 (d, *J* = 13.2 Hz, 1H), 3.56 (d, *J* = 13.2 Hz, 1H), 2.70 (t, *J* = 7.2 Hz, 2H), 2.16 (s, 3H), 1.55-1.47 (m, 2H), 1.35-1.28 (m, 2H), 1.26-1.20 (m, 4H), 0.86 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.0, 137.7, 137.1, 136.4, 133.7, 130.9, 130.8, 130.3, 130.1, 128.9, 128.8, 128.7, 128.6, 128.5, 128.3, 128.0, 126.7, 122.1, 58.4, 56.3, 54.5, 31.6, 27.8, 26.8, 22.5, 20.9, 19.2, 14.0. HRMS (ESI): Mass calculated for $C_{29}H_{33}N_4$ $[M+H]^+$ 437.2700, found, $[M+H]^+$, 437.2702.

Biological assays

Materials and methods for antimicrobial activity

Streptomycin and Ciprofloxacin (Sigma) were used as positive control against bacteria. Ketoconazole (Himedia, Mumbai) was used as positive control against fungi.

Tested microbes: The following bacteria and fungi were used for the experiment. Bacteria: *Salmonella paratyphi-B, Pseudomonas aeruginosa* MTCC 741, *Klebsiella pneumonia* MTCC 109, *Micrococcus luteus* MTCC 106, *Salmonella typhimurium* MTCC 1251, *Proteus vulgaris* MTCC 1771, *Shigella flexneri* MTCC 1457, *Enterobacter aerogenes* MTCC 111, *Staphylococcus epidermidis* MTCC 3615, *Staphylococcus aureus* MTCC 96 and *Staphylococcus aureus* (MRSA-methicillin resistant). The reference cultures were obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India-160 036; fungi: *Candida albicans* MTCC 227 and *Malassesia pachydermatis*. All the other cultures were obtained from the Department of Microbiology, Christian Medical College, Vellore, Tamil Nadu, India.

Preparation of inoculums

Bacterial inoculums were prepared by growing cells in Mueller Hinton broth (MHB) (Himedia) for 24 h at 37°C. The filamentous fungi were grown on sabouraud dextrose agar (SDA) slants at 28°C for 10 days and the spores were collected using sterile doubled distilled water and homogenized. Yeast was grown on sabouraud dextrose broth (SDB) at 28°C for 48-72 h.

Disc diffusion assay

Antimicrobial activities were carried out using well method.[28] Petri plates were prepared with 20 ml of sterile Mueller Hinton Agar (MHA) (Hi-media, Mumbai). The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min and a specific amount of synthesized compound at 1mg/well was added to each well separately. Negative control was prepared using respective solvents. Streptomycin was used as positive control against bacteria. Ketoconazole was used as positive control for fungi. The plates were incubated for 24 h at 37°C for bacteria and for 48 h at 28°C for fungi. Zones of inhibition were recorded in millimetres and the experiment was repeated twice.

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration studies of eight compounds were performed according to the standard reference methods for antibacterial activity.[29] The required concentrations (500

µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL) of the compound were dissolved in DMSO (2%), and diluted to give serial two-fold dilutions that were added to each medium in 96 well plates. An inoculum of 100µl from each well was inoculated. The antifungal agent Ketoconazole for fungi and Streptomycin and Ciprofloxacin for bacteria was included in the assay as positive controls. For fungi, the plates were incubated for 48 to 72 hours at 28°C and for bacteria the plates were incubated for 24 h at 37°C. The MIC for fungi was defined as the lowest extract concentration, showing no visible fungal growth after incubation time. 5 µl of tested broth was placed on the sterile MHA plates for bacteria and incubated at respective temperature. The MIC for bacteria was determined as the lowest concentration of the compound inhibiting the visual growth of the test cultures on the agar plate.

Cytotoxic properties

A549 lung adenocarcinoma cancer cell line was obtained from National Institute of Cell Sciences, Pune. A549 cell line was maintained in complete tissue culture medium Dulbecco's Modified Eagle's Medium with 10 % Fetal Bovine Serum and 2mM L-Glutamine, along with antibiotics (about 100 International Unit/mL of penicillin, 100 µg/mL of streptomycin) with the pH adjusted to 7.2. The cytotoxicity was determined according to the literature method[30] with some changes. Cells (5000 cells/well) were seeded in 96 well plates containing medium with different concentrations such as 50, 40, 30, 20, 10 and 5 µg/mL. The cells were cultivated at 37 °C with 5% CO_2 and 95% air in 100% relative humidity. After various durations of cultivation, the solution in the medium was removed. An aliquot of 100 µL of medium containing 1 mg/mL of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (Sigma) was loaded in the plate. The cells were cultured for 4 h and then the solution in the medium was removed. An aliquot of 100 µL of DMSO was added to the plate, which was shaken until the crystals were dissolved. The cytotoxicity against cancer cells was determined by measuring the absorbance of the converted dye at 540 nm in an Enzyme linked immune sorbant assay reader. Cytotoxicity of each sample was expressed as the half maximal inhibitory concentration (IC_{50}) value. The IC_{50} value is the concentration of test sample that causes 50% inhibition of cell growth, averaged from three replicate experiments.

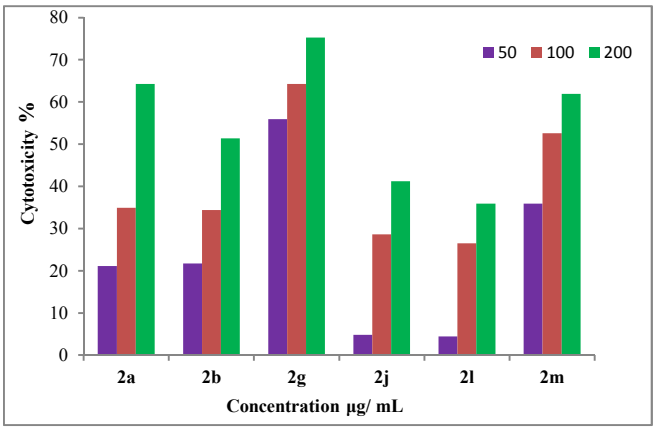
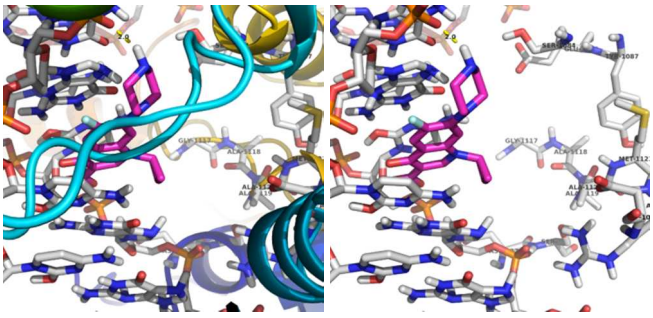


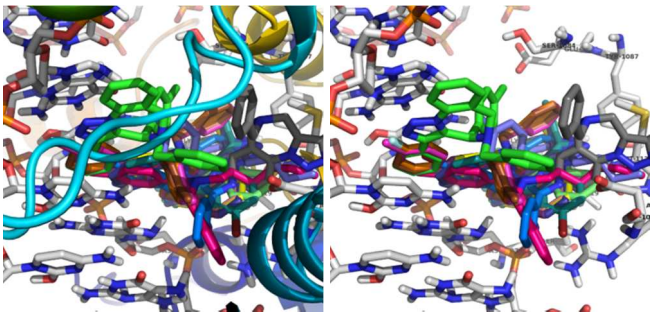
Fig 5 Comparison of anticancer activity of synthesized compounds against A549 cancer cell line

Molecular docking studies

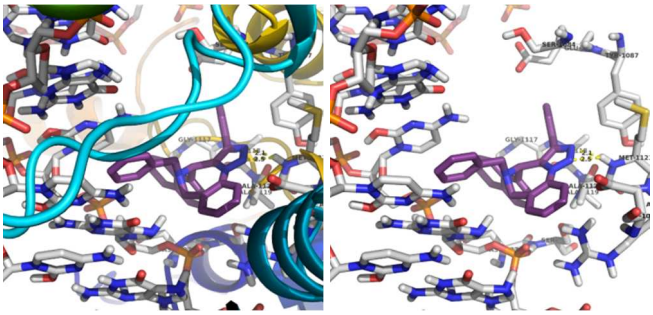
Molecular docking studies were performed using the AutoDock Tools (ADT) version 1.5.6 and AutoDock version 4.2.5.1 docking program. Three dimensional structure of DNA Gyrase (PDB ID: 2XCS) and Anaplastic Lymphoma Kinase (ALK) receptor (PDB ID: 2XP2) receptor were obtained from the Protein Data Bank. The co-crystallized ligand in the receptor structure was removed. Then, the water molecules present with the crystal were deleted, the polar hydrogen atoms were added, lower occupancy residue structures were deleted, and any incomplete side chains were replaced using the ADT. Gasteiger



Docking mode of Ciprofloxacin in the active site of 2XCS



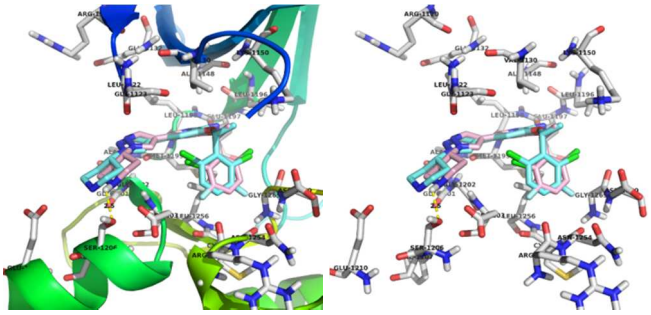
Docking mode of all the compounds in the active site of 2XCS



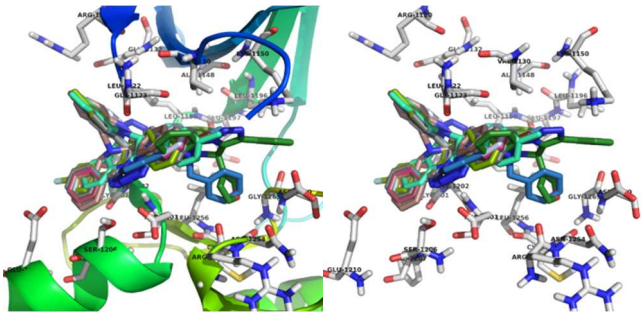
Docking mode of the most binding energy compound 2g in the active site of 2XCS

Fig. 6.Docking simulation in the active site of 2XCS receptor

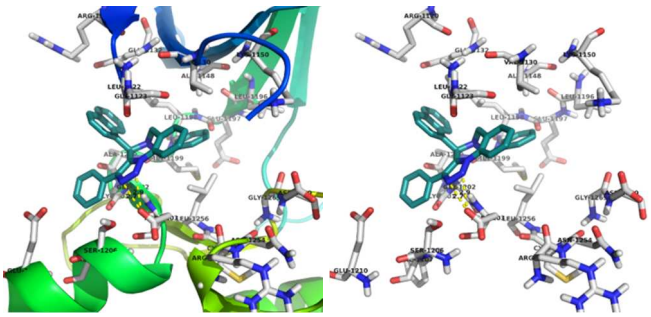
charges were added to each atom, and merged the non-polar hydrogen atoms to the protein structure. The distance between donor and acceptor atoms that form a hydrogen bond was defined as 1.9 Å with a tolerance of 0.5 Å, and the acceptor–hydrogen–donor angle was not less than 120˚. Then, the structures were saved in PDBQT file format for further studies in ADT.A grid box centred on 6.116, 43.906, 40.763 and 29.697, 47.794, 8.863 with dimension of 50 × 60 × 50 Å3 and 40 × 40 × 40 Å3 with 0.375 Å spacing was created around the binding site of co-crystallised ligand on 2XCS and 2XP2 respectively. The centre of the box was set at co-crystallised ligand centre and grid energy calculations were carried out. Default parameters were used for the AutoDock docking calculation and 50 docked conformations were generated for each compound. Genetic algorithms was used to calculate the energy of the binding interactions. The outputs were exported to PyMOL for visual inspection of the binding modes and interactions of the compounds with amino acid residues in the active sites.



Docking mode of Crizotinib in the active site of 2XP2



Docking mode of all the compounds in the active site of 2XP2



Docking mode of the most binding energy compound 2g in the active site of 2XP2

Fig 7 Docking simulation in the active site of 2XP2 receptor

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