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Original article

Ultrasound promoted one pot synthesis of novel fluorescent triazolyl spirocyclic oxindoles using DBU based task specific ionic liquids and their antimicrobial activity

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ABSTRACT

Spirocyclic oxindoles and triazolyl derivatives posses remarkable biological activities. In present work, we have described an efficient one pot four-component domino reaction of 1-(prop-2-ynyl)indoline-2,3-dione, cyclic 1,3-diketones, malononitrile and various aryl azides in DBU based ionic liquids [DBU-H]OAc and [DBU-Bu]OH under ultrasonic irradiation for the construction of heterocycles, comprising spiro-oxindole, 2-amino-4*H*-pyran, and 1,2,3-triazoles substructures. The antimicrobial activity of all compounds has been investigated against six microbial strains. All compounds showed good antimicrobial activity. All newly synthesized compounds exhibit fluorescence in methanol with large stoke shift.

1. Introduction

Infectious diseases caused by microbes such as bacteria and fungi are one of the leading causes of morbidity and mortality and the major reason for the increase in microbial infections is the resistance developed by these microbial organisms, particularly Gram-positive bacteria *Staphylococcus aureus* and species of the genus *Enterococcu* towards existing antimicrobial drugs [1]. Therefore development of alternative new more effective antimicrobial agents with new modes of action and a broad spectrum of activities is a one of the major challenges in drug discovery. Molecular hybridization involves combining two or more heterocyclic rings in a single molecule wherein combining units are derived from known bioactive molecules [2]. Pharmacophore hybridization is believed to be analogous to conventional combination therapy wherein the two drugs are covalently linked and available as a single entity [3].

Multicomponent reactions (MCRs) offer valuable strategies for the hybridization of molecules with several advantages e.g. elimination of intermediate steps, diversity, high efficiency, selectivity and minimal waste production [4–9]. MCRs using task specific ionic liquid (TSIL) with dual role of reaction media and catalyst as well as alternative sources of energy such as ultrasonic/microwave irradiation have gained much attention as efficient synthetic tools for synthesis of complex novel molecules/hybrids in the realm of green chemistry [10–13]. DBU based TSILs exhibit basicity similar to DBU and are accompanied with the general features of ILs. However they are less explored in organic synthesis as compared to other ionic liquids [14].

Spirocyclic oxindoles are privileged structures with diverse biological activities including antibacterial, antifungal, anticonvulsant, antiviral, and antiproliferative [15]. They act as potent inhibitors of monoamine oxidase in human urine and rat tissues [16] and acetylcholinesterase [17], antagonist of *in vitro* receptor binding by atrial natriuretic peptide [18] and possess a wide range of CNS activities [19]. Spiro oxindole ring system is also a part of many alkaloids with biological activities such as paraherquamide A which possess antiparasitic activity and antinematodal properties (Fig. 1.) [20]. Pyrans and fused pyran derivatives are biologically interesting compounds with activities such as antibacterial [21], antifungal [22], antitumor [23], hypotensive [24] and analgesic [25]. For example huajiaosimuline exhibits a selective cytotoxicity profile showing the greatest activity with estrogen receptor-positive ZR-

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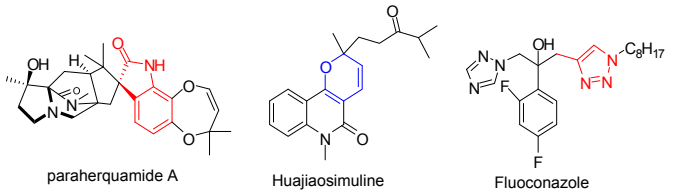


Fig. 1. Some representative compounds containing spirocyclic oxindole, pyran and 1,2,3-triazole moiety.

75-1 breast cancer cells (Fig. 1) [26]. 1,2,3-Triazoles are another privileged structures associated with a plethora of biological activities including antiHIV [27], antimicrobial [28], antiviral [29], antiproliferative [30], antibiotics [31], insecticides [32] and fungicidal [33]. Fluconazole is a well known antifungal drug consisting of 1,2,3-triazole moiety (Fig. 1).

Therefore, in continuation of our research interest in the synthesis of potentially bioactive heterocyclic compounds with diverse applications through hybridization [34], we decided to combine spirocyclic oxindole, 2-amino-4*H*-pyran and 1,2,3-triazoles in a single matrix through MCR approach in ionic liquids under ultrasonic irradiation and to study their biological activity and mode of action against pathogenic microorganisms.

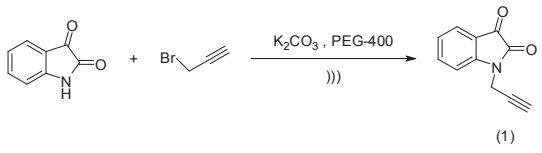
2. Results and discussion

2.1. Chemistry

We report herein an efficient multicomponent synthesis of novel fluorescent triazolyl spirocyclic oxindole derivatives (**2a**–**2o**) comprising spiro-oxindole, 2-amino-4*H*-pyran, and 1,2,3-triazole substructures by one pot four-component reaction of 1-(prop-2-ynyl)indoline-2,3-dione, cyclic 1,3-diketones such as dimedone/cyclohexan-1,3-dione/1,3-dimethylbarbituric acid, malononitrile and aryl azides in the presence of 10 mol% $CuSO_4.5H_2O$ and 20 mol% sodium ascorbate in ionic liquids [DBU-H]OAc and [DBU-Bu]OH under ultrasonic irradiation at room temperature. The antimicrobial activity and fluorescence properties of all new compounds were investigated.

Initially, 1-(prop-2-ynyl)indoline-2,3-dione, one of the component of MCR, was synthesized by the hitherto unreported procedure as outlined in Scheme 1.

The methodology for the proposed four-component reaction was established for the synthesis of **2a** using a model reaction comprising 1-(prop-2-ynyl)indoline-2,3-dione (1.0 mmol), malononitrile (1.0 mmol), dimedone (1.0 mmol) and 4-nitrophenyl azide (1.0 mmol) in presence of 10 mol% $CuSO_4.5H_2O$ and 20 mol% sodium ascorbate under different reaction conditions as shown in Table 1. There was no reaction when the above components were heated in ethanol (Table 1, entry **1**). The above reaction was then attempted in presence of [DBU-H]OAc (20 mol%) in ethanol. After workup (80 min), 55% of the desired product (2a) was obtained (Table 1, entry **2**). Reaction repeated in water and methanol in presence of



Scheme 1. Synthesis of 1-(prop-2-ynyl)indoline-2,3-dione.

Table 1
Optimization of reaction conditions for the proposed four component condensation in [DBU-H]OAc under ultrasonic irradiation.[a]

S. No.	Solvent	Base catalyst	Time (min)	Yield (%)
1.	Ethanol	–	120	–[b]
2.	Ethanol	[DBU-H]OAc (20 mol%)	80	55[c]
3.	Water	[DBU-H]OAc (20 mol%)	80	65[c]
4.	Methanol	[DBU-H]OAc (20 mol%)	120	60[c]
5.	–	[DBU-H]OAc (20 mol%)	20	92
6.	–	[DBU-H]OAc (30 mol%)	20	89
7.	–	[DBU-H]OAc (10 mol%)	50	75
8.	–	[DBU-H]OAc (20 mol%)	120	52[d]

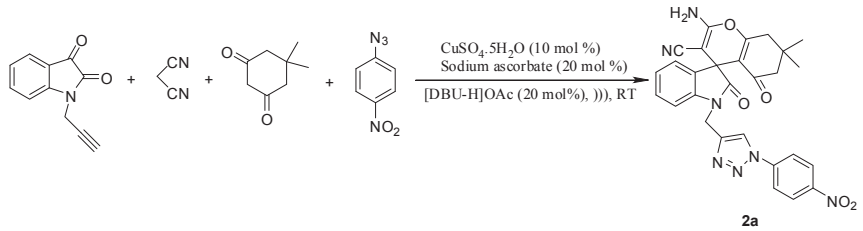
[a] Reaction was carried out using 1-(prop-2-ynyl)indoline-2,3-dione (1.0 mmol), dimedone (1.0 mmol), 4-nitrophenyl azide (1.0 mmol), malononitrile (1.0 mmol) in presence of $CuSO_4.5H_2O$ (10 mol %) and sodium ascorbate (20 mol %).
[b] No reaction.
[c] Mixture of products.
[d] The reaction was carried out using stirring at room temperature in absence of ultrasound irradiation.

[DBU-H]OAc (20 mol%) also gave poor yields of **2a** (Table 1, entries **3**, **4**). However, when the reaction was attempted in absence of any solvent using [DBU-H]OAc (20 mol%) with dual role of catalyst and medium under ultrasonic irradiation at room temperature, the reaction time reduced dramatically to 20 min and yielded 92% of the desired product **2a** after a simple workup (Scheme 2, Table 1, entry **5**).

Higher amount of [DBU-H]OAc (30 mol%) did not affect the reaction time and product yield (Table 1, entry **6**) while lower amount of [DBU-H]OAc (10 mol%) resulted in a longer reaction time and inferior yield (Table 1, entry **7**). When the reaction was carried out in [DBU-H]OAc in absence of ultrasonic irradiation, the reaction was incomplete even after 120 min and gave a mixture of products with only 52% of the desired product **2a** (Table 1, entry **8**).

Thus, condensation of four-components using $CuSO_4.5H_2O$ (10 mol%) and sodium ascorbate (20 mol%) in 20 mol% of [DBU-H]OAc under ultrasonic irradiation at room temperature proved to be the optimum condition for this reaction. Subsequently reactions were carried out by changing aryl azides. All the reactions preceded smoothly for both electron rich and electron deficient aryl azides which afforded the desired products (**2b**–**2g**) in high yields (Table 2, Method **A**, entries **2**–**7**). Replacement of dimedone with cyclohexane-1,3-dione under otherwise identical conditions also afforded corresponding triazolyl spiro-oxindoles (**2h**–**2k**) in high yields (Table 2, Method **A**, entries **8**–**11**). Further we examined the scope of reaction by replacing dimedone with 1,3-dimethylbarbituric acid under otherwise identical conditions. Reactions proceeded smoothly and yielded corresponding triazolyl spiro oxindoles (**2l**–**2o**) in high yields (Table 2, Method **A**, entries **12**–**15**). These results have been summarized in Table 2 (Method **A**), Scheme 3.

After successful synthesis of triazolyl spiro-oxindoles in [DBU-H]OAc under ultrasonic irradiation, we decided to explore this reaction in presence of another DBU based ionic liquid [DBU-Bu]OH. Therefore, a reaction of 1-(prop-2-ynyl)indoline-2,3-dione (1.0 mmol), malononitrile (1.0 mmol), dimedone (1.0 mmol) and 4-nitrophenyl azide (1.0 mmol) was attempted in [DBU-Bu]OH (20 mol%) in the presence of 10 mol% $CuSO_4.5H_2O$ and 20 mol% sodium ascorbate. The reaction was complete in 15 min under ultrasonic irradiation at room temperature and yielded 94% of the desired product **2a**. Higher amount of [DBU-Bu]OH did not affect the reaction time or yield of reaction, while use of 10 mol% of [DBU-Bu]OH resulted in longer reaction time. The reaction was incomplete even after 120 min when performed in absence of ultrasonic irradiation. Subsequently, reactions of other aryl azides were attempted which proceeded smoothly to yield corresponding



Scheme 2. Four component condensation for synthesis of spiro oxindole derivatives.

products (**2a**–**2g**) in high yields as shown in Table 2 (Method **B**, entries **1**–**7**). Also when cyclohexane-1,3-dione and 1,3-dimethylbarbituric acid were used in the place of dimedone, promising results were obtained with high yield of products (**2h**–**2o**) (Table 1, Method **B**, entries 8–15) Scheme 3.

The structures of all compounds (**2a**–**2o**) have been confirmed on the basis of their spectral data. IR spectrum of compound **2a** showed characteristic absorption bands at 3367 and 3161 cm^{-1} due to NH stretch of amino group. The ^1H NMR spectrum of compound **2a** showed singlet at δ 8.62 because of one triazolyl proton and another singlet at δ 7.25 corresponding to two NH_2 protons. The eight aromatic protons of **2a** were observed in the range of δ 8.28–6.85. Two methylene protons adjacent to nitrogen atom ($-NCH_aH_b$) of **2a** appeared as AB system and were observed as two sets of doublets at δ 5.03 and 4.93. The protons of two methylene groups adjacent to geminal dimethyl group also act as AB system and were observed as two sets of doublets at δ 2.51, 2.43 ($COCH_a.H_b$) and at δ 2.08, 1.99 ($CH_a.H_b$), respectively. The six protons of two geminal methyl groups were seen as two different singlets at δ 0.92 and δ 0.87, respectively. ^{13}C NMR spectra of compound **2a** showed twenty six signals corresponding to twenty six non-equivalent carbons. In ^{13}C NMR spectra signal at δ 195.28 accounted for the carbonyl carbon, while signal at δ 176.31 accounted for carbonyl carbon of oxindole ring system. The signal for spiro carbon of **2a** was observed at δ 49.85, while two methyl groups were observed at δ 27.63 and δ 26.90, respectively. HRMS of compound **2a** showed a molecular ion peak at 538.1819 [M^+ + H]. The position of NH_2 protons in the ^1H NMR spectra of all compounds depends upon the NMR solvent, when NMR was recorded in $CDCl_3$, NH_2 protons appeared in the region of δ 5.20–4.79, while in DMSO-d_6, NH_2 protons appeared in the region of δ 7.69–7.25.

Table 2
Synthesis of triazolyl spirocyclic oxindole derivatives under ultrasonic irradiation.

Entry	Ar	Product	Method A		Method B	
			Time (min)	Yield (%)	Time (min)	Yield (%)
1	4-$NO_2C_6H_4$	**2a**	20	92	15	94
2	3-ClC_6H_4	**2b**	40	87	20	88
3	4-BrC_6H_4	**2c**	30	90	15	93
4	4-MeC_6H_4	**2d**	45	89	20	87
5	4-$MeOC_6H_4$	**2e**	50	85	20	89
6	C_6H_5	**2f**	35	81	20	90
7	4-F,3-ClC_6H_3	**2g**	40	90	25	82
8	4-$NO_2C_6H_4$	**2h**	20	88	15	92
9	4-BrC_6H_4	**2i**	25	93	10	88
10	4-MeC_6H_4	**2j**	35	84	20	92
11	4-$MeOC_6H_4$	**2k**	40	81	25	90
12	4-$NO_2C_6H_4$	**2l**	25	90	15	89
13	4-BrC_6H_4	**2m**	30	87	15	92
14	4-MeC_6H_4	**2n**	35	92	20	91
15	4-$MeOC_6H_4$	**2o**	45	86	20	92

Method A: Reactions performed using task specific ionic liquid [DBU-H]OAc.
Method B: Reactions performed using task specific ionic liquid [DBU-Bu]OH.

The recovery of reaction medium and catalyst is an important aspect of green chemistry. Therefore, recyclability of [DBU-Bu]OH was investigated. After completion of the reaction for the synthesis of **2a** in [DBU-Bu]OH, water (5 mL) was added to the reaction mixture. The ionic liquid dissolved in the water, and the solution was filtered to isolate the product and washed again with water (5 mL). Water was evaporated from water-ionic liquid mixture and dried under reduced pressure. The recovered ionic liquid was reused to study its catalytic activity in subsequent runs for the synthesis of **2a**. No appreciable loss in the yield of **2a** was observed after three cycles as **2a** was obtained in 90%, 88% and 85% yield after first, second and third cycle, respectively.

The plausible reaction mechanism for the synthesis of triazolyl spirocyclic oxindole derivatives has been shown in Scheme 4. Initially, *Huiseng* 1,3-dipolar cycloaddition takes place between 1-(prop-2-ynyl)indoline-2,3-dione (**1**) and aryl azide in presence of Cu(1) generated *in situ* leading to formation of a triazole derivative (**1A**) as an intermediate. The formation of **1A** was confirmed by comparing with an authentic sample on TLC. This is followed by Knoevenagel condensation with malononitrile in task specific ionic liquid to give another intermediate **1B**. The unsaturated nitrile derivative so formed (1B) undergo subsequent reactions with 1,3-diketone in presence of ionic liquid to give the desired triazolyl spirocyclic oxindole derivatives (**2**).

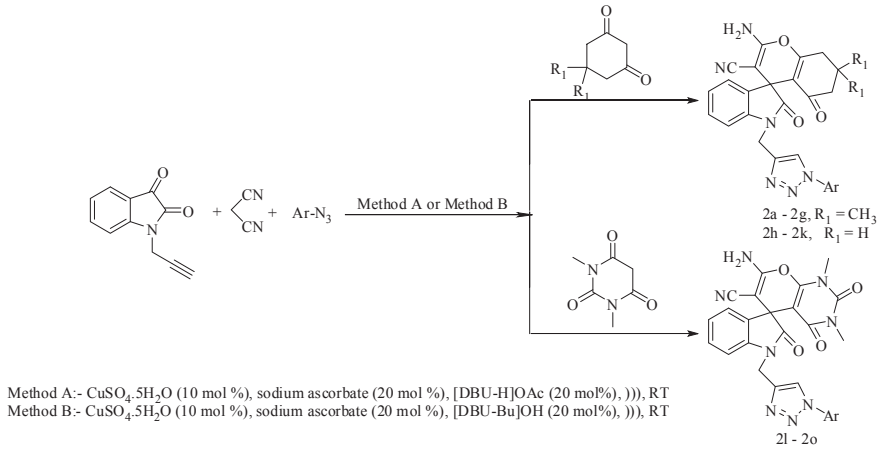
In order to provide evidence for this plausible mechanism, compound **1A** was synthesized using 4-nitrophenyl azide. It was then reacted with malononitrile in the presence of [DBU-Bu]OH under ultrasonic irradiation at room temperature to give compound **1B** after 5 min. Dimedone was added to the reaction mixture and the desired product **2a** was obtained in 92% yield after 10 min.

2.2. Biological screening of novel triazolyl spirocyclic oxindoles derivatives

2.2.1. In vitro antibacterial activity

Antibacterial activity of all new compounds (**2a**–**2o**) was evaluated against four bacterial strains. Two Gram-positive bacteria (*S. aureus* MTCC 96 and *Bacillus subtilis* MTCC 121); two Gram-negative bacteria (*Escherichia coli* MTCC 1652 and *Pseudomonas aeruginosa* MTCC 741) were used in the present study for evaluation of antibacterial activity of all new compounds. The results of the antibacterial activity evaluation reveal that all compounds possessed good Gram-positive antibacterial activity against *S. aureus* and *B. subtilis*. However, none of the compounds exhibited activity against Gram-negative (*E. coli* and *P. aeruginosa*) bacteria. The results of antibacterial activity of all compounds are shown in Table 3 and Fig. 2. All compounds showed diameter of growth of inhibition zone in the range of 13.6–20.6 mm against *S. aureus* bacteria, and in the range of 15.6–22.3 mm against *B. subtilis* bacteria.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of all compounds (**2a**–**2o**) was measured against Gram-positive bacteria. All compounds showed MIC values in the range of 32–256 μg/mL against *S. aureus* and in



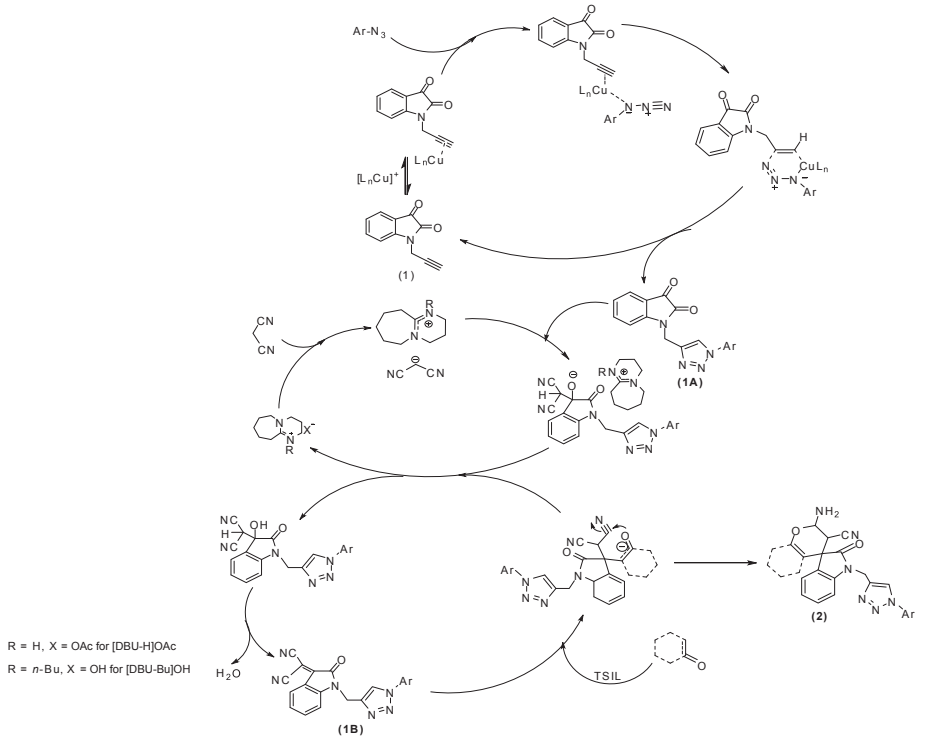
Scheme 3. Synthesis of triazolyl spiro-oxindoles in presence of task specific ionic liquids under ultrasonic irradiation at room temperature.

the range of 16–128 μg/mL against *B. subtilis* bacteria (Table 4). All compounds showed MBC values in the range of 64–512 μg/mL against *S. aureus* and in the range of 64–256 μg/mL against *B. subtilis* bacteria (Table 4).

The results of antibacterial activity (Tables 3 and 4) revealed that among all novel compounds, **2m** was found to be most active against Gram-positive bacteria with a diameter of growth of inhibition zone of 20.6 mm against *S. aureus* and 22.3 mm against *B. subtilis*. Compound **2m** showed MIC value of 32 μg/mL and MBC value of 64 μg/mL against *S. aureus*, while it showed MIC value of 16 μg/mL and MBC value of 64 μg/mL against *B. subtilis*. The structure activity relationship (SAR) study of these compounds revealed that compounds **2b**, **2c**, **2g**, **2i** and **2m** having halogen atom in their moiety showed better activity as compared to other compounds.

2.2.2. *In vitro antifungal activity*

Antifungal activity of all compounds (**2a**–**2o**) was evaluated by selecting two yeast strains *Candida albicans* (MTCC 227) *and Saccharomyces cerevisiae* (MTCC 170) on the basis of their clinical importance. The diameter of growth of inhibition zone (mm) and MIC (μg/mL) of all compounds was determined using amphotericin-B as a standard drug. The results of antifungal activity of all compounds (**2a**–**2o**) are shown in Table 5, Fig. 3. It can be seen from Table 5 that all compounds showed good antifungal activity. The diameter of growth of inhibition zone was observed in the range of 12.3–16.6 mm for yeast *C. albicans* and in the range of 13.6–17.6 mm for yeast *S. cerevisiae* for compounds **2a**–**2o**. All compounds showed MIC in the range of 128–512 μg/mL for *C. albicans* and in the range of 64–512 μg/mL against *S. cerevisiae*. Compound **2e** showed highest activity against yeast *C. albicans* with



Scheme 4. Plausible mechanism for synthesis of triazolyl spirocyclic oxindoles.

Table 3
Antibacterial activity (diameter of growth of inhibition zone) of all compounds.

Entry	Product	Diameter of growth of inhibition zone (mm)[a]			
		Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*
1	**2a**	16.6	17.3	–	–
2	**2b**	19.3	20.6	–	–
3	**2c**	19.3	22.2	–	–
4	**2d**	14.3	15.6	–	–
5	**2e**	15.6	17.3	–	–
6	**2f**	14.6	16.3	–	–
7	**2g**	19.6	21.3	–	–
8	**2h**	17.6	18.6	–	–
9	**2i**	18.6	20.3	–	–
10	**2j**	13.6	16.3	–	–
11	**2k**	14.0	15.6	–	–
12	**2l**	15.3	18.6	–	–
13	**2m**	20.6	22.3	–	–
14	**2n**	14.3	16.3	–	–
15	**2o**	15.3	17.6	–	–
16	Ciprofloxacin	26.6	24.0	25.0	22.0

– No activity.
[a] Values, including diameter of the well (8 mm), are means of three replicates.

Table 4
Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of compounds (**2a**-**2o**) against Gram positive bacteria.

Entry	Product	MIC (μg/mL)		MBC (μg/mL)	
		Staphylococcus aureus	*Bacillus subtilis*	*Staphylococcus aureus*	*Bacillus subtilis*
1	**2a**	128	128	128	256
2	**2b**	64	32	128	128
3	**2c**	32	16	128	64
4	**2d**	128	32	256	256
5	**2e**	128	128	256	256
6	**2f**	128	128	128	256
7	**2g**	64	32	128	128
8	**2h**	128	128	256	256
9	**2i**	64	32	64	128
10	**2j**	256	128	512	128
11	**2k**	64	32	512	128
12	**2l**	128	64	512	128
13	**2m**	32	16	64	64
14	**2n**	128	128	256	128
15	**2o**	256	128	512	256
16	Ciprofloxacin	6.25	6.25	6.25	6.25

diameter of growth of inhibition zone of 16.6 mm and MIC value of 128 μg/mL. While compound **2o** was found to be most active against *S. cerevisiae* with diameter of growth of inhibition zone of 17.6 mm and MIC value of 64 μg/mL. Compounds **2e**, **2k**, **2o** showed MIC value of 64 μg/mL against *S. cerevisiae* lower than the standard drug amphotericin-B. It can be inferred from the results of antifungal activity that the presence of methoxy group in molecules (**2e**, **2k**, **2o**) enhances the antifungal activity of these compounds.

The molecular basis of the antifungal activity of azole antifungal drugs is the inhibition of ergosterol biosynthesis which is necessary to maintain fungal cell membrane integrity and permeability. Indeed, azole antifungals inhibit the cytochrome P-450 lanosterol 14α-demethylase (CYP51) which is responsible for the oxidative removal of the 14α-methyl group of lanosterol [35]. In order to understand the possible mechanism of antifungal activity, the docking of most potent compound **2e** was performed at active site of CYP51 (PDB ID 1EA1). The docking studies were performed using autodock 4 programe with default parameters. The crystallographic structure of Cytochrome P450 14α-sterol demethylase (MTCYP51) with bound inhibitor (fluconazole) was downloaded from protein data bank (PDB ID 1EA1). Fluconazole was re-docked into the active site in order to check the validity of docking protocol and satisfactory results were obtained. The binding site for CYP51 inhibitors is located at residues Phe22, Tyr76, Met79, Phe83, Lys74, Phe255, Ala256, His259, Leu321, Ile322, Ile323, His430, Met433, Val434, Val435, Met253 and the heme [36]. The binding mode of compound **2e** at the active site of CYP51 is shown in Fig. 4. The nitrogen atom of amino group of **2e** coordinates with iron atom of Hem460, while nitrogen atom of triazole ring formed a hydrogen bond with Lys74 amino acid residue and is surrounded by Met79, Ile322, Leu324, Met325, Tyr76, His259, Ala256, Arg96, Met253 amino acid residues. These interactions may be responsible for the antifungal activity of compound **2e**. However, we cannot refute other mechanisms which are responsible for antifungal activity and only experiments on isolated CYP51 could confirm above hypothesis.

2.2.3. Fluoresecne properties

Novel fluorescent molecules are potential candidates as fluorescent probes, for imaging in clinical diagnostics and biomedical research. We observed that all compounds showed strong fluorescence in methanol when excited at wavelength 266 nm as shown in Fig. 5. The results of photophysical properties of all triazolyl spirocyclic oxindoles are summarized in Table 6. Moreover,

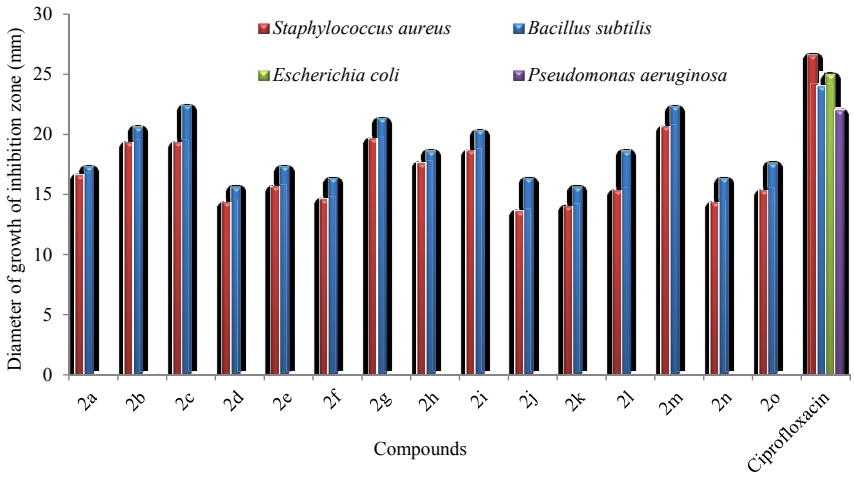


Fig. 2. Graphical representation of diameter of growth of inhibition zone (mm) of compounds (**2a**–**2o**) against bacterial strains.

Table 5
Antifungal activity of all compounds (**2a**–**2o**).

Entry	Product	Diameter of growth of inhibition zone (mm)		MIC (μg/mL)	
		Candida albicans	*Saccharomyces cerevisiae*	*Candida albicans*	*Saccharomyces cerevisiae*
1	**2a**	14.3	15.3	256	256
2	**2b**	12.3	13.6	512	256
3	**2c**	13.3	14.6	512	128
4	**2d**	12.6	14.0	512	256
5	**2e**	16.6	15.6	128	64
6	**2f**	13.3	14.3	256	256
7	**2g**	13.6	14.3	256	256
8	**2h**	12.3	13.6	512	256
9	**2i**	12.6	14.3	512	256
10	**2j**	14.3	15.6	128	128
11	**2k**	16.0	16.3	128	64
12	**2l**	12.6	13.6	512	512
13	**2m**	13.3	15.3	256	128
14	**2n**	12.3	14.3	512	256
15	**2o**	14.6	17.6	128	64
16	Amphotericin-B	17.6	18.3	100	100

the UV–visible spectra of all compounds contain intense absorption maxima in the range of 239–287 nm. These compounds exhibit emission band in the range of 366–417 nm with stokes shift in the range of 97–156 nm.

3. Conclusion

In conclusion, We have developed an efficient synthesis of novel structurally diverse fluorescent triazolyl spirocyclic oxindole derivatives by one pot four-component domino reaction in presence of [DBU-H]OAc and [DBU-Bu]OH under ultrasonic irradiation at room temperature. The antimicrobial activity of all compounds was evaluated against six microbial strains. Compound **2m** was found to be most potent antibacterial agent against *S. aureus* and against *B. subtilis*. All compounds showed good antifungal activity. Compounds **2e**, **2k**, **2o** showed MIC values lower than the standard drug amphotericin-B against *S. cerevisiae*. The docking studies of compound **2e** at the active site of cytochrome P-450 lanosterol 14α-demethylase (CYP51) were also performed. The compounds exhibit strong fluorescence in methanol with large stokes shift.

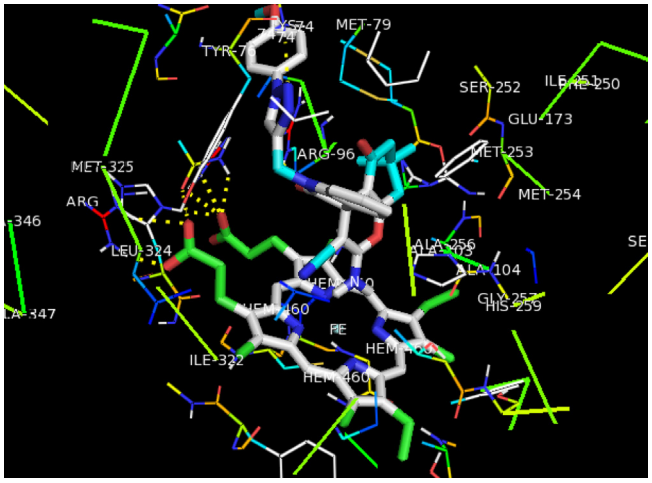


Fig. 4. Binding mode of compound **2e** at the active site of CYP51 (PDB ID: 1EA1).

4. Experimental

All chemicals were commercial and were purchased from Sigma–Aldrich, Spectrochem and were used as received. F_{254} Precoated aluminum plates with silica gel 60 from Merck were used to monitor reaction progress. Ultrasonic bath (54 KHz, 300 W, 3 L, capacity) of Throughclean ultrasonic Pvt. Ltd. (India) was used for reactions. Melting points were measured on Buchi M-560 melting point apparatus and are uncorrected. IR (KBr) spectra were recorded on Perkin Elmer FTIR spectrophotometer and the values are expressed as ν_{max} cm^{-1}. The NMR (^1H and ^{13}C) spectra were recorded on Jeol JNM ECX-400P at 400 MHz and 100 MHz, respectively. The chemical shift values are recorded on δ scale and the coupling constants (J) are in Hertz. The mass spectra were recorded on Agilent 6520-QTOF LCMS having ESI source in positive mode. Ultraviolet–visible (UV–Vis) absorption spectra were recorded on Analytikjena specord 250 spectrophotometer. The fluorescence spectra were measured at Cary Eclipse Fluorescence spectrophotometer.

4.1. Preparation of ionic liquid [DBU-H]OAc

[DBU-H]OAc was prepared by reaction of DBU with acetic acid by reported procedure [37].

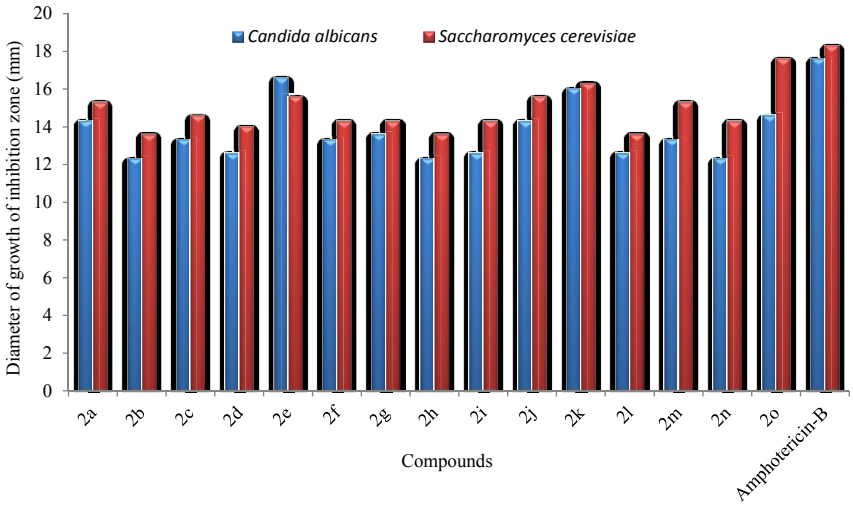


Fig. 3. Graphical representation of diameter of growth of inhibition zone (mm) of compounds (**2a**–**2o**) against yeast strains.

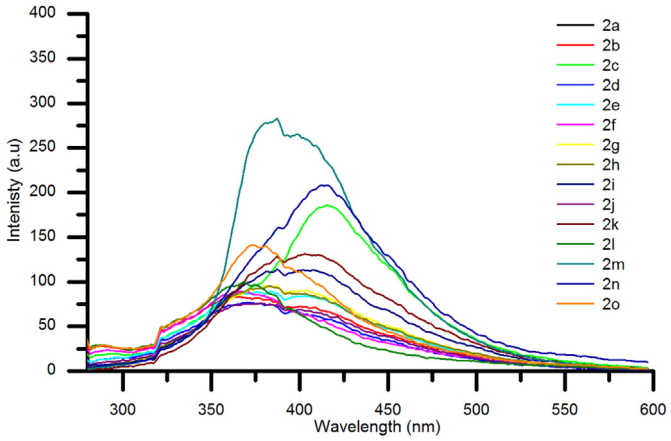


Fig. 5. Fluorescence spectra of compounds (**2a**–**2o**) in methanol.

4.2. Preparation of ionic liquid [DBU-Bu]OH

Firstly [DBU-Bu]Br was prepared by reaction of DBU with *n*-butyl bromide in cyclohexane by reported procedure [38]. In second step, KOH (1.2 eq) was added to a solution of [DBU-Bu]Br in dichloromethane. After 48 h of stirring at room temperature, the reaction mixture was filtered through a sintered funnel. The filtrate was concentrated on a rotary evaporator to yield a viscous liquid. Dichloromethane was added to the residue to cause precipitation of any remaining KBr. The precipitate was filtered through a sintered funnel and the filtrate concentrated again on rotary evaporator to obtain 92% of [DBU-Bu]OH.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.26–3.15 (m, 3H), 2.41–2.19 (m, 5H), 1.58–1.46 (m, 9H), 1.37–1.07 (m, 5H), 0.75–0.69 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 175.45, 49.33, 45.37, 36.83, 29.59, 28.84, 28.71, 28.30, 27.91, 25.58, 25.24, 20.11, 13.64; IR (ν_{max} cm^{-1}, KBr) = 3445, 2928, 1634, 1485, 1326.

4.3. Procedure for synthesis of 1-(prop-2-ynyl)indoline-2,3-dione

A mixture of isatin (1.0 mmol), propargyl bromide (1.2 mmol), K_2CO_3 (2.0 mmol) and PEG 400 (5 mL) was placed in a 50 mL round-bottomed flask. The reaction mixture was sonicated at room temperature for 4 h. After completion of reaction as monitored by TLC using ethyl acetate: petroleum ether (30:70, v/v) as eluent, water (10 mL) was added to the reaction mixture. The precipitate formed was collected by filtration at pump, washed with water to afford pure 1-(prop-2-ynyl)indoline-2,3-dione (M.p. 156–158 °C) [39] in 92% yield as orange solid.

Table 6
Photophysical data of all compounds (**2a**–**2o**) in methanol.

Product	λ_{abs} (nm)	$\varepsilon \times 10^5$ (L mol^{-1} cm^{-1})	λ_{em} (nm)	Stoke shift ($\Delta\nu$) nm
2a	239	0.29	373	134
2b	241	0.28	366	125
2c	261	0.43	417	156
2d	262	0.60	368	106
2e	264	0.75	378	114
2f	270	0.71	367	97
2g	287	0.25	384	97
2h	269	0.19	385	116
2i	269	0.78	398	129
2j	265	0.92	377	112
2k	269	0.93	405	136
2l	270	0.61	369	99
2m	269	0.41	385	116
2n	257	0.25	413	156
2o	270	0.55	377	107

4.4. General procedure for the domino synthesis of triazolyl spirocyclic oxindole derivatives (*2a*–*2o*) under ultrasonic irradiation in presence of [DBU-H]OAc (Method *A*)

A mixture of 1-(prop-2-ynyl)indoline-2,3-dione (1.0 mmol), dimedone/cyclohexane-1,3-dione/1,3-dimethyl barbituric acid (1.0 mmol), malononitrile (1.0 mmol), aryl azide (1.0 mmol), $CuSO_4.5H_2O$ (10 mol%), sodium ascorbate (20 mol%) and 20 mol% [DBU-H]OAc was placed in a 50 mL round bottomed flask. The reaction mixture was sonicated for appropriate time until the complete disappearance of starting materials. The progress of the reaction was monitored by TLC using ethyl acetate: petroleum ether (40:60, v/v) as eluent. After completion of the reaction as indicated by TLC, water (10 mL) was added to the reaction mixture. The precipitate formed was collected by filtration at pump and washed with water several times to remove Cu (II) from product. The product thus obtained was recrystallized from ethanol to yield pure products (**2a**–**2o**) in high yields as mentioned in Table 2 (Method **A**). The products were characterized by IR, ^{1}H NMR, ^{13}C NMR and Mass spectra.

4.5. General procedure for the synthesis of triazolyl spirocyclic oxindole derivatives (*2a*–*2o*) under ultrasonic irradiation in presence of [DBU-Bu]OH (Method *B*)

A mixture of 1-(prop-2-ynyl)indoline-2,3-dione (1.0 mmol), dimedone/cyclohexane-1,3-dione/1,3-dimethyl barbituric acid (1.0 mmol), malononitrile (1.0 mmol), aryl azide (1.0 mmol), $CuSO_4.5H_2O$ (10 mol%), sodium ascorbate (20 mol%) and 20 mol% [DBU-Bu]OH was placed in a 50 mL round bottomed flask. The reaction mixture was sonicated for appropriate time until the complete disappearance of starting materials. The progress of the reaction was monitored by TLC using ethyl acetate: petroleum ether (40:60, v/v) as eluent. After completion of the reaction as indicated by TLC, water (10 mL) was added to the reaction mixture. The precipitate formed was collected by filtration at pump and washed with water several times to remove Cu (II) from product. The product thus obtained was recrystallized from ethanol to yield pure products (**2a**–**2o**) (Table 2, Method **B**). The products were characterized by IR, ^{1}H NMR, ^{13}C NMR and Mass spectra.

4.6. Spectral data for compounds (*2a*–*2o*)

4.6.1. 2-Amino-7,7-dimethyl-1′-((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)-2′,5-dioxo-5,6,7,8-tetrahydrospiro[chromene-4,3′-indoline]-3-carbonitrile (*2a*)

White Solid, M.pt.: 270 °C (decomp), ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.62 (s, 1H, triazolyl-H), 8.28 (d, 2H, J = 8.8 Hz, ArH), 7.97 (d, 2H, J = 8.8 Hz, ArH), 7.25 (s, 2H, $-NH_2$), 7.12–7.08 (t, 1H, J = 8.8 Hz, ArH), 6.96–6.85 (m, 3H, ArH), 5.03 and 4.93 (AB system, J = 16.12 Hz, 2H, $-NCH_a.H_b$), 2.51 and 2.43 (AB system, J = 17.61 Hz, 2H, $-CH_a.H_b$), 2.08 and 1.99 (AB system, J = 16.12 Hz, 2H, $-CH_a.H_b$), 0.92 (s, 3H, CH_3), 0.87 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 195.28, 176.31, 164.69, 158.71, 146.62, 144.18, 141.64, 140.64, 133.47, 128.43, 125.60, 123.05, 122.88, 121.86, 120.40, 120.08, 117.57, 110.47, 108.77, 56.86, 49.85, 46.35, 35.23, 31.96, 27.63, 26.90; IR (ν_{max} cm^{-1}, KBr) = 3367, 3161, 1708, 1669, 1611, 1497; HRMS (ESI) [M^+ + H] calcd. for $C_{28}H_{23}N_7O_5$: 538.1838, found: 538.1819 [M^+ + H].

*4.6.2. 2-Amino-1′-((1-(3-chlorophenyl)-1H-1,2,3-triazol-4-yl) methyl)-7,7-dimethyl-2′,5-dioxo-5,6,7,8-tetrahydrospiro[chromene-4,3′-indoline]-3-carbonitrile (**2b**)*

White Solid, M.pt.: 176 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.33 (s, 1H, triazolyl-H), 7.73 (s, 1H, ArH), 7.56 (d, 1H, J = 7.36 Hz, ArH), 7.32–7.25 (m, 2H, ArH), 7.15–7.11 (t, 1H, J = 7.32 Hz, ArH), 6.98–6.95 (m, 2H, ArH), 6.76 (d, 1H, J = 8.04 Hz, ArH), 5.16 (s, 2H, $-NCH_2$), 4.88 (s, 2H, $-NH_2$), 2.53 and 2.43 (AB system, J = 17.56 Hz, 2H, $-CH_a.H_b$), 2.21 and 2.12 (AB system, J = 16.88 Hz, 2H, $-CH_a.H_b$), 1.08 (s, 3H, CH_3), 1.02 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 195.30, 176.36, 164.22, 158.30, 141.61, 140.34, 139.09, 138.96, 137.89, 135.33, 132.49, 131.45, 130.63, 129.26, 128.44, 123.48, 123.10, 120.52, 118.17, 119.21, 109.39, 50.51, 46.70, 40.92, 36.56, 32.28, 28.61, 27.52; IR (ν_{max} cm^{-1}, KBr): =3371, 3120, 1702, 1661, 1611, 1540; HRMS (ESI) [M$^+$ + H] calcd. for $C_{28}H_{23}ClN_6O_3$: 527.1598, found: 527.1590 [M$^+$ + H].

*4.6.3. 2-Amino-1′-((1-(4-bromophenyl)-1H-1,2,3-triazol-4-yl) methyl)-7,7-dimethyl-2′,5-dioxo-5,6,7,8-tetrahydrospiro[chromene-4,3′-indoline]-3-carbonitrile (**2c**)*

White Solid, M.pt.: 240 °C (decomp), ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.42 (s, 1H, triazolyl-H), 7.63 (s, 2H, $-NH_2$), 7.26 (s, 1H, ArH), 7.11–7.07 (t, 1H, J = 7.32 Hz, ArH), 6.97 (d, 2H, J = 7.32 Hz, ArH), 6.92–6.85 (m, 4H, ArH), 5.01 and 4.89 (AB system, J = 16.12 Hz, 2H, $-NCH_a.H_b$), 2.51 and 2.44 (AB system, J = 17.6 Hz, 2H, $-CH_a.H_b$), 2.08 and 1.99 (AB system, J = 16.12 Hz, 2H, $-CH_a.H_b$), 0.92 (s, 3H, CH_3), 0.88 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 195.80, 176.74, 165.24, 159.48, 142.38, 136.21, 134.02, 133.38, 133.25, 128.99, 128.90, 123.60, 123.35, 122.33, 121.91, 118.21, 116.04, 110.28, 109.31, 57.31, 50.42, 46.92, 35.91, 32.53, 28.18, 27.48; IR (ν_{max} cm^{-1}, KBr): =3363, 3160, 1707, 1670, 1610, 1497; HRMS (ESI) [M$^+$ + H] calcd. for $C_{28}H_{23}BrN_6O_3$: 571.1093, found: 571.1089 [M$^+$ + H].

*4.6.4. 2-Amino-7,7-dimethyl-2′,5-dioxo-1′-((1-p-tolyl-1H-1,2,3-triazol-4-yl)methyl)-5,6,7,8-tetrahydrospiro[chromene-4,3′-indoline]-3-carbonitrile (**2d**)*

White Solid, M.pt.: 250 °C (decomp), ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.29 (s, 1H, triazolyl-H), 7.56 (d, 2H, J = 8.24 Hz, ArH), 7.21–7.14 (m, 3H, ArH), 7.01–6.96 (m, 2H, ArH), 6.82 (d, 1H, J = 7.8 Hz, ArH), 5.20 (s, 2H, $-NH_2$), 5.08 (s, 2H, $-NCH_2$), 2.55 and 2.45 (AB system, J = 17.84 Hz, 2H, $-CH_a.H_b$), 2.33 (s, 3H, CH_3), 2.23 and 2.14 (AB system, J = 16.48 Hz, 2H, $-CH_a.H_b$), 1.10 (s, 3H, CH_3), 1.04 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 195.20, 176.55, 164.18, 158.41, 141.70, 138.48, 134.76, 132.55, 131.90, 130.08, 129.16, 128.40, 126.92, 123.37, 123.04, 121.02, 120.15, 111.52, 109.45, 50.46, 46.73, 40.85, 36.61, 32.20, 28.56, 27.46, 20.98; IR (ν_{max} cm^{-1}, KBr): =3327, 3210, 1724, 1673, 1611, 1518; HRMS (ESI) [M$^+$ + H] calcd. for $C_{29}H_{26}N_6O_3$: 507.2144, found: 507.2158 [M$^+$ + H].

*4.6.5. 2-Amino-1′-((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl) methyl)-7,7-dimethyl-2′,5-dioxo-5,6,7,8-tetrahydrospiro[chromene-4,3′-indoline]-3-carbonitrile (**2e**)*

White Solid, M.pt.: 214 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.19 (s, 1H, triazolyl-H), 7.55 (d, 2H, J = 6.6 Hz, ArH), 7.13–7.11 (m, 1H, ArH), 6.95–6.88 (m, 4H, ArH), 6.79 (d, 1H, J = 8.04 Hz, ArH), 5.17 (s, 2H, $-NCH_2$), 4.79 (s, 2H, $-NH_2$), 3.75 (s, 3H, $-OCH_3$), 2.52 and 2.42 (AB system, J = 17.84 Hz, 2H, $-CH_a.H_b$), 2.20 and 2.11 (AB system, J = 16.08 Hz, 2H, $-CH_a.H_b$), 1.07 (s, 3H, CH_3), 1.01 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 195.23, 176.17, 164.67, 159.24, 158.93, 141.89, 133.48, 129.91, 128.52, 128.48, 123.04, 122.76, 121.47, 121.40, 121.03, 117.66, 114.89, 110.50, 108.74, 56.80, 55.53, 49.89, 46.38, 35.48, 31.97, 27.62, 27.00; IR (ν_{max} cm^{-1}, KBr): =3393, 3157, 1702, 1671, 1601, 1520; HRMS (ESI) [M$^+$ + H] calcd. for $C_{29}H_{26}N_6O_4$: 523.2093, found: 523.2105 [M$^+$ + H].

*4.6.6. 2-Amino-7,7-dimethyl-2′,5-dioxo-1′-((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)-5,6,7,8 tetrahydrospiro[chromene-4,3′-indoline]-3-carbonitrile (**2f**)*

White Solid, M.pt.: 164 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.19 (s, 1H, triazolyl-H), 7.66 (d, 2H, J = 7.32 Hz, ArH), 7.39–7.35 (m, 3H, ArH), 7.30–7.27 (m, 1H, ArH), 7.15–7.11 (m, 1H, ArH), 6.97–6.92 (m, 1H, ArH), 6.78 (d, 1H, J = 7.32 Hz, ArH), 5.18 (s, 2H, $-NCH_2$), 4.83 (s, 2H, $-NH_2$), 3.75 (s, 3H, $-CH_3$), 2.52 and 2.42 (AB system, J = 17.6 Hz, 2H, $-CH_a.H_b$), 2.21 and 2.12 (AB system, J = 16.88 Hz, 2H, $-CH_a.H_b$), 1.08 (s, 3H, CH_3), 1.02 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 195.18, 176.64, 171.84, 168.23, 164.12, 158.30, 141.71, 137.05, 132.50, 130.08, 128.81, 129.24, 128.42, 127.78, 123.72, 121.03, 120.24, 111.60, 109.50, 50.50, 46.34, 40.89, 36.63, 32.36, 28.61, 27.53; IR (ν_{max} cm^{-1}, KBr): =3308, 3153, 1685, 1670, 1610, 1353; HRMS (ESI) [M$^+$ + H] calcd. for $C_{28}H_{24}N_6O_3$: 493.1988, found: 493.1992 [M$^+$ + H].

*4.6.7. 2-Amino-1′-((1-(3-chloro-4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-7,7-dimethyl-2′,5-dioxo-5,6,7,8-tetrahydrospiro [chromene-4,3′-indoline]-3-carbonitrile (**2g**)*

White Solid, M.pt.: 270 °C (decomp), ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.28 (s, 1H, triazolyl-H), 7.80–7.78 (m, 1H, ArH), 7.57–7.53 (m, 1H, ArH), 7.16–7.11 (m, 2H, ArH), 6.98–6.95 (m, 2H, ArH), 6.76 (d, 1H, J = 7.32 Hz, ArH), 5.16 (s, 2H, $-NCH_2$), 4.90 (s, 2H, $-NH_2$), 2.53 and 2.44 (AB system, J = 17.56 Hz, 2H, $-CH_a.H_b$), 2.22 and 2.12 (AB system, J = 16.12 Hz, 2H, $-CH_a.H_b$), 1.08 (s, 3H, CH_3), 1.02 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 195.30, 176.18, 164.20, 158.24, 156.36, 144.09, 141.43, 133.61, 132.46, 123.50, 123.12, 122.73, 122.23, 122.07, 121.18, 119.86, 117.51, 117.28, 116.69, 111.60, 109.35, 50.53, 46.67, 40.92, 36.49, 32.29, 28.61, 27.53; IR (ν_{max} cm^{-1}, KBr): = 3325, 3304, 3113, 1710, 1668, 1612, 1508; HRMS (ESI) [M$^+$ + H] calcd. for $C_{28}H_{22}ClFN_6O_3$: 545.1504 found: 545.1512 [M$^+$ + H].

*4.6.8. 2-Amino-1′-((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl) methyl)-2′,5-dioxo-5,6,7,8-tetrahydrospiro[chromene-4,3′-indoline]-3-carbonitrile (**2h**)*

White Solid, M.pt.: 267 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.74 (s, 1H, triazolyl-H), 8.39 (d, 2H, J = 8.24 Hz, ArH), 8.08 (d, 2H, J = 8.28 Hz, ArH), 7.34 (s, 2H, $-NH_2$), 7.24–6.98 (m, 4H, ArH), 5.24 and 5. 04 (AB system, J = 15 .56 Hz, 2H, $-NCH_a.H_b$), 2.68–2.61 (m, 2H, $-CH_2$), 2.26–2.18 (m, 2H, $-CH_2$), 1.94–1.92 (m, 2H, $-CH_2$); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 195.65, 176.55, 166.75, 158.55, 146.56, 144.38, 141.76, 140.49, 137.89, 133.34, 132.42, 128.06, 125.62, 123.68, 121.86, 120.06, 117.61, 111.76, 108.17, 56.02, 46.44, 39.08, 26.88, 19.86; IR (ν_{max} cm^{-1}, KBr): =3365, 3110, 1707, 1661, 1611, 1520; HRMS (ESI) [M$^+$ + H] calcd. for $C_{26}H_{19}N_7O_5$: 510.1525 found: 510.1532 [M$^+$ + H].

*4.6.9. 2-Amino-1′-((1-(4-bromophenyl)-1H-1,2,3-triazol-4-yl) methyl)-2′,5-dioxo-5,6,7,8-tetrahydrospiro[chromene-4,3′-indoline]-3-carbonitrile (**2i**)*

White Solid, M.pt.: 250 °C (decomp), ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.53 (s, 1H, triazolyl-H), 7.74–7.69 (m, 3H, ArH), 7.35 (s, 2H, $-NH_2$), 7.22–7.18 (m, 1H, ArH), 7.10 (d, 2H, J = 7.32 Hz, ArH), 7.02–6.95 (m, 2H, ArH), 5.11 and 5.01 (AB system, J = 16.12 Hz, 2H, $-NCH_a.H_b$), 2.67–2.66 (m, 2H, $-CH_2$), 2.26–2.20 (m, 2H, $-CH_2$), 1.94–1.91 (m, 2H, $-CH_2$); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 195.21, 176.08, 166.66, 158.76, 141.66, 135.63, 133.56, 132.70, 128.22, 126.42, 125.33, 123.18, 122.68, 121.93, 121.04, 117.44, 116.51, 111.42, 108.50, 56.89, 46.30, 36.24, 25.70, 19.82; IR (ν_{max} cm^{-1}, KBr): =3345, 3120, 1710, 1652, 1607, 1497 HRMS (ESI) [M$^+$ + H] calcd. for $C_{26}H_{19}BrN_6O_3$: 543.0780 found: 543.0786 [M$^+$ + H].

*4.6.10. 2-Amino-2′,5-dioxo-1′-((1-4-tolyl-1H-1,2,3-triazol-4-yl) methyl)-5,6,7,8-tetrahydrospiro [chromene-4,3′-indoline]-3-carbonitrile (**2j**)*

White Solid, M.pt.: 248 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.43 (s, 1H, triazolyl-H), 7.63 (s, 2H, $-NH_2$), 7.35–7.19 (m, 4H, ArH), 7.10–6.99 (m, 4H, ArH), 5.11 and 4.99 (AB system, J = 12.44 Hz, 2H, $-NCH_aH_b$), 2.68–2.66 (m, 2H, $-CH_2$), 2.49–2.23 (m, 5H, $-CH_2$, CH_3), 2.07–1.92 (m, 2H, $-CH_2$); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 195.45, 176.28, 166.56, 158.95, 147.38, 143.35, 142.02, 138.16, 134.25, 133.61, 130.25, 128.39, 123.08, 122.53, 121.03, 119.69, 117.65, 111.43, 108.10, 56.82, 46.46, 36.29, 35.52, 20.51, 19.72; IR (ν_{max} cm^{-1}, KBr): =3325, 3292, 1725, 1667, 1611; HRMS (ESI) [M^+ + H] calcd. For $C_{27}H_{22}N_6O_3$: 479.1831, found: 479.1841 [M^+ + H].

*4.6.11. 2-Amino-1′-((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl) methyl)-2′,5-dioxo-5,6,7,8-tetrahydrospiro[chromene-4,3′-indoline]-3-carbonitrile (**2k**)*

White Solid, M.pt.: 197 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.38 (s, 1H, triazolyl-H), 7.66 (d, J = 8.08 Hz, 2H, ArH), 7.35 (s, 2H, $-NH_2$), 7.19–6.98 (m, 6H, ArH), 5.15 and 5.01 (AB system, J = 14.92 Hz, 2H, $-NCH_aH_b$), 3.76 (s, 3H, OCH_3), 2.68–2.66 (m, 2H, $-CH_2$), 2.24–2.22 (m, 2H, $-CH_2$), 1.99–1.92 (m, 2H, $-CH_2$); ^{13}C NMR (100 MHz, DMSO-d6): δ = 195.29, 176.20, 166.30, 159.38, 158.62, 141.70, 133.48, 130.04, 128.10, 123.20, 122.85, 121.48, 121.05, 117.58, 115.13, 114.43, 113.67, 113.29, 108.47, 56.98, 55.58, 46.40, 35.45, 26. 74, 19.71; IR (ν_{max} cm^{-1}, KBr): =3345, 3192, 1715, 1611, 1597; HRMS (ESI) [M^+ + H] calcd. for $C_{27}H_{22}N_6O_4$: 495.1780 found: 495.1786 [M^+ + H].

*4.6.12. 7′-Amino-1′,3′-dimethyl-1-((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)-2,2′,4′-trioxo-1′,2′,3′,4′-tetrahydrospiro [indoline-3,5′-pyrano[2,3-d]pyrimidine]-6′-carbonitrile (**2l**)*

White Solid, M.pt.: 252 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.48 (s, 1H, triazolyl-H), 7.74 (d, J = 8.18 Hz, 2H, ArH), 7.55 (s, 2H, $-NH_2$), 7.29–7.02 (m, 6H, ArH), 5.12 and 5.02 (AB system, J = 14.90 Hz, 2H, $-NCH_aH_b$), 3.26 (s, 3H, $-NCH_3$), 3.05 (s, 3H, $-NCH_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 182.28, 179.12, 160.22, 157.48, 150.54, 145.84, 140.29, 129.76, 134.44, 132.50, 130.72, 128.34, 124.80, 122.46, 121.45, 120.30, 119.33, 117.65, 108.78, 101.28, 46.56, 34.08, 31.54, 28.67; IR (ν_{max} cm^{-1}, KBr): =3370, 3150, 1710, 1696, 1520; HRMS (ESI) [M^+ + H] calcd. for $C_{26}H_{19}N_9O_6$: 554.1536 found: 554.1540 [M^+ + H].

*4.6.13. 7′-Amino-1-((1-(4-bromophenyl)-1H-1,2,3-triazol-4-yl) methyl)-1′,3′-dimethyl-2,2′,4′-trioxo-1′,2′,3′,4′-tetrahydrospiro [indoline-3,5′-pyrano[2,3-d]pyrimidine]-6′-carbonitrile (**2m**)*

White Solid, M.pt.: 215 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.58 (s, 1H, triazolyl-H), 7.82–7.74 (m, 4H, ArH), 7.68 (s, 2H, $-NH_2$), 7.23–7.20 (m, 2H, ArH), 7.03–6.97 (m, 2H, ArH), 5.13 and 5.04 (AB system, J = 16.12 Hz, 2H, $-NCH_aH_b$), 3.36 (s, 3H, $-NCH_3$), 3.03 (s, 3H, $-NCH_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 181.47, 176.22, 162.43, 156.88, 151.44, 146.87, 140.89, 128.42, 132.33, 131.53, 130.58, 125.87, 123.88, 122.65, 121.55, 120.45, 119.23, 118.55, 107.88, 104.98, 45.56, 33.91, 30.67, 28.40; IR (ν_{max} cm^{-1}, KBr): = 3340, 3112, 1720, 1686, 1501; HRMS (ESI) [M^+ + H] calcd. for $C_{26}H_{19}BrN_8O_4$: 587.0790 found: 587.0801 [M^+ + H].

*4.6.14. 7′-Amino-1′,3′-dimethyl-2,2′,4′-trioxo-1-((1-4-tolyl-1H-1,2,3-triazol-4-yl)methyl)-1′,2′,3′,4′-tetrahydrospiro[indoline-3,5′-pyrano[2,3-d]pyrimidine]-6′-carbonitrile (**2n**)*

White Solid, M.pt.: 215 °C (decomp), ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.49 (s, 1H, triazolyl-H), 7.69 (s, 2H, $-NH_2$), 7.63 (d, J = 8.24 Hz, 2H, ArH), 7.33 (d, J = 8.24 Hz, 2H, ArH), 7.23–7.19 (m, 2H, ArH), 7.01–6.97 (m, 2H, ArH), 5.13 and 5. 08 (AB system, J = 16.04 Hz, 2H, $-NCH_aH_b$), 3.38 (s, 3H, $-NCH_3$), 3.03 (s, 3H, $-NCH_3$), 2.33 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 181.18, 176.14, 160.72, 158.68, 152.57, 149,89, 142.19, 139.66, 134.64, 133.21, 130.72, 129.14, 124.40, 123.06, 121.60, 120.65, 119.53, 117.83, 109.18, 100.18, 47.66, 36.02, 30.36, 28.47, 21.05; IR (ν_{max} cm^{-1}, KBr): = 3356, 3102, 1709, 1689, 1489; HRMS (ESI) [M^+ + H] calcd. for $C_{27}H_{22}N_8O_4$: 523.1842 found:523.1850 [M^+ + H].

*4.6.15. 7′-Amino-1′,3′-dimethyl-2,2′,4′-trioxo-1-((1-4-methoxyl-1H-1,2,3-triazol-4-yl)methyl)-1′,2′,3′,4′-tetrahydrospiro[indoline-3,5′-pyrano[2,3-d]pyrimidine]-6′-carbonitrile (**2o**)*

White Solid, M.pt.: 205 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.43 (s, 1H, triazolyl-H), 7.68–7.66 (m, 4H, $-NH_2$, ArH), 7.23–7.19 (m, 2H, ArH), 7.08–6.97 (m, 4H, ArH), 5.13 and 5 .03 (AB system, J = 15.4 Hz, 2H, $-NCH_aH_b$), 3.78 (s, 3H, $-OCH_3$), 3.38 (s, 3H, $-NCH_3$), 3.03 (s, 3H, $-NCH_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 175.55, 159.74, 158.92, 158.08, 152.17, 149.61, 142.06, 141.29, 132.41, 129.72, 128.59, 123.85, 122.72, 122.25, 121.55, 121.20, 117.75, 116.80, 114.79, 108.5, 56.87, 55.42, 46.73, 29.25, 27.53; IR (ν_{max} cm^{-1}, KBr): =3356, 3109, 1715, 1686, 1519; HRMS (ESI) [M^+ + H] calcd. for $C_{27}H_{22}N_8O_5$: 539.1791 found: 539.1805 [M^+ + H].

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.03.016.

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