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NEW APPROACH FOR THE SYNTHESIS OF SPIRO INDOLINONE INCORPORATED 1,2,4-TRIAZOLO[1,5-A]QUINOLINE DERIVATIVES AND THEIR PHARMACOLOGICAL SCREENING

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Abstract
An efficient and simple synthetic protocol for one-pot three-component reactions of isatin, enaminones and active methylene furnishing highly functionalised spiro indolinone incorporated 1,2,4-triazolo[1,5-a]quinolines are described as a new conventional synthetic route in the presence of $_{L}$-proline as a catalyst in moderate yields. All the synthesized compounds have been characterized by FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectrometry. Compound **4a-l** were evaluated for their *in vitro* antibacterial bioassay against a cluster of pathogenic strains of bacteria and fungi, *in vitro* antitubercular and antimalarial activity against *Mycobacterium tuberculosis* H37Rv strain and *Plasmodium falciparum* respectively. Compounds **4b** and **4k** exhibited excellent antitubercular and antimalarial potency. The cytotoxicity of the synthesized compounds was tested using a brine shrimp bioassay.

Keywords
Spiro compounds, 1,2,4-triazolo[1,5-a]quinoline, isatin and biological activities.

1. Introduction

After HIV and Cancer, world's one of the most dangerous infectious diseases malaria is transmitted to humans body by the infected female *Anopheles* mosquito.[I] Infection of malaria caused by protozoan parasite of the *Plasmodium* genus viz. *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium malariae*, *Plasmodium vivax*, and *Plasmodium knowlesi*, out of which *P. falciparum* is considered as the most virulent form.[II] Resistance to tuberculosis (TB) drugs is rapidly growing worldwide with huge number of cases of multi-drug resistant TB (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) recorded annually. Both are instigated by *Mycobacterium tuberculosis* (Mtb) strains that are resistant to well-known drugs in the treatment of TB, due to the very poor treatment outcome of MDR-TB and XDR-TB, more efficient treatment is urgently needed.[III]

The pharmacological evaluation of spiro compounds have been motivating field in medicinal chemistry for a long time. Isatin (1*H*-indole-2,3-dione) is a versatile heterocyclic compound with huge possibility of chemical variation at C-3, C-5 and at N-1 position.[IV] The isatin

scaffold demonstrates a wide range of biological and pharmacological activities such as antitumor,[V] antiviral,[VI] anti-HIV,[VII] antifungal,[VIII] anti-Parkinson's disease[IX] and anticonvulsants.[X]

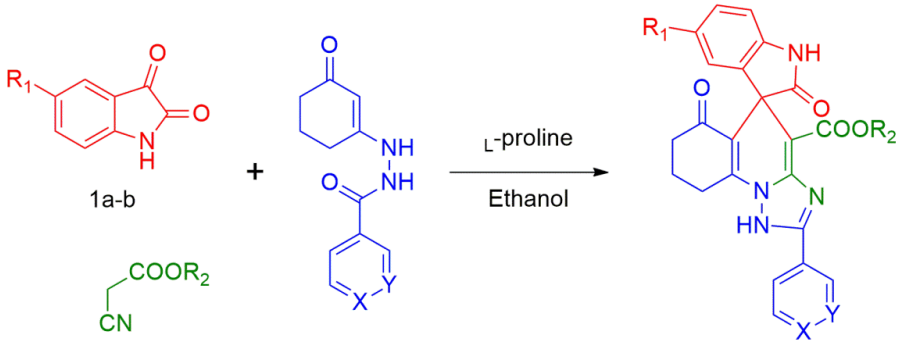
Quinoline is a widespread structural scaffold in numerous marketed drugs such as antibacterial, antimalarial agents, HIV-1 integrase inhibitors,[XI] and other compounds of pharmaceutical interest.[XII] Fused 1,2,4-triazoles are used as antithrombotic, anti-inflammatory, antibacterial, herbicidal, antiproliferative,[XIII-XVII] JAK2 inhibitor,[XVIII] cardiovascular agents.[XIX] Compounds containing the 1,2,4-triazole and quinoline as a fused motifs have been shown to possess anticonvulsant[XX-XXII] and antibacterial[XIV] activity.

Multi-component reactions (MCRs) are essential chemical conversions because of structural variety, usable simplicity and complexity of the molecules. Multi-component reactions have key advances for synthesis of particular products with molecular diversity which have fascinated much awareness as a facial means by synthetic chemists. Therefore, the developments of novel multi–component reactions have been interest of chemists.[XXIII-XXV]

In continuation to our work on quinoline,[XXVI-XXXII] herein we report a synthesis of spiro indolinone incorporated 1,2,4-triazolo[1,5-a]quinoline derivatives by reacting substituted isatin, enaminones and active methylene in the presence of catalytic amounts (5 mol%) of L-proline under ethanol as solvent. This reaction produces spiro derivatives in excellent yield and within a short period of time.

2. Chemistry

The synthetic approach adopted to obtain the targeted isatin incorporated 1,2,4-triazolo[1,5-a]quinoline derivatives is depicted in **Scheme 1**. The starting material *N*'-(3-oxocyclohexyl) isonicotinohydrazide **(3a)** and *N*'-(3-oxocyclohexyl) nicotinohydrazide **(3b)** was prepared by refluxing different hydrazide and 1,3-cyclohexadion using acetic acid as catalyst under aqueous condition.[XXXIII] The targeted compounds alkyl 5'-(un)substituted-2',6-dioxo-2-substituted-6,7,8,9-tetrahydro-1H-spiro[1,2,4-triazolo[1,5-a]quinoline-5,3'-indoline]-4-carboxylate **4a-l** were synthesized by cyclocondensation reaction of isatin **1a-b**, methyl 2-cyanoacetate **2a** or ethyl 2-cyanoacetate **2b** or isopropyl 2-cyanoacetate **2c** and enaminone **3a-b** in absolute ethanol using L-proline as an amphoteric catalyst[27].



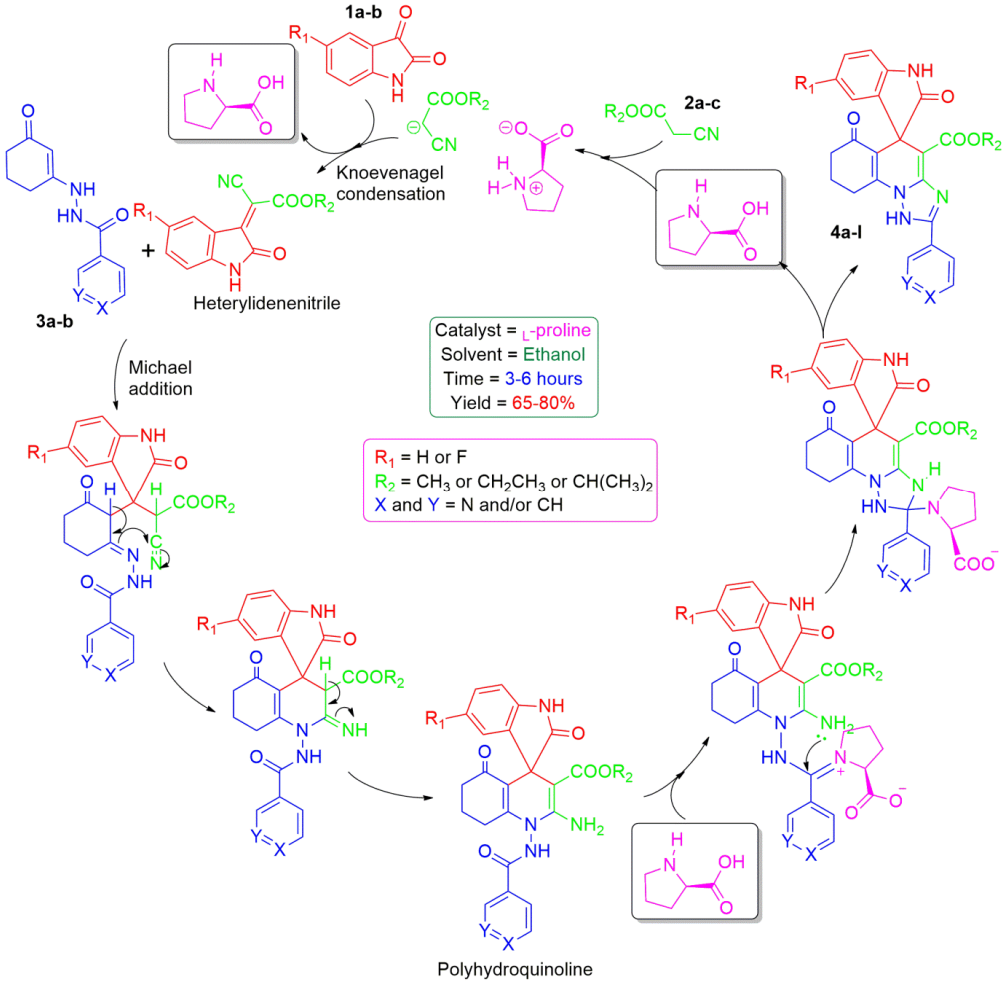
Entry	R_1	R_2	X	Y	Time (h)	Yield[a] (%)
4a	H	CH_3	N	CH	3.5	76
4b	H	CH_2CH_3	N	CH	3.5	80
4c	H	$CH(CH_3)_2$	N	CH	4.0	70
4d	H	CH_3	CH	N	4.5	78
4e	H	CH_2CH_3	CH	N	4.0	77
4f	H	$CH(CH_3)_2$	CH	N	4.5	68
4g	F	CH_3	N	CH	5.0	79

4h	F	CH_2CH_3	N	CH	5.5	72
4i	F	$CH(CH_3)_2$	N	CH	4.5	72
4j	F	CH_3	CH	N	4.0	70
4k	F	CH_2CH_3	CH	N	5.5	68
4l	F	$CH(CH_3)_2$	CH	N	6.0	65

[a] Isolated yields

Scheme 1 Synthesis of alkyl 5'-(un)substituted-2',6-dioxo-2-substituted-6,7,8,9-tetrahydro-1H-spiro[1,2,4-triazolo[1,5-a]quinoline-5,3'-indoline]-4-carboxylate **4a-l**.

A plausible mechanism for the reaction is outlined in **Scheme 2**. The reaction occurs in one pot primary construction of the heterylidenenitrile from the knoevenagel condensation between substituted isatin **1a-b** and activemethylene **2a-c** by loss of water molecule having the electron-poor ylidenic C=C bond, michael addition of enaminones **3a-b** to the ylidenic bond to form an acyclic intermediate which undergoes cyclization by nucleophilic attack of the -NH group on the electron deficient cyano carbon, followed by tautomerisation to form polyhydroquinoline intermediate. After intra-molecular keto-amine cyclization reaction of polyhydroquinoline intermediate to give 1,2,4-triazolo[1,5-a]quinoline derivative **4a–l**.



Scheme 2. Plausible mechanism for the synthesis of isatin incorporated 1,2,4-triazolo[1,5-a]quinoline.

3. Results and discussion

3.1. Analytical results

The structures of the synthesized compounds were confirmed by ^{1}H and ^{13}C NMR, FT-IR, mass spectrometry and elemental analysis. The IR spectra of compounds **4a-l** exhibited characteristic absorption bands for all the compounds were observed in the range of 3386-3396 and 3210-3286 cm^{-1} corresponding to asymmetrical and symmetrical stretching of NH group. The absorption band around 2986-3048 cm^{-1} is due to aromatic C-H stretching. The characteristic absorption band in the range of 1694-1699 and 1647-1652 cm^{-1} due to the presence of –C=O stretching. In ^{1}H NMR spectra of compounds **4a-l**, a sharp singlet peak around δ 4.96-5.01 ppm was attributed to -NH proton of 1,2,4-triazole ring. Another singlet peak of –NH proton of isatin was appeared around δ 9.91-9.99 ppm. The data from ^{13}C NMR spectral studies is also in accordance with the suggested structures. Mass spectra of title compounds showed expected molecular ion peak M^{+} corresponding with proposed molecular mass, which confirmed the chemical structures.

3.2 Biological section

3.2.1. Antibacterial activity

The *in vitro* antimicrobial screening of targeted compounds **4a-l** at minimal inhibitory concentration (MIC) were carried out by broth micro dilution method according to National Committee for Clinical Laboratory Standards (NCCLS).[XXXIV] The study of antibacterial screening data (**Table 1**) reveals that all the compounds **4a-l** demonstrates moderate to very good inhibitory activity.

Table 1. *In vitro* antimicrobial activity (MIC, μM) of compounds **4a-l**.

Entry	Gram-positive bacteria			Gram-negative bacteria			Fungi	
	S.P.	C.T.	B.S.	S.T.	V.C.	E.C.	C.A.	A.F.
	MTCC 1936	MTCC 449	MTCC 441	MTCC 98	MTCC 3906	MTCC 443	MTCC 227	MTCC 3008
4a	**217**	**217**	**544**	544	1088	544	2176	2176
4b	**132**	**211**	**211**	**211**	528	**132**	**528**	1056
4c	**205**	**410**	**205**	410	513	**205**	**1026**	>1026
4d	435	**435**	1088	435	**136**	1088	**544**	>2176
4e	**211**	**264**	**422**	264	**211**	1056	**528**	1056
4f	256	**410**	**256**	513	513	410	2052	2052
4g	418	**523**	**418**	523	523	**209**	>2094	2094
4h	1017	1017	**508**	**203**	**127**	406	**1017**	1017
4i	395	**395**	**197**	395	395	247	1978	>1978
4j	418	**418**	**523**	523	523	523	>2094	2094
4k	508	**406**	**406**	**203**	**203**	**127**	>2034	2034
4l	**197**	**197**	**395**	247	395	**197**	1978	>1978
A	286	715	715	286	286	286	n. t. [a]	n. t.
B	154	154	154	154	154	154	n. t.	n. t.
C	150	301	150	75	75	75	n. t.	n. t.
D	31	313	310	31	31	31	n. t.	n. t.
E	n. t.	n. t.	n. t.	n. t.	n. t.	n. t.	107	107
F	n. t.	n. t.	n. t.	n. t.	n. t.	n. t.	1417	283

S.P.: *Streptococcus pneumoniae*, C.T.: *Clostridium tetani*, B.S.: *Bacillus subtilis*, S.T.: *Salmonella typhi*, V.C.: *Vibrio cholera*, E.C.: *Escherichia coli*, C.A.: *Candida albicans*, A.F.: *Aspergillus fumigatus*, MTCC: Microbial Type Culture Collection. A: Ampicillin, B: Chloramphenicol, C: Ciprofloxacin, D: Norfloxacin, E: Nystatin, F: Griseofulvin, [a]n.t.: not tested.

The compound **4b** (132 μM) and **4k** (127 μM) showed maximum activity against *E. coli*, although compound **4d** (136 μM) and **4h** (127 μM) showed maximum activity against *V. cholera*. Majority of the compounds display excellent activity against gram positive bacteria *B. subtilis* and *C. tetani* as compared to ampicillin (715 μM), while compounds **4a** (217 μM), **4c** (205 μM), **4e** (205 μM) and **4l** (197 μM) showed similar potency to that of the standard drugs against *S. pneumoniae*. The compounds **4b** (211 μM), **4h** (203 μM), and **4k** (203 μM) displayed comparatively good activities against *S. typhi*. Compounds **4a** (217 μM), **4b** (211 μM) and **4l** (197 μM) showed maximum potency as that of standard drugs against *C. tetani*. While compounds **4b** (211 μM), **4c** (205 μM) and **4i** (197 μM) showed most potency as that of standard drugs against *B. subtilis*. The compounds **4b** illustrated highest activity in inhibiting gram positive bacteria i.e. 132 μM against *S. pneumoniae*. Remaining other compounds are moderate or less active against all gram positive and gram negative bacteria.

3.2.2. *Antifungal activity*

The result of antifungal study (**Table 1**) of the synthesized quinoline based 1,2,4-triazolo[1,5-a]quinoline derivatives revealed that all the compounds have poor activity against *A. fumigates*. Where as in comparison with standard fungicidal griseofulvin (MIC = 1417 μM), compounds **4b**, **4d**, **4e** contributed excellent antifungal activity against *C. albicans*; While compounds **4c**, **4h** illustrated similar potency against *C. albicans*. Remaining compounds showed weak antifungal potency than nystatin and griseofulvin.

3.2.3. Antituberculosis activity

The encouraging results from the antibacterial activity provoked us to decide screening of the title compounds for their *in vitro* antituberculosis activity against *M. Tuberculosis H37Rv* strain using Lowenstein-Jensen medium (conventional method) as described by Rattan.[XXXV] The bioassay results achieved for the usefulness of all the synthesized analogues against *M. tuberculosis* H37Rv is summarized in **Table 2**. Rifampicin and Isoniazid were used as the reference drugs. The outcome of the result revealed that, compounds **4b** (R_1 = H, R_2 = CH_3, R_3 = H), **4e** (R_1 = H, R_2 = CH_3, R_3 = H), **4h** (R_1 = H, R_2 = CH_3, R_3 = H) and **4k** (R_1 = H, R_2 = CH_3, R_3 = H) were found to possess outstanding activity (i.e. 98%, 98%, 95% and 99% at 250 μg/mL respectively) against *M. tuberculosis* H37Rv. The compound **4k** shows high potency against *M. tuberculosis* i.e. MIC = 50 μM as compared to rifampicin i.e. MIC = 48 μM at 99% inhibition. Compound **4b** MIC = 105 μM and **4e** MIC = 211 μM exhibited better inhibition of 98%. Also, compounds **4h** MIC = 203 μM displayed moderate inhibition of 95% (**Table 3**). Compound **4k** was emerging out as the most potent member of the series and opens up a new door to optimize this series for new class of antituberculer agent.

Table 2. *In vitro* antituberculosis activity (% Inhibition) of **4a-l** against *M. tuberculosis* H37Rv (at concentration 250 μg/mL).

Entry	% Inhibition	Entry	% Inhibition
4a	38%	**4h**	**95%**
4b	**98%**	4i	87%
4c	88%	4j	66%
4d	60%	**4k**	**99%**
4e	**98%**	4l	80%
4f	61%	Isoniazid	99%
4g	56%	Rifampicin	98%

Table 3. *In vitro* antituberculosis activity of title compounds exhibiting higher % inhibition against *M. tuberculosis* H37Rv (MICs, μM).

Entry	% Inhibition	MIC (μM)
4b	98	105
4e	98	211
4h	95	203
4k	99	50
Rifampicin	98	48
Isoniazid	99	1

3.2.4. Antimalarial activity

All the synthesized compounds **4a-l** was evaluated for their *in vitro* antimalarial activity against chloroquine and quinine sensitive strain of *P. falciparum*. All experiments were performed in duplicate and a mean value of IC_{50} is mentioned in **Table 4**.

Table 4. *In vitro* antimalarial activity of compounds **4a-l**.

Entry	IC_{50} (μM)	Entry	IC_{50} (μM)
4a	4.026	4h	1.790
4b	**0.295**	4i	2.512
4c	**0.246**	4j	1.340
4d	2.873	**4k**	**0.122**
4e	**0.422**	**4l**	**0.454**
4f	3.058	**Chloroquine**	0.062
4g	4.586	**Quinine**	0.826

The compounds **4b**, **4c**, **4e**, **4k** and **4l** were found to have IC_{50} in the range of 0.122 to 0.454 μM against *P. falciparum* strain. These compounds displayed excellent activity against *P. falciparum* strain as compared to quinine IC_{50} = 0.826 μM. Furthermore compounds **4k** was found to possess moderate activity i.e. IC_{50} = 0.122 μM as compared to chloroquine. Remaining all other compounds was found to be less active against *P. falciparum* strain.[XXXVI-XXXVIII]

3.2.5. Cytotoxicity (brine shrimp lethality bioassay)

The *in vitro* lethality test was done using brine shrimp eggs i.e. Artemia cysts. The Brine shrimp lethality bioassay is well thought out as a useful tool for preliminary toxicity assessment of bioactive compounds. Active compounds were again screened for their cytotoxicity by using the protocol of Meyer *et al*.[XXXIX] The LC_{50} values obtained for the six compounds those exhibited highest antitubercular activity and anti malarial activity are shown in **Table 5**. These six compounds were found to be less toxic when compared with the standard drug etoposide. Compounds **4k** (LC_{50} = 76.85 μM), **4h** (LC_{50} = 58.96 μM) and **4b** (LC_{50} = 60.27 μM) were exhibited comparatively less toxicity; Compounds **4e** (LC_{50} = 44.23 μM), **4c** (LC_{50} = 30.62 μM) and **4l** (LC_{50} = 28.58 μM) were possessed moderate toxicity as compared to all the tested compounds.

Table 5 Effect of compounds on brine shrimp lethality bioassay.

Entry	Concentration (μg/mL)	Log (Conc.)	No. of nauplii taken	No. of nauplii dead	% of Mortality	LC_{50} (μg/mL)	LC_{50} (μM)
4b	5	0.699	10	0	0	28.54	60.27
	10	1.000	10	2	20		
	20	1.301	10	3	30		
	30	1.477	10	4	40		
	40	1.602	10	6	60		

	50	1.699	10	8	80		
4c	5	0.699	10	2	20	14.92	30.62
	10	1.000	10	3	30		
	20	1.301	10	5	50		
	30	1.477	10	7	70		
	40	1.602	10	9	90		
	50	1.699	10	10	100		
4e	5	0.699	10	1	10	20.93	44.23
	10	1.000	10	3	30		
	20	1.301	10	4	40		
	30	1.477	10	6	60		
	40	1.602	10	7	70		
	50	1.699	10	8	80		
4h	5	0.699	10	0	0	28.98	58.96
	10	1.000	10	1	10		
	20	1.301	10	2	20		
	30	1.477	10	4	40		
	40	1.602	10	6	60		
	50	1.699	10	9	90		
4k	5	0.699	10	0	0	37.77	76.85
	10	1.000	10	1	10		
	20	1.301	10	2	20		
	30	1.477	10	4	40		
	40	1.602	10	5	50		
	50	1.699	10	7	70		
4l	5	0.699	10	2	20	14.45	28.58
	10	1.000	10	4	40		
	20	1.301	10	5	50		
	30	1.477	10	7	70		
	40	1.602	10	8	80		
	50	1.699	10	10	100		
Etoposide	-	-	-	-	-	7.46	12.67

3.3. Structure-activity relationship (SAR)

The results of the biological broadcast showed that the activity was considerably affected by introducing fluorinated isatin at C-4 position and nicotinamide nucleus at N-1 position on polyhydroquinoline scaffold (Fig.1).

We seemed that the fluorine group existing at R_1 position in isatin moiety at C-4 position, ethyl group at R_2 position and nicotinamide group at N-1 position in polyhydroquinoline nucleus exhibited excellent antimalarial activity against *P. falciparum* strain as compared to quinine as well as highest % inhibition and MIC against *M. tuberculosis* as compared to rifampicin. Also it showed improve antibacterial activity against *E. coli*. Without any substitution at R_1 position and ethyl group at R_2 position revealed highest activity against all gram positive bacteria and also active against *M. tuberculosis* H37Rv and *P. falciparum* strain, similarly it gives moderate active against *C. albicans*. The replacement of hydrogen with electron withdrawing fluoro groups at R_1 position increased antimalarial activity. It can be concluded that hydrogen and fluoro at R_1, nicotinamide and isonicotinamide groups at N-1 position and ethyl group at R_2 position of polyhydroquinoline ring are observed to be essentially responsible for deviation in biological potency. The isopropyl group present at R_2 position in polyhydroquinoline ring demonstrate higher cytotoxicity against brine shrimp

eggs i.e. Artemia cysts. But hydrogen and fluoro group existing at R_1 position and ethyl group at R_2 position displayed inferior cytotoxicity against brine shrimp eggs i.e. Artemia cysts.

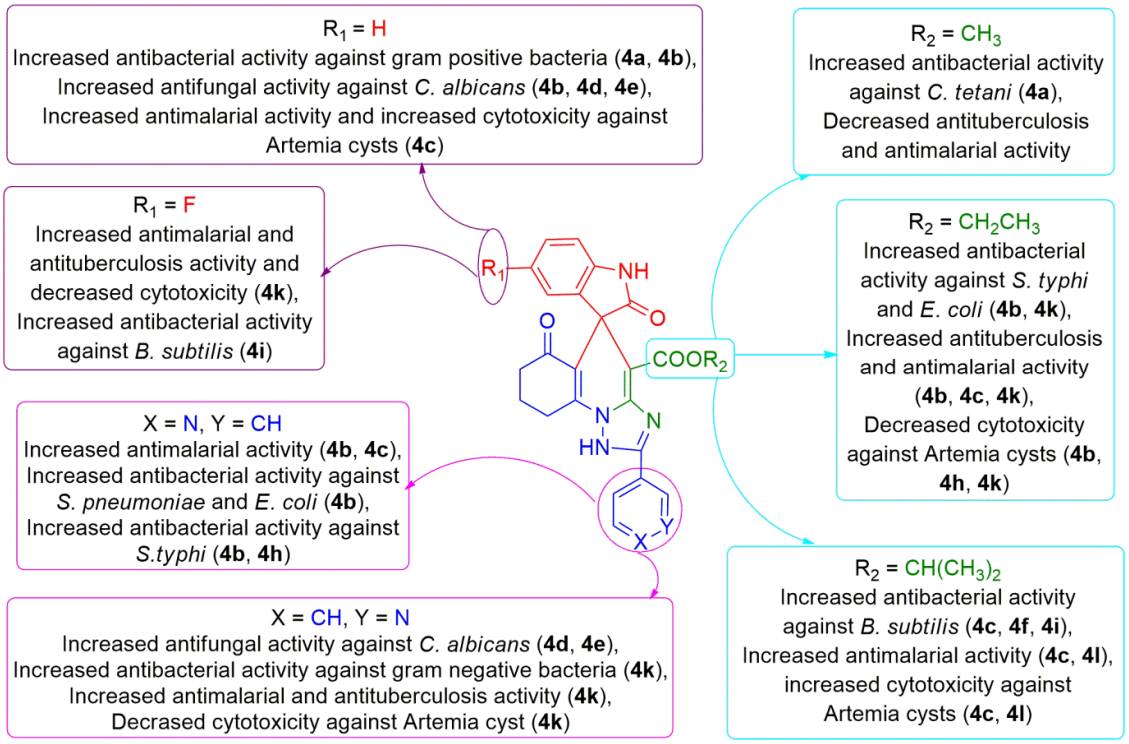


Figure 1: Structure–activity relationships for antimicrobial, antituberculosis, antimalarial and cytotoxicity activity of the synthesized compounds **4a-l**.

4. Conclusion

We have developed an eco-friendly route for the spiro indolinone incorporated 1,2,4-triazolo[1,5-a]quinoline derivatives **4a-l** *via* three-component cyclocondensation of isatin **1a-b**, activemethylene **2a-c** and enaminones **3a-b** using L-proline as the catalyst and ethanol as a solvent. The method utilizes readily available reactant, inexpensive catalyst and affords spiro derivatives in high yields. Furthermore, this work contributes to validate the choice of the isatin incorporated 1,2,4-triazolo[1,5-a]quinoline motifs, as a model, useful to design new antimicrobial, antitubercular and antimalarial compounds. This synthetic approach permits the insertion of potent bioactive nuclei in a single scaffold through an easy way. Subsequently, such type of compound would represent a productive template for further development of more biologically active scaffold and that deserve further investigation and derivatization in order to discover the scope and limitation of its biological activities.

5. Experimental section

5.1. Chemistry

All reactions were performed with commercially available reagents. They were used without further purification. The solvents used were of analytical grade. All reactions were monitored by thin-layer chromatography (TLC) on aluminium plates coated with silica gel 60 F_{254}, 0.25 mm thickness (Merck). Detection of the components was made by exposure to iodine vapours or UV light. Melting points were taken in melting point apparatus µThermoCal$_{10}$ (Analab Scientific Pvt. Ltd, India) and are uncorrected. The IR spectra were recorded on a FTIR MB 3000 spectrophotometer (ABB Bomem Inc., Canada/Agaram Industries, Chennai) using Zn-

Se Optics (490-8500 cm^{-1}) and only the characteristic peaks are reported in cm^{-1}. Mass spectra were recorded on Shimadzu LCMS 2010 spectrometer (Shimadzu, Tokyo, Japan) purchased under PURSE program of DST at Sardar Patel University, Vallabh Vidyanagar. ^{1}H and ^{13}C Nuclear Magnetic Resonance spectra were recorded in DMSO-d_6 on a Bruker Avance 400F (MHz) spectrometer (Bruker Scientific Corporation Ltd., Switzerland) using residual solvent signal as an internal standard at 400 MHz and 100 MHz respectively. Chemical shifts are reported in parts per million (ppm). Splitting patterns were designated as follows: s, singlet; d, doublet; dd, doublet of doublet and m, multiplet. The elemental analysis was carried out by using Perkin-Elmer 2400 series-II elemental analyzer (Perkin-Elmer, USA) and all compounds are within ± 0.4% of the theoretical compositions.

*5.1.1. Synthesis of the N'-(3-oxocyclohexyl) isonicotinohydrazide **(3a)** and N'-(3-oxocyclohexyl) nicotinohydrazide **(3b)** (substituted enaminones)*

Cyclohexane-1, 3-dione (10 mmol), isoniazid or nicotinohydrazide (10 mmol), acetic acid (5 mol %) and methanol (10 mL) were charged in a 50mL round bottom flask with mechanical stirrer and condenser. The reaction mixture was refluxed for 2-3 h. After the completion of reaction (checked by TLC), the substituted enaminones (**3a-b**) were filtered and washed with deionised water. The further purification was carried out by leaching in equal volume ratio of water and methanol (10:10 ml) to obtain the pure solid sample.

*5.1.2. General procedure for the synthesis of alkyl 5'-(un)substituted-2',6-dioxo-2-substituted-6,7,8,9-tetrahydro-1H-spiro[1,2,4-triazolo[1,5-a]quinoline-5,3'-indoline]-4-carboxylate **4a-l***

A 50 mL round bottom flask, fitted with a reflux condenser, was charged with a mixture of 5-(un)substituted isatin **1a-b** (1 mmol), methyl 2-cyanoacetate or ethyl 2-cyanoacetate (1 mmol) or isopropyl 2-cyanoacetate **2a-c**, substituted enaminones **3a-b** (1 mmol), and catalytic amount of L-proline (0.1 mmol) in ethanol (10 mL). The reaction mixture was refluxed for an appropriate time period till the completion of the reaction as indicated by TLC (Table 1). After the completion of reaction, the reaction mixture was cooled to room temperature and stirred magnetically for further 10 min. The solid mass separated was collected by filtration, washed well with ethanol (10 mL) and crystallized from hot chloroform: methanol (1:1) mixture. The physicochemical and spectroscopic characterization data of the synthesized compounds **4a-l** are given below.

*5.1.2.1. methyl 2',6-dioxo-2-(pyridin-4-yl)-6,7,8,9-tetrahydro-1H-spiro[[1,2,4]triazolo[1,5-a]quinoline-5,3'-indoline]-4-carboxylate **4a***

Yield 76%; m.p. 225–227°C; IR (ν_{max}, cm^{-1}): 3390, 3283, 3039, 1694, 1649; ^{1}H NMR (400 MHz, DMSO-d_6): 1.62-2.15 (m, 6H), 3.23 (s, 3H), 5.01 (s, 1H), 6.62 (d, 1H, J = 5.6 Hz), 6.77 (t, 1H, J = 7.2 Hz), 6.98-7.02 (m, 1H), 7.21 (s, 1H), 7.85-7.95 (m, 2H), 8.80-8.87 (m, 2H), 9.93 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 20.5, 25.3, 37.1, 49.0, 52.2, 78.4, 108.1, 114.7, 120.2, 121.9, 127.6, 138.3, 138.8, 144.1, 150.8, 153.1, 153.9, 165.7, 168.1, 181.7, 193.7; ESI-MS (m/z): Calcd. 441.44, found 441.71 (M^+); Anal. Calcd. (%) for $C_{24}H_{19}N_5O_4$: C, 65.30; H, 4.34; N, 15.86; Found: C, 65.02; H, 4.26; N, 15.98.

*5.1.2.2. ethyl 2',6-dioxo-2-(pyridin-4-yl)-6,7,8,9-tetrahydro-1H-spiro[[1,2,4]triazolo[1,5-a]quinoline-5,3'-indoline]-4-carboxylate **4b***

Yield 80%; m.p. 238-240°C; IR (ν_{max}, cm^{-1}): 3392, 3285, 3041, 1698, 1650; ^{1}H NMR (400 MHz, DMSO-d_6): 0.84 (t, 3H, J = 6.8 Hz), 1.64-2.67 (m, 6H), 3.69-3.72 (m, 2H), 4.99 (s, 1H), 6.62 (d, 1H, J = 7.6 Hz), 6.77 (t, 1H, J = 7.6 Hz), 6.99-7.03 (m, 1H), 7.18 (s, 1H), 7.94-7.95 (m, 2H), 8.85-8.87 (m, 2H), 9.93 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 13.5, 20.6, 36.9, 49.3, 58.7, 78.3, 108.3, 114.5, 120.5, 121.9, 127.4, 138.6, 138.9, 144.3, 150.7, 153.1, 153.6, 165.5, 168.4, 181.7, 193.9; ESI-MS (m/z): Calcd. 455.47, found 455.68 (M^+); Anal. Calcd. (%) for $C_{25}H_{21}N_5O_4$: C, 65.93; H, 4.65; N, 15.38; Found: C, 65.84; H, 4.72; N, 15.22.

5.1.2.3. isopropyl 2',6-dioxo-2-(pyridin-4-yl)-6,7,8,9-tetrahydro-1H-spiro[[1,2,4]triazolo[1,5-a]quinoline-5,3'-indoline]-4-carboxylate ***4c***

Yield 70%; m.p. 232-234°C; IR (ν_{max}, cm^{-1}): 3394, 3279, 2986, 1697, 1648; ^{1}H NMR (400 MHz, DMSO-d_6): 0.59 (d, 3H, J = 6.0 Hz), 1.04 (d, 3H, J = 6.4 Hz), 1.76-2.68 (m, 6H), 4.62 (t, 1H, J = 6.0 Hz), 4.99 (s, 1H), 6.62 (d, 1H, J = 7.6 Hz), 6.77 (d, 1H, J = 7.2 Hz), 6.99-7.03 (m, 1H), 7.16 (d, 1H, J = 6.8 Hz), 7.94-7.96 (m, 2H), 8.86-8.87 (m, 2H), 9.91 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 20.7, 21.1, 21.5, 25.2, 37.2, 49.1, 66.0, 78.8, 108.0, 114.6, 120.6, 122.2, 127.1, 138.2, 138.7, 143.9, 150.9, 153.2, 153.7, 165.6, 168.4, 181.9, 193.9; ESI-MS (m/z): Calcd. 469.49, found 469.97 (M^+); Anal. Calcd. (%) for $C_{26}H_{23}N_5O_4$: C, 66.51; H, 4.94; N, 14.92; Found: C, 66.72; H, 5.12; N, 14.69.

5.1.2.4. methyl 2',6-dioxo-2-(pyridin-3-yl)-6,7,8,9-tetrahydro-1H-spiro[[1,2,4]triazolo[1,5-a]quinoline-5,3'-indoline]-4-carboxylate ***4d***

Yield 78%; m.p. 248-250°C; IR (ν_{max}, cm^{-1}): 3391, 3285, 3044, 1696, 1650; ^{1}H NMR (400 MHz, DMSO-d_6): 1.81-2.70 (m, 6H), 3.24 (s, 3H), 4.97 (s, 1H), 6.64 (d, 1H, J = 7.6 Hz), 6.78 (t, 1H, J = 7.6 Hz), 6.98-7.02 (m, 1H), 7.23 (d, 1H, J = 7.2 Hz), 7.64 (dd, 1H, J = 4.8 Hz, J = 7.6 Hz), 8.40 (d, 1H, J = 8.0 Hz), 8.84 (d, 1H, J = 3.6 Hz), 9.22 (s, 1H), 9.93 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 20.6, 25.1, 37.3, 50.5, 78.6, 108.2, 113.4, 114.8, 121.1, 122.5, 127.2, 136.6, 139.4, 149.2, 153.6, 153.9, 154.5, 156.8, 165.5, 168.3, 181.8, 193.8; ESI-MS (m/z): Calcd. 441.44, found 441.59 (M^+); Anal. Calcd. (%) for $C_{24}H_{19}N_5O_4$: C, 65.30; H, 4.34; N, 15.86; Found: C, 65.02; H, 4.62; N, 15.71.

5.1.2.5. ethyl 2',6-dioxo-2-(pyridin-3-yl)-6,7,8,9-tetrahydro-1H-spiro[[1,2,4]triazolo[1,5-a]quinoline-5,3'-indoline]-4-carboxylate ***4e***

Yield 77%; m.p. 237-240°C; IR (ν_{max}, cm^{-1}): 3393, 3286, 3047, 1694, 1651; ^{1}H NMR (400 MHz, DMSO-d_6): 0.85 (t, 3H, J = 6.8 Hz), 1.74-2.65 (m, 6H), 3.71 (t, 2H, J = 6.8 Hz), 5.00 (s, 1H), 6.62 (d, 1H, J = 7.6 Hz), 6.78 (t, 1H, J = 7.2 Hz), 6.99-7.03 (m, 1H), 7.20 (d, 1H, J = 7.2 Hz), 7.64 (dd, 1H, J = 4.8 Hz, J = 8.0 Hz), 8.39 (d, 1H, J = 8.0 Hz), 8.84 (dd, 1H, J = 1.6 Hz, J = 4.8 Hz), 9.22 (s, 1H), 9.92 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 13.6, 20.6, 37.2, 49.4, 58.9, 78.2, 108.1, 111.3, 113.5, 114.5, 124.3, 127.2, 136.2, 139.1, 140.1, 149.3, 153.4, 154.5, 156.9, 165.4, 168.4, 181.2, 193.7; ESI-MS (m/z): Calcd. 455.47, found 455.81 (M^+); Anal. Calcd. (%) for $C_{25}H_{21}N_5O_4$: C, 65.93; H, 4.65; N, 15.38; Found: C, 65.78; H, 4.88; N, 15.61.

5.1.2.6. isopropyl 2',6-dioxo-2-(pyridin-3-yl)-6,7,8,9-tetrahydro-1H-spiro[[1,2,4]triazolo[1,5-a]quinoline-5,3'-indoline]-4-carboxylate ***4f***

Yield 68%; m.p. 229-232°C; IR (ν_{max}, cm^{-1}): 3396, 3276, 2986, 1698, 1647; ^{1}H NMR (400 MHz, DMSO-d_6): 0.59 (d, 3H, J = 6.0 Hz), 1.04 (d, 3H, J = 6.4 Hz), 1.80-2.69 (m, 6H), 4.62 (t, 1H, J = 6.4 Hz), 4.98 (s, 1H), 6.62 (d, 1H, J = 7.6 Hz), 6.77 (t, 1H, J = 7.2 Hz), 7.01 (t, 1H, J = 7.6 Hz), 7.17 (d, 1H, J = 7.2 Hz), 7.64 (dd, 1H, J = 5.2 Hz, J = 8.0 Hz), 8.39 (d, 1H, J = 8.0 Hz), 8.84 (d, 1H, J = 4.0 Hz), 9.21 (s, 1H), 9.91 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 20.5, 21.4, 21.7, 25.3, 37.2, 49.5, 66.2, 79.1, 108.5, 114.7, 124.1, 127.1, 136.1, 139.4, 140.2, 149.2, 153.6, 154.3, 156.7, 165.2, 168.5, 181.7, 193.7; ESI-MS (m/z): Calcd. 469.49, found 470.16 (M^+); Anal. Calcd. (%) for $C_{26}H_{23}N_5O_4$: C, 66.51; H, 4.94; N, 14.92; Found: C, 66.85; H, 4.81; N, 14.76.

5.1.2.7. methyl 5'-fluoro-2',6-dioxo-2-(pyridin-4-yl)-6,7,8,9-tetrahydro-1H-spiro[[1,2,4]triazolo[1,5-a]quinoline-5,3'-indoline]-4-carboxylate ***4g***

Yield 79%; m.p. 235-237°C; IR (ν_{max}, cm^{-1}): 3386, 3210, 3048, 1695, 1650; ^{1}H NMR (400 MHz, DMSO-d_6): 1.55 -2.13 (m, 6H), 3.26 (s, 3H), 4.98 (s, 1H), 6.61 (dd, 1H, J = 4.4 Hz, J = 8.4 Hz), 6.70 (dd, 1H, J = 4.0 Hz, J = 8.4 Hz), 6.80-6.85 (m, 1H), 6.94-6.96 (m, 1H), 7.82-7.96 (m, 1H), 8.78-8.87 (m, 2H), 9.99 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 20.8, 25.3, 37.2, 49.2, 52.4, 78.5, 108.2, 114.5, 124.0, 127.3, 136.2, 139.0, 144.2, 150.9, 153.5, 154.0, 156.7, 165.6, 168.7, 181.8, 193.8; ESI-MS (m/z): Calcd. 459.43, found 459.82 (M^+); Anal.

Calcd. (%) for $C_{24}H_{18}FN_5O_4$: C, 62.74; H, 3.95; N, 15.24; Found: C, 62.58; H, 3.83; N, 15.44.

5.1.2.8. ethyl 5'-fluoro-2',6-dioxo-2-(pyridin-4-yl)-6,7,8,9-tetrahydro-1H-spiro[[1,2,4]triazolo[1,5-a]quinoline-5,3'-indoline]-4-carboxylate ***4h***

Yield 72%; m.p. 232-234°C; IR (ν_{max}, cm^{-1}): 3394, 3286, 3047, 1699, 1652; ^{1}H NMR (400 MHz, DMSO-d_6): 1.06 (t, 3H, J = 6.8 Hz), 1.78-2.12 (m, 6H), 3.43-3.46 (m, 2H), 4.96 (s, 1H), 6.71 (dd, 1H, J = 4.4 Hz, J = 8.4 Hz), 6.93-6.97 (m, 1H), 7.42 (dd, 1H, J = 2.4 Hz, J = 8.0 Hz), 7.83 (dd, 2H, J = 1.6 Hz, J = 4.4 Hz), 8.80 (d, 2H, J = 6.0 Hz), 9.99 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 13.7, 20.8, 37.2, 49.3, 58.7, 78.1, 108.3, 111.1, 113.8, 114.7, 124.5, 127.3, 136.4, 139.5, 143.3, 150.5, 153.2, 154.6, 156.8, 165.5, 168.9, 182.2, 193.6; ESI-MS (m/z): Calcd. 473.46, found 473.75 (M^+); Anal. Calcd. (%) for $C_{25}H_{20}FN_5O_4$: C, 63.42; H, 4.26; N, 14.79; Found: C, 63.66; H, 3.98; N, 14.83.

5.1.2.9. isopropyl 5'-fluoro-2',6-dioxo-2-(pyridin-4-yl)-6,7,8,9-tetrahydro-1H-spiro[[1,2,4]triazolo[1,5-a]quinoline-5,3'-indoline]-4-carboxylate ***4i***

Yield 72%; m.p. 239-241°C; IR (ν_{max}, cm^{-1}): 3391, 3279, 2992, 1696, 1647; ^{1}H NMR (400 MHz, DMSO-d_6): 0.64 (d, 3H, J = 6.4 Hz), 1.05 (d, 3H, J = 6.0 Hz), 1.78-2.12 (m, 6H), 4.65 (t, 1H, J = 6.0 Hz), 5.01 (s, 1H), 6.59 (dd, 1H, J = 4.4 Hz, J = 8.4 Hz), 6.82-6.98 (m, 2H), 7.95-7.97 (m, 2H), 8.86 (d, 2H, J = 5.6 Hz), 9.97 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 20.7, 21.5, 21.7, 25.3, 37.0, 49.9, 66.7, 79.0, 108.1, 114.5, 124.3, 127.1, 136.3, 139.2, 143.3, 150.1, 152.9, 154.1, 156.7, 165.4, 168.9, 182.0, 193.6; ESI-MS (m/z): Calcd. 487.48, found 487.64 (M^+); Anal. Calcd. (%) for $C_{26}H_{22}FN_5O_4$: C, 64.06; H, 4.55; N, 14.37; Found: C, 64.29; H, 4.32; N, 14.49.

5.1.2.10. methyl 5'-fluoro-2',6-dioxo-2-(pyridin-3-yl)-6,7,8,9-tetrahydro-1H-spiro[[1,2,4]triazolo[1,5-a]quinoline-5,3'-indoline]-4-carboxylate ***4j***

Yield 70%; m.p. 230-232°C; IR (ν_{max}, cm^{-1}): 3387, 3269, 2992, 1696, 1647; ^{1}H NMR (400 MHz, DMSO-d_6): 1.79-2.75 (m, 6H), 3.26 (s, 3H), 4.98 (s, 1H), 6.61 (dd, 1H, J = 4.4 Hz, J = 8.4 Hz), 6.80-6.83 (m, 1H), 7.05 (dd, 1H, J = 2.8 Hz, J = 8.4 Hz), 7.65 (dd, 1H, J = 4.8 Hz, J = 7.6 Hz), 8.40 (d, 1H, J = 8.0 Hz), 8.85 (d, 1H, J = 3.6 Hz), 9.23 (d, 1H, J = 1.2 Hz), 9.98 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 20.7, 25.2, 37.0, 50.2, 78.4, 108.4, 113.5, 114.5, 124.1, 127.4, 136.4, 139.7, 149.5, 153.4, 153.6, 154.4, 156.9, 165.8, 168.8, 182.0, 193.9; ESI-MS (m/z): Calcd. 459.43, found 459.94 (M^+); Anal. Calcd. (%) for $C_{24}H_{18}FN_5O_4$: C, 62.74; H, 3.95; N, 15.24; Found: C, 62.53; H, 4.13; N, 15.11.

5.1.2.11. ethyl 5'-fluoro-2',6-dioxo-2-(pyridin-3-yl)-6,7,8,9-tetrahydro-1H-spiro[[1,2,4]triazolo[1,5-a]quinoline-5,3'-indoline]-4-carboxylate ***4k***

Yield 68%; m.p. 234-236°C; IR (ν_{max}, cm^{-1}): 3392, 3285, 3048, 1695, 1650; ^{1}H NMR (400 MHz, DMSO-d_6): 0.87 (t, 3H, J = 7.2 Hz), 1.79-2.70 (m, 6H), 3.72-3.75 (m, 2H), 4.98 (s, 1H), 6.60 (dd, 1H, J = 4.4 Hz, J = 8.4 Hz), 6.81-6.86 (m, 1H), 7.01 (dd, 1H, J = 2.4 Hz, J = 8.0 Hz), 7.64 (dd, 1H, J = 4.8 Hz, J = 7.6 Hz), 8.40 (d, 1H, J = 8.0 Hz), 8.85 (d, 1H, J = 3.6 Hz), 9.22 (d, 1H, J = 1.2 Hz), 9.98 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 13.6, 20.7, 25.3, 37.1, 49.5, 59.0, 78.3, 108.4, 111.0, 113.4, 114.4, 124.1, 127.4, 136.4, 139.5, 140.0, 149.5, 153.6, 154.4, 156.9, 159.2, 165.8, 168.6, 181.6, 193.9; ESI-MS (m/z): Calcd. 473.46, found 473.88 (M^+); Anal. Calcd. (%) for $C_{25}H_{20}FN_5O_4$: C, 63.42; H, 4.26; N, 14.79; Found: C, 63.68; H, 4.28; N, 14.57.

5.1.2.12. isopropyl 5'-fluoro-2',6-dioxo-2-(pyridin-3-yl)-6,7,8,9-tetrahydro-1H-spiro[[1,2,4]triazolo[1,5-a]quinoline-5,3'-indoline]-4-carboxylate ***4l***

Yield 65%; m.p. 226-228°C; IR (ν_{max}, cm^{-1}): 3386, 3210, 3047, 1697, 1651; ^{1}H NMR (400 MHz, DMSO-d_6): 0.65 (d, 3H, J = 6.4 Hz), 1.05 (d, 3H, J = 6.4 Hz), 1.78-2.69 (m, 6H), 4.64-4.67 (m, 1H), 4.96 (s, 1H), 6.59 (dd, 1H, J = 4.4 Hz, J = 8.4 Hz), 6.84-6.99 (m, 2H), 7.64-7.66 (m, 1H), 8.39 (d, 1H, J = 8.0 Hz), 8.85 (d, 1H, J = 3.2 Hz), 9.22 (d, 1H, J = 2.0 Hz), 9.96

(s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 20.6, 21.3, 25.2, 37.3, 49.8, 66.4, 79.0, 108.3, 113.6, 114.6, 124.1, 127.4, 136.5, 139.7, 140.3, 149.3, 153.7, 154.4, 157.1, 159.0, 165.7, 168.7, 181.9, 193.8; ESI-MS (m/z): Calcd. 487.48, found 487.79 (M^+); Anal. Calcd. (%) for $C_{26}H_{22}FN_5O_4$: C, 64.06; H, 4.55; N, 14.37; Found: C, 64.31; H, 4.29; N, 14.55.

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