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**Synthesis, *in-vitro* biological evaluation, and molecular docking study of novel spiro- β -
lactam-isatin hybrids**

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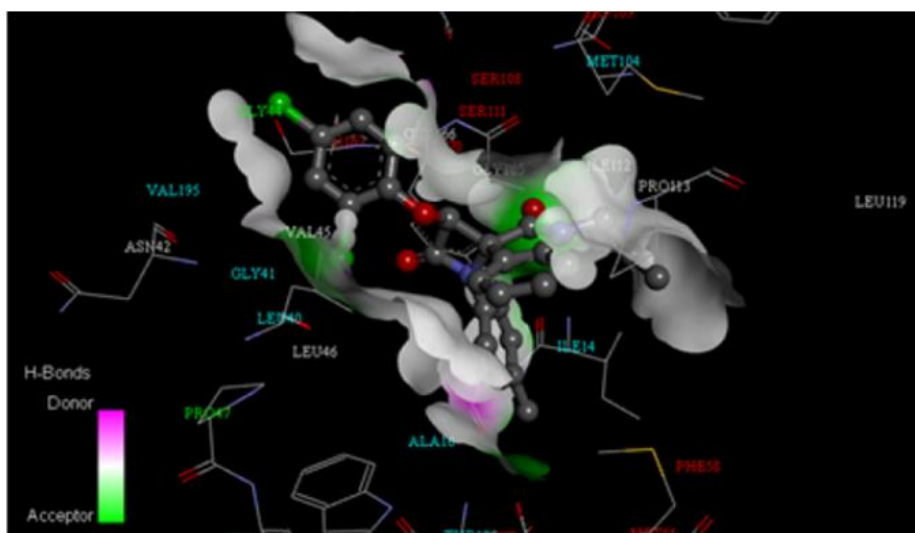
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Abstract

In our ongoing search for bioactive compounds, a class of novel spiro- β -lactam isatin hybrids has been synthesized through a [2+2] cycloaddition reaction from 1-allyl-3-(arylimino)indolin-2-one, ketenes and various aryloxy acetic acids. The formation of all cycloadducts was confirmed by FTIR, ^1H NMR, ^{13}C NMR, and mass spectroscopy as well as elemental analyses. The new β -lactams were subsequently evaluated for their biological activities demonstrating moderate to good activities against *P. falciparum* K1 strain. Among them, **4b** and **4e** lead to the best results with IC_{50} of 5.04 and 7.18 μM , respectively. The molecular docking simulation of **4b** with *P. falciparum* dihydrofolate reductase enzyme (PfDHFR) binding site presented several important intermolecular interactions. All the synthesized β -lactams were also evaluated for their antimicrobial activities against both Gram-positive (*S. aureus* ATCC 25923) and Gram-negative bacteria (*E. coli* ATCC 28922, *P. aeruginosa* ATCC 27853) but unfortunately MICs up to 200 $\mu\text{g}/\text{mL}$ were encountered in all cases.



Docked complex of compound **4b** in the binding pocket of PfDHFR (PDB ID: 1J3I)

Keywords: 2-azetidinone, isatin, in-silico study, antimalarial activities, Staudinger reaction.

Introduction

Malaria is an infectious disease killing thousands of people every year especially in Africa and caused by a parasite belonging to genus plasmodium and spread through the bite of the female mosquito of the genus Anopheles [1]. Various species of malaria parasites are pathogenic to human such as *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi* [2,3], and the most lethal human malaria is caused by *P. falciparum*. Some effective drugs are used in the treatment or prevention of malaria with different targets and mechanism of action. Quinolines (quinine, mefloquine), aminoquinolines (chloroquine, primaquine, amodiaquine), naphthoquinones (atovaquone), sesquiterpene trioxanes (artemisinin), sulfonamides (sulfadiazine, sulfadoxine), and antibiotics (clindamycin, tetracyclines) especially in combination with quinine have been mostly used for the treatment of malaria [2,4]. Unfortunately, the increase of antimalarial drug resistance limited the efficiency of classical treatments. Thus, although, the World Health Organization (WHO) recommend combination therapy of classical drugs [1], new and effective medications are needed to circumvent established mechanisms of resistance. In this context, the design and synthesis of new antimalarial agents as hybrid molecules combining two or more active pharmacophoric fragments constitute an interesting strategy [5–10] for which a diversity of natural products such as taxoids, peptides, carbohydrates and steroids have been hybridized by other pharmacologically active molecules such as β -lactams, anthraquinones, C₆₀-fullerenes, porphyrin and enediyne [11,12].

Four membered cyclic amides, 2-azetidinones, are commonly known as β -lactams. Among β -lactams, spiro- β -lactams have received special attention since some of their derivatives have

exhibited potential pharmaceutical applications including cholesterol absorption inhibition [13], antimalarial [14–17], antibacterial [18], antiviral [19], anti-HIV [14,15], antioxidant [20], anticancer and antiproliferative [20,21] activities. Moreover, spiro- β -lactams have been also used as synthons for synthesis of heterocycles and other organic compounds [22]. On the other hand, isatin (1H-indol-2,3-dione), a privileged structure, and its derivatives have received special attention among medicinal chemists since they can be used as starting materials for the synthesis of heterocycles with potential bioactivities [23–25]. During last decades, a large number of isatin derivatives have been reported to exhibit a wide range of biological activities [26–34].

Our research group previously reported the synthesis and characterization of some mono- and bis-spiroisatino β -lactams (**Fig.1**) [35] and designed some isatin-derived spiro β -lactams with aromatic and aliphatic spacers (**Fig.1**) which have exhibited similar activity towards cancer cells that of cisplatin used as positive control [21]. In the continuation of our studies, we would like to report herein the synthesis of some new spiro- β -lactam isatin hybrids involving a Staudinger reaction key step. We will also evaluate their antimalarial activity against *P. falciparum* K1 strain and their antibacterial activity against both Gram-positive and Gram-negative bacteria.

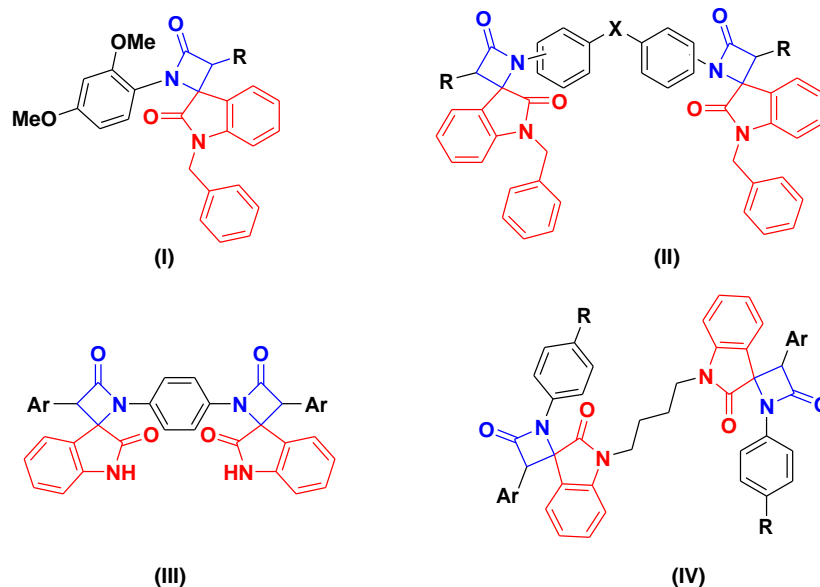


Fig 1. Examples of some isatin-derived spiro β -lactams previously reported by our group

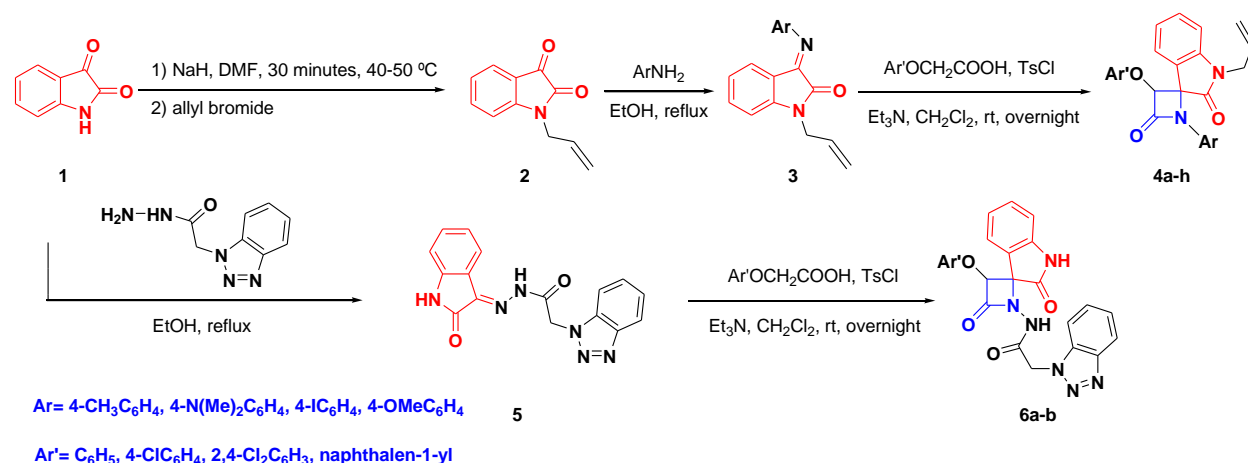
Results and Discussion

Chemistry

Owing to the importance and potential utility of the β -lactam, isatin and spiro structures, some new cycloadduct were synthesized and the influence of different substituents on the N-1, C-3 and C-4 positions of β -lactam ring was investigated. In this study, we have reported the synthesis of new spiro- β -lactams bearing an isatin moiety through a Staudinger cycloaddition reaction, as shown in **scheme 1**. Thus, the synthesis of compounds **4a-h** was achieved by treatment of isatin with sodium hydride and allyl bromide in *N,N*-dimethyl formamide (DMF) to lead to the formation of the expected allyl-isatin (**2**). The isatin-based imines (**3**) were subsequently synthesized through a condensation reaction of (**2**) with an appropriate aromatic amine in ethanol. Finally, the spiro- β -lactam adducts **4a-h** were obtained in good yields by reacting the different imines (**3**) with various acetic acid derivatives in the presence of *p*-toluenesulfonyl

chloride and Et₃N in CH₂Cl₂ at room temperature (**Scheme 1, Table 1**). Two other spiro- β -lactams (**6a-b**) were obtained from imine (**5**) by involving a [2+2] cycloaddition reaction. Thus, isatin was treated with 2-(1H-benzo[d][1,2,3]triazol-1-yl)acetohydrazide in EtOH to afford desired imine (**5**) which was subsequently treated with appropriate acetic acid derivatives in dry CH₂Cl₂ at room temperature in the presence of Et₃N and *p*-toluenesulfonyl chloride as acid activator to afford the expected spiro- β -lactams (**6a-b**).

The structure of all these new compounds was confirmed by elemental analyses and different spectroscopy techniques. As an example, the infrared spectra of **4e** exhibited the main characteristic band of β -lactam carbonyl group at 1766 cm⁻¹, while the signal at 1728 cm⁻¹ was assigned to the carbonyl group of isatin ring. Furthermore, the ¹H-NMR spectra of **4e** confirmed the structure of the cycloadduct since a singlet signal at 5.35 ppm was assigned to H-3 proton of a β -lactam ring and another one at 2.78 ppm to the two methyl groups attached to the nitrogen atom. The presence of the allylic protons was confirmed at 4.00-4.36 ppm as a multiplet as well as to vinylic ones presenting a signal at 4.99-5.08 (2H) and 5.72-5.87(1H) ppm. The ¹³C NMR spectrum of **4e** displayed two signals at 87.7 and 80.7 ppm related to C-3 and C-4 of the β -lactam ring, respectively. On the other hand, the signal at 159.2 ppm and 171.0 ppm can be unambiguously assigned to the carbonyl groups of the β -lactam and isatin rings, respectively.



Scheme.1. Synthetic pathway for the preparation of isatin-based spiro- β -lactams **4a-h**, **6a-b**

Table 1. Structures and isolated yields of β -lactams **4a-h**, **6a-b**

Cpd	Ar	Ar'	Yield (%)	Cpd	Ar	Ar'	Yield (%)
4a	4-CH ₃ C ₆ H ₄	C ₆ H ₅	85%	4f	4-N(CH ₃) ₂ C ₆ H ₄	C ₆ H ₅	77%
4b	4-CH ₃ C ₆ H ₄	2,4-Cl ₂ C ₆ H ₃	80%	4g	4-N(CH ₃) ₂ C ₆ H ₄	2,4-Cl ₂ C ₆ H ₃	70%
4c	4-IC ₆ H ₄	C ₆ H ₅	75%	4h	4-OCH ₃ C ₆ H ₄	C ₆ H ₅	79%
4d	4-N(CH ₃) ₂ C ₆ H ₄	1-naphtyl	84%	6a	-	4-ClC ₆ H ₄	75%
4e	4-N(CH ₃) ₂ C ₆ H ₄	4-ClC ₆ H ₄	77%	6b	-	2,4-Cl ₂ C ₆ H ₃	84%

The solid-state structure of **4e** was also investigated by X-ray single crystal diffraction study as depicted in **Fig. 2**. Despite several attempts to get better crystals of **4e** and a better data set, only poor-quality data were obtained (See supporting information). However, it was possible to solve the structure and all non-hydrogen atoms have been located unambiguously and their positions refined with anisotropic thermal parameters confirming the skeletal arrangement and geometry of **4e**.

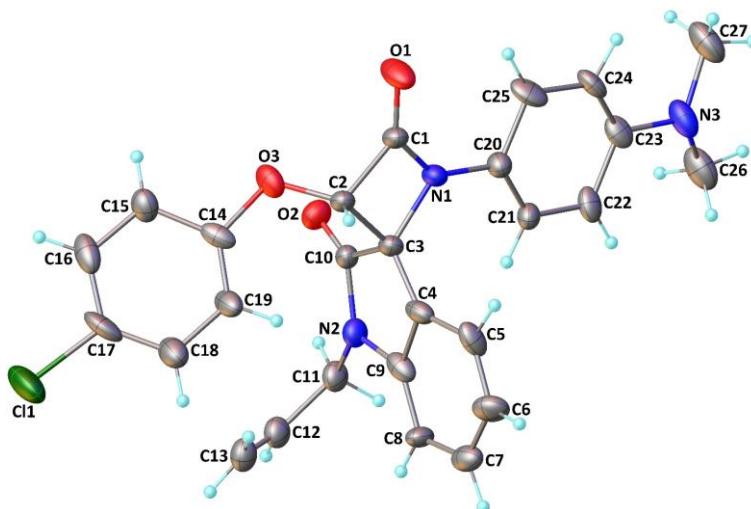


Fig. 2. A view of **4e** showing the atom-labelling scheme. Displacement ellipsoids are drawn at the 20% probability level and H atoms are shown as small spheres of arbitrary radii. Only major part of the disordered allyl moiety is shown for clarity.

Biological evaluations

Antimalarial screening

The antimalarial activity of the new molecules was successfully evaluated against *P. falciparum* K1 strain. The β -lactams **4a-g** showed moderate to good results with IC_{50} values ranging from 5.04 to up to 22.27 μ M, as outlined in **Table 2**. The compounds **4b** and **4e** exhibited the highest activity with IC_{50} values of 5.04 and 7.18 μ M, respectively. The structure activity relationship (SAR) analysis of the obtained results confirmed that the presence of electron withdrawing chlorine group on the phenyl ring at C-3 position lead to a positive effect on the antimalarial activity. The results demonstrated that as well as 4-chlorophenoxy and 2,4-dichlorophenoxy counterparts, the presence of the naphthyloxy moiety on the C-3 position of β -lactam ring in **4d** (IC_{50} =10.88 μ M) enhances both lipophilicity and antimalarial activity. These results is in agreement with our previous report [36]. Moreover, the comparison between compounds **4b**

(IC₅₀ =5.04 μM) and **4g** (IC₅₀ =16.48 μM) containing 2,4-dichlorophenoxy pendent at the C-3 position showed that the replacement of a *p*-tolyl moiety by more electron-rich *N,N*-dimethyl amino phenyl moiety on the N-1 position could reduce the inhibitory activity.

Amongst the **4d-g** compounds set possessing a *N,N*-dimethyl amino phenyl on N-1 position, derivative **4e** presenting a 4-chlorophenoxy counterpart on C-3 position (IC₅₀ =7.18 μM) demonstrated superior potency than compounds **4d** (IC₅₀ =10.88 μM), **4g** (IC₅₀ =16.48 μM) and **4f** (IC₅₀ =18.54 μM) with naphthalenyloxy, 2,4-dichlorophenoxy and phenoxy groups, respectively. Furthermore, amongst the compounds having phenoxy moiety on the C-3 position of the β-lactam (**4a**, **4c**, **4f** and **4h**), we observed that replacing of the electron withdrawing group with the electron rich ones on para position of phenyl group attached to N-1 decreased the antimalarial activity. On the other hand, the compound **4c** with iodophenyl counterparts exhibited IC₅₀ equal to 12.94 μM, while **4f** (*N,N*-dimethyl amino phenyl), **4a** (*p*-tolyl) and **4h** (phenoxy) lead to IC₅₀ of 18.54, 22.27 and 41.02 μM, respectively. The presence of the benzotriazole derivatives on the N-1 position of β-lactam ring had a negative effect on the antimalarial activity, the results suggesting that introduction of a bulky lipophilic substituted groups might affect the potency of the compounds.

Table 2. Antimalarial activity of spiro-β-lactams **4a-h**, **6a-b** against *P. falciparum* K1 Strain^a

Compound	IC ₅₀ (μM)	SD ^b	Compound	IC ₅₀ (μM)	SD
4a	22.27	4.65	4f	18.54	1.98
4b	5.04	0.28	4g	16.48	2.91
4c	12.92	0.25	4h	41.02	3.18
4d	10.88	0.58	6a	35.51	1.49
4e	7.18	0.65	6b	42.46	5.32

^a The standard antimalarial drug used was chloroquine with an IC₅₀ of 0.80 μM

^b Standard deviation

Antibacterial screening

All new compounds were then evaluated for their antibacterial activities against *E. coli* ATCC 28922, *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923 by determining the Minimal Inhibitory Concentrations (MIC) according to the NCCLS guidelines M7-A6 using the microbroth dilution methods [37]. Nevertheless, no compounds were able to demonstrate any significant antibacterial activity with MIC values greater than 200 $\mu\text{g/mL}$ (**Table 3**) whatever the considered bacterial strain.

Table 3. Antibacterial activity of new spiro- β -lactams

Compound	MIC (μM)	MIC (μM)	MIC (μM)
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>
4a	>200	>200	>200
4b	>200	>200	>200
4c	>200	>200	>200
4d	>200	>200	>200
4e	>200	>200	>200
4f	>200	>200	>200
4g	>200	>200	>200
4h	>200	>200	>200
6a	>200	>200	>200
6b	>200	>200	>200

Molecular Docking study

A computational ligand-target docking approach was carried out to disclose more details behind the inhibitory activity of compound **4b** with *P. falciparum* dihydrofolate reductase enzyme (PfDHFR) binding site. The best experimental conformation was found to possess a binding free energy of -7.13 kcal/mol as depicted in **Fig. 1**. Thus, **4b** was found to interact with key amino acids Ala16, Leu46, Ser11, Ile112, Ile164 and Val195. Furthermore, there are significant H-bonding interactions between oxygen of isatin and oxygen at C-3 position of the β -lactam ring with active sites Ile164 and Ser111, respectively. It should be noted that the presence of a 2,4-

dichlorophenoxy substituent led to the formation of hydrophobic interaction with amino acids Leu46 and Val195. Moreover, a stacking interaction between the phenyl rings and Ala16 and Ile112 located in the binding site of the enzyme was also observed. It is worth mentioning that the phenoxy substituted and *N*-substituted groups in the spiro- β -lactam-isatin scaffold could facilitate the orientation toward key residues in *P. falciparum* dihydrofolate reductase enzyme (PfDHFR) binding site. We were able to conclude that the introduction of a dichlorophenyl moiety lead to a favourable binding mode, resulted a suitable ligand-acceptor interaction. [37]

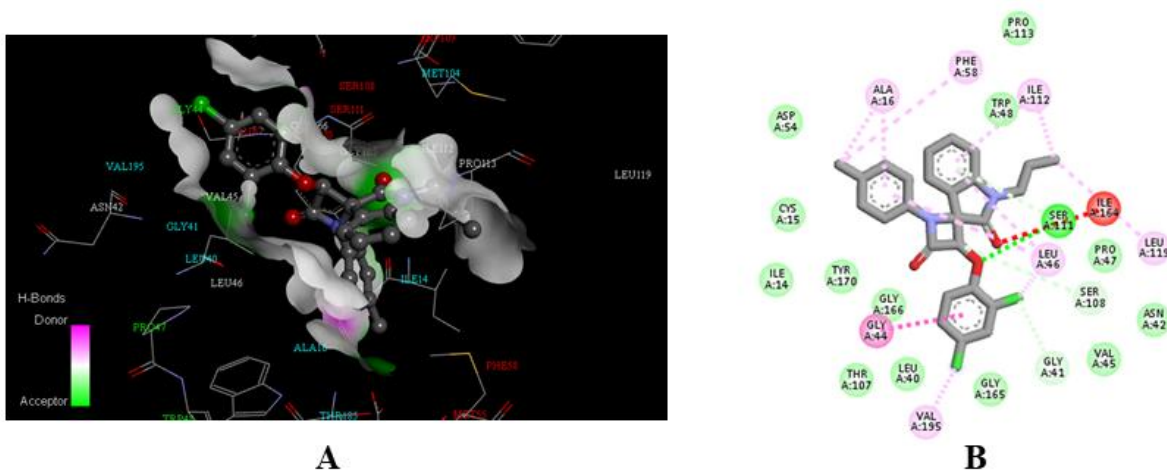


Figure 1. Docked complex of compound **4b** in the binding pocket of PfDHFR (PDB ID: 1J3I), **A:** the 3D view of the active site, **B:** the 2D view of the active site, (ΔG : - 7.13 kcal/mol)

Conclusion

Briefly, some spiro β -lactams **4a-h** and **2a-b** bearing the biological active isatin moiety have been synthesized, purified, well characterized and then evaluated for their biological activities. The new compounds have been prepared from imines derived from 1-allyl-3-(arylimino)indolin-2-one and ketenes of various acetic acid derivatives. These isatin-based β -lactams demonstrated moderate to good activity against *p. falciparum* K1 strain. The best results were obtained by **4b**

and **4e** with IC_{50} ranging from 5.04 to 7.18 μM , respectively. The *in-silico* results revealed that compound **4b** involved two favorable H-bonding through oxygens in carbonyl and ether moieties in the active site of *P. falciparum* dihydrofolate reductase enzyme. Further works are under current investigation to improve the structure of the best active compounds.

Experimental section

Materials

All reagents and organic solvents were purchased from Merck, Fluka, Sigma-Aldrich and Acros Organic companies. Et_3N and CH_2Cl_2 were dried over anhydrous CaH_2 and stored over 4Å molecular sieves before use. Thin layer chromatography (TLC) and column chromatography were performed on silica gel 254 and Merck Kieselgel (230-270 mesh), respectively. Structure determination of the synthesized compounds was achieved by using FT-IR, ^1H NMR, ^{13}C NMR, GC-Mass as well as Elemental analysis. ^1H NMR and ^{13}C NMR spectra were recorded on Bruker Avance DPX instrument (250 MHz for ^1H and 62.9 MHz for ^{13}C) in CDCl_3 or DMSO-d_6 with tetramethylsilane (TMS) as internal standard. All the chemical shifts were reported in ppm and coupling constants (J) are in Hz. FT-IR spectra were recorded on a Shimadzu FT-IR 8300 spectrophotometer in KBr pellets. Mass spectra analysis were carried out with a Shimadzu GC-MS QP 1000 EX device and elemental analysis were performed with a Thermo Finning Flah EA-1112 series. Moreover, the melting points (mp) were carried out in open capillaries with a Buchi 510 melting point device.

General experimental procedure for the synthesis of monobactams (**4a-h**, **6a-b**)

To a solution of isatin (5.00 mmol) in DMF (23 mL) was added sodium hydride (10.00 mmol) and the mixture was stirred for 30 minutes at 40-50°C. Allyl bromide (5.50 mmol) was then added and the reaction was allowed to proceed at room temperature until formation of N-allyl isatin (**2**). The crude product was poured into aqueous solution including NaCl, acidified with HCl (0.2 M), and the mixture was washed with 3×110 mL of ethyl acetate. The organic layer was dried over anhydrous Na₂SO₄ and the solvent evaporated under reduced pressure for more purification. After purification by short column chromatography, isatin derivative **2** (2.00 mmol) was treated with appropriate amine (2.00 mmol) in EtOH under reflux conditions to give imine (**3**).

For the synthesis of β -lactams (**4a-h**), to a solution of imine **3** (1.00 mmol) in dry CH₂Cl₂ (20 mL) was added corresponding acetic acid derivative (1.50 mmol), *p*-toluenesulfonyl chloride (1.50 mmol) as well as Et₃N (5.0 mmol) and let to stir at room temperature overnight. The crude mixture was washed with HCl 1M (20 mL), saturated NaHCO₃ (20 mL), and saturated sodium chloride (20 mL), respectively, and dried over anhydrous Na₂SO₄. Finally, the solvent was evaporated for more purification by column chromatography.

For the synthesis of imine **5**, a solution of isatin (2.00 mmol) and 2-(1H-benzo[d][1,2,3]triazol-1-yl)acetohydrazide was refluxed for appropriate time. After cooling the mixture, the precipitate was filtered and washed with EtOH several times to give pure Imine **5**. In the next step, we applied the same above procedure for synthesis of β -lactams (**6a-b**) from imine **5**.

N-Allyl-3-phenoxy-1-(*p*-tolyl)spiro[azetidine-2,3'-indoline]-2',4-dione (**4a**): White solid (Yield 85%); mp: 143-146 °C; IR (KBr, cm⁻¹): 1774 (CO β -lactam), 1720 (CO, amide); ¹H-NMR (CDCl₃, 250 MHz) δ 2.17 (3H, s, CH₃), 4.29-4.52 (2H, m, allylic CH₂N), 5.18-5.25 (2H, m, vinylic CH₂), 5.51 (1H, s, CH β -lactam ring), 5.71-5.92 (1H, m, vinylic CH), 6.54-6.59 (2H, m,

ArH), 6.78-6.85 (2H, m, ArH), 6.91-7.07 (6H, m, ArH), 7.16-7.25 (2H, m, ArH), 7.42 (1H, d, $J=7.25$ Hz, ArH); ^{13}C -NMR (CDCl_3 , 62.5 MHz) δ 20.9 (CH_3), 42.8($\text{CH}_2\text{-N}$), 77.6 (C-3), 76.7 (C-4), 109.7-157.01(aromatic and C=C vinylic), 160.1 (CO, β -lactam), 176.7 (CO amide); GC-MS $m/z = 410$ [M^+]; Anal. Calcd. for $\text{C}_{26}\text{H}_{22}\text{N}_2\text{O}_3$: C, 76.08; H, 5.40; N, 6.82%. Found: C, 75.90; H, 5.10; N, 6.70%.

1'-Allyl-3-(2,4-dichlorophenoxy)-1-(p-tolyl)spiro[azetidine-2,3'-indoline]-2',4-dione (4b): White solid (Yield 80%); mp: 138.4-141 °C; IR (KBr, cm^{-1}): 1759 (CO β -lactam), 1728 (CO, amide); ^1H -NMR (CDCl_3 , 250 MHz) δ 2.17 (3H, s, CH_3), 4.12-4.48 (2H, m, allylic CH_2N), 5.13-5.21 (2H, m, vinylic CH_2), 5.31 (1H, s, CH β -lactam ring), 5.52-5.67 (1H, m, vinylic CH), 6.90-6.97 (6H, m, ArH), 7.01-7.09 (1H, m, ArH), 7.16-7.20 (1H, m, ArH), 7.24-7.29 (2H, m, ArH), 7.33-7.40 (1H, m, ArH); ^{13}C -NMR (CDCl_3 , 62.5 MHz) δ 20.9 (CH_3), 43.0 ($\text{CH}_2\text{-N}$), 88.5 (C-3), 73.8 (C-4), 110.1-142.7 (aromatic and C=C vinylic), 162.1 (CO, β -lactam), 184.6 (CO, amide); GC-MS $m/z = 478$ [$^{35}\text{M}^+$], 480 [$^{37}\text{M}^+$]; Anal. Calcd. for $\text{C}_{26}\text{H}_{20}\text{Cl}_2\text{N}_2\text{O}_3$: C, 65.15; H, 4.21; N, 5.84%. Found: C, 65.65; H, 5.33; N, 5.90%.

1'-Allyl-1-(4-iodophenyl)-3-phenoxy spiro[azetidine-2,3'-indoline]-2',4-dione (4c): Grey solid (Yield 77%); mp: 179-184 °C; IR (KBr, cm^{-1}): 1774 (CO, β -lactam), 1712 (CO, Amide); ^1H -NMR (CDCl_3 , 250 MHz) δ 4.29-4.52 (2H, m, allylic CH_2N), 5.18-5.27 (2H, m, vinylic), 5.52 (1H, s, CH β -lactam ring), 5.73-5.88 (1H, m, vinylic CH), 6.56 (2H, dd, $J=7.7, 1.2$ Hz, ArH), 6.78-6.87 (3H, m, ArH), 6.93-7.07 (3H, m, ArH), 7.19-7.28 (3H, m, ArH), 7.39-7.47 (2H, m, ArH); ^{13}C -NMR (CDCl_3 , 62.5 MHz) δ 48.0 ($\text{CH}_2\text{-N}$), 86.5 (C-3), 70.9 (C-4), 106.7-147.5 (aromatic and C=C vinylic), 159.7 (CO, β -lactam), 176.5 (CO, amide); Anal. Calcd. for $\text{C}_{25}\text{H}_{19}\text{IN}_2\text{O}_3$: C, 57.49; H, 3.67; N, 5.36%. Found: C, 57.10; H, 3.66; N, 6.64%.

1'-Allyl-1-(4-(dimethylamino)phenyl)-3-(naphthalen-1-yloxy)spiro[azetidine-2,3'-indoline]-2',4-dione (4d): White solid (Yield 77%); mp: 155-158 °C; IR (KBr, cm⁻¹): 1766 (CO, β-lactam), 1704 (CO, amide); ¹H-NMR (CDCl₃, 250 MHz) δ 2.78 (6H, s, NCH₃), 4.30-4.51 (2H, m, allylic CH₂N), 5.14-5.20 (2H, m, vinylic CH₂), 5.66 (1H, s, CH β-lactam ring), 5.72-5.88 (1H, m, vinylic CH), 6.46-6.50 (2H, m, ArH), 6.70-6.72 (2H, m, ArH), 6.88-6.98 (3H, m, ArH), 7.23-7.37 (3H, m, ArH), 7.45-7.54 (3H, m, ArH), 7.60 (1H, d, *J*=8.0 Hz, ArH), 7.67-7.76 (1H, m, ArH); ¹³C-NMR (CDCl₃, 62.5 MHz) δ 40.56 (CH₃N, 6H), 42.8 (CH₂-N), 68.6 (C-4), 84.8 (C-3), 104.4-158.13 (aromatic carbons and C=C vinylic), 162.2 (CO, β-lactam), 179.0 (CO, amide); GC-MS *m/z* = 489 [M⁺]; Anal. Calcd. for C₃₁H₂₇N₃O₃: C, 76.05; H, 5.56; N, 8.58%. Found: C, 75.17; H, 5.99; N, 9.69%.

1'-Allyl-3-(4-chlorophenoxy)-1-(4-(dimethylamino)phenyl)spiro[azetidine-2,3'-indoline]-2',4-dione (4e): White solid (Yield 79%); mp: 186-192 °C; IR (KBr, cm⁻¹): 1766 (CO, β-lactam), 1728 (CO, amide); ¹H-NMR (CDCl₃, 250 MHz) δ 2.78 (6H, s, NCH₃), 4.00-4.36 (2H, m, allylic CH₂N), 4.99-5.08 (2H, m, vinylic CH₂), 5.35 (1H, s, CH β-lactam ring), 5.72-5.87 (1H, m, vinylic CH), 6.44-6.51 (4H, m, ArH), 6.85-7.12 (5H, m, ArH), 7.19-7.23 (1H, m, ArH), 7.32-7.40 (2H, m, ArH); ¹³C-NMR (CDCl₃, 62.5 MHz) δ 40.5 (CH₃N, 6H), 42.8 (CH₂-N), 80.7 (C-4), 87.7 (C-3), 110.0-155.1 (aromatic carbons and C=C vinylic), 159.2 (CO, β-lactam), 171.0 (CO, amide); GC-MS *m/z* = 473 [³⁵M⁺], 475 [³⁷M⁺]; Anal. Calcd. for C₂₇H₂₄ClN₃O₃: C, 68.42; H, 5.10; N, 8.47%. Found: C, 67.86; H, 5.65; N, 9.48%.

1'-Allyl-1-(4-(dimethylamino)phenyl)-3-phenoxy spiro[azetidine-2,3'-indoline]-2',4-dione (4f): Grey solid (Yield 74%); mp: 195 °C; IR (KBr, cm⁻¹): 1759 (CO, β-lactam), 1712 (CO, amide); ¹H-NMR (CDCl₃, 250 MHz) δ 2.85 (6H, s, NCH₃), 4.26-4.52 (2H, m, allylic CH₂N), 5.17-5.25

(2H, m, vinylic CH₂), 5.52 (1H, s, CH β -lactam ring), 5.73-5.88 (1H, m, vinylic CH), 6.54-6.59 (2H, m, ArH), 6.77-6.85 (2H, m, ArH), 6.92-7.07 (5H, m, ArH), 7.19-7.26 (3H, m, ArH), 7.42 (1H, d, $J=7.5$ Hz, ArH); ¹³C-NMR (CDCl₃, 62.5 MHz) δ 29.6 (CH₃N, 6H), 42.7 (CH₂-N), 72.5 (C-4), 85.1 (C-3), 103.1-155.4 (aromatic carbons and C=C vinylic), 161.9 (CO, β -lactam), 173.4 (CO, amide); GC-MS m/z = 439 [M⁺]; Anal. Calcd. for C₂₇H₂₅N₃O₃: C, 73.79; H, 5.73; N, 9.56%. Found: C, 70.10; H, 5.49; N, 9.30%.

1'-Allyl-3-(2,4-dichlorophenoxy)-1-(4-(dimethylamino)phenyl)spiro[azetidine-2,3'-indoline]-2',4-dione (4g): Gray solid (Yield 84%); mp: 180 °C; IR (KBr, cm⁻¹): 1766 (CO, β -lactam), 1728 (CO, amide); ¹H-NMR (CDCl₃, 250 MHz) δ 1.18 (6H, s, NCH₃), 4.09-4.48 (2H, m, allylic CH₂N), 5.12-5.20 (2H, m, vinylic CH₂), 5.30 (1H, s, CH β -lactam ring), 5.50-5.66 (1H, m, vinylic CH), 6.81 (1H, s, ArH), 6.84 (1H, s, ArH), 6.88 (1H, d, $J=7.8$ Hz, ArH), 6.92-6.97 (4H, m, ArH), 7.06 (2H, t, $J=7.5$ Hz, ArH), 7.24-7.27 (1H, m, ArH), 7.30-7.32 (1H, m, ArH); ¹³C-NMR (CDCl₃, 62.5 MHz) δ 28.2 (CH₃N, 6H), 44.5 (CH₂-N), 70.4 (C-4), 78.4 (C-3), 105.1-157.4 (aromatic carbons and C=C vinylic), 161.9 (CO, β -lactam), 173.5 (CO, amide); Anal. Calcd. for C₂₇H₂₄Cl₂N₃O₃: C, 63.79; H, 4.59; N, 8.27%. Found: C, 63.40; H, 4.28; N, 8.13%.

1'-Allyl-1-(4-methoxyphenyl)-3-phenoxy spiro[azetidine-2,3'-indoline]-2',4-dione (4h): Brown solid (Yield 75 %); mp: 124-128 °C; IR (KBr, cm⁻¹): 1774 (CO β -lactam), 1728 (CO, amide); ¹H-NMR (CDCl₃, 250 MHz) δ 3.71 (3H, s, OCH₃), 4.06-4.44 (2H, m, allylic CH₂N), 5.05-5.12 (2H, m, vinylic CH₂), 5.50 (1H, s, CH β -lactam ring), 5.79-5.95 (1H, m, vinylic CH), 6.60-6.65 (2H, m, ArH), 6.70-6.77 (2H, m, ArH), 6.95 (2H, d, $J=8.0$ Hz, ArH), 7.02-7.20 (5H, m, ArH), 7.26 (1H, s, ArH), 7.39-7.43 (1H, m, ArH); ¹³C-NMR (CDCl₃, 62.5 MHz) δ 42.8 (CH₃), 55.4 (CH₂-N), 87.8 (C-3), 85.2 (C-4), 110.1-157.0 (aromatic and C=C vinylic), 161.1 (CO, β -lactam),

169.6(CO, amide); GC-MS $m/z = 426 [M^+]$; Anal. Calcd. for $C_{26}H_{22}N_2O_4$: C, 73.23; H, 5.20; N, 6.57%. Found: C, 73.10; H, 5.33; N, 6.39%.

2-(1H-Benzo[d][1,2,3]triazol-1-yl)-N-(3-(4-chlorophenoxy)-2',4-dioxospiro[azetidine-2,3'-indolin]-1-yl)acetamide (6a): Yellow solid (Yield 84%); mp: 197-199 °C; IR (KBr, cm^{-1}): 1758 (CO, β -lactam), 1728 (CO, amide); 1H -NMR (DMSO- d_6 , 250 MHz) δ 5.39 (2H, s, CH_2N), 6.11 (1H, s, CH β -lactam), 7.03 (2H, d, $J=8.7$ Hz, ArH), 7.28-7.44 (4H, m, ArH), 7.56 (2H, q, $J=7.0$ Hz, ArH), 7.87 (1H, d, $J=8.0$ Hz, ArH), 8.06 (1H, d, $J=8.0$ Hz, ArH), 8.24 (1H, d, $J=8.0$ Hz, ArH), 8.41 (1H, d, $J=7.5$ Hz, ArH), 12.03 (1H, s, NH); ^{13}C -NMR (DMSO- d_6 , 62.5 MHz) δ 68.4 (CH_2-N), 88.7 (C-3), 80.8 (C-4), 110.9-163.2 (aromatic), 163.2 (CO, β -lactam), 171.3 (CO, amide), 168.5(CO, isatin); GC-MS $m/z = 488 [^{35}M^+]$, 500 [$^{37}M^+$]; Anal. Calcd. for $C_{24}H_{17}ClN_6O_4$: C, 58.96; H, 3.51; N, 17.19%. Found: C, 57.94; H, 3.32; N, 16.10%.

2-(1H-Benzo[d][1,2,3]triazol-1-yl)-N-(3-(2,4-dichlorophenoxy)-2',4-dioxospiro[azetidine-2,3'-indolin]-1-yl)acetamide (6b): Yellow solid (Yield 70%); mp: 198-199 °C; IR (KBr, cm^{-1}): 1766 (CO β -lactam), 1720 (CO, amide); 1H -NMR (DMSO- d_6 , 250 MHz) δ 5.53 (2H, s, CH_2N), 6.11 (1H, s, CH β -lactam ring), 7.22-7.31 (2H, m, ArH), 7.35-7.44 (2H, m, ArH), 7.52-7.58 (3H, m, ArH), 7.88 (1H, d, $J=8.2$ Hz, ArH), 8.07 (1H, d, $J=8.7$ Hz, ArH), 8.24 (1H, d, $J=8.2$ Hz, ArH), 8.42 (1H, d, $J=7.75$ Hz, ArH), 12.04 (1H, s, NH); ^{13}C -NMR (DMSO- d_6 , 62.5 MHz) δ 68.9 (CH_2-N), 87.7 (C-3), 75.7 (C-4), 110.8-152.3 (aromatic), 163.2 (CO, β -lactam), 178.8 (CO, amide), 168.0 (CO, isatin); Anal. Calcd. for $C_{24}H_{16}Cl_2N_6O_4$: C, 55.08; H, 3.08; N, 13.55%. Found: C, 54.90; H, 2.93; N, 14.13%.

Antibacterial activity procedure

Antibacterial activity determination (Minimal Inhibitory Concentration (MIC)) of novel β -lactams against *P. aeruginosa* ATCC 27853, *E. coli* ATCC 28922 and *S. aureus* ATCC 25923 was performed according to NCCLS guideline M7-A6 as described in the supporting information.

***In-vitro* antimalarial activity procedure**

The determination of *in-vitro* antimalarial activity of the new compounds was performed against *P. falciparum* strain K1 according a previously reported procedure [36,39] as described in the supporting information.

Computational studies

Molecular docking studies were performed using Auto-Dock software version 1.5.4, and the structure of tested compounds was created by HyperChem software version 8.0.10. The tested molecules energetically minimized by MM⁺ force field. The crystal structure of *P. falciparum* dihydrofolate reductase enzyme (PfDHFR PDB ID: 1J3I) was retrieved from the protein data Bank (<http://www.rcsb.org/>). All water molecules and ligands were deleted from the macromolecule and then, polar hydrogen atoms were added to the 1J3I. The active sites were explained in the x, y, z directions based on the center with x: 28.46, y:5.73, z: 62.35. All dockings were performed by the Lamarkian genetic algorithm (LGA) approach with grid sizes 25×25×25 (grid spacing 0.375 Å). The results were validated by redocking and comparing the original ligand inside the 1J3I utilizing root mean square difference (RMSD). The docking procedure was conducted according to previous report [40,41].

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References:

1. van Schalkwyk DA. History of antimalarial agents. In : eLS. John Wiley & Sons, Ltd: Chichester; 2015. p. 1–5.
<https://onlinelibrary.wiley.com/doi/10.1002/9780470015902.a0003624.pub3>
2. Uzor PF. Alkaloids from plants with antimalarial activity: A review of recent studies. Evidence-Based Complement Altern Med. 2020;2020:1–17.
<https://www.hindawi.com/journals/ecam/2020/8749083/>
3. Porta EOJ, Bofill Verdaguer I, Perez C, Banchio C, Ferreira de Azevedo M, Katzin AM, et al. Repositioning salirasib as a new antimalarial agent. Medchemcomm. 2019;10(9):1599–605. <http://xlink.rsc.org/?DOI=C9MD00298G>
4. Guerin PJ, Oliario P, Nosten F, Druilhe P, Laxminarayan R, Binka F, et al. Malaria: current status of control, diagnosis, treatment, and a proposed agenda for research and development. Lancet Infect Dis. 2002;2(9):564–73.
<https://linkinghub.elsevier.com/retrieve/pii/S1473309902003729>
5. Muregi FW, Ishih A. Next-generation antimalarial drugs: hybrid molecules as a new strategy in drug design. Drug Dev Res. 2009;71(1):20–32.
<https://onlinelibrary.wiley.com/doi/10.1002/ddr.20345>
6. Benoit-Vical F, Lelièvre J, Berry A, Deymier C, Dechy-Cabaret O, Cazelles J, et al. Trioxaquinones are new antimalarial agents active on all erythrocytic forms, including gametocytes. Antimicrob Agents Chemother. 2007;51(4):1463–72.
<https://journals.asm.org/doi/10.1128/AAC.00967-06>
7. Cosledan F, Fraisse L, Pellet A, Guillou F, Mordmuller B, Kremsner PG, et al. Selection

- of a trioxaquine as an antimalarial drug candidate. *Proc Natl Acad Sci*. 2008;105(45):17579–84. <http://www.pnas.org/cgi/doi/10.1073/pnas.0804338105>
8. Mahmud AW, Shallangwa GA, Uzairu A. In silico modeling of tetraoxane-8-aminoquinoline hybrids active against plasmodium falciparum. *Beni-Suef Univ J Basic Appl Sci*. 2020;9(1):19. <https://bjbas.springeropen.com/articles/10.1186/s43088-020-00044-0>
 9. Reiter C, Fröhlich T, Zeino M, Marschall M, Bahsi H, Leidenberger M, et al. New efficient artemisinin derived agents against human leukemia cells, human cytomegalovirus and plasmodium falciparum: 2nd generation 1,2,4-trioxane-ferrocene hybrids. *Eur J Med Chem*. 2015;97:164–72. <https://linkinghub.elsevier.com/retrieve/pii/S0223523415300179>
 10. Hans RH, Wiid IJF, van Helden PD, Wan B, Franzblau SG, Gut J, et al. Novel thiolactone–isatin hybrids as potential antimalarial and antitubercular agents. *Bioorg Med Chem Lett*. 2011;21(7):2055–8. <https://linkinghub.elsevier.com/retrieve/pii/S0960894X1100182X>
 11. Tietze LF, Bell HP, Chandrasekhar S. Natural product hybrids as new leads for drug discovery. *Angew Chemie Int Ed*. 2003;42(34):3996–4028. <https://onlinelibrary.wiley.com/doi/10.1002/anie.200200553>
 12. Pandey G, Dumbre SG, Khan MI, Shabab M, Puranik VG. A β -lactam-azasugar hybrid as a competitive potent galactosidase inhibitor. *Tetrahedron Lett*. 2006;47(45):7923–6. <https://linkinghub.elsevier.com/retrieve/pii/S0040403906017758>
 13. Benfatti F, Cardillo G, Gentilucci L, Tolomelli A. Synthesis and biological evaluation of unprecedented classes of spiro- β -lactams and azido- β -lactams as acyl-CoA:cholesterol acyltransferase inhibitors. *Bioorg Med Chem Lett*. 2007;17(7):1946–50. <https://linkinghub.elsevier.com/retrieve/pii/S0960894X07000777>
 14. Bártolo I, Santos BS, Fontinha D, Machado M, Francisco D, Sepodes B, et al. Spiro- β -lactam BSS-730A displays potent activity against HIV and plasmodium. *ACS Infect Dis*. 2021;7(2):421–34. <https://pubs.acs.org/doi/10.1021/acsinfecdis.0c00768>
 15. Alves NG, Bártolo I, Alves AJS, Fontinha D, Francisco D, Lopes SMM, et al. Synthesis and structure-activity relationships of new chiral spiro- β -lactams highly active against HIV-1 and plasmodium. *Eur J Med Chem*. 2021;219:113439. <https://linkinghub.elsevier.com/retrieve/pii/S0223523421002889>

16. Jarrahpour A, Shirvani P, Sinou V, Latour C, Brunel JM. Synthesis and biological evaluation of some new β -lactam-triazole hybrids. *Med Chem Res.* 2016;25(1):149–62. <http://link.springer.com/10.1007/s00044-015-1474-x>
17. Jarrahpour A, Ebrahimi E, De Clercq E, Sinou V, Latour C, Djouhri Bouktab L, et al. Synthesis of mono-, bis-spiro- and dispiro- β -lactams and evaluation of their antimalarial activities. *Tetrahedron.* 2011;67(45):8699–704. <https://linkinghub.elsevier.com/retrieve/pii/S0040402011013858>
18. Turos E, Long TE, Heldreth B, Leslie JM, Reddy GSK, Wang Y, et al. N-Thiolated β -lactams: A new family of anti-Bacillus agents. *Bioorg Med Chem Lett.* 2006;16(8):2084–90. <https://linkinghub.elsevier.com/retrieve/pii/S0960894X06001089>
19. Skiles JW, McNeil D. Spiro indolinone beta-lactams, inhibitors of poliovirus and rhinovirus 3C-proteinases. *Tetrahedron Lett.* 1990;31(50):7277–80. <https://linkinghub.elsevier.com/retrieve/pii/S0040403900885433>
20. Ranjbari S, Behzadi M, Sepehri S, Dadkhah Aseman M, Jarrahpour A, Mohkam M, et al. Investigations of antiproliferative and antioxidant activity of β -lactam morpholino-1,3,5-triazine hybrids. *Bioorg Med Chem.* 2020;28(8):115408. <https://linkinghub.elsevier.com/retrieve/pii/S0968089620302133>
21. Bashiri M, Jarrahpour A, Nabavizadeh SM, Karimian S, Rastegari B, Haddadi E, et al. Potent antiproliferative active agents: novel bis Schiff bases and bis spiro β -lactams bearing isatin tethered with butylene and phenylene as spacer and DNA/BSA binding behavior as well as studying molecular docking. *Med Chem Res.* 2021;30(1):258–84. <http://link.springer.com/10.1007/s00044-020-02659-5>
22. Dhooghe M, Thi HD. An update on the synthesis and reactivity of spiro-fused β -lactams. Opatz T, editor. *Arkivoc.* 2019;2018(6):314–47. <https://www.arkat-usa.org/arkivoc-journal/browse-arkivoc/ark.5550190.p010.808>
23. Silva JFM da, Garden SJ, Pinto AC. The chemistry of isatins: a review from 1975 to 1999. *J Braz Chem Soc.* 2001;12(3):273–324. http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0103-50532001000300002&lng=en&nrm=iso&tlng=en
24. Mohammadi S, Heiran R, Herrera RP, Marqués-López E. Isatin as a strategic motif for asymmetric catalysis. *ChemCatChem.* 2013;5(8):2131 – 2148.

25. Auria-Luna F, Marqués-López E, Mohammadi S, Heiran R, Herrera RP. New organocatalytic asymmetric synthesis of highly substituted chiral 2-oxospiro-[indole-3,4'-(1',4'-dihydropyridine)] derivatives. *Molecules*. 2015;20(9):15807–26.
26. Zhao X, Zhan X, Zhang H, Wan Y, Yang H, Wang Y, et al. Synthesis and biological evaluation of isatin derivatives containing 1,3,4-thiadiazole as potent α -glucosidase inhibitors. *Bioorg Med Chem Lett*. 2021;54:128447.
<https://linkinghub.elsevier.com/retrieve/pii/S0960894X21006740>
27. Bhagat K, Singh JV, Sharma A, Kaur A, Kumar N, Gulati HK, et al. Novel series of triazole containing coumarin and isatin based hybrid molecules as acetylcholinesterase inhibitors. *J Mol Struct*. 2021;1245:131085.
<https://linkinghub.elsevier.com/retrieve/pii/S0022286021012163>
28. Cheng R, Shi W, Yuan Q, Tang R, Wang Y, Yang D, et al. 5-Substituted isatin thiosemicarbazones as inhibitors of tyrosinase: Insights of substituent effects. *Spectrochim Acta Part A Mol Biomol Spectrosc*. 2021;255:119669.
<https://linkinghub.elsevier.com/retrieve/pii/S1386142521002456>
29. Liu W, Chen H, Zhang X, Zhang X, Xu L, Lei Y, et al. Isatin derivatives as a new class of aldose reductase inhibitors with antioxidant activity. *Med Chem Res*. 2021;30(8):1588–602. <https://link.springer.com/10.1007/s00044-021-02751-4>
30. Mishra R, Chaurasia H, Singh VK, Naaz F, Singh RK. Molecular modeling, QSAR analysis and antimicrobial properties of Schiff base derivatives of isatin. *J Mol Struct*. 2021;1243:130763. <https://linkinghub.elsevier.com/retrieve/pii/S0022286021008966>
31. Johansen MD, Shalini, Kumar S, Raynaud C, Quan DH, Britton WJ, et al. Biological and biochemical evaluation of isatin-isoniazid hybrids as bactericidal candidates against mycobacterium tuberculosis. *Antimicrob Agents Chemother*. 2021;65(8).
<https://journals.asm.org/doi/10.1128/AAC.00011-21>
32. Yakan H, Serdar Çavuş M, Kurt BZ, Muğlu H, Sönmez F, Güzel E. A new series of asymmetric bis-isatin derivatives containing urea/thiourea moiety: Preparation, spectroscopic elucidation, antioxidant properties and theoretical calculations. *J Mol Struct*. 2021;1239:130495. <https://linkinghub.elsevier.com/retrieve/pii/S0022286021006281>
33. Tumosienė I, Jonuškienė I, Kantminienė K, Mickevičius V, Petrikaitė V. Novel N-substituted amino acid hydrazone-isatin derivatives: Synthesis, antioxidant activity, and

- anticancer activity in 2D and 3D models in vitro. *Int J Mol Sci.* 2021;22(15):7799.
<https://www.mdpi.com/1422-0067/22/15/7799>
34. Emami S, Valipour M, Kazemi Komishani F, Sadati-Ashrafi F, Rasoulian M, Ghasemian M, et al. Synthesis, in silico, in vitro and in vivo evaluations of isatin aroylhydrazones as highly potent anticonvulsant agents. *Bioorg Chem.* 2021;112:104943.
<https://linkinghub.elsevier.com/retrieve/pii/S0045206821003205>
 35. Jarrahpour A, Khalili D. Synthesis of some mono- and bis-spiro- β -lactams of benzylisatin. *Tetrahedron Lett.* 2007;48(40):7140–7143.
<https://linkinghub.elsevier.com/retrieve/pii/S0040403907015298>
 36. Jarrahpour A, Heiran R, Sinou V, Latour C, Bouktab LD, Brunel JM, et al. Synthesis of new β -lactams bearing the biologically important morpholine ring and POM analyses of their antimicrobial and antimalarial activities. *Iran J Pharm Res.* 2019;18(1):34–48.
 37. National committee for clinical laboratory standards. methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 6th edn. approved standard M7-A6. NCCLS, Wayne, PA2003.
 38. Choowongkamon K, Theppabutr S, Songtawee N, Day NP, White NJ, Woodrow CJ, et al. Computational analysis of binding between malarial dihydrofolate reductases and anti-folates. *Malar J.* 2010;9(1):65.
<https://malariajournal.biomedcentral.com/articles/10.1186/1475-2875-9-65>
 39. Jarrahpour A, Ebrahimi E, Sinou V, Latour C, Brunel JM. Diastereoselective synthesis of potent antimalarial cis- β -lactam agents through a [2 + 2] cycloaddition of chiral imines with a chiral ketene. *Eur J Med Chem.* 2014;87:364–71.
 40. Shahraki O, Ghaznavi H, Akbarzadeh-T N, Shahraki S, Sheervalilou R, Kondori T. Chromium (III) complexes of 4,5-diazafluoren-9-one ligand as potential anti-proliferative agents: synthesis, characterization, DNA binding, molecular docking and in-vitro cytotoxicity evaluation. *Iran J Pharm Res.* 2021;20(3):618–35.
 41. Mahernia S, Hassanzadeh M, Adib M, Peytam F, Haghhighijoo Z, Iraj A, et al. The possible effect of microRNA-155 (miR-155) and BACE1 inhibitors in the memory of patients with down syndrome and alzheimer's disease: Design, synthesis, virtual screening, molecular modeling and biological evaluations. *J Biomol Struct Dyn.* 2021;1–13. <https://www.tandfonline.com/doi/full/10.1080/07391102.2021.1873861>

