Synthesis, *in-vitro* Biological Evaluation, and Molecular Docking Study of Novel spiro- β -lactam-isatin Hybrids

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Abstract

In our ongoing search for bioactive compounds, a class of novel spiro- β -lactam isatin hybrids have been obtained by [2+2] cycloaddition reaction from 1-allyl-3-(arylimino)indolin-2-one and ketenes from various aryloxy acetic acids. The formation of all cycloadducts were confirmed by FTIR, ^1H NMR, ^{13}C NMR, and Mass spectra as well as elemental analyses. The new β -lactams were evaluated for their biological activities. The compounds **4a-g** showed moderate to good activity against *P. falciparum* K1 strain and showed moderate to good activity. Among them, **4b** and **4e** demonstrated the best results with IC_{50} of 5.04 and 7.18, respectively. The molecular docking simulation of **4b** with *P. falciparum* dihydrofolate reductase enzyme (PfDHFR) binding site shown several intermolecular interactions that would be consider to design the future analogs of antimalarial agents. Moreover, all the synthesized β -lactams were evaluated against some gram positive and gram negative bacteria including *E. coli* ATCC 28922, *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923 at the MIC level of 200 $\mu\text{g/mL}$ and did not possess significant activity.

Introduction

Malaria is an infectious disease that kills thousands of people every year especially in Africa. It caused by parasite belong 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: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi* [2, 3], and the most fetal human malaria caused by *P. falciparum*. Some effective drugs are used in the treatment or prevention of malaria with different targets and mechanism of action. Quimolinomethanols (quinine, mefloquine), aminoquinolines (chloroquine, primaquine, amodiaquine), naphthoquinones (atovaquone), sesquiterpene trioxanes (artemisinin), sulfonamides (sulfadiazine, sulfadoxine), and antibiotics (clindamycin, tetracyclines) especially in combination with quinin have been mostly used for treatment of malaria [2, 4]. Unfortunately, antimalarial drug resistance has been complicated the treatment of malaria. Although, the World Health Organization (WHO) recommend combination therapy [1], but finding some new and effective medications is an urgent need because of increasing drug resistance. In this concept, such hybrid molecules by combination of two or more active pharmacophoric fragments have been developed as antimalarial agents [5–10]. The synthesis of hybrid molecules, as well as, evaluation of their biological activity [11, 12] such as anti-microbial [13–15], anti-cancer [16, 17], anti-inflammatory [18, 19], anti-malarial [20, 21], anti-hyperglycemic [22], anti-tubercular [23], anti-oxidant activities [24] and so on have been increasingly investigated for the last two decades. Based on the mentioned content, 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, C_{60} -fullerenes, porphyrin and enediyne and their properties have been evaluated [12, 25].

Four membered cyclic amides, 2-azetidinones, are commonly known as β -lactams. Well known biologically active compounds like cephalosporin and penicillin have the β -lactam ring in their structure [26]. Among β -lactams, spiro- β -lactams have received special attention since some of their derivatives

have exhibited potential pharmaceutical applications including cholesterol absorption inhibition [27], antimalarial [28–31], antibacterial [32], antiviral [33], anti-HIV [28, 29], antioxidant [34], anticancer and antiproliferative [34, 35] activities. Moreover, spiro- β -lactams have been also used as synthons for synthesis of heterocycles and other organic compounds [36].

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 [37]. During last decades, a large number of isatin derivatives have been reported to exhibit a wide range of biological activities such as α -glucosidase inhibitor [38], acetylcholinesterase inhibitor [39], tyrosinase inhibitor [40], and aldose reductase inhibitor [41], as well as antimicrobial [42], antitubercular [43], anticancer [44, 45], antioxidant [41, 45, 46], anticonvulsant [47], and antimalarial activities [48–50].

Considering the importance of two above motif parts, β -lactam and isatin, our research group previously reported the synthesis and characterization of some mono- and bis-spiroisatino β -lactams (**Fig.1**) [51]. Moreover, we recently design some isatin-derived spiro β -lactams with aromatic and aliphatic spacers (**Fig.1**) as well as investigation of their anticancer ability. As a result, several compounds exhibited similar activity to that of cisplatin as positive control [35]. Based on the above mention results, we would like to report the synthesis of some spiro- β -lactam isatin hybrids through Staudinger reaction. Also, we evaluated their antimalarial activity against *P. falciparum* K1 strain and their antibacterial activity against some broad-spectrum strains.

Results And Discussion

Chemistry

In this study we have reported some new spiro- β -lactams bearing isatin moiety by Staudinger cycloaddition reaction, as shown in **scheme 1**. For the synthesis of compounds **4a-h** firstly, isatin was treated with sodium hydride and allyl bromide in *N,N*-dimethyl formamide (DMF) to obtain allyl-isatin (**2**). Then, the isatin-based imines were synthesized by condensation reaction of allyl-isatin (**2**) and appropriate aromatic amine in Ethanol. Finally, the spiro- β -lactam adducts **4a-h** were obtained in good yield by the reaction of desired imine (**3**) with different acetic acid derivatives, *p*-toluenesulfonyl chloride and dry triethylamine (Et_3N) in dry CH_2Cl_2 at room temperature (**Scheme 1, Table 1**). Two other spiro- β -lactams (**6a-b**) were obtained from imine (**5**) by [2+2] cycloaddition reaction. In the first step, isatin was treated with 2-91-H-benzo[d][1,2,3]triazol-1-yl)acetohdrazide in EtOH to afford desired imine (**5**). Then, crude spiro- β -lactams (**6a-b**) were obtained by treatment of isatin-based imine (**5**) with appropriate acetic acid derivative, anhydrous Et_3N and *p*-toluenesulfonyl chloride as acid activator in dry CH_2Cl_2 at room temperature. These new spiro- β -lactams were purified by column chromatography to use for further investigations.

All new compounds were analyzed 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. Also, the ^1H -NMR spectra of **4e** confirmed the preparation of cycloadduct. A singlet signal at 5.35 ppm was assigned to H-3 proton of β -lactam ring and the other one at 2.78 ppm was attributed to two methyl groups attached to nitrogen. Allylic protons were appeared at 4.00-4.36 ppm as multiplate and multiplate signals at 4.99-5.08 (2H) and 5.72-5.87(1H) ppm were attributed to vinylic protons. Moreover, aromatic protons were observed at the appropriate position between 6.44-7.40 ppm. The ^{13}C NMR spectrum of **4e** displayed two signals at 87.7 and 80.7 ppm related to C-3 and C-4 of β -lactam ring, respectively. The signal at 159.2 ppm and the one at 171.0 ppm were assigned to carbonyl groups of β -lactam ring and isatin ring, respectively. The ^{13}C NMR spectra of **4e** also confirmed the presence of methyl and methylene carbons, as well as vinylic and aromatic carbons at 40.5, 42.8, and 110.0-155.1 ppm, respectively.

Table 1. The structures of synthesized β -lactams **4a-h**, **6a-b**

Compound	Ar	Ar'	Yield	Compound	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. The molecular structure of the compound is depicted in **Fig. 2**. Despite several attempts to get better crystals of **4e** and a better data set, only poor-quality data were obtained. However, it was possible to solve the structure without problems and all non-hydrogen atoms have been located unambiguously and their positions refined with anisotropic thermal parameters. The poor quality of the crystals and the additional presence of disorder led to relatively high R-values (see supporting information). The X-ray diffraction data led only to a poor structure model which, however, confirmed the skeletal arrangement and geometry of **4e**. The quality of the X-ray data does not allow a detailed discussion of molecular parameters.

Biological evaluations

The antimalarial activity of new molecules were 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 the presence of chlorine on the phenyl ring at C-3 position had a positive effect on antimalarial activity. Moreover, the comparison between compounds **4b** (IC_{50} = 5.04 μ M) and **4g** (IC_{50} = 16.48 μ M) containing 2,4-dichlorophenoxy pendent at C-3 position showed the replacement of methyl group with dimethyl amino group on para position of phenyl ring at N atom could reduce the inhibitory activity. Surprisingly, **4e** with 4-chlorophenoxy counterpart (IC_{50} = 7.18 μ M) in the compounds set containing of dimethyl anilino moiety demonstrated superior potency over **4d** (naphthalenyloxy), **4f** (phenoxy) and **4g** (2,4-dichlorophenoxy) compounds. According to the obtained data, the substituent at phenyl ring at N atom directly effect on activity of derivatives containing phenoxy moiety at C-3 position. The compound **4c** with iodophenyl, **4f** with dimethyl amino phenyl and **4a** with *p*-tolyl moiety showed IC_{50} of 12.92, 22.27 and 18.54 μ M, respectively. It suggested that introduction of bulky lipophilic substituted groups such as iodo substitute might affect the potency of compounds. Moreover, all new compounds were subsequently evaluated for their antibacterial activities against *E. coli* ATCC 28922, *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923. These spiro- β -lactams based isatin did not demonstrate significant antibacterial activity with MIC values greater than 200 μ g/mL.

Table 2. Antimalarial activity of new spiro- β -lactams against *p. falciparum* K1 Strain^a

Compound	IC_{50} (μ M) <i>p. falciparum</i> K1	Compound	IC_{50} (μ M) <i>p. falciparum</i> K1
4a	22.27	4f	18.54
4b	5.04	4g	16.48
4c	12.92	4h	N.D ^b
4d	10.88	6a	N.D ^b
4e	7.18	6b	N.D ^b

^a The standard antimalarial drug (SD) used was Chloroquine with IC_{50} of 0.80

^b Not determined

Molecular Docking study

A computational ligand-target docking approach were 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 has binding free energies of -7.13 kcal/mol as depicted in **Fig. 1**. The tested molecule was found to interact with key amino acids viz Ala 16, Leu 46, Ser11, Ile112, 164

and Val195. Based on the results, there are significant H-bonding interactions between oxygen of isatin and oxygen at C-3 position of β -lactam rings with active sites of Ile 164 and Ser111, respectively. It should be noted that the presence of 2,4-dichlorophenoxy substituted led to the formation of hydrophobic interaction with amino acids Leu 46 and Val195. Stacking interaction between phenyl rings and Ala 16 and Ile 112 located in the binding site of enzyme and phenyl moieties were 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 deduce that the introduction of dichlorophenyl pendant imposes a favorable binding mode, resulting in a suitable ligand-acceptor interaction.[52]

Conclusion

Briefly, some spiro β -lactams **4a-h** and **2a-b** bearing the biological active isatin moiety have been synthesized, purified and characterized by spectral analysis, 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 IC₅₀ of 5.04 and 7.18 μ M, respectively. The *in-silico* results revealed that compound **4b** in active site of *P. falciparum* dihydrofolate reductase enzyme involved two favorable H-bonding through oxygens in carbonyl and ether moieties. Further works with the aim of improvement structurally more active compounds are under investigation in our group. Furthermore, all compounds were evaluated for their antibacterial activity and none of them demonstrated significant activity against *E. coli* ATCC 28922, *P. aeruginosa* ATCC 27853, and *S. aureus* ATCC 25923 bacteria at the MIC levels of 200 μ g/mL.

Experimental

Materials

All reagents and organic solvents were purchased from Merck, Fluka, Sigma-Aldrich and Acros Organic companies. Et₃N and CH₂Cl₂ were dried over anhydrous CaH₂ and stored over molecular sieves before use. Thin layer chromatography (TLC) and column chromatography were performed on silica gel 254 and Merck Kieselgel (230-270 mesh), respectively. To determine the structure of synthesized compounds different methods were applied such as FT-IR, ¹HNMR, ¹³CNMR, GC-Mass as well as Elemental analysis. ¹HNMR and ¹³CNMR spectra were performed on Bruker Avance DPX instrument (250 MHz for ¹H and 62.9 MHz for ¹³C) in CDCl₃ or DMSO-d₆ with tetramethylsilane (TMS) as the 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. Also, the mass spectra were carried out with a Shimadzu GC-MS QP 1000 EX device, and elemental analysis was performed with a Thermo Fining Flash EA-1112 series. Moreover, the melting point (mp) was carried out in open capillaries with a Büchi 510 melting point device.

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

Firstly, to a solution of isatin (5.00 mmol) in DMF (23 mL) was added sodium hydride (10.00 mmol) and let to stir for 30 minutes at 40-50 °C. Then, allyl bromide (5.50 mmol) was added and the mixture either was allowed to proceed at room temperature until formation of N-allyl isatin (**2**). After that, the crude product was poured into aqueous solution including NaCl, acidified with HCl (0.2 M), and the mixture was washed with 110 mL ethyl acetate (EtOAc) for three times. 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 aromatic amine (2.00 mmol) in EtOH at reflux condition for appropriate time, and purified with EtOH to give imine (**3**). For the synthesis of β -lactams (**4a-h**), to a slution 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 stirr at room temperature overnight. The crud 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 purifaction by colum 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 percipate was filtered and wash 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**.

1'-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, CH₂N), 5.18-5.25 (2H, m, CH₂), 5.51 (1H, s, CH β -lactam ring), 5.71-5.92 (1H, m, CH), 6.54-6.59 (2H, m, Ar), 6.78-6.85 (2H, m, Ar), 6.91-7.07 (6H, m, Ar), 7.16-7.25 (2H, m, Ar), 7.42 (1H, d, *J*=7.25 Hz, Ar); ¹³C-NMR (CDCl₃, 62.5 MHz) δ 20.9 (CH₃), 42.8(CH₂-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 C₂₆H₂₂N₂O₃: 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 (CDCl₃, 250 MHz) δ 2.17 (3H, s, CH₃), 4.12-4.48 (2H, m, CH₂N), 5.13-5.21 (2H, m, CH₂), 5.31 (1H, s, CH β -lactam ring), 5.52-5.67 (1H, m, CH), 6.90-6.97 (6H, m, Ar), 7.01-7.09 (1H, m, Ar), 7.16-7.20 (1h, m, Ar), 7.24-7.29 (2H, m, Ar), 7.33-7.40 (1H, m, Ar); ¹³C-NMR (CDCl₃, 62.5 MHz) δ 20.9 (CH₃), 43.0 (CH₂-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 [³⁵M⁺], 480 [³⁷ M⁺]; Anal. Calcd. for C₂₆H₂₀Cl₂N₂O₃: 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): Gray solid (Yield 77 %); mp: 179-184 °C; IR (KBr, cm⁻¹): 1774 (CO, β -lactam), 1712 (CO, Amide); ¹H-NMR (CDCl₃, 250 MHz) δ 4.29-4.52 (2H, m, CH₂N), 5.18-5.27 (2H, m, CH₂), 5.52 (1H, s, CH β -lactam ring), 5.73-5.88 (1H, m, CH), 6.56 (2H, dd,

$J=7.7$, 1.2 Hz, Ar), 6.78-6.87 (3H, m, Ar), 6.93-7.07 (3H, m, Ar), 7.19-7.28 (3H, m, Ar), 7.39-7.47 (2H, m, Ar); ^{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 $\text{C}_{25}\text{H}_{19}\text{N}_2\text{O}_3$: 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 (CDCl_3 , 250 MHz) δ 2.78 (6H, s, CH_3N), 4.30-4.51 (2H, m, CH_2N), 5.14-5.20 (2H, m, CH_2), 5.66 (1H, s, CH β -lactam ring), 5.72-5.88 (1H, m, CH), 6.46-6.50 (2H, m, Ar), 6.70-6.72 (2H, m, Ar), 6.88-6.98 (3H, m, Ar), 7.23-7.37 (3H, m, Ar), 7.45-7.54 (3H, m, Ar), 7.60 (1H, d, $J=8.0$ Hz, Ar), 7.67-7.76 (1H, m, Ar); ^{13}C -NMR (CDCl_3 , 62.5 MHz) δ 40.56 (CH_3N , 6H), 42.8 ($\text{CH}_2\text{-N}$), 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 $\text{C}_{31}\text{H}_{27}\text{N}_3\text{O}_3$: 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 (CDCl_3 , 250 MHz) δ 2.78 (6H, s, CH_3N), 4.00-4.36 (2H, m, CH_2N), 4.99-5.08 (2H, m, CH_2), 5.35 (1H, s, CH β -lactam ring), 5.72-5.87 (1H, m, CH), 6.44-6.51 (4H, m, Ar), 6.85-7.12 (5H, m, Ar), 7.19-7.23 (1H, m, Ar), 7.32-7.40 (2H, m, Ar); ^{13}C -NMR (CDCl_3 , 62.5 MHz) δ 40.5 (CH_3N , 6H), 42.8 ($\text{CH}_2\text{-N}$), 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 [$^{35}\text{M}^+$], 475 [$^{37}\text{M}^+$]; Anal. Calcd. for $\text{C}_{27}\text{H}_{24}\text{ClN}_3\text{O}_3$: 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): Gray solid (Yield 74 %); mp: 195°C; IR (KBr, cm^{-1}): 1759 (CO, β -lactam), 1712 (CO, amide); ^1H -NMR (CDCl_3 , 250 MHz) δ 2.85 (6H, s, CH_3N), 4.26-4.52 (2H, m, CH_2N), 5.17-5.25 (2H, m, CH_2), 5.52 (1H, s, CH β -lactam ring), 5.73-5.88 (1H, m, CH), 6.54-6.59 (2H, m, Ar), 6.77-6.85 (2H, m, Ar), 6.92-7.07 (5H, m, Ar), 7.19-7.26 (3H, m, Ar), 7.42 (1H, d, $J=7.5$ Hz, Ar); ^{13}C -NMR (CDCl_3 , 62.5 MHz) δ 29.6 (CH_3N , 6H), 42.7 ($\text{CH}_2\text{-N}$), 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 $\text{C}_{27}\text{H}_{25}\text{N}_3\text{O}_3$: 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 (CDCl_3 , 250 MHz) δ 1.18 (6H, s, CH_3N), 4.09-4.48 (2H, m, CH_2N), 5.12-5.20 (2H, m, CH_2), 5.30 (1H, s, CH β -lactam ring), 5.50-5.66 (1H, m, CH), 6.81 (1H, s, Ar), 6.84 (1H, s, Ar), 6.88 (1H, d, $J=7.8$ Hz, Ar), 6.92-6.97 (4H, m, Ar), 7.06 (2H, t, $J=7.5$ Hz, Ar), 7.24-7.27 (1H, m, Ar), 7.30-7.32 (1H, m, Ar); ^{13}C -NMR (CDCl_3 , 62.5 MHz) δ 28.2 (CH_3N , 6H), 44.5 ($\text{CH}_2\text{-N}$), 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 $\text{C}_{27}\text{H}_{24}\text{Cl}_2\text{N}_3\text{O}_3$: 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, CH₃), 4.06-4.44 (2H, m, CH₂N), 5.05-5.12 (2H, m, CH₂), 5.50 (1H, s, CH β -lactam ring), 5.79-5.95 (1H, m, CH), 6.60-6.65 (2H, m, Ar), 6.70-6.77 (2H, m, Ar), 6.95 (2H, d, *J*=8.0 Hz, Ar), 7.02-7.20 (5H, m, Ar), 7.26 (1H, s, Ar), 7.39-7.43 (1H, m, Ar); 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 C₂₆H₂₂N₂O₄: 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-d₆, 250 MHz) δ 5.39 (2H, s, CH₂), 6.11 (1H, s, CH β -lactam), 7.03 (2H, d, *J*=8.7 Hz, Ar), 7.28-7.44 (4H, m, Ar), 7.56 (2H, q, *J*=7.0 Hz, Ar), 7.87 (1H, d, *J*=8.0 Hz, Ar), 8.06 (1H, d, *J*=8.0 Hz, Ar), 8.24 (1H, d, *J*=8.0 Hz, Ar), 8.41 (1H, d, *J*=7.5 Hz, Ar), 12.03 (1H, s, NH); ¹³C-NMR (DMSO-d₆, 62.5 MHz) δ 68.4 (CH₂-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 [³⁵M⁺], 500 [³⁷M⁺]; Anal. Calcd. for C₂₄H₁₇ClN₆O₄: 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-d₆, 250 MHz) δ 5.53 (2H, s, CH₂), 6.11 (1H, s, CH β -lactam ring), 7.22-7.31 (2H, m, Ar), 7.35-7.44 (2H, m, Ar), 7.52-7.58 (3H, m, Ar), 7.88 (1H, d, *J*=8.2 Hz, Ar), 8.07 (1H, d, *J*=8.7 Hz, Ar), 8.24 (1H, d, *J*=8.2 Hz, Ar), 8.42 (1H, d, *J*=7.75 Hz, Ar), 12.04 (1H, s, NH); ¹³C-NMR (DMSO-d₆, 62.5 MHz) δ 68.9 (CH₂-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 C₂₄H₁₆Cl₂N₆O₄: C, 55.08; H, 3.08; N, 13.55%. Found: C, 54.90; H, 2.93; N, 14.13%.

Antibacterial activity of new compounds

Antibacterial activity of novel β -lactams against three kinds of bacteria including *P. aeruginosa* ATCC 27853, *E. coli* ATCC 28922 and *S. aureus* ATCC 25923 was tested by the microbroth dilution method in sterile 96-well microplate. The minimal inhibitory concentration (MIC) was determined according to NCCLS guideline M7-A2. All experiments were performed according to previous reported method in our laboratory, as described in supporting information [53]. Unfortunately, none of tested compounds exhibited significant activity.

In-vitro antimalarial activity of new compounds

The *in-vitro* antimalarial activity of new compounds was evaluated against *P. falciparum* strain K1. We used previous reported method [54–59] for determination of antimalarial activity, as described in 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 docking procedure was conducted according to previous report.[60, 61]

Declarations

Acknowledgments

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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.

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Scheme

Scheme 1 is available in the Supplemental Files section.

Figures

Figure 1

Examples of some isatin-derived spiro β -lactams reported previously by our research group

Figure 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.

A

B

Figure 3

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)

Supplementary Files

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