C-5 Aryl Substituted Azaspirooxindolinones Derivatives: Synthesis and Biological Evaluation as Potential Inhibitors of Tec Family Kinases

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Abstract

The interleukin-2-inducible kinase (ITK) and Bruton tyrosine kinase (BTK) are two crucial Tec family kinase members with important roles in the development of hematopoietic malignancies, autoimmune disorders and other diseases in human. Thus, ITK and BTK are key targets for drug development. Spirooxindoles are important scaffolds for the synthesis of small molecules with broad and potent biological activities. In this study, we performed a structure-activity relationship study of a new series of 5'-(benzo[d][1,3]dioxol-5-yl)spiro[piperidine-4,3'-pyrrolo[2,3-b]pyridin]-2'(1'H)-one linked with N-acyl and C-5 aryl-substituted scaffolds in a panel of ITK and BTK cancer cell lines. Four compounds **11**, **12**, **14** and **15** showed high antiproliferative activity against ITK and BTK cell lines. Compounds **11** and **12** with a C-5 benzodioxole group and gem-dialkyl group attached to carbonyl on piperidine were highly effective in ITK-high Jurkat and CEM cell lines, and compound **14,** a biotin analogue, was identified as a good inhibitor of BTK-high RAMOS cells. Compound **15** with cyclopropyl group attached to carbonyl on piperidine also showed good activity in ITK and BTK cell lines.

Keywords: Antiproliferative activity; Bruton tyrosine kinase; CCRF-CEM cells; Interleukin-2-inducible kinase; Jurkat cells; K562 cells, RAMOS cells, Azaspirooxidole, Biotin, Suzuki-coupling reaction.

1. Introduction

Spirooxindoles, with heterocyclic motifs attached at the C-3 position of the oxindole core, are promising candidates in drug discovery and development. Reports suggest that spirooxidoles fused with different cyclic rings exhibit significant anticancer activity against selected cell lines. Likewise, spirooxindole-cyclopropane analogues [1], spirooxindole-pyrrolidine derivatives [2], spirooxindole-cyclohexane substituents fused with a pyrrole ring [3,4] and morpholine fused with 1,2,3-triazoles [5] exhibit a wide range of anticancer activity. The symmetrical trispirooxindole (JW67) is also effective against human SW480 colon adenocarcinoma cells through its effects on β-catenin levels [6]. Balan Balan *et al.* in 2020 reported that the substitution on piperidine nitrogen of spirooxindole moiety **V** results in derivates with nanomolar anticancer activity against HPK1 cell lines [7]. Further, C-5 substitution of spirooxindole derivatives of **VI** exhibited good anticancer activity [8]. The synthesised spirooxindole derivative such as

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MI-888 has been in preclinical research to treat human cancers [9].

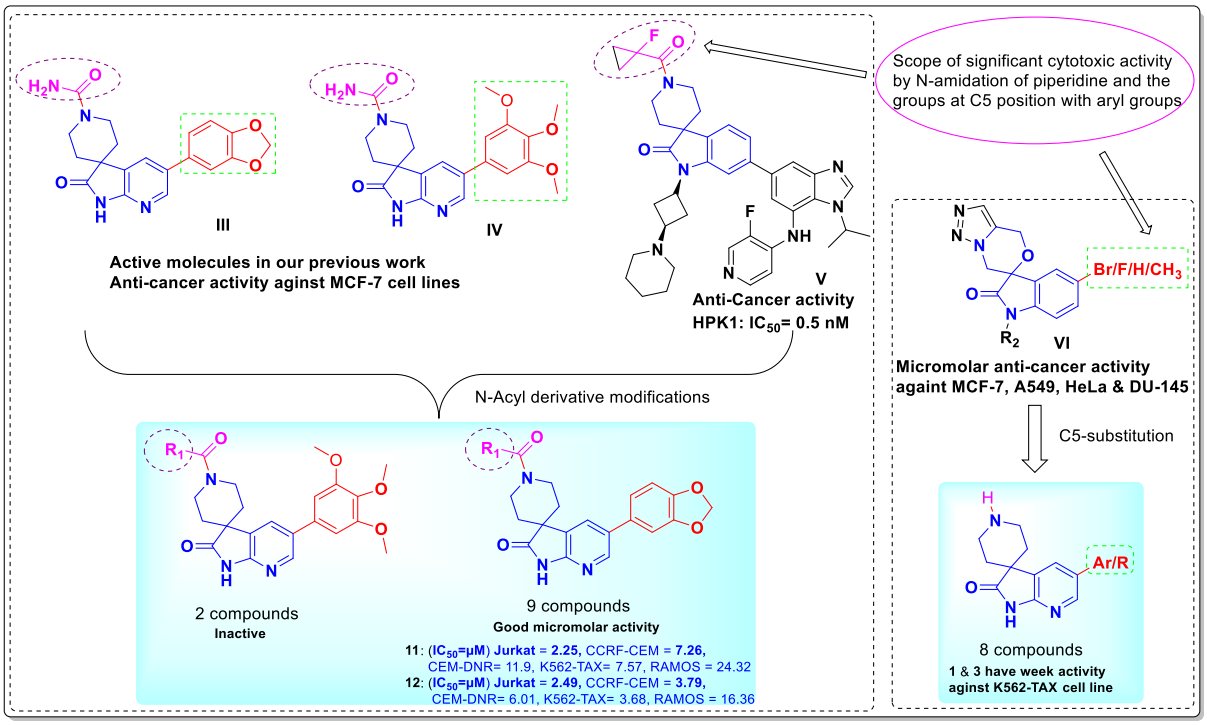


Fig. 1. Design of new series of azaspirooxindole analogues

The interleukin-2-inducible kinase (ITK) is a non-receptor protein tyrosine kinase that belongs to the Tec kinase family [10]. Although predominantly expressed in T-cell lineages, natural killer cells and mast cells are also known to express ITK [10]. By modulating calcium signalling, ITK controls the activity and translocation of transcription factors like NFAT and NFκB [11]. ITK drug discovery and development is becoming an attractive research area due to ITK's role in T-cell malignancies, autoimmune disorders and other human diseases [11–15]. Some prominent ITK-specific molecules developed since the early 2000s to target the active site include aminothiazoles, benzimidazoles, indoles, thienopyrazoles, imidazopyridines, and pyridones [16–22]. Other newly designed ITK inhibitors include aryl ketones by Mankind Pharma [23] and aminobenzothiazole by GlaxoSmithKline plc [24]. Furthermore, several diverse molecules such as 2-amino-5-(thioaryl)thiazoles, 2-amino-5-[(thiomethyl)aryl] thiazoles, (4 or 5-aryl)pyrazolyl-indoles, and benzimidazole derivatives have been reported as potent ITK inhibitors [25,26]. PRN694, a benzimidazol, is an ATP site-binding covalent inhibitor of ITK and receptor-like kinase but not Bruton tyrosine kinase (BTK) [27]. PRN694 is a potential drug for preventing colitis disease and psoriasis therapy [28].

Although there is tremendous interest in ITK as a drug target, only a few novel ITK inhibitors exist in preclinical and clinical trials [10]. Currently, ibrutinib is the only approved treatment for chronic lymphocytic leukaemia and mantle B-cell lymphoma in the European Union since 2012 [29]. Despite their biological significance, spirooxindoles and their

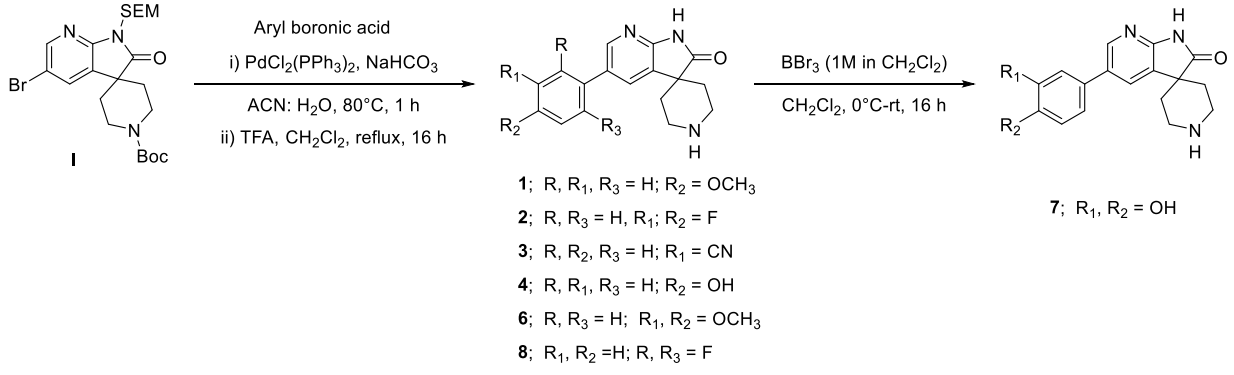
derivatives have not been explored extensively as anti-ITK compounds. Encouraged by the moderate activity of compounds **III** and **IV** against MCF-7 cell lines in our previous work [30] and structural optimisation of ibrutinib with benzodioxole group in another study [31], we herein report a structure-activity relationship study of two sets of azaspiroindolinones derivatives. First, a set of eight compounds (**1-8**) were synthesised to identify the activity of different hydrophilic groups substituted on aryl moiety on the C5 position of azaspirooxindolinone ring (Fig. 1). Next, the second series of eleven compounds (**9-19**) were prepared based on compound **III** and **IV** to evaluate the importance of carboxylic acid substituents on piperidine nitrogen by the conventional acid-amine coupling method. Altogether, we have synthesised nineteen new azaspiroindolinones and evaluated *in vitro* anticancer activity against a panel of ITK and BTK cell lines.

Results & Discussions

2.1 Chemistry

The key compounds **I**, **III** and **IV** were prepared as previously reported [30]. The 7-azaspirooxindole scaffold **I** can be easily substituted with functional groups at the C5 position by standard synthetic chemistry, allowing rapid exploration of the structure-activity relationship (SAR). Scheme **1** illustrates a two-step general synthetic route for the preparation of **1, 2, 3, 4, 6** and **8**. To synthesise these compounds, we performed Suzuki-coupling between compound **I** and commercially available aryl boronic acid in the presence of *trans*-$PdCl_2(PPh_3)_2$ catalyst and $NaHCO_3$ in acetonitrile-water at 90° C to give the intermediate C-arylation compounds. These intermediates on further global deprotection of Boc and SEM groups with trifluoroacetic acid resulted in corresponding compounds. Further, de-methylation of compound **6** was attempted with BBr_3 in dichloromethane, giving compound **7** in moderate yield.

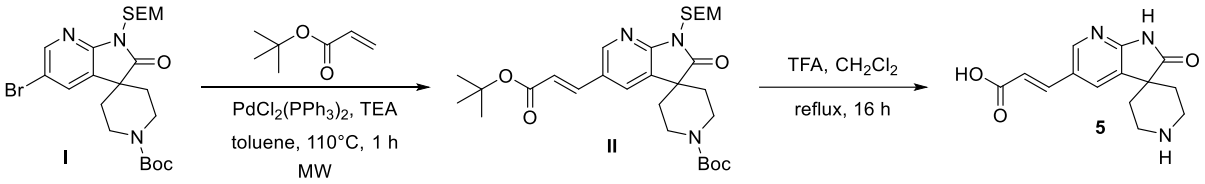
The Heck-coupling of arylbromo compound **I** and *tert*-butyl acrylate using *trans*-$PdCl_2(PPh_3)_2$ as the catalyst and triethylamine in toluene at 100° C under microwave irradiation yielded the intermediate **II** [32]. This intermediate **II** on further treatment with trifluoroacetic acid resulted in compound **5** (Scheme **2**) as a white solid.



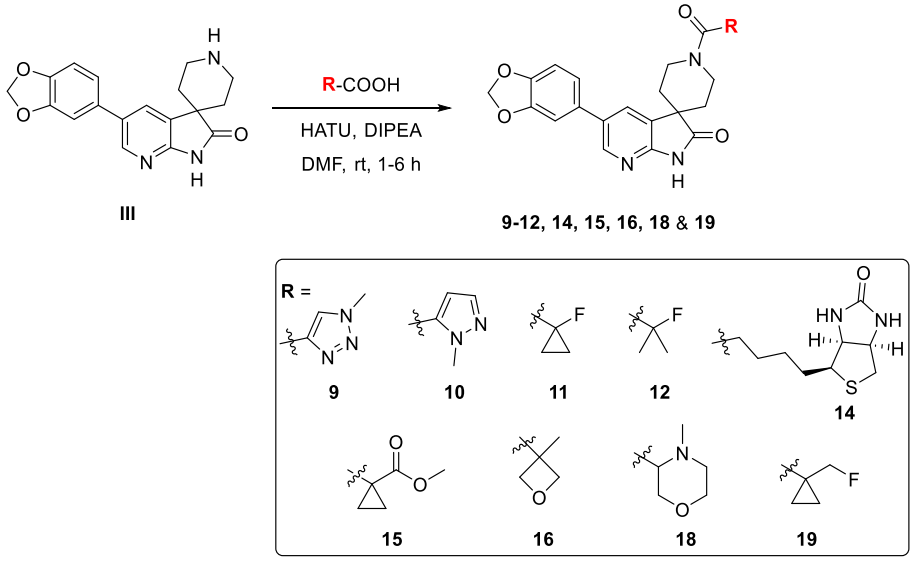
Scheme 1. Synthetic scheme for compounds **1**, **2**, **3**, **4**, **6**, **7** and **8**.

To synthesise compounds **9-12, 14, 15, 16, 18** and **19,** the key building block **III** was prepared with the same protocol reported in our previous work [30] and coupled with various carboxylic acids using HATU reagent, N, N-diisopropylethylamine in DMF at ambient temperature give the target compounds in good yield (Scheme **3**).

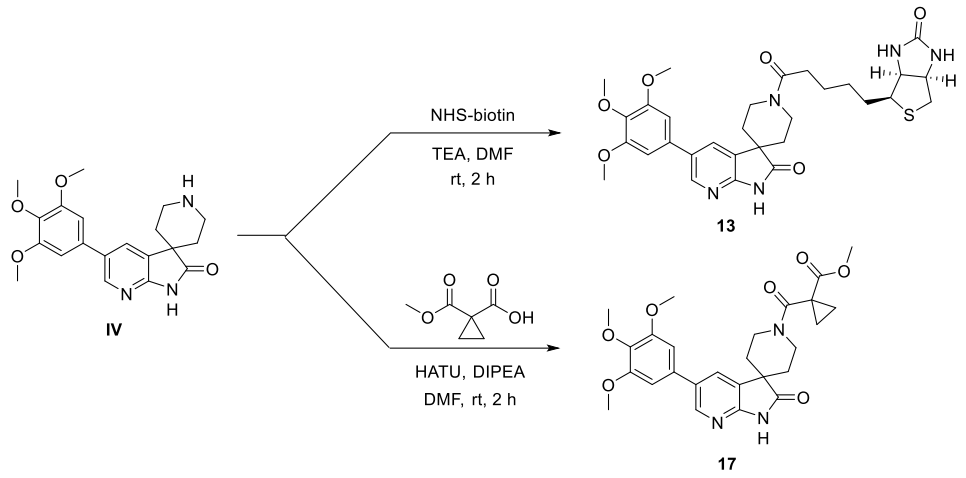
Substitution of compound **IV** with commercially available NHS-biotin using triethylamine base in DMF at room temperature gives compound **13** as a white solid. Similarly, **III** produces compound **14** as a white solid. The general acid-amine coupling of compound **IV** with 1-(methoxycarbonyl)cyclopropane-1-carboxylic acid with HATU/DIPEA obtained **17** in good yield. (Scheme **4**).



Scheme 2. Synthetic scheme of compound **5**.



Scheme 3. Synthetic scheme for compounds **9-12, 14, 15, 16, 18** and **19**.



Scheme 4. Synthetic scheme for compounds **13** and **17**.

2.2 Biology

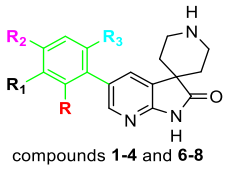
Nineteen derivatives were tested in a panel of cell lines consisting of ITK-high T-cell leukaemia lines [33,34], BTK-high B-cell leukaemia lines [33,35], ITK/BTK-null malignant lines, and two nonmalignant fibroblast lines. The compounds that did not result in 50% inhibition of cell proliferation when tested at a single dose of 50 μM were not processed further for dose-response analysis (Table 1, 2). For the cytotoxicity profiling, compounds are generally regarded as inactive if the IC_{50} is above 50 μM, weakly active above 30 μM, moderately active between 10 to 20 μM, and highly active below 10 μM. Based on this standard convention, the effect of six structurally similar compounds (active – **11, 12, 14, 15**; and inactive – **13** and **17**) were interesting to observe in ITK and BTK-high cell lines compared to ITK/BTK-null cells (Table 2). The active compounds were inactive in nonmalignant cells or showed weak activity in U2OS and HCT116 cells.

The SAR profiling indicates that group benzodioxole at the C5 position in compounds **11**, **12** and **15**, and 1-fluorocyclopropane in **11**, *gem*-dimethyl-1-fluoro in **12** and methyl-1-cyclopropane-1-carboxylate in **15** attached to carbonyl (C=O) are essential for biological activity (Table 2). The N-acyl analogue in 1-methyl-1H-1,2,3-triazole compound **9** did not enhance the antiproliferative activity. Compound **10** with 1-methyl-1H-1,2,3-triazole is inactive across all the cell lines. Compound **15** that possessed cyclopropyl attached with electron-withdrawing methyl ester group was active against most tested anticancer cell lines. This compound exhibited good anticancer activity against ITK high cell lines (IC_{50} = 14.8-28.8 μM), moderate activity in BTK high cell lines (IC_{50} =12.8-34.8 μM) and weak activity in ITK/BTK null cell lines (IC_{50} = 37.7-49.2 μM). Substitution of C5 position in **17** with 3,4,5-trimethoxy phenyl group affected compound activity in all anticancer cell lines. Similarly, compound **13,** a 3,4,5-trimethoxy phenyl-substituted at C5 analogue of active compound **14** (RAMOS IC_{50} = 9.0 μM; Jurkat IC_{50} = 26.0 μM)**,** did not possess any antiproliferative activity in tested cell lines. Increasing the ring size by introducing oxetane ring with the hydrophobic *tert*-methyl group at N-acyl carbonyl in **16** did not improve the activity against ITK cell lines (CCRF-CEM IC_{50} = 33.4 μM; CEM-DNR IC_{50} = 49.7 μM). Compound **18** with a six-membered N-methylmorpholine was inactive in all cell lines. This result

indicates that cyclopropyl or a similar *gem*-dimethyl group on N-acyl carbonyl of spiropiperidine ring is essential for the activity of this class of compounds. As expected, compound **19**, resulting from fluoromethylcyclopropane, was also moderately active in ITK high cell lines (CCRF-CEM IC_{50} = 25.5 µM; CEM-DNR IC_{50} = 28.9 µM).

Surprisingly, structural activity relationship profiling for C5 aryl substituent motifs with electron-rich atoms/groups such as 3,4-difluorophenyl (**2**), 2,6-difluorophenyl (**8**), 4-hyrdoxy phenyl analogue (**4**), 3,4-dimethoxyphenyl (**6**) and 3,4-dihydroxyphenyl substituent **7** without any group on the piperidine nitrogen were inactive against all cell lines. Compound **1** with 3-methoxyphenyl and compound **3** with 3-cyanophenyl group were poorly active against K562-tax cells. The C5 alkyl substitution compound **5** with a double bond is inactive in all cell lines.

Table 1. IC_{50} values of compounds **1-4** and **6-8** in cancer and non-cancer cell lines. Mean ± SD of at least 3 independent experiments**.**



compounds **1-4** and **6-8**

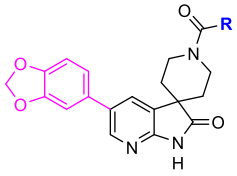
ıpd	ł, R3	R1	R2	IC50 (µM)										
				BJ, MRC-5	A549	HCT116	CT116 p53-/-	U2OS	RAMOS	K562	62-TAX	M-DNR	CRF-CEM	Jurkat
					ITK/BTK null				BTK high			ITK high		
1	*H*	*OMe*	*H*	>50	>50	>50	>50	>50	>50	>50	44.5	>50	>50	>50
2	*H*	*F*	*F*	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
3	*H*	*CN*	*H*	>50	>50	>50	>50	>50	>50	>50	46.2	>50	>50	>50
4	*H*	*H*	*OH*	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
6	*H*	*OMe*	*OMe*	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
7	*H*	*OH*	*OH*	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
8	F	H	H	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50

3. Conclusion

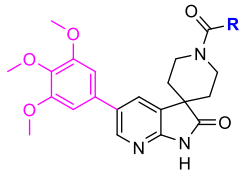
We have reported two sets of azaspiroindolinone derivatives and reported high activity of 4 structurally similar compounds against ITK and BTK positive cell lines. The active compounds **11** and **12** displayed a higher activity against ITK positive lines and moderate activity in BTK lines, whereas **14** was highly active in BTK than ITK cell lines. Members of the Tec kinase family share similarities in structure, consisting of PH domain, SH3 domain, SH2 domain and kinase domain [10]. The selectivity of active compounds against ITK and other members of the Tec kinase family is currently being planned for the identified active compounds. Further, coupling with aryl or alkyl groups on spiropiperidine amide (-NH) can generate derivatives with higher specificity against ITK and/or BTK. Nonetheless, given the role of immune cell-mediated human disorders [10], our findings provide new scaffolds for

stimulating the development of therapeutics directed against ITK and BTK.

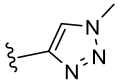
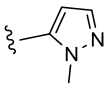
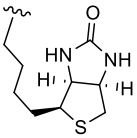
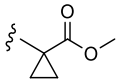
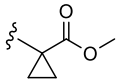
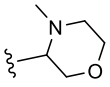
Table 2. IC_{50} values of compounds **9-19** in cancer and non-cancer cell lines. Mean ± SD of at least 3 independent experiments.



Compounds **9-12, 14-16, 18** & **19**



Compounds **13** &**17**

Cmpd.	Substituent (R)	IC_{50} (µM)										
		BJ, MRC-5	A549	HCT116	HCT116 p53-/-	U2OS	RAMOS	K562	K562-TAX	CEM-DNR	CCRF-CEM	Jurkat
			ITK/BTK null				BTK high			ITK high		
9		>50	>50	>50	>50	>50	>50	>50	22.4	43.2	45.9	>50
10		>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
11		>50	>50	>50	>50	36.2	24.3	>50	7.6	11.9	7.3	2.3
12		>50	>50	>50	>50	30.3	16.4	>50	3.9	6.0	3.8	2.5
13		>50	>50	>50	>50	>50	>50	>50	50	>50	>50	>50
14		>50	>50	>50	>50	>50	9.0	>50	>50	>50	29.8	26.0
15		>50	>50	37.7	41.6	49.2	24.0	34.8	12.5	20.5	15.2	14.8
16		>50	>50	>50	>50	>50	>50	>50	50	49.7	33.2	>50
17		>50	>50	>50	>50	>50	>50	>50	50	>50	>50	>50
18		>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
19		>50	>50	>50	>50	>50	>50	>50	30.7	28.9	25.5	>50

4.1 Experimental section

4.1 Chemistry

4.1.1 Materials and Methods

All chemicals (reagent grade) used were purchased from Combi-Blocks (USA), Johnson Matthey Co., Ltd., (USA) and Enamine Ltd. (Ukraine). All the solvents used for the reaction are LR grade. Analytical thin-layer chromatography (TLC.) was performed on

precoated silica gel 60 F_{254} plates, and visualisation on TLC was achieved by UV light. Flash column chromatography was undertaken on silica gel (100−200 mesh).^{1}H NMR was recorded on 400 or 500 MHz, and chemical shifts were quoted in parts per million (ppm) referenced to 0.0 ppm for tetramethylsilane. The following abbreviations were used to describe peak splitting patterns when appropriate: br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet. Coupling constants, *J*, were reported in the hertz unit (Hz). API LC-mass spectra were obtained on Agilent and Waters instruments. All the final compounds were purified on GRACE flash chromatography by using C18 reverse-phase columns. The mobile phase was a mixture of water (0.1% formic acid) and acetonitrile. Melting points were recorded on the Buchi M-560 instrument.

4.1.2 General method A

A solution mixture of arylbromo compound **I** (1.0 eq, 0.15 mmol), aryl boronic acid (1.5 eq.), $PdCl_2(PPh_3)_2$ (0.05 eq), X-Phos (0.1 eq.) and $NaHCO_3$ (2.0 eq) in acetonitrile: water (8:2) (4.0 mL) were de-gassed with argon for about 5 min. The resulting reaction mixture was maintained under stirring at 90 °C for 1-3 h. The reaction mixture was allowed to room temperature, diluted with ethyl acetate (25-30 mL), added 0.2 g of activated charcoal and filtered through celite. The filtrate was partitioned between water and ethyl acetate. The combined ethyl acetate layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The crude was purified by column chromatography over silica gel (100-200 mesh) using a solvent gradient mixture of 20-60% ethyl acetate in pet-ether as an eluent to afford the desired C-aryl compound. A mixture of the intermediate compound (0.1 mmol) and TFA (2.0 mL) in dichloromethane (2.0 mL) was heated to 60°C for 16 h and distilled off the volatiles under reduced pressure. The crude residue was purified by GRACE flash chromatography using a C18 column with 0.1% formic acid in water and acetonitrile as an eluent to obtain the final compound.

4.1.3 General method B

To a solution of acid (1.0 eq.), HATU (1.5 eq.) and DIPEA (3.0 eq.) in DMF (0.2 M) was added amine (1.2 eq.) at room temperature. The reaction mixture was stirred at room temperature for 2 h. The reaction mixture was partitioned between water (30 mL) and ethyl acetate (30 mL X 3). The combined organic layer was washed with water (50 mL), brine (50 mL), dried over anhydrous Na_2SO_4 and concentrated. The crude was purified by GRACE flash chromatography using a C18 column with 0.1% formic acid in water and acetonitrile as an eluent to give the final compound.

4.2. Synthesis of compounds 1, 2, 3, 4, 6 and 8 (Following general method A)

4.2.1 5'-(4-Methoxyphenyl)spiro[piperidine-4,3'-pyrrolo[2,3-b]pyridin]-2'(1'H)-one (**1**). Compound **1** was obtained from tert-butyl 5'-bromo-2'-oxo-1'-((2-(trimethylsilyl)ethoxy)methyl)-1',2'-dihydrospiro[piperidine-4,3'-pyrrolo[2,3-b]pyridine]-1-carboxylate and (4-methoxyphenyl)boronic acid as an off-white solid . Yield: 120 mg, 50%, MP: 232-238 °C, Pale brown solid. FT- IR (KBr): vmax 3777, 3473, 2842, 1722, 1591, 1474, 1199, 1124, 1016, 756, 665 cm-1. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 11.07 (brs, 1H), 8.21 (s, 1H), 7.88 (s, 1H), 7.39-7.35 (m, 2H), 7.25-7.21 (m, 1H), 3.96 (s, 3H), 3.46-3.49 (m, 2H), 3.33-3.30 (m, 2H), 2.03-1.98 (m, 4H). ^{13}C NMR: (100 MHz, DMSO-d_6): 28.52, 43.42, 55.52, 111.72, 120.90, 126.47, 127.94, 129.21, 130.22, 131.91, 146.69, 154.76, 156.16, and 179.82. LC-MS (ES-API): *m/z* = 310.3 $(M+H)^+$.

4.2.2 5'-(3,4-Difluorophenyl)spiro[piperidine-4,3'-pyrrolo[2,3-b]pyridin]-2'(1'H)-one (**2**).

Compound **2** was obtained from tert-butyl 5'-bromo-2'-oxo-1'-((2-(trimethylsilyl)ethoxy)methyl)-1',2'-dihydrospiro[piperidine-4,3'-pyrrolo[2,3-b]pyridine]-1-carboxylate and (3,4-difluorophenyl)boronic acid as a white solid. Yield: 80 mg, 65%, MP: 253-255 °C. FT-IR (KBr): vmax 3447, 3022, 2812, 1711, 1610, 1466, 1202, 1126, 836, 656 cm-1. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.28 (d, J = 2.0 Hz, 1H), 7.88 (d, J = 2.0 Hz, 1H), 7.43 (d, J = 6.8 Hz, 2H), 6.88 (d, J = 6.8 Hz, 2H), 3.82-3.75 (m, 2H), 3.45-3.39 (m, 2H), 3.25-3.09 (m, 4H). ^{13}C NMR: (100 MHz, DMSO-d6): 30.27, 40.67, 44.77, 116.96, 128.80, 129.02, 130.16, 130.70, 133.68, 145.76, 155.58, 158.78 and 181.64. LC-MS (ES-API): m/z = 296.3 $(M+H)^+$.

4.2.3 3-(2'-Oxo-1',2'-dihydrospiro[piperidine-4,3'-pyrrolo[2,3-b]pyridin]-5'-yl)benzonitrile (**3**). Compound **3** was obtained from tert-butyl 5'-bromo-2'-oxo-1'-((2-(trimethylsilyl)ethoxy)methyl)-1',2'-dihydrospiro[piperidine-4,3'-pyrrolo[2,3-b]pyridine]-1-carboxylate and (3-cyanophenyl)boronic acid as a white solid. Yield: 43 mg, 36%. MP: 201-208 °C. FT-IR (KBr): vmax 3492, 3402, 3079, 2229, 1726, 1605, 1467, 1233, 1186, 1126, 794, 763, 531 cm-1. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.41 (d, J = 2.0 Hz, 1H), 7.98 (d, J = 2.0 Hz, 1H), 7.23-7.19 (m, 3H), 7.04 (d, J = 4.0 Hz, 2H), 3.87 (s, 3H), 3.79 (s, 3H), 3.51-3.36 (m, 4H), 2.12-2.02 (m, 4H). ^{13}CNMR: (125 MHz, DMSO-d6): 28.42, 43.47, 55.59, 110.47, 112.25, 118.84, 127.33, 129.57, 130.14, 130.41, 144.63, 148.57, 149.16, 154.86 and 179.76. LC-MS (ES-API): m/z = 305.3 (M+H)+

4.2.4 5'-(4-Hydroxyphenyl)spiro[piperidine-4,3'-pyrrolo[2,3-b]pyridin]-2'(1'H)-one (**4**) Compound **4** was obtained from tert-butyl 5'-bromo-2'-oxo-1'-((2-(trimethylsilyl)ethoxy)methyl)-1',2'-dihydrospiro[piperidine-4,3'-pyrrolo[2,3-b]pyridine]-1-carboxylate and (4-hydroxyphenyl)boronic acid as a white solid. Yield: 40 mg, 35%. MP: 318-321 °C, Brown solid. FT-IR (KBr): vmax 3447, 3022, 2812, 1711, 1684, 1610, 1466, 1442, 1202, 1126, 836, 718, 658 cm-1. ^{1}H-NMR (400 MHz, CD_3OD): δ 8.28 (d, J = 2.0 Hz, 1H), 7.88 (d, J = 2.0 Hz, 1H), 7.45-7.41 (m, 2H), 6.90-6.87 (m, 2H), 3.82-3.75 (m, 2H), 3.45-3.39 (m, 2H), 2.25-2.09 (m, 4H). ^{13}C NMR: (100 MHz, CD_3OD): 30.27, 40.67, 44.77, 116.96, 128.80, 129.02, 130.16, 130.70, 133.68, 145.76, 155.58, 158.78, and 181.64. LC-MS (ES-API): m/z = 296.3 $(M+H)^+$.

4.2.5 5'-(3,4-Dimethoxyphenyl)spiro[piperidine-4,3'-pyrrolo[2,3-b]pyridin]-2'(1'H)-one (**6**) *(Scheme 1)*: Compound **6** was obtained from tert-butyl 5'-bromo-2'-oxo-1'-((2-(trimethylsilyl)ethoxy)methyl)-1',2'-dihydrospiro[piperidine-4,3'-pyrrolo[2,3-b]pyridine]-1-carboxylate and (3,4-dimethoxyphenyl)boronic acid as a white solid. Yield: 150 mg, 75%, MP: 272-275 °C. FT-IR (KBr): vmax 3117, 2993, 2851, 1673, 1615, 1539, 1470, 1329, 1204, 1138, 838, 802, 721 cm-1. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 11.30 (brs, 1H), 8.75 (brs, 1H), 8.41 (d, J = 2.0 Hz, 1H), 7.98 (d, J = 2.0 Hz, 1H), 7.23-7.19 (m, 2H), 7.04 (d, J = 8.5 Hz, 1H), 3.86 (s, 3H), 3.79 (s, 3H), 3.51-3.46 (m, 2H), 3.40-3.36 (m, 2H), 2.11-2.02 (m, 4H). ^{13}C NMR: (125 MHz, DMSO-d_6): δ 28.42, 43.47, 55.44, 110.47, 112.25, 118.84, 127.33, 129.57, 130.14, 130.41, 144.63, 148.57, 149.16, 154.86, and 179.76. LC-MS (ES-API): m/z = 340.0 $(M+H)^+$.

4.2.6. 5'-(2,6-Difluorophenyl)spiro[piperidine-4,3'-pyrrolo[2,3-b]pyridin]-2'(1'H)-one (**8**) *(Scheme 1)*: Compound **8** was obtained from tert-butyl 5'-bromo-2'-oxo-1'-((2-(trimethylsilyl)ethoxy)methyl)-1',2'-dihydrospiro[piperidine-4,3'-pyrrolo[2,3-b]pyridine]-1-carboxylate and (2,6-difluorophenyl)boronic acid as a white solid. Yield: 75.0 mg, 61%, MP: 280-284 °C. White solid. FT-IR (KBr): vmax 3094, 1730, 1602, 1467, 1321, 1223, 772, 669 cm-1. ^{1}H-NMR

(500 MHz, DMSO-d_6): δ 11.38 (s, 1H), 8.86 (s, 1H), 8.46 (s, 1H), 8.10 (s, 1H), 7.87 (t, J = 10.0 Hz, 1H), 7.57-7.51 (m, 2H), 3.52-3.41 (m, 4H), 2.11-1.98 (m, 4H). ^{13}C NMR: (125 MHz, DMSO-d_6): δ 28.77, 44.18, 116.18, 116.32, 118.36, 118.49, 123.91, 127.80, 128.67, 130.27, 135.61, 145.70, 156.28, and 180.15. LC-MS (ES-API): *m/z* = 316.1 $(M+H)^+$.

4.3 3-(2'-Oxo-1',2'-dihydrospiro[piperidine-4,3'-pyrrolo[2,3-b]pyridin]-5'-yl)acrylic acid (**5**): A solution mixture of arylbromo tert-butyl 5'-bromo-2'-oxo-1'-((2-(trimethylsilyl)ethoxy)methyl)-1',2'-dihydrospiro[piperidine-4,3'-pyrrolo[2,3-b]pyridine]-1-carboxylate **I** (1.0 eq, 0.15 mmol), *t*-butylacrylate (1.5 eq.), $PdCl_2(PPh_3)_2$ (0.05 eq), triethylamine (2.0 eq) in DMF (5.0 mL) were stirred at 100 °C for 1 h under MW irradiation. The reaction mixture was diluted with water, extracted with ethyl acetate twice. The combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The crude was purified by column chromatography over silica gel (100-200 mesh) using a solvent gradient mixture of 40% ethyl acetate in pet-ether as an eluent afforded desired compound. This compound (0.1 mmol) on de-protection with TFA (2.0 mL) in dichloromethane (2.0 mL) at 60°C for 10 h. Distilled off the volatiles under reduced pressure. The crude residue was purified by GRACE flash chromatography using C18 column with 0.1% formic acid in water and acetonitrile as an eluent to obtain the title compound. Yield 64%, MP: 280-284 °C. White solid. FT-IR (KBr): vmax 3217, 3015, 2824, 1726, 1675, 1624, 1481, 1404, 1212, 1139, 976, 720, 666 cm-1. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 12.39 (brs, 1H), 11.50 (s, 1H), 8.71 (brs, 1H), 8.40 (s, 1H), 8.23 (s, 1H), 7.61 (d, J = 16.0 Hz, 1H), 7.65 (d, J = 16.0 Hz, 1H), 3.48-3.36 (m, 4H), 2.08-1.93 (m, 4H). ^{13}C NMR: (125 MHz, DMSO-d6): δ 28.21, 39.16 (merged in DMSO signal), 43.63, 118.43, 124.75, 127.64, 128.96, 141.07, 148.86, 157.39, 167.58, and 179.59. LC-MS (ES-API): m/z = 274.0 $(M+H)^+$.

4.4 5'-(3,4-Dihydroxyphenyl)spiro[piperidine-4,3'-pyrrolo[2,3-b]pyridin]-2'(1'H)-one (**7**): To a solution of **6** (250 mg) in dichloromethane (10 mL) was cooled to 0 °C and added a solution of BBr_3 (5.0 eq.) (1.0 M in DCM) drop wise. The resulting reaction mixture was stirred at ambient temperature for 16 h under N_2. The resulting mixture was quenched with methanol (2.0 mL) and concentrated. The residue was alkaline with $NaHCO_3$ solution, extracted with ethyl acetate (30 mL x 3). The combined organic phase was washed with brine (50 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give a residue, which was purified by GRACE flash chromatography using C18 column with 0.1% formic acid in water and acetonitrile as an eluent to obtain the title compound as a brown solid. Yield: 50%, M.P: 255-258°C. FT-IR (KBr): vmax 3094, 1703, 1602, 1467, 1321, 1223, 772, 669 cm-1. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 11.13 (s, 1H), 8.39 (s, 1H), 8.24 (s, 1H), 7.91 (s, 1H), 7.04 (s, 1H), 6.92 (s, 1H), 6.81 (s, 1H), 3.40-3.21 (m, 4H), 1.91-1.83 (m, 4H). ^{13}C NMR: (125 MHz, DMSO-d_6): δ 30.25, 44.93, 75.91, 114.50, 116.68, 118.01, 128.47, 129.16, 129.66, 131.10, 144.29, 145.85, 146.37, 154.99, and 180.60. LC-MS (ES-API): m/z = 312.0 (M+H)+.

4.5. Synthesis of compounds 9 **to** 18 **(Following general method B)**

4.5.1 5'-(Benzo[d][1,3]dioxol-5-yl)-1-(1-methyl-1H-1,2,3-triazole-4-carbonyl)spiro[piperidine-4,3'-pyrrolo[2,3-b]pyridin]-2'(1'H)-one (**9**): Compound **9** was obtained from 5'-(benzo[d][1,3]dioxol-5-yl)spiro[piperidine-4,3'-pyrrolo[2,3-b]pyridin]-2'(1'H)-one and 1-methyl-1H-1,2,3-triazole-4-carboxylic acid as a white solid. Yield: 55 mg, 41%, MP: 215-217 °C. FT-IR (KBr): vmax 3421, 3099, 1716, 1643, 1465, 1213, 1033, 806, 669 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6): δ

8.51 (s, 1H), 8.35 (d, *J* = 2.0 Hz, 1H), 8.17 (d, *J* = 2.0 Hz, 1H), 7.34 (d, *J* = 2.4 Hz, 1H), 7.19 (dd, *J* = 1.6, 8.0 Hz, 1H), 6.98 (d, *J* = 8.0 Hz, 1H), 6.05 (s, 2H), 4.40-4.33 (m, 2H), 4.10 (s, 3H), 4.03-3.98 (m, 2H), 1.91-1.86 (m, 4H). ^{13}C NMR: (100 MHz, DMSO-d_6): *δ* 31.93, 32.86, 36.93, 38.09, 42.22, 45.90, 101.60, 107.59, 109.11, 120.56, 128.73, 129.60, 130.15, 130.43, 132.07, 143.58, 144.60, 147.25, 148.46, 155.45, 160.28, and 180.87. LC-MS (ES-API): *m/z* = 433.1 $(M+H)^+$.

4.5.2. 5'-(Benzo[d][1,3]dioxol-5-yl)-1-(1-methyl-1H-pyrazole-5-carbonyl)spiro[piperidine-4,3'-pyrrolo[2,3-b]pyridin]-2'(1'H)-one (**10**): Compound **10** was obtained from 5'-(benzo[d][1,3]dioxol-5-yl)spiro[piperidine-4,3'-pyrrolo[2,3-b]pyridin]-2'(1'H)-one and 1-fluorocyclopropane-1-carboxylic acid as a white solid. Yield: 60 mg, 34%. MP: 312-315 °C. FT-IR (KBr): vmax 3412, 2935, 2711, 1706, 1633, 1458, 1230, 1031, 786, 669 cm^{-1}. ^{1}H-NMR (500 MHz, DMSO-d_6): *δ* 11.16 (s, 1H), 8.34 (s, 1H), 8.15 (s, 1H), 7.48 (s, 1H), 7.32 (s, 1H), 7.18 (d, *J* = 7.5 Hz, 1H), 7.00 (d, *J* = 7.5 Hz, 1H), 6.53 (s, 1H), 6.06 (s, 2H), 4.10-3.75 (m, 7H), 1.95-1.80 (m, 4H). ^{13}C NMR: (125 MHz, DMSO-d_6): δ 31.31, 37.60, 42.52, 45.23, 101.14, 106.24, 107.09, 108.66, 120.09, 128.03, 129.73, 129.99, 131.61, 135.47, 137.38, 144.19, 146.79, 147.97, 154.96, 160.47 and 180.28. LC-MS (ES-API): *m/z* = 432.1 $(M+H)^+$.

4.5.3. 5'-(Benzo[d][1,3]dioxol-5-yl)-1-(1-fluorocyclopropane-1-carbonyl)spiro[piperidine-4,3'-pyrrolo[2,3-b]pyridin]-2'(1'H)-one (**11**): Compound **11** was obtained from 5'-(benzo[d][1,3]dioxol-5-yl)spiro[piperidine-4,3'-pyrrolo[2,3-b]pyridin]-2'(1'H)-one and 1-methyl-1H-pyrazole-5-carboxylic acid as an off-white white solid. Yield: 60 mg, 47%, MP: 235-240 °C. FT-IR (KBr): vmax 3428, 3097, 1715, 1645, 1469, 1214, 1305, 802, 808, 671, 518 cm^{-1}. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 11.17 (s, 1H), 8.35 (s, 1H), 8.16 (s, 1H), 7.33 (s, 1H), 7.19 (d, *J* = 7.5 Hz, 1H), 6.99 (d, *J* = 8.0 Hz, 1H), 6.05 (s, 2H), 4.10-3.80 (m, 4H), 1.96-1.78 (m, 4H), 1.31-1.22 (m, 4H). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.47 (s, 1H), 8.30 (s, 1H), 7.64 (s, 1H), 6.98-6.96 (m, 2H), 6.90 (d, *J* = 8.0 Hz, 1H), 6.02 (s, 2H), 4.22-4.89 (m, 4H), 2.05-1.94 (m, 4H), 1.40-1.25 (m, 4H). ^{13}C NMR: (125 MHz, DMSO-d_6): δ 10.72, 45.29, 77.34, 79.18, 101.12, 107.07, 108.63, 120.07, 128.15, 129.65, 129.96, 131.57, 144.14, 146.76, 147.97, 154.95, 164.99, 165.14, and 180.34. LC-MS (ES-API): *m/z* = 410.1 $(M+H)^+$.

4.5.4. 5'-(Benzo[d][1,3]dioxol-5-yl)-1-(2-fluoro-2-methylpropanoyl)spiro[piperidine-4,3'-pyrrolo[2,3-b]pyridin]-2'(1'H)-one (**12**): Compound **12** was obtained from 5'-(benzo[d][1,3]dioxol-5-yl)spiro[piperidine-4,3'-pyrrolo[2,3-b]pyridin]-2'(1'H)-one and 2-fluoro-2-methylpropanoic acid a white solid. Yield: 55 mg, 44%. MP: 249-253 °C. FT-IR (KBr): vmax 3425, 3089, 2985, 1722, 1629, 1458, 1217, 1031, 806, 655 cm^{-1}. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 11.16 (s, 1H), 8.34 (d, *J* = 1.5 Hz, 1H), 8.21 (brs, 1H), 7.63 (d, *J* = 2.0 Hz, 1H), 6.98-6.96 (m, 2H), 6.90 (d, *J* = 8.0 Hz, 1H), 6.02 (s, 2H), 4.22-4.18 (m, 3H), 3.91-3.89 (m, 1H), 2.03-1.99 (m, 2H), 1.94-1.88 (m, 2H), 1.69 (s, 3H), 1.65 (s, 3H). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.30 (d, *J* = 2.0 Hz, 1H), 8.21 (s, 1H), 7.63 (d, *J* = 2.0 Hz, 1H), 6.98-6.96 (m, 3H), 6.90 (d, *J* = 8.0 Hz, 1H), 6.02 (s, 2H), 4.19-4.18 (m, 3H), 3.91-3.89 (m, 1H), 2.03-1.99 (m, 2H), 1.93-1.90 (m, 2H), 1.67 (d, *J* = 21.5 Hz, 6H). ^{13}C NMR: (125 MHz, DMSO-d_6): 25.66, 25.85, 45.31, 95.91, 97.34, 101.11, 107.12, 108.61, 120.10, 128.18, 129.66, 129.94, 131.57, 144.12, 146.75, 147.95, 154.94, 169.19 and 180.35. LC-MS (ES-API): *m/z* = 412.1 $(M+H)^+$.

4.5.5 (3aS,4S,6aR)-4-(5-Oxo-5-(2'-oxo-5'-(3,4,5-trimethoxyphenyl)-1',2'-dihydrospiro[piperidine-4,3'-pyrrolo[2,3-b]pyridin]-1-yl)pentyl)tetrahydro-1H-thieno[3,4-d]imidazol-2(3H)-one (**13**): A solution of 5'-(3,4,5-

trimethoxyphenyl)spiro[piperidine-4,3'-pyrrolo[2,3-b]pyridin]-2'(1'H)-one **III** (0.271 mmol), 2,5-dioxopyrrolidin-1-yl 5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanoate (0.271 mmol) and triethylamine (0.541 mmol) in DMF (2.0 mL) were stirred at room temperature for 16 h. The reaction mixture was partitioned between ethyl acetate (30 ml x 2) and water (50 mL). The separated organic layer was washed with brine, dried over anhyd. Na_2SO_4 and concentrated. The crude was purified by GRACE flash chromatography using C18 column with 0.1% formic acid in water and acetonitrile as an eluent afforded the title compound as a white solid. Yield: 62 mg, 46%, M.P: 242-246°C. FT-IR (KBr): vmax 3404, 2927, 1712, 1598, 1458, 1230, 1116, 1012, 839, 667 cm^{-1}. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 11.12 (s, 1H), 8.41 (d, *J* = 1.5 Hz, 1H), 8.10 (s, 1H), 6.90 (s, 2H), 6.45 (s, 1H), 6.36 (s, 1H), 4.31-4.29 (m, 1H), 4.15-4.13 (m, 1H), 4.01-3.68 (m, 13H), 3.14-3.12 (m, 1H), 2.84-2.78 (m, 1H), 2.58-2.56 (m, 1H), 2.48-2.32 (m, 2H), 1.91-1.72 (m, 4H), 1.68-1.47 (m, 4H), 1.40-1.36 (m, 2H). ^{13}C NMR: (125 MHz, DMSO-d_6): 24.93, 28.16, 28.39, 31.47, 32.24, 36.44, 45.21, 55.50, 56.07, 59.17, 60.05, 61.05, 104.22, 128.30, 129.75, 130.49, 133.30, 137.08, 144.68, 153.25, 155.24, 162.70, 170.61 and 180.44. LC-MS (ES-API): *m/z* = 596.2 (M+H)+.

4.5.6. (3aS,4S,6aR)-4-(5-(5'-(Benzo[d][1,3]dioxol-5-yl)-2'-oxo-1',2'-dihydrospiro[piperidine-4,3'-pyrrolo[2,3-b]pyridin]-1-yl)-5-oxopentyl)tetrahydro-1H-thieno[3,4-d]imidazol-2(3H)-one (**14**) : Compound **14** was prepared by using the same procedure of compound **13** from 5'-(benzo[d][1,3]dioxol-5-yl)spiro[piperidine-4,3'-pyrrolo[2,3-b]pyridin]-2'(1'H)-one **III** and 2,5-dioxopyrrolidin-1-yl 5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanoate as a white solid. Yield: 68 mg, 51%, M.P: 185-187°C. FT-IR (KBr): vmax 3435, 3273, 2922, 1712, 1618, 1456, 1222, 1026, 931, 794, 675 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.12 (s, 1H), 8.34 (s, 1H), 8.10 (s, 1H), 7.32-6.98 (m, 3H), 6.45-6.32 (m, 2H), 6.05 (s, 2H), 4.30-4.15 (m, 2H), 3.90-3.79 (m, 4H), 3.12 (s, 1H), 2.83-2.81 (m, 1H), 2.59-2.37 (m, 3H), 1.83-1.39 (m, 10H). ^{13}C NMR: (125 MHz, DMSO-d_6): 24.95, 28.14, 28.38, 31.45, 32.12, 32.21, 36.52, 45.34, 55.49, 59.16, 61.03, 101.12, 107-08, 108.64, 120.06, 128.28, 129.60, 129.91, 131.60, 144.09, 146.76, 147.97, 154.97, 162.68, 170.64 and 180.41. LC-MS (ES-API): *m/z* = 550.1 $(M+H)^+$.

4.5.7. Methyl 1-(5'-(benzo[d][1,3]dioxol-5-yl)-2'-oxo-1',2'-dihydrospiro[piperidine-4,3'-pyrrolo[2,3-b]pyridine]-1-carbonyl)cyclopropane-1-carboxylate (**15**): Compound **15** was obtained from 5'-(benzo[d][1,3]dioxol-5-yl)spiro[piperidine-4,3'-pyrrolo[2,3-b]pyridin]-2'(1'H)-one and 1-(methoxycarbonyl)cyclopropane-1-carboxylic acid as a pale yellow solid. Yield: 68 mg, 49%. M.P: 280-284°C. FT-IR (KBr): vmax 3425, 3099, 2956, 1722, 1633, 1458, 1222, 1149, 1029, 837, 659 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.45 (brs, 1H), 8.30 (d, *J* = 2.0 Hz, 1H), 7.62 (d, *J* = 2.0 Hz, 1H), 7.97-7.95 (m, 2H), 6.91 (d, *J* = 8.0 Hz, 1H), 6.02 (s, 2H), 4.20-4.17 (m, 1H), 4.05-4.00 (m, 1H), 3.92-3.91 (m, 1H), 3.82-3.78 (m, 1H), 3.76 (s, 3H), 2.02-1.88 (m, 4H), 1.58-1.52 (m, 2H), 1.43-1.38 (m, 2H). ^{13}C NMR: (100 MHz, DMSO-d_6): 15.31, 15.53, 28.21, 31.20, 31.35, 37.06, 45.15, 52.45, 101.13, 107.05, 108.66, 120.05, 128.17, 129.55, 129.96, 131.62, 144.16, 146.77, 147.97, 154.96, 165.44, 171.52 and 180.34. LC-MS (ES-API): *m/z* = 450.1 $(M+H)^+$.

4.5.8. 5'-(Benzo[d][1,3]dioxol-5-yl)-1-(3-methyloxetane-3-carbonyl)spiro[piperidine-4,3'-pyrrolo[2,3-b]pyridin]-2'(1'H)-one (**16**): Compound **16** was obtained from 5'-(benzo[d][1,3]dioxol-5-yl)spiro[piperidine-4,3'-pyrrolo[2,3-b]pyridin]-2'(1'H)-one and 3-methyloxetane-3-carboxylic acid as an off-white solid. Yield: 48 mg, 37%. M.P: 281-285°C. FT-IR (KBr): vmax 3427, 2877, 1722, 1624, 1456, 1219, 1029, 829, 657 cm^{-1}. ^{1}H-

NMR (400 MHz, DMSO-d_6): δ 11.14 (s, 1H), 8.37 (s, 1H), 8.17 (s, 1H), 7.32 (s, 1H), 7.17 (d, J = 8.0 Hz, 2H), 7.00 (d, J = 8.0 Hz, 1H), 6.06 (s, 2H), 4.87 (dd, J = 5.6, 16.8 Hz, 2H), 4.32-4.28 (m, 2H), 3.93-3.20 (m, 6H), 1.95-75 (m, 4H), 1.59 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 23.00, 31.39, 31.73, 36.59, 43.95, 45.17, 78.27, 78.75, 101.12, 107.05, 108.64, 120.06, 128.08, 129.72, 129.93, 131.59, 144.12, 146.76, 147.96, 154.94, 172.32, and 180.30. LC-MS (ES-API): m/z = 422.1 $(M+H)^+$.

4.5.9. Methyl 1-(2'-oxo-5'-(3,4,5-trimethoxyphenyl)-1',2'-dihydrospiro[piperidine-4,3'-pyrrolo[2,3-b]pyridine]-1-carbonyl)cyclopropane-1-carboxylate (**17**): Compound **17** was obtained from 5'-(3,4,5-trimethoxyphenyl)spiro[piperidine-4,3'-pyrrolo[2,3-b]pyridin]-2'(1'H)-one and 1-(methoxycarbonyl)cyclopropane-1-carboxylic acid as a white solid. Yield: 70 mg, 52%. MP: 240-242 °C. FT-IR (KBr): vmax 3439, 3236, 2939, 1724, 1633, 1456, 1220, 1130, 1016, 837, 657, 557 cm^{-1}. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 11.17 (s, 1H), 8.40 (d, J = 2.0 Hz, 1H), 8.04 (d, J = 1.5 Hz, 1H), 6.90 (s, 2H), 4.04-4.01 (m, 1H), 3.92-3.67 (m, 15H), 1.97-1.79 (m, 4H), 1.45-1.33 (m, 4H). ^{13}C NMR: (125 MHz, DMSO-d_6): 15.40, 15.52, 28.21, 31.15, 31.49, 37.02, 40.91, 45.02, 52.43, 56.05, 60.03, 104.29, 128.13, 129.71, 130.54, 1333.32, 137.10, 144.73, 153.24, 155.20, 165.48, 171.52, and 180.35. LC-MS (ES-API): m/z = 496.1 $(M+H)^+$.

4.5.10. 5'-(Benzo[d][1,3]dioxol-5-yl)-1-(4-methylmorpholine-3-carbonyl)spiro[piperidine-4,3'-pyrrolo[2,3-b]pyridin]-2'(1'H)-one (**18**): Compound **18** was obtained 5'-(3,4,5-trimethoxyphenyl)spiro[piperidine-4,3'-pyrrolo[2,3-b]pyridin]-2'(1'H)-one and 4-methylmorpholine-3-carboxylic acid as a white solid. Yield: 72 mg, 52% MP: 287-290 °C. White solid. FT-IR (KBr): vmax 3423, 3172, 1707, 1653, 1467, 1386, 1232, 1039, 842, 557 cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 11.18 (bs, 1H), 8.35 (s, 1H), 8.13 (s, 1H), 7.31 (s, 1H), 7.16 (t, J = 5.5 Hz, 1H), 7.0 (d, J = 8.5 Hz, 1H), 6.06 (s, 2H), 4.18-3.49 (m, 9H), 3.07-2.61 (m, 4H), 1.93-1.78 (m, 4H). ^{13}C NMR: (125 MHz, DMSO-d_6): δ 31.26, 32.02, 32.38, 37.96, 40.83, 44.93, 52.87, 66.01, 101.17, 107.14, 108.69, 120.16, 127.97, 129.53, 131.62, 144.30, 146.81, 147.97, 154.99, 163.02, and 180.14. LC-MS (ES-API): m/z = 451.1 $(M+H)^+$.

4.5.11. 5'-(Benzo[d][1,3]dioxol-5-yl)-1-(1-(fluoromethyl)cyclopropane-1-carbonyl)spiro[piperidine-4,3'-pyrrolo[2,3-b]pyridin]-2'(1'H)-one (**19**): Compound **19** was obtained 5'-(3,4,5-trimethoxyphenyl)spiro[piperidine-4,3'-pyrrolo[2,3-b]pyridin]-2'(1'H)-one and 1-(fluoromethyl)cyclopropane-1-carboxylic acid as an off-white solid. Yield: 52 mg, 40%. MP: 259-263 °C. FT-IR (KBr): vmax 3427, 2914, 1720, 1631, 1465, 1226, 1035, 933, 806, 669 cm^{-1}. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 11.15 (s, 1H), 8.34 (s, 1H), 8.13 (s, 1H), 7.32 (s, 1H), 7.17 (d, J = 7.5 Hz, 1H), 6.99 (d, J = 8.0 Hz, 1H), 6.05 (s, 2H), 4.47 (d, J = 48.5 Hz, 2H), 3.90 (brs, 4H), 1.79 (brs, 4H), 1.03 (s, 2H), 0.90 (s, 2H). ^{13}C NMR: (125 MHz, DMSO-d_6): δ 10.17, 25.26, 25.46, 31.62, 38.26, 45.34, 87.77, 89.12, 101.13, 107.09, 108.64, 120.10, 128.19, 129.70, 129.93, 131.62, 144.11, 146.76, 147.96, 154.98, 168.81 and 180.36. LC-MS (ES-API): m/z = 424.1 $(M+H)^+$.

4.6 Cell lines

RAMOS, A549, HCT116 (parental and $p53^{-/-}$), CCRF-CEM, K562, U2OS, BJ and MRC-5 cell lines were obtained from ATCC (Middlesex, UK) and maintained according to recommendations. Multidrug-resistant sublines (CEM-DNR, K562-TAX) expressing the LRP and P-glycoprotein transporter proteins were derived and cultured as previously described [36]. Jurkat cells were purchased from DSMZ (Braunschweig, Germany) and maintained according to recommendations. All cell lines

were maintained in a standard 5% CO2/ atmospheric air humidified incubator at 37° C. Cell lines were routinely tested for mycoplasma contamination and authenticated biweekly or monthly.

4.7 Cytotoxicity assay

The cytotoxicity activity of all 19 compounds was tested under *in vitro* conditions using a 3-day standard 3-(4,5-dimethylthiazol-2yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium reduction assay in 384-well plates on a robotic high-throughput screening platform (HighResBio, Boston, MA) as described elsewhere [36]. The IC_{50} values were calculated from the respective dose−response curves of compounds with Dotmatics (San Diego, CA, USA)

Authors declaration

The authors declare no conflicting interests.

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Supplementary data

NMR, LC-MS and FT-IR spectra of all compounds are provided as Supplementary Data.

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