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Short communication

Extending the N-linked aminopiperidine class to the mycobacterial gyrase domain: Pharmacophore mapping from known antibacterial leads

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ABSTRACT

Bacterial DNA gyrase is a well-established and clinically validated target to develop novel antibacterial. Our effort was designated to search for synthetically better compounds with possibility of hit to lead development. With this as objective, a series of 1-(2-(4-aminopiperidin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one derivatives were designed by molecular hybridization strategy and synthesized following nine step reaction to yield activity in low nanomolar range and commendable antibacterial activities. Compound 1-(4-fluorophenyl)-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl) urea (**35**) emerged as the most promising inhibitor with an IC_{50} of 78 nM against *Mycobacterium tuberculosis* DNA gyrase enzyme, with MTB MIC of 0.62 μM, and not cytotoxic at 50 μM in eukaryotic cell line.

1. Introduction

Human negligence and lack of novel therapeutic agents have allowed *Mycobacterium tuberculosis* (*Mtb*), the etiological agents of Tuberculosis (TB) to resurge in more deadly drug resistant forms, with the recently identified totally drug resistant strains rendering complete resistance to currently available drug regime [1].The current TB drug discovery pipeline, though presently insufficient to address the unmet need of treatment, has few promising reports on leads that have the potential to become future drug candidate. The ones in the most advanced stages include the fluoroquinolones (FQ), specifically gatifloxacin and moxifloxacin, which are currently being evaluated in phase 2 and 3 clinical trials respectively [2]. This class of drugs acts by inhibiting DNA gyrase, the sole topoisomerase in *Mtb* that introduce negative supercoils into DNA and regulate the super helical state of the bacterial chromosomes [3]. These drugs predominately bind to the GyrA subunit of DNA gyrase, thereby trapping the gyrase-DNA complex which further resulted in oxidative damage that ultimately led to the bacterial cell death and has been found effective against both replicating and non-replicating, persistent mycobacterium strains [4]. The reports on moxifloxacin, also suggested that DNA gyrase may be a good target for reducing the length of TB treatment regimens. However, in spite of the quite successful progress made by the FQ class of analogues, the emerging resistance to the FQ leads to grave concern and drives the quest for newer, safer, and more effective TB treatment options [5]. Novel bacterial type II topoisomerase inhibitors (NBTIs) are promising class of topoisomerase inhibitor that are structurally and mechanistically different from the FQ class and hence may not be impacted by target mutations that cause resistance to FQ [6]. These classes of drugs have made significant progress in the recent years exhibiting broad spectrum antibacterial potency with many promising candidates being evaluated at various stages of drug discovery program. Their overall chemical structure, topology incorporates an N-linked '4-aminopiperidine' linker fused between a bicyclic left hand nucleus (LHS) and an aryl/heteroaryl right-hand-core (RHS). NBTIs, though well explored as a probable class for developing newer antibacterial leads; scarcely anything have been

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achieved with regard to exploiting this class as a potential lead for developing novel antimycobacterial agents [7]. Recent success in the design of many mycobacterial gyrase inhibitors in the earlier reports on antibacterial analogues of the same chemical class encouraged us to evaluate the inhibitory potential of NBTIs towards the mycobacterial gyrase domain. This is in consideration to the fact that these compounds act on a clinically proven target through a novel mechanism of inhibition with potency against fluoroquinolone-resistant isolates. In this study we disclose a new class of mycobacterial DNA gyrase inhibitor developed *via* molecular hybridization of previously reported antibacterial leads [6–7]. The designed molecules were subsequently synthesized and evaluated *in vitro* for their ability to inhibit DNA gyrase enzyme and whole cell MTB as important steps towards the derivation of structure–activity relationship.

2. Resultsand discussion

2.1. Design and synthesis

With target-based/phenotypic screening approaches offering few tangible successes in discovering novel antitubercular drugs, the concept of molecular hybridization could be of significant use to generate newer scaffold as potential antimycobacterial leads [8]. Molecular hybridization approach is an emerging structural modification tool involving adequate fusion of the two or more pharmacophoric units derived from previously reported leads/drugs in the design of new hybrid architecture that could maintain preselected characteristics of the original template [9]. We therefore envisaged that re-engineering the previously reported NBTIs could deliver a new scaffold/lead with better antimycobacterial activity *via* inhibition of the gyrase domain. The design strategy utilized for developing the inhibitor has been sketched in Fig. 1. It was decided to retain the 1-(2-(4-aminopiperidin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one scaffold in our initial structure-activity exploration as it was understood to be an important requisite in retaining the gyrase inhibitory potential. Since fluoro and methoxy groups at 7th position of the 1,5-naphthyridin-2(1H)-one core significantly improved the gyrase inhibition of the previously reported antibacterial NBTIs, these were also retained in our studies. Various carbamide/thiocarbamide derivatives were introduced as right hand core to increase stability and also to evaluate the steric and electronic effects on the antimycobacterial potency. The N-linked aminopiperidine based analogues have made significant progress in recent years as potential antibacterial leads. Research group from GSK have also found success by extending the above antibacterial aminopiperidine class to the antimicrobial target as well, with many compounds exhibiting promising inhibition of MTB [10].

Synthesis of the compounds started with the construction of 7-fluoro1-5-naphthyridin-2(1H)-one *via* a Heck coupling reaction of 2-chloro-5-fluoropyridin-3-amine (**5**) with butyl acrylate. Though various literatures have explored a variety of conditions and reagents to afford the Heck product, the protocol reported by Voight et al. was the most beneficial as it underwent an in-situ cyclization of the so obtained Heck product butyl 3-(3-amino-5-fluoropyridin-2-yl)acrylate to give the desired 7-fluoro1,5-naphthyridin-2(1H)-one (**6**) in good yield [11]. The ethyl bridge that connected the naphthyridinone core to the aminopiperidine linker was introduced at N-1 position by alkylating the so obtained 7-fluoro-1,5-naphthyridin-2(1H)-one (**6**) with bromoethanol in presence of Cs_2CO_3. Small amounts of the O-alkylated product obtained were removed by column chromatography. Compound **7** on further treatment with trifluoromethanesulfonic anhydride and pyridine afforded the corresponding triflate (**8**) in good yield. This was further condensed with 4-N-Boc-aminopiperidine *via* a SnAr displacement to obtain the nitrogen-linked analog (**9**). Subsequent Boc deprotection afforded the scaffold **11** in good yields. Concordantly, the so obtained *tert*-butyl (1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)carbamate (**9**) was also treated with sodium methoxide in methanol under reflux to introduce the methoxy substituent at the 7th position of 1,5-naphthyridin-2(1H)-one core *via* nucleophilic displacement of the fluoro group. This was subsequently subjected to Boc deprotection in a similar fashion to the fluoro analogue to give the desired product (**12**). The final library was then assembled by treating the obtained scaffolds **11**and **12** with the desired isocyanates/isothiocyanates to afford compounds **13**–**52** in excellent yields.

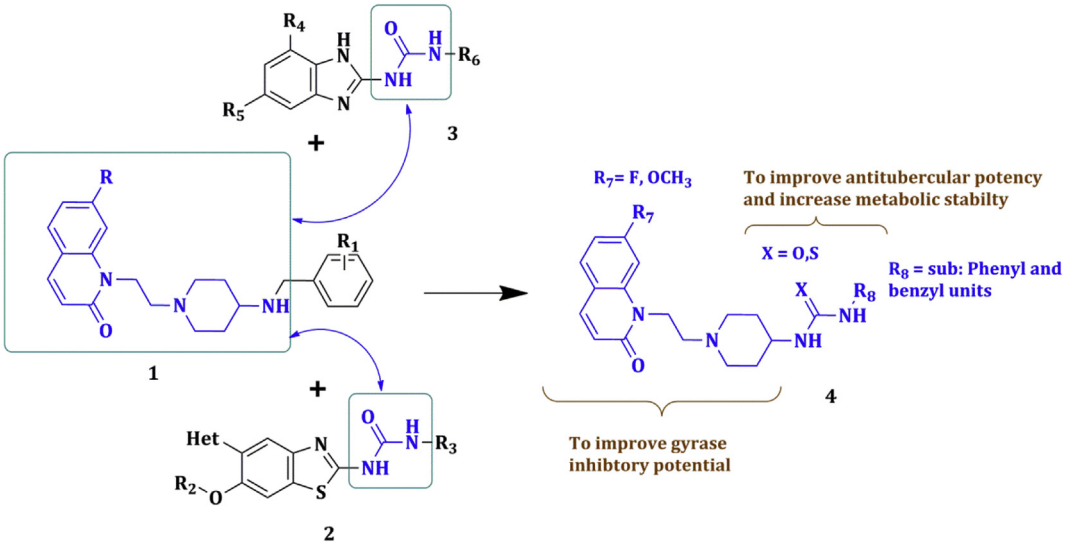


Fig. 1. Strategy employed for designing the lead. Chemical structure of previously reported synthetic inhibitors of DNA gyrasebearing1,5-naphthyridin-2(1H)-one core (**1**), carbamide side chain (**2**–**3**) and the inhibitor designed through molecular hybridization (**4**).

2.2. Pharmacological evaluations

All the forty compounds synthesized were screened for their enzyme inhibition studies using MTB DNA gyrase kit (Inspiralis, Norwich) [12]. Preliminary screening was performed at concentrations of 500, 125, 31.3, 7.8, and 1.95 μM and those which were active were further tested at 250, 62.5, 15.6, 3.9 and 0.97 μM concentrations. Finally molecules that exhibited more than 60% inhibitory activity at 0.97 μM were further screened at lower concentrations of 0.48, 0.24, 0.12, and 0.06 μM to ensure the activity profile. Among the entire series of forty compounds, thirteen compounds showed IC_{50}s less than 0.97 μM. While the most active compound **35** showed an IC_{50} of 78 nM which possessed electronegative group (fluorine) and the chlorine compound **36** exhibited an IC_{50} of 92 nM which indicated that the substitution of electronegative groups at the *para* position of the phenyl ring and the most electropositive methoxy group at 7th position of 1,5-naphthyridin-2(1H)-one could ensure excellent inhibitory property. Compounds **34** and **40** with hydrogen and methyl group at *para* position of phenyl group exhibited IC_{50} of about 10 μM and lost their inhibitory potential by a factor of hundred, thus marking the importance of the electronegative groups at this position. Eleven other compounds (**13**, **16**, **20**, **22**–**24**, **26**, **39**, **43**, **46** and **51**) showed IC_{50}s in between 0.2 and 0.6 μM which signified the importance of aminopiperidine moiety in the inhibition of DNA gyrase enzyme. Novobiocin and moxifloxacin were considered as positive controls as they have been shown to be potent inhibitors of DNA supercoiling of mycobacterial DNA gyrase. All the compounds in this study showed better enzyme supercoiling inhibitions compared to standard moxifloxacin drug whose IC_{50} was 11.2 μM and has been considered as one of the potent DNA gyrase inhibitor till date. Similarly the other broad spectrum standard drug novobiocin showed an IC_{50} of 46 nM. Dose-dependent inhibition profile of the most active compound **35** at different inhibitor concentrations along with standard novobiocin is illustrated in Fig. 2.

The compounds were further screened for their *in vitro* antimycobacterial activity against *M. tuberculosis* H37Rv strain by microplate Alamar blue assay method [13]. As these compounds were mostly hydrophilic, the *in vitro* antimycobacterial activities were in commendable range of 0.49–30.3 μM. Ethambutol (MIC: 15.31 μM), isoniazid (MIC: 0.66 mM), moxifloxacin (MIC: 1.2 μM) and novobiocin (MIC: >200 μM) were considered as standard drugs for comparison in this assay. Compared to the first-line antitubercular drugs like ethambutol and isoniazid, the most active compounds **35** showed a better MIC of 0.62 μM. Twelve compounds (**13**, **16**, **20**, **22**, **26**, **29**, **36**, **42**–**43**, **46**, **48** and **51**) exhibited good antimycobacterial inhibitory profiles with MIC less than 2 μM. These compounds could be considered as promising antitubercular leads with best *in vitro* DNA gyrase enzyme inhibitory profile.

The eukaryotic cell safety profile of all the compounds was observed by testing their *in vitro* cytotoxicity against RAW 264.7 cell (Mouse leukaemic monocyte macrophage cell line) at 100 μM concentration by using 4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazoliumbromide (MTT) assay [14]. All 40 molecules showed lesser cytotoxicity within a range of 19–45% as shown in Table 1. The most promising anti-TB compound **35** showed only 31.5% cytotoxicity with selectivity index of >161. The R and R′ dimethoxy substituted groups comparatively showed lesser toxicity when compared to other compounds in the series. Novobiocin was used as standard that exhibited 29.44% inhibition.

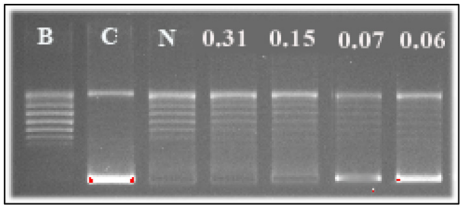


Fig. 2. Depicting the supercoiling assay picture of compound **35** at four different concentrations of 0.31, 0.15, 0.07, 0.06 μM and novobiocin as standard where R-Relaxed DNA substrate +DMSO; C-Relaxed DNA substrate.

3. Conclusion

By combining molecular hybridization strategy with biological assays we could successfully re-engineer the previously reported antibacterial leads that exhibited promising attributes of synthetic accessibility, excellent *in vitro* enzyme inhibition profiles, and antitubercular activity. With the urgent requirement of new anti-TB agents, we believe that the present class of DNA gyrase inhibitors reported in this paper could provide an interesting potential for further optimization to yield novel drugs aimed at combating the ever-increasing bacterial infections Scheme 1.

4. Experimental section

4.1. Chemistry

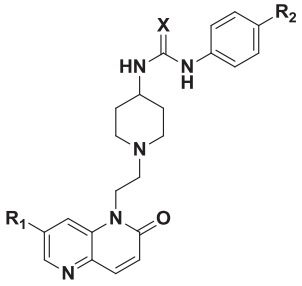
4.1.1. General

All commercially available chemicals and solvents were used without further purification. TLC experiments were performed on alumina-backed silica gel 60 F_{254} plates (Merck, Darmstadt, Germany). Homogeneity of the compounds was monitored by thin layer chromatography (TLC) on silica gel 60 F_{254} coated on aluminium plates, visualized by UV light and $KMnO_4$ treatment. All ^1H and ^{13}C NMR spectra were recorded on a Bruker AM-300 (300.12 MHz, 75.12 MHz) NMR spectrometer, Bruker BioSpin Corp, Germany. Chemical shifts were reported in ppm (δ) with reference to the internal standard TMS. The signals were designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Molecular weights of the synthesized compounds were checked by LCMS 6100B series Agilent Technology. Elemental analyses were carried out on an automatic Flash EA 1112 Series, CHN Analyzer (Thermo).

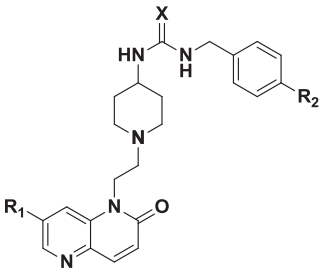
4.1.2. General procedure for synthesis of 7-fluoro-1,5-naphthyridin-2(1H)-one (6)

To a stirred mixture of substituted2-chloro-5-fluoropyridin-3-amine (**5**) (2 g, 13.65 mmol) and butyl acrylate (2.09 g, 16.38 mmol) in cumene (10 mL) at room temperature, was added N-methyldicyclohexylamine (7.99 g, 40.94 mmol), followed by catalytic amounts of tri *tert*-butyl phosphoniumtetrafluroborate (0.158 g, 0.546 mmol). This reaction mixture was purged for 5 min with argon followed by the addition of $Pd(OAc)_2$ (0.061 g, 0.273 mmol). The reaction mixture was heated at 150 °C for 4 h (monitored by TLC & LCMS for completion), and the reaction mixture was filtered through celite bed and solvent was evaporated under reduced pressure. The reaction mixture was further extracted with ethyl acetate (3 × 40 mL). The combined organic extracts were washed with brine (2 × 40 mL) and water (3 × 50 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography using hexane: ethyl acetate as eluent to give 7-fluoro-1, 5-naphthyridin-2(1H)-one (**6**) (1.23 g, 53%) as brown solid. M.p: 282–284 °C. ^1H NMR [300 MHz, DMSO-d_6]: δ_H12.31 (s, 1H), 8.21–6.95 (m, 4H). ^{13}C NMR [100 MHz, DMSO-d_6] δ_c: 163.5, 157.9, 142.8, 140.5, 132.3, 127.1, 123.2, 110.2 ESI-MS *m/z* 165 $(M+H)^+$. Anal Calcd for $C_8H_5FN_2O$: C, 58.54; H, 3.07; N, 17.07; Found: C, 58.58; H, 3.12; N, 17.03.

Table 1
In vitro inhibitory activities of synthesized compounds.



Cmpd 13- 40



Cmpd 41 - 52

Cmpd.	R_1	X	R_2	MTB supercoiling assay (IC_{50}) μM	MTB MIC μM	RAW 264.7 cytotoxicity at 100 μM (% inhib.)	Selective index (CC_{50}/MIC)
13	F	S	H	0.5 ± 0.22	1.83	41.43	>54.64
14	F	S	F	7.1 ± 0.35	6.80	38.54	>14.70
15	F	S	Cl	3.72 ± 0.25	8.52	40.46	>11.73
16	F	S	NO_2	0.28 ± 0.14	0.91	39.57	>109.89
17	F	S	$COCH_3$	11.3 ± 0.67	21.22	36.49	>4.71
18	F	S	OCH_3	1.8 ± 0.34	1.71	30.83	>58.48
19	F	S	CH_3	3.6 ± 0.72	3.54	38.58	>28.24
20	F	O	H	0.44 ± 0.1	1.90	42.95	>52.63
21	F	O	F	3.12 ± 0.66	3.64	39.58	>27.47
22	F	O	Cl	0.3 ± 0.05	1.29	25.69	>77.51
23	F	O	NO_2	0.25 ± 0.09	3.77	38.56	>26.52
24	F	O	$COCH_3$	0.2 ± 0.15	2.91	40.65	>34.0.36
25	F	O	OCH_3	2.2 ± 0.55	3.23	25.49	>30.95
26	F	O	CH_3	0.67 ± 0.12	1.84	29.46	>54.34
27	OCH_3	S	H	5.2 ± 0.89	7.14	37.56	>14.00
28	OCH_3	S	F	4.1 ± 0.43	3.42	38.45	>29.23
29	OCH_3	S	Cl	2.3 ± 0.69	1.65	32.57	>60.60
30	OCH_3	S	NO_2	3.9 ± 0.25	6.46	35.78	>15.47
31	OCH_3	S	$COCH_3$	3.1 ± 0.42	6.89	38.56	>14.51
32	OCH_3	S	OCH_3	8.6 ± 0.24	13.19	41.47	>7.58
33	OCH_3	S	CH_3	3.8 ± 0.17	3.45	38.83	>28.95
34	OCH_3	O	H	10.4 ± 0.44	14.82	45.78	>6.74
35	OCH_3	O	F	0.078 ± 0.02	0.62	31.54	>161.29
36	OCH_3	O	Cl	0.092 ± 0.05	1.85	28.46	>54.05
37	OCH_3	O	NO_2	1.91 ± 0.3	3.94	38.56	>25.38
38	OCH_3	O	$COCH_3$	11.6 ± 0.78	6.60	39.45	>15.15
39	OCH_3	O	OCH_3	0.2 ± 0.06	3.78	29.45	>26.45
40	OCH_3	O	CH_3	10.9 ± 0.72	30.30	39.56	>3.30
41	F	S	H	1.56 ± 0.31	7.10	46.56	>14.08
42	F	S	Cl	1.9 ± 0.77	1.64	43.67	>60.97
43	F	S	OCH_3	0.4 ± 0.43	1.66	41.32	>60.24
44	F	O	H	1.5 ± 0.31	7.37	27.45	>13.56
45	F	O	Cl	2.9 ± 0.23	3.70	42.67	>27.02
46	F	O	OCH_3	0.41 ± 0.2	1.89	32.11	>52.91
47	OCH_3	S	H	1.87 ± 0.54	7.50	33.64	>13.33
48	OCH_3	S	Cl	1.53 ± 0.22	1.60	29.45	>62.50
49	OCH_3	S	OCH_3	3.2 ± 0.81	12.97	19.44	>7.71
50	OCH_3	O	H	1.87 ± 0.26	13.42	28.34	>7.45
51	OCH_3	O	Cl	0.45 ± 0.04	1.65	40.88	>60.60
52	OCH_3	O	OCH_3	2.76 ± 0.35	3.35	39.52	>29.85
Moxifloxacin				11.2 ± 0.23	1.2	ND	ND
Novobiocin				46 ± 0.24	>200	29.44	ND
Ethambutol				ND	9.84	ND	ND

ND indicated not determined.

*4.1.3. General procedure for synthesis of 7-fluoro-1-(2-hydroxyethyl)-1,5-naphthyridin-2(1H)-one (**7**)*

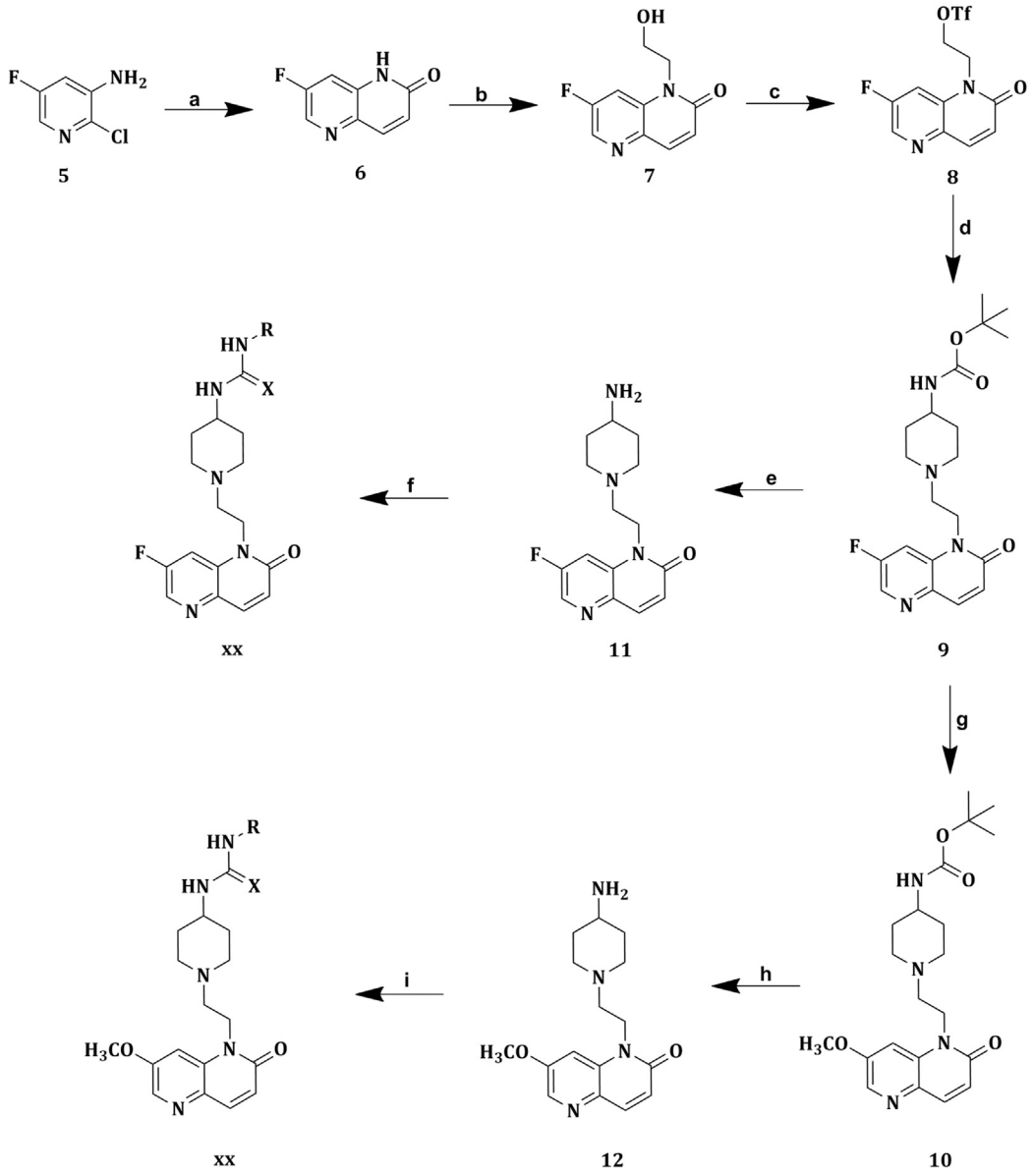
To a solution corresponding 7-fluoro-1,5-naphthyridin-2(1H)-one (**6**) (1 g, 6.09 mmol) in dry N,N′ dimethyl formamide (10 mL) was added Cs_2CO_3 (4.95 g, 15.23 mmol) and followed by 2-bromoethanol (0.913 g, 7.31 mmol) and heated in sealed tube at 120 °C for 4 h (monitored by TLC & LCMS for completion). The reaction mixture was then filtered through celite and washed with dichloromethane. The filtrate was concentrated under reduced pressure. The reaction mixture was further extracted with ethyl acetate (3 × 40 mL). The combined organic extracts were washed with brine (2 × 40 mL) and water (3 × 50 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane: ethyl acetate as eluent to give the corresponding 7-fluoro-1-(2-hydroxyethyl)-1,5-naphthyridin-2(1H)-one (**7**) (0.953 g, 75%) as pale yellow solid. M.p: 241–243 °C. ^{1}H NMR [300 MHz, DMSO-d_6] δ_H: 8.14–6.52 (m, 4H), 3.74–3.47 (m, 5H). ^{13}C NMR [DMSO-d_6] δ_C: 162.3, 157.8, 147.5, 140.1, 132.3, 125.6, 123.2, 110.1, 64.5, 46.2. ESI-MS *m*/*z* 209 $(M+H)^+$. Anal Calcd for $C_{10}H_9FN_2O_2$: C, 57.69; H, 4.36; N, 13.46; Found: C, 57.72; H, 4.38; N, 13.45.

*4.1.4. General procedure for synthesis of 2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl trifluoromethanesulfonate (**8**)*

A solution of 7-fluoro-1-(2-hydroxyethyl)-1,5-naphthyridin-2(1H)-one (7) (1 g, 4.80 mmol) in dichloromethane (10 mL) and pyridine (1.45 g, 14.41 mmol)was cooled to −20 °C. To this was added a solution of trifluromethanesulphonic anhydride (1.49 g, 5.28 mmol) in dichloromethane (10 mL) drop wise. The resultant reaction mixture was stirred at the same temperature for half an hour. Completion of the reaction was confirmed by TLC. Reaction mixture was then warmed to room temperature and given sodium bicarbonate (3 × 20 mL), water (3 × 20 mL) and brine (3 × 20 mL) washes. The organic layer was dried over magnesium sulphate and concentrated under reduced pressure to get a brownish thick liquid. The crude product was purified by silica gel column chromatography using hexane: ethyl acetate as eluent to give the corresponding 2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl trifluoromethanesulfonate (8) (01.23 g, 73%) as Yellow oil. ^{1}H NMR [DMSO-d_6] δ_H: 8.23–6.95 (m, 4H), 3.81–3.64 (m, 4H). ^{13}C NMR [DMSO-d_6] δ: 162.8, 157.8, 145.2, 140.2, 132.9, 125.6, 123.5, 119.2, 110.1, 61.2, 42.5. ESI-MS *m*/*z*: 341 $(M+H)^+$. Anal Calcd for $C_{11}H_8F_4N_2O_4S$: C, 38.83; H, 2.37; N, 8.23; Found: C, 38.85; H, 2.35; N, 8.27.

*4.1.5. General procedure for synthesis of tert-butyl (1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)carbamate (**9**)*

To a solution of corresponding 2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl trifluoromethanesulfonate (**8**) (1 g, 2.93 mmol) in dry N,N′ dimethyl formamide (10 mL) was added K_2CO_3 (1.22 g, 8.82 mmol) and followed by *tert*-butyl piperidin-4-ylcarbamate (0.706 g, 3.52 mmol) and was heated in sealed tube at 100 °C for 4 h (monitored by TLC & LCMS for completion). The reaction mixture was then filtered through celite and washed with dichloromethane. The filtrate was concentrated under reduced pressure. The reaction mixture was further extracted with ethyl acetate (3 × 40 mL). The combined organic extracts were washed with brine (2 × 40 mL) and water (3 × 50 mL), dried (MgSO4) and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane: ethyl acetate as eluent to give the corresponding *tert*-butyl (1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl) carbamate (**9**) (0.724 g, 63%) as pale yellow solid. M.p: 210–212 °C. ^{1}H NMR [DMSO-d_6] δ_H: 10.32 (s, 1H), 8.21–6.73 (m, 4H), 4.63–1.39 (m, 13H), 1.38 (s, 9H). ^{13}C NMR [DMSO-d_6] δ: 162.3, 157.8, 156.2, 146.2, 140.1, 132.5, 125.6, 123.1, 110.2, 80.2, 55.2, 52.3 (2C), 50.2, 49.5, 30.2 (2C), 27.6 (3C).ESI-MS *m*/*z*: 391 $(M+H)^+$. Anal Calcd for $C_{20}H_{27}FN_4O_3$: C, 61.52; H, 6.97; N, 14.35; Found: C, 61.55; H, 6.93; N, 14.37.



Reagents and conditions: a. Butyl acrylate, $Pd(OAc)_2$, $HP(t\text{-}Bu)_3BF_4$, Cummene 150°C; b. Bromoethanol, Cs_2CO_3, DMF, 80 °C; c.Tf_2O, TEA, DCM, 0 °C - rt; d. *tert*-butyl piperidin-4-ylcarbamate, K_2CO_3, DMF, 100 °C; e.HCl/Dioxane, 0 °C - rt; f. R_2NCS or R_2NCO, TEA, DCM ,0 °C - rt; g. Sodium methoxide, methanol, 65 °C; h. HCl/Dioxane, 0 °C - rt; i. R_2NCS or R_2NCO, TEA, DCM ,0 °C - rt;

Scheme 1. Synthetic protocol adopted to achieve the designed ligands.

*4.1.6. General procedure for synthesis of tert-butyl (1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl) carbamate (**10**)*

A solution of corresponding *tert*-butyl (1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)carbamate (**9**) (2.0 g, 5.12 mmol) in methanol (10 mL) at room temperature was added sodium methoxide (0.553 g, 10.2 mmol). The reaction mixture was heated at 65 °C for 3 h (monitored by TLC & LCMS for completion), and solvent evaporated under reduced pressure. The reaction mixture was further extracted with ethyl acetate (3 × 40 mL). The combined organic extracts were washed with brine (2 × 40 mL) and water (3 × 50 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography using hexane: ethyl acetate as eluent to give the corresponding *tert*-butyl (1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl) carbamate (**10**) (1.35 g, 65%) as pale yellow solid. M.p: 232–234 °C. ^{1}H NMR [DMSO-d_6]: 10.32 (s, 1H), 8.21–6.73 (m, 4H), 4.63–1.39 (m, 16H), 1.38 (s, 9H). ^{13}C NMR [DMSO-d_6] δ_c: 161.5, 156.7, 155.2, 146.2, 136.1, 132.5, 125.6, 119.1, 105.2, 80.3, 56.2, 55.2, 52.3 (2C), 50.2, 49.8, 30.2 (2C), 27.6 (3C).ESI-MS *m/z*: 403 $(M+H)^+$. Anal Calcd for $C_{21}H_{30}N_4O_4$: C, 62.67; H, 7.51; N, 13.92; Found: C, 62.61; H, 7.56; N, 13.90.

*4.1.7. General procedure for synthesis of substituted 1-(2-(4-aminopiperidin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**11**–**12**)*

A solution of corresponding substituted *tert*-butyl (1-(2-(2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)carbamate (**9**–**10**) (1 eq) in dichloromethane (10 mL) was cooled to 0 °C followed by drop wise addition of HCl in dioxane (2 mL) and stirred for 1 h. After completion of the reaction (monitored by TLC & LCMS), quenched with ice and concentrated under reduced pressure. The residue was partitioned between ethyl acetate (20 mL) and water (20 mL). Aqueous layer was basified with sodium carbonate solution and extracted with ethyl acetate (3 × 20 mL) and washed with water (3 × 10 mL) and brine (3 × 10 mL). Organic layer was dried over anhydrous sodium sulphate, filtered, and concentrated under reduced pressure to give the corresponding 1-(2-(4-aminopiperidin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**11**–**12**) in good yield.

*4.1.7.1. 1-(2-(4-Aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**11**).* The compound was synthesized according to the general procedure described above by using *tert*-butyl(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)carbamate (**9**) (2.0 g, 5.12 mmol) and HCl in dioxane (10 mL) to afford **11** (1.25 g, 84%) as pale yellow solid. M.p: 232–234 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 8.26–6.72 (m, 4H), 6.56 (s, 2H), 4.35–1.38 (m, 13H). ^{13}C NMR [DMSO-d$_6$] δ: 162.8, 155.9, 145.6, 140.2, 132.9, 125.1, 123.4, 109.7, 55.2, 52.6 (2c), 50.3, 45.2, 33.5 (2C). ESI-MS *m*/*z*: 291 (M+H)$^+$. Anal Calcd for $C_{15}H_{19}FN_4O$: C, 62.05; H, 6.60; N, 19.30; Found: C, 62.01; H, 6.64; N, 19.31.

*4.1.7.2. 1-(2-(4-Aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**12**).* The compound was synthesized according to the general procedure by utilizing *tert*-butyl (1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl) carbamate (**10**) (2.0 g, 4.97 mmol) and HCl in dioxane (10 mL) to afford **12** (1.35 g, 88%) as Yellow solid. M.p: 225–227 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 8.11–6.63 (m, 4H), 6.42 (s, 2H), 4.48 (t, J = 7.2 Hz, 2H), 3.92 (s, 3H), 3.65–1.36 (m, 11H). ^{13}C NMR [DMSO-d$_6$] δ: 162.3, 155.6, 145.7, 136.2, 132.8, 125.4, 119.2, 107.2, 56.2, 54.5, 52.3 (2C), 58.5, 46.2, 33.2 (2C) ESI-MS *m*/*z*: 303 (M+H)$^+$. Anal Calcd for $C_{16}H_{22}N_4O_2$: C, 63.55; H, 7.33; N, 18.53; Found: C, 63.58; H, 7.31; N, 18.55.

*4.1.8. General procedure for the synthesis of substituted 1-(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-phenylthiourea (**13**–**19**)*

A solution of 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**11**) (0.344 mmol) in dichloromethane (3 mL) was cooled to 0 °C followed by the addition of triethylamine (1.03 mmol). Corresponding substituted phenyl isothiocyanate was added to the reaction mixture and was stirred in room temperature for 12 h (monitored by TLC & LCMS for completion). The reaction mixture was then washed with water (3 × 5 mL) and brine (3 × 5 mL). Organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane: ethyl acetate as eluent to give the corresponding substituted1-(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-phenylthiourea (**13**–**19**)in good yields.

*4.1.8.1. 1-(1-(2-(7-Fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-phenylthiourea (**13**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**11**) (0.1 g, 0.344 mmol) and phenyl isothiocyanate (0.055 g, 0.413 mmol) to afford **13** (0.085 g, 58%) as white solid. M.p: 165–168 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 10.33 (b, 1H), 7.98–6.69 (m, 9H), 4.33 (t, J = 6.6 Hz, 2H), 4.05 (b, 1H), 2.81–1.22 (m, 11H). ^{13}C NMR [DMSO-d$_6$] δ_c: 178.1, 160.2, 157.9, 146.2, 140.3, 139.5, 133.8, 130.3 (2C), 129.2, 127.7 (2C), 125.2, 124.8, 109.5, 56.6, 54.2, 52.8 (2C), 47.6, 30.3 (2C). ESI-MS *m*/*z*: 426 (M+H)$^+$. Anal Calcd for $C_{22}H_{24}FN_5OS$: C, 62.10; H, 5.68; N, 16.46. Found: C, 62.11; H, 5.66; N, 16.45.

*4.1.8.2. 1-(1-(2-(7-Fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-(4-fluorophenyl)thiourea (**14**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**11**) (0.1 g, 0.344 mmol) and 4 fluro phenyl isothiocyanate (0.063 g, 0.413 mmol) to afford **14** (0.112 g, 70%) as pale yellow solid. M.p: 184–186 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 10.37 (b, 1H), 7.95–6.67 (m, 8H), 4.35 (t, J = 6.9 Hz, 2H), 4.06 (b, 1H), 2.92–1.24 (m, 11H). ^{13}C NMR [DMSO-d$_6$] δ: 178.9, 162.5, 161.7, 157.8, 145.3, 140.2, 135.6, 132.8, 130.8 (2C), 125.6, 123.8, 116.2 (2C), 109.5, 56.8, 54.5, 52.9 (2C), 47.5, 30.6 (2C). ESI-MS *m*/*z*: 444 (M+H)$^+$. Anal Calcd for $C_{22}H_{23}F_2N_5OS$: C, 59.58; H, 5.23; N, 15.79. Found: C, 59.60; H, 5.20; N, 15.80.

*4.1.8.3. 1-(4-Chlorophenyl)-3-(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)thiourea (**15**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**11**) (0.1 g, 0.344 mmol) and 4-chloro phenyl isothiocyanate (0.069 g, 0.413 mmol) to afford **15** (0.102 g, 64%)as brown solid. M.p: 188–190 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 10.37 (b, 1H), 7.92–6.55 (m, 8H), 4.30 (t, J = 6.6 Hz, 2H), 4.12 (b, 1H), 2.88–1.29 (m, 11H). ^{13}C NMR [DMSO-d$_6$] δ_c: 178.1, 161.7, 157.8, 145.9, 140.2, 137.2, 134.5, 132.8, 131.9 (2C), 130.3 (2C), 125.6, 123.8, 109.6, 56.8, 54.5, 52.3 (2C), 47.5, 30.3 (2C).ESI-MS *m*/*z*: 460 (M+H)$^+$. Anal Calcd for $C_{22}H_{23}ClFN_5OS$: C, 57.45; H, 5.04; N, 15.23. Found: C, 57.47; H, 5.07; N, 15.29.

*4.1.8.4. 1-(1-(2-(7-Fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-(4-nitrophenyl)thiourea (**16**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**11**) (0.1 g, 0.344 mmol) and 4-nitro phenyl isothiocyanate (0.074 g, 0.413 mmol) to afford **16** (0.138 g, 85%)as yellow solid. M.p: 205–207 °C. ^{1}H NMR [300 MHz, DMSO-d$_6$]: δ_H9.97 (b, 1H), 8.57–6.82 (m, 8H), 4.34 (t, J = 6.3 Hz, 2H), 4.09 (b, 1H), 2.93–1.28 (m, 11H). ^{13}C NMR [DMSO-d$_6$] δ: 178.9, 160.7, 157.3, 146.4, 142.3, 142.1, 141.6, 139.9, 132.8, 124.4 (2C), 123.9 (2C), 120.1, 109.3, 54.94, 53.16, 52.2 (2C), 50.6, 30.7 (2C). ESI-MS *m*/*z*: 471 (M+H)$^+$. Anal Calcd for $C_{22}H_{23}FN_6O_3S$: C, 56.16; H, 4.93; N, 17.86; Found: C, 56.18; H, 4.90; N, 17.87.

*4.1.8.5. 1-(4-Acetylphenyl)-3-(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)thiourea (**17**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**11**) (0.1 g, 0.344 mmol) and 4-acetyl phenyl isothiocyanate (0.073 g, 0.413 mmol) to afford **17** (0.122 g, 76%)as white solid. M.p: 188–190 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 10.49 (b, 1H), 8.38–6.75 (m, 8H), 4.62 (t, J = 7.5 Hz, 2H), 4.15 (b, 1H), 3.63–3.02 (m, 3H), 2.52 (s, 3H), 2.43–1.18 (m, 8H). ^{13}C NMR [DMSO-d$_6$] δ_c: 197.5, 178.9, 160.1, 157.8, 145.3, 143.4, 140.2, 138.3, 132.5, 130.2 (2C), 127.8 (2C), 125.3, 109.8, 55.8, 54.2, 53.5, 52.9 (2C), 47.8, 30.2 (2C), 25.8. ESI-MS *m*/*z*: 468 (M+H)$^+$. Anal Calcd for $C_{24}H_{26}FN_5O_2S$: C, 61.65; H, 5.60; N, 14.98; Found: C, 61.63; H, 5.57; N, 14.97.

*4.1.8.6. 1-(1-(2-(7-Fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl) piperidin-4-yl)-3-(4-methoxyphenyl)thiourea (**18**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**11**) (0.1 g, 0.344 mmol) and 4 methoxyphenyl isothiocyanate (0.068 g, 0.413 mmol) to afford **18** (0.126 g, 80%) as pale yellow solid. M.p: 130–132 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 10.45 (b, 1H), 8.25–6.75 (m, 8H), 4.33 (t, J = 6.0 Hz, 2H), 4.05 (b, 1H), 3.85 (s, 3H), 2.84–1.31 (m, 11H). ^{13}C NMR [DMSO-d$_6$] δ_c: 178.2, 161.2, 160.5, 157.8, 145.7, 140.2, 132.3, 129.5, 128.2 (2C), 125.6, 123.5, 115.1 (2C), 110.1, 56.2, 55.9, 52.5, 51.9 (2C), 48.5, 30.3 (2C). ESI-MS m/z: 456 (M+H)$^+$. Anal Calcd for $C_{23}H_{26}FN_5O_2S$: C, 60.64; H, 5.75; N, 15.37. Found: C, 60.69; H, 5.70; N, 15.35.

*4.1.8.7. 1-(1-(2-(7-Fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl) piperidin-4-yl)-3-(p-tolyl)thiourea (**19**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**11**) (0.1 g, 0.344 mmol) and 4-methyl phenyl isothiocyanate (0.062 g, 0.413 mmol) to afford **19** (0.118 g, 78%) as white solid. M.p: 181–183 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 10.42 (b, 1H), 7.94–6.58 (m, 8H), 4.31 (t, J = 6.9 Hz, 2H), 4.15 (b, 1H), 2.92–1.31 (m, 14H). ^{13}C NMR [DMSO-d$_6$] δ_c: 178.1, 160.2, 157.8, 145.8, 140.2, 138.5, 136.7, 132.8, 130.3 (2C), 126.7 (2C), 125.2, 123.8, 109.5, 56.8, 54.2, 52.8 (2C), 47.6, 30.3 (2C), 22.4. ESI-MS m/z: 440 (M+H)$^+$. Anal Calcd for: $C_{23}H_{26}FN_5OS$: C, 62.85; H, 5.96; N, 15.93. Found: C, 62.87; H, 5.95; N, 15.92.

*4.1.9. General procedure for the synthesis of substituted 1-(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-phenylurea (**20**–**26**)*

A solution of 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**11**) (0.344 mmol) in dichloromethane (3 mL) was cooled to 0 °C followed by the addition of triethylamine (1.03 mmol). Corresponding substituted phenyl isocyanate (0.413 mmol) was added to the reaction mixture and was stirred in room temperature for 12 h (monitored by TLC &LCMS for completion). The reaction mixture was then washed with water (3 × 5 mL) and brine (3 × 5 mL). Organic layer was dried (MgSO4) and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane: ethyl acetate as eluent to give the corresponding substituted1-(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-phenylurea (**20**–**26**) in good yield.

*4.1.9.1. 1-(1-(2-(7-Fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl) piperidin-4-yl)-3-phenylurea (**20**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**11**) (0.1 g, 0.344 mmol) and phenyl isocyanate (0.049 g, 0.413 mmol) to afford **20** (0.107 g, 76%) as Pale yellow solid. M.p: 212–214 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 10.36 (b, 1H), 10.28 (b, 1H), 7.95–6.67 (m, 9H), 4.64–1.38 (m, 13H). ^{13}C NMR [DMSO-d$_6$] δ_c: 160.7, 157.8, 155.6, 145.3, 140.2, 138.9, 132.9, 130.3 (2C), 129.2, 125.6, 123.4, 120.6 (2C), 109.8, 54.6, 52.8 (2C), 47.9, 46.8, 30.2 (2C). ESI-MS m/z: 410 (M+H)$^+$. Anal Calcd for $C_{22}H_{24}FN_5O_2$: C, 64.53; H, 5.91; N, 17.10. Found: C, 64.55; H, 5.96; N, 17.07.

*4.1.9.2. 1-(1-(2-(7-Fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl) piperidin-4-yl)-3-(4-fluorophenyl)urea (**21**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**11**) (0.1 g, 0.344 mmol) and 4-fluro phenyl isocyanate (0.056 g, 0.413 mmol) to afford **21** (0.122 g, 82%) as pale yellow solid. M.p: 239–241 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 10.12 (b, 1H), 9.99 (b, 1H), 8.63–6.84 (m, 8H), 4.36–1.42 (m, 13H). ^{13}C NMR [DMSO-d$_6$] δ_c: 161.8, 160.7, 155.8, 154.9, 145.9, 140.2, 136.7, 132.2, 125.5, 123.7, 120.2 (2C), 116.2 (2C), 109.8, 54.5, 52.3 (2C), 47.8, 46.7, 30.3 (2C). ESI-MS m/z: 428 (M+H)$^+$. Anal Calcd for $C_{22}H_{23}F_2N_5O_2$: C, 61.82; H, 5.42; N, 16.38; Found: C, 61.83; H, 5.44; N, 16.43.

*4.1.9.3. 1-(4-Chlorophenyl)-3-(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)urea (**22**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**11**) (0.1 g, 0.344 mmol) and 4-chloro phenyl isocyanate (0.063 g, 0.413 mmol) to afford **22** (0.133 g, 87%) as brown solid. M.p: 275–277 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 10.11 (b, 1H), 10.05 (b, 1H), 8.65–6.82 (m, 8H), 4.33–1.37 (m, 13H). ^{13}C NMR [DMSO-d$_6$] δ_c: 160.7, 157.8, 155.8, 145.9, 140.2, 138.6, 134.7, 132.3, 130.4 (2C), 125.6, 123.7, 120.5 (2C), 109.7, 54.2, 52.8 (2C), 47.9, 46.5, 30.4 (2C) ESI-MS m/z: 444 (M+H)$^+$. Anal Calcd for $C_{22}H_{23}ClFN_5O_2$: C, 59.53; H, 5.22; N, 15.78; Found: C, 59.57; H, 5.20; N, 15.76.

*4.1.9.4. 1-(1-(2-(7-Fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl) piperidin-4-yl)-3-(4-nitrophenyl)urea (**23**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**11**) (0.1 g, 0.344 mmol) and 4-nitro phenyl isocyanate (0.067 g, 0.413 mmol) to afford **23** (0.097 g, 62%) as pale yellow solid. M.p: 261–263 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 10.12 (b, 1H), 9.99 (b, 1H), 8.63–6.83 (m, 8H), 4.36–1.42 (m, 13H). ^{13}C NMR [DMSO-d$_6$] δ_c: 160.9, 155.2, 154.8, 144.3, 143.3, 142.9, 139.7, 132.8, 125.2, 124.9 (2C), 123.8, 120.2 (2C), 109.5, 54.5, 52.8 (2C), 47.8, 46.9, 30.2 (2C). ESI-MS m/z: 455 (M+H)$^+$. Anal Calcd for$C_{22}H_{23}FN_6O_4$: C, 58.14; H, 5.10; N, 18.49; Found: C, 58.17; H, 5.13; N, 18.45.

*4.1.9.5. 1-(4-Acetylphenyl)-3-(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)urea (**24**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**11**) (0.1 g, 0.344 mmol) and 4-acetyl phenyl isocyanate (0.066 g, 0.413 mmol) to afford **24** (0.104 g, 67%)as white solid. M.p: 224–226 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 10.56 (b, 1H), 10.44 (b, 1H), 8.39–6.72 (m, 8H), 4.68 (t, J = 7.8 Hz, 2H), 3.78–3.09 (m, 3H), 2.53 (s, 3H), 2.49–1.17 (m, 8H). ^{13}C NMR [DMSO-d$_6$] δ_c: 196.1, 161.2, 156.8, 154.2, 145.1, 143.2, 140.1, 136.7, 131.3, 129.1 (2C), 124.5, 123.8, 120.2 (2C), 109.8, 54.5, 51.4 (2C), 47.6, 45.5, 29.6 (2C), 26.2. ESI-MS m/z: 452 (M+H)$^+$. Anal Calcd for $C_{24}H_{26}FN_5O_3$: C, 63.85; H, 5.80; N, 15.51; Found: C, 63.86; H, 5.88; N, 15.49.

*4.1.9.6. 1-(1-(2-(7-Fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl) piperidin-4-yl)-3-(4-methoxyphenyl)urea (**25**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**11**) (0.1 g, 0.344 mmol) and 4-methoxyphenyl isocyanate (0.062 g, 0.413 mmol) to afford **25** (0.117 g, 77%) as white solid. M.p: 196–198 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 10.16 (b, 1H), 10.03 (b, 1H), 8.57–6.73 (m, 8H), 4.37 (t, J = 6.6 Hz, 2H), 3.81 (s, 3H), 4.32–1.38 (m, 11H). ^{13}C NMR [DMSO-d$_6$] δ_c: 160.7, 159.7, 157.8, 155.3, 145.2, 140.1, 131.7, 130.8, 125.6, 123.8, 120.2 (2C), 115.6 (2C), 109.5, 56.2, 54.2, 52.3 (2C), 47.8, 46.7, 30.3 (2C). ESI-MS m/z: 440 (M+H)$^+$. Anal Calcd for $C_{23}H_{26}FN_5O_3$: C, 62.86; H, 5.96; N, 15.94; Found: C, 62.88; H, 5.95; N, 15.99.

*4.1.9.7. 1-(1-(2-(7-Fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl) piperidin-4-yl)-3-(p-tolyl)urea (**26**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**11**) (0.1 g, 0.344 mmol) and 4-methyl phenyl isocyanate (0.054 g,

0.413 mmol) to afford **26** (0.120 g, 82%) as brown solid. M.p: 242–244 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 10.39 (b, 1H), 10.26 (b, 1H), 7.88–6.65 (m, 8H), 4.40 (t, J = 7.2 Hz, 2H), 3.05–1.36 (m, 14H). ^{13}C NMR [DMSO-d$_6$] δ_c: 160.7, 157.2, 155.4, 145.8, 140.2, 137.9, 137.2, 132.7, 130.2 (2C), 125.6, 123.8, 120.8 (2C), 109.8, 54.6, 52.5 (2C), 47.8, 46.5, 30.2 (2C), 20.8. ESI-MS m/z 424 (M+H)$^+$. Anal Calcd for: $C_{23}H_{26}FN_5O_2$: C, 65.23; H, 6.19; N, 16.54. Found: C, 65.25; H, 6.18; N, 16.55.

*4.1.10. General procedure for the synthesis of substituted 1-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-phenylthiourea (**27**–**33**)*

A solution of 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**12**) (0.33 mmol) in dichloromethane (1 mL) was cooled to 0 °C followed by the addition of triethylamine (0.99 mmol). Corresponding substituted phenyl isothiocyanate (0.397 mmol) was added to the reaction mixture and was stirred in room temperature for 12 h (monitored by TLC & LCMS for completion). The reaction mixture was washed with water (3 × 5 mL) and brine (3 × 5 mL). Organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane: ethyl acetate as eluent to give the corresponding substituted1-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-phenylthiourea (**27**–**33**)in good yields.

*4.1.10.1. 1-(1-(2-(7-Methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-phenylthiourea (**27**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**12**) (0.1 g, 0.330 mmol) and phenyl isothiocyanate (0.055 g, 0.397 mmol) to afford **27** (0.142 g, 82%) as white solid. M.p: 178–180 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 10.33 (b, 1H), 7.92–6.65 (m, 9H), 4.34 (t, J = 6.6 Hz, 2H), 4.09 (b, 1H), 3.82 (s, 3H), 2.86–1.23 (m, 11H). ^{13}C NMR [DMSO-d$_6$] δ_c: 178.2, 160.5, 157.8, 145.2, 141.3, 138.5, 134.8, 130.3 (2C), 129.7, 126.7 (2C), 125.2, 124.8, 110.5, 56.6, 55.3, 53.2, 52.8 (2C), 47.6, 30.3 (2C). ESI-MS m/z: 438 (M+H)$^+$. Anal Calcd for $C_{23}H_{27}N_5O_2S$: C, 63.13; H, 6.22; N, 16.01. Found: C, 63.11; H, 6.25; N, 16.09.

*4.1.10.2. 1-(4-Fluorophenyl)-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)thiourea (**28**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**12**) (0.1 g, 0.330 mmol) and 4-fluro phenyl isothiocyanate (0.060 g, 0.397 mmol) to afford **28** (0.108 g, 72%) as yellow solid. M.p: 165–167 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 10.35 (b, 1H), 7.98–6.65 (m, 8H), 4.33 (t, J = 7.2 Hz, 2H), 4.05 (b, 1H), 3.84 (s, 3H), 2.97–1.26 (m, 11H). ^{13}C NMR [DMSO-d$_6$] δ_c: 178.9, 162.5, 161.7, 157.8, 145.3, 140.2, 135.6, 132.8, 130.8 (2C), 125.6, 123.8, 116.2 (2C), 109.5, 56.8, 55.7, 54.5, 52.9 (2C), 47.5, 30.6 (2C). ESI-MS m/z: 456 (M+H)$^+$. Anal Calcd for $C_{23}H_{26}FN_5O_2S$: C, 60.64; H, 5.75; N, 15.37. Found: C, 60.68; H, 5.72; N, 15.39.

*4.1.10.3. 1-(4-Chlorophenyl)-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)thiourea (**29**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**12**) (0.1 g, 0.330 mmol) and 4-chloro phenyl isothiocyanate (0.067 g, 0.397 mmol) to afford **29** (0.121 g, 77%) as brown solid. M.p: 167–169 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 10.46 (b, 1H), 8.01–6.71 (m, 8H), 4.30 (t, J = 6.3 Hz, 2H), 4.15 (b, 1H), 3.91 (s, 3H), 2.91–1.35 (m, 11H). ^{13}C NMR [DMSO-d$_6$] δ_c: 178.1, 161.7, 157.8, 145.9, 140.2, 137.2, 134.5, 132.8, 131.9 (2C), 130.3 (2C), 125.6, 123.8, 109.6, 56.8, 55.4, 54.5, 52.3 (2C), 47.5, 30.3 (2C).ESI-MS m/z: 473 (M+H)$^+$. Anal Calcd for $C_{23}H_{26}ClN_5O_2S$: C, 58.53; H, 5.55; N, 14.84. Found: C, 58.57; H, 5.50; N, 14.89.

*4.1.10.4. 1-(1-(2-(7-Methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-(4-nitrophenyl)thiourea (**30**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**12**) (0.1 g, 0.330 mmol) and 4-nitro phenyl isothiocyanate (0.071 g, 0.397 mmol) to afford **30** (0.099 g, 62%)as yellow solid. M.p: 229–231 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 10.03 (b, 1H), 8.62–6.87 (m, 8H), 4.41 (t, J = 6.3 Hz, 2H), 4.18 (b, 1H), 3.95 (s, 3H), 3.05–1.42 (m, 11H). ^{13}C NMR [DMSO-d$_6$] δ_c: 178.9, 160.7, 157.3, 146.4, 142.3, 142.1, 141.6, 139.9, 132.8, 124.4 (2C), 123.9 (2C), 120.1, 109.3, 55.9, 54.94, 53.16, 52.2 (2C), 50.6, 30.7 (2C). ESI-MS m/z: 483 (M+H)$^+$. Anal Calcd for $C_{23}H_{26}N_6O_4S$: C, 57.25; H, 5.43; N, 17.42. Found: C, 57.21; H, 5.42; N, 17.38.

*4.1.10.5. 1-(4-Acetylphenyl)-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)thiourea (**31**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**12**) (0.1 g, 0.330 mmol) and 4-acetyl phenyl isothiocyanate (0.070 g, 0.397 mmol) to afford **31** (0.11 g, 69%) as white solid. M.p: 194–196 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 10.35 (b, 1H), 8.25–6.62 (m, 8H), 4.41 (t, J = 7.8 Hz, 2H), 4.18 (b, 1H), 3.85 (s, 3H), 3.54–2.99 (m, 3H), 2.56 (s, 3H), 2.44–1.23 (m, 8H). ^{13}C NMR [DMSO-d$_6$] δ_c: 197.8, 177.5, 162.3, 155.3, 143.9, 143.5, 138.5, 136.2, 132.8, 130.2 (2C), 125.2 (2C), 124.9, 119.2, 105.8, 56.5, 54.2, 53.5, 51.5 (2C), 47.5, 30.2 (2C), 25.6.ESI-MS m/z: 480 (M+H)$^+$. Anal Calcd for $C_{25}H_{29}N_5O_3S$: C, 62.61; H, 6.09; N, 14.60; Found: C, 62.66; H, 6.11; N, 14.56.

*4.1.10.6. 1-(1-(2-(7-Methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-(4-methoxyphenyl)thiourea (**32**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**12**) (0.1 g, 0.330 mmol) and 4-methoxyphenyl isothiocyanate (0.065 g, 0.397 mmol) to afford **32** (0.125 g, 81%) as brown solid. M.p: 121–123 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 10.32 (b, 1H), 7.83–6.58 (m, 8H), 4.49 (t, J = 6.3 Hz, 2H), 4.12 (b, 1H), 3.85 (s, 3H), 3.75 (s, 3H), 2.93–1.41 (m, 11H). ^{13}C NMR [DMSO-d$_6$] δ_c: 178.3, 163.5, 160.2, 155.8, 143.3, 136.8, 132.3, 131.6, 128.1 (2C), 125.6, 119.2, 115.6 (2C), 105.2, 55.8 (2C), 55.2, 54.3, 52.8 (2C), 50.2, 30.5 (2C). ESI-MS m/z: 468 (M+H)$^+$. Anal Calcd for $C_{24}H_{29}N_5O_3S$: C, 61.65; H, 6.25; N, 14.98. Found: C, 61.63; H, 6.27; N, 14.97.

*4.1.10.7. 1-(1-(2-(7-Methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-(p-tolyl)thiourea (**33**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**12**) (0.1 g, 0.330 mmol) and 4-methyl phenyl isothiocyanate (0.059 g, 0.397 mmol) to afford **33** (0.115 g, 77%) as white solid. M.p: 134–136 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 10.37 (b, 1H), 7.89–6.52 (m, 8H), 4.29 (t, J = 6.9 Hz, 2H), 4.11 (b, 1H), 3.75 (s, 3H), 2.92–1.31 (m, 14H). ^{13}C NMR [DMSO-d$_6$] δ_c: 180.2, 163.3, 155.6, 142.9, 136.2, 135.3, 134.9, 132.1, 130.3 (2C), 126.8 (2C), 124.4, 119.2, 104.3, 56.4, 55.8, 53.8, 53.1 (2C), 50.1, 30.8 (2C), 22.6. ESI-MS m/z 452 (M+H)$^+$. Anal. Calcd. for: $C_{24}H_{29}N_5O_2S$: C, 63.83; H, 6.47; N, 15.51. Found: C, 63.82; H, 6.45; N, 15.55.

*4.1.11. General procedure for the synthesis of substituted 1-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-phenylurea (**34**–**40**)*

A solution of 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**12**) (0.33 mmol) in dichloromethane (1 mL) was cooled to 0 °C followed by the addition of triethylamine

(0.99 mmol). Corresponding substituted phenyl isocyanate (0.397 mmol) was added to the reaction mixture and was stirred in room temperature for 12 h (monitored by TLC & LCMS for completion). The reaction mixture was washed with water (3 × 5 mL) and brine (3 × 5 mL). Organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane: ethyl acetate as eluent to give the corresponding substituted1-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-phenylthiourea (**34**–**40**) in good yield.

*4.1.11.1. 1-(1-(2-(7-Methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl) piperidin-4-yl)-3-phenylurea (**34**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**12**) (0.1 g, 0.330 mmol) and phenyl isocyanate (0.047 g, 0.397 mmol) to afford **34** (0.102 g, 73%) as yellow solid. M.p: 186–188 °C. ^{1}H NMR [DMSO-d_6] δ_H: 10.46 (b, 1H), 10.38 (b, 1H), 7.92–6.65 (m, 9H), 4.67 (t, J = 6.9 Hz, 2H), 3.85 (s, 3H), 3.01–1.42 (m, 11H). ^{13}C NMR [DMSO-d_6] δ_c: 161.7, 156.8, 155.6, 145.3, 140.2, 136.9, 132.9, 130.3 (2C), 129.2, 125.6, 123.4 (2C), 120.6, 106.8, 56.2, 53.6, 52.8 (2C), 47.9, 46.8, 30.2 (2C). ESI-MS m/z: 422 $(M+H)^+$. Anal. Calcd. for $C_{23}H_{27}N_5O_3$: C, 65.54; H, 6.46; N, 16.62. Found: C, 65.55; H, 6.40; N, 16.67.

*4.1.11.2. 1-(4-Fluorophenyl)-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)urea (**35**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**12**) (0.1 g, 0.330 mmol) and 4-fluro phenyl isocyanate (0.059 g, 0.397 mmol) to afford **35** (0.117 g, 81%) as pale yellow solid. M.p: 209–211 °C. ^{1}H NMR [DMSO-d_6] δ_H: 10.42 (b, 1H), 10.35 (b, 1H), 7.98–6.65 (m, 8H), 4.52 (t, J = 6.6 Hz, 2H), 3.82 (s, 3H), 3.48–1.38 (m, 11H). ^{13}C NMR [DMSO-d_6] δ_c: 163.1, 160.7, 155.8, 154.9, 145.9, 136.7, 134.9, 132.2, 125.5, 120.2 (2C), 119.1, 116.2 (2C), 106.8, 56.5, 54.5, 52.3 (2C), 49.5, 46.7, 30.3 (2C). ESI-MS m/z: 440 $(M+H)^+$. Anal Calcd for $C_{23}H_{26}FN_5O_3$: C, 62.86; H, 5.96; N, 15.94. Found: C, 62.82; H, 5.99; N, 15.91.

*4.1.11.3. 1-(4-Chlorophenyl)-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)urea (**36**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**12**) (0.1 g, 0.330 mmol) and 4-chloro phenyl isocyanate (0.061 g, 0.397 mmol) to afford **36** (0.128 g, 85%) as pale brown solid. M.p: 206–208 °C. ^{1}H NMR [DMSO-d_6] δ_H: 10.49 (b, 1H), 10.41 (b, 1H), 7.96–6.68 (m, 8H), 4.49 (t, J = 6.3 Hz, 2H), 3.85 (s, 3H), 3.25–1.39 (m, 11H). ^{13}C NMR [DMSO-d_6] δ_c: 161.7, 156.8, 155.8, 145.9, 138.6, 135.7, 132.9, 132.3, 130.4 (2C), 125.6, 120.5 (2C), 119.5, 106.7, 56.2, 54.2, 52.8 (2C), 47.9, 46.5, 30.4 (2C). ESI-MS m/z: 456 $(M+H)^+$. Anal Calcd for $C_{23}H_{26}ClN_5O_3$: C, 60.59; H, 5.75; N, 15.36. Found: C, 60.57; H, 5.78; N, 15.38.

*4.1.11.4. 1-(1-(2-(7-Methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl) piperidin-4-yl)-3-(4-nitrophenyl)urea (**37**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**12**) (0.1 g, 0.330 mmol) and 4-nitro phenyl isocyanate (0.065 g, 0.397 mmol) to afford **37** (0.092 g, 60%) as yellow solid. M.p: 221–223 °C. ^{1}H NMR [DMSO-d_6] δ_H: 10.52 (b, 1H), 10.43 (b, 1H), 8.15–6.68 (m, 8H), 4.52 (t, J = 6.9 Hz, 2H), 3.86 (s, 3H), 3.36–1.48 (m, 11H). ^{13}C NMR [DMSO-d_6] δ_c: 162.9, 155.2, 154.8, 144.3, 143.3, 142.9, 135.7, 132.8, 125.2, 124.9 (2C), 120.2 (2C), 119.2, 106.5, 56.2, 54.1, 52.8 (2C), 47.8, 46.9, 30.2 (2C). ESI-MS m/z: 467 $(M+H)^+$. Anal Calcd for $C_{23}H_{26}N_6O_5$: C, 59.22; H, 5.62; N, 18.02. Found: C, 59.25; H, 5.58; N, 18.08.

*4.1.11.5. 1-(4-Acetylphenyl)-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)urea (**38**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**12**) (0.1 g, 0.330 mmol) and 4-acetyl phenyl isocyanate (0.063 g, 0.397 mmol) to afford **38** (0.12 g, 78%) as white solid. M.p: 230–232 °C. ^{1}H NMR [DMSO-d_6] δ_H: 10.59 (b, 1H). 10.42 (b, 1H), 8.34–6.70 (m, 8H), 4.68 (t, J = 6.6 Hz, 2H), 4.05 (s, 3H), 3.73–3.05 (m, 3H), 2.51 (s, 3H), 2.45–1.15 (m, 8H). ^{13}C NMR [DMSO-d_6] δ_c: 196.1, 161.0, 156.8, 154.2, 145.0, 140.8, 136.7, 135.1, 131.3, 129.6 (2C), 125.4, 120.9 (2C), 116.5, 104.9, 57.0, 52.0, 51.48 (2C), 51.0, 47.6, 29.2 (2C), 26.2. ESI-MS m/z: 464 $(M+H)^+$. Anal Calcd for $C_{25}H_{29}N_5O_4$: C, 64.78; H, 6.31; N, 15.11; Found: C, 64.77; H, 6.29; N, 15.14.

*4.1.11.6. 1-(1-(2-(7-Methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl) piperidin-4-yl)-3-(4-methoxyphenyl)urea (**39**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**12**) (0.1 g, 0.330 mmol) and 4-methoxyphenyl isothiocyanate (0.059 g, 0.397 mmol) to afford **39** (0.114 g, 76%) as yellow solid. M.p: 217–219 °C. ^{1}H NMR [DMSO-d_6] δ_H: 10.42 (b, 1H), 10.53 (b, 1H), 7.88–6.63 (m, 8H), 4.51 (t, J = 6.9 Hz, 2H), 3.85 (s, 3H), 3.81 (s, 3H), 3.27–1.39 (m, 11H). ^{13}C NMR [DMSO-d_6] δ_c: 162.7, 159.7, 157.8, 155.3, 145.2, 136.1, 131.7, 130.8, 125.6, 120.2 (2C), 119.5, 115.6 (2C), 106.5, 56.2 (2C), 54.2, 52.3 (2C), 47.8, 46.7, 30.3 (2C). ESI-MS m/z: 452 $(M+H)^+$. Anal Calcd for $C_{24}H_{29}N_5O_4$: C, 63.84; H, 6.47; N, 15.51. Found: C, 63.88; H, 6.43; N, 15.55.

*4.1.11.7. 1-(1-(2-(7-Methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl) piperidin-4-yl)-3-(p-tolyl)urea (**40**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**12**) (0.1 g, 0.330 mmol) and 4-methyl phenyl isocyanate (0.053 g, 0.397 mmol) to afford **40** (0.103 g, 72%) as pale brown solid. M.p: 225–227 °C. ^{1}H NMR [DMSO-d_6] δ_H: 10.42 (b, 1H), 10.36 (b, 1H), 7.83–6.66 (m, 8H), 4.42 (t, J = 7.5 Hz, 2H), 3.87 (s, 3H) 3.12–1.39 (m, 14H). ^{13}C NMR [DMSO-d_6] δ_c: 161.7, 157.2, 155.4, 145.8, 137.9, 137.2, 135.3, 132.7, 130.2 (2C), 125.6, 120.8 (2C), 119.2, 106.8, 56.2, 54.6, 52.5 (2C), 47.8, 46.5, 30.2 (2C), 20.8. ESI-MS m/z: 436 $(M+H)^+$. Anal Calcd for $C_{24}H_{29}N_5O_3$: C, 66.19; H, 6.71; N, 16.08. Found: C, 66.22; H, 6.78; N, 16.12%.

*4.1.12. General procedure for the synthesis of substituted 1-benzyl-3-(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)thiourea (**41**–**43**)*

A solution of 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**11**) (0.344 mmol) in dichloromethane (3 mL) was cooled to 0 °C followed by the addition of triethylamine (1.02 mmol). Corresponding substituted benzyl isothiocyanate (0.413 mmol) was added to the reaction mixture and was stirred in room temperature for 12 h (monitored by TLC & LCMS for completion). The reaction mixture was washed with water (3 × 5 mL) and brine (3 × 5 mL). Organic layer was dried over ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane: ethyl acetate as eluent to give the corresponding substituted1-benzyl-3-(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)thiourea (**41**–**43**) in good yields.

*4.1.12.1. 1-Benzyl-3-(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)thiourea (**41**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**11**) (0.1 g, 0.344 mmol) and benzyl isothiocyanate (0.062 g, 0.413 mmol) to afford **41** (0.108 g, 71%) as pale yellow solid. M.p:

174–176 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 8.68–6.82 (m, 9H), 5.52 (b, 1H), 5.26 (b, 1H), 4.36–4.12 (m, 4H), 2.89–1.25 (m, 11H). ^{13}C NMR [DMSO-d$_6$] δ_c: 179.8, 160.7, 157.3, 145.3, 139.8, 137.2, 132.7, 128.3 (2C), 127.8 (2C), 127.5, 124.6, 123.8, 109.8, 54.2, 53.4, 52.8 (2C), 47.3, 43.5, 30.2 (2C). ESI-MS *m/z*: 440 $(M+H)^+$. Anal Calcd for $C_{23}H_{26}FN_5OS$: C, 62.85; H, 5.96; N, 15.93; Found: C, 62.82; H, 5.95; N, 15.95.

*4.1.12.2. 1-(4-Chlorobenzyl)-3-(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)thiourea (**42**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**11**) (0.1 g, 0.344 mmol) and 4-chloro benzyl isothiocyanate (0.075 g, 0.413 mmol) to afford **42** (0.132 g, 80%) as yellow solid. M.p: 163–165 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 8.65–6.66 (m, 8H), 5.51 (b, 1H), 5.32 (b, 1H), 4.27–4.07 (m, 4H), 2.76–1.19 (m, 11H). ^{13}C NMR [DMSO-d$_6$] δ_c: 179.8, 160.7, 157.8, 145.2, 140.3, 137.8, 135.2 (2C), 132.9, 132.5, 129.5 (2C), 125.6, 123.5, 109.8, 55.4, 53.5, 51.5 (2C), 47.8, 46.5, 30.2 (2C). ESI-MS *m/z*: 474 $(M+H)^+$. Anal Calcd for $C_{23}H_{25}ClFN_5OS$: C, 58.28; H, 5.32; N, 14.78; Found: C, 58.30; H, 5.30; N, 14.75.

*4.1.12.3. 1-(1-(2-(7-Fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl) piperidin-4-yl)-3-(4-methoxybenzyl)thiourea (**43**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**11**) (0.1 g, 0.344 mmol) and 4-methoxy benzyl isothiocyanate (0.074 g, 0.413 mmol) to afford **43** (0.128 g, 79%)as brown solid. M.p: 133–135 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 8.52–6.73 (m, 8H), 5.34 (b, 1H), 5.21 (b, 1H), 4.29–4.05 (m, 4H), 3.69 (s, 3H), 2.81–1.12 (m, 11H). ^{13}C NMR [DMSO-d$_6$] δ_c: 179.8, 160.6, 158.0, 157.3, 145.6, 139.9, 132.7, 129.8 (2C), 129.6, 125.6, 123.9, 113.6 (2C), 109.3, 55.7, 54.9, 53.6, 52.8 (2C), 47.8, 45.2, 30.3 (2C). ESI-MS *m/z*: 470 $(M+H)^+$. Anal Calcd for$C_{24}H_{28}FN_5O_2S$: C, 61.39; H, 6.01; N, 14.91; Found: C, 61.35; H, 6.05; N, 14.93.

*4.1.13. General procedure for the synthesis of substituted 1-benzyl-3-(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)urea (**44**–**46**)*

A solution of 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**11**) (0.344 mmol) in dichloromethane (3 mL) was cooled to 0 °C followed by the addition of triethylamine (1.03 mmol). Corresponding substituted benzyl isocyanate (0.413 mmol) was added to the reaction mixture and was stirred in room temperature for 12 h (monitored by TLC & LCMS for completion). The reaction mixture was washed with water (3 × 5 mL) and brine (3 × 5 mL). Organic layer was dried over ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane: ethyl acetate as eluent to give the corresponding substituted1-benzyl-3-(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)urea (**44**–**46**) in good yield.

*4.1.13.1. 1-Benzyl-3-(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)urea (**44**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**11**) (0.1 g, 0.344 mmol) and benzyl isocyanate (0.055 g, 0.413 mmol) to afford **44** (0.116 g, 80%) as yellow solid. M.p: 180–182 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 8.71–6.84 (m, 9H), 6.12 (b, 1H), 5.84 (b, 1H), 4.36–4.12 (m, 4H), 2.89–1.25 (m, 11H). ^{13}C NMR [DMSO-d$_6$] δ_c: 161.6, 158.1, 157.3, 144.2, 139.9, 137.2, 132.7, 128.3 (2C), 127.8 (2C), 127.2, 124.6, 123.9, 109.4, 53.1, 52.4 (2C), 51.2, 48.1, 45.5, 31.3 (2C). ESI-MS *m/z*: 424 $(M+H)^+$. Anal Calcd for $C_{23}H_{26}FN_5O_2$: C, 65.23; H, 6.19; N, 16.54; Found: C, 65.21; H, 6.17; N, 16.53.

*4.1.13.2. 1-(4-Chlorobenzyl)-3-(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)urea (**45**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**11**) (0.1 g, 0.344 mmol) and 4-chloro benzyl isocyanate (0.069 g, 0.413 mmol) to afford **45** (0.105 g, 66%) as yellow solid. M.p: 215–217 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 8.77–6.86 (m, 8H), 6.11 (b, 1H), 5.89 (b, 1H), 4.32–4.11 (m, 4H), 2.88–1.21 (m, 11H). ^{13}C NMR [DMSO-d$_6$] δ_c: 160.7, 158.6, 157.4, 144.5, 139.9, 138.7, 137.5 (2C), 132.9, 132.7, 128.3 (2C), 124.8, 123.5, 109.8, 53.3, 52.8 (2C), 52.5, 46.5, 42.6, 31.3 (2C). ESI-MS *m/z*: 458 $(M+H)^+$. Anal Calcd for $C_{23}H_{25}ClFN_5O_2$: C, 60.33; H, 5.50; N, 15.29; Found: C, 60.35; H, 5.53; N, 15.27.

*4.1.13.3. 1-(1-(2-(7-Fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl) piperidin-4-yl)-3-(4-methoxybenzyl)urea (**46**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**11**) (0.1 g, 0.344 mmol) and 4-methoxy benzyl isocyanate (0.067 g, 0.413 mmol) to afford **46** (0.125 g, 80%) as brown solid. M.p: 137–139 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 8.67–6.81 (m, 8H), 6.84 (b, 1H), 5.81 (b, 1H), 4.34–4.08 (m, 4H), 3.71 (s, 3H), 2.83–1.14 (m, 11H). ^{13}C NMR [DMSO-d$_6$] δ_c: 160.6, 160.4, 158.0, 157.3, 139.9, 134.4, 133.1, 132.8 (2C), 132.7, 128.3, 123.9, 113.6 (2C), 109.3, 55.0, 53.1, 52.4 (2C), 52.2, 46.0, 42.2, 32.3 (2C). ESI-MS *m/z*: 454 $(M+H)^+$. Anal Calcd for $C_{24}H_{28}FN_5O_3$: C, 63.56; H, 6.22; N, 15.44; Found: C, 63.59; H, 6.25; N, 15.41.

*4.1.14. General procedure for the synthesis of substituted 1-benzyl-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl) piperidin-4-yl)thiourea (**47**–**49**)*

A solution of 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**12**) (0.330 mmol) in dichloromethane (1 mL) was cooled to 0 °C followed by the addition of triethylamine (0.99 mmol). Corresponding substituted benzyl isothiocyanate (0.397 mmol) was added to the reaction mixture and was stirred in room temperature for 12 h (monitored by TLC & LCMS for completion). The reaction mixture was washed with water (3 × 5 mL) and brine (3 × 5 mL). Organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane: ethyl acetate as eluent to give the corresponding substituted1-benzyl-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)thiourea (**47**–**49**) in good yield.

*4.1.14.1. 1-Benzyl-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)thiourea (**47**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**12**) (0.1 g, 0.330 mmol) and benzyl isothiocyanate (0.059 g, 0.397 mmol) to afford **47** (0.108 g, 77%) as pale yellow solid. M.p: 149–151 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 7.79–7.61 (m, 2H), 7.37 (b, 1H), 7.31 (b, 1H), 7.23–6.65 (m, 7H), 4.68 (s, 2H), 4.42 (t, $J = 6.6$ Hz, 2H), 3.91 (s, 3H), 3.08–1.25 (m, 11H). ^{13}C NMR [DMSO-d$_6$] δ_c: 180.91, 161.9, 155.6, 146.2, 138.2, 136.2, 132.4, 129.1 (2C), 126.2 (2C), 125.9, 124.5, 119.1, 105.2, 56.2, 54.9, 54.1, 52.5 (2C), 51.2, 48.5, 30.2 (2C). ESI-MS *m/z*: 452 $(M+H)^+$. Anal Calcd for $C_{24}H_{29}N_5O_2S$: C, 63.83; H, 6.47; N, 15.51. Found: C, 63.86; H, 6.44; N, 15.55.

*4.1.14.2. 1-(4-Chlorobenzyl)-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)thiourea (**48**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**12**) (0.1 g, 0.330 mmol) and 4-chloro benzyl isothiocyanate (0.072 g, 0.397 mmol) to afford**48** (0.114 g, 71%) as white

solid. M.p: 151–153 °C. ^{1}H NMR [DMSO-d$_6$]: δ_H8.28–0.7.85 (m, 2H), 7.70 (b, 1H), 7.47 (b, 1H), 7.41–6.64 (m, 6H), 4.64 (s, 2H), 4.38 (t, J = 7.2 Hz, 2H), 3.98 (s, 3H), 2.91–1.33 (m, 11H). ^{13}C NMR [DMSO-d$_6$] δ_c: 180.2, 161.5, 155.6, 146.2, 137.1, 136.5, 134.7 (2C), 132.5, 132.1, 129.2 (2C), 125.1, 119.2, 105.8, 56.2, 55.7, 54.5, 52.5 (2C), 51.2, 50.1, 30.2 (2C). ESI-MS m/z487 (M+H)$^+$. Anal Calcd for $C_{24}H_{28}ClN_5O_2S$: C, 59.31; H, 5.81; N, 14.41; Found: C, 59.41; H, 5.79; N, 14.45.

*4.1.14.3. 1-(1-(2-(7-Methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl) ethyl)piperidin-4-yl)-3-(4-methoxybenzyl)thiourea (**49**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**12**) (0.1 g, 0.330 mmol) and 4-methoxy benzyl isothiocyanate (0.071 g, 0.397 mmol) to afford **49** (0.122 g, 77%) as brown solid. M.p: 184–186 °C. ^{1}H NMR [DMSO-d$_6$]: δ_H8.2–7.87 (m, 2H), 7.58 (b, 1H), 7.43 (b, 1H), 7.45–6.61 (m, 6H), 4.57 (s, 2H), 4.35 (t, J = 6.6 Hz, 2H), 3.98 (s, 3H), 395 (s, 3H), 2.87–1.35 (m, 11H). ^{13}C NMR [DMSO-d$_6$] δ_c: 180.5, 161.5, 159.5, 155.1, 146.2, 135.3, 132.5, 130.2 (2C), 129.5, 125.3, 119.1, 115.2 (2C), 104.1, 56.2 (2C), 55.8, 54.5, 52.5 (2C), 51.2, 50.3, 30.2 (2C). ESI-MS m/z: 482 (M+H)$^+$. Anal Calcd for $C_{25}H_{31}N_5O_3S$: C, 62.35; H, 6.49; N, 14.54; Found: C, 62.38; H, 6.42; N, 14.56.

*4.1.15. General procedure for the synthesis of substituted 1-benzyl-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl) piperidin-4-yl)urea (**50**–**52**)*

A solution of 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**12**) (0.33 mmol) in dichloromethane (1 mL) was cooled to 0 °C followed by the addition of triethylamine (0.99 mmol). Corresponding substituted benzyl isocyanate (0.397 mmol) was added to the reaction mixture and was stirred in room temperature for 12 h (monitored by TLC & LCMS for completion). The reaction mixture was washed with water (3 × 5 mL) and brine (3 × 5 mL). Organic layer was dried over ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane: ethyl acetate as eluent to give the corresponding substituted1-benzyl-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)urea (**50**–**52**) in good yield.

*4.1.15.1. 1-Benzyl-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)urea (**50**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**12**) (0.1 g, 0.330 mmol) and benzyl isocyanate (0.052 g, 0.397 mmol) to afford **50** (0.105 g, 73%) as pale yellow solid. M.p: 184–186 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 10.75 (b, 1H), 10.52 (s, 1H), 7.92–6.67 (m, 9H), 4.56 (s, 2H), 4.41 (t, J = 7.2 Hz, 2H), 3.87 (s, 3H), 3.28–1.36 (m, 11H). ^{13}C NMR [DMSO-d$_6$] δ_c: 163.1, 157.8, 155.3, 145.7, 138.1, 136.1, 132.3, 129.1 (2C), 127.1 (2C), 126.8, 125.1, 119.2, 104.2, 56.2, 54.5, 52.3 (2C), 51.3, 48.5, 45.6, 30.1 (2C). ESI-MS m/z: 436 (M+H)$^+$. Anal Calcd for $C_{24}H_{29}N_5O_3$: C, 66.19; H, 6.71; N, 16.08. Found: C, 66.22; H, 6.75; N, 16.05.

*4.1.15.2. 1-(4-Chlorobenzyl)-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)urea (**51**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**12**) (0.1 g, 0.330 mmol) and 4-chloro benzyl isocyanate (0.066 g, 0.397 mmol) to afford **51** (0.129 g, 83%) as pale brown solid. M.p: 169–171 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 10.65 (b, 1H), 10.32 (s, 1H), 8.03–6.62 (m, 8H), 4.51 (s, 2H), 4.43 (t, J = 6.3 Hz, 2H), 3.86 (s, 3H), 3.31–1.42 (m, 11H). ^{13}C NMR [DMSO-d$_6$] δ_c: 161.2, 158.2, 155.6, 145.6, 137.2, 135.9, 135.1 (2C), 132.5, 131.9, 129.5 (2C), 125.2, 119.1, 105.1, 56.2, 54.5, 52.5 (2C), 51.5, 48.5, 45.6, 30.2 (2C). ESI-MS m/z: 470 (M+H)$^+$. Anal Calcd for $C_{24}H_{28}ClN_5O_3$: C, 61.34; H, 6.01; N, 14.90; Found: C, 61.39; H, 6.01; N, 14.97.

*4.1.15.3. 1-(1-(2-(7-Methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl) ethyl)piperidin-4-yl)-3-(4-methoxybenzyl)urea (**52**).* The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**12**) (0.1 g, 0.330 mmol) and 4-methoxy benzyl isocyanate (0.064 g, 0.397 mmol) to afford**52** (0.119 g, 77%)as brown solid. M.p: 185–187 °C. ^{1}H NMR [DMSO-d$_6$] δ_H: 10.44 (b, 1H), 10.29 (s, 1H), 8.12–6.71 (m, 8H), 4.65 (s, 2H), 4.48 (t, J = 6.6 Hz, 2H), 3.92 (s, 3H), 3.87 (s, 3H), 3.35–1.43 (m, 11H). ^{13}C NMR [DMSO-d$_6$] δ_c: 162.1, 159.7, 158.1, 155.6, 146.2, 136.6, 132.8, 130.7 (2C), 129.1, 124.8, 119.2, 115.2 (2C), 105.1, 56.2 (2C), 54.6, 52.5 (2C), 51.2, 48.6, 45.6, 30.2 (2C). ESI-MS m/z: 466 (M+H)$^+$. Anal Calcd for $C_{25}H_{31}N_5O_4$: C, 64.50; H, 6.71; N, 15.04; Found: C, 64.55; H, 6.69; N, 15.09.

4.2. M. tuberculosis DNA supercoiling assay

MTB DNA supercoiling assay was performed using hetero tetramer gyrase enzyme containing GyrA and GyrB subunits together, by using a MTB DNA supercoiling assay kit (Inspiralis Limited, Norwich) [8].The assay was performed in 1.5 mL Eppendorf tubes in 30 μL reaction volume consisting of assay buffer (50 mM HEPES. KOH (pH 7.9), 6 mM magnesium acetate, 100 mM potassium glutamate, 4 mM DTT, 2 mM spermidine, 1 mM ATP, and 0.05 mg/mL of albumin). This mixture was together incubated with 1U of MTB DNA gyrase with 0.5 μg of relaxed pBR322, a substrate and at varied test compound concentrations for 30 min at 37 °C, to calculate their IC_{50} value. Eventually the reaction was quenched by the addition of 30 μL of chloroform: isoamyl alcohol in a ratio of 24:1 and STEB buffer (40% sucrose, 100 mM Tris–HCl (pH 8.0), 100 mM EDTA and 0.5 mg/mL bromophenol blue) in equal volumes. Later the samples were subjected to brisk vortex for obtaining a homogenous state, centrifuged and loaded onto 1% Agarose gel in 1× TAE buffer. Staining with ethidium bromide continued for 10 min and de staining for 10 min with 1× TAE buffer was followed. Further, samples were analysed using Image lab software (Bio-Rad) and relative quantification was done based on the control sample. Novobiocin which was used as a positive control in this assay.

4.3. In vitro MTB MABA assay

All the compounds were further screened for their *in vitro* antimycobacterial activity against *M. tuberculosis* H37Rv strain (obtained from National Institute of Research in Tuberculosis, Chennai, India) by microplate alamar blue assay method [9] from 25 to 0.78 μg/mL. In brief, the inoculum was prepared from fresh LJ medium which was re-suspended in 7H9-S medium (7H9 broth, 0.1% casitone, 0.5% glycerol, supplemented oleic acid, albumin, dextrose, and catalase [OADC]), adjusted to a McFarland tube No. 1, and diluted 1:20; for each 100 μL was used as inoculum. Each drug stock solution was thawed and diluted in 7H9-S by four-fold the final highest concentration tested. Serial two-fold dilutions of each drug were prepared directly in a sterile 96-well flat-bottomed micro titre plates using 100 μL of 7H9-S. A growth control containing no antibiotic and a sterile control were also prepared for each plate. In order to avoid evaporation during incubation of 7 days, sterile water was added to all perimeter wells. The plate was covered, sealed in plastic bags and incubated at 37 °C in normal atmosphere. After 7 days of incubation, 30 μL of alamar blue solution was added to each well, and the plate was further re-incubated overnight. Subsequently, a change in colour from blue (oxidized state) to pink (reduced condition) indicated growth of

bacteria, and the MIC was defined as the lowest concentration of drug that prevented this change in colour.

4.4. In vitro cytotoxicity screening

Eukaryotic RAW 264.7 mouse macrophages cells were used to test the cytotoxic activity of all the compounds [10]. Toxicity was measured by incubating the test compounds in 96 flat-bottomed well plates containing cell counts of 5×10^5 at different concentrations, with 5% CO_2 and 95% O_2atmosphere for 48 h at 37 °C [15]. About 4 h before the end of incubation period 10 μL of MTT reagent (10 mg mL^{-1}) was added, and the plate was centrifuged at 1200 rcf for about 3 min to obtain a clear supernatant. The supernatant was removed, and subsequently to each well 200 μL of DMSO was added to dissolve the so formed formazan crystals. The absorbance was measured at a wavelength of 560 nm on Perkin Elmer Victor X3 microplate reader against the blank after a span of 10 min. Assays were performed in triplicates for each concentration of drug to minimize the error rate. Cytotoxicity of each compound was expressed as % inhibition at that particular concentration.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.08.018.

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