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Optimization of potency and pharmacokinetics of tricyclic indole derived inhibitors of HCV NS5B polymerase. Identification of ester prodrugs with improved oral pharmacokinetics

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ABSTRACT

HCV infections are the leading causes for hepatocellular carcinoma and liver transplantation in the United States. Recent advances in drug discovery have identified direct acting antivirals which have significantly improved cure rates in patients. Current efforts are directed towards identification of novel direct acting antiviral targeting different mechanism of actions which could become part of all oral therapies. We recently disclosed the identification of a novel tricyclic indole derived inhibitors of HCV NS5B polymerase that bound to the enzyme close to the active site. In this manuscript we describe further optimization of potency and pharmacokinetics (PK) of these inhibitors to identify compounds in low nM potency against gt-1b. These analogs also demonstrate excellent PK in rats and monkeys when administered as a dimethyl ethyl amino ester prodrug.

1. Introduction

Hepatitis C virus is the primary causative agent for non-A, non B hepatitis.[1] More than 80% of all infections turn chronic at the risk of developing severe liver disease resulting in hepatocellular carcinoma.[2] Recent advances in development of direct acting antivirals have significantly improved SVR (sustained virologic response) in patients providing a new hope for cure in infected patients.[3] Currently discovery of new treatments are directed towards interferon sparing all oral therapies that are well tolerated with shorter duration of treatment. Direct acting antivirals derived from inhibition of different mechanism are combined to achieve high rates of cure.[3]

The HCV NS5B RNA dependent RNA polymerase has evolved as a primary target for identification of novel inhibitors for drug intervention. Proof of concept for this target was initially demonstrated by Nesbuvir (HCV-796)[4] which demonstrated significant viral load reduction in humans. However, it was discontinued from further development due to adverse side effects. Other non-nucleoside inhibitors such as filbuvir,[5] ANA598,[6] VCH-759[7] VX222, BI207127 and ABT333 are currently undergoing clinical evaluation and have demonstrated robust viral load reductions in humans. Nucleoside derived inhibitors such as Sofusbuvir[8] have also demonstrated excellent efficacy and being evaluated as a component of all oral regimens.

We recently disclosed the discovery of a tricyclic indole derived HCV NS5B polymerase inhibitor that bound to the enzyme close to the active site[9] Analogs derived from this scaffold demonstrated excellent enzyme inhibition and modest cellular activity and PK in animals. In this manuscript we disclose further optimization of potency and PK leading to compounds with low single digit replicon activity and excellent oral exposures.

2. Chemistry

Compounds described in this manuscript were synthesized following synthetic routes shown in following schemes. Scheme 1 outlines the synthesis of tricyclic indole and functionalization at the N1 nitrogen.

Alkylation of 2-bromo-5-fluorophenol with diethyl acetal of bromoacetaldehyde followed by cyclization of the resulted ether

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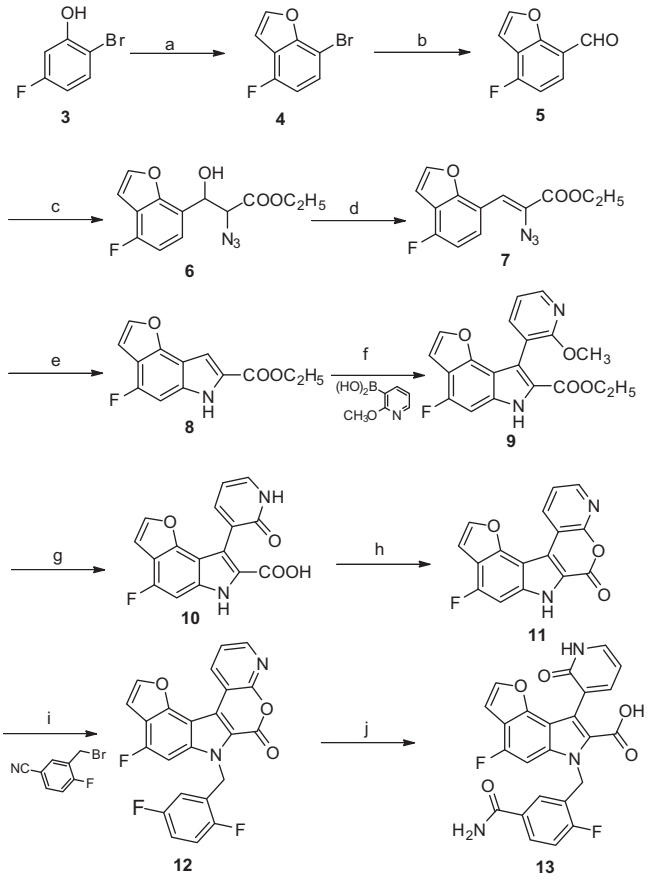
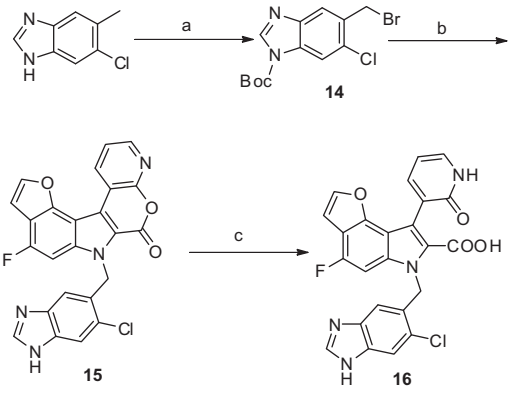


Scheme 1. Reagents and conditions: (a) (i) Cs_2CO_3, $(C_2H_5O)_2CH–CH_2Br$, DMF, 150 °C; (ii) PPA, toluene, reflux, 51%; (b) BuLi, ether, −78 °C, DMF, 94%; (c) $N_3CH_2COOC_2H_5$, DBU, LiCl, THF, rt, 81%; (d) CH_3SO_2Cl, Et_3N, −78 °C → rt, 86%; (e) Xylenes reflux, 70%; (f) (i) NIS, DMF, 93%; (ii) $Pd(PPh_3)_2Cl_2$, K_2CO_3, H_2O/DME, 80%; (g) (i) HCl, dixoane reflux; (ii) aq LiOH, THF reflux, 85%; (h) EDCI, Et_3N, DMF, 78%; (i) Cs_2CO_3, DMF, 51%;(j) aq LiOH, THF reflux, 81%.

with polyphosphoric acid yielded furan **4**.[10] Halogen metal exchange with butyl lithium followed by reaction with DMF formed aldehyde **5**, which was condensed with ethyl azido acetate using DBU and LiCl to form azido alcohols **6** as a mixture of diastereomers. The mixtures of alcohols were eliminated using methanesulfonyl chloride and triethylamine to form azido ester[11] **7** which was cyclized to indole **8** by refluxing in xylenes.[12]On cooling to room temperature, indole **8** crystallized from the reaction mixture which was filtered. Iodination of indole **8** with NIS resulted in selective iodination at C(3) positions which was coupled with 2-methoxy pyridin-3-yl boronic acid to yield **9**.[13] The methyl ether of **9** was demethylated with 4 M HCl in dioxane to form the pyridone which was further saponified using aqueous lithium hydroxide to form carboxylic acid **10**. Treatment of resultant acid with EDCI induced an intramolecular cyclization to form tetracyclic lactone **11**. N-alkylation of **11** with 3-(bromomethyl)-4-fluorobenzonitrile in DMF resulted in *N*-alkylated lactone intermediate which was further hydrolyzed using aqueous lithium hydroxide to form desired inhibitor **13**. 7-Fluoro substituted indole was synthesized starting from 2-bromo-4 fluorophenol and using similar reaction conditions to assemble tricyclic indole of type **9**. It was alkylated with 5-fluorobenzyl bromine and hydrolyzed with LiOH. It was further demethylated using 4 M HCl in dioxane as previously described.

N1 substituents that were not readily available were synthesized using methods shown below. The synthesis of imidazole **16**



Scheme 2. Reagents and conditions: (a) (i) Boc_2O, DMAP; (ii) NBS, Bz_2O_2, CCl_4 reflux, 54%; (b) (i) **11,** Cs_2CO_3, DMF; (ii) TFA, CH_2Cl_2, 76%; (c) aq LiOH, THF, 48%.

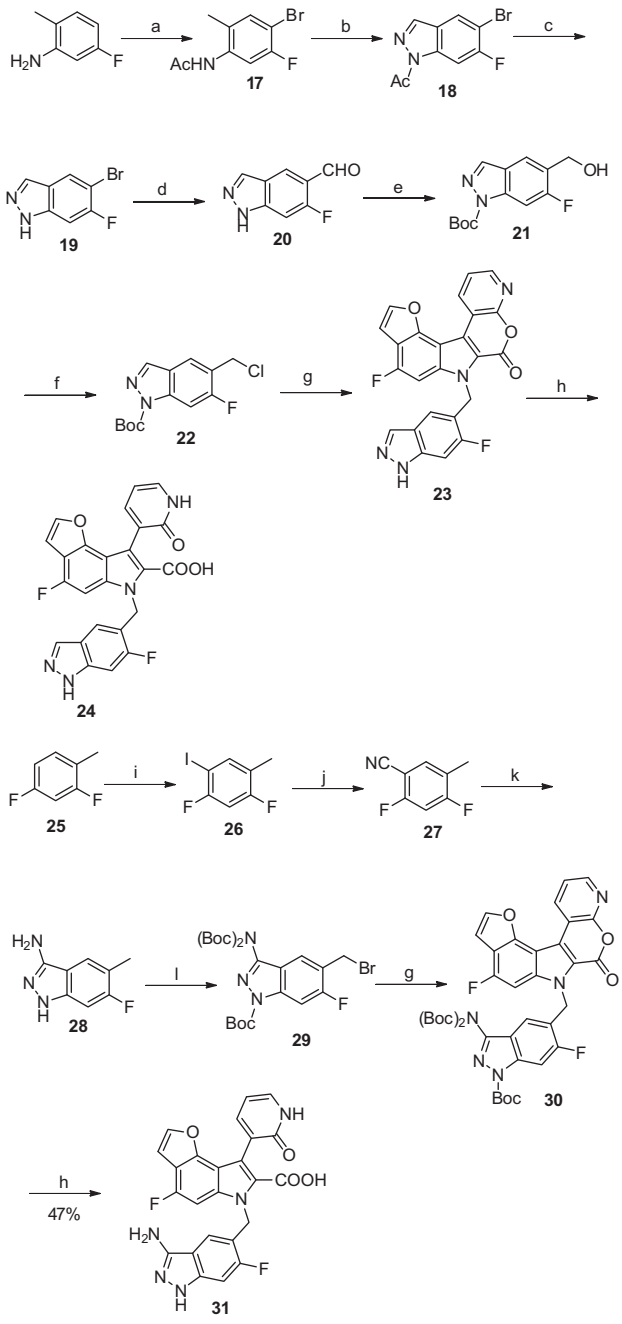
is shown in Scheme 2. Commercially available 4-chloro-5-methylbenzene-1,2-diamine was refluxed with trimethyl orthoformate to form 6-chloro-5-methyl-1*H*-benzo[*d*]imidazole.[14] The imidazole nitrogen was protected with Boc group and the resulting compound was brominated using NBS and benzoyl peroxide to yield bromide **14**, which was converted to imidazole derivative **16** as previously described.

Scheme 3 outlines the syntheses of tricyclic indole analogs derived from N1 indazole and 3-aminoindazole. Acetylation of 5-fluoro-2-methylaniline with acetic anhydride formed acetanilide **17** which was selectively brominated at the 4-position using bromine in acetic acid. Compound **17** was diazotized with isoamyl nitrite in the presence of potassium acetate which underwent cyclization to form acetylated indazole **18**. Refluxing **18** with a solution of aq HCl resulted in deacetylation to form 1*H*-indazole **19**.[15] Halogen metal exchange of **19** followed by reaction with DMF resulted in formation of aldehyde **20**. The indazole **20** was protected with Boc group and reduced to alcohol **21** by treatment with sodium borohydride in excellent yield. This two-step sequence effectively homologated the aryl bromide **19** to benzylic derivative **21**. Treatment of alcohol **21** with methanesulfonyl chloride and triethylamine resulted in formation of benzylic chloride **22** which was converted to the indazole analog **24** using chemistry previously described.

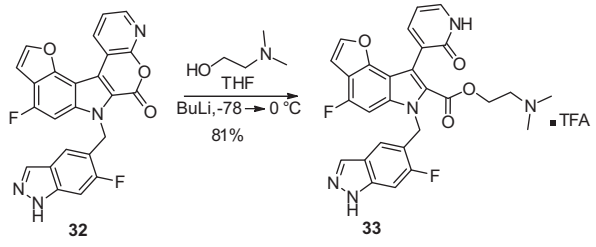
Amino indazole derived inhibitor **31** was synthesized starting from 2,4-difluorotoluene. Treatment with NIS in TFA resulted in regioselective iodination to yield iodide **26**.[16] Palladium catalyzed cyanation of **26** with zinc cyanide and $(PPh_3)_4Pd$ installed the nitrile group forming 2,4-difluoro-5-methylbenzonitrile (**27**). Refluxing a solution of **27** with hydrazine formed 3-amino indazole derivative **28** which was exhaustively Boc-protected using Boc_2O and DMAP. The resultant tri-Boc protected compound was brominated using NBS and benzoyl peroxide to form bromo compound **29** which was converted to inhibitor **31** following similar procedures outlined in Scheme 1.

The methodology used for the syntheses of ester prodrugs is shown in Scheme 4. Deprotonation of 2,2-dimethylamino ethanol with butyl lithium followed by reaction with lactone **32** and purification using reverse phase HPLC resulted in ester derivative **33** as the corresponding trifluoro acetate salt.

Synthesized analogs were initially evaluated in an enzyme assay to determine IC_{50}. Compounds demonstrating excellent enzyme inhibition were evaluated in the replicon based cellular assay to determine their ability to reduce HCV viral RNA levels by 50% to generate EC_{50}. Selected compounds displaying good enzyme potencies and cellular activities were evaluated for oral exposure in rats using the rapid rat assay.[17] Compounds



Scheme 3. Reagents and conditions: (a) (i) Ac_2O, toluene, (ii) Br_2, acetic acid 82%;(b) isoamyl nitrite, CH_3COOK, $CHCl_3$ 18-crown-6; (c) aq HCl reflux; (d) (i) NaH, THF, 0 °C (ii) BuLi, −78 °C then DMF, 86%; (e) (i) Boc_2O, DMAP (ii) $NaBH_4$, MeOH, 68%; (f) CH_3SO_2Cl, Py, CH_2Cl_2, 59%; (g) (i) **11**, Cs_2CO_3, DMF, 96%; (ii) HCl; dioxane (h) aq LiOH, THF; (i) NIS, TFA, 77%; (j) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 42%; (k) N_2H_4, Isopropnol, reflux, 10%; (l) (i) Boc_2O, DMAP; (ii) NBS, Bz_2O_2, 56%.



Scheme 4.

demonstrating acceptable oral exposures in rats were further evaluated in full PK in rats and monkeys. Synthesized prodrugs were evaluated in rats and monkeys using similar paradigm monitoring both the concentration of the ester prodrug and the acid metabolite in plasma.

3. Results and discussion

We recently disclosed the discovery of 4–5 furan fused tricyclic indole derivatives of type **34** as potent inhibitors of HCV polymerase that bound to the enzyme close to the active site.[9,18] Even though compounds in this class had excellent enzyme inhibition activity, they demonstrated modest cellular potencies. Moreover, they have an embedded furan ring that could potentially lead to reactive metabolites resulting from CYP mediated oxidation of the benzofuran. It is well established that furans readily undergo P_{450} mediated oxidation forming 2-butene-1,4-dials which are further conjugated by proteins and glutathione. Eventhough benzofurans are metabolized differently compared to furans, given the plethora of toxicity literature of furans early investigation of oxidative metabolites deemed prudent.[19]

Microsomal incubation of **34** resulted in formation of minor metabolites with mass consistent to **38** and **40** (Fig. 1). It is conceivable that **40** could be formed from the opening of epoxide intermediate **37** by water. Structure **38** was envisioned to be formed from compound **34** via oxidation of pyridone ring followed by rearrangement leading to pyrrole derivative. To further confirm the formation of reactive intermediate analog **34** was incubated with human microsomes in the presence of methoxyl amine. Metabolites resulting from addition of methoxyl amine and glutathione **39** and **41** were observed in MS at low concentrations indicating potential formation of epoxide intermediate **37**. We therefore needed methods to suppress the formation of this intermediate. One of strategies widely adapted to suppress CYP mediated oxidations is the introduction of strategically placed fluorine atoms. The incorporation of fluorine atom not only minimizes oxidation of C–H at the carbon bearing the fluorine atom but also reduces metabolism at carbons in the vicinity. Based on this premise we synthesized C(6) and C(7) fluorinated analogs **35** and **36**.

The C(7) fluoro analog **35** (IC_{50} = 17 nM, EC_{50} = 2600 nM) demonstrated a comparable enzyme activity to the desfluoro analog **34** (IC_{50} = 31 nM, EC_{50} = 160 nM) however, the cellular activity of the fluorinated analog was ~15-fold less active than hydrogen derivative. Whereas, the C(6) fluorinated derivative **36** (IC_{50} = 2 - nM, EC_{90} = 90 nM) retained similar potency to the desfluoro analog **34**. Furthermore, incubation of **36** with human microsomes suppressed oxidation metabolites and did not form any glutathione addition products. Thus, the introduction of fluorine at C(6) had the beneficial effect of minimizing oxidation metabolite at the furan ring, yet retaining cellular potency. We therefore decided to develop **36** as our new lead and optimize potency by modification of N_1 moieties.

Previous SAR studies in the desfluoro series of inhibitors had demonstrated that the introduction of 2-fluoro-5-carbamoyl benzyl N_1 moiety improved cellular activity of the resulting inhibitors.[9] We therefore incorporated this moiety in the C(6) fluoroindole series resulting in analog **13** (IC_{50} = 4 nM, EC_{50} = 15 - nM). This modification resulted in improvement in cellular potency. Evaluation of **13** in full rat PK demonstrated a moderate exposure with an AUC = 1.7 μM h when dosed at 3 mg/kg and a low bioavailability of 1.5%. Analysis of plasma samples for metabolites demonstrated no glutathione adducts further validating microsomal incubation results. Having identified potent inhibitors in the cellular assay we investigated modification of N_1 substitu-

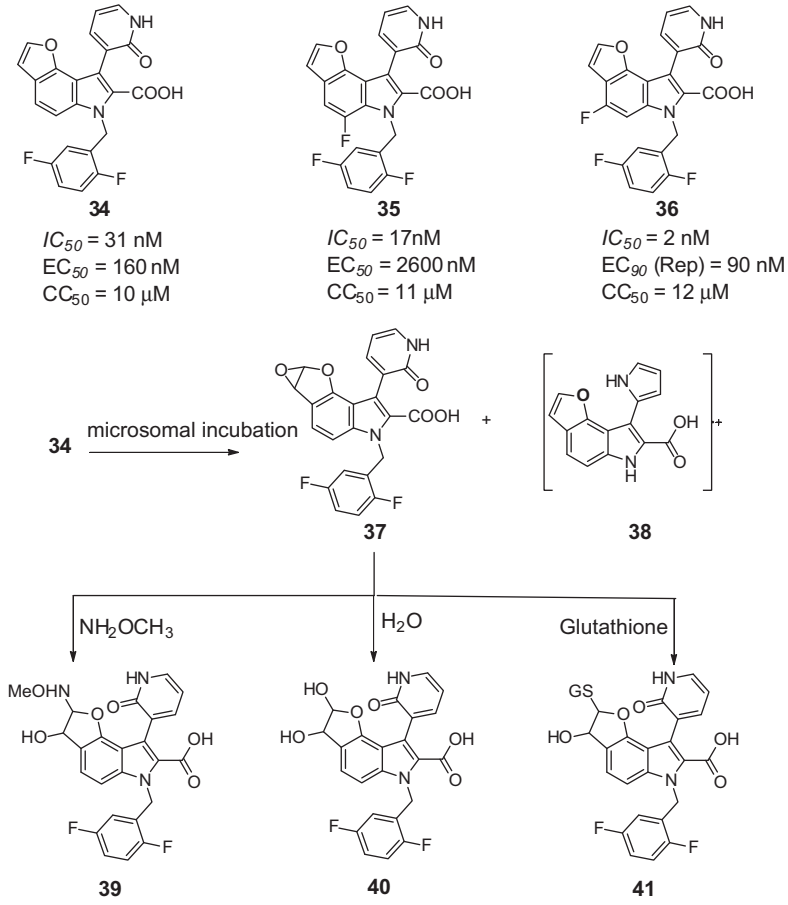


Figure 1. Microsomal incubation results of benzofuran derived inhibitors.

tion to improve oral PK in animals. We reasoned the poor oral exposure of **13** resulted from reduced absorption. We hypothesized the presence of primary amide potentially contributed to reduced oral absorption. We therefore replaced the carboxamide functionality with bicyclic heterocyclic isosteres containing hydrogen bond donors. These results are summarized in Table 1.

Replacement of *N*-2-fluoro-5-carbamoyl benzyl group in analog **13** with benzimidazole moiety resulted in analog **42** (IC_{50} = 5 nM; EC_{50} = 30 nM) with similar binding and cellular activity as amide analog. Introduction of fluorine at 5 position of benzimidazole resulted in analog **43** (IC_{50} = 3 nM; EC_{50} = 7 nM) with improved cellular potency compared to **42**. Replacement of fluorine with chlorine was also equally well tolerated with the resulting analog **16** having similar activity to the fluoro derivative **43**. We next evaluated the incorporation of a series of regioisomeric indazoles at N1. Incorporation of 3-amino-6-fluoro-1*H*-indazolylmethyl group at N1 resulted in very potent derivative **31** (IC_{50} = 4 nM; EC_{50} = 3 nM) with excellent enzyme binding and cellular potencies. Evaluation of this compound for oral PK in rats and monkeys showed modest exposure in rats AUC = 2.1 μM and no exposure in cynomologus monkeys AUC = 0 μM h.

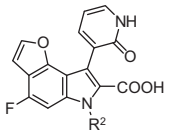
Introduction of regioisomeric amino indazole was also tolerated resulting in compound **44** (IC_{50} = 5 nM; EC_{50} = 12 nM) with a modest decrease in cellular activity. *N*-methylation of indazole nitrogen in **31** also resulted in an analog with poor cellular activity (IC_{50} = 6 nM; EC_{50} = 20 nM). However the incorporation of 6-fluoro-1*H*-indazole resulted in analogs **24** with good enzyme and cellular potencies (IC_{50} = 3 nM; EC_{50} = 4 nM). It was also very reassuring to find it was well absorbed in rats with an AUC = 9.3 μM h and monkeys with AUC = 1.0 μM h. The corresponding chloro (**48**) and methyl analogs (**49**) were also well tolerated and demonstrated adequate exposure in rats.

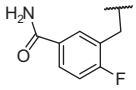
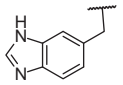
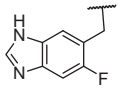
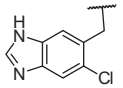
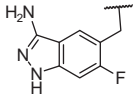
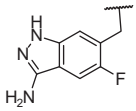
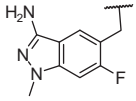
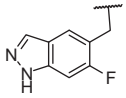
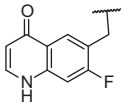
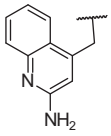
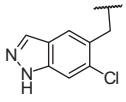
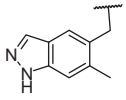
Replacement of N_1 group with 6-(7-fluoro-4-oxo-1,4-dihydroquinolinyl)methyl moiety and 6-((2-fluoroquinolin-3-yl)methyl resulted in analogs **46** (IC_{50} = 3 nM; EC_{50} = 7 nM) and **47** (IC_{50} = 4 - nM; EC_{50} = 6 nM) with excellent binding and cellular activities. The amino quinoline derivative **47** showed good oral exposure in rats (AUC_{0-6h} = 24 μM h) but was poorly absorbed in monkeys (AUC_{0-6h} = 0.4 μM h).

It was clear from synthesized analogs that most analogs demonstrated poor exposure in monkeys. Our efforts to boost PK in rats by making carboxylate salt **50** resulted in modest improvement in oral AUC (Table 2). We therefore explored the synthesis of prodrugs to enhance oral exposure. As an initial screen we synthesized novel ester prodrugs of analog **16** and evaluated them in rats. Analog **16** was chosen as a potential candidate for prodrug investigation based on its potency and ease of synthesis. Synthesized compounds were dosed orally at 10 mg/kg in rats and the AUC of both the parent ester and the corresponding acid were evaluated integrating area under the curve over 0–6 h post dosing.

As shown in the Table 2, esters derived from both primary and secondary alcohols were synthesized. In addition, alcohols chosen for ester formation contained both lipophilic and hydrophilic groups to modulate aqueous and lipid solubilities. Thus, introduction of ethyl ester resulted in analog **51** (AUC_{0-6h} = 0.0 μM h), which displayed poor PK for both the parent ester and the acid metabolite. The introduction of methoxyethyl ester resulted in analog **52** with the acid metabolite showing an AUC_{0-6h} = 1.1 μM h. This was

Table 1
Structure activity relationship of bicyclic heterocycles substitution at N_1.



Entry	R^2	Gt-1b IC_{50} (nM)	Gt-1b EC_{50} (nM)	CC_{50} (μM)	AUC_{0-6h} (μM h) rat
13		4	15	2.5	1.7*
42		5	30	2.0	9.3
43		3	7	50	7.7
16		6	8	25	1.2
31		4	3	1	2.1
44		5	12	2	3.6
45		6	20	2	7.2
24		3	4	51	9.3
46		3	7	2	
47		4	6	50	24
48		3	5	50	5.1
49		3	2	–	2.1

Compounds were orally dosed in Sprague Dawley rats at 10 mg/kg in MC and cynomolgus monkeys at 3 mg/kg in 0.4% MC.

partially better than dosing the ethyl ester **51**, however it was no better to the carboxylic acid **16**. Even though the PK was not improved compared to the corresponding acid analog it was clear from this study that synthesized esters were substrates of esterases and were being hydrolyzed to yield desirable acid. We next evaluated secondary aliphatic esters derived from 2-methyl benzyl alcohol and 2-indanol resulting in esters **53** (AUC_{0-6h} = 0.2 μM h) and **54** (AUC_{0-6h} = 0 μM h). Both these esters displayed poor PK in rats. A comparison of ester prodrugs **50**–**54** revealed the oxygen containing prodrug **52** had better AUC for the acid metabolite than aliphatic esters **51**, **53** and **54**. We next decided to explore amine containing esters and dose them as corresponding amine salts.

Dimethylamino ethyl ester prodrug had a profound effect on exposure of these inhibitors in rats. Pharmacokinetic studies of dimethylamino ethyl ester derivative **55** resulted in marked improvement in AUC_{0-6h} of the acid. The exposure for the metabolite acid increased from 1.1 to 32.5 μM h. This was ~30-fold improvement in exposure of **16**. Homologation of dimethylamino ethyl ester to dimethylamino propyl ester resulted in analog **56** which demonstrated poor AUC_{0-6h} = 0.7 μM h for the acid metabolite. However

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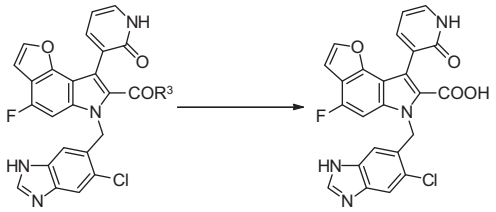
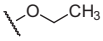
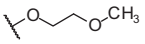
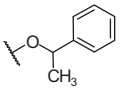
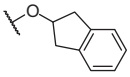
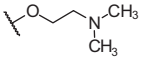
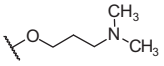
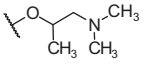
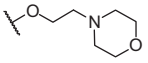
Table 2
Effect of ester prodrugs on AUC of analog 16.



Entry	R^3	AUC_{0-6h}-prodrug (μM h)	AUC_{0-6h}-acid (μM h)
50		–	2.1
51		0.1	0
52		0.2	1.1
53		0.3	0.2
54		0.0	0.0
55		0.5	32.5
56		9.1	0.7
57		0.8	32.6
58		0.2	5.5

Esters 55–58 were orally dosed in Sprague Dawley rats at 10 mg/kg in 0.4% MC as the corresponding trifluoroacetate salts.

the exposure for the ester prodrug was good (AUC_{0-6h} = 9.1 μM h). This clearly indicated that the hydrolysis of the propyl ester was rate limiting and was inefficiently converted to the acid. One could speculate the inefficient hydrolysis of **56**–**16** was probably because **56** was a poor substrate for the enzyme responsible for hydrolyzing the ester. We further explored other prodrugs by incorporating 1-dimethylamino 2-proponol and 2-morpholino ethanol yielding **57** (AUC_{0-6h} = 32.6 μM h) and **58** (AUC_{0-6h} = 5.5 μM h) indicating both were efficiently converted to the corresponding acid in vivo. It was clear from this screening that polar esters were preferred and dimethylamino ethyl and 1-dimethylamino 2-propyl esters were most desirable. Since the introduction of 1-dimethylamino 2-propyl ester resulted in the compound with a stereo center, we decided to utilize dimethylamino ethyl ester for further investigation.

Having identified dimethyl amino ethyl ester as a desirable prodrug we introduced this in various analogs and evaluated these compounds for full PK in rats and monkeys. Table 3 summarizes the improvement in exposure of the parent by incorporation of dimethyamino ethyl ester prodrugs. Various analogs with desirable cellular potencies were converted to the corresponding prodrug following procedure outline in Scheme 4 and dosed in rats at 10 mg/kg in 0.4% methylcellulose. The concentration of the acid metabolite was monitored in plasma over 24 h. It was very encouraging to observe a marked improvement in exposure of all derivatives on administering the prodrug.

Synthesis of dimethylamino ethyl ester prodrug of **43** resulted in compound **59** that improved the oral exposure in rats from AUC_{0-6h} = 7.7 to 159 μM h over 0–24 h. Similarly the formation of dimethyl amino ethyl ester prodrug of **16** resulted in ~175 fold improvement in PK rising the AUC_{0-6h} from 1.2 μM h to AUC_{0-24-h} = 208 μM h. A similar beneficial effect was observed in the indazole analogs **31, 24** and **49**. The 3-aminoindazole derivative **31** (AUC_{0-6h} = 2.1 μM h) had demonstrated an inadequate exposure to be projected as a potential QD drug whereas the dimethylamino ethyl ester product improved exposure (AUC_{0-24h} = 118 μM h) making it suitable for QD dosing. Similarly analogs **33** and **61**, prodrugs of **24** and **49** improved AUC from 9.3 and 5.1 μM h to 222 and 79 μM h respectively. Ester **62**, prodrug of compound **47** resulted in 30-fold improvement in exposure with an AUC_{0-24-h} = 651 μM h.The prodrugs were also dosed in monkeys at 3 mg/kg and assayed for concentration of ester and acid metabolite in the plasma (Table 3). It is evident from the table that in most cases the exposure was greatly improved compared to dosing the carboxylic acid as the sodium salt. In almost all cases the AUC_{0-24h} were between 10 and 20 μM h with exposures of acid metabolites sustaining over 24 h supporting potential once a day dosing in humans.

4. Conclusions

We recently disclosed the identification of HCV NS5B polymerase inhibitors that bound to the enzyme close to the active site, derived from 6*H*-furo[2,3-e]indole-7-carboxylic acid core. Initial lead characterized by compounds of type **34** (IC_{50} = 31 nM, EC_{50} = 160 - nM) demonstrated excellent enzyme inhibition with modest replicon cellular potencies and pharmacokinetics. In addition, the tricyclic indole core incorporated a fused furan moiety which was a potential structural alert leading to reactive metabolites derived from cytochrome P_{450} mediated oxidations.

Therefore, we first interrogated analog **34** for formation of reactive species by incubating it with microsomes in the presence and absence of glutathione and *N*-methoxylamine. Although the conversions were low, we could clearly detect glutathione and methoxy amine trapped products in mass spectrum. This required further modification of the lead to eliminate or minimize these undesired metabolites. We therefore investigated introduction of fluorine atoms in the ring to reduce oxidation.

Incorporation of fluorine at C(6) position was well tolerated resulting in compound **36** (IC_{50} = 2 nM, IC_{50} = 90 nM). It was also determined that introduction of C(6) fluorine atom suppressed oxidation products thus generating further interest in optimizing of the tricyclic indole lead.

Previous studies had identified incorporation of 2-fluoro-5-carbamoyl benzyl group improved cellular potency**.** Incorporation of this N_1 group in **36** resulted in analog **13** (IC_{50} = 4 nM, IC_{50} = 15 - nM, AUC_{0-24h} = 1.7 μM h). However inhibitor **13** was poorly absorbed when administered orally in rats at 3 mg/kg. In an attempt to improve PK retaining the improved cellular activity of **13** we explored introduction of novel bicyclic hetreocycles at N_1 that mimicked amide funtional groups. This had a profound impact on the cellular activities of our compounds. The introduction of benzimidazole, and indazole moieties resulted in analogs **16, 31** and **24** that had improved potency to analog **13**. These analogs demonstrated modest PK in rats however had suboptimal PK in monkeys. We therefore explored ester prodrugs and identified dimethyl amino ethyl ester that greatly improved PK. These ester derivatives were orally well absorbed and efficiently converted to the corresponding acid metabolite in vivo, thus greatly improving PK across species. The exact processing of the prodrug is still being investigated. Due to the similarity to choline one could speculate the role of choline esterases mediating the hydrolysis of these prodrugs. Thus we have optimized the potency and PK of our initial tricyclic indole leads to identify analogs with excellent potency and PK.

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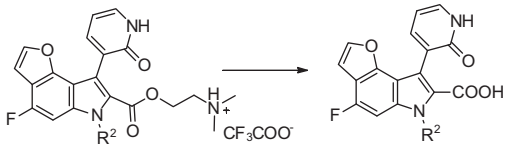
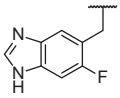
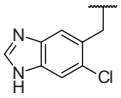
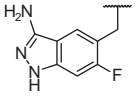
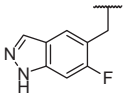
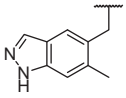
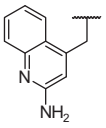
Table 3
Effect of dimethylaminoethyl ester prodrug on exposure of potent ticyclic inhibitors of HCV.



Entry	R^2	Gt-1b EC_{50} (nM)	AUC_{0-24h} (μM h) Acid[a] (Rat)	AUC_{0-24h} (μM h) Acid[a] (Mky)
59		6	159	14.6
55		13	208	14
60		4	118	4.2
33		6	222	33
61		2	79	10.7
62		7	651	17

[a] Dosed orally to cynomolgus monkeys (3 mg/kg) and Sprauge Dawley (10 mg/kg) in 0.4% MC. AUC represents area under the curve from 0 to 24 h.

5. Experimental

5.1. General

Dry solvents were purchased from Aldrich or Acros and used without further purification. Other solvents or reagents were used as obtained except when otherwise noted. Analytical thin layer chromatographies (TLC) were performed on pre-coated silica gel plates available from Analtech. Column chromatography were performed using Merck silica gel 60 (particle size 0.040–0.055 μm, 230–400 mesh), or using Biotage or Isco chromatographic systems with pre-packed silica columns. Purification of target compounds were done on reverse phase HPLC C_{18} column. Elution was achieved using water, and acetonitrile or water containing 0.1% TFA and THF system. (Column:Waters:Delta Pk, P/No 11805, Wat 011805, 300 mm × 30 mm (L/ID) C18, 15 μM, 300 Å, 343K16006 (W): 30 mL/min flow; 30–70% ramp water/acetonitrile; 0 → 40 min). Visualization was accomplished with UV light or by staining with basic $KMnO_4$ solution, methanolic H_2SO_4 or Vaughn's reagent. NMR spectra were recorded in $CDCl_3$ or D_6-DMSO unless otherwise noted either in a 400 or 500 MHz (^{1}H NMR), or 100 or 125 MHz (^{13}C NMR). Mass spectra were obtained using electron spray ionization methods. Where applicable ^{13}C NMRs are reported as fluorine coupled spectra unless otherwise indicated.

5.1.1. 7-Bromo-4-fluorobenzofuran (4)

A solution of 2-Bromo-5-fluorophenol (228.00 g, 1.19 mol), Potassium carbonate (247.47 g, 1.79 mol) in *N*,*N*-Dimethylformamide (3.00 L) was treated with 2-Bromo-1,1-diethoxyethane (197.54 mL, 1.31 mol) and heated at 135 °C for 7 h. The reaction mixture was concentrated in vacuo and extracted with EtOAc (3 × 2 L). The combined organic layers were washed with aq NaOH (2 M, 4 L). The organic layer was dried ($MgSO_4$), filtered, concentrated in vacuo to yield 1-Bromo-2-(2,2-diethoxyethoxy)-4-fluorobenzene (362.00 g; Yield = 98%) that was used as it is in the next step. ^{1}H NMR (400 MHz, D_6-DMSO) δ 7.58 (d, 1H, J = 6.8 & 8.8 Hz), 7.11 (dd, 1H, J = 2.8 & 10.8 Hz), 6.76 (dt, 1H, J = 2.2 & 8.1 Hz), 4.81 (t, 1H, J = 5.1 Hz), 4.03 (d, 2H, J = 5.1 Hz), 3.73–3.56 (m, 4H), 1.13 (t, 6H, J = 6.6 Hz).

A solution of 1-Bromo-2-(2,2-diethoxyethoxy)-4-fluorobenzene (352.00 g, 1.15 mol) in toluene (2500 mL, 2.3 mol) was treated with polyphosphoric acid (370.00 g, 3.4 mol) and heated at reflux for 5 h. The reaction mixture was concentrated in vacuo diluted with water (3 L) and the extracted with EtOAc (4 L). The organic layer was washed with aq NaOH (2 L), filtered, concentrated in vacuo and purified by distillation at reduced pressure to yield (**4**) 7-bromo-4-fluorobenzofuran (125.00 g; Yield = 51%. bp 80 °C (1 mm/Hg) as a colorless liquid which solidified at rt. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, 1H, J = 2.2 Hz), 7.39 (dd, 1H J = 5.1 & 3.7 Hz), 6.94 (d, 1H, J = 2.2 Hz), 6.86 (t, 1H, J = 8.8 Hz).

5.1.2. 4-Fluorobenzofuran-7-carbaldehyde (5)

A solution of 7-bromo-4-fluorobenzofuran (**4**) (124.12 g, 577.25 mmol) in ether (2.0 L) was cooled to −78 °C and treated drop wise with a solution of 2.5 M of *n*-Butyllithium in Hexane (235.5 mL) and stirred at −78 °C for 15 min. To this reaction mixture was added *N*,*N*-Dimethylformamide (89.393 mL, 1.15 mol) and stirred at −78 °C for 30 min. The reaction mixture was quenched with methanol (23.383 mL, 577.25 mmol) and warmed to rt. It was diluted with ether (300 ml) and the organic layer was washed with water (300 mL). The seperated organic layer was dried ($MgSO_4$) filtered, concentrated in vacuo to yield 4-Fluor-

obenzofuran-7-carbaldehyde (**5**) (89.00 g; Yield = 93.9%). Analysis of the product by ^{1}H NMR indicated that the reaction mixture was pure. It was therefore taken to the next step as it is. ^{1}H NMR 10.35 (s, 1H), 7.81 (dd, 1H, J = 5.2 & 8.0 Hz), 7.76 (d, 1H, J = 2.0 Hz), 7.07 (t, 1H, J = 8.8 Hz), 6.95 (d, 1H, J = 2.2 Hz).

5.1.3. Ethyl 2-azido-3-(4-fluorobenzofuran-7-yl)-3-hydroxypropanoate (6)

To a solution of 4-fluorobenzofuran-7-carbaldehyde (**5**, 12.71 g, 77.45 mmol), lithium chloride (6.567 g, 154.9 mmol) and diazabicyclo[5.4.0]undec-7-ene (23.16 mL, 154.9 mmol) was added a solution of ethyl azidoacetate (20.00 g, 154.9 mmol; added as a 30% solution in CH_2Cl_2) at 0 °C and stirred for 2–3 h. The completion of the reaction was followed by TLC (EtOAc/Hexanes 1:4). On complete consumption of starting material it was diluted with ethyl acetate (1 L) and washed with water and aqueous HCl (1 M, 400 mL). The combined organic layers were dried ($MgSO_4$), filtered, concentrated in vacuo and dried ($MgSO_4$). The organic layer was concentrated in vacuo and purified by chromatography SiO_2 (EtOAc/Hexanes) to yield (**6**) ethyl 2-azido-3-(4-fluorobenzofuran-7-yl)-3-hydroxypropanoate (18.3 g; Yield = 81%) as a colorless oil and mixture of diastereomers.

5.1.4. (*Z*)-Ethyl 2-azido-3-(4-fluorobenzofuran-7-yl)acrylate (7)

A solution of (**6**) ethyl-2-azido-3-(4-fluorobenzofuran-7-yl)-3-hydroxypropanoate (15.7 g, 53.5 mmol) and methanesulfonyl chloride (8.29 mL, 107 mmol) in methylene chloride (87.7 mL, 1.37 mmol) at −30 °C was treated drop wise with a solution of triethylamine (52.2 mL, 375.0 mmol) in methylene chloride (100 mL). The reaction mixture was stirred at −30 °C for 3 h, diluted with aq. saturated sodium bicarbonate and methylene chloride (400 mL). The organic layer was separated and washed with water, aq HCl (1 M) and brine. The organic layer was dried ($MgSO_4$), filtered, concentrated in vacuo, and purified by chromatography (SiO_2, 10% EtOAc in (1:1) Hexanes/CH_2Cl_2) to yield (**7**) (Z)-ethyl 2-azido-3-(4-fluorobenzofuran-7-yl)acrylate (12.6 g; Yield = 86%). ^{1}H NMR (400 MHz, D_6-DMSO) δ 8.14 (dd, 1H, J = 5.6 & 8.8 Hz), 8.10 (d, 1H, J = 2.2 Hz), 7.19 (s, 1H), 7.13 (t, 1H, J = 8.8 Hz), 7.10 (d, 1H, J = 2.2 Hz), 4.32 (q, 2H, J = 6.6 Hz), 1.32 (t, 3H, J = 6.6 Hz).

5.1.5. Ethyl 4-fluoro-6*H*-furo[2,3-*e*]indole-7-carboxylate (8)

150 mL of Xylenes was heated at 165 °C. To this boiling solution was added drop wise a solution of (*Z*)-ethyl 2-azido-3-(4-fluorobenzofuran-7-yl)acrylate (**7**, 11.2 g, 40.7 mmol) in Xylenes (70.00 mL, 189.4 mmol). The reaction mixture was stirred for additional 20.0 min and allowed to cool to rt. when colorless solid (**8**) Ethyl 4-fluoro-6*H*-furo[2,3-*e*]indole-7-carboxylate precipitated (7.00 g; Yield = 69.6%). The solid was filtered and washed with hexanes and dried. ^{1}H NMR (400 MHz, D_6-DMSO) δ 12.31 (s, 1H), 7.99 (d, 1H, J = 1.7 Hz), 7.30 (d, 1H, J = 2.5 Hz), 7.12–7.08 (m, 2H), 4.34 (q, 2H, J = 6.8 Hz), 1.33 (t, 3H, J = 6.8 Hz).

5.1.6. Ethyl-4-fluoro-8-(2-methoxypyridin-3-yl)-6*H*-furo[2,3-*e*]indole-7-carboxylate (9)

A solution of Ethyl-4-fluoro-6*H*-furo[2,3-*e*]indole-7-carboxylate (**8**, 15.88 g, 64.23 mmol) in DMF (100.00 mL) was treated with *N*-Iodosuccinimide (15.90 g, 70.66 mmol) and stirred at rt for 2 h. The reaction mixture was diluted with water (1000 mL) and extracted in EtOAc (1000 mL). The organic layer was washed with water (1000 mL), aq sodium thiosulfate (5% aq soln 1 L) and dried ($MgSO_4$). The organic layer was dried ($MgSO_4$), filtered, concentrated in vacuo to yield ethyl-4-fluoro-8-iodo-6*H*-furo[2,3-*e*]indole-7-carboxylate (22.30 g; Yield = 93%) as a buff colored solid used in the next reaction. ^{1}H NMR (400 MHz, D_6-DMSO) δ 12.53 (s, 1H), 8.07 (d, 1H, J = 1.8 Hz), 7.14 (d, 1H, J = 10.4 Hz), 7.11 (d, 1H, J = 2.4 Hz), 4.35 (q, 2H, J = 7.3 Hz), 1.37 (t, 3H, J = 7.3 Hz).

A solution of ethyl-4-fluoro-8-iodo-6*H*-furo[2,3-*e*]indole-7-carboxylate (22.000 g, 58.962 mmol), 2-methoxypyridin-3-ylboronic acid (13.527 g, 88.444 mmol), $(PPh_3)_2PdCl_2$ (4.13 g, 5.88 mmol) in 1,2-dimethoxyethane (250.0 mL) was degassed for 2 min and stirred at rt for 15 min. The yellow reaction mixture was treated with a solution of potassium carbonate (30.53 g, 220.9 mmol) in water (250.0 mL) and stirred at 90 °C for 3 h. The yellow reaction turned orange dark with the disappearance of starting material (TLC). The reaction mixture was diluted with EtOAc (1000 mL) and washed with aq. NaOH (500 mL, 1 M), dried ($MgSO_4$), filtered, concentrated in vacuo, and purified by chromatography SiO_2 (THF/Hexanes 0–60%) to yield (**9**) ethyl-4-fluoro-8-(2-methoxypyridin-3-yl)-6*H*-furo[2,3-*e*]indole-7-carboxylate (16.65 g; Yield = 80%) as pale brown solid. ^{1}H NMR (400 MHz, D_6-DMSO), δ 12.53 (s, 1H), 8.20 (dd, 1H, J = 1.8 & 5.2 Hz), 7.85 (d, 1H, J = 2.2 Hz), 7.79 (dd, 1H, J = 1.6 & 7.2 Hz), 7.14 (d, 1H, J = 9.8 Hz), 7.08–7.04 (m, 1H), 7.04 (d, 1H, J = 2.1 Hz), 4.15 (q, 2H, J = 6.8 Hz), 3.72 (s, 3H), 1.08 (t, 3H, J = 7.3 Hz)

5.1.7. 4-Fluoro-8-(2-oxo-1,2-dihydropyridin-3-yl)-6*H*-furo[2,3-*e*]indole-7-carboxylic acid (10)

A solution of (**9**) ethyl 4-fluoro-8-(2-methoxypyridin-3-yl)-6*H*-furo[2,3-*e*]indole-7-carboxylate (4.50 g, 12.7 mmol) in methanol (10.00 mL, 246.9 mmol) was treated with a solution of 4 M Hydrogen chloride in dioxane (100 mL) and heated at 90 °C for 3 h in a pressure tube. The reaction mixture was concentrated in vacuo and the residue was purified by chromatography SiO_2 (THF/Hexanes 0–100%) to obtain ethyl 4-fluoro-8-(2-oxo-1,2-dihydropyridin-3-yl)-6*H*-furo[2,3-*e*]indole-7-carboxylate as a colorless solid. The reaction was repeated such that a total of 17 g of ethyl 4-fluoro-8-(2-methoxypyridin-3-yl)-6*H*-furo[2,3-*e*]indole-7-carboxylate was completely used up.

A solution of ethyl 4-fluoro-8-(2-oxo-1,2-dihydropyridin-3-yl)-6*H*-furo[2,3-*e*]indole-7-carboxylate (810.00 mg, 2.38 mmol) in water (25.00 mL, 1388 mmol), THF (25.00 mL, 346.7 mmol) and methanol (25.00 mL, 780.2 mmol) was treated with lithium hydroxide monohydrate (499.41 mg, 11.901 mmol) and heated at 80 °C for 1 h. As the reaction proceeds a colorless solid precipitates out. Analysis of the reaction mixture with TLC (acetone/hexanes 70%) showed disappearance of starting material. The reaction mixture was acidified, filtered and dried in vacuo to yield (**10**) 4-fluoro-8-(2-oxo-1,2-dihydropyridin-3-yl)-6*H*-furo[2,3-*e*]indole-7-carboxylic acid (627.00 mg; Yield = 85%) as colorless solid used in the next reaction.

5.1.8. 4-Fluorofuro[2,3-*e*]pyrido[3′,2′:5,6]pyrano[3,4-*b*]indol-7(6h)-one (11)

A solution of (**10**) 4-fluoro-8-(2-oxo-1,2-dihydropyridin-3-yl)-6*H*-furo[2,3-*e*]indole-7-carboxylic acid (8.00 g, 25.6 mmol) and *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (9.82 g, 51.2 mmol) in *N*,*N*-Dimethylformamide (153.85 mL) was treated with triethylamine (35.71 mL, 256.2 mmol) and stirred overnight at rt. The mixture was concentrated in vacuo to remove the DMF and triethyl amine and the resulting residue was diluted with methanol (100.00 mL) when a colorless solid seperated out. It was filtered and dried to yield (**11**) 4-Fluorofuro[2,3*e*]pyrido[3′,2′:5,6]pyrano[3,4-*b*]indol-7(6h)-one (5.90 g; Yield = 78%) ^{1}H NMR (400 MHz, D_6-DMSO) δ 13.23 (s, 1H), 9.10 (dd, 1H, J = 1.6 & 7.2 Hz), 8.43 (dd, 1H, J = 2.0 & 4.4 Hz), 8.19 (d, 1H, J = 2.4 Hz), 7.59 (dd, 1H, J = 4.8 & 7.6 Hz) 7.32 (m, 2H). LR–MS (ESI): calcd for $C_{16}H_8FN_2O_3$ $[M+H]^+$ 295.1; found 295.

5.1.9. 4-Fluoro-6-[(2-fluoro-5-cyanophenyl)methyl]furo[2,3-*e*]pyrido[3,4-*B*]indol-7(6*H*)-one (12)

A suspension of lactone **11** (230 mg, 0.79 mmol), 3-(bromomethyl)-4-fluorobenzonitrile (334 mg, 1.56 mmol) in DMF (5.0 mL, 64.4 mmol) was treated with Cs_2CO_3 (510 mg, 1.56 mmol) and stirred at rt for 2 h. The reaction mixture was diluted with water (50 mL) and extracted with EtOAc (300 mL). The organic layer was separated and concentrated in vacuo. The residue was diluted with methanol (20 mL) when a colorless solid separated out. The colorless solid was filtered and concentrated in vacuo to yield **12** (170 mg, 51%). ^{1}H NMR (400 MHz, D_6-DMSO) 9.30 (d, 1H, J = 7.3 Hz), 8.48 (br s, 1H), 8.29 (bs, 1H), 7.85 (bs, 1H), 7.70–7.63 (m, 2H), 7.52 (t, 1H, J = 9.8 Hz), 7.30 (bs, 1H), 7.26 (bd, 1H, J = 5.0 Hz), 6.07 (s, 2H). LR–MS (ESI): calcd for $C_{24}H_{12}F_2N_3O_3$ $[M+H]^+$ 428.4; found 428.1 $[M+1]^+$

5.1.10. 6-(5-Carbamoyl-2-fluorobenzyl)-4-fluoro-8-(2-oxo-1,2-dihydropyridin-3-yl)-6*H*-furo[2,3-*e*]indole-7-carboxylic acid (13)

A solution of **12** (90 mg, 0.21 mmol) in THF (20 mL) was treated with $LiOH{\cdot}H_2O$ (36 mg, 0.84 mmol) and heated at reflux for 14 h. The reaction mixture was acidified with aqueous HCl (1 M soln) and stirred fot 10 min A colorless solid separated out which was filtered and used as it is. (yield 80 mg, 81%) ^{1}H NMR (400 MHz, D_6-DMSO), δ 12.93 (s, 1H), 11.74 (s, 1H), 7.92 (d, 1H, J = 2.4 Hz), 7.87 (s, 1H), 7.82–7.78 (m, 1H), 7.64 (dd, 1H, J = 2.1 & 6.8 Hz), 7.47 (d, 1H, J = 10.8 Hz), 7.40 (d, 1H, J = 7.6 Hz), 7.31–7.27 (m, 3H), 7.07 (d, 1H, J = 2.4 Hz), 6.32 (t, 1H, J = 6.8 Hz), 5.92 (s, 2H); ^{13}C NMR (125 MHz, D_6-DMSO) δ 166.5, 162.4, 162.3, 161.4, 160.3, 154.9, 153.0, 149.0, 148.9, 144.1, 140.5, 135.9, 135.8, 134.3, 130.7, 130.6, 128.3, 128.2, 127.98, 127.94, 126.69, 126.66, 125.7, 125.3, 125.2, 115.8, 115.1, 114.9, 110.8, 110.6, 109.7, 104.9, 103.2, 92.7, 92.5, 42.44, 42.41; LR–MS (ESI): calcd for $C_{24}H_{16}F_2N_3O_5$ $[M+H]^+$ 464.1; found 464.0.

5.1.11. *tert*-Butyl-5-(bromomethyl)-6-chloro-1*H* benzo[*d*]imidazole-1-carboxylate (14)

A solution of *tert*-butyl-6-chloro-5-methyl-1*H*-benzo[*d*]imidazole-1-carboxylate[14] (2.00 g, 7.5 mmol) in CCl_4, was treated with NBS (1.74 g, 9.75 mmol) and benzoyl peroxide (273 mg, 1.13 mmol) and heated at reflux for 14 h. The reaction mixture was concentrated in vacuo and purified by chromatography (SiO_2, EtOAc/Hexanes) to yield **14** (1.4 g, 54%) as a viscous oil.

5.1.12. 6-((6-Chloro-1*H*-benzo[*d*]imidazol-5-yl)methyl)-4-fluorofuro[2,3-*e*]pyrido[3′,2′,5,6]pyrano[3,4-*b*]indol-7(6*H*)-one (15)

A solution of **11** (843 mg, 2.86 mmol), **14** (1.10 g, 3.2 mmol), and Cs_2CO_3 (1.17 g, 3.58 mmol) in DMF (20.0 mL) was stirred at rt for 12 h and concentrated in vacuo The residue was dissolved in EtOAc (300 mL) and washed with brine, dried ($MgSO_4$), filtered concentrated in vacuo and purified by chromatography (SiO_2, EtOAc/Hexanes) to yield N-alkylated compound. It was further dissolved in CH_2Cl_2 (20 mL) and treated with TFA (20 mL) and stirred at rt for 0.5 h. The reaction mixture was concentrated in vacuo to yield **15** a colorless solid with poor solubility in most solvents that was used in the next reaction without further purification (1.00 g, 76%).

5.1.13. 6-((6-Chloro-1*H*-benzo[*d*]imidazol-5-yl)methyl)-4-fluoro-8-(2-oxo-1,2-dihydropyridin-3-yl)-6*H*-furo[2,3-*e*]indole-7-carboxylic acid (16)

A suspension of **15** (200 mg, 0.43 mmol) in THF (10 mL) and water (10 mL) was treated with $LiOH{\cdot}H_2O$ (100 mg, 2.38 mmol) and stirred at rt for 2 h. The reaction mixture was quenched with aq HCl (1 M, 5 mL) and concentrated in vacuo. The residue was purified by reverse phase HPLC (water containing 0.01% TFA and CH_3CN) to yield **16** (100 mg, 48%) as a colorless solid ^{1}H NMR (400 MHz, D_6-DMSO) δ 9.32 (s, 1H), 8.07 (s, 1H), 7.98 (d, 1H, J = 2.2 Hz), 7.70 (dd, 1H, J = 2.2 & 7.2 Hz), 7.45 (dd, 1H, J = 1.4 & 6.6 Hz), 7.42 (d, 1H, J = 11 Hz), 7.11 (d, 1H, J = 2.2 Hz), 6.56 (s, 1H), 6.37 (t, 1H, J = 6.6 Hz), 6.06 (s, 2H). ^{13}C NMR (125 MHz, D_6-DMSO) δ 162.3, 161.3, 158.2, 157.9, 155.1, 153.1, 149.0, 148.9, 144.2, 142.6, 140.5, 135.7, 135.6, 134.4, 134.0, 133.1, 131.7, 126.72, 126.65, 125.7, 116.0, 115.6, 111.6, 110.9, 110.7, 109.8, 104.8, 103.3, 92.7, 92.5, 46.5; MS (LRMS, ESI) Calcd for $C_{24}H_{15}ClFN_4O_4$ $[M+H]^+$ 477.1; Found 477.3.

5.1.14. *N*-(4-Bromo-5-fluoro-2-methylphenyl)acetamide (17)

A solution of 5-fluoro-2-methylaniline (25 g, 200 mmol) in toluene (250 mL) was treated with Ac_2O (25 mL, 226 mmol) and heated at reflux for 1 h. The reaction mixture was concentrated in vacuo when a colorless solid precipitated out which was filtered and washed with ether and hexanes. The colorless solid was taken in acetic acide (150 mL) and treated with a solution of Br_2 (9.6 mL, 186 mmol) in acetic acid (20 mL) and stirred at rt for 12 h. The reaction mixture was diluted with water when a colorless solid **17** (40 g, 81.6%) precipitated out. It was filtered and extensively washed.

5.1.15. 1-(5-bromo-6-fluoro-1*H*-indazol-1-yl)ethanone (18)

A solution of **17** (10.00 g, 40.6 mmol) in $CHCl_3$ (100 mL) was treated with Ac_2O (11.5 mL, 122 mmol), KOAc (8.00 g, 81.5 mmol), 18-crown-6 (540 mg, 2.04 mmol) amyl nitrite (12.3 mL, 871 mmol) and heated at 65 °C for 12 h. The reaction mixture was cooled to rt and treated with EtOAc (500 mL) washed with water and dried ($MgSO_4$), filtered concentrated in *vacuo* and purified by chromatography (SiO_2, EtOAc/Hexanes) to yield **18** as a colorless solid. ^{1}H NMR (400 MHz, D_6-DMSO) δ 8.41 (s, 1H), 8.29 (d, 1H, J = 6.6 Hz), 8.08 (d, 1H, J = 9.5 Hz), 2.69 (s, 3H).

5.1.16. 5-Bromo-6-fluoro-1*H*-indazole (19)

A solution of **18** (5.0 g, 19.5 mmol) was taken in aqueous HCl (3 M, 100 mL) and methanol (20 mL) and heated at 90 °C for 3 h. The reaction mixture was cooled to rt and basified with aqueous NaOH (1 M). A colorless solid precipitated out which was filtered and dried to yield 5-bromo-6-fluoro-1*H*-indazole as a colorless solid.

5.1.17. 6-Fluoro-1*H*-indazole-5-carbaldehyde (20)

A solution of **19** (3.5 g, 16.28 mmol) in THF (200 mL) was treated with NaH (60% mineral oil, 1.17 g) at 0 °C and stirred at rt for 20 min. The reaction mixture was cooled to −78 °C and treated with BuLi (2.5 M in Hexanes, 8.14 mL) dropwise. The mixture was stirred at temperature for 30 min treated with DMF (64 mmols) and slowly warmed to rt when the viscous solution turn homogenous and stirring was efficient. Analysis of TLC (40% EtOAc/Hexanes) indicated complete conversion of starting material to product. The reaction mixture was acidified with aqueous 1 M HCl and taken up in EtOAc (500 mL) washed with aqueous HCl (1 M, 100 mL), brine (100 mL) dried ($MgSO_4$), filtered concentrated in vacuo to yield **20** (2.3 g, 86%) ^{1}H NMR (400 MHz, D_6-DMSO) δ 13.52 (s, 1H), 10.16 (s, 1H), 8.37 (d, 1H, J = 6.7), 8.32 (s, 1H), 7.46 (d, 1H, J = 11.6 Hz).

5.1.18. *tert*-Butyl-5-(chloromethyl)-6-fluoro-1*H*-indazole-1-carboxylate (22)

A solution of the crude product **20** (2.3 g, 14.0 mmol) was take in THF (100 mL) was treated with Boc_2O (3.55 g, 16.28 mmol) and DMAP (300 mg) and stirred at rt for 3 h. The reaction mixture was concentrated in vacuo and the residue purified by chromatography (SiO_2, EtOAc/Hexanes) to yield *tert*-butyl-6-fluoro-1*H*-inadzole-1-

carboxylate (3.55 g, 81%). The boc-protected compound was taken in methanol (50 mL) and cooled to 0 °C and treated with $NaBH_4$ (1.02 g, 26.9 mmol) and stirred at that temperature for 1 h. The reaction mixture was diluted with aq HCl (200 mL) and extracted into EtOAc (200 mL). The combined organic layers were dried ($MgSO_4$), filtered concentrated in vacuo and purified by chromatography (SiO_2, EtOAc/Hexanes) to yield alcohol (3.00 g, 84%) as a colorless solid. The alcohol (3.00 g, 11.27 mmol) was taken in CH_2Cl_2 (50 mL) at 0 °C and treated with pyridine (4.56 mL, 56.33 mmol) and methanesulfonyl chloride (1.31 mL, 16.90 mmol) and stirred at rt for 16 h. The reaction mixture was concentrated in vacuo and the residue was dissolved in EtOAc (300 mL). The organic layer was washed with aq HCl (1 M, 100 mL), brine (100 mL) dried ($MgSO_4$) filtered concentrated in vacuo and purified by chromatography (SiO_2, EtOAc/Hexanes) to yield *tert*-butyl 5-(chloromethyl)-6-fluoro-1*H*-indazole-1-carboxylate (1.9 g, 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.91 (d, 1H, J = 10.3 Hz), 7.78 (d, 1H, J = 7.2 Hz), 4.73 (s, 2H), 1.72 (s, 9H).

5.1.19. 6-((1-(*tert*-butoxycarbonyl)-6-fluoro-1*H*-indazol-5-yl)methyl)-4-fluorofuro[2,3-*e*]pyrido[3′,2′,5,6]pyrano[3,4-*b*]indol-7(6*H*)-one (23)

A suspension of **11** (900 mg, 3.05 mmol) in DMF (40 mL) was treated with *tert*-butyl 5-(chloromethyl)-6-fluoro-1*H*-indazole-1-carboxylate (**22**, 1.088 g, 3.82 mmol) and Cs_2CO_3 (1.50 g, 4.58 mmol) and stirred overnight at rt. The reaction mixture was concentrated in vacuo extracted with CH_2Cl_2 (600 mL). The combined organic layer was washed with water, dried ($MgSO_4$), filtered concentrated in vacuo and purified by chromatography (THF/Hexanes) to yield (1.60 g, 96.4%). The product was dissolved in CH_2Cl_2 (40 mL) and TFA (40 mL) and stirred at rt for 1 h. The reaction mixture was concentrated in vacuo and used in the next reaction as it is.

5.1.20. 4-Fluoro-6-((6-fluoro-1*H*-indazol-5-yl)methyl)-8-(2-oxo-1,2-dihydropyridin-3-yl)-6*H*-furo[2,3-*e*]indole-7-carboxylic acid (24)

A suspension of **23** (900 mg, 2.04 mmol) in THF (40 mL) and water (40 mL) was treated with $LiOH{\cdot}H_2O$ (426 mg, 10.2 mmol) and stirred at rt for 3.5 h. The reaction mixture was acidified with aqueous HCl and concentrated in vacuo. The residue was directly purified on reverse phase HPLC (CH_3CN, water containing 0.01% TFA) to yield **24** as a colorless solid. ^{1}H NMR (500 MHz, D_6-DMSO) δ 13.06 (bs, 2H), 11.78 (s, 1H), 7.96 (s, 1H), 7.94 (d, 1H, J = 2.5 Hz), 7.69 (dd, 1H, J = 2.0 & 7.0 Hz), 7.49 (d, 1H, J = 11.0 Hz), 7.43 (dd, 1H, J = 1.6 & 6.5 Hz), 7.38 (d, 1H, J = 10.5 Hz), 7.08 (d, 1H, J = 2.0 Hz), 7.03 (d, 1H, J = 7.0 Hz), 6.35 (t, 1H, J = 7.0 Hz), 5.99 (s, 2H). ^{13}C NMR (125 MHz, D_6-DMSO) δ 162.5, 161.4, 160.0, 158.0, 154.8, 152.9, 149.0, 148.9, 144.0, 140.5, 135.7, 135.6, 134.3, 133.5, 126.91, 126.88, 125.8, 119.6, 119.4, 119.3, 119.03, 118.98, 115.5, 110.7, 110.5, 109.7, 104.9, 103.2, 95.9, 95.7, 92.9, 92.7, 42.91, 42.87; MS (LRMS, ESI) Calcd for $C_{24}H_{15}F_2N_4O_4$ $[M+1]^+$ 461.1; Found 461.3.

5.1.21. 1,5-Difluoro-2-iodo-4-methylbenzene (26)

A solution of 2,4-difluorotoluene (4.72 g, 36.8 mmol) in TFA (12.3 mL, 159.5 mmol) was treated with NIS (9.59 g, 42.6 mmol) at 0 °C and stirred at room temperature overnight. The reaction mixture was concentrated in vacuo and the residue was dissolved in hexanes. The organic layer was washed with aqueous sodium thiosulfate (3 × 30 mL) and brine (3 × 30 mL). The organic layer was separated dried ($MgSO_4$), filtered concentrated in vacuo and purified by bulb to bulb distillation to yield **26** (7.2 g, 77%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (t, 1H, J = 8.0 Hz), 6.79 (dd, 1H, J = 8.7, 8.4 Hz), 2.2 (s, 3H).

5.1.22. 2,4-Difluoro-5-methylbenzonitrile (27)

A solution of 1,5-difluoro-2-iodo-4-methylbenzene (**26,** 7.11 g, 28.0 mmol), $Zn(CN)_2$ (1.97 g, 16.8 mmol) and $Pd(PPh_3)_4$ (3.23 g, 2.8 mmol) in DMF (40 mL) was heated at 90 °C for 1.5 h. The reaction mixture was concentrated in vacuo and the residue was taken in water (400 mL) and extracted with ether (400 mL). The combined organic layer was washed with excess aq ammonium hydroxide solution. The separated organic layers were dried and purified b chromatography to yield purified product which contained triphenylphospine oxide. The crude mixture was further purified by sublimation (45 °C, 1 mm/Hg) to yield **27** (1.8 g, 42%) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (t, 1H, J = 8.0 Hz), 6.92 (t, 1H, J = 9.2 Hz), 2.27 (s, 3H).

5.1.23. 6-Fluoro-5-methyl-1*H*-indazol-3-amine (28)

A solution of **27** (1.40 g, 9.1 mmol), hydrazine (0.7 mL, 22.30 mmol) in isopropanol (50 mL, 653 mmol) was heated at reflux for 24 h. The reaction mixture was concentrated in vacuo and the residue was treated with aq. $Na_2S_2O_3$ (200 mL) and extracted with EtOAc (200 mL). The organic layer was dried ($MgSO_4$), filtered concentrated in vacuo and the residue was purified by chromatography (SiO_2, Acetone/Hexanes) to yield 6-fluoro-5-methyl-1*H*-indazol-3-amine (360 mg, 25%). ^{1}H NMR 11.26 (s, 1H), 7.60 (d, 1H, J = 7.6 Hz), 6.92 (d, 1H, 10.8 Hz), 5.27 (s, 2H), 2.22 (s, 3H).

5.1.24. *tert*-Butyl-5-(bromomethyl)-3-(bis-(tert-butoxycarbonyl)amino)-6-fluoro-1*H*-indazole-1-carboxylate (29)

A solution of **28** (330 mg, 1.99 mmol) and Boc_2O (2.61 g, 12 mmol) and DMAP (48.8 mg, 0.39 mmol) in acetonitrile (15 mL) was stirred at reflux for 2 h and concentrated in vacuo. The residue was purified by chromatography (SiO_2, EtOAc/Hexanes) to yield *tert*-butyl 5-(bromomethyl)-3-(bis-(tert-butoxycarbonyl)amino)-6-fluoro-1*H*-indazole-1-carboxylate (640 mg, 68%) as a viscous oil that was used in the next step without further purification.

A solution of N-Boc-protected compound (630 mg, 1.35 mmol), NBS (337 mg, 1.89 mmol) and benzoylperoxide (66 mg, 0.3 mmol) in CCl_4 (20 mL, 207 mmol) was heated at reflux for 3 h. The reaction mixture was concentrated in vacuo and the residue was dissolved in EtOAc (300 mL). The organic layer was washed with aqueous NaS_2O_3, dried ($MgSO_4$), filtered concentrated in vacuo and purified by chromatography (SiO_2, EtOAc/Hexanes) to yield **29** (610 mg, 83%) as a colorless oil which also contained unreacted starting material.

5.1.25. 5-((3-(Bis(tert-butoxycarbonyl)amino)-1-(tert-butoxycarbonyl)-6-fluoro-1*H*-indazole-5-yl)methyl)-4-fluorofuro[2,3-*e*]pyrido[3′,2′,5,6]pyrano[3,4-*b*]indol-7(6*H*)-one (30)

A suspension of **11** (294 mg, 1.00 mmol), **29** (592 mg, 1.09 mmol) and Cs_2CO_3 (488.7 mg, 1.5) in DMF (20 mL) was stirred a rt for 4 h and concentrated in vacuo. The residue was dissolved in EtOAc (200 mL) and washed with aq HCl (100 mL), brine (100 mL), dried ($MgSO_4$), filtered, concentrated in vacuo and purified by chromatography (SiO_2, Acetone/Hexanes) to yield **30** (490 mg, 65%) as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD), δ 9.17 (dd, 1H, J = 1.5 & 7.3 Hz), 8.34 (dd, 1H, J = 5.2 & 2.2 Hz), 7.95–7.92 (m, 2H), 7.45 (dd, 1H, J = 5.1 & 8.1 Hz), 7.27 (d, 1H, J = 10.3 Hz), 7.08 (d, 1H, J = 2.2 Hz), 6.71 (d, 1H, J = 6.6 Hz), 6.08 (s, 2H), 1.68 (s, 9H), 1.05 (s, 18H).

5.1.26. 6-((3-Amino-6-fluoro-1*H*-indazol-5-yl)methyl)-4-fluoro-8-(2-oxo-1,2-dihydropyridin-3-yl)-6*H*-furo[2,3-*e*]indole-7-carboxylic acid (31)

A solution of **30** (490 mg, 0.65 mmol) was treated with 4 M soln of HCl in dioxane (25 mL) and stirred at rt for 4 h. The reaction mixture was concentrated in vacuo and used as it is in the next step.

The crude solid (100 mg, 0.22 mmol) in THF (4.00 mL) and water (4.00 mL) was treated with $LiOH{\cdot}H_2O$ (64 mg, 1.53 mmol) and stirred at rt for 12 h. The reaction mixture was concentrated in vacuo and purified by reverse phase HPLC (water containing 0.01% TFA and CH_3CN) to yield **31** as a colorless solid (48 mg, 47%) as a colorless solid. ^{1}H NMR (400 MHz, D_6-DMSO) δ ^{1}H NMR 13.04 (b, 1H), 11.78 (bs, 1H), 7.92 (d, 1H, J = 2.4 Hz), 7.70 (dd, 1H, J = 6.8 & 2.5 Hz), 7.44 (d, 1H, J = 14.8 Hz), 7.43–7.41 (m, 1H), 7.13 (d, 1H, J = 7.6 Hz), 7.08 (d, 1H, J = 2.4 Hz), 7.03 (d, 1H, J = 11.2 Hz), 6.35 (t, 1H, J = 6.8 Hz), 5.91 (s, 2H). ^{13}C NMR (125 MHz, D_6-DMSO). δ 162.5, 161.5, 160.7, 158.8, 154.8, 152.8, 149.0, 148.9, 148.7, 143.9, 140.6, 140.6, 140.5, 135.9, 135.8, 134.2, 127.0, 126.9, 125.9, 119.55, 119.49, 116.37, 116.2, 111.0, 110.6, 110.4, 109.80, 109.79, 104.9, 103.2, 92.7, 43.10, 43.05. MS (LRMS, ESI) Calcd for $C_{24}H_{16}F_2N_5O_4$ $[M+1]^+$ 475.4; Found 475.3.

5.1.27. 2-(Dimethylamino)ethyl 4-fluoro-6-((6-fluoro-1*H*-indazol-5-yl)methyl)-8-(2-oxo-1,2-dihydropyridin-3-yl)-6*H*-furo[2,3-*e*]indole-7-carboxylate (33)

A solution of *N*,*N* dimethylamino ethanol (603 μL, 6.00 mmol) in THF (1.6 mL) at −20 °C was treated with a solution of BuLi (2.5 M in Hexanes, 2 mL) and stirred for 15 min. To this solution was added **32** (700 mg, 1.54 mmol) and stirred at rt for 45 min. The reaction mixture was quenched with TFA and concentrated in vacuo. The residue was purified by reverse phase HPLC to isolate the TFA salt **33** as a colorless solid. The TFA salt was further converted to HCl salt by adding aq HCl to the salt and concentrating in vacuo (730 mg). ^{1}H NMR (500 MHz, D_6-DMSO), δ, 12.20 (s, 1H), 10.45 (s, 1H), 7.99 (s, 1H), 7.99 (d, 1H, J = 2.5 Hz), 7.92 (dd, 1H, J = 7.0 & 2.0 Hz), 7.59 (d, 1H, J = 10.5 Hz), 7.53 (d, 1H, J = 6.5 & 2.0 Hz), 7.39 (d, 1H, J = 11.0 Hz), 7.17 (d, 1H, J = 7.0 Hz), 7.13 (d, 1H, J = 2.0 Hz), 6.48 (t, 1H, J = 6.5 Hz), 5.95 (s, 2H), 4.49 (bt, 2H, J = 5.0 Hz), 3.28 (dd, 2H, J = 9.5 & 4.5 Hz), 2.72 (s, 3H), 2.71 (s, 3H). ^{13}C NMR (125 MHz, D_6-DMSO), δ, 161.3, 160.8, 159.9, 158.0, 155.1, 153.2, 148.8, 148.7, 144.2, 140.9, 139.2, 139.0, 135.9, 135.8, 134.6, 133.5, 125.62, 125.59, 124.6, 119.43, 119.38, 119.28, 119.1, 118.9, 115.3, 111.1, 110.9, 109.2, 105.6, 103.3, 96.0, 95.8, 93.1, 92.9, 58.6, 54.8, 43.29, 43.25, 42.2; MS (LRMS, ESI) Calcd for $C_{28}H_{24}F_2N_5O_4$ $[M+H]^+$ 532.2; Found 532.4.

5.1.28. 6-(2,5-Difluorobenzyl)-5-fluoro-8-(2-oxo-1,2-dihydropyridin-3-yl)-6*H*-furo[2,3-*e*]indole-7-carboxylic acid (35)

The synthesis of 7-fluoro indole derivative was initiated from 2-bromo-4-fluorophenol and converted to substituted tricyclic indole of type **9** using similar reactions shown in Scheme 1. Thus obtained regioisomeric indole of type **9** was alkylated with 2,5 difluorobenzyl bromide and demethylated using 4 M HCl in dioxane.[9] ^{1}H NMR (400 MHz, D_6-DMSO), δ above 12 (b, 1H) 11.75 (s, 1H), 7.89 (s, 1H), 7.69 (d, 1H, J = 5.9 Hz), 7.42–7.37 (m, 2H), 7.33–7.27 (m, 1H), 7.16–7.12 (m, 1H), 6.95 (s, 1H), 6.38–6.31 (m, 2H), 5.97 (d, 2H).

5.1.29. 6-(2,5-Difluorobenzyl)-4-fluoro-8-(2-oxo-1,2-dihydropyridin-3-yl)-6*H*-furo[2,3-*e*]indole-7-carboxylic acid (36)

^{1}H NMR (400 MHz, D_6-DMSO), δ above 12 (1H), 11.75 (s, 1H), 7.93 (d, 1H, J = 2.0 Hz), 7.66 (dd, 1H, J = 2.4 & 7.5 Hz), 7.48 (d, 1H, J = 10.8 Hz), 7.41–7.39 (m, 1H), 7.30 (dt, 1H, J = 4.8 & 8.2 Hz), 7.17–7.12 (m, 1H), 7.08 (d, 1H, J = 2.0 Hz), 6.37–6.33 (m, 1H), 6.31 (t, 1H, J = 6.8 Hz), 5.90 (s, 2H).

5.1.30. 6-((1*H*-Benzo[*d*]imidazol-5-yl)methyl)-4-fluoro-8-(2-oxo-1,2-dihydropyridin-3-yl)-6*H*-furo[2,3-*e*]indole-7-carboxylic acid (42)

^{1}H NMR (500 MHz, D_6-DMSO) δ, 13.08 (s, 1H), 11.82 (s, 1H), 9.46 (s, 1H), 7.99 (d, 1H, J = 2.0 Hz), 7.80 (d, 1H, J = 8.5 Hz), 7.66 (dd, 1H, J = 6.5 & 2.0 Hz), 7.52 (d, 1H, J = 10.5 Hz), 7.51 (s, 1H), 7.44 (dd, 1H, J = 6.5 & 2.0 Hz), 7.39 (d, 1H, J = 9.5 Hz), 7.07 (d, 1H, J = 2.0 Hz), 6.35 (t, 1H, J = 7.0 Hz), 6.07 (s, 2H). ^{13}C NMR (125 MHz, D_6-DMSO) δ 162.6, 161.4, 154.9, 152.9, 149.0, 148.9, 144.1, 140.7, 140.5, 136.3, 135.5, 135.4, 134.4, 131.0, 130.0, 126.69, 126.67, 125.7, 124.5, 115.7, 114.6, 111.9, 110.7, 110.5, 109.8, 104.9, 103.2, 92.9, 92.7, 47.6; LR–MS (ESI): calcd for $C_{24}H_{16}FN_4O_4$ $[M+H]^+$ 443.12; found 443.22.

5.1.31. 4-Fluoro-6-((6-fluoro-1*H*-benzo[*d*]imidazol-5-yl)methyl)-8-(2-oxo-1,2-dihydropyridin-3-yl)-6*H*-furo[2,3-*e*]indole-7-carboxylic acid·HCl (43)

^{1}H NMR (500 MHz, D_6-DMSO) δ 13.07 (s, 1H), 11.82 (s, 1H), 9.32 (s, 1H), 7.96 (s, 1H), 7.78 (d, 1H, J = 9.0 Hz), 7.67 (d, 1H, J = 6.5 Hz), 7.52 (s, 1H, J = 10.5 Hz), 7.44 (d, 1H, J = 6.0 Hz), 7.09 (s, 1H), 6.96 (d, 1H, J = 6.0 Hz), 6.35 (t, 1H, J = 6.5 Hz), 6.07 (s, 2H). ^{13}C NMR (125 MHz, D_6-DMSO) δ 162.4, 161.3, 158.4, 156.4, 155.0, 153.0, 149.0, 148.9, 144.2, 141.8, 140.5, 135.7, 135.6, 134.4, 131.0, 130.9, 128.1, 126.7, 126.6, 125.7, 124.6, 124.4, 116.0, 112.60, 112.56, 110.8, 110.6, 109.8, 104.9, 103.2, 101.3, 101.1, 92.8, 92.6, 42.87, 42.82; MS (LRMS, ESI) Calcd for $C_{24}H_{15}F_2N_4O_4$ $[M+1]^+$ 461.1; Found 461.2.

5.1.32. 6-((3-Amino-5-fluoro-1*H*-indazol-6-yl)methyl)-4-fluoro-8-(2-oxo-1,2-dihydropyridin-3-yl)-6*H*-furo[2,3-*e*]indole-7-carboxylic acid (44)

^{1}H NMR (400 MHz, D_6-DMSO), δ, 11.76 (b, 1H), 11.23 (bs, 1H), 7.94 (d, 1H, J = 2.0 Hz), 7.67 (dd, 1H, J = 2.0 & 6.8 Hz), 7.51 (dd, 1H, J = 10.4 Hz), 7.46 (d, 1H, J = 10.8 Hz), 7.44 (s, 1H, J = 2.8 & 7.2 Hz), 7.09 (d, 1H, J = 2.4 Hz), 6.36 (d, 1H, J = 6.8 Hz), 6.34 (dd, 1H, J = 6.8 & 2.4 Hz), 5.98 (s, 2H). ^{13}C NMR (125 MHz, D_6-DMSO), δ, 162.4, 161.3, 154.9, 153.9, 152.9, 152.1, 149.0, 149.0, 148.9, 144.1, 140.5, 138.1, 135.7, 135.6, 134.3, 126.84, 126.81, 125.72, 125.71, 125.35, 125.19, 115.6, 112.14, 112.06, 110.7, 110.5, 109.68, 109.66, 107.11, 107.07, 104.8, 104.5, 104.4, 103.2, 92.8, 92.6, 43.14, 43.10. MS (LRMS, ESI) Calcd for $C_{24}H_{16}F_2N_5O_4$ $[M+1]^+$ 475.4; Found 475.3.

5.1.33. 6-((3-Amino-6-fluoro-1-methyl-1*H*-indazol-5-yl)methyl)-4-fluoro-8-(2-oxo-1,2-dihydropyridin-3-yl)-6*H*-furo[2,3-*e*]indole-7-carboxylic acid (45)

^{1}H NMR (500 MHz, D_6-DMSO), δ, 12.90 (b, 1H), 11.76 (bs, 1H), 7.92 (dd, 1H, J = 2.0 & 0.5 Hz), 7.70 (d, 1H, J = 6 & 1 Hz), 7.43 (d, 1H, J = 11.0 Hz), 7.43–7.42 (m, 1H), 7.23 (d, 1H, J = 11.5 Hz), 7.10 (d, 1H, J = 7.5 Hz), 7.07 (dd, 1H, J = 0.5 & 1 Hz), 6.35 (t, 1H, J = 6.5 Hz), 5.91 (s, 2H), 5.4 (b, 2H), 3.65 (s, 3H). ^{13}C NMR (125 MHz, D_6-DMSO), δ 162.45, 161.43, 160.72, 158.79, 154.74, 152.80, 149.00, 148.91, 148.09, 143.87, 140.54, 140.43, 140.32, 135.84, 135.74, 134.19, 126.92, 126.89, 125.88, 119.72, 119.66, 116.04, 115.90, 115.51, 110.82, 110.54, 110.34, 109.78, 104.87, 103.19, 94.56, 94.34, 92.82, 92.63, 43.01, 42.96, 34.53. MS (LRMS, ESI) Calcd for $C_{25}H_{18}F_2N_5O_4$ $[M+1]^+$ 490.1; Found 490.4.

5.1.34. 4-Fluoro-6-((6-fluoro-1*H*-indazol-5-yl)methyl)-8-(2-oxo-1,2-dihydropyridin-3-yl)-6*H*-furo[2,3-*e*]indole-7-carboxylic acid (46)

^{1}H NMR (500 MHz, D_6-DMSO), δ 12.52–11.32 (bm, 3H), 7.98 (dd, 2H, J = 7.5 & 5.0 Hz), 7.67 (dd, 1H, J = 2.0 & 7.2 Hz), 7.51–7.50 (bm,

2H), 7.43 (dd, 1H, J = 6.5 & 1.5 Hz), 7.40 (d, 1H, J = 11.0 Hz), 7.09 (d, 1H, J = 2.5 Hz), 6.34 (t, 1H, J = 6.5 Hz), 6.08 (d, 1H, J = 7.5 Hz), 5.99 (s, 2H). ^{13}C NMR (125 MHz, D_6-DMSO), δ, 175.0, 162.5, 162.4, 161.3, 160.5, 154.9, 153.0, 149.0, 148.9, 144.1, 140.6, 140.4, 140.3, 140.2, 135.8, 135.7, 134.3, 126.7, 126.6, 125.7, 124.82, 124.77, 122.6, 122.5, 121.8, 115.8, 110.8, 110.6, 109.7, 108.5, 104.9, 104.1, 103., 103.7, 103.2, 92.8, 92.6, 42.48, 42.45, MS (LRMS, ESI) Calcd for $C_{26}H_{16}F_2N_3O_5$ $[M+1]^+$ 488.1; Found 488.2.

5.1.35. 6-((2-Aminoquinolin-4-yl)methyl)-4-fluoro-8-(2-oxo-1,2-dihydropyridin-3-yl)-6*H*-furo[2,3-*e*]indole-7-carboxylic acid (47)

^{1}H NMR (500 MHz, D_6-DMSO), δ, 14.22 (s, 1H), 11.83 (s, 1H), 8.82 (bs, 1H), 8.28 (d, 1H, J = 8.5 Hz), 8.05 (bs, 1H), 7.98 (s, 1H), 7.87 (t, 1H, J = 7.5 Hz), 7.78 (d, 1H, J = 8.0 Hz), 7.71 (d, 1H, J = 5.5 Hz), 7.63 (t, 1H, J = 7.5 Hz), 7.53 (d, 1H, J = 11.0 Hz), 7.46 (d, 1H, J = 5.5 Hz), 7.12 (s, 1H), 6.37 (t, 1H, J = 6.5 Hz), 6.32 (s, 2H), 5.94 (s, 1H). ^{13}C NMR (125 MHz, D_6-DMSO), δ, 162.3, 161.3, 155.2, 153.7, 153.3, 152.4, 149.1, 149.0, 144.3, 140.7, 136.04, 135.94, 135.5, 134.5, 132.6, 126.3, 126.2, 125.7, 124.9, 124.7, 118.8, 117.6, 116.7, 111.0, 110.9, 110.0, 108.1, 104.8, 103.3, 92.8, 92.6, 46.3, MS (LRMS, ESI) Calcd for $C_{26}H_{18}FN_4O_4$ $[M+1]^+$ 469.1; Found 469.2.

5.1.36. 4-Fluoro-6-((6-methyl-1*H*-indazol-5-yl)methyl)-8-(2-oxo-1,2-dihydropyridin-3-yl)-6*H*-furo[2,3-*e*]indole-7-carboxylic acid (49)

^{1}H NMR (500 MHz, D_6-DMSO), δ, 12.87 (b, 2H), 11.78 (b, 1H), 7.95 (d, 1H, J = 2.5 Hz), 7.77 (s, 1H), 7.74 (dd, 1H, J = 7.0 & 2.0 Hz), 7.44 (dd, 1H, J = 6.6 & 2.0 Hz), 7.40 (s, 1H), 7.34 (d, 1H, J = 10.5 Hz), 7.08 (d, 1H, J = 2.0 Hz), 6.39 (s, 1H), 6.37 (t, 1H, J = 6.5 Hz), 5.91 (s, 2H), 2.54 (s, 3H). ^{13}C NMR (125 MHz, D_6-DMSO), δ, 162.4, 161.4, 154.8, 152.9, 149.0, 148.9, 144.0, 140.5, 139.2, 135.8, 135.7, 134.3, 133.6, 132.8, 129.6, 127.08, 127.06, 125.9, 121.1, 115.4, 114.9, 110.7, 110.5, 110.3, 109.7, 104.9, 103.3, 92.9, 92.7, 46.3, 19.5, MS (LRMS, ESI) Calcd for $C_{25}H_{18}FN_4O_4$ $[M+1]^+$ 457.1; Found 457.2.

5.1.37. 2-(Dimethylamino)ethyl 6-((6-chloro-1*H*-benzo[*d*]imidazol-5-yl)methyl)-4-fluoro-8-(2-oxo-1,2-dihydropyridin-3-yl)-6*H*-furo[2,3-*e*]indole-7-carboxylate (55)

^{1}H NMR (500 MHz, D_6-DMSO), 12.08 (s, 1H), 9.58 (s, 1H), 8.63 (s, 1H), 8.09 (d, 1H, J = 2.5 Hz), 7.93 (dd, 1H, J = 7.0 and 2 Hz), 7.90 (s, 1H), 7.56 (dd, 1H, J = 2.0 & 6.5 Hz), 7.47 (d, 1H, J = 10.5 Hz), 7.14 (d, 1H, J = 2.0 Hz), 6.54 (s, 1H), 6.50 (t, 1H, J = 7.0 Hz), 5.99 (s, 2H), 4.02 (bt, 2H), 3.27 (bt, 2H, J = 4.0 Hz), 2.73 (s, 6H). ^{13}C NMR (125 MHz, D_6-DMSO), 161.4, 160.60 158.1,157.8, 155.4, 153.4, 148.9, 148.8, 144.4, 143.0, 141.1, 136.1, 136.0, 134.8, 130.2, 127.9, 127.3, 125.9, 125.3, 124.6, 115.8, 112.0, 111.3, 111.1, 109.3, 105.6, 103.4, 92.9, 92.7, 58.6, 55.1, 46.8, 42.5. MS (LRMS, ESI) Calcd for $C_{28}H_{24}ClFN_5O_4$ $[M+H]^+$ 548.2; Found 548.4.

5.1.38. 2-(Dimethylamino)ethyl-4-fluoro-6-((6-fluoro-1*H*-benzo[*d*]imidazol-5-yl)methyl)-8-(2-oxo-1,2-dihydropyridin-3-yl)-6*H*-furo[2,3-*e*]indole-7-carboxylate (59)

^{1}H NMR (400 MHz, D_6-DMSO) δ 10.49 (s, 1H), 9.43 (s, 1H), 7.99 (d, 1H, J = 2.2 Hz), 7.86 (dd, 1H, J = 2.0 & 7.20 Hz), 7.80 (d, 1H, J = 9.5 Hz), 7.60 (d, 1H, J = 10.3 Hz), 7.51 (dd, 1H, J = 2.0 & 6.4 Hz), 7.11 (d, 1H, J = 2.0 Hz), 7.10 (d, 1H, J = 6.6 Hz), 6.45 (t, 1H, J = 6.6 Hz), 6.01 (s, 2H), 4.45 (t, 2H, J = 5.1Hz), 3.25 (bq, 2H, J = 4.4 Hz), 2.69 (s, 3H), 2.68 (s, 3H).

5.1.39. 2-(Dimethylamino)ethyl 6-((3-amino-6-fluoro-1*H*-indazol-5-yl)methyl)-4-fluoro-8-(2-oxo-1,2-dihydropyridin-3-yl)-6*H*-furo[2,3-*e*]indole-7-carboxylate (60)

^{1}H NMR (500 MHz, D_6-DMSO) δ 12.16 (s, 1H), 10.51 (s, 1H), 7.98 (d, 1H, J = 2.5 Hz), 7.90 (dd, 1H, J = 7.0 & 2.0 Hz), 7.54 (d, 1H, J = 10.5 Hz), 7.51 (dd, 1H, J = 6.5 & 2.0 Hz), 7.46 (d, 1H, J = 7.0 Hz), 7.34 (d, 1H, J = 10.5 Hz), 7.12 (d, 1H, J = 2.0 Hz), 6.46 (t, 1H, J = 7.0 Hz), 5.90 (s, 2H), 4.48 (bt, 2H, J = 4.5 Hz), 3.27 (bt, 2H), 2.70 (s, 6H). ^{13}C NMR (125 MHz, D_6-DMSO), δ, 161.3, 160.7, 155.2, 153., 148.9, 148.8, 144.2, 141.5, 141.4, 141.0, 136.2, 136.1, 134.6, 125.26, 125.23, 124.8, 120.75, 120.71, 120.69, 119.9, 119.78, 119.75, 115.8, 111.1, 110.9, 109.43, 109.33, 109.31, 109.28, 105.5, 103.3, 96.8, 96.6, 93.0, 92.8, 58.5, 54.8, 43.22, 43.17, 42.2. MS (LRMS, ESI) Calcd for $C_{28}H_{25}F_2N_6O_4$ $[M+H]^+$ 547.2; Found 547.4

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