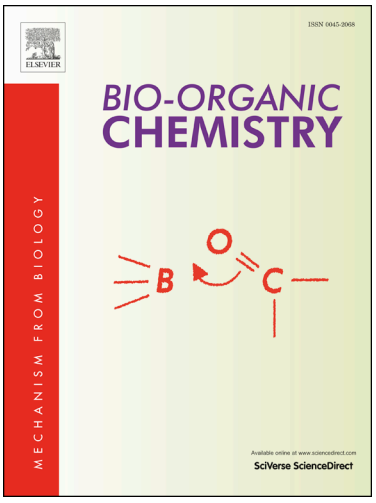

Design, synthesis and biological evaluation of *Helicobacter pylori* inosine 5′-monophosphate dehydrogenase (*Hp*IMPDH) inhibitors. Further optimization of selectivity towards *Hp*IMPDH over human IMPDH2

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Design, synthesis and biological evaluation of *Helicobacter pylori* inosine 5′-monophosphate dehydrogenase (*Hp*IMPDH) inhibitors. Further optimization of selectivity towards *Hp*IMPDH over human IMPDH2

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Abstract

Inosine 5′-monophosphate dehydrogenase (IMPDH, EC 1.1.1.205) catalyzes a crucial step in guanine nucleotide biosynthesis, thereby governing cell proliferation. In contrast to mammalian IMPDHs, microbial IMPDHs are relatively less explored as potential targets for antimicrobial drug discovery. In continuation with our previous work, here we report the discovery of moderately potent and highly selective *Helicobacter pylori* IMPDH (*Hp*IMPDH) inhibitors. The present study is mainly focused around our previously identified, modestly potent and relatively nonselective (for *Hp*IMPDH over human IMPDH2) hit molecule **IX** (**16i**). In an attempt to optimize the selectivity for the bacterial enzyme, we screened a set of 48 redesigned new chemical entities (NCEs) belonging to 5-aminoisobenzofuran-1(3*H*)-one series for their *in vitro Hp*IMPDH and human IMPDH2 inhibition. A total of 12 compounds (hits) demonstrated ≥70% *Hp*IMPDH inhibition at 10 μM concentration; none of the hits were active against *h*IMPDH2. Compound **24** was found to be the most potent and selective molecule (*Hp*IMPDH IC_{50} = 2.21 μM) in the series. The study reaffirmed the utility of 5-aminoisobenzofuran-1(3*H*)-one as a promising scaffold with great potential for further development of potent and selective *Hp*IMPDH inhibitors.

Keywords: *Helicobacter pylori*, IMPDH, *Hp*IMPDH, *h*IMPDH2, 5-aminoisobenzofuran-1(3*H*)-one

Abbreviations: PUD: Peptic ulcer disease; MALT: gastric mucosa-associated lymphoid tissue; XMP: xanthosine 5′-monophosphate; IMP: inosine 5′-monophosphate; DPPA: diphenylphosphoryl azide; TEA: triethyl amine; EEDQ: N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; HBTU: hexafluorophosphate benzotriazole tetramethyl uranium; NMM: N-methylmorpholine; EDCI: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; DMAP: 4-(N,N-dimethylamino)pyridine; DIPEA: Di-isopropylethylamine

1. Introduction

Approximately 50% of the world's population is infected with *Helicobacter pylori* (*H. pylori*), one of the most prevalent gram-negative bacterial pathogens, mostly residing in human gastric mucosa [1]. The bacillus finds a home in the highly acidic environment of the stomach, where very few other microorganisms can survive. The deadly pathogen is associated with various gastric complications such as chronic gastritis, peptic ulcer disease (PUD), gastric mucosa-associated lymphoid tissue (MALT) lymphoma and gastric carcinoma [1-3]. *H. pylori* is classified as a class I gastric carcinogen by the World Health Organization (WHO). Around 2.9% gastric cancers occur in *H. pylori*-infected individuals [4]. Treatment of the infection is extremely challenging due to its survival in a hostile acidic environment. Various methodo-logies investigated in the recent past such as drug repurposing campaigns of approved drugs [4,5], randomized controlled trials [6], natural products therapy [7,8], and many others are likely to offer potential alternatives for the treatment of *H. pylori*. Newer molecules with novel mechanism(s) of action are needed for contributing significantly in, not only treating but also eliminating, *H. pylori* from the body.

Inosine 5′-monophosphate dehydrogenase (IMPDH) presents an interesting, yet largely unexploited target for antimicrobial drug development. Rapid proliferation is an important characteristic of many microbial infections, which is supported by the expansion of guanine nucleotide pool by the rapidly-dividing cells. The enzyme IMPDH (EC 1.1.1.205) catalyzes the oxidation of inosine 5′-monophosphate (IMP) to xanthosine 5′-monophosphate (XMP) with concomitant reduction of nicotinamide adenine dinucleotide (NAD^+), which is a crucial step in the de novo biosynthesis of guanine nucleotides. Inhibition of IMPDH certainly leads to a fall in the supply of XMP and reduces the formation of GMP and supplementary guanine derivatives (guanine nucleotide pool). The major consequence of IMPDH inhibition is an overall reduction in intracellular GTP and the 'guanylate pool' (GMP, GDP, GTP and dGTP)

which blocks proliferation. Also, IMPDH has historically been a fascinating target for immunosuppressive, antiviral and anticancer drug development. The differences in the affinity for the substrates, structural features and kinetic properties dictate the design of selective and potent inhibitors for prokaryotic and eukaryotic IMPDHs [9-11].

Just like other microbes, *Cryptosporidium parvum* (*C. parvum*), a protozoan parasite causing diarrhoea and malnutrition especially in children, is dependent on IMPDH for its supply of guanine nucleotides to spread and sustain the infection. In fact, *C. parvum* IMPDH (*Cp*IMPDH) inhibitor development is an active area of research. Several selective and potent *Cp*IMPDH inhibitors are reported in the literature [12,13]. Also, it is well documented that *Cp*IMPDH, being structurally similar to many bacterial IMPDHs, is likely to be inhibited by the similar, if not same, set of compounds inhibiting other bacterial IMPDHs [9,12]. Working on the similar lines in reverse direction, five series of *Cp*IMPDH-selective inhibitors (**I-V**, Fig. 1; linker with 3-4 atom spacer containing amide, urea, etc., between the two aryl groups) were screened for *in vitro* enzyme inhibitory activity against IMPDHs from *Bacillus anthraces* [13], *Mycobacterium tuberculosis* (*Mtb*) [14] and *Francisella tularensis* [15]. In addition, few *Cp*IMPDH inhibitors were found to be potent *H. pylori* IMPDH (*Hp*IMPDH) inhibitors; **C91** (**VI**, Fig. 1) was the most potent *Hp*IMPDH inhibitor reported so far (IC_{50} = 8 and 1.1 nM for *Cp*IMPDH and *Hp*IMPDH, respectively) [12,16]. Very recently, our group has reported 3-aryldiazenyl indoles (**VII**, Fig. 1) [17] and 3-carboxamido indoles (**VIII**, Fig. 1) as new series of *Hp*IMPDH inhibitors [18].

Known *Hp*- and *Cp*IMPDH inhibitors possess common structural features, namely, two aromatic rings (Ar^1 and Ar^2) connected with a suitable linker. All the reported *Hp*IMPDH inhibitors have a three-to-four C spacer (mostly acetamide). This information is crucial for the

design and development of species-selective and potent microbial IMPDH inhibitors. Several investigators are working on the IMPDH inhibitor development programmes [12-24]. Our research group has contributed recently on the design and development of potent and selective prokaryotic (including *H. pylori*) and eukaryotic IMPDH inhibitors [10,17-24].

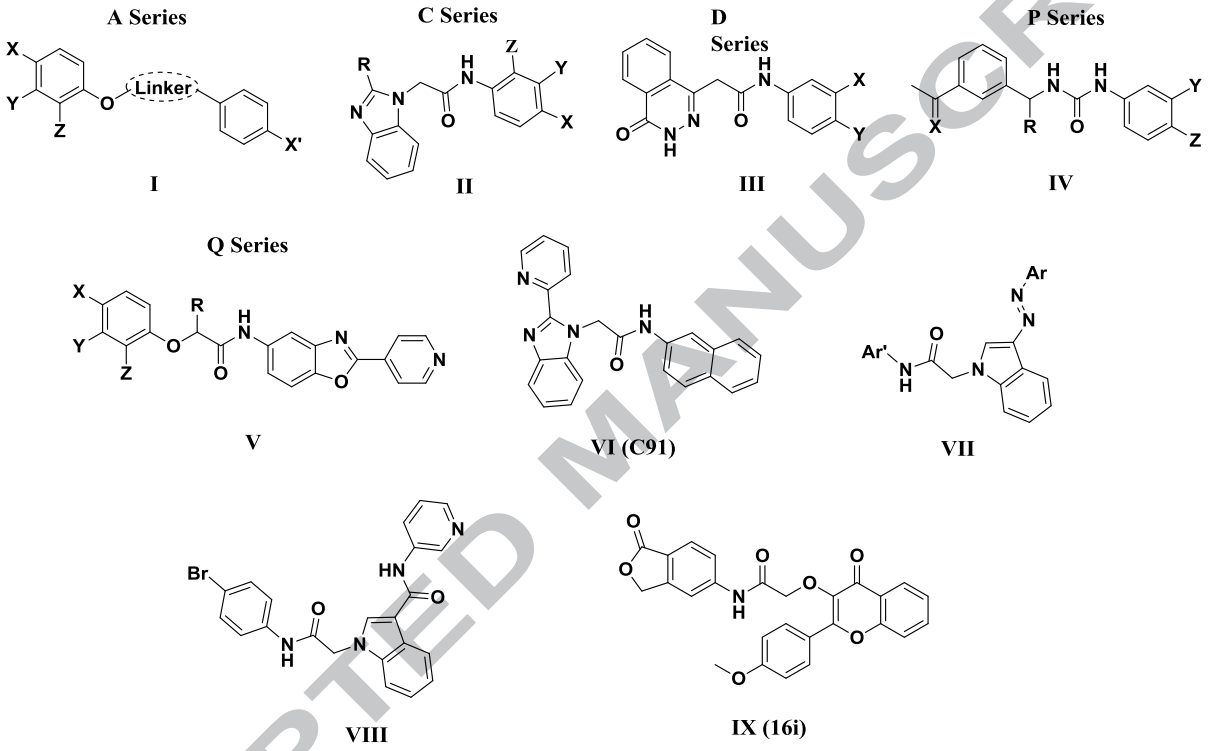


Fig. 1. Reported *C. parvum* and *H. pylori* IMPDH inhibitors

Our previous report on *Hp*IMPDH inhibitors led to the identification of moderately potent hit **16i** (**IX**, Fig. 1) displaying higher *Hp*IMPDH (99.89% inhibition at 10 μM) and moderate human IMPDH2 (*h*IMPDH2) inhibition (56.81% at 10 μM). The hit **16i** was relatively nonselective for *Hp*IMPDH [22]. We started off with the sole objective of modulating the selectivity of **16i** for the bacterial enzyme over the human counterpart by systematic structure-activity and selectivity exploration. In the present study, we report the design and synthesis of 5-aminoisobenzofuran-1(3*H*)-one derivatives possessing similar

pharmacophoric features as the reported *Hp*IMPDH inhibitors (Fig. 1). The synthesized compounds were further screened in *in vitro* biochemical assays (*Hp*IMPDH and *h*IMPDH2 enzyme inhibition). In addition, we believe that the availability of structurally diverse, potent and selective *Hp*IMPDH inhibitors would stimulate the scientific community's interest in this field, attempting to eliminate deadly *H. pylori* from the masses. Here, we report our initial stint with the design and development of structurally novel, moderately potent and highly selective *Hp*IMPDH inhibitors. The hits are likely to be explored further for improving potency, molecular, physicochemical, pharmacokinetic and toxicity profiles.

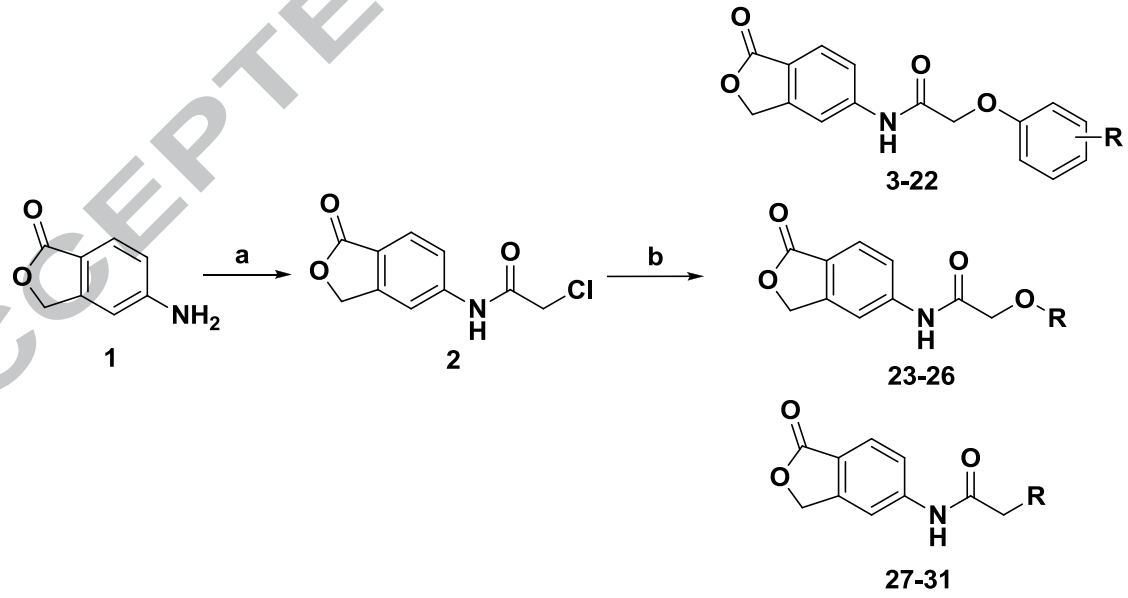
2. Results and Discussion

2.1. Chemistry

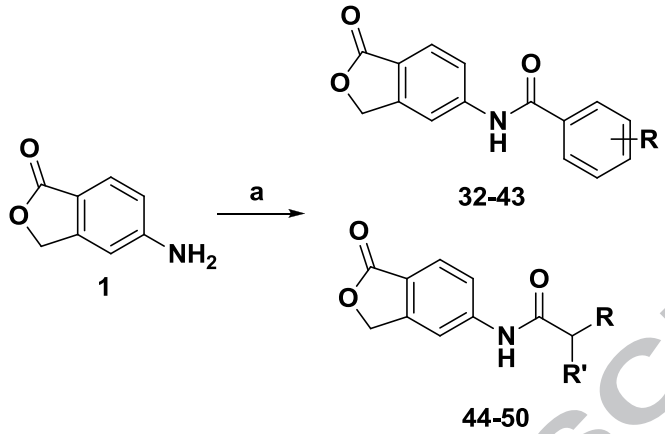
In the present study, two series of phthalide derivatives – series I and II – were designed and synthesized as outlined in Schemes 1 and 2, respectively. The emphasis was placed on the pharmacophoric requirements for IMPDHs in general, and microbial IMPDHs in particular. The two aryl groups (Ar^1 and Ar^2) with varying linkers, conformationally flexible versus rigid, short (2-3 atoms) versus long (4-5 atoms), etc., were tried. One of the aryl groups (Ar^1) was fixed as a 5-substitued phthalide substructure in all the designed molecules, based on our previous experience with *Hp*IMPDH inhibitors design strategy [22]. Several derivatives of 5-aminoisobenzofuran-1(3*H*)-one (**1**) were synthesized using conventional amide bond formation reaction [25]. Numerous amide-coupling reagents were employed, namely, diphenylphosphoryl azide (DPPA)/triethylamine (TEA), N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ)/pyridine, hexafluorophosphate benzotriazole tetramethyl uronium (HBTU)/N-methylmorpholine (NMM), TBTU/HOBt, HATU/DIPEA, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI)/4-(N,N-dimethylamino)pyridine (DMAP)/HOBt, etc. None of these reagents worked. Hence, we

resorted to chloroacetyl chloride (series I, Scheme 1) and substituted acid chloride (series II, Scheme 2) as the activated acid. The choice of chloroacetyl chloride as acyl halide was based on the linker present in the known *Hp*IMPDH inhibitor, **C91** (**VII**, Figure 1).

In case of series I – ethers (**3-26**, Table 1) and amines (**27-31**, Table 1), the chloroacetamide intermediate **2** was synthesized using **1** and chloroacetyl chloride in dry THF in presence of TEA at 0 °C, followed by overnight alkylation using anhydrous K_2CO_3/DMF (General procedure A) as a solvent to yield the title compounds (**3-31**). The series II amides (**32-50**, Table 2) were prepared using **1** and various substituted acid chlorides in presence of pyridine and THF (General procedure B) as a solvent to yield the title compounds (**32-50**). The reactions were quite clean and posed little or no problem for purification by selective precipitation and/or column chromatography.



Scheme 1. Reagents and conditions: (a) chloroacetyl chloride, TEA, THF, 0 ^{o}C to RT, over-night; (b) substituted phenol or substituted amine, K_2CO_3, DMF, RT, 16 hrs.



Scheme 2. Reagents and conditions**:** (a) substituted acid chlorides, pyridine, THF, 0 ºC to RT, overnight.

2.2. Biological evaluation

All the synthesized compounds (**3-50**, Tables 1 and 2) were screened for *in vitro* *Hp*IMPDH and *h*IMPDH2 inhibition at 10 μM concentration. The % inhibition data are given in Tables 1 and 2. Followed by the initial screening, hits with ~70% or higher inhibition (Figures 2 and 3) were further taken up for IC_{50} determination (**3**, **9**, **10**, **15**, **16**, **23**, **24**, **30**, **47-50**). To generate the concentration-response curves, initial rates of reaction were plotted against their corresponding logarithmic concentrations in case of *Hp*IMPDH. The IC_{50} data for *Hp*IMPDH are given in Tables 1 and 2. Concentration-response curve for the most potent compound **24** is given in the Fig. 2.

From series I (ether derivatives, **3-26**, Table 1), a total of eight compounds (**3**, **9**, **10**, **15**, **16**, **23**, **24** and **30**, Fig. S112, *Supplementary Data* section) exhibited ~70% *Hp*IMPDH inhibition. Compound **24** showed highest % inhibition. The IC_{50}s of the hits ranged from 2.21 – 20.9 μM. The substituents on the aryl ring (*o*-, *m*-, *p*-) ranged from very strongly deactivating (-NO_2), strongly deactivating (Ac, formyl, etc.) to activating (-CH_3). The -COOMe substituent at the *p*-position yielded slightly more potent compound (**10**) compared to the one at the *o*-position (**3**). The corresponding *n*-propyl (**11**) and *n*-butyl (**12**) esters were

considerably less active, mostly emphasizing the limited size in the binding pocket of the enzyme. Shifting *m*-CH_3 substituent (**6**) to *p*-position (**9**) increased enzyme inhibitory activity. Similarly, replacing *p*-CH_3 (**9**) with *p*-Cl (**15**) retained the activity. Any further combination of the two substituents *o,m*-, *o,p*- *m,p*- or *o,m,p*- led to either retention (**16**), slight decrease (**17-19**, **21-22**) or substantial decrease (**20**) in % *Hp*IMPDH inhibition. Replacing the substituted Ph ring on the ether O with naphthyl ring led to substantial increase in enzyme inhibitory activity. Of the two such compounds, one with 2-naphthyl ether (**24**) was slightly more potent than its 1-naphthyl counterpart (**23**). Further substituents such as quinolin-8-yl (**25**) led to slight decrease in activity while additional substitution on the quinoline ring (**26**) abolished the activity, further demonstrating limited tolerance to bulk at this end of the molecule.

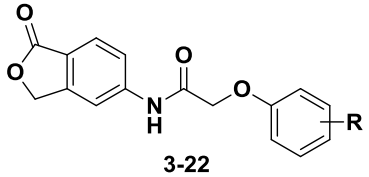
Interestingly, all the secondary (**27-29**), aromatic (**30**) and tertiary amines (**31**) exhibited >50% *Hp*IMPDH inhibition, with compound **30** consisting of 3-formyl-1*H*-indol-1-yl as the second aryl feature being better than others in the series (% inhibition ~70% at 10 μM concentration). The modest activity (~50% inhibition) may arise from the protonated form (wherever possible) of the amine compensating the binding energy via electrostatic interaction. This is particularly important in view of the fact that the *H. pylori* resides in a highly acidic environment where most of these amines will be highly protonated. This subseries containing

the amino functional group needs further exploration in terms of potency and selectivity for *Hp*IMPDH over *h*IMPDH2.

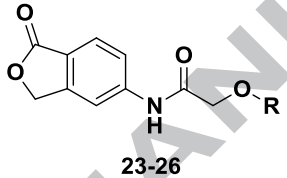
In series II of phthalide derivatives (**32-50**, Table 2, Fig. S113, *Supplementary Data* section), the spacer between 5-substituted phthalide substructure and other aryl (pharmacophoric) feature was reduced to 2-3 atoms (Scheme 2) which helped in understanding the importance of the spacer length. In the first subgroup (**32-43**, Table 2), a

conformationally rigid, two-atom spacer was tried. Compound **32** displayed moderated inhibition (61.34%). Addition of substituents, irrespective of electron-donating (e.g., **36**) or withdrawing (e.g., **33**), smaller (e.g., **35**) or bulky (e.g., **34**) nature of the substituent, maintained similar, albeit less, potency compared to **32** (50-57%). Compared to 3- or 4-methoxy substituents (**36** and **37**), the 2,5-$(OCH_3)_2$ substituents resulted in complete loss of enzyme inhibitory activity (**40**).

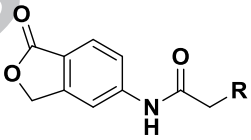
Table 1. Biological activities of compound **3-31** against *Hp*IMPDH



3-22



23-26



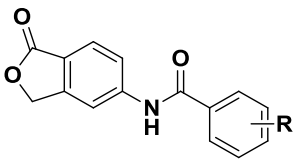
27-31

Code	-R	*Hp*IMPDH		*h*IMPDH2
		% Inhibition[a]	IC_{50} (µM)[a]	% Inhibition[a]
C91	-	-	0.197 ± 0.08[b]	Inactive[c]
IX (16i)[d]	-	99.89 ± 2.12	1.88 ± 0.07	56.81 ± 0.95
3	2-methylcarboxylate	69.7 ± 2.65	11.9 ± 0.05	< 0.1
4	2-nitro	1.2 ± 1.03	n.d.	< 0.1
5	2-acetyl	4.4 ± 1.06	n.d.	< 0.1
6	3-methyl	50.6 ± 1.85	n.d.	< 0.1
7	4-nitro	52.8 ± 2.35	n.d.	< 0.1
8	4-acetyl	6.2 ± 1.13	n.d.	< 0.1
9	4-methyl	70.4 ± 1.74	12.9 ± 0.21	< 0.1
10	4-methylcarboxylate	78.8 ± 3.82	8.9 ± 0.03	< 0.1
11	4-n-propylcarboxylate	14.1 ± 2.69	n.d.	< 0.1
12	4-n-butylcarboxylate	12.6 ± 2.12	n.d.	< 0.1
13	4-formyl	3.3 ± 1.06	n.d.	< 0.1
14	4-acetamide	22.6 ± 1.36	n.d.	< 0.1
15	4-chloro	71.6 ± 2.65	11.5 ± 0.07	< 0.1

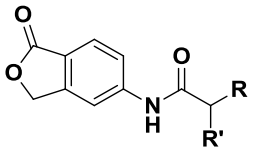
16	4-chloro-3-methyl	69.6 ± 1.78	15.7 ± 0.06	< 0.1
17	4-chloro-2-nitro	61.6 ± 2.96	n.d.	< 0.1
18	4-chloro-2,5-dimethyl	54.1 ± 3.32	n.d.	< 0.1
19	3-methyl-4-nitro	60 ± 4.12	n.d.	< 0.1
20	4-acetyl-2-methoxy	16.1 ± 2.09	n.d.	< 0.1
21	4-formyl-2-methoxy	55.1 ± 3.78	n.d.	< 0.1
22	2-isopropyl-5-methyl	64.6 ± 2.31	n.d.	< 0.1
23	naphthalen-1-yl	76.9 ± 4.8	12.7 ± 0.07	< 0.1
24	naphthalen-2-yl	87.2 ± 3.96	2.21 ± 0.02*	< 0.1
25	quinolin-8-yl	62.3 ± 2.6	n.d.	< 0.1
26	5-chloro-7-iodoquinolin-8-yl	19.1 ± 1.02	n.d.	< 0.1
27	*N*-methyl-*N*-phenylamino	51.6 ± 2.89	n.d.	< 0.1
28	*N*-ethyl-*N*-phenylamino	56.3 ± 1.96	n.d.	< 0.1
29	*N*-(4-fluorophenyl)amino	54.2 ± 4.89	n.d.	< 0.1
30	3-formyl-1*H*-indol-1-yl	71.2 ± 2.95	20.9 ± 0.11*	< 0.1
31	Morpholino	58.4 ± 2.59	n.d.	< 0.1

[a]All the data are expressed as ± SD (average of n=3). IC_{50} values were determined for compounds with % inhibition ~70% or more at 10 µM concentration; n.d. = not determined; [b] Ref. 17,18. The literature IC_{50} as reported in Ref. 12 was 1.1 nM; [c] Ref. 12; [d] Ref. 22; * Inhibition data obtained by monitoring absorbance of NADH; [c] Ref. 22

Table 2. Biological activity of compound **32-50** against *Hp*IMPDH



32-43



44-50

Code	**-R**	**-R′**	***Hp*IMPDH**		***h*IMPDH2**
			% Inhibition[a]	**IC_{50} (µM)[a]**	**% Inhibition[a]**

32	H	-	61.3 ± 1.50	n.d.	< 0.1
33	4-nitro	-	57.1 ± 2.53	n.d.	< 0.1
34	4-phenyl	-	50.1 ± 2.95	n.d.	< 0.1
35	4-methyl	-	51.3 ± 3.56	n.d.	< 0.1
36	3-methoxy	-	56.3 ± 3.62	n.d.	< 0.1
37	4- methoxy	-	57.1 ± 1.68	n.d.	< 0.1
38	3-benzyloxy	-	57.5 ± 1.35	n.d.	< 0.1
39	3-trifluoromethyl	-	55 ± 1.65	n.d.	< 0.1
40	2,5-dimethoxy	-	-	Inactive*	< 0.1
41	2-chloro-5-fluoro	-	51.9 ± 3.12	n.d.	< 0.1
42	2,4-dichloro	-	54.5 ± 2.65	n.d.	< 0.1
43	3-bromo-4-methoxy	-	50.9 ± 1.23	n.d.	< 0.1
44	Phenyl	H	54.9 ± 3.96	n.d.	< 0.1
45	Phenyl	cyclopentyl	56 ± 3.25	n.d.	< 0.1
46	Phenyl	$-CH_2-CH_2-$	52.2 ± 2.24	n.d.	< 0.1
(±)-**47**	4-isobutylphenyl	CH_3	76.6 ± 4.60	10.7 ± 0.12	< 0.1
(±)-**48**	3-benzoylphenyl	CH_3	86.2 ± 1.99	7.2 ± 0.07	< 0.1
(±)-**49**	2-fluorobiphenyl-4-yl	CH_3	78.5 ± 2.10	8.6 ± 0.03	< 0.1
50	4-fluoronaphth-1-yl	H	73.2 ± 2.78	11.4 ± 0.09	< 0.1

[a]All the data are expressed as ± SD (average of n=3). IC_{50} values were determined for compounds with % inhibition ~70% or more at 10 μM concentration; n.d. = not determined. * Inhibition data obtained by monitoring absorbance of NADH.

In the second subgroup of series II (**44-50**), the spacer length was maintained at three atoms with addition of one more (cyclo)alkyl group, especially -CH_3 (in order to search for potential 'magic' methyl effect), was attempted. The base compound (**44**) exhibited moderate inhibition of *Hp*IMPDH. The placement of a cycloalkyl group on the α-carbon next to amide (**45-46**) did not have any impact on the enzyme inhibition. Placing bulky substituents on the aryl ring along with the α-methyl group (racemic mixture) increased the activity notably (**47** versus **44**). Placing 3-benzoyl group on the second aryl ring led to further increase in % *Hp*IMPDH inhibition yielding relatively potent compound in this subgroup of series II (**48**, 86.21%, IC_{50} = 7.20 ± 0.07 μM). Other two compounds **49** and **50**, in line with series I results, maintained higher *Hp*IMPDH potency.

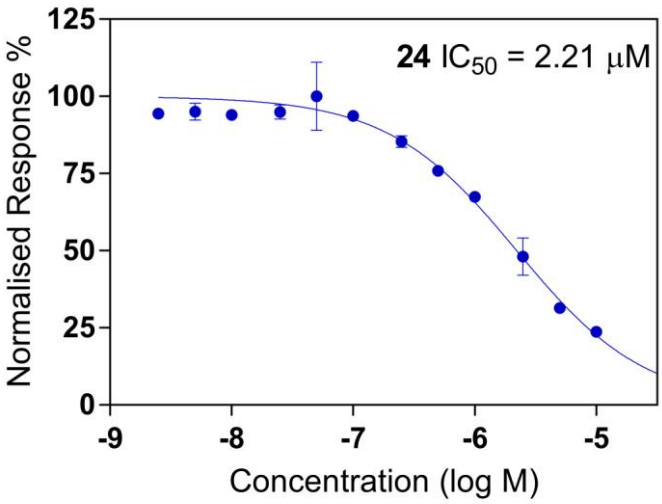


Fig. 2. Concentration response curve of compound **24**. The data was fitted using four-parameter Hill equation and each data point represents mean of triplicate

On the similar lines, all the synthesized molecules were evaluated for *h*IMPDH2 inhibition at 10 μM concentration, to address the issue of selectivity for the microbial

enzyme. None of the tested molecules exhibited significant *h*IMPDH2 inhibition. The results of % *h*IMPDH2 inhibition are listed in Tables 1 and 2. This undoubtedly confirmed the gain in selectivity of the hits for *Hp*IMPDH over *h*IMPDH2 due to extensive SAR investigations. Compound **24** turned out to be the most, yet moderately potent and selective, hit of the series. **C91**, the most potent *Hp*IMPDH known so far, was also selective over the human enzyme. Compound **24** shared 2-naphthyl substructure with **C91**, along with similar four-atom linker. The flexible alignment of **24** onto **C91** is shown in Fig. 3. As seen from the alignment, the 5-aminoisobenzofuran-1(3*H*)-one substructure was aligned onto the 2-naphthyl substituent in **C91** and vice versa for the 2-naphthyl group of **24**, which was found aligned onto the benzimidazole substructure of **C91** in an attempt to align the –NHCO- functionality of the linkers. The ether O in **24** linker was nicely aligned onto the pyridine N from **C91** (H-bond acceptor feature). Additionally, the pyridine ring was protruding from the superimposed bulk from both the molecules. Nonetheless, the alignment gave us the insight for future design strategies based on 5-aminoisobenzofuran-1(3*H*)-one core structure. In addition, the docked pose of **24** into the cofactor-binding site of *Hp*IMPDH homology model revealed crucial drug-receptor interactions (Figure 4). While the proximal end of **24**, i.e., the aminoisobenzofuranone ring O, formed one H-bond with Val224 (aminoisobenzofuranone core) and a cation-π interaction (Lys71), the rest of **24** interacted with the cofactor-binding site residues via mostly hydrophobic interactions, particularly the distal 2-naphthyl group. The π-π stacking interactions with IMP underlined the importance of 2-napthyl group in potency and possibly, selectivity. The lower activity of **23** (Table 1), in view of the proposed binding mode, could be attributed to the disturbed π-π stacking interaction with IMP. The methodical details of the docking analyses along with the relevant references in the *Supplementary Data* section. The clues obtained from the flexible alignment and the docking studies are crucial in driving further design of potent and selective IMPDH inhibitors.

Overall, our systematic and concerted efforts in developing potent and selective *Hp*IMPDH inhibitors yielded fruitful outcomes in the form of moderately potent and selective hits. These hits can certainly act as structurally novel starting points for the design of newer and selective *Hp*IMPDH inhibitors.

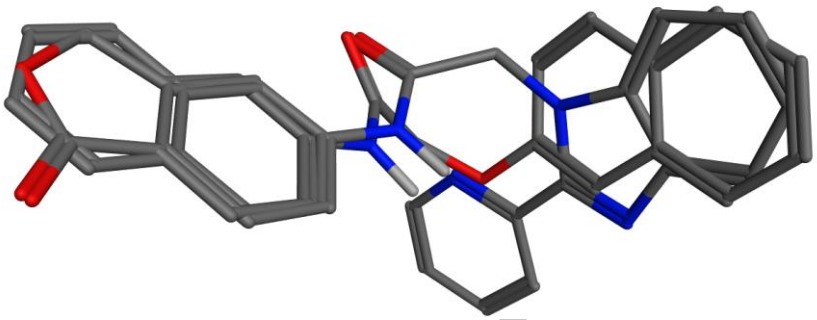


Fig. 3. Flexible alignment of **24** onto **C91**. The capped-stick structures are shown in element color.

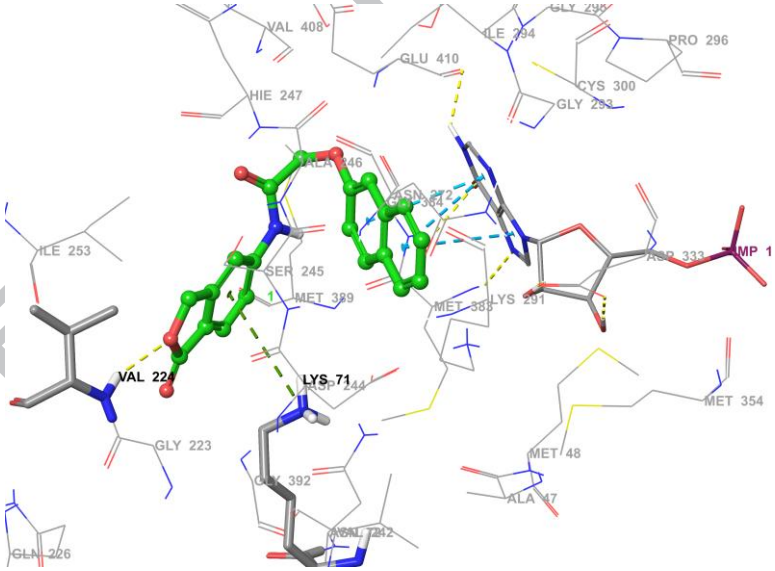


Fig 4. Binding mode of **24** into the cofactor-binding pocket of *Hp*IMPDH homology model. The ligand is shown as ball-and-stick model, substrate (IMP) as stick model while the enzyme residues are shown as line models colored by the element. Yellow dotted lines indicate H-bond interaction with Val224, dark-

green dotted lines indicate cation-π interaction of Lys71 and the amino-isobenzofuranone core while the light-green dotted lines indicate the π-π stacking interaction between the naphthyl ring of **24** and the purine ring of **IMP**.

2.3. Computational studies

2.3.1. Calculation/prediction of molecular and physicochemical property profiles of the title compounds

All the designed compounds (series I and II) were evaluated for their calculated/predicted molecular and physicochemical property profiles to check their drug likeliness and identify potential issues, if any, related to potentially poor pharmacokinetics well before the synthesis. Table 1S (*Supplementary Data*) lists all the abovementioned property categories for all the molecules. As given in Table S1, none of the designed molecules exhibited abnormal values and were within the commonly accepted ranges for drugs and drug-like molecules. These molecules were then taken up for syntheses and biological evaluation.

2.3.2. In silico predicted toxicity profiles of the hits

Given the importance of toxicity issues in drug discovery and development, it is imperative that one should avoid problematic substructures contributing to the toxicity issues such as carcinogenicity, mutagenicity, genotoxicity, reproductive toxicity, etc. The field of predictive toxicity has come a long way. Keeping this in mind, we initiated the predicted toxicity profiling of the hits to eliminate problematic structures right before they are evaluated further down the drug discovery funnel. The details of the predictive toxicity models, as implemented in Case Ultra ICH M7 bundle are provided in Table 2S

(*Supplementary Data*). The results of the *in silico* toxicity predictions for the mutagenicity, genotoxicity and carcinogenicity end-points are given in Table 3.

The results compare the hits (**3**, **9**, **10**, **15**, **16**, **23**, **24**, **30**, **47-50**) from the two series along with the most potent *Hp*IMPDH inhibitor **C91** in terms of mutagenicity, genotoxicity and carcinogenicity, three major preclinical toxicity end-points. In case of bacterial mutagenicity (ICH M7) models, most of the hits, except **C91** and **23**, were negative in at least three of the five models, with the overall result negative. Further investigation revealed the presence of benzimidazole (**C91**) and 1-naphthyl (**23**) substructures led to either positive of inconclusive outcome. For all practical purposes, the inconclusive was considered positive. In case of genotoxicity models, the results were mixed. Models such as GT3_A8J predicted all hits as positive for potential genotoxicity. These results may be interpreted cautiously and can be of great help in identifying potential trouble-makers early in the discovery phase. Similarly, the carcinogenicity predictions were also mixed, e.g., for hit **40**, of the six models, three predicted it as positive and the remaining ones as negative. Compound **C91** has been predicted as positive by all the models. Nonetheless, the medicinal chemist's experience and intuition are likely to help in interpreting these predictions in combination with the structural analysis (presence/absence of a potential alert, such as Michael acceptor).

3. Conclusions

In the present study, a novel series of *Hp*IMPDH inhibitors was designed, synthesized and evaluated for potency and selectivity over the human enzyme. Systematic structure-activity trends in the series led to the discovery of moderately potent and selective *Hp*IMPDH inhibitors. The probing of the microbial IMPDH binding site and the results thereof, could go a long way in *Hp*IMPDH inhibitor discovery given the relative dearth of chemotypes in this field. Overall, a total of 13 hits displayed ~70% inhibition or more. The top-ranked

compound of the series **24** exhibited moderate potency (IC_{50} = 2.21 µM) and high selectivity over human IMPDH2. The outcome of the present investigation led to further refinement of the microbial,

in particular, *Hp*IMPDH inhibitor requirements. The results clearly disclosed the potential of 5-aminoisobenzofuran-1(3*H*)-one as a lead scaffold which can be exploited for further design and development of safer, selective and potent, not only *H. pylori* but also, other microbial IMPDH inhibitors.

Table 3. *In silico* toxicity predictions for the hit molecules

Sr. No.	Model Name	C91	3	9	10	15	16	23	24	30	40	47	48	49	50
Bacterial Mutagenicity Models (ICH M7)															
1	GT1_A7B	#	-	-	-	-	-	+	#	-	-	-	-	#	-
2	GT1_AT_ECOLI	#	#	#	#	#	#	+	+	#	#	#	#	#	#
3	GT_EXPERT	-	-	-	-	-	-	+	-	+	-	-	-	-	+
4	PHARM_ECOLI	$	-	-	-	-	-	-	-	-	-	-	-	-	$
5	PHARM_SALM	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Genotoxicity Models															
6	GT2_A7U	+	+	#	#	#	#	+	+	+	#	#	#	#	#
7	GT2_A7V	$	#	#	#	#	#	+	#	+	-	-	#	-	$
8	GT2_A7W	+	+	-	-	-	#	+	+	+	-	-	-	#	+
9	GT2_A7X	#	+	-	-	-	-	+	#	+	-	-	-	-	$
10	GT2_A8H	$	-	-	-	-	#	$	-	#	$	#	-	-	$
11	GT3_A7S	$	-	-	-	-	#	-	-	-	-	-	-	+	$
12	GT3_A7T	#	-	-	#	+	+	+	-	#	-	-	-	-	+
13	GT3_A8J	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14	GT4_A7N	+	#	#	#	#	#	+	+	+	+	#	#	#	+
Carcinogenicity Models															
15	AF1	+	+	-	-	-	-	-	+	$	-	+	-	-	+
16	AF2	+	+	+	-	+	+	+	+	+	+	+	+	+	+
17	AF3	+	-	-	-	-	-	-	+	+	-	-	-	-	$
18	AF4	+	+	+	+	+	-	+	+	+	+	+	+	+	+
19	AFU	+	+	-	-	-	-	-	+	$	-	+	-	-	$
20	AFV	+	+	+	+	+	+	+	+	+	+	+	-	+	+
21	AFW	+	-	-	-	-	-	-	+	$	-	-	-	-	$

+ Positive
- Negative
Inconclusive
$ Out of domain

4. Experimental

4.1. Chemistry

All the chemicals were purchased from vendors such as Acros Organics (Geel, Belgium), Alfa Aesar (Karlsruhe, Germany), Spectrochem (Mumbai, India), Sigma Aldrich (Steinheim, Germany) or Merck (Darmstadt, Germany) and used without further purification unless otherwise indicated. All the reactions were carried out under dry N_2 atmosphere and progress of the reaction was monitored by thin layer chromatography (TLC) using an aluminium plate coated with silica gel 60 F_{254} (Merck Millipore, Billerica, MA, USA). All the title compounds were purified by column chromatography packed with silica gel of 230-400 mesh, 60 Å of Merck using a mixture of DCM/MeOH as eluent. Melting points were determined on Veego VMP DS melting point apparatus (General Trading Co., India) and are uncorrected. Purity of all the compounds was determined using HPLC and found to be >95%. HPLC analysis was performed on Agilent 1220 Infinity system (Santa Clara, CA, USA). An isocratic mobile phase consisting of (A) acetonitrile and (B) water (80:20, v/v) was used with a C_{18} Kromasil column (15 cm × 4.6 mm, 5 µm particle size, 100 Å pore size), flow rate of 1 mL/min. The ^{1}H-NMR spectra were recorded in DMSO-d_6 or $CDCl_3$ with tetramethylsilane (TMS) as an internal standard, on Bruker Advance 400 (400 MHz); chemical shifts were expressed in δ ppm. Mass spectra (MS) were recorded on a Shimadzu 8040 LC-MS/MS system (Japan), using electrospray ionization (ESI) mode.

4.1.1. 2-Chloro-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)acetamide (**2**) [22]

To a stirred mixture of 5-aminoisobenzofuran-1(3*H*)-one (**1**) (3.01 g, 20.1 mmol) and triethylamine (TEA) (3.36 mL, 24.12 mmol) in dry THF was added chloroacetyl chloride (1.76 mL, 22.11 mmol) dropwise at 0 °C. After completion of the reaction, triethylammonium chloride was filtered off and the filtrate was concentrated under vacuum

and quenched with water. The solid obtained was filtered at suction and washed to neutral filtrate, air-dried to give **2** as light-brown solid. Yield: 90%; mp: 218-220 °C.

4.1.2. General procedure A. Synthesis of N-(1-Oxo-1,3-dihydroisobenzofuran-5-yl)aceta-*mide derivatives (**3-31**)* [22]

A solution containing **2** (0.1 g, 0.44 mmol), anhydrous K_2CO_3 (0.18 g, 1.33 mmol) and appropriate hydroxyl/amino compound (0.44 mmol) in dry DMF (3 mL) was stirred for 16 hrs at 25 °C. After completion of the reaction, the reaction mixture was quenched with brine (50 mL). The precipitated product was filtered off, washed with water and dried under vacuum. The crude product was further purified by column chromatography using DCM:MeOH (95:5) as mobile phase.

*4.1.3. General procedure B. Synthesis of N-(1-Oxo-1,3-dihydroisobenzofuran-5-yl)substituted amides (**32-50**)*

To a mixture of 5-amino-isobenzofuran-1(3*H*)-one (**1**, 1 eq.) and pyridine (2 eq.) in THF was added substituted acid chloride (1 eq.) at 0 °C. The mixture was stirred overnight at RT for 24 hrs. After completion of the reaction, the reaction mixture was concentrated and crude product obtained was further purified by column chromatography using DCM:MeOH (95:5) as mobile phase.

4.1.4. Methyl 2-(2-oxo-2-(1-oxo-1,3-dihydroisobenzofuran-5-ylamino)ethoxy)benzoate (**3**)

The title compound was synthesized from **2** (0.1 g, 0.44 mmol), K_2CO_3 (0.18 g, 1.33 mmol) and methyl 2-hydroxybenzoate (0.067 g, 0.44 mmol) as described in the general procedure A to yield **3** as a white solid. Yield: 68%; TLC R_f = 0.57 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 210-212 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 10.78 (s, 1H), 8.30 (s, 1H), 7.99 (d, *J* = 8.0 Hz, 1H), 7.88 (d, *J* = 8.3 Hz, 1H), 7.76 (d, *J* = 8.3 Hz, 1H), 7.56 (t, *J* = 7.3 Hz, 1H), 7.11 (t, *J* = 7.6 Hz, 1H), 6.97 (d, *J* = 8.3 Hz, 1H), 5.29 (s, 2H), 4.73 (s, 2H), 4.00 (s, 3H); MS (ESI) *m/z*: 342 $[M+H]^+$.

4.1.5. 2-(2-Nitrophenoxy)-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)acetamide (**4**)

The title compound was synthesized from **2** (0.1 g, 0.44 mmol), K_2CO_3 (0.18 g, 1.33 mmol) and 2-nitrophenol (0.062 g, 0.44 mmol) as described in the general procedure A to yield **4** as a light-yellow solid. Yield: 63%; TLC R_f = 0.39 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 270-272 °C; MS (ESI) *m/z*: 329 $[M+H]^+$.

4.1.6. 2-(2-Acetylphenoxy)-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)acetamide (**5**)

The title compound was synthesized from **2** (0.1 g, 0.44 mmol), K_2CO_3 (0.18 g, 1.33 mmol) and 1-(2-hydroxyphenyl)ethanone (0.06 g, 0.44 mmol) as described in the general procedure A to yield **5** as an off-white solid. Yield: 64%; TLC R_f = 0.46 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 266-268 °C; MS (ESI) *m/z*: 326 $[M+H]^+$.

4.1.7. N-(1-Oxo-1,3-dihydroisobenzofuran-5-yl)-2-(3-tolyloxy)acetamide (**6**)

The title compound was synthesized from **2** (0.1 g, 0.44 mmol), K_2CO_3 (0.18 g, 1.33 mmol) and *m*-cresol (0.048 g, 0.44 mmol) as described in the general procedure A to yield **6** as a white solid. Yield: 62%; TLC R_f = 0.56 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 290-292 °C; MS (ESI) *m/z*: 298 $[M+H]^+$.

4.1.8. 2-(4-Nitrophenoxy)-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)acetamide (**7**)

The title compound was synthesized from **2** (0.1 g, 0.44 mmol), K_2CO_3 (0.18 g, 1.33 mmol) and 4-nitrophenol (0.062 g, 0.44 mmol) as described in the general procedure A to yield **7** as a light-yellow solid. Yield: 68%; TLC R_f = 0.41 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 244-246 °C; MS (ESI) *m/z*: 329 $[M+H]^+$.

4.1.9. 2-(4-Acetylphenoxy)-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)acetamide (**8**)

The title compound was synthesized from **2** (0.1 g, 0.44 mmol), K_2CO_3 (0.18 g, 1.33 mmol) and 1-(4-hydroxyphenyl)ethanone (0.06 g, 0.44 mmol) as described in the general procedure A to yield **8** as a light brown solid. Yield: 78%; TLC R_f = 0.47 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: >300 °C; MS (ESI) *m/z*: 326 $[M+H]^+$.

4.1.10. N-(1-Oxo-1,3-dihydroisobenzofuran-5-yl)-2-(4-tolyloxy)acetamide (**9**)

The title compound was synthesized from **2** (0.1 g, 0.44 mmol), K_2CO_3 (0.18 g, 1.33 mmol) and *p*-cresol (0.048 g, 0.44 mmol) as described in the general procedure A to yield **9** as a white solid. Yield: 69%; TLC R_f = 0.53 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 210-212 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 10.57 (s, 1H), 8.06 (s, 1H), 7.80 (d, *J* = 8.4 Hz, 1H), 7.71 (d, *J* = 8.5 Hz, 1H), 7.10 (d, *J* = 8.1 Hz, 2H), 6.88 (d, *J* = 8.1 Hz, 2H), 5.36 (s, 2H), 4.71 (s, 2H), 2.22 (s, 3H); MS (ESI) *m/z*: 298 $[M+H]^+$.

4.1.11. Methyl 4-(2-oxo-2-(1-oxo-1,3-dihydroisobenzofuran-5-ylamino)ethoxy)benzoate (**10**)

The title compound was synthesized from **2** (0.1 g, 0.44 mmol), K_2CO_3 (0.18 g, 1.33 mmol) and methyl 4-hydroxybenzoate (0.067 g, 0.44 mmol) as described in the general procedure A to yield **10** as an off-white solid. Yield: 65%; TLC R_f = 0.56 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 226-228 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 10.7(s, 1H), 8.03 (s, 1H), 7.96 – 7.88 (m, 2H), 7.78 (d, *J* = 8.4 Hz, 1H), 7.68 (d, *J* = 8.5 Hz, 1H), 7.13 – 7.06 (m, 2H), 5.35 (s, 2H), 4.88 (s, 2H), 3.80 (s, 3H); MS (ESI) *m/z*: 342 $[M+H]^+$.

4.1.12. Propyl 4-(2-oxo-2-(1-oxo-1,3-dihydroisobenzofuran-5-ylamino)ethoxy)benzoate (**11**)

The title compound was synthesized from **2** (0.1 g, 0.44 mmol), K_2CO_3 (0.18 g, 1.33 mmol) and propyl 4-hydroxybenzoate (0.080 g, 0.44 mmol) as described in the general procedure A to yield **11** as a white solid. Yield: 69%; TLC R_f = 0.59 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 280-282 °C; MS (ESI) *m/z*: 370 $[M+H]^+$.

4.1.13. Butyl 4-(2-oxo-2-(1-oxo-1,3-dihydroisobenzofuran-5-ylamino)ethoxy)benzoate (**12**)

The title compound was synthesized from **2** (0.1 g, 0.44 mmol), K_2CO_3 (0.18 g, 1.33 mmol) and butyl 4-hydroxybenzoate (0.086 g, 0.44 mmol) as described in the general procedure A to yield **12** as a white solid. Yield: 71%; TLC R_f = 0.63 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 180-182 °C; MS (ESI) *m/z*: 384 $[M+H]^+$.

4.1.14. 2-(4-Formylphenoxy)-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)acetamide (**13**)

The title compound was synthesized from **2** (0.1 g, 0.44 mmol), K_2CO_3 (0.18 g, 1.33 mmol) and 4-hydroxybenzaldehyde (0.054 g, 0.44 mmol) as described in the general procedure A to yield **13** as an off-white solid. Yield: 72%; TLC R_f = 0.54 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 290-292 °C; MS (ESI) *m/z*: 312 $[M+H]^+$.

4.1.15. 2-(4-Acetamidophenoxy)-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)acetamide (**14**)

The title compound was synthesized from **2** (0.1 g, 0.44 mmol), K_2CO_3 (0.18 g, 1.33 mmol) and N-(4-hydroxyphenyl)acetamide (0.067 g, 0.44 mmol) as described in the general procedure A to yield **14** as an off-white solid. Yield: 63%; TLC R_f = 0.51 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 214-216 °C; MS (ESI) *m/z*: 341 $[M+H]^+$.

4.1.16. 2-(4-Chlorophenoxy)-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)acetamide (**15**)

The title compound was synthesized from **2** (0.1 g, 0.44 mmol), K_2CO_3 (0.18 g, 1.33 mmol) and 4-chlorophenol (0.057 g, 0.44 mmol) as described in the general procedure A to yield **15** as a white solid. Yield: 68%; TLC R_f = 0.55 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 178-180 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 10.60 (s, 1H), 8.05 (s, 1H), 7.80 (d, *J* = 8.4 Hz, 1H), 7.70 (d, *J* = 7.6 Hz, 1H), 7.35 (d, *J* = 8.8 Hz, 2H), 7.02 (d, *J* = 8.8 Hz, 2H), 5.36 (s, 2H), 4.78 (s, 2H); MS (ESI) *m/z*: 318 $[M+H]^+$.

4.1.17. 2-(4-Chloro-3-methylphenoxy)-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)acetamide (**16**)

The title compound was synthesized from **2** (0.1 g, 0.44 mmol), K_2CO_3 (0.18 g, 1.33 mmol) and 4-chloro-3-methylphenol (0.063 g, 0.44 mmol) as described in the general procedure A to yield **16** as a white solid. Yield: 65%; TLC R_f = 0.57 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 284-286 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 10.58 (s, 1H), 8.05 (s, 1H), 7.80 (d, *J* = 8.4 Hz, 1H), 7.70 (d, *J* = 8.4 Hz, 1H), 7.32 (d, *J* = 8.8 Hz, 1H), 7.02 (s, 1H), 6.85 (d, *J* = 8.9 Hz, 1H), 5.36 (s, 2H), 4.76 (s, 2H), 2.28 (s, 3H); MS (ESI) *m/z*: 332 $[M+H]^+$.

4.1.18. 2-(4-Chloro-2-nitrophenoxy)-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)acetamide (**17**)

The title compound was synthesized from **2** (0.1 g, 0.44 mmol), K_2CO_3 (0.18 g, 1.33 mmol) and 4-chloro-2-nitrophenol (0.077 g, 0.44 mmol) as described in the general procedure A to yield **17** as an off-white solid. Yield: 66%; TLC R_f = 0.48 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 292-294 °C; MS (ESI) *m/z*: 363 $[M+H]^+$.

4.1.19. 2-(4-Chloro-3,5-dimethylphenoxy)-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)acetamide (**18**)

The title compound was synthesized from **2** (0.1 g, 0.44 mmol), K_2CO_3 (0.18 g, 1.33 mmol) and 4-chloro-3,5-dimethylphenol (0.069 g, 0.44 mmol) as described in the general procedure A to yield **18** as a white solid. Yield: 72%; TLC R_f = 0.61 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 234-236 °C; MS (ESI) *m/z*: 346 $[M+H]^+$.

4.1.20. 2-(3-Methyl-4-nitrophenoxy)-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)acetamide (**19**)

The title compound was synthesized from **2** (0.1 g, 0.44 mmol), K_2CO_3 (0.18 g, 1.33 mmol) and 3-methyl-4-nitrophenol (0.068 g, 0.44 mmol) as described in the general procedure A to yield **19** as a light yellow solid. Yield: 67%; TLC R_f = 0.42 (DCM:MeOH, 95:5); purity (HPLC): 98.08%; mp: 242-244 °C; MS (ESI) *m/z*: 343 $[M+H]^+$.

4.1.21. 2-(4-Acetyl-2-methoxyphenoxy)-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)acetamide (**20**)

The title compound was synthesized from **2** (0.1 g, 0.44 mmol), K_2CO_3 (0.18 g, 1.33 mmol) and 1-(4-hydroxy-3-methoxyphenyl)ethanone (0.074 g, 0.44 mmol) as described in the general procedure A to yield **20** as a white solid. Yield: 66%; TLC R_f = 0.44 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 228-230 °C; MS (ESI) *m/z*: 356 $[M+H]^+$.

4.1.22. 2-(4-formyl-2-methoxyphenoxy)-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)acetamide (**21**)

The title compound was synthesized from **2** (0.1 g, 0.44 mmol), K_2CO_3 (0.18 g, 1.33 mmol) and 4-hydroxy-3-methoxybenzaldehyde (0.067 g, 0.44 mmol) as described in the general procedure A to yield **21** as an off-white solid. Yield: 67%; TLC R_f = 0.51 (DCM:MeOH, 95:5); purity (HPLC): 98.20%; mp: 190-192 °C; MS (ESI) *m/z*: 342 $[M+H]^+$.

4.1.23. 2-(2-isopropyl-5-methylphenoxy)-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)acetamide (**22**)

The title compound was synthesized from 2 (0.1 g, 0.44 mmol), K_2CO_3 (0.18 g, 1.33 mmol) and 2-isopropyl-5-methylphenol (0.067 g, 0.44 mmol) as described in the general procedure A to yield 22 as an off-white solid. Yield: 64%; TLC R_f = 0.58 (DCM:MeOH, 95:5); purity (HPLC): 98.62%; mp: 130-132 °C; MS (ESI) *m/z*: 340 $[M+H]^+$.

4.1.24. 2-(Naphthalen-1-yloxy)-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)acetamide (**23**)

The title compound was synthesized from **2** (0.1 g, 0.44 mmol), K_2CO_3 (0.18 g, 1.33 mmol) and naphthalen-1-ol (0.064 g, 0.44 mmol) as described in the general procedure A to yield **23** as a brown solid. Yield: 63%; TLC R_f = 0.50 (DCM:MeOH, 95:5); purity (HPLC): 98.52%; mp: 212-214 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 10.74 (s, 1H), 8.35 – 8.28 (m, 1H), 8.08 (s, 1H), 7.92 – 7.85 (m, 1H), 7.81 (d, *J* = 8.5 Hz, 1H), 7.73 (d, *J* = 8.5 Hz, 1H), 7.59 – 7.48 (m, 3H), 7.41 (t, *J* = 8.0 Hz, 1H), 6.93 (d, *J* = 7.6 Hz, 1H), 5.37 (s, 2H), 4.99 (s, 2H); MS (ESI) *m/z*: 334 $[M+H]^+$.

4.1.25. 2-(Naphthalen-2-yloxy)-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)acetamide (**24**)

The title compound was synthesized from **2** (0.1 g, 0.44 mmol), K_2CO_3 (0.18 g, 1.33 mmol) and naphthalen-2-ol (0.064 g, 0.44 mmol) as described in the general procedure A to yield **24** as a white solid. Yield: 66%; TLC R_f = 0.48 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 290-292 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 10.60 (s, 1H), 8.08 (s, 1H), 7.90 – 7.76 (m, 4H), 7.74 (d, *J* = 8.4 Hz, 1H), 7.45 (t, *J* = 7.5 Hz, 1H), 7.38 – 7.28 (m, 3H), 5.36 (s, 2H), 4.90 (s, 2H); MS (ESI) *m/z*: 334 $[M+H]^+$.

4.1.26. N-(1-Oxo-1,3-dihydroisobenzofuran-5-yl)-2-(quinolin-8-yloxy)acetamide (**25**)

The title compound was synthesized from **2** (0.1 g, 0.44 mmol), K_2CO_3 (0.18 g, 1.33 mmol) and quinolin-8-ol (0.064 g, 0.44 mmol) as described in the general procedure A to yield **25** as a brown solid. Yield: 69%; TLC R_f = 0.42 (DCM:MeOH, 95:5); purity (HPLC): 98.75%; mp: 190-192 °C; MS (ESI) *m/z*: 335 $[M+H]^+$.

4.1.27. 2-(5-Chloro-7-iodoquinolin-8-yloxy)-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)acetamide (**26**)

The title compound was synthesized from **2** (0.1 g, 0.44 mmol), K_2CO_3 (0.18 g, 1.33 mmol) and 5-chloro-7-iodoquinolin-8-ol (0.135 g, 0.44 mmol) as described in the general procedure A to yield **26** as a brown solid. Yield: 72%; TLC R_f = 0.44 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 228-230 °C; MS (ESI) *m/z*: 495 $[M+H]^+$.

4.1.28. 2-(Methyl(phenyl)amino)-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)acetamide (**27**)

The title compound was synthesized from **2** (0.1 g, 0.44 mmol), K_2CO_3 (0.18 g, 1.33 mmol) and *N*-methylaniline (0.047 g, 0.44 mmol) as described in the general procedure A to yield **27** as an off-white solid. Yield: 69%; TLC R_f = 0.43 (DCM:MeOH, 95:5); purity (HPLC): 98.74%; mp: 240-242 °C; MS (ESI) *m/z*: 297 $[M+H]^+$.

4.1.29. 2-(Ethyl(phenyl)amino)-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)acetamide (**28**)

The title compound was synthesized from **2** (0.1 g, 0.44 mmol), K_2CO_3 (0.18 g, 1.33 mmol) and *N*-ethylaniline (0.054 g, 0.44 mmol) as described in the general procedure A to yield **28** as an off-white solid. Yield: 68%; TLC R_f = 0.43 (DCM:MeOH, 95:5); purity (HPLC): 98.43%; mp: 138-140 °C; MS (ESI) *m/z*: 311 $[M+H]^+$.

4.1.30. 2-(4-Fluorophenylamino)-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)acetamide (**29**)

The title compound was synthesized from **2** (0.1 g, 0.44 mmol), K_2CO_3 (0.18 g, 1.33 mmol) and 4-fluoroaniline (0.049 g, 0.44 mmol) as described in the general procedure A to

yield **29** as an off-white solid. Yield: 65%; TLC R_f = 0.41 (DCM:MeOH, 95:5); purity (HPLC): 98.99%; mp: 284-286 °C; MS (ESI) *m/z*: 301 $[M+H]^+$.

4.1.31. 2-(3-Formyl-1H-indol-1-yl)-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)acetamide (**30**)

The title compound was synthesized from **2** (0.1 g, 0.44 mmol), K_2CO_3 (0.18 g, 1.33 mmol) and 1*H*-indole-3-carbaldehyde (0.064 g, 0.44 mmol) as described in the general procedure A to yield **30** as a white solid. Yield: 61%; TLC R_f = 0.48 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 290-292 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 11.01 (s, 1H), 9.95 (s, 1H), 8.34 (s, 1H), 8.12 (d, *J* = 7.5 Hz, 1H), 7.99 (s, 1H), 7.81 (d, *J* = 8.3 Hz, 1H), 7.67 (d, *J* = 8.4 Hz, 1H), 7.56 (d, *J* = 7.8 Hz, 1H), 7.34 – 7.22 (m, 2H), 5.32 (d, *J* = 18.3 Hz, 4H); MS (ESI) *m/z*: 335 $[M+H]^+$.

4.1.32. 2-Morpholino-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)acetamide (**31**)

The title compound was synthesized from **2** (0.1 g, 0.44 mmol), K_2CO_3 (0.18 g, 1.33 mmol), morpholine (0.039 g, 0.44 mmol) as described in the general procedure A to yield **31** as an off-white solid. Yield: 62%; TLC R_f = 0.42 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 160-162 °C; MS (ESI) *m/z*: 277 $[M+H]^+$.

4.1.33. N-(1-Oxo-1,3-dihydroisobenzofuran-5-yl)benzamide (**32**)

The title compound was synthesized from **1** (0.2 g, 1.34 mmol), pyridine (0.22 mL, 2.68 mmol) and benzoyl chloride (0.19 g, 1.34 mmol) as described in the general procedure B to yield **32** as an off-white solid. Yield: 68%; TLC R_f = 0.49 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 146-148 °C; MS (ESI) *m/z*: 254 $[M+H]^+$.

4.1.34. 4-Nitro-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)benzamide (**33**)

The title compound was synthesized from **1** (0.2 g, 1.34 mmol), pyridine (0.22 mL, 2.68 mmol) and 4-nitrobenzoyl chloride (0.25 g, 1.34 mmol) as described in the general procedure B to yield **33** as a off-white solid. Yield: 57%; TLC R_f = 0.46 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 288-290 °C; MS (ESI) *m/z*: 299 $[M+H]^+$.

4.1.35. N-(1-Oxo-1,3-dihydroisobenzofuran-5-yl)biphenyl-4-carboxamide (**34**)

The title compound was synthesized from **1** (0.2 g, 1.34 mmol), pyridine (0.22 mL, 2.68 mmol) and biphenyl-4-carbonyl chloride (0.29 g, 1.34 mmol) as described in the general procedure B to yield **34** as an off-white solid. Yield: 64%; TLC R_f = 0.53 (DCM:MeOH, 95:5); purity (HPLC): 97.10%; mp: 212-214 °C; MS (ESI) *m/z*: 330 $[M+H]^+$.

4.1.36. 4-Methyl-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)benzamide (**35**)

The title compound was synthesized from **1** (0.2 g, 1.34 mmol), pyridine (0.22 mL, 2.68 mmol) and 4-methylbenzoyl chloride (0.20 g, 1.34 mmol) as described in the general procedure B to yield **35** as a yellow solid. Yield: 62%; TLC R_f = 0.51 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 234-236 °C; MS (ESI) *m/z*: 268 $[M+H]^+$.

4.1.37. 4-Methoxy-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)benzamide (**36**)

The title compound was synthesized from **1** (0.2 g, 1.34 mmol), pyridine (0.22 mL, 2.68 mmol) and 4-methoxybenzoyl chloride (0.23 g, 1.34 mmol) as described in the general procedure B to yield **36** as a white solid. Yield: 53%; TLC R_f = 0.47 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 232-234 °C; MS (ESI) *m/z*: 284 $[M+H]^+$.

4.1.38. 3-Methoxy-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)benzamide (**37**)

The title compound was synthesized from **1** (0.2 g, 1.34 mmol), pyridine (0.22 mL, 2.68 mmol) and 3-methoxybenzoyl chloride (0.23 g, 1.34 mmol) as described in the general procedure B to yield **37** as a white solid. Yield: 59%; TLC R_f = 0.46 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 224-226 °C; MS (ESI) *m/z*: 284 $[M+H]^+$.

4.1.39. 3-(Benzyloxy)-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)benzamide (**38**)

The title compound was synthesized from **1** (0.2 g, 1.34 mmol), pyridine (0.22 mL, 2.68 mmol) and 3-(benzyloxy)benzoyl chloride (0.33 g, 1.34 mmol) as described in the general procedure B to yield **38** as a white solid. Yield: 68%; TLC R_f = 0.49 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 138-140 °C; MS (ESI) *m/z*: 360 $[M+H]^+$.

4.1.40. N-(1-Oxo-1,3-dihydroisobenzofuran-5-yl)-3-(trifluoromethyl)benzamide (**39**)

The title compound was synthesized from **1** (0.2 g, 1.34 mmol), pyridine (0.22 mL, 2.68 mmol) and 3-(trifluoromethyl)benzoyl chloride (0.28 g, 1.34 mmol) as described in the general procedure B to yield **39** as a white solid. Yield: 63%; TLC R_f = 0.52 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 242-244 °C; MS (ESI) *m/z*: 322 $[M+H]^+$.

4.1.41. 2,5-Dimethoxy-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)benzamide (**40**)

The title compound was synthesized from **1** (0.2 g, 1.34 mmol), pyridine (0.22 mL, 2.68 mmol) and 2,5-dimethoxybenzoyl chloride (0.27 g, 1.34 mmol) as described in the general procedure B to yield **40** as a green solid. Yield: 72%; TLC R_f = 0.43 (DCM:MeOH, 95:5); purity (HPLC): 98.36%; mp: 218-220 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 10.34 (s, 1H), 8.30 (s, 1H), 7.85 (d, *J* = 8.2 Hz, 1H), 7.79 (d, *J* = 3.2 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.11 – 7.04 (m, 1H), 7.00 (d, *J* = 9.0 Hz, 1H), 5.29 (s, 2H), 4.05 (s, 3H), 3.84 (s, 3H); MS (ESI) *m/z*: 314 $[M+H]^+$.

4.1.42. 2-Chloro-5-fluoro-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)benzamide (**41**)

The title compound was synthesized from **1** (0.2 g, 1.34 mmol), pyridine (0.22 mL, 2.68 mmol) and 2-chloro-5-fluorobenzoyl chloride (0.26 g, 1.34 mmol) as described in the general procedure B to yield **41** as a off-white solid. Yield: 57%; TLC R_f = 0.55 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 198-200 °C; MS (ESI) *m/z*: 306 $[M+H]^+$.

4.1.43. 2,4-Dichloro-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)benzamide (**42**)

The title compound was synthesized from **1** (0.2 g, 1.34 mmol), pyridine (0.22 mL, 2.68 mmol) and 2,4-dichlorobenzoyl chloride (0.28 g, 1.34 mmol) as described in the general procedure B to yield **42** as a white solid. Yield: 69%; TLC R_f = 0.53 (DCM:MeOH, 95:5); purity (HPLC): 98.32%; mp: 188-190 °C; MS (ESI) *m/z*: 323 $[M+H]^+$.

4.1.44. 3- Bromo-4-methoxy-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)benzamide (**43**)

The title compound was synthesized from **1** (0.2 g, 1.34 mmol), pyridine (0.22 mL, 2.68 mmol) and 3-bromo-4-methoxybenzoyl chloride (0.33 g, 1.34 mmol) as described in the general procedure B to yield **43** as an off-white solid. Yield: 58%; TLC R_f = 0.49 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 256-258 °C; MS (ESI) *m/z*: 363 $[M+H]^+$.

4.1.45. N-(1-Oxo-1,3-dihydroisobenzofuran-5-yl)-2-phenylacetamide (**44**)

The title compound was synthesized from **1** (0.2 g, 1.34 mmol), pyridine (0.22 mL, 2.68 mmol) and 2-phenylacetyl chloride (0.21 g, 1.34 mmol) as described in the general procedure B to yield **44** as a light-yellow solid. Yield: 67%; TLC R_f = 0.49 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 132-134 °C; MS (ESI) *m/z*: 268 $[M+H]^+$.

4.1.46. 2-Cyclopentyl-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)-2-phenylacetamide (**45**)

The title compound was synthesized from **1** (0.2 g, 1.34 mmol), pyridine (0.22 mL, 2.68 mmol) and 2-cyclopentyl-2-phenylacetyl chloride (0.30 g, 1.34 mmol) as described in the general procedure B to yield **45** as a white solid. Yield: 61%; TLC R_f = 0.52 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 190-192 °C; MS (ESI) *m/z*: 336 $[M+H]^+$.

4.1.47. N-(1-Oxo-1,3-dihydroisobenzofuran-5-yl)-1-phenylcyclopropanecarboxamide (**46**)

The title compound was synthesized from **1** (0.2 g, 1.34 mmol), pyridine (0.22 mL, 2.68 mmol) and 1-phenylcyclopropanecarbonyl chloride (0.24 g, 1.34 mmol) as described in the general procedure B to yield **46** as a light-yellow solid. Yield: 63%; TLC R_f = 0.49 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 120-122 °C; MS (ESI) *m/z*: 294 $[M+H]^+$.

4.1.48. (±)-2-(4-Isobutylphenyl)-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)propanamide (**47**)

The title compound was synthesized from **1** (0.2 g, 1.34 mmol), pyridine (0.22 mL, 2.68 mmol) and (±)-2-(4-isobutylphenyl)propanoyl chloride (0.30 g, 1.34 mmol) as described in the general procedure B to yield **47** as an off-white solid. Yield: 64%; TLC R_f = 0.55 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 152-154 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 10.53 (s, 1H), 8.04 (s, 1H), 7.75 (d, *J* = 8.4 Hz, 1H), 7.62 (d, *J* = 8.4 Hz, 1H), 7.28

(d, *J* = 7.6 Hz, 2H), 7.10 (d, *J* = 7.6 Hz, 2H), 5.33 (s, 2H), 3.84 (q, *J* = 7.0 Hz, 1H), 2.38 (d, *J* = 7.1 Hz, 2H), 1.78 (m, 1H), 1.40 (d, *J* = 6.8 Hz, 3H), 0.82 (d, *J* = 6.5 Hz, 6H); MS (ESI) *m/z*: 338 $[M+H]^+$.

4.1.49. (±)-2-(3-benzoylphenyl)-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)propanamide (**48**)

The title compound was synthesized from **1** (0.2 g, 1.34 mmol), pyridine (0.22 mL, 2.68 mmol) and (±)-2-(3-benzoylphenyl)propanoyl chloride (0.37 g, 1.34 mmol) as described in the general procedure B to yield **48** as a white solid. Yield: 61%; TLC R_f = 0.52 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 68-70 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 10.67 (s, 1H), 8.06 (s, 1H), 7.83 (s, 1H), 7.77 – 7.64 (m, 5H), 7.62 – 7.58 (d, 2H), 7.55 (t, *J* = 7.6 Hz, 3H), 5.34 (s, 2H), 4.01 (s, 1H), 1.48 (s, 3H); MS (ESI) *m/z*: 386 $[M+H]^+$.

4.1.50. (±)-2-(2-fluorobiphenyl-4-yl)-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)propanamide (**49**)

The title compound was synthesized from **1** (0.2 g, 1.34 mmol), pyridine (0.22 mL, 2.68 mmol) and (±)-2-(2-fluorobiphenyl-4-yl)propanoyl chloride (0.35 g, 1.34 mmol) as described in the general procedure B to yield **49** as an off-white solid. Yield: 62%; TLC R_f = 0.57 (DCM:MeOH, 95:5); purity (HPLC): 98.68%; mp: 184-186 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 10.71 (s, 1H), 8.05 (s, 1H), 7.77 (d, *J* = 8.4 Hz, 1H), 7.65 (d, *J* = 8.4 Hz, 1H), 7.55 – 7.41 (m, 5H), 7.39 (d, *J* = 7.2 Hz, 1H), 7.38 – 7.27 (m, 2H), 5.34 (s, 2H), 3.34 (s, 1H), 1.47 (d, *J* = 6.9 Hz, 3H); MS (ESI) *m/z*: 376 $[M+H]^+$.

4.1.51. 2-(4-Fluoronaphthalen-1-yl)-N-(1-oxo-1,3-dihydroisobenzofuran-5-yl)acetamide (**50**)

The title compound was synthesized from **1** (0.2 g, 1.34 mmol), pyridine (0.22 mL, 2.68 mmol) and 2-(4-fluoronaphthalen-1-yl)acetyl chloride (0.30 g, 1.34 mmol) as described in the general procedure B to yield **50** as a light-brown solid. Yield: 58%; TLC R_f = 0.47 (DCM:MeOH, 95:5); purity (HPLC): >99%; mp: 170-172 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.17 (d, 1H), 7.98 (s, 2H), 7.73 – 7.64 (m, 2H), 7.61 (dt, *J* = 2.3, 6.0 Hz, 2H), 7.42 (dd, *J* =

5.2, 7.8 Hz, 1H), 7.15 (dd, *J* = 7.7, 10.1 Hz, 1H), 7.12 – 7.05 (m, 1H), 5.19 (s, 2H), 4.17 (s, 2H); MS (ESI) *m/z*: 336 $[M+H]^+$.

4.2. Biological Evaluation

4.2.1. In vitro HpIMPDH inhibition assay

A total of 48 molecules were screened at 10 µM concentration in the *in vitro* assay. The assay was performed in 200 µL final volume in Black96F well plate (Tarsons Products Pvt. Ltd., Kolkata, India) with a reaction buffer composed of 50 mM Tris-HCl (pH 8.6), 100 mM KCl and 1 mM dithiothreitol (DTT). Assays were performed using 100 nM *Hp*IMPDH in presence or absence of test compounds. The assay mixture was incubated for 10 min at 37 °C and the reaction was initiated by adding 250 µM of IMP and 300 µM of NAD^+ (substrate buffer). The assay was allowed to proceed at 37 °C for 45 min. Generated NADH was measured by reading the fluorescence (λ_{ex} = 340 nm, λ_{em} = 440 nm) at an interval of 1 min using PerkinElmer EnVision Multilabel Reader (Waltham, Massachusetts, U.S.). **C91** (1 µM) was used as positive control and DMSO as vehicle control. For IC_{50} determination, a total of 10 concentrations ranging from 50 nM to 25 µM in triplicates, were used. Enzyme inhibition and IC_{50} values were expressed in % inhibition and µM, respectively [22].

4.2.2. In vitro hIMPDH2 inhibition assay

The *h*IMPDH2 was purchased from NovoCIB SAS (Lyon, France). A total of 26 molecules were screened at 10 µM concentration in the *in vitro* assay. The assay was performed in 96-well plates (Tarsons, 980040) (200 µL final volume) with reaction buffer containing 100 mM Tris–HCl (pH 8.6), 100 mM KCl and 5 mM DTT, 4% v/v DMSO plus or minus test compound and 0.15 mU (0.003 mg/mL) of purified *h*IMPDH2 enzyme per well (from 1.5 mg/mL stock concentration). The final volume of the enzyme stock solution per well was 2 µL. It was insignificant to cause any change in the final assay buffer composition. The reaction was initiated by the addition of (substrate buffer) 0.2 mM of IMP and 0.2 mM of

NAD^+ and the assay was allowed to proceed at 37 °C for 30 min. The generated NADH was measured by recording the absorbance at 340 nm. At this wavelength, a background of <0.1 optical density (OD) was observed with negligible crosstalk between wells. Mycophenolic acid (10 µM) was used as a positive control and DMSO as a vehicle control. Enzyme inhibition at 10 µM concentration was expressed in % [21-24].

4.2.3. Data analysis

The *Hp*IMPDH or *h*IMPDH2 inhibition by the title compounds was investigated by monitoring the change in the initial velocity of NADH formation (absorbance or fluorescence) for 10 min. after start of the reaction. To calculate the IC_{50} values, initial rate of reaction (slope) was plotted against concentrations of the hits and the IC_{50} values were calculated for each compound by fitting the data in Equation (1) with the help of GraphPad Prism version 6.0 (La Jolla, California, USA).

$$Y = Bottom + (Top-Bottom)/(1+10\text{^}((LogIC_{50}-X)*HillSlope)) \quad (1)$$

4.3. Computational studies

4.3.1. Hardware and software

All the molecular modeling studies were performed on HP laptop (Intel® Core™ i7-5500U CPU @ 2.40 GHz, RAM 4 GB) running Windows 8.1 Home Basic Operating System. Schrödinger Small-Molecule Drug Discovery Suite Release 2016-1 (Schrödinger, LLC, New York, NY 2016-1) [26], and the products included therein were used for molecular modeling studies. In silico preclinical toxicity end points (mutagenicity, genotoxicity, and carcinogenicity) predictions were carried out using CASE Ultra Q6 (1.6.2.3) software [27-29].

4.3.2. In silico property predictions

4.3.2.1. Ligand preparation

The molecular structures were built in *Maestro* and prepared using the *LigPrep* module [26]. The default setting, as implemented in the tool, were used except the pH 7.4, instead of default 7.0±2.0, for assigning the correct ionization states. The prepared structures were used for property calculation/predictions.

4.3.2.2. In silico property predictions

CASE Ultra software was used for predicting the preclinical toxicity end-points and *QikProp* module as implemented in Schrödinger Small-Molecule Drug Discovery Suite 2016-1, was used for predicting the molecular, physicochemical and preliminary pharmacokinetic properties of the hits to understand their drug-likeness potential. CASE Ultra is a quantitative structure–activity relationship (QSAR) software for predicting toxicity of chemicals. The models as implemented in CASE Ultra cover a wide variety of preclinical toxicity end-points and are claimed to be the largest collection of high-quality computational models developed in collaboration with USFDA. Table S1 (*Supplementary Data*) lists the CASE Ultra models used in this investigation. The molecular, physicochemical, and pharmacokinetic property predictions are given in Table S2 (*Supplementary Data*).

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Appendix A. Supplementary Data

The spectral characterization data, ^{1}H-NMR and mass spectra of the NCEs along with HPLC chromatograms, calculated/predicted molecular, physicochemical and preliminary pharmacokinetic properties (Table S1) and details of toxicity prediction models (Table S2), are given.

Conflict of Interest

The authors declare no potential conflict of interests.

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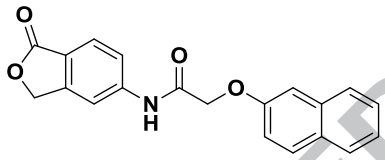
Graphical Abstract

Design, synthesis and biological evaluation of *Helicobacter pylori* inosine 5′-monophosphate dehydrogenase (*Hp*IMPDH) inhibitors. Further optimization of selectivity towards *Hp*IMPDH over human IMPDH2



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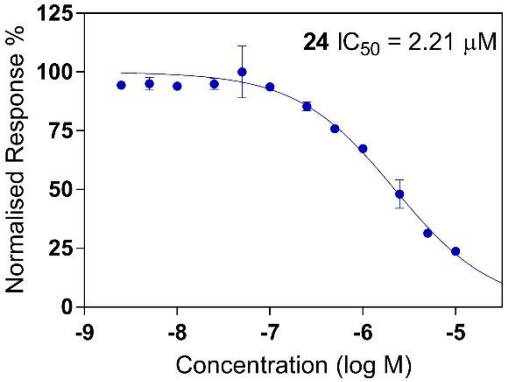
Inhibition: *Hp*IMPDH: ~87%
*h*IMPDH2: <0.1%
(@ 10 μM)



Highlights

- Discovery of a novel, moderately potent and selective *Hp*IMPDH inhibitor
- Drug-like and relatively nontoxic hit amenable to further structural modifications identified
- Discovery of 5-aminoisobenzofuran-1(3*H*)-one as a novel and promising scaffold for *Hp*IMPDH inhibitor development