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Original article

3-Biphenylimidazo[1,2-*a*]pyridines or [1,2-*b*]pyridazines and analogues, novel *Flaviviridae* inhibitors

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ABSTRACT

Using Ttou 84 as starting point, a novel class of biphenyl derivatives of imidazo[1,2-*a*]pyridine and imidazo[1,2-*b*]pyridazine was designed to optimize the inhibitory properties on the replication of the bovine viral diarrhoea virus (BVDV) and hepatitis C virus (HCV). Three sites of pharmacomodulation were chosen i.e. positions 2, 3 and 6 on the central heterocyclic core structure. From the 49 analogues tested, only compound **18j** (3-(2′-hydroxybiphen-3-yl)-2-(2-methoxyphenyl)-6-(thien-3-yl)imidazo[1,2-*b*]pyridazine) showed antiviral activity in the HCV replicon system reminiscent of selective inhibition (60–70% inhibition). Compound **4f** (3-(biphen-3-yl)-2-(4-fluorophenyl)-6-phenylthioimidazo[1,2-*a*]pyridine) proved to be the most selective inhibitor of BVDV replication and showed no or only marginal cross-resistance with known inhibitors of pestivirus replication. The cross-resistance profile of **4f** might indicate that **4f** does not interact with the same binding site as BPIP, VP32947, AG110 or LZ37. From 42 analogues tested against both viruses, QSAR studies were discussed in regard to BVDV antiviral activity.

1. Introduction

The bovine viral diarrhoea virus (BVDV) and hepatitis C virus (HCV), for which BVDV was once considered to be a surrogate [1], are two viral pathogens that belong to the family of *Flaviviridae* [2]. BVDV, together with the classical swine fever virus (CSFV), are representatives of the genus *Pestivirus*. These viruses cause important diseases in livestock (i.e. BVDV in cattle and CSFV in pigs) regardless of the availability of efficacious vaccines and the implementation of elaborate eradication or control programs [3–5]. An alternative approach to combat BVDV and CSFV infections could be the use of antiviral agents that specifically inhibit the replication of the virus [6,7]. Although likely not suited to treat large herds, it may be important to have selective broad-spectrum anti-*Pestivirus* compounds at hand, for example as a mean to rapidly control CSFV outbreaks [6,7].

Worldwide, 170 million people are chronic carriers of HCV. This virus is a major cause of cirrhosis and primary hepatocellular carcinoma, and the main reason for liver transplantations among adults in Western countries [8]. The current therapy for hepatitis C virus infection consists of the combination of pegylated interferon-α, the nucleoside analogue ribavirin and a virus-specific protease inhibitor Telaprevir or Boceprevir [9]. This therapy is only effective in about 60–70% of patients that suffer from chronic HCV infection, and is associated with important side effects. A potential concern is the emergence of drug-resistant virus variants. The ultimate goal in HCV therapy is to evolve to an all-oral, interferon–sparse combination of direct-acting antivirals against HCV with minimal adverse effects and against which no drug-resistance develops during treatment [10,11]. Hence, there is still a need to diversify the portfolio of direct-acting antiviral drugs, especially drugs with novel mechanisms of action.

In the course of our studies on the evaluation of the chemical and pharmacological properties of imidazoazines, we previously identified a novel series of compounds with selective antiviral activity against the human cytomegalovirus (hCMV) and varicella-zoster virus (VZV); viral pathogens that belong to the α- and β-*Herpesviridae* respectively [12,13]. In our search for selective inhibitors of virus replication, we decided to evaluate the antiviral

Abbreviations: CCR2, CC chemokine receptor 2; CCL2, CC chemokine ligand 2; CCR5, CC chemokine receptor 5; TLC, thin layer chromatography; BVDV, bovine viral diarrhoea virus; HCV, hepatitis C virus; EC_{50}, 50% effective concentration; CC_{50}, 50% cytotoxic concentration; QSAR, quantitative structure–activity relationship.

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properties of around 120 compounds from our chemical library against BVDV and HCV. Five hits were identified from this library and a pharmacomodulation studies was initiated on the most potent compound against HCV (Ttou 84, EC_{50} = 2.64 μg mL^{-1}, CC_{50} = 36 μg mL^{-1}, Scheme 1), leading to a novel series of biphenyl derivatives of imidazo[1,2-*a*]pyridine and [1,2-*b*]pyridazine. The synthesis of this new series of compounds, its biological evaluation and a QSAR study is subject of the present article.

2. Chemistry

49 compounds were obtained in this program. From the hit compound Ttou 84 (Fig. 1), three sites for pharmacomodulation were chosen at positions 2, 3 and 6 of the heterocycle; the two series imidazo[1,2-*a*]pyridine and imidazo[1,2-*b*]pyridazine were studied concomitantly. Various groups were introduced at site I (aryl, amine, thioether) and site II (hydrogen, alkyl, ester, aryl). The biphenyl part of site III was first studied in *o*, *m* and *p* configuration, and the terminal phenyl subsequently replaced by other biaryl groups.

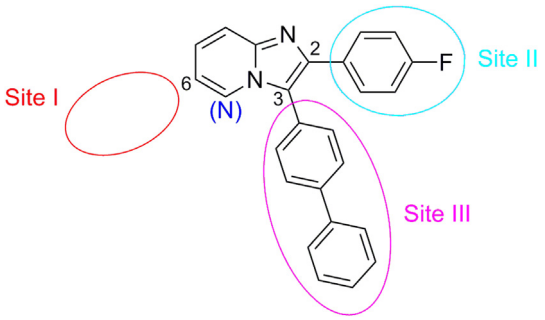


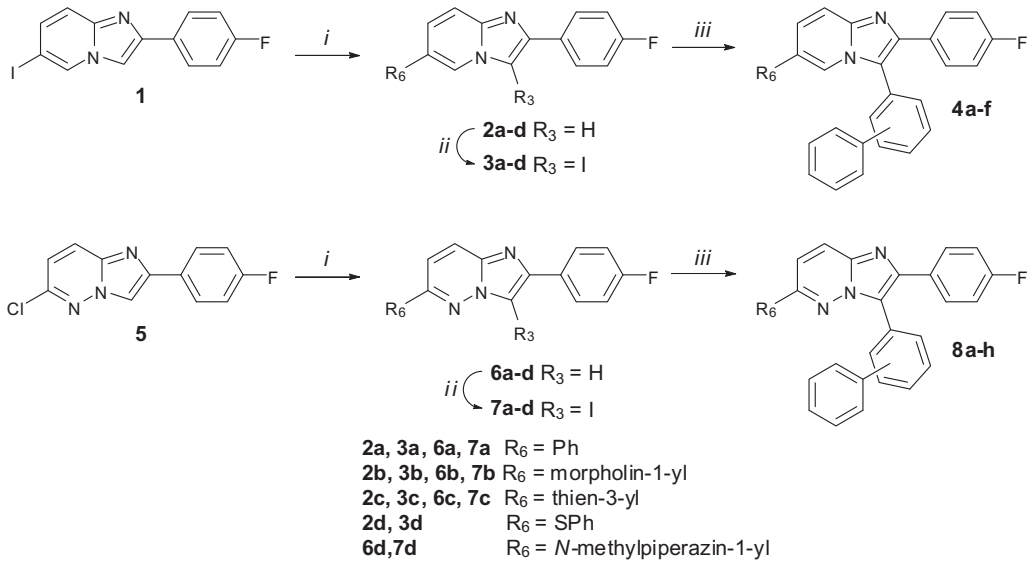
Fig. 1. Structure of Ttou 84 and pharmacomodulation sites.

2.1. Pharmacomodulation at site I

In a first approach, we decided to study the influence of the substituent in position 6 concomitantly with the evaluation of the biphenyl substitution in position 3, and this in the two imidazoazines series (Scheme 1). The same synthetic strategy was adopted in both series with a first metallo-catalyzed cross-coupling reaction in position 6 of the heterocycle, followed by an iodination in position 3 (using *N*-iodosuccinimide in acetonitrile) and a Suzuki cross-coupling reaction in position 3 in traditional conditions (1.2 eq of boronic acid, 2 mol% of $Pd(PPh_3)_4$, 2 eq of Na_2CO_3 in DME/H_2O). Different metallo-catalyzed cross-coupling reactions were performed on position 6 in order to introduce variability at this position: Suzuki cross-coupling reactions, and copper-catalyzed cross-couplings of amines and thiophenol. Two series of compounds **4a**–**f** and **8a**–**h** were obtained.

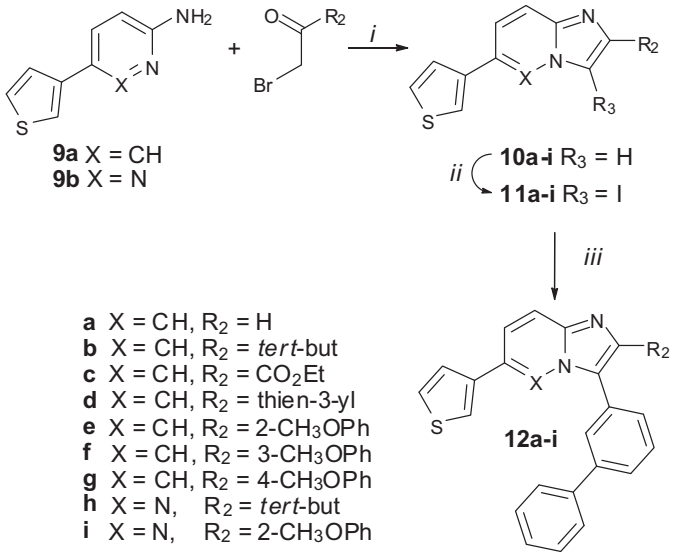
2.2. Pharmacomodulation at site II

Depending on the biological results previously obtained in the study of site I, we decided to continue the pharmacomodulation program with a thienyl group in position 6 (Scheme 2). This thienyl group was introduced directly on the starting aminopyridine or aminopyridazine. The condensation of these aminothienylazines



Reagents and conditions: (*i*) phenyl or thien-3-ylboronic acids (1.2 eq), $Pd(PPh_3)_4$ (2 mol%), Na_2CO_3 (2 eq)/DME, H_2O; or morpholine (1.1 eq), CuI (15 mol%), K_3PO_4 (2 eq), ethylene glycol (2 eq)/2-propanol (1 mL); or thiophenol (1.2 eq), CuI (5 mol%), K_2CO_3 (2 eq), *N*,*N*'-dimethylethylenediamine (15 mol%)/2-propanol (1 mL); or amine, reflux. (*ii*) NIS (1.5 eq)/CH_3CN. (*iii*) biphenylboronic acids (1.2 eq), $Pd(PPh_3)_4$ (2 mol%), Na_2CO_3 (2 eq)/DME, H_2O.

Scheme 1. Pharmacomodulation in positions 3 and 6 of the 2-(4-fluorophenyl)imidazoazines **1** and **5**.



Reagents and conditions: (*i*) EtOH, reflux (*ii*) NIS (1.5 eq)/CH_3CN (*iii*) biphen-3-ylboronic acid (1,2 eq), $Pd(PPh_3)_4$ (2 mol%), Na_2CO_3 (2 eq)/DME,H_2O.

Scheme 2. Pharmacomodulation in position 2 of the 6-thien-3-ylimidazo[1,2-*a*]pyridine and [1,2-*b*]pyridazine.

with convenient α-halogenocarbonyl compounds in refluxing ethanol led to the imidazoazines diversely functionalized in position 2. Like previously, an iodination and a Suzuki cross-coupling reaction in position 3 led to compounds **12a**–**i**.

2.3. Pharmacomodulation at site III

First, we studied the influence of substituting different positions of the terminal phenyl in the biphenyl side group. Subsequently, the terminal phenyl was replaced by various heteroaromatic groups. This functionalization was performed starting from the 3-(3-bromophenyl)imidazo[1,2-*a*]pyridines **15a**–**b** leading to compounds **16a**–**p**, or starting from the 3-(3-bromophenyl)-6-thienylimidazo[1,2-*b*]pyridazines **17b**–**c** leading to compounds **18a**–**j** (Scheme 3). The 3-bromophenyl present in the intermediates **15a**–**b** and **17b**–**c** could not be introduced in position 3 of the heterocycle through a Suzuki cross-coupling reaction, as autocondensation of the boronic acid on the 3-bromophenyl occurred. We thus synthesized the convenient α-halogenocarbonyl compounds **14a**–**c** that were condensed to the appropriate aminopyridine **13** or 3-amino-6-thienylpyridazine **9b**. Finally, the substitution of the intermediates **15a**–**b** and **17b**–**c** was performed using a Suzuki cross-coupling reaction.

3. Results and discussion

3.1. Anti-HCV activity

The biphenyl compounds were evaluated for selective antiviral activity in the HCV subgenomic replicon system (genotype 1b) in Huh 5-2 cells [14]. The adverse effect of the compounds on the host cell metabolism was quantified in parallel with the antiviral effect to allow correct interpretation of the data. As per definition, only compounds that produce significant inhibition of viral replication (>>70% inhibition) at concentrations that do not elicit an antimetabolic effect on the host cells (cell viability >>90%) can considered to be selective inhibitors of HCV replication in this replicon system.

Table 1 summarizes the antiviral data. For all of the compounds, except for compound **18j**, the antiviral activity that was observed in the Huh 5-2 replicon system clearly could be attributed to an adverse effect of the compound on the host cell metabolism, quite frequently yielding low selectivity index values ($SI = CC_{50}/EC_{50}$). Therefore, these compounds could not be considered selective inhibitors of the replication of HCV, even though an EC_{50} value could frequently be derived from the dose–response curves. For compound **18j**, a SI of >40 was obtained and an inhibitory effect of 60–70% on virus replication could be observed at concentrations that do not significantly affect the host cell metabolism. The SI of **18j** was thus improved compared to the SI of the parent compound Ttou 84.

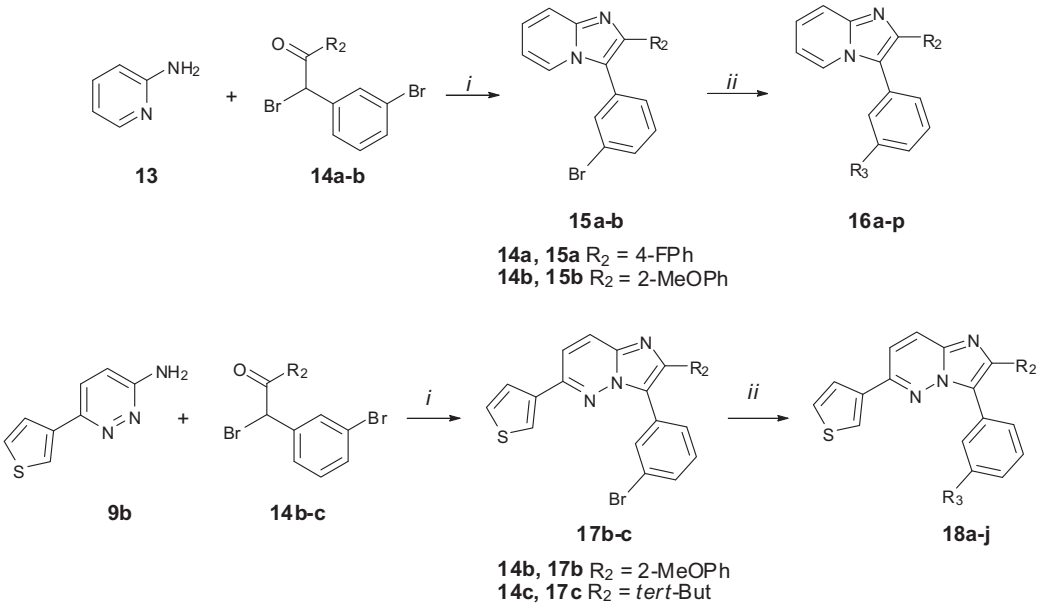
3.2. Anti-BVDV activity

The biphenyl compounds were then evaluated for selective inhibition of BVDV (strain NADL)-induced CPE formation as assessed by the MTS method in MDBK cells (Table 1).

The nature of the biphenyl group appears to largely influence the anti-BVDV activity in the two imidazoazines series as follows: biphen-3-yl > biphen-2-yl > biphen-4-yl (see, for examples, compounds **4a**–**b**, **8a**–**b** and **8c**–**e**). This influence cannot be attributed only to the adverse effect on the host cell as seen for compound **8a** with an SI of 20. The study was then pursued using a biphen-3-yl group in position 3 of the scaffold.

3.2.1. Pharmacomodulation at site I

For position 6, the thien-3-yl substituted derivatives **4e** and **8f** produced EC_{50} values amongst the lowest in the two series of imidazoazines (EC_{50} = 0.74–1.7 μg mL^{-1}). The 6-thiophenyl derivative **4f** and the 6-phenyl compound **8a** showed also interesting anti-BVDV activities (EC_{50} = 2.0 and 1.23 μg mL^{-1}, respectively). The best SI were obtained in the imidazo[1,2-*a*]pyridine series, for the 6-thien-3-yl and 6-thiophenyl derivatives **4e**–**f** (SI > 45).



Reagents and conditions: (*i*) EtOH, reflux (*ii*) $R_3B(OH)_2$ (1.2 eq), $Pd(PPh_3)_4$ (2 mol%), Na_2CO_3 (2 eq)/DME, H_2O; or amine (1.2 eq), Pd_2dba_3 (0.5 mol%), BINAP (1.5 mol%), NaOtBu (1.4 eq)/toluene.

Scheme 3. Pharmacomodulation of the biphenyl group in the imidazo[1,2-*a*]pyridine and imidazo[1,2-*b*]pyridazine series.

3.2.2. Pharmacomodulation at site II

With regard to the 2-substitution pattern, the study was performed on the 3-(biphen-3-yl)-6-(thien-3-yl)imidazo[1,2-*a*]pyridine scaffold. A total loss of activity was observed with all the substituents tested (hydrogen, *tertio*-butyl, ester, and 2-, 3-, or 4-methoxyphenyl) in **12a**–**g**, except for compound **12d** with a thien-3-yl group in the 2-position ($EC_{50} = 6.6$ μg mL^{-1}). Only two substituents were evaluated in the imidazo[1,2-*b*]pyridazine series, both without success.

3.2.3. Pharmacomodulation at site III

The study was performed on the 6-unsubstituted imidazo[1,2-*a*]pyridine and the 6-(thien-3-yl)imidazo[1,2-*b*]pyridazine series. Most compounds showed a pronounced cytotoxic effect on the host cells with SI-values ranging between 1.4 and 7.1. Only two derivatives combined activity with a low cytotoxic effect, e.g. the 2-(4-fluorophenyl)-3-(4′-methoxybiphen-3-yl)imidazo[1,2-*a*]pyridine **16d** and 2-*tertio*-butyl-3-(3′-hydroxybiphen-3-yl)-6-(thien-3-yl) imidazo[1,2-*b*]pyridazine **18d** (SI of 36 and 26.7, respectively).

3.2.4. Characterization of the anti-BVDV activity

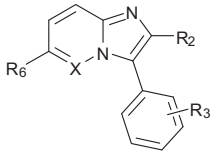
Out of 42 analogues, **4f** (Scheme 4) was identified as the most potent and selective inhibitor of BVDV (strain NADL)-induced cytopathic effect (CPE) formation as assessed by the MTS method. In a multiple infection cycle assay in MDBK cells, it was shown that the compound inhibited virus-induced CPE formation in a dose-dependent manner (Fig. 2A). Based on the data represented in Fig. 2A, the 50% effective concentration (EC_{50}) was determined to be 4.0 ± 1.3 μg mL. The anti-BVDV activity of **4f** was further confirmed by means of RT-qPCR to assess its effect at the level of viral RNA synthesis (Fig. 2A) and determination of infectious virus yield to quantify the inhibitory effect at the level of the production of progeny infectious virus particles (Fig. 2B). Comparable inhibition patterns for CPE reduction, viral RNA synthesis and infectious virus yield were observed. The dose–response effect of the compound on the number of RNA copies that are released as progeny virions by treated, infected cells allowed to calculate an EC_{50} of 2.7 ± 0.4 μg mL while the effect on the number of infectious virus particles, released by treated, infected cells, was about two-fold less pronounced, i.e. 8.0 ± 0.2 μg mL. The concentration of **4f** that reduced the proliferation of exponentially growing MDBK cells by 50% (50% cytostatic concentration or CC_{50}) was >100 μg mL (Fig. 2A). Hence, a SI of 19–37 was calculated. Compound **4f** largely retained its antiviral potency when evaluated on the replication of BPIP-resistant virus ($EC_{50} = 6.0 \pm 1.1$ μg mL or 1.5-fold change compared to WT), AG110-resistant virus ($EC_{50} = 3.9 \pm 0.8$ μg mL or a 0.9-fold change compared to WT) and was about 2-fold less active against LZ37 resistant virus ($EC_{50} = 8.8 \pm 1.9$ μg mL or 2.2-fold change compared to WT).

3.3. QSAR study

The QSAR study described here is based on 42 derivatives presenting an EC_{50} for BVDV. The QSAR software (Discovery Studio_ 2.5, Accelrys, Inc., San Diego, CA) presents 179 physicochemical descriptors: electronic, spatial, shadow, shape and thermodynamic indices. After several filters (analyzing the correlation matrix, eliminating the highly correlated descriptors), and eliminating descriptors with too wide range of training data (>100), only 26 2D or 3D descriptors were retained as dependent on EC_{50} for BVDV by Genetic Function Approximation (GFA) algorithms.

The QSAR equation with the regression statistics is given in Fig. 3. The best predictive result is obtained with the lower log EC_{50}. As the coefficient in the equation is positive for Estate Keys as "Sums of electrotopological state values for CH_3 or CH atoms" (ES_Sum_sCH_3 and _aaCH, respectively), the lower the number of

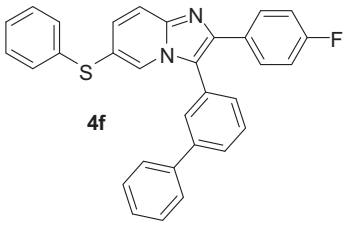
Table 1
Inhibitory effects of biphenylimidazoazines and analogues on cytopathogenic effect induction by the BVDV in MDBK cells and on HCV subgenomic replicon replication in Huh 5-2 cells.



Cpd	X	R_6	R_2	R_3	BVDV			HCV		
					CC_{50}[a] (μg mL^{-1})	EC_{50}[b] (μg mL^{-1})	SI[c]	CC_{50}[a] (μg mL^{-1})	EC_{50}[b] (μg mL^{-1})	SI[c]
4a	CH	Ph	4-F–Ph	3-Ph	>50	12	>4	56.8	52.2	1.09
4b	CH	Ph	4-F–Ph	4-Ph	30	>30	>1	11.4	4.5	2.5
4c	CH	Morpholin-1-yl	4-F–Ph	3-Ph	>50	>50	>1	11.1	6.7	1.7
4d	CH	Morpholin-1-yl	4-F–Ph	4-Ph	>50	>50	>1	15.6	6.7	2.3
4e	CH	Thien-3-yl	4-F–Ph	3-Ph	>33	0.74	>45	>112	>112	>1
4f	CH	SPh	4-F–Ph	3-Ph	>100	2.0	>50	>106	>106	>1
8a	N	Ph	4-F–Ph	3-Ph	25	1.23	20	15.9	11.3	1.4
8b	N	Ph	4-F–Ph	4-Ph	>50	>50	>1	45.3	ND	ND
8c	N	Morpholin-1-yl	4-F–Ph	2-Ph	31	11	3	11.1	11.1	1
8d	N	Morpholin-1-yl	4-F–Ph	3-Ph	12	4	3	11.1	ND	ND
8e	N	Morpholin-1-yl	4-F–Ph	4-Ph	>50	>50	>1	33.3	ND	ND
8f	N	Thien-3-yl	4-F–Ph	3-Ph	13	1.7	8	13.4	11.2	1.2
8g	N	Thien-3-yl	4-F–Ph	4-Ph	>50	>50	>1	>112	>112	>1
8h	N	*N*-Methylpiperazin-1-yl	4-F–Ph	4-Ph	>50	>50	>1	45.3	ND	ND
12a	CH	Thien-3-yl	H	3-Ph	>100	>100	>1	>142	89.5	> 1.6
12b	CH	Thien-3-yl	*tert*-But	3-Ph	>100	62.6	>1.6	>123	42.5	> 2.4
12c	CH	Thien-3-yl	CO_2Et	3-Ph	67.7	>67.7	>1	>118	53.1	> 2.2
12d	CH	Thien-3-yl	Thien-3-yl	3-Ph	>100	6.6	>15.2	>115	64.5	> 1.8
12e	CH	Thien-3-yl	2-CH_3OPh	3-Ph	>100	68.6	>1.5	56.8	17.5	3.3
12f	CH	Thien-3-yl	3-CH_3OPh	3-Ph	>100	15.0	>6.7	85.2	52.4	1.6
12g	CH	Thien-3-yl	4-CH_3OPh	3-Ph	>100	22.7	>4.4	>109	56.8	> 1.9
12h	N	Thien-3-yl	*tert*-But	3-Ph	>100	37.9	>2.6	63.2	22.7	2.8
12i	N	Thien-3-yl	2-CH_3OPh	3-Ph	10.4	3.4	3.1	97.4	14.7	6.6
16a	CH	H	4-F–Ph	3-Ph	ND	ND	ND	60.4	34.9	1.7
16b	CH	H	4-F–Ph	3-(2-CH_3OPh)	8.5	2.7	3.2	29.7	10.7	2.8
16c	CH	H	4-F–Ph	3-(3-CH_3OPh)	11.5	3.1	3.7	50.2	4.3	11.8
16d	CH	H	4-F–Ph	3-(4-CH_3OPh)	>100	2.8	>36	34.8	1.63	21.3
16e	CH	H	4-F–Ph	3-(4-OH–Ph)	ND	ND	ND	69.5	13.5	5.1
16f	CH	H	4-F–Ph	3-(4-Cl–Ph)	12.7	3.0	4.3	57.5	5.9	9.7
16g	CH	H	4-F–Ph	3-(4-F–Ph)	5.4	3.9	1.4	54.5	10.3	5.3
16h	CH	H	4-F–Ph	3-(Pyridin-4-yl)	7.0	0.98	7.1	45.5	20.2	2.3
16i	CH	H	4-F–Ph	3-(Fur-2-yl)	ND	ND	ND	24.7	12.8	1.9
16j	CH	H	4-F–Ph	3-(Fur-3-yl)	5.6	1.7	3.2	66.2	19.2	3.4
16k	CH	H	4-F–Ph	3-(Thien-3-yl)	7.0	2.3	3.0	56.7	11.1	5.1
16l	CH	H	4-F–Ph	3-(Benzo[*b*]fur-2-yl)	ND	ND	ND	54.9	28	2
16m	CH	H	4-F–Ph	3-(*N*-Methylpiperazin-1-yl)	ND	ND	ND	60.1	21.8	2.8
16n	CH	H	2-CH_3OPh	3-(2-OH–Ph)	14.4	7.55	1.9	95.8	48.9	2
16o	CH	H	2-CH_3OPh	3-(3-OH–Ph)	ND	ND	ND	40.3	3.5	11.4
16p	CH	H	2-CH_3OPh	3-(4-OH–Ph)	>100	17.7	>5.6	43.8	18.8	2.3
18a	N	Thien-3-yl	*tert*-But	3-(fur-2-yl)	>100	>100	1	41.3	8.6	4.8
18b	N	Thien-3-yl	*tert*-But	3-(fur-3-yl)	4.5	1.7	2.7	55.1	8.7	6.3
18c	N	Thien-3-yl	*tert*-But	3-(4-OH–Ph)	>100	14.2	7.1	15	5	3
18d	N	Thien-3-yl	*tert*-but	3-(3-OH–Ph)	>100	3.7	26.7	20	3.9	5.2
18e	N	Thien-3-yl	*tert*-But	3-(2-OH–Ph)	>100	>100	1.0	75.3	30.1	2.5
18f	N	Thien-3-yl	2-CH_3OPh	3-(fur-2-yl)	ND	ND	ND	54.3	15.1	3.6
18g	N	Thien-3-yl	2-CH_3OPh	3-(fur-3-yl)	>100	>100	1.0	15.7	11.1	1.4
18h	N	Thien-3-yl	2-CH_3OPh	3-(4-OH–Ph)	>100	>100	1.0	22.1	2.4	9.2
18i	N	Thien-3-yl	2-CH_3OPh	3-(3-OH–Ph)	>100	>100	1.0	78.5	3.8	20.8
18j	N	Thien-3-yl	2-CH_3OPh	3-(2-OH–Ph)	16.2	1.36	12	>205.0	4.8	43

ND = not determined.
[a] Minimum cytotoxic concentration required to reduce the overall cell metabolism by 50%.
[b] Minimum concentration required to reduce the virus-produced luciferase reporter gene activity by 50%.
[c] Selectivity index (ratio of CC_{50} to EC_{50}).

CH_3, and the smaller the structure (with the less number of CH with two aromatic bonds), the lower the EC_{50}. Concerning the molecular properties, the pKa of ionisable sites should be as "high" as possible. Concerning the molecular properties counter, the number of hydrogen bond accepting group should be also as "high" as possible. The topological descriptor depending on the connectivity indices: CHI_V_3_P (Kier and Hall valence-modified connectivity index amount of branching, ring structures present, and flexibility) derives from the 2D topology of the molecule. To reduce the EC_{50}, it should be as high as possible. "Dipole" 3D descriptors indicate the strength and the orientation behaviour of a molecule in an electrostatic field, and here, the higher is the value, the lower is EC_{50}. The equations showed occurrences of 3D Jurs descriptors suggesting the importance of charge distribution (Jurs_RNCG showed that the relative negative charge should be as small as possible) and surface areas (Jurs_RNCS and Jurs_WNSA_3 dependant on the total



Scheme 4. Structure of **4f**.

molecular solvent-accessible surface area, and both should be small values, and so suggesting compact structure).

The 3D-QSAR model (Discovery Studio® 2.5, Accelrys), defines the critical regions (steric or electrostatic) of imidazo[1,2-*a*]pyridine or [1,2-*b*]pyridazine derivatives affecting the cytopathogenic effect induction by the BVDV. It was a Partial Least Squares (PLS) model built on 400 independent variables ($N = 42$, R-square = 0.71). A contour plot of the electrostatic field region favourable (in blue) or unfavourable (in red) against the BVDV is shown in Fig. 4 with the visible superposition of one active compound **4f** and one inactive **18a**. The energy grids corresponding to the favourable (in green) or unfavourable (orange) steric effects against the BVDV are shown in Fig. 5 with the superposition of **4f** and **18a**. A good active derivative should have strong Van der Waals attraction in the green areas and a polar group in the blue electrostatic potential areas (which are dominant near the second phenyl ring substituted on C-3).

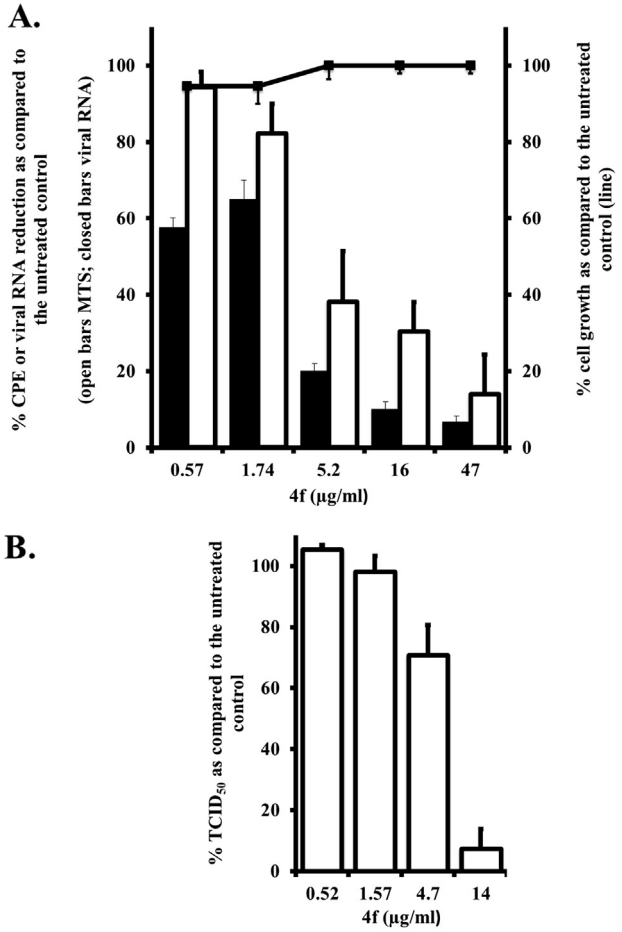


Fig. 2. (A) Effect of **4f** on BVDV (NADL)-induced CPE formation in MDBK cells (open bars), on release of extracellular viral RNA (filled bars) and on the proliferation of exponentially growing MDBK cells (line). (B) Inhibitory effect of **4f** on infectious virus yield (open bars).

Several key features of the 3D-QSAR contour map are predicted to increase BVDV antiviral activity:

- a more positive environment (as alkyl group) at C-7 but also C-6 (instead of an aromatic substituent in site I) (electronic study)
- reinforce negative environment on para position of the phenyl group at the C-2 position (for example, a para diaromatic group in site II) (electronic study)
- a more negative environment on the meta or para phenyl group at the C-3 position (electronegative group on para or meta on the terminal phenyl in site III) (electronic study)
- alkyl group on the position C-7 or on C-6 (in site I) (steric study)
- more bulk group near the meta or para position on the 3′-biphenyl group in site III (steric study)

4. Conclusions

Using hit Ttou 84 as starting point, a novel series of biphenyl derivatives of imidazo[1,2-*a*]pyridine and [1,2-*b*]pyridazine was synthesized in an attempt to optimize this compound class for inhibitory properties on the replication of the bovine viral diarrhoea virus and hepatitis C virus.

Following evaluation of 49 analogues in the HCV subgenomic replicon system, all except one compound have proven to be aspecific inhibitors of HCV replication. Only compound **18j** displayed some properties in the HCV replicon system suggesting selective inhibition of HCV replication. However, the antiviral activity was not very pronounced (60–70% inhibition).

Additional optimization of the biphenylimidazo[1,2-*a*]pyridine or [1,2-*b*]pyridazine scaffold might result in a more potent inhibitor of HCV replication. Given the fact that no cross-resistance was observed with BPIP-resistant BVDV, this class of compounds has the potential to inhibit-HCV replication by a yet unknown mechanism. Namely, we previously demonstrated that BPIP-analogues could be further optimized to exert specific anti-HCV inhibitory activity [15,16]. One analogue of BPIP is currently under clinical investigation, i.e. GS9190 [17].

The biphenyl compounds were then evaluated for selective inhibition of BVDV (strain NADL)-induced CPE formation as assessed by the MTS method in MDBK cells. Compound **4f** proved to be the most selective inhibitor of BVDV replication as corroborated by different methods. Furthermore, **4f** did not or only marginally showed cross-resistance with known inhibitors of the *Pestivirus* replication. Previously, we and others reported compounds with very different chemical structures that all appear to interact with a single drug-binding pocket within the finger domain region of the BVDV RdRp with two separate but potentially overlapping binding sites rather than two distinct drug-binding pockets [18]. The cross-resistance profile of **4f** might indicate that **4f** interact with a potentially new binding site in the RdRp, different from the binding site of BPIP [19], VP32947 [20], AG110 [21] or LZ37 [18], or could have a completely different, potentially unknown, target. However, selection of **4f** resistant virus in conjunction with a thorough geno- and phenotypic analysis are in order to better understand the mechanism by which **4f** exerts its anti-BVDV and anti-HCV activity.

5. Experimental section

5.1. General remarks

All solvents were anhydrous reagents from commercial sources. Unless otherwise noted, all chemicals and reagents were obtained commercially and used without purification. NMR spectra were recorded at 200 MHz (^{1}H) or 50 MHz (^{13}C) on a Bruker DPX instrument. The chemical shifts are reported in parts per million (ppm, δ)

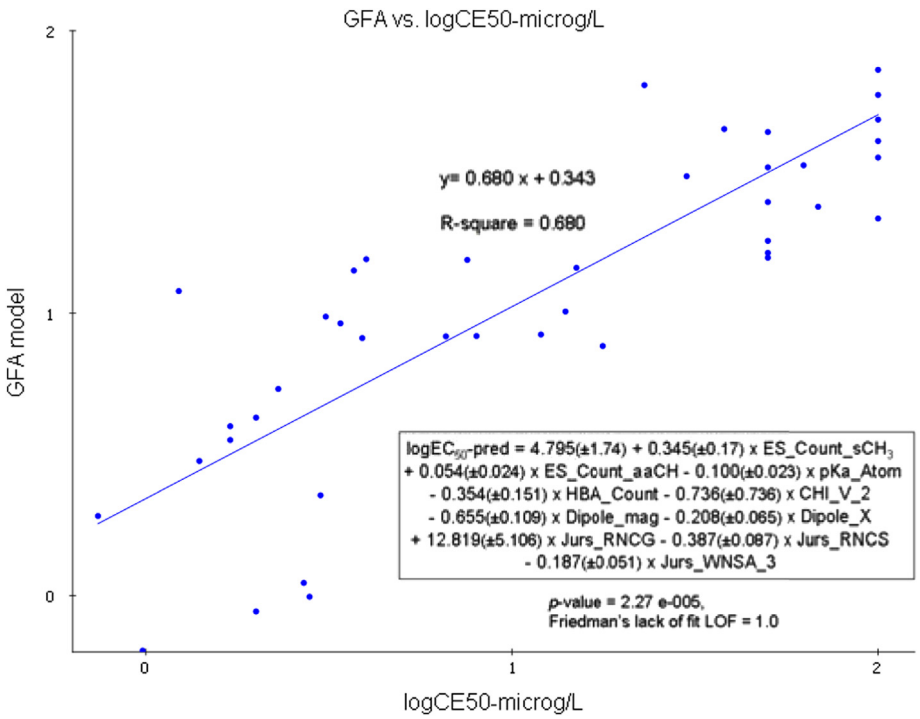


Fig. 3. Line plot of predicted log EC_{50} activity versus log EC_{50} of the BVDV of imidazo[1,2-*a*]pyridine or [1,2-*b*]pyridazine derivatives with the regression statistics and the QSAR equation by genetic function approximation (GFA) process and statistics.

relative to residual deuterated solvent peaks. The possible inversion of two values in the NMR spectra is expressed by an asterisk. Known compounds were prepared according to literature procedures: 2-(4-fluorophenyl)-6-iodoimidazo[1,2-*a*]pyridine (**1**) [22], 6-chloro-2-(4-fluorophenyl)imidazo[1,2-*b*]pyridazine (**5**) [23], 2-(4-fluorophenyl)-6-phenylimidazo[1,2-*a*]pyridine (**2a**) [24], 2-(4-fluorophenyl)-6-morpholinoimidazo[1,2-*a*]pyridine (**2b**) [22], 2-(4-fluorophenyl)-6-(thien-3-yl)imidazo[1,2-*a*]pyridine (**2c**) [24], 2-(4-fluorophenyl)-6-phenylimidazo[1,2-*b*]pyridazine (**6a**) [23], 2-(4-fluorophenyl)-6-(thien-3-yl)imidazo[1,2-*b*]pyridazine (**6c**) [23], 2-amino-5-(thien-3-yl)pyridine (**9a**) [25], 3-amino-6-(thien-3-yl)pyridazine (**9b**) [26], 6-(thien-3-yl)imidazo[1,2-*a*]pyridine (**10a**) [24].

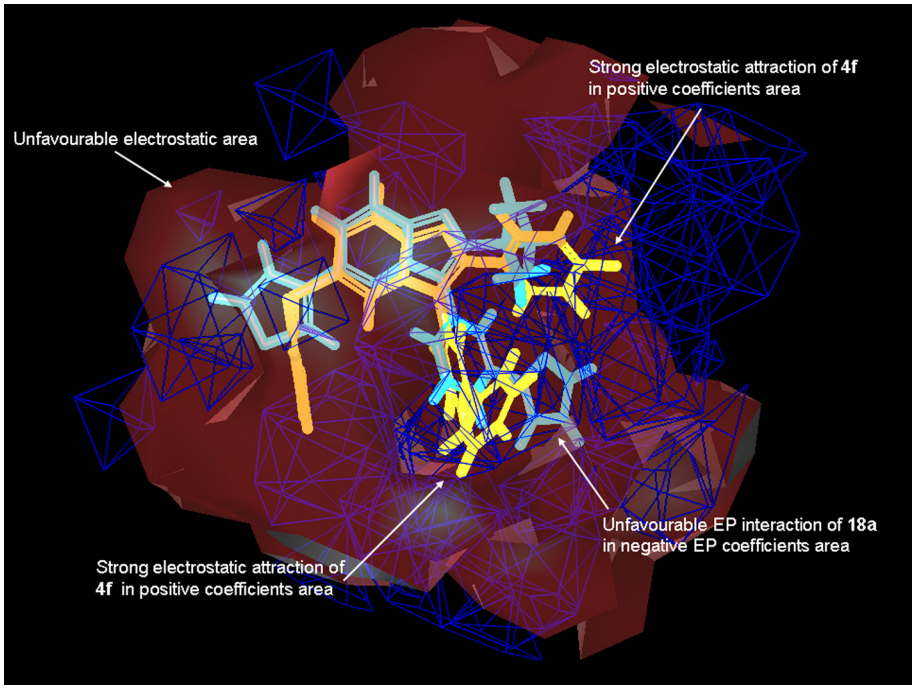


Fig. 4. Isosurface of the 3D-QSAR model coefficients on Electrostatic Potential (EP) grids with positive electrostatic potential in red solid representation and the negative area is represented in blue triangle mesh representation for the aligned molecular structures of 42 imidazo[1,2-*a*]pyridine or [1,2-*b*]pyridazine derivatives. Derivatives **4f** and **18a** are shown in yellow stick and blue stick representation, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

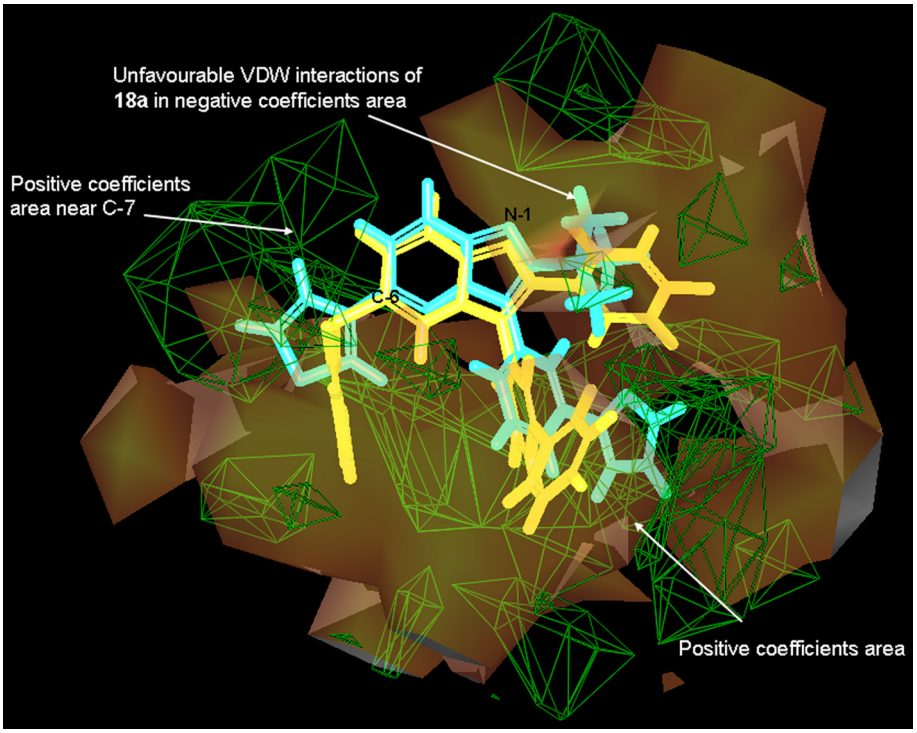


Fig. 5. Isosurface of the 3D-QSAR model coefficients on Van der Waals (VDW) grids. The green triangle mesh representation indicates positive coefficients; the orange solid surface indicates negative coefficients. Derivatives **4f** and **18a** are shown in yellow stick and blue stick representation, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

5.2. Chemistry

5.2.1. 2-(4-Fluorophenyl)-6-phenylsulfanylimidazo[1,2-a]pyridine (**2d**)

2-(4-Fluorophenyl)-6-iodoimidazo[1,2-*a*]pyridine **1** (338 mg, 1 mmol), copper(I) iodide (9.5 mg, 5 mol%), and K_2CO_3 (198 mg, 2 mmol) were added to a screw-capped test tube. The tube was evacuated and backfilled with argon. *N*,*N*′-dimethylethylenediamine (16 μl, 15 mol%), thiophenol (132 mg, 1.2 mmol), and isopropanol (1 mL) were added successively by syringe at room temperature. The tube was sealed with a Teflon-lined cap and the reaction mixture was heated at 112 °C for 48 h. After cooling at room temperature, the suspension was diluted with CH_2Cl_2 and filtered on Celite®. The solvent was removed with the aid of a rotary evaporator to give a residue that was purified by column chromatography on silica gel eluting with CH_2Cl_2–CH_3OH (98/02 v/v) to give a white product (83% yield). Mp 134–135 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.31 (dd, 1H, J = 1.7–0.9 Hz, H-5), 7.95 (dd, 2H, J = 8.8–5.4 Hz, F–Ph-2,6), 7.82 (s, 1H, H-3), 7.63 (d, 1H, J = 9.4 Hz, H-8), 7.35–7.27 (m, 5H, Ph), 7.23 (dd, 1H, J = 9.4–1.7 Hz, H-7), 7.17 (t, 2H, J = 8.8 Hz, F–Ph-3,5). ^{13}C NMR (50 MHz, $CDCl_3$) δ 163.4 (J = 245 Hz), 146.2, 145.2, 136.5, 130.5, 130.2 (J = 1 Hz), 129.9, 129.8, 129.2, 128.4 (J = 8.1 Hz), 127.6, 119.9, 118.3, 116.3 (J = 21.5 Hz), 108.7.

5.2.2. 2-(4-Fluorophenyl)-3-iodo-6-phenylimidazo[1,2-a]pyridine (**3a**)

Method A: A mixture of the conveniently substituted 2-(4-fluorophenyl)imidazo[1,2-*a*]pyridine (0.4 mmol), *N*-iodosuccinimide (0.6 mmol), and acetonitrile (1 mL) was stirred 3 h at room temperature. The resulting solid was filtered off and washed with 10 mL of acetonitrile. The compound was obtained in 77% yield and used without further purification. Mp > 260 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.42 (m, 1H, H-5), 8.12 (dd, 2H, J = 8.9–5.4 Hz, F–Ph-2,6), 7.77 (m, 1H, H-8), 7.70–7.45 (m, 6H, Ph, H-7), 7.23 (t, 2H, J = 8.9 Hz, F–Ph-3,5).

5.2.3. 2-(4-Fluorophenyl)-3-iodo-6-(morpholin-1-yl)imidazo[1,2-a]pyridine (**3b**)

Method A (88% yield). Mp 233–234 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.06 (dd, 2H, J = 8.9–5.4 Hz, F–Ph-2,6), 7.64 (d, 1H, J = 2.2 Hz, H-5), 7.58 (d, 1H, J = 9.7 Hz, H-8), 7.20 (t, 2H, J = 8.9 Hz, F–Ph-3,5), 7.19 (dd, 1H, J = 9.7–2.2 Hz, H-7), 3.96 (t, 4H, J = 4.8 Hz, mor), 3.19 (t, 4H, J = 4.8 Hz, mor).

5.2.4. 2-(4-Fluorophenyl)-3-iodo-6-(thien-3-yl)imidazo[1,2-a]pyridine (**3c**)

Method A (95% yield). Mp 246–247 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.45 (m, 1H, H-5), 8.10 (dd, 2H, J = 8.8–5.4 Hz, F–Ph-2,6), 7.74 (d, 1H, J = 9.3 Hz, H-8), 7.63–7.52 (m, 3H, H-7, th-2,4), 7.46 (m, 1H, th-5), 7.23 (t, 2H, J = 8.8 Hz, F–Ph-3,5).

5.2.5. 2-(4-Fluorophenyl)-3-iodo-6-(phenylsulfanyl)imidazo[1,2-a]pyridine (**3d**)

Method A. The residue was purified by chromatography (neutral alumina, CH_2Cl_2) to obtain **3d** in 58% yield. Mp 173–175 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.42 (s, 1H, H-5), 8.08 (dd, 2H, J = 8.7–5.4 Hz, F–Ph-2,6), 7.61 (d, 1H, J = 9.4 Hz, H-8), 7.37–7.26 (m, 5H, Ph), 7.29 (d, 1H, J = 9.4 Hz, H-7), 7.22 (t, 2H, J = 8.7 Hz, F–Ph-3,5).

5.2.6. 3-(Biphen-3-yl)-2-(4-fluorophenyl)-6-phenylimidazo[1,2-a]pyridine (**4a**)

Method B: A screw-cap test tube was charged, under argon, with the conveniently substituted 3-iodoimidazo[1,2-*a*]pyridine or 3-(3-bromophenyl)imidazo[1,2-*a*]pyridine or 3-(3-bromophenyl)imidazo[1,2-*b*]pyridazine (0.5 mmol), biphenylboronic acid (0.6 mmol), *tetrakis*(triphenylphosphine)palladium (2 mol %), and Na_2CO_3

(1.05 mmol) in 1,2-dimethoxyethane (2 mL) and water (1 mL). The screw-cap test tube was sealed with a cap and the reaction mixture was stirred magnetically at 100 °C for 4 h. After cooling, the suspension was taken up in water, extracted with CH_2Cl_2 three times. The organic layer was dried with $MgSO_4$, and evaporated to dryness. The residue was chromatographed on neutral alumina eluting with CH_2Cl_2 to give 98% of **4a**. Mp 153–154 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.20 (dd, 1H, J = 1.8–1 Hz, H-5), 7.84 (dd, 1H, J = 9.3–1 Hz, H-8), 7.82–7.69 (m, 5H, F–Ph-2,6, biPh), 7.63–7.30 (m, 12H, H-7, Ph, biPh), 7.05 (t, 2H, J = 8.9 Hz, F–Ph-3,5). ^{13}C NMR (50 MHz, $CDCl_3$) δ 163.1 (J = 246 Hz), 144.5, 143.4, 142.8, 140.6, 137.9, 133.7, 130.8, 130.5, 130.3 (J = 8 Hz), 130.0, 129.7, 129.5, 128.6, 128.5, 128.4, 127.9, 127.7, 127.5, 126.6, 121.1, 117.8, 115.9 (J = 21.3 Hz). Anal. Calcd for $C_{31}H_{21}FN_2$: C, 84.52; H, 4.81; N, 6.36. Found: C, 84.77; H, 4.62; N, 6.51.

5.2.7. 3-(Biphen-4-yl)-2-(4-fluorophenyl)-6-phenylimidazo[1,2-a] pyridine (**4b**)

Method B. The residue was chromatographed on silica gel eluting with CH_2Cl_2–CH_3OH (99.5/0.5 v/v) to give **4b** (98% yield). Mp 215–216 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.23 (m, 1H, H-5), 7.86–7.71 (m, 7H, F–Ph-2,6, H-8, biPh), 7.62–7.41 (m, 11H, Ph, biPh, H-7), 7.05 (t, 2H, J = 8.7 Hz, F–Ph-3,5). ^{13}C NMR (50 MHz, $CDCl_3$) δ 163.0 (J = 245 Hz), 144.8, 142.8, 142.3, 140.6, 138.0, 131.6, 130.8 (J = 3.3 Hz), 130.4 (J = 8 Hz), 129.7, 129.6, 128.9, 128.9, 128.4, 127.6, 126.3, 121.5, 121.1, 117.9, 115.9 (J = 21 Hz). Anal. Calcd for $C_{31}H_{21}FN_2$: C, 84.52; H, 4.81; N, 6.36. Found: C, 84.38; H, 5.02; N, 6.39.

5.2.8. 3-(Biphen-3-yl)-2-(4-fluorophenyl)-6-(morpholin-1-yl) imidazo[1,2-a]pyridine (**4c**)

Method B. The residue was chromatographed on silica gel eluting with CH_2Cl_2–CH_3OH (99.5/0.5 v/v) to give **4c** (49% yield). Mp 198–199 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.77 (m, 1H, biPh), 7.72–7.57 (m, 7H, F–Ph-2,6, H-8, biPh), 7.53–7.41 (m, 5H, H-5, biPh), 7.17 (dd, 1H, J = 9.7–2.2 Hz, H-7), 7.01 (t, 2H, J = 8.8 Hz, F–Ph-3,5), 3.87 (m, 4H, mor), 3.04 (m, 4H, mor). ^{13}C NMR (50 MHz, $CDCl_3$) δ 162.4 (J = 245 Hz), 142.8, 142.0, 141.7, 140.3, 130.6, 130.5 (J = 3.5 Hz), 130.3, 129.8, 129.7, 129.5 (J = 7.6 Hz), 129.4, 129.2, 129.1, 128.0, 127.8, 127.2, 121.4, 117.6, 115.4 (J = 21.3 Hz), 108.9, 66.8, 50.7. Anal. Calcd for $C_{29}H_{24}FN_3O$: C, 77.49; H, 5.38; N, 9.35. Found: C, 77.86; H, 5.16; N, 9.36.

5.2.9. 3-(Biphen-4-yl)-2-(4-fluorophenyl)-6-(morpholin-1-yl) imidazo[1,2-a]pyridine (**4d**)

Method B. The residue was chromatographed on silica gel eluting with CH_2Cl_2–CH_3OH (99.5/0.5 v/v) to give **4d** (63% yield). Mp 191–192 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.85–7.65 (m, 7H, F–Ph-2,6, H-8, biPh), 7.58–7.30 (m, 6H, H-5, biPh), 7.18 (dd, 1H, J = 9.6–2.2 Hz, H-7), 7.03 (t, 2H, J = 8.9 Hz, F–Ph-3,5), 3.89 (m, 4H, mor), 3.05 (m, 4H, mor). ^{13}C NMR (50 MHz, $CDCl_3$) δ 162.7 (J = 245 Hz), 142.3, 141.9, 140.5, 140.4, 131.2, 130.7, 130.0 (J = 8 Hz), 129.4, 129.0, 128.6, 128.3, 127.4, 121.8, 121.5, 117.8, 115.6 (J = 21.5 Hz), 109.1, 67.0, 51.0. Anal. Calcd for $C_{29}H_{24}FN_3O$: C, 77.49; H, 5.38; N, 9.35. Found: C, 77.36; H, 5.49; N, 9.28.

5.2.10. 3-(Biphen-3-yl)-2-(4-fluorophenyl)-6-(thien-3-yl)imidazo [1,2-a]pyridine (**4e**)

Method B. (95% yield). Mp 164–166 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.21 (dd, 1H, J = 0.7 Hz, H-5), 7.78–7.71 (m, 5H, F–Ph-2,6, H-8, biPh), 7.61–7.58 (m, 3H, th-2,4, biPh), 7.53–7.43 (m, 7H, H-7, biPh), 7.29 (m, 1H, th-5), 7.04 (t, 2H, J = 8.8 Hz, F–Ph-3,5). ^{13}C NMR (50 MHz, $CDCl_3$) δ 162.6 (J = 245 Hz), 144.0, 142.9, 142.0, 140.2, 138.3, 130.3, 130.2, 130.1 (J = 3 Hz), 129.8 (J = 8 Hz), 129.5, 129.0, 128.0, 127.9, 127.2, 127.1, 125.9, 125.5, 122.3, 121.2, 121.0, 119.9, 117.5, 115.4 (J = 21 Hz). Anal. Calcd for $C_{29}H_{19}FN_2S$: C, 78.00; H, 4.29; N, 6.27. Found: C, 77.82; H, 4.59; N, 6.14.

5.2.11. 3-(Biphen-3-yl)-2-(4-fluorophenyl)-6-(phenylsulfanyl) imidazo[1,2-a]pyridine (**4f**)

Method B. The residue was chromatographed on silica gel eluting with CH_2Cl_2–petroleum spirit (50/50 v/v) to give **4f** (52% yield). Mp 153–154 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.22 (s, 1H, H-5), 7.80–7.66 (m, 6H, F–Ph-2,6, biPh), 7.62–7.41 (m, 6H, H-8, biPh), 7.30–7.20 (6H, H-7, Ph), 7.04 (t, 2H, J = 8.7 Hz, F–Ph-3,5). ^{13}C NMR (50 MHz, $CDCl_3$) δ 162.7 (J = 246 Hz), 143.8, 142.9, 140.2, 136.0, 130.3, 130.1, 129.9 (J = 8 Hz), 129.6, 129.4, 129.3, 129.2, 129.0, 128.2, 127.9, 127.2, 127.0, 126.4, 121.2, 119.5, 117.9, 115.5 (J = 21 Hz). Anal. Calcd for $C_{31}H_{21}FN_2S$: C, 78.79; H, 4.48; N, 5.93. Found: C, 78.97; H, 4.49; N, 6.04.

5.2.12. 2-(4-Fluorophenyl)-6-(morpholin-1-yl)imidazo[1,2-b] pyridazine (**6b**)

A mixture of 6-chloro-2-(4-fluorophenyl)imidazo[1,2-*b*]pyridazine (4 mmol) and morpholine (10 mL) was refluxed for 24 h. After cooling, the mixture was diluted with water (50 mL), the resulting solid was filtered off and washed with water to give **6b** (87% yield). Mp 202–203 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.98 (s, 1H, H-3), 7.91 (dd, 2H, J = 8.7–5.4 Hz, F–Ph-2,6), 7.79 (d, 1H, J = 9.8 Hz, H-8), 7.16 (t, 2H, J = 8.7 Hz, F–Ph-3,5), 6.84 (d, 1H, J = 9.8 Hz, H-7), 3.90 (t, 4H, J = 4.9 Hz, mor), 3.52 (t, 4H, J = 4.9 Hz, mor). ^{13}C NMR (50 MHz, $CDCl_3$) δ 163.0 (J = 245 Hz, F–Ph-4), 155.4 (C-6), 143.6 (C-2), 137.2 (C-8a), 130.4 (F–Ph-1), 127.6 (J = 8 Hz, F–Ph-2,6), 125.9 (C-8), 116.1 (J = 21 Hz, F–Ph-3,5), 112.8 (C-3), 110.2 (C-7), 66.9 (mor), 46.9 (mor).

5.2.13. 2-(4-Fluorophenyl)-6-(N-methylpiperazin-1-yl)imidazo[1,2-b]pyridazine (**6d**)

A mixture of 6-chloro-2-(4-fluorophenyl)imidazo[1,2-*b*]pyridazine (4 mmol) and *N*-methylpiperazine (25 mL) was refluxed for 24 h. After cooling, the mixture was diluted with water (150 mL), the resulting solid was filtered off and washed with water to give **6d** (78% yield). Mp 190–191 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.96 (s, 1H, H-3), 7.90 (dd, 2H, J = 9–5.4 Hz, F–Ph-2,6), 7.74 (d, 1H, J = 9.9 Hz, H-8), 7.15 (t, 2H, J = 9 Hz, F–Ph-3,5), 6.85 (d, 1H, J = 9.9 Hz, H-7), 3.57 (m, 4H, pip), 2.59 (m, 4H, pip), 2.40 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 162.9 (J = 246 Hz, F–Ph-4), 155.3 (C-6), 143.3 (C-2), 137.1 (C-8a), 130.5 (J = 3 Hz, F–Ph-1), 127.5 (J = 8 Hz, F–Ph-2,6), 125.7 (C-8), 116.0 (J = 21 Hz, F–Ph-3,5), 112.8 (C-3), 110.6 (C-7), 56.8 (pip), 46.5 (pip, CH_3).

5.2.14. 2-(4-Fluorophenyl)-3-iodo-6-phenylimidazo[1,2-b] pyridazine (**7a**)

Method A (96% yield). Mp 265–266 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.22–8.10 (m, 4H, Ph-2,6, F–Ph-2,6), 8.02 (d, 1H, J = 9.4 Hz, H-8), 7.65 (d, 1H, J = 9.4 Hz, H-7), 7.63–7.58 (m, 3H, Ph-3,4,5), 7.25 (t, 2H, J = 8.7 Hz, F–Ph-3,5).

5.2.15. 2-(4-Fluorophenyl)-3-iodo-6-(morpholin-1-yl)imidazo[1,2-b]pyridazine (**7b**)

Method A (93% yield). Mp 235–236 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.11 (dd, 2H, J = 8.9–5.4 Hz, F–Ph-2,6), 7.73 (d, 1H, J = 9.8 Hz, H-8), 7.19 (t, 2H, J = 8.9 Hz, F–Ph-3,5), 6.88 (d, 1H, J = 9.8 Hz, H-7), 3.92 (4H, mor), 3.61 (4H, mor).

5.2.16. 2-(4-Fluorophenyl)-3-iodo-6-(thien-3-yl)imidazo[1,2-b] pyridazine (**7c**)

Method A (78% yield). Mp > 260 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.47 (m, 1H, Th-2), 8.20 (d, 1H, J = 9.6 Hz, H-8), 8.19 (m, 2H, F–Ph-2,6), 7.92 (d, 1H, J = 9.6 Hz, H-7), 7.90–7.79 (m, 2H, Th-4,5), 7.40 (t, 2H, J = 8.9 Hz, F–Ph-3,5).

5.2.17. 2-(4-Fluorophenyl)-3-iodo-6-(N-methylpiperazin-1-yl) imidazo[1,2-b]pyridazine (**7d**)

Method A (62% yield). Mp 234–235 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.11 (dd, 2H, J = 8.7–5.4 Hz, F–Ph-2,6), 7.69 (d, 1H, J = 9.7 Hz, H-8),

7.19 (t, 2H, J = 8.7 Hz, F–Ph-3,5), 6.89 (d, 1H, J = 9.7 Hz, H-7), 3.67 (m, 4H, pip), 2.61 (m, 4H, pip), 2.45 (s, 3H, CH_3).

*5.2.18. 3-(Biphen-3-yl)-2-(4-fluorophenyl)-6-phenylimidazo[1,2-b] pyridazine (**8a**)*

Method B (75% yield). Mp 190–191 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.21 (d, 1H, J = 9.5 Hz, H-8), 8.04–7.99 (m, 3H, Ph-2,6, biPh), 7.83 (dd, 2H, J = 8.9–5.4 Hz, F–Ph-2,6), 7.75–7.35 (m, 12H, H-7, Ph-3,4,5, biPh), 7.12 (t, 2H, J = 8.9 Hz, F–Ph-3,5). ^{13}C NMR (50 MHz, $CDCl_3$) δ 162.9 (J = 246 Hz), 151.4, 142.8, 141.6, 140.8, 138.2, 135.7, 130.4 (J = 8 Hz), 130.2, 130.1, 129.4, 129.3, 129.1, 129.1, 128.9, 127.6, 127.5, 127.2, 127.0, 125.3, 125.1, 116.5, 115.6 (J = 21 Hz). Anal. Calcd for $C_{30}H_{20}FN_3$: C, 81.61; H, 4.57; N, 9.52. Found: C, 81.74; H, 4.33; N, 9.64.

*5.2.19. 3-(Biphen-4-yl)-2-(4-fluorophenyl)-6-phenylimidazo[1,2-b] pyridazine (**8b**)*

Method B (98% yield). Mp 233–234 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.13 (d, 1H, J = 9.5 Hz, H-8), 8.01 (m, 2H, Ph-2,6), 7.85–7.73 (m, 8H, biPh, F–Ph-2,6), 7.62 (d, 1H, J = 9.5 Hz, H-7), 7.57–7.43 (m, 6H, Ph-3,4,5, biPh), 7.11 (t, 2H, J = 8.8 Hz, F–Ph-3,5). ^{13}C NMR (50 MHz, $CDCl_3$) δ 162.5 (J = 246 Hz), 151.7, 143.3, 141.5, 140.9, 138.6, 136.0, 131.2, 130.7 (J = 7.5 Hz), 130.4, 129.4, 129.3, 128.0, 127.6, 127.5, 127.4, 125.7, 125.3, 116.7, 115.9 (J = 21.5 Hz). Anal. Calcd for $C_{30}H_{20}FN_3$: C, 81.61; H, 4.57; N, 9.52. Found: C, 81.94; H, 4.41; N, 9.55.

*5.2.20. 3-(Biphen-2-yl)-2-(4-fluorophenyl)-6-(morpholin-1-yl) imidazo[1,2-b]pyridazine (**8c**)*

Method B (30% yield). Mp 179–180 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.76 (d, 1H, J = 9.8 Hz, H-8), 7.61–7.44 (m, 6H, F–Ph-2,6, biPh), 7.10–6.90 (m, 7H, F–Ph-3,5, biPh), 6.75 (d, 1H, J = 9.8 Hz, H-7), 3.79 (m, 4H, mor), 3.33 (m, 4H, mor). ^{13}C NMR (50 MHz, $CDCl_3$) δ 162.6 (J = 245 Hz), 154.7, 143.5, 141.3, 141.0, 135.9, 132.7, 130.7, 130.6, 129.8, 129.3 (J = 8 Hz), 128.3, 128.0, 127.9, 127.1, 125.5, 125.0, 115.5 (J = 21 Hz), 109.6, 66.8, 46.7. Anal. Calcd for $C_{28}H_{23}FN_4O$: C, 74.65; H, 5.15; N, 12.44. Found: C, 74.52; H, 5.33; N, 12.80.

*5.2.21. 3-(Biphen-3-yl)-2-(4-fluorophenyl)-6-(morpholin-1-yl) imidazo[1,2-b]pyridazine (**8d**)*

Method B (48% yield). Mp 125–126 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.92 (s, 1H, biPh), 7.90 (d, 1H, J = 9.8 Hz, H-8), 7.72 (dd, 2H, J = 8.8–5.4 Hz, F–Ph-2,6), 7.61 (m, 5H, biPh), 7.51–7.38 (m, 3H, biPh), 7.07 (t, 2H, J = 8.8 Hz, F–Ph-3,5), 6.91 (d, 1H, J = 9.8 Hz, H-7), 3.85 (m, 4H, mor), 3.50 (m, 4H, mor). ^{13}C NMR (50 MHz, $CDCl_3$) δ 162.9 (J = 246 Hz), 155.0, 141.6, 141.2, 141.1, 136.4, 130.9, 130.4 (J = 8 Hz), 129.8, 129.4, 129.2, 127.9, 127.3, 126.1, 125.0, 115.8 (J = 21 Hz), 110.2, 66.8, 46.8. Anal. Calcd for $C_{28}H_{23}FN_4O$: C, 74.65; H, 5.15; N, 12.44. Found: C, 74.71; H, 5.29; N, 12.46.

*5.2.22. 3-(Biphen-4-yl)-2-(4-fluorophenyl)-6-(morpholin-1-yl) imidazo[1,2-b]pyridazine (**8e**)*

Method B (92% yield). Mp 213–214 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.88 (d, 1H, J = 9.9 Hz, H-8), 7.75–7.68 (m, 8H, F–Ph-2,6, biPh), 7.52 (m, 2H, biPh), 7.43 (m, 1H, biPh), 7.07 (t, 2H, J = 8.8 Hz, F–Ph-3,5), 6.90 (d, 1H, J = 9.9 Hz, H-7), 3.87 (m, 4H, mor), 3.50 (m, 4H, mor). ^{13}C NMR (50 MHz, $CDCl_3$) δ 162.8 (J = 245 Hz), 155.0, 141.4, 141.0, 140.8, 136.6, 131.0, 130.9, 130.4 (J = 8 Hz), 129.3, 128.4, 127.9, 127.4, 127.3, 126.1, 124.8, 115.7 (J = 21 Hz), 110.0, 66.8, 46.8. Anal. Calcd for $C_{28}H_{23}FN_4O$: C, 74.65; H, 5.15; N, 12.44. Found: C, 74.80; H, 5.37; N, 12.31.

*5.2.23. 3-(Biphen-3-yl)-2-(4-fluorophenyl)-6-(thien-3-yl)imidazo [1,2-b]pyridazine (**8f**)*

Method B (83% yield). Mp 197–198 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.07 (d, 1H, J = 9.4 Hz, H-8), 7.95 (m, 1H, BiPh), 7.89 (dd, 1H, J = 3–1.3 Hz, Th-2), 7.79 (dd, 2H, J = 8.8–5.4 Hz, F–Ph-2,6), 7.76–7.68 (m, 3H, th-4, biPh), 7.63–7.58 (m, 3H, biPh), 7.55 (d, 1H, J = 9.4 Hz, H-7), 7.51–7.35 (m, 4H, Th-5, biPh), 7.10 (t, 2H, J = 8.8 Hz, F–Ph-3,5). ^{13}C NMR (50 MHz, $CDCl_3$) δ 162.8 (J = 246 Hz), 154.3, 147.6, 142.8, 141.5, 140.7, 138.2, 138.1, 130.3 (J = 8.1 Hz), 129.3, 129.2, 129.1, 128.9, 127.6, 127.4, 127.1, 127.0, 126.1, 125.3, 124.9, 124.7, 116.5, 115.6 (J = 21 Hz). Anal. Calcd for $C_{28}H_{18}FN_3S$: C, 75.15; H, 4.05; N, 9.39. Found: C, 74.98; H, 4.13; N, 9.46.

*5.2.24. 3-(Biphen-4-yl)-2-(4-fluorophenyl)-6-(thien-3-yl)imidazo [1,2-b]pyridazine (**8g**)*

Method B (84% yield). Mp 218–219 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.07 (d, 1H, J = 9.4 Hz, H-8), 7.90 (dd, 1H, J = 3–1.3 Hz, Th-2), 7.82–7.73 (m, 8H, F–Ph-2,6, biPh), 7.70 (dd, 1H, J = 5.1–1.3 Hz, Th-4), 7.58–7.43 (m, 3H, biPh), 7.52 (d, 1H, J = 9.4 Hz, H-7), 7.46 (dd, 1H, J = 5.1–3 Hz, Th-5), 7.10 (t, 2H, J = 8.8 Hz, F–Ph-3,5). ^{13}C NMR (50 MHz, $CDCl_3$) δ 163.1 (J = 246 Hz), 147.8, 143.1, 141.4, 140.8, 138.5, 131.1, 130.7, 130.6 (J = 8 Hz), 129.3, 128.0, 127.6, 127.5, 127.3, 126.5, 125.6, 125.1, 116.7, 115.9 (J = 21 Hz). Anal. Calcd for $C_{28}H_{18}FN_3S$: C, 75.15; H, 4.05; N, 9.39. Found: C, 75.26; H, 4.03; N, 9.24.

*5.2.25. 3-(Biphen-4-yl)-2-(4-fluorophenyl)-6-(4-methylpiperazin-1-yl)imidazo[1,2-b]pyridazine (**8h**)*

Method B (92% yield). Mp 242–243 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.82 (d, 1H, J = 9.8 Hz, H-8), 7.78–7.69 (m, 8H, F–Ph-2,6, biPh), 7.51 (m, 2H, biPh), 7.43 (m, 1H, biPh), 7.06 (t, 2H, J = 8.8 Hz, F–Ph-3,5), 6.91 (d, 1H, J = 9.8 Hz, H-7), 3.56 (t, 2H, J = 4.9 Hz, pip), 2.58 (t, 2H, J = 4.9 Hz, pip), 2.39 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 162.8 (J = 245 Hz), 154.8, 141.5, 140.8, 136.6, 131.4, 130.9, 130.4 (J = 8 Hz), 129.2, 128.6, 127.9, 127.4, 127.3, 126.0, 124.7, 115.7 (J = 21 Hz), 110.2, 54.9, 46.4. Anal. Calcd for $C_{29}H_{26}FN_5$: C, 75.14; H, 5.65; N, 15.11. Found: C, 45.46; H, 5.37; N, 14.82.

*5.2.26. 2-tert-Butyl-6-(thien-3-yl)imidazo[1,2-a]pyridine (**10b**)*

Method C: A mixture of 2-amino-5-(thien-3-yl)pyridine (2.27 mmol), the conveniently substituted compound α-halogenocarbonyle (2.73 mmol), and 1,2-dimethoxyethane (5 mL) was stirred overnight at room temperature. The solvent was removed under reduced pressure, the resulting residue was then dissolved in ethanol (5 mL) and refluxed overnight. After cooling and concentration, the residue was suspended in water, make alkaline with Na_2CO_3 and extracted with CH_2Cl_2. After drying with $MgSO_4$, the organic layers were evaporated to dryness. The residue was chromatographed on neutral alumina eluting with CH_2Cl_2 to give **10b** in 94% yield. Mp 119–120 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.06 (m, 1H, H-5), 7.45 (d, 1H, J = 9.3 Hz, H-8), 7.43–7.12 (m, 5H, Th-2,4,5, H-7, H-3), 1.35 (m, 9H, $3CH_3$). ^{13}C NMR (50 MHz, $CDCl_3$) δ 158.0 (C-8a), 144.8 (C-2), 138.6 (Th-3), 127.2 (Th-2), 125.9 (Th-5), 124.3 (C-7), 122.4 (C-5), 121.4 (C-6), 120.6 (Th-4), 117.2 (C-8), 107.6 (C-3), 32.7 (C-^{t}Bu), 30.6 (3 CH_3).

*5.2.27. Ethyl 6-(thien-3-yl)imidazo[1,2-a]pyridin-2-carboxylate (**10c**)*

Method C (69% yield). Mp 125–126 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.28 (s, 1H, H-5), 8.15 (s, 1H, H-3), 7.61 (d, 1H, J = 9.5 Hz, H-8), 7.40 (m, 3H, Th-2,4, H-7), 7.27 (dd, 1H, J = 1.2–4.9 Hz, Th-5), 4.38 (q, J = 7.1 Hz, 2H, CH_2), 1.37 (t, 3H, J = 7.1 Hz, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 163.6 (C=O), 144.7 (C-8a), 137.7 (C-2), 137.5 (Th-3), 127.7 (Th-2), 126.9 (Th-5), 125.9 (C-7), 123.5 (C-6), 122.9 (C-5), 121.8 (Th-4), 119.1 (C-3), 117.8 (C-8), 61.5 (CH_2), 14.8 (CH_3).

*5.2.28. 2,6-di(Thien-3-yl)imidazo[1,2-a]pyridine (**10d**)*

Method C (66% yield). Mp > 300 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.38 (s, 1H, H-5), 7.95 (d, 1H, J = 2.9 Hz, Th-2), 7.82 (s, 1H, H-3), 7.75 (d, 1H, J = 9.5 Hz, H-8), 7.58–7.49 (m, 4H, H-7, Th-2,4, Th-4′), 7.45–7.37 (m, 1H, Th-5, Th-5′). ^{13}C NMR (50 MHz, $CDCl_3$) δ 145.8, 142.2, 136.6, 127.6, 126.7, 126.3, 126.1, 122.6, 122.4, 121.4, 121.1, 117.4, 113.7.

*5.2.29. 2-(2-Methoxyphenyl)-6-(thien-3-yl)imidazo[1,2-a]pyridine (**10e**)*

Method C (54% yield). Mp 94–95 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.48 (d, 1H, J = 7.7 Hz, Ph-6), 8.23 (s, 1H, H-5), 8.17 (s, 1H, H-3), 7.61 (d, 1H, J = 9.3 Hz, H-8), 7.40 (m, 2H, Th-2,4), 7.35 (dd, 1H, J = 9.3–1.6 Hz, H-7), 7.29 (m, 2H, Th-5, Ph-4), 7.14 (t, 1H, J = 7.7 Hz, Ph-5), 6.98 (d, 1H, J = 7.7 Hz, Ph-3), 3.95 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 157.2, 144.0, 142.0, 138.6, 129.1, 127.3, 126.0, 125.0, 122.7, 122.5, 121.7, 121.4, 120.8, 117.4, 113.5, 111.3, 55.8.

*5.2.30. 2-(3-Methoxyphenyl)-6-(thien-3-yl)imidazo[1,2-a]pyridine (**10f**)*

Method C (47% yield). Mp 130–131 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.32 (dd, 1H, J = 1.7–0.9 Hz, H-5), 7.89 (s, 1H, H-3), 7.68 (dd, 1H, J = 9.4–0.9 Hz, H-8), 7.59 (m, 1H, Ph-2), 7.55 (m, 1H, Ph-6), 7.50–7.34 (m, 5H, H-7, Th-2,4,5, Ph-5), 6.93 (m, 1H, Ph-4), 3.93 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 160.5 (Ph-3), 146.5 (C-8a), 145.2 (C-2), 138.4 (Th-3), 135.5 (Ph-1), 130.2 (Ph-5), 127.5 (Th-2), 126.1 (Th-5), 125.4 (C-7), 122.5 (C-5), 122.3 (C-6), 121.1 (Th-4), 118.9 (Ph-6), 117.8 (C-8), 114.6 (Ph-4), 111.3 (Ph-2), 109.2 (C-3), 55.8 (CH_3).

*5.2.31. 2-(4-Methoxyphenyl)-6-(thien-3-yl)imidazo[1,2-a]pyridine (**10g**)*

Method C (75% yield). Mp 212–213 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.35 (dd, 1H, J = 1.6–0.9 Hz, H-5), 7.93 (m, 2H, Ph-2,6), 7.84 (s, 1H, H-3), 7.68 (d, 1H, J = 9.3 Hz, H-8), 7.48 (m, 3H, H-7, Th-2,4), 7.38 (dd, 1H, J = 3.7–2.7 Hz, Th-5), 7.02 (m, 2H, Ph-3,5), 3.89 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 160.2 (Ph-4), 145.6 (C-8a), 144.6 (C-2), 138.3 (Th-3), 132.8 (Ph-1), 127.8 (Ph-2,6), 127.5 (Th-2), 126.1 (Th-5), 125.9 (C-7), 122.7 (C-5, C-6), 121.3 (Th-4), 117.1 (C-8), 114.6 (Ph-3,5), 108.1 (C-3), 55.8 (CH_3).

*5.2.32. 2-tert-Butyl-6-(thien-3-yl)imidazo[1,2-b]pyridazine (**10h**)*

Method C (82% yield). Mp 97–98 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.88 (d, 1H, J = 9.4 Hz, H-8), 7.82 (dd, 1H, J = 2.9–1.3 Hz, Th-2), 7.76 (s, 1H, H-3), 7.66 (dd, 1H, J = 5.1–1.3 Hz, Th-4), 7.42 (dd, 1H, J = 5.1–2.9 Hz, Th-5), 7.32 (d, 1H, J = 9.4 Hz, H-7), 1.44 (s, 9H, 3 CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 158.2 (C-6), 147.8 (C-2), 138.7 (C-8a), 138.3 (Th-3), 127.5 (Th-5), 126.5 (Th-4), 125.3 (C-8), 124.8 (Th-2), 116.1 (C-7), 112.3 (C-3), 33.1 (C-^{t}Bu), 30.7 (3 CH_3).

*5.2.33. 2-(2-Methoxyphenyl)-6-(thien-3-yl)imidazo[1,2-b]pyridazine (**10i**)*

Method C. The compound was not isolated and used directly in reaction.

*5.2.34. 3-Iodo-6-(thien-3-yl)imidazo[1,2-a]pyridine (**11a**)*

Method A (98% yield). Mp 128–129 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.33 (dd, 1H, J = 1.6–0.9 Hz, H-5), 7.74 (s, 1H, H-2), 7.72 (dd, 1H, J = 9.3–0.9 Hz, H-8), 7.59–7.50 (m, 3H, Th-2,5, H-7), 7.43 (dd, 1H, J = 4.9–1.4 Hz, Th-5).

*5.2.35. 3-Iodo-2-tert-butyl-6-(thien-3-yl)imidazo[1,2-a]pyridine (**11b**)*

Method A (65% yield). Mp 129–130 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.31 (m, 1H, H-5), 7.55 (dd, 1H, J = 9.2–0.5 Hz, H-8), 7.36 (m, 3H, H-7, Th-2,5), 7.29 (dd, 1H, J = 4.7–1.4 Hz, Th-4), 1.56 (m, 9H, 3CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 157.2 (C-8a), 145.8 (C-2a), 138.5 (Th-3), 127.4 (Th-2), 126.2 (Th-5), 125.1 (C-7), 122.8 (C-5), 122.6 (C-6), 121.3 (Th-4), 117.5 (C-8), 57.9 (C-3), 33.8 (C–^{t}Bu), 30.6 (3 CH_3).

*5.2.36. Ethyl 3-iodo-6-(thien-3-yl)imidazo[1,2-a]pyridin-2-carboxylate (**11c**)*

Method A (96% yield). ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.43 (s, 1H, H-5), 7.83 (d, 1H, J = 9.5 Hz, H-8), 7.65 (dd, 1H, J = 9.5–1.6 Hz, H-7), 7.60–7.50 (m, 2H, Th-2,5), 7.43 (dd, 1H, J = 5.1–1.2 Hz, Th-4), 4.53 (q, 2H, J = 7.1 Hz, CH_2), 1.54 (t, 3H, J = 7.1 Hz, CH_3).

*5.2.37. 3-Iodo-2,6-di(thien-3-yl)imidazo[1,2-a]pyridine (**11d**)*

Method A (71% yield). Mp > 300 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.41 (s, 1H, H-5), 8.16 (m, 1H, J = 2.9–1.3 Hz, Th-2′), 7.95 (m, 1H, J = 5.0–1.3 Hz, Th-4′), 7.69 (d, 1H, J = 9.3 Hz, H-8), 7.59–7.43 (m, 5H, Th-5′, H-7, Th-2,4,5).

*5.2.38. 3-Iodo-2-(2-methoxyphenyl)-6-(thien-3-yl)imidazo[1,2-a]pyridine (**11e**)*

Method A (99% yield). Mp > 95 °C degradation. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.39 (m, 1H, J = 1.7 Hz, H-5), 7.82 (dd, 1H, J = 9.3–0.9 Hz, H-8), 7.60 (dd, 1H, J = 9.3–1.7 Hz, H-7), 7.58–7.46 (m, 4H, Th-2,5, Ph-4,6), 7.44 (dd, 1H, J = 5.0–1.4 Hz, Th-4), 7.12 (td, 1H, J = 6.9–1 Hz, Ph-5), 7.08 (d, 1H, J = 7.8 Hz, Ph-3), 3.93 (s, 3H, CH_3).

*5.2.39. 3-Iodo-2-(3-methoxyphenyl)-6-(thien-3-yl)imidazo[1,2-a]pyridine (**11f**)*

Method A (99% yield). Mp 168–169 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.42 (m, 1H, H-5), 7.83 (d, 1H, J = 9.1 Hz, H-8), 7.65–7.40 (m, 7H, H-7, Th-2,4,5, Ph-2,5,6), 7.02 (m, 1H, Ph-4), 3.96 (s, 3H, CH_3).

*5.2.40. 3-Iodo-2-(4-methoxyphenyl)-6-(thien-3-yl)imidazo[1,2-a]pyridine (**11g**)*

Method A (99% yield). Mp 185–186 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.41 (m, 1H, H-5), 7.95 (m, 2H, Ph-2,6), 7.78 (dd, 1H, J = 9.2–0.8 Hz, H-8), 7.62–7.51 (m, 3H, H-7, Th-2,5), 7.44 (dd, 1H, J = 5.0–1.4 Hz, Th-4), 7.07 (m, 2H, Ph-3,5), 3.92 (s, 3H, CH_3).

*5.2.41. 3-Iodo-2-tert-butyl-6-(thien-3-yl)imidazo[1,2-b]pyridazine (**11h**)*

Method A (58% yield). Mp 159–160 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.97 (dd, 1H, J = 2.9–1.3 Hz, Th-2), 7.91 (d, 1H, J = 9.3 Hz, H-8), 7.85 (dd, 1H, J = 5.1–1.3 Hz, Th-4), 7.50 (dd, 1H, J = 5.1–2.9 Hz, Th-5), 7.46 (d, 1H, J = 9.3 Hz, H-7), 1.62 (s, 9H, 3 CH_3).

*5.2.42. 3-Iodo-2-(2-methoxyphenyl)-6-(thien-3-yl)imidazo[1,2-b]pyridazine (**11i**)*

Method A (54% yield). Mp 166–167 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.16 (d, 1H, J = 9.5 Hz, H-8), 8.05 (m, 1H, MeOPh-6), 7.86 (m, 1H, Th-2), 7.63 (d, 1H, J = 9.5 Hz, H-7), 7.56–7.48 (m, 3H, MeOPh-4, Th-4,5), 7.19–7.08 (m, 2H, MeOPh-3,5), 3.95 (s, 3H, CH_3).

*5.2.43. 3-(Biphen-3-yl)-6-(thien-3-yl)imidazo[1,2-a]pyridine (**12a**)*

Method B (82% yield). Mp = 75–76 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.59 (s, 1H, H-5), 7.82 (m, 2H, biPh), 7.77 (d, 1H, J = 9.3 Hz, H-8), 7.73–7.30 (m, 11H, BiPh, H-7, H-2, Th-2,4,5). ^{13}C NMR (50 MHz, $CDCl_3$) δ 143.0, 140.8, 138.7, 133.5, 130.2, 129.4, 128.2, 127.6, 127.5, 127.2, 126.3, 125.2, 122.7, 121.4, 120.4, 118.6. Anal. Calcd for $C_{23}H_{16}N_2S$: C, 78.38; H, 4.58; N, 7.95. Found: C, 78.59; H, 4.61; N, 7.85.

*5.2.44. 3-(Biphen-3-yl)-2-tert-butyl-6-(thien-3-yl)imidazo[1,2-a]pyridine (**12b**)*

Method B (62% yield). Mp 160–161 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.83–7.34 (m, 14H, H-7, H-8, Th-2,5, BiPh), 7.20 (dd, 1H, J = 4.8–1.5 Hz, Th-4), 1.41 (s, 9H, 3CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 153.4, 142.8, 142.4, 140.6, 139.0, 132.3, 131.3, 131.1, 130.0, 129.4, 128.2, 127.6, 127.2, 126.3, 124.6, 121.8, 120.9, 120.6, 120.4, 117.3. Anal. Calcd for $C_{27}H_{24}N_2S$: C, 79.37; H, 5.92; N, 6.86. Found: C, 79.11; H, 5.97; N, 6.96.

*5.2.45. Ethyl 3-(biphen-3-yl)-6-(thien-3-yl)imidazo[1,2-a]pyridine-2-carboxylate (**12c**)*

Method B (48% yield). Mp 83–84 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.19 (s, 1H, H-5), 7.88–7.40 (m, 13H, H-8, H-7, Th-2,5, BiPh), 7.26

(dd, 1H, J = 4.4–1.5 Hz, Th-4), 4.40 (q, 2H, J = 7.1 Hz, CH_2), 1.35 (t, 3H, J = 7.1 Hz, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 163.6, 144.0, 142.3, 140.7, 138.0, 134.0, 130.0, 129.9, 129.8, 129.3, 128.9, 128.7, 128.1, 127.6, 127.2, 126.1, 123.8, 121.9, 120.7, 119.4, 61.4, 14.7. Anal. Calcd for $C_{26}H_{20}N_2O_2S$: C, 73.56; H, 4.75; N, 6.60. Found: C, 73.84; H, 4.62; N, 6.54.

*5.2.46. 3-(Biphen-3-yl)-2,6-di(thien-3-yl)imidazo[1,2-a]pyridine (**12d**)*

Method B (81% yield). Mp 90–91 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.15 (s, 1H, H-5), 7.84–7.25 (m, 17H, H-8, H-7, Th, Th′, BiPh), 7.56–7.48 (m, 4H), 7.45–7.36 (m, 4H), 7.30–7.25 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 143.7, 142.8, 140.1, 139.8, 138.2, 135.1, 130.3, 129.9, 129.7, 129.1, 128.3, 128.0, 127.2, 127.1, 127.0, 125.9, 125.6, 125.5, 123.1, 122.3, 121.0, 120.8, 120.0, 117.2. Anal. Calcd for $C_{27}H_{18}N_2S_2$: C, 74.62; H, 4.17; N, 6.45. Found: C, 74.77; H, 4.12; N, 6.76.

*5.2.47. 3-(Biphen-3-yl)-2-(2-methoxyphenyl)-6-(thien-3-yl) imidazo[1,2-a]pyridine (**12e**)*

Method B (36% yield). Mp 110–111 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.51 (s, 1H, H-5), 7.79 (dd, 1H, J = 9.3–0.8 Hz, H-8), 7.69 (dd, 1H, J = 7.5–1.7 Hz, MeOPh-6), 7.66–7.34 (m, 13H, H-7, Th-2,5, MeOPh-4, BiPh), 7.31 (dd, 1H, J = 5.6–1.4 Hz, Th-4), 7.08 (td, 1H, J = 7.5–1.0 Hz, MeOPh-5), 6.88 (d, 1H, J = 8.2 Hz, MeOPh-3), 3.41 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 157.1, 144.5, 142.3, 141.9, 141.0, 139.0, 132.5, 131.6, 129.8, 129.3, 128.3, 128.0, 127.5, 127.4, 127.1, 126.3, 125.0, 124.0, 123.4, 122.3, 121.1, 120.1, 118.2, 111.4, 55.2. Anal. Calcd for $C_{30}H_{22}N_2OS$: C, 78.57; H, 4.84; N, 6.11. Found: C, 78.46; H, 4.92; N, 6.31.

*5.2.48. 3-(Biphen-3-yl)-2-(3-methoxyphenyl)-6-(thien-3-yl) imidazo[1,2-a]pyridine (**12f**)*

Method B (16% yield). Mp 127–128 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.21 (s, 1H, H-5), 7.82–7.75 (m, 17H, H-8, H-7, Th-2,4,5, BiPh, MeOPh-2,5,6), 6.86 (m, 1H, MeOPh-4), 3.74 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 160.0, 144.4, 143.3, 143.1, 140.6, 138.7, 135.8, 130.8, 130.6, 130.0, 129.8, 129.4, 128.2, 127.5, 127.4, 126.3, 125.5, 122.4, 121.9, 121.2, 120.9, 120.3, 118.0, 114.8, 112.9, 55.5. Anal. Calcd for $C_{30}H_{22}N_2OS$: C, 78.57; H, 4.84; N, 6.11. Found: C, 78.80; H, 4.77; N, 6.28.

*5.2.49. 3-(Biphen-3-yl)-2-(4-methoxyphenyl)-6-(thien-3-yl) imidazo[1,2-a]pyridine (**12g**)*

Method B (50% yield). Mp 205–206 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.21 (m, 1H, H-5), 7.80–7.40 (m, 15H, H-8, H-7, Th-2,5, MeOPh-2,6, BiPh), 7.29 (m, 1H, Th-4), 6.88 (d, 2H, J = 8.9 Hz, MeOPh-3,5), 3.84 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 159.7, 144.5, 143.4, 143.0, 140.6, 138.8, 131.0, 130.6, 129.9, 129.8, 129.7, 129.4, 128.2, 128.1, 127.6, 127.4, 127.1, 126.2, 125.3, 122.2, 121.1, 121.0, 120.1, 117.7, 114.3, 55.7. Anal. Calcd for $C_{30}H_{22}N_2OS$: C, 78.57; H, 4.84; N, 6.11. Found: C, 78.62; H, 4.85; N, 6.18.

*5.2.50. 3-(Biphen-3-yl)-2-tert-butyl-6-(thien-3-yl)imidazo[1,2-b] pyridazine (**12h**)*

Method B (69% yield). Mp 171–172 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.01 (d, 1H, J = 9.4 Hz, H-8), 7.79–7.39 (m, 12H, H-7, Th-2,4, BiPh), 7.34 (dd, 1H, J = 5.1–2.9 Hz, Th-5), 1.44 (s, 9H, 3 CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 154.1, 147.5, 141.5, 141.4, 139.0, 136.7, 132.1, 131.4, 131.2, 129.5, 129.2, 128.1, 127.8, 127.7, 127.2, 126.7, 125.3, 124.7, 115.8, 34.7, 31.8. Anal. Calcd for $C_{26}H_{23}N_3S$: C, 76.25; H, 5.66; N, 10.26. Found: C, 76.34; H, 5.74; N, 10.19.

*5.2.51. 3-(Biphen-3-yl)-2-(2-methoxyphenyl)-6-(thien-3-yl) imidazo[1,2-b]pyridazine (**12i**)*

Method B (38% yield). Mp 174–175 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.08 (d, 1H, J = 9.4 Hz, H-8), 7.91 (dd, 1H, J = 3.0–1.3 Hz, Th-2), 7.82–7.30 (m, 14H, H-7, Th-4,5, BiPh, MeOPh-4,6), 7.13 (t, 1H, J = 7.4 Hz, MeOPh-5), 6.96 (d, 1H, J = 8.4 Hz, MeOPh-3), 3.45 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 157.5, 147.9, 142.5, 141.5, 141.3, 138.9, 138.5, 132.5, 130.9, 130.4, 129.4, 129.0, 128.2, 128.1, 127.9, 127.6, 127.5, 126.9, 126.8, 126.7, 126.1, 125.2, 124.5, 121.6, 116.3, 111.8, 55.6. Anal. Calcd for $C_{29}H_{21}N_3OS$: C, 75.79; H, 4.61; N, 9.14. Found: C, 75.68; H, 4.77; N, 9.19.

*5.2.52. 2-Bromo-2-(3-bromophenyl)-4-fluoroacetophenone (**14a**)*

Method D. A mixture of 2-(3-bromophenyl)-4-fluoroacetophenone (2.6 g, 8.9 mmol) and 30% aqueous solution of HBr (0.1 mL) in $CHCl_3$ (25 mL) is added dropwise with bromine (0.46 mL, 8.9 mmol) in $CHCl_3$ (2.5 mL). After stirring at room temperature overnight, the reaction mixture is washed with a 5% aqueous solution of sodium thiosulfate and then with water. The organic phase is dried with $MgSO_4$, filtered and evaporated to dryness. The residue is purified by column chromatography on silica gel eluting with CH_2Cl_2–petroleum ether (10/90 v/v) to give **14a** in 72% yield. Mp 102–103 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.07 (dd, 2H, J = 8.8–5.3 Hz, F–Ph-2,6), 7.74 (t, 1H, J = 1.9 Hz, Br–Ph-2), 7.53–7.48 (m, 2H, Br–Ph-4,6), 7.28 (dd, 1H, J = 7.7–8 Hz, Br–Ph-5), 7.18 (t, 2H, J = 8.8 Hz, F–Ph-3,5), 6.28 (s, 1H, CH).

*5.2.53. 2-Bromo-2-(3-bromophenyl)-2-methoxyacetophenone (**14b**)*

Method D (56% yield). The compound was not isolated and used directly in reaction.

*5.2.54. 1-Bromo-1-(3-bromophenyl)-3,3-dimethylbutan-2-one (**14c**)*

Method D (93% yield). Oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.74 (m, 1H, Br–Ph-2), 7.50–7.42 (m, 2H, Br–Ph-4,6), 7.21 (t, 1H, J = 7.9 Hz, Br–Ph-5), 5.81 (s, 1H, CH), 1.22 (s, 9H, 3 CH_3).

*5.2.55. 3-(3-Bromophenyl)-2-(4-fluorophenyl)imidazo[1,2-a] pyridine (**15a**)*

Method C (70% yield). Mp 123–124 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.99 (d, 1H, J = 7.0 Hz, H-5), 7.73–7.61 (m, 5H, F–Ph-2,6, H-8, Br–Ph-2,4), 7.50–7.37 (m, 2H, Br–Ph-5,6), 7.27 (ddd, 1H, J = 8.9–7.0–1.2 Hz, H-7), 7.00 (t, 2H, J = 8.8 Hz, F–Ph-3,5), 6.82 (t, 1H, J = 7.0–1.0 Hz, H-6). ^{13}C NMR (50 MHz, $CDCl_3$) δ 163.1 (J = 246 Hz), 145.6, 142.7, 133.8, 132.7, 131.8, 134.4 (J = 8 Hz), 130.1, 128.2 (J = 3 Hz), 125.8, 124.1, 123.7, 119.8, 118.2, 115.7 (J = 20 Hz), 113.3.

*5.2.56. 3-(3-Bromophenyl)-2-(2-methoxyphenyl)imidazo[1,2-a] pyridine (**15b**)*

Method C (17% yield). Oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.18 (d, 1H, J = 6.9 Hz, H-5), 7.71 (d, 1H, J = 8.9 Hz, H-8), 7.64 (dd, 1H, J = 7.5–1.4 Hz, MeOPh-6), 7.58 (s, 1H, BrPh-2), 7.49 (m, 1H, BrPh-4), 7.38–7.18 (m, 4H, H-7, Br–Ph-5,6, MeOPh-4), 7.04 (t, 1H, J = 7.4 Hz, MeOPh-5), 6.86–6.77 (m, 2H, H-6, MeOPh-3), 3.40 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 157.0, 145.5, 141.7, 133.5, 132.6, 132.2, 131.4, 131.0, 130.2, 128.4, 125.2, 123.7, 124.5, 123.2, 121.3, 118.5, 113.2, 111.5, 55.3.

*5.2.57. 3-(Biphen-3-yl)-2-(4-fluorophenyl)imidazo[1,2-a]pyridine (**16a**)*

Method B (81% yield). Mp 64–65 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.06 (d, 1H, J = 6.9 Hz, H-5), 7.79–7.71 (m, 5H, F–Ph-2,6, H-8, biPh), 7.68–7.58 (m, 3H, BiPh), 7.53–7.40 (m, 4H, BiPh), 7.26 (m, 1H, J = 9.0–6.9–1.2 Hz, H-7), 7.03 (m, 2H, J = 8.8 Hz, F–Ph-3,5), 6.79 (td, 1H, J = 6.9–1.2 Hz, H-6). ^{13}C NMR (50 MHz, $CDCl_3$) δ 163.0 (J = 245 Hz), 145.4, 143.2, 142.2, 140.8, 130.9 (J = 3 Hz), 130.8, 130.7, 130.4 (J = 8 Hz), 130.1, 129.8, 129.6, 128.4, 128.3, 127.7, 125.6, 124.0, 121.3, 118.1, 115.9 (J = 21 Hz), 113.1. Anal. Calcd for $C_{25}H_{17}FN_2$: C, 82.40; H, 4.70; N, 7.69. Found: C, 82.59; H, 4.77; N, 7.58.

*5.2.58. 2-(4-Fluorophenyl)-3-(2′-methoxybiphen-3-yl)imidazo[1,2-a]pyridine (**16b**)*

Method B (89% yield). Mp 146–147 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.14 (dd, 1H, J = 6.9–1.2 Hz, H-5), 7.81–7.66 (m, 5H, F–Ph-2,6, H-8, BiPh), 7.60 (td, 1H, J = 7.4–0.7 Hz, BiPh-5), 7.43–7.36 (m, 3H, BiPh), 7.26 (m, 1H, J = 9.5–6.8–1.2 Hz, H-7), 7.12–7.00 (m, 4H, F–Ph-3,5, BiPh-3′,5′), 6.81 (td, 1H, J = 6.9–6.8–1.2 Hz, H-6), 3.82 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 162.9 (J = 245 Hz), 156.9, 150.2, 145.4, 142.0, 140.4, 132.5, 131.3, 130.9 (J = 3 Hz), 130.6, 130.5, 130.4, 130.1, 129.9, 129.7 (J = 8 Hz), 129.6, 125.5, 124.1, 121.6, 118.0, 115.8 (J = 21 Hz), 112.9, 111.8, 56.1. Anal. Calcd for $C_{26}H_{19}FN_2O$: C, 79.17; H, 4.86; N, 7.10. Found: C, 79.35; H, 4.92; N, 7.28.

*5.2.59. 2-(4-Fluorophenyl)-3-(3′-methoxybiphen-3-yl)imidazo[1,2-a]pyridine (**16c**)*

Method B (71% yield). Mp 69–70 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.06 (d, 1H, J = 6.9 Hz, H-5), 7.78–7.70 (m, 5H, H-8, F–Ph-2,6, BiPh), 7.62 (t, 1H, J = 7.5 Hz, BiPh-5), 7.43 (m, 1H, BiPh-5′), 7.35 (d, 1H, J = 7.7 Hz, BiPh), 7.27–6.91 (m, 6H, H-7, BiPh-2′,4′,6′, F–Ph-2,5), 6.77 (t, 1H, J = 6.9 Hz, H-6), 3.85 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 163.0 (J = 245 Hz), 160.6, 145.5, 143.1, 142.3, 142.2, 130.9 (J = 3 Hz), 130.8, 130.7, 130.6, 130.5, 130.4, 130.0 (J = 8 Hz), 128.3, 125.5, 123.9, 121.3, 120.1, 118.1, 115.9 (J = 21 Hz), 113.8, 113.4, 113.1, 55.9. Anal. Calcd for $C_{26}H_{19}FN_2O$: C, 79.17; H, 4.86; N, 7.10. Found: C, 79.28; H, 4.85; N, 7.16.

*5.2.60. 2-(4-Fluorophenyl)-3-(4′-methoxybiphen-3-yl)imidazo[1,2-a]pyridine (**16d**)*

Method B (77% yield). Mp 175–176 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.04 (d, 1H, J = 6.8 Hz, H-5), 7.78–7.67 (m, 5H, H-8, F–Ph-2,6, BiPh-2′,6′), 7.63 (d, 1H, J = 7.5 Hz, BiPh-5), 7.53 (m, 2H, BiPh), 7.43 (dt, 1H, J = 7.5–1.5 Hz, BiPh), 7.24 (ddd, 1H, J = 8.9–6.8–1.2 Hz, H-7), 7.07–6.96 (m, 4H, F–Ph-3,5, BiPh-3′,5′), 6.77 (td, 1H, J = 6.8–1.2 Hz, H-6), 3.86 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 163.0 (J = 245 Hz), 160.1, 145.4, 142.8, 142.2, 133.2, 130.9 (J = 3 Hz), 130.7, 130.5 (J = 8 Hz), 129.4, 129.3, 128.7, 127.8, 125.5, 124.0, 121.4, 118.1, 115.8 (J = 21 Hz), 114.9, 113.0, 55.9. Anal. Calcd for $C_{26}H_{19}FN_2O$: C, 79.17; H, 4.86; N, 7.10. Found: C, 79.33; H, 4.91; N, 7.08.

*5.2.61. 2-(4-Fluorophenyl)-3-(4′-hydroxybiphen-3-yl)imidazo[1,2-a]pyridine (**16e**)*

Method B (65% yield). Mp 262–263 °C. ^{1}H NMR (200 MHz, DMSO) δ 9.63 (s, 1H, OH), 8.11 (d, 1H, J = 6.3 Hz, H-5), 7.79–7.53 (m, 8H, H-8, F–Ph-2,6, BiPh), 7.41–7.30 (m, 2H, H-7, BiPh), 7.17 (t, 2H, J = 8.5 Hz, F–Ph-3,5), 6.95–6.84 (m, 3H, H-6, BiPh-3′,5′). ^{13}C NMR (50 MHz, DMSO) δ 162.6 (J = 243 Hz), 158.5, 145.1, 142.6, 141.4, 131.8, 131.3, 131.1, 130.8, 130.4 (J = 8 Hz), 129.6, 129.0, 128.9, 127.7, 126.4, 125.0, 121.7, 117.9, 116.8, 116.3 (J = 21 Hz), 113.9. Anal. Calcd for $C_{25}H_{17}FN_2O$: C, 78.93; H, 4.50; N, 7.36. Found: C, 79.09; H, 4.52; N, 7.28.

*5.2.62. 3-(4′-Chlorobiphen-3-yl)-2-(4-fluorophenyl)imidazo[1,2-a]pyridine (**16f**)*

Method B (82% yield). Mp 116–117 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.01 (d, 1H, J = 6.8 Hz, H-5), 7.74–7.50 (m, 7H, H-8, F–Ph-2,6, BiPh), 7.48 (d, 2H, J = 8.6 Hz, BiPh-2′,6′), 7.38 (m, 2H, J = 8.6 Hz, BiPh-3′,5′), 7.22 (dd, 1H, J = 9–6.8 Hz, H-7), 6.99 (t, 2H, J = 8.7 Hz, F–Ph-3,5), 6.76 (t, 1H, J = 6.8 Hz, H-6). ^{13}C NMR (50 MHz, $CDCl_3$) δ 163.0 (J = 246 Hz), 145.5, 142.3, 142.0, 139.2, 134.5, 131.0, 130.8, 130.4 (J = 8 Hz), 130.3, 129.7, 129.6, 128.9, 128.1, 125.6, 123.8, 121.1, 118.1, 115.9 (J = 21 Hz), 113.1. Anal. Calcd for $C_{25}H_{16}ClFN_2$: C, 75.28; H, 4.04; N, 7.02. Found: C, 75.41; H, 4.23; N, 6.94.

*5.2.63. 3-(4′-Fluorobiphen-3-yl)-2-(4-fluorophenyl)imidazo[1,2-a]pyridine (**16g**)*

Method B (58% yield). Mp 114–115 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.04 (d, 1H, J = 6.8 Hz, H-5), 7.76–7.60 (m, 6H, H-8, F–Ph-2,6, BiPh), 7.57 (dd, 2H, J = 8.8–5.3 Hz, BiPh-2′,6′), 7.47 (m, 1H, BiPh), 7.26 (dd, J = 9.0–6.8 Hz, H-7), 7.16 (t, 2H, J = 8.8 Hz, BiPh-3′-5′), 7.02 (t, 2H, J = 8.8 Hz, F–Ph-3,5), 6.80 (td, 1H, J = 6.8–1.1 Hz, H-6). ^{13}C NMR (50 MHz, $CDCl_3$) δ 163.3 (J = 246 Hz), 163.0 (J = 245 Hz), 145.5, 142.2, 142.1, 136.9 (J = 3 Hz), 130.9, 130.8, 130.3 (J = 8 Hz), 130.0, 129.7, 129.3 (J = 8 Hz), 128.2, 125.5, 123.9, 121.2, 118.1, 116.4 (J = 21 Hz), 115.9 (J = 21 Hz), 113.1. Anal. Calcd for $C_{25}H_{16}F_2N_2$: C, 78.52; H, 4.22; N, 7.33. Found: C, 78.64; H, 4.18; N, 7.40.

*5.2.64. 3-[3-(Pyridin-4-yl)phenyl]-2-(4-fluorophenyl)imidazo[1,2-a]pyridine (**16h**)*

Method B (81% yield). Mp 119–120 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.59 (d, 2H, J = 5.3 Hz, Py-3,5), 7.96 (dd, 1H, J = 6.8–1.1 Hz, H-5), 7.72–7.57 (m, 6H, F–Ph-2,6, H-8, Ph), 7.47 (dd, 1H, J = 7.4–1.1 Hz, Ph), 7.41 (d, 2H, J = 5.3 Hz, Py-2,6), 7.16 (ddd, 1H, J = 9.0–6.8–1.1 Hz, H-7), 6.96 (t, 2H, J = 8.8 Hz, F–Ph-3,5), 6.71 (t, 1H, J = 6.8 Hz, H-6). ^{13}C NMR (50 MHz, $CDCl_3$) δ 162.9 (J = 246 Hz), 150.9, 147.8, 145.5, 142.4, 140.1, 131.7, 131.2, 131.1, 130.7 (J = 3 Hz), 130.3 (J = 8 Hz), 129.7, 128.1, 125.6, 123.7, 122.1, 120.7, 118.1, 115.9 (J = 21 Hz), 113.2. Anal. Calcd for $C_{24}H_{16}FN_3$: C, 78.89; H, 4.41; N, 11.50. Found: C, 78.94; H, 4.37; N, 11.58.

*5.2.65. 3-[3-(Fur-2-yl)phenyl]-2-(4-fluorophenyl)imidazo[1,2-a]pyridine (**16i**)*

Method B (82% yield). Oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.01 (dd, 1H, J = 6.8–1.2 Hz, H-5), 7.85–7.81 (m, 2H, BiPh), 7.75–7.68 (m, 3H, H-8, F–Ph-2,4), 7.58 (dd, 1H, J = 8.6–7.7 Hz, Ph-5), 7.51 (dd, 1H, J = 1.8–0.7 Hz, Fur-5), 7.33 (dt, 1H, J = 7.7–1.3 Hz, Ph), 7.26 (dd, 1H, J = 9.1–6.8 Hz, H-7), 7.01 (t, 2H, J = 8.9 Hz, F–Ph-3,5), 6.79 (td, 1H, J = 6.8–1.2 Hz, H-6), 6.72 (dd, 1H, J = 3.4–0.7 Hz, Fur-3), 6.52 (dd, 1H, J = 3.4–1.8 Hz, Fur-4). ^{13}C NMR (50 MHz, $CDCl_3$) δ 162.9 (J = 245 Hz), 153.6, 145.4, 143.2, 142.1, 132.9, 130.8, 130.7, 130.3 (J = 8 Hz), 130.2, 126.3, 125.6, 124.9, 124.0, 121.1, 118.0, 115.9 (J = 21 Hz), 113.1, 112.5, 106.5. Anal. Calcd for $C_{23}H_{15}FN_2O$: C, 77.95; H, 4.27; N, 7.90. Found: C, 78.04; H, 4.21; N, 7.88.

*5.2.66. 3-[3-(Fur-3-yl)phenyl]-2-(4-fluorophenyl)imidazo[1,2-a]pyridine (**16j**)*

Method B (94% yield). Mp 121–122 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.98 (d, 1H, J = 6.9 Hz, H-5), 7.75–7.64 (m, 5H, F–Ph-2,6, H-8, Ph), 7.60 (m, 1H, Fur-2), 7.54 (t, 1H, J = 7.7 Hz, Ph-5), 7.48 (m, 1H, Fur-4), 7.33 (dt, 1H, J = 7.3–1.6 Hz, Ph), 7.21 (ddd, 1H, J = 9.0–6.9–0.8 Hz, H-7), 6.99 (t, 2H, J = 8.7 Hz, F–Ph-3,5), 6.75 (t, 1H, J = 6.9 Hz, H-6), 6.68 (dd, 1H, J = 1.8–0.9 Hz, Fur-5). ^{13}C NMR (50 MHz, $CDCl_3$) δ 162.7 (J = 245 Hz), 145.2, 144.4, 142.0, 139.3, 134.3, 130.7, 130.6, 130.1 (J = 8 Hz), 129.6, 128.2, 126.8, 126.1, 125.3, 123.7, 120.9, 117.9, 115.7 (J = 21 Hz), 112.8, 109.0. Anal. Calcd for $C_{23}H_{15}FN_2O$: C, 77.95; H, 4.27; N, 7.90. Found: C, 78.12; H, 4.28; N, 7.91.

*5.2.67. 3-[3-(Thien-3-yl)phenyl]-2-(4-fluorophenyl)imidazo[1,2-a]pyridine (**16k**)*

Method B (89% yield). Mp 159–160 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.04 (d, 1H, J = 6.9 Hz, H-5), 7.79–7.69 (m, 5H, F–Ph-2,6, H-8, Ph), 7.61 (t, 1H, J = 7.5 Hz, Ph-5), 7.51 (dd, 1H, J = 2.7–1.2 Hz, Th-2), 7.47 (m, 3H, Th-4,5, Ph), 7.28 (ddd, 1H, J = 9.0–6.9–1.3 Hz, H-7), 7.02 (t, 2H, J = 8.9 Hz, F–Ph-3,5), 6.81 (td, 1H, J = 6.9–1.1 Hz, H-6). ^{13}C NMR (50 MHz, $CDCl_3$) δ 162.9 (J = 245 Hz), 145.4, 142.1, 141.8, 137.8, 130.9 (J = 3 Hz), 130.8, 130.7, 130.3 (J = 8 Hz), 129.9, 127.5, 127.3, 126.6, 125.5, 123.9, 121.6, 121.2, 118.0, 115.8 (J = 21 Hz), 113.0. Anal. Calcd for $C_{23}H_{15}FN_2S$: C, 74.57; H, 4.08; N, 7.56. Found: C, 74.76; H, 4.16; N, 7.48.

*5.2.68. 3-[3-(Benzo[b]fur-2-yl)phenyl]-2-(4-fluorophenyl)imidazo[1,2-a]pyridine (**16l**)*

Method B (76% yield). Mp 121–122 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.06–8.01 (m, 3H, H-5, Ph-2,4), 7.77–7.60 (m, 5H, F–Ph-2,6, H-8,

Bzfur-4,7), 7.54 (m, 1H, Ph), 7.44 (dt, 1H, J = 7.7–1.3 Hz, Ph-6), 7.38–7.24 (m, 3H, H-7, Bzfur-5,6), 7.09 (d, 1H, J = 0.8 Hz, Fur-3), 7.00 (t, 2H, J = 8.7 Hz, F–Ph-3,5), 6.83 (dd, 1H, J = 6.8–1.1 Hz, H-6). ^{13}C NMR (50 MHz, $CDCl_3$) δ 163.0 (J = 246 Hz), 155.6, 155.4, 145.5, 142.3, 132.6, 131.4, 131.0, 131.9, 130.7 (J = 3 Hz), 130.3 (J = 8 Hz), 129.6, 127.4, 126.0, 125.7, 125.3, 124.0, 123.8, 121.7, 120.8, 118.1, 115.9 (J = 21 Hz), 113.2, 111.9, 102.9. Anal. Calcd for $C_{27}H_{17}FN_2O$: C, 80.18; H, 4.24; N, 6.93. Found: C, 80.46; H, 4.14; N, 7.12.

*5.2.69. 3-[3-(4-Methylpiperazin-1-yl)phenyl]-2-(4-fluorophenyl) imidazo[1,2-a]pyridine (**16m**)*

A screw-cap test tube was charged under argon, with **15a** (184 mg, 0.5 mmol), $Pd_2(dba)_3$ (2.3 mg, 0.5 mol %), BINAP (5 mg, 1.5 mol %), and NaO^{t}Bu (67 mg, 0.7 mmol). A Teflon septum was attached, and the tube was evacuated and backfilled with argon. The evacuated/backfield sequence was repeated an additional time. Then, *N*-methylpiperazine (67 μl, 0.6 mmol) and toluene (2 mL) were added by syringe under argon. The screw-cap test tube was sealed with a cap and the reaction mixture was stirred magnetically at 80 °C for 6 days. After cooling, the suspension was taken up in water, extracted with dichloromethane three times. The organic layer was dried with $MgSO_4$, and evaporated to dryness. The residue was chromatographed on neutral alumina with CH_2Cl_2 to give 81% of **16m**. Mp 132–133 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.96 (dd, 1H, J = 6.9–1.3 Hz, H-5), 7.71 (dd, 2H, J = 8.9–5.5 Hz, F–Ph-2,6), 7.68 (dd, 1H, J = 9 Hz, H-8), 7.44 (t, 1H, J = 7.8 Hz, Ph-5), 7.17 (ddd, 1H, J = 9–6.7–1.3 Hz, H-7), 7.08–6.88 (m, 5H, F–Ph-3,5, Ph), 6.70 (ddd, 1H, J = 6.9–6.7–1.1 Hz, H-6), 3.20 (t, 4H, J = 5.2–4.9 Hz, Pi-2,6), 2.54 (t, 4H, J = 5.2–4.9 Hz, Pi-3,5), 2.32 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 162.8 (J = 245 Hz), 152.7, 145.2, 141.7, 131.0, 130.9, 130.9, 130.2 (J = 8 Hz), 125.3, 124.1, 122.0, 121.9, 118.3, 118.0, 117.0, 115.8 (J = 21 Hz), 112.9, 55.4, 49.2, 46.6. Anal. Calcd for $C_{24}H_{23}FN_4$: C, 74.59; H, 6.00; N, 14.50. Found: C, 74.41; H, 6.28; N, 14.64.

*5.2.70. 3-(2′-Hydroxybiphen-3-yl)-2-(2-methoxyphenyl)imidazo [1,2-a]pyridine (**16n**)*

Method B (75% yield). Mp 234–235 °C. ^{1}H NMR (200 MHz, MeOH) δ 8.26 (d, 1H, J = 6.9 Hz, H-5), 7.52–7.26 (m, 6H), 7.14 (m, 1H, H-7), 7.07–6.85 (m, 4H), 6.78–6.65 (m, 4H, H-6, BiPh-3′,5′, MeOPh-3), 3.22 (s, 3H, CH_3). ^{13}C NMR (50 MHz, MeOH) δ 156.8, 154.1, 144.5, 139.6, 139.5, 131.7, 130.4, 129.8, 129.6, 129.5, 128.9, 128.7, 128.5, 127.8, 127.2, 125.2, 123.6, 123.1, 122.9, 120.5, 119.8, 116.5, 115.8, 112.7, 111.1, 54.4. Anal. Calcd for $C_{26}H_{20}N_2O_2$: C, 79.57; H, 5.14; N, 8.15. Found: C, 79.74; H, 5.22; N, 8.37.

*5.2.71. 3-(3′-Hydroxybiphen-3-yl)-2-(2-methoxyphenyl)imidazo [1,2-a]pyridine (**16o**)*

Method B (75% yield). Mp 274–275 °C. ^{1}H NMR (200 MHz, MeOH) δ 8.11 (d, 1H, J = 6.9 Hz, H-5), 7.42 (d, 1H, J = 9.1 Hz, H-8), 7.38–6.97 (m, 9H), 6.85–6.57 (m, 6H), 3.13 (s, 3H, CH_3). ^{13}C NMR (50 MHz, MeOH) δ 157.9, 157.2, 145.1, 142.5, 142.4, 140.4, 132.3, 131.0, 130.4, 130.3, 129.8, 128.3, 128.1, 127.3, 125.8, 124.0, 123.4, 123.3, 121.2, 118.9, 117.2, 115.0, 114.4, 113.4, 111.6, 55.1. Anal. Calcd for $C_{26}H_{20}N_2O_2$: C, 79.57; H, 5.14; N, 8.15. Found: C, 79.82; H, 5.37; N, 8.12.

*5.2.72. 3-(4′-Hydroxybiphen-3-yl)-2-(2-methoxyphenyl)imidazo [1,2-a]pyridine (**16p**)*

Method B (71% yield). Mp 275–276 °C. ^{1}H NMR (200 MHz, MeOH) δ 8.09 (d, 1H, J = 6.9 Hz, H-5), 7.38–7.15 (m, 6H), 7.10–7.00 (m, 5H), 6.79–6.54 (m, 5H), 3.08 (s, 3H, CH_3). ^{13}C NMR (50 MHz, MeOH) δ 157.4, 145.0, 142.4, 132.2, 130.3, 129.8, 128.5, 127.7, 127.2, 126.8, 126.1, 124.1, 123.6, 121.1, 117.0, 116.1, 113.6, 111.6, 55.0. Anal. Calcd for $C_{26}H_{20}N_2O_2$: C, 79.57; H, 5.14; N, 8.15. Found: C, 79.74; H, 5.07; N, 8.11.

*5.2.73. 3-(3-Bromophenyl)-2-(2-methoxyphenyl)-6-(thien-3-yl) imidazo[1,2-b]pyridazine (**17b**)*

Method C (19% yield). Mp 199–200 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.00 (m, 1H, MeO–Ph-6), 7.99 (d, 1H, J = 9.4 Hz, H-8), 7.84 (dd, 1H, J = 2.8–0.9 Hz, Th-2), 7.71–7.66 (m, 2H, Th-4, Br–Ph-2), 7.53 (m, 1H, Th-5), 7.49–7.37 (m, 4H, H-7, Br–Ph-4,6, MeOPh-4), 7.23 (t, 1H, J = 7.9 Hz, Br–Ph-5), 7.11 (t, 1H, J = 7.4–7.1 Hz, MeOPh-5), 6.91 (d, 1H, J = 8.2 Hz, MeOPh-3), 3.43 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 157.2, 147.9, 142.5, 138.6, 132.6, 132.4, 132.0, 130.9, 130.7, 130.0, 127.8, 127.6, 125.3, 124.0, 122.4, 121.6, 116.6, 111.8, 55.5.

*5.2.74. 3-(3-Bromophenyl)-2-tert-butyl-6-(thien-3-yl)imidazo[1,2-b]pyridazine (**17c**)*

Method C (64% yield). Mp 135–136 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.84 (d, 1H, J = 9.4 Hz, H-8), 7.60–7.57 (m, 2H, Th-2, BrPh-2), 7.52 (dd, 1H, J = 7.3–1.7 Hz, BrPh-6), 7.35 (dd, 1H, J = 5.0–1.2 Hz, Th-4), 7.33–7.24 (m, 3H, H-7, BrPh-4,5), 7.18 (dd, 1H, J = 5.0–2.9 Hz, Th-5), 1.31 (s, 9H, 3 CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 153.4, 146.7, 137.9, 135.9, 134.5, 133.0, 131.4, 130.5, 129.4, 126.4, 125.7, 124.5, 124.1, 122.9, 121.8, 115.3, 33.8, 31.0.

*5.2.75. 3-[3-(Fur-2-yl)phenyl]-2-tert-butyl-6-(thien-3-yl)imidazo [1,2-b]pyridazine (**18a**)*

Method B (97% yield). Mp 152–153 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.01 (d, 1H, J = 9.4 Hz, H-8), 7.85–7.81 (m, 2H, Ph-2,4), 7.73 (dd, 1H, J = 2.9–1.2 Hz, Th-2), 7.58 (t, 1H, J = 7.4 Hz, Ph-5), 7.51 (d, 1H, J = 1.8 Hz, Fur-5), 7.49 (dd, 1H, J = 5.1–1.2 Hz, Th-4), 7.42 (d, 1H, J = 9.4 Hz, H-7), 7.39 (d, 1H, J = 7.4 Hz, Ph-6), 7.32 (dd, 1H, J = 5.1–2.9 Hz, Th-5), 6.73 (d, 1H, J = 3.3 Hz, Fur-3), 6.52 (dd, 1H, J = 3.3–1.8 Hz, Fur-4), 1.43 (s, 9H, 3 CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 153.5, 153.2, 146.8, 142.1, 138.1, 135.9, 131.3, 130.7, 128.4, 127.1, 126.4, 126.0, 124.5, 124.4, 124.0, 123.7, 115.2, 111.6, 105.2, 33.9, 31.1. Anal. Calcd for $C_{24}H_{21}N_3OS$: C, 72.15; H, 5.30; N, 10.52. Found: C, 72.42; H, 5.23; N, 10.62.

*5.2.76. 3-[3-(Fur-3-yl)phenyl]-2-tert-butyl-6-(thien-3-yl)imidazo [1,2-b]pyridazine (**18b**)*

Method B (99% yield). Mp 144–145 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.96 (d, 1H, J = 9.4 Hz, H-8), 7.79 (s, 1H, Fur-2), 7.68 (dd, 1H, J = 2.9–1.2 Hz, Th-2), 7.63–7.58 (m, 2H, Ph-2,4), 7.51 (t, 1H, J = 7.4 Hz, Ph-5), 7.48 (m, 1H, Fur-5), 7.44 (dd, 1H, J = 5.0–1.2 Hz, Th-4), 7.37 (dt, 1H, J = 7.4–1.5 Hz, Ph-6), 7.36 (d, 1H, J = 9.4 Hz, H-7), 7.26 (dd, 1H, J = 5.0–2.9 Hz, Th-5), 6.74 (dd, 1H, J = 1.8–0.9 Hz, Fur-4), 1.41, (s, 9H, 3 CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 153.2, 146.7, 143.6, 138.5, 138.1, 135.9, 132.1, 131.5, 130.4, 129.1, 128.5, 126.4, 126.0, 125.9, 125.8, 124.5, 124.4, 124.0, 115.1, 108.6, 33.9, 31.1. Anal. Calcd for $C_{24}H_{21}N_3OS$: C, 72.15; H, 5.30; N, 10.52. Found: C, 72.28; H, 5.29; N, 10.57.

*5.2.77. 3-(4′-Hydroxybiphen-3-yl)-2-tert-butyl-6-(thien-3-yl) imidazo[1,2-b]pyridazine (**18c**)*

Method B (19% yield). Mp > 300 °C. ^{1}H NMR (200 MHz, DMSO) δ 9.60 (s, 1H, OH), 8.20 (m, 1H, Th-2), 8.17 (d, 1H, J = 9.5 Hz, H-8), 7.77 (d, 1H, J = 9.5 Hz, H-7), 7.77–7.69 (m, 2H, Ph), 7.63 (dd, 1H, J = 4.9–3 Hz, Th-5), 7.59 (m, 1H, Ph), 7.57 (d, 2H, J = 8.4 Hz, HOPh-2,6), 7.43–7.39 (m, 2H, Th-4, Ph), 6.86 (d, 1H, J = 8.4 Hz, HOPh-3,5), 1.32 (s, 9H, 3 CH_3). ^{13}C NMR (50 MHz, DMSO) δ 157.6, 152.6, 147.1, 140.2, 138.1, 135.7, 131.5, 130.6, 130.0, 129.4, 129.0, 128.0, 126.4, 125.9, 125.3, 124.2, 116.1, 34.1, 31.3. Anal. Calcd for $C_{26}H_{23}N_3OS$: C, 73.38; H, 5.45; N, 9.87. Found: C, 73.51; H, 5.39; N, 10.02.

*5.2.78. 3-(3′-Hydroxybiphen-3-yl)-2-tert-butyl-6-(thien-3-yl) imidazo[1,2-b]pyridazine (**18d**)*

Method B (83% yield). Mp 268–269 °C. ^{1}H NMR (200 MHz, DMSO) δ 9.59 (s, 1H, OH), 8.17 (m, 2H, Th-2, H-8), 7.79–7.10 (m, 10H,

H-7, Th-4,5, BiPh), 6.79 (d, 1H, J = 8.0 Hz, BiPh-4′), 1.33 (s, 9H, 3 CH_3). ^{13}C NMR (50 MHz, DMSO) δ 158.2, 152.7, 147.1, 141.4, 140.3, 138.1, 135.8, 131.6, 131.0, 130.4, 130.1, 129.1, 128.0, 127.1, 125.9, 125.3, 124.0, 117.7, 116.2, 115.0, 113.7, 34.1, 31.3. Anal. Calcd for $C_{26}H_{23}N_3OS$: C, 73.38; H, 5.45; N, 9.87. Found: C, 73.45; H, 5.51; N, 9.98.

*5.2.79. 3-(2′-Hydroxybiphen-3-yl)-2-tert-butyl-6-(thien-3-yl) imidazo[1,2-b]pyridazine (**18e**)*

Method B (61% yield). Mp 271–272 °C. ^{1}H NMR (200 MHz, DMSO) δ 9.69 (s, 1H, OH), 8.22 (s, 1H, Th-2), 8.16 (d, 1H, J = 9.4 Hz, H-8), 7.75 (d, 1H, J = 9.4 Hz, H-7), 7.71–7.55 (m, 4H, Th-5, HOPh-6, Ph-2,4), 7.49–7.42 (m, 2H, Th-4, Ph-5), 7.35 (d, 1H, J = 8.2 Hz, Ph-6), 7.19 (t, 1H, J = 7.2 Hz, HOPh-4), 6.99–6.86 (m, 2H, HOPh-3,5), 1.34 (s, 9H, 3CH_3). ^{13}C NMR (50 MHz, DMSO) δ 158.7, 152.7, 147.0, 138.6, 138.1, 135.7, 132.6, 130.6, 130.4, 130.0, 129.4, 129.0, 128.2, 128.0, 127.3, 126.0, 125.9, 125.3, 124.3, 119.9, 116.4, 116.1, 34.1, 31.3. Anal. Calcd for $C_{26}H_{23}N_3OS$: C, 73.38; H, 5.45; N, 9.87. Found: C, 73.55; H, 5.28; N, 9.91.

*5.2.80. 3-[3-(Fur-2-yl)phenyl]-2-(2-methoxyphenyl)-6-(thien-3-yl) imidazo[1,2-b]pyridazine (**18f**)*

Method B (94% yield). Mp = 195–196 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.19 (m, 1H, MeOPh-6), 8.10 (d, 1H, J = 9.4 Hz, H-8), 7.92 (dd, 1H, J = 2.9–1.3 Hz, Th-2), 7.76 (dd, 1H, J = 5.1–1.3 Hz, Th-4), 7.68 (m, 2H, Fur-5, Ph), 7.51 (d, 1H, J = 9.4 Hz, H-7), 7.56–7.38 (m, 5H, MeOPh-4, Ph), 7.11 (t, 1H, J = 7.4 Hz, MeOPh-5), 6.93 (d, 1H, J = 8.2 Hz, MeOPh-3), 6.58 (d, 1H, J = 3.4 Hz, Fur-3), 6.50 (dd, 1H, J = 3.4–1.8 Hz, Fur-4), 3.46 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 156.7, 153.8, 147.2, 141.9, 138.1, 137.7, 131.7, 130.4, 130.0, 129.8, 128.2, 127.5, 126.7, 126.0, 125.6, 125.3, 124.5, 124.0, 123.4, 122.8, 120.8, 115.7, 111.6, 111.1, 104.8, 55.9. Anal. Calcd for $C_{27}H_{19}N_3O_2S$: C, 72.14; H, 4.26; N, 9.35. Found: C, 72.33; H, 4.12; N, 9.41.

*5.2.81. 3-[3-(Fur-3-yl)phenyl]-2-(2-methoxyphenyl)-6-(thien-3-yl) imidazo[1,2-b]pyridazine (**18g**)*

Method B (99% yield). Mp = 196–197 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.07 (d, 1H, J = 9.4 Hz, H-8), 7.86 (m, 2H, Th-2, MeO–Ph-6), 7.73–7.62 (m, 4H, Ph-2,4, Th-4, Fur-2), 7.50–7.40 (m, 6H, H-7, Th-5, Fur-5, Ph-5,6, MeOPh-4), 7.12 (t, 1H, J = 7.5 Hz, MeOPh-5), 6.93 (d, 1H, J = 8.2 Hz, MeOPh-3), 6.60 (s, 1H, Fur-4), 3.44 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 156.7, 147.2, 143.6, 141.5, 138.3, 138.1, 137.7, 131.9, 131.7, 130.1, 129.8, 128.3, 127.0, 126.8, 126.2, 126.0, 125.9, 125.3, 124.8, 124.4, 123.7, 120.8, 115.6, 111.1, 108.5, 54.9. Anal. Calcd for $C_{27}H_{19}N_3O_2S$: C, 72.14; H, 4.26; N, 9.35. Found: C, 72.46; H, 4.08; N, 9.52.

*5.2.82. 3-(4′-Hydroxybiphen-3-yl)-2-(2-methoxyphenyl)-6-(thien-3-yl)imidazo[1,2-b]pyridazine (**18h**)*

Method B (53% yield). Mp 223–224 °C. ^{1}H NMR (200 MHz, DMSO) δ 9.61 (s, 1H, OH), 8.42 (m, 1H, Ph-6), 8.29 (d, 1H, J = 9.5 Hz, H-8), 7.91 (d, 1H, J = 9.5 Hz, H-7), 7.77–7.42 (m, 8H, MeOPh-4, Ph-2,3,4,5, Th-2,4,5), 7.31 (d, 2H, J = 8.4 Hz, HOPh-2,6), 7.09 (m, 2H, MeOPh-3,5), 6.81(d, 2H, J = 8.4 Hz, HOPh-3,5), 3.35 (s, 3H, CH_3). ^{13}C NMR (50 MHz, DMSO) δ 158.2, 157.7, 148.3, 142.2, 140.9, 138.8, 138.4, 132.5, 131.7, 131.0, 130.8, 129.7, 129.1, 128.6, 127.3, 127.1, 126.9, 126.7, 126.4, 126.3, 124.9, 121.7, 117.5, 116.8, 112.7, 55.8. Anal. Calcd for $C_{26}H_{23}N_3OS$: C, 73.24; H, 4.45; N, 8.84. Found: C, 73.46; H, 4.39; N, 8.91.

*5.2.83. 3-(3′-Hydroxybiphen-3-yl)-2-(2-methoxyphenyl)-6-(thien-3-yl)imidazo[1,2-b]pyridazine (**18i**)*

Method B (89% yield). Mp 155–156 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.08 (d, 1H, J = 9.6 Hz, H-8), 7.76 (m, 2H, MeOPh-6, Th-2), 7.61–7.16 (m, 10H), 6.98–6.82 (m, 5H), 3.36 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 157.3, 156.8, 147.5, 141.9, 140.5, 140.4, 137.6, 137.3, 131.7, 130.0, 129.7, 129.3, 128.3, 127.5, 126.8, 126.4, 126.3, 125.8, 124.8, 124.6, 122.4, 120.7, 118.2, 116.3, 114.8, 114.2, 111.2, 54.7. Anal. Calcd for $C_{29}H_{21}N_3O_2S$: C, 73.24; H, 4.45; N, 8.84. Found: C, 73.38; H, 4.35; N, 8.92.

*5.2.84. 3-(2′-Hydroxybiphen-3-yl)-2-(2-methoxyphenyl)-6-(thien-3-yl)imidazo[1,2-b]pyridazine (**18j**)*

Method B (67% yield). Mp 257–258 °C. ^{1}H NMR (200 MHz, DMSO) δ 9.52 (s, 1H, OH), 8.37 (m, 1H, Th-2), 8.23 (d, 1H, J = 9.4 Hz, H-8), 7.85 (d, 1H, J = 9.4 Hz, H-7), 7.71–7.33 (m, 8H), 7.11–6.79 (m, 6H, MeOPh-3,4,5, HOPh-3,4,5), 3.29 (s, 3H, CH_3). ^{13}C NMR (50 MHz, DMSO) δ 156.9, 154.6, 147.5, 141.3, 138.7, 138.1, 137.5, 131.7, 130.5, 130.2, 129.5, 129.2, 128.9, 128.2, 127.9, 127.8, 126.7, 126.5, 126.1, 126.0, 125.9, 124.1, 120.8, 119.7, 116.6, 116.3, 112.0, 55.0. Anal. Calcd for $C_{29}H_{21}N_3O_2S$: C, 73.24; H, 4.45; N, 8.84. Found: C, 73.49; H, 4.51; N, 8.74.

5.3. Biology

5.3.1. Cells and viruses

Madin–Darby bovine kidney (MDBK) cells were grown in MEM (Gibco) supplemented with 5% heat-inactivated FCS (Integro). BVDV strain NADL was provided by Dr P Kerkhofs (CODA/SERVA, Ukkel, Belgium) and Huh-5-2 HCV subgenomic replicon-containing cells were provided by Prof R Bartenschlager (University of Heidelberg, Heidelberg, Germany). BPIP-resistant virus, AG110-resistant virus, LZ37-resistant virus were selected as described previously [18,19,21].

5.3.2. Anti-BVDV assay

Assays were performed as described previously [18,19,21]. In brief, MDBK cells were seeded at a density of 5×10^3 per well in 96-well cell culture plates in MEM-FCS. Following 24 h incubation at 37 °C and 5% CO_2, medium was removed and 5-fold serial dilutions of the test compounds were added in a total volume of 100 µl, after which the BVDV inoculum (MOI = 2) was added to each well. This inoculum resulted in a greater than 90% destruction of the cell monolayer after 3 days of incubation at 37 °C. Uninfected cells and cells receiving virus without compound were included in each assay plate. After 5 days, medium was removed, and 90 µl of MEM-FCS supplemented with 10 µl of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium/phenazinemethosulfate (MTS/PMS) solution (Promega, Leiden, The Netherlands) was added to each well. Following a 2 h incubation period at 37 °C, the optical density of each well was read at 490 nm in a microplate reader (signal to noise ratio = 5). The percentage CPE was calculated. The 50% effective concentration was derived from the dose–response curves as the concentration of compound that offered 50% protection of the cells against virus-induced cytopathic effect and was calculated using linear interpolation.

5.3.3. Anti-HCV assay in Huh 5-2 cells

The anti-HCV assay was performed as described previously [27,28]. In brief, Huh 5-2 cells were seeded at a density of 5×10^3 per well in a 96-well white View plate (Packard, Canberra, Canada) in complete DMEM supplemented with 250 µg mL G418. Following incubation for 24 h at 37 °C (5% CO_2), medium was removed and 3-fold serial dilutions in complete DMEM (without G418) of the test compounds were added in a total volume of 100 µl. After 4 days of incubation at 37 °C, cell culture medium was removed and luciferase activity was quantified using the Steady-Glo luciferase assay system (Promega, Leiden, The Netherlands); the luciferase signal was measured using a Luminoskan Ascent (Thermo, Vantaa, Finland). The 50% effective concentration was defined as the concentration of compound that reduced the luciferase signal by 50%.

5.3.4. Cytotoxicity assay

MDBK or Huh 5-2 cells were seeded at a density of 5×10^3 cells per well of a 96-well plate in MEM-FCS; 24 h later, serial dilutions of the test compounds were added. Cells were allowed to proliferate for 3 days at 37 °C, after which the overall cell metabolic activity, which is representative for the number of cells, was quantified by means of the MTS/PMS method (Promega). The 50% cytostatic concentration was defined as the concentration that inhibited the proliferation of exponentially growing cells by 50% and was calculated using linear interpolation.

5.3.5. Molecular modelling

Computational results were obtained using software programs from Accelrys Software Inc. The molecules were built and minimized in molecular package (Discovery Studio® 2.5.5, Accelrys, San Diego, CA) by CHARMm with CFF partial charge estimation method. The 3D structures were generated and optimized using a CHARMm forcefield with a root mean squared (RMS) difference of energy gradient of 0.1 kcal/mol. The GFA model in QSAR protocol was used with a population size of 100 and 5000 maximum generations, in linear model form. The descriptors correlation matrix is given in Supplementary data.

For the 3D-QSAR model, all the structures were superimposed by choosing imidazo-pyridine/pyridazine scaffold, with N-1, N-4, and C-7 as tethers to superimpose. The grid spacing was 2.5 Å. All the parameters have been left to the system defaults.

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Appendix A. Supplementary material

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