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ARTICLE TYPE

Design and Synthesis of Imidazo[1,2-α][1,8]naphthyridine Derivatives as Anti-HCV Agents via Direct C-H Arylation†

Shengdian Huang,[§a] Jie Qing,[§b,c] Shuo Wang,[a] Huan Wang,[a] Linqi Zhang*[b] and Yefeng Tang*[a]

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RO8191 represents a newly identified small-molecule IFN-α-substitute that displays potent anti-HCV activity. In this communication, we reported the design and synthesis of two series of imidazo[1,2-α][1,8]naphthyridine derivatives as RO8191's analogues via direct C-H arylation approach. Notably, by adjusting the reaction conditions, we could achieve the two series of analogues via regioselective single- and double-arylations, respectively. The anti-HCV activities of the synthesized compounds were evaluated with the HCV cell culture system, and the preliminary results showed that some of them displayed promising anti-HCV activities.

Since its discovery in 1980s, hepatitis C virus (HCV) infection has evolved into one of the most aggressive diseases in the world.[1] It is estimated that 170 million individuals are infected with HCV, and many of them developed into severe live diseases, such as chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma, which led to significant annul death.[2] Over the past decades, the combination of pegylated interferon-α (IFN-α) and ribavirin has been used as the standard clinical therapy against chronic HCV infection.[3] However, the efficiency of this therapy is compromised by several issues, including the modest sustained virological response (SVR), lack of compliance and severe side effects.[4] Although two recently approved protease inhibitors, telaprevir and boceprevir, significantly improved the cure rates of HCV infection,[5] many patients cannot tolerate the IFN-based therapy and remain untreated. Thus, there exists a high unmet need to develop IFN-free treatment regimens.

Recently, a small molecule named RO8191 (**1**) was identified by the scientists from Chugai Pharmaceutical Co. Ltd. through high throughput screening, which displays remarkable anti-HCV activity.[6] The mode of action study revealed that RO8191 exerted its anti-viral function by directly interacting with the type 1 IFN receptor to drive IFN-stimulated genes (ISG) expression, which then induced the anti-viral response of innate immune system. Therefore, RO8191 could be potentially utilized as IFN substitute in various IFN-α-based anti-viral regimens.

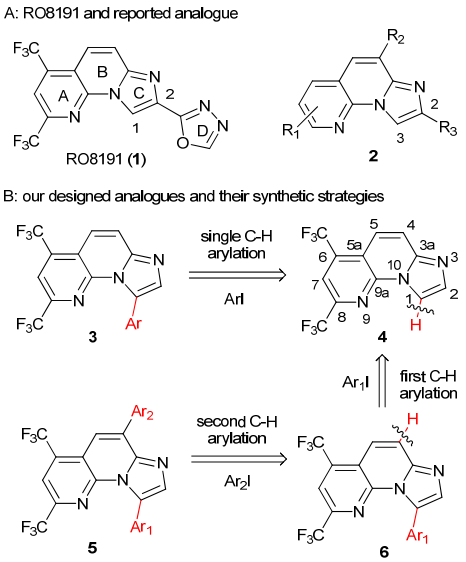

Fig.1 A: Structure of RO8191 and reported analogue (**2**). B: Our designed analogues (**3** and **5**) and their synthetic strategies.

Attracted by its appealing nature, GSK performed a preliminary structure-activity relationship (SAR) study on RO8191. Over 100 analogues were reported, among which the structural variants on the A, B, C and D rings of RO8191 were systematically modified.[7] In parallel with this work, we also launched a program with the long-term objective to develop new leads with more potent anti-HCV activity and favourable drug properties than RO8191. For this end, several series of RO8191 analogues were designed and synthesized,[8] among which, structure **3** was particularly notable, since it bear a hetereocycle moiety on the C1 instead of C2 position of C ring, thus representing a new type of RO8191 analogue that has never been explored so far. Herein we report the efficient synthesis of a library of heterocycles bearing structure **3** via the direct C-H arylation approach. Moreover, we also discovered that simply by adjusting the reaction conditions we could achieve another series of RO8191's analogues, as represented by structure **5**, via

[a]*The Comprehensive AIDS Research Center, and The Department of Pharmacology and Pharmaceutical Sciences, School of Medicine, Tsinghua University, Beijing 100084, China. Fax: +86 10 62797732; Tel: +86 10 62798236; E-mail: yefengtang@tsinghua.edu.cn;*

[b]*The Comprehensive AIDS Research Center, and The Department of Basic Medical Sciences, School of Medicine, Tsinghua University, Beijing 100084, China.*

[c]*Tsinghua-Peking Center for Life Sciences, School of Life Sciences, Tsinghua University, Beijing 100084, P.R. China*

[§]These authors contributed equally to this work

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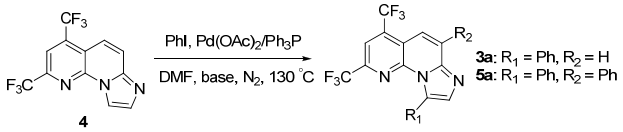
sequential arylations of C1-H and C4-H bonds. The anti-HCV activities of the synthesized compounds were evaluated using HCV cell culture system, which showed that some of them displayed promising results.

Over the past decades, transition metal-catalyzed C-H activation/functionalization has grown into a powerful tool for direct construction of carbon-carbon (C-C) bonds and carbon-heteroatom (C-X) bonds from simple C-H bonds.[9] It not only shortens the steps of synthetic route, but also revolutionizes the ways by which chemists think about the chemical reactivity and contrive the chemical synthesis, and thus it has found increasing applications in both academy and industry.[10] We envisioned that the key structural element of **3**, featured a heterobiaryl scaffold, represented an ideal platform for implementing the direct C-H arylation strategy. Indeed, both intramolecular and intermolecular biaryl couplings via C-H arylation have been well documented, although the intermolecular versions are usually more challenging, mainly because of the inherent reactivity and selectivity issues. In our scenario, although there are multiple C-H bonds (C1-H, C2-H, C4-H, C5-H and C7-H) could be potentially activated, we envisioned that the favourable electronic nature of substrate **4**[11] as well as the potential chelation effect of N-9[12] might facilitate the C-H arylation to occur selectively at the C1 position.

To test the above hypothesis, substrate **4** was prepared according to the literature procedure (see Supporting Information).[11] With **4** in hands, we initiated our study by performing the reaction with the conditions (PhI, $Pd(OAc)_2/Ph_3P$, KOAc, DMF, 130 °C, 3 h) employed in previous studies.[13] To our delight, the desired product **3a** did generate in modest yield (36%, entry 1, table 1), whose structure was unambiguously confirmed by the X-ray crystallographic study (Fig. 2).[14] Notably, increasing the reaction time and temperature had little influence on the outcomes, leaving substantial amount of **4** recovered. Given that the base usually played a crucial role in such type of transformation,[15] we then evaluated several commonly used bases in our case. Gratifyingly, it was shown that while AgOAc, K_3PO_4 and Cs_2CO_3 afforded inferior results (entries 2-4), K_2CO_3 gave a slightly improved yield (45%, entry 5). More strikingly, Ag_2CO_3 displayed superior reactivity to provide **4** in an excellent yield (85%, entry 6). Interestingly, we found that when 2.0 equiv. of PhI and Ag_2CO_3 were employed, a new product (24%) was generated along with **3a** (60%), whose structure was assigned to be the double-arylation product **5a** (entry 7) based on spectroscopic studies (for details, see Supporting Information) and mechanism rationalization.[16] This outcome, albeit unexpected, stimulated us to explore the possibility of accessing another series of RO8191's analogues via the double C-H arylation approach. To achieve this goal, we turned to identify the conditions that could lead to **5a** as the major product. Pleasingly, after several tries, we found that simply increasing the usage of Ag_2CO_3 to 4.0 equivalents could dramatically improve the yield of **5a** to 89%. These discoveries were particularly notable, since it enabled the divergent synthesis of two different series of analogues of RO8191 from a common precursor, simply by adjusting the usage of the base and aryl iodide in the reaction.

Table 1. Condition screening of C-H arylation of **4**



Entry[a,b]	PhI (equiv.)	Base (equiv.)	Products	Yield[c] (%)
1	1.3	KOAc (1.0)	**3a**	36
2	1.3	AgOAc (1.0)	**3a**	30
3	1.3	K_3PO_4 (1.0)	**3a**	21
4	1.3	Cs_2CO_3 (1.0)	**3a**	34
5	1.3	K_2CO_3 (1.0)	**3a**	45
6	**1.3**	**Ag_2CO_3 (1.0)**	**3a**	**85**
7	2.0	Ag_2CO_3 (2.0)	**3a**	60
			5a	24
8	**2.0**	**Ag_2CO_3** (4.0)	**5a**	**89**

[a]10% $Pd(OAc)_2/Ph_3P$ were used. [b]The reaction was run with 0.3 mmol of **4** in 1.0 ml DMF. [c]Refers to isolated yield.

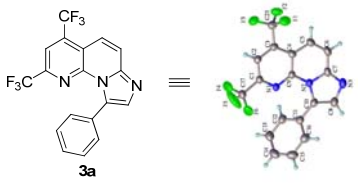
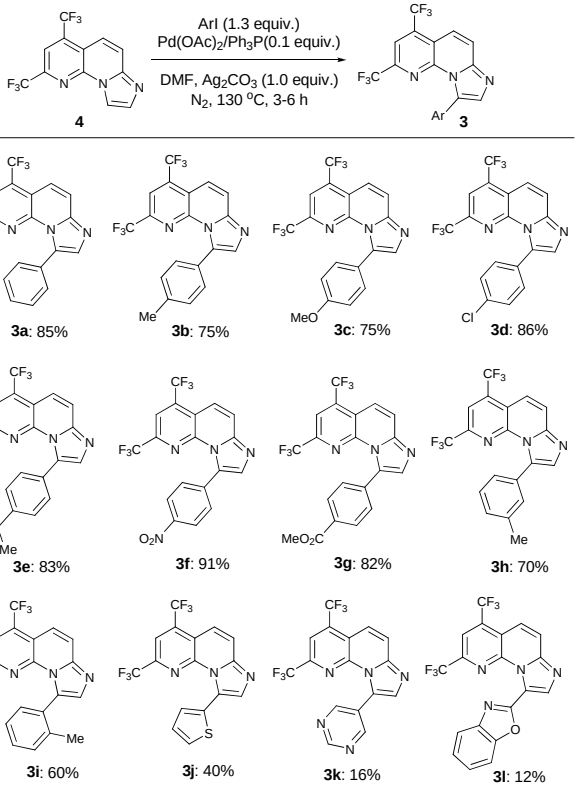


Fig. 2: X-ray crystal structure of **3a** with ellipsoids set at 50% probability

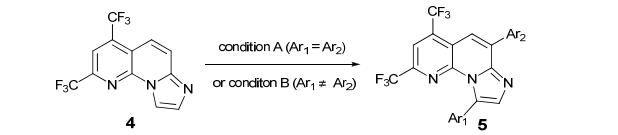
Table 2. Scope of single C-H arylation[a,b]



[a]The reaction was run with 0.164 mmol of **4** in 1.0 ml DMF. [b]Refers to isolated yield.

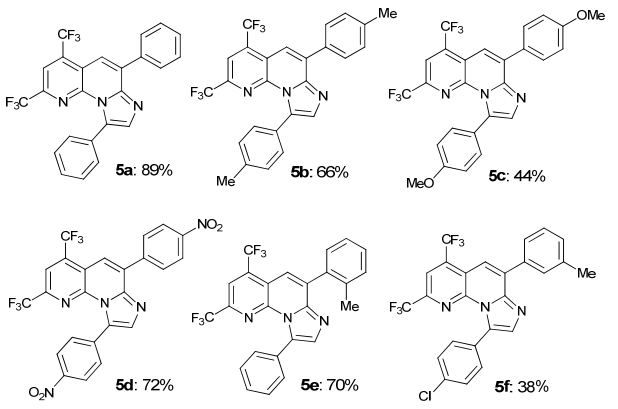
With the optimized conditions in hand, we then turned to explore the generality of the above transformations. First of all, the single C-H arylation was examined. As shown in Table 2, various aryl iodides bearing electron–withdrawing or electron-donating groups could undergo the desired transformations smoothly, affording the corresponding products **3a-g** in good to excellent yields. Generally, the electron-deficient substrates (4-Cl, 4-$COCH_3$, 4-NO_2 and 4-CO_2CH_3, **3d-g**) provided better results than those electro-rich ones (4-CH_3 and 4-CH_3O, **3b-c**). In addition, it was found that the steric effect had some influences on the results, with the *ortho*-substituted substrate **3i** giving a lower yield than the corresponding *para*- or *meta*-substituted ones (**3b** and **3h**). To further extend the substrate scope, some heterocyclic aryl iodides were also tried in the reaction, including 2-iodothiophene, 5-iodopyrimidine and 2-iodobenzo[d]oxazole. However, only moderate to low yields of the corresponding products (**3j-l**) were obtained.

Table 3. Scope of double C-H arylation[a-c]



Condition A: ArI (2.0 equiv.), $Pd(OAc)_2/Ph_3P$ (0.1 equiv.), Ag_2CO_3 (4.0 equiv.), DMF, 130 °C, 6-12 h

Condition B: Ar_1I (1.3 equiv.), $Pd(OAc)_2/Ph_3P$ (0.1 equiv.), Ag_2CO_3 (1.0 equiv.), DMF, 130 °C, 3 h; then Ar_2I (2.0 equiv.), Ag_2CO_3 (2.0 equiv.), 6-12 h



[a]The reaction was run with 0.164 mmol of **4** in 1.0 ml DMF. [b]Condition A were employed for synthesis of **5a-d** and condition B for **5e-f**; [c]Refers to isolated yield.

With the synthesis of mono-arylation product **3** secured, we next moved to synthesize the second series of analogues **5** via double C-H arylations. As shown in table 3, under the optimal conditions (entry 8, Table 1), several substituted aryl iodides, including 4-CH_3-, 4-CH_3O- and 4-NO_2-iodobenzenes, underwent the double arylations smoothly to afford the corresponding products **5b-d** in good to excellent yields. Notably, this transformation could be further extended to the synthesis of those compounds bearing two different aryl moieties on the C-1 and C-4 position. As the proof-of-concept cases, **5e** and **5f** were prepared by employing a slightly modified procedure, in which the two different aryl iodides were sequentially introduced into the reaction mixtures (for details, see Supporting Information). Notably, the operationally simple, one-pot double C-H arylations could be potentially applied to the rapid generation of a library of RO8191's analogues with remarkably structural complexity and diversity, which is very crucial for the further biomedical investigations.[17]

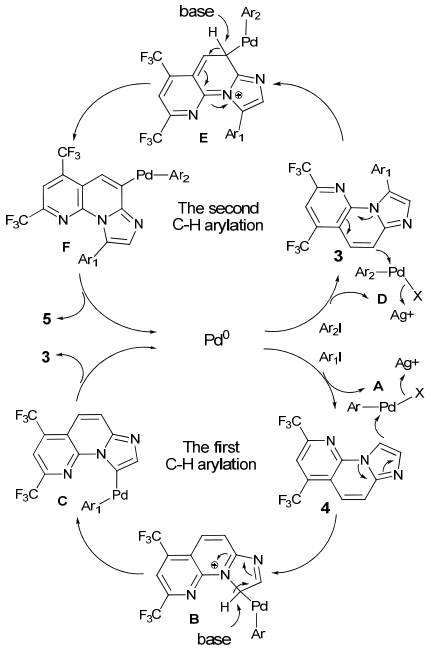


Fig. 3: The proposed mechanisms of single- and double C-H activation/arylations

On the basis of the aforementioned experimental results, the plausible mechanisms of the titled transformations are proposed, as depicted in the Figure 3. Thus, oxidative addition of Pd(0) with Ar_1I afforded the aryl-palladium halide species **A**, which then underwent the electrophilic palladation with **4** to generate the intermediate **B**. Deprotonation of **B** with the action of base led to the formation of **C**, which subsequently underwent the reductive elimination to give the single-arylation product **3** and Pd(0) catalyst. Apparently, when the second Ar_2I and excess amounts of base were employed, **3** could further undergo the second C-H arylation on C-4 position to provide **5**, via a similar catalytic cycle as described above.

Table 4. Anti-viral activity of **3a-l** and **5a-f** against HCV genotype 2a JFH-1 virus

No. of compounds	EC_{50} (μM)[a,b]	No. of compounds	EC_{50} (μM)[a,b]
3a	12.0	**3k**	>20
3b	9.9	**3l**	>20
3c	10.3	**5a**	>20
3d	11.1	**5b**	>20
3e	12.0	**5c**	4.1
3f	8.2	**5d**	>20
3g	13.8	**5e**	>20
3h	2.6	**5f**	>20
3i	2.8	**RO8191**	0.18
3j	8.2		

[a] Inhibitory concentration that reduced viral replication by 50%. B Each data point represents the average of three replicates in cell culture and the values of EC_{50} was plotted by the GraphPad Prism 5 software.

To determine the inhibitory activities of the two series of RO8191 analogues, we prepared the HCV cell culture system (HCVcc-hRluc-JFH1), HCV genotype 2a JFH-1 virus containing a humanized Rellina luciferase reporter gene (for experimental details, see Supporting Information).[18] The anti-HCV activities of the synthesized analogues were then evaluated using the HCV cell culture system, with RO8191 as positive control. The results were summarized in Table 4. As shown, most of the single arylation products (**3a-i**) displayed moderate inhibitory activity against HCV (JFH-1, genotype 2a), with EC_{50} values ranged from 2.8 to 13.8 μM. Among them, **3h** and **3i**, which bear meta- or otho-substituted aryl moiety, exhibited more potent activities than the para-substituted compounds (**3a-g**). Unexpectedly, **3j-l**, which have heterocyclic moiety on the C1 position, displayed lower or no anti-viral activity. Furthermore, except for **5c**, all of the double arylation products (**5a**, **5b** and **5d-f**) were proved to be inactive at 20 μM concentration.

Conclusion

In summary, we have developed an efficient approach for the synthesis of two series of imidazo[1,2-a][1,8]naphthyridine derivatives as the analogues of RO8191, a newly discovered small-molecule IFN-α-substitute. The key element of the approach features a novel Pd-catalyzed regioselective single- or double-arylation. Preliminary biological evaluations revealed that some of the synthesized compounds displayed promising anti-HCV activities. Our investigations enrich the structure-activity relationship study on RO8191, and provide informative clue for the design and synthesis of next generation of RO8191's analogues as anti-HCV agents, which is underway in our laboratory and will be reported in due course.

Acknowledgements

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Graphic Abstract

Two series of analogues of RO1891, a potent anti-HCV agent, were synthesized via Pd-catalyzed regioselective single and double C-H arylations, respectively.