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Article

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# Discovery of chromane containing hepatitis C virus (HCV) NS5A inhibitors with improved potency against resistance-associated variants

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**ABSTRACT:** The discovery of potent and pan-genotypic HCV NS5A inhibitors faces many challenges including the significant diversity among genotypes, substantial potency shift conferred on some key resistance-associated variants, inconsistent SARs between different genotypes and mutants, and the lacking of models of inhibitor/protein complexes for rational inhibitor design. As part of ongoing efforts on HCV NS5A inhibition at Merck, we now describe the discovery of a novel series of chromane containing NS5A inhibitors. SAR studies around the "Z" group of the tetracyclic indole scaffold explored fused bicyclic rings as alternates to the phenyl group of elbasvir (**1**, MK-8742) and identified novel chromane and 2,3-dihydrobenzofuran derivatives as "Z" group replacements offered good potency across all genotypes. This effort, incorporating the C-1 fluoro substitution at the tetracyclic indole core, led to the discovery of a new series of NS5A inhibitors, such as compound **14** and **25-28**, with significantly improved potency against resistance-associated variants, such as GT2b, GT1a Y93H, and GT1a L31V. Compound **14** also showed reasonable PK exposures in preclinical species (rat and dog).

## INTRODUCTION

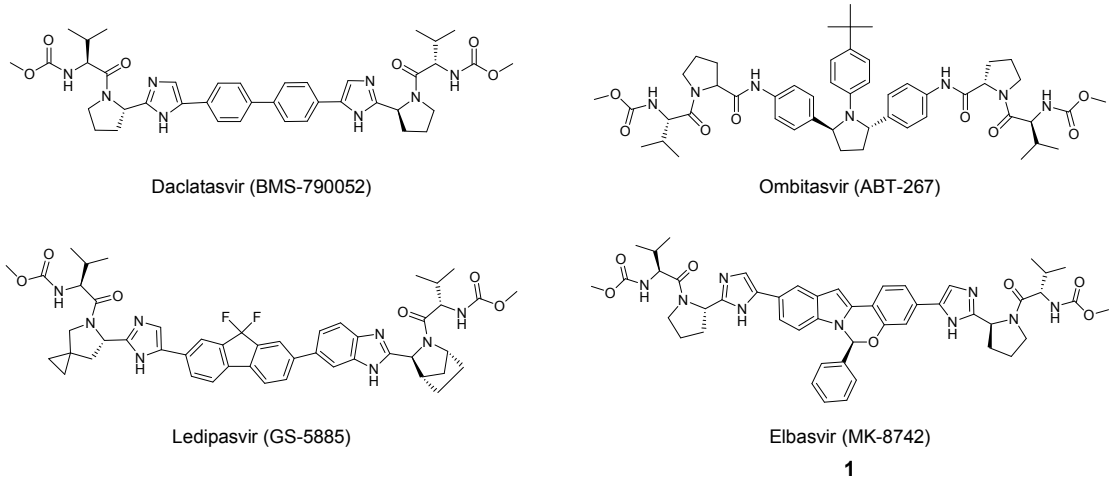
Hepatitis C virus (HCV) infection comprises both acute and chronic forms that result from contact with infected blood in most cases. Approximately 20% of infections are spontaneously cleared within 6 months with no symptoms; however, the majority lead to chronic infections with dire consequences over a period of 25-30 years.[1] The chronic infection often leads to liver diseases including fibrosis, cirrhosis, and ultimately hepatocellular carcinoma. It is estimated that 130-170 million people worldwide are infected with HCV, with 9 million in the United States and western Europe.[2,3] Currently 40-50% of all liver transplants in the United States are due to HCV infection.[4] Globally approximate 500,000 individuals die every year from HCV related liver diseases.[2] Until 2013, treatment of HCV infection was focused on interferon/ribavirin based therapy and was limited by both efficacy (~50% cure rate) and tolerability.[5] Addition of HCV protease inhibitors, such as telaprevir or boceprevir, to the therapy improved cure rates to 70~80% for genotype 1 patients,[5] but tolerability issues, such as flu-like symptoms, still remained due to the retention of interferon. Current HCV therapy with interferon-free, all oral direct-acting antiviral agents (DAAs) has significantly improved on efficacy, treatment duration, and adverse effects. Each DAA targets at one of the HCV nonstructural (NS) proteins that are important in replication including the NS3/4A protease, NS5A phosphoprotein,[6-8] and NS5B polymerase.

NS5A has no known enzymatic activity, but it is essential for HCV RNA replication[7] and virus assembly.[9] NS5A inhibition is therefore an important component in regimens for the treatment of HCV infection. However, early NS5A inhibitors suffered from a low genetic barrier to resistance and demonstrated weak activity against non-GT1 and GT1a resistance-associated variants (RAVs).[10] Subsequent inhibitors have improved activity against RAVs; however, the need for next-generation NS5A inhibitors with increased potency against all genotypes as well as NS5A RAVs are needed.[11] To date, five NS5A inhibitors have been approved for HCV treatment, either alone or in combination with

other DAAs: daclatasvir (BMS-790052),[10,12] ledipasvir (GS-5885),[13] ombitasvir (ABT-267),[14] elbasvir (**1**, MK-8742),[15,16] and velpatasvir (GS-5816).[17]

HCV has seven major genotypes (GT1-7, GT1-3 account for 70-90% of all HCV infections depending on the region), with multiple subtypes identified for each.[18-20] Optimization of the potency profiles of NS5A inhibitors against broad genotypes and mutants faces unique challenges owing to this significant diversity among genotypes and the substantial fold of losses in potency conferred by several key clinically-relevant RAVs. The absence of robust biochemical assays and models of inhibitor/NS5A protein complexes provided additional challenges to the medicinal chemistry efforts. SAR studies were therefore primarily driven by potency data generated in replicon cells. SARs may not transfer between different genotypes/mutants and often SARs improving the potency against one genotype/mutant may hurt the potency against others. Previous research efforts at Merck have identified the tetracyclic indole-based inhibitor elbasvir (**1**) shown in Figure 1.[15,16] Continued efforts sought to explore the impact of structural changes to compound **1**, with the aim of determining if an inhibitor with a 'flat' potency profile could be obtained. In particular, a "flat" potency profile is defined by a minimal potency shift (~10x) between GT1a and other genotypes and RAVs, such as GT2b (bearing the resistance-associated methionine at position 31), GT1a Y93H, and GT1a L31V, which were the least sensitive RAVs of compound **1**. Due to the lack of X-ray structures of inhibitor/protein complexes, rational designs of NS5A inhibitors are not possible. Our strategy was to explore every region of the structure of compound **1** and identify SARs that could improve the potency against GT2b, GT1a Y93H, and GT1a L31V together, and maintain the good potency against the other genotypes and mutants already achieved by compound **1**. Towards this end, we have reported SAR studies directed toward the "core",[21-24] "Z" group,[25,26] "cap",[27] and proline[28] modifications (Figure 2). SAR studies around the "Z = aryl groups"[26] yielded NS5A inhibitors with reduced potency shifts from GT1a to GT2b, GT1a Y93H, and GT1a L31V which had not been observed in our efforts around the alternative "core", "Z = alkyl group",[25] "cap", and proline modifications, clearly indicating the value of "Z =

aromatic group" plays in the potency profiles of the tetracyclic indole based NS5A inhibitors. In a different study, we identified another important SAR that the C-1 fluoro substitution on the tetracyclic indole core could improve the potency against both GT2b and GT1a Y93H simultaneously.[29] These key SAR achievements overcame some of the challenges we mentioned above and set the foundation and direction for our future efforts. Although compounds with "flat" or near "flat" potency profiles have not been identified, we continued our efforts around the "Z group" modifications with the C-1 fluoro substituted tetracyclic indole core (Figure 2), and these efforts have led to the discovery of more potent HCV NS5A inhibitors with significantly reduced potency shift against NS5A RAVs. In this report, we disclose a novel series of chromane containing NS5A inhibitors with near "flat" potency profiles as well as reasonable PK exposure in both rat and dog.




**1**

**Figure 1**. Approved HCV NS5A inhibitors.

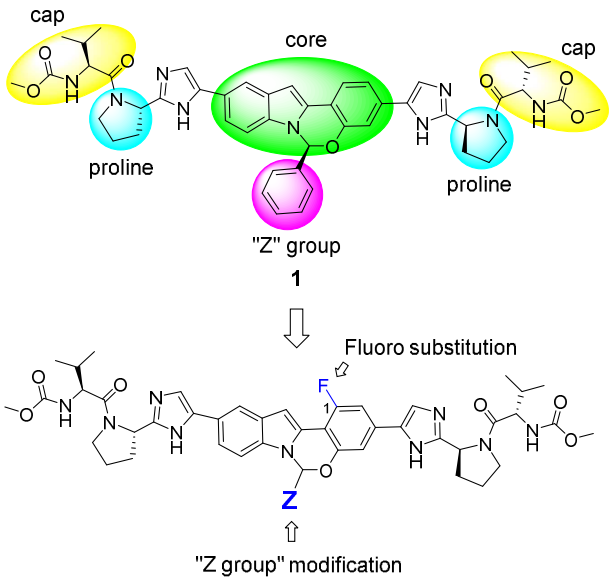



**Figure 2**. "Z group" modification strategy using the C-1 fluoro substituted tetracyclic indole core.

## RESULTS AND DISCUSSION

During our previous SAR studies, efforts trying to differentiate the meta- and para-substitution on the phenyl group of compound **1** led to the conclusion that substitutions at both positions were tolerated.[26] For example, both compounds **2a** and **2b** (Figure 3) showed good potency against all genotypes tested (Table 1), indicating good tolerance of biaryl rings in this region. With these data in hand, we sought to explore the impact of 3,4-fused bicyclic groups, as "Z groups", with the goal of improving the potency against RAVs (Figure 3).

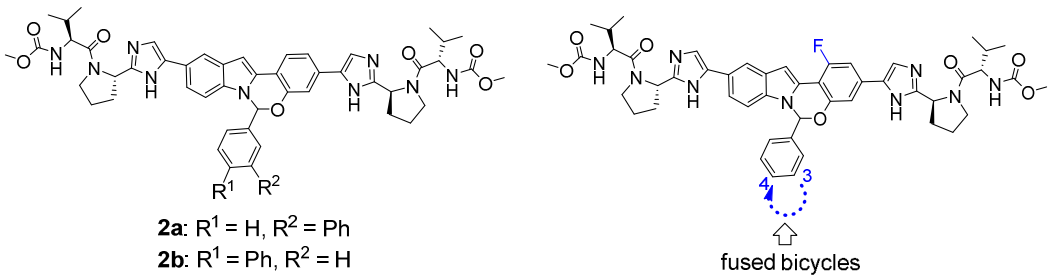



**Figure 3**. Bi-phenyl substituted aminal derivatives of compound **1** and strategy for fused bicyclic group substitutions.

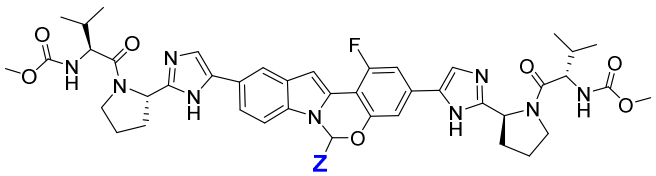
Table 1. *In vitro* potency profiles of compounds **1**-**2b**.

| ID | Replicon $EC_{90}$ (nM) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | GT1a | GT1b | GT2a | GT2b | GT3a | GT4a | GT1a Y93H | GT1a L31V |
| **1** | 0.006 | 0.006 | 0.019 | 11 | 0.12 | 0.016 | 28 | 1 |
| **2a**[a] | 0.005 | 0.006 | 0.004 | 3.8 | 0.3 | 0.02 | 21 | 1 |
| **2b**[a] | < 0.02 | < 0.02 | < 0.2 | 2.6 | < 0.2 | < 0.02 | 4.6 | < 2 |

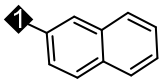
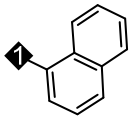
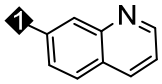
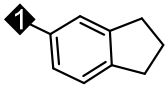
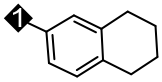
[a]Mixture of diastereomers at the aminal carbon.

Compounds with different fused bicyclic rings as "Z group" were prepared and their *in vitro* potency profiles against selected replicons are summarized in Table 2. As anticipated, naphthalen-2-yl derivative **3** showed good potency against all genotypes tested. In contrast, naphthalen-1-yl derivative **4**, with a second phenyl ring fused at the ortho and meta position, showed reduced potency against both GT2b and GT1a Y93H, confirming the limited space around the ortho position of the phenyl group that was also noticed previously.[26] Introducing a nitrogen to the distal phenyl ring of the naphthalen-2-yl group led to the quinolin-7-yl analog **5**, which was similar in activity to compound **3**, except for a ~10x loss in GT3a potency. Fused alkyl rings were also introduced between the meta and para positions of the phenyl group. Both the 2,3-dihydro-1H-inden-5-yl derivative **6** and the 5,6,7,8-tetrahydronaphthalen-2-yl derivative **7** showed good potency against all genotypes tested, with their potency profiles similar to compound **3**.

Table 2. Bicyclic ring substituted aminal derivatives of compound **1**




| ID[a] | Z | Replicon $EC_{90}$ (nM) | | | | |
|---|---|---|---|---|---|---|
| | | GT1a | GT2b | GT3a | GT1a Y93H | GT1a L31V |

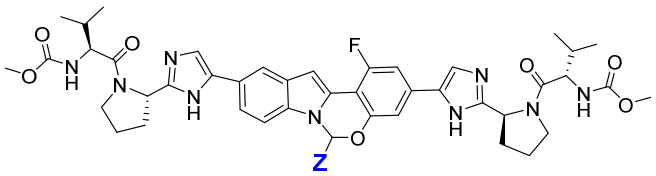
| | | | | | | |
|---|---|---|---|---|---|---|
| **3** |  | 0.02 | 1 | 0.04 | 7 | 0.2 |
| **4** |  | 0.01 | 31 | 0.1 | 32 | 0.09 |
| **5** |  | 0.01 | 3 | 0.3 | 4 | 0.2 |
| **6** |  | 0.004 | 3 | 0.01 | 2 | nt[b] |
| **7** |  | 0.005 | 2 | 0.01 | 2 | nt |

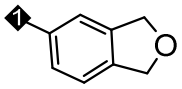
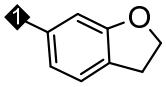
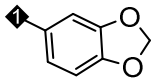
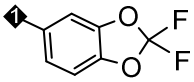
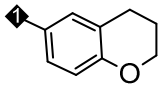
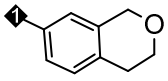
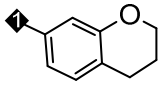
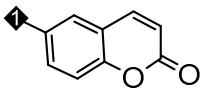
[a]Active isomer at the aminal carbon. [b]nt = not tested.

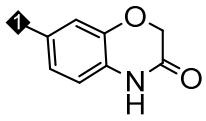
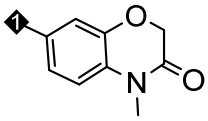
Encouraged by the data in Table 2, we incorporated a SAR identified from earlier studies into the fused Z-groups shown above. Previously, we determined that alkoxy substitutions on either the meta- or para-position of the phenyl group could improve the potency against GT2b and GT1a Y93H.[26] Thus, we incorporated oxygen atom(s) into the distal ring of the fused "Z groups" found in compounds **6** and **7**. Incorporating one oxygen atom into the cyclopentyl ring of compound **6** yielded derivatives **8** and **9** (Table 3), with both compounds showing potency similar to compound **6**. Benzo[d][1,3]dioxole derivative **10** lost 10-fold of its potency against GT3a than compound **9**. Interestingly, introducing a 2,2-difluoro substitution to the benzo[d][1,3]dioxole moiety brought the potency against GT3a back, and compound **11** is similar in activity to compound **9** against all the genotypes tested. Introducing one oxygen atom to the cyclohexyl group of compound **7** produced chromane or isochromane derivatives **12-14**. While compounds **12** and **13** showed similar potency to compound **7**, compound **14** showed improved potency against GT2b, GT1a Y93H, and GT1a L31V**,** with all $EC_{90}$ values obtained in the sub-nM range. This provided the impetus to introduce other bicyclic groups with more structure diversity. Compound **15**, which contains a 2H-chromen-2-one moiety, was 10x weaker in potency

against GT1a Y93H than **14**, and so was the 2H-benzo[b][1,4]oxazin-3(4H)-one derivative **16**. Introducing a methyl group to the amide nitrogen of **16** produced **17**, which was more potent against GT2b, GT3a, and GT1a Y93H than **16**.

Table 3. Oxygenated-bicyclic ring substituted aminal derivatives of compound **1**




| ID[a] | Z | Replicon $EC_{90}$ (nM) | | | | |
|---|---|---|---|---|---|---|
| | | GT1a | GT2b | GT3a | GT1a Y93H | GT1a L31V |
| **8** |  | 0.003 | 2 | 0.02 | 5 | nt[b] |
| **9**[c] |  | 0.02 | 3 | 0.1 | 6 | 0.5 |
| **10** |  | 0.008 | 3 | 1 | 4 | 0.2 |
| **11** |  | 0.004 | 5 | 0.1 | 8 | 0.5 |
| **12** |  | 0.003 | 7 | 0.02 | 4 | nt |
| **13** |  | 0.003 | 4 | 0.02 | 2 | nt |
| **14** |  | 0.003 | 0.3 | 0.01 | 0.5 | 0.02 |
| **15** |  | 0.008 | 10 | 0.1 | 8 | nt |

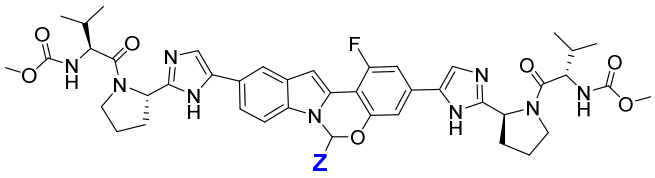
| | | | | | | |
|---|---|---|---|---|---|---|
| **16** |  | 0.01 | 22 | 0.4 | 32 | nt |
| **17** |  | 0.002 | 2 | 0.01 | 3 | nt |

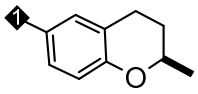
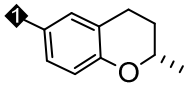
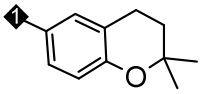
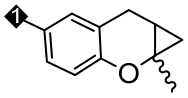
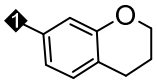
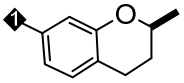
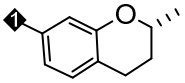
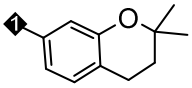
[a]Active isomer at the aminal carbon unless noted separately. [b]nt = not tested. [c]Mixture of diastereomers at the aminal carbon.

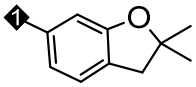
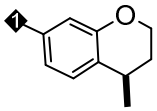
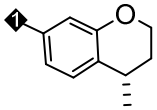
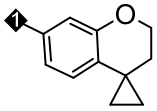
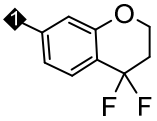
Given the encouraging profile of compounds such as **14**, we explored minor modifications around chromane and 2,3-dihydrobenzofuran moieties, aiming to further improve the potency and block any potential metabolism. Compounds with substituted chromane or 2,3-dihydrobenzofuran were prepared and their *in vitro* potency profiles are summarized in Table 4. Introducing a methyl group to the C-2 position of compound **12** produced two diastereomers, compounds **18** and **19**, which are similar in activity to **12**, and so was the corresponding gem-di-Me analog **20**. Introducing a fused cyclopropyl and a methyl group to **12** led to compound **21,** which showed potency similar to **12** as well. It seems that methyl substitutions around the chromane moiety of compound **12** had minimum effect on the potency; we turned our attention to the modification of compound **14**. A similar SAR was observed for methylations at the C-2 position of the chromane moiety of **14**. C-2 mono-methylation derivatives **22** and **23**, as well as the corresponding gem-dimethyl derivative **24** all showed similar or slightly weaker potency profiles than **14**. However, the 2,2-dimethyl-2,3-dihydrobenzofuran derivative **25** showed improved potency against GT2a and GT1a Y93H, as compared to compound **24**. The C-4 methylated chromane derivatives (compounds **26** and **27)** of **14** may have slightly better potency against GT1a Y93H than **14**, even though the differences are within the assay variation. This potency improvement against GT1a Y93H was also observed for the corresponding spiro-cyclopropyl analog **28**, which was 5x more potent against GT1a Y93H than **14**. Replacing the spiro-cyclopropyl group of

compound **28** with a di-fluoro group leads to compound **29**, which is similar in activity to compound **28**, except a 7x loss in activity against GT1a Y93H.

Table 4. Substituted chromane and 2,3-dihydrobenzofuran containing NS5A inhibitors




| ID[a] | Z | Replicon $EC_{90}$ (nM) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | GT1a | GT1b | GT2b | GT3a | GT4a | GT1a Y93H | GT1a L31V |
| **18**[b] |  | 0.005 | Nt[c] | 7 | 0.02 | nt | 2 | nt |
| **19**[b] |  | 0.004 | nt | 7 | 0.01 | nt | 3 | nt |
| **20** |  | 0.005 | nt | 2 | 0.03 | nt | 0.9 | nt |
| **21**[d] |  | 0.005 | nt | 5 | 0.03 | nt | 1 | nt |
| **14** |  | 0.003 | 0.003 | 0.3 | 0.01 | 0.002 | 0.5 | 0.02 |
| **22**[b] |  | 0.007 | 0.005 | 0.7 | 0.009 | 0.007 | 0.9 | 0.04 |
| **23**[b] |  | 0.003 | 0.005 | 0.9 | 0.01 | 0.005 | 0.7 | 0.02 |
| **24** |  | 0.005 | nt | 2 | 0.02 | nt | 1 | nt |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **25** |  | 0.003 | 0.004 | 0.5 | 0.005 | 0.003 | 0.2 | 0.02 |
| **26**[b] |  | 0.004 | 0.002 | 0.2 | 0.006 | 0.003 | 0.2 | 0.007 |
| **27**[b] |  | 0.004 | 0.002 | 0.2 | 0.01 | 0.003 | 0.2 | 0.01 |
| **28** |  | 0.005 | 0.004 | 0.2 | 0.01 | 0.006 | 0.1 | 0.006 |
| **29** |  | 0.002 | 0.002 | 0.2 | 0.007 | 0.002 | 0.7 | 0.01 |

[a]Active isomer at the aminal carbon. [b]Absolute stereochemistry at the chromane ring assigned arbitrarily. [c]nt = not tested. [d]Mixture of diastereomers at the chromane ring.

Using the $EC_{90}$ values in Table 4, the calculated potency shifts from GT1a for selected compounds are listed in Table 5. Compound **14** showed < 10-fold of shift for genotypes 1b, 3a, 4a, and L31V. Its potency shift for GT2b and GT1a Y93H from GT1a are 100x and 167x, respectively. Compound **25** showed similar potency shift to **14**. The potency shift of compounds **26** and **27** against both GT2b and GT1a Y93H were improved to 50x. Compound **28** showed the most "flat" potency profile among these compounds with potency shifts against GT2b and GT1a Y93H of 40x and 20x, respectively.

Table 5. Calculated potency shifts from GT1a $EC_{90}$ for compounds **14** and **25-28**.

| ID | Calculated potency shifts relative to GT1a $EC_{90}$ | | | | | | |
|---|---|---|---|---|---|---|---|
| | GT1a | GT1b | GT2b | GT3a | GT4a | GT1a Y93H | GT1a L31V |
| **1** | 1x | 1x | 1800x | 20x | 3x | 4700x | 167x |
| **14** | 1x | 1x | 100x | 3x | 1x | 167x | 7x |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **25** | 1x | 1x | 167x | 2x | 1x | 67x | 7x |
| **26** | 1x | 1x | 50x | 2x | 1x | 50x | 2x |
| **27** | 1x | 1x | 50x | 3x | 1x | 50x | 3x |
| **28** | 1x | 1x | 40x | 2x | 1x | 20x | 1x |

With the encouraging potency improvement against RAVs as shown in Table 5, we turned our attention to the pharmacokinetic (PK) profiles of these compounds. The rat or dog PK profiles of selected compounds are listed in Table 6. Compound **14** showed good po (oral) AUC (area under curve), low total clearance, reasonable half-life and $C_{24h}$ in both preclinical species. Although its bioavailability in rat is low, it was higher (16%) in dog. Due to its sub-nM $EC_{90}$ values against all the genotypes tested, we believe the PK profile of compound **14** is acceptable. Compounds **25**, which has a 2,2-dimethyl-2,3-dihydrobenzofuran moiety, showed reduced exposure in rat relative to compound **14**. Compounds **28**, which has a spiro-cyclopropyl substituent at the C-4 of the chromane moiety, showed much reduced AUC (po), $C_{max}$, and $C_{24h}$ than Compound **14**, potentially due to the increased total clearance. Compound **14** was tested in an oral single rising dose PK study (Figure 4), and the results are summarized in Table 7. In this study, compound **14** showed a good linear dose-exposure relationship between 10 mpk and 100 mpk. However, when the dose was increased to 300 mpk, no additional improvement on the exposure of **14** was observed, indicating the exposure reached a plateau between 100-300 mpk. We also noticed a 3-fold difference between the AUC (po) at 10 mpk obtained in the screening PK study (Table 6) and the single rising dose PK study (Table 7). Since the protocols of both PK studies were the same, this exposure variation was potentially caused by the salt form difference of the samples used in the PK studies.

Table 6. Pharmacokinetic profiles of compounds **14**, **25**, and **28**[a,b]

| | IV | PO |
|---|---|---|

| ID | Species | Dose (mpk) | $T_{1/2}$ (h) | CL (mL/min/Kg) | Dose (mpk) | AUC (μM.h) | $C_{max}$ (nM) | $C_{24h}$ (nM) | F% |
|---|---|---|---|---|---|---|---|---|---|
| **14**[c] | Rat | 2 | 5.8 | 8 | 10 | 1.35 | 86 | 17 | 5 |
| **25**[c] | Rat | 2 | 8.4 | 7 | 10 | 0.71 | 58 | 9 | 3 |
| **28**[c] | Rat | 2 | 8.5 | 15 | 10 | 0.24 | 29 | 1 | 2 |
| **14**[d] | Dog | 1 | 11.4[e] | 2 | 5 | 6.92 | 1224 | 51 | 16 |

[a]Male Wistar Han rats or Beagle dogs were used. Compound was dosed IV in 60% PEG200 and PO in 10% TWEEN. [b]For the PK profile of compound **1**, see reference 15. [c]2TFA salt. [d]2HCl salt. [e]IV mean residence time.

Table 7. Rat oral single rising dose PK profile of compound **14**[a,b]

| Dose (mpk) | AUC (μM.h) | $C_{max}$ (μM) | $T_{max}$ (h) | MRT (h) |
|---|---|---|---|---|
| 10 | 0.42 | 0.04 | 5.0 | 8.5 |
| 100 | 5.9 | 0.35 | 5.0 | 13 |
| 300 | 4.9 | 0.40 | 8.0 | 11 |

[a]Male Wistar Han rats were used. Compound was doses in 10% TWEEN. [b]2HCl salt.

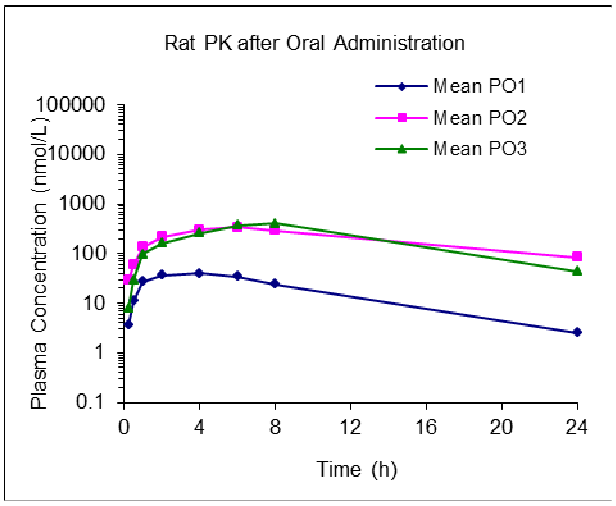



**Figure 4**. Rat oral single rising dose PK study of compound **14**. (PO1: 10 mpk; PO2: 100 mpk; PO3: 300 mpk.)

Potency profiles of compounds **14** and **28** against additional GT1a and GT1b mutants are summarized in Table 8. As NS5A inhibitors are likely to be developed in combination with other DAAs such as NS3 inhibitors, we investigated the impact of compounds **14** and **28** on NS3 RAVs. As anticipated, compounds **14** and **28** retained their potency and were not shifted against prototypical GT1a NS3 RAVs, such as GT1a R155K and GT1a V36M+R155K double substitution and GT1b D168Y, suggesting an absence of cross resistance. Both compounds showed a relatively larger potency shift (300~400x) against GT1a Q30D and compound **28** also showed a relatively larger potency shift (300x) against GT1a Y93N.

Table 8. *In vitro* potency profiles of compounds **14** and **24** against selected GT1a and GT1b mutants

| Genotypes | **14** | | **28** | |
|---|---|---|---|---|
| | $EC_{90}$ (nM) | Shift[a] | $EC_{90}$ (nM) | Shift[a] |
| 1a | 0.003 | 1x | 0.003 | 1x |
| 1a Q30R | 0.02 | 7x | 0.03 | 10x |
| 1a Q30D | 0.9 | 300x | 1.2 | 400x |
| 1a R155K | 0.0001 | 1x | nt[b] | - |
| 1a R155K :V36M | 0.001 | 1x | nt | - |
| 1a Y93N | 0.2 | 67x | 0.9 | 300x |
| 1b Y93H | 0.07 | 23x | 0.02 | 7x |
| 1b L31V | 0.005 | 2x | 0.005 | 2x |
| 1b D168Y | 0.002 | 1x | nt | - |

[a]Shift calculated from GT1a $EC_{90}$ value. [b]nt = not tested.

The *in vitro* cell toxicity of compounds **1** and **14** were assessed by using Huh7 cells in a Click-it EdU plus cell count assay. $CC_{50}$ values of both compounds **1** and **14** were determined to be >50 μM, indicating that, for both compounds, no cell toxicity was observed in this assay.

Recently numerous models have been developed to explain subsets of SAR data with conflicting results.[30,31] Effort has been initiated in Merck trying to explain the potency improvement achieved by these chromane containing tetracyclic indole based HCV NS5A inhibitors, as well as other potent HCV NS5A inhibitors we identified, either by using any of these existing models or by building a new model. The results will be reported in due course.

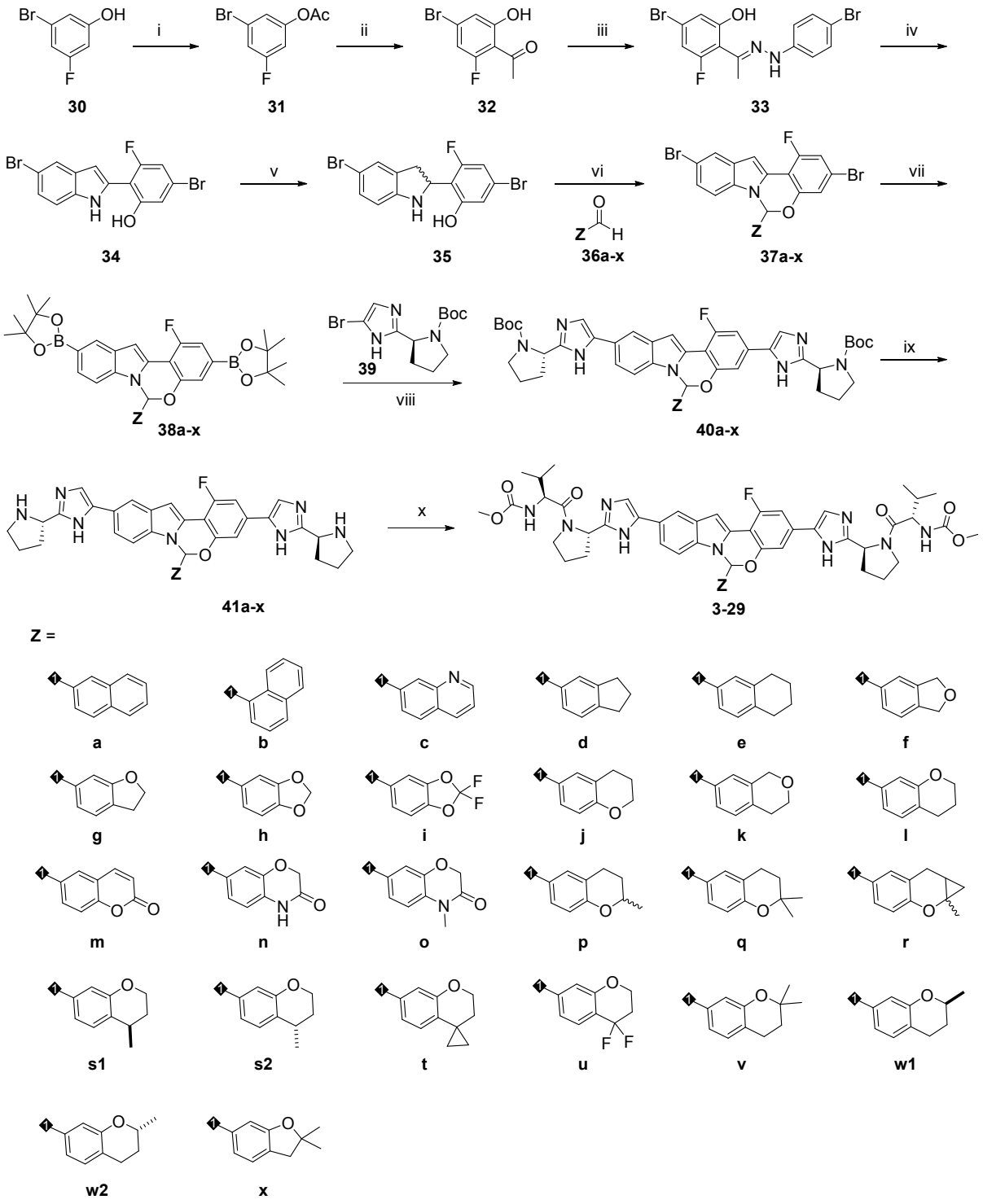
## CONCLUSION

In spite of the challenges faced by the discovery of pan-genotypic HCV NS5A inhibitors caused by the significant diversity among genotypes/mutants and the lacking of inhibitor/protein complexes X-ray structures for rational inhibitor design, we have discovered potent and pan-genotypic NS5A inhibitors with near "flat" potency profiles as well as reasonable PK exposure. SAR studies around the fused bicyclic groups at the aminal carbon, denoted as the "Z groups," with the C-1 fluoro substituted tetracyclic indole core led to the discovery of novel chromane and 2,3-dihydrobenzofuran containing HCV NS5A inhibitors with significantly improved potency against NS5A RAVs, such as GT2b, GT1a Y93H, and GT1a L31V. Modifications on the chromane moiety, such as methylation or cyclopropylation on the C-4 carbon, further improved the potency against GT1a Y93H. Compounds with near "flat" potency profiles, such as compounds **14** and **25-28**, have been identified. Among these compounds, compound **14** stands out due to its reasonable PK profiles in both rat and dog. Its bioavailability in rat is relatively low, but its bioavailability in dog is higher and its exposure in rat is proportional with increasing doses up to 100 mpk. Compound **14** also showed good potency profiles

against a select panel of GT1a and GT1b mutants as well as NS3 RAVs. No cell toxicity was observed for compound **14** in an *in vitro* cell toxicity assessment assay using Huh7 cells.

## CHEMISTRY

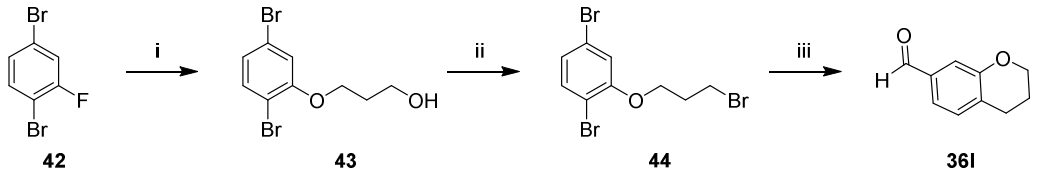
Compounds with different "Z group" were prepared based on the chemistry outlined in Scheme 1. Acetylation of 3-bromo-5-fluorophenol **30** gave 3-Bromo-5-fluorophenyl acetate **31** which underwent an $AlCl_3$-promoted rearrangement to give 1-(4-bromo-2-fluoro-6-hydroxyphenyl)ethan-1-one **32**. Fisher indole synthesis of compound **32** afforded indole **34** which was subsequently reduced to indoline **35** under zinc/TFA conditions. Treatment of **35** with an aldehyde **36a-x** under acidic conditions induced ring cyclization to afford the tetracyclic indoline intermediate which was oxidized to indole **37a-x** under DDQ condition[16]. **37a-x** was then converted to bis-pinacol boronate **38a-x** which underwent Suzuki coupling reaction with *tert*-butyl (*S*)-2-(5-bromo-1H-imidazol-2-yl)pyrrolidine-1-carboxylate (**39**)[16] to afford bis-Boc intermediate **40a-x**. Treatment of **40a-x** under acidic conditions afforded diamine **41a-x**. The (methoxycarbonyl)-L-valine group was installed under BOP-mediated amide coupling conditions. The mixture of diastereomers was separated by chiral SFC to yield the individual compounds for biological evaluation where indicated. The data for the more active isomers, which was determined to be the *S*-isomer in the case of compound **1**, are reported unless noted separately.




**Scheme 1** Preparation of compounds with different "Z groups"

Reagents and conditions: (i) AcCl, DIPEA, DCM, rt, 16 h; (ii) $AlCl_3$, DCM, 0 $^oC$, 30 min; then concentrated, 140 $^oC$, 3 h; (iii) 4-bromophenyl hydrazine HCl, AcOH/EtOH (1:10), reflux, 6 h; (iv) Polyphosphoric acid, xylenes, 110 $^oC$, 3 h; (v) Zn, TFA, 75 $^oC$, 24 h, (vi) ZCHO, TFA, $CH_3CN$, 25 $^oC$, 6 h; then DDQ, toluene, 115 $^oC$; (vii) Bis(pinacolato)diboron, Pd(dppf)$Cl_2$, KOAc, dioxane, 90 $^oC$, 16 h; (viii) **39**, Pd(dppf)$Cl_2$, $K_2CO_3$, dioxane/$H_2O$ (10:1), 90 $^oC$, 16 h; (ix) HCl, MeOH, 0.5 h. (x) (methoxycarbonyl)-L-valine, BOP, DIPEA, DMF, 10 h; then chiral SFC separation.

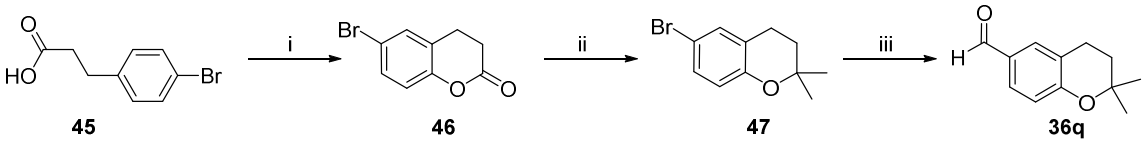
Aldehydes **36a-x** were either purchased or prepared. Chromane-7-carbaldehyde (**36l**) was prepared based on the chemistry outlined in Scheme 2. 1,4-Dibromo-2-fluorobenzene (**42**) was treated with ethylene glycol and *t*-BuOK afforded alcohol **43**; which was converted to bromide **44** under $PBr_3$ conditions. Treatment of compound **44** with one equivalent of *n*-BuLi promoted the ring closure of the chromane. Without isolation of the intermediate, addition of a 2nd equivalent of *n*-BuLi followed by DMF afforded chromane-7-carbaldehyde (**36l**).




**Scheme 2** Preparation of aldehyde **36l**.

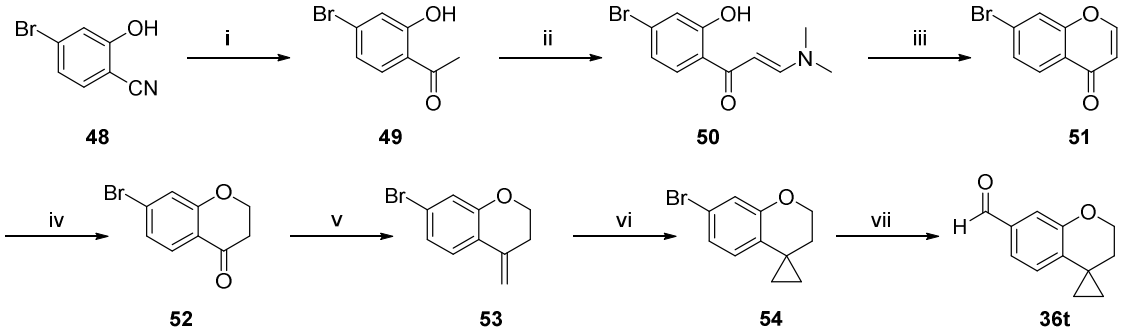
Reagents and conditions: (i) Ethylene glycol, NMP, *t*-BuOK, 88%; (ii) $PBr_3$, 100 $^oC$, 2 h, 98%; (iii) *n*-BuLi, THF, -78 $^oC$, 30 min, then *n*-BuLi, DMF, -78 $^oC$ to 25 $^oC$, 1 h, 47%.

The preparation of 2,2-Dimethylchromane-6-carbaldehyde (**36q**) was outlined in Scheme 3. PIFA promoted ring closure of acid **45** led to lactone **46**, which was converted to compound **47** under MeLi conditions. Lithium-halogen exchange of **47** followed by addition of DMF afforded the desired aldehyde **36q**. The preparation of spiro[chromane-4,1'-cyclopropane]-7-carbaldehyde (**36t**) was outlined in Scheme 4. 4-Bromo-2-hydroxybenzonitrile (**48**) was treated with MeMgBr to produce methyl ketone **49**, which was condensed with N, N-dimethylformamide to give compound **50**. Acid promoted ring closure of **50** afforded **51**, which was reduced, under DIBAl-H conditions, to give ketone **52**. Wittig reaction of **52** and methyltriphenylphosphonium bromide led to olefin **53**. Cyclopropylation of **53** lead to compound **54** which was converted to aldehyde **36t** under the *n*-BuLi/DMF conditions.




**Scheme 3** Preparation of aldehyde **36q**.

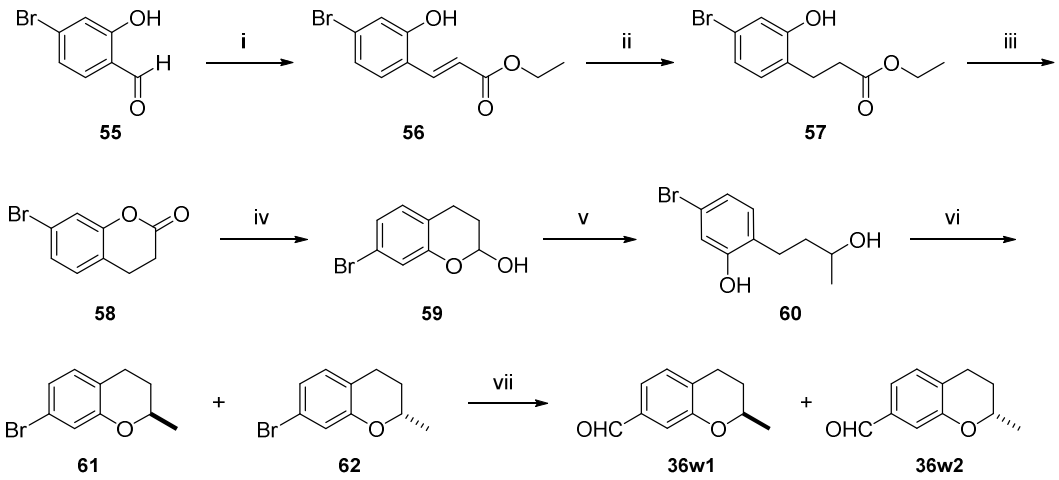
Reagents and conditions: (i) [Bis(trifluoroacetoxy)iodo]benzene (PIFA), TFA, $BF_3 \cdot Et_2O$, 40-60 $^oC$, 48 h, 25%; (ii) MeLi, THF, 0 $^oC$, 16 h, 34%; (iii) *n*-BuLi, THF, DMF, -78 $^oC$, 1 h, 69%.




**Scheme 4** Preparation of aldehyde **36t**.

Reagents and conditions: (i) MeMgBr, THF, -78 $^oC$, 1h; then HCl, water, 25 $^oC$, 24h, 73%; (ii) N, N-dimethylformamide, toluene, reflux, 16 h, 75%; (iii) HCl, DCM, reflux, 2 h, 98%; (iv) DIBAL, THF, -78 $^oC$, 1 h, 64%; (v) Methyltriphenylphosphonium bromide, *n*-BuLi, 25 $^oC$, 1 h, 42%; (vi) $CH_2I_2$, $ZnEt_2$, DCM, TFA, 0 $^oC$, then **53**, 25 $^oC$, 16 h, 45%; (vii) *n*-BuLi, THF, DMF, -78 $^oC$, 1 h, 46%.

The preparation of **36w1** and **36w2** were outline in Scheme 5. Wittig reaction of **55** produced **56** which was then reduced, under Ru catalyzed hydrogenation, to give ester **57**. TsOH promoted ring closure of **57** afforded lactone **58**, which was reduced to semi-ketal **59** under DIBAl-H condition. Treatment of **59** with MeMgBr produced compound **60**. Intra-molecular Mitsunobu reaction of compound **60** afforded the racemic ring closure product, which was resolved by chiral SFC to give enantiomers **61** and **62**. Both **61** and **62** were converted to the corresponding aldehydes **36w1** and **36w2** under the *n*-BuLi-DMF conditions. The stereochemistry of **36w1** and **36w2** were assigned arbitrarily.




**Scheme 5** Preparation of aldehydes **36w1** and **36w2**.

Reagents and conditions: (i) $Ph_3$=$CHCO_2Et$, DCM, 88%; (ii) $Ru(PPh_3)_3Cl$, MeOH, $H_2$, 25 $^oC$, 16 h; (iii) TsOH $\cdot H_2O$, Toluene, 88%; (iv) DIBAl-H, -78 $^oC$, 2 h, 71%; (v) MeMgBr, 25 $^oC$, 2 h, 61%; (vi) $PPh_3$, DIAD, DCM, 0 $^oC$, 1 h, 72%, then chiral SFC separation; (vii) **61**, *n*-BuLi, THF, -78 $^oC$, DMF, 1 h, afforded **36w1** in 87%. **36w2** was obtained from **62** in the same manner.

For the preparation of additional chromane aldehyde derivatives, please see the experimental section.

## Experimental

Reagents were purchased from commercial suppliers and were used without further purification unless otherwise specified. Normal phase column chromatography was carried out using prepacked silica gel cartridges for use on the Isco CombiFlash Companion. Analytical thin layer chromatography (TLC) visualization was performed using 254 nm wavelength ultraviolet light. Preparative HPLC purifications (reverse-phase) were performed using a Gilson 215 Liquid Handler typically with a Phenomenex Luna C18(2) 100×21.2mm 10 micron column. A 15 min run (14 mL/min, 10% to 90% MeCN/$H_2O$, with 0.1%TFA) with UV detection at 254 nm or 210 nm was typically used.

SFC separations were carried out on a Thar SFC instrumentation. Column was specified in each separation; mobile phase A, $CO_2$, mobile phase B, MeOH, back pressure 100 bar, flow rate 25-50 mL/min, detection at 210−350 nm.

Nuclear magnetic resonance spectra were recorded on a Varian 400 or 600 MHz spectrometer. Residual solvent signal was used as internal standard for chemical shift assignments. Spectra were taken in the indicated solvent at ambient temperature, and the chemical shifts are reported in parts per million (ppm (δ)) relative to the lock of the solvent used. Resonance patterns are recorded with the following notations: br (broad), s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet).

The purity of the final compounds was determined based on LCMS data acquired on Agilent 1200 Series HPLC equipped with DAD & 6110 single quadrupole MSD & ELSD with Agilent TC-C18, 50×2.1mm, 5μm for acid methods, Waters X-Bridge ShieldRP18, 50x2.1mm 5μm for basic methods. All final compounds were >95% pure by this method. HRMS analysis was carried on Waters Acquity UPLC and Waters Xevo G2 QTof. Column: Acquity UPLC BEH C18 1.7um, flow rate 0.8 mL/min, Mobile phase A: ammonium formate solution, 5mM in water + 0.05% ammonium Hydroxide, Mobile phase B: 0.025% ammonium hydroxide in acetonitrile, Gradient: 5% to 98% B in A.

**Virology Assay**

The tabulated activity data are mean values of at least two experiments, and in general, the replicon assay exhibits a maximum of 3-fold variation. Detailed replicon biological assays were described previously.[30,31] Briefly, the resistance-associated variants in NS5A were each introduced into GT 1a-H77, GT 1b-Con1, or chimeric replicons. In transient assays, the replicon containing the variant was transfected via electroporation into a Huh7 derived cell line. The percent inhibition of HCV replicon replication as determined by decreased luciferase signal was calculated for each compound concentration, and the $EC_{90}$ values were calculated as described above.

**PK Assessment**

Rat and dog PK studies (24 h): Compounds were dosed in overnight fasted rats and dogs via IV and PO administration. Two animals were included in each dosing group. The IV dose was administered in 60% PEG200 at 2 mg/kg in rats and 1 mg/kg in dogs whereas oral dose was administrated in 10% Tween at 10 mg/kg in rats and 5 mg/kg in dogs. Blood was collected up to 24 hours and plasma was obtained by centrifugation of the blood. Compound concentrations in plasma were detected by LC/MS analysis.

**Mammalian Cytotoxicity Assay**

The Click-it EdU Alexa Fluor 488 HCS assay kit ( Life technologies, C10351) was used to assess the potential cytotoxicity in mammalian cells using a modified version of the manufacturer's protocol. For the assay, Huh7 cells were seeded at 1,200 per well of a 384-well poly-D-lysine coated plates (Greiner, 781946) in 25 μl of culture medium (Life Technologies) and treated with a 20-point twofold dilution series of compounds. Cells and compounds are incubated for 72 hours, with Edu added for the last 18-20 hours. The plates were then fixed, permeabilized and stained with Hoechst as

per manufacturer's protocol. Alexa Fluor 488 dye was added to the EdU by way of the click-it reaction as described in the manufacturer's protocol. Plates were scanned on the Acumen eX3 (TTP LabTech). Hoechst was detected using the 405 laser with the 420-500nm bandpass filter, and EdU-AF488 was detected using the 488 laser with the 500-530 nm bandpass filter.

Huh7 cells containing HCV replicon were grown based on literature method.[34] The authentication of the cell line is accomplished by the ability to grow in selective culture conditions with the antibiotic G418. Controls are included in each assay run to confirm the functioning of the assay. Cells are routinely checked for mycoplasma by using the LONZA kit, MycoAlert Assay.

Compounds **3**-**13**, **15**-**29** were prepared in similar methods as compound **14**.

*Methyl [(1S)-1-({(2S)-2-[5-(1-fluoro-10-{2-[(2S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}pyrrolidin-2-yl]-1H-imidazol-5-yl}-6-naphthalen-2-ylindolo[1,2-c][1,3]benzoxazin-3-yl)-1H-imidazol-2-yl]pyrrolidin-1-yl}carbonyl)-2-methylpropyl]carbamate (**3**).* The more active isomer at the aminal carbon and the absolute stereochemistry at the aminal carbon was not defined. $^{1}$H-NMR (400MHz, $CD_3OD$) δ: 7.99 (s, 1 H), 7.82-7.74 (m, 3H), 7.67 (m, 2H), 7.61 (d, J = 8.4 Hz, 1H), 7.46-7.41 (m, 3H), 7.40-7.20 (m, 6H), 5.24- 5.14 (m, 2 H), 4.23- 4.18 (m, 2 H), 4.06 (m, 2 H), 3.86 (m, 2 H), 3.63 (s, 6 H), 2.53- 2.47 (m, 2 H), 2.26- 2.01 (m, 8 H), 0.93-0.84 (m, 12 H). MS (ESI) m/z calcd for $C_{53}H_{57}FN_9O_7$ $[M+H]^+$, 950.4; found, 950.5.

*methyl [(1S)-1-({(2S)-2-[5-(1-fluoro-10-{2-[(2S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}pyrrolidin-2-yl]-1H-imidazol-5-yl}-6-naphthalen-1-ylindolo[1,2-c][1,3]benzoxazin-3-yl)-1H-imidazol-2-yl]pyrrolidin-1-yl}carbonyl)-2-methylpropyl]carbamate (**4**).* The more active isomer at the aminal carbon and the absolute stereochemistry at the aminal carbon was not defined. $^{1}$H-NMR (400MHz, $CD_3OD$) δ: δ 8.63 (d, J = 8.4 Hz, 1H), 8.36 (s, 1 H), 8.06 (s, 1 H), 7.90 (d, J = 8.0 Hz, 1H),

7.82 (d, J = 8.0 Hz, 1H), 7.71 (s, 1 H), 7.68 (m, 2H), 7.58 (d, J = 8.0 Hz, 1H), 7.42 (d, J = 1.6 Hz, 1H), 7.29-7.23 (m, 3H), 7.11 (1, 3H), 7.03 (s, 1H), 6.13 (d, J = 7.2 Hz, 1H), 5.23- 5.06 (m, 2 H), 4.20 (d, J = 7.2 Hz, 1H), 4.11 (d, J = 7.2 Hz, 1H), 4.13- 4.00 (m, 2 H), 3.86-3.77 (m, 2 H), 3.60 (s, 6 H), 2.56- 2.47 (m, 2 H), 2.24- 1.92 (m, 8 H), 0.91-0.76 (m, 12 H). MS (ESI) *m/z* calcd for C53H57FN9O7 $[M+H]^+$, 950.4; found, 950.5.

*methyl [(1S)-1-({(2S)-2-[5-(1-fluoro-10-{2-[(2S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}pyrrolidin-2-yl]-1H-imidazol-5-yl}-6-quinolin-7-ylindolo[1,2-c][1,3]benzoxazin-3-yl)-1H-imidazol-2-yl]pyrrolidin-1-yl}carbonyl)-2-methylpropyl]carbamate (**5**)*. The more active isomer at the aminal carbon and the absolute stereochemistry at the aminal carbon was not defined. $^1$H-NMR (400MHz, $CD_3OD$) δ: 8.76 (d, J =1.2 Hz, 1 H), 8.38 (d, J =8 Hz, 1 H), 8.11 (s, 1 H), 8.00- 7.98 (m, 2 H), 7.86 (s, 1 H), 7.77 (m, 1 H), 7.64 (s, 1 H), 7.57- 7.55 (s, 1 H), 7.34- 7.29 (m, 3H), 7.23 (s, 1 H), 5.25- 5.13 (m, 2 H), 4.22- 4.06 (m, 4 H), 3.88- 3.77 (m, 2 H), 3.64- 6.63 (m, 6 H), 2.59- 2.46 (m, 2 H), 2.26- 1.95 (m, 8 H), 0.97-0.83 (m, 12 H). MS (ESI) *m/z* calcd for C52H56FN10O7 $[M+H]^+$, 951.4; found, 951.4.

*Methyl [(1S)-1-{[(2S)-2-{5-[6-(2,3-dihydro-1H-inden-5-yl)-1-fluoro-10-{2-[(2S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}pyrrolidin-2-yl]-1H-imidazol-5-yl}indolo[1,2-c][1,3]benzoxazin-3-yl]-1H-imidazol-2-yl}pyrrolidin-1-yl]carbonyl}-2-methylpropyl]carbamate (**6**)*. The more active isomer at the aminal carbon and the absolute stereochemistry at the aminal carbon was not defined. $^1$H-NMR (400MHz, $CD_3OD$) δ: 8.03 (s, 1 H), 7.86 (s, 1 H), 7.73 (s, 1 H), 7.55 (s, 1 H), 7.46 (dd, J =8.0, 1.2 Hz, 1 H), 7.32-7.21 (m, 4 H), 7.10 (d, J =7.6 Hz, 1 H), 6.86 (s, 1 H), 6.78 (d, J =7.6 Hz, 1 H), 5.25- 5.15 (m, 2 H), 4.20 (m, 2 H), 4.10 (m, 2 H), 3.86- 3.82 (m, 2 H), 3.64 (s, 6 H), 2.82- 2.74 (m, 4 H), 2.56- 2.49 (m, 2 H), 2.26- 1.97 (m, 10 H), 0.92-0.89 (m, 12 H). MS (ESI) *m/z* calcd for C52H59FN9O7 $[M+H]^+$, 940.4; found, 940.5.

*methyl [(1S)-1-{[(2S)-2-{5-[1-fluoro-10-{2-[(2S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}pyrrolidin-2-yl]-1H-imidazol-5-yl}-6-(5,6,7,8-tetrahydronaphthalen-2-yl)indolo[1,2-c][1,3]benzoxazin-3-yl]-1H-imidazol-2-yl}pyrrolidin-1-yl]carbonyl}-2-methylpropyl]carbamate (**7**).* The more active isomer at the aminal carbon and the absolute stereochemistry at the aminal carbon was not defined. $^1$H-NMR (400MHz, $CD_3OD$) δ: 8.01 (s, 1 H), 7.87 (s, 1 H), 7.74 (s, 1 H), 7.44 (m, 2 H), 7.29- 7.20 (m, 4 H), 6.95 (d, J =8.0 Hz, 1 H), 6.75- 6.69 (m, 2 H), 5.25- 5.17 (m, 2 H), 4.22 (m, 2 H), 4.09 (m, 2 H), 3.86 (m, 2 H), 3.65 (s, 6 H), 2.68- 2.48 (m, 7 H), 2.28- 2.03 (m, 8 H), 1.72 (m, 4 H), 0.94 (m, 12 H). MS (ESI) *m/z* calcd for C53H61FN9O7 $[M+H]^+$, 954.4; found, 954.5.

*methyl [(1S)-1-{[(2S)-2-{5-[6-(1,3-dihydro-2-benzofuran-5-yl)-1-fluoro-3-{2-[(2S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}pyrrolidin-2-yl]-1H-imidazol-5-yl}indolo[1,2-c][1,3]benzoxazin-10-yl]-1H-imidazol-2-yl}pyrrolidin-1-yl]carbonyl}-2-methylpropyl]carbamate(**8**).* The more active isomer at the aminal carbon and the absolute stereochemistry at the aminal carbon was not defined. $^1$H-NMR (400MHz, $CD_3OD$) δ: 8.02 (s, 1 H), 7.77 - 7.74 (m, 2 H), 7.64 (s, 1 H), 7.47 (m, 1 H), 7.33 - 7.29 (m, 2 H), 7.25 - 7.20 (m, 1 H), 7.18- 6.92 (m, 2 H), 6.95 (m, 2 H), 5.24 (m, 2 H), 4.96 - 4.87 (m, 4 H), 4.20 (t, J =8 Hz, 2 H), 4.08 (m, 2 H), 3.84 (t, J =7.6 Hz, 2 H), 3.63 (s, 6 H), 2.56 – 2.48 (m, 2 H), 2.25 - 2.20 (m, 8 H), 0.96 - 0.86 (m, 12 H). MS (ESI) *m/z* calcd for C51H57FN9O8 $[M+H]^+$, 942.4; found, 942.5.

*methyl [(1S)-1-{[(2S)-2-{5-[6-(2,3-dihydro-1-benzofuran-6-yl)-1-fluoro-10-{2-[(2S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}pyrrolidin-2-yl]-1H-imidazol-5-yl}indolo[1,2-c][1,3]benzoxazin-3-yl]-1H-imidazol-2-yl}pyrrolidin-1-yl]carbonyl}-2-methylpropyl]carbamate (**9**).* Mixture of diastereomers at the aminal carbon. $^1$H-NMR (400MHz, $CD_3OD$) δ: 8.02 (s, 1 H), 7.83 (s, 1 H), 7.71 (s, 1 H), 7.51-7.33 (m, 2 H), 7.25-7.12 (m, 4 H), 7.06 (d, J =8 Hz, 1 H), 6.42 – 6.37 (m, 2 H),

5.25 - 5.22 (m, 2 H), 4.45 (t, J =8.8 Hz, 2 H), 4.21 (m, 2 H), 4.06 (m, 2 H), 3.83 (m, 2 H), 3.64 (s, 6 H), 3.09 (t, J =8.8 Hz, 2 H), 2.52 (m, 2 H), 2.30 - 2.00 (m, 8 H), 0.96 - 0.80 (m, 12 H). MS (ESI) *m/z* calcd for C51H57FN9O8 $[M+H]^+$, 942.4; found, 942.4.

*methyl [(1S)-1-{[(2S)-2-{5-[6-(1,3-benzodioxol-5-yl)-1-fluoro-10-{2-[(2S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}pyrrolidin-2-yl]-1H-imidazol-5-yl}indolo[1,2-c][1,3]benzoxazin-3-yl]-1H-imidazol-2-yl}pyrrolidin-1-yl]carbonyl}-2-methylpropyl]carbamate(**10**).* The more active isomer at the aminal carbon and the absolute stereochemistry at the aminal carbon was not defined. $^1$H-NMR (400MHz, $CD_3OD$) δ: 7.98 (s, 1 H), 7.82 (s, 1H), 7.73 (s, 1 H), 7.49-7.46 (m, 2 H), 7.33-7.25 (m, 3 H), 7.19 (s, 1 H), 6.68 (d, J =8 Hz, 1 H), 6.58 (d, J =1.2 Hz, 1 H), 6.38 (d, J =8 Hz, 1 H), 5.89 (s, 2 H), 5.25-5.15 (m, 2 H), 4.23-4.19 (t, J =8 Hz, 2 H), 4.09-4.07 (m, 2 H), 3.87-3.83 (m, 2 H), 3.64 (s, 6 H), 2.56-2.47 (m, 2 H), 2.26-2.00 (m, 8 H), 0.97-0.87 (m, 12 H). MS (ESI) *m/z* calcd for C50H55FN9O9 $[M+H]^+$, 944.4; found, 944.4.

*methyl [(1S)-1-{[(2S)-2-{5-[6-(2,2-difluoro-1,3-benzodioxol-5-yl)-1-fluoro-10-{2-[(2S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}pyrrolidin-2-yl]-1H-imidazol-5-yl}indolo[1,2-c][1,3]benzoxazin-3-yl]-1H-imidazol-2-yl}pyrrolidin-1-yl]carbonyl}-2-methylpropyl]carbamate (**11**).* The more active isomer at the aminal carbon and the absolute stereochemistry at the aminal carbon was not defined. $^1$H-NMR (400MHz, $CD_3OD$) δ: 8.05 (s, 1 H), 7.88 (s, 1H), 7.76 (s, 1 H), 7.70 (s, 1 H), 7.53 (dd, d= 8.0Hz, 1.2 HZ, 1 H), 7.42-7.22 (m, 4 H), 7.06 (d, J =8.4 Hz, 1 H), 6.98 (d, J =1.2 Hz, 1 H), 6.60 (dd, d= 8.4 Hz, 1.2 HZ, 1 H), 5.25-5.15 (m, 2 H), 4.23-4.19 (m, 2 H), 4.09-4.07 (m, 2 H), 3.87-3.83 (m, 2 H), 3.64 (s, 6 H), 2.55-2.47 (m, 2 H), 2.26-2.02 (m, 8 H), 0.97-0.86 (m, 12 H). MS (ESI) *m/z* calcd for C50H53F3N9O9 $[M+H]^+$, 980.4; found, 980.4.

*methyl [(1S)-1-{[(2S)-2-{5-[6-(3,4-dihydro-2H-chromen-6-yl)-1-fluoro-10-{2-[(2S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}pyrrolidin-2-yl]-1H-imidazol-5-yl}indolo[1,2-c][1,3]benzoxazin-3-yl]-1H-imidazol-2-yl}pyrrolidin-1-yl]carbonyl}-2-methylpropyl]carbamate* ***(12)***. The more active isomer at the aminal carbon and the absolute stereochemistry at the aminal carbon was not defined. $^1$H-NMR (400MHz, $CD_3OD$) δ: 8.00 (s, 1 H), 7.87 (s, 1 H), 7.72 (s, 1 H), 7.33 - 7.30 (m, 2 H), 7.31 (m, 1 H), 7.19 - 7.14 (m, 3 H), 6.73 (m, 2 H), 6.64 (m, 1 H), 5.24 - 5.16 (m, 2 H), 4.20 (t, *J* = 8.4 Hz, 2 H), 4.12 - 4.07 (m, 4 H), 3.93 - 3.76 (m, 2 H), 3.63 (s, 6 H), 2.61 (m, 2 H), 2.59 - 2.49 (m, 2 H), 2.31 - 2.21 (m, 2 H), 2.24 - 2.14 (m, 4 H), 2.06 (m, 2 H), 1.89 (m, 2 H), 0.99 – 0.89 (m, 12 H). MS (ESI) *m/z* calcd for C52H59FN9O8 $[M+H]^+$, 956.4; found, 956.5.

*methyl [(1S)-1-{[(2S)-2-{5-[6-(3,4-dihydro-1H-isochromen-7-yl)-1-fluoro-10-{2-[(2S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}pyrrolidin-2-yl]-1H-imidazol-5-yl}indolo[1,2-c][1,3]benzoxazin-3-yl]-1H-imidazol-2-yl}pyrrolidin-1-yl]carbonyl}-2-methylpropyl]carbamate* ***(13)***. The more active isomer at the aminal carbon and the absolute stereochemistry at the aminal carbon was not defined. $^1$H-NMR (400MHz, $CD_3OD$) δ: 7.93 (s, 1 H), 7.82 (s, 1 H), 7.70 (s, 1 H), 7.44 (s, 1 H), 7.30 - 7.11 (m, 3 H), 7.00 (J =7.6 Hz,1 H), 6.71 (m, 2 H), 5.25 – 5.18 (m, 2 H), 4.58 - 4.53(m, 2 H), 4.24 - 4.20 (m, 2 H), 4.07 (m, 2 H), 3.90 – 3.88 (m, 4 H), 3.82 (s, 6 H), 2.72 (s, 2 H), 2.53 (m, 2 H), 2.25 – 2.00 (m, 9 H), 0.96 – 0.86 (m, 12 H). MS (ESI) *m/z* calcd for C52H59FN9O8 $[M+H]^+$, 956.4; found, 956.5.

*Dimethyl ((2S,2'S)-((2S,2'S)-((6-(chroman-7-yl)-1-fluoro-6H-benzo[5,6][1,3]oxazino[3,4-a]indole-3,10-diyl)bis(1H-imidazole-5,2-diyl))bis(pyrrolidine-2,1-diyl))bis(3-methyl-1-oxobutane-1,2-diyl))dicarbamate* ***(14)***. To a mixture of **41l** (355 mg, 0.45 mmol), (methoxycarbonyl)-L-valine (0.99 mmol) and DIPEA (3.6 mmol) in DMF (5 mL) was added BOP (1.0 mmol). The resulting mixture was stirred at room temperature until LCMS indicated **41l** was consumed up. The mixture was purified by

Pre-HPLC to give a mixture of diastereomers of compound **14** and its other isomer (210 mg, 48.8 % yield). MS (ESI) m/z ($M+H^+$): 956.5. The mixture were separated by chiral SFC to give **14** (69 mg, 33%) and its other diastereomer . SFC condition: Chiralpak AS-H, 250×20 mm, 5 μm, Mobile phase: 40% of EtOH (0.05%DEA) in $CO_2$, Flow rate: 50 mL/min , wavelength: 220 nm. Compound **14**: $^1$H-NMR (400MHz, MeOH-$d_4$) δ: 7.92 (s, 1 H), 7.81 (s, 1 H), 7.69 (s, 1 H), 7.41 - 7.46 (m, 2 H), 7.25 - 7.28 (m, 2 H), 7.14 (m, 1 H), 7.08 - 7.09 (m, 1 H), 6.88 - 6.90 (m, 1 H), 6.40 - 6.42 (m, 1 H), 6.29 (s, 1 H), 5.17 - 5.23 (m, 2 H), 4.20 - 4.24 (m, 2 H), 4.00 - 4.08 (m, 4 H), 3.85 - 3.89 (m, 2 H), 3.63 (s, 6 H), 2.62 - 2.64 (m, 2 H), 2.48 - 2.57 (m, 2 H), 2.21 - 2.32 (m, 2 H), 2.05 - 2.17 (m, 6 H), 0.86 - 0.96 (m, 12 H). $^{13}$C-NMR (600MHz, MeOH-$d_4$) δ: 174.3, 174.2, 161.4, 159.7, 159.5, 156.4, 152.4, 152.3, 150.7, 149.5, 136.9, 136.4, 135.7, 131.3, 130.6, 128.3, 125.5, 122.0, 120.7, 119.4, 119.2, 117.2, 115.7, 114.8, 111.9, 111.6, 107.9, 107.8, 103.8, 85.3, 67.5, 59.9, 55.2, 55.0, 52.8, 32.5, 32.4, 31.1, 26.3, 25.5, 23.1, 19.9, 18.2. HRMS (ESI+) *m/z* calcd for C52H59FN9O8 $[M+H]^+$, 956.4471; found, 956.4495.

*Methyl [(1S)-1-{[(2S)-2-{5-[1-fluoro-10-{2-[(2S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}pyrrolidin-2-yl]-1H-imidazol-5-yl}-6-(2-oxo-2H-chromen-7-yl)indolo[1,2-c][1,3]benzoxazin-3-yl]-1H-imidazol-2-yl}pyrrolidin-1-yl]carbonyl}-2-methylpropyl]carbamate (**15**).* The more active isomer at the aminal carbon. The absolute stereochemistry at the aminal carbon was not defined. MS (ESI) *m/z* calcd for C52H55FN9O9 $[M+H]^+$, 968.4; found, 968.7.

*methyl [(1S)-1-{[(2S)-2-{5-[1-fluoro-3-{2-[(2S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}pyrrolidin-2-yl]-1H-imidazol-5-yl}-6-(3-oxo-3,4-dihydro-2H-1,4-benzoxazin-7-yl)indolo[1,2-c][1,3]benzoxazin-10-yl]-1H-imidazol-2-yl}pyrrolidin-1-yl]carbonyl}-2-methylpropyl]carbamate (**16**).* The more active isomer at the aminal carbon. The absolute stereochemistry at the aminal carbon was not defined. $^1$H-NMR (400MHz, $CD_3OD$) δ: 8.00 (s, 1H), 7.84 (s, 1H), 7.74 (s, 1H), 7.53−7.31 (m, 2H), 7.24−7.17 (m, 2H), 6.78−6.76 (m, 1H), 6.59−6.56 (m,

2H), 5.26−5.18 (m, 2H), 4.47 (s, 2H), 4.25−4.21 (m, 2H), 4.09 (s, 2H), 3.90−3.88 (m, 2H), 3.65 (s, 6H), 2.65−2.54 (m, 2H), 2.10−2.05 (m, 8H), 2.10−2.05 (m, 8H), 0.95−0.88 (m, 12H). MS (ESI) *m/z* calcd for C51H56FN10O9 $[M+H]^+$, 971.4; found, 971.4.

*methyl [(1S)-1-{[(2S)-2-{5-[1-fluoro-3-{2-[(2S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}pyrrolidin-2-yl]-1H-imidazol-5-yl}-6-(4-methyl-3-oxo-3,4-dihydro-2H-1,4-benzoxazin-7-yl)indolo[1,2-c][1,3]benzoxazin-10-yl]-1H-imidazol-2-yl}pyrrolidin-1-yl]carbonyl}-2-methylpropyl]carbamate (**17**).* The more active isomer at the aminal carbon and the absolute stereochemistry at the aminal carbon was not defined. $^1$H-NMR (400MHz, $CD_3OD$) δ: 7.82−7.72 (m, 2H), 7.34−7.12 (m, 6H), 7.05−6.78 (m, 2H), 6.55−6.49 (m, 2H), 5.16−5.09 (m, 2H), 4.60 (s, 2H), 4.37−4.20 (m, 4H), 4.09−3.76 (m, 4H), 3.45−3.28 (m, 6H), 3.09 (s, 3H), 2.26−2.00 (m, 8H), 1.27−1.12 (m, 6H), 0.93−0.89 (m, 6H). MS (ESI) *m/z* calcd for C52H58FN10O9 $[M+H]^+$, 985.4; found, 985.4.

*methyl [(1S)-1-{[(2S)-2-{5-[1-fluoro-3-{2-[(2S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}pyrrolidin-2-yl]-1H-imidazol-5-yl}-6-(2-methyl-3,4-dihydro-2H-chromen-6-yl)indolo[1,2-c][1,3]benzoxazin-10-yl]-1H-imidazol-2-yl}pyrrolidin-1-yl]carbonyl}-2-methylpropyl]carbamate (**18**).* Compounds **18**, **19**, and other two less potent diastereomers were separated by SFC. Conditions: Chiralpak AS-H 250×20 mm, 5 μm, Mobile phase: 40% of EtOH (0.05% DEA) in $CO_2$, Flow rate: 50 mL/min, wavelength: 220 nm. $^1$H-NMR (400MHz, $CD_3OD$) δ: 7.95 (s, 1H), 7.78 (s, 1H), 7.71 (s, 1H), 7.43−7.28 (m, 3H), 7.17−7.13 (m, 3H), 6.76−6.71 (m, 2H), 6.62−6.59 (m, 1H), 5.24−5.14 (m, 2H), 4.22−4.19 (m, 2H), 4.07−4.02 (m, 3H), 3.87−3.85 (m, 2H),3.68 (s, 6H), 2.67−2.46 (m, 4H), 2.23−1.91 (m, 9H), 1.51−1.59 (m, 1H), 1.29 (d, *J*=6.4 Hz, 3H), 0.92−0.86 (m, 12H). MS (ESI) *m/z* calcd for C53H61FN9O8 $[M+H]^+$, 970.5; found, 970.7.

*methyl [(1S)-1-{[(2S)-2-{5-[1-fluoro-3-{2-[(2S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}pyrrolidin-2-yl]-1H-imidazol-5-yl}-6-(2-methyl-3,4-dihydro-2H-chromen-6-yl)indolo[1,2-c][1,3]benzoxazin-10-yl]-1H-imidazol-2-yl}pyrrolidin-1-yl]carbonyl}-2-methylpropyl]carbamate (**19**).* Compounds **18**, **19**, and other two less potent diastereomers were separated by SFC. Conditions: Chiralpak AS-H 250×20 mm, 5 μm, Mobile phase: 40% of EtOH (0.05% DEA) in $CO_2$, Flow rate: 50 mL/min, wavelength: 220 nm. $^1$H-NMR (400MHz, $CD_3OD$) δ: 7.93 (s, 1H), 7.70 (s, 2H), 7.40−7.35 (m, 3H), 7.30−7.11 (m, 3H), 6.78−6.73 (m, 2H), 6.62−6.60 (m, 1H), 5.24−5.15 (m, 2H), 4.20 (s, 2H), 4.07−4.02 (m, 3H), 3.85 (s, 2H), 3.85 (s, 6H), 2.75−2.67 (m, 1H), 2.57−2.43 (m, 3H), 2.24−1.90 (m, 9H), 1.58−1.48 (m, 1H) 1.28 (d, *J*=6.0 Hz, 3H), 0.97−0.83 (m, 12H). MS (ESI) *m/z* calcd for C53H61FN9O8 $[M+H]^+$, 970.5; found, 970.5.

*methyl [(1S)-1-{[(2S)-2-{5-[6-(2,2-dimethyl-3,4-dihydro-2H-chromen-6-yl)-1-fluoro-10-{2-[(2S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}pyrrolidin-2-yl]-1H-imidazol-5-yl}indolo[1,2-c][1,3]benzoxazin-3-yl]-1H-imidazol-2-yl}pyrrolidin-1-yl]carbonyl}-2-methylpropyl]carbamate (**20**).* The more active isomer at the aminal carbon and the absolute stereochemistry at the aminal carbon was not defined. $^1$H-NMR (400MHz, $CD_3OD$) δ: 8.00 (s, 1H), 7.86 (s, 1H), 7.73 (s, 1H), 7.45−7.43 (m, 2H), 7.33−7.24 (m, 1H), 7.19−7.17 (m, 3H), 6.78−6.74 (m, 2H), 6.61−6.59 (m, 1H), 5.24−5.15 (m, 2H), 4.22−4.20 (m, 2H), 4.18−4.07 (m, 2H), 3.86−3.82 (m, 2H), 3.86 (s, 6H), 2.65−2.50 (m, 4H), 2.26−1.99 (m, 8H), 1.73−1.70 (m, 2H), 1.24 (s, 6H), 0.98−0.85 (m, 12H). MS (ESI) *m/z* calcd for C54H63FN9O8 $[M+H]^+$, 984.5; found, 984.5.

*methyl [(1S)-1-{[(2S)-2-{5-[1-fluoro-10-{2-[(2S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}pyrrolidin-2-yl]-1H-imidazol-5-yl}-6-(1a-methyl-1,1a,7,7a-tetrahydrocyclopropa[b]chromen-5-yl)indolo[1,2-c][1,3]benzoxazin-3-yl]-1H-imidazol-2-yl}pyrrolidin-1-yl]carbonyl}-2-methylpropyl]carbamate (**21**).* The more active isomer at the aminal

carbon and the absolute stereochemistry at the aminal carbon was not defined. Mixture of diastereomers at the chromane ring. $^1$H-NMR (400MHz, $CD_3OD$) δ: 8.00−7.98 (m, 1H), 7.80 (s, 1H), 7.72 (s, 1H), 7.43−7.39 (m, 2H), 7.32−7.30 (m, 1H), 7.21−7.17 (m, 3H), 6.80−6.63 (m, 3H), 5.24−5.15 (m, 2H), 4.22−4.18 (m, 2H), 4.07−3.84 (m, 2H), 3.63 (s, 2H), 3.28 (s, 6H), 2.99−2.80 (m, 1H), 2.76−2.46 (m, 3H), 2.25−2.01 (m, 8H),1.47 (s, 3H), 1.24 (s, 1H), 0.96−0.80 (m, 12H), 0.76−0.75 (m, 1H), 0.55−0.53 (m, 1H). MS (ESI) *m/z* calcd for C54H61FN9O8 $[M+H]^+$, 982.5; found, 982.5.

*methyl [(1S)-1-{[(2S)-2-{5-[1-fluoro-10-{2-[(2S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}pyrrolidin-2-yl]-1H-imidazol-5-yl}-6-(2-methyl-3,4-dihydro-2H-chromen-7-yl)indolo[1,2-c][1,3]benzoxazin-3-yl]-1H-imidazol-2-yl}pyrrolidin-1-yl]carbonyl}-2-methylpropyl]carbamate (**22**).* $^1$H-NMR (400MHz, $CD_3OD$) δ: *δ* 7.85 (s, 1H), 7.61 (s, 1H), 7.40−7.20 (m, 5H), 7.04−6.90 (m, 3H), 6.47−6.45 (m, 1H), 6.28 (s, 1H), 5.24−5.13 (m, 2H), 4.25−4.23 (m, 2H), 4.08−3.88 (m, 6H), 3.65 (s, 6H), 2.73−2.34 (m, 3H), 2.26−1.93 (m, 9H), 1.30−1.24 (m, 4H), 0.99−0.90 (m, 12H). MS (ESI) *m/z* calcd for C53H61FN9O8 $[M+H]^+$, 970.5; found, 970.5.

*methyl [(1S)-1-{[(2S)-2-{5-[1-fluoro-10-{2-[(2S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}pyrrolidin-2-yl]-1H-imidazol-5-yl}-6-(2-methyl-3,4-dihydro-2H-chromen-7-yl)indolo[1,2-c][1,3]benzoxazin-3-yl]-1H-imidazol-2-yl}pyrrolidin-1-yl]carbonyl}-2-methylpropyl]carbamate (**23**).* $^1$H-NMR (400MHz, $CD_3OD$) δ: 7.87 (s, 1H), 7.64 (s, 1H), 7.43−7.39 (m, 2H), 7.26−7.20 (m, 3H), 7.07−7.00 (m, 2H), 6.92−6.90 (m, 1H), 6.49−6.48 (m, 1H), 6.23 (s, 1H), 5.24−5.12 (m, 2H), 4.23−4.21 (m, 2H), 4.01−3.99 (m, 4H), 3.87−3.86 (m, 2H), 3.64−3.58 (m, 6H), 2.75−2.40 (m, 3H), 2.26−1.92 (m, 9H), 1.60−1.35 (m, 1H), 1.26−1.23 (m, 3H), 0.97−0.88 (m, 12H). MS (ESI) *m/z* calcd for C53H61FN9O8 $[M+H]^+$, 970.5; found, 970.5.

*methyl [(1S)-1-{[(2S)-2-{5-[6-(2,2-dimethyl-3,4-dihydro-2H-chromen-7-yl)-1-fluoro-10-{2-[(2S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}pyrrolidin-2-yl]-1H-imidazol-5-yl}indolo[1,2-c][1,3]benzoxazin-3-yl]-1H-imidazol-2-yl}pyrrolidin-1-yl]carbonyl}-2-methylpropyl]carbamate (**24**).* $^1$H-NMR (400MHz, $CD_3OD$) δ: 8.03 (s, 1H), 7.80−7.74 (m, 2H), 7.53−7.48 (m, 2H), 7.37−7.19 (m, 4H), 6.98−6.96 (m, 1H), 6.50−6.49 (m, 1H), 6.27 (s, 1H), 5.25−5.18 (m, 2H), 4.24−4.08 (m, 6H), 3.86−3.37 (m, 6H), 2.69−2.16 (m, 12H), 1.73−1.70 (m, 2H), 1.28−0.86 (m, 18H). MS (ESI) *m/z* calcd for $C_{54}H_{63}FN_9O_8$ $[M+H]^+$, 984.5; found, 984.5.

*methyl [(1S)-1-{[(2S)-2-{5-[6-(2,2-dimethyl-2,3-dihydro-1-benzofuran-6-yl)-1-fluoro-10-{2-[(2S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}pyrrolidin-2-yl]-1H-imidazol-5-yl}indolo[1,2-c][1,3]benzoxazin-3-yl]-1H-imidazol-2-yl}pyrrolidin-1-yl]carbonyl}-2-methylpropyl]carbamate (**25**).* $^1$H-NMR (400MHz, $CD_3OD$) δ: 8.02 (s, 1H), 7.85 (s, 1H), 7.74 (s, 1H), 7.53−7.47 (m, 2H), 7.35−7.26 (m, 3H), 7.20−7.19 (m, 1H), 7.01−6.99 (m, 1H), 6.39−6.29 (m, 2H), 5.25−5.20 (m, 2H), 4.22−4.18 (m, 2H), 4.08−4.06 (m, 2H), 3.86−3.82 (m, 2H), 3.61 (s, 6H), 2.92 (s, 2H), 2.55−2.51 (m, 2H), 2.26−2.05 (m, 8H), 1.35−1.33 (m, 6H), 0.97−0.86 (m, 12H). MS (ESI) *m/z* calcd for $C_{53}H_{61}FN_9O_8$ $[M+H]^+$, 970.5; found, 970.5.

*methyl [(1S)-1-{[(2S)-2-{5-[1-fluoro-10-{2-[(2S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}pyrrolidin-2-yl]-1H-imidazol-5-yl}-6-(4-methyl-3,4-dihydro-2H-chromen-7-yl)indolo[1,2-c][1,3]benzoxazin-3-yl]-1H-imidazol-2-yl}pyrrolidin-1-yl]carbonyl}-2-methylpropyl]carbamate (**26**).* $^1$H-NMR (400MHz, $CD_3OD$) δ: 8.02 (s, 1H), 7.87 (s, 1H), 7.73 (s, 1H), 7.52−7.48 (m, 1H), 7.48−7.46 (m, 1H), 7.34−7.25 (m, 3H), 7.19−7.18 (m, 1H), 7.05−7.04 (m, 1H), 6.48−6.46 (m, 1H), 6.31 (s, 1H), 5.23−5.18 (m, 2H), 4.23−4.18 (m, 2H), 4.08−4.02 (m, 4H), 3.87−3.81 (m, 2H), 3.63 (s, 6H), 2.83−2.82 (m, 1H), 2.55−2.51 (m, 2H), 2.26−1.97 (m, 9H), 1.63−1.53 (m, 1H),

1.22−1.20 (d, 3H), 0.93−0.86 (m, 12H). MS (ESI) *m/z* calcd for C53H61FN9O8 $[M+H]^+$, 970.5; found, 970.5.

*methyl [(1S)-1-{[(2S)-2-{5-[1-fluoro-10-{2-[(2S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}pyrrolidin-2-yl]-1H-imidazol-5-yl}-6-(4-methyl-3,4-dihydro-2H-chromen-7-yl)indolo[1,2-c][1,3]benzoxazin-3-yl]-1H-imidazol-2-yl}pyrrolidin-1-yl]carbonyl}-2-methylpropyl]carbamate (**27**).* $^1$H-NMR (400MHz, $CD_3OD$) δ: 8.02 (s, 1H), 7.86 (s, 1H), 7.74 (s, 1H), 7.55−7.50 (m, 2H), 7.48−7.19 (m, 4H), 7.06−7.04 (m, 1H), 6.48−6.47 (m, 1H), 6.29 (s, 1H), 5.23−5.15 (m, 2H), 4.22−4.18 (m, 2H), 4.09−4.02 (m, 4H), 4.01−3.82 (m, 2H), 3.64 (s, 6H), 2.84−2.82 (m, 1H), 2.57−2.50 (m, 2H), 2.26−1.96 (m, 9H), 1.70−1.50 (m, 1H), 1.21−1.19 (m, 3H), 0.93−0.86 (m, 12H). MS (ESI) *m/z* calcd for C53H61FN9O8 $[M+H]^+$, 970.5; found, 970.5.

*methyl [(1S)-1-{[(2S)-2-{5-[6-(2,3-dihydrospiro[chromene-4,1'-cyclopropan]-7-yl)-1-fluoro-10-{2-[(2S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}pyrrolidin-2-yl]-1H-imidazol-5-yl}indolo[1,2-c][1,3]benzoxazin-3-yl]-1H-imidazol-2-yl}pyrrolidin-1-yl]carbonyl}-2-methylpropyl]carbamate (**28**).* $^1$H-NMR (400MHz, $CD_3OD$) δ: 8.00 (s, 1H), 7.84 (s, 1H), 7.71 (s, 1H), 7.51−7.45 (m, 2H), 7.34−7.17 (m, 4H), 6.54 (d, *J*=8.0 Hz, 1H), 6.40−6.37 (m, 1H), 6.32−6.31 (m, 1H), 5.20−5.14 (m, 2H), 4.20−4.14 (m, 6H), 3.81−3.79 (m, 2H), 3.61 (s, 6H), 2.70−2.60 (m, 2H), 2.50−1.98 (m, 8H), 1.73−1.71 (m, 2H), 0.94−0.77 (m, 16H). MS (ESI) *m/z* calcd for C54H61FN9O8 $[M+H]^+$, 982.5; found, 982.5.

*methyl [(1S)-1-{[(2S)-2-{5-[6-(4,4-difluoro-3,4-dihydro-2H-chromen-7-yl)-1-fluoro-10-{2-[(2S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}pyrrolidin-2-yl]-1H-imidazol-5-yl}indolo[1,2-c][1,3]benzoxazin-3-yl]-1H-imidazol-2-yl}pyrrolidin-1-yl]carbonyl}-2-methylpropyl]carbamate (**29**).* More active isomer at the aminal carbon and its absolute stereochemistry was not defined. $^1$H-NMR

(400MHz, $CD_3OD$) δ: 8.06 (s, 1H), 7.79 (s, 1H), 7.75 (s, 1H), 7.69 (s, 1H), 7.57 (dd, J = 10, 2 Hz, 1H), 7.49 (s, 1H), 7.48 (s, 1H), 7.38 (d, J=10 Hz, 1H), 7.29 (s, 1H), 7.20 (d, J=5Hz, 1H), 6.75 (dd, J=5, 2 Hz, 1H), 6.41 (s, 1H), 5.29 (t, J=10 Hz, 1H), 5.21 (t, J=10 Hz, 1H), 4.28 (m, 4H), 4.14 (m, 1H), 4.08 (m, 1H), 3.91 (m, 2H), 3.69 (s, 6H), 2.60 (m, 1H), 2.48 (m, 1H), 2.42 (m, 2H), 2.30 (m, 2H), 2.15-2.25 (m, 4H), 2.07 (m, 2H), 1.06-0.92 (m, 12H). MS (ESI) *m/z* calcd for C52H57F3N9O8 $[M+H]^+$, 992.4; found, 992.7.

Preparation of compounds **31-35** have been reported. For detailed procedures, see reference 16. Aldehydes **36a-k, m-o** were purchased from commercial resources.

*Chromane-7-carbaldehyde (**36l**)*. To a solution of compound **44** (16.6 g, 50.6 mmol) in dry THF (300 mL) was added *n*-BuLi (20 mL, 2.5 M, 50.6 mmol) at -78 $^oC$ dropwise, and stirred at this temperature for 30 min. Then, another batch of *n*-BuLi (20 mL, 2.5 M, 50.6 mmol) was added dropwise. After stirring for 15 min, a solution of DMF (7.3 g, 100 mmol) in THF (20 mL) was added dropwise. The mixture was warmed up to rt slowly and stirred for 30 min. Saturated aqueous $NH_4Cl$ was added to quench the reaction. The mixture was extracted with EA. The organic phase was washed with brine, dried over $Na_2SO_4$, and concentrated *in vacuo*. Purification through silica gel column chromatography gave **36l** as a white solid (4.1 g, 47 %). $^1$H-NMR ($CDCl_3$) δ: 9.85 (s, 1 H), 7.30 - 7.32 (m, 1 H), 7.23-7.24 (m, 1 H), 7.13-7.15 (m, 1 H), 4.17 - 4.20 (t, *J* = 5.2 Hz, 2 H), 1.98 - 2.03(m, 2 H).

*2-Methylchromane-6-carbaldehyde(**36p**)*. To a mixture of 3-(4-bromophenyl)propanoic acid (70.2 g, 308 mmol) and $BF_3{\cdot}OEt_2$ (66 g, 462 mmol) in TFA was added PIFA (198.6 g, 462 mmol) dropwise. The mixture was stirred at 40 $^oC$ for 45 hours, and then TFA was removed. The mixture was poured into $H_2O$, extracted with EA. The organic layer was washed with $NaHCO_3$ solution and brine, dried over $Na_2SO_4$. After filtration and concentration, the residue was purified by silica gel column

chromatography (PE:EA=20:1~10:1) to give 6-bromochroman-2-one ( 33 g, 47.4%). To 6-bromochroman-2-one (3.41 g, 14.956mmol) in THF was added $CH_3MgBr$ (32.9 mmol) at 0 $^oC$. The mixture was stirred at 0~20 $^oC$ for 16 h, and then the mixture was quenched with saturated $NH_4Cl$ solution. The aqueous layer was extracted with EA. The organic layer was washed with $H_2O$ and brine, dried over $Na_2SO_4$. After filtration and concentration, the residue was purified by $SiO_2$ column (PE:EA= 20:1~5:1) to give 4-bromo-2-(3-hydroxybutyl)phenol (2.65 g, 72.6%). To a mixture of 4-bromo-2-(3-hydroxybutyl)phenol (3.05 g, 12.5 mmol) and $PPh_3$ (4.912 g, 18.75 mmol) in DCM was added DIAD (4.84 g, 18.75 mmol) dropwise at 0 $^oC$. The mixture was stirred at 0~20 $^oC$ for 2 h, then the reaction was quenched with $H_2O$, extracted with EA. The organic layer was washed with $H_2O$ and brine, dried over $Na_2SO_4$. After filtration and concentration, the residue was purified by $SiO_2$ column (PE:EA=1:0~30:1) to give 6-bromo-2-methylchromane (1.7 g, 60.3%). To 6-bromo-2-methylchromane (2.7 g, 11.947 mmol) in THF was added *n*-BuLi (13.75 mmol) at -78 $^oC$. The mixture was stirred at -78 $^oC$ for 5min, and then DMF (1.3 g, 18 mmol) was added dropwise. 30 min later, reaction mixture was quenched with $NH_4Cl$, extracted with EA. The organic layer was washed with $H_2O$ and brine, dried over $Na_2SO_4$, After filtration and concentration, the residue was purified by $SiO_2$ column (PE:EA=100:1~30:1) to give **36p** (1.9 6 g, 93.3%) which was used without separating the two enantiomers. Isomers were separated at the final compound stage.

*4-Methylchromane-7-carbaldehyde (**36q**).* To a solution of compound **47** (390 mg, 1.64 mmol) in THF (10 mL) was added *n*-BuLi (2.5 M in hexane, 1.96 mL, 4.92 mmol) dropwise at -78 ℃ under nitrogen. The mixture was stirred at -78 ℃ for 10 min. Then DMF (0.76 ml, 9.83 mmol) was added. The resultant mixture was stirred at -78 ℃ for 1 h. The reaction was quenched by addition of aq. $NH_4Cl$. The aqueous layer was extracted with EA. The organic layer was washed with brine, dried over sodium sulfate, filtered and concentrated under vacuum, purified by pre-TLC eluting with PE : EA = 20 : 1 to give compound **36q** (200 mg, 69%) as an yellow oil. $^1$H-NMR (400 MHz, $CDCl_3$): δ 9.76 (s,

1H), 7.54-7.56 (m, 2H), 6.79 (d, *J* = 8.8 Hz, 1H), 2.77 (t, *J* = 6.4 Hz, 2H), 1.78 (t, *J* = 6.4 Hz, 2H), 1.30 (s, 6H).

*1a-Methyl-1,1a,7,7a-tetrahydrocyclopropa[b]chromene-5-carbaldehyde(**36r**).* A trimethylaluminum solution (2.0 M in toluene, 20 mL, 40 mmol) was added dropwise to $Cp_2TiCl_2$ (5 g, 20 mmol) under nitrogen at 0℃. After the addition, the resulting red solution was stirred at room temperature for 24 h, and then was cooled to -78 $^oC$. A solution of 6-bromochroman-2-one (**46**, 4.48 g, 20 mmol) in THF (20 mL) was added dropwise over 5-10 min. After the addition, the reaction mixture was allowed to warm to room temperature and stirred for about 30 min. 50 drops of aq 1M NaOH was added over 10 min, stirring was continued until gas evolution stopped. The reaction mixture was filtered, concentration *in vacuo*, and purified by silica gel column chromatography eluted with PE to give 6-bromo-2-methylenechromane (2 g, 45%) as yellow oil. $^1$H-NMR ($CDCl_3$ 400 MHz): δ 7.17-7.24 (m, 2H), 6.75 (d, J = 8.8 Hz, 1H), 4.56 (s, 1H), 4.16 (s, 1H), 2.76 (t, J = 6.8 Hz, 2H), 2.54 (t, J = 6.8 Hz, 2H). $CH_2I_2$ (12.44 g, 46.4 mmol) was added dropwise to a stirred solution of $ZnEt_2$ (1 M in hexane, 23.2 mL, 23.2 mmol) in dichloromethane (23 mL) at -78 ℃ under nitrogen. After the addition was completed, the reaction mixture was warmed up to 0 $^oC$, and stirred at 0 ℃ for 15 min resulting in the formation of a white precipitate. TFA (2.646 g, 23.2 mmol) was added to the mixture resulting in the rapid formation of a homogeneous colourless solution which was allowed to stir at 0 ℃ for 15 min. Then a solution of 6-bromo-2-methylenechromane (2.6 g, 11.6 mmol) in dichloromethane (10 mL) was added. The resultant mixture was stirred at room temperature for 16 h. Then saturated $Na_2EDTA$ (50 mL) was added. The resultant mixture was vigorously stirred for 5 min, then diluted with dichloromethane (100 mL) and saturated aq. $NaHCO_3$ (100 mL). The aqueous layer was extracted with dichloromethane. The organic layer was washed with brine, dried over sodium sulfate, filtered and concentrated under vacuum, and purified by silica gel column chromatography eluted with PE to give 5-bromo-1a-methyl-1,1a,7,7a-tetrahydrocyclopropa[b]chromene (1 g, 36%) as a white solid. $^1$H-NMR

(400 MHz, $CDCl_3$): δ 7.11-7.16 (m, 2H), 6.65 (d, J = 8.4 Hz, 1H), 3.08-3.13 (m, 1H), 2.86-2.90 (m, 1H), 1.54 (s, 3H), 1.20-1.25 (m, 1H), 0.92-0.95 (m, 1H), 0.54-0.58 (m, 1H). To a solution of 5-bromo-1a-methyl-1,1a,7,7a-tetrahydrocyclopropa[b]chromene (0.8 g, 3.36 mmol) in THF (20 mL) was added *n*-BuLi (2.5 M in hexane, 4 mL, 10 mmol) dropwise at -78 ℃ under nitrogen. The mixture was stirred at -78 ℃ for 5 min. Then DMF (1.54 mL, 20 mmol) was added. The resultant mixture was stirred at -78 ℃ for 1 h. The reaction was quenched by addition aq. $NH_4Cl$, extracted with EA. The organic layer was washed with brine, dried over sodium sulfate, filtered and concentrated *in vacuo*, purified by silica gel column chromatography eluted with PE: EA = 100: 1 to give 1a-methyl-1,1a,7,7a-tetrahydrocyclopropa[b]chromene-5-carbaldehyde (**36r**, 0.3 g, 48%) as an yellow oil. $^1$H-NMR (400 MHz, $CDCl_3$): δ 9.75 (s, 1H), 7.50-7.57 (m, 2H), 6.82 (d, J = 8.4 Hz, 1H), 3.11-3.16 (m, 1H), 2.95-2.99 (m, 1H), 1.54 (s, 3H), 1.23-1.28 (m, 1H), 0.88-0.94 (m, 1H), 0.58-0.62 (m, 1H).

*4-Methylchromane-7-carbaldehyde (**36s1** and **36s2**)*. A solution of 7-bromo-4-methylenechromane (3.2 g, 14.3 mmol) and $PtO_2$ (3.2 g) in ethyl acetate (50 mL) was stirred under hydrogen balloon at room temperature for 2 h, filtered and concentrated under vacuum. The residue was purified by silica gel column chromatography eluted with PE: EA = 100: 1 to give 7-bromo-4-methylchromane (2.5 g, 77%) as light yellow oil. $^1$H-NMR (400 MHz, $CDCl_3$): δ 6.93-6.99 (m, 3H), 4.10-4.20 (m, 2H), 2.83-2.91 (m, 1H), 2.00-2.08 (m, 1H), 1.67-1.72 (m, 1H), 1.65-1.66 (m, 3H). 7-bromo-4-methylchromane was separated by chiral SFC to give (*R*)-7-bromo-4-methylchromane and (*S*)-7-bromo-4-methylchromane. Conditions of SFC separation: column: AD-3 (150x20 MM), co-solvent: MeOH (0.05% DEA) 5-40%. Flow rate 50 mL/min, wavelength: 210 nm. The stereochemistry were assinged abitrarily. To a solution of (*R*)-7-bromo-4-methylchromane (1 g, 4.42 mmol) in THF (10 mL) was added *n*-BuLi (2.5 M in hexane, 5.3 mL, 13.27 mmol) dropwise at -78 ℃ under nitrogen. The mixture was stirred at -78 ℃ for 10 min. Then DMF (2 ml, 26.55 mmol) was added. The resultant mixture was stirred at -78 ℃ for 1 h. The reaction was quenched by addition aq. $NH_4Cl$, extracted with EA.

The organic layer was washed with brine, dried over sodium sulfate, filtered and concentrated under vacuum to give (*R*)-4-methylchromane-7-carbaldehyde (**36s1**) (0.7 g, 90%) as a yellow oil, which was directly used in the next step. (*S*)-4-methylchromane-7-carbaldehyde (**36s2**) was prepared from (*S*)-7-bromo-4-methylchromane by the same method as **36s1**.

*Spiro[chromane-4,1'-cyclopropane]-7-carbaldehyde (**36t**).* To a solution of compound **54** (0.77 g, 3.24 mmol) in THF (13 mL) was added *n*-BuLi (2.5 M in hexane, 2 mL, 5 mmol) dropwise at -78 ℃ under nitrogen. The mixture was stirred at -78 ℃for 5 min. Then DMF (1 ml, 13 mmol) was added. The resultant mixture was stirred at -78 ℃ for 1 h. The reaction was quenched by addition aq. $NH_4Cl$, extracted with EA. The organic layer was washed with brine, dried over sodium sulfate, filtered and concentrated under vacuum, purified by pre-TLC eluted with PE: EA = 20: 1 to give compound **36t** (0.28 g, 46%) as yellow oil.

*4,4-Difluorochromane-7-carbaldehyde (**36u**).* Deoxofluor (10 mL, 50 mmol) was added to a solution of 7-bromochroman-4-one (**52,** 1.13 g, 5 mmol) in DCM (2 mL) and EtOH (0.1 mL) in a sealed tube. The sealed tube was heated at 40 ℃ for 24 h. The resultant mixture was poured into ice-water. Aq. $Na_2CO_3$ was added to adjust pH = 9. The aqueous layer was extracted with DCM. The organic layer was washed with brine, dried over sodium sulfate, filtered and concentrated *in vacuo*, purified by silica gel column chromatography eluted with PE to give 7-bromo-4,4-difluorochromane (0.774 g, 62%) as an yellow oil. $^1$H-NMR (400 MHz, $CDCl_3$): δ 7.42 (d, J = 8.4 Hz, 1H), 7.11 (d, J = 8.8 Hz, 1H), 7.06 (s, 1H), 4.34 (t, J = 5.6 Hz, 2H), 2.39-2.49 (m, 2H). To a solution of 7-bromo-4,4-difluorochromane (0.7 g, 2.8 mmol) in THF (10 mL) was added *n*-BuLi (2.5 M in hexane, 3.5 mL, 8.75 mmol) dropwise at -78 ℃ under nitrogen. The mixture was stirred at -78 ℃ for 5 min. Then DMF (1.4 mL, 18.2 mmol) was added. The resultant mixture was stirred at -78 ℃ for 1 h. The reaction was quenched by addition aq. $NH_4Cl$, and extracted with EA. The organic layer was washed with brine, dried over

sodium sulfate, filtered and concentrated *in vacuo*, purified by silica gel column chromatography eluted with PE:EA = 20:1 to give 4,4-difluorochromane-7-carbaldehyde (**36u**) (0.33 g, 59%) as an yellow oil. $^1$H-NMR (400 MHz, $CDCl_3$): δ 9.96 (s, 1H), 7.74 (d, J = 8.0 Hz, 1H), 7.50 (d, J = 8.0 Hz, 1H), 7.37 (s, 1H), 4.40 (t, J = 6.0 Hz, 2H), 2.45-2.54 (m, 2H).

*2,2-Dimethylchromane-7-carbaldehyde(**36v**).* To a solution of 7-bromochroman-2-one (**58**, 2.26 g, 10 mmol) in THF (30 mL) was added MeLi (1.6 M in ether, 15.6 mL, 25 mmol) dropwise at 0 ℃ under nitrogen. The mixture was stirred at room temperature for 3 h, re-cooled in ice bath, and aq. 1M HCl (2.5 mL) was added dropwise. The layers were separated and the aqueous layer was extracted with EA. The organic layer was washed with brine, dried over sodium sulfate, filtered and concentrated *in vacuo*. The residue was dissolved in toluene (30 mL), $TsOH{\cdot}H_2O$ (95 mg, 0.5 mmol) was added, and the mixture was refluxed for 16 h in a Dean-Stark apparatus. After removal of the solvent *in vacuo*, purification of the residue by column chromatography on silica gel eluted with PE: EA = 300: 1 gave 7-bromo-2,2-dimethylchromane (0.71 g, 30%) as yellow oil. To a solution of 7-bromo-2,2-dimethylchromane (590 mg, 2.46 mmol) in THF (10 mL) was added n-BuLi (2.5 M in hexane, 3 mL, 7.5 mmol) dropwise at -78 ℃ under nitrogen. The mixture was stirred at -78 ℃ for 10 min. Then DMF (1.2 mL, 15.6 mmol) was added. The resultant mixture was stirred at -78 ℃ for 1 h. The reaction was quenched by addition of 1N aq. HCl, extracted with EA. The organic layer was washed with brine, dried over sodium sulfate, filtered and concentrated *in vacuo*. Purification of the residue by silica gel column chromatography eluted with PE: EA = 20:1 gave 2,2-dimethylchromane-7-carbaldehyde (**36v**) (300 mg, 64.5%) as yellow oil.

*(S)-2-methylchromane-7-carbaldehyde (**36w1**).* To a solution of compound **61** (1.108 g, 4.9 mmol) in THF (50 mL) was added *n*-BuLi (2.5 M in hexane, 6 mL, 15 mmol) dropwise at -78 ℃ under nitrogen. The mixture was stirred at -78 ℃ for 10 min. Then DMF (2.3 mL, 30 mmol) was added. The resultant

mixture was stirred at -78 ℃ for 1 h. The reaction was quenched by addition of aq. $NH_4Cl$, and extracted with EA. The organic layer was washed with brine, dried over sodium sulfate, filtered and concentrated *in vacuo*, purified by silica gel column chromatography eluted with PE: EA = 20: 1 to give compound **36w1** (0.75 g, 87% yield) as yellow oil.

*(R)-2-methylchromane-7-carbaldehyde (**36w2**).* Prepared from **62** in the same method as **36w1**.

*2,2-Dimethyl-2,3-dihydrobenzofuran-6-carbaldehyde (**36x**).* prepared in a similar manner as **36v**.

*3,10-dibromo-6-(chroman-7-yl)-1-fluoro-6H-benzo[5,6][1,3]oxazino[3,4-a]indole (**37l**).* To a mixture of **36l** (20 g, 0.12 mol) and **35** (38 g, 0.12 mol) in anhydrous $CH_3CN$ (400 mL) was added TFA (0.1 mL) at rt. The reaction mixture was stirred at 25 $^oC$ for 6 h; the solid was collected by filtration and washed with $CH_3CN$ to give 3,10-dibromo-6-(chroman-7-yl)-1-fluoro-12,12a-dihydro-6H-benzo[5,6][1,3]oxazino[3,4-a]indole (48 g, 87.8% yield). The solution of 3,10-dibromo-6-(chroman-7-yl)-1-fluoro-12,12a-dihydro-6H-benzo[5,6][1,3]oxazino[3,4-a]indole (48 g, 98.6 mmol) in dry toluene (450 mL) was added DDQ (33.6 g, 0.15 mol). After refluxing for 2h, the solvent was removed and the residue was diluted with EtOAc. The organic layer was washed with saturated $NaS_2O_3$ aqueous solution and brine, dried over $Na_2SO_4$, filtered and concentrated. The residue was washed with MeOH and filtered to give compound **37l** (45 g, 93% yield). $^1$H-NMR (400 MHz, $CDCl_3$) δ: 7.79 (s, 1 H), 7.20 - 7.22 (m, 1 H), 7.07 (s, 1 H), 6.97 - 7.01 (m, 3 H), 6.90 - 6.92 (m, 1 H), 6.39 - 6.45 (m, 1 H), 6.39 (s, 1 H), 4.08 - 4.11 (t, $J$ = 4.8 Hz, 1 H), 2.68 - 2.71 (t, $J$ = 6.4 Hz, 2 H), 1.90 - 1.95 (m, 2 H).

*6-(Chroman-7-yl)-1-fluoro-3,10-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indole (**38l**).* To a solution of **37l** (900 mg, 1.7 mmol) in 1,4-dioxane was added bis pinacol borate (2.0 mmol) and $Pd(dppf)Cl_2$ (0.09 mmol) and KOAc (5.1 mmol). The

reaction mixture was stirred under $N_2$ and heated to 110 °C for overnight. After that, the solvent was removed under vacuum, and the residue was purified by column chromatography with silica gel to afford **38l** (700 mg, 66 % yield). MS (ESI) *m/z* calcd for C36H41B2FNO6 $[M+H]^+$, 623.3; found, 624.

*Di-tert-butyl 2,2'-((6-(chroman-7-yl)-1-fluoro-6H-benzo[5,6][1,3]oxazino[3,4-a]indole-3,10-diyl)bis(1H-imidazole-5,2-diyl))(2S,2'S)-bis(pyrrolidine-1-carboxylate) (**40l**).* A suspension of **38l** (1140 mg, 1.83 mmol), **39** (2.2 mmol), $Pd(dppf)_2Cl_2$ (0.1 mmol), $Na_2CO_3$ (5.5 mmol) in THF/$H_2O$ (10:1, 10 mL) was refluxed at 75 $^oC$ overnight under $N_2$ protection. After that, the mixture was filtered, the filtrate was diluted with water (50 mL) and extracted with ETOAC (100 mL). The organic layer was washed with brine and dried over anhydrous sodium sulfate. After concentrated under vacuum, the residue was purified by column chromatography (PE / ETOAC = 8:1 to 5:1) to afford **40l** (400 mg, 26%). MS (ESI) *m/z* calcd for C48H53FN7O6 $[M+H]^+$, 841.4; found, 842.

*6-(Chroman-7-yl)-1-fluoro-3,10-bis(2-((S)-pyrrolidin-2-yl)-1H-imidazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indole HCl salt (**41l**).* Compound **40l** (400 mg, 0.48 mmol) was added to a solution of HCl/$CH_3OH$ (5 mL, 3M). The mixture was stirred at room temperature for 2-3 hr. When the reaction completed (monitored by LCMS), the mixture was concentrated under vacuum to give compound **41l** which was used in the next step without further purification. MS (ESI) *m/z* calcd for C38H37FN7O2 $[M+H]^+$, 641.29; found, 642.

*3-(2,5-Dibromophenoxy)propan-1-ol (**43**)*. To a mixture of compound **42** (20 g, 78.7 mmol), ethylene glycol (110 mL) and NMP (10 mL) was added *t*-BuOK (31.2 g, 280 mmol) slowly. The mixture was stirred at 100 $^oC$ for overnight. The mixture was poured into ice water, extracted with EA. The organic extract was washed with brine, dried over anhydrous $Na_2SO_4$, filtered, and concentrated. The

residue was purified by $SiO_2$ chromatography to give **43** as a colorless oil (21 g, 87.5 %). MS (ESI) m/z calcd for C9H12Br2O2 [M +H]+, 307.9; found, 311.0.

*1,4-Dibromo-2-(3-bromopropoxy)benzene (**44**)*. To a solution of compound **43** (17 g, 54.8 mmol) in toluene (200 mL) was added $PBr_3$ (7.4 g, 27.4 mmol) at 0 °C. After the addition was completed, the mixture was heated to 100 ℃ for 2 h. TLC showed the compound **43** disappeared. The mixture was poured into ice-water and extracted with EA. The organic phase was washed with $NaHCO_3$ and brine, dried over $Na_2SO_4$, and concentrated *in vacuo*. Purification through silica gel column chromatography gave **44** as a colorless oil (18 g, 98 %). $^1$H-NMR ($CDCl_3$) δ: 7.35 - 7.37 (d, *J* = 8.4 Hz, 1 H), 7.01 - 7.02 (m, 1 H), 6.95 - 6.98 (m, 1 H), 4.11-4.14 (t, *J* = 5.6 Hz, 2 H), 3.63 - 3.66 (t, *J* = 6.4 Hz), 2.32 - 2.38 (m, 2 H).

*7-Bromo-4-methylchromane(**46**).* To a solution of 3-(4-bromophenyl)propanoic acid (**45**, 37 g, 162.3 mmol) and PIFA (104.67 g, 243.4 mmol) in TFA (500 mL) was added $BF_3{\cdot}OEt_2$ (34.57 g, 243.4 mmol) dropwise at room temperature under nitrogen. The mixture was stirred at 40-60 ℃ for 48 h, concentrated under vacuum. The residue was dissolved in ethyl acetate, and aq. NaOH was added to adjust pH = 13, and then filtered. The filtrate was extracted with ethyl acetate. The organic layers was washed with brine, dried over sodium sulfate, and concentrated under vacuum, purified by silica gel column chromatography eluted with PE: EA = 10: 1 to give compound **46** (9 g, 24.5%) as light yellow solid.

*7-Bromo-4-methylchromane (**47**).* To a solution of compound **46** (1.12 g, 5 mmol) in THF (10 mL) was added MeLi (1.6 M in ether, 7.8 mL, 12.5 mmol) dropwise at 0 ℃ under nitrogen. The mixture was stirred at room temperature for 16 h, re-cooled with ice-water bath, and aq. HCl (1M, 2.5 mL) was added dropwise. The layers were separated and the aqueous layer was extracted with EA. The organic

layer was washed with brine, dried over sodium sulfate, filtered and concentrated under vacuum. The residue was dissolved in toluene (20 mL), $TsOH \cdot H_2O$ (19 mg) was added, and the mixture was refluxed for 16 h in a Dean-Stark apparatus. After removal of the solvent under vacuum, the residue was purified by silica gel column chromatography eluted with PE: EA = 100: 1 to give compound **47** (0.4 g, 33.6%) as yellow oil. $^1$H-NMR (400 MHz, $CDCl_3$): δ 7.07-7.09 (m, 2H), 6.57 (d, *J* = 8.4 Hz, 1H), 2.66 (t, *J* = 6.8 Hz, 2H), 1.70 (t, *J* = 6.8 Hz, 2H), 1.24 (s, 6H).

*1-(4-Bromo-2-hydroxyphenyl)ethan-1-one (**49**).* To a solution of 4-bromo-2-hydroxybenzonitrile **48** (48 g, 244 mmol) in THF (1500 mL) was added MeMgBr (3 M in diethyl ether, 450 mL, 1350 mmol) dropwise under nitrogen at -40 ℃ for 30 min. The mixture was stirred at -40 ℃ for 1 h, then at room temperature for 16 h. Water (1000 mL) and conc. HCl (200 mL) were added and stirred at room temperature for 24 h. The aqueous layer was extracted with EA. The organic layer was washed with saturated $NaHCO_3$, brine, dried over sodium sulfate, filtered and concentrated under vacuum to give compound **49** (48 g, 73%) as a yellow solid. $^1$H-NMR (400 MHz, $CDCl_3$): δ 12.32 (s, 1H), 7.55 (d, *J* = 8.4 Hz, 1H), 7.15 (s, 1H), 7.01 (d, *J* = 8.4 Hz, 1H), 2.59 (s, 3H).

*(E)-1-(4-Bromo-2-hydroxyphenyl)-3-(dimethylamino)prop-2-en-1-one (**50**).* To a solution of compound **49** (48 g, 224 mmol) in dry toluene (500 mL) was added N, N-dimethylformamide (53.3 g, 448 mmol) at room temperature. The mixture was stirred under reflux `condition` for 16 h, then cooled to rt, and a crystalline was formed. The crystalline was collected by filtration and washed with toluene to give the first batch **(**37 g) of compound **50** as a yellow solid. The filtrate was concentrated under vacuum, purified by silica gel column chromatography eluted with PE: EA = 5: 1 to give the second batch (8 g) of compound **50** (total yield: 74.6%). $^1$H-NMR (400 MHz, $CDCl_3$): δ 7.85 (d, *J* = 12.0 Hz, 1H), 7.50 (d, *J* = 8.8 Hz, 1H), 7.26 (s, 1H), 7.09 (s, 1H), 6.92 (d, *J* = 8.8 Hz, 1H), 5.66 (d, *J* = 12.0 Hz, 1H), 3.18 (s, 3H), 2.95 (s, 3H).

*7-Bromo-4H-chromen-4-one (**51**).* To a solution of compound **50** (37 g, 137.5 mmol) in dichloromethane (400 mL) was added conc. HCl (115 mL, 1.375 mol) at room temperature. The mixture was refluxed for 2 h, extracted with dichloromethane. The organic layer was washed with saturated $NaHCO_3$, brine, dried over sodium sulfate, filtered and concentrated under vacuum to give compound **51** (31 g, 98% yield) as a light yellow solid. $^1$H-NMR (400 MHz, $CDCl_3$): δ 8.04 (d, $J$ = 8.8 Hz, 1H), 7.80 (d, $J$ = 6.4 Hz, 1H), 7.63 (s, 1H), 7.50 (d, $J$ = 8.8 Hz, 1H), 6.32 (d, $J$ = 6.4 Hz, 1H).

*7-bromochroman-4-one (**52**).* To a solution of compound **51** (32.6 g, 145.5 mmol) in THF (600 mL) was added DIBAl-H (1 M in toluene, 437 mL, 437 mmol) dropwise under nitrogen at -78 ℃ for 30 min. The mixture was stirred at -78 ℃ for 1 h. The resultant mixture was poured into 500 mL of 1 M aq. HCl, and extracted with EA. The organic layer was washed with brine, dried over sodium sulfate. After filtration and concentration, the residue was purified by silica gel column chromatography eluted with PE: EA = 20: 1 to give compound **52** (21 g, 63.8%) as a yellow solid. $^1$H-NMR (400 MHz, $CDCl_3$): δ 7.72 (d, $J$ = 8.0 Hz, 1H), 7.13-7.17 (m, 2H), 4.52 (t, $J$ = 6.4 Hz, 1H), 2.79 (d, $J$ = 6.4 Hz, 1H).

*7-Bromo-4-methylenechromane (**53**).* To a solution of methyltriphenylphosphonium bromide (40.45 g, 111.31 mmol) in THF (500 mL) was added *n*-BuLi (2.5 M in hexane, 45.3 mL, 111.31 mmol) dropwise at 0 ℃ under nitrogen. The mixture was stirred at 0 ℃ for 1 h and then a solution of compound **52** (21.34 g, 94.62 mmol) in THF (100 mL) was added. The mixture was stirred at 0 ℃ for 1 h, then at room temperature for another 1 h. The reaction was quenched by addition of aq. $NH_4Cl$, and extracted with EA. The organic layer was washed with brine, dried over sodium sulfate, filtered and concentrated under vacuum, purified by silica gel column chromatography eluted with PE: EA = 50: 1 to give compound **53** (8.8 g, 41.6%) as yellow oil. $^1$H-NMR (400 MHz, $CDCl_3$): δ 7.38 (d, $J$ =

8.8 Hz, 1H), 6.97-6.99 (m, 2H), 5.47 (s, 1H), 4.89 (s, 1H), 4.20 (t, *J* = 5.6 Hz, 2H), 2.64 (t, *J* = 5.6 Hz, 2H).

*7-Bromospiro[chromane-4,1'-cyclopropane]* ***(54)***. $CH_2I_2$ (17.17 g, 66 mmol) was added dropwise to a stirred solution of $ZnEt_2$ (1 M in hexane, 33 mL, 33 mmol) in dichloromethane (33 mL) at -78 ℃ under nitrogen, and the mixture was stirred at 0 ℃ for 15 min resulting in the formation of a white precipitate. TFA (3.77 mL, 33 mmol) was added to the mixture resulting in the rapid formation of a homogeneous colourless solution which was allowed to stir at 0 ℃ for 15 min. Then a solution of compound **53** (3.7 g, 16.5 mmol) in dichloromethane (10 mL) was added. The resultant mixture was stirred at room temperature for 16 h. Then saturated $Na_2EDTA$ (70 mL) was added. The resultant mixture was vigorously stirred for 5 min, then diluted with dichloromethane (100 mL) and saturated $NaHCO_3$ (100 mL). The aqueous layer was extracted with dichloromethane. The organic layer was washed with brine, dried over sodium sulfate, filtered and concentrated under vacuum, purified by silica gel column chromatography eluted with PE: EA = 50:1 to give compound **54** (2 g, 51%) as a white solid. $^1$H-NMR (400 MHz, $CDCl_3$): δ 6.89-6.94 (m, 2H), 6.47 (d, *J* = 8.4 Hz, 1H), 4.25 (t, *J* = 5.2 Hz, 2H), 1.82 (t, *J* = 5.2 Hz, 2H), 1.00 (t, *J* = 5.2 Hz, 2H), 0.82 (t, *J* = 5.2 Hz, 2H).

*Ethyl (E)-3-(4-bromo-2-hydroxyphenyl)acrylate* ***(56)***. To a solution of compound **55** (25.5 g, 126 mmol) in DCM (250 mL) was added $Ph_3P{=}CHCO_2Et$ (48.3 g, 138.6 mmol) in portions. The mixture was stirred at room temperature under nitrogen for 1 h. The reaction was complete detected by TLC (PE: EA = 5: 1). The solvent was removed *in vacuo*. The residue was purified by silica gel column chromatography eluted with PE: EA = 10: 1 to give compound **56** (30 g, 88%) as a white solid. $^1$H-NMR (400 MHz, $CDCl_3$): δ 7.95 (d, J = 16.4 Hz, 1H), 7.58-7.69 (br, 1H), 7.48-7.52 (m, 1H), 7.26-7.32 (m, 2H), 6.62 (d, J = 16.4 Hz, 1H), 4.29 (q, J = 7.2 Hz, 2H), 1.35 (t, J = 7.2 Hz, 3H).

*Ethyl 3-(4-bromo-2-hydroxyphenyl)propanoate (**57**):* A solution of compound **56** (30 g, 111 mmol) and $Ru(PPh_3)_3Cl$ (3 g, 3.24 mmol) in MeOH (1000 mL) was stirred under hydrogen (50 psi) at room temperature for 16 h, concentrated *in vacuo* to give the crude **57** which was directly used in the next step.

*7-Bromochroman-2-one (**58**).* Compound **57** (30 g, 110 mmol) was dissolved in toluene (250 mL), $TsOH \cdot H_2O$ (419 mg, 2.2 mmol) was added, and the mixture was refluxed for 16 h in a Dean-Stark apparatus. After removal of the solvent *in vacuo*, purification of the residue by column chromatography on silica gel eluted with PE: EA = 10: 1 to give compound **58** (22 g, 88%) as a white solid. $^1$H-NMR (400 MHz, $CDCl_3$): δ 7.11-7.16 (m, 2H), 6.70 (s, 1H), 2.88 (t, J = 6.4 Hz, 2H), 2.54 (t, J = 6.4 Hz, 2H).

*7-Bromochroman-2-ol (**59**).* To a solution of compound **58** (16 g, 70.8 mmol) in DCM (200 mL) was added DIBAl-H (1 M in toluene, 85 mL, 85 mmol) dropwise under nitrogen at -78 ℃ for 15 min. The mixture was stirred at -78 ℃ for 2 h. The resultant mixture was poured into 200 mL of 1 M aq. HCl, and extracted with EA. The organic layer was washed with brine, dried over sodium sulfate, filtered and concentrated *in vacuo*, purified by silica gel column chromatography eluted with PE: EA = 20: 1 to give compound **59** (11.5 g, 71%) as a white solid. $^1$H-NMR (400 MHz, $CDCl_3$): δ6.81-6.98 (m, 3H), 5.54 (S, 1H), 2.79-2.99 (m, 1H), 2.49-2.64 (m, 1H), 1.85-2.01 (m, 2H).

*5-Bromo-2-(3-hydroxybutyl)phenol (**60**):* To a solution of compound **59** (11.5 g, 50.4 mmol) in THF (200 mL) was added MeMgBr (3 M in $Et_2O$, 50.4 mL, 151 mmol) dropwise at 0 ℃ under nitrogen. The mixture was stirred at 0 ℃ for 20 min. The resultant mixture was allowed to warm to room temperature and stirred for 2 h. The reaction was quenched by addition 1N aq. HCl, and extracted with EA. The organic layer was washed with brine, dried over sodium sulfate, filtered and

concentrated *in vacuo*, purified by silica gel column chromatography eluted with PE : EA = 10 : 1 to give compound **60** (7.5 g, 61%) as a white solid. $^1$H-NMR (400 MHz, $CDCl_3$): δ 6.89-7.06 (m, 3H), 3.69-3.79 (m, 1H), 2.77-2.87 (m, 1H), 2.59-2.62 (m, 1H), 1.61-1.83 (m, 2H), 1.16-1.25 (m, 3H).

*(S)-7-bromo-2-methylchromane (**61**) and (R)-7-bromo-2-methylchromane (**62**).* To a solution of compound **60** (8.2 g, 33.6 mmol) and $PPh_3$ (9.69 g, 37 mmol) in DCM (150 mL) was added DIAD (7.3 mL, 37 mmol) dropwise at 0℃.The mixture was stirred at 0 ℃ for 1 h. the reaction was quenched by addition of ice water, and then extracted with DCM. The organic layer was washed with brine, dried over sodium sulfate, filtered and concentrated *in vacuo*, purified by silica gel column chromatography eluted with PE to give a mixture of **61** and **62** (5.5 g, 72%) as yellow oil, which was separated by SFC to give compound **61** and **62**. The stereochemistry of **61** and **62** were assigned arbitrarily. Condition of SFC: column: AD-3 (150*20 MM), co-solvent: MeOH (with 0.05% DEA, 5-40%), flow rate: 50 ml/min.

## Supporting Information

$^1$H-NMR spectra of compounds **3**-**14**, **16**-**29**

$^{13}$C-NMR spectra of compound **14**

SMILES molecular formula strings of compounds **1**-**29**

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## Abbreviations used

BOP ((Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate), DEA (diethyl amine), DIAD (Diisopropyl azodicarboxylate), DIBAl-H (Diisobutylaluminum hydride solution ),

DIEA or DIPEA (N, N-diisopropylethylamine), EtOAc or EA (ethyl acetate), HATU (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b] pyridinium 3-oxid hexafluorophosphate), IPA (isopropyl alcohol), IV (intravenously), MeOH (methanol), mpk (mg per kg), PE (Petroleum ether), PIFA ([Bis(trifluoroacetoxy)iodo]benzene ), PO (by mouth, orally, from the Latin "per os"), SFC (Supercritical Fluid Chromatography)

**References and notes**


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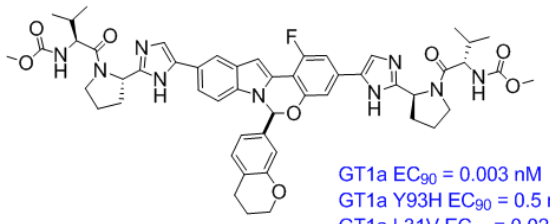
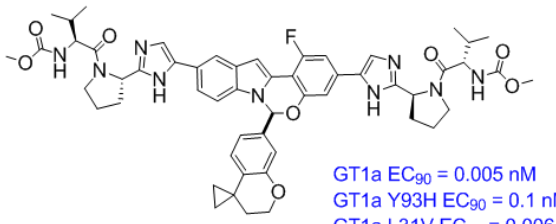
Table of Contents graphic



**14**

GT1a $EC_{90}$ = 0.003 nM
GT1a Y93H $EC_{90}$ = 0.5 nM
GT1a L31V $EC_{90}$ = 0.02 nM
GT2b $EC_{90}$ = 0.3 nM



**28**

GT1a $EC_{90}$ = 0.005 nM
GT1a Y93H $EC_{90}$ = 0.1 nM
GT1a L31V $EC_{90}$ = 0.006 nM
GT2b $EC_{90}$ = 0.2 nM