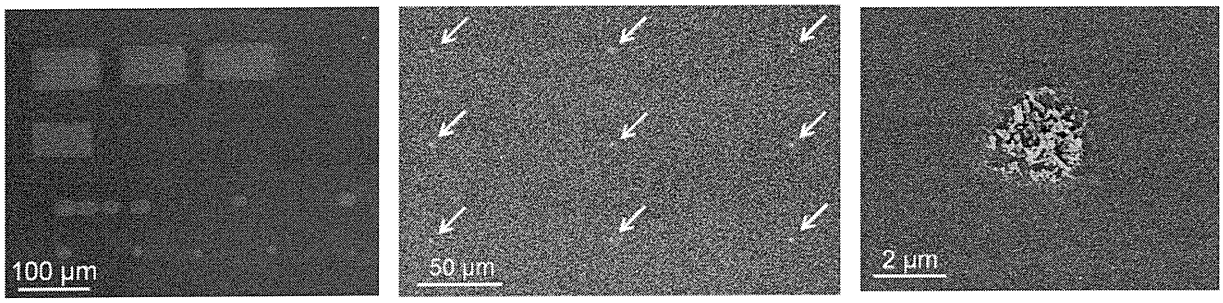

Fig. 2. EB-patterned PDA NP monolayers at different scales. In (b), the spots are indicated by arrows.

length of the square and the mean gap width between the squares are 9.7 and 3.1 μm, respectively. The side length of the square hole and the thickness of the bar of the grid are 7.5 and 5 μm, respectively, as given in the data sheet. The measured side length of the square is 2.2 μm larger than that of the hole, and the gap width is 1.9 μm less than the bar thickness in the original mask. This may arise from the diffraction effect at the mask edges since the mask was put on the substrate without any pressure during the UV irradiation. These results suggest that the patterning of PDA NP monolayers can be made within a resolution of 1.1 μm, considering that the difference in the square size of 2.2 μm is produced on both sides of the square.

Next, we attempted patterning based on polymerization of DA NPs using EB irradiation. EB polymerization was employed to obtain a PDA film of good quality.[12] We irradiated EBs of different shapes to the DA NP-coated substrate in the SEM chamber. We took out the substrate from the chamber and the substrate was rinsed with acetone to remove the DA NPs. Then, it was put in the chamber again for SEM observation. Figure 2(a) shows the SEM image of the PDA NPs patterned with rectangular EBs of different spot sizes. The shape of the PDA monolayer corresponds to the scan area of the EB. Reflection absorption spectroscopy was made for a rectangle PDA monolayer with a dimension of $100 \times 150\,\mu m^2$, produced by EB polymerization. The spectrum is shown in Fig. 3. The characteristic peaks of PDA at 601 and 663 nm are observed, which are absent in DA NPs, indicating that the DA NPs are certainly polymerized by EB exposure.

Finally, we examined the formation of a small PDA NP spot using narrow EB of a few nanometers in diameter. Figure 2(b) shows a large-area SEM image involving nine PDA NP spots, which correspond to the EB-irradiated positions (shown with arrow). The DA NPs out of the EB-irradiated spots are rinsed off. Figure 2(c) shows the fine image of the PDA NP spot. The diameter is approximately 2.5 μm. The spot was much broader than expected, although the EB diameter was much smaller, in the order of a few nanometers. The possible causes of the broadening are (i) scattered electrons from the substrate, (ii) defocused EB beam due to less contrast of the substrate, (iii) secondary electrons generated from substrate surface, and (iv) local heating of the substrate by EB irradiation. To form a nanometer-size PDA NP spot, optimization of parameters, such as the EB intensity, acceleration voltage, and focusing, are necessary.

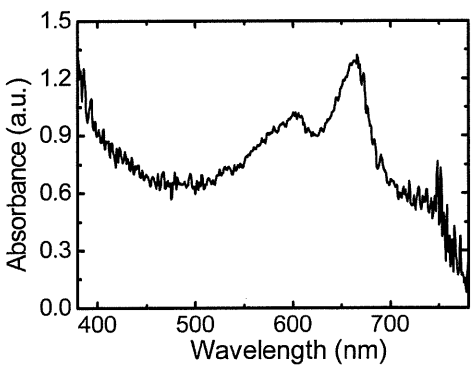

Fig. 3. Reflection absorption spectrum of an EB-patterned PDA NP monolayer.

In summary, we have shown a simple micropatterning method of PDA NP monolayers on the basis of UV or EB polymerization of DA. The UV patterning can be made within a resolution of 1.1 μm. The EB irradiation also brings about the polymerization, and patterned PDA NP monolayers were formed. However, the smallest spot size of PDA NPs produced by the EB polymerization was 2.5 μm in diameter even when a narrow EB was used. Optimization of the EB irradiation is for future work, in order to obtain nanometer-size PDA NP spots.

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バイオ分野における表面プラズモンの利用

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バイオ分野における表面プラズモンの利用

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金属ナノ粒子中の局在表面プラズモンを用いた生体検出や医療への応用について，最近の話題を二つ紹介する．一つは表面プラズモン共鳴による表面増強ラマン散乱を使った生体物質などの検出や生体組織のイメージングである．もう一つは，それを用いた近赤外光の高効率利用である．高コントラストな生体組織の近赤外イメージングやがんの光熱治療が研究されている．

Keywords : localized surface plasmon, surface enhanced Raman scattering, biosensor, two photon luminescence, photothermal cancer therapy, nanoparticle

1. まえがき

バイオ分野で最も広く用いられてきた表面プラズモンの利用方法に，有機分子やたんぱく質，核酸などの検出がある．これは，表面近傍の物質の有無により表面プラズモンの共鳴条件が変化することを利用している[1,2]．図1(a)に示すように検出対象分子（アナライト）に相互作用をもつ分子（リガンド）を表面に固定化し，そこへの分子の結合や吸着を反射率や散乱光強度の変化としてプローブする．蛍光色素などで標識（ラベル）する必要がない検出方法である．すでに多くの市販品もあり，生化学や遺伝子工学の分野では欠くことのできないツールとなっている．

近年，バイオ，医療，環境などの分野で表面プラズモン(Surface Plasmon : SP)の利用がさらに進んでいる．一つは，表面増強ラマン散乱(Surface-Enhanced Raman Scattering : SERS) を利用した増感やイメージングである．ラマン散乱は振動分光の一種であり，官能基や分子構造に応じて，それら固有の振動数だけシフトした散乱光が生じる現象である．それを分光すると分子種の同定やその電子状態，結合や吸着状態などのさまざまな知見を得ることができる．SERS はリガンドを用いずに物質の検出ができるため，細菌やウイルスなどの病原体を迅速に検出できる可能性がある（図1(b)）．また，リガンドを用いた物質などの検出においても SERS は有効である．この方法では，図1(c)に示すように，SERS を発生する色素分子を標識として用い，アナライトの高感度検出を行う．この色素は SERS タグ，SERS レポーターなどとも呼ばれるが，本稿ではこの色素を SERS 色素と呼ぶことにする．SERS が観測されるように入射光電場を増強させる必要があるため，SERS 色素を金属ナノ粒子に固定化して用いる．一度に多くのアナ

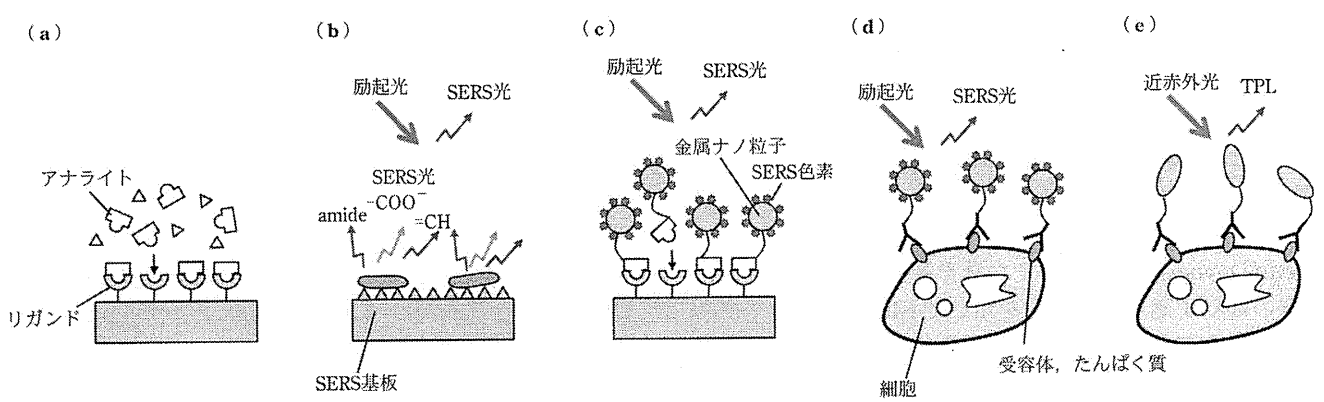

図1　(a)非標識バイオセンシング方法．アナライトがリガンドに結合した際の反射率，散乱光強度の変化などを測定する．(b)SERS 基板を用いた物質や微生物，ウイルスの検出．この方法ではリガンドを用いる必要がない．(c)SERS 色素を使った高感度なバイオセンシング．(d)SERS 色素を使った細胞のイメージング．SERS 色素を固定化した金属ナノ粒子に抗体などを修飾し，細胞表面の受容体，たんぱく質に特異的に結合させる．(e)TPL を使った細胞のイメージング．

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ライトを検出する方法（多重検出）に適しているなど，通常の蛍光標識に対していくつかの長所を有する．また，リガンドを基板に固定化している必要はなく，例えば細胞表面にリガンドが存在する場合にも有効である．これを利用した細胞のイメージングが多数報告されている（図1(**d**)）．

もう一つの表面プラズモン共鳴の利用方法に，近赤外光の高効率利用がある．波長700～900 nmの近赤外領域の光は，生体組織での吸収が比較的小さく透過率が高い．これは，水や血液中のヘモグロビンの吸収がこの波長帯で小さいことによる．そのため，生体組織中へ光を照射することが可能となる．これを利用すると金属ナノ粒子中の表面プラズモンによる局所的な発熱を利用した細胞の加工や破壊ができる．がん治療を目指した動物実験がいくつか報告されている．また，金属ナノ粒子からの多光子蛍光を使った高コントラストの生体組織のイメージングも可能であるため，これらを組み合わせることにより，光熱治療の新しい方法が開発されてようとしている（図1(**e**)）．本稿では，金や銀などのナノ粒子中の表面プラズモンを使った生体検出や医療への応用について，最近の話題を紹介する．

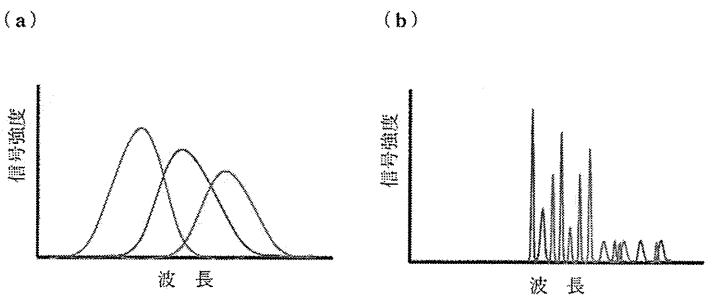

図2　蛍光スペクトル(a)とSERSスペクトル(b)の比較を模式的に表した図．蛍光スペクトルではバンド幅が広いため3種類（青，緑，赤）の蛍光が重なり分離が難しいが，SERSスペクトルはバンド幅が狭いためそれらの分離が容易である．

2.　表面増強ラマン散乱

2.1　非標識検出

一般にラマン散乱光は非常に弱いが，金属表面に分子が吸着した際には強いラマン散乱が生じることがある[3]．これを表面増強ラマン散乱（SERS）と呼ぶ．特に粗い金属表面や金属ナノ粒子の会合構造で強い散乱光が得られる．増強は局在表面プラズモンによる電場増強効果（物理的な効果）と電子移動による振動増強（化学的な効果）の二つの寄与が考えられている．一般に前者は10^2～10^{10}の増強が，後者では10～10^2の増強があると考えられている．

一つの応用例にSERS基板がある．これは試料を基板の上に塗布するだけで，強いラマン散乱分光を可能にする基板である．金属ナノ粒子を固定化したもの，周期構造を用いたもの，金属ナノ構造を表面に構築したものなどがある．SERSのバイオ分野への応用では，SERS基板を使った物質や病原体の分析が考えられる．

Van Duyneらは，ラテックス球を基板上に配列してそこに銀の薄膜を蒸着した基板を用いてSERSを用いてグルコースの濃度を測定している[4,5]．銀の表面をエチレングリコールの自己組織化単分子膜でコートし親和性の問題と酸化の問題を解決し，銀表面でも安定性に検出できるようにした．血漿中で濃度0～450 mg/dLの間で十分な定量性が確保されている．グルコースなどの生体内物質の低侵襲な検査法となる可能性がある．

また，近年SERSを用いた病原体の検出が報告されている．Tripらは斜め蒸着したナノロッドで構築した構造を使って，アデノウイルス，ライノウイルス，HIV，RSウイルス，インフルエンザウイルスなどの検出を行っている[6]．ウイルスを構築している核酸やアミノ基などの振動ピークが観測されており，それぞれの構成比がウイルスにより異なるので，原理的にはこれらの分析が可能である．Alexanderらは市販のSERS基板を用いて迅速なウイルス検出や診断の可能性を示した[7]．腫瘍ウイルス，牛痘ウイルス，水疱性口内炎ウイルスの検出結果を示した．ウイルス粒子数は基板上で1 mm^2当たり100以下でも検出が可能となった．得られたスペクトルの差はあまり大きいものではないため，統計的な手法を使ってウイルス種を決定している．

SERSを使った細菌の検出も報告されている．Irudayarajらは溶液中に形成した銀ナノ粒子集合体に細菌を吸着させてスペクトルの測定を行った[8]．この集合体を用いることにより，SERSの増強度10^8程度が見積もられている．大腸菌（O 157），ネズミチフス菌，黄色ブドウ球菌の検出が行われている．濃度は，1 mL中の個体数100程度であった．この他にも，バクテリアの検出は大腸菌の検出を中心としてグラム陽性菌やグラム陰性菌，炭疽菌類，枯草菌類などの検出の報告がある[9～12]．

2.2　SERS標識による高感度検出

図1(c)や図1(d)に示すように，金や銀などの金属ナノ粒子にSERSを示す色素を固定化して物質の検出を行う方法がある．SERS色素を用いた標識は，蛍光色素を使った標識に対して以下の長所がある[13]．①複数の色素を使っても励起光源は1台のレーザーでよい．一方，蛍光色素は分子により励起スペクトルが異なるため，多重検出には複数の光源が必要である．②SERS色素のほうが蛍光色素より種類が多い．③ラマンバンドの半値幅は非常に狭いので，多種の標識を容易に識別することができる．④蛍光色素は金属表面に近づくと消光するため，表面プラズモンを有効に使うことが難しい．図2に①および③を模式的に示した．このように，SERS色素による標識は多重化においてメリットが大きい．

DNAの多重化検出の手順の例を図3に示す．あらかじめ塩基配列aをもつプローブDNAを固定化した基板を用意しておく．もし，塩基配列a′b′をもつターゲットDNAが試料中に存在する場合には，塩基配列a′の部分が基板上のプローブDNAに結合する．次にSERS色素でラベルさ

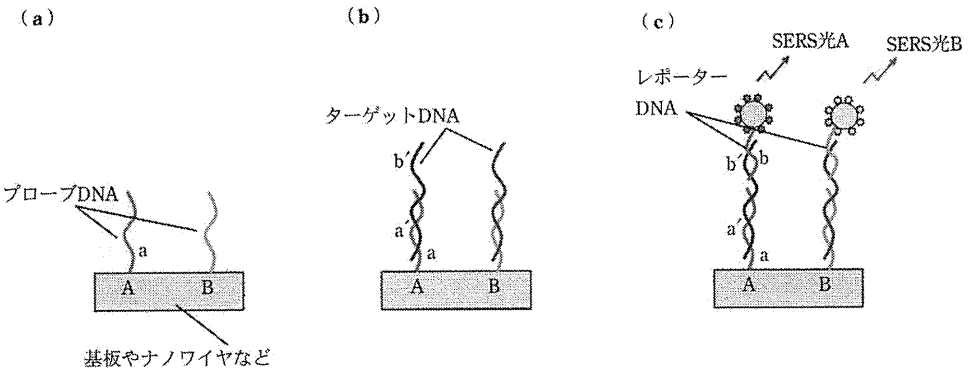

図3　多重DNA検出の説明図．(a)基板やナノワイヤにプローブDNAを結合．ここでは，DNA(A)とDNA(B)の2種類について考える．例えば，プローブDNA(A)の塩基配列をaとする．(b)塩基配列a′-b′をもつターゲットDNAがプローブDNAに結合する．(c)塩基配列bをもつリポーターDNAがプローブDNAのb′部分に結合する．リポーターDNAの末端にはSERS色素を固体化した金属ナノ粒子が結合している．異なるSERS色素の種類を用いることにより，DNA(A)とDNA(B)を空間的に区別できなくても，スペクトルから区別が可能であり，ターゲットDNAの有無がわかる．

れた銀ナノ粒子を末端に結合したレポーターDNA(塩基配列bをもつ)を用意し，それを反応させると固定化される．複数の種類のターゲットDNAがあり，それに応じてプローブDNAも複数用意した場合，それらが空間的に区別できないときでも（図3ではDNA(A)とDNA(B)の2種類を考えている），SERS色素のスペクトルの違いによりターゲットDNAの有無を識別することができる．

MirkinらはCy 3やTMRなど8種類の色素でラベルしたDNAを用意し，それらに特異的に結合するDNAの検出を行った[13]．このままではSERS信号が弱いので，銀で染色することにより色素からの明瞭なSERS信号が得られている．この方法を用いて低濃度（20 fM）でのDNA検出に成功している．Kimらは，病原体由来のDNAの多重検出に成功している[14]．プローブDNAを金ナノワイヤ上に結合して，SERS色素とレポーターDNAをもつナノ粒子が固定化されている様子がSEM（Scanning Electron Microscope)像で示されている．また，PorterらはSERSによる標識をした金ナノ粒子によるサンドイッチアッセイ法を使いPSA（Prostate Specific Antigen）の検出を行い，検出限界1 pg/mL程度の高感度測定を実現している[15]．PSAは前立腺がんの診断に用いる腫瘍マーカーの一種である．0.1 ng/mLが検出できれば実用的であるため，この結果は感度的には十分な値である．

近年では，標識された金属ナノ粒子をがんなどの特定の組織のイメージングに用いる例が多く報告されている．Kimらは直径200 nmのシリカ粒子を直径数nmの銀粒子で覆い，さらにラマン散乱を示す色素を固定し，シリカで覆った「SERSドット」を開発した[16]．その最外層のシリカ層に抗体を固定化することにより，ドットを細胞上のたんぱく質などに特異的に結合させることができる．抗体を固定化したSERSドットが結合したがん細胞の顕微鏡イメージやその領域のSERSスペクトルを測定している．また，Sunらは，銀のクラスタをSERS活性色素でラベルした構造を利用して，前立腺細胞中のPSAの分布をイメージングすることに成功している[17]．

Nieらは同様の方法で，マウス生体内のがん細胞に結合した金ナノ粒子に固定化された色素からのSERSスペクトルを観察した[18]．直径60～80 nmの金粒子をコアとして，その表面にラマン色素およびPEG（ポリエチレングリコール）を被覆したものを用いている．この金ナノ粒子からの信号強度を同様の目的で用いられる量子ドットからの蛍光強度と比べている．その結果，SERS色素が固定化された金ナノ粒子からの信号は量子ドットのそれと比較して約200倍強いことが示された．また，金ナノ粒子の表面に上皮成長因子受容体（Epidermal Growth Factor Receptor：EGFR）に対し特異性をもつ抗体をリガンドとして固定化した．これをがん細胞に暴露したところ，頭頸部悪性腫瘤細胞からは強いラマン散乱光が得られたのに対して，非小細胞肺がん細胞からはラマン散乱光が得られなかった．前者は細胞表面にEGFRを提示しているのに対して，後者はそれが少ないためである．さらに抗体を修飾した金ナノ粒子をその悪性腫瘤が移植されたマウスに注射し観察したところ，4～6時間後には腫瘤にSERS粒子が徐々に集まり，強いラマン散乱光が観察されている．入射光には生体組織での光の透過性が高い近赤外光（波長785 nm）を用いたため，生体の外から照射した光でSERSの検出を行っている．一方，肝臓からのラマン散乱光は，抗体で標識しない金ナノ粒子を用いた場合とほぼ同じ強度であった．これらの結果から，この手法が生体内の組織の選択的なイメージングや低侵襲な検査に有用な手法であることがわかる．

3.　近赤外光の高効率利用

金属ナノ粒子中の表面プラズモン共鳴を利用して生体組織を観察するもう一つの利点に，近赤外光を高効率に利用できる点がある．血液などによる吸収を最小限に抑えることができる波長領域であり，光熱治療などへの応用が期待

される．さらに，近赤外光励起の多光子過程を利用した分光やイメージングは高い空間分解能とコントラストを実現する．これらの特徴を生かした研究が報告されているので，以下に紹介する．

金ナノロッドを使った生体組織の2光子蛍光（Two Photon Luminescence：TPL）によるイメージングはWangらにより初めて報告されている[19]．金ナノロッドは，長軸方向に強い近赤外の表面プラズモン共鳴吸収をもつ．金は伝導体からdバンドへの遷移，あるいは，バンド内の遷移による蛍光が生じ，さらに共鳴時に生ずる強い局所電場によりTPLが観察される．研究ではマウスの耳たぶの血管中に金ナノロッドを注入し，そのTPL像を観察した．量子ドットによるイメージングと比べて約1/20の粒子数で明瞭な像が得られている．Ben-Yakarらは，金ナノロッド中のTPLを使って，皮膚がんの細胞のイメージングを行った[20]．金ナノロッドにはあらかじめEGFR抗体を修飾していたものを用いている．2光子自己蛍光像では，細胞全体が明るく観察されるのに対して，TPL像では細胞表面の輪郭に沿って明るく観察されている．これは，がん細胞の表面に過剰に存在するEFFRにナノロッドが選択的に結合しているためであり，細胞のもつ特徴が像に表れている．また，自己蛍光ではイメージングに64 μWの入射光強度が必要であったが，TPL像では0.14 μWで十分な明るさの像が得られている．

金属ナノ粒子によるイメージングは，細胞の観察だけでなくその破壊にも用いることができる．El-Sayedら[21]は，EGFR抗体で修飾をした球状の金ナノ粒子およびナノロッドを用意し，通常の皮膚角化細胞(HaCat)，口腔がん細胞（HOC 313）および舌がん細胞（HSC 3）に結合させた．暗視野顕微鏡観察によりHOC 313およびHSC 3では細胞表面が金ナノ粒子で覆われ，その形が明瞭にイメージングされるのに対して，正常なHaCat細胞ではそのような像は観察されなかった．がん細胞では細胞膜にEGFRが提示されており，金ナノ粒子が結合したためである．波長800 nmの光を照射したところ，がん細胞では光強度80 mWで破壊されたのに対して，HaCat細胞では破壊が起こり始める照射強度は120 mWであった．金ナノ粒子の有無がこの光強度の違いの理由と考えられる．すなわち，正常細胞とがん細胞の選択的な破壊を実現しており，がんの光熱治療への利用が期待される．

このような光熱効果は，細胞の加工ツールとしても利用され始めようとしている[22]．金ナノ粒子を固定化した先端を直径1 μm程にとがらせたマイクロキャピラリを使って，そこへパルスレーザーを導波して照射することにより，細胞の損傷の小さい加工ツールとなることが示されている．また，Zharnovらは，黄色ブドウ球菌の細胞壁にあるたんぱく質（Protein A）に抗体を結合させ，金ナノ粒子を固定化した．そして，パルスレーザーを用いて選択的に黄色ブドウ球菌を殺菌している[23]．熱レンズ顕微鏡と分光分析により殺菌のメカニズムが光照射による金ナノ粒子周辺の瞬間的な温度上昇に伴う泡の発生によることを明らかにしている．

ナノロッド以外に近赤外領域に強い共鳴ピークをもつ金属ナノ粒子に，コア-シェル構造をもつナノ粒子がある．HalasとWestら[24]が用いたコア-シェル構造は，コア径は直径約120 nmのシリカでできており，シェルは厚さ12 nmの金ナノ粒子の層である．その表面は，PEGで覆われている．金ナノ粒子では520 nm付近に表面プラズモンの共鳴ピークが現れるが，このコア-シェル粒子では波長800 nmにピークが現れる．腫瘍をもつマウスにそのナノ粒子を静脈注射すると腫瘍組織に粒子が集まることが観察された．この様子が，組織の深さ方向の分布を調べることができる光コヒーレンストモグラフィ（Optical Coherence Tomography：OCT)像で明瞭に観察されている．さらに，近赤外光を腫瘍部分に照射することにより，マウスの生存期間が長くなることが確かめられ，光熱治療の効果が現れているとしている．

4. むすび

バイオや医療の分野での金属ナノ粒子中の表面プラズモン共鳴の利用方法の研究例を紹介した．ここ数年，この分野の研究は急速に進んでいる．将来，がんをはじめとする各種の疾患の診断や治療の有力なツールとなることが期待される．

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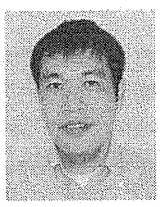

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Fused heterocyclic amido compounds as anti-hepatitis C virus agents

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ABSTRACT

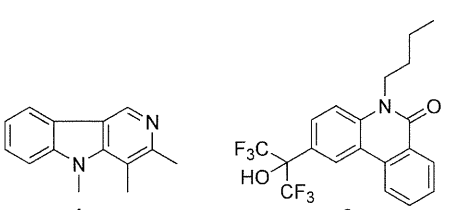
We identified a fused heteroaromatic amido structure based on the phenanthridine skeleton as a superior scaffold for candidate drugs with potent anti-HCV activity. Among the compounds synthesized, a phenanthridine analogue with a 1,3-dioxolyl group (**24**) possessed the most potent anti-HCV activity (EC_{50} value: 50 nM), with acceptable cytotoxicity. The structural development and structure–activity relationships of these compounds are described.

1. Introduction

Hepatitis C virus (HCV: a member of the *Flaviviridae* family) is thought to be a major cause of human hepatitis,[1,2] and it is estimated that at least 170 million people worldwide are chronically infected with this virus.[3] Most infections become persistent, and about 60% of cases progress to chronic liver disease, which in turn can lead to cirrhosis, hepatocellular carcinoma, and liver failure.[4,5] Currently, no vaccine is available to prevent HCV infection, and the standard treatment for chronic hepatitis C consists of pegylated interferon (IFN)-α in combination with the nucleoside analogue ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide). However, the virus cannot be eliminated from approximately half of infected patients treated with these agents.[6] In addition, the side effects of these agents are sometimes serious and unacceptable to patients. A number of molecules are being studied in clinical trials,[7] but none of them has yet been approved. Therefore, alternative agents for the treatment and prevention of HCV infection are urgently needed.

Recently, we have succeeded in the development of antiviral agents with polyphyletic skeletons.[8–16] Among them, several fused-heterocyclic compounds, including 3,4,5-trimethyl-γ-carboline (**1**)[9] and 5-butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)phenanthridin-6(5*H*)-one (**2**),[12] exhibited anti-flavivirus activity (Fig. 1). The γ-carboline analogue **1** showed powerful inhibitory activity for bovine viral diarrhea virus (BVDV) replication, with an EC_{50} value of 3.5 nM. Although BVDV belongs to the *Flaviviridae* family, as HCV does,[17] and is thought to be a surrogate model for HCV,[18–20] compound **1** showed only very weak activity against HCV. On the other hand, phenanthridine analogue **2**, which has a fused heterocyclic amido structure, exhibited moderate, dose-dependent HCV replication-inhibitory activity,[12] supporting the idea that phenanthridine analogues could be lead compounds in the development of anti-HCV drugs.

In this paper, we describe the synthesis of a series of fused heterocyclic amido compounds based on the structure of phenanthridine **2**, as well as the evaluation of their anti-HCV activity, and the creation of potent anti-HCV agents possessing one or more methoxy group(s) and/or a 1,3-dioxolyl group.



1
anti-BVDV agent
EC_{50} = 3.5 nM

2
anti-HCV agent
EC_{50} = 3.2 μM

Figure 1. Antiviral activity of fused heterocyclic compounds.

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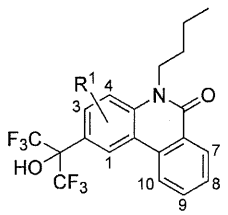
2. Results and discussion

2.1. Electronic effects of substituents

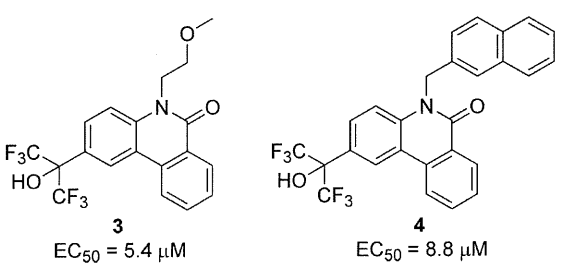
Our previous study[12] on the anti-HCV activity of phenanthridine analogues indicated that a 1,1,1,3,3,3-hexafluoro-2-hydroxypropyl group at the 2-position is essential for inhibition of HCV replication. As for the substituent on the amido nitrogen, a butyl group is appropriate in terms of size and electric charge. Indeed, introduction of a polar methoxyethyl (**3**) or bulky 2-naphthyl (**4**) group at this position did not result in improvement of anti-HCV activity in further investigations (Fig. 2). On the basis of these findings, we designed new phenanthridine analogues (derived from **2**) modified at the 3- or 4-position with three kinds of substituents (F, Me and OMe) possessing different properties, and examined their antiviral activity. These compounds were synthesized as shown in Scheme 1. Briefly, *N*-butylaniline derivatives **28a–f**, prepared from the corresponding anilines, were treated with hexafluoroacetone trihydrate to give 1,1,1,3,3,3-hexafluoro-2-hydroxypropyl derivatives **29a–f**, which were condensed with 2-iodobenzoyl chloride. The resulting anilides **30a–f** were cyclized in the presence of Pd catalyst to give compounds **5–10**.

As shown in Table 1, introduction of substituents at the 4-position enhanced the anti-HCV activity in the order of fluoro (**6**) < methyl (**8**) < methoxy (**10**), whereas introduction of substituents at the 3-position did not improve the anti-HCV activity, compared with unsubstituted **2**. The analogues with a non-polar methyl group, that is, compounds **7** and **8**, showed almost the same anti-HCV activity as the analogues substituted at the corresponding site (3- or 4-position) with an electron-withdrawing fluoro group, that is, compounds **5** and **6**, respectively. On the other hand, analogues with an electron-donating methoxy group (compounds **9** and **10**) exhibited higher anti-HCV activity than the corresponding methyl- and/or fluoro-substituted analogues. As regards cytotoxicity, electron-withdrawing character of the substituent seems to be unfavorable, that is, introduction of a fluoro group at the 4-position (**6**) resulted in an increase of the cytotoxicity compared to other 4-substituted compounds, **8** and **10**. These results suggest that the substituent effect is determined more by electronic character than by the steric factor, and an electron-donating group is favorable for anti-HCV activity. Therefore, we selected the methoxy group as a fixed substituent for further structural development studies.

Table 1
Effects of substituents of phenanthridine on anti-HCV activities and cytotoxicities



	R^1	EC_{50} (μM)	CC_{50} (μM)
2	H	3.2	15.8
5	3-F	5.3	25.5
6	4-F	2.4	12.1
7	3-CH_3	5.6	28.9
8	4-CH_3	2.1	31.9
9	3-OCH_3	3.4	>40
10	4-OCH_3	1.1	22.3



3 EC_{50} = 5.4 μM

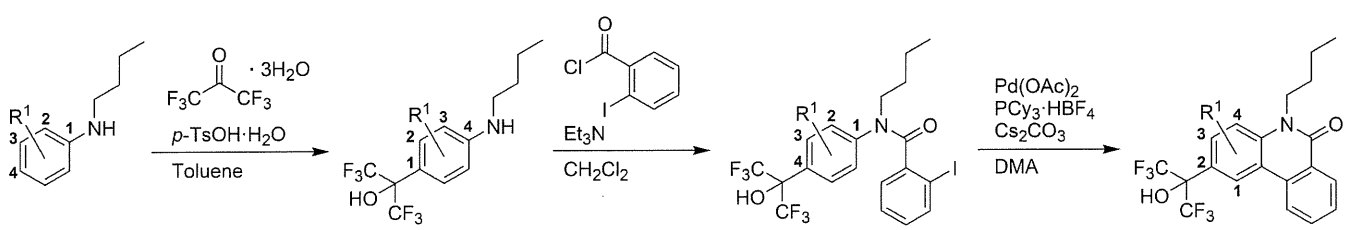
4 EC_{50} = 8.8 μM

Figure 2. Antiviral efficacy of **3** and **4** against HCV proliferation.

2.2. Regioisomers of methoxyl-substituted analogues

Based on our previous structure–activity relationship studies of anti-BVDV γ-carboline analogues, which indicated that regioselective methyl substitution dramatically influenced the antiviral activity,[8,9] we next investigated the effect of methoxy substitution. For this purpose, we synthesized all regioisomers of methoxy-substituted phenanthridine analogues **9–15** by using the same method as in Scheme 1 (see Scheme 2).

The anti-HCV activity and cytotoxicity of prepared compounds **9–15** are summarized in Table 2. The effect of methoxylation on the anti-HCV activity seems to be dependent on the position at which the methoxy group is introduced. Introduction of a methoxy group at positions 1, 7, 9 and 10 decreased the anti-HCV activity compared to methoxy-unsubstituted analogue **2**, whereas introduction at positions 3, 4 and 8 increased the activity. The 8-methoxylated analogue (**13**) showed the most potent anti-HCV activity among this series of compounds, with the EC_{50} value of 0.98 μM. As for cytotoxicity, the introduction of the methoxy group at positions



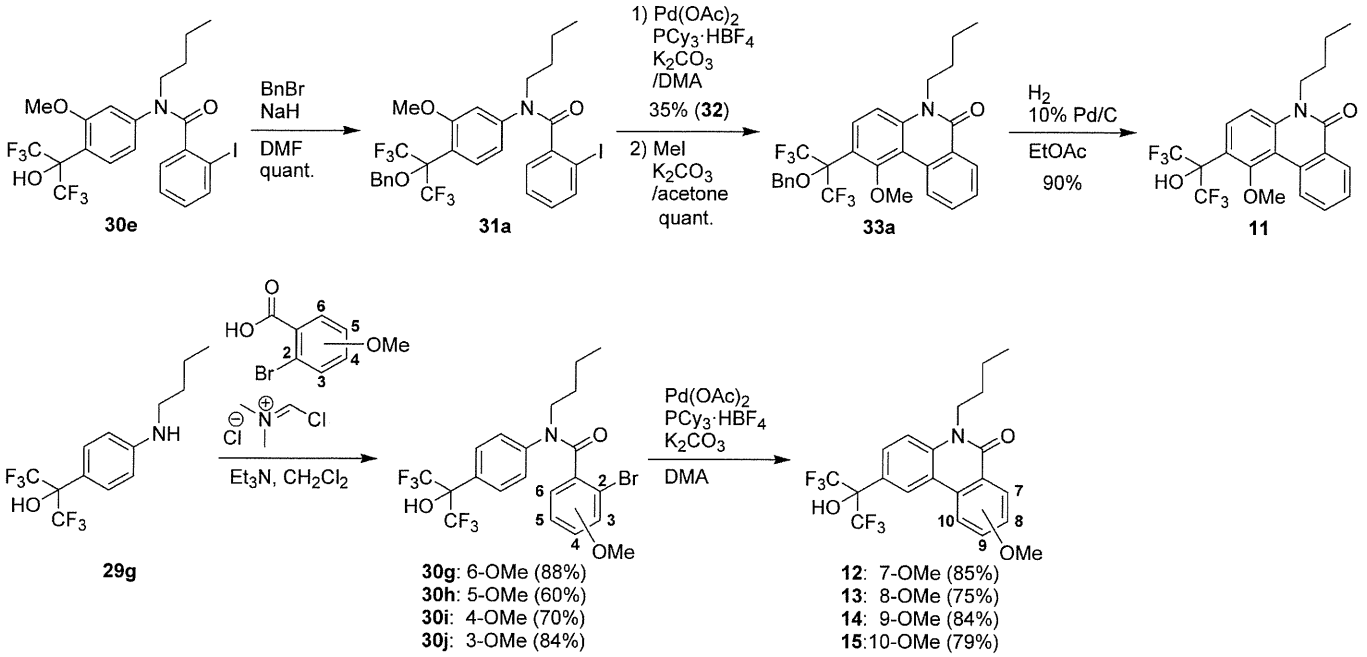
28a: R^1 = 3-F
28b: R^1 = 2-F
28c: R^1 = 3-Me
28d: R^1 = 2-Me
28e: R^1 = 3-OMe
28f: R^1 = 2-OMe

29a: R^1 = 2-F (37%)
29b: R^1 = 3-F (74%)
29c: R^1 = 2-Me (39%)
29d: R^1 = 3-Me (93%)
29e: R^1 = 2-OMe (35%)
29f: R^1 = 3-OMe (76%)

30a: R^1 = 3-F (85%)
30b: R^1 = 2-F (87%)
30c: R^1 = 3-Me (81%)
30d: R^1 = 2-Me (85%)
30e: R^1 = 3-OMe (quant.)
30f: R^1 = 2-OMe (82%)

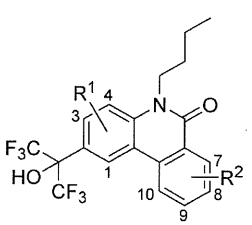
5: R^1 = 3-F (36%)
6: R^1 = 4-F (73%)
7: R^1 = 3-Me (37%)
8: R^1 = 4-Me (81%)
9: R^1 = 3-OMe (27%)
10: R^1 = 4-OMe (73%)

Scheme 1. Synthesis of **5–10**.



Scheme 2. Synthesis of **11–15**.

Table 2
Anti-HCV activities and cytotoxicities of regioisomers of methoxyphenanthridine **9–15**



	R^1	R^2	EC_{50} (μM)	CC_{50} (μM)
2	H	H	3.2	15.8
11	1-OCH_3	H	7.2	25.3
9	3-OCH_3	H	3.4	>40
10	4-OCH_3	H	1.1	22.3
12	H	7-OCH_3	9.96	12.6
13	H	8-OCH_3	0.98	9.6
14	H	9-OCH_3	16.6	28.9
15	H	10-OCH_3	5.0	6.9

7, 8 and 10 increased the cytotoxicity, while introduction at other positions decreased the cytotoxicity or had no effect.

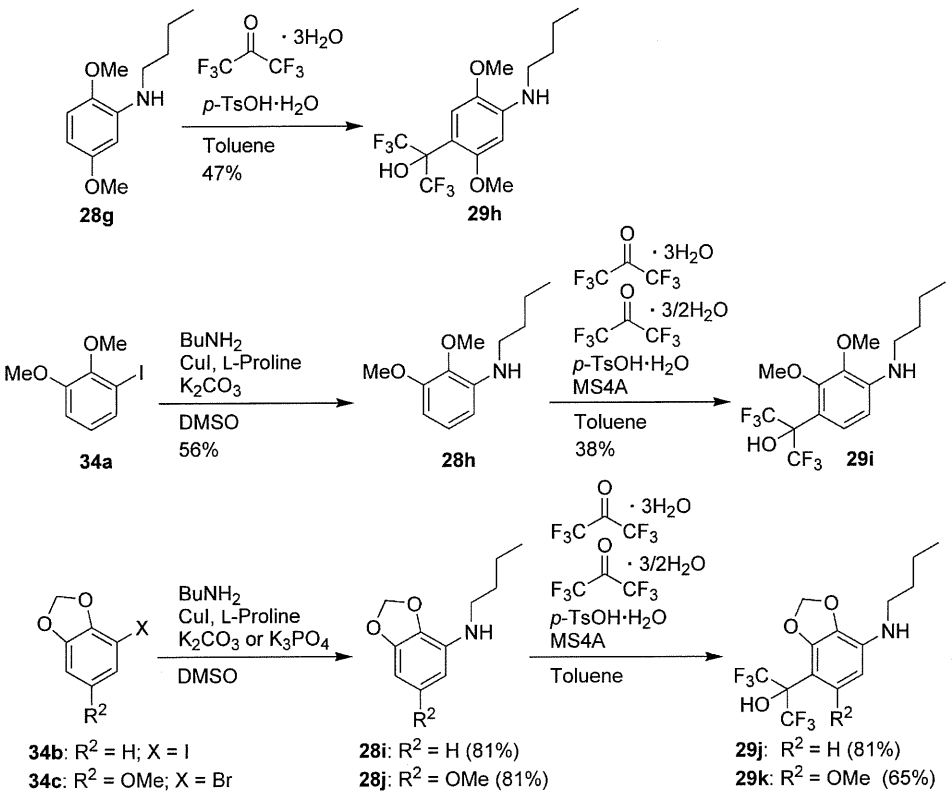
2.3. Dimethoxyl- and trimethoxyl-substituted analogues

The results for the regioisomers of the monomethoxy-substituted analogues indicate that the introduction of a methoxy group at the 3, 4, or 8 position of the phenanthridine skeleton enhanced the anti-HCV activity, compared to unsubstituted analogue **2**. In addition, introduction of a methoxy group at the 1-position seems to have potential for the reduction of cytotoxicity, without affecting the anti-HCV activity. On the basis of these results, we expected that dimethoxylation and/or trimethoxylation at a combination of the 1, 3, 4 and 8-positions might lead to more potent anti-HCV agents. Various compounds, except for analogues possessing methoxy groups at both the 1 and 3 positions, were prepared by means of a method similar to that used for the synthesis of **5–10**, as illustrated in Schemes 4 and 5. The commercially unavailable aniline **28h** was prepared by means of metal-catalyzed reaction, as shown in Scheme 3. Briefly, 1-iodo-2,3-dimethoxybenzene (**34a**), prepared from 1,2-dimethoxybenzene by the literature method,[21] was coupled with butylamine by amino acid-promoted CuI-catalyzed C–N bond formation reaction.[22]

As shown in Table 3, among the dimethoxylated compounds, 3,8-disubstituted (**18**) and 4,8-disubstituted analogues (**19**) exhibited anti-HCV activity several-fold higher than that of the monomethoxy compounds: the EC_{50} values of **18** and **19** were 0.18 and 0.51 μM, respectively. Two other dimethoxylated compounds, 1,4-disubstituted (**16**) and 3,4-disubstituted (**17**) analogues, showed anti-HCV potency similar to that of the monomethoxy compounds. On the other hand, trimethoxylated compounds **20** and **21** showed only a slight improvement in the anti-HCV activity compared to the monomethoxy compounds. As for cytotoxicity, analogues possessing a methoxy group at the 1-position, **16** and **20**, exhibited almost no cytotoxicity, while other polymethoxylated compounds were more cytotoxic than the monomethoxylated compounds. These results suggest that introduction of two methoxy groups on the same phenyl ring is unfavorable for anti-HCV activity: *n*,4-dimethoxy analogues (*n* = 1 or 3: **16** and **17**, respectively) show weaker anti-HCV activity than 4-methoxy analogue **10**, and *n*,4,8-trimethoxy analogues (*n* = 1 or 3: **20** and **21**, respectively) show weaker anti-HCV activity than 4-methoxy (**10**) or 8-methoxy (**13**) analogues. In addition, it was suggested that introduction of a methoxy group at the 1-position reduced the cytotoxicity.

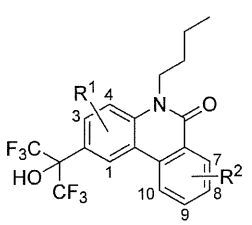
2.4. [1,3]Dioxolophenanthridine analogues

Although 3,8-(**18**) and 4,8-dimethoxyphenanthridine (**19**) analogues showed potent anti-HCV activity, which seems to arise from a synergistic effect of the methoxy groups, 3,4,8-trimethoxy-substituted phenanthridine analogue (**21**) showed enhancement of only the cytotoxicity, but not the anti-HCV activity. This result suggests that the unfavorable effect of steric hindrance between adjacent methoxy groups at the 3 and 4 positions is larger than the cumulative antiviral activity-enhancing effect of these methoxy groups. This hypothesis prompted us to examine the introduction of a 1,3-dioxolyl group (so-called methylenedioxy group)



Scheme 3. Synthesis of **29h–k**.

Table 3
Anti-HCV activities and cytotoxicities of dimethoxy and trimethoxyphenanthridine **16–21**



	R^1	R^2	EC_{50} (μM)	CC_{50} (μM)
16	1,4-$(OCH_3)_2$	H	5.7	>100
17	3,4-$(OCH_3)_2$	H	2.6	10.4
18	3-OCH_3	8-OCH_3	0.18	7.6
19	4-OCH_3	8-OCH_3	0.51	11.1
20	1,4-$(OCH_3)_2$	8-OCH_3	6.1	>100
21	3,4-$(OCH_3)_2$	8-OCH_3	1.8	6.8

instead of the adjacent methoxy structure of analogue (**21**), and we prepared various related analogues with a 1,3-dioxolyl group as illustrated in Schemes 3, 6 and 7.

As expected, the compounds with a 1,3-dioxolyl group at the 3/4-positions, that is, compounds **23** and **24**, exhibited potent anti-HCV activity, with EC_{50} values of 0.29 and 0.050 μM, respectively (Table 4). As with the dimethoxylated compounds, introduction of a methoxy group at the 8-position enhanced the anti-HCV activity. Although introduction of a methoxy group at the 1-position in this series of compounds, **26** and **27**, greatly decreased the cytotoxicity, it did not affect the anti-HCV activity. The compound possessing a 1,3-dioxolyl group at the 8/9-positions (**22**) showed moderate anti-HCV activity with almost no cytotoxicity, whereas introduction of an additional 1,3-dioxolyl group at the 3/4-positions (**25**) resulted in enhancement of the cytotoxicity.

3. Conclusion

We have developed a series of potent anti-HCV agents consisting of fused heterocyclic amido structure with good selectivity for virus replication-inhibitory activity over cytotoxicity. Among them, compound **24** showed the most potent anti-HCV activity, having an EC_{50} value of 50 nM, with a selectivity index (SI: CC_{50}/EC_{50}) value of 128.

4. Experimental

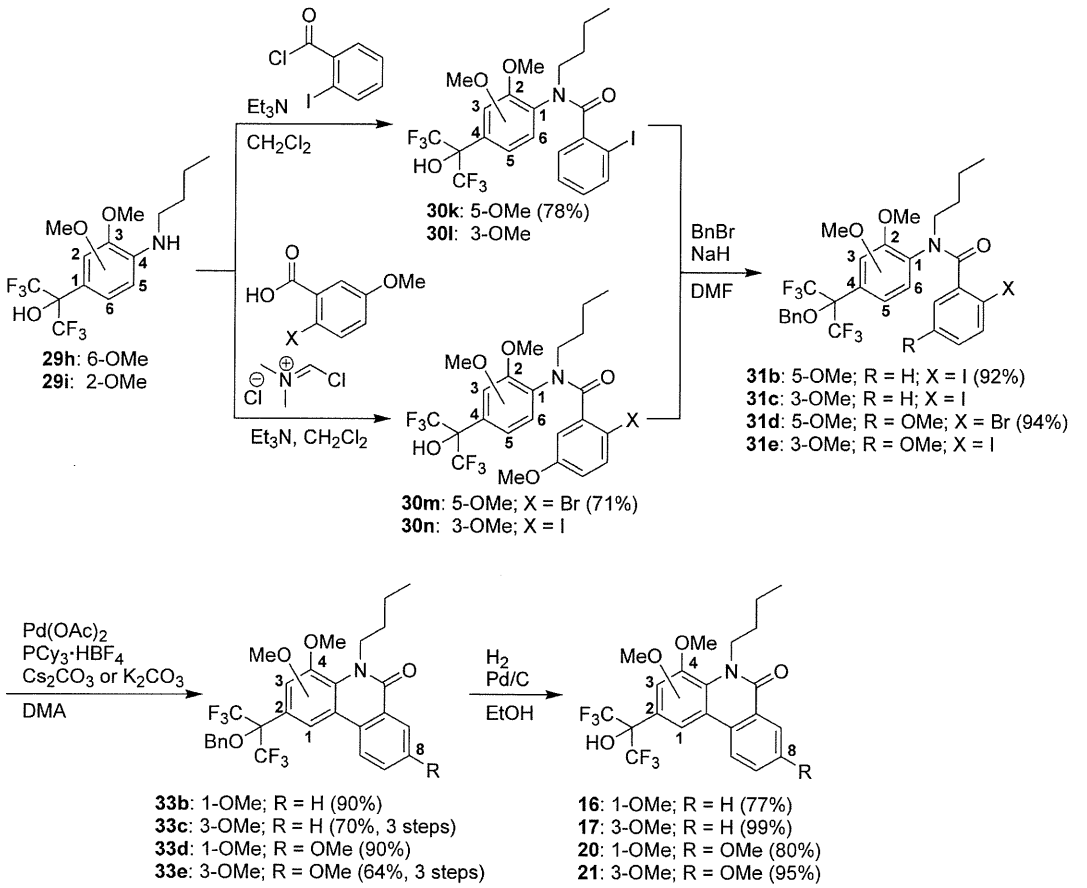
4.1. General comments

Melting points were determined by using a Yanagimoto hot-stage melting point apparatus and are uncorrected. ^1H NMR spectra were recorded on a JEOL JNM-GX500 (500 MHz) spectrometer. Chemical shifts are expressed in δ (ppm) values with tetramethylsilane (TMS) as an internal reference. Mass spectra (MS) and high-resolution mass spectra (HRMS) were recorded on a JEOL JMS-DX303 spectrometer. Elemental analyses were carried out in the Microanalytical Laboratory, Faculty of Pharmaceutical Sciences, The University of Tokyo, and were within ±0.4% of the theoretical values.

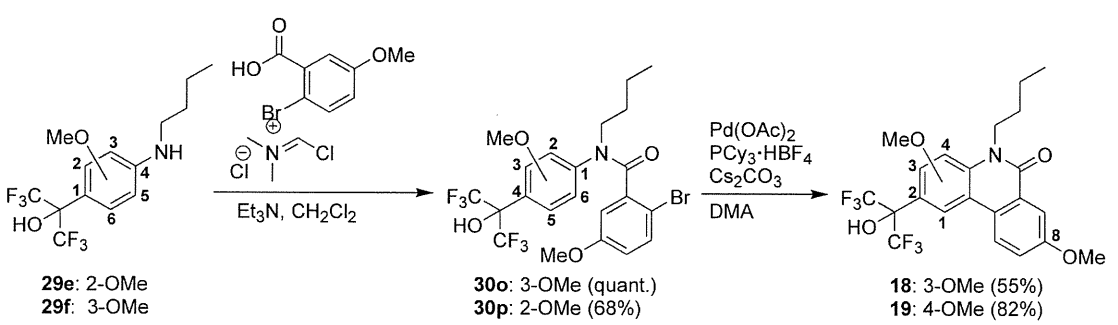
4.2. Chemistry

4.2.1. General procedure A: synthesis of phenylhexafluoropropanol analogues

4.2.1.1. 2-(4-Butylamino-2-fluorophenyl)-1,1,1,3,3,3-hexafluoropropan-2-ol (29a). To a mixture of hexafluoroacetone trihydrate (707 mg, 3.21 mmol) and toluene (5.0 ml) were added *N*-butyl-3-fluoroaniline (**28a**) (358 mg, 2.14 mmol) and *p*-TsOH·H_2O (81.5 mg, 428 μmol), then the mixture was stirred at 120 °C for 10 h. After cooling to room temperature, the mixture was diluted with ethyl acetate and then washed with satd $NaHCO_3$



Scheme 4. Synthesis of **16**, **17**, **20**, **21**.



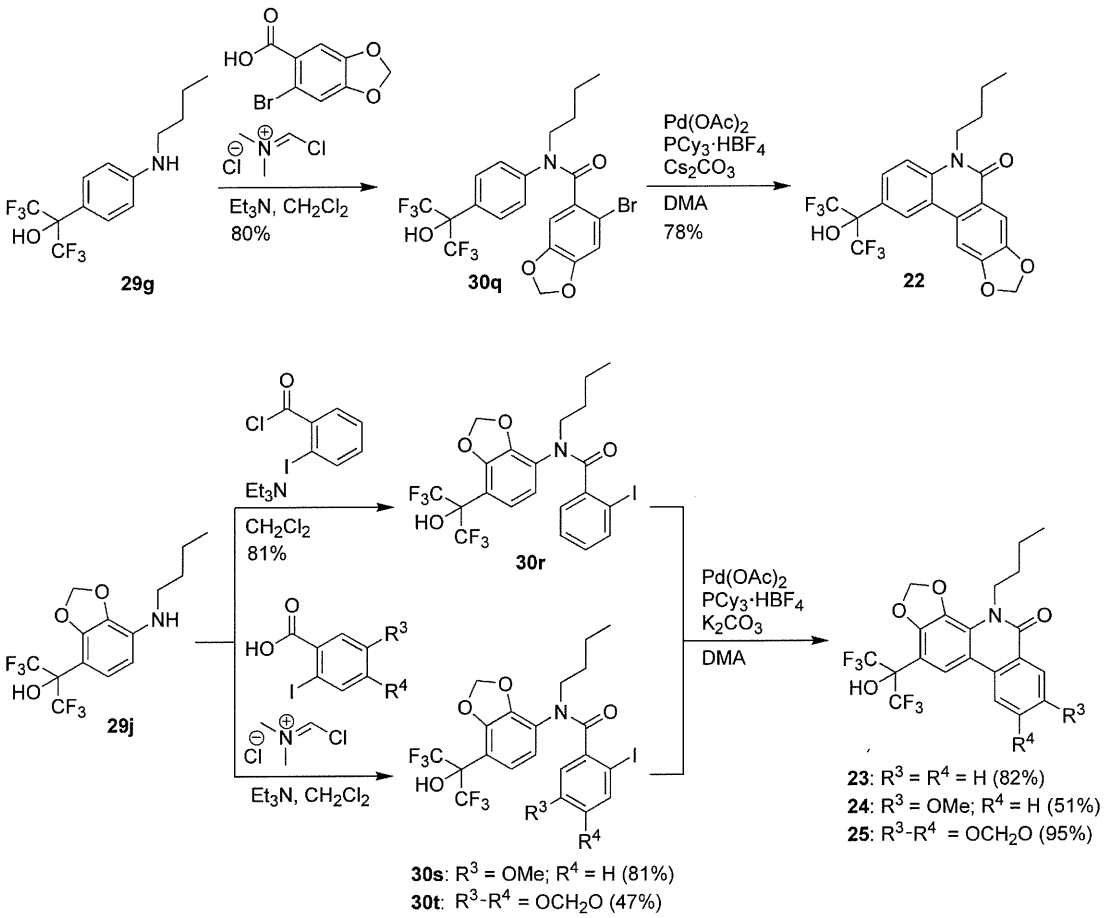
Scheme 5. Synthesis of **18**, **19**.

aqueous solution and brine, and dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (*n*-hexane/AcOEt = 1:0–2:1) to give **29a** (263 mg, 0.790 mmol, 37%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40–7.24 (m, 1H), 6.41–6.38 (m, 1H), 6.31–6.27 (m, 1H), 4.30 (br s, 1H), 4.02 (br s, 1H), 3.11 (t, 2H, J = 6.1 Hz), 1.61 (quin, 2H, J = 7.3 Hz), 1.43 (sext, 2H, J = 7.3 Hz), 0.97 (t, 3H, J = 7.3 Hz); MS (FAB) m/z 334 $(M+H)^+$.

4.2.1.2. 2-(4-Butylamino-3-fluorophenyl)-1,1,1,3,3,3-hexafluoropropan-2-ol (29b). Prepared from *N*-butyl-2-fluoroaniline (**28b**) in accordance with the general procedure A. Colorless oil (74%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33–7.29 (m, 2H), 6.71–6.67 (m, 1H), 4.06 (br s, 1H), 3.70 (br s, 1H), 3.19–3.12 (m, 2H), 1.65 (quin, 2H, J = 7.3 Hz), 1.44 (sext, 2H, J = 7.3 Hz), 0.97 (t, 3H, J = 7.3 Hz); MS (FAB) m/z 334 $(M+H)^+$.

4.2.1.3. 2-(4-Butylamino-2-methylphenyl)-1,1,1,3,3,3-hexafluoropropan-2-ol (29c). Prepared from *N*-butyl-3-methylaniline (**28c**) in accordance with the general procedure A. Colorless oil (39%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50 (d, 1H, J = 7.9 Hz), 7.09 (d, 1H, J = 7.9 Hz), 7.07 (s, 1H), 3.52 (br s, 1H), 3.03 (t, 2H, J = 7.3 Hz), 2.37 (s, 3H), 1.65 (quin, 2H, J = 7.3 Hz), 1.52 (s, 1H), 1.44 (sext, 2H, J = 7.3 Hz), 0.96 (t, 3H, J = 7.3 Hz); MS (FAB) m/z 330 $(M+H)^+$.

4.2.1.4. 2-(4-Butylamino-3-methylphenyl)-1,1,1,3,3,3-hexafluoropropan-2-ol (29d). Prepared from *N*-butyl-2-methylaniline (**28d**) in accordance with the general procedure A. Colorless oil (93%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 (d, 1H, J = 8.5 Hz), 7.33 (s, 1H), 6.61 (d, 1H, J = 8.5 Hz), 3.65 (br s, 1H), 3.32 (br s, 1H), 3.17 (t, 2H, J = 7.3 Hz), 2.15 (s, 3H), 1.66 (quin, 2H, J = 7.3 Hz), 1.45 (sext, 2H, J = 7.3 Hz), 0.98 (t, 3H, J = 7.3 Hz); MS (FAB) m/z 330 $(M+H)^+$.



Scheme 6. Synthesis of **22–25**.

4.2.1.5. 2-(4-Butylamino-2-methoxyphenyl)-1,1,1,3,3,3-hexafluoropropan-2-ol (29e). Prepared from *N*-butyl-3-methoxyaniline (**28e**) in accordance with the general procedure A. Colorless oil (35%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 (s, 1H), 7.27 (d, 1H, J = 8.5 Hz), 6.26 (dd, 1H, J = 8.5, 2.4 Hz), 6.18 (d, 1H, J = 2.4 Hz), 4.15 (br s, 1H), 3.91 (s, 1H), 3.12 (m, 2H), 1.61 (quin, 2H, J = 7.3 Hz), 1.43 (sext, 2H, J = 7.3 Hz), 0.97 (t, 3H, J = 7.3 Hz); MS (FAB) m/z 346 $(M+H)^+$.

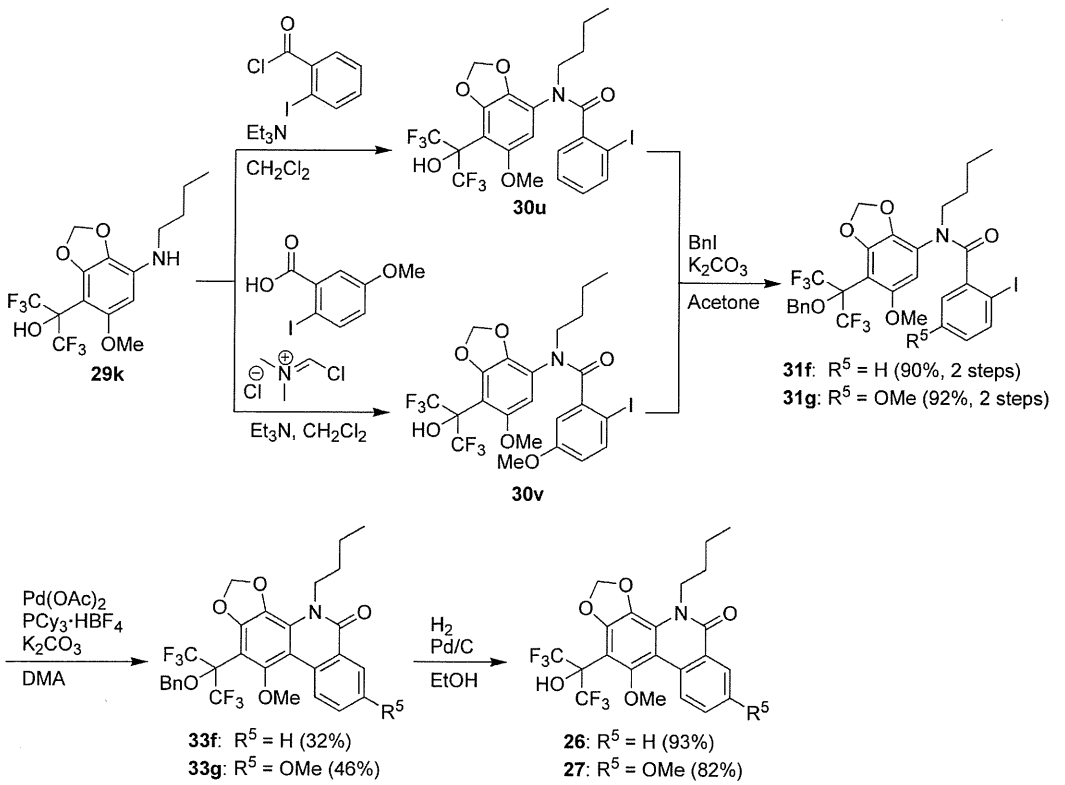
4.2.1.6. 2-(4-Butylamino-3-methoxyphenyl)-1,1,1,3,3,3-hexafluoropropan-2-ol (29f). Prepared from *N*-butyl-2-methoxyaniline (**28f**) in accordance with the general procedure A. Colorless oil (76%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.16 (d, 1H, J = 8.5 Hz), 7.05 (s, 1H), 6.58 (d, 1H, J = 8.5 Hz), 4.36 (br s, 1H), 3.95 (br s, 1H), 3.85 (s, 3H), 3.14 (t, 2H, J = 7.3 Hz), 1.64 (quin, 2H, J = 7.3 Hz), 1.44 (sext, 2H, J = 7.3 Hz), 0.96 (t, 3H, J = 7.3 Hz); MS (FAB) m/z 346 $(M+H)^+$.

4.2.1.7. 2-(4-Butylamino-2,5-dimethoxyphenyl)-1,1,1,3,3,3-hexafluoropropan-2-ol (29h). Prepared from 2,5-dimethoxy-*N*-butylaniline (**28g**) in accordance with the general procedure A. Colorless oil (47%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74 (s, 1H), 6.80 (s, 1H), 6.21 (s, 1H), 4.46 (br s, 1H), 3.92 (s, 3H), 3.80 (s, 3H), 3.14 (m, 2H), 1.66 (quin, 2H, J = 7.3 Hz), 1.45 (sext, 2H, J = 7.3 Hz), 0.98 (t, 3H, J = 7.3 Hz); MS (FAB) m/z 376 $(M+H)^+$.

4.2.1.8. 2-(4-Butylamino-2,3-dimethoxyphenyl)-1,1,1,3,3,3-hexafluoropropan-2-ol (29i). Prepared from **28b** in accordance with a slight modification of the general procedure A. To a mixture of hexafluoroacetone sesquihydrate (309 mg, 1.60 mmol) and hexafluoroacetone trihydrate (352 mg, 1.60 mmol) were added a solution of **28h** (167 mg, 0.800 mmol) in toluene (1.6 mL), *p*-TsOH·H_2O (15.2 mg, 79.9 μmol), and MS4A (600 mg), and the mixture was stirred at 120 °C for 10 h. After cooling to room temperature, the mixture was diluted with ethyl acetate, and then filtered off under suction and washed with ethyl acetate. The filtrate was washed with water and brine, and dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (*n*-hexane/CH_2Cl_2 = 7:1) to give **29i** (115 mg, 0.306 mmol, 38%) as a colorless oil. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.00 (s, 1H), 7.18 (d, J = 9.2 Hz, 1H), 6.41 (d, J = 9.2 Hz, 1H), 5.52 (t, J = 6.1 Hz, 1H), 3.79 (s, 3H), 3.63 (s, 3H), 3.08 (td, J = 6.1, 6.7 Hz, 2H), 1.52 (tt, J = 6.7, 7.3 Hz, 2H), 1.34 (qt, J = 7.3, 7.3 Hz, 2H), 0.90 (t, J = 7.3 Hz, 3H); MS (FAB) m/z 375 $(M)^+$.

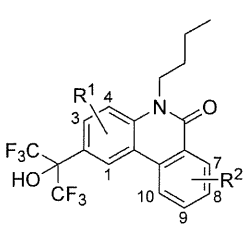
4.2.1.9. 4-(Butylamino)benzo[*d*][1,3]dioxole-2-(1,1,1,3,3,3-hexafluoro)propan-2-ol (29j). Prepared from **28i** in accordance with the synthesis of **29i**. White solid (81%); mp 60.5–62.0 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.19 (s, 1H), 6.93 (d, J = 9.2 Hz, 1H), 6.33 (d, J = 9.2 Hz, 1H), 5.91 (s, 2H), 5.47 (t, J = 6.7 Hz, 1H), 3.08 (td, J = 6.7, 7.3 Hz, 2H), 1.52 (tt, J = 7.3, 7.9 Hz, 2H), 1.34 (qt, J = 7.3, 7.9 Hz, 2H), 0.90 (t, J = 7.3 Hz, 3H); MS (FAB) m/z 359 $(M)^+$.

4.2.1.10. 4-(Butylamino)-6-methoxybenzo[*d*][1,3]dioxole-2-(1,1,1,3,3,3-hexafluoro)propan-2-ol (29k). Prepared from **28j** in accordance with the synthesis of **29i**. Yellow amorphous solid (65%); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.55–7.85 (br, 1H), 5.98 (br s, 1H), 5.84 (s, 2H), 5.40–5.80 (br, 1H), 3.72 (br s, 2H), 3.13 (td, J = 6.7, 6.7 Hz [with NH–CH_2 coupling], 2H), 1.50 (tt, J = 6.7, 7.3 Hz, 2H), 1.33 (qt, J = 6.7, 7.3 Hz, 2H), 0.89 (t, J = 7.3 Hz, 3H); MS (FAB) m/z 389 $(M)^+$.



Scheme 7. Synthesis of **26** and **27**.

Table 4
Anti-HCV activities and cytotoxicities of phenanthridine analogues with 1,3-dioxolyl group (**22–27**)



	R^1	R^2	EC_{50} (μM)	CC_{50} (μM)	SI[a]
23	3,4-(OCH_2O)	H	0.29	24.2	83.3
22	H	8,9-(OCH_2O)	2.3	>100	>43.1
26	1-OCH_3 3,4-(OCH_2O)	H	9.8	93.9	9.56
24	3,4-(OCH_2O)	8-OCH_3	0.050	6.4	128
27	1-OCH_3 3,4-(OCH_2O)	8-OCH_3	12.0	>100	>8.31
25	3,4-(OCH_2O)	8,9-(OCH_2O)	1.73	15.9	9.18

[a] Selectivity index (CC_{50}/EC_{50}).

4.2.2. 3-Iodo-1,2-dimethoxybenzene (34a)

To a solution of veratrol (1.80 g, 13.0 mmol) in THF (10.0 mL) was added *n*-butyllithium (1.65 M hexane solution, 8.70 mL, 14.4 mmol) at −10 °C, and the mixture was stirred at the room temperature for 2 h. After the reaction mixture was cooled to −45 °C, to this was added a solution of I_2 (3.63 g, 14.3 mmol) in THF (10.0 mL), and the mixture was stirred at the room temperature for 1.5 h. The reaction was quenched by the addition of saturated aqueous NH_4Cl solution and then extracted with ethyl acetate. The combined organic extracts were washed with 10% aqueous $Na_2S_2O_3$ solution, brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (*n*-hexane to *n*-hexane/ethyl acetate = 29:1) to give **34a** (2.44 g, 9.25 mmol, 71%) as a white solid. Mp 35.0–35.5 °C; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.33 (dd, *J* = 7.9, 1.2 Hz, 1H), 6.87 (dd, *J* = 7.9, 1.2 Hz, 1H), 6.78 (dd, *J* = 7.9, 7.9 Hz, 1H), 3.84 (s, 3H), 3.81 (s, 3H); MS (FAB) *m/z* 264 $(M)^+$.

4.2.3. *N*-Butyl-2,3-dimethoyxaniline (28h)

To a mixture of **34a** (396 mg, 1.50 mmol) and K_2CO_3 (415 mg, 3.00 mmol) were added a solution of CuI (85.7 mg, 0.450 mmol) and L-proline (104 mg, 0.900 mmol) in DMSO (2.0 mL) and *n*-butylamine (600 μl, 6.07 mmol), and the mixture was stirred at 90 °C for 15 h. After cooling to room temperature, the mixture was diluted with ethyl acetate, and then washed with water and brine. The organic layer was dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (*n*-hexane/ethyl acetate/CH_2Cl_2 = 50:1:1 to 30:1:1) to give **28h** (174 mg, 0.833 mmol, 56%) as a colorless oil. ^1H NMR ($CDCl_3$, 500 MHz) δ 6.85–6.96 (m, 3H), 3.87 (s, 3H), 3.83 (s, 3H), 3.15 (t, *J* = 7.3 Hz, 2H), 1.65 (tt, *J* = 7.3, 7.3 Hz, 2H), 1.38 (qt, *J* = 7.3, 7.3 Hz, 2H), 0.90 (t, *J* = 7.3 Hz, 3H); MS (FAB) *m/z* 209 $(M)^+$, 210 $(M+H)^+$.

4.2.4. 4-Iodobenzo[*d*][1,3]dioxole (34b)

To a solution of **34a** (792 mg, 3.00 mmol) in CH_2Cl_2 (10.0 mL) was added BBr_3 (1.0 M CH_2Cl_2 solution, 14.0 mL, 14.0 mmol) at −78 °C under Ar atmosphere, and the mixture was stirred at the room temperature. After 42 h, the reaction mixture was poured into ice, and volatile materials were removed under reduced pressure. The residue was extracted with ethyl acetate. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was dissolved in DMF (30 mL), and to this were added Cs_2CO_3 (1.00 g, 3.07 mmol) and CH_2I_2 (250 mL, 3.10 mmol) and the mixture was stirred at 120 °C for 1 h under Ar atmosphere. After the reaction mixture was cooled to room temperature, DMF was removed under reduced pressure. To the residue were added ethyl acetate and water, and the resulting insoluble material was removed by filtration and washed with ethyl acetate. The filtrate was extracted with ethyl

acetate, and the combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (*n*-hexane/ethyl acetate = 50:1) to give **34b** [563 mg, 2.27 mmol, 76% (2 steps)] as a white solid. Mp 35.5–37.0 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.11 (dd, J = 1.2, 8.6 Hz, 1H), 6.75 (dd, J = 1.2, 7.9 Hz, 1H), 6.78 (dd, J = 7.9, 8.6 Hz, 1H), 5.99 (s, 2H); MS (FAB) *m/z* 248 $(M)^+$.

4.2.5. 4-(Butylamino)benzo[*d*][1,3]dioxole (28i)

Prepared from **34b** in accordance with the synthesis of **28h**. Colorless oil (81%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.73 (t, J = 7.9 Hz, 1H), 6.36–6.53 (m, 2H), 5.92 (s, 2H), 3.19 (t, J = 6.7 Hz, 2H), 1.64 (tt, J = 6.7, 8.0 Hz, 2H), 1.40 (qt, J = 7.3, 8.0 Hz, 2H), 0.92 (t, J = 7.3 Hz, 3H); MS (FAB) *m/z* 193 $(M+H)^+$.

4.2.6. 4-(Butylamino)-6-methoxybenzo[*d*][1,3]dioxole (28j)

Prepared from 3-bromo-5-methoxybenzo[*d*][1,3]dioxole (**34c**)[23] in accordance with the synthesis of **28h**. Pale yellow oil (81%); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 5.89 (d, J = 2.5 Hz, 1H), 5.81 (s, 2H), 5.77(d, J = 2.5 Hz, 1H), 5.14 (t, J = 6.1 Hz, 1H), 3.61 (s, 3H), 3.05 (dt, J = 6.1, 6.7 Hz, 2H), 1.47 (tt, J = 6.7, 7.3 Hz, 2H), 1.32 (qt, J = 7.3, 7.3 Hz, 2H), 0.88 (t, J = 7.3 Hz, 3H); MS (FAB) *m/z* 223 $(M)^+$.

4.2.7. General procedure B: synthesis of benzamide analogues (I)

4.2.7.1. *N*-Butyl-*N*-[3-fluoro-4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)phenyl]-2-iodobenzamide (30a). To a solution of **29a** (100 mg, 300 μmol) in CH_2Cl_2 (5.0 mL) were added dropwise 2-iodobenzoyl chloride (88.0 mg, 330 μmol) and Et_3N (82.5 μl, 750 μmol) at 0 °C, then the mixture was stirred for 3 h, and was allowed to warm to room temperature. After cooling to 0 °C, the mixture was diluted with ethyl acetate and then water was added. The organic layer was separated and washed with brine, and dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (*n*-hexane/AcOEt = 1:0–1:1) to give **30a** (144 mg, 256 μmol, 85%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.90–6.80 (m, 7H), 4.05–3.80 (m, 2H), 3.50 (br s, 1H), 1.76–1.57 (m, 2H), 1.57–1.35 (m, 2H), 1.02–0.83 (m, 3H); MS (FAB) *m/z* 436 $(M+H)^+$.

4.2.7.2. *N*-Butyl-*N*-[2-fluoro-4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)phenyl]-2-iodobenzamide (30b). Prepared from **29b** in accordance with the general procedure B. White solid (87%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54 (d, 2H, J = 7.3 Hz), 7.52–7.25 (m, 3H), 7.02–6.93 (m, 2H), 6.89–6.81 (m, 1H), 4.20–4.00 (m, 1H), 3.73–3.50 (m, 1H), 3.50–3.37 (m, 1H), 1.72–1.53 (m, 2H), 1.53–1.37 (m, 2H), 0.96 (t, 3H, J = 7.3 Hz); MS (FAB) *m/z* 436 $(M+H)^+$.

4.2.7.3. *N*-Butyl-*N*-[4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-3-methylphenyl]-2-iodobenzamide (30c). Prepared from **29c** in accordance with the general procedure B. White solid (81%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 (d, 2H, J = 7.9 Hz), 7.10–6.95 (m, 5H), 6.80–6.77 (m, 1H), 4.85 (br s, 1H), 3.93–3.82 (m, 2H), 2.23 (s, 3H), 1.70–1.55 (m, 2H), 1.49–1.30 (m, 2H), 0.93 (t, 3H, J = 7.3 Hz); MS (FAB) *m/z* 432 $(M+H)^+$.

4.2.7.4. *N*-Butyl-*N*-[4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-2-methylphenyl]-2-iodobenzamide (30d). Prepared from **29d** in accordance with the general procedure B. White solid (85%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69 (d, 1H, J = 7.9 Hz), 7.48 (s, 1H), 7.28 (s, 2H), 6.96 (dd, 1H, J = 7.9, 7.3 Hz), 6.82–6.76 (m, 2H), 4.47–4.38 (m, 2H), 3.18–3.10 (m, 1H), 2.38 (s, 3H), 1.83–1.76 (m, 1H),1.67–1.52 (m, 1H), 1.52–1.30 (m, 2H), 0.95 (t, 3H, J = 7.3 Hz); MS (FAB) *m/z* 432 $(M+H)^+$.

4.2.7.5. *N*-Butyl-*N*-[4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-3-methoxyphenyl]-2-iodobenzamide (30e). Prepared from **29e** in accordance with the general procedure B. White solid (quant.); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 (d, 2H, J = 7.9 Hz), 7.41 (d, 1H, J = 9.1 Hz), 7.21–6.97 (m, 2H), 6.95–6.83 (m, 3H), 6.80 (s, 1H), 4.85 (br s, 1H), 4.12–3.85 (m, 2H), 3.77 (s, 3H), 1.72–1.60 (m, 2H), 1.52–1.37 (m, 2H), 0.96 (t, 3H, J = 7.3 Hz); MS (FAB) *m/z* 448 $(M+H)^+$.

4.2.7.6. *N*-Butyl-*N*-[4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-2-methoxyphenyl]-2-iodobenzamide (30f). Prepared from **29f** in accordance with the general procedure B. White solid (82%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.63 (d, 2H, J = 7.9 Hz), 7.30 (d, 1H, J = 8.5 Hz), 7.14 (s, 1H), 7.06 (d, 1H, J = 8.5 Hz), 6.99–6.92 (m, 2H), 6.80–6.77 (m, 1H), 4.71 (br s, 1H), 4.22–4.07 (m, 1H), 3.85 (s, 1H), 3.48–3.34 (m, 1H), 1.72–1.51 (m, 2H), 1.51–1.32 (m, 2H), 0.94 (t, 3H, J = 7.3 Hz); MS (FAB) *m/z* 576 $(M+H)^+$.

4.2.7.7. *N*-Butyl-*N*-[4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-2,5-dimethoxyphenyl]-2-iodobenzamide (30k). Prepared from **29h** in accordance with the general procedure B. White solid (78%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.67 (d, 1H, J = 7.9 Hz), 7.34 (br s, 1H), 7.04 (dd, 1H, J = 7.3, 6.7 Hz), 7.01 (s, 1H), 6.93 (d, 1H, J = 7.3 Hz), 6.91 (s, 1H), 6.87 (dd, 1H, J = 7.9, 6.7 Hz), 4.30–4.20 (m, 1H), 3.83 (s, 3H), 3.80 (s, 3H), 3.44–3.33 (m, 1H), 1.75–1.50 (m, 2H), 1.50–1.40 (m, 2H), 0.96 (t, 3H, J = 7.3 Hz); MS (FAB) *m/z* 606 $(M+H)^+$.

4.2.7.8. *N*-Butyl-*N*-[7-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)benzo[*d*][1,3]dioxol-4-yl]-2-iodobenzamide (30r). Prepared from **29j** in accordance with the general procedure B. White solid (81%); mp 113.0–115.0 °C; ^{1}H NMR (DMSO-d_6, 500 MHz, 50 °C) δ 8.52 (s, 1H), 7.70 (d, J = 7.9 Hz, 1H), 7.20 (dd, J = 7.3, 7.9 Hz, 1H), 7.12 (d, J = 7.9 Hz, 1H), 6.96 (dd, J = 7.3, 8.6 Hz, 1H), 6.86 (d, J = 8.6 Hz, 1H), 5.98 (s, 2H), 3.79 (t, J = 6.7 Hz, 2H), 1.54 (tt, J = 6.7, 8.0 Hz, 2H), 1.39 (dd, J = 7.3, 8.0 Hz, 2H), 6.86 (t, J = 7.3 Hz, 3H); MS (FAB) *m/z* 590 $(M+H)^+$.

4.2.8. General procedure C: synthesis of benzamide analogues (II)

4.2.8.1. *N*-Butyl-2-bromo-*N*-[4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)phenyl]-6-methoxybenzamide (30g). To a solution of 2-bromo-6-methoxybenzoic acid (104 mg, 397 μmol) in CH_2Cl_2 (3.0 mL) were added chloromethlenedimethyliminium chloride (50.8 mg, 397 μmol) at 0 °C under Ar atmosphere, then the mixture was stirred at room temperature for 1 h. At 0 °C, 2-(4-butylaminophenyl)-1,1,1,3,3,3-hexafluoropropan-2-ol (**29g**)[24] (50.0 mg, 159 μmol) and Et_3N (166 μl, 1.19 mmol) then the mixture was stirred for 3 h, and was allowed to warm to room temperature. At 0 °C, the mixture was diluted with ethyl acetate and then water was added. The organic layer was separated and washed with brine, and dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (*n*-hexane/AcOEt = 1:0–1:1) to give **30g** (73.5 mg, 139 μmol, 88%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50 (d, 1H, J = 8.5 Hz), 7.30 (d, 1H, J = 8.5 Hz), 6.96–6.93 (m, 2H), 6.51 (d, 1H, J = 7.3 Hz), 4.20–4.08 (m, 1H), 4.06 (br s, 1H), 4.75–4.67 (m, 1H), 3.65 (s, 3H), 1.81–1.54 (m, 2H), 1.52–1.34 (m, 2H), 0.94 (t, 3H, J = 7.3 Hz); MS (FAB) *m/z* 528 $(M+H)^+$.

4.2.8.2. *N*-Butyl-2-bromo-*N*-[4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)phenyl]-5-methoxybenzamide (30h). Prepared from **29g** and 2-bromo-5-methoxybenzoic acid in accordance with the general procedure C. White solid (60%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27 (d, 1H, J = 9.1 Hz), 7.21 (d, 1H, J = 8.5 Hz), 7.13 (s, 1H), 7.07 (d, 1H, J = 8.5 Hz), 6.56 (d, 1H, J = 3.1 Hz), 6.53

(dd, 1H, J = 9.1, 3.1 Hz), 4.25 (br s, 1H), 4.25–4.12 (m, 1H), 3.85 (s, 3H), 3.56 (s, 3H), 3.50–3.35 (m, 1H), 1.72–1.50 (m, 2H), 1.50–1.35 (m, 2H), 0.94 (t, 3H, J = 7.3 Hz); MS (FAB) m/z 558 $(M+H)^+$.

4.2.8.3. *N*-Butyl-2-bromo-*N*-[4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)phenyl]-4-methoxybenzamide (30i). Prepared from **29g** and 2-bromo-4-methoxybenzoic acid in accordance with the general procedure C. White solid (70%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70–7.47 (m, 2H), 7.32–7.10 (m, 3H), 7.10–6.85 (m, 1H), 6.70–6.48 (m, 1H), 4.05–3.78(m, 3H), 3.70 (s, 3H), 1.70–1.50 (m, 2H), 1.50–1.33 (m, 2H), 0.99–0.78 (m, 3H); MS (FAB) m/z 528 $(M+H)^+$.

4.2.8.4. *N*-Butyl-2-bromo-*N*-[4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)phenyl]-3-methoxybenzamide (30j). Prepared from **29g** and 2-bromo-3-methoxybenzoic acid in accordance with the general procedure C. White solid (84%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51 (d, 1H, J = 8.5 Hz), 7.00 (dd, 1H, J = 7.9, 7.9 Hz), 6.66 (d, 1H, J = 7.9 Hz), 6.59 (d, 1H, J = 7.9 Hz), 4.05–3.90 (m, 2H), 3.80 (s, 3H), 1.73–1.63 (m, 2H), 1.50–1.35 (m, 2H), 0.94 (t, 3H, J = 7.3 Hz); MS (FAB) m/z 528 $(M+H)^+$.

4.2.8.5. 2-Bromo-*N*-butyl-*N*-[4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-2,5-dimethoxyphenyl]-5-methoxybenzamide (30m). Prepared from **29h** and 2-bromo-5-methoxybenzoic acid in accordance with the general procedure C. White solid (71%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 (br s, 1H), 7.26 (d, 1H, J = 9.2 Hz), 6.97 (s, 1H), 6.92 (s, 1H), 6.58 (dd, 1H, J = 9.2, 3.1 Hz), 6.55 (d, 1H, J = 3.1 Hz), 4.28–4.18 (m, 1H), 3.83 (s, 3H), 3.79 (s, 3H), 3.59 (s, 3H), 3.48–3.35 (m, 1H), 1.72–1.50 (m, 2H), 1.50–1.37 (m, 2H), 0.96 (t, 3H, J = 7.3 Hz); MS (FAB) m/z 588 $(M+H)^+$.

4.2.8.6. 2-Bromo-*N*-butyl-*N*-[4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-3-methoxyphenyl]-5-methoxybenzamide (30o). Prepared from **29e** and 2-bromo-5-methoxybenzoic acid in accordance with the general procedure C. White solid (quant.); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42 (d, 1H, J = 8.5 Hz), 7.30 (d, 2H, J = 9.2 Hz), 7.13 (br s, 1H), 6.92 (d, 1H, J = 8.5 Hz), 6.80 (s, 1H), 6.63 (d, 1H, J = 9.2 Hz), 6.50 (s, 1H), 4.10–3.85 (m, 1H), 3.77 (s, 3H), 3.61 (s, 3H), 1.70–1.59 (m, 2H), 1.50–1.38 (m, 2H), 0.96 (t, 3H, J = 7.3 Hz); MS (FAB) m/z 478 $(M+H)^+$.

4.2.8.7. 2-Bromo-*N*-butyl-*N*-[4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-2-methoxyphenyl]-5-methoxybenzamide (30p). Prepared from **29f** and 2-bromo-5-methoxybenzoic acid in accordance with the general procedure C. White solid (68%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27 (d, 1H, J = 9.1 Hz), 7.21 (d, 1H, J = 8.5 Hz), 7.13 (s, 1H), 7.07 (d, 1H, J = 8.5 Hz), 6.56 (d, 1H, J = 3.1 Hz), 6.53 (dd, 1H, J = 9.1, 3.1 Hz), 4.25 (br s, 1H), 4.25–4.12 (m, 1H), 3.85 (s, 3H), 3.56 (s, 3H), 3.50–3.35 (m, 1H), 1.72–1.50 (m, 2H), 1.50–1.35 (m, 2H), 0.94 (t, 3H, J = 7.3 Hz); MS (FAB) m/z 558 $(M+H)^+$.

4.2.8.8. 6-Bromo-*N*-butyl-*N*-[4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)phenyl]benzo[*d*][1,3]dioxole-5-carboxamide (30q). Prepared from **29g** and 6-bromobenzo[*d*][1,3]dioxole-5-carboxylic acid[25] in accordance with the general procedure C. White solid (80%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70–7.50 (m, 2H), 7.25–7.13 (m, 2H), 6.83–6.77 (m, 1H), 6.50–6.45 (m, 1H), 5.85 (s, 1H), 4.50–4.30 (m, 1H), 4.00–3.80 (m, 2H), 1.70–1.50 (m, 2H), 1.50–1.25 (m, 2H), 1.05–0.80 (m, 3H); MS (FAB) m/z 542 $(M+H)^+$.

4.2.8.9. *N*-Butyl-*N*-[7-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)benzo[*d*][1,3]dioxol-4-yl]-2-iodo-5-methoxbenzamide (30s). Prepared from **29j** and 2-iodo-5-methoxybenzoic acid[26] in accordance with the general procedure C. White solid (99%); mp 123.0–125.5 °C; ^{1}H NMR (DMSO-d_6, 500 MHz, 1:0.3 mixture of amide conformers) δ 8.74 (s, 0.3H), 8.66 (s, 1H), 7.78 (d, J = 9.2 Hz, 0.3H), 7.55 (d, J = 9.2 Hz, 1H), 7.22 (d, J = 9.2 Hz, 0.3H), 7.07 (d, J = 9.2 Hz, 0.3H), 6.97 (d, J = 9.2 Hz, 1H), 6.95 (d, J = 3.1 Hz, 0.3H), 6.85 (d, J = 9.2 Hz, 1H), 6.84 (dd, J = 3.1, 9.2 Hz, 0.3H), 6.63 (d, J = 3.1 Hz, 1H), 6.59 (dd, J = 3.1, 9.2 Hz, 1H), 6.13 (br s, 0.6H), 5.99 (br s, 2H), 3.72–3.82 (m, [2+0.6]H), 3.57 (s, [3+0.9]H), 1.52 (tt, J = 7.3, 8.0 Hz, [2+0.6]H), 1.38 (qd, J = 7.3, 8.0 Hz, [2+0.6]H), 0.90 (t, J = 7.3 Hz, [3+0.9]H); MS (FAB) m/z 620 $(M+H)^+$.

4.2.8.10. *N*-Butyl-*N*-[7-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)benzo[*d*][1,3]dioxol-4-yl]-6-iodobenzo[*d*][1,3]dioxole-5-carboxamide (30t). Prepared from **29j** and 6-iodobenzo[*d*][1,3]dioxole-5-carboxylic acid[27] in accordance with the general procedure C. Pale yellow foam (47%); ^{1}H NMR (DMSO-d_6, 500 MHz, 1:0.3 mixture of amide conformers) δ 8.75 (s, 0.3H), 8.68 (s, 1H), 7.45 (s, 0.3H), 7.24 (s, 1H), 7.21 (d, J = 9.2 Hz, 0.3H), 7.05 (d, J = 9.2 Hz, 0.3H), 7.01 (s, 0.3H), 6.99 (d, J = 9.2 Hz, 1H), 6.85 (d, J = 9.2 Hz, 1H), 6.69 (s, 1H), 6.12 (br s, 1.2H), 6.01 (s, 2H), 5.93 (s, 2H), 3.65–3.85 (br, 2H), 3.35–3.55 (br, 0.6H), 1.50 (tt, J = 7.3, 7.3 Hz, [2+0.6]H), 1.35 (qd, J = 7.3, 7.3 Hz, [2+0.6]H), 0.87 (t, J = 7.3 Hz, [3+0.9]H); MS (FAB) m/z 634 $(M+H)^+$.

4.2.9. General procedure D: synthesis of benzyloxyhexafluoropropane analogues

4.2.9.1. *N*-[4-(2-Benzyloxy-1,1,1,3,3,3-hexafluoropropan-2-yl)-*N*-butyl-3-methoxyphenyl]-2-iodobenzamide (31a). To a solution of **30e** (41.0 mg, 71.3 μmol) in DMF (3.0 mL) was added 55% NaH (3.73 mg, 85.5 μmol) at 0 °C under Ar atmosphere, then the mixture was stirred for 0.5 h at room temperature. At 0 °C, benzyl bromide (12.7 μl, 107 μmol) was added, then the mixture was stirred for 3 h, and allow to warm to room temperature. At 0 °C, the mixture was diluted with ethyl acetate and then water was added. The organic layer was separated and washed with brine, and dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (*n*-hexane/ethyl acetate = 1:0–2:1) to give **31a** (48.5 mg, 71.3 μmol, quant.) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75–7.70 (m, 1H), 7.40–7.28 (m, 6H), 7.15–7.05 (m, 1H), 6.95–6.80 (m, 3H), 6.76 (s, 1H), 4.70 (s, 2H), 4.10–3.75 (m, 2H), 3.61 (s, 3H), 1.77–1.70 (m, 2H), 1.55–1.38 (m, 2H), 1.03–0.90 (m, 3H); MS (FAB) m/z 666 $(M+H)^+$.

4.2.9.2. *N*-[4-(2-Benzyloxy-1,1,1,3,3,3-hexafluoropropan-2-yl)-2,5-dimethoxyphenyl]-*N*-butyl-2-iodobenzamide (31b). Prepared from **30k** in accordance with the general procedure D. White solid (92%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.67 (d, 1H, J = 7.9 Hz), 7.45–7.25 (m, 5H), 7.01 (dd, 1H, J = 7.3, 6.7 Hz), 6.96 (s, 1H), 6.92 (d, 1H, J = 7.3 Hz), 6.90–6.80 (m, 2H), 4.56 (d, 1H, J = 12.1 Hz), 4.39 (d, 1H, J = 12.1 Hz), 4.25–4.15 (m, 1H), 3.65 (s, 3H), 3.45–3.32 (m, 1H), 3.41 (s, 3H), 1.75–1.50 (m, 2H), 1.50–1.37 (m, 2H), 0.96 (t, 3H, J = 7.3 Hz); MS (FAB) m/z 696 $(M+H)^+$.

4.2.9.3. *N*-[4-(2-Benzyloxy-1,1,1,3,3,3-hexafluoropropan-2-yl)-2,5-dimethoxyphenyl]-2-bromo-*N*-butyl-5-methoxybenzamide (31d). Prepared from **30m** in accordance with the general procedure D. White solid (94%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45–7.28 (m, 5H), 7.24 (d, 1H, J = 8.5 Hz), 6.93 (s, 1H), 6.84 (s, 1H), 6.57 (dd, 1H, J = 8.5, 3.0 Hz), 6.54 (d, 1H, J = 3.0 Hz), 4.58 (d, 1H, J = 11.6 Hz), 4.36 (d, 1H, J = 11.6 Hz), 4.25–4.18 (m, 1H), 3.35 (s, 3H), 3.55 (s, 3H), 3.48–3.35 (m, 1H), 3.41 (s, 3H), 1.75–1.50 (m, 2H), 1.50–1.40 (m, 2H), 0.96 (t, 3H, J = 7.3 Hz); MS (FAB) m/z 598 $(M+H)^+$.

4.2.9.4. *N*-[7-(2-Benzyloxy-1,1,1,3,3,3-hexafluoropropan-2-yl)-6-methoxybenzo[*d*][1,3]dioxol-4-yl]-*N*-butyl-2-iodobenzamide (31f). Prepared from **29k** in accordance with the general pro-

cedure B and subsequent the general procedure D. Yellow oil [90% (2 steps)]; ^{1}H NMR (DMSO-d_6, 500 MHz, 1:0.15 mixture of amide conformers) δ 7.74 (d, J = 8.0 Hz, 1H), 7.26–7.46 (m, [5+1.2]H), 7.18–7.26 (m, [1+0.15]H), 7.15 (dd, J = 1.2, 7.3 Hz, 1H), 7.00 (ddd, J = 1.8, 7.3, 8.0 Hz, 1H), 6.73 (s, 0.15H), 6.44 (s, 1H), 6.07 (s, 0.3H), 5.90 (s, 2H), 4.65 (s, 0.3H), 4.37 (s, 2H), 3.75–3.95 (br, 2H), 3.76 (s, 0.3H), 3.55 (s, [3+0.45]H), 1.59 (tt, J = 7.3, 8.0 Hz, [2+0.3]H), 1.40 (qd, J = 7.3, 8.0 Hz, [2+0.3]H), 0.92 (t, J = 7.3 Hz, [3+0.45]H); MS (FAB) m/z 709 $(M)^+$, 710 $(M+H)^+$.

4.2.9.5. *N*-[7-(2-Benzyloxy-1,1,1,3,3,3-hexafluoropropan-2-yl)-6-methoxybenzo[*d*][1,3]dioxol-4-yl]-*N*-butyl-2-iodo-5-methoxybenzamide (31g). Prepared from **29k** and 2-iodo-5-methoxybenzoic acid in accordance with the synthesis of **30s** and subsequent the general procedure D. Yellow solid [92% (2 steps)]; mp 123.0–125.5 °C; ^{1}H NMR (DMSO-d_6, 500 MHz, 1:0.135 mixture of amide conformers) δ 7.90 (d, J = 8.6 Hz, 0.135H), 7.59 (d, J = 8.6 Hz, 1H), 7.25–7.43 (m, [5+0.675]H), 7.01 (d, J = 3.1 Hz, 0.135H), 6.97 (dd, J = 3.1, 8.6 Hz, 0.135H), 6.75 (s, 0.135H), 6.68 (d, J = 3.1 Hz, 1H), 6.61 (dd, J = 3.1, 8.6 Hz, 1H), 6.47 (s, 1H), 6.07 (br s, 0.27H), 5.89 (br s, 2H), 4.64 (s, 0.27H), 4.37 (s, 1H), 3.75–3.90 (br, 2H), 3.80 (s, 0.405H), 3.79 (s, 0.405H), 3.76 (s, 0.27H), 3.56 (s, 3H), 3.55 (s, 3H), 1.60 (tt, J = 7.3, 8.0 Hz, [2+0.27]H), 1.41 (qd, J = 7.3, 8.0 Hz, [2+0.27]H), 0.92 (t, J = 7.3 Hz, [3+0.405]H); MS (FAB) m/z 739 $(M)^+$.

4.2.10. General procedure E: synthesis of phenanthridine analogues by Pd-catalyzed cyclization

4.2.10.1. 2-(2-Benzyloxy-1,1,1,3,3,3-hexafluoropropan-2-yl)-5-butyl-1,4-dimethoxyphenanthridin-6(5*H*)-one (33b). To a solution of **31b** (50.0 mg, 71.9 μmol) in DMA (3.0 mL) were added $PCy_3{\cdot}HBF_4$ (13.2 mg, 35.9 μmol), Cs_2CO_3 (141 mg, 431 μmol) and $Pd(OAc)_2$ (4.04 mg, 18.0 μmol) under Ar atmosphere, then the mixture was stirred for 3 h at 120 °C. At 0 °C, the mixture was diluted with ethyl acetate and then water was added. The organic layer was separated and washed with brine, and dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (*n*-hexane/AcOEt = 1:0–2:1) to give **33b** (36.7 mg, 41.0 μmol, 90%) as a white solid. Mp 98.0–100.0 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.90 (d, J = 8.5 Hz, 1H), 8.50 (d, J = 7.9 Hz, 1H), 7.72 (dd, J = 8.5, 7.3 Hz, 1H), 7.59 (dd, J = 7.9, 7.3 Hz, 1H), 7.47–7.25 (m, 5H), 7.12 (s, 1H), 4.85–4.80 (m, 1H), 4.60–4.52 (m, 1H), 4.52–4.40 (m, 1H), 4.40–4.25 (m, 1H), 3.61 (s, 3H), 3.51 (s, 3H), 1.95–1.75 (m, 2H), 1.42 (sext, J = 7.3 Hz, 2H), 0.97 (t, J = 7.3 Hz, 3H); MS (FAB) m/z 568 $(M+H)^+$; HRMS (FAB) calcd for $C_{29}H_{28}F_6NO_4$ 568.1923; found: 568.1891 $(M+H)^+$.

4.2.10.2. 2-(2-Benzyloxy-1,1,1,3,3,3-hexafluoropropan-2-yl)-5-butyl-3,4-dimethoxyphenanthridin-6(5*H*)-one (33c). Prepared from **29i** in accordance with the general procedure B and subsequent the general procedures D and E. Pale yellow oil (70%, 3 steps). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.26–8.31 (m, 1H), 8.06 (s, 1H), 7.56–7.61 (m, 2H), 7.43–7.50 (m, 5H), 7.34–7.40 (m, 1H), 4.77 (s, 2H), 4.47 (t, J = 7.3 Hz, 2H), 3.94 (s, 3H), 3.77 (s, 3H), 1.68 (tt, J = 7.3, 7.9 Hz, 2H), 1.31 (qt, J = 7.3, 7.9 Hz, 2H), 0.91 (t, J = 7.3 Hz, 3H); MS (FAB) m/z 568 $(M+H)^+$.

4.2.10.3. 2-(2-Benzyloxy-1,1,1,3,3,3-hexafluoropropan-2-yl)-5-butyl-1,4,8-trimethoxyphenanthridin-6(5*H*)-one (33d). Prepared from **31d** in accordance with the general procedure E. White solid (90%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.84 (d, J = 9.2 Hz, 1H), 7.93 (d, J = 3.1 Hz, 1H), 7.46–7.26 (m, 6H), 7.07 (s, 1H), 4.85–4.80 (m, 1H), 4.60–4.52 (m, 1H), 4.52–4.40 (m, 1H), 4.40–4.30 (m, 1H), 3.96 (s, 3H), 3.60 (s, 3H), 3.52 (s, 3H), 1.95–1.75 (m, 2H), 1.43 (sext, J = 7.3 Hz, 2H), 0.98 (t, J = 7.3 Hz, 3H); MS (FAB) m/z 598 $(M+H)^+$; HRMS (FAB) calcd for $C_{30}H_{30}F_6NO_5$ 598.2028; found: 598.2045 $(M+H)^+$.

4.2.10.4. 2-(2-Benzyloxy-1,1,1,3,3,3-hexafluoropropan-2-yl)-5-butyl-3,4,8-trimethoxyphenanthridin-6(5*H*)-one (33e). Prepared from **29i** and 2-iodo-5-methoxybenzoic acid in accordance with the synthesis of **30s** and subsequent the general procedures D and E. Pale yellow oil (64%, 3 steps). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.97 (s, 1H), 7.71 (d, J = 2.5 Hz, 1H), 7.41–7.51 (m, 5H), 7.32 (d, J = 9.2 Hz, 1H), 7.19 (dd, J = 2.5, 9.2 Hz, 1H), 4.75 (s, 2H), 4.48 (t, J = 8.0 Hz, 2H), 3.92 (s, 3H), 3.88 (s, 3H), 3.77 (s, 3H), 1.68 (tt, J = 7.3, 8.0 Hz, 2H), 1.32 (qt, J = 7.3, 7.3 Hz, 2H), 0.91 (t, J = 7.3 Hz, 3H); MS (FAB) m/z 598 $(M+H)^+$.

4.2.10.5. 11-(2-Benzyloxy-1,1,1,3,3,3-hexafluoropropan-2-yl)-4-butyl-10-methoxy-[1,3]dioxolo[4,5-*c*]phenanthridin-5(4*H*)-one (33f). Prepared from **31f** in accordance with the general procedure E. White solid (32%); mp 165.0–167.0 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.68 (d, J = 8.6 Hz, 1H), 8.33 (dd, J = 1.2, 7.9 Hz, 1H), 7.84 (ddd, J = 1.2, 7.4, 8.6 Hz, 1H), 7.61 (dd, J = 7.4, 7.9 Hz, 1H), 7.31–7.44 (m, 5H), 6.15 (s, 1H), 6.05 (s, 1H), 4.64 (d, J = 9.8 Hz, 1H), 4.59 (d, J = 9.8 Hz, 1H), 4.42–4.52 (m, 1H), 4.29–4.40 (m, 1H), 3.55 (s, 3H), 1.62–1.79 (m, 2H), 1.36 (qt, J = 7.3, 7.3 Hz, 2H), 0.93 (t, J = 7.3 Hz, 3H); HRMS (FAB) calcd for $C_{29}H_{26}F_6NO_5$ 582.1715; found: 582.1704 $(M+H)^+$.

4.2.10.6. 11-(2-Benzyloxy-1,1,1,3,3,3-hexafluoropropan-2-yl)-4-butyl-7,10-dimethoxy-[1,3]dioxolo[4,5-*c*]phenanthridin-5(4*H*)-one (33g). Prepared from **31g** in accordance with the general procedure E. White solid (46%); mp 134.5–136.0 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.63 (d, J = 9.2 Hz, 1H), 7.77 (d, J = 3.1 Hz, 1H), 7.45 (dd, J = 3.1, 9.2 Hz, 1H), 7.31–7.43 (m, 5H), 6.14 (s, 1H), 6.03 (s, 1H), 4.63 (d, J = 9.8 Hz, 1H), 4.57 (d, J = 9.8 Hz, 1H), 4.43–4.52 (m, 1H), 4.31–4.41 (m, 1H), 3.90 (s, 3H), 3.54 (s, 3H), 1.70 (ttd, J = 6.7, 7.3, 7.3 Hz, 2H), 1.36 (qt, J = 7.3, 7.3 Hz, 2H), 0.93 (t, J = 7.3 Hz, 3H); MS (FAB) m/z 611 $(M)^+$, 612 $(M+H)^+$.

4.2.10.7. 5-Butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-3-fluorophenanthridin-6(5*H*)-one (5). Prepared from **30a** in accordance with the general procedure E. White solid (36%); mp 182.0–184.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.65 (d, J = 9.7 Hz, 1H), 8.58 (d, J = 7.9 Hz, 1H), 7.94 (dd, J = 9.1, 8.5 Hz, 1H), 7.77 (dd, J = 9.7, 7.9 Hz, 1H), 7.63 (dd, J = 7.9, 7.9 Hz, 1H), 7.29 (d, J = 9.1 Hz, 1H), 4.66 (s, 1H), 4.37 (t, J = 7.3 Hz, 2H), 1.77 (quin, J = 7.3 Hz, 2H), 1.51 (sext, J = 7.3 Hz, 2H), 1.02 (t, J = 7.3 Hz, 3H); MS (FAB) m/z 436 $(M+H)^+$; Anal. Calcd for $C_{20}H_{16}F_7NO_2$: C, 55.18; H, 3.70; N, 3.22. Found: C, 55.18; H, 3.78; N, 3.37.

4.2.10.8. 5-Butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-4-fluorophenanthridin-6(5*H*)-one (6). Prepared from **30b** in accordance with the general procedure E. White solid (73%); mp 188.5–190.0 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.47 (d, J = 9.8 Hz, 1H), 8.46 (s, 1H), 8.21 (d, J = 7.9 Hz, 1H), 7.75 (dd, J = 8.5, 7.9 Hz, 1H), 7.60 (dd, J = 9.8, 8.5 Hz, 1H), 7.60 (s, 1H), 4.98 (s, 1H), 4.46 (s, 2H), 1.82 (quin, J = 7.3 Hz, 2H), 1.47 (sext, J = 7.3 Hz, 2H), 0.99 (t, J = 7.3 Hz, 3H); MS (FAB) m/z 436 $(M+H)^+$; Anal. Calcd for $C_{20}H_{16}F_7NO_2$: C, 55.18; H, 3.70; N, 3.22. Found: C, 54.80; H, 3.75; N, 3.40.

4.2.10.9. 5-Butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-3-methylphenanthridin-6(5*H*)-one (7). Prepared from **30c** in accordance with the general procedure E. White solid (37%); mp 64.0–67.0 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8,56 (d, J = 7.9 Hz, 1H), 8.29 (d, J = 7.9 Hz, 1H), 7.77 (dd, J = 7.9, 7.9 Hz, 1H), 7.64 (dd, J = 7.9, 7.9 Hz, 1H), 7.58 (d, J = 7.9 Hz, 1H), 7.19 (d,

J = 7.9 Hz, 1H), 4.03 (t, J = 7.3 Hz, 2H), 2.98 (s, 3H), 1.56 (s, 1H), 1.56 (quin, J = 7.3 Hz, 2H), 1.13 (sext, J = 7.3 Hz, 2H), 0.82 (t, J = 7.3 Hz, 3H); HRMS (FAB) calcd for $C_{21}H_{20}F_6NO_2$ 432.1398; found: 432.1350 $(M+H)^+$.

4.2.10.10. 5-Butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-4-methylphenanthridin-6(5*H*)-one (8). Prepared from **30d** in accordance with the general procedure E. White solid (81%); mp 133.0–135.0 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 8.48 (s, 1H), 8.47 (d, J = 7.9 Hz, 1H), 8.21 (d, J = 7.9 Hz, 1H), 7.73 (dd, J = 7.9, 7.9 Hz, 1H), 7.61 (s, 1H), 7.57 (dd, J = 7.9, 7.9 Hz, 1H), 4.45 (t, J = 7.3 Hz, 2H), 4.43 (s, 1H), 2.69 (s, 3H), 1.65 (quin, J = 7.3 Hz, 2H), 1.26 (sext, J = 7.3 Hz, 2H), 0.87 (t, J = 7.3 Hz, 3H); MS (FAB) m/z 432 $(M+H)^+$; Anal. Calcd for $C_{21}H_{19}F_6NO_2$: C, 58.47; H, 4.44; N, 3.25. Found: C, 58.37; H, 4.50; N, 3.24.

4.2.10.11. 5-Butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-3-methoxyphenanthridin-6(5*H*)-one (9). Prepared from **30e** in accordance with the general procedure E. White solid (27%); mp 101.0–104.0 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 8.52 (d, J = 7.9 Hz, 1H), 8.47 (s, 1H), 8.12 (d, J = 8.6 Hz, 1H), 7.79 (dd, J = 8.6, 6.7 Hz, 1H), 7.59 (dd, J = 7.9, 6.7 Hz, 1H), 6.98 (s, 1H), 4.38 (t, J = 7.3 Hz, 2H), 4.12 (s, 3H), 1.82 (quin, J = 7.3 Hz, 2H), 1.54 (sext, J = 7.3 Hz, 2H), 1.05 (t, J = 7.3 Hz, 3H); HRMS (FAB) calcd for $C_{21}H_{20}F_6NO_3$ 448.1347; found: 448.1350 $(M+H)^+$.

4.2.10.12. 5-Butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-4-methoxyphenanthridin-6(5*H*)-one (10). Prepared from **30f** in accordance with the general procedure E. White solid (73%); mp 91.0–95.0 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 8.50 (d, J = 7.9 Hz, 1H), 8.26 (s, 1H), 8.22 (d, J = 7.9 Hz, 1H), 7.75 (dd, J = 7.9, 7.3 Hz, 1H), 7.59 (dd, J = 7.9, 7.3 Hz, 1H), 7.36 (s, 1H), 4.54 (t, J = 7.3 Hz, 1H), 4.03 (s, 1H), 3.97 (s, 3H), 1.84 (quin, J = 7.3 Hz, 2H), 1.44 (sext, J = 7.3 Hz, 2H), 0.99 (t, J = 7.3 Hz, 3H); MS (FAB) m/z 448 $(M+H)^+$; Anal. Calcd for $C_{21}H_{19}F_6NO_3 \cdot 1/2H_2O$: C, 55.30; H, 4.42; N, 3.07. Found: C, 55.27; H, 4.46; N, 3.09.

4.2.10.13. 5-Butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-7-methoxyphenanthridin-6(5*H*)-one (12). Prepared from **30g** in accordance with the general procedure E. White solid (85%); mp 226.0–230.0 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 8.64 (s, 1H), 8.37 (d, J = 9.1 Hz, 1H), 7.82 (d, J = 8.5 Hz, 1H), 7.48 (d, J = 2.4 Hz, 1H), 7.37 (d, J = 8.5 Hz, 1H), 7.10 (dd, J = 9.1, 2.4 Hz, 1H), 5.09 (br s, 1H), 4.26 (t, J = 7.3 Hz, 2H), 3.94 (s, 3H), 1.72 (quin, J = 7.3 Hz, 2H), 1.44 (sext, J = 7.3 Hz, 2H), 0.95 (t, J = 7.3 Hz, 3H); MS (FAB) m/z 448 $(M+H)^+$; Anal. Calcd for $C_{21}H_{19}F_6NO_3 \cdot 3/4H_2O$: C, 54.73; H, 4.48; N, 3.04. Found: C, 54.71; H, 4.51; N, 2.91.

4.2.10.14. 5-Butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-8-methoxyphenanthridin-6(5*H*)-one (13). Prepared from **30h** in accordance with the general procedure E. White solid (75%); mp 195.0–197.5 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 8.59 (s, 1H), 8.18 (d, J = 9.2 Hz, 1H), 7.94 (d, J = 3.0 Hz, 1H), 7.79 (d, J = 9.2 Hz, 1H), 7.44 (d, J = 9.2 Hz, 1H), 7.34 (dd, J = 9.2, 3.0 Hz, 1H), 4.59 (s, 1H), 4.38 (t, J = 7.3 Hz, 2H), 1.77 (quin, J = 7.3 Hz, 2H), 1.52 (sext, J = 7.3 Hz, 2H), 1.02 (t, J = 7.3 Hz, 3H); MS (FAB) m/z 448 $(M+H)^+$; Anal. Calcd for $C_{21}H_{19}F_6NO_3 \cdot H_2O$: C, 54.20; H, 4.55; N, 3.01. Found: C, 53.89; H, 4.12; N, 2.96.

4.2.10.15. 5-Butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-9-methoxyphenanthridin-6(5*H*)-one (14). Prepared from **30i** in accordance with the general procedure E. White solid (84%); mp 85.5–87.0 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 8.51 (s, 1H), 7.83 (d, J = 9.1 Hz, 1H), 7.81 (d, J = 7.9 Hz, 1H), 7.60 (dd, J = 7.9, 7.9 Hz, 1H), 7.33 (d, J = 9.1 Hz, 1H), 7.06 (d, J = 7.9 Hz, 1H), 5.22 (br s, 1H), 4.20 (t, J = 7.3 Hz, 2H), 4.04 (s, 3H), 1.68 (quin, J = 7.3 Hz, 2H), 1.48 (sext, J = 7.3 Hz, 2H), 0.99 (t, J = 7.3 Hz, 3H); MS (FAB) m/z 448 $(M+H)^+$; Anal. Calcd for $C_{21}H_{19}F_6NO_3 \cdot H_2O$: C, 54.20; H, 4.55; N, 3.01. Found: C, 54.32; H, 4.54; N, 3.13.

4.2.10.16. 5-Butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-10-methoxyphenanthridin-6(5*H*)-one (15). Prepared from **30j** in accordance with the general procedure E. White solid (79%); mp 175.5–177.0 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 9.75 (s, 1H), 8.16 (d, J = 7.9 Hz, 1H), 7.84 (d, J = 9.2 Hz, 1H), 7.50 (dd, J = 7.9, 7.9 Hz, 1H), 7.38 (d, J = 9.2 Hz, 1H), 7.22 (d, J = 7.9 Hz, 1H), 4.68 (br s, 1H), 4.30 (t, J = 7.3 Hz, 2H), 4.01 (s, 3H), 1.74 (quin, J = 7.3 Hz, 2H), 1.49 (sext, J = 7.3 Hz, 2H), 1.00 (t, J = 7.3 Hz, 3H); MS (FAB) m/z 448 $(M+H)^+$; Anal. Calcd for $C_{21}H_{19}F_6NO_3$: C, 56.38; H, 4.28; N, 3.13. Found: C, 56.15; H, 4.41; N, 3.14.

4.2.10.17. 5-Butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-3,8-dimethoxyphenanthridin-6(5*H*)-one (18). Prepared from **30o** in accordance with the general procedure E. White solid (55%); mp 184.5–186.5 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 8.38 (s, 1H), 8.04 (d, J = 8.5 Hz, 1H), 7.93 (d, J = 3.1 Hz, 1H), 7.33 (dd, J = 8.5, 3.1 Hz, 1H), 7.32 (s, 1H), 4.56 (t, J = 7.3 Hz, 2H), 4.34 (s, 1H), 3.97 (s, 3H), 3.95 (s, 3H), 1.85 (quin, J = 7.3 Hz, 2H), 1.45 (sext, J = 7.3 Hz, 2H), 0.99 (t, J = 7.3 Hz, 3H); MS (FAB) m/z 478 $(M+H)^+$; Anal. Calcd for $C_{22}H_{21}F_6NO_4$: C, 55.35; H, 4.43; N, 2.93. Found: C, 55.17; H, 4.39; N, 2.93.

4.2.10.18. 5-Butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-4,8-dimethoxyphenanthridin-6(5*H*)-one (19). Prepared from **30p** in accordance with the general procedure E. White solid (82%); mp 177.0–181.0 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 8.18 (s, 1H), 8.14 (d, J = 9.1 Hz, 1H), 7.92 (d, J = 3.1 Hz, 1H), 7.38 (dd, J = 8.5, 3.1 Hz, 1H), 7.23 (br s, 1H), 6.98 (s, 1H), 4.39 (t, J = 7.3 Hz, 2H), 4.11 (s, 3H), 3.96 (s, 3H), 1.82 (quin, J = 7.3 Hz, 2H), 1.55 (sext, J = 7.3 Hz, 2H), 1.05 (t, J = 7.3 Hz, 3H); MS (FAB) m/z 478 $(M+H)^+$; HRMS (FAB) calcd for $C_{22}H_{22}F_6NO_4$ 478.1453; found: 478.1412 $(M+H)^+$.

4.2.10.19. 5-Butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-[1,3]dioxolo[4,5-*j*]phenanthridin-6(5*H*)-one (22). Prepared from **30q** in accordance with the general procedure E. White solid (78%); mp 214.5–215.5 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 8.43 (s, 1H), 7.84 (s, 1H), 7.79 (d, J = 9.1 Hz, 1H), 7.55 (s, 1H), 7.41 (d, J = 9.1 Hz, 1H), 6.13 (s, 2H), 4.45 (s, 1H), 4.31 (t, J = 7.3 Hz, 2H), 1.75 (quin, J = 7.3 Hz, 2H), 1.50 (sext, J = 7.3 Hz, 2H), 1.01 (t, J = 7.3 Hz, 3H); MS (FAB) m/z 462 $(M+H)^+$; Anal. Calcd for $C_{21}H_{17}F_6NO_4$: C, 54.67; H, 3.71; N, 3.04. Found: C, 54.45; H, 3.72; N, 3.12.

4.2.10.20. 4-Butyl-11-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-[1,3]dioxolo[4,5-*c*]phenanthridin-5(4*H*)-one (23). Prepared from **30r** in accordance with the general procedure E. White solid. Mp 186.5–188.5 °C; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.98 (s, 1H), 8.32 (dd, J = 7.9, 1.2 Hz, 1H), 8.27 (s, 1H), 8.26 (d, J = 7.9 Hz, 1H), 7.85 (td, J = 7.3, 1.2 Hz, 1H), 7.62 (t, J = 7.3 Hz, 1H), 6.19 (s, 2H), 4.43 (t, J = 7.9 Hz, 2H), 1.68 (tt, J = 7.3, 7.9 Hz, 2H), 1.37 (qt, J = 7.3, 7.3 Hz, 2H), 0.93 (t, J = 7.3 Hz, 3H); HRMS (FAB) calcd for $C_{21}H_{18}F_6NO_4$ 462.1140; found: 462.1169 $(M+H)^+$.

4.2.10.21. 4-Butyl-11-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-7-methoxy-[1,3]dioxolo[4,5-*c*]phenanthridin-5(4*H*)-one (24). Prepared from **30s** in accordance with the general procedure E. White solid (51%); mp 196.0–197.5 °C; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.94 (s, 1H), 8.20 (d, J = 9.2 Hz, 1H), 8.16 (s, 1H), 7.74 (d, J = 3.1 Hz, 1H), 7.46 (dd, J = 9.2, 3.1 Hz, 1H), 6.16 (s, 2H), 4.44 (t, J = 7.9 Hz, 2H), 3.89 (s, 3H), 1.68 (tt, J = 7.3, 7.9 Hz, 2H), 1.36 (qt,

J = 7.3, 7.9 Hz, 2H), 0.92 (t, J = 7.9 Hz, 3H); HRMS (FAB) calcd for $C_{22}H_{20}F_6NO_5$ 492.1246; found: 492.1257 $(M+H)^+$.

4.2.10.22. 4-Butyl-12-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-bis[1,3]dioxolo[4,5-*c*:4',5'-*j*]phenanthridin-5(4*H*)-one (25). Prepared from **30t** in accordance with the general procedure E. White solid (95%); mp 236.0–237.0 °C; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.89 (s, 1H), 8.10 (s, 1H), 7.70 (s, 1H), 7.63 (s, 1H), 6.22 (s, 2H), 6.16 (s, 2H), 4.40 (t, J = 7.9 Hz, 2H), 1.66 (tt, J = 7.3, 7.9 Hz, 2H), 1.35 (qt, J = 7.3, 7.3 Hz, 2H), 0.92 (t, J = 7.3 Hz, 3H); HRMS (FAB) calcd for $C_{22}H_{18}F_6NO_6$ 506.1038; found: 506.1010 $(M+H)^+$.

4.2.11. General procedure F: synthesis of phenanthridine analogues via debenzylation by hydrogenation

4.2.11.1. 5-Butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-3,4,8-trimethoxyphenanthridin-6(5*H*)-one (21). To a solution of **33e** (19.2 mg, 32.1 μmol) in ethanol (0.5 mL) was added 10% Pd/C (2.2 mg), and the mixture was stirred for 3 h at room temperature under an H_2 atmosphere. The reaction mixture was filtered through a pad of Celite®, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel chromatography (*n*-hexane/ethyl acetate = 9:1) to give **21** (95%) as a white solid. Mp 108.0–110.0 °C; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.72 (s, 1H), 8.40 (s, 1H), 8.21 (d, J = 9.2 Hz, 1H), 7.75 (d, J = 3.1 Hz, 1H), 7.48 (dd, J = 9.2, 3.1 Hz, 1H), 4.50 (t, J = 7.3 Hz, 2H), 3.90 (s, 3H), 3.89 (s, 3H), 3.73 (s, 3H), 1.67 (tt, J = 7.3, 7.3 Hz, 2H), 1.28 (qt, J = 7.3, 7.3 Hz, 2H), 0.89 (t, J = 7.3 Hz, 3H); HRMS (FAB) calcd for $C_{23}H_{24}F_6NO_5$ 508.1559; found: 508.1549 $(M+H)^+$.

4.2.11.2. 5-Butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-1-methoxyphenanthridin-6(5*H*)-one (11). Prepared from **33a** in accordance with the general procedure F. White solid (90%); mp 123.0–123.5 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 8.88 (d, J = 8.5 Hz, 1H), 8.59 (d, J = 6.7 Hz, 1H), 8.27 (br s, 1H), 7.79 (dd, J = 8.5, 6.7 Hz, 1H), 7.73 (d, J = 9.8 Hz, 1H), 7.65 (dd, J = 8.5, 6.7 Hz, 1H), 7.29 (d, J = 9.8 Hz, 1H), 4.48–4.40 (m, 1H), 4.35–4.4.22 (m, 1H), 3.86 (s, 3H), 1.90–1.73 (m, 2H), 1.60–0.97 (m, 2H), 1.03 (t, J = 7.3 Hz, 3H); MS (FAB) *m/z* 448 $(M+H)^+$; HRMS (FAB) calcd for $C_{21}H_{20}F_6NO_3$ 448.1347; found: 448.1394 $(M+H)^+$.

4.2.11.3. 5-Butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-1,4-dimethoxyphenanthridin-6(5*H*)-one (16). Prepared from **33b** in accordance with the general procedure F. White solid (77%); mp 134.0–134.5 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 8.80 (d, J = 7.9 Hz, 1H), 8.53 (d, J = 7.9 Hz, 1H), 8.39 (br s, 1H), 7.74 (dd, J = 7.9, 6.7 Hz, 1H), 7.63 (dd, J = 7.9, 6.7 Hz, 1H), 7.18 (s, 1H), 4.55–4.45 (m, 1H), 4.40–4.30 (m, 1H), 3.92 (s, 3H), 3.78 (s, 3H), 1.92–1.80 (m, 1H), 1.44 (sext, J = 7.3 Hz, 2H), 1.00 (t, J = 7.3 Hz, 3H); MS (FAB) *m/z* 478 $(M+H)^+$; HRMS (FAB) calcd for $C_{22}H_{22}F_6NO_4$ 478.1453; Found: 478.1420 $(M+H)^+$.

4.2.11.4. 5-Butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-3,4-dimethoxyphenanthridin-6(5*H*)-one (17). Prepared from **33c** in accordance with the general procedure F. White solid (99%); mp 106.0–107.0 °C; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.76 (s, 1H), 8.50 (s, 1H), 8.33 (d, J = 8.6 Hz, 1H), 8.28 (d, J = 7.3 Hz, 1H), 7.87 (t, J = 8.6 Hz, 1H), 7.64 (t, J = 7.3 Hz, 2H), 4.49 (t, J = 7.3 Hz, 2H), 3.91 (s, 3H), 3.73 (s, 3H), 1.67 (tt, J = 7.3, 7.3 Hz, 2H), 1.28 (qt, J = 7.3, 7.3 Hz, 2H), 0.89 (t, J = 7.3 Hz, 3H); HRMS (FAB) calcd for $C_{22}H_{22}F_6NO_4$ 478.1453; found: 478.1445 $(M+H)^+$.

4.2.11.5. 5-Butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-1,4,8-trimethoxyphenanthridin-6(5*H*)-one (20). Prepared from **33d** in accordance with the general procedure F. White solid (80%); mp 156.0–157.0 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 8.73 (d, J = 9.2 Hz, 1H), 8.42 (br s, 1H), 7.96 (d, J = 3.0 Hz, 1H), 7.31 (dd, J = 9.2, 3.0 Hz, 1H), 7.13 (s, 1H), 4.55–4.45 (m, 1H), 4.40–4.30 (m, 1H), 3.97 (s, 3H), 3.92 (s, 3H), 3.76 (s, 3H), 1.92–1.80 (m, 1H), 1.45 (sext, J = 7.3 Hz, 2H), 1.00 (t, J = 7.3 Hz, 3H); MS (FAB) *m/z* 508 $(M+H)^+$; HRMS (FAB) calcd for $C_{23}H_{24}F_6NO_5$ 508.1559; found: 508.1605 $(M+H)^+$.

4.2.11.6. 4-Butyl-11-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-10-methoxy-[1,3]dioxolo[4,5-*c*]phenanthridin-5(4*H*)-one (26). Prepared from **33f** in accordance with the general procedure F. White solid (93%); mp 160.0–161.5 °C; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.67 (d, J = 8.6 Hz, 1H), 8.35 (s, 1H), 8.31 (d, J = 8.0 Hz, 1H), 7.81 (dd, J = 7.3, 8.6 Hz, 1H), 7.59 (dd, J = 7.3, 8.0 Hz, 1H), 6.10 (s, 2H), 4.22–4.55 (br, 2H), 3.54 (s, 3H), 1.66 (tt, J = 7.3, 7.9 Hz, 2H), 1.35 (qt, J = 7.3, 7.9 Hz, 2H), 0.92 (t, J = 7.3 Hz, 3H); HRMS (FAB) calcd for $C_{22}H_{20}F_6NO_5$ 492.1246; found: 492.1261 $(M+H)^+$.

4.2.11.7. 4-Butyl-11-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-7,10-dimethoxy-[1,3]dioxolo[4,5-*c*]phenanthridin-5(4*H*)-one (27). Prepared from **33c** in accordance with the general procedure F. White solid (82%); mp 138.5–140.0 °C; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.62 (d, J = 9.2 Hz, 1H), 8.34 (s, 1H), 7.76 (d, J = 3.1 Hz, 1H), 7.43 (dd, J = 3.1, 9.2 Hz, 1H), 6.08 (s, 2H), 4.28–4.53 (br, 2H), 3.89 (s, 3H), 3.53 (s, 3H), 1.66 (tt, J = 7.3, 7.3 Hz, 2H), 1.35 (qt, J = 7.3, 7.3 Hz, 2H), 0.92 (t, J = 7.3 Hz, 3H); HRMS (FAB) calcd for $C_{23}H_{22}F_6NO_6$ 522.1351; found: 522.1361 $(M+H)^+$.

4.2.12. *N*-[4-(2-Benzyloxy-1,1,1,3,3,3-hexafluoropropan-2-yl)-*N*-butyl-3-hydroxyphenyl]-2-iodobenzamide (32)

To a solution of **31a** (40.0 mg, 60.1 μmol) in DMA (3.0 mL) were added dropwise $PCy_3{\cdot}HBF_4$ (5.53 mg, 15.0 μmol), Cs_2CO_3 (68.6 mg, 210 μmol) and $Pd(OAc)_2$ (1.75 mg, 7.81 μmol) under Ar atmosphere, then the mixture was stirred for 2 h at 165 °C. At 0 °C, the mixture was diluted with ethyl acetate and then water was added. The organic layer was separated and washed with brine, and dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (*n*-hexane/AcOEt = 1:0–2:1) to give **32** (11.0 mg, 21.0 μmol, 35%) as a white solid. ^1H NMR (500 MHz, $CDCl_3$) δ 9.26 (d, J = 8.5 Hz, 1H), 8.92 (s, 1H), 8.63 (d, J = 7.9 Hz, 1H), 7.74 (dd, J = 8.5, 7.3 Hz, 1H), 7.60 (dd, J = 7.9, 7.3 Hz, 1H), 7.53 (d, 1H, J = 9.2 Hz), 7.46 (s, 5H), 7.11 (d, 1H, J = 9.2 Hz), 4.83 (s, 2H), 4.45–4.35 (m, 2H), 1.82 (quin, 2H, J = 7.3 Hz), 1.54 (sext, J = 7.3 Hz, 2H), 1.04 (t, J = 7.3 Hz, 3H); MS (FAB) *m/z* 524 $(M+H)^+$.

4.2.13. 2-(2-Benzyloxy-1,1,1,3,3,3-hexafluoropropan-2-yl)-5-butyl-1-methoxyphenanthridin-6(5*H*)-one (33a)

To a solution of **32** (10.0 mg, 19.1 μmol) in CH_2Cl_2 (0.5 mL) were added dropwise methyl iodide (1.78 μl, 28.7 μmol) and K_2CO_3 (3.96 mg, 28.7 μmol) at 0 °C, then the mixture was stirred for 3 h and was allowed to warm to room temperature. At 0 °C, the mixture was diluted with ethyl acetate and then water was added. The organic layer was separated and washed with brine, and dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (n-hexane/AcOEt = 1:0–2:1) to give **33a** (13.5 mg, 19.1 μmol, quant.) as a white solid. ^1H NMR (500 MHz, $CDCl_3$) δ 8.91 (d, J = 7.9 Hz, 1H), 8.56 (d, J = 6.7 Hz, 1H), 7.78 (dd, J = 7.9, 7.3 Hz, 1H), 7.71 (d, J = 9.2 Hz, 1H), 7.62 (dd, 1H, J = 7.3, 6.7 Hz), 7.41 (s, 5H), 7.21 (d, 1H, J = 9.2 Hz), 4.76–4.25 (m, 2H), 3.69 (s, 3H), 1.82–1.74 (m, 2H), 1.52 (sext, J = 7.3 Hz, 2H), 1.02 (t, J = 7.3 Hz, 3H); MS (FAB) *m/z* 538 $(M+H)^+$.

4.2.14. 2-(1,1,1,3,3,3-Hexafluoro-2-hydroxypropan-2-yl)-5-(2-methoxyethyl)phenanthridin-6(5*H*)-one (3)

Prepared from *N*-[4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)phenyl]-2-iodobenzamide and 2-chloroethyl methyl ether in

accordance with the literature method.[28] White solid (84%); mp 174.0–178.0 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.65 (s, 1H), 8.52 (d, J = 7.9 Hz, 1H), 8.29 (d, J = 8.5 Hz, 1H), 7.83 (d, J = 9.2 Hz, 1H), 7.76 (dd, J = 8.5, 8.5 Hz, 1H), 7.67 (d, J = 9.2 Hz, 1H), 7.61 (dd, J = 8.5, 7.9 Hz, 1H), 4.60 (t, J = 5.5 Hz, 2H), 3.38 (s, 3H); MS (FAB) m/z 420 $(M+H)^+$; HRMS (FAB) calcd for $C_{19}H_{16}F_6NO_3$ 420.1034; found: 420.0997 $(M+H)^+$.

4.2.15. 2-(1,1,1,3,3,3-Hexafluoro-2-hydroxypropan-2-yl)-5-(2-naphthylmethyl)phenanthridin-6(5*H*)-one (4)

Prepared from *N*-[4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)phenyl]-2-iodobenzamide and 2-(bromomethyl)naphthalene in accordance with the literature method.[28] White solid (48%); mp 128.0–129.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.68 (s, 1H), 8.64 (d, J = 7.9 Hz, 1H), 8.34 (d, J = 8.5 Hz, 1H), 7.85–7.77 (m, 3H), 7.73–57 (m, 4H), 7.49–7.33 (m, 4H), 5.76 (br s, 2H), 4.39 (s, 1H); MS (FAB) m/z 502 $(M+H)^+$; Anal. Calcd for $C_{27}H_{17}F_6NO_2 \cdot 2/3H_2O$: C, 63.16; H, 3.60; N, 2.73. Found: C, 63.16; H, 3.66; N, 2.79.

4.3. Bioassay

Subgenome HCV RNA replicon cells containing the luciferase gene (LucNeo#2)[29] were cultured and maintained in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum, antibiotics, and 1 mg/ml geneticin (Wako, Osaka, Japan). For anti-HCV assays, the cells were suspended (50,000 cells/ml) in the culture medium without geneticin and inoculated into a mictotiter plate. After incubation for 24 h, the cells were further incubated with fresh culture medium containing various concentrations of test compounds. After 3 days, the cells were washed with phosphate-buffered saline and treated with a cell-lysis solution for 10 min by intermittent shaking. The cell lysate (25 μl) was transferred to a white microtiter plate. One hundred microlitre of a luciferase assay reagent (Promega, Madison, WI) was added into each well, and its luciferase activity was measured by a luminometer. For cell viability assay, 10 μl of a tetrazolium dye solution (TetraColor One®, Seikagaku Biobusiness Corp., Tokyo, Japan) was added into each well. After incubation for 1 h, specific absorbance (450 nm) was measured by a microplate reader.

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