Bioorganic & Medicinal Chemistry 24 (2016) 160–178

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

8-Hydroxyquinoline-based inhibitors of the Rce1 protease disrupt Ras membrane localization in human cells

Idrees Mohammed [a,†], Shahienaz E. Hampton [a,†], Louise Ashall [a,†], Emily R. Hildebrandt [c,†], Robert A. Kutlik [b], Surya P. Manandhar [c], Brandon J. Floyd [b], Haley E. Smith [a], Jonathan K. Dozier [d], Mark D. Distefano [d], Walter K. Schmidt [c], Timothy M. Dore [a,b,*]

[a] New York University Abu Dhabi, PO Box 129188, Abu Dhabi, United Arab Emirates
[b] Department of Chemistry, University of Georgia, Athens, GA 30602, United States
[c] Department of Biochemistry & Molecular Biology, University of Georgia, Athens, GA 30602, United States
[d] Department of Chemistry, University of Minnesota, Minneapolis, MN 55455, United States

ARTICLE INFO

Article history:
Received 26 October 2015
Revised 23 November 2015
Accepted 29 November 2015
Available online 30 November 2015

Keywords:
Ras converting enzyme (Rce1)
Protease inhibitors
Ras mislocalization

ABSTRACT

Ras converting enzyme 1 (Rce1) is an endoprotease that catalyzes processing of the C-terminus of Ras protein by removing -aaX from the CaaX motif. The activity of Rce1 is crucial for proper localization of Ras to the plasma membrane where it functions. Ras is responsible for transmitting signals related to cell proliferation, cell cycle progression, and apoptosis. The disregulation of these pathways due to constitutively active oncogenic Ras can ultimately lead to cancer. Ras, its effectors and regulators, and the enzymes that are involved in its maturation process are all targets for anti-cancer therapeutics. Key enzymes required for Ras maturation and localization are the farnesyltransferase (FTase), Rce1, and isoprenylcysteine carboxyl methyltransferase (ICMT). Among these proteins, the physiological role of Rce1 in regulating Ras and other CaaX proteins has not been fully explored. Small-molecule inhibitors of Rce1 could be useful as chemical biology tools to understand further the downstream impact of Rce1 on Ras function and serve as potential leads for cancer therapeutics. Structure–activity relationship (SAR) analysis of a previously reported Rce1 inhibitor, NSC1011, has been performed to generate a new library of Rce1 inhibitors. The new inhibitors caused a reduction in Rce1 in vitro activity, exhibited low cell toxicity, and induced mislocalization of EGFP-Ras from the plasma membrane in human colon carcinoma cells giving rise to a phenotype similar to that observed with siRNA knockdowns of Rce1 expression. Several of the new inhibitors were more effective at mislocalizing K-Ras compared to a potent farnesyltransferase inhibitor (FTI), which is significant because of the preponderance of K-Ras mutations in cancer.

1. Introduction

Small molecule mediated inhibition of oncogenic Ras signaling is an emerging trend in the anticancer drug discovery field.[1,2] Overall, it is estimated that about 30% of human cancers involve activating Ras mutations.[3] Ras proteins are membrane-associated small GTPases that mediate signal transduction events related to growth, differentiation, cytoskeletal organization, and membrane trafficking. Ras has a characteristic CaaX motif (where C is cysteine, a is an aliphatic amino acid, and X is one of several amino acids) at its C-terminus, which interacts sequentially with farnesyltransferase (FTase), Ras converting enzyme 1 endoprotease (Rce1), and isoprenylcysteine carboxyl methyltransferase (ICMT). All Ras isoforms localize to the plasma membrane where they are poised to mediate their signaling effects.[4–6] There are multiple approaches to modulating Ras signaling. Chemotherapeutic targeting of mutant Ras proteins with guanine nucleotide mimics is perceived as impractical due to the picomolar binding affinity of Ras for GTP and GDP and the availability of GTP and GDP in micromolar concentrations within the cell.[7,8] Also, Ras proteins do not have accessible pockets on their surface. Despite these difficulties, a few recent studies report allosteric[9–12] and covalent inhibitors[13,14] of mutant K-Ras. Additional approaches include inhibition of downstream effectors (e.g., kinases) and proteins essential for transformative growth in the presence of oncogenic Ras.[15]

* Corresponding author.
E-mail address: timothy.dore@nyu.edu (T.M. Dore).
† These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bmc.2015.11.043
0968-0896/

An alternative strategy to inhibit oncogenic Ras signaling involves disruption of the Ras maturation process, which subsequently leads to the protein's mislocalization. Farnesyltransferase inhibitors (FTIs) progressed to late stage clinical trials (e.g., Tipifarnib, Lonafarnib, Salisarib), but the overall efficacy in patients with solid tumors was far less than expected, mainly due to alternative geranylgeranylation of K-Ras and N-Ras isoforms.[16] Studies in cell culture showed that FTIs disrupt Ras localization. Ras is also mislocalized in the absence of Rce1 protease or ICMT activities.[17,18] The deletion of the gene encoding Rce1 markedly sensitizes tumor cells to FTI treatment,[19] and the elimination of ICMT in fibroblasts blocks oncogenic K-Ras mediated transformation.[20] Mice lacking the Rce1 gene die in early stages of embryonic development,[17] whereas tissue-specific knockouts display context specific effects. Loss of Rce1 from heart tissue results in lethal cardiomyopathy, whereas a liver-specific knockout appears healthy and has normal hematopoietic function.[21]

Selective and potent inhibitors of Rce1 would be useful to further investigate the physiological role of Rce1 in regulating Ras and other CaaX proteins and to explore their potential as an anticancer chemotherapeutic strategy. Known Rce1 inhibitors[22,23] range from substrate mimics to small molecules.[24–29] Selective inhibition of the Rce1 protease, however, continues to be a challenging problem. The ideal inhibitor must avoid simultaneous inhibition of the functionally related and evolutionarily distinct CaaX protease sterile mutant 24 (Ste24).[30] Ste24 is essential for the maturation of lamin A, and defects in Ste24 activity, either by mutation or inhibition, lead to the development of laminopathies, such as progeria, muscular dystrophy, and lipodystrophy.[31,32]

Rce1 is an integral membrane protein localized to the endoplasmic reticulum (ER).[33,34] The crystal structure of the human Rce1 (*Hs*Rce1) has not yet been elucidated, although topology data on Rce1 from *Saccharomyces cerevisiae* (*Sc*Rce1)[35] and the crystal structure of a homolog from the archaea *Methanococcus maripaludis*[36] are available. Originally thought to be a cysteine protease,[37] then a metalloprotease,[38] the crystal structure of the archaea homolog suggests that it proteolyzes Ras through a novel mechanism involving an active site glutamate.[36] The possibility of a cysteine protease mechanism has been eliminated,[35] whereas a glutamate and two histidines are essential for proteolytic activity,[39,40] supporting the latter two mechanistic hypotheses. The sequence of Rce1 from *M. maripaludis* (*Mm*Rce1) is only 15% identical to that of human Rce1 (*Hs*Rce1),[36] so one should be cautious in assigning structure and function of the human enzyme based on *Mm*Rce1's structure.

NSC1011 (**1**, Fig. 1) inhibits *Hs*Rce1 in an in vitro proteolysis assay (IC_{50} = 9 μM)[29] and mislocalizes a GFP-Ras2p reporter in yeast cells.[41] In this report, we present an investigation of the structure–activity relationships (SAR) of NSC1011 to improve potency and selectivity against *Hs*Rce1. Additionally, we show the ability of NSC1011 and some of its derivatives to mislocalize EGFP-Ras isoforms in a human colon carcinoma cell line (HCT-116).

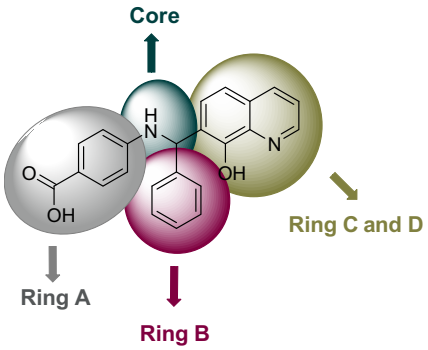
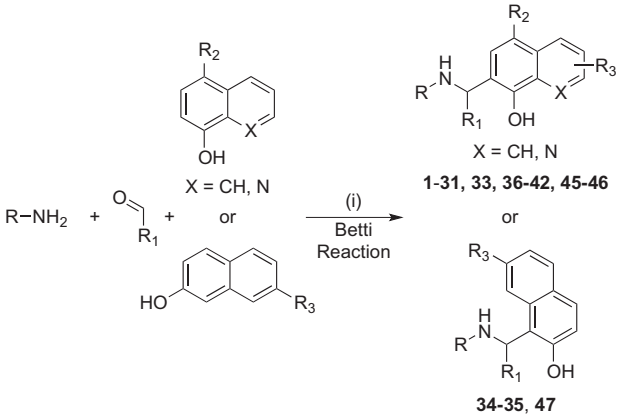


Figure 1. NSC1011 (**1**) and ring identification used to describe the SAR.



Scheme 1. General synthetic approach to NSC1011 analogs. Reagents and conditions: (i) See the Section 4 for details of various reaction conditions.

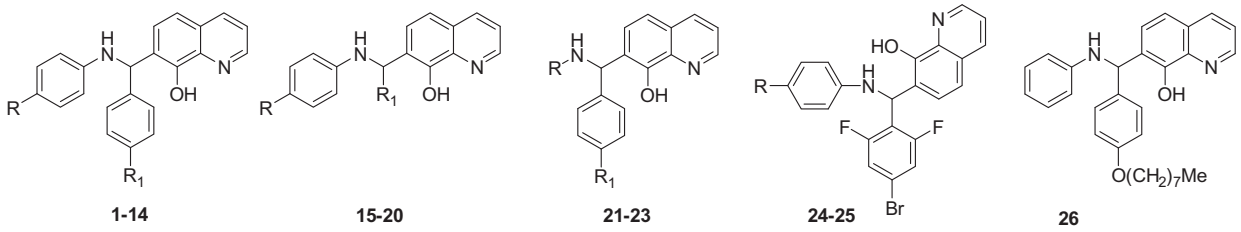
2. Results and discussion

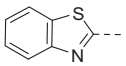
2.1. Chemistry

The preparation of the majority of the compounds was carried out using the classical Betti reaction or its modified versions (Scheme 1). The classical Betti reaction is a one pot multicomponent reaction between an amine, an aldehyde, and an electron rich bicyclic phenol.[42–46] The lead molecule NSC1011 (**1**, Table 1) was synthesized by the reaction between *p*-aminobenzoic acid, benzaldehyde, and 8-hydroxyquinoline in ethanol at room temperature in the presence of a catalytic amount of pyridine for 10–15 days stirring to afford **1** in about 16% yield (protocol A). Heating the reaction to 120 °C in ethanol (protocol B) led to a decrease in reaction time to 12 h and an improvement in yield to 55%. If carried out under microwave conditions at 140 °C, the reaction time could be reduced to approximately 15 min (protocol C) with higher yield (65%). Compounds **2**–**6**, **9**–**10**, **15**–**18**, **21**, **24**–**25**, **37**–**38**, and **40**–**42** were prepared by following any one of four protocols (A-D). Compounds **7** and **8** (NSC 1013 and 84093, respectively) were acquired from the National Cancer Institute's Developmental Therapeutics Program (DTP).

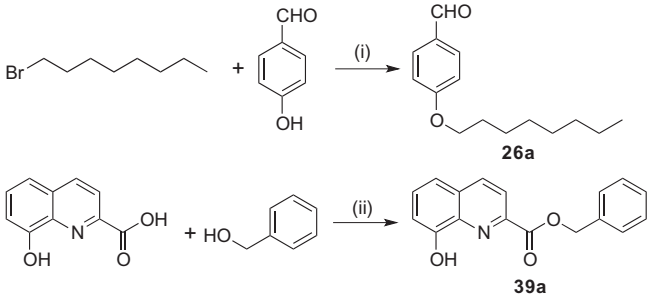
Ester derivatives **11**–**14** and **19** were prepared by refluxing benzocaine, the corresponding aldehyde, and 8-hydroxyquinoline in a 10% aqueous solution of NaCl instead of ethanol.[47] Benzamide derivative **22** was successfully obtained in 70% yield by refluxing a mixture of benzamide, benzaldehyde, and 8-hydroxyquinoline in dichloroethane in the presence of catalytic *p*-toluenesulfonic acid.[48] Synthesis of imine analog **23** required heating a mixture of ammonium carbamate, benzaldehyde (2 equiv), and 8-hydroxyquinoline in ethanol at 125 °C for 12–15 h.[49] Octyloxy analog **26** required the initial preparation of the aldehyde intermediate **26a**[50] by reacting *p*-hydroxybenzaldehyde with octyl bromide in the presence of potassium carbonate and catalytic potassium iodide (Scheme 2). Subsequently, a mixture of **26a**, aniline, and 8-hydroxyquinoline was neatly heated at 130 °C for 24 h to afford the Betti reaction product **26**. Compound **39** was synthesized by first reacting 8-hydroxyquinoline-2-carboxylic acid and benzylic alcohol in the presence of triphenylphosphine and diisopropylazodicarboxylate in THF solvent at 0 to 10 °C in a modified Mitsunobu procedure[51] to generate intermediate hydroxyquinoline

Table 1
Enzymatic results for compounds synthesized, **1–26**

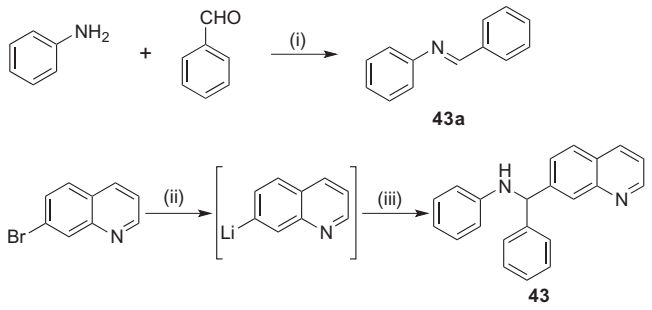


No.	R	R_1	*Hs*Rce1 percent activity remaining[a] (10 μM)	*Hs*Rce1 IC_{50}[b] (μM)
1[c]	CO_2H	H	25 ± 0.05	6.9 ± 1.06
2	H	H	39 ± 1.3	8.9 ± 1.08
3	CO_2H	CN	76 ± 3.1	16 ± 1.1
4	CO_2H	Me	32 ± 3.6	7.1 ± 1.0
5	CO_2H	Br	23 ± 1.3	6.7 ± 1.1
6	CO_2H	F	32 ± 0.01	8.2 ± 1.1
7[d]	H	NO_2	85 ± 5.0	11 ± 1.2
8[d]	Me	H	54 ± 5.2	8.8 ± 1.1
9	CN	H	56 ± 0.2	nd
10	NO_2	Br	64 ± 1.2	nd
11	CO_2Et	H	52 ± 3.2	nd
12	CO_2Et	CF_3	89 ± 3.8	nd
13	CO_2Et	F	63 ± 0.4	nd
14	CO_2Et	Cl	71 ± 0.6	nd
15	CO_2H	2-Pyridine	90 ± 5.2	38 ± 1.1
16	CO_2H	3-Pyridine	70 ± 1.8	14 ± 1.0
17	CO_2H	Cyclohexyl	34 ± 4.8	9.8 ± 1.1
18	H	Cyclohexyl	59 ± 0.03	nd
19	CO_2Et	Cyclopentyl	82 ± 1.2	nd
20	H	H	82 ± 0.49	nd
21		H	83 ± 5.7	nd
22	Ph(CO)	H	81 ± 1.1	nd
23	PhCH	H	73 ± 2.1	nd
24	H	—	76 ± 1.7	nd
25	CO_2H	—	64 ± 3.7	nd
26	—	—	89 ± 3.5	nd

[a] The percent activity remaining values are the averages of replicates from one assay in the presence of inhibitor.
[b] nd = not determined.
[c] Compound **1** (NSC1011) has previously been tested and data published,[29] however it was resynthesized, retested, and included here for comparative purposes.
[d] Compounds **7** and **8** were acquired from the NCI DTP and are included for SAR comparison.



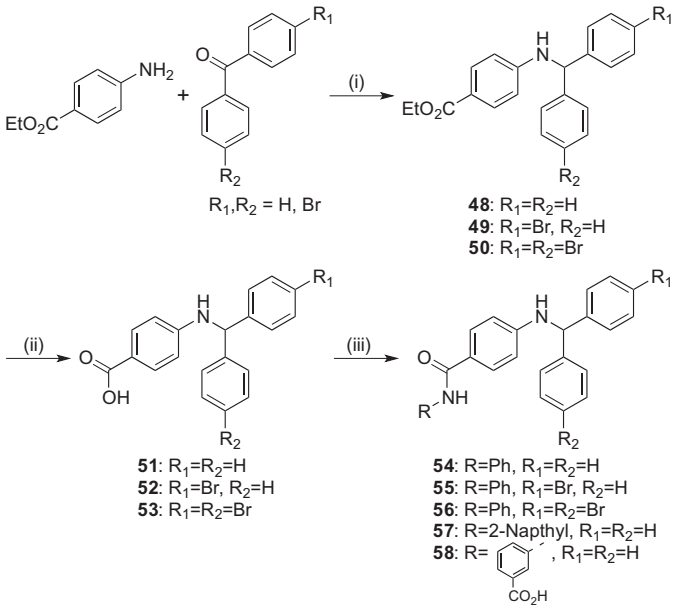
Scheme 2. Synthesis of intermediates **26a** and **39a**. Reagents and conditions: (i) K_2CO_3, cat. KI, acetone, reflux; (ii) PPh_3, DIAD, THF, 0 °C to rt, 1 h.



Scheme 3. Synthetic approach to **43**. Reagents and conditions: (i) neat, 60 °C; (ii) *n*-BuLi, THF, −78 °C; (iii) **43a**, THF, −78 °C to rt, 3 h.

39a[52] (Scheme 2), which was subsequently heated neatly with 8-hydroxyquinoline and benzaldehyde in the presence of catalytic pyridine at 120 °C to afford compound **39** in 15% yield. Quinoline derivative **43** was prepared in two steps (Scheme 3). The intermediate imine **43a** was synthesized by heating a mixture of aniline and benzaldehyde neatly at 60 °C. Without further purification, **43a** was added to the lithiated quinoline derivative, which was generated by reacting 7-bromoquinoline with *n*-butyllithium at −78 °C, to afford **43** in 7% yield.

The 1-naphthol derivative **27** was synthesized in 95% yield by reacting 1-naphthol, aniline, and benzaldehyde in ethanol for 12–24 h at room temperature in the presence of a catalytic amount of pyridine. The same conditions were used to yield the other 1-naphthol derivatives **28–31**, **33**, and **45–46**, with the exception of **31**, which was carried out in DMSO at room temperature by stirring for 36–48 h. In contrast to the 1-naphthols, 2-naphthol derivatives **34** and **35** required heating the respective reaction mixtures



Scheme 4. Synthesis of analogs **48**–**58**. Reagents and conditions: (i) (a) i. $TiCl_4$, DCM; 0 °C–t; (b) $NaCNBH_3$, MeOH; (ii), 5 N NaOH (aq), dioxane/EtOH, rt; (iii) *R*-NH_2, COMU, DIPEA, DMF, 0 °C–rt.

at 100 °C. The diastereomeric 2-naphthol derivative **47** was synthesized by heating the reactants neatly at 85 °C for 12 h. The product precipitated out as a crystalline solid upon discharge of ethanol and cooling to room temperature.[53] Derivative **32** was prepared in modest yield by treating NSC1011 (**1**) with iodomethane in the presence of potassium hydroxide.

The diphenyl compounds, **48**–**58** were prepared by reductive amination of the appropriate benzophenone and benzocaine in the presence of titanium(IV) chloride and sodium cyanoborohydride (Scheme 4).[54] Esters **48**–**50** were then hydrolyzed to acids **51**–**53** and the subsequent amidations with aniline, amino-naphthalene, and 3-aminobenzoic acid were carried out to give **54**–**58**. Similarly, naphthyl analog **44** was prepared via reductive amination using 2-benzoylnapthalene and aniline.

All derivatives synthesized were tested as racemic mixtures in the biological assays.

2.2. Biological assays

2.2.1. CaaX proteolysis assay determines efficacy of Rce1 inhibition

An established in vitro fluorescence-based assay was used to measure the CaaX protease activity of the human Rce1 (*Hs*Rce1) following treatment with the inhibitors.[29,55,56] The assay is based on the Rce1-dependent cleavage of a quenched fluorogenic K-Ras4b-derived peptide using membranes derived from yeast expressing *Hs*Rce1. Inhibitors were added at 10 μM and the percentage of remaining *Hs*Rce1 activity determined by comparison of initial rates (Tables 1–3). The lead compound NSC1011 (**1**) was resynthesized and included for comparison with the new derivatives. From the set of derivatives synthesized, certain structure–activity relationships were apparent.

At 10 μM, NSC1011 (**1**) reduced the activity of *Hs*Rce1 to 25% of the uninhibited control (Table 1). NSC1008 (**2**), which lacks the 4-carboxylic acid functionality on ring A (Fig. 1), was slightly less potent than NSC1011 (**1**), showing a slight increase in the remaining activity (39%). Other derivatives showed that a 4-carboxyate on the A-ring was slightly more inhibitory than an unsubstituted A-ring (e.g., **17** vs **18**, **24** vs **25**, **45** vs **46**). Replacement of the 4-carboxylate in the A-ring by either a methyl (**8**), a cyano (**9**), nitro (**10**), or alkyl ester (**11**–**14**, **19**, **32**) was not tolerated. Replacing the A-ring with a benzothioazolyl (**21**), benzoyl (**22**), or benzyl (**23**) group was detrimental to the inhibition of Rce1 proteolysis.

Some substituents in the *para*-position of the B-ring were tolerated well (Table 1); analogs containing 4-bromo (**5**), 4-fluoro (**6**), and 4-methyl (**4**) groups dropped the activity of Rce1 to 23–32% of the uninhibited control, whereas analogs containing 4-nitro (**7**), 4-cyano (**3**), or 4-octyloxy (**26**) groups did not inhibit *Hs*Rce1 activity significantly. Replacement of the B-ring phenyl group with 2-pyridyl (**15**), 3-pyridyl (**16**), or trihalogen-substituted rings designed to take advantage of halogen bonding (**24**, **25**),[57,58] led to no significant reduction in Rce1 activity. Complete removal of the B-ring (**20**) had no significant effect on Rce1 proteolysis activity, although analogs with an aliphatic cyclic moiety such as a cyclohexyl group (**17**–**19**) showed similar activity to NSC1011 (**1**).

Replacing the hydroxyquinoline moiety with a 1-naphthol group (**27**–**31**, **33**, **45**–**46**) provided compounds with slightly better in vitro activities against *Hs*Rce1 than NSC1011 (**1**) (Table 2). In this series of analogs, A-ring substitutions (**28**–**31**, **45**–**46**) induced similar levels of diminished Rce1 activity, and a bromine at the 4-position of the 1-naphthol ring system (**33**) retained inhibitory activity. In contrast, 2-naphthol derivatives **34**, **35**, and **47** were weaker inhibitors, and replacement of the quinoline with a phenyl ring (**51**) led to an inactive compound (Table 3).

In general, adding substituents to the quinoline had a detrimental effect on the inhibitory properties relative to NSC1011 (**1**). Substitutions at the 2-position of the hydroxyquinoline moiety (**36**, **37**, **38**, **39**) or a ring extension (**42**) did not reduce *Hs*Rce1 activity relative to the unsubstituted parent compound NSC1008 (**2**) (Table 2). Derivatives with halogen substitutions at the 5-position of the hydroxquinoline ring system were relatively inactive. The 8-hydroxy group is critical for inhibition of *Hs*Rce1; compounds lacking it (**43**, **44**) or possessing an 8-methoxy group (**32**) have little inhibitory activity. This suggests that a hydrogen bond donor is necessary for inhibition of proteolysis.

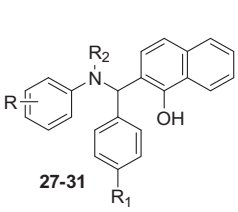
Diphenyl analogs **48** and **51** were synthesized to investigate the importance of the bicyclic ring system (rings C and D). Neither the ethyl ester **48** nor the acid derivative **51** showed any effect upon inhibition of the enzyme, and similarly, *para*-bromo substitution on either one or both of the A- and B-rings (**49**, **50**, **52**, **53**) resulted in relatively inactive compounds (Table 3). Extension of these diphenyl derivatives via amidation at the *para*-carboxy moiety of ring A to yield phenyl amide counterparts **54**–**58** also did not provide compounds with significant inhibitory activity against *Hs*Rce1.

The IC_{50} values of a selection of analogs (**1**–**8**, **15**–**17**, **27**–**31**, **33**, **45**, and **46**) were measured in the in vitro proteolysis assay (Fig. 2).[29,55,56] The set included thirteen derivatives that inhibited the activity of Rce1 to less than 40% of the control in the single-point assay and some of the less active analogs for comparison. The IC_{50} values are generally consistent with the single-point data, and indicate that the 1-naphthol derivatives **27**, **29**, and **31** (IC_{50} = 4.9, 4.2, and 3.9 μM, respectively) are marginally more potent compared to the corresponding 8-hydroxyquinoline analogs **1**, **2**, and **5** (IC_{50} = 6.9, 8.9, and 6.7 μM, respectively) in the biochemical assay. The inhibitory activity of the 1-naphthol derivatives infers that *Hs*Rce1 may not be a zinc metalloprotease (8-hydroxyquinoline scaffold chelates zinc, whereas 1-naphthol scaffold does not), which agrees with structural data reported for *Mm*Rce1.[36]

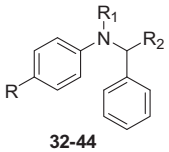
2.2.2. Analysis of inhibitor toxicity in human cells

To further investigate the role of Rce1 inhibitors in human cell lines and their effect on the localization of Ras isoforms, our secondary biological screening strategy was to remove any potentially cytotoxic compounds. Effective inhibitors, as determined by the IC_{50} values in the CaaX proteolysis assay, were tested in HCT-116

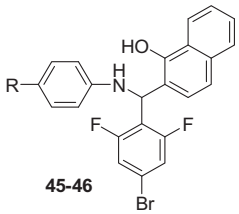
Table 2
Enzymatic results for compounds **27–47**



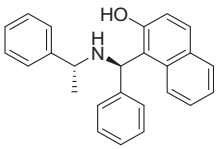
27-31



32-44



45-46



47

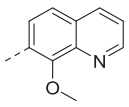
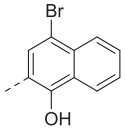
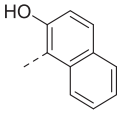
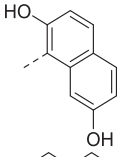
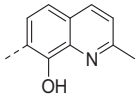
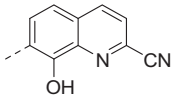
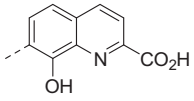
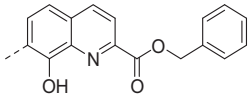
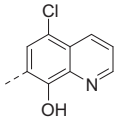
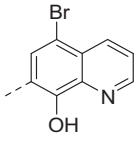
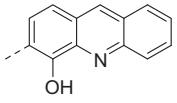
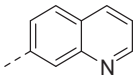
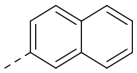
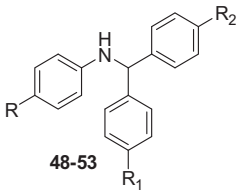
No.	R	R_1	R_2	*Hs*Rce1 percent activity remaining[a] (10 μM)	*Hs*Rce1 IC_{50}[b] (μM)
27	H	H	H	17 ± 0.4	4.9 ± 1.1
28	*p*-*t*-Butyl	H	H	38 ± 0.47	5.0 ± 1.1
29	*p*-CO_2H	H	H	19 ± 2.4	4.2 ± 1.1
30	*m*-CO_2H	H	H	23 ± 5.5	5.3 ± 1.1
31	*p*-CO_2H	Br	H	23 ± 1.1	3.9 ± 1.0
32	CO_2Me	H		65 ± 2.1	nd
33	H	H		27 ± 3.2	5.4 ± 1.1
34	H	H		59 ± 0.65	nd
35	H	H		51 ± 2.9	nd
36	H	H		83 ± 6.9	nd
37	H	H		83 ± 0.1	nd
38	H	H		77 ± 3.9	nd
39	H	H		75 ± 2.9	nd
40	H	H		68 ± 0.7	nd
41	H	H		73 ± 3.5	nd
42	H	H		51 ± 2.0	nd

Table 2 (*continued*)

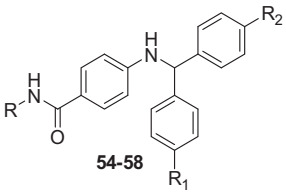
No.	R	R_1	R_2	*Hs*Rce1 percent activity remaining[a] (10 μM)	*Hs*Rce1 IC_{50}[b] (μM)
43	H	H		82 ± 0.3	nd
44	H	H		77 ± 2.8	nd
45	H	—	—	45 ± 0.32	9.4 ± 1.1
46	CO_2H	—	—	21 ± 2.4	3.8 ± 1.1
47	—	—	—	63 ± 0.08	nd

[a] The % activity remaining values are the averages of replicates from one assay in the presence of inhibitor.
[b] nd = not determined.

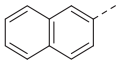
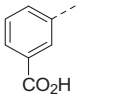
Table 3
Enzymatic results for compounds **48–58**



48-53



54-58

No.	R	R_1	R_2	*Hs*Rce1 % activity remaining[a] (10 μM)	*Hs*Rce1 IC_{50}[b] (μM)
48	CO_2Et	H	H	82 ± 4.4	nd
49	CO_2Et	Br	H	79 ± 3.4	nd
50	CO_2Et	Br	Br	83 ± 7.6	nd
51	CO_2H	H	H	90 ± 3.6	nd
52	CO_2H	Br	H	86 ± 0.2	nd
53	CO_2H	Br	Br	83 ± 2.6	nd
54	Ph	H	H	78 ± 1.0	nd
55	Ph	Br	H	78 ± 2.5	nd
56	Ph	Br	Br	80 ± 4.8	nd
57		H	H	89 ± 3.5	nd
58		H	H	72 ± 2.2	nd

[a] The % activity remaining values are the averages of replicates from one assay in the presence of inhibitor.
[b] nd = not determined.

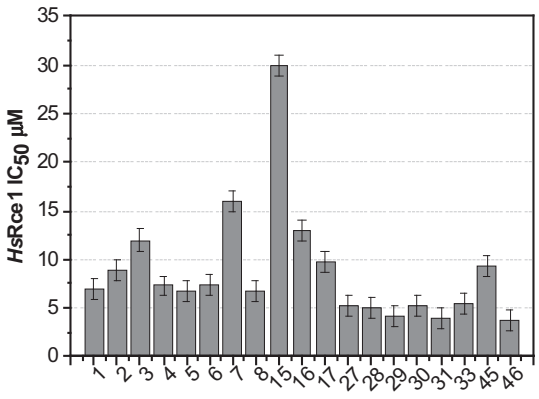


Figure 2. *Hs*Rce1 IC_{50} values. Inhibitors were evaluated using the fluorescence-based CaaX proteolysis assay measuring the percent activity remaining.

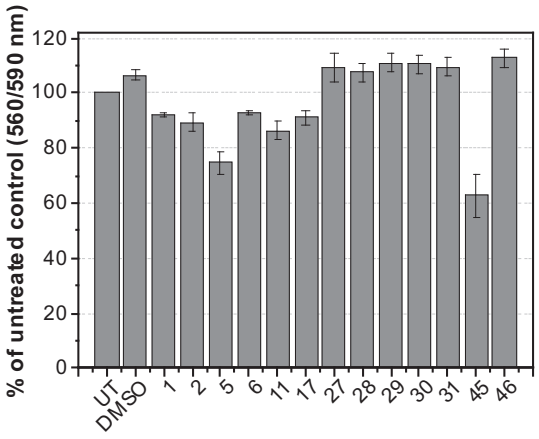


Figure 3. Rce1 inhibitor toxicity in human cells. Compounds were added to cells at 25 μM then incubated for 20 h. UT = untreated, DMSO = DMSO (0.4%) treated cells. Cell viability was measured using the CellTiter-Blue assay (Promega). Each column is the average of three independent experiments. Error bars indicate SEM.

human colon carcinoma cells to initially assess the levels of toxicity in mammalian cell lines (Fig. 3 and Supplemental Fig. 1). The majority of compounds appeared relatively non-toxic after treatment of cells for 20 h at 25 μM causing less than 10% cell death compared to DMSO-treated (0.4%) and untreated cells. Compounds **5** and **45** caused approximately 25% and 40% cell death, respectively.

2.2.3. Cell-based assay of Ras mislocalization

Previous reports have shown that EGFP-Ras constructs are mislocalized from the plasma membrane (PM) in both $Rce1^{-/-}$ cells[19] and in response to FTIs.[59] To quantitatively investigate the impact of Rce1 inhibitors on the plasma membrane (PM) localization of Ras, HCT-116 cells were transiently transfected with EGFP conjugated to the N-terminus of H-, N-, or K-Ras (K-Ras4b), treated with compounds, and the cross-sectional fluorescence intensity of Ras analyzed similarly to the method previously described for yeast.[41] Prior to testing the Rce1 inhibitors, the impact of reduced Rce1 expression on EGFP-Ras localization was validated in HCT-116 cells using an siRNA knock-down approach (Fig. 4). Approximately 60–75% knock-down of Rce1 RNA levels was observed, and this led to mislocalization of all three Ras isoforms, albeit to different degrees, which nonetheless confirmed the expected phenotype.

Live cell analysis in HCT-116 cells showed that EGFP constructs of H-, N-, and K-Ras were localized to the PM in untreated (data not shown) and DMSO-treated cells (Fig. 5). Quantification of each EGFP-Ras isoform in DMSO-treated conditions, revealed that ~50% of the total fluorescence was detected within 1 μm of the PM, as defined by the threshold boundaries in cross-sectional

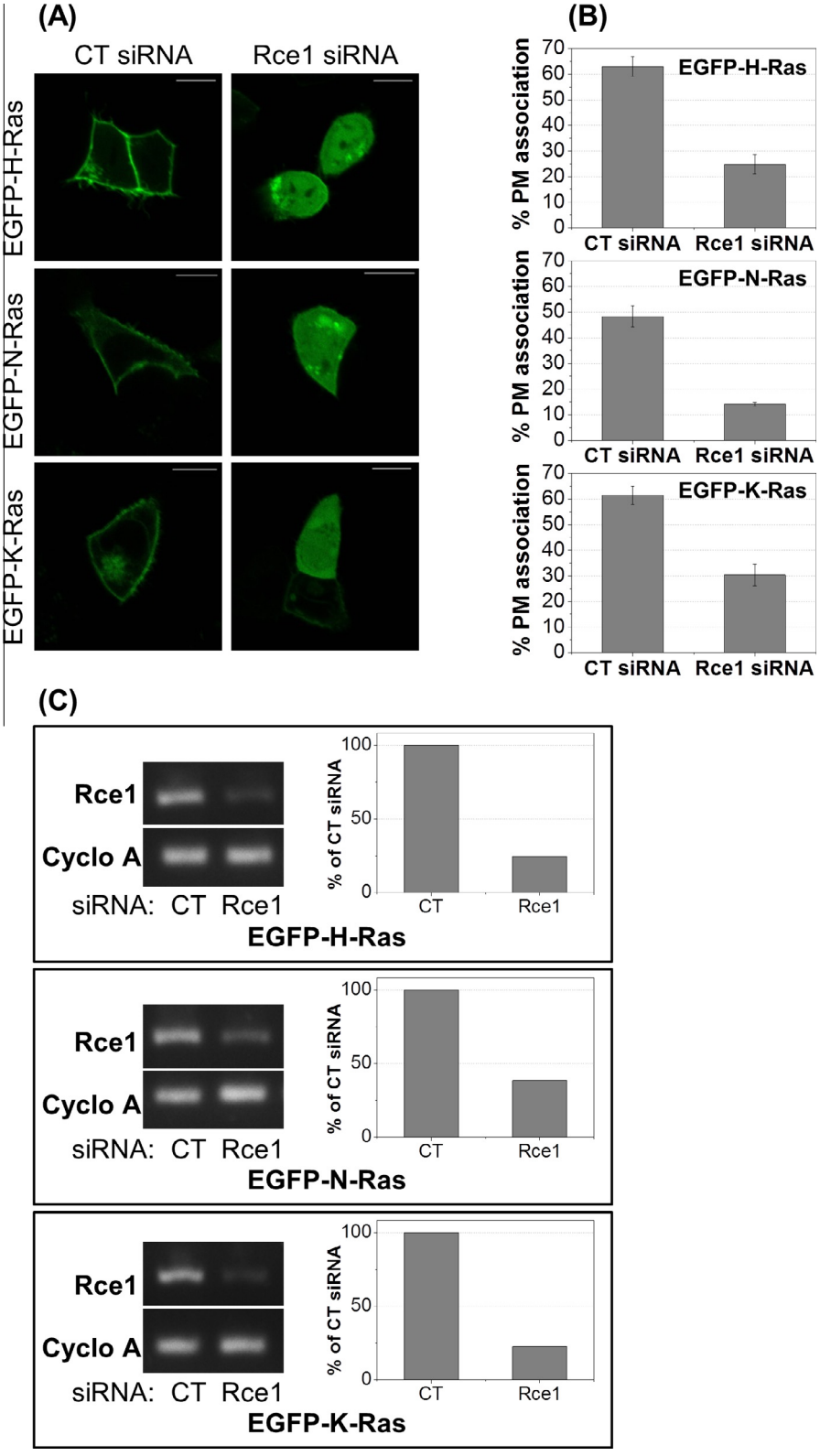


Figure 4. Rce1 siRNA knock-down mislocalizes EGFP-Ras isoforms in human cells. (A) HCT-116 cells were transfected with either control (CT) or Rce1 siRNA and with either EGFP-H-, N-, or K-Ras. Cells were imaged by confocal microscopy 24 h post-transfection. Images show representative cells. Scale bars = 10 μm. (B) Quantification of EGFP-H-, N-, and K-Ras mislocalization (see Section 4 for method). ~30 cells per condition were analyzed. (C) HCT-116 cells were transfected with either CT or Rce1 siRNA and with either EGFP-H-, N-, or K-Ras. Cells were imaged by confocal microscopy 24 h post-transfection, then lysed for mRNA analysis by semi-quantitative RT-PCR for Rce1 and cyclophilin A expression. Graphs report the densitometric analysis of the gels.

fluorescence profiles of individual cells (see Section 4 for details). As proof of principle, cells were treated with 2 μM of the FTI L744,832,[60] and a strong cytosolic localisation was observed for H-Ras and N-Ras (<10% of the total fluorescence at the PM). This localization was consistent with previous reports.[61] Additionally, the relatively higher abundance of K-Ras at the PM (~35%) was also

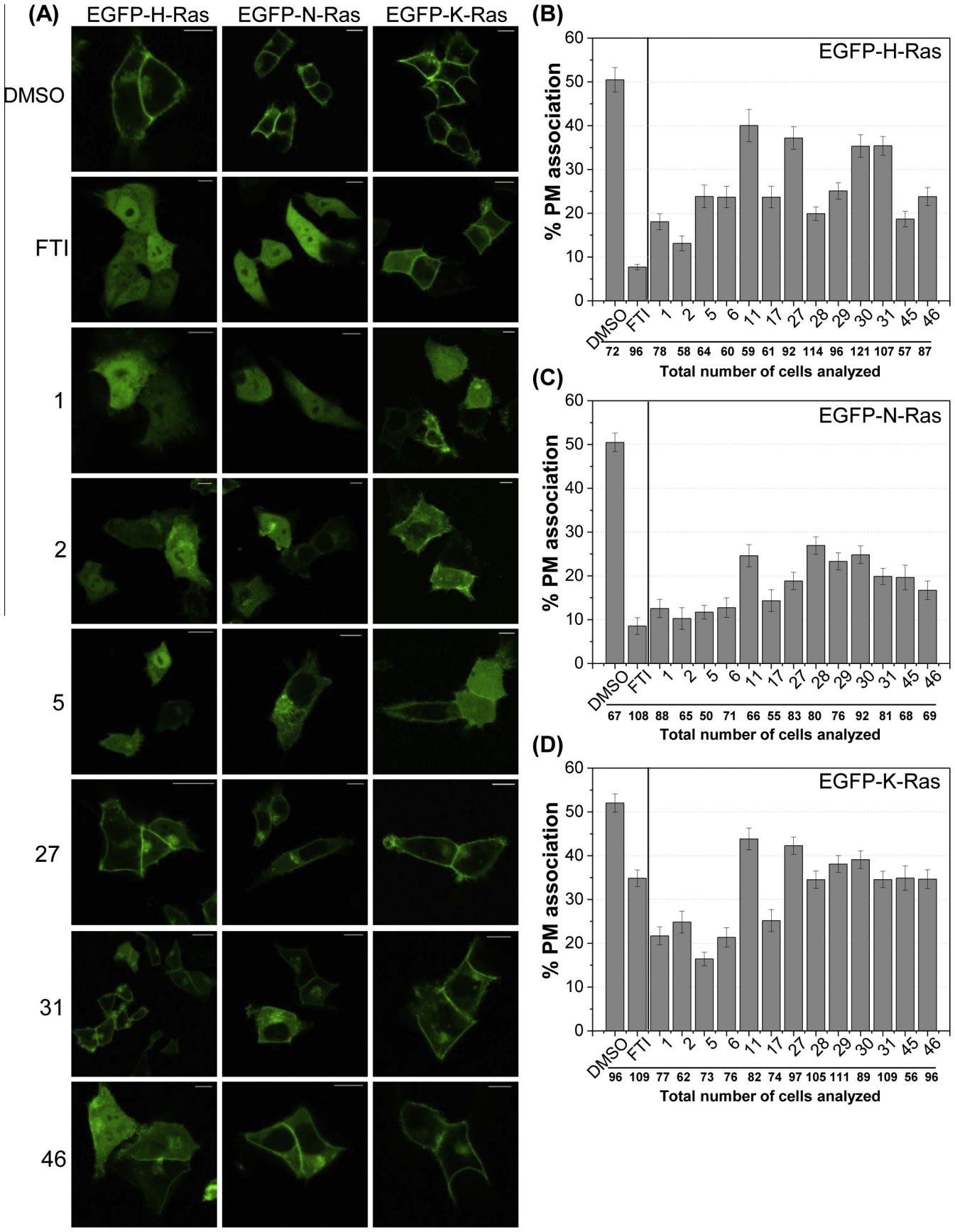


Figure 5. Rce1 inhibitors mislocalize H-, N-, and K-Ras in human cells. (A) HCT-116 cells were transiently transfected with EGFP fusion constructs of H-, N-, or K-Ras, and treated with either DMSO, FTI (2 μM), or Rce1 inhibitors (25 μM) for 20 h. Images show representative cells. Scale bars = 10 μm. (B–D) Quantification of H-, N-, and K-Ras mislocalization (see Section 4 for method). Percent PM association is reported with SEM bars.

consistent, as K-Ras can be alternatively prenylated by geranylgeranyl transferase (GGTase), which enables proper localization of K-Ras.[59]

Compounds **1**, **2**, **5**, **6**, **11**, **17**, **27**–**31**, **45**, and **46** were tested to quantify the level of EGFP-Ras PM association (Fig. 5B–D). These compounds were selected because they reduced the activity of Rce1 to <40% of the uninhibited control in the CaaX proteolysis assay and showed low cell toxicity (<40% cell death after 20-h treatment). Compound **11** was also included from the 8-hydroxyquinoline series even though it only reduced Rce1 activity to 52% in the proteolysis assay. Data showed that compound **2** was the most effective in mislocalizing H-Ras and N-Ras, with PM association as low as 14% and 10%, respectively. Rce1 inhibitors **1**, **2**, **5**, **6**, and **17** were more effective at mislocalizing K-Ras than the FTI, and the lowest PM association (16%) for K-Ras was observed with compound **5**.

The mislocalization experiments demonstrate that the 8-hydroxyquinoline derivatives (**1**, **2**, **5**, **6**, and **17**) are marginally better at causing stronger cytoplasmic localization and reduced PM association of Ras as compared to the 1-napththol derivatives (**27**–**31**, **45**, and **46**). It is possible that 8-hydroxyquinoline derivatives have better cell permeability and availability on the endoplasmic reticulum membrane (ER) as compared to the 1-naphthol derivatives. Interestingly, the Rce1 inhibitors differentially mislocalized the Ras isoforms; greater mislocalization was observed for N-Ras as compared to H-Ras and K-Ras. This was also reflected in the results observed following Rce1 siRNA knockdown. It is not surprising that the compounds were least effective at mislocalizing K-Ras; others report that in *Rce1*-deficient mouse embryonic fibroblasts (MEFs), GFP-K-Ras4B still exhibits significant PM localization, although at lower levels compared to those observed in the wild-type MEFs.[19,59,62] Similarly, it has been shown that deubiquitination of Rce1 by ubiquitin-specific protease 17 (USP17) reduces Rce1 activity in MEF cells, resulting in mislocalization of H-Ras and N-Ras, but not K-Ras.[63] K-Ras mislocalization is less affected by Rce1 inhibition or genetic knockout, indicating that downstream processing of K-Ras to the PM differs from that of H- and N-Ras. The fact that several of the compounds were better at mislocalizing K-Ras than the FTI is noteworthy, because activating mutations in K-Ras are the most frequently observed in cancer relative to the other isoforms.[3] The alternative prenylation by geranylgeranyl transferases (GGTases) when FTases are blocked is attributed to the inability of FTIs to significantly impact K-Ras function and the poor performance of FTIs in clinical trials.[16,64,65]

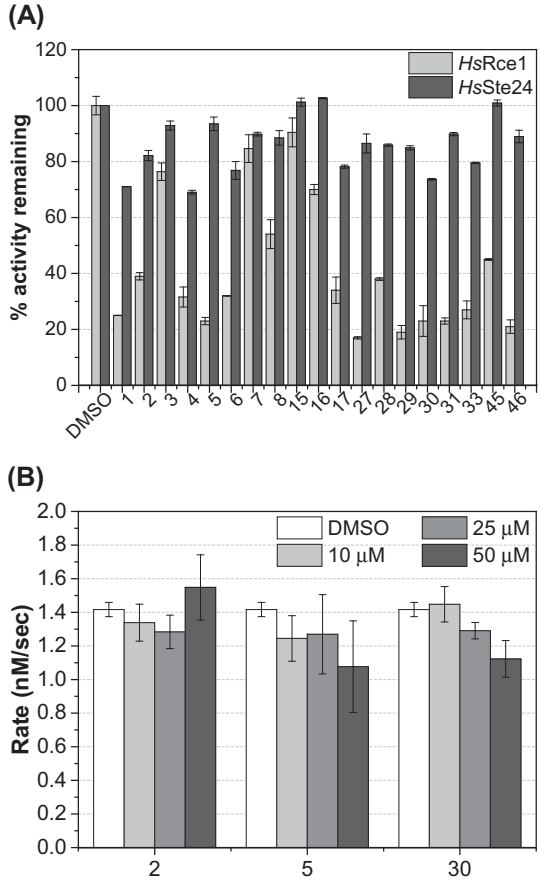


Figure 6. Efficacies of Rce1 inhibitors for Ste24 and FTase. (A) Compounds were evaluated at 10 μM using the fluorescence-based CaaX proteolysis assay measuring the percent activity remaining. Yeast cells expressed either *Hs*Rce1 or *Hs*Ste24. Error bars show SD. (B) Indicated compounds were evaluated at 10, 25, and 50 μM using the fluorescence-based FTase assay. Error bars show SD.

2.2.4. Inhibitors show selectivity for Rce1

A key requirement of an inhibitor of Rce1 is selectivity toward Rce1 over the functionally similar Ste24 protease. This is important due to the role that Ste24 plays in the maturation of lamin A, where inhibition of Ste24 leads to laminopathies.[66] Compounds **1**–**8**, **15**–**17**, **27**–**31**, **33**, **45**, and **46** were tested in a single-point (10 μM) CaaX proteolysis assay[29,55,56] using membranes derived from yeast expressing human Ste24 enzyme[67] (Fig. 6A). We found that the majority of the compounds tested did not cause strong inhibition of Ste24. We also tested whether or not the Rce1 inhibitors inhibit farnesyltransferase (FTase). Compounds **2**, **5**, and **30**, which have representative structures and activities against Rce1, were chosen and tested in a fluorescence-based FTase assay.[68,69] The data showed that FTase activity is not inhibited at concentrations as high as 50 μM (Fig. 6B). These data suggest that the identified inhibitors of Rce1 do not inhibit the other known CaaX protease and the upstream enzyme in the Ras maturation pathway, strengthening the argument that the efficacy of the compounds in human cells results from the intracellular inhibition of Rce1 and not inhibition of FTase.

3. Conclusion

We prepared derivatives of the Rce1 inhibitor NSC1011, some of which showed IC_{50} values in the low micromolar range in an in vitro fluorescent proteolysis assay. Compounds identified as inhibitors in the biochemical screen of Rce1 activity also caused the mislocalization of EGFP-labeled Ras in HCT-116 cells, which is a human colon carcinoma cell line. Compounds possessing a naphthol group instead of the hydroxyquinoline were more potent in the in vitro proteolysis assay, whereas the quinolinol derivatives were better able to mislocalize EGFP-tagged Ras in the cellular assay. The inhibitors were reasonably non-toxic in the short term (<20 h), and they were more effective at disrupting the localization of N-Ras, by comparison to H-Ras and K-Ras. One might have expected to see similar levels of mislocalization among the isoforms, but using an siRNA to knock down Rce1 expression, we observed Ras mislocalization phenotypes among N-Ras, H-Ras, and K-Ras similar to the ones observed with the Rce1 inhibitors. Differential impact on the PM localization of the Ras isoforms is also observed in cells lacking the Rce1 gene, indicating that K-Ras is localized to the PM by a different path than H-Ras and N-Ras.[19,59,62] Several Rce1 inhibitors were more effective at mislocalizing K-Ras compared to the FTI, which is an intriguing result given the preponderance of activating K-Ras mutations in certain forms of cancer.[3] The results of this study further support the need for potent Rce1 inhibitors to better understand the biology and role of Rce1 in the processing of the Ras isoforms. In addition to observing the mislocalization of Ras isoforms, more study is required to

determine the effect of the Rce1 inhibitors on Ras activity and signaling pathways downstream of Ras. Rce1 inhibition in the presence of oncogenic K-Ras in mouse haematopoietic cells worsens the myeloproliferative disease compared to oncogenic K-Ras alone.[70] It will therefore be interesting to test the effect of Rce1 inhibition in other disease systems, which may be context dependent. Selective and potent Rce1 inhibitors would significantly aid these investigations.

4. Material and methods

4.1. Chemistry

4.1.1. Materials

All reagents and solvents were purchased from commercial sources and used without further purification unless otherwise noted.

4.1.2. Instrumentation

^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded on either a 600 MHz, 500 MHz, or 400 MHz spectrometer. Chemical shifts (δ) are expressed in ppm. High Resolution Mass spectroscopy (HRMS) was performed on Agilent Technologies 6540 UHD Accurate-Mass QTOF LC/MS system equipped with Agilent 1260 Infinity series HPLC. Purity of the final compounds was determined to be >95% (unless otherwise stated) using either PerkinElmer 2400 Series II elemental analyzer on CHN mode or reversed-phase HPLC. Elemental analysis values are reported as percentages. For HPLC purity, analysis was performed on either Agilent 1290 Infinity series uHPLC (Method-A) or Agilent 1260 Infinity series HPLC (Method-B) using a ZORBAX Extend-C18, Rapid Resolution HT (1.8 μm particle size, 50 × 2.1 mm dimensions) column with quantitation by area under the curve (AUC) at 254 nm (Agilent Diode Array Detector). We applied either of the following two methods for the analysis: Method-A (mobile phase A: water (pH 8.2 using aq NH_4OH) and mobile phase B: acetonitrile) starting from 5% B and ramping to 100% B over 10 min followed by 100% B hold for 11 min, then ramping to 5% B over 12 min and equilibrating prior to the next run; and Method-B (mobile phase A: water (with 0.1% formic acid) and mobile phase B: acetonitrile) starting from 5% B and ramping to 100% B over 10 min followed by 100% B hold for 11 min, then ramping to 5% B over 13 min and equilibrating prior to the next run. The injection volume was 1 μL and the flow rate was 0.3 mL/min with maximum pressure of about 600 Bar. Samples were dissolved in acetonitrile or they were initially dissolved in approximately 50 μL of DMSO and the resulting solution diluted with acetonitrile. X-ray diffraction data were collected at 100 K on a Bruker APEX II DUO diffractometer.

4.1.3. Synthetic procedures and characterization data

General procedures for the synthesis of compounds **1**–**6**, **9**–**10**, **15**–**18**, **21**, **24**–**25**, **37**–**38**, and **40**–**42**: Protocol A, B, C, or D was followed.

4.1.3.1. Protocol A. *4.1.3.1.1. 4-(((8-Hydroxyquinolin-7-yl)(phenyl)methyl)amino)benzoic acid (**1**).* *p*-Aminobenzoic acid (0.27 g, 2.0 mmol) and benzaldehyde (0.21 g, 2.0 mmol) were suspended in ethanol (10 mL) followed by stirring at room temperature for about 5 min. To the mixture, 8-hydroxyquinoline (0.29 g, 2.0 mmol) was added followed by the addition of pyridine (0.2 mL). The reaction mixture was stirred at room temperature for 10–15 days. The reaction was monitored by TLC (3:2 acetonitrile/water) and LC–MS. A light yellow cream colored precipitate was isolated upon filtration, which was washed several times with ethanol to afford pure compound **1** as a cream colored amorphous solid (0.12 g, 0.32 mmol, 16%). ^{1}H NMR (600 MHz, DMSO-d_6, δ): 12.00 (br s, 1H), 10.11 (br s, 1H), 8.86 (dd, J = 1.4 Hz, 4.1 Hz, 1H), 8.28 (dd, J = 1.4 Hz, 8.3 Hz, 1H), 7.60 (d, J = 8.8 Hz, 2H), 7.54 (m, 2H), 7.39 (d, J = 8.2 Hz, 3H), 7.33 (t, J = 7.5 Hz, 2H), 7.25 (m, 2H), 6.66 (d, J = 8.7 Hz, 2H), 6.24 (d, J = 7.2 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6, δ): 167.4, 151.6, 149.9, 148.3, 142.0, 138.1, 136.0, 130.9, 128.4, 127.6, 127.4, 127.0, 126.1, 124.5, 121.8, 117.6, 117.6, 111.8, 53.9; HRMS-ESI (m/z): $[M-C_7H_6NO_2]^+$ calcd for $C_{23}H_{18}N_2O_3$, 234.09189; found 234.09182. Anal. Calcd for $C_{23}H_{18}N_2O_3$: C, 74.58; H, 4.90; N, 7.56. Found: C, 74.43; H, 4.72; N, 7.47.

4.1.3.2. Protocol B. *4.1.3.2.1. 4-(((4-Bromophenyl)(8-hydroxyquinolin-7-yl)methyl)amino)benzoic acid (**5**).* *p*-Aminobenzoic acid (0.27 g, 2.0 mmol), and *p*-bromobenzaldehyde (0.37 g, 2.0 mmol) were suspended in ethanol (5 mL) followed by stirring at room temperature for about 5 min. To the mixture, 8-hydroxyquinoline (0.29 g, 2.0 mmol) was added and the reaction mixture was refluxed, and heated up to 120 °C for about 12 h. The reaction was monitored by TLC (3:2 acetonitrile/water,) and LC–MS. The reaction mixture was later allowed to cool down and a cream colored precipitate was isolated upon filtration, which was washed several times with ethanol to afford pure compound **5** as a light cream colored amorphous solid (0.31 g, 35%). ^{1}H NMR (600 MHz, DMSO-d_6, δ): 12.02 (br s, 1H), 10.19 (br s, 1H), 8.86 (dd, J = 1.4 Hz, 4.1 Hz, 1H), 8.28 (dd, J = 1.4 Hz, 8.3 Hz, 1H), 7.62 (d, J = 8.8 Hz, 2H), 7.52 (m, 4H), 7.40 (d, J = 8.6 Hz, 1H), 7.33 (d, J = 8.4 Hz, 2H), 7.26 (d, J = 7.1 Hz, 1H), 6.66 (d, J = 8.7 Hz, 2H), 6.21 (d, J = 7.1 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6, δ): 167.3, 151.5, 150.0, 148.4, 141.5, 138.1, 136.1, 131.3, 130.9, 129.6, 127.7, 126.0, 124.0, 121.9, 120.1, 117.8, 117.7, 111.8, 53.5; HRMS-ESI (m/z): $[M-C_7H_6NO_2]^+$ calcd for $C_{23}H_{17}BrN_2O_3$, 312.00240 and 314.00035, found 312.00230 and 314.00042. Anal. Calcd for $C_{23}H_{17}BrN_2O_3$: C, 61.48; H, 3.81; N, 6.23. Found: C, 61.32; H, 3.62; N, 6.19. HPLC (Method B) R_t = 9.787 min (>95%).

4.1.3.3. Protocol C. *4.1.3.3.1. 7-((Benzo[d]thiazol-2-ylamino)(phenyl)methyl)quinolin-8-ol (**21**).* 2-Aminobenzothiazole (0.30 g, 2.0 mmol), and benzaldehyde (0.21 g, 2.0 mmol) were taken in a 10-mL microwave reaction vessel equipped with protective septum cap, and charged with minimal volume of ethanol (not more than 2.5 mL). To the mixture, 8-hydroxyquinoline (0.29 g, 2.0 mmol) was added, and the reaction vessel was subjected to microwave heating at 140 °C in a Discovery-CEM closed reaction vessel microwave system, equipped with stirring, for about 15 min. After completion of the reaction, which was detected by LC–MS, the reaction mixture was allowed to cool down, and the solvents were removed under reduced pressure. The residual solid was taken into a 50-mL beaker and heated in the presence of hexane, and washed several times with ethanol to afford pure compound **21** as a colorless amorphous solid (0.69 g, 90%). ^{1}H NMR (600 MHz, DMSO-d_6, δ): 10.11 (br s, 1H), 8.95 (d, J = 8.3 Hz, 1H), 8.86 (d, J = 3.9 Hz, 1H), 8.30 (d, J = 8.2 Hz, 1H), 7.66 (d, J = 7.8 Hz, 1H), 7.56 (m, 2H), 7.43 (d, J = 8.6 Hz, 1H), 7.35 (m, 5H), 7.23 (t, J = 7.3 Hz, 1H,), 7.18 (t, J = 7.5 Hz, 1H), 7.00 (t, J = 7.5 Hz, 1H), 6.88 (d, J = 8.1 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6, δ): 165.5, 152.2, 149.7, 148.4, 142.1, 138.1, 136.1, 130.5, 128.4, 127.7, 127.0, 126.1, 125.5, 124.4, 121.9, 121.1, 120.9, 118.3, 118.3, 117.5, 55.1; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{23}H_{17}N_3OS$ 384.11706, found 384.11726 and $[M-C_7H_6NO_2]^+$ calcd for $C_{23}H_{17}N_3OS$, 234.09189, found 234.09189. Anal. Calcd for $C_{23}H_{17}N_3OS$: C, 72.04; H, 4.47; N, 10.96. Found: C, 71.66; H, 4.34; N, 10.82; HPLC (Method B) R_t = 9.427 min (>95%).

4.1.3.4. Protocol D. *4.1.3.4.1. 3-(Phenyl(phenylamino)methyl)acridin-4-ol (**42**).* Aniline (0.37 g, 4.0 mmol), and benzaldehyde

(0.42 g, 4.0 mmol) were dissolved in ethanol (10 mL) followed by stirring at room temperature for about 5 minutes. To the mixture, acridin-4-ol (0.78 g, 4.0 mmol) and catalytic pyridine (2 drops) was added and the reaction mixture was refluxed up to 100 °C for about 24 h. The reaction was monitored by TLC (3:2 acetonitrile/water) and LC–MS. The reaction mixture was later allowed to cool down and a dark brownish yellow precipitate was isolated upon gravity filtration, which was washed several times with ethanol to afford pure compound as an amorphous solid (0.79 g, 42%). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 8.70 (s, 1H), 8.19 (d, J = 8.7 Hz, 1H), 7.99 (d, J = 8.4 Hz, 1H), 7.77 (t, J = 7.5 Hz, 1H), 7.61 (d, J = 8.9 Hz, 1H), 7.54 (m, 3H), 7.49 (d, J = 8.9 Hz, 1H), 7.33 (t, J = 7.7 Hz, 2H), 7.25 (m, 2H), 7.11 (t, J = 7.5 Hz, 2H), 6.68 (m, 3H), 6.23 (s, 1H), 4.53 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 148.3, 147.7, 147.3, 142.7, 140.3, 136.1, 130.5, 129.3, 129.1, 128.9, 128.5, 127.6, 127.5, 127.4, 126.14, 126.10, 126.03, 122.9, 118.5, 117.9, 113.8, 56.8; HRMS-ESI (m/z): calculated for fragment ion $[M-C_6H_6N]^+$ 284.10754 found 284.10760. Anal. Calcd for $C_{26}H_{20}N_2O$: C, 82.95; H, 5.36; N, 7.44. Found: C, 82.47; H, 5.07; N, 7.28.

*4.1.3.4.2. 7-(Phenyl(phenylamino)methyl)quinolin-8-ol (**2**).* Protocol A, amorphous solid (0.13 g, 20%). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 8.74 (m, 1H), 8.55 (br s, 1H), 8.10 (d, J = 8.2 Hz, 1H), 7.61 (d, J = 8.6 Hz, 1H), 7.48 (d, J = 7.7 Hz, 2H), 7.39 (m, 1H), 7.31 (m, 3H), 7.25 (m, 1H), 7.10 (t, J = 7.9 Hz, 2H), 6.68 (t, J = 7.3 Hz, 1H), 6.63 (d, J = 7.9 Hz, 2H), 6.14 (d, J = 4.0 Hz, 1H), 4.47 (d, J = 2.9 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$, δ): 149.1, 148.2, 147.6, 142.7, 138.5, 136.2, 129.3, 128.9, 127.8, 127.6, 127.5, 126.8, 124.3, 121.9, 118.1, 117.9, 113.8, 57.0; HRMS-ESI (m/z): $[M-C_6H_6N]^+$ calcd for $C_{22}H_{18}N_2O$, 234.09189, found 234.09185. Anal. Calcd for $C_{22}H_{18}N_2O$: C, 80.96; H, 5.56; N, 8.58. Found: C, 81.02; H, 5.40; N, 8.60; HPLC (Method A) R_t = 4.086 min (>95%).

*4.1.3.4.3. 4-(((4-Cyanophenyl)(8-hydroxyquinolin-7-yl)methyl) amino)benzoic acid (**3**).* Protocol A, pale yellow amorphous solid (0.879 g, 44%). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 12.08 (br s, 1H), 10.30 (br s, 1H), 8.88 (dd, J = 4.2 Hz, 1.5 Hz, 1H), 8.30 (dd, J = 8.3 Hz, 1.5 Hz, 1H), 7.82 (d, J = 8.3 Hz, 2H), 7.66–7.56 (m,5H), 7.49 (d, J = 8.6 Hz, 1H), 7.41 (d, J = 8.6 Hz, 1H), 7.34 (d, J = 7.3 Hz, 2H), 6.69 (d, J = 8.8 Hz, 2H), 6.34 (d, J = 7.2 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 167.8, 151.8, 150.7, 149.0, 148.2, 138.6, 136.6, 133.0, 131.5, 128.8, 128.3, 126.5, 124.0, 122.5, 119.3, 118.6, 118.3, 112.4, 110.3, 54.4; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{24}H_{17}N_3O_3$ 396.1343, found 396.1365. Anal. Calcd for $C_{24}H_{17}N_3O_3$: C, 72.90; H, 4.33; N, 10.63. Found: C, 72.63; H, 3.94; N, 10.42.

*4.1.3.4.4. 4-(((8-Hydroxyquinolin-7-yl)(p-tolyl)methyl)amino) benzoic acid (**4**).* Protocol A, yellow amorphous solid (0.416 g, 22%). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 12.00 (br s, 1H), 10.10 (br s, 1H), 8.83 (dd, J = 4.1 Hz, 1.5 Hz, 1H), 8.26 (dd, J = 8.3 Hz, 1.3 Hz, 1H), 7.58 (d, J = 8.8 Hz, 2H), 7.53–7.48 (m, 2H), 7.37–7.51, (m, 1H), 7.26–7.20 (m,3H), 7.12–7.10 (m, 2H), 6.63 (d, J = 8.7 Hz, 2H), 6.17 (d, J = 7.0 Hz, 1H), 2.23 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 167.9, 152.2, 150.3, 148.8, 139.5, 138.6, 136.6, 136.5, 131.4, 129.4, 128.1, 127.8, 126.6, 125.2, 122.3, 118.02, 118.00 112.2, 54.1, 21.1. Anal. Calcd for $C_{24}H_{20}N_2O_3$: C, 74.98; H, 5.24; N, 7.29. Found: C, 74.99; H, 4.85; N, 7.15.

*4.1.3.4.5. 4-(((4-Fluorophenyl)(8-hydroxyquinolin-7-yl)methyl) amino)benzoic acid (**6**).* Protocol A, off-white powder (0.409 g, 53%). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 12.02 (br s, 1H), 10.17 (br s, 1H), 8.85 (d, J = 2.9 Hz, 1H), 8.27 (d, J = 8.2 Hz, 1H), 7.60 (d, J = 8.7 Hz, 2H), 7.55–7.49 (m, 2H), 7.41–7.38 (m, 3H), 7.26 (d, J = 7.2 Hz, 1H), 7.15 (t, J = 8.8 Hz, 2H), 6.64 (d, J = 8.6 Hz, 2H), 6.21 (d, J = 7.1 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$, δ): 167.8, 161.7 (d, ^1J (^{19}F, ^{13}C) = 243.3 Hz, CF_{ar}), 152.0, 150.5, 148.9, 138.6 (d, ^4J (^{19}F, ^{13}C) = 3.0 Hz, CF_{ar}), 136.6, 131.4, 129.8 (d, ^3J (^{19}F, ^{13}C) = 8.2 Hz, CF_{ar}),, 128.2, 126.4, 124.8, 122.3, 118.2, 118.1, 115.6 (d, ^2J (^{19}F, ^{13}C) = 21.3 Hz, CF_{ar}), 112.3, 53.8; ^{19}F NMR (470 MHz, DMSO-d_6, δ): −60.9; HRMS-ESI (m/z): $[M-C_7H_6NO_2]^+$ calcd for $C_{23}H_{17}FN_2O_3$ 252.08247, found 252.08319; Anal. Calcd for $C_{23}H_{17}FN_2O_3$: C, 71.13; H, 4.41; N, 7.21. Found: C, 70.88; H, 4.21; N, 7.25.

*4.1.3.4.6. 4-(((8-Hydroxyquinolin-7-yl)(phenyl)methyl)amino) benzonitrile (**9**).* Protocol C, amorphous solid (0.35 g, 50%). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 8.77 (dd, J = 1.5 Hz, 4.2 Hz, 1H), 8.61 (br s, 1H), 8.12 (dd, J = 1.4 Hz, 8.3 Hz, 1H), 7.48 (d, J = 8.6 Hz, 1H), 7.43 (m, 3H), 7.34 (m, 5H), 7.28 (m, 1H), 6.60 (d, J = 8.8 Hz, 2H), 6.18 (d, J = 5.3 Hz, 1H), 5.05 (d, J = 5.2 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$, δ): 150.6, 149.3, 148.5, 141.3, 138.4, 136.3, 133.8, 129.1, 128.0, 127.5, 126.4, 122.7, 122.2, 120.5, 118.4, 114.6, 113.3, 99.6, 56.5; HRMS-ESI (m/z): $[M-C_7H_5N_2]^+$ calcd for $C_{23}H_{17}N_3O$ 234.09189, found 234.09168. Anal. Calcd for: C, 78.61; H, 4.88; N, 11.96. Found: C, 78.03; H, 4.68; N, 12.09.

*4.1.3.4.7. 7-((4-Bromophenyl)((4-nitrophenyl)amino)methyl) quinolin-8-ol (**10**).* Protocol C, amorphous solid (0.40 g, 45%). ^{1}H NMR (600 MHz, acetone-d_6, δ): 9.16 (br s, 1H), 8.85 (dd, J = 1.5 Hz, 4.2 Hz, 1H), 8.31 (dd, J = 1.5 Hz, 8.3 Hz, 1H), 8.01 (d, J = 9.3 Hz, 2H), 7.55 (m, 4H), 7.45 (m, 3H), 7.10 (d, J = 6.3 Hz, 1H), 6.83 (d, J = 9.2 Hz, 2H), 6.42 (d, J = 6.3 Hz, 1H); ^{13}C NMR (150 MHz, acetone-d_6, δ): 154.2, 150.7, 149.6, 141.7, 139.1, 138.9, 137.1, 132.6, 130.6, 129.0, 127.1, 126.7, 123.5, 123.2, 121.8, 119.1, 113.0, 55.7; HRMS-ESI (m/z): $[M-C_6H_5N_2O_2]^+$ calcd for C_{22}-$H_{16}BrN_3O_3$ 312.00240 and 314.00035, found 312.00243 and 314.00054; HPLC (Method B) R_t = 11.240 min (>95%).

*4.1.3.4.8. 4-(((8-Hydroxyquinolin-7-yl)(pyridin-2-yl)methyl) amino)benzoic acid (**15**).* Protocol A, amorphous solid (0.562 g, 65%). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 12.05 (br s, 1H), 10.28 (br s,1H), 8.87 (dd, J = 4.0, 1.3 Hz, 1H), 8.57 (d, J = 4.0 Hz, 1H), 8.26 (dd, J = 8.3, 1.3 Hz, 1H), 7.78 (td, J = 7.8, 1.7 Hz, 1H), 7.61 (d, J = 8.7 Hz, 2H), 7.56–7.49, (m, 3H), 7.39–7.27 (m,3H), 6.71 (d, J = 8.6 Hz, 2H), 6.33 (d, J = 7.0 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6, δ): 168.1, 160.6, 151.8, 150.8, 149.6, 149.0, 138.8, 137.8, 136.7, 131.6, 128.4, 126.9, 124.7, 123.2, 122.8, 122.5, 121.8, 118.3, 112.5, 55.5; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{22}H_{17}N_3O_3$ 372.1348, found 372.1349.

*4.1.3.4.9. 4-(((8-Hydroxyquinolin-7-yl)(pyridin-3-yl)methyl) amino)benzoic acid (**16**).* Protocol A, pink amorphous solid solid (1.05 g, 57%). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 12.06 (br s, 1H), 10.26 (br s, 1H), 8.83 (d, J = 3.1 Hz, 1H), 8.61 (d, J = 1.0 Hz, 1H), 8.44 (d, J = 4.0 Hz, 1H), 8.26 (d, J = 8.0 Hz, 1H), 7.74–7.70 (m, 1H), 7.63–7.60 (m, 2H), 7.56–7.50 (m, 2H), 7.40–7.32, (m, 3H) 6.66 (d, J = 8.6 Hz, 2H), 6.25 (d, J = 7.2 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6, δ): 167.8, 151.9, 149.5, 148.9, 148.7, 138.6, 137.8, 136.6, 135.6, 131.5, 126.9, 128.7, 126.2, 124.1, 124.0, 122.4, 118.5, 118.3, 112.4, 52.7; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{22}H_{17}N_3O_3$ 372.1343, found 372.1341. Anal. Calcd for $C_{22}H_{17}N_3O_3$: C, 71.15; H, 4.61; N, 11.31. Found: C, 70.82; H, 4.24; N, 11.15.

*4.1.3.4.10. 4-((Cyclohexyl(8-hydroxyquinolin-7-yl)methyl)amino) benzoic acid (**17**).* Protocol B, off-white amorphous solid (0.406 g, 54%). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 11.89 (br s, 1H), 9.87 (br s, 1H), 8.86 (dd, J = 4.2, 1.6 Hz, 1H), 8.26 (dd, J = 8.3, 1.6 Hz, 1H), 7.54–7.51 (m, 3H), 7.46 (d, J = 8.5 Hz, 1H), 7.36 (d, J = 8.6 Hz, 1H), 6.99 (d, J = 8.2 Hz, 1H), 6.59 (d, J = 8.7 Hz, 2H), 4.77 (t, J = 8.4 Hz, 1H), 2.11 (d, J = 11.6 Hz, 1H), 1.80–1.61 (m, 4H), 1.29–1.05 (m, 6H); ^{13}C NMR (150 MHz, DMSO-d_6, δ): 167.8, 152.8, 150.6, 148.6, 138.3, 136.4, 131.4, 127.8, 126.3, 125.3, 122.0, 118.1, 117.2, 111.5, 55.1, 42.9, 30.4, 29.6, 26.12, 26.10; HRMS-ESI (m/z): $[M-C_7H_6NO_2]^+$ calcd for $C_{23}H_{24}N_2O_3$ 240.13884, found 240.13960. Anal. Calcd for $C_{23}H_{24}N_2O_3$: C, 73.38; H, 6.43. N, 7.44. Found: C, 73.28; H, 6.34; N, 7.42.

*4.1.3.4.11. 7-(Cyclohexyl(phenylamino)methyl)quinolin-8-ol (**18**).* Protocol B, off-white amorphous solid (0.257 g, 39%). ^{1}H NMR (600 MHz, DMSO-d_6, δ): 9.74 (br s, 1H), 8.84 (d, J = 2.7 Hz, 1H), 8.24 (d, J = 8.2 Hz, 1H), 7.51–7.49 (m, 2H), 7.32 (d, J = 8.5 Hz,

1H), 6.90 (t, J = 7.8 Hz, 2H), 6.56 (d, J = 7.9 Hz, 2H), 6.36 (t, J = 7.2 Hz, 1H), 6.13 (d, J = 8.4 Hz, 1H), 4.71 (t, J = 8.3 Hz, 1H), 2.12 (d, J = 11.6 Hz, 1H), 1.78–1.72 (m, 2H), 1.64–1.57 (m, 2H), 1.32–1.00 (m, 6H); ^{13}C NMR (150 MHz, DMSO-d_6, δ): 150.1, 148.5, 148.0, 137.7, 135.9, 128.6, 127.1, 126.1, 125.7, 121.3, 117.3, 115.1, 112.1, 54.6, 42.7, 29.9, 29.2, 26.1, 25.8, 25.7; HRMS-ESI (m/z): $[M-C_6H_6N]^+$ calcd for $C_{22}H_{24}N_2O$ 240.13884, found 240.13909. Anal. Calcd for $C_{22}H_{24}N_2O$: C, 79.48; H, 7.28; N, 8.43. Found: C, 79.09; H, 7.14; N, 8.36.

*4.1.3.4.12. 7-((4-Bromo-2,6-difluorophenyl)(phenylamino)methyl) quinolin-8-ol (**24**).* Protocol A, amorphous solid (0.26 g, 30%). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 8.73 (m, 1H), 8.56 (bs, 1H), 8.10 (m, 1H), 7.71 (d, J = 8.6 Hz, 1H), 7.40 (m, 1H), 7.30 (d, J = 8.05 Hz, 1H), 7.15 (m, 2H), 7.05 (m, 2H), 6.72 (m, 3H), 6.54 (d, J = 8.5 Hz, 1H), 4.77 (d, J = 8.6 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$, δ): 160.9 (dd, ^1J (^{19}F, ^{13}C) = 252.6 Hz, ^3J (^{19}F, ^{13}C) = 9.3 Hz, CF_{ar}), 149.2, 148.1, 146.4, 138.0, 135.9, 129.3, 127.7, 126.1, 121.8, 121.3, 120.9 (t, ^3J (^{19}F, ^{13}C) = 12.8 Hz, CBr_{ar}), 118.4, 117.5 (t, ^2J (^{19}F, ^{13}C) = 17.5 Hz, CC_{ar}), 117.4, 115.8 (distorted dd, ^2J (^{19}F, ^{13}C) = 23.9 Hz, CH_{ar}), 113.6, 47.5; HRMS-ESI (m/z): $[M-C_6H_6N]^+$ calcd for $C_{22}H_{15}BrF_2N_2O$ 347.98356 and 349.98151, found 347.98341 and 349.98180. Anal. Calcd for $C_{22}H_{15}BrF_2N_2O$: C, 59.88; H, 3.43; N, 6.35. Found: C, 59.76; H, 3.16; N, 6.32.

*4.1.3.4.13. 4-(((4-Bromo-2,6-difluorophenyl)(8-hydroxyquinolin-7-yl)methyl)amino)benzoic acid (**25**).* Protocol A, amorphous solid (0.17 g, 18%). ^{1}H NMR (600 MHz, DMSO-d_6, δ): 12.08 (br s, 1H), 10.18 (br s, 1H), 8.83 (m, 1H), 8.29 (d, J = 8.2 Hz, 1H), 7.65 (d, J = 8.1 Hz, 3H), 7.53 (m, 1H), 7.46 (d, J = 8.0 Hz, 2H), 7.39 (d, J = 8.5 Hz, 1H), 7.34 (d, J = 6.5 Hz, 1H), 6.60 (d, J = 8.2 Hz, 2H), 6.35 (d, J = 6.4 Hz, 1H); ^{13}C NMR (125 MHz, acetone-d_6, δ): 167.7, 162.2 (dd, ^1J (^{19}F, ^{13}C) = 252.9 Hz, ^3J (^{19}F, ^{13}C) = 9.0 Hz, CF_{ar}), 152.23, 152.17, 150.5, 149.4, 139.0, 137.0, 132.4, 129.0, 127.1, 123.1, 122.1 (merged t, CBr_{ar}), 119.9, 118.2, 116.8, 116.5 (merged t, CC_{ar}), 112.9 (distorted d, CH_{ar}), 47.5; HRMS-ESI (m/z): $[M-C_7H_6NO_2]^+$ calcd for $C_{23}H_{15}BrF_2N_2O_3$ 347.98356 and 349.98151, found 347.98338 and 349.98151; R_t HPLC (Method-B) = 9.873 min (>95%).

*4.1.3.4.14. 8-Hydroxy-7-(phenyl(phenylamino)methyl)quinoline-2-carbonitrile (**37**).* Protocol D, amorphous solid (0.44 g, 25%). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 10.56 (s, 1H), 8.54 (d, J = 8.4 Hz, 1H), 8.00 (d, J = 8.4 Hz, 1H), 7.81 (d, J = 8.5 Hz, 1H), 7.53 (d, J = 8.6 Hz, 1H), 7.42 (m, 2H), 7.33 (t, J = 7.8 Hz, 2H), 7.24 (t, J = 7.3 Hz, 1H), 7.01 (t, J = 7.9 Hz, 2H), 6.65 (d, J = 8.1 Hz, 2H), 6.52 (m, 2H), 6.18 (d, J = 7.1 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6, δ): 150.1, 147.7, 142.3, 138.5, 138.0, 130.6, 129.6, 128.8, 128.41, 128.37, 128.0, 127.4, 126.9, 123.9, 117.8, 117.7, 116.3, 112.9, 54.0; HRMS-ESI (m/z): calculated for fragment ion $[M-C_6H_6N]^+$ 259.08714 found 259.08709. Anal. Calcd for $C_{23}H_{17}N_3O$: C, 78.61; H, 4.88; N, 11.96. Found: C, 77.59; H, 4.51; N, 11.63.

*4.1.3.4.15. 8-Hydroxy-7-(phenyl(phenylamino)methyl)quinoline-2-carboxylic acid (**38**).* Protocol D, amorphous solid (0.044 g, 3%). ^{1}H NMR (600 MHz, DMSO-d_6, δ): 12.91 (br s, 1H), 10.45 (br s, 1H), 8.50 (d, J = 8.5 Hz, 1H), 8.11 (d, J = 8.5 Hz, 1H), 7.78 (d, J = 8.6 Hz, 1H), 7.52 (d, J = 8.6 Hz, 1H), 7.54 (m, 2H), 7.33 (t, J = 7.8 Hz, 2H), 7.23 (t, J = 7.4 Hz, 1H), 7.01 (m, 2H), 6.67 (d, J = 7.8 Hz, 2H), 6.54 (m, 1H), 6.50 (t, J = 7.3 Hz, 1H), 6.18 (d, J = 7.2 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6, δ): 165.5, 150.7, 148.3, 144.8, 142.9, 138.8, 136.6, 129.5, 129.2, 128.8, 127.8, 127.4, 127.1, 120.2, 118.0, 116.8, 114.3, 113.4, 54.6; HRMS-ESI (m/z): calculated for fragment ion $[M-C_6H_6N]^+$ 278.08172 found 278.08172.

*4.1.3.4.16. 5-Chloro-7-(phenyl(phenylamino)methyl)quinolin-8-ol (**40**).* Protocol D, amorphous solid (0.09 g, 5%). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 8.77 (d, J = 3.9 Hz, 1H), 8.52 (br s, 1H), 8.45 (d, J = 8.4 Hz, 1H), 7.74 (s, 1H), 7.48 (m, 3H), 7.33 (t, J = 7.7 Hz, 2H), 7.26 (m, 1H), 7.12 (t, J = 8.1 Hz, 2H), 6.71 (t, J = 7.3 Hz, 1H), 6.63 (d, J = 7.9 Hz, 2H), 6.10 (s, 1H), 4.42 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$, δ): 148.5, 148.0, 147.2, 141.9, 138.7, 133.3, 129.2, 128.8, 127.6, 127.4, 126.1, 125.5, 124.8, 122.3, 120.9, 118.0, 113.6, 56.7; HRMS-ESI (m/z): calculated for fragment ion $[M-C_6H_6N]^+$ 268.05292 found 268.05287.

*4.1.3.4.17. 5-Bromo-7-(phenyl(phenylamino)methyl)quinolin-8-ol (**41**).* Protocol D, amorphous solid (0.2 g, 10%). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 8.73 (d, J = 3.9 Hz, 1H), 8.62 (br s, 1H), 8.40 (d, J = 8.5 Hz, 1H), 7.92 (s, 1H), 7.47 (m, 3H), 7.33 (t, J = 7.5 Hz, 2H), 7.25 (m, 1H), 7.12 (t, J = 7.9 Hz, 2H), 6.71 (t, J = 7.3 Hz, 1H), 6.63 (d, J = 8.0 Hz, 2H), 6.10 (s, 1H), 4.42 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 148.9, 148.7, 147.4, 142.1, 139.1, 136.0, 129.8, 129.4, 129.0, 127.8, 127.6, 127.0, 125.7, 122.8, 118.3, 113.8, 110.6, 56.9; HRMS-ESI (m/z): calculated for fragment ion $[M-C_6H_6N]^+$ 312.00240 found 312.00245. Anal. Calcd for $C_{22}H_{17}BrN_2O$: C, 65.20; H, 4.23; N, 6.91. Found: C, 65.01; H, 4.09; N, 6.74.

4.1.3.5. General procedure for the synthesis of compounds 11–14 and 19. *4.1.3.5.1. Ethyl 4-(((8-hydroxyquinolin-7-yl)(phenyl) methyl)amino)benzoate (**11**).* Benzocaine (0.331 g, 2.0 mmol), benzaldehyde (0.212 g, 2.0 mmol) and 8-hydroxyquinoline (0.275 g, 2.0 mmol) were suspended in a 10% aqueous solution of NaCl (2 mL). This was heated to 100 °C overnight. Upon cooling, the resultant precipitate was filtered and washed with ethanol to yield **11** as a white amorphous solid (0.365 g, 46%). ^{1}H NMR (600 MHz, DMSO-d_6, δ): 10.13 (br s, 1H), 8.87 (d, J = 2.7 Hz, 1H), 8.29 (d, J = 8.2 Hz, 1H), 7.64 (d, J = 8.7 Hz, 2H), 7.60–7.52 (m, 2H), 7.40–7.32 (m, 6H), 7.34 (t, J = 7.7 Hz, 3H), 7.25 (t, J = 7.2 Hz, 1H), 6.69 (d, J = 8.5 Hz, 2H), 6.26 (d, J = 7.1 Hz, 1H), 4.17 (q, J = 7.0 Hz, 2H), 1.23 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6, δ): 165.7, 151.9, 149.9, 148.3, 141.9, 138.1, 136.0, 130.7, 128.4, 127.6, 127.4, 127.0, 126.1, 124.4, 121.8, 117.6, 116.8, 111.9, 59.5, 53.9, 14.3; HRMS-ESI (m/z): $[M-C_9H_{10}NO_2]^+$ calcd for $C_{25}H_{22}N_2O_3$ 302.07927, found 302.08118. Anal. Calcd for $C_{25}H_{22}N_2O_3$: C, 75.36; H, 5.57; N, 7.03. Found: C, 75.62; H, 5.27; N, 7.03.

*4.1.3.5.2. Ethyl 4-(((8-hydroxyquinolin-7-yl)(4-(trifluoromethyl) phenyl)methyl)amino)benzoate (**12**).* White solid (0.439 g, 47%). ^{1}H NMR (600 MHz, DMSO-d_6, δ): 10.27 (br s, 1H), 8.88 (d, J = 8.2 Hz, 1H), 8.30 (d, J = 8.2 Hz, 1H), 7.71 (d, J = 8.1 Hz, 2H), 7.65 (d, J = 8.6 Hz, 2H), 7.61 (d, J = 8.0 Hz, 2H), 7.57 (dd, J = 8.2, 4.1 Hz, 1H), 7.50 (d, J = 8.6 Hz, 1H), 7.42–7.41 (m, 2H), 6.71 (d, J = 8.5 Hz, 2H), 6.35 (d, J = 7.1 Hz, 1H), 4.18 (q, J = 7.1 Hz, 2H), 1.24 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6, δ): 165.7, 151.7, 150.1, 148.5, 146.7, 138.1, 136.1, 130.8, 128.1, 127.8, 126.0, 125.4, 123.6, 122.0, 117.8, 117.1, 112.0, 59.6, 53.7, 14.3; ^{19}F NMR (564 MHz, DMSO-d_6, δ): −60.9; HRMS-ESI (m/z): $[M-C_9H_{10}NO_2]^+$ calcd for $C_{26}H_{21}F_3N_2O_3$ 302.07927, found 302.08118. Anal. Calcd for $C_{26}H_{21}F_3N_2O_3$: C, 66.95; H, 5.54; N, 6.01. Found: C, 66.69; H, 4.35; N, 5.91.

*4.1.3.5.3. Ethyl 4-(((4-fluorophenyl)(8-hydroxyquinolin-7-yl) methyl)amino)benzoate (**13**).* Off-white powder (0.459 g, 55%). ^{1}H NMR (600 MHz, DMSO-d_6, δ): 10.14 (br s, 1H), 8.87 (d, J = 2.7 Hz, 1H), 8.29 (d, J = 8.2 Hz, 1H), 7.64 (d, J = 8.6 Hz, 2H), 7.55 (dd, J = 8.1, 4.0 Hz, 1H), 7.51 (d, J = 8.6 Hz, 1H), 7.43–7.40 (m, 3H), 7.13 (d, J = 7.1 Hz, 1H), 7.17 (t, J = 8.8 Hz, 2H), 6.68 (d, J = 8.5 Hz, 2H), 6.25 (d, J = 7.1 Hz, 1H), 4.17 (q, J = 7.0 Hz, 2H), 1.23 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6, δ): 165.7, 161.2 (d, ^1J (^{19}F, ^{13}C) = 222.7 Hz, CF_{ar}), 151.0, 148.4, 146.7, 138.0 (d, ^4J (^{19}F, ^{13}C) = 3.5 Hz, CF_{ar}), 136.1, 130.7, 129.3 (d, ^3J (^{19}F, ^{13}C) = 8.0 Hz, CF_{ar}), 127.7, 125.9, 124.2, 121.9, 117.7, 116.9, 115.1 (d, ^2J (^{19}F, ^{13}C) = 21.4 Hz, CF_{ar}), 112.6, 111.9, 59.5, 53.3, 14.3; HRMS-ESI (m/z): $[M-C_9H_{10}NO_2]^+$ calcd for $C_{25}H_{21}FN_2O_3$ 252.08247, found 252.08135. Anal. Calcd for $C_{25}H_{21}FN_2O_3$: C, 72.10; H, 5.08; N, 6.73. Found: C, 71.72; H, 4.83; N, 6.69.

*4.1.3.5.4. Ethyl 4-(((4-chlorophenyl)(8-hydroxyquinolin-7-yl) methyl)amino)benzoate (**14**).* Off-white powder (0.366 g, 42%).

^{1}H NMR (600 MHz, DMSO-d_6, δ): 10.20 (br s, 1H), 8.87 (d, 1H, J = 2.7 Hz), 8.30 (d, 1H, J = 8.2 Hz), 7.65 (d, 2H, J = 8.6 Hz), 7.56 (dd, 1H, J = 8.2, 4.1 Hz), 7.49 (d, 1H, J = 8.5 Hz), 7.40 (s, 5H), 7.35 (d, 1H, J = 7.1 Hz), 6.70 (d, 2H, J = 8.5 Hz), 6.25 (d, 1H, J = 7.1 Hz), 4.18 (q, 2H, J = 7.0 Hz), 1.23 (t, 3H, J = 7.1 Hz); ^{13}C NMR (150 MHz, DMSO-d_6, δ): 166.2, 152.2, 150.5, 148.9, 141.4, 138.6, 136.6, 132.1, 131.3, 129.7, 128.9, 128.2, 126.5, 124.4, 122.4, 118.2, 117.5, 112.4, 60.1, 53.9, 14.8; HRMS-ESI (m/z): $[M-C_9H_{10}NO_2]^+$ calcd for $C_{25}H_{21}ClN_2O_3$ 268.05292, found 268.05451. Anal. Calcd for $C_{25}H_{21}ClN_2O_3$: C, 69.36; H, 4.89; N, 6.47. Found: C, 68.99; H, 4.67; N, 6.36.

*4.1.3.5.5. Ethyl 4-((cyclopentyl(8-hydroxyquinolin-7-yl)methyl) amino)benzoate (**19**).* White powder (0.296 g, 38%). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 9.89 (br s, 1H), 8.86 (br s, 1H), 8.25 (d, 1H, J = 8.1 Hz), 7.55–7.51 (m, 4H), 7.36 (d, 1H, J = 8.4 Hz), 7.12 (d, 1H, J = 7.2 Hz), 6.61 (d, 2H, J = 7.7 Hz), 4.79 (t, 1H, J = 8.3 Hz), 4.14–4.12 (m, 2H), 2.41–2.37 (m, 1H), 2.01–1.94 (m, 1H), 1.70–1.44 (m, 5H), 1.32–1.24 (m, 2H), 1.20 (m, 3H); ^{13}C NMR (150 MHz, DMSO-d_6, δ): 165.7, 152.2, 149.8, 148.2, 137.8, 135.9, 130.7, 127.3, 125.7, 121.5, 117.8, 115.9, 112.4, 59.4, 53.9, 45.5, 30.5, 28.8, 25.0, 24.7, 14.3; HRMS-ESI (m/z): $[M-C_9H_{10}NO_2]^+$ calcd for $C_{24}H_{26}N_2O_3$ 226.12319, found 226.12274. Anal. Calcd for $C_{24}H_{26}N_2O_3$: C, 73.82; H, 6.71; N, 7.17. Found: C, 73.66; H, 6.54; N, 7.15.

4.1.3.6. General procedure for the synthesis of compounds 27–31, 33, and 45–46. *4.1.3.6.1. 2-(Phenyl(phenylamino)methyl)naphthalen-1-ol (**27**).* Aniline (0.37 g, 4.0 mmol), and benzaldehyde (0.42 g, 4.0 mmol) were dissolved in ethanol (10 mL) followed by stirring at room temperature for about 5 min. To the mixture, 1-naphthol (0.57 g, 4.0 mmol) was added followed by the addition of catalytic pyridine (0.2 mL) and the reaction was stirred at room temperature for 24 h. The reaction was monitored by TLC (3:2 acetonitrile/water) and LC–MS. A colorless precipitate was isolated upon filtration, which was washed several times with ethanol to afford pure compound **27** as a colorless amorphous solid (1.2 g, 95%). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 10.82 (s, 1H), 8.27 (m, 1H), 7.75 (m, 1H), 7.46 (m, 2H), 7.41 (m, 2H), 7.31 (m, 4H), 7.17 (m, 2H), 7.08 (d, J = 8.4 Hz, 1H), 6.90 (t, J = 7.4 Hz, 1H), 6.83 (d, J = 7.7 Hz, 2H), 5.59 (s, 1H), 4.23 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$, δ): 152.7, 146.8, 141.8, 134.1, 129.6, 129.5, 128.6, 127.9, 127.5, 126.6, 126.3, 125.8, 125.4, 122.5, 121.8, 119.8, 117.7, 116.7, 66.5; HRMS-ESI (m/z): $[M-C_6H_6N]^+$ calcd for $C_{23}H_{19}NO$ 233.09664, found 233.09661. Anal. Calcd for $C_{23}H_{19}NO$: C, 84.89; H, 5.89; N, 4.30. Found: C, 84.73; H, 5.91; N, 4.45.

*4.1.3.6.2. 2-(((4-(tert-Butyl)phenyl)amino)(phenyl)methyl)naphthalen-1-ol (**28**).* Crystals (1.29 g, 85%), mp 157–158 °C (hexane). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 11.11 (s, 1H), 8.27 (m, 1H), 7.74 (m, 1H), 7.46 (m, 2H), 7.41 (m, 2H), 7.34 (m, 3H), 7.30 (m, 1H), 7.20 (d, J = 7.5 Hz, 2H), 7.09 (d, J = 8.3 Hz, 1H,), 6.80 (d, J = 7.6 Hz, 2H), 5.56 (s, 1H), 4.17 (s, 1H), 1.24 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$, δ): 152.6, 144.5, 144.1, 141.8, 133.8, 129.2, 128.3, 127.7, 127.3, 126.3, 126.2, 126.1, 125.6, 125.1, 122.3, 119.5, 117.5, 116.2, 66.8, 34.1, 31.4; HRMS-ESI (m/z): $[M-C_{10}H_{14}N]^+$ calcd for $C_{27}H_{27}NO$ 233.09664, found 233.09670. Anal. Calcd for $C_{27}H_{27}NO$: C, 85.00; H, 7.13; N, 3.67. Found: C, 84.32; H, 7.05; N, 3.64.

*4.1.3.6.3. 4-(((1-Hydroxynaphthalen-2-yl)(phenyl)methyl)amino) benzoic acid (**29**).* Amorphous solid (0.35 g, 24%). ^{1}H NMR (600 MHz, DMSO-d_6, δ): 12.00 (s, 1H), 9.64 (s, 1H), 8.25 (d, J = 7.95 Hz, 1H), 7.79 (m, 1H), 7.63 (d, J = 8.8 Hz, 2H), 7.44 (m, 6H), 7.32 (m, 2H), 7.23 (m, 2H), 6.70 (d, J = 8.8 Hz, 2H), 6.34 (d, J = 7.1 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6, δ): 167.4, 151.8, 149.4, 142.5, 133.4, 130.9, 128.3, 127.6, 127.4, 126.9, 125.9, 125.4, 125.2, 125.0, 123.7, 122.1, 119.8, 117.5, 111.9, 54.0; HRMS-ESI (m/z): $[M-C_7H_6NO_2]^+$ calcd for $C_{24}H_{19}NO_3$ 233.09664, found 233.09618. Anal. Calcd for $C_{24}H_{19}NO_3$: C, 78.03; H, 5.18; N, 3.79. Found: C, 78.30; H, 5.24; N, 3.79.

*4.1.3.6.4. 3-(((1-Hydroxynaphthalen-2-yl)(phenyl)methyl)amino) benzoic acid (**30**).* Amorphous solid (0.32 g, 22%). ^{1}H NMR (600 MHz, DMSO-d_6, δ): 12.59 (bs, 1H), 9.68 (s, 1H), 8.24 (d, J = 8.0 Hz, 1H); 7.78 (d, J = 7.7 Hz, 1H); 7.39 (m, 9H), 7.21 (m, 1H), 7.12 (m, 2H), 6.91 (m, 1H), 6.74 (d, J = 7.1 Hz, 1H); 6.28 (d, J = 7.0 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6, δ): 167.8, 149.4, 148.1, 142.9, 133.4, 131.3, 128.8, 128.3, 127.6, 127.4, 126.8, 125.8, 125.5, 125.2, 125.0, 123.9, 122.1, 119.7, 117.2, 117.2, 113.9, 54.5; HRMS-ESI (m/z): $[M-C_7H_6NO_2]^+$ calcd for $C_{24}H_{19}NO_3$ 233.09664, found 234.09696. Anal. Calcd for $C_{24}H_{19}NO_3$: C, 78.03; H, 5.18; N, 3.79. Found: C, 77.88; H, 5.35; N, 3.70.

*4.1.3.6.5. 4-(((4-Bromophenyl)(1-hydroxynaphthalen-2-yl)methyl) amino)benzoic acid (**31**).* The reaction was performed in DMSO solvent and followed the same procedure as discussed for compound **26**. Amorphous solid (0.98 g, 55%). ^{1}H NMR (600 MHz, DMSO-d_6, δ): 12.10 (bs, 1H), 9.71 (bs, 1H), 8.26 (d, J = 8.0 Hz, 1H), 7.79 (d, J = 7.7 Hz, 1H), 7.65 (d, J = 8.1 Hz, 2H), 7.43 (m, 8H), 7.22 (d, J = 6.9 Hz, 1H), 6.70 (d, J = 8.1 Hz, 2H), 6.32 (d, J = 6.9 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6, δ): 167.5, 151.7, 149.5, 142.0, 133.6, 131.3, 131.0, 129.7, 127.7, 126.1, 125.3, 125.2, 123.3, 122.2, 120.0, 120.0, 117.8, 112.7, 112.0, 53.6; HRMS-ESI (m/z): $[M-C_7H_6NO_2]^+$ calcd for $C_{24}H_{18}BrNO_3$ 311.00715 and 313.00511, found 311.00690 and 313.00503. Anal. Calcd for $C_{24}H_{18}BrNO_3$: C, 64.30; H, 4.05; N, 3.12. Found: C, 64.48; H, 4.23; N, 3.25.

*4.1.3.6.6. 4-Bromo-2-(phenyl(phenylamino)methyl)naphthalen-1-ol (**33**).* Amorphous solid (1.45 g, 90%). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 11.07 (s, 1H), 8.30 (d, J = 8.4 Hz, 1H), 8.11 (d, J = 8.4 Hz, 1H), 7.59 (m, 1H), 7.51 (m, 1H), 7.36 (m, 6H), 7.20 (m, 2H), 6.94 (t, J = 7.4 Hz, 1H), 6.84 (d, J = 7.8 Hz, 2H), 5.51 (s, 1H), 4.22 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$, δ): 152.5, 146.3, 141.1, 131.9, 129.5, 129.4, 129.4, 128.7, 127.7, 127.7, 126.9, 126.6, 125.9, 122.8, 121.9, 118.4, 116.5, 112.3, 66.2; HRMS-ESI (m/z): $[M-C_6H_6N]^+$ calcd for $C_{23}H_{18}BrNO$ 311.00715 and 313.00511, found 311.00811 and 313.00622. Anal. Calcd for $C_{23}H_{18}BrNO$: C, 68.33; H, 4.49; N, 3.46. Found: C, 68.18; H, 4.23; N, 3.38.

*4.1.3.6.7. 2-((4-Bromo-2,6-difluorophenyl)(phenylamino)methyl)-naphthalen-1-ol (**45**).* Amorphous solid (1.49 g, 85%). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 10.02 (s, 1H), 8.33 (m, 1H), 7.74 (m, 1H), 7.48 (m, 2H), 7.24 (m, 3H), 7.12 (d, J = 8.0 Hz, 2H), 6.93 (m, 3H), 6.74 (d, J = 8.5 Hz, 1H), 6.42 (d, J = 10.5 Hz, 1H), 4.57 (d, J = 10.7 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$, δ): 161.4 (dd, ^1J (^{19}F, ^{13}C) = 252.2 Hz, ^3J (^{19}F, ^{13}C) = 9.2 Hz, CF_{ar}), 152.9, 145.0, 134.5, 129.8, 127.4, 126.9, 125.6, 125.6, 124.3, 122.6, 122.4 (t, ^3J (^{19}F, ^{13}C) = 12.8 Hz, CBr_{ar}), 122.2, 119.4, 117.1, 116.3 (distorted dd, ^2J (^{19}F, ^{13}C) = 26.2 Hz, CH_{ar}), 115.5, 115.1 (t, ^2J (^{19}F, ^{13}C) = 17.0 Hz, CC_{ar}), 53.2; HRMS-ESI (m/z): $[M-C_6H_6N]^+$ calcd for $C_{23}H_{16}BrF_2NO$ 346.98831 and 348.98626, found 346.98740 and 348.98551. Anal. Calcd for $C_{23}H_{16}BrF_2NO$: C, 62.74; H, 3.66; N, 3.18. Found: C, 62.54; H, 3.33; N, 3.17.

*4.1.3.6.8. 4-(((4-Bromo-2,6-difluorophenyl)(1-hydroxynaphthalen-2-yl)methyl)amino)benzoic acid (**46**).* *p*-Aminobenzoic acid (0.68 g, 5.0 mmol), and 4-bromo-2,6-difluorobenzaldehyde (1.10 g, 5.0 mmol) were suspended in ethanol (about 15 mL) followed by stirring at room temperature for about 5 min. To the mixture, 1-naphthol (0.72 g, 5.0 mmol) was added and the resulting mixture was stirred at room temperature for about 24 h. A cream colored precipitate was isolated upon filtration, which was washed several times with ethanol and warm hexane to afford pure **37** as a light cream colored amorphous solid (0.67 g, 28%). ^{1}H NMR (600 MHz, DMSO-d_6, δ): 12.07 (bs, 1H), 9.67 (bs, 1H), 8.20 (m, 1H), 7.81 (m, 1H), 7.66 (d, J = 8.9 Hz, 2H), 7.56 (d, J = 8.6 Hz, 1H), 7.46 (m, 4H), 7.41 (d, J = 8.6 Hz, 1H), 7.29 (d, J = 6.8 Hz, 1H), 6.64 (d, J = 8.8 Hz, 2H), 6.42 (d, J = 6.8 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6, δ): 167.4, 160.6 (dd, ^1J (^{19}F, ^{13}C) = 252.4 Hz, ^3J (^{19}F, ^{13}C) = 9.3 Hz, CF_{ar}), 151.2, 149.5, 133.7, 131.1, 127.6, 125.9, 125.1, 125.0, 124.9, 121.7,

121.3, 120.6 (t, ^3J (^{19}F, ^{13}C) = 13.0 Hz, CBr_{ar}), 119.0, 118.0, 117.5 (t, ^2J (^{19}F, ^{13}C) = 17.4 Hz, CC_{ar}), 115.7 (distorted d, ^2J (^{19}F, ^{13}C) = 29.3, CH_{ar}), 111.5, 46.3; HRMS-ESI (*m*/*z*): $[M-C_7H_6NO_2]^+$ calcd for $C_{24}H_{16}BrF_2NO_3$ 346.98831 and 348.98626, found 346.98806 and 348.98620. Anal. Calcd for $C_{24}H_{16}BrF_2NO_3$: C, 59.52; H, 3.33; N, 2.89. Found: C, 59.33; H, 3.10; N, 2.86.

4.1.3.7. General procedure for the synthesis of compounds 34–35. *4.1.3.7.1. 1-(Phenyl(phenylamino)methyl)naphthalen-2-ol (**34**).* Aniline (0.18 g, 2.0 mmol), and benzaldehyde (0.21 g, 2.0 mmol) were dissolved in ethanol (5 mL) followed by stirring at room temperature for about 5 min. To the mixture, 2-naphthol (0.29 g, 2.0 mmol) and 2–3 drops of pyridine were added and the reaction mixture was refluxed at 100 °C for about 12 h. The reaction was monitored by TLC (3:2 acetonitrile/water) and LC–MS. The reaction mixture was later allowed to cool down and a colorless precipitate was isolated upon filtration, which was washed with ethanol to afford pure compound **34** as a colorless amorphous solid (0.51 g, 80%). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 11.49 (s, 1H), 7.78 (m, 2H), 7.74 (d, *J* = 8.9 Hz, 1H), 7.48 (m, 2H), 7.36 (m, 3H), 7.29 (m, 2H), 7.15 (m, 3H), 6.92 (t, *J* = 7.4 Hz, 1H), 6.77 (d, *J* = 7.7 Hz, 2H), 6.17 (s, 1H), 4.14 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$, δ): 156.4, 146.9, 141.2, 131.7, 130.2, 129.6, 129.6, 129.3, 129.2, 128.8, 128.2, 127.0, 123.0, 122.1, 121.6, 120.2, 116.5, 113.9, 63.0; HRMS-ESI (*m*/*z*): $[M-C_6H_6N]^+$ calcd for $C_{23}H_{19}NO$ 233.09664, found 233.09656; HPLC (Method B) R_t = 10.972 min (>95%).

*4.1.3.7.2. 1-(Phenyl(phenylamino)methyl)naphthalene-2,7-diol (**35**).* Amorphous solid (0.50 g, 74%). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 11.51 (s, 1H), 7.65 (m, 2H), 7.44 (d, *J* = 7.0 Hz, 2H), 7.31 (m, 3H), 7.15 (t, *J* = 7.6 Hz, 2H), 7.06 (s, 1H), 6.98 (m, 1H), 6.92 (t, *J* = 7.2 Hz, 1H), 6.87 (d, *J* = 8.3 Hz, 1H), 6.76 (d, *J* = 7.8 Hz, 2H), 6.00 (s, 1H), 5.00 (s, 1H), 4.12 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$, δ): 157.1, 154.4, 146.9, 140.9, 133.2, 131.1, 130.0, 129.7, 129.6, 128.8, 128.2, 124.7, 122.1, 117.8, 116.5, 114.4, 112.6, 104.7, 63.0; HRMS-ESI (*m*/*z*): $[M-C_6H_6N]^+$ calcd for $C_{23}H_{19}NO_2$ 249.09155, found 249.09185. Anal. Calcd for $C_{23}H_{19}NO_2$: C, 80.92; H, 5.61; N, 4.10. Found: C, 80.45; H, 5.42; N, 3.93.

4.1.3.8. General procedure for the synthesis of compounds 48–50. *4.1.3.8.1. Ethyl 4-(benzhydrylamino)benzoate (**48**).* Benzophenone (0.500 g, 2.74 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and $TiCl_4$ (1 M solution in CH_2Cl_2, 1.1 mL, 1.1 mmol) was added to it. The mixture was cooled to 0 °C followed by the addition of benzocaine (0.996 g, 6.03 mmol) in dry CH_2Cl_2 (10 mL) and stirred for about 3 h at room temperature under N_2. The reaction was then quenched with $NaBH_3CN$ (1 M solution in THF, 3.29 mL, 3.29 mmol) followed by the addition of 10 mL of methanol and stirring for an additional 3 h at room temperature. The mixture was made basic (pH ~ 10) using 10% w/v aqueous NaOH and then filtered. The filtrate was extracted with CH_2Cl_2, and the organic extract was washed with brine and dried ($MgSO_4$). The crude product was purified by flash chromatography (0–10% EtOAc/hexane) to give the product as a white amorphous solid (0.626 g, 73%). ^{1}H NMR (600 MHz, DMSO-d_6, δ): 7.65 (d, *J* = 8.9 Hz, 2H), 7.39 (m, 4H), 7.34 (t, *J* = 7.7 Hz, 4H), 7.29 (d, *J* = 7.2 Hz, 1H), 7.25 (t, *J* = 7.3 Hz, 2H), 6.72 (d, *J* = 8.8 Hz, 2H), 5.79 (d, *J* = 7.2 Hz, 1H), 4.19 (q, *J* = 7.0 Hz, 2H), 1.25 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, δ): 165.8, 151.8, 142.6, 130.6, 128.5, 127.3, 127.0, 116.7, 112.1, 60.2, 59.5, 14.3; HRMS-ESI (*m*/*z*): $[M-C_9H_{10}NO_2]^+$ calcd for $C_{22}H_{21}NO_2$ 167.08608, found 167.08041; $[M+Na]^+$ calcd for $C_{22}H_{21}NO_2$ 354.14670, found 234.14412. Anal. Calcd for $C_{22}H_{21}NO_2$: C, 79.73; H, 6.39; N, 4.23. Found: C, 79.38; H, 6.23; N, 4.17.

*4.1.3.8.2. Ethyl 4-(((4-bromophenyl)(phenyl)methyl)amino)benzoate (**49**).* White amorphous solid (0.310 g, 28%). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 7.64 (d, *J* = 8.9 Hz, 2H), 7.54 (d, *J* = 8.4, 2H), 7.38–7.37 (m, 6H), 7.30–7.26 (m, 2H), 6.69 (d, *J* = 8.8 Hz, 2H), 5.80 (d, *J* = 7.2 Hz, 1H), 4.19 (q, *J* = 7.8 Hz, 2H), 1.25 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, δ): 166.2, 152.1, 142.6, 142.5, 131.9, 131.1, 130.0, 129.0, 127.9, 127.7, 120.6, 117.4, 112.7, 60.0, 59.9, 14.8; HRMS-ESI (*m*/*z*): $[M+H]^+$ calcd for $C_{22}H_{20}BrNO_2$ 410.07557, found 410.07401; HPLC (Method B) R_t = 7.374 min (>95%).

*4.1.3.8.3. Ethyl 4-((bis(4-bromophenyl)methyl)amino)benzoate (**50**).* White amorphous solid (0.250 g, 37%). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 7.65 (d, *J* = 8.8 Hz, 2H), 7.55 (d, *J* = 8.4 Hz, 4H), 7.33–7.28 (m, 5H), 6.70 (d, *J* = 8.7 Hz, 2H), 5.82 (d, *J* = 7.3 Hz, 1H), 4.19 (q, *J* = 7.1 Hz, 2H), 1.25 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, δ): 166.2, 152.0, 142.0, 132.0, 131.2, 130.1, 120.9, 117.7, 112.7, 60.1, 59.2, 14.8; HRMS-ESI (*m*/*z*): $[M]^+$ calcd for $C_{22}H_{19}Br_2NO_2$ 486.97825, found 486.98092; HPLC (Method B) R_t = 7.929 min (>95%).

4.1.3.9. General procedure for the synthesis of compounds 51–53. *4.1.3.9.1. 4-(Benzhydrylamino)benzoic acid (**51**).* Ester, **48** (1.54 g, 4.65 mmol) was dissolved in dioxane (5 mL) followed by addition of 5 M aqueous NaOH (5 mL) and ethanol (5 mL). The mixture was stirred at 80 °C for 8 h. The crude mixture was concentrated yielding a white precipitate in the aqueous layer. Water was added until the precipitate completely dissolved. The mixture was then made acidic (pH 6) with 3 N HCl (aq) from which the desired product precipitated. This was filtered, washed with hexane, and dried to give the product as a cream-colored amorphous solid (1.24 g, 88%). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 12.0 (bs, 1H), 7.61 (d, *J* = 8.9 Hz, 2H), 7.39–7.37 (m, 4H), 7.35–7.32 (m, 4H), 7.25–7.19 (m, 3H), 6.68 (d, *J* = 8.8 Hz, 2H), 5.76 (d, *J* = 7.1 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6, δ): 167.9, 152.1, 143.2, 131.3, 129.0, 127.8, 127.5, 118.1, 112.5, 60.7; HRMS-ESI (*m*/*z*): $[M+Na]^+$ calcd for $C_{20}H_{17}NO_2$ 326.11570; found 326.11580; HPLC (Method B) R_t = 5.431 min (>90%).

*4.1.3.9.2. 4-(((4-Bromophenyl)(phenyl)methyl)amino) benzoic acid (**52**).* Cream-colored amorphous solid (0.175 g, 73%). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 12.0 (bs, 1H), 7.62 (d, *J* = 8.7 Hz, 2H), 7.54 (d, *J* = 8.4 Hz, 2H), 7.38–7.31 (m, 6H), 7.27–7.21 (m, 2H), 6.68 (d, *J* = 8.7 Hz, 2H), 5.79 (d, *J* = 7.2 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6, δ): 167.9, 151.9, 142.7, 142.6, 131.8, 131.3, 130.0, 129.0, 127.9, 127.7, 120.6, 118.3, 112.6, 60.0; HRMS-ESI (*m*/*z*): $[M+Na]^+$ calcd for $C_{20}H_{16}BrNO_2$ 404.02621; found 404.02610; HPLC (Method B) R_t = 6.514 min (>95%).

*4.1.3.9.3. 4-((bis(4-Bromophenyl)methyl)amino)benzoic acid (**53**).* Cream-colored amorphous solid (0.110 g, 61%). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 12.1 (bs, 1H), 7.63 (d, *J* = 8.8 Hz, 2H), 7.56–7.54 (m, 4H), 7.34–7.32 (m, 4H), 7.22 (d, *J* = 7.3 Hz ,1H), 6.67 (d, *J* = 8.8 Hz, 2H), 5.81 (d, *J* = 7.3 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6, δ): 167.8, 151.7, 142.1, 131.9, 131.4, 130.1, 120.8, 118.5, 112.6, 59.3; HRMS-ESI (*m*/*z*): $[M]^+$ calcd for $C_{20}H_{15}Br_2NO_2$ 458.94695; found 458.94454; HPLC (Method B) R_t = 6.113 min (>90%).

4.1.3.10. General procedure for the synthesis of compounds 54–58. *4.1.3.10.1. 4-(Benzhydrylamino)-N-phenylbenzamide (**54**).* Acid **51** (0.100 g, 0.33 mmol) was dissolved in DMF (5 mL). The solution was cooled to 0 °C and COMU (0.142 g, 0.33 mmol) was added, followed by DIPEA (0.11 mL, 0.66 mmol). Aniline (0.150 mL, 1.65 mmol) was then added and the mixture stirred for 3 h, during which time the temperature was allowed to rise to room temperature. Upon reaction completion, CH_2Cl_2 (20 mL) was added to the mixture, which was washed successively with saturated sodium bicarbonate and brine (twice). The organic portion was then dried over $MgSO_4$ and concentrated under reduced pressure. The crude mixture was purified by flash chromatography (0–50% EtOAc/hexane) to give the product as a cream-colored amorphous solid (0.065 g, 52%). ^{1}H NMR

(500 MHz, DMSO-d_6, δ): 9.75 (s, 1H), 7.73–7.69 (m, 4H), 7.36–7.33 (m, 4H), 7.31–7.28 (m, 3H), 7.26–7.23 (m, 2H), 7.10 (d, J = 7.2 Hz, 1H), 7.05–7.02 (m, 1H), 6.74 (d, J = 8.8 Hz, 2H), 5.79 (d, J = 7.1 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6, δ): 165.8, 151.2, 143.4, 140.2, 129.4, 129.0, 128.9, 127.8, 127.5, 123.4, 122.3, 120.5, 112.6, 60.8; HRMS-ESI (m/z): [M+H]$^+$ calcd for $C_{26}H_{22}N_2O$ 379.18104; found 379.18043; HPLC (Method B) R_t = 6.389 min (>95%).

*4.1.3.10.2. 4-(((4-Bromophenyl)(phenyl)methyl)amino)-N-phenylbenzamide (**55**).* Light brown amorphous solid (0.070 g, 53%). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 9.76 (s, 1H), 7.73–7.69 (m, 4H), 7.55–7.53 (m, 2H), 7.41–7.34 (m, 6H), 7.31–7.25 (m, 3H), 7.11 (d, J = 7.3 Hz, 1H), 7.05–7.02 (m, 1H), 6.73 (d, J = 8.8 Hz, 2H), 5.81 (d, J = 7.2 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6, δ): 165.8, 164.2, 151.0, 142.8, 140.2, 131.8, 130.1, 129.5, 129.0, 128.9, 127.9, 127.7, 123.4, 122.6, 120.6, 120.5, 112.6, 60.1; HRMS-ESI (m/z): [M+H]$^+$ calcd for $C_{26}H_{21}BrN_2O$ 457.09155, 459.08950; found 457.09321, 459.09173; HPLC (Method B) R_t = 6.919 min (>95%).

*4.1.3.10.3. 4-((Bis(4-bromophenyl)methyl)amino)-N-phenylbenzamide (**56**).* Brown amorphous solid (0.030 g, 43%). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 7.67–7.65 (m, 3H), 7.59–7.57 (m, 2H), 7.48–7.46 (m, 4H), 7.33 (t, J = 8.4 Hz, 2H), 7.19–7.17 (m, 4H), 7.12–7.09 (m, 1H), 6.55–6.53 (m, 2H), 6.50 (d, J = 4.1 Hz, 1H), 4.57 (d, J = 4.2 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6, δ): 165.3, 149.5, 140.4, 138.3, 132.2, 129.1, 129.0, 128.7, 124.1, 124.0, 121.9, 120.0, 113.0, 61.4; HRMS-ESI (m/z): [M+H]$^+$ calcd for $C_{26}H_{20}Br_2N_2O$ 537.00002; found 537.00067; HPLC (Method B) R_t = 7.392 min (>90%).

*4.1.3.10.4. 4-(Benzhydrylamino)-N-(naphthalen-2-yl)benzamide (**57**).* Cream-colored amorphous solid (0.200 g, 25%). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 9.97 (s, 1H), 8.40 (s, 1H), 7.85–7.75 (m, 6H), 7.48–7.34 (m, 10H), 7.25 (t, J = 7.3 Hz, 2H), 7.13 (d, J = 7.2 Hz, 1H), 6.76 (d, J = 8.7 Hz, 1H), 5.80 (d, J = 7.1 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6, δ): 166.0, 151.3, 143.3, 137.8, 133.9, 130.1, 129.5, 129.0, 128.4, 127.9, 127.8, 127.7, 127.5, 126.7, 124.9, 122.2, 121.4, 116.3, 112.6, 60.8; HRMS-ESI (m/z): [M+H]$^+$ calcd for $C_{30}H_{24}N_2O$ 429.19669; found 429.19666, [2M+H]$^+$ calcd for $C_{60}H_{48}N_4O_2$ 857.38555; found 857.38571; HPLC (Method B) R_t = 7.029 min (>95%).

*4.1.3.10.5. 3-(4-(Benzhydrylamino)benzamido)benzoic acid (**58**).* Cream-colored amorphous solid (0.050 g, 18%). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 12.96, 1.3 Hz, 1H), 7.44–7.40 (m, 5H), 7.36–7.33 (m, 4H), 7.26–7.23 (m, 2H), 7.13 (d, J = 7.15 Hz, 1H), 6.74 (d, J = 8.8 Hz, 2H), 5.80 (d, J = 7.2 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6, δ): 167.8, 165.9, 151.3, 143.3, 140.4, 131.6, 129.5, 129.1, 129.0, 127.8, 127.5, 124.5, 124.2, 121. 9, 121.3, 112.6, 60.8; HRMS-ESI (m/z): [M+H]$^+$ calcd for $C_{27}H_{22}N_2O_3$ 423.17087; found 423.17208, [2M+H]$^+$ calcd for $C_{54}H_{44}N_4O_6$ 845.33391; found 845.33656; HPLC (Method B) R_t = 7.980 min (>90%).

4.1.3.11. Procedures for the synthesis of compounds 20, 22, 23, 26, 32, 36, 39, 43–44, and 47. *4.1.3.11.1. 7-((Phenylamino)methyl) quinolin-8-ol (**20**).* Aniline (0.37 g, 4.0 mmol) and paraformaldehyde (0.12 g, 4.0 mmol) were dissolved in ethanol (10 mL) followed by stirring at room temperature for about 5 min. To the mixture, 8-hydroxyquinoline (0.58 g, 4.0 mmol) was added and the reaction mixture was refluxed at 120 °C (Note: the reaction temperature was achieved through gradual increase during the course of 3 h) for about 12 h. The reaction was monitored by TLC (3:2 acetonitrile/water) and LC–MS. The reaction mixture was allowed to cool and a colorless precipitate was isolated upon filtration, which was washed several times with ethanol to afford pure compound **20** as a colorless amorphous solid (0.52 g, 52%). ^{1}H NMR (600 MHz, acetone-d_6, δ): 8.96 (br s, 1H), 8.81 (d, J = 3.8 Hz, 1H), 8.26 (d, J = 8.2 Hz, 1H), 7.60 (d, J = 8.4 Hz, 1H), 7.50 (m, 1H), 7.36 (d, J = 8.4 Hz, 1H), 7.06 (m, 2H), 6.72 (d, J = 7.9 Hz, 2H), 6.56 (t, J = 7.2 Hz, 1H), 5.52 (br s, 1H), 4.59 (distorted doublet, 2H); ^{13}C NMR (150 MHz, acetone-d_6, δ): 150.5, 149.8, 149.1, 139.0, 137.0, 129.8, 128.6, 128.6, 122.7, 122.5, 118.3, 117.4, 113.5, 42.4; HRMS-ESI (m/z): [M−C_6H_6N]$^+$ calcd for $C_{16}H_{14}N_2O$ 158.06059, found 158.06038; HPLC (Method-B) R_t = 6.980 min (>95%).

*4.1.3.11.2. N-((8-Hydroxyquinolin-7-yl)(phenyl)methyl)benzamide (**22**).* Benzamide (0.67 g, 5.5 mmol), benzaldehyde (0.53 g, 5.0 mmol), and 8-hydroxyquinoline (0.72 g, 5.0 mmol) were dissolved in dichloroethane (12 mL). To the mixture, *p*-toluenesulfonic acid (95 mg, 0.5 mmol) was added as catalyst and the reaction mixture was refluxed at 125 °C for 12 h. After completion of the reaction, which was detected by LC–MS, the reaction mixture was allowed to cool. A colorless precipitate was isolated upon filtration, which was taken in ethanol and heated for 15 min. The resulting solid was filtered and washed with ethanol to afford pure compound **22** as a colorless amorphous solid (1.22 g, 70%). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 8.73 (m, 1H), 8.14 (m, 2H), 7.88 (d, J = 7.3 Hz, 2H), 7.55 (d, J = 8.5 Hz, 1H), 7.49 (m, 1H), 7.40 (m, 7H), 7.30 (m, 2H), 7.23 (m, 1H), 6.76 (d, J = 8.8 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$, δ): 166.6, 149.4, 148.4, 141.6, 138.6, 136.3, 134.7, 131.7, 128.9, 128.8, 128.7, 127.9, 127.5, 127.3, 127.4, 122.6, 122.1, 118.4, 55.7; HRMS-ESI (m/z): [M+H]$^+$ calcd for $C_{23}H_{18}N_2O_2$ 355.14465, found 355.14484 and [M−C_7H_6NO]$^+$ calcd for $C_{23}H_{18}N_2O_2$ 234.09189, found 234.09171. Anal. Calcd for $C_{23}H_{18}N_2O_2$: C, 77.95; H, 5.12; N, 7.90. Found: C, 77.48; H, 4.97; N, 7.98; HPLC (Method B) R_t = 8.960 min (>95%).

*4.1.3.11.3. (E)-7-((Benzylideneamino)(phenyl)methyl)quinolin-8-ol (**23**).* Ammonium carbamate (1.17 g, 15.0 mmol), 8-hydroxyquinoline (0.72 g, 5.0 mmol), and benzaldehyde (1.06 g, 10.0 mmol) were suspended in ethanol (20 mL). The resulting mixture was refluxed at 125 °C for 12 h. After completion of the reaction, which was detected by LC–MS, the solvent was removed through rotary evaporation, and the resulting residue was extracted with a dichloromethane/water mixture. The organic extracts were concentrated in a rotary evaporator and the residual solid was taken in a 50-mL beaker, suspended in hexane, and heated to remove any unreacted starting materials. The resulting free flowing yellow powder was compound **23** (0.090 g, 30%). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 9.15 (bs, 1H), 8.76 (m, 1H), 8.56 (s, 1H), 8.07 (m, 1H), 7.85 (m, 2H), 7.65 (d, J = 8.6 Hz, 1H), 7.51 (d, J = 7.5 Hz, 2H), 7.38 (m, 4H), 7.30 (m, 3H), 7.22 (m, 1H), 6.28 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$, δ): 161.9, 149.4, 148.3, 143.6, 138.9, 136.3, 136.1, 131.2, 128.8, 128.7, 128.6, 127.9, 127.8, 127.6, 127.2, 125.3, 121.7, 118.0, 71.8; HRMS-ESI (m/z): [M−C_7H_6N]$^+$ calcd for $C_{23}H_{18}N_2O$ 234.09189, found 234.09163. Anal. Calcd for $C_{23}H_{18}N_2O$: C, 81.63; H, 5.36; N, 8.28. Found: C, 81.10; H, 5.20; N, 8.23.

*4.1.3.11.4. 4-(Octyloxy)benzaldehyde (**26a**)*[50]. 4-Hydroxybenzaldehyde (0.61 g, 5 mmol) was dissolved in acetone. Potassium carbonate (0.759 g, 5.5 mmol) was added and the resulting mixture was stirred for 5 minutes at room temperature. To the mixture, 1-bromooctane (0.965 g, 5 mmol) and potassium iodide (0.83 g, 5 mmol) were added, followed by refluxing for 6 h. TLC showed the completion of the reaction. Solvent was removed in vacuo and the resulting residue was extracted with ethyl acetate (2 × 10 mL), treated with brine, and dried over sodium sulfate. Flash chromatography using 5% ethyl acetate in hexane afforded a gummy colorless compound (0.94 g, 80%). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 9.88 (s, 1H), 7.83 (d, J = 8.7 Hz, 2H), 6.99 (d, J = 8.7 Hz, 2H), 4.04 (t, J = 6.5 Hz, 2H), 1.81 (quin., J_1 = 6.6 Hz, J_2 = 7.2 Hz, 2H), 1.46 (quin., J_1 = 6.8 Hz, J_2 = 7.6 Hz, 2H), 1.32 (m, 8H), 0.89 (t, J = 6.8 Hz, 3H).

*4.1.3.11.5. 7-((4-(Octyloxy)phenyl)(phenylamino)methyl)quinolin-8-ol (**26**).* A mixture of aniline (0.186 g, 2.0 mmol), aldehyde **26a** (0.47 g, 2.0 mmol), and 8-hydroxyquinoline (0.29 g, 2.0 mmol) was heated neatly up to 130 °C for about 24 h. The reaction was

monitored by TLC (4:1 acetonitrile/water) and LC–MS. Reverse phase chromatography (C18 column) using 4:1 acetonitrile/water afforded compound **26** as a colorless gummy solid (0.045 g, 5%). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 8.74 (d, J = 3.4 Hz, 1H), 8.10 (d, J = 8.3 Hz, 1H), 7.62 (d, J = 8.6 Hz, 1H), 7.38 (m, 3H), 7.30 (d, J = 8.6 Hz, 1H), 7.10 (t, J = 7.9 Hz, 2H), 6.83 (d, J = 8.6 Hz, 2H), 6.67 (t, J = 7.3 Hz, 1H), 6.61 (d, J = 8.0 Hz, 2H), 6.08 (s, 1H), 4.42 (br s, 1H), 3.90 (t, J = 6.5 Hz, 2H), 1.74 (p, J_1 = 6.6 Hz, J_2 = 7.1 Hz, 2H), 1.41 (p, J_1 = 6.8 Hz, J_2 = 7.8 Hz, 2H), 1.28 (m, 8H), 0.87 (t, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 158.4, 148.9, 148.0, 147.5, 138.2, 136.0, 134.4, 129.1, 128.5, 127.6, 126.6, 124.4, 121.6, 117.8, 117.6, 114.6, 113.5, 68.0, 56.3, 31.8, 29.36, 29.29, 29.25, 26.1, 22.7, 14.1; HRMS-ESI (m/z): $[M-C_6H_6N]^+$ calcd for $C_{30}H_{34}N_2O_2$ 362.21200 found 362.21204. Anal. Calcd for $C_{30}H_{34}N_2O_2$: C, 79.26; H, 7.54; N, 6.16. Found: C, 78.51; H, 7.48; N, 5.34.

*4.1.3.11.6. Methyl 4-(((8-methoxyquinolin-7-yl)(phenyl)methyl) amino)benzoate (**32**).* Compound **1** (0.100 g, 0.270 mmol) and KOH (0.068 g, 1.22 mmol) were suspended in DMF (5 mL). To this was added MeI (0.038 g, 0.270 mmol) and the mixture allowed to stir at room temperature overnight. The solvent was removed *in vacuo* and the crude residue purified by flash chromatography (EtOAc/hexane 0–10%) to yield **31** as white gummy solid (0.035 g, 33%). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 8.93 (d, J = 2.5 Hz, 1H), 8.13 (d, J = 8.32 Hz, 1H), 7.80 (d, J = 8.6 Hz, 2H), 7.56 (q, J = 8.5 Hz, 2H), 7.42–7.38 (m, 3H), 7.33 (t, J = 7.6 Hz, 2H), 6.58 (d, J = 8.7 Hz, 2H), 6.28 (d, J = 5.4 Hz, 1H), 4.87 (d, J = 5.1 Hz, 1H), 3.98 (s, 3H), 3.81 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6, δ): 167.2, 153.5, 150.8, 149.7, 142.8, 141.9, 136.2, 134.0, 131.5, 129.4, 128.8, 127.7, 127.6, 125.7, 123.7, 121.3, 119.0, 112.3, 62.5, 56.7, 51.5; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{25}H_{22}N_2O_3$ 399.17087, found 399.17381. HPLC (Method B) R_t = 8.76 min (>95%).

*4.1.3.11.7. 2-Methyl-7-(phenyl(phenylamino)methyl)quinolin-8-ol (**36**).* Aniline (0.465 g, 5.0 mmol) and benzaldehyde (0.53 g, 5.0 mmol) were dissolved in ethanol (10 mL) followed by stirring at room temperature for about 5 minutes. To the mixture, 2-methyl-8-quinolinol (0.795 g, 5.0 mmol) and catalytic pyridine (2 drops) was added and the reaction mixture was refluxed up to 100 °C for about 24 h. The reaction was monitored by TLC (3:2 acetonitrile/water) and LC–MS. Ethanol was removed by rotary evaporation to afford a crude solid, which was purified by flash chromatography using 2% ethyl acetate in hexane. The column fractions containing the compound were kept overnight in the hood to obtain needle shaped crystals (0.92 g, 54%). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 7.97 (d, J = 8.4 Hz, 1H), 7.51 (d, J = 8.5 Hz, 1H), 7.48 (m, 2H), 7.31 (m, 2H), 7.24 (m, 3H), 7.10 (m, 2H), 6.67 (m, 1H), 6.63 (m, 2H), 6.12 (d, J = 3.4 Hz, 1H), 4.49 (s, 1H), 2.69 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 157.3, 148.6, 147.7, 142.8, 137.8, 136.2, 129.3, 128.8, 127.6, 127.4, 125.8, 125.7, 124.2, 122.7, 117.86, 117.78, 113.8, 57.0, 25.1; HRMS-ESI (m/z): $[M-C_6H_6N]^+$ calcd for $C_{23}H_{20}N_2O$ 248.10754, found 248.10762. Anal. Calcd for $C_{23}H_{20}N_2O$: C, 81.15; H, 5.92; N, 8.23. Found: C, 80.88; H, 5.89; N, 8.17. HPLC (Method-B) R_t = 2.770 (>95%).

*4.1.3.11.8. Benzyl 8-hydroxyquinoline-2-carboxylate (**39a**).* [52] 8-Hydroxyquinoline-2-carboxylic acid (0.945 g, 5 mmol), triphenyl phosphine (1.965 g, 7.5 mmol), and benzyl alcohol (0.54 g, 5 mmol) were dissolved in THF (50 mL) and the resulting mixture was stirred at 0 °C in an ice bath. Diisopropyl azodicarboxylate (1.515 g, 7.5 mmol) was added dropwise over a period of 5 min and stirring was continued for 30 minutes. Solvents were removed in vacuo and the resultant residue solid was triturated with diethyl ether (repeated 3 times) to remove most of the triphenyl phosphine oxide by-product as a solid. The filtrate was treated with brine and dried over sodium sulfate. Reverse phase chromatography (C18 column) using 40% acetonitrile in water afforded a light yellow amorphous material (0.56 g, 40%). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 8.69 (br s, 1H), 8.25 (m, 1H), 8.16 (m, 1H), 7.55 (m, 1H), 7.50 (m, 2H), 7.41 (m, 2H), 7.36 (m, 2H), 7.22 (d, J = 8.5 Hz, 1H), 5.49 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 165.0, 153.6, 145.4, 138.0, 137.4, 135.6, 130.5, 129.9, 128.9, 128.76, 128.71, 121.8, 117.8, 111.4, 67.9; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{17}H_{13}NO_3$ 280.09682, found 280.09732. HPLC (Method-B) R_t = 5.466 (>95%).

*4.1.3.11.9. Benzyl 8-hydroxy-7-(phenyl(phenylamino)methyl) quinoline-2-carboxylate (**39**).* A mixture of **39a** (0.42 g, 1.5 mmol), aniline (0.14 g, 1.5 mmol), and benzaldehyde (0.16 g, 1.5 mmol) was heated neatly at 120 °C for 12 h. Reverse phase column chromatography (C18 column) using 40% acetonitrile in water afforded a light yellow solid compound. The solid compound was again washed with ethanol to afford a light yellow amorphous compound (0.1 g, 15%). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 8.88 (br s, 1H), 8.20 (d, J = 8.5 Hz, 1H); 8.14 (d, J = 8.5 Hz, 1H), 7.76 (d, J = 8.5 Hz, 1H), 7.48 (m, 4H), 7.40 (t, J = 7.6 Hz, 2H), 7.35 (m, 2H), 7.31 (t, J = 7.7 Hz, 2H), 7.24 (t, J = 3.6 Hz, 1H), 7.10 (t, J = 8.1 Hz, 2H), 6.68 (t, J = 7.3 Hz, 1H), 6.62 (d, J = 7.9 Hz, 2H), 6.15 (s, 1H), 5.47 (s, 2H), 4.48 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$, δ): 165.0, 150.2, 147.5, 145.6, 142.4, 137.9, 137.3, 135.6, 129.36, 129.32, 129.0, 128.91, 128.89, 128.75, 128.68, 127.6, 125.6, 121.6, 118.0, 117.8, 113.7, 67.9, 56.9; HRMS-ESI (m/z): $[M-C_6H_6N]^+$ calcd for $C_{30}H_{24}N_2O_3$ 368.12867 found 368.12867.

*4.1.3.11.10. N-(Phenyl(quinolin-7-yl)methyl)aniline (**43**).* Aniline (0.37 g, 4.0 mmol) and benzaldehyde (0.42 g, 4.0 mmol) were heated together at 60 °C for 4 h to generate imine **43a**. In a separate vessel, *n*-butyl lithium (2.5 mL of 1.6 M in hexane, 0.256 g, 4 mmol) was added slowly via a dry syringe at −78 °C to a solution of 7-bromoquinoline (0.416 g, 2 mmol) in THF. The mixture was stirred at -78 °C for 45 minutes. A solution of **43a** (0.362 g, 2 mmol) in dry THF was then added slowly via syringe and the reaction mixture was allowed to warm up to room temperature. The reaction was continued for 3 h followed by the addition of an aqueous saturated solution of ammonium chloride. Solvents were removed in vacuo and extracted with ethyl acetate. Flash chromatography using 30% ethyl acetate in hexane afforded **43** as a light cream-colored amorphous solid (0.045 g, 7%). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 8.88 (dd, J_1 = 4.2 Hz, J_2 = 1.7 Hz, 1H), 8.12 (m, 2H), 7.77 (d, J = 8.4 Hz, 1H), 7.58 (dd, J_1 = 8.4 Hz, J_2 = 1.7 Hz, 1H), 7.40 (m, 2H), 7.34 (m, 3H), 7.26 (m, 1H), 7.10 (m, 2H), 6.69 (t, J = 7.3 Hz, 1H), 6.59 (d, J = 8.0 Hz, 2H), 5.70 (d, J = 3.8 Hz, 1H), 4.37 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 150.7, 148.5, 147.1, 144.5, 142.4, 135.8, 129.2, 128.9, 128.4, 127.69, 127.65, 127.60, 127.5, 126.3, 121.1, 117.9, 113.6, 63.0; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{22}H_{18}N_2$ 311.15482 found 311.15479. HPLC (Method-A) R_t = 8.786 (>95%).

*4.1.3.11.11. N-(Naphthalen-2-yl(phenyl)methyl)aniline (**44**).* 2-Naphthylphenyl ketone (0.232 g, 1 mmol) was dissolved in dry dichloromethane and $TiCl_4$ (1.1 mL of 1 M solution in CH_2Cl_2, 0.208 g, 1.1 mmol) was added to it. The mixture was cooled to 0 °C followed by the addition of aniline (0.195 g, 2.1 mmol) and stirred for about 3 h at room temperature under N_2. The reaction mixture was then quenched with $NaBH_3CN$ (1.2 mL of 1 M solution in THF, 0.0754 g, 1.2 mmol) followed by the addition of 2 mL of methanol and stirred for an additional 1 hour at the same temperature. The mixture was made basic (pH ~10) using 5 M aqueous solution of NaOH and filtered through celite. The filtrate was extracted with ethyl acetate (2 × 5 mL), organic layers washed with brine and dried over sodium sulfate. Flash chromatography using hexane afforded a colorless amorphous solid (0.26 g, 85%). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 7.84 (s, 1H), 7.79 (m, 3H), 7.45 (m, 3H), 7.39 (m, 2H), 7.32 (m, 2H), 7.26 (m, 1H), 7.11 (m, 2H), 6.69 (m, 1H), 6.58 (m, 2H), 5.65 (d, J = 3.6 Hz, 1H), 4.30 (d, J = 3.3 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 147.6, 143.0, 140.4, 133.7, 133.0, 129.4, 129.0, 128.8, 128.2, 127.9, 127.7, 126.4, 126.14,

126.10, 125.9, 117.9, 113.7, 63.5; HRMS-ESI (m/z): $[M-C_6H_6N]^+$ calcd for $C_{23}H_{19}N$ 217.10173, found 217.10178. Anal. Calcd for $C_{22}H_{17}BrN_2O$: C, 89.28; H, 6.19; N, 4.53. Found: C, 88.83; H, 5.97; N, 4.47. HPLC (Method-B) R_t = 7.261 (>95%).

*4.1.3.11.12. 1-((R)-Phenyl(((R)-1-phenylethyl)amino)methyl)-naphthalen-2-ol (**47**).* *R*-Phenethylamine (0.66 g, 5.5 mmol) and benzaldehyde (0.58 g, 5.5 mmol) were stirred at room temperature for about 5 min. To the mixture, 2-naphthol (0.72 g, 5.0 mmol) was added and the reaction mixture was heated neatly at 85 °C for about 12 h under nitrogen atmosphere. The reaction was monitored by TLC (5% methanol in dichloromethane) and LC–MS. After 12 h heating, the reaction was dispersed at room temperature with ethanol (5 mL) and kept undisturbed for 12 h. A colorless crystalline solid settled down, which was filtered and washed several times with ethanol and hexane to afford compound **47** (0.79 g, 45%) as a colorless crystalline solid, mp 154–155 °C (ethanol), literature mp 155–156 °C (EtOAc–hexane).[53] ^{1}H NMR (600 MHz, $CDCl_3$, δ): 13.70 (s, 1H), 7.71 (m, 2H), 7.36 (m, 4H), 7.19 (m, 10H), 5.45 (s, 1H), 3.88 (m, 1H), 2.27 (d, J = 9.6 Hz, 1H), 1.48 (d, J = 6.8 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$, δ): 157.28, 143.09, 141.49, 132.59, 129.71, 129.05, 128.94, 128.74, 128.68, 127.95, 127.88, 127.67, 126.68, 126.36, 122.37, 121.09, 120.05, 113.06, 60.27, 56.63, 22.96; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{25}H_{23}NO$ 354.18579, found 354.18593 and $[M - C_8H_{10}N]^+$ calcd for $C_{25}H_{23}NO$ 233.09664, found 233.09681. Anal. Calcd for $C_{25}H_{23}NO$: C, 84.95; H, 6.56; N, 3.96. Found: C, 84.61; H, 6.54; N, 3.98.

4.1.4. X-ray diffractometry

Data were recorded on Bruker APEX-II DUO diffractometer using Cu $K\alpha$ radiation (wavelength = 1.54178 Å) and a graphite monochromator.

4.1.4.1. X-ray crystal structure of 28. CCDC deposition number 1437906. The structure was refined using the program SHELXL-2014-1. Crystal data: $C_{27}H_{27}NO$, M = 381.49, triclinic, space group $P-1$, a = 9.1165(2) Å, b = 11.0940(3) Å, c = 11.2998(3) Å; α = 76.621(1)°, β = 72.212(1)°, γ = 72.021(1)°; V = 1023.14(5) Å^3; Z = 2; T = 100.01 K; μ = 0.571 mm^{-1}; Crystal size = 0.10 × 0.05 × 0.05 mm^3. Of 16,805 reflections measured to theta range 4.157 to 66.621°, 3,507 were independent (R_{int} = 0.0000). Final R1 = 0.0350 ($I > 2\sigma(I)$), wR2 = 0.0924.

4.1.4.2. X-ray crystal structure of 36. CCDC deposition number 1437907. The structure was refined using the program SHELXL 2014-1. Crystal data: $C_{23}H_{20}N_2O$, M = 340.41, monoclinic, space group P 1 2$_1$/n 1, a = 14.0529(7) Å, b = 5.5734(3) Å, c = 22.5326(11) Å; α = 90°, β = 99.465(2)°, γ = 90°; V = 1740.78(15) Å^3; Z = 4; T = 100 K; μ = 0.627 mm^{-1}; Crystal size = 0.18 × 0.18 × 0.07 mm^3. Of 52,480 reflections measured to theta range 3.469–66.655°, 3,080 were independent (R_{int} = 0.0000). Final R1 = 0.0345 ($I > 2\sigma(I)$), wR2 = 0.0908.

4.2. Biological assays

4.2.1. Yeast strains

The yeast strain used in this study was SM3614 (*MAT***a** *trp1 leu2 ura3 his4 can1 ste24::LEU2 rce1::TRP1*).[71] Plasmid-bearing versions of the strain were generated according to published methods.[72] Strains were routinely grown at 30 °C using synthetic complete dropout (SC–Ura) medium.[73] The over-expression plasmid encoding human Rce1p (pWS335) has been reported.[39] The over-expression plasmid encoding human ZmpSte24 (pWS1275; 2μ URA3 P_{PGK}-HA-HsSTE24) was constructed by conventional cloning methods starting with previously described plasmid pWS183.[39]

4.2.2. In vitro fluorescence-based CaaX proteolysis assay

An established fluorescence-based assay was used to monitor CaaX protease activity in the presence of inhibitors.[29,55,56] The assay involved the mixing of a quenched fluorogenic substrate with yeast membranes enriched for the appropriate CaaX protease. The Rce1 fluorogenic substrate ABZ-KSKTKC(farnesyl)Q_LIM and the Ste24 substrate ABZ-KSKTKC(farnesyl)VIQ_L were purchased from AnaSpec (San Jose, CA) and are based on the K-Ras4b C-terminal sequence. ABZ is 2-aminobenzoic acid, and Q_L is (*S*)-2-amino-6-((2,4-dinitrophenyl)amino)hexanoic acid. The substrate was diluted from a 1-mM stock prepared in 4% DMSO. Membranes used as the source of protease activity were isolated from yeast according to our reported methods.[25,39,67] The membranes and substrate were diluted with assay buffer (200 mM Tris, pH 7.5, 200 mM NaCl, 1 mg/mL BSA) immediately before use. Compounds were prepared as 50X stocks in DMSO. Assay assembly involved the dispensing of 50-μL aliquots of diluted membranes into the wells of a black, clear-bottom, 96-well microplate. This was followed by the addition of each compound to duplicate wells and a 15-min pretreatment incubation at 30 °C. After pretreatment, the assay was initiated by adding 50 μL of diluted substrate, resulting in final membrane and substrate concentrations of 0.25 mg/mL and 20 μM substrate, respectively. The sample fluorescence was measured every 30–60 s over a 60-min time course at 30 °C using a BioTek Synergy HT microplate fluorometer equipped with a 320/420-nm excitation/emission filter set. The collected data were exported to Microsoft Excel, graphed as a function of fluorescence vs time, and initial velocities determined. These values were used to calculate the percentage activities relative to a DMSO-treated sample, which was included as a control in each reaction set. To correct for compound-induced effects on fluorescence readout, samples were completely proteolyzed with trypsin (10 μg/mL) at the end of each experiment to determine the maximum possible fluorescence in the presence and absence (i.e., DMSO control) of compounds. These values were used to normalize raw reaction rates. IC_{50} experiments were conducted using a range of compound concentrations (0.25–250 μM final); the final DMSO concentration was the same in all reactions. IC_{50} values calculated with Prism 6.0 (GraphPad Software, Inc.) from values obtained for initial velocities.

4.2.3. Fluorescence-based FTase assay

To determine whether the compounds are inhibitors of the protein farnesyltransferase (FTase), a previously reported fluorescence-based FTase assay[68] was adapted for use in a microtiter plate format.[69] Briefly, 100 μL of a concentrated enzyme assay solution (5 μM Dansyl-GCVLS, 12.5 mM DTT, 125 mM Tris·HCl, pH 7.5, 25 mM $MgCl_2$, 25 μM $ZnCl_2$, 0.5% octyl-β-D-glucopyranoside, 25 μM FPP) was added to wells of a black 96-well plate (Nunc #237105). To each well, water (117.5 μL) was also added. Varying volumes of the different inhibitors dissolved in DMSO were added to the wells followed by addition of DMSO to a final volume of 12.5 μL DMSO in each well. Three different inhibitor concentrations (10, 25, 50 μM) were tested in the assay. The plate was first screened for initial fluorescence using a Beckman Coulter DTX 880 Multimode Detector using the A340/10 filter for excitation at 340 nm and F535/25 filter to monitor for emission. Afterward, 20 μL of enzyme solution (62.5 nM rat FTase, 50 mM Tris·HCl, pH 7.5, 50 μM $ZnCl_2$, 5 mM $MgCl_2$, 20 mM KCl, 1 mM DTT, 1 mg/mL BSA) was added to each well to initiate the enzymatic reaction; final assay volume was 250 μL. Fluorescence was monitored in the same way as for the initial fluorescence measurement but taking a fluorescence reading every 40 s for a total of 1 h. Fluorescence data was exported to Microsoft Excel and the rate was determined by converting the rate obtained in the first

10 min of the reaction from fluorescence intensity units (FIU/s) to nM/s using the equation $v_i = RP/\Delta F$, where v_i is the rate of the reaction in nM/s, R is the rate of the reaction in FIU/s, P is the amount of product in nM and ΔF is the difference in fluorescence intensity between the start of the assay and the maximal fluorescence intensity obtained.

4.2.4. Materials for cell based assays

Tissue culture medium was supplied by Sigma, and fetal bovine serum (FBS) were obtained from PAA laboratories. All other chemicals were supplied by Sigma unless stated otherwise.

4.2.5. Plasmids

All plasmids were propagated using *Escherichia coli* DH5α and purified using the GenElute HP plasmid maxiprep kit (Sigma). EGFP-H-Ras, EGFP-N-Ras, and EGFP-K-Ras4b where obtained from Mark Philips (NYU).

4.2.6. Cell culture

HCT-116 cells (received from Rana Al Assah, NYUAD) were grown in Dulbecco's Minimal Essential Medium (D6429) plus 10% FBS, 1% non-essential amino acids (NEAA), and maintained at 37 °C, 5% CO_2. For confocal fluorescence microscopy, HCT-116 cells were plated on 24-well glass-bottom plates (Greiner Bio-One) at 4×10^4 cells per well in 0.5 mL medium. The cells were transfected with the appropriate plasmid 24 h post-seeding using TrueFect (United Biosystems) in accordance with the manufacturer's recommendations. The optimised ratio of DNA/TrueFect used for transfection of HCT-116 cells in a 24-well plate was 0.5 μg DNA per 1 μL TrueFect. For CellTiter-Blue assays, HCT-116 cells were plated on 96-well plates (Corning) at 7×10^3 cells per well in 100 μL medium. For Rce1 siRNA knock-down confocal microscopy and RT-PCR experiments, HCT-116 cells were plated on 35-mm glass-bottom dishes (Greiner Bio-One) at 2×10^5 cells per dish in medium (2 mL). The cells were transfected with 100 pmol of either *Silencer*® Cy™3-labeled Negative Control No. 1 siRNA (Life Technologies) or *AccuTarget*™ Premade siRNA Rce1 (Bioneer) and 1 μg of either EGFP-H-Ras, EGFP-N-Ras, or EGFP-K-Ras4b using Lipofectamine® RNAiMAX transfection reagent (3 μL) and Opti-MEM® (Life technologies) in accordance with the manufacturer's recommendations 24 h post-plating.

4.2.7. Treatment of cells with compounds

Cells were transfected with plasmid as described above then treated with the stated concentration of FTI L-744,832 (Calbiochem), Rce1 inhibitors, or DMSO as the vehicle control. Cells were then incubated at 37 °C, 5% CO_2 for an additional 20 h before imaging or determining cell viability.

4.2.8. Cell viability assay

The CellTiter-Blue assay (Promega) was used to assess cell viability of HCT-116 cells following treatment with Rce1 inhibitors. Cells were plated and treated with compounds as described above. CellTiter-Blue was added to the cells 20 h post-treatment per manufacturer's instructions. After 4 h of incubation, fluorescence was measured using 560/590 nm filters on a BioTek Synergy microplate reader. Three independent experiments were performed, averaged, and plotted with SEM bars. The data is represented relative to untreated cells.

4.2.9. Confocal microscopy

Live cell imaging was carried out on HCT-116 cells in 24-well glass-bottom plates (for testing compounds) or 35-mm glass-bottom dishes (for Rce1 siRNA experiments) in a humidified CO_2 incubator (at 37 °C, 5% CO_2) 24 h post-transfection, using an Olympus IX81 with a 30X oil immersion objective (NA = 1.05). Excitation of EGFP was performed using an Argon ion laser at 488 nm. Emitted light was reflected through a 500–600 nm filter from a dichroic mirror. Data capture and extraction was carried out with FluoView10-ASW version 4.0 software (Olympus). At least 40 cells were imaged per condition. The relative distribution of EGFP-Ras isoforms was determined with Fiji image analysis platform[74] and analyzed by a method adapted from Manandhar et al.[41] A ROI line was drawn across the cell to measure the fluorescence intensity. The fluorescence intensity values were normalised (0 to 1) and used to calculate the percentage of the total fluorescence associated with the PM (% PM association). The PM width was set to 1 μm, (which was the average of several EGFP-Ras PM localized cells) and mislocalized fluorescence was defined as any fluorescence 1 μm or more from the PM.

4.2.10. Rce1 siRNA knock-down and RT-PCR

Following imaging by confocal microscopy, cells were lysed and the RNA extracted using the High Pure RNA isolation kit (Roche) according to manufacturer's instructions. The RNA concentration and purity was quantified using a NanoDrop 2000c UV–Vis spectrophotometer (Thermo Scientific). The absorbance was measured at 260 and 280 nm. All RNA was of sufficient purity with a A260/A280 ratio between 1.9–2.1 used in the reverse transcriptase reaction. Total RNA from each sample (2 μg) was reverse transcribed using the SuperScript® VILO™ cDNA synthesis kit (Life Technologies). For PCR amplification the cDNA samples were diluted 10-fold and 5 μL of each cDNA sample was used per reaction combined with a mastermix consisting of 1× NH_4 reaction buffer (Bioline), 80 μM dNTPs (Bioline), 2 mM $MgCl_2$, 20 pmol of each primer (5′-3′) Rce1 forward GTCCTGGTGGTGTCCAGTCT, Rce1 reverse GGAACACCAGCTCCTCTGTC, cyclophilin A forward CACCGTGTTCTTCGACATTG, cyclophilin A reverse GCCATCCAACCACTCAGTCT and 1 U Biotaq DNA polymerase (Bioline), made up to a final volume of 25 μL with dH_2O. The cDNA was amplified using MultiGene™ OptiMax thermal cycler (Labnet International) with cycling parameters as follows: initial denaturation at 94 °C for 2 min, 30 cycles of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min, and hold at 72 °C for 5 min. Products were resolved by 1.2% agarose gel electrophoresis containing SYBR® Safe DNA gel stain (Life Technologies) against HyperLadder™ 50 bp (Bioline).

Acknowledgements

We thank the National Institutes of Health (GM67092) for partial support of this work. We also thank Matteo Lusi and Liang Li for performing X-ray crystallographic structure determinations, Rana Al Assah for providing HCT-116 cells, and Mark Philips for providing the EGFP-Ras constructs. The research was carried out using Core Technology Platform resources at New York University Abu Dhabi and mass spectrometry resources at the Proteomics and Mass Spectrometry Core Facility at the University of Georgia.

Supplementary data

Supplementary data (Figure S1 showing toxicity data, Figures S2 and S3 showing ORTEP diagram for compounds **28** and **36**, proteolysis assay IC_{50} determinations, and NMR spectra) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.11.043.

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