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Lead identification to generate 3-cyanoquinoline inhibitors of insulin-like growth factor receptor (IGF-1R) for potential use in cancer treatment

Lori M. Miller [a,*], Scott C. Mayer [a,*], Dan M. Berger [c], Diane H. Boschelli [c], Frank Boschelli [d], Li Di [a], Xuemei Du [c], Minu Dutia [c], Middleton B. Floyd [c], Mark Johnson [b], Cynthia Hess Kenny [c], Girija Krishnamurthy [c], Franklin Moy [b], Susan Petusky [a], Diane Tkach [d], Nancy Torres [c], Biqi Wu [c], Weixin Xu [b]

[a] Wyeth Research, Chemical & Screening Sciences, Princeton, NJ, USA
[b] Wyeth Research, Chemical & Screening Sciences, Cambridge, MA, USA
[c] Wyeth Research, Chemical & Screening Sciences, Pearl River, NY, USA
[d] Wyeth Research, Oncology, Pearl River, NY, USA

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ABSTRACT

Insulin-like growth factor receptor (IGF-1R) is a growth factor receptor tyrosine kinase that acts as a critical mediator of cell proliferation and survival. Inhibitors of this receptor are believed to provide a new target in cancer therapy. We previously reported an isoquinolinedione series of IGF-1R inhibitors. Now we have identified a series of 3-cyanoquinoline compounds that are low nanomolar inhibitors of IGF-1R. The strategies, synthesis, and SAR behind the cyanoquinoline scaffold will be discussed.

Studies of the insulin-like growth factor receptor tyrosine kinase (IGF-1R) have shown that it is both overexpressed in human cancer cells and involved in the production of new tumors.[1] Therefore, it is believed that inhibitors of IGF-1R may impede tumor growth and provide new cancer therapeutics.[2] Efforts to design new IGF-1R inhibitors are complicated by an 84–85% homology between IGF-1R and insulin receptor (IR) in the kinase domain.[3] Our group previously reported an isoquinolinedione series of compounds that inhibited IGF-1R (Fig. 1).[4] Here we disclose another series of compounds, the cyanoquinolines (Fig. 2, originally from our in-house MEK equity),[5] which show better inhibitory activity than what was observed in the isoquinolinedione series of compounds.

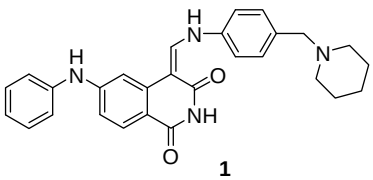

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Figure 1. Representative compound from the isoquinolinedione series.

The cyanoquinoline series was found in a high-throughput screen (HTS) using a Lance enzyme assay.[6] Input from structural biology and data mining provided additional information about the characteristics of this lead series.

Evidence of stoichiometric binding was obtained using the changes in the intrinsic fluorescence of the protein in the presence of increasing concentrations of the inhibitor, with K_D value estimated to be less than 10 nM (Fig. 3).[7] Assuming 1:1 binding model for the non-hyperbolic curve, it is estimated that 38% of the protein binds to the inhibitor. A co-crystal structure of **2**, shown in Figure

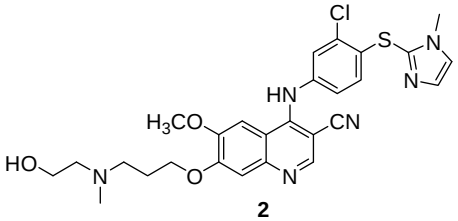

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Figure 2. Representative compound from the cyanoquinoline series.

* Corresponding authors. Tel.: +1 732 274 4426; fax: +1 732 274 4505.
E-mail addresses: millerl9@wyeth.com (L.M. Miller), mayers@wyeth.com (S.C. Mayer).

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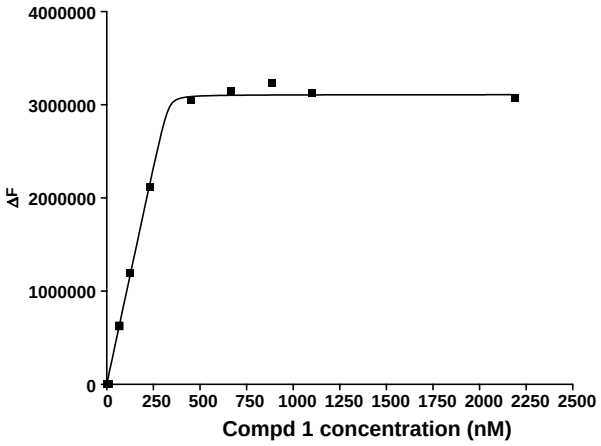


Figure 3. Binding isotherm of the interactions of compound **1** with IGF-1R. The symbols represent the changes in the intrinsic fluorescence of the protein as a function of the increasing concentration of the compound. The solid line represents the data fitted to a 1:1 binding model for the interactions of compound **1** with the protein. The non-hyperbolic binding isotherm is consistent with tight binding and K_D value <10 nM.

4, showed binding similar to what was found in molecular modeling studies. Figure 4 shows that the key interaction between IGF-1R and **1** is through the backbone NH of Met1082 and the quinoline core nitrogen of **2**, with the imidazole headgroup also breaking the conserved interaction between K1033 and E1050.[8]

Compounds in the corporate database with the same core structure as **2** gave some insight as to which regions of the molecule would be important for inhibitory activity. Table 1 shows that these areas are the 2-thio-imidazole head piece (**8** vs **9** and **10**), chlorine substitution at R^3 (**8** vs **7**) and the basic amine in R^2 (**2**–**6**, **8** vs **11** and **12**). Additionally, methylation of the N-linkage provided equal potency (**5** vs **6**). Using this information, additional libraries (compounds **13**–**42**) were prepared to expand our knowledge of the SAR, with **29** being one of the most potent compounds made in this series.

Out of the additional libraries made (Table 1, compounds **13**–**42**), **19** and **22** showed that a basic amine in the tailpiece region of the molecule was necessary for inhibitory activity. Many different tertiary amines were tolerated at this position, as shown in Table 1. Also, halogenation at R^3 was crucial for biological activity (**16** and **17** vs **18**). The most interesting SAR was found when changes were

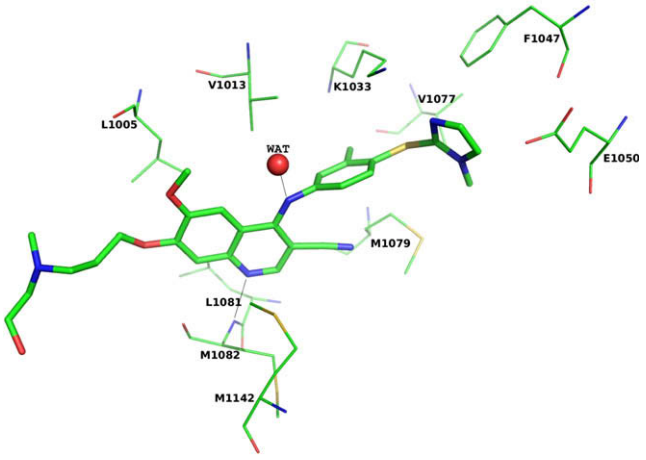
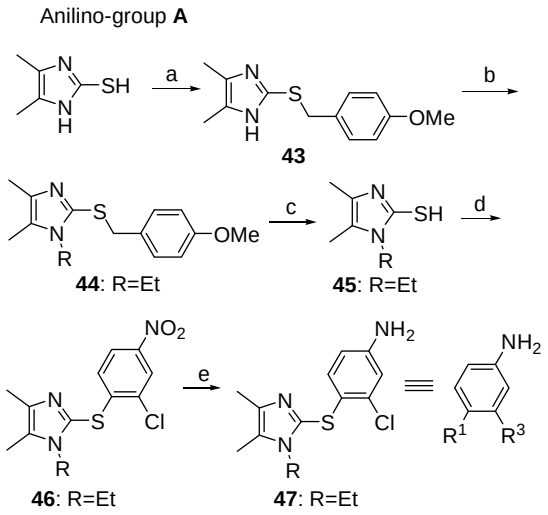


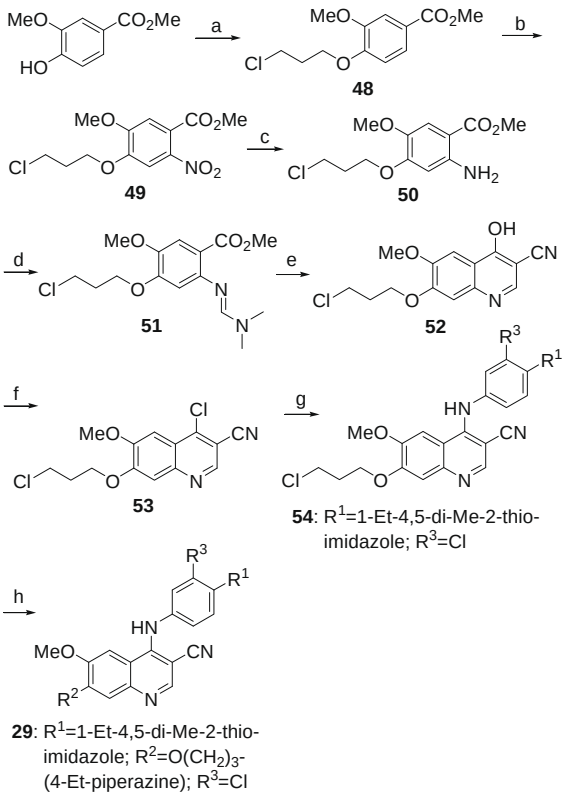
Figure 4. Co-crystal structure of compound **2** in IGF-1R. The key interaction is between Met1082's backbone NH and the quinoline core nitrogen of **2**.[8]



Scheme 1. Reagents: (a) DIEA, 4-OMe-BnCl, NMP; (b) i—NaH; ii—RX, DMF; (c) TFA, toluene; (d) i—NaH; ii—3-Cl-4-F-nitrobenzene, DMF; (e) Fe, NH_4Cl, MeOH, H_2O.

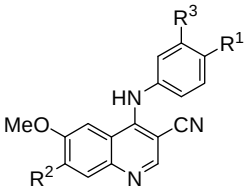
made to the 2-thio-imidazole head piece. Multiple alkyl substitution on this heterocycle, as first investigated by other kinase programs such as MEK,[5] afforded a 6- to 15-fold increase in potency (**5** vs **13** and **16**, **3** vs **29**). Despite the aqueous solubility challenges of this series (i.e., ~2 mg/mL at pH 7.4 for **29**), a cellular myeloid assay showed encouraging data, with an IC_{50} of 90 nM for **29**.[9]

The compounds in the cyanoquinoline series made to date have been more selective for IR over IGF1-R [i.e., the selectivity ratio



Scheme 2. Reagents: (a) 3-Cl-propyl Br, Cs_2CO_3, acetone; (b) HNO_3; (c) Fe, NH_4Cl, MeOH, H_2O; (d) DMF·DMA; (e) *n*-BuLi, CH_3CN, THF; (f) $POCl_3$, DMF (cat.); (g) anilino-group **A**, EtO–ethanol, pyr·HCl; (h) R^2 amine, DMF, NaI.

Table 1
IGFR inhibitory activity



Compound	R^1	R^2	R^3	IGFR IC_{50}[a] (μM)
2[b]	1-Me-2-thio-imidazole	$O(CH_2)_3NMe(CH_2)_2OH$	Cl	0.140 ± 0.088
3[c]	1-Me-2-thio-imidazole	$O(CH_2)_3$-(4-Et-piperazine)	Cl	0.050 ± 0.011
4[c]	1-Me-2-thio-imidazole	$O(CH_2)_3$-pyrrolidine	Cl	0.088
5[c]	1-Me-2-thio-imidazole	$NH(CH_2)_3NMe_2$	Cl	0.151 ± 0.008
6[c]	1-Me-2-thio-imidazole	$NMe(CH_2)_3NMe_2$	Cl	0.15
7[c]	1-Me-2-thio-imidazole	$O(CH_2)_3$-morpholine	H	1.00
8[c]	1-Me-2-thio-imidazole	$O(CH_2)_3$-morpholine	Cl	0.066
9[c]	Thio-phenol	$O(CH_2)_3$-morpholine	H	1.09
10[c]	Thio-2-pyridine	$O(CH_2)_3$-morpholine	Cl	>10
11[c]	1-Me-2-thio-imidazole	OMe	Cl	0.847
12[c]	1-Me-2-thio-imidazole	4-Me-piperazine	Cl	0.6
13	1,4,5-tri-Me-2-thio-imidazole	$NH(CH_2)_3NMe_2$	Cl	0.026 ± 0.008
14	1-Bn-4,5-di-Me-2-thio-imidazole	$NH(CH_2)_3NMe_2$	Cl	0.065 ± 0.034
15	1,5-di-Me-2-thio-benzimidazole	$NH(CH_2)_3NMe_2$	Cl	0.291
16	1-Et-4,5-di-Me-2-thio-imidazole	$NH(CH_2)_3NMe_2$	Cl	0.010 ± 0.004
17	1,4,5-tri-Me-2-thio-imidazole	$NH(CH_2)_3NMe_2$	Br	0.030
18	1,4,5-tri-Me-2-thio-imidazole	$NH(CH_2)_3NMe_2$	H	0.394
19	1-Et-4,5-di-Me-2-thio-imidazole	$NH(CH_2)_3Ph$	Cl	13.7 ± 4.98
20	1-Et-4,5-di-Me-2-thio-imidazole	$NH(CH_2)_3$-morpholine	Cl	0.020 ± 0.011
21	1-Et-4,5-di-Me-2-thio-imidazole	$NH(CH_2)_3N(CH_2CH_2OH)_2$	Cl	0.026 ± 0.007
22	1-Et-4,5-di-Me-2-thio-imidazole	$O(CH_2)_3NHSO_2Ph$	Cl	3.88 ± 1.079
23	1-Et-4,5-di-Me-2-thio-imidazole	$O(CH_2)_3N(t\text{-Bu})CH_2CH_2OH$	Cl	0.024
24	1-Et-4,5-di-Me-2-thio-imidazole	$O(CH_2)_3NMe_2$	Cl	0.030
25	1-Et-4,5-di-Me-2-thio-imidazole	$O(CH_2)_3$-(4-Et carbamyl-piperazine)	Cl	0.045 ± 0.005
26	1-Et-4,5-di-Me-2-thio-imidazole	$O(CH_2)_3$-(2-Et-piperidine)	Cl	0.032 ± 0.008
27	1-Et-4,5-di-Me-2-thio-imidazole	$O(CH_2)_3$-(4-Et-carboxy-piperidine)	Cl	0.135 ± 0.01
28	1-Et-4,5-di-Me-2-thio-imidazole	$O(CH_2)_3$-$NHSO_2Me$	Cl	0.031 ± 0.006
29	1-Et-4,5-di-Me-2-thio-imidazole	$O(CH_2)_3$-(4-Et-piperazine)	Cl	0.009 ± 0.003
30	1-Et-4,5-di-Me-2-thio-imidazole	$O(CH_2)_3$-(4-Et-OH-piperazine)	Cl	0.011
31	1-Et-4,5-di-Me-2-thio-imidazole	$O(CH_2)_3NMe(CH_2)_2OH$	Cl	0.014
32	1-Et-4,5-di-Me-2-thio-imidazole	$O(CH_2)_3$-(3-OH-pyrrolidine)	Cl	0.020
33	1-Et-4,5-di-Me-2-thio-imidazole	$O(CH_2)_3$-pyrrolidine	Cl	0.067
34	1-Et-4,5-di-Me-2-thio-imidazole	$O(CH_2)_3$-piperazine-Et carbamate	Cl	0.017
35	1-Et-4,5-di-Me-2-thio-imidazole	$O(CH_2)_3NMeCH_2CHOHCH_2OH$	Cl	0.014
36	1-Et-4,5-di-Me-2-thio-imidazole	$O(CH_2)_3$-morpholine	Cl	0.015 ± 0.003
37	1-Et-4,5-di-Me-2-thio-imidazole	$O(CH_2)_3NMeCH_2CH_2$-(1,3-dioxolane)	Cl	0.013
38	1-Et-4,5-di-Me-2-thio-imidazole	$O(CH_2)_3$-(4-OH-piperidine)	Cl	0.047
39	1-Et-4,5-di-Me-2-thio-imidazole	$O(CH_2)_3$-(4-Ac-piperazine)	Cl	0.016
40	1-Et-4,5-di-Me-2-thio-imidazole	$NH(CH_2)_3$-(4-Me-piperazine)	Cl	0.016 ± 0.002
41	1-Pr-4,5-di-Me-2-thio-imidazole	$NH(CH_2)_3NMe_2$	Cl	0.189 ± 0.032
42	1-*i*-Pr-4,5-di-Me-2-thio-imidazole	$NH(CH_2)_3NMe_2$	Cl	0.079 ± 0.008

[a] Concentration inducing 50% inhibition of IGFR ($N = 2–3$).[4]
[b] See Ref. 8.
[c] See Ref. 4.

(IC_{50} IR inhibition/IC_{50} IGFR inhibition) for **29** is 0.17].[6] While disappointing, this result was not unexpected due to the homology between the two receptors.

The preparation of the compounds is shown in Schemes 1 and 2. The anilino-group **A** (Scheme 1) was created by reaction of 4,5-dimethyl-1H-imidazole-2-thiol and 4-methoxy benzyl chloride followed by the addition of sodium hydride and the appropriately substituted alkyl halide. After removal of the protecting group with TFA, the compound was subjected to sodium hydride and 3-chloro-4-fluoro-nitrobenzene, and then further reduced to the aniline with iron in the presence of ammonium chloride to provide the anilino-group **A**.

The cyanoquinoline core for R^2 O-linkage (Scheme 2) was completed after methyl vanillate was reacted with 3-chloro-propyl bromide in the presence of cesium carbonate, followed by nitration with HNO_3. This nitro group was reduced with iron in the presence of ammonium chloride, and the aniline was heated with DMF·DMA. The cyanoquinoline core was formed after cyclization with the lithium anion of acetonitrile, and the resulting intermediate was converted to the chloride with $POCl_3$. The chloride was reacted with the anilino-group **A** in the presence of pyridine HCl, followed by the reaction of the R^2 amine in the presence of NaI to afford the final O-linked products (see Table 1).[10] Generation of products with the R^2 N-linkage has been previously reported.[11]

To conclude, a series of cyanoquinoline compounds were found to be low nanomolar inhibitors of IGF-1R. These compounds had better potency than our previously reported isoquinolinedione compounds. While SAR studies focused on three different areas of the molecule, we found the most significant improvement in activity with multiple alkyl substitutions on the 2-thio-imidazole headpiece. Compound **29** had encouraging activity in a cellular myeloid assay, but overall the cyanoquinoline compounds were

more selective for IR instead of IGF-1R. The goal for future analogs will be to maintain potency while achieving selectivity for IGF-1R over IR.

Acknowledgments

We are grateful to the Discovery Analytical Chemistry department of Wyeth Research for elemental analyses, ^{1}H NMR, and mass spectroscopy and Dr. John A. Butera, Dr. John Ellingboe, Ms. Annette Banker, Ms. Joan Sabalski, and Mr. Gengcheng Yang for scientific discussions.

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6. IGF-1R and IR kinase assays: The catalytic domains of IGF-1R or IR were cloned described in Pautsch et al.[12] into pAcG2T [BD Biosciences (San Jose, CA)]. GST-IGF-1R and GST-IR fusion proteins were isolated by glutathione bead capture, eluted, incubated in 20 mM Tris–HCl, pH 7.5, 0.2 M NaCl, 0.01 M $MgCl_2$ and 7.5 mM ATP for 12 min. followed by thrombin cleavage, purification on a glutathione column and MonoQ fractionation. IGF-1R or IR catalytic domains phosphorylated on all three tyrosines in the activation loop as determined by mass spectrometry were then purified on a Superdex 200 column. This 'tris' phosphorylated protein was used in enzyme assays at 0.6 ng per reaction in 50 mM Hepes, pH 7.5, 0.01 M $MgCl_2$ with 150 μg/mL of bovine serum albumin (Sigma #A-8918) and peptide at a final concentration of 1 μg/mL (Biotin-NH_2-TRDIYETDYYRK-OH) for 90 min at room temperature. The final ATP concentration was 100 μM.
7. (a) Jamieson, E. R.; Jacobson, M. P.; Barnes, C. M.; Chow, C. S.; Lippard, S. J. *J. Biol. Chem.* **1999**, *274*, 12346; (b) The changes in the intrinsic fluorescence of the protein (at 1 μM) were measured in the absence and presence of various concentrations (0–2200 nM) of compound **2**, using an excitation wavelength of 295 nm and emission at 347 nm. The fluorescence emission spectra of the protein were recorded from 310 to 580 nm using the Jobin Yvon Spex fluorometer. The reaction buffer contained 50 mM Hepes, pH 7.5, and 10 mM $MgCl_2$. Increasing concentrations of the inhibitor quenched the fluorescence and the changes were used to determine the binding affinity.
8. (a) Xu, W.; Dwyer, B.; Moy, F.; Mayer, S.; Boschelli, F.; Tkach, D.; Bean, K.; Mosyak, L.; Wilhelm, J. Presented at the 5th Annual Meeting of Protein Kinase Targets & Structure-Based Design, Boston, MA, June 2007; poster session.; (b) RCSB Protein Data Bank (PDB) deposition number 3F5P.
9. IGF1-R dependent proliferation assay: Full-length IGF1-R was cloned from a human placenta cDNA library (Invitrogen) and inserted into pIRESneo2 (BD Biosciences). IL-3 dependent FDCP1 myeloid cells (ATCC) were transformed with or an IGF1-R expression clone pIRESneo2-IGFR clone (NM_000875). G418 resistant cells were selected by limiting dilution. IGF1-R expressing clones that acquired IL-3 independence in the presence of IGF1 (10 ng/mL) were identified from these clones. For 96-well proliferation assays, 2000 cells were plated per well on day 1 with 10 ng/mL IGF1, compound was added on day 2, and relative cell number was determined by addition of CellTiter-Glo (Promega). IC_{50}s were determined using model 63 of the LSW Excel plug-in.
10. *Experimental:* The synthesis of compound **29** described here serves as a representative example of the synthetic methodology used in this paper.
Step 1: 2-(4-Methoxy-benzylsulfanyl)-4,5-dimethyl-1H-imidazole (**43**). To a stirred solution of 4,5-dimethyl-1H-imidazole-2-thiol (2.00 g, 15.6 mmol) in NMP (50 mL) was added DIEA (2.72 mL, 16.4 mmol) and 5-methoxybenzyl chloride (2.72 mL, 16.4 mmol). The reaction mixture was stirred at rt overnight, diluted with water, and the precipitate was collected via vacuum filtration to give the desired product (3.14 g, 12.6 mmol, 81%) as a white solid; 1H NMR (DMSO-d_6) δ 1.97 (2, 6H), 3.67 (s, 3H), 4.06 (s, 2H), 6.78 (d, J = 8.7 Hz, 2H), 7.14 (d, J = 8.7 Hz, 2H), 11.67 (s, 1H); mass spectrum [(+) ESI], m/z 249 $(M+H)^+$.
Step 2: 1-Ethyl-2-(4-methoxy-benzylsulfanyl)-4,5-dimethyl-1H-imidazole (**44**). To a stirred solution of 2-(4-methoxy-benzylsulfanyl)-4,5-dimethyl-1*H*-imidazole (3.14 g, 12.6 mmol) in DMF (30 mL) at 0 °C was added NaH (0.56 g, 13.9 mmol). After 15 min at 0 °C iodoethane (1.11 mL, 13.9 mmol) was added slowly, the reaction mixture was stirred 15 min at 0 °C, then warmed to rt and stirred overnight. The reaction was quenched with MeOH (20 mL), diluted with water (10 mL) and extracted with EtOAc (3× 50 mL). The organic layer was washed with brine (50 mL), dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography (0–5% MeOH: $CHCl_3$ gradient) to afford the product (2.9 g, 12.6 mmol, 83%) as an oil; ^{1}H NMR (DMSO-d_6) δ 0.96 (t, J = 14.5 Hz, 3H), 1.97–2.03 (m, 2H), 2.69 (s, 3H), 2.84 (s, 3H), 3.67 (s, 3H), 4.04 (s, 2H), 6.78 (d, J = 8.7 Hz, 2H), 7.10 (d, J = 8.7 Hz, 2H), 11.64 (s, 1H); mass spectrum [(+) ESI], m/z 277 $(M+H)^+$.
Step 3: 1-Ethyl-4,5-dimethyl-1H-imidazole-2-thiol (**45**). To a stirred solution of 1-ethyl-2-(4-methoxy-benzylsulfanyl)-4,5-dimethyl-1*H*-imidazole (3.48 g, 12.6 mmol) in toluene (10 mL) was added TFA (10 mL). The reaction mixture was heated at 100 °C for 60 h. The solution was concentrated and purified by column chromatography (0–5% MeOH/$CHCl_3$ gradient) to afford the product (1.51 g, 9.66 mmol, 77%) as an oil; ^{1}H NMR (DMSO-d_6) δ 1.09 (t, J = 14.3 Hz, 3H), 1.96 (s, 3H), 2.06 (s, 3H), 3.88 (q, J = 19.5 Hz, 2H), 7.90 (s, 1H); mass spectrum [(+) ESI], m/z 157 $(M+H)^+$.
Step 4: 2-(2-Chloro-4-nitro-phenylsulfanyl)-1-ethyl-4,5-dimethyl-1H-imidazole (**46**). To a stirred solution of 1-ethyl-4,5-dimethyl-1*H*-imidazole-2-thiol (1.51 g, 9.66 mmol) in DMF (20 mL) at 0 °C was added NaH (0.425 g, 10.6 mmol). After 15 min at 0 °C, 3-chloro-4-fluoronitrobenzene (0.1.70 g, 9.66 mmol) was added slowly, the reaction mixture was stirred at 0 °C, 30 min, warmed to rt and stirred overnight. The reaction was diluted with water (20 mL) and extracted with EtOAc (3× 50 mL). The organic layer was washed with brine (50 mL), dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography (0–10% MeOH/$CHCl_3$ gradient) to afford the product (2.2 g, 7.06 mmol, 73%) as an oil; ^{1}H NMR (DMSO-d_6) δ 1.06 (t, J = 14.5 Hz, 3H), 2.10 (s, 3H), 2.18 (s, 3H), 3.88 (q, J = 21.7 Hz, 2H), 6.61 (d, J = 8.9 Hz, 1H), 8.04 (dd, J = 2.4, 8.9 Hz, 1H), 8.29 (s, 1H); mass spectrum [(+) ESI], m/z 312 $(M+H)^+$.
Step 5: 3-Chloro-4-(1-ethyl-4,5-dimethyl-1H-imidazol-2-ylsulfanyl)-phenylamine (anilino-group **A**, **47**). To a stirred solution of 2-(2-chloro-4-nitro-phenylsulfanyl)-1-ethyl-4,5-dimethyl-1*H*-imidazole (2.20 g, 7.06 mmol) in MeOH:H_2O (5:1, 54 mL) was added iron (1.24 g, 22.3 mmol) followed by NH_4Cl (1.24 g, 34.2 mmol). The reaction mixture was heated to reflux for 2 h, cooled to rt, diluted with water (20 mL) and EtOAc (60 mL), filtered through celite and washed with EtOAc. The layers were separated, the organic layer was washed with brine (60 mL), dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography (0–5% MeOH: $CHCl_3$) to afford the product (1.4 g, 4.97 mmol, 71%) as a solid; ^{1}H NMR (DMSO-d_6) δ 0.98 (t, J = 14.5 Hz, 3H), 1.99 (s, 3H), 2.08 (s, 3H), 3.89 (q, J = 21.7 Hz, 2H), 5.47 (s, 2H), 6.68–6.74 (m, 3H); mass spectrum [(+) ESI], m/z 282 $(M+H)^+$.
Step 6: Methyl 4-(3-chloropropoxy)-3-methoxybenzoate (**48**). To a stirred solution of methyl vanillate (2.00 g, 11.0 mmol) in acetone (50 mL) was added Cs_2CO_3 (3.94 g, 12.1 mmol) followed by 3-chloro-1-propyl bromide (2.16 mL, 22.0 mmol). The solution was stirred at rt for 3 days. The solution was then diluted with H_2O (50 mL) and extracted with Et_2O (2× 200 mL). The organic layer was washed with brine, dried ($MgSO_4$), and concentrated to afford the product (0.290 g, 85%) as a solid; ^{1}H NMR (DMSO-d_6) δ 2.10–2.20 (m, 2H), 3.74 (t, J = 6.5 Hz, 2H), 3.77 (s, 6H), 4.12 (t, J = 6.0 Hz, 2H), 7.06 (d, J = 8.5 Hz, 1H), 7.41 (d, J = 2.0 Hz, 1H), 7.53 (dd, J = 2.1, 8.5 Hz, 1H); mass spectrum [(+) ESI], m/z 259 $(M+H)^+$.
Step 7: Methyl 4-(3-chloropropoxy)-5-methoxy-2-nitrobenzoate (**49**). To a cooled flask of HNO_3 (12 mL) at 0 °C was slowly added methyl 4-(3-chloropropoxy)-3-methoxybenzoate (2.75 g, 10.6 mmol) portion wise over 15 min. The solution was stirred at this temperature for 0.5 h and then at rt for 1 h. The solution was cooled back down to 0 °C and diluted H_2O (~10 mL). The mixture was stirred for 0.5 h, and a yellowish orange oily solid came out. The mixture was extracted with Et_2O, and the resulting organic layer washed with brine, dried ($MgSO_4$), and concentrated to afford the product (3.03 g, 94%); ^{1}H NMR (DMSO-d_6) δ 2.12–2.21 (m, 2H), 3.73 (t, J = 6.5 Hz, 2H), 3.78 (s, 3H), 3.88 (s, 3H), 4.20 (t, J = 6.1 Hz, 2H), 7.28 (s, 1H), 7.63 (s, 1H); mass spectrum [(+) ESI], m/z 326 $(M+Na)^+$.
Step 8: Methyl 2-amino-4-(3-chloropropoxy)-5-methoxybenzoate (**50**). To a stirred solution of methyl 4-(3-chloropropoxy)-5-methoxy-2-nitrobenzoate (3.03 g, 9.98 mmol) in MeOH:H_2O (5:1, 48 mL) was added iron (2.23 g, 39.9 mmol) followed by NH_4Cl (2.13 g, 39.9 mmol). The solution was heated to reflux overnight. The solution was cooled to rt and diluted with EtOAc (100 mL) and H_2O (30 mL). The mixture was filtered through celite, and the filtrate layers were separated. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated. The residue was purified with Biotage Flash 40 (10–30% EtOAc/petroleum ether gradient) to afford the product (2.07 g, 76%); ^{1}H NMR (DMSO-d_6) δ 2.11–2.19 (m, 2H), 3.60 (s, 3H), 3.70 (s, 3H), 3.73 (t, J = 6.5 Hz, 2H), 4.00 (t, J = 6.1 Hz, 2H), 6.35 (s, 1H), 6.37 (s, 2H), 7.09 (s, 1H); mass spectrum [(+) ESI], m/z 274 $(M+H)^+$.
Step 9: (E)-Methyl 4-(3-chloropropoxy)-2-[(dimethyl-amino)methyleneamino]-5-methoxybenzoate (**51**). To a flask with methyl 2-amino-4-(3-chloropropoxy)-5-methoxy-benzoate (2.07 g, 7.56 mmol) was added DMF·DMA (12 mL). The solution was heated to reflux for 4 h. The solvent was remove via high vacuum, and the resulting residue was passed through florasil, eluting with CH_2Cl_2, and then concentrated to afford the product (2.47 g, 99%); ^{1}H NMR (DMSO-d_6) δ 2.08–2.17 (m, 2H), 2.83–2.95 (br s, 6H), 3.64 (s, 3H), 3.68 (s, 3H), 3.73 (t, J = 6.5 Hz, 2H), 4.07 (t, J = 6.0 Hz, 2H), 6.44 (s, 1H), 7.11 (s, 1H), 7.43 (s, 1H); mass spectrum [(+) ESI], m/z 329 $(M+H)^+$.

Step 10: 7-*(3-Chloropropoxy)-4-hydroxy-6-methoxyquino-line-3-carbonitrile* (**52**). To a stirred solution of *n*-BuLi in hexanes (6.76 mL, 2.5 M solution) at −78 °C was added a solution of acetonitrile (0.88 mL, 16.9 mmol) in THF (20 mL) dropwise. The reaction mixture was stirred ~15 min at this temperature. Then a solution of (*E*)-methyl4-(3-chloropropoxy)-2-[(dimethylamino) methyleneamino]-5-methoxybenzoate (2.52 g, 7.66 mmol) in THF (20 mL) was added dropwise over 20 min. The mixture was kept at −78 °C for 2 h and then removed dry ice bath for 0.5 h. After replacing the dry ice bath, HOAc (2.19 mL, 38.3 mmol) was added dropwise, and the mixture was warmed to rt overnight. The solvent was removed via high vacuum, and the residue was diluted with H_2O (~20 mL). The resulting yellow precipitate was filtered off and washed with excess H_2O and Et_2O to afford the product (1.90 g g, 85%); ^{1}H NMR (DMSO-d_6) δ 2.15–2.24 (m, 2H), 3.77 (t, *J* = 6.5 Hz, 2H), 3.81 (s, 3H), 4.13 (t, *J* = 6.0 Hz, 2H), 7.01 (s, 1H), 7.41 (s, 1H), 8.40 (s, 1H), 11.74–12.47 (br s, 1H); mass spectrum [(+) ESI], *m*/*z* 293 $(M+H)^+$.

Step 11: 4-Chloro-7-(3-chloropropoxy)-6-methoxyquino-line-3-carbonitrile (**53**). A stirred solution of 7-(3-chloropropoxy)-4-hydroxy-6-methoxyquinoline-3-carbo-nitrile (1.90 g, 6.49 mmol) in $POCl_3$ (10 mL) with a few drops DMF (~20) was heated to 110 °C for 1 h. After cooling to rt, the solution was concentrated and azetroped with toluene. The residue was poured over ice/H_2O and neutralized with satd $NaHCO_3$. The precipitate was collected via vacuum filtration, washed with excess H_2O and Et_2O. It was purified with Biotage Flash 40 (20–40% EtOAc/petroleum ether gradient) to afford the product (1.39 g, 69%); ^{1}H NMR (DMSO-d_6) δ 2.21–2.29 (m, 2H), 3.78 (t, *J* = 6.4 Hz, 2H), 3.98 (s, 3H), 4.32 (t, *J* = 6.0 Hz, 2H), 7.42 (s, 1H), 7.54 (s, 1H), 8.95 (s, 1H); mass spectrum [(+) ESI], *m*/*z* 311/313 $(M+H)^+$.

Step 12: 4-[3-Chloro-4-(1-ethyl-4,5-dimethyl-1H-imidazol-2-ylsulfanyl)-phenylamino]-7-(3-chloro-propoxy)-6-meth-oxy-quinoline-3-carbonitrile (**54**). To a stirred solution of 4-chloro-7-(3-chloro-propoxy)-6-methoxy-quinoline-3-carbonitrile (0.298 g, 0.958 mmol) in ethoxyethanol (6 mL) was added 3-chloro-4-(1-ethyl-4,5-dimethyl-1H-imidazol-2-ylsulfanyl)-phenylamine (0.270 g, 0.958 mmol) and pyridine HCl (0.122 g, 1.05 mmol). The mixture was heated at 120 °C overnight. The solution was cooled to rt, quenched with satd $NaHCO_3$ solution (10 mL), stirred at rt for 30 min and the precipitate was collected via vacuum filtration. The solid was absorbed on silica and purified by flash chromatography (0–3% MeOH/$CHCl_3$ gradient) to afford the product (0.240 g, 0.431 mmol, 45%) as a solid; mass spectrum [(+) ESI], *m*/*z* 557 $(M+H)^+$.

Step 13: 4-({3-Chloro-4-[(1-ethyl-4,5-dimethyl-1H-imidazol-2-yl) sulfanyl]phenyl} amino)-7-[3-(4-ethyl-1-piperazinyl)propoxy]-6-methoxy-3-quinolinecarbonitrile (**29**). To a stirred solution of 4-[3-chloro-4-(1-ethyl-4,5-dimethyl-1H-imidazol-2-ylsulfanyl)-phenylamino]-7-(3-chloro-propoxy)-6-methoxy-quinoline-3-carbonitrile (0.300 g, 0.539 mmol) in DMF (2 mL) was added 1-ethyl-piperazine (0.137 mL, 1.08 mmol) and NaI (0.008 g, 0.0539 mmol). The solution was heated at 100 °C overnight. The solution was cooled to rt and diluted with brine (2 mL) and H_2O (2 mL). The precipitate was collected via vacuum filtration and washed with H_2O and Et_2O to afford the product (0.290 g, 85%) as a solid, mp 229–230 °C; ^{1}H NMR (DMSO-d_6) δ 0.93 (t, *J* = 7.2 Hz, 3H), 1.04 (t, *J* = 7.1 Hz, 3H), 1.85–1.95 (m, 2H), 2.07 (s, 3H), 2.15 (s, 3H), 2.22–2.43 (m, 12H), 3.86 (s, 3H), 3.90 (dd, *J* = 7.1, 14.4 Hz, 2H), 4.14 (t, *J* = 6.4 Hz, 2H), 6.58 (d, *J* = 8.6 Hz, 1H), 7.06 (dd, *J* = 2.3, 8.7 Hz, 1H), 7.29 (s, 1H), 7.30 (d, *J* = 2.3 Hz, 1H), 7.61 (s, 1H), 8.45 (s, 1H), 9.46 (s, 1H); mass spectrum [(+) ESI], *m*/*z* 634/636 $(M+H)^+$.

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