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Discovery of aminoquinolines as a new class of potent inhibitors of heat shock protein 90 (Hsp90): Synthesis, biology, and molecular modeling

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ABSTRACT

The molecular chaperone Hsp90 plays important roles in maintaining malignant phenotypes. Recent studies suggest that Hsp90 exerts high-affinity interactions with multiple oncoproteins, which are essential for the growth of tumor cells. As a result, research has focused on finding Hsp90 probes as potential and selective anticancer agents. In a high-throughput screening exercise, we identified quinoline **7** as a moderate inhibitor of Hsp90. Further hit identification, SAR studies, and biological investigation revealed several synthetic analogs in this series with micromolar activities in both fluorescent polarization (FP) assay and a cell-based Western blot (WB) assay. These compounds represent a new class of Hsp90 inhibitors with simple chemical structures.

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1. Introduction

Heat shock protein 90 (Hsp90) has emerged as an important biological target that modulates a variety of cellular processes including cell maturation, stability, and conformational maintenance of signature cancer proteins such as Akt, Raf-1, HER-2/ErbB2, and p53.[1] Reports indicate that Hsp90 from stress-induced cells exhibits a higher affinity for small molecule inhibitors relative to normal cells as a result of increased refolding requirements of its mutated or altered clients.[2,3] Thus, identification of selective tumor-specific Hsp90 inhibitors could lead to the specific targeting of cancer cells and circumvent systemic toxicities. The antibiotic geldanamycin (**1a**) and its synthetic analogs (17-AAG, 17-DMAG, **1b**, **1c**)[4] were the first small molecules found to bind competitively to the ATP-binding pocket in the Hsp90 N-terminal domain and thereby prevent the ATP-mediated conformational change needed for protein–protein interactions of Hsp90 with its client polypeptides. While geldanamycin analogs are showing promising anticancer activity in clinical trials,[5]several other compounds either from natural or synthetic sources have also been identified (Fig. 1). Notable among these is the natural product radicicol (**2**),[6] which exhibits potent in vitro Hsp90 inhibitory activity, but lacks in vivo efficacy. The first reported synthetic class of Hsp90 inhibitors was the purine-scaffold series, represented by PU3 (**4**).[7] The purine-scaffold was subsequently developed into the clinical agents PU-H71 (**5**)[8] and CNF2024 (**6**).[9] Very recently, the pyrazole/isoxazole class of compounds (**3a** and **3b**) was discovered from high-throughput screening.[10] All these known analogs utilize the same binding pocket as geldanamycin on Hsp90 (Fig. 1).[8b,11,13]

Two strategies have proven useful for identifying Hsp90 inhibitors. On one hand, structure-based design strategies were employed to develop potent analogs[8,9,12] with X-ray templates of Hsp90–ligand complexes.[13] On the other hand, high-throughput screening (HTS) methods are being investigated to find novel chemical scaffolds.[14] In the present study, we employed a fluorescence polarization (FP) assay[15] to identify a new class of Hsp90 inhibitors by HTS. The aminoquinoline **7** was identified as a marginally active Hsp90 inhibitor hit. Structure validation, re-synthesis, and structure–activity relationship (SAR) studies led to several aminoquinoline analogs as low micromolar inhibitors of Hsp90. The best compound **10** exhibits low micromolar activities in both primary FP and cell-based Western blot (WB) assays (Fig. 2). Hit identification, synthesis, SAR and bio-structural analysis of the quinoline derivatives are described in detail below.

2. Results and discussion

Various bioassays have been developed to identify novel inhibitors of Hsp90.[16] Most of these assays are amenable to HTS, since they report the interaction of a small molecule with recombinant Hsp90-α or -β. These proteins are derived from yeast or human

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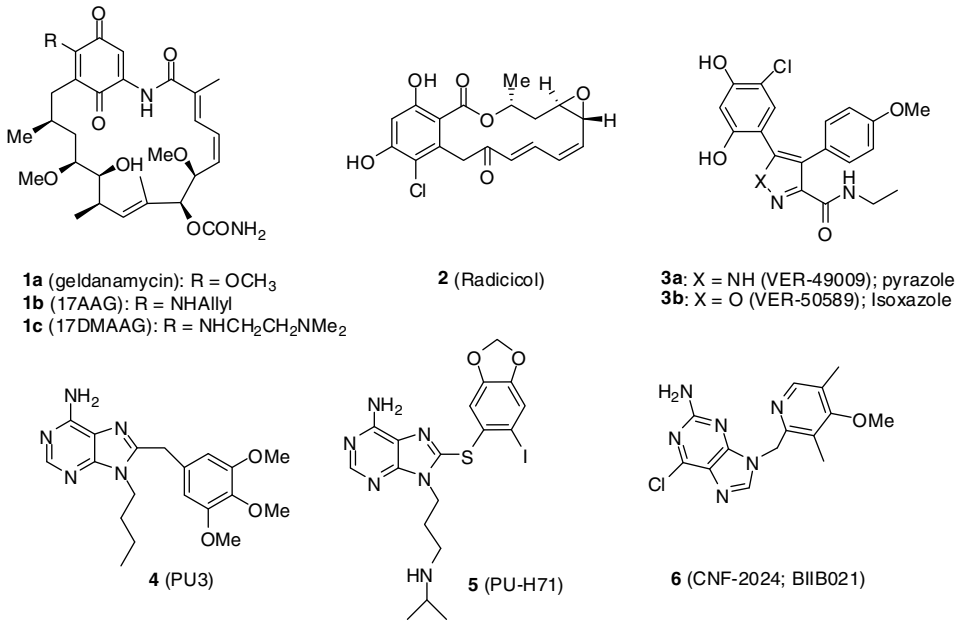


Figure 1. Structures of known Hsp90 inhibitors and purine analogs.

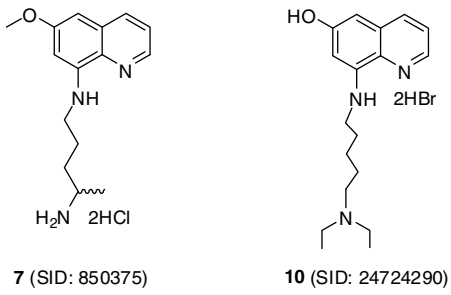


Figure 2. Hsp90 hit compound **7** and the most active analog **10**.

normal cells, respectively, in which Hsp90 is found to be in a latent, low-affinity form, compared to the high-affinity state present in tumor cells.[17] One way to determine the therapeutically relevant state of Hsp90 is to develop an assay that makes use of human cancer cell derived lysates, instead of recombinant protein. The former permits direct measurement of the interaction between small molecule and tumor-specific Hsp90. Such an assay is capable of leading to identification of molecules that are specific for tumor cell Hsp90. By employing a recently developed FP assay for HTS,[15] we have identified a highly promising compound, namely **7**, for follow-up by chemistry and molecular modeling.[18]

2.1. Hit identification

In a high-throughput screening, it has become common for molecules with known biological activities to emerge as modulators of novel targets.[19] One example in the present work is the anti-malarial agent quinocide-dihydrochloride, namely 8-(4-aminopentylamino)-6-methoxyquinoline-dihydrochloride (**7**),[20] which appeared as a novel scaffold in the modulation of Hsp90 (Fig. 2). This compound showed activities in the low micromolar range in both FP and WB assays (Table 1). The FP assay measures the interaction of Cy3b-conjugated geldanamycin with Hsp90 in tumor cell lysates for identifying ATP-binding inhibitors. Hsp90 uniquely stabilizes the Her2/Hsp90 association.[21] Addition of Hsp90 inhibitors to cancer cells induces the proteasomal degradation of a small subset of proteins involved in signal transduction such as Raf1 kinase, Akt, and certain transmembrane tyrosine kinases such as Her2. Thus, Her2 degradation in cells is a functional read-out of Hsp90 inhibition. Correlation between Hsp90 binding and Her2 degrada-

Table 1
Bioactivity of aminoquinolines in FP and WB assays

ID	Compound	IC_{50}[a] (μM) (FP assay)	IC_{50}[b] (μM) (Western blot)
5	PU-H71	0.043	0.06–0.13
7	850375	5.8	5.0
8a	TG2-193-3	40	NA[c]
8c	TG2-205	40	NA
8	24724348	4.9	1.6
9	24724372	29	6.0
10	24724290	1.3 (0.73)[d]	1.0 (2–3)[d]
11	24724310	2.4	6.0
18a	AS-222	>50	NA
18d	AS-217	>50	NA
19a	24724364	3.1	NA
19b	24724377	8.0	NA
19c	24724374	40.0	13
19d	JM1-156b	>50	25
19e	24724347	>50	NA
19f	24724365	>50	NA
19g	24724375	14.4	NA
19h	24724350	44	25
19i	24724357	>50	NA
19j	24724376	>50	NA
19k	24724346	2.1	25
19l	24724369	14	NA
19m	24724351	>50	NA
20a	JM1-138b	7.6	NA
20b	JM1-152c	<1.6	NA
20c	JM1-156c	<1.6	NA
20d	JM1-135c	<1.6	NA
20f	JM1-147d	<1.6	20
20g	24724309	<1.0	20
20h	24724292	0.8	30
20i	24724291	<1.6	NA
20j	JM1-128a	1.8	NA
20k	24724363	1.1	NA

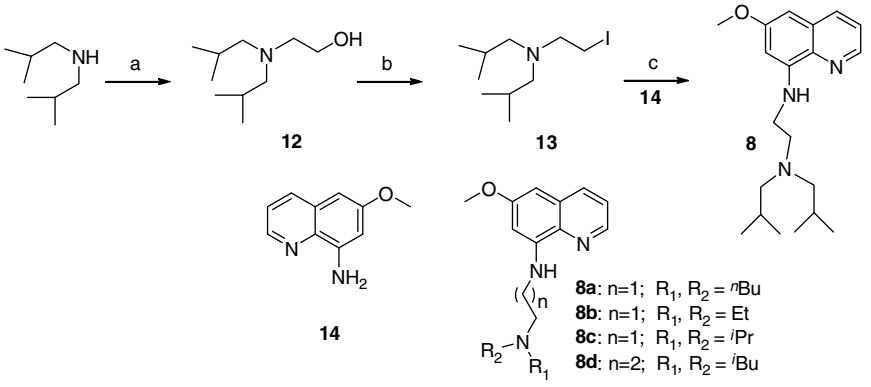
[a] Mean of two determinations.
[b] Mean of three determinations.
[c] NA, not active.
[d] Values in parentheses are from re-synthesized **10**.

tion in cancer cells is indicative of a selective biological effect in these cells via Hsp90. We used a WB-based assay to measure the cellular level of Her2 protein in MCF-7 breast cancer cell lysates collected after 24 h of compound treatment. The original hit compound **7** was re-synthesized in both neutral and dihydrochloride salt forms as previously reported.[22] Surprisingly, both synthesized forms of **7** showed only moderate biological activity (IC_{50}: ~30 μM in FP assay) in both assays by comparison to the original sample (SID 850375, collected from Discovery Partner International (DPI) as part of MLSCN). As the purities of both synthetic and original DPI sample for **7** were checked by LC/MS, the original DPI sample showed about 60% purity, while the re-synthesized compound was about 97% in purity. The biological activity of the original sample of **7** was believed to arise partially from degradation products present in the original DPI library sample, which will be detailed in a separate publication. Nevertheless, the structure of **7** serves as a starting point for analog identification.

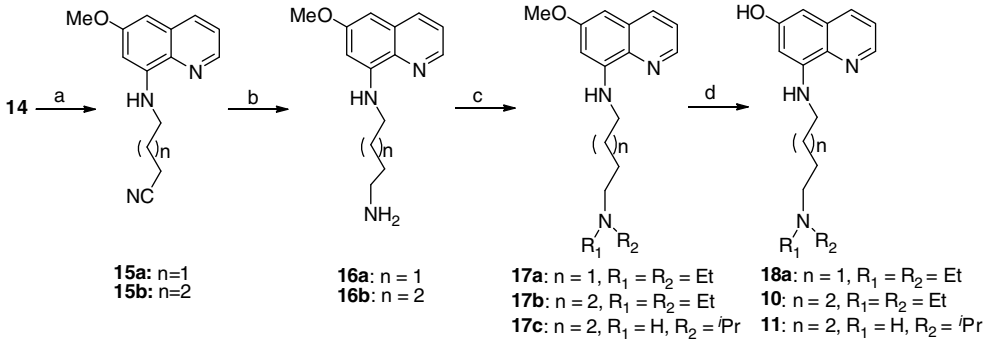
One of the advantages of compound **7** is that many analogs of this scaffold (aminoquinolines) are available for SAR from the National Cancer Institute (NCI) collection. To take advantage of this collection, we performed 2D-tanimoto similarity searching on a structural database consisting of 250,000 compounds available from the NCI.[23] 2D descriptors were assigned to the NCI database using the MDL public keys SciTegic's Extended Connectivity Fingerprints (ECFP_6) available in Pipeline Pilot.[24] Threshold similarity was set to 60% to introduce alternative scaffolds into the pool. Thirty-five compounds were identified and ordered from NCI and tested in both FP and WB assays. In addition, design and synthesis for some aminoquinolines were also executed through Schemes 1 and 2. These compounds were segregated into 2-subclasses; namely, 8-alkylaminoalkyl-6-methoxy-quinolines and 8-alkylaminoalkyl-6-hydroxyl-quinolines as shown in Figure 3. Table 1 summarizes estimated in vitro binding affinities to Hsp90 as well as the ability to prevent Hsp90 interaction with Her2 in the WB assay, leading to the degradation of Her2.

2.2. Chemistry and SAR-analyses of aminoquinolines

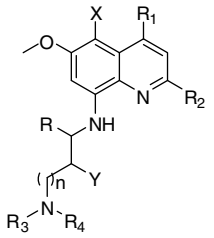
Compound **8** with a 2-carbon linker between the quinoline moiety and diisobutyl amine is the only active compound in the series. Replacing the isobutyl groups on the terminal amine of **8** with *n*-dibutyl, diethyl, and diisopropyl units (**8a**–**c**) (Scheme 1) eliminates the activities in both FP and WB assays. Likewise, **19a** (with *sec*-butyl group) and **19b** (with one ethyl and one *sec*-pentyl groups) show activity only in the FP assay, but fail to show a response in WB assay, indicating the importance of diisobutyl groups on the terminal nitrogen. Increasing the chain length to 3–4 carbons while swapping the alkyl groups on the terminal nitrogen also eliminates activity (cf. **19c**–**d**), suggesting the importance of the 2-carbon linker. Likewise **9** with a 3-carbon linker, an extra hydroxyl group on the middle carbon and a methyl group at the quinoline C-2 position shows sixfold less activity in the FP assay and threefold less in the WB assay compared to **8**. Thus, longer the linkers appear to impair activity in this series. Likewise **19k** with a 3-carbon linker, but with additional appendages (anilino, PhN) at the quinoline C-5 position shows mixed effects. Compound **19k** shows twofold increased activity in FP assay and threefold decreased activity compared to **8**, which suggests that a 2–3 carbon linker is optimal for the 6-methoxy quinoline series. Supporting this interpretation, compounds **19d**–**e**, **19h**–**j** with 4–5 carbon tethers

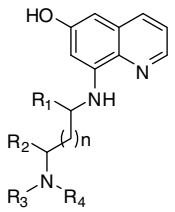


Scheme 1. Synthesis of 6-methoxy-aminoquinoline derivatives. Reagents and conditions: (a) Bromoethanol, Et_3N, THF, reflux; (b) Imidazole (3 equiv), PPh_3 and I_2 (1.5 equiv), Et_2O/MeCN; (c) excess Et_3N, MeOH/DMF (2:1), 135 °C, 2 days.



Scheme 2. Syntheses of 6-hydroxyl-aminoquinoline derivatives. Reagents and conditions: (a) $Br(CH_2)_2(CH_2)_nCN$, Et_3N, EtOH, reflux, 3 days; (b) LAH, THF, 0 °C–rt;(c) CH_3CHO, $NaBH(OAc)_3$; (d) 48% aq HBr, reflux.





8: n=0, R = R_1 = R_2 = H; R_3 = R_4 = *s*-Bu; X = Y = H
9: n=1, R = R_1 = R_2 = H; R_3 = R_4 = *s*-Bu, X = H, Y = OH
19a: n = 0, R = R_1 = R_2 = H; R_3 = H, R_4 = *s*-Bu; X = Y = H
19b: n = 0, R = R_1 = R_2 = H; R_3 = Et, R_4 = s-Pentyl; X = Y = H
19c: n = 1, R = R_1 = R_2 = H; R_3 = H, R_4 = *3*-pentyl; X = Y = H
19d: n = 2, R = R_1 = R_2 = H; R_3 = R_4 = H; X = Y = H
19e: n = 3, R = R_1 = R_2 = H; R_3 = H, R_4 = *t*-pentyl; X = Y = H
19f: n = 2, R = R_1 = Me, R_2 = H, R_3 = ^{n}Pr, R_4 = H; X = Y = H
19g: n = 2, R = Me, R_1 = R_2 = H, R_3 = ^{i}Pr, R_4 = H; X = Y = H
19h: n = 2, R = Me, X = OMe, R_1 = R_2 = Y = H, R_3 = ^{i}Pr, R_4 = H
19i: n = 2, R = Me, R_1 = R_2 = Y = H, X = *p*-Cl-PhO, R_3 = R_4 = H
19j: n=3, R = H, R_1 = R_2 = Y = H, X = *p*-Meo-PhO, R_3 = ^{i}Pr, R_4 = H
19k: n = 1, R = R_1 = R_2 = H, X = PhN, Y = H, R_3 = R_4 = Et
19l: n =1, R_1 = H, R_2 = OMe, X = Y = H, R_3 = R_4 = Et
19m: n =1, R_1= Cl, R_2 = H, X = Y = H, R_3 = R_4 = *i*-pr

10: n=3, R_1 = R_2 = H, R_3 = R_4 = Et
11: n=3, R_1 = R_2 = H, R_3 = ^{i}Pr, R_4 = H
20a: n = 1, R_1 = R_2 = H, R_3 = R_4 = H
20b: n = 1, R_1 = H, R_2 = Et, R_3 = R_4 = H
20c: n = 2, R_1 = R_2 = R_3 = R_4 = H
20d: n = 2, R_1 = H, R_2 = Me, R_3 = R_4 = H
20e: n=2, R_1 = R_2 = H, R_3 = R_4 = Et
20f: n = 3, R_1 = H, R_2 = Me, R_3 = R_4 = H
20g: n = 3, R_1 = R = R_3 = R_4 = H
20h: n =1, R_1= Me, R_2 = H, R_3 = R_4 = Me
20i: n = 2, R_1 = Me, R_2 = H, R_3 = ^{i}Pr, R4 = H
20j: n = 2, R_1 = Me, R_2 = R_3 = R_4 = H
20k: n = 2, R_1 = Me, R_2 = H, R_3 = R_4 = Et

Figure 3. Structure of quinoline analogs obtained from NCI collection[25] and synthesis.

were found to be inactive or much less active compared to **8**. Substitutions around the quinoline moiety also resulted in complete loss of activity (cf. **19l**–**m**, **19f**–**19j**).

From the 6-hydroxyl-8-aminoquinilone series (Figs. 2 and 3 and Scheme 2) only two compounds **10** and **11** show promising activity. In this series, contrary to the methoxy quinoline series, the longer linkers promote activity. Compound **10** exhibits nearly twice the binding affinity of **11** in the FP assay and eightfold more active in the WB assay. The difference between these two compounds is only the alkyl groups on the terminal nitrogen. The former (compound **10**) possesses two ethyl groups compared to the latter (compound **11**), which incorporates only one isopropyl group. This result is consistent with the findings for the methoxy quinoline series and clearly suggests that di- alkyl substituents on the terminal nitrogen are very important for activity, especially in the WB assay. The latter observation also operates when **10** is compared with **20a**–**g**, which do not bear substituents on the terminal nitrogen and show no activities in the WB assay, despite the fact **20a**–**g** proved to be highly active in the competitive-binding assay (Table 1).

To assure that the activities observed with the most active compounds (**8**, **10**, and **11**) were truly due to the structures assigned by NCI, the compounds were re-synthesized in our laboratory as shown in Schemes 1 and 2. Compounds **8**, **10**, and **11** have very similar structures to hit compound **7** with only slight changes in the length of the linker, alkyl groups on the amine tail and the free hydroxy group instead of methoxy group in some cases.

Compound **8** was prepared as shown in Scheme 1. Diisobutylamine was reacted with bromoethanol to obtain 2-(*N*,*N*-diisobutyl)-ethanol **12**, which was treated with triphenyl phosphine and iodine in the presence of imidazole to provide 2-(*N*,*N*-diisobutyl)-ethyl iodide **13**. Condensation of **13** with 6-methoxy-8-aminoquinoline **14** in the presence of triethylamine provided compound **8**. Similarly, derivatives **8a**–**c** with different alkyl groups on the amine nitrogen and a longer chain analog **8d** were also obtained in the same way.

The syntheses of **10** and **11** were carried out as shown in Scheme 2. 6-Methoxy-8-amino-quinolin **14** was treated with excess bromoalkylnitrile in a mixed solvent of triethylamine and methanol under refluxing for more than 48 h to obtain nitrile compound **15** in low to moderate yields. Reduction of **15** with lithium aluminum hydride (LAH) afforded the terminal amine **16**, which

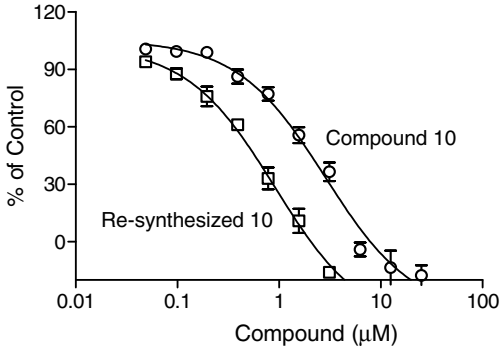


Figure 4. The Hsp90 inhibition activity of the resynthesized compound **10** was confirmed in a FP assay. Increasing concentrations of compound **10** from the compound library or resynthesized batch were added to the cy3B-geldanamycin/MCF7 cell lysate (Hsp90) reaction and the FP readout was recorded. Results from a representative of three independent experiments are shown.

was further alkylated by reductive-amination with either acetaldehyde or acetone to deliver **17a**–**c**. Deprotection of methoxy analogs **17** delivered hit compounds **10**, **11**, and their analogs **18a**.

As shown in Figure 4, the resynthesized **10** was highly active in FP assay with an IC_{50} around 0.73 μM in a representative experiment. Importantly, this compound appeared to be able to enter cells and exerted a potent activity to induce Her2 degradation with an average IC_{50} of 1.0 μM (0.8 μM in a representative assay as shown in Fig. 5).

2.3. Discussion

An ideal small molecule probe should bind effectively to tumor Hsp90, after which a conformational change will lead to disruption of the Hsp90-client protein interaction and client degradation as observed in Western blots. In the present study, Western blots have been performed for Her2 degradation, to monitor client protein interaction with Hsp90. Her2 shows greatest sensitivity, and it was selected as diagnostic in the current study. Compounds **20b**–**k** have shown potent activity in the FP assay with IC_{50} values in the low micromolar range, but failed to demonstrate activity in the WB assay.[26] Poor membrane permeability might account for the different behaviors in the cell-free and cell-based assay. (Table 1).

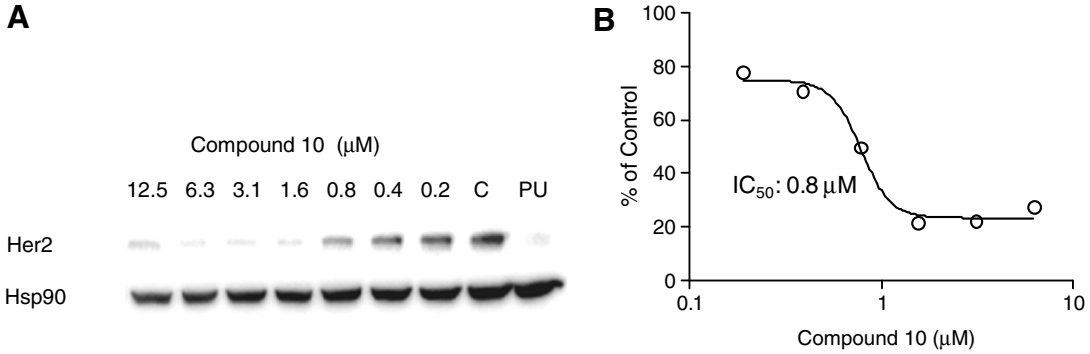


Figure 5. Compound **10**-induced Her2 degradation. (A) Western blot assay. MCF7 cells were treated with test compounds for 24 h before being lysed for Western blot analysis with antibodies to Her 2 and Hsp90. (B) The dose-curve of compound **10** in Her2 degradation assay. The density of each band in Western blot from (A) was quantified. Data were normalized to total Hsp90 protein level and then normalized to the vehicle (DMSO) control well. Data shown are from one representative of 6 independent experiments. Compound **5** was used as a positive control and was tested at 60–130 nM.

Among all the analogs, **8**–**11** obtained from NCI showed promising activity in both FP and WB assays. Compounds **8** and **9** both feature a 6-methoxy group on the quinoline moiety, while **10** and **11** share a common scaffold with 6-hydroxyl substitution. Compounds **8** and **9** were obtained as dihydrochloride salts and, **10** and **11** were available as dihydrobromide salts. These analogs not only inhibited the binding of labeled geldanamycin to Hsp90 with IC_{50} values in the range of 1–5 μM (except **9** which showed IC_{50} = 29 μM) but also induced degradation of Her2 with activities in the low micromolar range. Resynthesised compound **10** exhibits activity in both FP and WB assays, however, synthesized versions of **8** and **11** had only marginal activities in either the FP or WB assays. It is difficult to pin down causes of the discrepancy, when both samples showed similar analytic data. However, minor undetectable impurities present in the NCI sample due to long-term storage in DMSO could potentially account for the discrepancy. Gratifyingly, resynthesized compound **10** shows consistent activities in both FP assay and cell-based WB assay with IC_{50} values in low micromolar range (~1 μM).

2.4. Biostructural analysis

We have hypothesized that aminoquinolines bind to the N-terminal region based on their activity in the in vitro FP assay, which suggests that they bind competitively with the known N-terminal inhibitor, geldanamycin. It is possible that the aminoquinolines are able to influence the N-terminal ATP-binding site from an allosteric location. However, no such site has been identified at this time, though through space communication between the N-terminal and C-terminal regions is being explored. The binding modes of five N-terminal Hsp90 inhibitor classes have been solved by X-ray crystallography and were used as a guide to model the aminoquinolines into the N-terminal ATP-binding site.[11–13,8b] Despite the wide conformational and functional diversity, they share three common pharmacophore sites: hydrophobic cluster, hydrogen-bonding acceptor, and hydrogen-bonding donor. Hsp90 inhibitors interact with a hydrophobic cluster consisting of residues M84, L93, F124, Y125, and V136 (Numbering based on Protein Databank (PDB) code: 2BRE[27]). (Fig. 6). The two other common pharmacophore sites involve an H-bond donor and an H-bond acceptor (T171 and D79, respectively). Other hydrogen-bonding interactions are observed as well, but these two appear most consistently in analysis of all available co-crystals (Fig. 7).

The GLIDE protocol (Schrodinger Inc.)[28] was used to dock **10** into the ATP-binding pocket of Hsp90. No constraints were implemented during the docking calculation. Receptor-site coordinates were derived from the Protein DataBank of 2BRE. Rendering for all pictures was generated using PyMol (DeLano Scientific).[29]

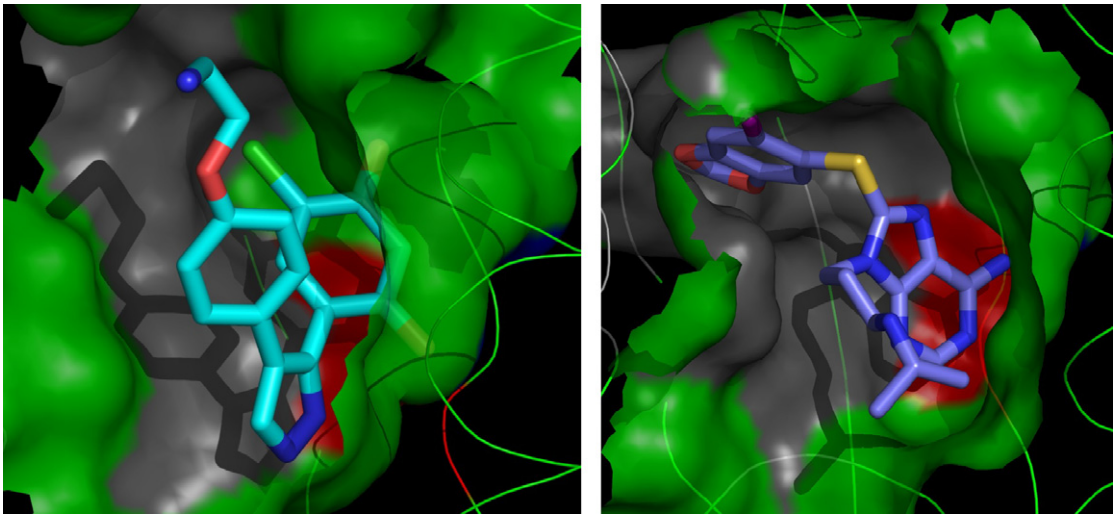


Figure 6. 3D representations of the hydrophobic and hydrogen-bonding interactions with the pyrazole (cyan) and purine (blue) inhibitors (PDB ID: 2BRE, 2FWZ, respectively). Receptor represented as a molecular surface, where the hydrophobic region is highlighted in gray, the hydrogen-bonding region in red/blue. As shown in the figure, the pyrazole interacts only with the surface of the hydrophobic cluster while the purine buries a benzodioxazole deep into the hydrophobic cluster.

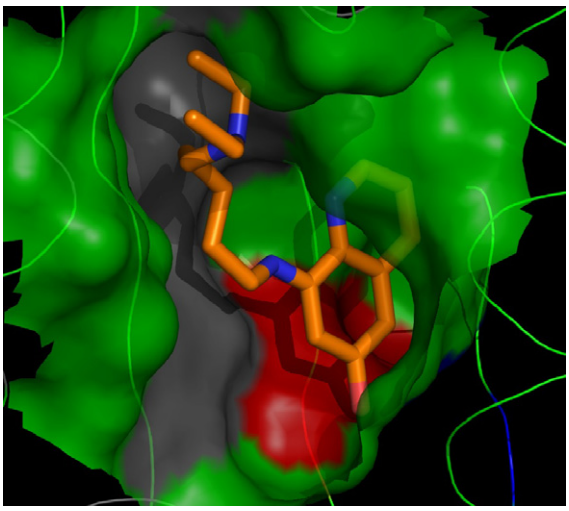


Figure 7. Docking of the **10** (red) into the ATP N-terminal-binding site of Hsp90. Docking results show that the compound makes contacts with key pharmacophoric centers (gray—hydrophogic region, red/blue—hydrogen-bonding region observed in Hsp90 crystal structures).

3. Conclusions

In an HTS campaign, we identified compound **7** as an inhibitor of Hsp90 with modest activity. SAR-study yielded 3 highly active derivatives (**8**, **10**, and **11**) by FP assay, which all show IC_{50} values in low micromolar range. These compounds also enhance degradation of the Hsp90 client proteins, for instance, Her2, as demonstrated by a cell-based Western blot assay. However, after resynthesis and biological testing of synthesized materials only one compound, namely **10**, demonstrated consistent activities in both FP assay and WB assay with low micromolar (1 μM) activities. The use of compound **10** as a lead for further SAR exploration could yield optimized and therapeutically useful molecules.

4. Experimental

4.1. 2-(Diisobutylamino)ethyl iodide (13)

To a solution of 2-(diisobutylamino)ethanol (**12**) (3 mmol) in ether and acetonitrile (3:1, 12 ml) was sequentially added imidazole (0.6 g, 9 mmol) followed by triphenyl phospine (1.2 g, 4.5 mmol) and iodine (1.5 g, 4.5 mmol) at 0 °C. The resulting reaction mixture was stirred for a further 30 min. The reaction was quenched with $Na_2S_2O_3$ solution and the reaction mixture portioned between ether and water. The organic layer was separated and after the usual work-up, the crude product was purified by silica gel chromatography to give the title compound **13** (60% yield). ^{1}H NMR: (400 MHz, $CDCl_3$): δ 3.09 (t, J = 8.4 Hz, 2H), 2.74 (t, J = 8.0 Hz, 2H), 2.12 (d, J = 7.2 Hz, 4H), 1.64 (m, 2H), 0.86 (d, J = 6.4 Hz, 12H).

4.2. N^1,N^1-Diisobutyl-N^2-(6-methoxyquinolin-8-yl)ethane-1,2-diamine (8)

To a solution of 6-methoxy-8-amino quinoline (**14**) (106 mg, 0.6 mmol) and **13** (213 mg, 0.8 mmol) in methanol and dimethyl formamide (2:1, 3 ml) was added triethylamine (0.254 ml, 1.8 mmol) and the reaction mixture heated at 135 °C for 48 h. Saturated $NaHCO_3$ was added to quench the reaction and the product extracted with dichloromethane. The organic layer was subjected to a usual work-up to provide the crude product. Silica gel chromatography yielded the title compound **8** as a yellow liquid (35% yield). ^{1}H NMR (400 MHz, MeOH-d_4): δ 8.44 (dd, J = 4.2, 1.6 Hz, 1H), 7.97 (dd, J = 8.2, 1.6 Hz, 1H), 7.30 (dd, J = 8.2, 4 Hz, 1H), 6.43 (d, J = 2.4 Hz, 1H), 6.25 (d, J = 2.4 Hz, 1H), 3.84 (s, OCH_3, 3H), 3.21 (t, J = 6 Hz, 2H), 2.68 (t, J = 6 Hz, 2H). 2.15 (d, J = 6.8 Hz, 4H), 1.72 (m, 2H), 0.88 (d, J = 6.8 Hz, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.6, 146.5, 144.7, 144.6, 135.9, 134.6, 129.8, 122.0, 96.7, 92.0, 64.3, 55.3, 54.2, 41.2, 26.9, 21.2, 21.1. HRMS calcd for $C_{20}H_{32}N_3O$ [M+H]: 330.25399; found: 330.25355 (Δ−1.33). Anal. Calcd for $C_{20}H_{31}N_3O$: C, 72.91; H, 9.48; N, 12.75; observed: C, 73.19; H, 9.47; N, 12.75.

4.3. Synthesis of dihydrochloride salt of 8

A solution of **8** (10 mg) in dichloromethane (4 ml) was purged with hydrochloride gas for 30 min. Evaporation of the solvent provided the title compound (100% yield). ^{1}H NMR (400 MHz, MeOH-d_4): δ 8.87 (dd, J = 8.4, 1.2 Hz, 1H), 8.80 (dd, J = 5.2, 1.2 Hz, 1H), 7.19 (dd, J = 8.6, 5.2 Hz, 1H), 7.05 (d, J = 2.4 Hz, 1H), 6.93 (d, J = 2 Hz, 1H), 3.97 (s, OCH_3, 3H), 3.79 (t, J = 6.8 Hz, 2H), 3.67 (t, J = 6.8 Hz, 2H). 3.19 (d, J = 6.8 Hz, 4H), 2.20 (m, 2H), 1.07 (d, J = 6.8 Hz, 12H). Anal. Calcd for $C_{20}H_{35}Cl_2N_3O_3$ ($2H_2O$): C, 54.79; H, 8.51; N, 9.58; observed: C, 54.66; H, 7.98; N, 9.35.

4.4. Compounds 8a–8d were synthesized by using the same method as for synthesis of compound 8

4.4.1. N^1,N^1-Dibutyl-N^2-(6-methoxyquinolin-8-yl)ethane-1,2-diamine (8a)

Yield 26%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.53 (dd, J = 4, 1.6 Hz, 1H), 7.89 (dd, J = 8, 1.6 Hz, 1H), 7.27 (dd, J = 8.2, 4 Hz, 1H), 6.45 (d, broad triplet, J = 5.2 Hz, NH), 6.33 (d, J = 2.8 Hz, 1H), 6.27 (d, J = 2.4 Hz, 1H), 3.87 (s, OCH_3, 3H), 3.30 (dd, J = 12, 6 Hz, 2H), 2.80 (t, J = 6.4 Hz, 2H). 2.49 (t, J = 7.2 Hz, 4H), 1.43 (m, 4H), 1.32 (m, 4H), 0.87 (t, J = 7.2 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.6, 146.3, 144.7, 135.8, 134.7, 128.9, 122.0, 96.8, 92.1, 55.4, 54.2, 52.9, 41.2, 29.4, 20.8, 14.2. HRMS calcd for $C_{20}H_{32}N_3O$ [M+H]: 330.25399; found: 330.25355 (Δ−1.33). Anal. Calcd for $C_{20}H_{31}N_3O$: C, 72.91; H, 9.48; N, 12.75; observed: C, 72.67; H, 9.58; N, 11.55.

4.4.2. N^1,N^1-Diethyl-N^2-(6-methoxyquinolin-8-yl)ethane-1,2-diamine (8b)

Yield 25%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.53 (dd, J = 4.2, 2 Hz, 1H), 7.89 (dd, J = 8.4, 1.6 Hz, 1H), 7.28 (dd, J = 8.2, 4.4 Hz, 1H), 6.37 (t, J = 4.4 Hz, NH), 6.33 (d, J = 2.4 Hz, 1H), 6.28 (d, J = 2.4 Hz, 1H), 3.87 (s, OCH_3, 3H), 3.34 (dd, J = 12.2, 6.8 Hz, 1H), 2.82 (t, J = 6.8 Hz, 2H). 2.62 (q, J = 7.2 Hz, 4H), 1.06 (t, J = 7.2 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.6, 146.2, 144.7, 135.8, 134.8, 129.9, 122.0, 96.8, 92.2, 55.4, 51.7, 47.2, 41.2, 11.8. HRMS calcd for $C_{16}H_{24}N_3O$ [M+H]: 274.19139; found: 274.10107. Anal. Calcd for $C_{16}H_{23}N_3O$: C, 70.30; H, 8.48; N, 15.37; observed: C, 70.10; H, 8.23; N, 15.06 (Δ−1.16).

4.4.3. N^1,N^1-Diisopropyl-N^2-(6-methoxyquinolin-8-yl)ethane-1,2-diamine (8c)

Yield 25%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.52 (dd, J = 4.2, 1.2 Hz, 1H), 7.89 (dd, J = 8.4, 1.6 Hz, 1H), 7.27 (dd, J = 8.4, 4.4 Hz, 1H), 6.48 (br s, NH), 6.32 (d, J = 2.4 Hz, 1H), 6.27 (d, J = 2.4 Hz, 1H), 3.87 (s, OCH_3, 3H), 3.21 (br s, 2H), 3.06 (t, J = 6 Hz, 2H). 2.82 (t, J = 6 Hz, 2H), 1.04 (d, J = 6 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.6, 146.4, 144.7, 135.8, 134.8, 129.9, 121.9, 96.8, 92.1, 55.5, 48.6, 43.8, 43.2, 21.0. HRMS calcd for $C_{18}H_{28}N_3O$ [M+H]: 302.22269; found: 302.22235 (Δ−1.12).

4.4.4. N^1,N^1-Diisobutyl-N^3-(6-methoxyquinolin-8-yl)propane-1,3-diamine (8d)

Yield 35%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.52 (dd, J = 4.2, 2 Hz, 1H), 7.90 (dd, J = 8.4, 1.6 Hz, 1H), 7.28 (dd, J = 8.4, 4.4 Hz, 1H), 6.33 (d,

J = 2.4 Hz, 1H), 6.30 (d, J = 2.8 Hz, 1H), 6.13 (t, J = 5.6 Hz, NH), 3.87 (s, OCH_3, 3H), 3.31 (dd, J = 7, 5.2 Hz, 2H), 2.46 (t, J = 6.8 Hz, 2H). 2.07 (d, J = 7.2 Hz, 4H), 1.86 (m, 2H), 1.70 (m, 2H), 0.88 (d, J = 6.4 Hz, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.7, 146.3, 144.5, 144.4, 135.6, 134.9, 129.9, 122.0, 96.7, 91.9, 64.2, 55.4, 53.2, 41.9, 27.2, 26.8, 21.2, 21.1. HRMS calcd for $C_{21}H_{34}N_3O$ [M+H]: 344.26964; found: 344.26901 (Δ−1.83). Anal. Calcd for $C_{21}H_{33}N_3O$: C, 73.43; H, 9.68; N, 12.01; observed: C, 73.34; H, 9.88; N, 12.01.

4.4.5. 4-(6-Methoxyquinolin-8-ylamino)butanenitrile (15)

6-Methoxy-8-amino-quinoline (**14**) (1.5 mmol, 261.3 mg) and 4-bromobutanenitrile (2.25 mmol, 333.0 mg) were refluxed in a mixture of Et_3N (2 ml) and MeOH (1 ml) for 24 h. More 4-bromobutanenitrile (2.25 mmol, 333.0 mg) was added and the mixture refluxed for another 48 h. The mixture was concentrated to give a dark oily residue which was purified by chromatography using hexane/EtOAc (2:1) as eluent to afford compound **15a** (148 mg, 40%). ^1H NMR (400 MHz, $CDCl_3$): δ 8.54 (dd, J = 1.6 Hz, 4.0 Hz, 1H), 7.95 (dd, J=1.6 Hz, 8.4 Hz, 1H), 7.33 (dd, J = 8.0 Hz, 4.4 Hz, 1 H), 6.40 (d, J = 2.8 Hz, 1H), 6.32 (d, J = 2.8 Hz, 1H), 6.20 (m, 1H), 3.90 (s, 3H), 3.49 (q, J = 6.4 Hz, 2H), 2.53 (t, J = 7.2 Hz, 2H), 2.11 (quintet, J = 6.8 Hz, 2H).

4.5. Similar procedure was employed to make 15b

4.5.1. Compound 15b

Yield 41% . ^1H NMR (400 MHz, $CDCl_3$): δ 8.54 (dd, J = 1.6 Hz, 4.4 Hz, 1H), 7.94 (dd, J = 1.6 Hz, 8.4 Hz, 1H), 7.32 (dd, J = 4.0 Hz, 8.4 Hz, 1H), 6.37 (d, J = 2.8 Hz, 1H), 6.29 (d, J = 2.8 Hz, 1H), 6.12 (m, 1H), 3.90 (s, 3H), 3.35 (q, J = 6.0 Hz, 2H), 2.42 (t, J = 7.2 Hz, 2H), 1.84-1.95 (m, 4H). Anal. Calcd for $C_{15}H_{17}N_3O$: C, 70.56; H, 6.71; N, 16.46; Found: C, 70.63; H, 6.75; N, 16.48.

4.5.2. N^1,N^1-Diethyl-N^4-(6-methoxyquinolin-8-yl)butane-1,4-diamine (17b)

To a solution of compound **15a** (1.0 mmol, 1 equiv, 255.31 mg) in THF (5 ml) was added LAH (2.0 mmol, 2 equiv, 1.0 M in THF) dropwise at −78 °C. Reaction mixture was kept at this temperature for an additional 2 h, which was then allowed to slowly warm up to room temperature. The reaction was quenched by addition of $NaHCO_3$ (satd aq), extracted by EtOAc (3× 5 ml), combined the organic layer and washed with brine. Dried over Na_2SO_4 and evaporated off the solvent to obtain crude compound **16b** as an oily residue, which was subjected to the next step without further purification. To a solution of crude compound **16b** in 5 ml of CH_2Cl_2 at 0 °C was added acetaldehyde (85 μL, 1.52 mmol). Reaction mixture was kept at 0 °C for about 30 min, and then $NaBH(OAc)_3$ (350 mg, 1.65 mmol) was added to the above mixture. The resulting solution was allowed to warm to room temperature for another 3 h. The mixture was diluted with CH_2Cl_2 (30 ml) and sequentially washed with 10% $NaHCO_3$ and brine. Drying over sodium sulfate followed by filtration and concentration provided a residue that was purified by silica gel chromatography to obtain the product **17b** (90 mg, 43%). ^1H NMR (400 MHz, MeOH-d_4): δ 8.61 (dd, J = 3.9, 1.6 Hz, 1H), 8.13 (dd, J = 8.6, 1.6 Hz, 1H), 7.36 (dd, J = 8.6, 3.9 Hz, 1H), 6.86 (d, J = 2.3 Hz, 1H), 6.83 (d, J = 2.3 Hz, 1H), 3.88 (s, 3H), 3.37 (quartet, J = 7.0 Hz, 2H), 2.47 (quartet, J = 7.0 Hz, 4H), 2.35 (t, J = 7.8 Hz, 2H), 1.55–1.48 (m, 2H), 1.41-1.33 (m, 2H), 1.26-1.21 (m, 2H), 0.96 (t, J = 7.0 Hz, 6H). MS, m/z ($C_{19}H_{29}N_3O$): calcd, 315.2; found, 316.4 (MH).

4.6. Similar procedure was employed to get 17a and 17c

4.6.1. Compound 17a

^1H NMR (400 MHz, $CDCl_3$): δ 8.53 (dd, J = 1.6 Hz, 4.4 Hz, 1H), 7.92 (dd, J = 1.6, 8.4 Hz, 1H), 7.29 (dd, J = 4.0, 8.0 Hz, 1H), 6.34 (d, J = 2.4 Hz, 1H), 6.29 (d, J = 2.4 Hz, 1H), 6.13 (br, 1H), 3.89 (s, 3H), 2.48–2.58 (m, 6H), 1.77 (quintet, J = 8.0 Hz, 2H), 1.60–1.68 (m, 4H), 1.03 (t, J = 7.2 Hz, 6H). Compound **17c** (5.3 mg, 27%). ^1H NMR (400 MHz, $CDCl_3$): δ 8.52 (dd, J = 1.6, 4.0 Hz, 1H), 7.91 (dd, J = 1.6, 8.4 Hz, 1H), 7.28 (dd, J = 4.4, 8.4 Hz, 1H), 6.33 (d, J = 2.8 Hz, 1H), 6.26 (d, J = 2.4 Hz, 1H), 6.07 (br, 1m), 3.88 (s, 3H), 3.26 (q, J = 6.8 Hz, 2H), 2.78 (septuplet, J = 6.4 Hz, 1H), 2.61 (t, J = 6.8 Hz, 2H), 1.77 (quintet, J = 7.2 Hz, 2H), 1.46–1.59 (m, 4H), 1.03 (d, J = 6.0 Hz, 6H).

4.6.2. 8-(5-(Diethylamino)pentylamino)quinolin-6-ol dihydrobromide (10)

A solution of **17b** (10 mg, 0.031 mmol) in HBr (1 ml, 48% aq) was heated up to 120 °C in microwave initiator for 2.5 h. Cooled down the reaction mixture, evaporated off the solvent by genevac DD-4X evaporator. The resulting dark brown residue was purified by silica gel chromatography to provide the title compound **10** (8 mg, 55%) as a pale brown solid. ^1H NMR (400 MHz, DMSO-d_6): δ 9.12 (br s, 1H), 8.60 (d, J = 3.9 Hz, 1H), 8.30 (d, J = 7.8 Hz, 1H), 7.56 (dd, J = 7.8, 4.6 Hz, 1H), 6.50 (s, 1H), 6.43 (s, 1H), 3.21 (t, J = 7.0 Hz, 2H), 3.08 (quintet, J = 7.0 Hz, 4H), 3.03–2.98 (m, 2H), 1.74–1.62 (m, 4H), 1.47-1.39 (m, 2H), 1.16 (t, J = 7.0 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 158.5, 143.9, 142.0, 133.0, 132.9 130.7, 122.5, 100.4, 92.4, 54.5, 51.3, 46.8, 28.1, 24.4, 23.5, 9.2, 9.1. EI, m/z ($C_{18}H_{27}N_3O$): calcd, 301.4; found: 302.3 (M+H). Anal. Calcd for $C_{18}H_{29}Br_2N_3O{\cdot}H_2O$: C, 44.92; H, 6.49; N, 8.73; observed: C, 45.50; H, 6.26; N, 8.59.

4.7. Similar procedure was employed to get 11 and 18a

4.7.1. 8-(5-(Isopropylamino)pentylamino)quinolin-6-ol (11)

(5.9 mg, 47%) ^1H NMR (400 MHz, MeOH-d_4): δ 8.59–8.62 (m, 2H), 7.71 (dd, J = 5.2, 8.4 Hz, 1H), 6.70 (s, 1H), 3.22–3.29 (m, 3H), 2.94 (t, J = 8.0 Hz, 2H), 1.79 (quintet, J = 7.6 Hz, 2H), 1.68 (quintet, J = 8.0 Hz, 2H), 1.53-1.59 (m, 2H), 1.22 (d, J = 6.8 Hz, 6H).

HRMS calcd for $C_{17}H_{25}N_3O$: 287.19976; observed: 288.20691(M+1).

4.7.2. 8-(4-(Diethylamino)butylamino)quinolin-6-ol (18a)

^1H NMR (400 MHz, MeOH-d_4): δ 8.36 (dd, J = 1.6 Hz, 4.0 Hz, 1H), 7.83 (dd, J = 1.6 Hz, 8.4 Hz, 1H), 7.23 (dd, J = 4.4 Hz, 8.4 Hz, 1H), 6.27 (d, J = 2.0 Hz, 1H), 6.24 (d, J= 2.4 Hz, 1H), 3.08–3.16 (m, 6H), 1.76–1.83 (m, 4H), 1.20 (t, J = 7.6 Hz, 6H). MS, m/z ($C_{17}H_{25}N_3O$): calcd, 287.20; found: 288.40 (M+H).

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