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Discovery of a Potent Anti-tumor Agent through Regioselective Mono-*N*-acylation of 7*H*-Pyrrolo[3,2-*f*]quinazoline-1,3-diamine

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Abstract

7*H*-Pyrrolo[3,2-*f*]quinazoline-1,3-diamine (**1**) is a privileged chemical scaffold with significant biological activities. However, the currently accessible chemical space derived from **1** is rather limited. Here we expanded the chemical space related to **1** by developing efficient methods for regioselective monoacylation at *N*¹, *N*³ and *N*⁷, respectively. With this novel methodology, a focused library of mono-*N*-acylated pyrroloquinazoline-1,3-diamines were prepared and screened for anti-breast cancer activity. The structure-activity relationship (SAR) results showed that *N*³-acylated compounds were in general more potent than *N*¹-acylated compounds while *N*⁷-acylation significantly reduced their solubility. Among the compounds evaluated, **7f** possessed 8-fold more potent activity than **1** in MDA-MB-468 cells. More importantly, **7f** was not toxic to normal human cells. These results suggest that **7f** is a novel compound as a potential anti-breast cancer agent without harming normal cells.

Introduction

Phenotypic screening played a fundamental role in the history of pharmacology by providing new molecular entities (NME) for drug discovery and chemical probes to study biological pathways.¹⁻⁴ A recent analysis of FDA-approved drugs between 1999 and 2008 showed that the phenotypic screening method bears a prominent position in discovering the first-in-class drugs in the era of modern molecular biology.⁵ This renaissance of phenotypic screening in drug discovery^{1, 6} requires our access to novel chemical space in order to sustain further discoveries.⁷ Privileged chemical scaffolds are potential ligands to a diverse array of receptors.^{8, 9} Therefore, novel chemical space derived from these scaffolds can provide the basis to discover novel bioactive compounds with drug-like properties, perhaps with a unique mechanism of action.⁹⁻¹³

7*H*-Pyrrolo[3,2-*f*]quinazoline-1,3-diamine (**1**, Figure 1) and its derivatives, originally synthesized as antifolates in the 1970s,¹⁴ have been shown to possess a variety of biological activities including antibacterial, anticancer and antiparasitic activity.¹⁴⁻¹⁷ Antiviral activity against herpes simplex virus (HSV) has also been reported.¹⁸ The biochemical targets for these compounds include dihydrofolate reductase (DHFR) from various species,^{16, 19} thrombin receptors,^{20, 21} and protein tyrosine phosphatase 1B (PTP1B).^{22, 23} The wide spectrum bioactivity of **1** is not due to the so-called “frequent hitter”²⁴ phenotype. Instead, these bioactivities are specific because a survey of the target-based and phenotypic screening assays involving **1** in PubChem showed it was only active in 35/528 or 6.6% of

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Electronic supplementary information. Additional experimental procedures, supporting Figures, ¹H NMR and ¹³C NMR spectra.

the assays (Table S1),²⁵ suggesting that this particular chemotype is a privileged scaffold being intrinsically useful for different biological targets.^{8, 9}

The significant biological activities associated with **1** spurred a great deal of interest in the synthesis and biological evaluation of its derivatives.^{16, 20, 23, 26} However, most of the modifications were based on *N*⁷-alkylation. To the best of our knowledge, nothing is known about the chemical space related to the regioselectively mono-*N*-acylated pyrrolo[3,2-*f*]quinazoline-1,3-diamines. The regioselectively mono-*N*-acylated compounds are expected to significantly modulate the electrostatics of the structural core as evidenced by the dramatically different molecular electrostatic potential (MEP) surfaces, calculated at HF/6-31G** level of theory,²⁷ of **2a**, **4a** and **7a** compared with that of **1** (Figure 1). Since electrostatic complementarity between ligands and receptors contributes a critical component to the overall binding interaction,²⁸⁻³⁰ these differences in electrostatics may provide leads for novel biological activities with a distinct mechanism of action. The significant challenge to rapidly synthesize these compounds could be the underlying reason for this unexplored chemical space because of the presence of multiple competing reactive sites in **1**. Herein, we describe our strategies to achieve regioselective mono-*N*-acylation of **1** at *N*¹, *N*³ and *N*⁷ (Figure 1), which significantly expand the accessible chemical space derived from **1**. From this focused library of new compounds, a potent and nontoxic anti-breast cancer compound **7f** was discovered by a phenotypic anti-cancer screen. These results illustrate the significant biological utility of this expanded chemical space.

Results and discussion

It is predicted that the proton attached to *N*⁷ is most acidic, however, the *pK*_as of the protons attached to *N*¹ and *N*³ are probably comparable. To further investigate this point, the structure of **1** was optimized at HF/6-31G** level of theory and the Mulliken atomic charges³¹ were calculated.²⁸ Consistent with the prediction, *N*⁷ is least negatively charged among the three ionizable nitrogen atoms (Figure 1). *N*¹ is slightly less charged than *N*³, suggesting that the order of *pK*_a is *N*⁷ < *N*¹ < *N*³. Therefore, we speculated that *N*⁷-H could be selectively deprotonated and acylated. Indeed, when **1**, prepared from a reported procedure with slight modifications in 82% yield from 5-aminoindole (see Supporting Information),³² was deprotonated by NaH followed by treatment with acetic anhydride (**3a**), compound **2a** was obtained in 78% yield (entry 1, Table 1). The diagnostic loss of *N*⁷-H at 11.55 ppm and loss of a triplet at 7.43 ppm attributed to C⁸-H in **1** clearly supported that the acetyl group was attached to *N*⁷. A few different anhydrides (**3b-3d**) were used and the corresponding *N*⁷-acylated products were obtained in comparable yields (entries 2-4, Table 1). Due to the limited commercial availability of anhydrides and loss of an acyl equivalent during reactions with anhydrides, we investigated the utility of *N*-hydroxysuccinimide (NHS) esters as the acylating reagents. Both aliphatic and aromatic carboxylic NHS esters were found to react readily to give the *N*⁷ acylated compounds in good to excellent yields (entries 5-6, Table 1). The discovery of NHS esters as efficient acylating agents substantially expands the variety of *N*⁷ acylated compounds that can be prepared through this route because of easy access to carboxylic acids. In general, the *N*⁷ acylated compounds **2** are poorly soluble in common organic solvents or water. Therefore, most of the products were not purified by column chromatography, but they were all found to be >95% pure based on ¹H NMR analyses (see Supporting Information). In the case of **2a-2c**, the solubility was so limited that high-quality ¹³C NMR spectra could not be obtained.

*N*¹ and *N*³ were predicted to be more nucleophilic than *N*⁷, however, all attempts to direct acetylation of either *N*¹ or *N*³ in compound **1** with Ac₂O failed to provide selectively mono-*N*-acetylated products. After considerable experimentation, we discovered that treatment of **2a** with NaH gave *N*¹-acetylated product **4a** in 24% yield (Table 2). The regioselectivity of

this reaction was confirmed by the positive nuclear Overhauser effect (NOE) between H_a and H_b, H_a and H_c observed in **4a**. The mechanism for this transformation presumably involves an intermolecular acetyl transfer from N⁷ of one molecule to N^I of another molecule followed by cleavage of N⁷-acetyl group from the latter molecule. The major byproduct generated from this reaction was the deacetylated compound **1**, which was isolated in 74% yield. The combined yields of **1** and **4a** accounted for nearly quantitative recovery of **2a**. The absence of N³-acylated product from this reaction supported the prediction of pK_a order of N^I < N³ (Figure 1) and illustrated that subtle differences in pK_a can be synthetically exploited. All the aliphatic acylated substrates **2a-2e** were successfully converted into N^I-acylated products **4a-4e** in 24-38% isolated yields (Table 2). In the case of aromatic acylated compound **4f**, it was obtained in 29% yield existing as a 1:1 mixture of two inseparable yet clearly NMR-distinguishable tautomers **4f** and **4f** in DMSO-*d*₆.³³ Different bases exerted a great effect on the yield of this acyl transfer reaction. For example, the use of LDA gave 0% yield of **4a** while 45% yield of **4a** was obtained if LiHMDS was employed as a base (see Table S2). Similarly, 50% yield of **4f** and **4f** was obtained when LiHMDS was used as the base.

To achieve selective N³ acylation, a more elaborate and indirect scheme was designed (Table 3). The N^I amine was temporarily converted into a less nucleophilic hydroxyl group to give **5** in quantitative yield through acid hydrolysis.^{26, 34} Then the nucleophilic N³ in **5** was selectively acylated by treating with either an anhydride or NHS ester to generate compounds **6** in good to excellent yields (Table 3). With the acylated intermediates **6** in hand, the N^I amine was regenerated using an S_NAr displacement reaction between ammonia (NH₃/MeOH) and activated benzotriazole adducts generated between **6** and BOP³⁵ to provide **7a-7f** in moderate to good yields (Table 3). Therefore, the hydroxyl group in **5** served as a temporary protecting group for N^I amine. With this set of methods (Tables 1-3), we successfully completed regioselective monoacylation of N^I, N³ and N⁷ of **1**.

To further demonstrate the versatility of the synthetic methods for selective acylation, we investigated the possibility of selective bisacylation to prepare compounds **4a**, **8a** and **8b**, which are N^I, N³, N³, N⁷-, and N^I, N⁷-bisacetylated, respectively (Scheme 1). We found that selective deprotonation of N⁷-H of **7a** by NaH followed by treatment with Ac₂O gave **8a** in 81% yield. Similarly, compound **8b** was obtained from **4a** by NaH/Ac₂O in 53% yield. As was the case for compounds **2**, treatment of **8a** with LiHMDS resulted in selective acetyl transfer from N⁷ to N^I to afford **4a**, which existed as a 10:1 mixture of tautomers **4a** and **4a** in DMSO-*d*₆. Finally, a synthesis of N^I, N³, N⁷-triacylated compound **9** was accomplished in 71% yield by heating a mixture of **1** and Ac₂O at 100 °C for 2 h (Scheme 1). Compound **9** was presented as a 3.4:1 mixture of two tautomers **9** and **9** in DMSO-*d*₆.

To illustrate the biological utility of the expanded chemical space derived from **1**, we carried out a phenotypic antiproliferative assay with this focused library of new compounds in breast cancer cell lines. The N⁷-acylated compounds **2**, **8a-8b** and **9** were found to be inappropriate for biological testing because of poor solubility in DMSO or aqueous buffers. Two triple negative breast cancer (TNBC) cell lines, MDA-MB-231 and MDA-MB-468, were selected to evaluate potential anticancer activity of **4** and **7** by an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay.^{36, 37} TNBC represents a unique subtype of breast cancer clinically characterized by the lack of expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) and carries poor prognosis.^{38, 39} Current treatment options for TNBC are limited to conventional cytotoxic chemotherapeutics without much success and novel nontoxic agents are in dire need.⁴⁰ The antiproliferative activities of **4** and **7** in MDA-MB-231 and MDA-MB-468 cells are presented in Table 4. The biological activities of compound **1** and an FDA-approved breast cancer drug doxorubicin (Dox) were also included for comparison. In

general, compounds **7** are more potent than **4**(**4d-4f** vs **7d-7f**). In the series of N^I -acylated compounds **4**, the potency followed the order of **1** > **4f** > **4e** > **4b** > **4c** > **4a** > **4d**, while an order of **7f** > **7e** > **1** > **7b** > **7c** > **7d** $\approx$ **7a** was observed in the series of N^B -acylated compounds **7**. These potency differences indicate the complex interplay of regiochemistry, electronics and sterics in contributing to their anticancer activity. While the bulky groups in **4e-f** and **7e-f** rendered them more potent, the sterics *per se* did not predict this potency improvement because the bulky isopropyl group in **4d** and **7d** did not provide more benefit than the corresponding less bulky **4a-4c** and **7a-7c**. Instead, the opposite was observed. Compound **4a** was the only bisacetylated compound tested for antiproliferative activity in MDA-MB-231 and MDA-MB-468 cells. However, no significant activity was observed (Table 1).

Among the tested compounds, **7f** displayed superior activity in both MDA-MB-231 (GI_{50} = 1.60 μ M) and MDA-MB-468 (GI_{50} = 0.44 μ M) cells and was $\sim$ 3-8-fold more potent than **1** (Table 4). One of the limitations of current therapeutics for TNBC is their toxicity to normal cells. Therefore, we investigated if **7f** was toxic to normal human mammary epithelial cells (HMEC). We found that no toxicity in HMEC was observed up to 5 μ M after continuous 72 h incubation (Figure 2) although some toxicity was seen at 10 μ M. If the drug treatment was reduced to 24 h, no toxicity was observed even at 10 μ M (Figure 2). Reduction of drug exposure time in cancer cells did not significantly decrease activity, further demonstrating **7f**'s selective sensitivity in cancer cells (Figure S1). In contrast, the cytotoxic chemotherapeutic agent Dox started to present toxic effect in HMEC even at 0.1 μ M (Figure 2). These results suggest that **7f** is a potential novel nontoxic anti-TNBC agent.

Compound **1** was known to be a human DHFR inhibitor, which is a potential mechanism contributing to its antiproliferative activity in TNBC cells.^{16, 19} Based on the orientation of an N^I -alkylated **1** bound to DHFR from *Candida albicans* and its structural similarity to human DHFR,⁴¹ it was predicted that the bulky naphthyl group in **7f** would not be accommodated in the human DHFR binding pocket (Figure S2). Indeed, we found that **7f** did not inhibit human DHFR up to 10 μ M concentration (Figure S2), indicating that the potent antiproliferative activity of **7f** in TNBC cells is independent of DHFR inhibition. We recently showed that small molecule inhibitors of CREB (cyclic-AMP response element binding protein) are potential novel anticancer agents.^{36, 42} Therefore, we also tested if **7f** was able to inhibit CREB-mediated gene transcription using a CREB reporter assay in HEK 293T cells.⁴³ However, no inhibition was observed up to 10 μ M concentration (Figure S3), excluding the CREB pathway as a potential target of **7f**.

Conclusions

In conclusion, we have developed efficient methods to selectively monoacylate N^I , N^B and N^7 of compound **1**. The distinct differences in the nucleophilicity and pK_a of the nitrogen atoms were exploited to achieve regioselectivity. These methods significantly expand the accessible chemical space of this privileged chemical scaffold for phenotypic screening. Similar strategies should be applicable to other heterocycles with multiple potentially competing reactive sites. To demonstrate the biological utility of the newly developed chemical space, a phenotypic anti-cancer screen identified **7f** as a potent and nontoxic anti-breast cancer agent independent of DHFR or CREB inhibition.

Experimental section

General

The solvents used for each reaction were purified from the Glass Contout solvent purification system. All the other chemicals were used as provided without further

purification unless otherwise stated. Melting points were determined in capillary tubes using Mel-Temp and are uncorrected. NMR spectra were recorded at 400 MHz (^1H NMR) and 100 MHz (^{13}C NMR). Chemical shifts (δ) are reported in ppm relative to the residual CHCl_3 (^1H , 7.26 ppm, ^{13}C , 77.0 ppm) or DMSO (^1H , 2.50 ppm, ^{13}C , 39.5 ppm). The following abbreviations were used to describe the splitting pattern of individual peaks if applicable: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. The coupling constants (J) were reported in Hertz (Hz). Silica gel flash chromatography was performed using 230–400 mesh silica gel (EMD). The mass spectra were obtained from an LTQ Orbitrap Discovery mass spectrometer (Thermo Scientific, West Palm Beach, FL) with electrospray operated either in positive or negative mode. All final compounds were confirmed to be of >95% purity based on HPLC (Waters) analysis using an XBridge C18 column (4.6 $\times$ 150 mm) and detected at 254 nm (due to the poor solubility, compound **2a–2d** were not evaluated by HPLC). The mobile phases for HPLC are water and acetonitrile, both of which contain 0.1% TFA (for compounds **2e**, **4e**, **7e** which contain a TBS group, 0.01% TFA was used due to instability of these compounds in 0.1% TFA).

Representative procedure for N^7 -acylation: synthesis of (1,3-Diamino-7H-pyrrolo[3,2-f]quinazolin-7-yl)ethanone (2a)—NaH (20.0 mg, 60% in mineral oil, 0.50 mmol) was added to a stirred solution of **1** (90.0 mg, 0.45 mmol) in dry DMF (5 mL) at 25 $^\circ\text{C}$ under Ar atmosphere. The reaction mixture was stirred for 1 h, when Ac_2O (47.3 L, 0.50 mmol) was added and the mixture was stirred for another 3 h. The solvent was removed and the residue was treated with water. The solid was collected by filtration and dried in vacuum. Then it was treated with DCM (2 mL) and collected by filtration to give the desired product **2a** as a yellowish solid (85.0 mg, 78%); mp 202–204 $^\circ\text{C}$. ^1H NMR (400 MHz, DMSO- d_6) δ 8.56 (d, J = 8.8 Hz, 1 H), 7.99 (d, J = 3.6 Hz, 1 H), 7.39 (d, J = 4.0 Hz, 1 H), 7.18 (d, J = 9.2 Hz, 1 H), 6.90 (s, 2 H), 5.92 (s, 2 H), 2.69 (s, 3 H); HRMS (ESI) Calcd for $\text{C}_{12}\text{H}_{12}\text{N}_5\text{O}^+$ ($\text{M} + \text{H}$) $^+$ 242.10364; Found 242.10313.

Representative procedure for N^1 -acylation: synthesis of N-(3-Amino-7H-pyrrolo[3,2-f]quinazolin-1-yl)acetamide (4a)—Method A: NaH (6.0 mg, 0.15 mmol) was added to a stirred suspension of **2a** (33 mg, 0.137 mmol) in dry DMF (3 mL) under argon atmosphere at 25 $^\circ\text{C}$. The reaction mixture was stirred at 25 $^\circ\text{C}$ for 2 h, when a few drops of water was added to quench the reaction. Then the solvents were removed and the residue was purified by column chromatography on silica gel, eluting with 15:1 DCM:MeOH containing 1% DIPEA to give a sticky solid, which was treated with water (1 mL) at room temperature for 1 h. Then the solid was collected by filtration to give the desired product **4a** (8.0 mg, 23%) as a yellowish solid. Method B: A suspension of **2a** (40 mg, 0.166 mmol) in dry DMF under argon atmosphere was cooled to 0 $^\circ\text{C}$. LiHMDS (182 μL , 1 M, 0.182 mmol) was added dropwise to the suspension. The reaction mixture was stirred for 40 min at 0 $^\circ\text{C}$ and then a few drops of water was added to quench the reaction. Then the solvents were removed in vacuo and the residue was purified by column chromatography on silica gel, eluting with 15:1 DCM:MeOH containing 1% DIPEA to give compound **4a** (18 mg, 45%) as a yellowish solid: mp 236–238 $^\circ\text{C}$. ^1H NMR (400 MHz, DMSO- d_6) δ 11.61 (s, 1 H), 10.22 (s, 1 H), 7.80 (d, J = 8.8 Hz, 1 H), 7.43 (brs, 1 H), 7.17 (d, J = 8.8 Hz, 1 H), 6.70 (brs, 1 H), 6.38 (s, 2 H), 2.20 (s, 3 H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 169.7, 159.0, 157.0, 152.1, 130.6, 124.8, 121.2, 119.9, 118.8, 109.2, 103.5, 23.5; HRMS (ESI) Calcd for $\text{C}_{12}\text{H}_{12}\text{N}_5\text{O}^+$ ($\text{M} + \text{H}$) $^+$ 242.10364; Found 242.10361.

3-Amino-7H-pyrrolo[3,2-f]quinazolin-1-ol (5)—A suspension of **1** (200 mg, 1.0 mmol) in 6 N aqueous HCl (10.0 mL) was heated under reflux for overnight. The reaction mixture was adjusted to pH = 10–11 with 10.0 N NaOH and the resulting black solution was stirred for 1 h. Then the pH was adjusted to 6–7 and the precipitate was collected by filtration to

give the desired product **5** (200 mg, 99%) as a brown solid: mp 284–286 °C. ¹H NMR (400 MHz, DMSO-*d*₆) 11.34 (s, 1 H), 10.95 (brs, 1 H), 7.64 (d, *J* = 8.8 Hz, 1 H), 7.41 (t, *J* = 2.4 Hz, 1 H), 7.10 (s, 1 H), 6.97 (d, *J* = 8.4 Hz, 1 H), 6.17 (s, 2 H); ¹³C NMR (100 MHz, DMSO-*d*₆) 162.7, 150.9, 142.9, 131.8, 127.0, 123.9, 119.2, 115.2, 107.6, 102.5; HRMS (ESI) Calcd for C₁₀H₉N₄O⁺ (M + H)⁺ 201.07709; Found 201.07708.

Representative procedure for N³-acylation: synthesis of N-(1-Hydroxy-7H-pyrrolo[3,2-f]quinazolin-3-yl)acetamide (6a)—A suspension of **5** (50 mg, 0.25 mmol) in Ac₂O (3 mL) was stirred at 110 °C for 1.5 h. The reaction mixture was cooled to room temperature. The excess of acetic anhydride was removed and the residue was treated with DCM (3 mL). The solid was collected by filtration to give the desired product **6a** (50 mg, 83%) as a brown solid, which was used for the next step without further purification: mp 266–268 °C. ¹H NMR (400 MHz, DMSO-*d*₆) 11.95 (s, 1 H), 11.61 (s, 1 H), 11.49 (s, 1 H), 7.83 (d, *J* = 8.4 Hz, 1 H), 7.55 (t, *J* = 2.8 Hz, 1 H), 7.23–7.20 (m, 2 H), 2.17 (s, 3 H); ¹³C NMR (100 MHz, DMSO-*d*₆) 173.4, 160.4, 144.8, 144.7, 132.8, 127.3, 123.7, 119.6, 119.0, 111.1, 103.0, 23.8; HRMS (ESI) Calcd for C₁₂H₉N₄O₂[−] (M - H)[−] 241.07310; Found 241.07294.

Representative procedure for NN³-acylation: synthesis of N-(1-Amino-7H-pyrrolo[3,2-f]quinazolin-3-yl)acetamide (7a)—BOP (83.1 mg, 0.188 mmol) and DBU (32.3 L, 0.216 mmol) were added to a stirred solution of **6a** (35.0 mg, 0.144 mmol) in dry DMF (3 mL). The resulting reaction mixture was stirred for 4 h, when NH₃ (7 N in MeOH, 0.82 mL, 5.7 mmol) was added. The reaction mixture was stirred at room temperature for 16 h. The solvents were removed and the residue was purified by column chromatography on silica gel, eluting with 1.5:1 EtOAc:THF containing 1% DIPEA to give a yellow solid, which was further treated with DCM (2 mL) and collected by filtration to give the desired compound **7a** (17.0 mg, 48%) as a yellowish solid: mp 260–262 °C. ¹H NMR (400 MHz, DMSO-*d*₆) 11.79 (s, 1 H), 9.76 (s, 1 H), 7.84 (d, *J* = 8.4 Hz, 1 H), 7.56 (brs, 1 H), 7.26 (d, *J* = 8.8 Hz, 1 H), 7.23 (brs, 1 H), 7.11 (brs, 2 H), 2.25 (s, 3 H); ¹³C NMR (100 MHz, DMSO-*d*₆) 169.7, 161.9, 152.5, 148.4, 131.9, 125.6, 120.0, 119.6, 119.4, 102.5, 24.6; HRMS (ESI) Calcd for C₁₂H₁₂N₅O⁺ (M + H)⁺ 242.10364; Found 242.10359.

Synthesis of N-(7-Acetyl-1-amino-7H-pyrrolo[3,2-f]quinazolin-3-yl)acetamide (8a)—NaH (40 mg, 60% in mineral oil, 1.0 mmol) was added to a stirred solution of **7a** (220 mg, 0.91 mmol) in dry DMF (25 mL) at 25 °C under Ar atmosphere. The reaction mixture was stirred for 1 h, when Ac₂O (95 L, 1.0 mmol) was added and the mixture was stirred for another 1 h. The white solid was collected by filtration and washed with DCM (2 mL). Then the solid was treated with H₂O (6 mL). The solid was collected by filtration to give **8a** as a gray solid (210 mg, 81%): mp 261–263 °C. ¹H NMR (400 MHz, DMSO-*d*₆) 9.84 (s, 1 H), 8.73 (d, *J* = 9.2 Hz, 1 H), 8.12 (d, *J* = 4.0 Hz, 1 H), 7.56 (d, *J* = 4.0 Hz, 1 H), 7.46 (d, *J* = 9.2 Hz, 1 H), 7.38 (brs, 2 H), 2.73 (s, 3 H), 2.28 (s, 3 H); HRMS (ESI) Calcd for C₁₄H₁₄N₅O₂⁺ (M + H)⁺ 284.11420; Found 284.11410.

Synthesis of N,N'-(7H-Pyrrolo[3,2-f]quinazoline-1,3-diyl)diacetamide (4a) and N-(3-acetamido-2,7-dihydro-1H-pyrrolo[3,2-f]quinazolin-1-ylidene)acetamide (4a'')—A suspension of **8a** (94 mg, 0.332 mmol) in dry DMF (9.4 mL) under Ar atmosphere was cooled to 0 °C. LiHMDS (365 μL, 1 M in THF/ethylbenzene, 0.365 mmol) was added dropwise to the suspension. The reaction mixture was stirred at 0 °C for 40 min, when a few drops of water was added to the reaction mixture to quench the reaction. The solvents were removed *in vacuo* and the residue was purified by column chromatography on silica gel, eluting with 2:1-1:1 DCM:THF containing 1% DIPEA to give a yellowish compound (40 mg, 43%), which existed as a 10:1 tautomeric mixture of **4a** and **4a''** in

DMSO- d_6 : mp 220–222 °C. ^1H NMR (400 MHz, DMSO- d_6) 15.60 (s, 0.1 H), 11.90 (s, 1 H), 11.74 (s, 0.1 H), 11.49 (s, 0.1 H), 10.49 (s, 1 H), 10.46 (s, 1 H), 8.03 (d, J = 8.8 Hz, 1 H), 7.93 (d, J = 8.8 Hz, 0.1 H), 7.68 (t, J = 2.0 Hz, 0.1 H), 7.59 (t, J = 2.4 Hz, 1.1 H), 7.47 (d, J = 8.8 Hz, 1 H), 7.26 (d, J = 8.4 Hz, 0.1 H), 6.88 (brs, 1 H), 2.30 (s, 3 H), 2.29 (s, 0.3 H), 2.24 (s, 3 H), 2.16 (s, 0.3 H); ^{13}C NMR (100 MHz, DMSO- d_6) 184.5, 171.8, 170.2, 169.2, 156.9, 154.9, 151.8, 150.5, 145.7, 142.6, 133.0, 132.3, 126.8, 125.8, 123.1, 122.1, 120.9, 119.8, 119.3, 118.9, 111.6, 110.3, 105.9, 104.3, 28.8, 24.5, 23.8; HRMS (ESI) Calcd for $\text{C}_{14}\text{H}_{14}\text{N}_5\text{O}_2^+$ ($\text{M} + \text{H}$) $^+$ 284.11420; Found 284.11412.

Synthesis of N-(7-Acetyl-3-amino-7H-pyrrolo[3,2-f]quinazolin-1-yl)acetamide (8b)—NaH (4.4 mg, 60% in mineral oil, 0.11 mmol) was added to a stirred solution of **4a** (24.1 mg, 0.1 mmol) in dry DMF (1 mL) at 25 °C under Ar atmosphere. The reaction mixture was stirred for 1 h, when Ac_2O (11 L, 0.11 mmol) was added and the mixture was stirred for another 3 h. The solvent was removed and the residue was treated with THF (1.5 mL). The solid was collected by filtration and then treated with H_2O (1.5 mL). The solid was collected by filtration to give **8b** as a yellowish solid (15.0 mg, 53%): mp 232–234 °C. ^1H NMR (400 MHz, DMSO- d_6) 10.50 (s, 1 H), 8.69 (d, J = 9.2 Hz, 1 H), 7.94 (d, J = 3.6 Hz, 1 H), 7.36 (d, J = 9.2 Hz, 1 H), 6.90 (d, J = 2.8 Hz, 1 H), 6.68 (s, 2 H), 2.70 (s, 3 H), 2.22 (s, 3 H); ^{13}C NMR (100 MHz, DMSO- d_6) 170.1, 159.8, 158.0, 152.7, 129.7, 127.2, 124.4, 123.4, 121.9, 108.8, 108.7, 23.9, 23.7; HRMS (ESI) Calcd for $\text{C}_{14}\text{H}_{14}\text{N}_5\text{O}_2^+$ ($\text{M} + \text{H}$) $^+$ 284.11420; Found 284.11403.

Synthesis of N,N'-(7-Acetyl-7H-pyrrolo[3,2-f]quinazoline-1,3-diyl)diacetamide (9) and N-(3-acetamido-7-acetyl-2,7-dihydro-1H-pyrrolo[3,2-f]quinazolin-1-ylidene)acetamide (9')—A mixture of **1** (100 mg, 0.5 mmol) and Ac_2O (189 μL , 2.0 mmol) in DMF (6 mL) was stirred at 100 °C for 2 h. Then the reaction mixture was cooled to room temperature and the solid was collected by filtration, washed with DCM (2 mL) to give a yellowish compound (115 mg, 71%), which existed as a 3.4:1 tautomeric mixture of **9** and **9'** in DMSO- d_6 : mp > 300 °C. ^1H NMR (400 MHz, DMSO- d_6) 15.52 (s, 0.3 H), 11.60 (s, 0.3 H), 10.80 (s, 1 H), 10.61 (s, 1 H), 8.91 (d, J = 9.2 Hz, 1 H), 8.80 (d, J = 8.8 Hz, 0.3 H), 8.09 (d, J = 4.0 Hz, 1.3 H), 7.95 (d, J = 4.0 Hz, 0.3 H), 7.70 (d, J = 9.2 Hz, 1 H), 7.45 (d, J = 9.2 Hz, 0.3 H), 7.05 (d, J = 3.6 Hz, 1 H), 2.74 (s, 3 H), 2.73 (s, 1 H), 2.32 (s, 3 H), 2.31 (s, 1 H), 2.26 (s, 3 H), 2.17 (s, 1 H); HRMS (ESI) Calcd for $\text{C}_{16}\text{H}_{16}\text{N}_5\text{O}_3^+$ ($\text{M} + \text{H}$) $^+$ 326.12477; Found 326.12494.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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33. The tautomeric mixture became a single tautomer 4f upon treatment with an aqueous NaOH solution (see supporting information for its NMR spectra). In addition, all the active protons in 4f disappeared in its ^1H NMR due to H-D exchange with HDO generated from reaction of NaOH with $\text{DMSO-}d_6$. For the same reason, the signals from the residual solvents in both ^1H NMR and ^{13}C NMR spectra became very complicated.
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Figure 1.

The chemical structures (top) of **1** and regioselectively *N*-acetylated **2a**, **4a**, **7a** and their corresponding molecular electrostatic potential (MEP) surfaces (bottom). The MEP surfaces were calculated at HF/6-31G** level of theory and mapped onto their electron densities. The Mulliken atomic charges, also calculated at HF/6-31G** level of theory, on N^1 , N^3 and N^7 of **1** are indicated in the parentheses. All the surfaces were normalized from -50 kcal/mol to +50 kcal/mol.

Figure 2.

Compound **7f** was not toxic to normal human mammary epithelial cells (HMEC). HMEC cells were treated with different concentrations of Dox or **7f** for either 24 h or 72 h. For those cells treated with drugs for 24 h, the cells were further incubated in drug-free media for 48 h. At the end of the 72 h incubation period, the number of viable cells was quantified by an MTT assay. The data are presented as mean $\pm$ SD of a representative experiment performed in triplicates.

Scheme 1. Synthesis of bis- and tri-acetylated compounds **4a**, **8a-8b** and **9**.

Table 1Selective N^7 -acylation of **1**.^a

entry	acylating reagent	product		R	yield
1	(CH ₃ CO) ₂ O	3a	2a	Me	78
2	(CH ₃ CH ₂ CO) ₂ O	3b	2b	Et	78
3	(CH ₃ CH ₂ CH ₂ CO) ₂ O	3c	2c	Pr	73
4	[(CH ₃) ₂ CHCO] ₂ O	3d	2d	<i>i</i> Pr	78
5	TBSO(CH ₂) ₄ COOSu	3e	2e	TBSO(CH ₂) ₄	68
6		3f	2f		91

^aCompound **1** was treated with NaH (1.1 equiv) in DMF for 1 h, then an acylating reagent (1.1 equiv) was added. The yields refer to isolated yields.

Table 2

Synthesis of *N*^{*l*}-acylated **4** from **2**.^{*a*} ^{*a*}The reactions were carried out with compounds **2** (1.0 equiv) and NaH (1.1 equiv) in DMF. The yields refer to isolated yields.^{*b*}Obtained as a 1:1 mixture of two tautomers **4f** and **4f'** in DMSO-*d*₆.

Table 3

Synthesis of *N*²-acylated **7**.

entry	R	acylating reagent	6	yield ^c	7	yield ^c
1	Me	3a	6a	83	7a	48
2	Et	3b	6b	84	7b	44
3	Pr	3c	6c	85	7c	37
4	<i>i</i> Pr	3d	6d	67	7d	40
5	TBSO(CH ₂) ₄	3e	6e	62	7e	50
6		3f	6f	56	7f	25

^aThis step was carried out with compound **5** (1.0 equiv) and an anhydride in neat or an NHS ester (1.5 equiv) in DMF.

^bThis operation was executed with **6** (1.0 equiv), BOP (1.3 equiv) and DBU (1.5 equiv) for 4 h. Then NH₃ (7 N in MeOH) was added.

^cIsolated yields.

Table 4

Antiproliferative activities of **4** and **7** in MDA-MB-231 and MDA-MB-468 cells.^a

compd	GI ₅₀ (μM)		compd	GI ₅₀ (μM)	
	MDA-MB-231	MDA-MB-468		MDA-MB-231	MDA-MB-468
4a	32.62 ± 13.97	56.46 ± 17.97	7a	27.17 ± 11.40	53.54 ± 29.54
4b	18.66 ± 2.24	20.28 ± 7.40	7b	21.43 ± 9.86	24.41 ± 3.33
4c	26.86 ± 12.90	26.27 ± 4.83	7c	25.52 ± 9.93	27.37 ± 4.52
4d	>100	>100	7d	39.66 ± 22.46	29.80 ± 8.41
4e	15.64 ± 7.66	11.11 ± 1.73	7e	2.43 ± 0.13	2.24 ± 0.40
4f	8.46 ± 2.56	8.91 ± 1.28	7f	1.60 ± 0.51	0.44 ± 0.14
4a	>100	>100	1	4.13 ± 0.54	3.34 ± 0.93
			Dox	0.15 ± 0.57	0.085 ± 0.029

^aThe GI₅₀ values, which represent the concentrations needed to inhibit the growth of the cancer cells by 50% during a 72 h incubation period, are presented as mean ± SD of at least two independent experiments performed in duplicates. If the GI₅₀ did not reach at the highest concentration tested at 100 μM, it is designated as >100.