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Synthesis and antitumor activities of a series of novel quinoxalinhydrazides

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Abstract—Recently, we discovered a novel class of anticancer compounds with remarkable potency in a panel of cancer cell lines. A prototype compound, **SC144**, showed significant in vivo efficacy in mice xenograft models of human breast cancer cells. Herein, we report on a new synthetic route to **SC144** and the synthesis of several of its analogues in order to understand required features for activity. A one-step coupling of 7-fluoro-4-chloropyrrolo[1,2-*a*]quinoxaline with pyrazin-2-carbohydrazide improved the yield significantly. Although several of the analogues showed significant activities, modification of the heteroacyl moiety had a dramatic effect on potency.

1. Introduction

Recent advances in targeted therapeutics coupled with new approaches in target identification have accelerated the need to design small-molecule compounds with drug-like properties.[1] Such molecules normally satisfy the Lipinski's rule-of-five and should preferably be active orally.[2,3] For targeted therapeutics against cancer, identification of lead compounds with novel mechanisms of action, low toxicity, and enhanced activity profiles is of paramount importance. Previously, we discovered that some of our small-molecule HIV-1 integrase inhibitors exhibited remarkable cytotoxicity, which prompted us to seek to understand their pharmacological properties.[4] Although HIV-1 integrase has no cellular homologue, its inhibitors may, however, inhibit other enzymes with similar active site chemistry.[5] Our extensive modifications of some of those original leads[4] resulted in the discovery of a very promising analogue, **SC144**, with desirable drug-like properties.[6] Although the elucidation of the mechanism of action of these compounds is under active investigation in our laboratory, we were interested in developing a coherent structure–activity relationship amongst these novel compounds. Therefore, in an effort to understand important features for the remarkable antitumor activity of **SC144**, we prepared a series of novel analogues to understand the effect of substitution on the 2-carbohydrazide moiety.

2. Results and discussion

2.1. Chemistry

The synthesis of compounds designated as **SC153–159** has been accomplished starting from the commercially available 1-(2-aminophenyl)pyrrole **1**. Compound **1** was treated with triphosgene in toluene under reflux to give **2** in quantitative yield. The lactam obtained was subsequently transformed into 4-chloro-1*H*-pyrrolo[1,2-*a*]quinoxaline **3** by treatment with phosphoryl chloride. Reaction of **3** with hydrazine monohydrate in DMF afforded 1-(*H*-pyrrolo[1,2-*a*]quinoxalin-4-yl)hydrazine **4**. The hydrazine derivative was reacted with the appropriate carboxylic acid in the presence of EDC/DMAP to give **SC155–159** (Scheme 1). The preparation of **SC153** and **SC154** was accomplished by a condensation step, using a procedure identical to that

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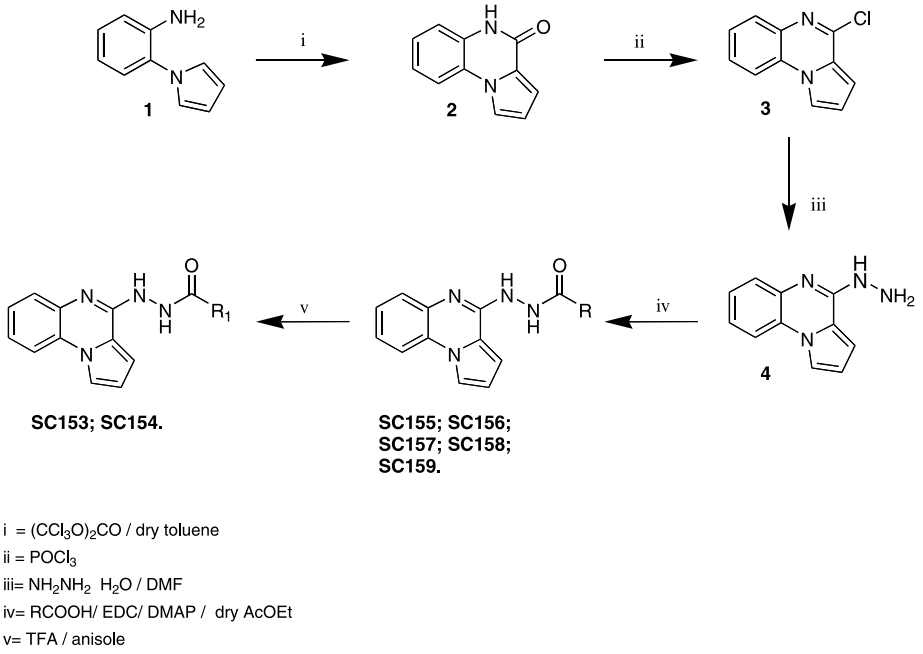


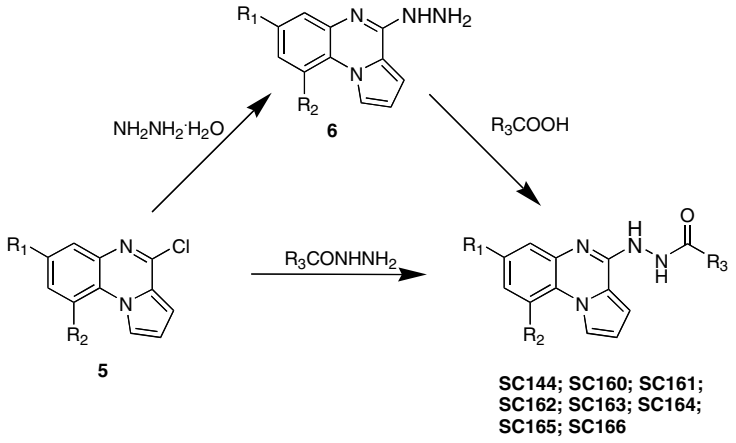
Scheme 1.

described for the preceding compounds but using the appropriate *N*-Boc-aminoacid, followed by a deprotection step by treatment with trifluoroacetic acid and anisole (Scheme 1).

Compounds **SC144** and **SC160–166** were prepared by reacting an appropriate chloro derivative of **5** with hydrazine monohydrate to give pure fluoro-4-hydrazinopyrrolo[1,2-*a*]quinoxalines **6**. The subsequent N-acylation step with selected commercially available carboxylic acids was performed using 2,2′-dipyridyldisulfide and triphenylphosphine as a condensing system.[6] A more convenient approach for the synthesis of **SC144** and **SC160–166** (as hydrochloride salts) was discovered by direct reaction of chloro derivatives of **5** with acylhydrazides in ethanol under reflux (Scheme 2).[7] Acylhydrazides were prepared by the reaction of specific methyl carboxylates with hydrazine monohydrate in refluxing ethanol.[8]

2.2. Antitumor activity

All new compounds were tested in four human cancer cell lines, a breast cancer cell, MDA-MB-435, and three colon cancer cells. Compound **SC153** was moderately active against colon cancer lines and inactive against the breast cancer line. All other indole analogues (**SC154–SC159**) were inactive at 20 μM. In the pyridino and pyrazino derivatives, the position of the nitrogen atom on the ring appeared to be important for activity. For example, **SC160** and **SC162** were inactive, whereas **SC161** was highly active in all cell lines (Fig. 1). In all the cell lines we tested, **SC161**, with IC_{50} values that range from 0.3 to 4 μM,



Scheme 2.

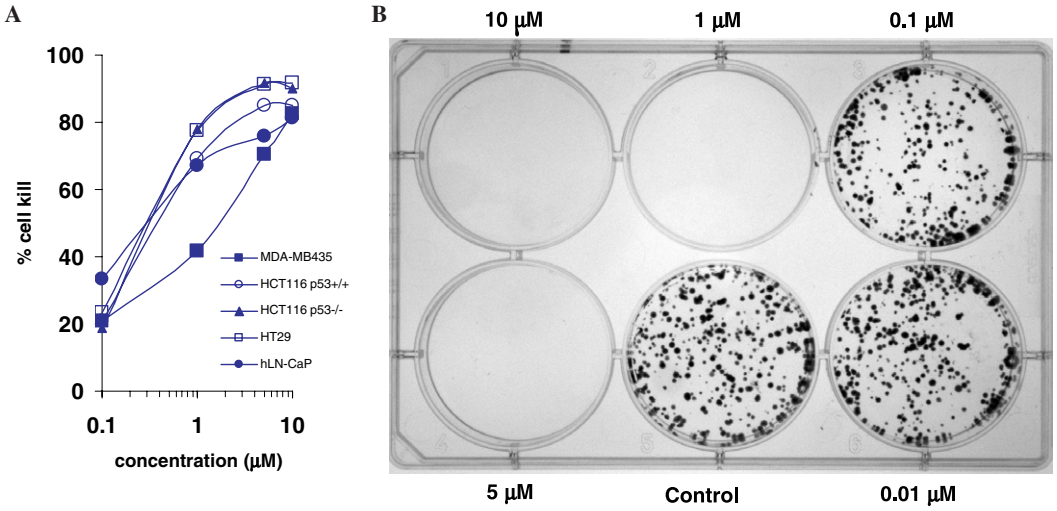


Figure 1. Compound **SC161** shows remarkable activity in a panel of cell lines. (A) The IC_{50} values of **SC161** range from 0.3 to 4 μM in representative breast, colon, and prostate cell lines using MTT assay (B) **SC161** completely block colony formation at doses $\geqslant 1$ μM.

was more potent than the previously described **SC144**.[6] Interestingly, in colony formation assays at doses above 1 μM, **SC161** completely abolished cell growth (Fig. 1). All compounds satisfy Lipinski's rule-of-five[3] as calculated using ADMET Predictor™ (Simulations Plus Inc.) (Table 1). For example, **SC161** has a molecular weight of 322.3 (MW < 500), calculated log *P* of 1.82 (*c*log *P* < 5), two hydrogen bond donors (HBD < 5), and six hydrogen bond acceptors (HBA < 10). In addition, **SC161**, with only three rotatable bonds, is very compact. The predicted acidic and basic pK_a values are also listed in Table 1. Compound **SC166** with IC_{50} values of 8–15 μM was also active, but significantly less so than **SC161**. In summary, while the substitution at the 2-carbohydrazide moiety had a profound effect on activity, the position of the fluorine atom on the benzo fused ring of pyrroloquinoxaline did not seem to greatly influence activity.

3. Experimental

All reactions were carried out under a nitrogen atmosphere. Reaction progress was monitored by TLC on silica gel plates (Merck 60, F_{254}, 0.2 mm). Organic solutions were dried over $MgSO_4$. Evaporation refers to removal of solvent on a rotary evaporator under reduced pressure. Melting points were measured using a Gallenkamp apparatus and are uncorrected. IR spectra were recorded as thin films on Perkin-Elmer 398 and FT 1600 spectrophotometers. ^{1}H NMR spectra were recorded on a Brüker 300-MHz spectrometer with TMS as an internal standard: chemical shifts are expressed in δ values (ppm) and coupling constants (*J*) in Hz. Mass spectral data were determined by direct insertion at 70 eV with a VG70 spectrometer. Merck silica gel (Kieselgel 60/230–400 mesh) was used for flash chromatography columns. Elemental analyses were performed on a Perkin-Elmer 240C element analyzer, and the results are within ±0.4% of the theoretical values. Yields refer to purified products and are not optimized.

3.1. General procedure for the preparation of compounds SC155–159

The preparation of **1***H*-indole-**2**-carboxylic acid *N*′-pyrrolo[**1**,**2**-*a*]quinoxalin-**4**-yl-hydrazide (**SC155**) is reported as a representative example.

To a stirred solution of EDC (94 mg, 0.49 mmol) and DMAP (cat.) in ethyl acetate (15 mL), 1-(*H*-pyrrolo- [1,2-*a*]quinoxalin-4-yl)hydrazine **4** (77 mg, 0.39 mmol) and 2-indolecarboxylic acid (63 mg, 0.39 mmol) were added portionwise within 15 min. The resulting mixture was stirred at room temperature for 24 h, then shaken with sodium bicarbonate saturated solution and water. Evaporation of the dried extract gave a residue, which was crystallized to give **SC155** as a white solid (82 mg, 62% yield); mp 186 °C (dichloromethane/light petroleum); IR (KBr) 3255, 1680 cm^{-1}; ^{1}H NMR (DMSO-d_6) 6.75 (s, 1H), 7.05 (m, 1H), 7.20 (m, 4H), 7.40 (m, 3H), 7.65 (m, 1H), 8.10 (m, 1H), 8.35 (s, 1H), 9.55 (br s, 1H), 10.65 (br s, 1H), 11.80 (br s, 1H). MS (CI) *m*/*z* 342 (MH^+). Anal. ($C_{20}H_{15}N_5O$) C, H, N.

3.1.1. 1*H*-Indole-5-carboxylic acid *N*′-pyrrolo[1,2-*a*]quinoxalin-4-yl-hydrazide (SC156). Following the identical procedure to that described for **SC155**, but using 2-indolecarboxylic acid (63 mg, 0.39 mmol), **SC156** was obtained as a white solid (69 mg, 52% yield); mp 160 °C (dichloromethane/light petroleum); IR (KBr) 3250, 1680 cm^{-1}; ^{1}H NMR (acetone-d_6) 6.60 (d, 1H, $J = 3.6$ Hz), 6.75 (t, 1H, $J = 3.6$ Hz), 7.23 (d, 1H, $J = 3.6$ Hz), 7.29 (m, 2H), 7.51 (m, 3H), 7.85 (d, 1H, $J = 8.5$ Hz), 8.03 (m, 1H), 8.20 (m, 1H), 8.39 (s, 1H),

9.60 (br s, 1H), 10.70 (br s, 1H), 11.45 (br s, 1H). MS (CI) *m*/*z* 342 (MH^+). Anal. ($C_{20}H_{15}N_5O$) C, H, N.

3.1.2. 1*H*-Indole-6-carboxylic acid *N*′-pyrrolo[1,2-*a*]quinoxalin-4-yl-hydrazide (SC157). Following a procedure identical to that described for **SC155**, but using 6-indolecarboxylic acid (63 mg, 0.39 mmol), **SC157** was obtained as a white solid (17 mg, 13% yield); mp 198.5 °C (dichloromethane/light petroleum); IR (KBr) 3245, 1685 cm^{-1}; ^1H NMR (acetone-d_6) 6.55 (m, 1H), 6.85 (m, 1H), 7.28 (m, 1H), 7.28 (m, 3H), 7.45 (m, 1H), 7.60 (d, 1H, J = 8.1 Hz), 8.70 (m, 2H), 8.15 (s, 1H), 8.39 (m, 1H), 9.44 (br s, 1H), 10.55 (br s, 1H), 11.51 (br s, 1H). MS (CI) *m*/*z* 342 (MH^+). Anal. ($C_{20}H_{15}N_5O$) C, H, N.

3.1.3. 1*H*-Indole-3-carboxylic acid *N*′-pyrrolo[1,2-*a*]quinoxalin-4-yl-hydrazide (SC158). Following a procedure identical to that described for **SC155**, but using 3-indolecarboxylic acid (63 mg, 0.39 mmol), **SC158** was obtained as a white solid (42 mg, 32% yield); mp 162.5 °C (dichloromethane/light petroleum); IR (KBr) 3250, 1685 cm^{-1}; ^1H NMR ($CDCl_3$) 6.80 (m, 1H), 6.90 (t, 1H, J = 3.3 Hz), 7.08 (d, 1H, J = 3.2 Hz), 7.30–7.60 (m, 4H), 7.48 (m, 1H), 7.58 (m, 1H), 7.90 (m, 2H), 8.10 (m, 1H), 8.11 (s, 1H), 8.30 (m, 1H), 9.20 (br s, 1H), 10.25 (br s, 1H), 11.60 (br s, 1H). MS (CI) *m*/*z* 342 (MH^+). Anal. ($C_{20}H_{15}N_5O$) C, H, N.

3.1.4. 2-Methoxy-*N*′-(*H*-pyrrolo[1,2-*a*]quinoxalin-4-yl)benzohydrazide (SC159). Following a procedure identical to that described for **SC155**, but using 2-methoxybenzoic acid (60 mg, 0.39 mmol), **SC159** was obtained as a white solid (98 mg, 75% yield); mp 204.5 °C (dichloromethane/light petroleum); IR (KBr) 3200, 1675 cm^{-1}; ^1H NMR (DMSO-d_6) 3.90 (s, 3H), 6.70 (m, 1H), 7.15 (m, 5H), 7.45 (m, 2H), 7.75 (m, 1H), 8.00 (m, 1H), 8.25 (br s, 1H), 9.75 (br s, 1H), 10.30 (br s, 1H). MS (CI) *m*/*z* 333 (MH^+). Anal. ($C_{19}H_{16}N_4O_2$) C, H, N.

3.1.5. Thiazolidine-4-carboxylic acid *N*′-pyrrolo[1,2-*a*]quinoxalin-4-yl-hydrazide (SC153). Starting from *N*-Boc-thiazolidine-4-carboxylic acid (90 mg, 0.39 mmol), *tert*-butyl-4-[(2-pyrrolo[1,2-*a*]quinoxalin-4-ylhydrazino)carbonyl]-1,3- thiazolidine-3-carboxylate was obtained as a solid, after crystallization (hexanes). The solid obtained was added to a stirred mixture of TFA (2 mL) and anisole (2 mL) at 0 °C. The reaction mixture was allowed to reach to room temperature and stirred for a further 50 min. Evaporation of the volatiles by azeotropization with toluene (3 × 3 mL) gave **SC153** as a pale yellow solid (66 mg, 55% yield based on compound **4**). mp 162 °C (ethyl acetate/hexanes); IR (KBr) 3255, 1690 cm^{-1}; ^1H NMR (methanol-d_4) 3.15 (dd, 1H, J = 10.9, 4.9) 3.30 (dd, 1H, J = 10.9, 7.1 Hz), 4.11 (0.5 of ABq, 1H, J = 9.7 Hz), 4.25 (0.5 of ABq, 1H, J = 9.7 Hz), 4.45 (dd, 1H, J = 7.1, 4.9 Hz), 6.92 (m, 1H), 7.41 (m, 3H), 7.71 (d, 1H, J = 7.4 Hz), 8.09 (d, 1H, J = 9.3 Hz), 8.38 (m, 1H), 10.40 (br s, 1H), 11.20 (br s, 1H). MS (CI) *m*/*z* 314 (MH^+). Anal. ($C_{15}H_{15}N_5OS$) C, H, N.

3.1.6. 3-Amino-propionic acid *N*′-pyrrolo[1,2-*a*]quinoxalin-4-yl-hydrazide (SC154). Following a procedure identical to that described for **SC153**, but using *N*-Boc-*R*-alanine (74 mg, 0.39 mmol), **SC154** was obtained as a white solid (92 mg, 88% yield based on compound **4**). Mp 164.5 °C (dichloromethane/light petroleum); IR (KBr) 3255, 1680 cm^{-1}; ^1H NMR (DMSO-d_6) 2.80 (m, 2H) 3.20 (m, 2H), 7.05 (m, 1H), 7.50 (m, 2H), 7.95 (m, 2H), 8.30 (m, 1H), 8.60 (m, 1H), 10.70 (br s, 1H), 11.25 (br s, 1H). MS (CI) *m*/*z* 270 (MH^+). Anal. ($C_{14}H_{15}N_5O$) C, H, N.

3.2. General procedure for the preparation of compounds SC144 and SC160–166 (hydrochlorides)

The preparation of *N*′-(**7**-fluoropyrrolo[**1**,**2**-*a*]quinoxalin-**4**-yl)pyrazine-**2**-carbohydrazidehydrochloride (**SC144.HCl**) is reported as a representative example.

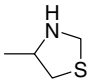
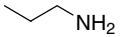
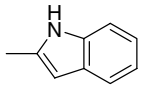
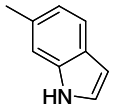
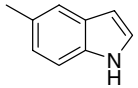
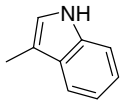
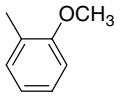
A mixture of 7-fluoro-4-chloropyrrolo[1,2-*a*]quinoxaline (200 mg, 0.90 mmol) and pyrazin-2-carbohydrazide (125 mg, 0.90 mmol) in EtOH (2 mL) was refluxed for 5 h and then chilled overnight. The product was collected by filtration, washed with cold EtOH, and dried in vacuo to give pure **SC144.HCl** (257 mg, 80 % yield). mp 282 °C (dec.) (methanol/ethyl acetate); IR (KBr) 3255, 1690 cm^{-1}; ^1H NMR (DMSO-d_6) 3.75 (br s, 1H), 6.96 (m, 1H), 7.35 (t, 1H, J = 8.7 Hz), 7.68 (m, 2H), 8.30 (dd, 1H, J = 8.7, 4.8 Hz), 8.60 (s, 1H), 8.82 (m, 1H), 8.94 (d, 1H, J = 2.7 Hz), 9.22 (s, 1H), 11.67 (br s, 1H). MS (CI) *m*/*z* 323 (MH^+). Anal. ($C_{16}H_{12}ClFN_6O$) C, H, N.

3.2.1. *N*′-(7-Fluoropyrrolo[1,2-*a*]quinoxalin-4-yl)pyridine-2-carbohydrazide hydrochloride (SC160.HCl). Following the same procedure described for compound **SC144.HCl**, but using picolinohydrazide (124 mg, 0.90 mmol), **SC160.HCl** was obtained as a yellow solid (263 mg; 82% yield). mp > 280 °C (dec.) (ethanol/ethyl acetate); IR (KBr) 3245, 1750 cm^{-1}; ^1H NMR (CD_3OD) (ppm): 8.80 (dd, 2H, J = 6.2, 1.4 Hz); 8.45 (m, 1H); 8.18 (dd, 1H, J = 9.2, 4.9 Hz); 8.03 (d, 2H, J = 6.0); 7.60 (m, 1H); 7.47 (dd, 1H, J = 9.2, 2.7 Hz); 7.28 (m, 1H); 7.0 (dd, 1H J = 4.2, 2.8 Hz). MS (CI) *m*/*z* 322 (MH^+). Anal. ($C_{17}H_{13}ClFN_5O$) C, H, N.

3.2.2. *N*′-(9-Fluoropyrrolo[1,2-*a*]quinoxalin-4-yl)pyrazine-2- carbohydrazide hydrochloride (SC161.HCl). Following the same procedure described for compound **SC144.HCl**, but using 4-chloro-9-fluoropyrrolo[1,2-*a*]quinoxaline (200 mg, 0.90 mmol), **SC161.HCl** was obtained as a yellow solid (303 mg; 94% yield). mp 248 °C (dec.) (ethanol/ethyl acetate); IR (KBr) 3250, 1680 cm^{-1}; ^1H NMR (DMSO-d_6) (ppm): 10.90 (br s, 1H); 9.80 (br s, 1H); 9.20 (s, 1H); 8.90 (s, 1H); 8.80 (s, 1H); 8.10 (m, 1H); 7.20 (m, 5H); 6.80 (s, 1H). MS (CI) *m*/*z* 323 (MH^+). Anal. ($C_{16}H_{12}ClFN_6O$) C, H, N.

3.2.3. *N*′-(7-Fluoropyrrolo[1,2-*a*]quinoxalin-4-yl)nicotinyl hydrazide hydrochloride (SC162.HCl). Following the same procedure described for compound **SC144.HCl**, but using nicotinohydrazide (124 mg, 0.90 mmol),

Table 1. Cytotoxicity and physicochemical properties of quinoxalinhydrazides

Compound	R	R_1	IC_{50}[a] (μM)				Selected physicochemical properties[b]						
			MDA-MB- 435	HCT 116 $p53^{+/+}$	HCT 116 $p53^{-/-}$	HT 29	MW	Acid-pred pK_a	Basic-pred pK_a	S+ log *P*	N_FrRotB	HDB	HBA
SC153			>20	15 ± 3	10 ± 2	14 ± 1	313.4	10.31	6.17, 3.71, −1.06, −3.85	1.21	4	3	5
SC154			>20	>20	>20	>20	269.3	10.4	8.54, 4.14, −0.81, −3.78	0.71	5	3	5
SC155			>20	>20	>20	>20	341.4	9.48; 11.93	4.15, 1.54, −1.19, −3.94	3.21	3	3	4
SC156			>20	>20	>20	>20	341.4	9.86; 14.18	4.13, 1.59, −0.69, −3.77	3.20	3	3	4
SC157			>20	>20	>20	>20	341.4	9.87; 14.50	4.17, 2.05, −0.59, −3.64	3.18	3	3	4
SC158			>20	>20	>20	>20	341.4	9.75; 12.75	4.21, 2.11, −0.90, −3.80	3.19	3	3	4
SC159			>20	>20	>20	>20	332.3	9.69	3.92, −1.10, −4.17	3.18	4	2	5

Compound	R_1	R_2	R_3	IC_{50} (μM)					Selected physicochemical properties						
				MDA-MB-435	HCT 116 $p53^{+/+}$	HCT 116 $p53^{-/-}$	HT 29	hLN-CaP	MW	Acid-pred pK_a	Basic-pred pK_a	S+log *P*	N_Fr RotB	HDB	HBA
SC160	F	H		>20	>20	>20	17	>20	321.3	9.3	3.69, 0.66, −1.32, −3.58	2.50	3	2	5
SC161	H	F		3 ± 2	0.4 ± 0.01	0.3 ± 0.07	0.3 ± 0.06	0.4	322.3	9.08	3.31, 0.91, −0.97, −2.79, −5.20	1.82	3	2	6

SC162	F	H		>20	>20	>20	NT	321.3	9.5	3.84, 2.37, −0.75, −3.13		2.16	3				2	5						
SC163	F	H		>20	>20	>20	>20	NT		338.3	9.24	3.59, −1.28, −3.90				3.18	3	2	4					
SC164	F	H		>20	>20	>20	>20	NT		326.3	9.52	3.65, −1.28, −3.85				3.10	3	2	4					
SC165	F	H		>20	>20	>20	>20	NT		310.3	9.27	3.49, −1.43, −4.11				2.61	3	2	5					
SC166	F	H		15 ± 1	8 ± 1	11± 1	13 ± 2	NT		372.3	8.9	3.55, 1.18, −0.93, −2.73, −4.88				2.83	3	2	6					
SC144	F	H		4 ± 0.1	0.6 ± 0.07	0.9 ± 0.04	0.9 ± 0.06	0.4 ± 0.06		322.3	9.08	3.52, 0.97, −0.95, −2.79, −5.21				1.79	3	2	6					

[a] Cytotoxic concentration (IC_{50}) is defined as drug concentration causing a 50% decrease in cell population using MTT assay as described in the experimental section. MDA-MB-435: breast cancer cells,

SC162.HCl was obtained as a white solid (231 mg; 72% yield). Mp > 250 °C (dec.) (ethanol/ethyl acetate); IR (KBr) 3200, 1750 cm^{-1}; ^1H NMR(DMSO-d_6) (ppm): 10.90 (br s, 1H); 9.80 (br s, 1H); 9.12 (s, 1H); 8.77 (s, 1H); 8.32 (m, 2H); 8.15 (m, 1H); 7.59 (m, 1H); 7.15 (m, 2H); 6.77 (s, 1H); 4.10 (m, 2H). *m/z* 322 (MH^+). Anal. ($C_{17}H_{13}ClFN_5O$) C, H, N.

3.2.4. *N′*-(7-Fluoropyrrolo[1,2-*a*]quinoxalin-4-yl)2′-fluorobenzoylhydrazide hydrochloride (SC163.HCl). Following the same procedure described for compound **SC144.HCl**, but using 2-fluorobenzohydrazide (139 mg; 0.90 mmol), **SC163.HCl** was obtained as a white solid (222 mg; 66% yield). Mp 247 °C (dec.) (ethanol/ethyl acetate); IR (KBr) 3250, 1675 cm^{-1}; ^1H NMR (DMSO-d_6) (ppm): 10.45 (br s, 1H); 9.75 (br s, 1H); 8.30 (s, 1H); 8.15 (m, 1H), 7.80 (m, 1H); 7.60 (m, 1H); 7.35 (m, 2H); 7.20 (m, 2H); 6.78 (m, 1H); 4.09 (m, 2H). *m/z* 339 (MH^+). Anal. ($C_{18}H_{13}ClFN_4O$) C, H, N.

3.2.5. *N′*-(7-Fluoropyrrolo[1,2-*a*]quinoxalin-4-yl)thiophene-2-carbohydrazide hydrochloride (SC164.HCl). Following the same procedure described for compound **SC144.HCl**, but using thiophene-2-carbohydrazide (128 mg; 0.90 mmol), **SC164.HCl** was obtained as a white solid (251 mg; 77% yield). Mp 261 °C (dec.) (ethanol/ethyl acetate); IR (KBr) 3245, 1680 cm^{-1}; ^1H NMR (CD_3OD) (ppm): 8.41 (m, 1H); 8.18 (dd, 1H, J = 9.3, 5.0 Hz); 7.89 (dd, 1H, J = 3.8, 1.1 Hz); 7.82 (d, 1H, J = 4.9); 7.57 (m, 1H); 7.49 (dd, 1H, J = 9.3, 2.8) 7.28 (m, 1H); 7.21 (dd, 1H, J = 4.9, 3.8) 6.98 (dd, 1H, J = 4.2, 2.8 Hz). *m/z* 327 (MH^+). Anal. ($C_{16}H_{12}ClFN_4SO$) C, H, N.

3.2.6. *N′*-(7-Fluoropyrrolo[1,2-*a*]quinoxalin-4-yl)furan-2-carbohydrazide hydrochloride (SC165.HCl). Following the same procedure described for compound **SC144.HCl**, but using furan-2-carbohydrazide (113 mg; 0.90 mmol), **SC165.HCl** was obtained as a pale yellow solid (243 mg; 78% yield). Mp 256 °C (dec.) (ethanol/ethyl acetate); IR (KBr) 3240, 1700 cm^{-1}; ^1H NMR (CD_3OD) (ppm): 8.48 (m, 1H); 8.25 (m, 1H); 7.85 (m, 1H); 7.65 (dd, 1H J = 4.3, 1.2 Hz); 7.55 (dd, 1H J = 9.2, 2.7 Hz); 7.35 (m, 2H); 7.05 (m, 1H); 6.73 (m, 1H). *m/z* 311 (MH^+). Anal. ($C_{16}H_{12}ClFN_4O_2$) C, H, N.

3.2.7. *N′*-(7-Fluoropyrrolo[1,2-*a*]quinoxalin-4- yl)quinoxaline-2-carbohydrazide hydrochloride (SC166.HCl). Following the same procedure described for compound **SC144.HCl**, but using quinoxaline-2-carbohydrazide (170 mg; 0.90 mmol), **SC166.HCl** was obtained as a pale yellow solid (327 mg; 89% yield). Mp 280 °C (dec.) (ethanol/ethyl acetate); IR (KBr) 3255, 1750 cm^{-1}; ^1H NMR (CD_3OD) (ppm): 9.55 (s, 1H); 8.47 (m, 1H); 8.28 (m, 1H); 8.20 (m, 2H); 7.97 (m, 2H); 7.68 (dd, 1H, J = 4.5, 1.2); 7.44 (dd, 1H, J = 9.3, 2.5); 7.32 (m, 1H); 7.04 (dd, 1H, J = 4.2, 2.5). *m/z* 373 (MH^+). Anal. ($C_{20}H_{14}ClFN_6O$) C, H, N.

3.3. Cell culture

Human breast cancer cells MDA-MB-435 and colon cancer HT29, p53 mutant were purchased from the

American Type Cell Culture (Manassas, VA). The HCT116 $P53^{+/+}$ and HCT116 $P53^{-/-}$ cells were kindly provided by Dr. Bert Vogelstein (Johns Hopkins Medical Institutions, Baltimore, MD). Human prostate cancer cells, LNCaP, were kindly provided by Richard Cote (University of Southern California Keck School of Medicine). Cells were maintained as monolayer cultures in RPMI 1640 media supplemented with 10% fetal bovine serum (Gemini-Bioproducts, Woodland, CA) and 2 mmol/L L-glutamine at 37 °C in a humidified atmosphere of 5% CO_2. To remove the adherent cells from the flask for passaging and counting, cells were washed with PBS without calcium or magnesium, incubated with a small volume of 0.25% trypsin–EDTA solution (Sigma–Aldrich, St. Louis, MO) for 5–10 min, and washed with culture medium and centrifuged. All experiments were performed using cells at exponential growth stage. Cells were routinely checked for mycoplasma contamination using a PCR-based assay (Stratagene, Cambridge, UK).

3.4. Drugs

Stock solutions (10 mM) of all compounds were prepared in DMSO and stored at −20 °C. Further dilutions were made fresh in PBS or cell-culture media.

3.5. Cytotoxicity assays

Cytotoxicity was assessed by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as previously described.[9] Briefly, cells were seeded in 96-well microtiter plates and allowed to attach. Cells were subsequently treated with continuous exposure to the corresponding drug for 72 h. An MTT solution (at a final concentration of 0.5 mg/mL) was added to each well, and cells were incubated for 4 h at 37 °C. After removal of the medium, DMSO was added and the absorbance was read at 570 nm. All assays were done in triplicate. The IC_{50} was then determined for each drug from a plot of log (drug concentration) versus percentage of cells killed.

3.6. Colony formation assay

Colony formation assays were also performed to confirm the activity of these compounds as described.[10] Briefly, cells were plated in 6-well plates at a density of 100 cells/well and allowed to attach. The next day, serial dilutions of the corresponding compounds were added and allowed to incubate for 24 h. After exposure, cells were washed in PBS and cultured in free media until colonies were formed (8–10 days). Cells were subsequently washed, fixed with a 1% glutaraldehyde solution for 30 min, and stained with a solution of crystal violet (2%) for 30 min. After staining, cells were thoroughly washed with water. Colonies were imaged on the Versa-Doc Imaging System (Bio-Rad) and counted using the Quantity One quantitation software package (Bio-Rad). The data reported represent means of at least three independent experiments.

References and notes

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