

Synthesis and Antimicrobial Studies of 4-[3-(3-Fluorophenyl)-4-formyl-1H-pyrazol-1-yl]benzoic Acid and 4-[3-(4-Fluorophenyl)-4-formyl-1H-pyrazol-1-yl]benzoic Acid as Potent Growth Inhibitors of Drug-Resistant Bacteria

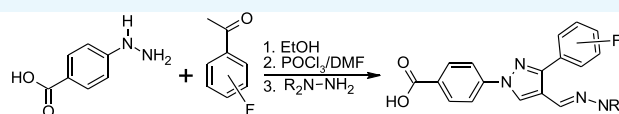
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Supporting Information

ABSTRACT: Microbial resistance to antibiotics is an urgent and worldwide concern. Several pyrazole-derived hydrazones were synthesized by using benign reaction conditions. Several of these molecules are potent growth inhibitors of drug-resistant strains of *Staphylococcus aureus* and *Acinetobacter baumannii* with minimum inhibitory concentration values as low as 0.39 $\mu\text{g}/\text{mL}$. Furthermore, these molecules are nontoxic to human cells at high concentrations. Some of these molecules were tested for their ability to disrupt the bacterial membrane by using the SYTO-9/propidium iodide (BacLight) assay.



- Efficient synthesis of novel molecules
- Potent growth inhibitors MRSA
- Non-toxic to mammalian cell
- Computational studies for drug-likeness

INTRODUCTION

Antibiotic resistance is escalating to dangerously high levels all over the world. Without an imperative and coordinated action, we are heading for a post-antibiotic era, in which common infections and minor injuries could kill a person. Scarcity of effective antibiotics to treat antibiotic-resistance infections is putting modern medicine such as chemotherapy, surgeries, and organ transplantation at risk. To prevent and control the spread of antibiotic resistance, investment in research and development of new antibiotics, vaccines, and diagnostic tools is urgent.¹

Staphylococcus aureus, a Gram-positive bacterium, is a leading cause of infection in US healthcare facilities. Methicillin-resistant *S. aureus* (MRSA) is resistant to several antibiotics. In the community, MRSA often causes skin infections as well as more serious infections such as pneumonia (lung infection). Untreated MRSA can cause sepsis and even death. In healthcare settings, such as hospitals and nursing homes, this drug-resistant bacterium can cause bloodstream infections, pneumonia, and surgical site infections. Approximately, 2% of the general population and 5% of patients in the US carry MRSA in their nares or on their skin. More than 119 000 bloodstream staphylococcal infections occurred, and nearly 20 000 people died with this infection in 2017.² *Acinetobacter baumannii*, a Gram-negative bacterium, can cause a variety of diseases including pneumonia or blood or wound infections. This bacterium may colonize in patients without causing infection or symptoms in healthy people. *Acinetobacter*

may cause severe problems to people with weakened immune systems, chronic lung disease, or diabetes. *A. baumannii* is often resistant to commonly prescribed antibiotics.³ Since 2003, the beginning of the Iraq war, several US service members have been infected by *A. baumannii* making an enormous problem in veterans. So, this bacteria is also called Iraqibacter.^{4,5} In 2017, the World Health Organization (WHO) has released a list of 12 drug-resistant bacteria that pose the greatest threat to human health and for which new antibiotics are desperately needed. Carbapenem-resistant *A. baumannii* (CRAB) is on top of the list.^{6,7} CRAB infection is associated with high morbidity and mortality as well as it has the potential to cause outbreaks and spread resistance.⁸ It is very challenging to find novel molecules that can inhibit the growth of nonfermenting bacteria such as *A. baumannii* and *P. aeruginosa*, which are often multidrug resistant.⁹ *A. baumannii* has a highly impermeable outer membrane that prevents the uptake of most molecules including several antibiotics.¹⁰

RESULTS AND DISCUSSION

In our pursuit to find novel small-molecule heterocycles,^{11–15} we have reported the synthesis and antimicrobial activities of several pyrazole-derived hydrazones.^{16–19} Based on our reported molecules, we designed and synthesized fluorine-

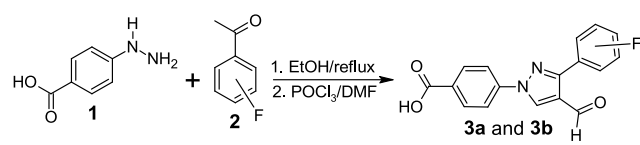
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substituted aldehydes to synthesize hydrazone derivatives for antimicrobial studies. Fluorine substitution has been extensively studied in drug discovery to enhance the biological activity and increase the chemical and metabolic stability of the resultant molecules.²⁰ The starting materials, fluorophenyl derivatives (**1**), were synthesized in the multigram scale by using our established procedure.^{17,18} Reactions of 4-hydrazinobenzoic acid with fluorophenyl acetophenones (**2**) afford the hydrazone derivatives. The evaporation of the solvent followed by the reaction with POCl₃/DMF affords the aldehyde derivatives (**3a** and **3b**) in a one-pot reaction (Scheme 1). Reactions of the aldehyde derivatives with

Scheme 1. Synthesis of Fluorophenyl-Derived Pyrazole Aldehyde



different hydrazines afforded the target molecules. All of the synthesized molecules were tested against a total of 14 Gram-positive and Gram-negative bacterial strains.

The first compound, *N*-methyl-*N*-phenyl derivative (**4**), showed moderate growth inhibition of the tested bacteria. *N,N*-Bisphenyl derivative (**5**) showed very good activity against a methicillin-sensitive *S. aureus* strain with an MIC value of 3.125 $\mu\text{g}/\text{mL}$ concentration. This molecule also showed excellent activity against methicillin-resistant *S. aureus* strains: *S. aureus* ATCC 33591 (Sa91), *S. aureus* ATCC 33592 (Sa92), *S. aureus* ATCC 700699 (Sa99) with MIC value as low as 0.78 $\mu\text{g}/\text{mL}$. Potent growth inhibition of these three MRSA strains is very significant as they have SCC *mec* II or III genomic islands conferring multidrug resistance.²¹ This compound (**5**) inhibited the growth of *S. epidermidis* 700296 (Se) with an MIC value of 3.125 $\mu\text{g}/\text{mL}$. *S. epidermidis* is an opportunistic pathogen, which is a part of normal human microbiota.²² Last but not the least, this novel molecule (**5**) inhibited the growth of *B. subtilis* with an MIC value of 1.56 $\mu\text{g}/\text{mL}$, making it a good candidate for further testing against *B. anthracis*, a potential biological warfare agent.²³ *N*-Benzyl-*N*-phenyl derivative (**6**) was found to be very potent across all of the tested Gram-positive strains with an MIC value as low as 1.56 $\mu\text{g}/\text{mL}$. *N,N*-Dibenzyl derivative (**7**) showed less potency against the staphylococcal species but inhibited the growth of *B. subtilis* at 1.56 $\mu\text{g}/\text{mL}$ concentration. Chloro substitution on the phenyl hydrazone moiety decreased the activity of the molecule (**8**) with an MIC value as low as 3.125 $\mu\text{g}/\text{mL}$ against MRSA. Bromo substitution (**9** and **10**) also showed similar results with decreased MIC values. Bisfluoro-substituted derivative (**11**) showed activity with an MIC value of 3.125 $\mu\text{g}/\text{mL}$ against the multi-drug-resistant strain, *S. aureus* ATCC 33592 (Sa92). Bischloro substitution (**12**) increased the activity of the resultant compound several fold. This compound (**12**) is the most potent compound in the series with an MIC value of 0.39 $\mu\text{g}/\text{mL}$ against the multi-resistant staph strain (Sa92). 3-Chloro-2-fluoro derivative (**13**) also showed very good activity against these antibiotic-resistant strains with an MIC value as low as 0.78 $\mu\text{g}/\text{mL}$. Polyfluorinated derivatives (**14** and **15**) showed moderate activity against the tested strains as did the 4-trifluoromethyl phenyl derivative (**16**). 4-Nitro substitution (**17**) showed

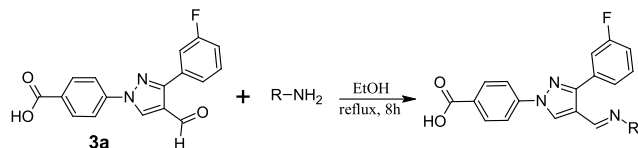
strong activity against some of the tested strains with an MIC value of 0.78 $\mu\text{g}/\text{mL}$. The addition of carboxylic acid (**18**) and heterocycles (**19**, **20**, and **21**) completely ceased the activity of the resultant hydrazone derivatives (Table 1).

Likewise, the 4-fluorophenyl-derived aldehyde (**3b**) was treated with different hydrazine derivatives to synthesize the corresponding hydrazones (Table 2). Due to solubility issues, we could not characterize and test several compounds of this series. Decreased solubility in dimethyl sulfoxide solvent may be due to the symmetrical nature of the 4-fluorophenyl substituent. Nevertheless, the following compounds were synthesized in good yields, and these compounds were tested for their growth inhibition properties against 14 bacterial strains. *N*-Methyl-*N*-phenyl derivative (**22**) showed moderate activity with an MIC value as low as 6.25 $\mu\text{g}/\text{mL}$ against one of the MRSA strains. *N,N*-Diphenyl derivative (**23**) showed very potent activity with an MIC value as low as 0.78 $\mu\text{g}/\text{mL}$. This compound (**23**) also inhibited the growth of *B. subtilis* with an MIC value of 1.56 $\mu\text{g}/\text{mL}$ concentration. Surprisingly, no activity was observed against *S. epidermidis*. *N*-Benzyl-*N*-phenyl derivative (**24**) failed to show any significant activity against the tested strains of bacteria. *N,N*-Dibenzyl derivative (**25**) showed moderate activity against some of the strains with an MIC value as low as 3.125 $\mu\text{g}/\text{mL}$. Fluoro- and bromo-substituted derivatives (**26**, **27**, and **28**) showed moderate activity against the tested strains. 3-Chloro-2-fluorophenyl-substituted derivative (**29**) showed good activity against the two strains of MRSA with an MIC value as low as 1.56 $\mu\text{g}/\text{mL}$. Cyano-substituted derivative (**30**) showed moderate activity against the tested Gram-positive strains of bacteria, and no activity was observed for the nitro derivative (**31**). Similar to the previous class of compounds, carboxylic acid and other substitution (**32–35**) did not show any significant potency against the tested strains. We have reported several pyrazole derivatives as anti-MRSA agents,^{16,17,19,24} but compound **13** is the most potent compound found to date with an MIC of 0.39 $\mu\text{g}/\text{mL}$.

All of the above compounds were tested against the seven Gram-negative bacterial strains. Some of these compounds showed moderate activity against *A. baumannii* strains, but none of the compounds showed any activity against the other four strains of Gram-negative bacteria: *Escherichia coli* ATCC 25922 (Ec), *E. aerogenes* ATCC 13048 (Ea), *Pseudomonas aeruginosa* 27833 (Pa), and *Klebsiella pneumoniae* ATCC 700603 (Kp). Only three derivatives (**8**, **9**, and **10**) of 3-fluorophenyl-derived aldehyde showed moderate activity against the three strains of *A. baumannii* with the lowest MIC value being 3.125 $\mu\text{g}/\text{mL}$. 4-Fluorophenyl-derived hydrazones (**3**) also showed moderate activity against the strains of *A. baumannii* (Table 3).

Although hydrazones possess greater intrinsic hydrolytic stability than the corresponding imines but still these molecules can be converted into the starting materials by reacting with water under the physiological condition (Scheme 2).²⁵ The antimicrobial activity of the compounds, hydrazones, may due to the hydrolyzed products, aldehydes, and hydrazines. To test this possibility, the starting materials of one of the most potent molecules (i.e., **12**) were tested against *S. aureus* ATCC 700699 (Sa99). The aldehyde derivative (**3a**) did not show any activity, but the hydrazine (**36**) showed weak growth inhibition with an MIC value of 25 $\mu\text{g}/\text{mL}$. The reaction mixture (**3a** and **36**) also showed the similar activity of hydrazine. Thus, we can conclude that the antimicrobial

Table 1. Synthesis and Antimicrobial Activity of 3-Fluorophenyl-Substituted Hydrazone Derivatives, Gram-Positive Bacteria: Antibiotic Susceptible Strain *S. aureus* ATCC 25923 (Sa), and Antibiotic-Resistant Strains: *S. aureus* BAA-2312 (Sa12), *S. aureus* ATCC 33591 (Sa91), *S. aureus* ATCC 700699 (Sa99), *S. aureus* ATCC 33592 (Sa92), *S. epidermidis* 700296 (Se), *B. subtilis* ATCC 6623 (Bs), and NA = No Activity



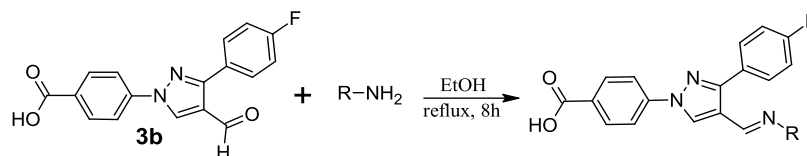
compnds	R	MIC Values ($\mu\text{g/mL}$)						
		Sa	Sa12	Sa91	Sa99	Sa92	Se	Bs
4		12.5	25	25	25	12.5	12.5	12.5
5		3.125	3.125	3.125	1.56	0.78	3.125	1.56
6		1.56	1.56	1.56	1.56	1.56	1.56	1.56
7		12.5	6.25	6.25	3.125	3.125	NA	1.56
8		6.25	12.5	12.5	3.125	6.25	12.5	12.5
9		12.5	12.5	12.5	3.125	6.25	12.5	12.5
10		12.5	12.5	12.5	6.25	6.25	12.5	12.5
11		12.5	12.5	12.5	6.25	3.125	12.5	6.25
12		3.125	3.125	3.125	0.78	0.39	3.125	1.56
13		3.125	12.5	6.25	3.125	0.78	6.25	1.56
14		12.5	12.5	12.5	6.25	6.25	25	12.5
15		12.5	12.5	12.5	6.25	3.125	25	6.25
16		12.5	12.5	6.25	6.25	6.25	12.5	12.5
17		3.125	NA	NA	3.125	0.78	NA	0.78
18		NA	NA	NA	NA	NA	NA	NA
19		NA	NA	NA	NA	NA	NA	NA
20		NA	NA	NA	NA	NA	NA	NA
21		NA	NA	NA	NA	NA	NA	NA
	Vancomycin	0.39	0.78	1.56	0.39	0.39	3.125	0.39

activity of the compounds is due to their hydrazone functional groups not due to their possible hydrolyzed products.

Compounds showing potent antimicrobial activity were tested against a human embryonic kidney (HEK293) cell line for their in vitro toxicity. Potent compounds did not show any noticeable cytotoxicity at the MIC concentration. IC_{50} values for the compounds are more than $10 \mu\text{g/mL}$. High IC_{50} values

and low MIC values for compounds 5, 12, and 23 indicate suitability for further drug development (Figure 1). Furthermore, these new compounds were submitted to the National Cancer Institute (NCI) for cytotoxicity studies against NCI-60 cancer cell lines. None of the compounds showed any substantial growth inhibition activity against these cancer cell lines at $10 \mu\text{M}$ concentration. Hence, these

Table 2. Synthesis and Antimicrobial Activity of 4-Fluorophenyl-Substituted Hydrazone Derivatives, Gram-Positive Bacteria: Antibiotic Susceptible Strain *S. aureus* ATCC 25923 (Sa), and Antibiotic-Resistant Strains: *S. aureus* BAA-2312 (Sa12), *S. aureus* ATCC 33591 (Sa91), *S. aureus* ATCC 700699 (Sa99), *S. aureus* ATCC 33592 (Sa92), *S. epidermidis* 700296 (Se), *B. subtilis* ATCC 6623 (Bs), and NA = No Activity



comps	R	MIC Values (ug/mL)						
		Sa	Sa 12	Sa 91	Sa99	Sa 92	Se	Bs
22		12.5	NA	12.5	12.5	6.25	12.5	12.5
23		1.56	3.125	NA	1.56	0.78	NA	1.56
24		NA	NA	NA	NA	NA	NA	NA
25		NA	NA	NA	6.25	3.125	NA	6.25
26		12.5	25	25	12.5	12.5	25	25
27		12.5	12.5	12.5	6.25	3.125	25	12.5
28		12.5	12.5	25	6.25	6.25	12.5	12.5
29		6.25	3.125	12.5	1.56	1.56	12.5	3.125
30		12.5	NA	NA	6.25	12.5	NA	25
31		NA	NA	NA	NA	NA	NA	NA
32		NA	NA	NA	NA	NA	NA	NA
33		NA	NA	NA	NA	NA	NA	NA
34		NA	NA	NA	NA	NA	NA	NA
35		NA	NA	NA	NA	NA	NA	NA
Vancomycin		0.39	0.78	1.56	0.39	0.39	3.125	0.39

fluorophenyl-derived pyrazole derivatives (4–35) are nontoxic to human cell lines.

The bacterial membrane is one of the key targets of antibacterial agents. Antibiotics targeting bacterial membranes could be the key to solve bacterial resistance to antibiotics.²⁶ Seven potent compounds (8, 21, 22, 30, 31, 32, and 33) were tested for their membrane permeability for the *A. baumannii* ATCC 19606 strain by using the SYTO/9-PI assay (Figure 2). Compounds 21 and 22 have shown better membrane disruption ability than the positive control, Colistin (C).

Calculated Physicochemical Properties. Physicochemical properties of the six most potent compounds (5, 6, 12, 13, 23, and 29) were calculated by using the SwissAMDE, a freely available online software (<http://www.swissadme.ch/index>.

php). The *n*-octanol/water partition coefficient (*ilogP*) is an important parameter for the drug design and development. The *ilogP* values are mostly <4.0, which are within the acceptable range (Table 4). Topological total surface area of these molecules is less than 140 Å, which indicates the potential for very good passive transport through the cell membranes.²⁷

Correlations of antimicrobial activity and the energy of lowest unoccupied molecular orbital (LUMO) orbitals have been reported previously.^{26,28} We calculated the energy of highest occupied molecular orbital (HOMO), LUMO orbital and HOMO–LUMO energy gap by using the density functional theory calculation (see the Supporting Information).

Table 3. MIC Values of Compounds against Gram-Negative Bacterial Strains: *A. baumannii* ATCC 19606 (Type Strain, AB06), *A. baumannii* ATCC BAA-1605 (Ab05), *A. baumannii* ATCC 747 (Ab47), *E. coli* ATCC 25922 (Ec), *E. aerogenes* ATCC 13048 (Ea), *P. aeruginosa* 27833 (Pa), *K. pneumoniae* ATCC 700603 (Kp), and NA = No Activity

compounds	MIC ($\mu\text{g/mL}$)						
	Ab06	Ab47	Ab05	Ec	Ea	Pa	Kp
8	6.25	12.5	6.25	NA	NA	NA	NA
9	6.25	12.5	12.5	NA	NA	NA	NA
10	3.125	6.25	6.25	NA	NA	NA	NA
26	12.5	>25	25	NA	NA	NA	NA
27	6.25	25	12.5	NA	NA	NA	NA
30	12.5	12.5	>25	NA	NA	NA	NA
Colistin	3.125	1.56	3.125				

Scheme 2. Possible Hydrolysis of a Hydrazone to the Corresponding Aldehyde (3a) and Hydrazine (36)

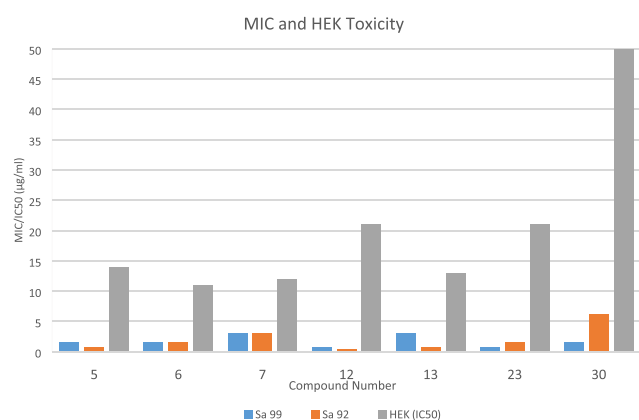
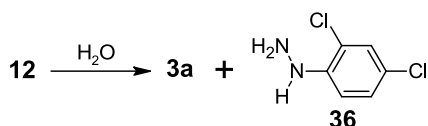


Figure 1. Cytotoxicity studies of some potent compounds.

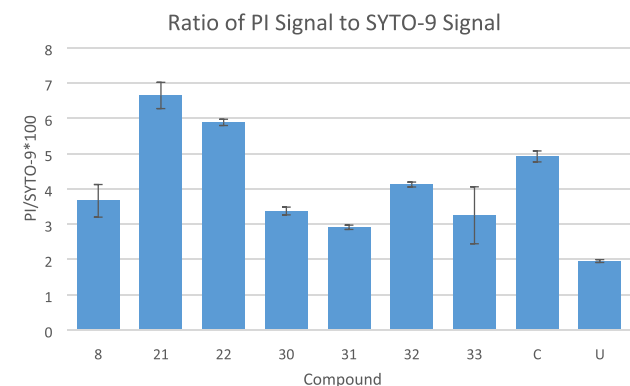


Figure 2. Membrane disruption: ratios of PI to SYTO-9 signals with Colistin 6.25 $\mu\text{g/mL}$ (C) and untreated controls (U). Bars indicate the standard deviation of the duplicate samples.

Unfortunately, for our molecules, we did not find any good correlation.

Table 4. Calculated Physicochemical Properties

compounds	$\log P$	TPSA
5	3.91	70.72
6	3.77	70.72
13	3.56	79.51
14	3.48	79.51
23	4.05	70.72
29	3.45	79.51

CONCLUSIONS

In summary, we have efficiently synthesized several potential antibiotics by using benign reaction conditions and readily available starting materials. Several of these molecules have been found to be potent growth inhibitors of MRSA and *B. subtilis*. These molecules are significantly less toxic to human cell lines, which make them potential candidates for further antibiotic development. Design, synthesis, and antimicrobial studies of novel molecules based on the lead structures of this manuscript are in progress and will be reported soon.

EXPERIMENTAL SECTION

General Consideration. All of the reactions were carried out under the air atmosphere in round-bottom flasks. Solvents for reactions, recrystallizations and deuterated solvents for ^1H and ^{13}C NMR spectroscopy were purchased from Fisher Scientific and Oakwood chemical.

Synthesis of Hydrazone Derivatives.^{16,17,24} A mixture of 4-hydrazinobenzene (1, 1.597 g, 10.5 mmol) and 1-(3-fluorophenyl)ethanone (2, 1.381 g, 10 mmol) in ethanol was refluxed for 8 h. The solvent was removed under reduced pressure, and the hydrazone derivative was further dried in vacuo. The dried product was subjected to the next reaction without further isolation or purification. The fluorophenyl-derived product in anhydrous *N,N*-dimethyl formamide (30 mL) was stirred for 15 min to dissolve the solid material completely. The clear solution was chilled in an ice bath, and phosphorous oxychloride (POCl_3 , 4.67 mL, 50 mmol) was added dropwise. The reaction mixture was brought to an ambient temperature after 30 min and then heated for 8 h at 80 $^\circ\text{C}$. After the completion of the reaction, the hot reaction mixture was decanted onto the ice in a beaker and the mixture was stirred for 12 h to precipitate the product. The solid product was filtered and washed with water repeatedly until the filtrate was clear. The aldehyde product (3a and 3b) was further dried in vacuo to get the pure pyrazole-derived aldehyde in a very good overall yield.

MIC Studies.^{17,24} All of the synthesized compounds were tested for antimicrobial properties, and the minimum inhibitory concentration (MIC) was determined by broth microdilution plate-based technique as per Clinical and Laboratory Standards Institute (CLSI) procedures. In short, bacterial strains were streaked onto Tryptic Soy Agar (TSA) or TSA with 5% sheep blood plates. The streaked plates were incubated at 35 $^\circ\text{C}$ overnight. Isolated colonies were suspended in 0.9% NaCl sterile saline to match a 0.5 MacFarland standard. The suspension was further diluted 1:100 with the Mueller Hinton broth to an approximate 1×10^6 CFU/mL concentration. Compounds were dissolved in dimethyl sulfoxide (DMSO) to a 2 mg/mL concentration and further diluted serially 1:2 in microplates such that after the addition of bacteria solution in broth, the final concentrations began at 50 $\mu\text{g/mL}$. The inhibition of bacterial growth or

viability was determined using resazurin as a colorimetric assay.²⁹ Resazurin solution was added with the bacterial suspension to 8 $\mu\text{g}/\text{mL}$ final concentration, and the microplates were incubated at 35 °C for 20–24 h to read them visually. Negative controls (bacteria without inhibitors), technical control (bacteria plus serially diluted DMSO), and positive controls (bacteria plus a serially diluted antibiotic) were included on every plate. The MIC value was the lowest concentration where there was no color change of resazurin from purple (resorufin) to red (resazurin) (evidence of the bacterial growth).

Cytotoxicity Studies. In vitro toxicity studies of the potent compounds were accomplished according to our reported modified procedure.^{17,24} HEK293 cells were counted using a Countess automated cell counter, and 4000 cells per well were seeded in black 96-well plates. Compounds were serially diluted starting at 50 $\mu\text{g}/\text{mL}$, added in triplicate, and incubated for 24 h. Resazurin solution (5%) for a final volume of 240 μL in each well was added after 24 h. After the addition of 5% resazurin, plates were incubated for 4 and 6 h before taking the readings for cell viability. Fluorescence was measured at 544 nm (excitation) and 590 nm (emission) using a BMG Labtech Fluostar Optima plate reader.

SYTO-9/PI Studies. BacLight assay (Invitrogen) was used to assess the membrane permeability of the bacterium *A. baumannii* ATCC 19606 following the treatment with our pyrazole-derived compounds using the reported procedure^{26,30} with a slight modification described below. SYTO-9 is a green fluorescent protein, which freely permeates into all bacterial cells and increases in fluorescent intensity when bound to DNA. This provides a baseline measurement of the total bacteria found in a single well, which can then be compared to the fluorescent intensity of propidium iodide (PI). Propidium iodide is much less permeable to the cell membrane but also increases in fluorescent intensity once it enters the cell and binds to DNA. PI is expected to only show a strong signal when significant membrane damage is present, so our compound treatments can be compared to negative controls and we can conclude if membrane damage has or has not occurred. In earlier experiments, our compounds have demonstrated a bleaching effect on the PI signal (results not shown), therefore we must remove our compounds from the cell suspension by centrifugation prior to reading our plates.

Ab 19606 was grown in cation adjusted Mueller Hinton broth at 37 °C with shaking to reach the log phase growth and an OD 600 nm of 0.2. The cultures were centrifuged at 10 000g for 10 min, the cell pellet was washed once with sterile water, resuspended so that the final optical density of the cell suspension was 0.2 OD, and aliquoted into separate wells of a 24-well-plate (780 $\mu\text{L}/\text{well}$), then test compounds were added (20 $\mu\text{L}/\text{well}$) at the MIC concentration as well as double the MIC and two 1:2 dilution factors below MIC. Suspensions were incubated at 37 °C with shaking for 1 h and then moved to individual microcentrifuge tubes and were centrifuged at 10 000g for 10 min, washed once with sterile water, and resuspended in 800 μL of water. A 1:1 mixture of SYTO-9 (1 $\mu\text{g}/\text{mL}$) and propidium iodide (1 mg/mL) was added to the suspension (3 $\mu\text{L}/\text{mL}$) and mixed well. The mixture (100 μL) was added to each well of a black opaque 96-well plate, and the plates were incubated in the dark for 15 min at room temperature. Green fluorescence (SYTO-9) was read at 530 nm, and red fluorescence (PI) was read at 645 nm (excitation

wavelength, 485 nm). The ratio of green to red fluorescence was expressed as a percentage of the control.

EXPERIMENTAL DATA

4-[3-(3-Fluorophenyl)-4-formyl-pyrazol-1-yl]benzoic Acid (3a). Yellow solid, ¹H NMR, 300 MHz (DMSO-*d*₆): δ 9.98 (s, 1H), 9.47 (s, 1H), 8.10 (s, 4H), 7.82 (d, *J* = 8.4 Hz, 2H), 7.58–7.51 (m, 1H), 7.32 (t, *J* = 9.42 Hz, 1H); ¹³C NMR (75 MHz, DMSO-*d*₆): δ 184.9, 166.9, 162.5 (¹*J* = 241.6 Hz), 151.7 (⁴*J* = 2.51 Hz), 141.8, 136.8, 133.6 (³*J* = 8.53 Hz), 131.3, 131.0 (³*J* = 8.3 Hz), 130.1, 125.2 (⁴*J* = 2.6 Hz), 123.09, 119.36, 116.63 (²*J* = 20.8 Hz), 115.77 (²*J* = 23 Hz). HRMS (ESI-FTMS, *m/z*): calcd for C₁₇H₁₁FN₂O₃ [M + H]⁺ 311.0826, found 311.0825. Yield (2.8 g, 90%).

4-{3-(3-Fluorophenyl)-4-[(*E*)-(2-methyl-2-phenylhydrazinylidene)methyl]-1*H*-pyrazol-1-yl]benzoic Acid (4). Light yellow solid, ¹H NMR, 300 MHz (DMSO-*d*₆): δ 8.99 (s, 1H), 8.10 (s, 4H), 7.62–7.54 (m, 4H), 7.36–7.22 (m, 4H), 6.87–6.83 (m, 1H), 2.49 (s, 3H); ¹³C NMR (75 MHz, DMSO-*d*₆): δ 167.3, 162.7 (¹*J* = 242.0 Hz), 150.0, 147.7, 142.4, 135.4 (³*J* = 8.2 Hz), 131.3, 131.1 (³*J* = 8.4 Hz), 129.4, 129.2, 127.3, 124.8, 124.5, 120.4 (³*J* = 14.1 Hz), 118.4, 115.7, 115.5, 115.2, 114.9, 32.8. HRMS (ESI-FTMS, *m/z*): calcd for C₂₄H₁₉FN₄O₂ [M + H]⁺ 415.1564, found 415.1563. Yield (377 mg, 91%).

4-[4-[(*E*)-(Diphenylhydrazono)methyl]-3-(3-fluorophenyl)pyrazol-1-yl]benzoic Acid (5). Yellow Solid, ¹H NMR, 300 MHz (DMSO-*d*₆): δ 9.09 (s, 1H), 8.13–8.05 (m, 4H), 7.46–7.41 (m, 7H), 7.27–7.15 (m, 8H); ¹³C NMR (75 MHz, DMSO-*d*₆): δ 167.1, 162.5 (¹*J* = 242.3 Hz), 150.3, 143.2, 142.4, 134.9 (³*J* = 8.3 Hz), 131.3, 130.9 (³*J* = 8.4 Hz), 130.6, 130.4, 129.6, 129.0, 128.0 (³*J* = 7.4 Hz), 124.9, 124.7, 122.5, 119.2, 118.5, 115.7 (²*J* = 20.9 Hz), 115.3 (²*J* = 22.4 Hz). HRMS (ESI-FTMS, *m/z*): calcd for C₂₉H₂₁FN₄O₂ [M + H]⁺ 477.1721, found 477.1725. Yield (419 mg, 88%).

4-[4-[(*E*)-(Benzyl(phenyl)hydrazono)methyl]-3-(3-fluorophenyl)pyrazol-1-yl]benzoic Acid (6). White solid, ¹H NMR, 300 MHz (DMSO-*d*₆): δ 9.02 (s, 1H), 8.14–8.05 (m, 4H), 7.47–7.18 (m, 13H), 7.06 (d, *J* = 7.5 Hz, 1H), 6.90 (t, *J* = 7.1 Hz, 1H), 5.29 (s, 2H); ¹³C NMR (75 MHz, DMSO-*d*₆): δ 167.1, 162.6 (¹*J* = 242.6 Hz), 149.9 (⁴*J* = 2.3 Hz), 147.7, 142.5, 136.4, 134.9 (³*J* = 8.3 Hz), 131.3, 131.0 (³*J* = 8.4 Hz), 129.5, 129.3, 128.9, 127.5, 127.1, 126.5, 125.5, 124.3, 120.6, 120.0, 118.6, 115.6 (²*J* = 20.7 Hz), 114.9, 114.6, 49.0. HRMS (ESI-FTMS, *m/z*): calcd for C₃₀H₂₄FN₄O₂ [M + H]⁺ 492.1950, found 492.1954. Yield (437 mg, 89%).

4-[4-[(2,2-Dibenzylhydrazino)methyl]-3-(3-fluorophenyl)pyrazol-1-yl]benzoic Acid (7). White solid, ¹H NMR, 300 MHz (DMSO-*d*₆): δ 13.08 (br s, 1H), 8.78 (s, 1H), 8.11–8.03 (m, 4H), 7.61–7.15 (m, 13H), 7.09 (s, 1H), 6.97 (d, *J* = 7.4 Hz, 1H), 4.32 (s, 4H); ¹³C NMR (75 MHz, DMSO-*d*₆): δ 167.1, 162.5 (¹*J* = 242.4 Hz), 149.4, 142.5, 138.2, 134.9 (³*J* = 8.1 Hz), 131.3, 130.9 (³*J* = 8.2 Hz), 129.0, 128.8, 127.8, 127.5, 125.9, 124.0, 123.8, 120.4, 118.5, 115.4 (²*J* = 20.9 Hz), 114.6 (²*J* = 22.2 Hz), 58.2. HRMS (ESI-FTMS, *m/z*): calcd for C₃₁H₂₆FN₄O₂ [M + H]⁺ 506.2107, found 506.2107. Yield (465 mg, 92%).

4-[4-[(*E*)-(3-Chlorophenyl)hydrazono]methyl]-3-(3-fluorophenyl)pyrazol-1-yl]benzoic Acid (8). Yellow Solid, ¹H NMR, 300 MHz (DMSO-*d*₆): δ 10.49 (s, 1H), 9.08 (s, 1H), 8.15–8.07 (m, 4H), 7.98 (s, 1H), 7.64–7.53 (m, 3H), 7.31 (t, *J* = 7.6 Hz, 1H), 7.18 (t, *J* = 7.9 Hz, 1H) 7.08 (s, 1H),

6.87 (d, $J = 8.04$, 1H), 6.72 (d, $J = 7.7$ Hz, 1H); ^{13}C NMR (75 MHz, DMSO- d_6); δ 167.1, 162.5 ($^1J = 242.0$ Hz), 150.1, 147.0, 142.4, 135.0, 134.4, 131.3, 130.9, 130.4, 129.0, 127.7, 124.9, 119.2, 118.4, 118.2, 115.8, 115.6, 115.3, 111.1 ($^2J = 37.7$ Hz). HRMS (ESI-FTMS, m/z): calcd for $\text{C}_{23}\text{H}_{17}\text{ClFN}_4\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 437.0991, found 437.0990. Yield (340 mg, 78%).

4-[4-[(E)-[(3-Bromophenyl)hydrazono]methyl]-3-(3-fluorophenyl)pyrazol-1-yl]benzoic Acid (9). Green solid, ^1H NMR, 300 MHz (DMSO- d_6); δ 10.46 (s, 1H), 9.07 (s, 1H), 8.10 (m, 5H), 7.61 (t, $J = 4.8$ Hz, 3H), 7.31 (s, 1H), 7.21 (s, 1H), 7.11 (d, $J = 7.6$ Hz, 1H), 6.92–6.85 (m, 2H); ^{13}C NMR (75 MHz, DMSO- d_6); δ 167.1, 162.6 ($^1J = 242$ Hz), 150.2 ($^4J = 2.2$ Hz), 147.2, 142.4, 135.1 ($^3J = 8.3$ Hz), 131.3, 131.0 ($^3J = 8.4$ Hz), 130.5, 129.0, 127.9, 125.0 ($^4J = 2.4$ Hz), 123.0, 121.2, 119.2, 118.6, 115.9, 115.6, 115.4, 114.2, 111.3, HRMS (ESI-FTMS, m/z): calcd for $\text{C}_{23}\text{H}_{16}\text{BrFN}_4\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 481.0494, found 481.0485. Yield (384 mg, 80%).

4-[4-[(E)-[(4-Bromophenyl)hydrazono]methyl]-3-(3-fluorophenyl)pyrazol-1-yl]benzoic Acid (10). ^1H NMR, 300 MHz (DMSO- d_6); δ 10.44 (s, 1H), 9.05 (s, 1H), 8.15–8.06 (m, 4H), 7.97 (s, 1H), 7.63–7.53 (m, 3H), 7.35–7.32 (m, 3H), 6.98–6.96 (m, 2H); ^{13}C NMR (75 MHz, DMSO- d_6); δ 167.1, 162.6 ($^1J = 241.9$ Hz), 150.1 ($^4J = 2.4$ Hz), 145.0, 142.4, 135.0 ($^3J = 8.3$ Hz), 132.0, 131.3, 131.1 ($^3J = 8.4$ Hz), 129.9, 129.0, 127.6, 124.9 ($^4J = 2.5$ Hz), 119.4, 118.5, 115.8 ($^2J = 21.1$ Hz), 115.4 ($^2J = 22.5$ Hz), 114.1, 109.7. HRMS (ESI-FTMS, m/z): calcd for $\text{C}_{23}\text{H}_{16}\text{BrFN}_4\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 481.0494, found 481.0485. Yield (393 mg, 82%).

4-[4-[(E)-[(2,5-Difluorophenyl)hydrazono]methyl]-3-(3-fluorophenyl)pyrazol-1-yl]benzoic Acid (11). Brownish yellow solid, ^1H NMR, 300 MHz (DMSO- d_6); δ 13.11 (br s, 1H), 9.04 (s, 1H), 8.13–8.05 (m, 4H), 7.46–7.18 (m, 13H), 7.04 (d, $J = 7.38$ Hz, 1H), 6.90 (t, $J = 7.02$ Hz, 1H), 5.29 (s, 2H); ^{13}C NMR (75 MHz, DMSO- d_6); δ 167.1, 162.6 ($^1J = 241.9$ Hz), 159.7 ($^1J = 234.8$ Hz), 150.3 ($^4J = 2.4$ Hz), 145.5 ($^1J = 234.8$ Hz), 142.4, 135.3 (t, $^3J = 11.9$ Hz), 134.9 ($^3J = 8.2$ Hz), 133.1, 131.3, 131.1 ($^3J = 8.4$ Hz), 129.0, 127.9, 124.9 ($^4J = 2.5$ Hz), 119.2, 118.6, 116.3 ($^2J = 20.5$ Hz), 115.8 ($^2J = 20.7$ Hz), 115.4 ($^2J = 22.4$ Hz), 103.9 ($^2J = 24.4$ Hz), 100.7 ($^2J = 29.3$ Hz). HRMS (ESI-FTMS, m/z): calcd for $\text{C}_{23}\text{H}_{15}\text{F}_3\text{N}_4\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 437.122, found 437.1221. Yield (332 mg, 76%).

4-[4-[(E)-[(2,4-Dichlorophenyl)hydrazono]methyl]-3-(3-fluorophenyl)pyrazol-1-yl]benzoic Acid (12). Light Yellow solid, ^1H NMR, 300 MHz (DMSO- d_6); δ 10.06 (s, 1H), 9.09 (s, 1H), 8.63 (s, 1H), 8.15–8.07 (m, 4H), 7.60 (m, 4H), 7.43 (s, 1H), 7.27 (t, $J = 8.9$ Hz, 2H); ^{13}C NMR (75 MHz, DMSO- d_6); δ 167.1, 162.6 ($^1J = 242.0$ Hz), 150.4 ($^4J = 2.2$ Hz), 142.4, 141.0, 134.9 ($^3J = 8.3$ Hz), 133.7, 131.1 ($^3J = 8.4$ Hz), 129.1, 128.9, 128.2, 127.6, 124.9 ($^4J = 2.6$ Hz), 122.3, 119.3, 118.6, 116.7, 115.8 ($^2J = 20.9$ Hz), 115.5, 115.3 ($^3J = 8.3$ Hz). HRMS (ESI-FTMS, m/z): calcd for $\text{C}_{23}\text{H}_{15}\text{Cl}_2\text{FN}_4\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 469.0628, found 469.0630. Yield (360 mg, 77%).

4-[4-[(E)-[(3-Chloro-2-fluorophenyl)hydrazono]methyl]-3-(3-fluorophenyl)pyrazol-1-yl]benzoic Acid (13). Yellow Solid, ^1H NMR, 300 MHz (DMSO- d_6); δ 10.42 (s, 1H), 9.09 (s, 1H), 8.31 (s, 1H), 8.15–8.06 (m, 4H), 7.61–7.56 (m, 3H), 7.44 (t, $J = 8.01$ Hz, 1H), 7.35 (t, $J = 2.82$ Hz, 1H), 7.07 (t, $J = 8.13$ Hz, 1H), 6.86 (t, $J = 7.95$ Hz, 1H); ^{13}C NMR (75 MHz, DMSO- d_6); δ 167.1, 162.6 ($^1J = 241.8$ Hz), 150.3 ($^4J = 2.5$ Hz), 144.7 ($^1J = 239.8$ Hz), 142.4, 135.3 ($^3J = 9.3$ Hz), 134.9 ($^3J = 8.2$ Hz), 133.2, 131.3, 131.1 ($^3J = 8.4$ Hz), 129.1, 127.8, 125.8 ($^4J = 4.0$ Hz), 124.9 ($^4J = 2.6$ Hz), 119.9 ($^2J = 14.2$ Hz), 119.2, 118.8, 118.6, 115.8 ($^2J = 20.5$ Hz),

115.4 ($^2J = 22.4$ Hz), 112.9. HRMS (ESI-FTMS, m/z): calcd for $\text{C}_{23}\text{H}_{15}\text{ClF}_2\text{N}_4\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 453.0924, found 453.0928.

4-[3-(3-Fluorophenyl)-4-[(E)-[(2,3,5,6-tetrafluorophenyl)hydrazono]methyl]pyrazol-1-yl]benzoic Acid (14). Light yellow solid, ^1H NMR, 300 MHz (DMSO- d_6); δ 13.11 (br s, 1H), 10.37 (s, 1H), 8.88 (s, 1H), 8.32 (s, 1H), 8.14–8.01 (m, 4H), 7.64–7.48 (m, 3H), 7.33–7.14 (m, 2H); ^{13}C NMR (75 MHz, DMSO- d_6); δ =167.0, 162.6 ($^1J_{\text{C-F}} = 241.8$ Hz), 150.4 ($^4J_{\text{C-F}} = 2.4$ Hz), 146.6 (dt, $^1J = 241.8$ Hz, $^3J = 10.6$ Hz), 142.3, 136.5 (dd, $^1J = 233.1$ Hz, $^2J = 20.1$ Hz), 135.3, 134.7 ($^3J_{\text{C-F}} = 8.3$ Hz), 131.3, 131.0 ($^3J_{\text{C-F}} = 8.4$ Hz), 129.1, 127.8, 125.7 (m), 124.9 ($^4J_{\text{C-F}} = 2.5$ Hz), 118.8, 118.5, 115.8 ($^2J = 21.0$ Hz), 115.5 ($^2J_{\text{C-F}} = 22.5$ Hz), 95.5 (t, $^2J = 24.1$ Hz). HRMS (ESI-FTMS, m/z): calcd for $\text{C}_{23}\text{H}_{13}\text{F}_5\text{N}_4\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 473.1031, found 473.1032. Yield (405 mg, 86%).

4-[3-(3-Fluorophenyl)-4-[(E)-[(2,3,4,5,6-pentafluorophenyl)hydrazono]methyl]pyrazol-1-yl]benzoic Acid (15). Brown yellow solid, ^1H NMR, 300 MHz (DMSO- d_6); δ 10.13 (s, 1H), 8.76 (s, 1H), 8.21 (s, 1H), 8.04 (s, 4H), 7.58–7.47 (m, 3H), 7.26 (t, $J = 7.62$, 1H); ^{13}C NMR (75 MHz, DMSO- d_6); δ 167.0, 162.5 ($^1J = 241.9$ Hz), 150.2 ($^4J = 2.3$ Hz), 142.2, 138.1 (m), 137.9 (m), 135.3, 134.6 ($^3J = 8.3$ Hz), 131.3 (m), 130.9 ($^3J = 8.4$ Hz), 129.0, 127.5, 124.8 ($^4J = 2.4$ Hz), 121.6 ($^3J = 10.2$ Hz), 118.9, 118.5 ($^3J = 9.8$ Hz), 115.7 ($^2J = 21.0$ Hz), 115.3 ($^2J = 22.6$ Hz). HRMS (ESI-FTMS, m/z): calcd for $\text{C}_{23}\text{H}_{12}\text{F}_6\text{N}_4\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 491.0937, found 491.0937. Yield (432 mg, 88%).

4-[3-(3-Fluorophenyl)-4-[(E)-[(4-(trifluoromethyl)phenyl)hydrazono]methyl]pyrazol-1-yl]benzoic Acid (16). ^1H NMR, 300 MHz (DMSO- d_6); δ 10.74 (s, 1H), 9.09 (s, 1H), 8.15–8.07 (m, 4H), 8.04 (s, 1H), 7.65–7.54 (m, 3H), 7.51 (d, $J = 8.5$ Hz, 2H), 7.33 (t, $J = 7.6$ Hz, 1H), 7.13 (d, $J = 8.4$ Hz, 2H); ^{13}C NMR (75 MHz, DMSO- d_6); δ 167.1, 162.6 ($^1J = 241.9$ Hz), 150.3 ($^4J = 2.3$ Hz), 148.6, 142.4, 135.0 ($^3J = 8.3$ Hz), 131.6, 131.3, 131.1 ($^3J = 8.4$ Hz), 129.1, 127.9, 125.5 (q, $^1J = 268.4$ Hz), 125.0 ($^4J = 2.6$ Hz), 119.1, 118.6, 118.4 (q, $^2J = 31.6$ Hz), 115.8 ($^2J = 20.8$ Hz), 115.5 ($^2J = 22.4$ Hz), 111.8. HRMS (ESI-FTMS, m/z): calcd for $\text{C}_{24}\text{H}_{16}\text{F}_4\text{N}_4\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 469.1282, found 469.1282. Yield (369 mg, 79%).

4-[3-(3-Fluorophenyl)-4-[(E)-(4-nitrophenyl)methyl]pyrazol-1-yl]benzoic Acid (17). Orange solid, ^1H NMR, 300 MHz (DMSO- d_6); δ 9.12 (s, 1H), 8.09 (s, 7H), 7.62–7.53 (m, 3H), 7.35–7.30 (m, 1H), 7.10–7.07 (m, 2H); ^{13}C NMR (75 MHz, DMSO- d_6); δ 167.2, 162.6 ($^1J = 242.3$ Hz), 150.9, 150.6, 142.1, 138.5, 134.8 ($^3J = 8.3$ Hz), 134.6, 131.3, 131.2, 131.1, 129.8, 128.4, 126.5, 125.0, 118.6, 115.9 ($^2J = 20.6$ Hz), 115.5 ($^2J = 22.2$ Hz), 111.4. HRMS (ESI-FTMS, m/z): calcd for $\text{C}_{23}\text{H}_{16}\text{FN}_5\text{O}_4$ [$\text{M} + \text{H}$] $^+$ 446.1259, found 446.1259. Yield (400 mg, 90%).

4-[(2E)-2-[[1-(4-Carboxyphenyl)-3-(3-fluorophenyl)pyrazol-4-yl]methylene]hydrazino]benzoic Acid (18). Yellowish solid, ^1H NMR, 300 MHz (DMSO- d_6); δ 10.75 (s, 1H), 9.08 (s, 1H), 8.11–8.05 (m, 5H), 7.79 (d, $J = 8.2$ Hz, 2H), 7.62 (d, $J = 7.8$ Hz, 3H), 7.32 (s, 1H), 7.05 (d, $J = 8.1$ Hz, 2H); ^{13}C NMR (75 MHz, DMSO- d_6); δ 167.7, 167.1, 162.6 ($^1J = 241.9$ Hz), 150.3, 149.2, 142.4, 135.0 ($^3J = 8.3$ Hz), 131.4 ($^2J = 15.5$ Hz), 131.1 ($^3J = 8.5$ Hz), 129.1, 127.9, 125.0 ($^4J = 2.5$ Hz), 120.5, 119.2, 118.6, 115.9, 115.7, 115.4, 111.4. HRMS (ESI-FTMS, m/z): calcd for $\text{C}_{24}\text{H}_{17}\text{FN}_4\text{O}_4$ [$\text{M} + \text{H}$] $^+$ 445.1306, found 445.1310. Yield (386 mg, 87%).

4-[3-(3-Fluorophenyl)-4-[(E)-1,2,4-triazol-4-yliminomethyl]pyrazol-1-yl]benzoic Acid (19). White solid, ^1H NMR, 300 MHz (DMSO- d_6): δ 9.33 (s, 1H), 9.12 (s, 2H), 8.97 (s, 1H), 8.19–8.08 (m, 4H), 7.72–7.69 (m, 2H), 7.57 (d, $J = 7.2$ Hz, 1H), 7.35 (t, $J = 8.1$ Hz, 1H); (done) ^{13}C NMR (75 MHz, DMSO- d_6): δ 167.0, 162.7 ($^1J = 242.3$ Hz), 152.3, 151.5, 142.0, 139.4, 133.8 ($^3J = 8.5$ Hz), 131.4, 130.6, 129.9, 125.3, 119.3, 116.5, 116.2, 115.7 ($^2J = 22.3$ Hz). HRMS (ESI-FTMS, m/z): calcd for $\text{C}_{19}\text{H}_{13}\text{FN}_6\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 377.1157, found 377.1160. Yield (270 mg, 72%).

4-[3-(3-Fluorophenyl)-4-[(E)-[2-(pyridin-2-yl)hydrazinylidene]methyl]-1H-pyrazol-1-yl]benzoic Acid (20). Light yellow solid, ^1H NMR (300 MHz, DMSO- d_6): 13.08 (be s, 1H), 11.18 (s, 1H), 9.02 (s, 1H), 8.45 (d, $J = 4.4$ Hz, 2H), 8.31 (s, 1H), 8.17 (d, $J = 8.2$ Hz, 2H), 8.07 (d, $J = 8.2$ Hz, 2H), 7.73–7.68 (m, 2H), 7.60–7.56 (m, 1H), 7.31 (t, $J = 7.7$ Hz, 1H), 6.82 (s, 1H); ^{13}C APT NMR (75 MHz, DMSO- d_6): 167.0, 162.7 (d, $^1J = 241.6$ Hz), 160.2, 158.7, 150.6, 142.4, 134.7, 134.6, 131.3, 131.2 (d, $^3J = 8.0$ Hz), 129.1, 127.9, 125.0, 119.0, 118.8, 116.0, 115.7, 115.4, 113.3. HRMS (ESI-FTMS, m/z): calcd for $\text{C}_{22}\text{H}_{16}\text{FN}_5\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 402.1360, found 402.1365. Yield (300 mg, 75%).

4-[3-(3-Fluorophenyl)-4-[(E)-(4-methylpiperazin-1-yl)iminomethyl]pyrazol-1-yl]benzoic Acid (21). White solid, ^1H NMR, 300 MHz (DMSO- d_6): δ 8.81 (s, 1H), 8.07 (s, 4H), 7.63–7.61 (m, 3H), 7.57–7.50 (m, 1H), 7.28 (t, $J = 2.2$ Hz, 1H), 3.08 (s, 4H), 2.5 (s, 4H), 2.2 (s, 3H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 167.1, 162.6 ($^1J = 241.8$ Hz), 149.9, 142.4, 135.1 ($^3J = 8.1$ Hz), 131.3, 131.1 ($^3J = 8.2$ Hz), 129.2, 127.6, 127.2, 124.8, 120.2, 118.5, 115.6 ($^2J = 21.0$ Hz), 115.2 ($^2J = 22.7$ Hz), 115.1, 54.3, 50.8, 45.9. HRMS (ESI-FTMS, m/z): calcd for $\text{C}_{22}\text{H}_{22}\text{FN}_5\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 408.1830, found 408.1835. Yield (321 mg, 79%).

4-[3-(4-Fluorophenyl)-4-formyl-pyrazol-1-yl]benzoic Acid (3b). Yellowish Solid, ^1H NMR, 300 MHz (DMSO- d_6): δ 12.37 (br s, 1H), 9.84 (s, 1H), 7.82 (d, $J = 8.6$ Hz, 2H), 7.65–7.58 (m, 2H), 7.46–7.39 (m, 1H), 7.31–7.28 (m, 2H), 7.15 (t, $J = 8.3$ Hz, 1H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 185.0, 166.9, 163.2 ($^1J = 245.0$ Hz), 152.2, 141.9, 136.5, 131.4, 131.3, 130.0, 127.9 ($^4J = 3.0$ Hz), 122.9, 119.3, 115.9 ($^2J = 21.4$ Hz). HRMS (ESI-FTMS, m/z): calcd for $\text{C}_{17}\text{H}_{11}\text{FN}_2\text{O}_3$ [$\text{M} + \text{H}$] $^+$ 311.0826, found 311.0825. Yield (2.88 g, 93%).

4-[3-(4-Fluorophenyl)-4-[(E)-[methyl(phenyl)hydrazono]methyl]pyrazol-1-yl]benzoic Acid (22). Yellowish solid, ^1H NMR, 300 MHz (DMSO- d_6): δ 9.0 (s, 1H), 8.14–8.06 (m, 4H), 7.84 (t, $J = 7.23$ Hz, 2H), 7.6 (s, 1H), 7.37–7.23 (m, 6H), 6.86 (t, $J = 7.05$ Hz, 1H), 2.5 (s, 3H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 167.1, 162.6 ($^1J = 244.0$ Hz), 150.5, 147.7, 142.5, 131.3, 130.8 ($^3J = 8.2$ Hz), 129.6, 129.5, 129.2, 128.9, 127.0, 124.6, 120.3, 118.4, 116.0 ($^2J = 21.3$ Hz), 114.9, 32.8. HRMS (ESI-FTMS, m/z): calcd for $\text{C}_{24}\text{H}_{19}\text{FN}_4\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 415.1565, found 415.1561. Yield (376 mg, 91%).

4-[4-[(E)-(Diphenylhydrazono)methyl]-3-(4-fluorophenyl)pyrazol-1-yl]benzoic Acid (23). Light Yellow solid, ^1H NMR, 300 MHz (DMSO- d_6): δ 9.07 (s, 1H), 8.08 (s, 4H), 7.63–7.58 (m, 2H), 7.44 (t, $J = 7.86$ Hz, 4H), 7.27–7.12 (m, 9H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 167.1, 162.6 ($^1J = 244.2$ Hz), 150.7, 143.2, 142.5, 131.3, 130.8 ($^3J = 8.2$ Hz), 130.4, 129.2 ($^4J = 3.0$ Hz), 128.9, 128.1, 127.8, 124.9, 122.5, 119.0, 118.4, 115.8 ($^2J = 21.3$ Hz). HRMS (ESI-FTMS, m/z): calcd for HRMS (ESI-FTMS, m/z): calcd for $\text{C}_{29}\text{H}_{21}\text{FN}_4\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 477.1721, found 477.1722. Yield (428 mg, 90%).

4-[4-[(2-Benzyl-2-phenyl-hydrazino)methyl]-3-(4-fluorophenyl)pyrazol-1-yl]benzoic Acid (24). Light yellow solid, ^1H NMR, 300 MHz (DMSO- d_6): δ 9.01 (s, 1H), 8.13–8.05 (m, 4H), 7.46–7.27 (m, 10H), 7.19 (d, $J = 7.1$ Hz, 2H), 7.12 (t, $J = 8.8$ Hz, 2H), 6.9 (t, $J = 7.1$ Hz, 1H), 5.28 (s, 2H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 167.1, 162.4 ($^1J = 244.0$ Hz), 150.4, 147.7, 142.5, 136.4, 131.3, 130.2 ($^3J = 8.2$ Hz), 129.5, 129.4, 129.1 ($^4J = 3.0$ Hz), 128.8, 127.6, 126.8, 126.6, 125.7, 120.6, 119.7, 119.3, 118.5, 115.9 ($^2J = 21.3$ Hz), 114.6, 56.4. HRMS (ESI-FTMS, m/z): calcd for HRMS (ESI-FTMS, m/z): calcd for $\text{C}_{30}\text{H}_{23}\text{FN}_4\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 491.1877, found 491.1875. Yield (441 mg, 90%).

4-[4-[(E)-(Dibenzylhydrazono)methyl]-3-(4-fluorophenyl)pyrazol-1-yl]benzoic Acid (25). Light yellow Solid, ^1H NMR, 300 MHz (DMSO- d_6 + CDCl_3): δ 8.77 (s, 1H), 8.06–8.05 (m, 4H), 7.35–7.29 (m, 12H), 7.09–7.04 (m, 3H), 4.55 (s, 4H); ^{13}C NMR (75 MHz, DMSO- d_6 + CDCl_3): δ 167.1, 162.7 ($^1J = 270$ Hz), 149.9, 142.6, 138.3, 131.4, 131.3, 130.1 ($^3J = 8.2$ Hz), 129.0, 128.6, 127.8, 127.5, 125.7, 124.2, 120.1, 118.4, 115.8 ($^2J = 21.4$ Hz), 58.2. HRMS (ESI-FTMS, m/z): calcd for HRMS (ESI-FTMS, m/z): calcd for $\text{C}_{31}\text{H}_{25}\text{FN}_4\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 505.2034, found 505.2044. Yield (463 mg, 92%).

4-[3-(4-Fluorophenyl)-4-[(E)-[(4-fluorophenyl)hydrazono]methyl]pyrazol-1-yl]benzoic Acid (26). Yellowish solid, ^1H NMR, 300 MHz (DMSO- d_6): δ 9.0 (s, 1H), 8.10 (s, 4H), 7.91 (s, 1H), 7.80 (t, $J = 7.9$ Hz, 2H), 7.36 (t, $J = 8.4$ Hz, 2H), 7.07–7.00 (m, 3H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 167.1, 162.7 ($^1J = 244$ Hz), 156.1 ($^1J = 232$ Hz), 150.5, 142.5, 142.4, 131.3, 130.9 ($^3J = 8.3$ Hz), 129.3 ($^4J = 2.9$ Hz), 129.1, 128.8, 127.1, 119.4, 118.4, 116.7 ($^2J = 22.7$ Hz), 115.9 ($^2J = 21.3$ Hz), 113.1 ($^3J = 7.2$ Hz). HRMS (ESI-FTMS, m/z): calcd for HRMS (ESI-FTMS, m/z): calcd for $\text{C}_{23}\text{H}_{16}\text{F}_2\text{N}_4\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 419.1314, found 419.1311. Yield (326 mg, 78%).

4-[4-[(E)-[(3-Bromophenyl)hydrazono]methyl]-3-(4-fluorophenyl)pyrazol-1-yl]benzoic Acid (27). Light Yellow Solid, ^1H NMR, 300 MHz (DMSO- d_6): δ 10.43 (s, 1H), 9.04 (s, 1H), 8.13–8.06 (m, 4H), 7.93 (s, 1H), 7.83–7.78 (m, 2H), 7.36 (t, $J = 8.8$ Hz, 2H), 7.17–7.09 (m, 2H), 6.86 (t, $J = 7.4$ Hz, 2H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 167.1, 162.7 ($^1J = 244.2$ Hz), 150.7, 147.2, 142.5, 131.4, 131.1, ($^3J = 8.2$ Hz), 130.6, 129.31, 129.3 ($^3J = 12.4$ Hz), 128.9, 127.8, 123.0, 121.2, 119.0, 118.5, 115.9 ($^2J = 21.4$ Hz), 114.2, 111.3. HRMS (ESI-FTMS, m/z): calcd for $\text{C}_{23}\text{H}_{16}\text{BrFN}_4\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 479.0513, found 479.0510. Yield (377 mg, 79%).

4-[4-[(E)-[(2,5-Difluorophenyl)hydrazono]methyl]-3-(4-fluorophenyl)pyrazol-1-yl]benzoic Acid (28). Light yellow solid, ^1H NMR, 300 MHz (DMSO- d_6): δ 10.33 (s, 1H), 8.85 (s, 1H), 8.28 (s, 1H), 8.12–8.04 (m, 5H), 7.83–7.78 (m, 2H), 7.34 (t, $J = 3\text{H}$), 7.50–7.10 (m, 1H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 167.0, 162.8 ($^1J = 244.2$ Hz), 150.9, 146.6 (m), 142.4, 138.4 ($^3J = 16.3$ Hz), 135.7, 131.3, 131.0 ($^3J = 8.2$ Hz), 129.0, 128.9 ($^4J = 3.0$ Hz), 127.5, 125.7, 118.7, 118.3, 115.9 ($^2J = 21.3$ Hz), 95.8, 95.5, 95.2. HRMS (ESI-FTMS, m/z): calcd for $\text{C}_{23}\text{H}_{13}\text{F}_3\text{N}_4\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 437.1219, found 437.1220. Yield (331 mg, 76%).

4-[4-[(E)-[(3-Chloro-2-fluorophenyl)hydrazono]methyl]-3-(4-fluorophenyl)pyrazol-1-yl]benzoic Acid (29). Light Yellow Solid, ^1H NMR, 300 MHz (DMSO- d_6): δ 10.39 (s, 1H), 9.05 (s, 1H), 8.25 (s, 1H), 8.09 (s, 4H), 7.78 (t, $J = 7.8$ Hz, 2H), 7.44–7.33 (m, 3H), 7.07 (t, $J = 8.1$ Hz, 1H), 6.85 (t, $J = 6.9$ Hz, 1H); ^{13}C NMR (75 MHz, DMSO- d_6): δ

167.1, 162.7 ($^1J = 244.2$ Hz), 150.8, 144.6 ($^1J = 239.8$ Hz), 142.4, 135.3 ($^3J = 9.1$ Hz), 133.3, 131.4, 130.9 ($^3J = 8.3$ Hz), 129.0 ($^4J = 2.9$ Hz), 129.0, 127.6, 125.8 ($^4J = 3.7$ Hz), 119.8 ($^3J = 14.1$ Hz), 119.0, 118.8, 118.5, 116.0 ($^2J = 21.4$ Hz), 112.9. HRMS (ESI-FTMS, m/z): calcd for $C_{23}H_{15}ClF_2N_4O_2$ [$M + H$] $^+$ 453.0924, 455.0897, found 453.0926, 455.0895. Yield (334 mg, 74%).

4-[4-[(E)-[(4-Cyanophenyl)hydrazono]methyl]-3-(4-fluorophenyl)pyrazol-1-yl]benzoic Acid (30). Greenish yellow solid, 1H NMR, 300 MHz (DMSO- d_6): δ 10.88 (s, 1H), 9.07 (s, 1H), 8.13–8.06 (m, 4H), 8.01 (s, 1H), 7.83–7.78 (m, 2H), 7.59 (d, $J = 8.7$ Hz, 2H), 7.36 (t, $J = 8.8$ Hz, 2H), 7.08 (d, $J = 8.6$ Hz, 2H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 167.1, 162.8 ($^1J = 244.3$ Hz), 150.9, 148.9, 142.4, 134.0, 132.8, 131.3, 131.0 ($^3J = 8.2$ Hz), 129.1, 127.9, 120.6, 118.7, 118.5, 116.0 ($^2J = 21.4$ Hz), 112.3, 99.4. HRMS (ESI-FTMS, m/z): calcd for $C_{24}H_{16}FN_5O_2$ [$M + H$] $^+$ 426.1367, found 426.1364. Yield (352 mg, 83%).

4-[3-(4-Fluorophenyl)-4-[(E)-[2-(4-nitrophenyl)hydrazinylidene]methyl]-1H-pyrazol-1-yl]benzoic Acid (31). Orange solid, 1H NMR, 300 MHz (DMSO- d_6): δ 11.23 (br s, 1H), 9.10 (s, 1H), 8.11–8.05 (m, 7H), 7.82–7.78 (m, 2H), 7.37 ($J = 8.7$ Hz, 2H), 7.07 (d, $J = 8.5$ Hz, 2H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 167.1, 162.8 ($^1J = 244.4$ Hz), 151.0, 150.9, 142.3, 138.5, 134.7, 131.3, 131.0 ($^3J = 8.3$ Hz), 129.1, 129.0 ($^4J = 3.0$ Hz), 128.2, 126.5, 118.5, 118.4, 116.0 ($^2J = 21.4$ Hz), 111.4. HRMS (ESI-FTMS, m/z): calcd for $C_{23}H_{16}FN_5O_4$ [$M + H$] $^+$ 446.1259, found 446.1265. Yield (396 mg, 89%).

3-[(2E)-2-[[1-(4-Carboxyphenyl)-3-(4-fluorophenyl)pyrazol-4-yl]methylene]hydrazino]benzoic Acid (32). Yellow solid, 1H NMR, 300 MHz (DMSO- d_6): δ 10.44 (s, 1H), 9.01 (s, 1H), 8.14–8.06 (m, 4H), 7.96 (s, 1H), 7.89–7.84 (m, 2H), 7.57 (s, 1H), 7.41–7.30 (m, 4H), 7.23 (m, 1H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 168.1, 167.1, 162.7 ($^1J = 244.2$ Hz), 150.6, 145.9, 142.5, 132.1, 131.3, 131.0 ($^3J = 8.3$ Hz), 130.3, 129.6, 129.2 ($^4J = 3.1$ Hz), 129.0, 127.8, 119.8, 119.1, 118.5, 116.4, 115.9 ($^2J = 21.3$ Hz), 112.7. HRMS (ESI-FTMS, m/z): calcd for $C_{24}H_{17}FN_4O_4$ [$M + H$] $^+$ 445.1307, found 445.1308. Yield (364 mg, 82%).

4-[(2E)-2-[[1-(4-Carboxyphenyl)-3-(4-fluorophenyl)pyrazol-4-yl]methylene]hydrazino]benzoic Acid (33). Yellow solid, 1H NMR, 300 MHz (DMSO- d_6): δ 12.71 (br s, 2H), 10.74 (s, 1H), 9.07 (s, 1H), 8.14–8.07 (m, 4H), 8.01 (s, 1H), 7.84–7.78 (m, 4H), 7.40–7.34 (m, 2H), 7.04 (d, $J = 8.6$ Hz, 2H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 167.7, 167.1, 162.8 ($^1J = 244.3$ Hz), 150.8, 149.2, 142.5, 131.7, 131.6, 131.3, 131.0 ($^3J = 8.3$ Hz), 129.1 ($^4J = 2.9$ Hz), 128.9, 127.6, 120.4, 119.0, 118.5, 116.0 ($^2J = 21.4$ Hz), 111.4. HRMS (ESI-FTMS, m/z): calcd for $C_{24}H_{17}FN_4O_4$ [$M + H$] $^+$ 445.1307, found 445.1308. Yield (381 mg, 86%).

4-[4-[(E)-[(2,4-Dioxoimidazolidin-1-yl)hydrazono]methyl]-3-(4-fluorophenyl)pyrazol-1-yl]benzoic Acid (34). White solid, 1H NMR, 300 MHz (DMSO- d_6): δ 11.26 (s, 1H), 9.02 (s, 1H), 8.15–8.05 (m, 4H), 7.89–7.85 (m, 2H), 7.73 (s, 1H), 7.33 (t, $J = 8.8$ Hz, 2H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 169.5, 167.0, 162.8 ($^1J = 244.5$ Hz), 153.7, 151.4, 142.3, 135.8, 131.3, 131.0 ($^3J = 8.3$ Hz), 129.2, 128.6, 128.3, 118.8, 117.8, 116.1 ($^2J = 21.3$ Hz), 49.0. HRMS (ESI-FTMS, m/z): calcd for $C_{20}H_{14}FN_5O_4$ [$M + H$] $^+$ 408.1103, found 408.1105. Yield (317 mg, 78%).

3-[(2E)-2-[[1-(4-Carboxyphenyl)-3-(4-fluorophenyl)pyrazol-4-yl]methylene]hydrazino]benzoic Acid (35).

White solid, 1H NMR, 300 MHz (DMSO- d_6): δ 11.02 (br s, 1H), 9.01 (s, 1H), 8.14–8.04 (m, 5H), 7.81–7.76 (m, 2H), 7.34 (t, $J = 8.79$ Hz, 2H), 3.67 (s, 3H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 167.0, 162.8 ($^1J = 244.3$ Hz), 154.2, 151.4, 142.4, 131.3, 131.0 ($^3J = 8.2$ Hz), 129.1, 128.7, 128.1, 118.8, 117.9, 116.0 ($^2J = 21.4$ Hz), 52.3. HRMS (ESI-FTMS, m/z): calcd for $C_{19}H_{15}FN_4O_4$ [$M + H$] $^+$ 383.1150, found 383.1157. Yield (267 mg, 70%).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b01967.

1H and ^{13}C NMR spectra of new compounds; computational data (PDF)

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Notes

The authors declare no competing financial interest.

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