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Quinolone-3-amidoalkanol: A New Class of Potent and Broad-Spectrum Antimicrobial Agent

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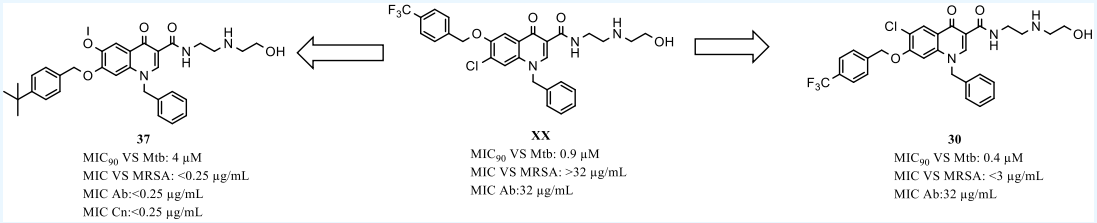

ABSTRACT: Herein, we describe 39 novel quinolone compounds bearing a hydrophilic amine chain and varied substituted benzyloxy units. These compounds demonstrate broad-spectrum activities against acid-fast bacterium, Gram-positive and -negative bacteria, fungi, and leishmania parasite. Compound **30** maintained antitubercular activity against moxifloxacin-, isoniazid-, and rifampicin-resistant *Mycobacterium tuberculosis*, while **37** exhibited low micromolar activities (<1 μg/mL) against World Health Organization (WHO) critical pathogens: *Cryptococcus neoformans*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*. Compounds in this study are metabolically robust, demonstrating % remnant of >98% after 30 min in the presence of human, rat, and mouse liver microsomes. Several compounds thus reported here are promising leads for the treatment of diseases caused by infectious agents.

1. INTRODUCTION

Tuberculosis (TB) is an airborne infectious disease that primarily affects the lungs. TB sometimes spreads from the lungs to affect other tissues such as the spleen, bone marrow, spine, and brain.[1] This disease, which in 2021 affected more than 10.6 million people with ill health and killed at least 1.5 million people, is currently the second (second to COVID-19) leading cause of death from a single infective agent.[2] TB has a large human reservoir, with one-third of the world's population currently harboring the asymptomatic form of the disease (latent TB).[3]

The treatment for any form of TB, be it drug-susceptible or drug-resistant, is complex and requires a combination of multiple drugs that are taken over a period of at least 4 months.[4] For example, treatment of drug-susceptible TB requires a daily intake of isoniazid, pyrazinamide, ethambutol, and rifampicin for 2 months followed by isoniazid and rifampicin for at least four additional months.[5] For treating drug-resistant TB, the World Health Organization (WHO) 2022 treatment guideline recommends the use of BPaLM (bedaquiline, pretomanid, linezolide, and moxifloxacin) for 6−9 months.[6] There is an apparent need to identify and develop novel antitubercular agents and treatment regimens to increase the drug weaponry against TB. Moreover, although TB affects all parts of the world, the burden is relatively high in low- to middle-income countries. With Russia, China, India, Bangladesh, Brazil, Nigeria, and South Africa being some of the worst affected countries.[2] As such, new antitubercular agents should be inexpensive to make.

In addition to TB, global public health is currently under threat by drug-resistant pathogenic bacteria, most of which have developed several mechanisms of resistance to overcome the efficacy of multiple drug classes.[7] Commonly encountered drug-resistant bacteria in community and/or clinical settings have been clustered and dubbed "ESKAPE" pathogens.[8] These bacteria are responsible for several clinical indications such as septicemia, ventilator-associated pneumonia (VAP), urinary tract infection (UTI), and skin and soft tissue infection.[9] They are collectively responsible for over 929,000 deaths in 2019 and an associated annual health care cost of over $2 billion dollars.[10]

Although the threat and burden posed by drug-resistant bacteria is postulated to get worst, only eight drugs are currently in the development pipeline against these pathogens, and they belong to just three drug classes: β-lactam/β-

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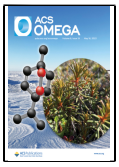

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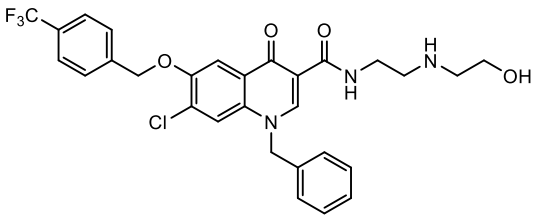
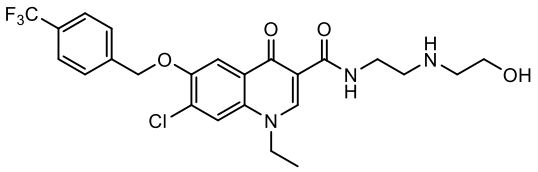


XX

MIC_{90} vs Mtb: 0.9 μM
MIC vs MRSA: >32 μg/mL
MIC vs Ab: 32 μg/mL



YY

MIC_{90} vs Mtb: 1.9 μM
MIC vs MRSA: 16 μg/mL
MIC vs Ab: 32 μg/mL

Figure 1. Previously identified hit compounds.

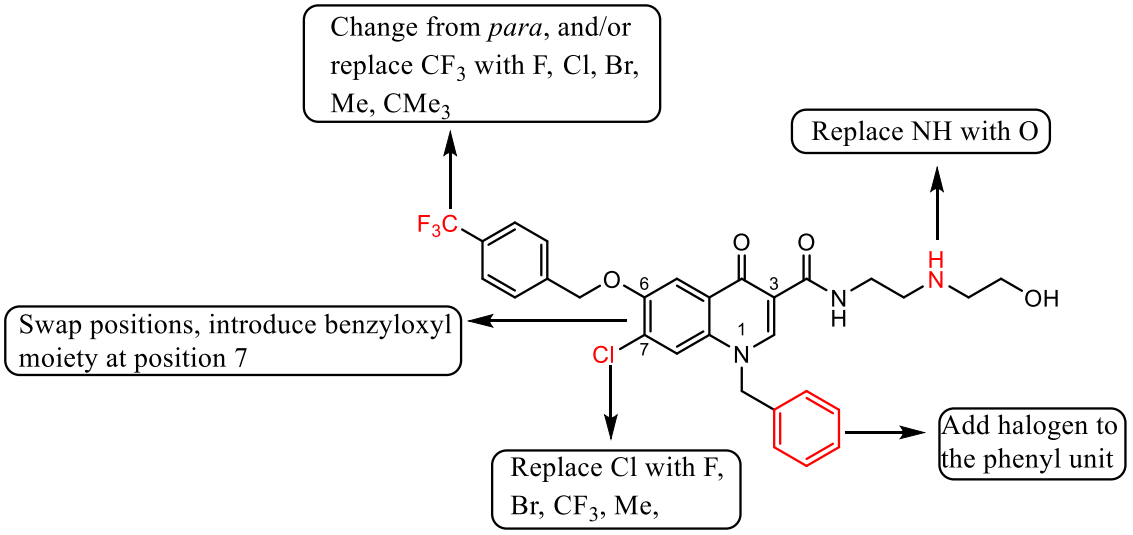


Figure 2. Synthetic plan to generate analogues for SAR.

lactamase inhibitors, aminoglycoside (plazomicin), and tetracycline (evaracycline).[11]

WHO recently classified drug-resistant bacteria into "critical, high, and medium" priority to emphasize where the discovery and development of new antibacterial agents are urgently needed. Critical priority pathogens include *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacteriaceae* (*Escherichia coli* and *Klebsiella pneumoniae*).[12] These Gram-negative pathogens are resistant to the latest generations of carbapenems and cephalosporins,[13] which are mainstay antibiotics. This has necessitated the use of colistin (a previously abandoned agent with toxicity concerns) as the last line of defense against these bacteria.[14] There is a pressing need to identify and develop new molecules active against these pathogenic bacteria.

In addition to bacterial infections, fungal diseases are also on the rise and are associated with drug resistance.[15] This has prompted the WHO to also classify pathogenic fungi into critical, high, and medium priority based on the need for new drugs. Within the critical priority group are *Candida albicans* and *Cryptococcus neoformans*.[16] Candidiasis, caused mostly by *C. albicans*, has a global mortality rate of 27−55%.[17] Cryptococcosis, caused by *C. neoformans* and *Cryptococcus gattii*, is accountable for at least 15% of all AIDS-associated deaths worldwide.[18] In sub-Saharan Africa, *C. neoformans*-HIV co-infection has more than 90% mortality rate.[19]

Current treatments for candidiasis and cryptococcosis are limited to three chemical classes: polyenes, azoles, and echinocandins.[20] Due to the high cost, complicated route of administration, and monitoring implications that come with the application of polyenes such as amphotericin B and flucytosine,[21] fluconazole is the sole treatment option for cryptococcosis in sub-Saharan Africa.[22] This has led to increased fluconazole resistance and treatment failures in this region.[23] There is therefore an apparent need to identify new and low-cost compounds with activity against the causal agents of cryptococcosis.

Leishmaniasis is a neglected parasitic disease that is caused by parasitic protozoans of the genus *Leishmania*.[24] There are approximately 1.3 million new cases of the disease and about 70,000 attributable deaths annually.[25] This disease presents in several forms: *Cutaneous leishmaniasis* (CL), which is characterized by ulceration of the skin; *Mucocutaneous leishmaniasis* (ML), which presents as destruction of the buccal and nasal cavities; and *Visceral leishmaniasis* (VL), which affects internal organs such as the liver, and spleen.[26] Both CL and ML are caused by multiple species and subspecies of leishmania parasites. They both cause serious disfigurement and disability.[27] *Leishmania infantum* and *Leishmania donovani* cause VL; this form of leishmaniasis is fatal if not promptly and appropriately treated.[28]

VL is treated using amphotericin B, pentavalent antimonials, or miltefosine depending on the region. These treatments have

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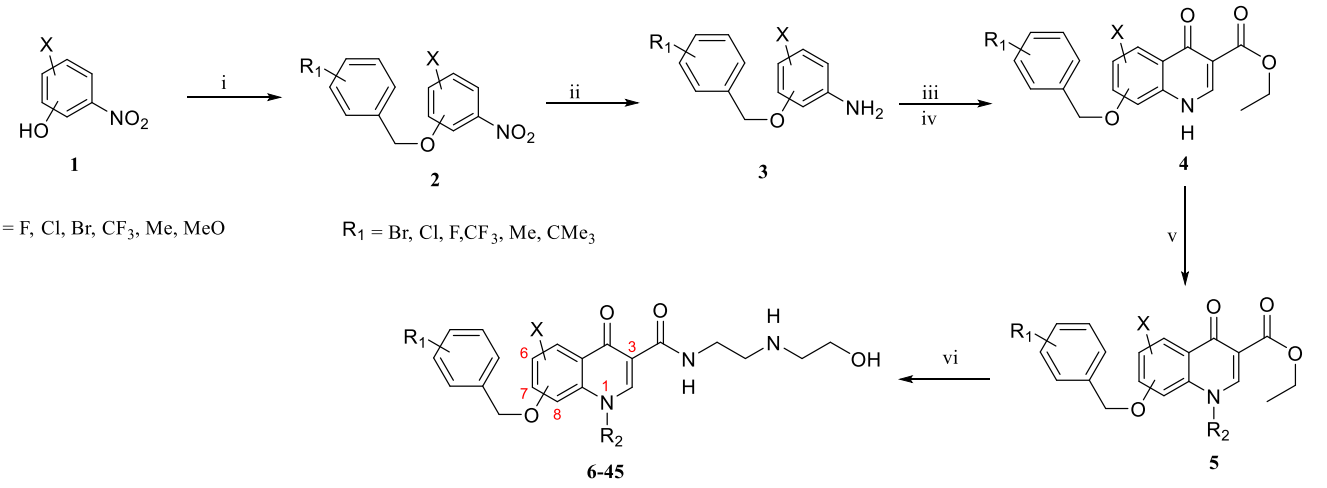
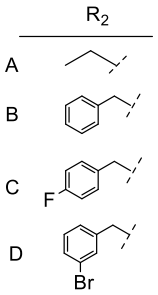
Scheme 1. Synthesis of Target Compounds[a]



6: R_1 = *p*-CF_3, R_2 = C, X = 7-Cl
7: R_1 = *p*-CF_3, R_2 = D, X = 7-Cl
8: R_1 = *p*-F, R_2 = A, X = 7-Cl
9: R_1 = *p*-F, R_2 = B, X = 7-Cl
10: R_1 = *p*-CMe_3, R_2 = A, X = 7-Cl
11: R_1 = *p*-CMe_3, R_2 = B, X = 7-Cl
12: R_1 = *p*-Cl, R_2 = A, X = 7-Cl
13: R_1 = *p*-Cl, R_2 = B, X = 7-Cl
14: R_1 = *m*-Me, R_2 = A, X = 7-Cl
15: R_1 = *m*-Cl, R_2 = B, X = 7-Cl
16: R_1 = *m*-CF_3, R_2 = A, X = 7-Cl
17: R_1 = *m*-CF_3, R_2 = B, X = 7-Cl
18: R_1 = *p*-CF_3, R_2 = B, X = 7-Br
19: R_1 = *p*-CF_3, R_2 = A, X = 7-CF_3
20: R_1 = *p*-Br, R_2 = A, X = 7-F
21: R_1 = *p*-Br, R_2 = B, X = 7-F
22: R_1 = *m*-Br, R_2 = A, X = 7-F
23: R_1 = *m*-Br, R_2 = B, X = 7-F
24: R_1 = *m*-Br, R_2 = C, X = 7-F
25: R_1 = *m*-Cl, R_2 = B, X = 7-F
26: R_1 = *p*-F, R_2 = A, X = 7-F
27: R_1 = *p*-F, R_2 = B, X = 7-F
28: R_1 = *p*-F, R_2 = C, X = 7-F
29: R_1 = *m*-Cl, R_2 = B, X = 7-Me
30: R_1 = *p*-CF_3, R_2 = B, X = 6-Cl
31: R_1 = *p*-CF_3, R_2 = B, X = 6,8-diCl
32: R_1 = *p*-CF_3, R_2 = A, X = 6-MeO
33: R_1 = *p*-CF_3, R_2 = B, X = 6-MeO
34: R_1 = *m*-CF_3, R_2 = A, X = 6-MeO
35: R_1 = *m*-CF_3, R_2 = B, X = 6-MeO
36: R_1 = *m*-CMe_3, R_2 = A, X = 6-MeO
37: R_1 = *m*-CMe_3, R_2 = B, X = 6-MeO
38: R_1 = *m*-Cl, R_2 = B, X = 6-MeO
39: R_1 = *m*-Br, R_2 = A, X = 6-MeO
40: R_1 = *m*-Br, R_2 = B, X = 6-MeO
41: R_1 = *m*-F, R_2 = A, X = 6-MeO
42: R_1 = *m*-F, R_2 = B, X = 6-MeO
43: R_1 = *m*-diF, R_2 = A, X = 6-MeO



[a]Reagents and conditions: (i) benzyl halide, K_2CO_3, acetone, reflux 12 h; (ii) Fe-powder, acetic acid, NH_4Cl, ethanol, reflux 12−48 h; (iii) ethanol, reflux 12 h; (iv) diphenyl ether, 240−250 °C, 15 min; (v) K_2CO_3, $CHCl_3$/THF (2:1), benzyl/alkyl halide (3 equiv), reflux, 36 h; (vi) amine (5 equiv), DBU (1.5 equiv), $CHCl_3$, reflux 48−72 h.

several shortcomings, including toxicities, poor pharmacokinetic properties, and cost.[29] Hence, there is a need to identify new compounds with anti-leishmania activity.

We previously identified compounds **XX** and **YY** (Figure 1), within a subseries of 10 compounds, with activity against *Mycobacterium tuberculosis* (Mtb), the causal agent of TB. These compounds also showed weak to moderate activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and *A. baumannii*.[30]

Herein, we execute extensive analogue synthesis with the aim of generating more active compounds and a structure−activity relationship for this series. The synthesized compounds were all screened for activity against *L. donovani*, Mtb, and cell toxicity. Compounds exhibiting low cell toxicity were selected and evaluated for activity against ESKAPE pathogens, *C. neoformans*, and *C. albicans*. Seven compounds were selected, and their ADME properties were evaluated in vitro.

2. RESULTS AND DISCUSSION

2.1. Chemistry. We previously identified antitubercular hit compounds (**XX** and **YY**) from a small series of compounds that differ only on the nature of substituents at position 3 of the quinolone nucleus. Herein, we aim to probe structural variations at different positions of the quinolone nucleus and evaluate how these iterative changes affect antitubercular activity. We generated several analogues of compounds **XX** and **YY** by varying substituents at different positions of the quinolone ring one at a time. In some instances, substituents at more than one position (6 and 7) were concurrently varied or their positions swapped (Figure 2). Starting at position 1, halogens were added to the phenyl moiety to give compounds **6** and **7**. At position 6, the *p*-CF_3 moiety was either changed to *m*-position or replaced with either electron-withdrawing (F, Cl, Br) or electron-donating (Me, CMe_3) moieties to produce compounds **8**−**17**. The Cl atom at position 7 was replaced with Br or CF_3 moiety to generate compounds **18** and **19**.

With a F/Me at position 7, the *p*-CF_3 moiety attached to the benzyloxy group at position 6 was replaced with either *m*- or *p*-F, Cl, and Br to afford compounds **20**−**29**.

Next, we swap substituents at positions 6 and 7, introducing a Cl at position 6 and *p*-CF_3 benzyloxy at position 7 to generate compounds **30** and **31**. Subsequently, the Cl at position 6 was replaced with an MeO moiety, while the *p*-CF_3 unit was changed to either *m*- or *p*-F, Cl, Br, Me, and CMe_3 moieties to generate compounds **32**−**43**. The hydrophilic amide chain at position 3 was also modified by replacing the secondary amine (−NH−) with an O atom as seen in compounds **44** and **45**.

To achieve all of these intended compounds, the reagents and reaction conditions presented in Scheme 1 were deployed.

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Table 1. Structures and Antitubercular and Cytotoxicity Activities of Target Compounds

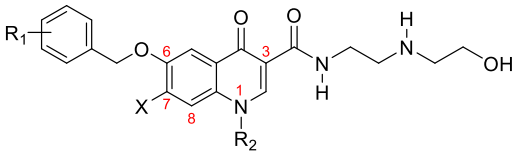
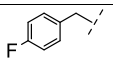
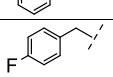
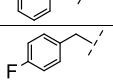
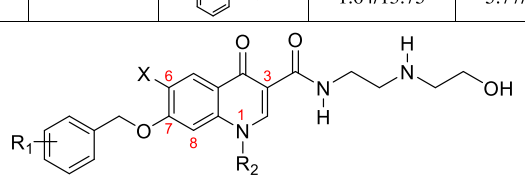
Entry	X	R_1	R_2	*Mtb* MIC_{90}[a] μM Day 7/14	*Mtb* MIC_{90}[b] μM Day 7/14	CC_{50} μM VS HEK293
						
6	7-Cl	*p*-CF_3		3.46/6.53	7.81/7.97	>32
7	7-Cl	*p*-CF_3		2.91/4.99	6.13/7.74	>32
8	7-Cl	*p*-F		3.62/8.46	>125/125	>32
9	7-Cl	*p*-F		2.65/3.87	8.09/7.81	>32
10	7-Cl	*p*-CMe_3		3.39/4.89	7.99/13.16	>32
11	7-Cl	*p*-CMe_3		2.47/3.38	7.6/15.4	>32
12	7-Cl	*p*-Cl		3.39/8.88	13.99/62.5	>32
13	7-Cl	*p*-Cl		6.05/7.81	3.91/10.71	>32
14	7-Cl	*m*-Me		8.93/15.63	31.25/62.5	>32
15	7-Cl	*m*-Cl		41.93/125	>125/>125	>32
16	7-Cl	*m*-CF_3		1.65/2.43	6.82/16.04	>32
17	7-Cl	*m*-CF_3		>125/>125	>125/>125	>32
18	7-Br	*p*-CF_3		0.96/3.13	3.13/2.64	<5
19	7-CF_3	*p*-CF_3		1.84/2.08	17.03/21.88	<5
20	7-F	*p*-Br		9.92/16.07	16.07/44.89	<5
21	7-F	*p*-Br		3.35/5.17	5.27/7.84	<5
22	7-F	*m*-Br		2.04/3.88	8.39/12.69	<5
23	7-F	*m*-Br		1.736/2.928	6.736/10.47	<5
24	7-F	*m*-Br		1.95/2.68	7.81/2.91	<5
25	7-F	*m*-Cl		3.14/4.69	4.69/9.18	<5
26	7-F	*p*-F		31.25/19.19	125/125	<5
27	7-F	*p*-F		2.71/5.77	13.79/5.43	<5
28	7-F	*p*-F		3.82/6.06	15.83/7.81	<5
29	7-Me	*m*-Cl		1.64/13.75	5.77/31.25	<5
						
30	6-Cl	*p*-CF_3		0.495/2.316	4.61/3.906	>32
31	6,8-diCl	*p*-CF_3		0.815/4.19	>125/3.325	>32
32	6-MeO	*p*-CF_3		19.24/63.15	31.25/92.5	>32

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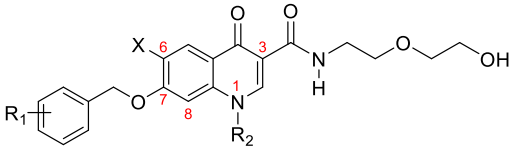
Table 1. continued

Entry	X	R_1	R_2	*Mtb* MIC_{90}[a] μM Day 7/14	*Mtb* MIC_{90}[b] μM Day 7/14	CC_{50} μM VS HEK293
33	6-MeO	*p*-CF_3		5.82/13.15	15.40/31.66	>32
34	6-MeO	*m*-CF_3		9.0/23.11	7.37/62.5	>32
35	6-MeO	*m*-CF_3		5.87/15.62	3.91/31.25	>32
36	6-MeO	*p*-CMe_3		17.49/48.98	62.5/125	>32
37	6-MeO	*p*-CMe_3		4.42/7.49	13.84/31.25	>32
38	6-MeO	*m*-Cl		5.47/14.53	15.63/31.25	>32
39	6-MeO	*m*-Br		31.25/15.63	33.25/32.5	>32
40	6-MeO	*m*-Br		15.63/15.63	31.25/62.5	>32
41	6-MeO	*m*-F		>125/62.5	62.5/125	>32
42	6-MeO	*m*-F		15.8/14.73	15.63/62.5	>32
43	6-MeO	*m*,*m*-diF		13.71/35.37	7.81/31.86	>32
						
44	6-Cl	*p*-CF_3		>125/ >125	>125/ >125	>32
45	6-Cl	*p*-CF_3		>125/ >125	>125/ >125	>32
Rifampicin	-	-	-	0.010/0.017	0.001/0.002	-

[a]Medium contained 7H9 CAS GLU Tx. [b]Medium contained 7H9 ADC GLU Tw.

Briefly, various benzyl halides were treated with nitrophenols (**1**) under basic condition to form nitrobenzyl ethers (**2**), which underwent $-NO_2$ reduction to form benzyloxyanilines (**3**) in good yields (64−97%). Subsequent conjugate addition with diethyl ethoxylmethylenemalonate and cyclization afforded intermediate **4** in moderate to good yields of 38−82%. Intermediate **4** was derivatized through consecutive *N*-alkylation and aminolysis to generate target compounds **6**−**45** in low to moderate yields (4−50%).

Analytical techniques, nuclear magnetic resonance (NMR) spectroscopy and high-resolution mass spectrometry (HRMS), were used to characterize and confirm the identity of target compounds. Chemical transformations were confirmed by the presence of the following general signatory peaks on ^1H and ^{13}C NMR of all target compounds: (i) a triplet signal at *ca.* 10.29 ppm on the ^1H NMR with the corresponding ^{13}C NMR signal at *ca.* 165.00 ppm suggests successful aminolysis of the ester at C-3 to an amide (−CONH−). (ii) A singlet signal appearing at *ca.* 5.3 ppm on the ^1H NMR and a ^{13}C NMR peak at *ca.* 68.94 ppm is attributed to the $-OCH_2-$ moiety introduced during the Williamson ether synthesis step. (iii) The signal appearing at ca. 4.5 ppm on the ^1H NMR as either a quintet or singlet is attributed to $-NCH_2-$ moiety, and it confirms complete deprotonation and *N*-alkylation of the quinolone intermediates. In addition, the ^1H NMR spectra of **32**−**43** display a singlet peak at *ca.* 3.88 ppm representing the methyl ($-CH_3$) of the MeO moiety at C-6. The ^{13}C NMR signal for this peak appears at about *ca.* 55.75 ppm. Finally, the −OH signal appears as a triplet peak at around *ca.* 4.2−4.5 ppm in all 1H NMR, except where there is exchange with solvent peaks. Molecular ion peaks were consistent with the molecular weight (MW) of the desired compounds (Supporting Materials). HPLC percent (%) purity of target compounds was determined in the range of 90−99%.

2.2. Antitubercular Activity and Cytotoxicity Evaluation. In vitro inhibitory activity of the target compounds against Mtb was done alongside rifampicin as a reference in two Middlebrook 7H9 media cultures (CAS GLU Tx and ADC GLU Tw). Noteworthy, the CAS growth medium contains amino acids (casine), while the ADC growth medium contains peptide (albumin); thus, the latter may introduce the protein binding factor in overall biological activity. Table 1 reports the antitubercular activity of the target compounds as the minimum inhibitory concentration required to inhibit 90% (MIC_{90}) of the bacteria population. Target compounds in this study were generally active against Mtb in both media, with the compounds displaying better activity in 7H9 CAS GLU Tx medium (MIC_{90} 2.47−19.24 μM) than the ADC GLU Tw medium (MIC_{90} 3.91 −62.5 μM). The reduced activity observed in ADC GLU Tw medium could be attributed to protein binding in this medium.

In comparison to compound **XX** (MIC_{90}: 0.9−2 μM), introducing halogens to the benzyl moiety at position 1 as seen in compounds **6** (MIC_{90}: 3−6 μM) and **7** (MIC_{90}: 3−5 μM) generally leads to analogues with a small reduction in antitubercular activity. Replacing the *p*-CF_3 group on the benzyloxyl moiety at position 6 with *p*-F, *p*-Cl, *p*-CMe_3, as is the case in compounds **8**−**12** (MIC_{90}: ∼3 μM), generally leads to analogues with comparable antitubercular activity to compound **XX** and **YY**. With the exception of compound **16** (MIC_{90}: 1−2 μM), compounds **14**, **15**, and **17** (MIC_{90}: 8−125 μM), having a *meta*-substituted benzyloxy moiety at position 6 generally showed lower antitubercular activity than their *para*-

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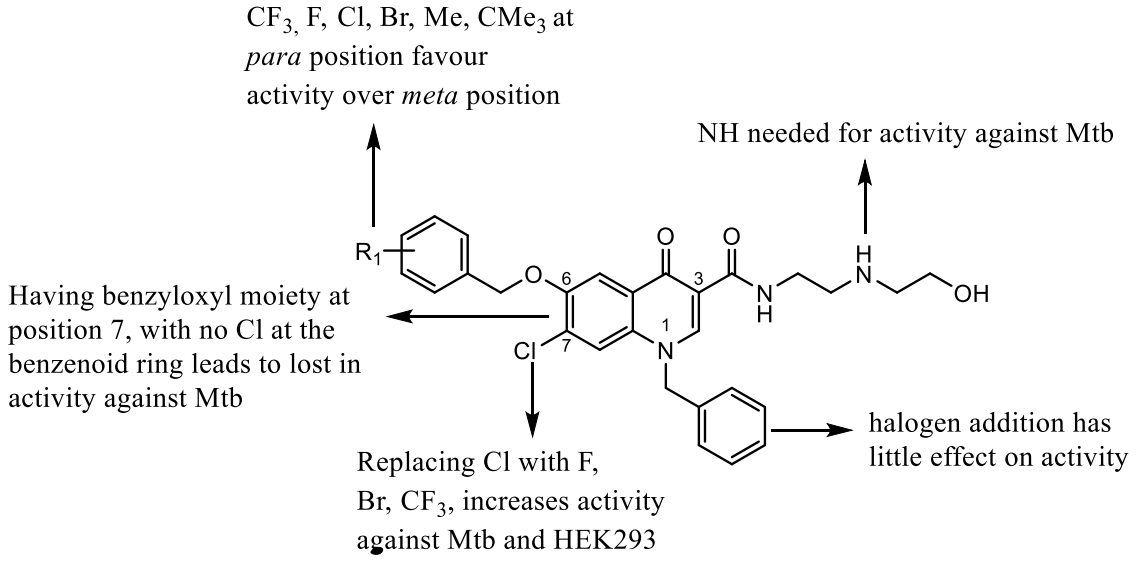

Figure 3. Summary of antitubercular SARS.

Table 2. Antibacterial (μg/mL), Antifungal (μg/mL), and Antileishmanial (μM) Activities of Selected Compounds[a]

entry	MRSa	Ec	Kp	Pa	Ab	Ca	Cn	Ld
6	2	<0.25	0.5	<0.25	1	>32	<0.25	>20
7	<0.25	<0.25	<0.25	<0.25	<0.25	>32	<0.25	>20
10	<0.25	<0.25	<0.25	<0.25	<0.25	16	<0.25	2.8
11	<0.25	<0.25	<0.25	<0.25	<0.25	>32	<0.25	3.0
13	32	<0.25	<0.25	<0.25	<0.25	>32	<0.25	>20
14	>32	<0.25	<0.25	<0.25	<0.25	>32	4	>20
30	<0.25	>32	>32	>32	>32	>32	<0.25	2.8
31	<0.25	>32	>32	>32	>32	>32	<0.25	7.1
37	<0.25	1	<0.25	<0.25	<0.25	4	<0.25	9.5
38	>32	<0.25	<0.25	<0.25	0.5	>32	4	>20
39	>32	<0.25	<0.25	<0.25	0.5	>32	<0.25	>20
40	<0.25	<0.25	<0.25	<0.25	0.5	<0.25	16	>20
41	>32	<0.25	<0.25	<0.25	<0.25	>32	>32	>20
42	>32	<0.25	<0.25	<0.25	<0.25	>32	<0.25	>20
43	>32	<0.25	<0.25	<0.25	<0.25	>32	>32	>20
colistin	-	0.12	0.25	0.25	0.25			
levofloxacin	0.9	<0.05	1.81	3.62	0.9	>32	>32	
vancomycin	1							
fluconazole						0.12	8	
amphoteric B								0.03

[a]MRSa = Methicilin resistant *S. aureus*, Ec = *E. coli*, Kp = *K. pneumoniea*, Pa = *P. aeruginosa*, Ab = *A. baumannii*, Ca = *C. albicans*, Cn = *C. neoformans*, Ld = *L. donovani*.

substituted analogues, compounds **8**−**12**. It is noteworthy to mention that compound **16** also showed strong cytotoxicity (CC_{50}: <5 μM) against HEK293 cells.

Replacing the −Cl atom at position 7 with F, Br, Me, and CF_3 gave compounds **18**−**29**, most of which showed very potent antitubercular activity (MIC_{90}: 0.9−3 μM). These compounds, however, also showed strong cytotoxicity (CC_{50}: < 5 μM) against HEK293 cells.

Swapping substituents at positions 6 and 7, i.e., introducing a Cl atom at position 6 and a *p*-CF_3 benzyloxy at position 7, afforded compound **30** (MIC_{90}: 0.4 μM) with improved antitubercular activity. Introducing two Cl atoms in the benzenoid ring at positions 6 and 8 to furnish compound **31** (MIC_{90}: 0.8−4 μM) did not have any significant shift in antitubercular activity. Replacing the Cl atom at position 6 of compound **30** with an MeO moiety to afford compound **33** (MIC_{90}: 5−13 μM) led to a drastic decline in antitubercular activity. Compounds **32**−**43**, generally exhibited moderate antitubercular activity in the range of 5−18 μM. Compounds wherein the benzenoid ring bears −Cl substituent (**6**−**16**, **30**, and **31**) were generally more active than their counterparts bearing −MeO (**32**−**43**) moiety, regardless of the nature of substitution at R_1 and/or R_2. This emphasizes that the presence of a Cl atom appended to the benzenoid ring is essential for antitubercular activity. Compounds **44** and **45**, wherein the hydrophilic amide chain now bears an O in the place of −NH−, were inactive (MIC_{90}: >125 μM). Suggesting the presence of the secondary amine at this part of the molecule is also essential for antitubercular activity.

Compound **30** was selected and screened against moxifloxacin (GyrA_S531L)-, isoniazid (KatG_W198)-, and rifampicin (RpoB_G88C)-resistant *Mtb* strains. The com-

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Table 3. In Vitro ADME Properties of Selected Compounds[a]

entry	solubility (μM)	log D	% remnant at 30 min (HLM/RLM/MLM)	CL_{int}, (μL/min/mg) (HLM/RLM/MLM)	permeability (log P_{app})
6	<5	2.9	89/>99/>99	<11.6/<11.6/<11.6	<−7
8	15	1.5	>99/98/>99	<11.6/<11.6/<11.6	<−7
10	25	1.6	>99/>99/>99	<11.6/<11.6/<11.6	−6.6
11	<5	4.2	97/>99/>99	<11.6/<11.6/<11.6	<−7
19	10	2.6	99/>99/>99	<11.6/<11.6/<11.6	<−7
30	<5	4.4	98/97/>99	<11.6/<11.6/<11.6	<−7
31	<5	3.7	>99/97/95	<11.6/<11.6/<11.6	−6.4
37	10	2.7	>99/98/99	<11.6/<11.6/<11.6	−6.8

[a]HLM = human liver microsomes, RLM = rat liver microsomes, MLM = mouse liver microsomes, CL_{int} = intrinsic clearance.

pound exhibited equipotent activity against all drug-resistant strains and showed no shift in MIC value from the wild-type (H37Rv) strain. This clearly suggests compounds in this series perturb targets other than those reported for moxifloxacins, isoniazid and rifampicin. Figure 3 gives a summary of the antitubercular SARs.

2.3. Antibacterial, Antifungal, and Antileishmanial Evaluation. The antibacterial activity of selected compounds was determined against *E. coli* (ATCC25922), multi-drug-resistant *K. pneumoniae* (ATCC700603), *A. baumannii* (ATCC19606), *P. aeruginosa* (ATCC27853), and MRSA (ATCC43300). Bacteria were cultured in cation-adjusted Mueller Hinton broth and then added to compounds containing wells in 384-well plates at a cell density of 5×10^5 CFU/mL to make a total volume of 50 μL per well.

These compounds were also screened for antifungal activity against *C. albicans* (ATCC90028) and *C. neoformans* (ATCC 208821). Fungi were cultured using Yeast Extract-Peptone Dextrose (YPD) agar and added to compounds containing wells in 384-well plates at a cell density of 2.5×10^3 CFU/mL to make a total volume of 50 μL per well.

Compounds were initially screened at a single-point concentration of 32 μg/mL, and if they exhibited >80% growth inhibition of any target, they were then subjected to an eight-point dose−response in the range of 32−0.25 μg/mL to generate minimum inhibitory concentration (MIC). The MIC is defined as the minimum concentration of a compound required to exhibit ≥80% growth inhibition. Colistin sulfate and vancomycin hydrochloride were included as reference against Gram-negative and Gram-positive bacteria, respectively. Fluconazole was used as a reference against *C. albicans* and *C. neoformans*. Hits are classified as any compound exhibiting an MIC value of ≤16 μg/mL.

In comparison to compounds **XX** and **YY**, which we previously reported to show moderate activity (MIC: 16−32 μg/mL) against MRSA and *A. baumannii*, the compounds investigated here for antibacterial and antifungal activities generally showed improved activities (MIC: <5 μg/mL). They demonstrated potent activity (MIC: <0.25 μg/mL) against Gram-negative bacteria (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, *A. baumannii*) on par with colistin (MIC: 0.1−0.25 μg/mL), the reference drug (Table 2). Compounds **6**, **7**, **10**, **11**, **30**, **31**, **37**, and **40** demonstrated low micromolar activity (MIC: 0.25−2 μg/mL) against MRSA, while compounds **13**, **38**, **39**, **41**, **42**, and **43** were inactive (MIC: >32 μg/mL).

With respect to antifungal activity, most of the compounds evaluated were not active against *C. albicans*. A few compounds, however, demonstrated potent to moderate activity, e.g., compound **10** exhibited moderate activity of 16 μg/mL, compound **37** showed a low MIC value of 4 μg/mL, and compound **40** exhibited potent activity (MIC: <0.25 μg/mL) against *C. albicans*, which is on par with fluconazole (MIC: 0.125 μg/mL). *C. neoformans* was more susceptible to this series of compounds, with most of the compounds exhibiting potent MIC values of <0.25 μg/mL. For example, compounds **6**, **7**, **10**, and **11** exhibited equipotent activity of MIC: <0.25 μg/mL against *C. neoformans*. This activity is superior to that of fluconazole which has an MIC value of 8 μg/mL.

Compound **37** is noteworthy as a broad-spectrum antimicrobial agent. This compound demonstrated low micromolar activities in the range of 0.25−4 μg/mL against all bacteria and fungi pathogens investigated in this study.

Compounds were initially screened against *L. donovani* at a single-point concentration of 20 μM. Majority of compounds did not inhibit *L. donovani* growth at this concentration, exhibiting relative fluorescence unit (RFU) comparable to that of dimethyl sulfoxide (DMSO), the negative control. A few compounds that exhibited RFU comparable to amphotericin B (although at a lower concentration) were subjected to dose−response analyses at lower concentrations to generate IC_{50} (compound concentration required to inhibit 50% of parasite growth) values as presented in Table 2. Compounds **10**, **11**, and **30** exhibited low micromolar activity (IC_{50}: <3 μM), while compounds **31** and **37** demonstrated moderate activity against *L. donovani*.

2.4. In Vitro DMPK Evaluation. Eight compounds were selected, and their physicochemical and ADME properties were evaluated (Table 3). The lipophilicity at pH 7.4 (log D) for all compounds evaluated was within the acceptable range of <5 for orally available drugs.[31] Five compounds (**6**, **8**, **10**, **19**, and **37**) had log D of <3, while three compounds (**11**, **30**, and **31**) had log D > 3. Comparing the structures and log D values of compound **10** (log D: 1.6) against **11** (log D: 4.2) suggests that the presence of an *N*-benzyl unit at position 1 of the quinolone nucleus leads to a significant increase in log D. All compounds having log D > 3 bear the *N*-benzyl unit.

These selected compounds were also evaluated for kinetic aqueous solubility at pH 6.5. All compounds had very low solubility of <30 μM, which is far lower than the threshold value of 150 μM for orally available drugs. Compound **10** showed the best solubility of 25 μM within this series, while *N*-benzylated analogues all had aqueous solubility < 5 μM. It is important to mention that poor solubility has been an issue reported for other quinolone lead compounds in different clinical indications.[32]

The compounds generally showed poor permeability of <−6 Log P_{app} in parallel artificial membrane assay (PAMPA). This result could be partly attributed to the poor aqueous solubility of these compounds since the PAMPA is carried out

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in 100% aqueous condition. Permeability value > −5 P_{app} has been observed to correlate with good oral bioavailability.[33]

The metabolic stability of the selected compounds was determined in the presence of human, rat, and mouse liver microsomes. All compounds evaluated showed good metabolic stability, with a % remnant of >95% in all three species after 30 min. This metabolic robustness is further confirmed in this species by the low intrinsic clearance value of <11 μL/min/mg demonstrated by all compounds.

3. CONCLUSIONS

Several analogues of **XX** and **YY** were obtained using relatively cheap synthetic techniques that do not require the use of a metal catalyst. These compounds demonstrated broad-spectrum activity against acid-fast bacterium, Gram-negative and -positive bacteria, fungi, and leishmania parasite. Selected compounds were also evaluated for ADME properties, wherein they demonstrated good metabolic stability, poor solubility, and permeability.

With regard to antitubercular activity, it could be observed that the presence of a Cl atom appended to the benzenoid ring and a secondary amine within the hydrophilic amide chain at position 3 of the quinolone ring are essential for activity. Structure−property analyses suggest that an *N*-benzyl unit at position 1 leads to a rapid increase in Log *D* and poor aqueous solubility. Compound **30** maintained activity against moxifloxacin-, isoniazid-, and rifampicin-resistant *M. tuberculosis* strains, this observation suggests that a possible new target is being perturbed by this compound series. Compound **37**, for example, has superior activity than fluconazole against *C. neoformans*.

The poor solubility and permeability highlight the need for future work aimed at solving these challenges. This could be tackled through more analogue synthesis or formulation studies.

4. MATERIALS AND METHODS

4.1. Equipment and Reagents. Chemicals and solvents used for all synthetic reactions were procured from various suppliers such as Sigma-Aldrich, Ace, Rochelle, and Ambeed and were used as supplied. Merck $60F_{254}$ silica gel plates supported on 0.20 mm thick aluminum sheets were used in thin-layer chromatography (TLC) to monitor the progress of the reactions. Recrystallized dry synthetic products (15−20 mg) were dissolved in DMSO-d_6 and analyzed using a Bruker Avance III 600 spectrophotometer at 600 MHz (for ^{1}H) and 151 MHz (for ^{13}C) to acquire the nuclear magnetic resonance (NMR) spectra data. Chemical shifts were referenced to the residual solvent signals (DMSO-d_6: 2.50 and 39.52 ppm for ^{1}H and ^{13}C, respectively) and are reported in parts per million (ppm). The Mestrenova 14.2.3 software was used to visualize and analyze the reported NMR data. Spin multiplicities assigned to ^{1}H spectral peaks are reported as s (singlet), d (doublet), t (triplet), dd (doublet of doublets), and m (multiplet). A Buchi B-545 melting point apparatus was used to determine melting points (°C) that are reported as observed for all target compounds. The Bruker micrOTOF-Q II mass spectrometer was used to record the high-resolution mass spectra (HRMS) using atmospheric pressure chemical ionization (APCI) in positive-ion mode. Percentage (%) purity of test compounds was determined on an Agilent 1200 series high-performance liquid chromatography (HPLC) system equipped with a quaternary pump and an Agilent 1200 series diode array detector. The analytes were eluted over a Venusil XBP C18 column (4.60 × 150 mm^2, 5 μm) as a stationary phase, while the mobile phase consisted initially of 30% acetonitrile (ACN) and 70% Milli-Q water at a flow rate of 1 mL/min. A solvent gradient program was used, allowing 15 and 5 min analysis and equilibration times, respectively, per sample. Therefore, ACN in the mobile phase was linearly increased to 85% for 5 min. A 20 μL solution (1 mM) of each test compound dissolved in ACN was injected into the HPLC system, and the eluent was monitored at a wavelength of 254 nm.

4.2. Chemical Synthesis and Spectral Data. *4.2.1. Synthesis of the Quinolone Nucleus.* Three grams of nitrophenol (**1**) were treated with appropriately substituted benzyl bromide (1.1 equiv) and K_2CO_3 (0.5 equiv) under refluxing acetone overnight. Acetone was removed *in vacuo*, deionized water was added, and the mixture was stirred for 10 min. The ensuing precipitate was filtered, triturated in petroleum ether, and dried to afford the benzyloxy-nitrobenzene intermediates (**2**). Intermediate **2** (4 g) was treated with 5 equivalence of reduced iron powder, NH_4Cl (1.2 equiv), 2 mL of acetic acid, and 50 mL of ethanol under reflux for 48 h. The complete reaction mixture was concentrated, and the crude was absorbed on silica gel (200 mL) and flushed with an ethyl acetate/hexane (4:1) solvent mixture to afford aniline intermediates (**3**) after evaporating the solvent *in vacuo*. Four grams of **3** were refluxed with diethyl ethoxylmethylenemalonate (1.2 equiv) in ethanol (10 mL) for 12 h. Ethanol was evaporated, followed by the addition of petroleum ether. The ensuing precipitate was filtered and air-dried overnight. The dry malonate product (10 g) was added to boiling diphenyl ether (70 mL) at 240 °C and allowed to reflux for 2−4 h. The mixture was cooled to room temperature and saturated with petroleum ether (20 mL) before it was filtered and washed three times. The filtered residue was air-dried to afford **4** in 38−82% yield (Scheme 1).

4.2.2. N-Alkylation. Intermediate **4** was treated with K_2CO_3 (2 equiv), appropriate aryl/alkyl halides (2.5 equiv), and $CHCl_3$/THF (2:1, v/v) solvent mixture under reflux for up to 48 h. After reaction completion as indicated by TLC, the solvent was removed *in vacuo*. Deionized water was added to the concentrated crude and stirred at room temperature for 10−15 min. The resultant precipitated was filtered and dried to furnish intermediates **5** in moderate to good yields of 48−78%.

4.2.3. Amidation. Intermediate **5** was treated with 2-(2-aminoethylamino)ethanol (5 equiv) in the presence of 1,8-diazabicyclo [5.4.0] undec-7-ene (DBU) (5 equiv) and chloroform as the solvent under reflux for 72 h. The solvent was evaporated in vacuo after reaction completion, and 50 mL of deionized water was added. The product was extracted with DCM (20 mL × 3), which was concentrated, and the resultant crude was recrystallized from an appropriate solvent to furnish target compounds in overall yields of 4−59%.

4.2.4. Compounds Characterization. 4.2.4.1. 7-Chloro-1-(4-fluorobenzyl)-N-{2-[(2-hydroxyethyl)amino]ethyl}-4-oxo-6-{[4-(trifluoromethyl)benzyl]oxy}-1,4-dihydroquinolone-3-carboxamide, 6. Yellow flakes, mp 220−221 °C, yield 16%, ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.04 (t, J 5.9 Hz, 1H, −CONH), 9.01 (s, 1H, −NCH), 8.05 (s, 1H, Ar-CH), 7.90 (s, 1H, Ar-CH), 7.80 (d, J = 8.2 Hz, 2H, Ar-CH), 7.75−7.67 (m, 2H, Ar-CH), 7.30 (dd, J = 8.6, 5.5 Hz, 2H, Ar-CH), 7.24−7.18 (m, 2H, Ar-CH), 5.79 (s, 2H, $-OCH_2$), 5.48 (s, 2H, $-NCH_2$),

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5.25 (s, 1H, −NH), 4.52 (d, J = 7.1 Hz, 1H, −OH), 3.70−3.62 (m, 4H, $-CH_2$), 3.12 (t, J = 6.3 Hz, 2H, $-CH_2$), 3.05−2.99 (m, 2H, $-CH_2$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.20, 164.87, 162.60, 160.90, 150.86, 148.12, 141.01, 133.70, 131.90, 128.86 (d, J^{CF_3} = 8.5 Hz), 128.60, 128.41, 127.72, 127.36, 125.50 (d, J^{CF_3} = 3.6 Hz), 119.84, 115.85, 110.63, 108.23, 69.51, 56.47, 55.00, 49.18, 46.70, 35.42. HRMS-APCI m/z calcd for $C_{29}H_{27}ClF_4N_3O_4$ $[M + H]^+$, 592.1621, found 592.1632. Purity (HPLC): 93%, R_t = 6.7 min.

4.2.4.2. 1-(3-Bromobenzyl)-7-chloro-N-{2-[(2-hydroxyethyl)amino]ethyl}-4-oxo-6-{[4-(trifluoromethyl)-benzyl]oxy}-1,4-dihydroquinolone-3-carboxamide, ***7****.* Off-white powder, mp 212−213 °C, yield 13%, ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.98 (t, J = 5.5 Hz, 1H, −CONH), 8.99 (s, 1H, −NCH), 8.01−7.93 (m, 2H, Ar-CH), 7.80 (d, J = 8.2 Hz, 2H, Ar-CH), 7.72 (d, J = 8.1 Hz, 2H, Ar-CH), 7.51 (d, J = 6.4 Hz, 2H, Ar-CH), 7.31 (dd, J = 11.4, 5.0 Hz, 1H, Ar-CH), 7.15 (d, J = 7.9 Hz, 1H, Ar-CH), 5.79 (s, 2H, $-OCH_2$), 5.47 (s, 2H, $-NCH_2$), 5.35 (s, 1H, −NH), 4.50 (s, 1H, −OH), 3.47 (t, J = 5.6 Hz, 2H, $-CH_2$), 3.41 (dd, J = 11.9, 6.0 Hz, 2H, $-CH_2$), 2.72 (t, J = 6.2 Hz, 2H, $-CH_2$), 2.63 (t, J = 5.7 Hz, 2H, $-CH_2$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.33, 163.82, 150.81, 148.20, 141.02, 138.59, 133.69, 131.16, 130.89, 129.45, 128.59, 128.33, 127.75, 127.41, 125.45 (d, J^{CF_3} = 3.0 Hz), 125.35, 125.08, 122.09, 119.56, 111.08, 108.35, 69.49, 60.27, 54.93, 51.40, 48.58, 38.74. HRMS-APCI m/z calcd for $C_{29}H_{27}BrClF_3N_3O_4$ $[M + H]^+$, 652.0820, found 652.0791. Purity (HPLC): 98%, R_t = 6.9 min.

4.2.4.3. 7-Chloro-1-ethyl-6-((4-fluorobenzyl)oxy)-N-(2-((2-hydroxyethyl)amino)ethyl)-4-oxo-1,4-dihydroquinolone-3-carboxamide, ***8****.* White powder, yield: 67%, mp: 203 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.98 (s, 1H, −CONH), 8.79 (s, 1H, −NCH), 8.10 (s, 1H, Ar-CH), 7.96 (s, 1H, Ar-CH), 7.56 (s, 2H, Ar-CH), 7.25 (s, 2H, Ar-CH), 5.33 (s, 2H, CH_2), 4.45 (d, J = 37.7 Hz, 3H, $-CH_2-$OH), 3.53−3.36 (m, 5H, $2CH_2$, NH), 2.66 (d, J = 51.3 Hz, 4H, $2CH_2$), 1.36 (s, 3H, CH_3). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.05, 163.93, 161.23, 150.80, 146.89, 133.24, 132.48, 129.63 (d, J^{CF_3} = 8.4 Hz), 128.50, 127.28, 119.02, 115.31 (d, J^{CF_3} = 21.6 Hz), 110.77, 108.35, 69.69, 60.37, 51.42, 48.66, 48.29, 38.74, 14.54. HRMS-APCI m/z calcd for $C_{23}H_{26}ClFN_3O_4$ $[M + H]^+$, 462.1590, found 462.1597. Purity (HPLC): 98%, R_t = 9.7 min.

4.2.4.4. 1-Benzyl-7-chloro-6-((4-fluorobenzyl)oxy)-N-(2-((2-hydroxyethyl)amino)ethyl)-4-oxo-1,4-dihydroquinolone-3-carboxamide, ***9****.* Gray powder, yield 69%, mp: 199−201 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.04 (t, J = 5.4 Hz, 1H, −CONH), 9.03 (s, 1H, −NCH), 7.99 (t, J = 8.6 Hz, 2H, Ar-CH), 7.58 (dd, J = 8.6, 5.6 Hz, 2H, Ar-CH), 7.40 (t, J = 7.5 Hz, 2H, Ar-CH), 7.33 (dd, J = 15.2, 7.8 Hz, 1H, Ar-CH), 7.31−7.22 (m, 4H, Ar-CH), 5.82 (s, 2H, CH_2), 5.36 (s, 2H, CH_2), 4.54 (s, 1H, OH), 3.51 (t, J = 5.6 Hz, 2H, CH_2), 3.45 (dt, J = 19.9, 10.0 Hz, 3H, CH_2, NH), 2.77 (t, J = 6.1 Hz, 2H, CH_2), 2.67 (dd, J = 13.1, 7.3 Hz, 2H, CH_2). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.17, 163.77, 150.79, 147.98, 135.66, 133.54, 133.20, 129.59 (d, J^{CF} = 8.3 Hz), 128.8, 128.07, 127.82, 127.27, 126.35, 119.55, 115.32, 115.17, 110.74, 108.12, 69.57, 60.11, 55.58, 51.25, 48.45, 38.67. HRMS-APCI m/z calcd for $C_{28}H_{28}ClFN_3O_4$ $[M + H]^+$, 524.1743, found 524.1747. Purity (HPLC): 96%, R_t = 10.2 min.

4.2.4.5. 6-{[4-(tert-Butyl)benzyl]oxy}-7-chloro-1-ethyl-N-{2-[(2-hydroxyethyl)amino]ethyl}-4-oxo-1,4-dihydroquinolone-3-carboxamide, ***10****.* Yellow flakes, mp 187−188 °C, yield 12%, ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.10 (t, J = 5.9 Hz, 1H, −CONH), 8.81 (s, 1H, −NCH), 8.14 (s, 1H, Ar-CH), 7.93 (s, 1H, Ar-CH), 7.46−7.41 (m, 4H, Ar-CH), 5.35−5.23 (m, 3H, $-OCH_2$ and −NH), 4.51 (q, J = 7.1 Hz, 2H, $-NCH_2$), 4.24 (q, J = 6.9 Hz, 1H, −OH), 3.71−3.63 (m, 4H, $-CH_2$), 3.12 (q, J = 7.1 Hz, 2H, $-CH_2$), 3.04−3.00 (m, 2H, $-CH_2$), 1.35 (t, J = 7.1 Hz, 3H, −Me), 1.28 (s, 9H, −Me). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.89, 164.90, 150.95, 150.44, 146.89, 133.00, 132.93, 128.55, 127.24, 127.11, 125.18, 119.12, 110.20, 107.88, 70.04, 56.28, 49.01, 48.29, 46.54, 35.18, 34.19, 30.96, 14.57. HRMS-APCI m/z calcd for $C_{27}H_{35}ClN_3O_4$ $[M + H]^+$, 500.2311, found 500.2322. Purity (HPLC): 95%, R_t = 6.7 min.

4.2.4.6. 1-Benzyl-6-{[4-(tert-butyl)benzyl]oxy}-7-chloro-N-{2-[(2-hydroxyethyl)amino]ethyl}-4-oxo-1,4-dihydroquinolone-3-carboxamide, ***11****.* Cream powder, mp 208−210 °C, yield 17%, ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.03 (t, J = 5.5 Hz, 1H, −CONH), 8.99 (s, 1H, −NCH), 7.96 (d, J = 8.0 Hz, 2H, Ar-CH), 7.51−7.18 (m, 9H, Ar-CH), 5.79 (s, 2H, $-OCH_2$), 5.29 (s, 2H, $-NCH_2$), 4.89−4.41 (m, 2H, −NH and −OH), 3.52 (t, J = 5.5 Hz, 2H, $-CH_2$), 3.48 (dd, J = 11.8, 5.9 Hz, 2H, $-CH_2$), 2.83 (t, J = 6.1 Hz, 2H, $-CH_2$), 2.73 (t, J = 5.5 Hz, 2H, $-CH_2$), 1.27 (s, 9H, −Me). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.29, 164.18, 151.11, 150.57, 148.08, 135.78, 133.59, 133.04, 128.97, 128.54, 128.25, 127.96, 127.39, 126.49, 125.30, 119.70, 110.76, 108.03, 70.22, 59.32, 55.73, 50.85, 48.17, 37.92, 34.31, 31.08. HRMS-APCI m/z calcd for $C_{32}H_{37}ClN_3O_4$ $[M + H]^+$, 562.2467, found 562.2459. Purity (HPLC): 95%, R_t = 7.0 min.

4.2.4.7. 7-Chloro-6-((4-chlorobenzyl)oxy)-1-ethyl-N-(2-((2-hydroxyethyl)amino)ethyl)-4-oxo-1,4-dihydroquinolone-3-carboxamide, ***12****.* Brown solid, mp 175−176 °C, yield 5%, ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.06 (t, J = 5.8 Hz, 1H, −CONH), 8.81 (s, 1H, −NCH), 8.15 (s, 1H, Ar-CH), 7.90 (s, 1H, Ar-CH), 7.55−7.46 (m, 4H, Ar-CH), 5.37 (s, 2H, $-OCH_2$), 5.10 (s, 1H, −NH), 4.50 (q, J = 7.0 Hz, 2H, $-NCH_2$), 4.23 (dd, J = 13.9, 6.9 Hz, 1H, −OH), 3.65−3.56 (m, 4H, $-CH_2$), 3.03 (q, J = 7.1 Hz, 2H, $-CH_2$), 2.94 (t, J = 5.2 Hz, 2H, $-CH_2$), 1.35 (t, J = 7.1 Hz, 3H, −Me). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.01, 164.80, 150.80, 147.07, 135.22, 133.27, 132.62, 129.19, 128.62, 128.59, 127.24, 119.26, 110.46, 108.29, 69.55, 57.25, 49.64, 48.42, 47.15, 36.07, 14.69. HRMS-APCI m/z calcd for $C_{23}H_{26}Cl_2N_3O_4$ $[M + H]^+$, 478.1295, found 478.1298. Purity (HPLC): 95%, R_t = 6.3 min.

4.2.4.8. 1-Benzyl-7-chloro-6-[(4-chlorobenzyl)oxy]-N-{2-[(2-hydroxyethyl)amino]ethyl}-4-oxo-1,4-dihydroquinolone-3-carboxamide, ***13****.* Off-white crystals, mp 207−208 °C, yield 4%, ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.99 (t, J = 5.5 Hz, 1H, −CONH), 8.99 (s, 1H, Ar-CH), 7.98−7.90 (m, 2H, Ar-CH), 7.57−7.17 (m, 9H, Ar-CH), 5.78 (s, 2H, $-OCH_2$), 5.33 (s, 2H, $-NCH_2$), 4.49 (d, J = 6.9 Hz, 1H, −OH), 3.47 (t, J = 5.6 Hz, 2H, $-CH_2$), 3.41 (dd, J = 11.9, 6.0 Hz, 1H, −NH), 2.75−2.67 (m, 4H, $-2CH_2$), 2.62 (q, J = 6.0 Hz, 2H, $-CH_2$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.30, 163.88, 150.84, 148.13, 135.79, 135.19, 132.63, 129.22, 128.98, 128.56, 127.97, 127.41, 126.50, 119.70, 110.91, 108.29, 69.56, 60.38, 55.75, 51.47, 48.65, 38.83. HRMS-APCI m/z calcd for $C_{28}H_{28}Cl_2N_3O_4$ $[M + H]^+$, 540.1451, found 540.1429. Purity (HPLC): 96%, R_t = 6.9 min.

4.2.4.9. 7-Chloro-1-ethyl-N-{2-[(2-hydroxyethyl)amino]-ethyl}-6-[(3-methylbenzyl]oxy)-4-oxo-1,4-dihydroquinolone-3-carboxamide, ***14****.* Brown solid, mp 141−142 °C, yield 11%, ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.06 (t, J = 5.7 Hz, 1H, −CONH), 8.80 (s, 1H, −NCH), 8.14 (s, 1H, Ar-CH), 7.92 (d,

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J = 7.1 Hz, 1H, Ar-CH), 7.35−7.23 (m, 4H, Ar-CH), 5.30 (d, J = 12.6 Hz, 2H, $-OCH_2$), 4.50 (q, J = 7.1 Hz, 2H, $-NCH_2$), 4.26−4.20 (m, 1H, −OH), 3.65−3.58 (m, 4H, $-CH_2$), 3.03 (t, J = 5.9 Hz, 2H, $-CH_2$), 2.93 (s, 2H, $-CH_2$), 2.32 (s, 3H, −Bz−Me), 1.35 (t, J = 7.1 Hz, 3H, −Me), 1.12 (m, 1H, −NH). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.03, 164.85, 151.04, 147.02, 137.72, 136.03, 133.18, 128.71, 128.48, 127.92, 127.26, 124.52, 119.42, 119.22, 110.43, 108.18, 70.28, 57.28, 49.59, 48.37, 47.17, 36.07, 21.13, 14.73. HRMS-APCI m/z calcd for $C_{24}H_{29}ClN_3O_4$ $[M + H]^+$, 458.1841, found 458.1802. Purity (HPLC): 86%, R_t = 6.2 min.

4.2.4.10. 1-Benzyl-7-chloro-6-((3-chlorobenzyl)oxy)-N-(2-((2-hydroxyethyl)amino)ethyl)-4-oxo-1,4-dihydroquinolone-3-carboxamide, ***15****.* Brown powder, yield: 66%, mp: 198−200 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.07−9.94 (m, 1H, −CONH), 8.99 (s, 1H, NCH), 8.04−7.88 (m, 2H, Ar-CH), 7.57 (d, J = 12.9 Hz, 1H, Ar-CH), 7.50−7.39 (m, 3H, Ar-CH), 7.39−7.33 (m, 2H, Ar-CH), 7.33−7.27 (m, 1H, Ar-CH), 7.22 (d, J = 7.4 Hz, 2H, Ar-CH), 5.79 (s, 2H, CH_2), 5.35 (d, J = 8.3 Hz, 2H, CH_2), 4.49 (s, 1H, OH), 3.46 (dd, J = 16.1, 10.6 Hz, 2H, CH_2), 3.40 (dt, J = 23.9, 11.9 Hz, 3H, CH_2, NH), 2.78−2.68 (m, 2H, CH_2), 2.63 (t, J = 5.7 Hz, 2H, CH_2). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.57, 164.15, 151.08, 148.42, 139.00, 136.06, 134.05, 133.44, 130.76, 129.26, 129.23, 128.46, 128.24, 127.69, 127.33, 126.77, 126.19, 120.01, 111.20, 108.53, 69.74, 60.62, 56.01, 51.72, 48.90, 38.90. HRMS-APCI m/z calcd for $C_{28}H_{28}Cl_2N_3O_4$ $[M + H]^+$, 540.4456, found 540.1462. Purity (HPLC): 96%, R_t = 10.6 min.

4.2.4.11. 7-Chloro-1-ethyl-N-(2-((2-hydroxyethyl)amino)-ethyl)-4-oxo-6-((3-(trifluoromethyl)benzyl)oxy)-1,4-dihydro-quinolone-3-carboxamide, ***16****.* White powder, yield 71%, mp: 196−198 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.97 (t, J = 5.5 Hz, 1H, CONH), 8.78 (d, J = 3.2 Hz, 1H, Ar-CH), 8.11 (s, 1H, Ar-CH), 7.97 (s, 1H, NCH), 7.88 (s, 1H, Ar-CH), 7.82 (d, J = 7.6 Hz, 1H, Ar-CH), 7.71 (d, J = 7.8 Hz, 1H, Ar-CH), 7.67 (t, J = 7.7 Hz, 1H, Ar-CH), 5.45 (s, 2H, CH_2), 4.47 (q, J = 7.1 Hz, 2H, CH_2), 4.40 (s, 1H, OH), 3.45 (t, J = 5.5 Hz, 2H, CH_2), 3.41−3.35 (m, 3H, NH, CH_2), 2.69 (t, J = 6.2 Hz, 2H, CH_2), 2.60 (t, J = 5.7 Hz, 2H, CH_2), 1.35 (t, J = 7.1 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.64, 163.50, 150.28, 146.54, 137.33, 132.97, 130.88, 129.19, 128.90, 128.17, 126.89, 125.09, 124.25, 123.29 (d, J^{CF_3} = 3.9 Hz), 118.68, 110.41, 107.96, 69.10, 59.94, 50.99, 48.22, 47.90, 38.90, 14.12. HRMS-APCI m/z calcd for $C_{24}H_{26}ClF_3N_3O_4$ $[M + H]^+$, 512.1593, found 512.1545. Purity (HPLC): 98%, R_t = 10.1 min.

4.2.4.12. 1-Benzyl-7-chloro-N-(2-((2-hydroxyethyl)amino)-ethyl)-4-oxo-6-((3-(trifluoromethyl)benzyl)oxy)-1,4-dihydro-quinolone-3-carboxamide, ***17****.* White powder, yield: 64%, mp: 215−216 ^{0}C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.98 (t, J = 5.2 Hz, 1H, CONH), 8.98 (s, 1H, NCH), 7.97 (t, J = 8.4 Hz, 2H, Ar-CH), 7.88 (s, 1H, Ar-CH), 7.81 (d, J = 7.4 Hz, 1H, Ar-CH), 7.75−7.64 (m, 2H, Ar-CH), 7.33 (dt, J = 7.0, 7.4 Hz, 3H-Ar-CH), 7.22 (d, J = 7.4 Hz, 2H-Ar-CH), 5.78 (s, 2H, CH_2), 5.44 (s, 2H, CH_2), 4.43 (s, 1H, OH), 3.47 (s, 2H, CH_2), 3.41 (dt, J = 20.8, 10.4 Hz, 3H, NH, CH_2), 2.72 (t, J = 6.1 Hz, 2H, CH_2), 2.63 (t, J = 5.6 Hz, 2H, CH_2). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.27, 163.81, 150.80, 148.08, 137.67, 135.69, 133.81, 131.30, 129.59, 128.90, 128.17, 127.91, 127.40, 126.46, 126.09, 123.73, 119.66, 110.93, 108.34, 69.55, 60.36, 55.73, 51.40, 48.59, 38.92. HRMS-APCI m/z calcd for $C_{29}H_{28}ClF_3N_3O_4$ $[M + H]^+$, 574.1700, found 574.1722. Purity (HPLC): 99%, R_t = 10.6 min.

4.2.4.13. 1-Benzyl-7-bromo-N-(2-((2-hydroxyethyl)-amino)ethyl)-4-oxo-6-((4-(trifluoromethyl)benzyl)oxy)-1,4-dihydroquinolone-3-carboxamide, ***18****.* White powder, yield: 52%, mp: 211 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.99 (t, J = 5.5 Hz, 1H, CONH), 8.97 (s, 1H, Ar-CH), 8.11 (s, 1H, NCH), 7.89 (s, 1H, Ar-CH), 7.79 (d, J = 8.2 Hz, 2H, Ar-CH), 7.72 (d, J = 8.1 Hz, 2H, Ar-CH), 7.36 (t, J = 7.5 Hz, 2H, Ar-CH), 7.30 (t, J = 7.3 Hz, 1H, Ar-CH), 7.21 (d, J = 7.4 Hz, 2H, Ar-CH), 5.78 (s, 2H, CH_2), 5.45 (s, 2H, CH_2), 4.52 (s, 1H, OH), 3.46 (dd, J = 11.0, 5.2 Hz, 5H, $2CH_2$, NH), 2.71 (t, J = 6.2 Hz, 2H, CH_2), 2.62 (t, J = 5.7 Hz, 2H, CH_2). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.35, 163.85, 151.53, 148.03, 140.99, 135.70, 133.97, 128.92, 127.92, 127.60, 126.42, 125.37, 122.80, 118.40, 110.79, 107.73, 69.49, 60.26, 55.73, 51.33, 48.52, 38.72. HRMS-APCI m/z calcd for $C_{29}H_{28}BrF_3N_3O_4$ $[M + H]^+$ 618.1145, found 618.1188. Purity (HPLC): 97%, R_t = 10.6 min.

4.2.4.14. 1-Ethyl-N-(2-((2-hydroxyethyl)amino)ethyl)-4-oxo-7-(trifluoromethyl)-6-((4-(trifluoromethyl)benzyl)oxy)-1,4-dihydroquinolone-3-carboxamide, ***19****.* Brown powder, yield: 47%, mp: 257−259 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.31 (s, 1H, CONH), 9.94 (d, J = 4.8 Hz, 1H, Ar-CH), 8.89 (s, 1H, NCH), 8.12 (d, J = 7.2 Hz, 1H, Ar-CH), 8.06 (s, 1H, Ar-CH), 7.80 (t, J = 8.9 Hz, 3H, Ar-CH), 5.54 (s, 2H, CH_2), 5.37 (s, 1H, OH), 4.58 (d, J = 7.0 Hz, 2H, CH_2), 3.45 (d, J = 15.3 Hz, 5H, $2CH_2$, NH), 2.70 (t, J = 5.9 Hz, 2H, CH_2), 2.61 (t, J = 5.5 Hz, 2H, CH_2), 1.38 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.10, 164.66, 164.08, 152.57, 152.03, 148.40, 148.17, 141.17, 132.64, 131.65, 131.51, 127.83, 125.81, 117.90, 111.52, 109.37, 69.67, 60.69, 51.76, 48.95, 48.75, 39.14, 14.91. HRMS-APCI m/z calcd for $C_{25}H_{26}F_6N_3O_4$ $[M + H]^+$ 546.1848, found 546.1809. Purity (HPLC): 95%, R_t = 8.9 min.

4.2.4.15. 6-((4-Bromobenzyl)oxy)-1-ethyl-7-fluoro-N-(2-((2-hydroxyethyl)amino)ethyl)-4-oxo-1,4-dihydroquinolone-3-carboxamide, ***20****.* Beige powder, yield: 47%, mp: 194 °C.^{1}H NMR (600 MHz, DMSO-d_6) δ 10.06 (t, J = 5.3 Hz, 1H, CONH), 8.79 (s, 1H, NCH), 7.94 (t, J = 10.4 Hz, 2H, Ar-CH), 7.62 (d, J = 8.3 Hz, 2H, Ar-CH), 7.46 (d, J = 8.3 Hz, 2H, Ar-CH), 5.32 (s, 2H, CH_2), 4.44 (q, J = 7.1 Hz, 2H, CH_2), 3.53 (d, J = 20.6 Hz, 6H, $2CH_2$, NH, OH), 2.86 (d, J = 14.2 Hz, 4H, $2CH_2$), 1.34 (t, J = 7.1 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.98, 154.25, 153.12, 146.86, 144.86, 135.41, 133.62, 131.37, 129.66, 124.29, 121.16, 110.39, 109.76, 105.20, 69.43, 48.42, 14.45. HRMS-APCI m/z calcd for $C_{23}H_{26}BrFN_3O_4$ $[M + H]^+$ 506.1037, found 506.1063. Purity (HPLC): 96%, R_t = 9.8 min.

4.2.4.16. 1-Benzyl-6-((4-bromobenzyl)oxy)-7-fluoro-N-(2-((2-hydroxyethyl)amino)ethyl)-4-oxo-1,4-dihydroquinolone-3-carboxamide, ***21****.* Beige powder, yield: 40%, mp: 235−237 °C.^{1}H NMR (600 MHz, DMSO-d_6) δ 9.98 (t, J = 7.6 Hz, 1H, CONH), 8.99 (s, 1H, NCH), 7.97 (t, J = 9.1 Hz, 1H, Ar-CH), 7.76 (d, J = 8.6 Hz, 1H, Ar-CH), 7.531 (dd, J = 8.6, 9.1 Hz, 2H, Ar-CH), 7.44 - 7.22 (m, 7H, Ar-CH), 5.73 (s, 2H, CH_2), 5.30 (s, 2H, CH_2), 4.49 (s, 1H, OH), 3.47 (d, J = 30.4 Hz, 5H, $2CH_2$, NH), 2.78 (s, 2H, CH_2), 2.69 (s, 2H, CH_2). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.10, 164.66, 161.08, 154.57, 152.03, 148.40, 148.17, 141.17, 132.64, 131.65, 131.51, 127.83, 125.81, 117.90, 115.76, 115.62, 111.52, 109.37, 70.67, 60.69, 51.76, 48.95, 48.75, 39.14. HRMS-APCI m/z calcd for $C_{28}H_{28}F_2N_3O_4$ $[M + H]^+$ 508.2042, found 508.2049. Purity (HPLC): 96%, R_t = 8.8 min.

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4.2.4.17. 6-((3-Bromobenzyl)oxy)-1-ethyl-7-fluoro-N-(2-((2-hydroxyethyl)amino)ethyl)-4-oxo-1,4-dihydroquinolone-3-carboxamide, ***22****.* Gray powder, yield: 45%, mp: 210−211 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.02 (t, J = 5.5 Hz, 1H, CONH), 8.99 (s, 1H, NCH), 7.97 (d, J = 9.3 Hz, 1H, Ar-CH), 7.69 (s, 1H, Ar-CH), 7.55 (t, J = 8.9 Hz, 1H, Ar-CH), 7.40−7.34 (m, 3H, Ar-CH), 5.74 (q, J = 7.6 Hz, 2H, CH_2), 5.32 (s, 2H, CH_2), 4.49 (s, 1H, OH), 3.46 (dd, J = 16.0, 10.4 Hz, 5H, NH, $2CH_2$), 2.71 (t, J = 6.2 Hz, 2H, CH_2), 2.62 (t, J = 5.7 Hz, 2H, CH_2), 2.08 (t, J = 7.6 Hz, 3H, CH_3).^{13}C NMR (151 MHz, DMSO-d_6) δ 173.48, 163.43, 147.40, 144.17, 138.64, 135.65, 130.51, 130.05, 127.83, 126.19, 126.09, 125.90, 124.76, 121.62, 111.52, 110.45, 68.94, 59.89, 55.55, 50.96, 40.14, 39.14, 14.03. HRMS-APCI m/z calcd for $C_{23}H_{26}BrFN_3O_4$ $[M + H]^+$ 506.1085, found 506.1063. Purity (HPLC): 98%, R_t = 9.0 min.

4.2.4.18. 1-Benzyl-6-((3-bromobenzyl)oxy)-7-fluoro-N-(2-((2-hydroxyethyl)amino)ethyl)-4-oxo-1,4-dihydroquinolone-3-carboxamide, ***23****.* Brown powder, yield: 37%, mp: 213 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.02 (t, J = 5.5 Hz, 1H, CONH), 8.99 (s, 1H, Ar-CH), 7.97 (d, J = 9.2 Hz, 1H, Ar-CH), 7.76 (d, J = 12.7 Hz, 1H, Ar-CH), 7.58−7.53 (m, 1H, Ar-CH), 7.40−7.34 (m, 3H, Ar-CH), 7.30 (t, J = 7.3 Hz, 2H, Ar-CH), 7.22 (t, J = 8.0 Hz, 3H, Ar-CH), 5.73 (s, 2H, CH_2), 5.32 (s, 2H, CH_2), 4.50 (s, 1H, OH), 3.48−3.45 (m, 5H, $2CH_2$, NH), 2.71 (t, J = 6.3 Hz, 2H, CH_2), 2.62 (t, J = 5.7 Hz, 2H, CH_2).^{13}C NMR (151 MHz, DMSO-d_6) δ 174.08, 163.82, 147.97, 138.72, 135.56, 130.90, 130.67, 130.53, 130.20, 128.80 (d, J^{CF} = 16.0 Hz), 127.88, 126.53, 121.63, 110.85, 69.33, 60.33, 55.75, 51.32, 48.54, 38.54 (most carbon peaks were not observed due to poor solubility). HRMS-APCI m/z calcd for $C_{28}H_{28}BrFN_3O_4$ $[M + H]^+$ 568.1204, found 568.1244. Purity (HPLC): 92%, R_t = 8.9 min.

4.2.4.19. 6-((3-Bromobenzyl)oxy)-7-fluoro-1-(4-fluorobenzyl)-N-(2-((2-hydroxyethyl)amino)ethyl)-4-oxo-1,4-dihydroquinolone-3-carboxamide, ***24****.* Beige powder, yield: 34%, mp: 179 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.32 (t, J = 5.6 Hz, 1H, CONH), 8.99 (s, 1H, NCH), 7.97 (d, J = 9.3 Hz, 2H, Ar-CH), 7.69 (s, 1H, Ar-CH), 7.54−7.47 (m, 3H, Ar-CH), 7.29 (dd, J = 9.5, 4.3 Hz, 4H, Ar-CH), 5.71 (s, 2H, CH_2), 5.32 (s, 2H, CH_2), 4.49 (s, 1H, OH), 3.44 (dd, J = 13.9, 10.4 Hz, 5H, $2CH_2$, NH), 2.70 (t, J = 6.4 Hz, 2H, CH_2), 2.61 (t, J = 5.7 Hz, 2H, CH_2). ^{13}C NMR (151 MHz, DMSO-d_6) δ 163.11, 160.11, 159.97, 147.68, 147.55, 138.51, 133.57, 131.54, 130.56, 130.42, 129.94, 129.44, 129.31, 128.49, 126.34, 125.91, 115.46, 115.25, 110.54, 109.41, 109.28, 108.66, 75. 23, 68.98, 59.93, 51.00, 48.21, 37.96 (carbon peaks are week due to poor solubility). HRMS-APCI m/z calcd for $C_{28}H_{27}BrF_2N_3O_4$ $[M + H]^+$ 586.1114 $[M + H]^+$, found 586.1116. Purity (HPLC): 90%, R_t = 8.1 min.

4.2.4.20. 1-Benzyl-6-((3-chlorobenzyl)oxy)-7-fluoro-N-(2-((2-hydroxyethyl)amino)ethyl)-4-oxo-1,4-dihydroquinolone-3-carboxamide, ***25****.* Beige powder, yield: 36%, mp: 201−203 °C.^{1}H NMR (600 MHz, DMSO-d_6) δ 10.02 (t, J = 5.4 Hz, 1H, CONH), 8.99 (s, 1H, NCH), 7.97 (d, J = 9.2 Hz, 1H, Ar-CH), 7.76 (d, J = 12.7 Hz, 1H, Ar-CH), 7.56 (d, J = 11.5 Hz, 1H, Ar-CH), 7.50−7.39 (m, 3H, Ar-CH), 7.35 (t, J = 7.5 Hz, 2H, Ar-CH), 7.29 (t, J = 7.3 Hz, 1H, Ar-CH), 7.22 (d, J = 7.5 Hz, 2H, Ar-CH), 5.73 (s, 2H, CH_2), 5.32 (s, 2H, CH_2), 4.49 (s, 1H, OH), 3.47 (t, J = 5.5 Hz, 4H, $2CH_2$), 2.71 (t, J = 6.2 Hz, 2H, CH2), 2.61 (dd, J = 13.1, 7.4 Hz, 3H, NH, CH_2). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.74, 163.48, 147.63, 154.57, 152.03, 148.40, 148.17, 141.17, 132.64, 131.65, 131.51, 127.65, 127.54, 126.81, 125.90, 124.76, 110.52, 109.43, 105.34, 105.18, 69.57, 59.95, 55.41, 51.02, 48.20, 38.40. HRMS-APCI m/z calcd for $C_{28}H_{28}ClFN_3O_4$ $[M + H]^+$ 524.1747, found 524.1746. Purity (HPLC): 98%, R_t = 8.9 min.

4.2.4.21. 1-Ethyl-7-fluoro-6-((3-fluorobenzyl)oxy)-N-(2-((2-hydroxyethyl)amino)ethyl)-4-oxo-1,4-dihydroquinolone-3-carboxamide, ***26****.* Beige powder, yield: 49%, mp: 198−200 °C.^{1}H NMR (600 MHz, DMSO-d_6) δ 10.03 (t, J = 5.4 Hz, 1H, CONH), 8.79 (s, 1H, NCH), 7.96 (dd, J = 30.9, 11.0 Hz, 2H, Ar-CH), 7.56 (dd, J = 8.0, 5.8 Hz, 2H, Ar-CH), 7.25 (t, J = 8.7 Hz, 2H, Ar-CH), 5.31 (s, 2H, CH_2), 4.44 (dd, J = 14.0, 6.9 Hz, 3H, $2CH_2$, OH), 3.46 (dd, J = 11.0, 5.5 Hz, 5H, $2CH_2$, NH), 2.70 (t, J = 6.2 Hz, 2H, CH_2), 2.62 (dd, J = 13.6, 7.9 Hz, 2H, CH_2), 1.35 (t, J = 7.1 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.74, 163.94, 161.01, 146.83, 133.51, 132.20, 130.02, 129.96, 124.42, 115.34, 115.20, 110.68, 109.75, 105.17, 105.01, 69.55, 60.18, 51.31, 48.55, 48.41, 38.61, 14.48. HRMS-APCI m/z calcd for $C_{23}H_{26}F_2N_3O_4$ $[M + H]^+$ 446.1886, found 446.1903. Purity (HPLC): 90%, R_t = 9.1 min.

4.2.4.22. 1-Benzyl-7-fluoro-6-((4-fluorobenzyl)oxy)-N-(2-((2-hydroxyethyl)amino)ethyl)-4-oxo-1,4-dihydroquinolone-3-carboxamide, ***27****.* White powder, yield: 39%, mp: 230−232 °C.^{1}H NMR (600 MHz, DMSO-d_6) δ 10.03 (t, J = 5.4 Hz, 1H, CONH), 8.99 (s, 1H, CNH), 7.97 (t, J = 9.7 Hz, 1H, Ar-CH), 7.75 (d, J = 9.7 Hz, 1H, Ar-CH), 7.53 (dd, J = 8.2, 5.7 Hz, 2H, Ar-CH), 7.40−7.16 (m, 7H, Ar-CH), 5.73 (s, 2H, CH_2), 5.29 (s, 2H, CH_2), 4.49 (s, 1H, OH), 3.46 (dd, J = 14.7, 9.3 Hz, 3H, CH_2, NH), 3.43−3.40 (m, 2H, CH_2), 2.71 (t, J = 6.1 Hz, 2H, CH_2), 2.67−2.58 (m, 2H, CH_2). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.48, 164.94, 164.24, 161.42, 154.23, 148.35, 135.99, 132.54, 130.46, 130.40, 129.25, 128.27, 126.88, 124.95, 115.76, 115.62, 111.23, 110.17, 70.00, 60.65, 56.13, 51.75, 48.94, 39.11. HRMS-APCI m/z calcd for $C_{28}H_{28}F_2N_3O_4$ $[M + H]^+$ 507.2042, found 508.2049. Purity (HPLC): 91%, R_t = 10.2 min.

4.2.4.23. 7-Fluoro-1-(4-fluorobenzyl)-6-((4-fluorobenzyl)oxy)-N-(2-((2-hydroxyethyl)amino)ethyl)-4-oxo-1,4-dihydroquinolone-3-carboxamide, ***28****.* Beige powder, yield: 30%, mp: 236 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.02 (t, J = 5.5 Hz, 1H, CONH), 8.99 (s, 1H, NCH), 7.97 (t, J = 9.0 Hz, 1H, Ar-CH), 7.78 (d, J = 9.0 Hz, 1H, Ar-CH), 7.60−7.46 (m, 4H, Ar-CH), 7.36−7.14 (m, 6H, Ar-CH), 5.71 (s, 2H, CH_2), 5.29 (s, 2H, CH_2), 4.47 (d, J = 20.5 Hz, 1H, OH), 3.51−3.43 (m, 5H, NH, $2CH_2$), 2.71 (t, J = 6.1 Hz, 2H, CH_2), 2.61 (dd, J = 13.2, 7.5 Hz, 2H, CH_2), 1.13 (solvent peak). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.07, 163.79, 147.83, 131.74, 130.02 (d, J^{CF} = 8.5 Hz), 128.80 (d, J^{CF} = 8.3 Hz), 124.56, 115.74, 115.60, 115.34, 115.20, 110.89, 69.58, 60.25, 54.96, 51.33, 48.53. 39.11 (most carbon peaks are not visible due to poor solubility). HRMS-APCI m/z calcd for $C_{28}H_{27}F_3N_3O_4$ $[M + H]^+$ 525.1948, found: 526.1963. Purity (HPLC): 90%, R_t = 10.4 min.

4.2.4.24. 1-Benzyl-6-((3-chlorobenzyl)oxy)-N-(2-((2-hydroxyethyl)amino)ethyl)-7-methyl-4-oxo-1,4-dihydroquinolone-3-carboxamide, ***29****.* Beige powder, yield: 29%, mp: 218 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.13 (t, J = 5.5 Hz, 1H, CONH), 8.94 (s, 1H, NCH), 7.75 (s, 1H, Ar-CH), 7.68 (s, 1H, Ar-CH), 7.55 (s, 1H, Ar-CH), 7.49−7.37 (m, 3H, Ar-CH), 7.35 (t, J = 7.5 Hz, 2H, Ar-CH), 7.28 (t, J = 7.3 Hz, 1H, Ar-CH), 7.22 (d, J = 7.5 Hz, 2H, Ar-CH), 5.75 (s, 2H, CH_2), 5.26 (d, J = 9.4 Hz, 2H, CH_2), 4.50 (s, 1H, OH), 3.47 (t, J = 5.5 Hz, 2H, CH_2), 3.40 (dd, J = 11.4, 5.5 Hz, 3H, NH, CH_2), 2.71 (t, J = 6.2 Hz, 2H, CH_2), 2.62 (t, J = 5.7 Hz, 2H,

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CH_2), 2.31 (s, 3H, CH_3). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.53, 164.15, 153.88, 147.00, 139.32, 136.01, 133.78, 133.51, 133.03, 130.32, 128.79, 127.74, 127.64, 126.80, 126.77, 126.41, 125.65, 119.37, 110.27, 105.34, 68.47, 60.26, 55.47, 51.36, 48.61, 38.67, 16.83. HRMS-APCI m/z calcd for $C_{29}H_{31}ClN_3O_4$ $[M + H]^+$ 520.1998, found 520.1977. Purity (HPLC): 98%, R_t = 10.5 min.

4.2.4.25. 1-Benzyl-6-chloro-N-(2-((2-hydroxyethyl)amino)-ethyl)-4-oxo-7-((4-(trifluoromethyl)benzyl)oxy)-1,4-dihydro-quinolone-3-carboxamide, ***30****.* White solid, mp 216−218 °C, yield 50%, ^1H NMR (600 MHz, DMSO-d_6) δ 9.93 (t, J = 5.6 Hz, 1H, CONH), 8.98 (s, 1H, NCH), 8.27 (s, 1H, Ar-CH), 7.69 (d, J = 8.2 Hz, 2H, Ar-CH), 7.56 (t, J = 9.2 Hz, 2H, Ar-CH), 7.40−7.14 (m, 6H, Ar-CH), 5.76 (s, 2H, OCH_2), 5.42 (s, 2H, NCH_2), 4.47 (s, 1H, OH), 3.47 (t, J = 5.7 Hz, 2H, CH_2), 3.40 (dd, J = 12.0, 6.2 Hz, 2H, CH_2), 2.70 (t, J = 6.2 Hz, 2H, CH_2), 2.62 (t, J = 5.7 Hz, 2H, CH_2). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.94, 163.70, 156.21, 148.83, 140.24, 139.55, 135.55, 128.86, 128.64, 128.43, 127.86, 127.65, 127.08, 126.63, 125.49, 125.47, 125.44, 125.02, 123.22, 121.89, 120.63, 111.65, 101.69, 69.64, 60.39, 55.69, 51.48, 48.67, 38.85. HRMS-APCI m/z calcd for $C_{29}H_{28}ClF_3N_3O_4$ $[M + H]^+$, 574.1715, found 574.1685. Purity (HPLC): 98%, R_t = 5.8 min.

4.2.4.26. 1-Benzyl-6,8-dichloro-N-(2-((2-hydroxyethyl)-amino)ethyl)-4-oxo-7-((4-(trifluoromethyl)benzyl)oxy)-1,4-dihydroquinolone-3-carboxamide, ***31****.* White solid, mp 237−239 °C, yield 42%, ^1H NMR (600 MHz, DMSO-d_6) δ 9.92 (t, J = 6.0 Hz, 1H, CONH), 9.02 (s, 1H, NCH), 7.96 (dd, J = 8.8, 7.8 Hz, 2H, Ar-CH), 7.78 (dd, J = 8.8, 7.8 Hz, 2H, Ar-CH), 7.51−7.18 (m, 6H, Ar-CH), 5.81 (s, 2H, OCH_2), 5.30 (td, J = 29.3, 24.2 Hz, 1H, OH), 5.12 (s, 2H, NCH_2), 3.68 (dd, J = 7.7, 4.5 Hz, 4H, $2CH_2$), 3.14 (t, J = 6.2 Hz, 2H, CH_2), 3.05−3.03 (m, 1H, CH_2). ^{13}C NMR (151 MHz, DMSO-d_6) δ 175.40, 164.98, 149.31, 148.51, 141.24, 138.53, 135.91, 133.57, 129.50, 129.09, 128.53, 127.86, 126.98, 125.82, 124.08, 120.30, 118.68, 112.97, 110.98, 73.85, 70.88, 56.81, 49.60, 47.07, 35.85. HRMS-APCI m/z calcd for $C_{29}H_{27}Cl_2F_3N_3O_4$ $[M + H]^+$, 608.1325, found 608.1290. Purity (HPLC): 90%, R_t = 6.8 min.

4.2.4.27. 1-Ethyl-N-{2-[(2-hydroxyethyl)amino]ethyl}-6-methoxy-4-oxo-7-{[4-(trifluoromethyl)benzyl]oxy}-1,4-dihydroquinolone-3-carboxamide, ***32****.* Cream flakes, mp 202−203 °C, yield 7%, ^1H NMR (600 MHz, DMSO-d_6) δ 10.29 (t, J = 5.9 Hz, 1H, −CONH), 8.74−8.72 (m, 1H, Ar-CH), 7.75 (dt, J = 7.4 Hz, 5H, Ar-CH), 7.29 (s, 1H, Ar-CH), 5.48 (s, 2H, $-OCH_2$), 5.05 (s, 1H, −NH), 4.49 (q, J = 7.1 Hz, 2H, $-NCH_2$), 3.91 (s, 3H, −OMe), 3.59 (dt, J = 12.2, 5.7 Hz, 5H, $2CH_2$ and −OH), 3.01 (t, J = 6.3 Hz, 2H, $-CH_2$), 2.92 (t, J = 5.4 Hz, 2H, $-CH_2$), 1.27 (t, J = 7.1 Hz, 3H, −Me). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.70, 164.93, 151.88, 147.45, 145.73, 140.79, 133.81, 128.44, 128.23, 128.07, 125.22 (q, J^{CF_3} = 3.7 Hz), 124.81, 123.03, 121.30, 109.92, 105.44, 100.27, 69.19, 57.20, 55.48, 49.49, 48.12, 47.09, 35.96, 14.19. HRMS-APCI m/z calcd for $C_{25}H_{29}F_3N_3O_5$ $[M + H]^+$, 508.2054, found 508.2051. Purity (HPLC): 100%, R_t = 6.2 min.

4.2.4.28. 1-Benzyl-N-{2-[(2-hydroxyethyl)amino]ethyl}-6-methoxy-4-oxo-7-{[4-(trifluoromethyl)benzyl]oxy}-1,4-dihydroquinolone-3-carboxamide, ***33****.* Cream powder, mp 223−224 °C, yield 4%, ^1H NMR (600 MHz, DMSO-d_6) δ 10.31 (t, J = 6.2 Hz, 1H, −CONH), 8.94 (s, 1H, −NCH), 8.76−8.72 (m, 1H, Ar-CH), 7.72−7.66 (m, 3H, Ar-CH), 7.54 (t, J = 9.1 Hz, 2H, Ar-CH), 7.29−7.23 (m, 3H, Ar-CH), 7.15 (d, J = 7.0 Hz, 2H, Ar-CH), 5.77 (s, 2H, $-OCH_2$), 5.36−5.24 (m, 3H, $-NH$, $-NCH_2$), 4.50 (q, J = 7.1 Hz, 1H, −OH), 3.89 (s, 3H, −OMe), 3.70 − 3.62 (m, 4H, $2CH_2$), 3.18−3.09 (m, 2H, CH_2), 3.05−3.00 (m, 2H, CH_2). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.13, 165.39, 151.96, 147.80, 147.13, 140.68, 135.86, 134.67, 128.82, 128.55, 128.36, 127.93, 127.86, 128.82, 127.70, 126.53, 125.42 (q, J^{CF_3} = 3.7 Hz), 121.68, 110.20, 105.68, 101.18, 69.16, 56.50, 55.77, 55.74, 49.20, 46.85, 35.43. HRMS-APCI m/z calcd for $C_{30}H_{31}F_3N_3O_5$ $[M + H]^+$, 570.2210, found 570.2192. Purity (HPLC): 90%, R_t = 6.3 min.

4.2.4.29. 1-Ethyl-N-{2-[(2-hydroxyethyl)amino]ethyl}-6-methoxy-4-oxo-7-{[3-(trifluoromethyl)benzyl]oxy}-1,4-dihydroquinolone-3-carboxamide, ***34****.* Brown solid, mp 195 °C, yield 18%, ^1H NMR (600 MHz, DMSO-d_6) δ 10.19 (t, J = 5.6 Hz, 1H, CONH), 8.72 (s, 1H, NCH), 7.92 (s, 1H, Ar-CH), 7.82 (d, J = 7.7 Hz, 1H, Ar-CH), 7.75−7.71 (m, 2H, Ar-CH), 7.68 (t, J = 7.8 Hz, 1H, Ar-CH), 7.29 (s, 1H, Ar-CH), 5.47 (s, 2H, $-OCH_2$), 4.48 (q, J = 7.0 Hz, 2H, $-NCH_2$), 4.36 (s, 1H, OH), 3.90 (s, 3H, $-CH_3$), 3.46 (d, J = 4.4 Hz, 2H, $-CH_2$), 3.39 (q, J = 6.1 Hz, 2H, $-CH_2$), 2.69 (t, J = 6.2 Hz, 2H, $-CH_2$), 2.61 (t, J = 5.7 Hz, 2H, $-CH_2$), 1.71 (s, 1H, −NH), 1.27 (t, J = 7.1 Hz, 3H, −Me). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.01, 164.35, 152.06, 147.63, 145.95, 137.78, 134.05, 131.87, 129.79, 129.37, 129.16, 124.86, 124.39 (q, J^{CF_3} = 3.9 Hz), 121.63, 110.54, 105.81, 100.47, 69.43, 60.39, 55.72, 51.51, 48.79, 48.31, 38.77, 14.41. HRMS-APCI m/z calcd for $C_{25}H_{29}F_3N_3O_5$ $[M + H]^+$, 508.2054, found 508.2047. Purity (HPLC): 99%, R_t = 6.2 min.

4.2.4.30. 1-Benzyl-N-{2-[(2-hydroxyethyl)amino]ethyl}-6-methoxy-4-oxo-7-{[3-(trifluoromethyl)benzyl]oxy}-1,4-dihydroquinolone-3-carboxamide, ***35****.* Cream powder, mp 156−157 °C, yield 5%, ^1H NMR (600 MHz, DMSO-d_6) δ 10.20 (t, J = 5.7 Hz, 1H, CONH), 8.93 (s, 1H, NCH), 7.80−7.57 (m, 5H, Ar-CH), 7.36−7.14 (m, 6H, Ar-CH), 5.75 (s, 2H, OCH_2), 5.29 (s, 2H, NCH_2), 4.48 (q, J = 7.0 Hz, 1H, OH), 3.88 (s, 3H, −OMe), 3.49 (dd, J = 10.9, 5.3 Hz, 2H, CH_2), 3.45 (dd, J = 12.0, 6.0 Hz, 2H, CH_2), 2.80−2.74 (m, 2H, CH_2), 2.68 (q, J = 5.5 Hz, 2H, CH_2), 1.26 (dd, J = 21.3, 14.2 Hz, 1H, NH). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.31, 164.57, 152.14, 147.82, 147.13, 137.56, 136.05, 134.79, 131.79, 129.83, 129.46, 129.25, 128.99, 128.01, 126.83, 125.14, 125.00, 124.25 (q, J^{CF_3} = 3.5 Hz), 121.92, 110.70, 105.92, 101.29, 69.61, 59.89, 55.86, 51.26, 48.57, 38.39. HRMS-APCI m/z calcd for $C_{30}H_{31}F_3N_2O_6$ $[M + H]^+$, 570.2210, found 570.2208. Purity (HPLC): 90%, R_t = 6.3 min.

4.2.4.31. 7-{[4-(tert-Butyl)benzyl]oxy}-1-ethyl-N-{2-[(2-hydroxyethyl)amino]ethyl}-6-methoxy-4-oxo-1,4-dihydroquinolone-3-carboxamide, ***36****.* Cream solid, mp 184−185 °C, yield 11%, ^1H NMR (600 MHz, DMSO-d_6) δ 10.23 (t, J = 6.0 Hz, 1H, CONH), 8.73 (d, J = 2.7 Hz, 1H, Ar-CH), 7.73−7.24 (m, 6H, Ar-CH), 5.31 (s, 2H, OCH_2), 4.86 (s, 1H, −NH), 4.48 (q, J = 7.1 Hz, 2H, $-NCH_2$), 4.35 (t, J = 5.7 Hz, 1H, −OH), 3.88 (s, 3H, −OMe), 3.58 (ddt, J = 25.0, 19.2, 6.2 Hz, 4H, $-CH_2$), 3.20 (ddd, J = 35.3, 20.2, 11.3 Hz, 2H, CH_2), 2.96 (d, J = 30.7 Hz, 2H, $-CH_2$), 1.32−1.22 (m, 12H, $4CH_3$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.01, 164.76, 152.47, 150.69, 147.72, 145.94, 134.14, 133.14, 127.96, 125.35, 121.30, 110.17, 105.56, 100.29, 70.25, 59.32, 55.65, 49.99, 48.37, 47.34, 37.66, 34.33, 31.09, 14.45. HRMS-APCI m/z calcd for $C_{28}H_{38}N_3O_5$ $[M + H]^+$, 496.2806, found 496.2791. Purity (HPLC): 91%, R_t = 6.4 min.

4.2.4.32. 1-Benzyl-7-{[4-(tert-butyl)benzyl]oxy}-N-{2-[(2-hydroxyethyl)amino]ethyl}-6-methoxy-4-oxo-1,4-dihydroquinolone-3-carboxamide, ***37****.* White solid, mp 214−215 °C, yield 9%, ^1H NMR (600 MHz, DMSO-d_6) δ 10.34 (dt, J =

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11.8, 6.0 Hz, 1H, CONH), 8.95 (s, 1H, NCH), 7.69 (d, J = 5.2 Hz, 1H, Ar-CH), 7.40−7.18 (m, 10H, Ar-CH), 5.81 (s, 2H, OCH_2), 5.22 (s, 1H, NH), 5.11 (s, 2H, NCH_2), 4.50 (d, J = 7.3 Hz, 1H, OH), 3.85 (s, 3H, OCH_3), 3.68 (dd, J = 24.7, 18.7 Hz, 4H, $2CH_2$), 3.15 (t, J = 6.1 Hz, 2H, CH_2), 3.05 (t, J = 5.2 Hz, 2H, CH_2), 1.26 (m, 1.42−1.13, 9H, $3CH_3$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.00, 165.37, 152.28, 150.58, 147.67, 146.92, 135.86, 134.63, 132.57, 128.79, 127.94, 127.78, 126.54, 125.14, 121.26, 110.01, 105.33, 100.70, 69.97, 56.26, 55.68, 55.52, 48.98, 46.80, 35.24, 34.17, 30.94. HRMS-APCI m/z calcd for $C_{33}H_{40}N_3O_5$ $[M + H]^+$, 558.2962, found 558.2954. Purity (HPLC): 93%, R_t = 6.2 min.

4.2.4.33. 1-Benzyl-7-[(3-chlorobenzyl)oxy]-N-{2-[(2-hydroxyethyl)amino]ethyl}-6-methoxy-4-oxo-1,4-dihydroquinolone-3-carboxamide, ***38****.* Brown powder, mp 187−188 °C, yield 9%, ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.31 (dt, J = 18.7, 6.2 Hz, 1H, CONH), 8.95 (s, 1H, Ar-CH), 8.74 (d, J = 4.6 Hz, 2H, Ar-CH), 7.74−7.69 (m, 1H, Ar-CH), 7.51−7.17 (m, 8H, Ar-CH), 5.77 (s, 2H, OCH_2), 5.21 (s, 2H, NCH_2), 4.51 (q, J = 6.9 Hz, 1H, OH), 3.92−3.84 (m, 3H, OCH_3), 3.71−3.60 (m, 4H, $2CH_2$), 3.18−3.07 (m, 2H, CH_2), 3.02 (dd, J = 9.9, 4.7 Hz, 2H, CH_2), 1.27 (dd, J = 25.8, 18.6 Hz, 1H, −NH). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.98, 165.20, 151.95, 147.64, 146.92, 138.26, 135.73, 134.53, 133.04, 130.33, 128.73, 127.96, 127.79, 127.23, 126.57, 126.07, 121.51, 110.06, 105.50, 101.01, 69.25, 56.41, 55.60, 49.09, 48.27, 46.68, 35.30. HRMS-APCI m/z calcd for $C_{29}H_{31}ClN_3O_5$ $[M + H]^+$, 536.1947, found 536.1943. Purity (HPLC): 92%, R_t = 6.2 min.

4.2.4.34. 7-[(3-Bromobenzyl)oxy]-1-ethyl-N-{2-[(2-hydroxyethyl)amino]ethyl}-6-methoxy-4-oxo-1,4-dihydroquinolone-3-carboxamide, ***39****.* Brown powder, mp 183−184 °C, yield 9%, ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.19 (t, J = 5.6 Hz, 1H, CONH), 8.72 (s, 1H, Ar-CH), 7.73 (d, J = 4.9 Hz, 2H, Ar-CH), 7.59−7.49 (m, 2H, Ar-CH), 7.39 (t, J = 7.8 Hz, 1H, Ar-CH), 7.26 (s, 1H, Ar-CH), 5.37 (s, 2H, OCH_2), 4.47 (q, J = 7.0 Hz, 3H, NCH_2 and OH), 3.90 (s, 3H, OCH_3), 3.46 (t, J = 5.7 Hz, 2H, CH_2), 3.39 (dt, J = 10.4, 5.2 Hz, 2H, CH_2), 2.69 (t, J = 6.2 Hz, 2H, CH_2), 2.61 (t, J = 5.7 Hz, 2H, CH_2), 1.28 (t, J = 7.1 Hz, 3H, CH_3), 1.05 (t, J = 7.0 Hz, 1H, NH). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.01, 164.38, 152.08, 147.62, 145.93, 139.03, 134.04, 130.99, 130.84, 130.63, 126.88, 121.78, 121.57, 110.52, 105.78, 100.40, 69.34, 60.36, 55.71, 51.48, 48.77, 48.32, 38.74, 14.43. HRMS-APCI m/z calcd for $C_{24}H_{29}BrN_3O_5$ $[M + H]^+$, 518.1285, found 518.1263. Purity (HPLC): 98%, R_t = 6.1 min.

4.2.4.35. 1-Benzyl-7-[(3-bromobenzyl)oxy]-N-{2-[(2-hydroxyethyl)amino]ethyl}-6-methoxy-4-oxo-1,4-dihydroquinolone-3-carboxamide, ***40****.* Brown solid, mp 139−141 °C, yield 13%, ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.20 (t, J = 5.9 Hz, 1H, CONH), 8.93 (s, 1H, NCH), 7.72 (d, J = 2.5 Hz, 1H, Ar-CH), 7.62−7.49 (m, 2H, Ar-CH), 7.38−7.16 (m, 8H, Ar-CH), 5.74 (s, 2H, OCH_2), 5.18 (s, 2H, NCH_2), 4.60−4.43 (m, 1H, OH), 3.88 (s, 3H, CH_3), 3.48 (t, J = 5.7 Hz, 2H, CH_2), 3.42 (dd, J = 12.0, 6.1 Hz, 2H, CH_2), 2.73 (dd, J = 11.1, 4.9 Hz, 2H, CH_2), 2.64 (q, J = 5.8 Hz, 2H, CH_2), 1.29 (t, J = 7.1 Hz, 1H, NH). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.18, 164.33, 151.98, 147.66, 146.97, 138.65, 135.90, 134.62, 130.99, 130.69, 130.27, 128.88, 127.91, 126.64, 121.75, 110.59, 105.76, 101.02, 69.32, 60.18, 55.72, 51.37, 48.67, 38.62. HRMS-APCI m/z calcd for $C_{29}H_{31}BrN_3O_5$ $[M + H]^+$, 580.1442, found 580.1438. Purity (HPLC): 92%, R_t = 6.3 min.

4.2.4.36. 1-Ethyl-7-[(3-fluorobenzyl)oxy]-N-{2-[(2-hydroxyethyl)amino]ethyl}-6-methoxy-4-oxo-1,4-dihydroquinolone-3-carboxamide, ***41****.* Brown powder, mp 191−192 °C, yield 17%, ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.20 (t, J = 5.6 Hz, 1H, CONH), 8.72 (s, 1H, Ar-CH), 7.72 (s, 1H, Ar-CH), 7.48 (td, J = 8.1, 6.2 Hz, 1H, Ar-CH), 7.37−7.34 (m, 2H, Ar-CH), 7.27 (s, 1H, Ar-CH), 7.22−7.17 (m, 1H, Ar-CH), 5.38 (s, 2H, OCH_2), 4.47 (q, J = 7.1 Hz, 3H, NCH_2 & OH), 3.90 (s, 3H, OCH_3), 3.46 (dd, J = 9.9, 4.2 Hz, 2H, CH_2), 3.39 (dd, J = 11.9, 6.1 Hz, 2H, CH_2), 2.70 (t, J = 6.2 Hz, 2H, CH_2), 2.61 (t, J = 5.7 Hz, 2H, CH_2), 1.28 (t, J = 7.1 Hz, 3H, CH_3), 1.05 (t, J = 7.0 Hz, 1H, NH). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.01, 164.38, 163.01, 161.40, 152.13, 147.64, 145.93, 139.09, 134.07, 130.65, 123.83, 121.57, 114.48, 110.52, 105.76, 100.37, 69.47, 60.34, 55.71, 51.48, 48.77, 48.31, 38.73, 14.45. HRMS-APCI m/z calcd for $C_{24}H_{29}FN_3O_5$ $[M + H]^+$, 458.2086, found 458.2070. Purity (HPLC): 98%, R_t = 6.0 min.

4.2.4.37. 1-Benzyl-7-[(3-fluorobenzyl)oxy]-N-{2-[(2-hydroxyethyl)amino]ethyl}-6-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***42****.* Brown solid, mp 205−207 °C, yield 24%, ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.30 (t, J = 6.0 Hz, 1H, CONH), 8.94 (s, 1H, Ar-CH), 8.74 (s, 1H, NCH), 7.73−7.69 (m, 1H, Ar-CH), 7.42−7.12 (m, 9H, Ar-CH), 5.78 (s, 2H, OCH_2), 5.23 (s, 2H, NCH_2), 4.51 (s, 1H, OH), 3.88 (s, 3H, $-OCH_3$), 3.66 (dd, J = 11.8, 5.9 Hz, 4H, CH_2), 3.11 (t, J = 6.4 Hz, 2H, CH_2), 3.03−2.99 (m, 2H, $-CH_2$), 1.27 (dd, J = 24.3, 17.1 Hz, 1H, NH). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.13, 165.31, 162.99, 161.36, 152.10, 147.80, 147.07, 138.75 (d, J^{CF} = 7.8 Hz), 135.90, 134.69, 130.63, 128.87, 127.92, 126.69, 123.59, 121.64, 114.88, 114.39, 114.25, 110.21, 105.64, 101.17, 69.43, 56.66, 55.75, 49.30, 46.84, 40.06, 35.53. HRMS-APCI m/z calcd for $C_{29}H_{31}FN_3O_5$ $[M + H]^+$, 520.2243, found 520.2243. Purity (HPLC): 90%, R_t = 6.1 min.

4.2.4.38. 7-[(3,5-Difluorobenzyl)oxy]-1-ethyl-N-{2-[(2-hydroxyethyl)amino]ethyl}-6-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***43****.* Brown crystals, mp 174−175 °C, yield 17%, ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.19 (t, J = 5.6 Hz, 1H, CONH), 8.72 (d, J = 6.9 Hz, 1H, Ar-CH), 7.73 (d, J = 4.9 Hz, 1H, Ar-CH), 7.32−7.17 (m, 4H, Ar-CH), 5.39 (s, 2H,OCH_2), 4.48 (q, J = 7.0 Hz, 2H, NCH_2), 4.35 (s, 1H, OH), 3.92 (s, 3H, $-OCH_3$), 3.46 (dd, J = 10.4, 4.7 Hz, 2H, CH_2), 3.40 (dd, J = 11.9, 6.1 Hz, 2H, CH_2), 2.70 (t, J = 6.2 Hz, 2H, CH_2), 2.62 (t, J = 5.7 Hz, 2H, CH_2), 1.29 (t, J = 7.1 Hz, 3H, CH_3), 1.06 (t, J = 7.0 Hz, 1H, NH). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.00, 164.36, 163.30, 161.67, 151.89, 147.61, 145.97, 140.87, 134.02, 121.73, 110.81 (d, J^{CF} = 5.3 Hz), 110.59 (d, J^{CF} = 15.3 Hz), 105.87, 103.52, 100.48, 68.94, 60.31, 55.75, 51.44, 48.74, 48.30, 38.70, 14.41. HRMS-APCI m/z calcd for $C_{24}H_{28}F_2N_3O_5$ $[M + H]^+$, 476.1992, found 476.1986. Purity (HPLC): 100%, R_t = 6.0 min.

4.2.4.39. 6-Chloro-1-ethyl-N-(2-(2-hydroxyethoxy)ethyl)-4-oxo-7-((4-(trifluoromethyl)benzyl)oxy)-1,4-dihydroquinolone-3-carboxamide, ***44****.* White solid, mp 159−161 °C, yield 40%, ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.91 (t, J = 5.5 Hz, 1H, CONH), 8.78 (s, 1H, NCH), 8.30 (s, 1H, Ar-CH), 7.79 (dd, J = 9.6, 8.2 Hz, 4H, Ar-CH), 7.40 (s, 1H, Ar-CH), 5.58 (s, 2H, CH_2), 4.48 (q, J = 7.1 Hz, 2H, NCH_2), 4.40 (t, J = 5.4 Hz, 1H, OH), 3.59−3.47 (m, 8H, $4CH_2$), 1.33 (t, J = 7.1 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.62, 163.64, 156.30, 147.59, 140.45, 138.86, 128.65, 127.70, 126.90, 125.26, 125.23, 121.70, 120.44, 111.34, 101.14, 71.97, 69.88, 69.09, 60.09, 48.15, 38.34, 13.88. HRMS-APCI m/z calcd for $C_{24}H_{25}ClF_3N_2O_5$ $[M + H]^+$, 513.1399, found 513.1397. Purity (HPLC): 97%, R_t = 9.0 min.

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*4.2.4.40. 1-Benzyl-6-chloro-N-(2-(2-hydroxyethoxy)ethyl)-4-oxo-7-((4-(trifluoromethyl)benzyl)oxy)-1,4-dihydroquinolone-3-carboxamide, **45**.* White solid, mp 221−223 °C, yield 49%, ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.90 (t, J = 5.5 Hz, 1H, CONH), 8.94 (s, 1H, NCH), 8.30 (s, 1H, Ar-CH), 7.70 (d, J = 8.1 Hz, 2H, Ar-CH), 7.59 (d, J = 8.1 Hz, 2H, Ar-CH), 7.40 − 7.19 (m, 6H, Ar-CH), 5.74 (s, 2H, OCH_2), 5.41 (s, 2H, NCH_2), 4.41 (t, J = 5.2 Hz, 1H, OH), 3.60−3.48 (m, 8H, $4CH_2$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.76, 163.52, 156.13, 148.56, 140.03, 139.42, 135.23, 128.61, 128.55, 127.64, 127.42, 126.90, 126.43, 125.19, 125.16, 121.78, 120.55, 111.45, 101.66, 71.97, 69.65, 69.05, 60.09, 55.78, 55.63, 38.38. HRMS-APCI m/z calcd for $C_{24}H_{25}ClF_3N_2O_5$ $[M + H]^+$, 575.1515, found 575.1574. Purity (HPLC): 98%, R_t = 9.6 min.

4.3. Antimycobacterial Evaluations. Antitubercular activity assay was performed using the broth microdilution method as described previously.[30] A 10 mL culture of Mtb pMSp12::GFP was grown in a Middlebrook 7H9 medium supplemented with either 0.03% casitone, 0.4% glucose, and 0.05% tyloxapol, or 0.03% albumine, 0.4% glucose, and 0.05% Tween 80, to an optical density (OD_{600}) of 0.6−0.7. The media cultures were diluted to 1:500.[34]

Twofold serial dilutions of the 10 mM test compounds were prepared into a 96-well microtiter plate and mixed with 50 μL of the diluted Mtb cultures. The plates were sealed and incubated at 37 °C under 5% CO_2 and optimal humidity. Their relative fluorescence excitation and emission were recorded at 485 and 520 nM, respectively, on days 7 and 14 using a plate reader (FLUOstar OPTIMA, BMG LABTECH). The dose−response curves, from which MIC_{90} values are calculated, were generated through the Levenberg−Marquardt damped least-squares method using the CDD Vault from Collaborative Drug Discovery data analysis software.

Rifampicin at twice the MIC_{90} and 5% DMSO were included as the minimum and maximum growth controls, respectively.

4.4. In Vitro Antibacterial and Antifungal Evaluation. These assays were performed as previously reported.[35] Eightfold serial dilutions of test samples containing less than 0.5% of DMSO were prepared in 384-well plates.

Using Cation-Adjusted Mueller Hinton broth (CAMHB) kept at 37 °C, bacteria were cultured overnight and then diluted 40-fold in fresh broth and further cultured for 1.5−3 h. After dilution, mid-log phase cultures were added to compound-containing wells at a cell density of 5 × 10^5 CFU/mL and a volume of 50 μL. Plates were incubated at 37 °C for 18 h without shaking.

Bacterial growth inhibition was determined using a Tecan M1000 Pro monochromator plate reader to measure absorbance at 600 nm (OD_{600}). This growth inhibition assay also included a negative control (media only) and positive control (bacteria without inhibitors) on the same plate as references. The minimum inhibitory concentration (MIC), the lowest concentration at which >80% of bacteria growth is inhibited, was determined following CLSI guidelines. Hits were classified by MIC ≤ 16 μg/mL in either replicate (n = 2).

C. albicans was cultured using Yeast Extract-Peptone Dextrose (YEPD) agar at 30 °C for 3 days. A suspension of (1−5) × 10^6 CFU/mL was diluted and added to compound-containing wells in 384-well plates to give a final cell density of 2.5 × 10^3 CFU/mL and a volume of 50 μL. Plates were sealed and incubated at 35 °C for 36 h without shaking. Resazurin (0.001% final concentration) was added, and the plates were further incubated for 2 h. Growth inhibition was determined by measuring absorbances at 630 nm (OD_{630}) with a Biotek Multiflo Synergy HTX plate reader.[36]

4.5. In Vitro Antileishmanial Evaluation. Wild-type *L. donovani* Bob (LdBob) promastigotes, which were derived from the *L. donovani* 1S2D strain,[37,38] were cultured in DME-L medium[39] that was supplemented with 5% heat-inactivated fetal bovine serum (Thermo Scientific HyClone), 100 μM xanthine, and 5 g of hemin/mL. All cultures were maintained at 26 °C, pH 7.4 with 5% CO_2. Inhibition assays were performed in quadruplicate in 96-well plates (Costar, Corning) in a final volume of 200 mL. Briefly, compounds were dissolved in DMSO and diluted twofold in DME-L from 50 μM to 50 nM. The control wells contained no drug and a final concentration of 0.0025% DMSO. Parasites were seeded on top of the diluted compounds in DME-L at a final concentration of 5 × 10^4 parasites/mL, incubated for 5 days, as above, and SYBR Green I fluorescence-based assays were performed as described.[40] Data were analyzed and graphed as detailed.[41]

4.6. In Vitro Cytotoxicity Evaluation. HEK293 cells were grown using Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). The cells were plated in compound-containing plates at a density of 5 × 10^3 cells per well and a volume of 50 μL. The plates were incubated for 20 h at 37 °C in 5% CO_2. Resazurin, 5 μL, was added to each well, and the plates were further incubated for 3 h.

Plates were excited at 560 nm, and the emission of each well was measured at 590 nm using a Tecan M1000 Pro monochromator plate reader. The CC_{50} (concentration required to inhibit 50% cell growth) value was generated by computing the inhibition values against the corresponding log (concentration) using a sigmoidal dose−response function with variable fitting values for the bottom, top, and slope. Any value with a > indicates samples with CC_{50} values above the maximum tested concentration (32 μg/mL).[42]

4.7. Solubility Determination. Aqueous solubility was determined at pH 6.5 in 96-well plate format using an adapted miniaturized shake-flask method. Briefly, 4 μL of a 10 mM stock in DMSO was added to a 96-well plate and evaporated using a GeneVac system. Phosphate-buffered saline (pH 6.5) was added to the compounds containing wells, after which the plate was incubated for 24 h at 25 °C while shaking. Samples were centrifuged at 3500 g for 15 min and then transferred to an analysis plate. For each sample, concentrations of 10−220 μM in DMSO were used to generate a calibration curve, which was included in the analysis plate. Analyses were performed by HPLC−DAD, and the solubility of each sample was determined from the corresponding calibration curve.[43]

4.8. Log $P_{7.4}$ Determination. Lipophilicity at pH 7.4 (Log D) was determined in 96-well plate format using a miniaturized shake-flask method. Equal volumes of 1-octanol and phosphate-buffered saline (pH 7.4) were added to each compound in a deep-well plate. The plate was vigorously shaken for 2 h at 25 °C, after which the phases were carefully separated and transferred to another plate. Analysis was performed by HPLC-UV (Agilent 1200 rapid resolution HPLC coupled to a diode array detector). Log D values were determined from the peak areas of the compound in octanol and buffer phases.[44]

4.9. Metabolic Stability Testing. The microsomal stability assay was performed using a single-point assay design wherein compounds were incubated at 1 μM in human (mixed

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gender, Xenotech), rat (male rat IGS, Xenotech), and mouse (male mouse CD1, Xenotech) liver microsomes (0.4 mg/mL) for 30 min at 37 °C. Reactions were quenched by adding ice-cold ACN containing internal standard. The samples were then centrifuged and analyzed by liquid chromatography and tandem mass spectrometry (LC-MS/MS) for the disappearance of the parent compound. Half-life, clearance, and hepatic excretion ratios were determined using standard equations.[45]

4.10. Permeability Assay. The PAMPA assay was performed following the literature procedure by Wohnslan and Faller with some modifications.[46] The assay was performed in triplicate using 96-well MultiScreen Filter plates (Millipore, 0.4 μM PCTE Membrane). Membrane filters precoated with 5% hexadecane in hexane were allowed to dry prior to the assay. Lucifer yellow (membrane integrity marker) was added to the apical wells of the precoated MultiScreen plate donor/solutions containing test compounds, while phosphate-buffered saline (pH 7.4) was added to the 96-well acceptor plate. The donor buffer was spiked using a test compound. The donor plate was slotted into the acceptor plate and incubated at room temperature for 4 h with gentle shaking (40−50 rpm). After incubation, samples from the acceptor wells and theoretical equilibrium wells were transferred to the analysis plate and matrix-matched with blank donor buffer. ACN containing internal standard (carbamazepine, 0.0236 μg/mL) was added to all samples, and they were analyzed by LC-MS/MS (Agilent Rapid Resolution HPLC, AB SCIEX 4500 MS). The normalized (analyte/internal) peak areas were used to calculate the apparent permeability (Papp), which was reported as Log P_{app}.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c01406.

^{1}H NMR, ^{13}C NMR, and APCI-HRMS spectral data for all target compounds (PDF)

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Notes

The authors declare no competing financial interest.

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