Journal of Medicinal Chemistry

pubs.acs.org/jmc Article

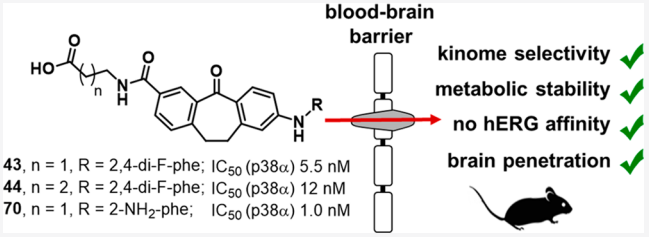
Design and Synthesis of Highly Selective Brain Penetrant p38α Mitogen-Activated Protein Kinase Inhibitors

Niklas M. Tormählen,[⊥] Mariella Martorelli,[⊥] Annette Kuhn, Florian Maier, Jamil Guezguez, Michael Burnet, Wolfgang Albrecht, Stefan A. Laufer, and Pierre Koch*

Cite This: https://doi.org/10.1021/acs.jmedchem.0c01773 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Stress-induced p38α mitogen-activated protein (MAP) kinase activation modulates cytokine overproduction and is associated with neuroinflammation and neurodegeneration. As a potential therapeutic approach, novel Skepinone-based p38α MAP kinase inhibitors were optimized to cross the blood−brain barrier via either amino acid transporters or hydrophobic diffusion. To enhance absorption from the oral route, we used methyl ester prodrugs of the active carboxy analogs. Of these, 3-(8-((2,4-difluorophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]-annulene-3-carboxamido)propanoic acid (**43**; p38α, IC_{50} = 5.5 nM) and 4-(8-((2,4-difluorophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamido)butanoic acid (**44**; p38α, IC_{50} = 12 nM) had brain-to-plasma ratios of 1.4 and 4.4, respectively. Compound **70**, 3-(8-((2-aminophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamido)-propanoic acid (p38α, IC_{50} = 1.0 nM), the Skepinone-N counterpart of **43**, was most present in the mouse brain (brain-to-plasma ratio of 4.7; 0.4 mg/kg p.o., 2 h, 580 nmol/kg). Compounds **43**, **44**, and **70** were p38α-MAP-kinase-selective, metabolically stable, hERG nonbinding, and able to modulate IL-6 and TNF-α production in cell-based assays.



INTRODUCTION

The p38α mitogen-activated protein (MAP) kinase catalyzes the transfer of the γ-phosphate of its natural cosubstrate ATP to the hydroxyl group of serine and threonine side chains of its substrates, thereby activating cell signaling cascades, in particular, during inflammation. This central role made it an early choice as a drug target for chronic inflammatory diseases as target-based screening evolved in the mid-1990s.[1] p38α MAP kinase is also expressed in glia and neurons, making it potentially relevant for central nervous system (CNS) disorders and associated neuroinflammatory responses.[2−8]

Increased activity of p38α MAP kinase was found in post-mortem brain tissue from Alzheimer's disease (AD) patients.[9,10] In microglia, p38α MAP kinase stimulates the release of the pro-inflammatory cytokines interleukin (IL)-1β and tumor necrosis factor α (TNF-α) in response to stressors including amyloid-β42[11] as well as during tau localization and neuronal plasticity.[12]

Given this association with CNS inflammation, three phase II trials on the selective p38α MAP kinase inhibitor Neflamapimod (**1a**, VX-745)[13] (Figure 1) for the treatment of AD were completed (NCT02423200, NCT02423122, and NCT03402659), and another one on brain inflammation in AD (NCT03435861) was launched in 2018.[14] In 2019, an additional phase II study began on the cognitive effects of Neflamapimod in early-stage Huntington's disease (NCT03980938). According to the sponsors, these trials yielded significant improvements in cognition using a dose of 40 mg of Neflamapimod three times daily versus b.i.d. (twice a day) or placebo (EIP Pharma, Inc., 13th Clinical Trials in

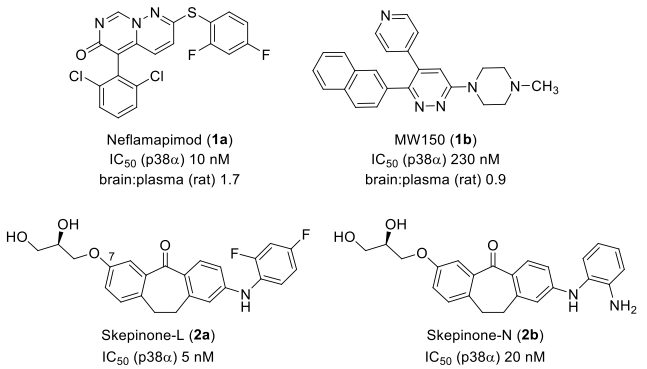


Figure 1. Structures of p38α MAP kinase inhibitors Neflamapimod (**1a**, VX-745),[15] MW150 (**1b**),[16] Skepinone-L (**2a**), and Skepinone-N (**2b**).

Special Issue: New Horizons in Drug Discovery - Understanding and Advancing Kinase Inhibitors

Received: October 9, 2020

Alzheimer's Disease (CTAD) meeting, AscenD-LB study). These data suggest that whereas there is potential to improve AD signs with compounds from this class, those optimized for peripheral diseases like rheumatoid arthritis (RA) require frequent dosing to exert central effects. When applied at 1.5 mg/kg in rats, Neflamapimod reached 33 ng/mL (76 nM) in plasma, which corresponds to 129 nmol/g in brain. This was the dose reported to improve results in the Morris water maze model.[15] At 4.5 mg/kg, the substance was able to influence hippocampal IL-1β, and exposure was reported to be 92 ng/mL in plasma corresponding to 359 nmol/kg in brain. These data suggest that depending on the parameter used, the effective dose corresponds to peak brain levels in the range of 13−36 times the IC_{50} value for p38α MAP kinase.

Using the same rationale, the brain penetrant pyridinylpyridazine-based p38α MAP kinase inhibitor MW150 (**1b**) was investigated in diverse animal models of neurologic disorders including AD and has now progressed to clinical trials.[16−20] When applied at 6 mg/kg, reported plasma concentrations are in the range of 390 to 650 ng/mL,[20] corresponding to brain concentrations of 1030 to 1693 nmol/kg. The effective dose recorded for APP/PS1 mice was 2.5 mg/kg, suggesting that at effective doses, the peak levels in brain would likely be two to three times the IC_{50} value for p38α MAP kinase.

In 2012, we reported the development of dibenzosuberone derivatives Skepinone-L (**2a**) and Skepinone-N (**2b**) as potent selective p38α MAP kinase inhibitors (Figure 1).[21,22] The prototypic compound Skepinone-L has been used as a probe to further investigate the role of p38α MAP kinase in various disorders.[23−27] X-ray analysis of this inhibitor complexed with the target enzyme suggests that it acts as a type I kinase inhibitor, inducing a glycine flip at the hinge region and binding hydrophobic region (HR) I and HR II (Figure 2).[21] Skepinone-L has served as a starting point for the design of type I 1/2 p38α MAP kinase inhibitors with improved binding kinetics.[28,29]

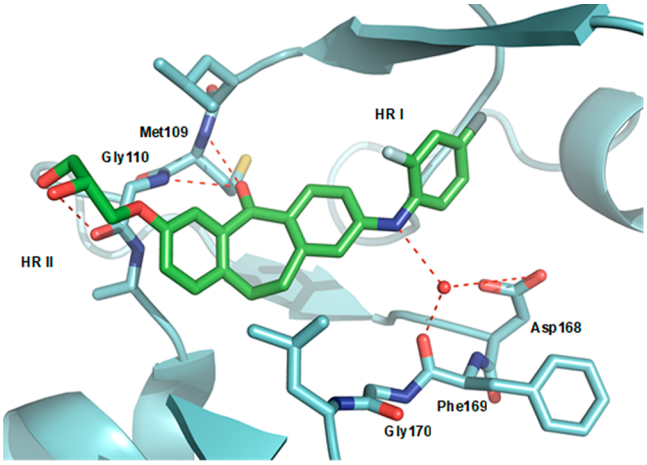


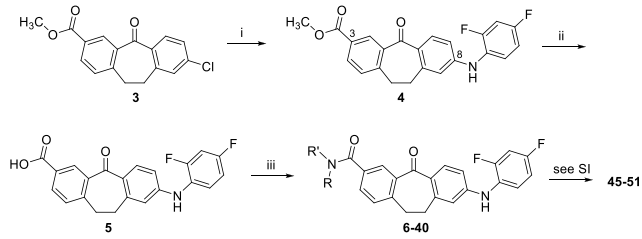
Figure 2. Binding mode of Skepinone-L (**2a**) within the ATP binding site of p38α MAP kinase (PDB code: 3QUE).

The aim of the present study was to identify selective and metabolically stable Skepinone-based inhibitors of p38α MAP kinase, which also have the ability to cross the blood−brain barrier (BBB) *in vivo* in mouse models. To this end, we employed amino acid transporters and passive diffusion mechanisms. The ether function at position C-7 of the dibenzosuberone core targeting the HR II was replaced by amide moieties to increase interaction possibilities with the enzyme. Lipophilic and basic moieties, (amino) acids, biogenic amines, amino alcohols, and glucosamine were introduced as amide moieties with the potential for active transport into the brain as amino acid or carbohydrate mimics. In many analogs, these mimics gave rise to terminal carboxy groups. Given that carboxy species may be poorly resorbed in the gut, we also formed ester prodrugs of these compounds to compare with the free acids in both enzyme inhibition and oral pharmacokinetic studies .

RESULTS AND DISCUSSION

Synthesis. The novel dibenzosuberone derivatives **6−51** (Skepinone-L series) and **54−72** (Skepinone-N series) were synthesized by a linear approach starting from the reported methyl 8-chloro-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]-annulene-3-carboxylate[30] (**3**). The amide moiety targeting HR II of p38α MAP kinase was introduced in the last steps of the synthetic sequences (Schemes 1 and 4). The synthesis of

Scheme 1. Synthesis of Dibenzosuberones 6−40 (Skepinone-L Series)[a]

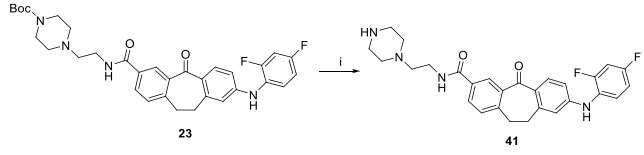


[a]Reagents and conditions: (i) 2,4-difluoroaniline, Cs_2CO_3, XPhos, $Pd(OAc)_2$, 1,4-dioxane/*t*-BuOH (5:1, v/v), 110 °C. (ii) KOH, MeOH, reflux temperature. (iii) CDI, DMF, 50 °C. For the detailed structures of compounds **6−51**, see Tables 1 and 2 and Table S1, SI.

dibenzosuberones **6−51** is depicted in Schemes 1−3 and Schemes S1−S6 (SI). In the first step, the amino function at position C-8 of the dibenzosuberone core was introduced by the coupling of **3** to 2,4-difluoroaniline under Buchwald−Hartwig conditions (Scheme 1). Basic hydrolysis of **4** resulted in dibenzosuberone **5** bearing a free carboxylic acid function in position C-3. Activation of the carboxylic acid with 1,1′-carbonyldiimidazole (CDI) and conversion with the respective amines resulted in compounds **6−40**. The detailed syntheses of dibenzosuberones **45−51** are depicted in Schemes S1−S6 (Supporting Information, SI).

The Boc group present in dibenzosuberone **23** was cleaved under acidic conditions to yield the corresponding amino derivative **41** (Scheme 2).

Scheme 2. Synthesis of 41[a]

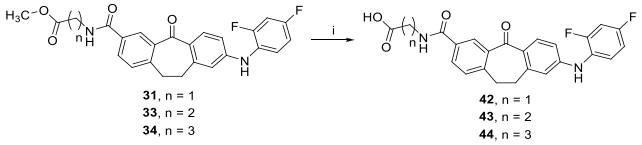


[a]Reagents and conditions: (i) 2,2,2-trifluoroacetic acid (TFA), dichloromethane (DCM), room temperature (rt).

https://doi.org/10.1021/acs.jmedchem.0c01773
J. Med. Chem. XXXX, XXX, XXX−XXX

Carboxylic acids **42**–**44** were obtained by treatment of the corresponding esters **31**, **33**, and **34** with methanolic potassium hydroxide solution at reflux temperature (Scheme 3).

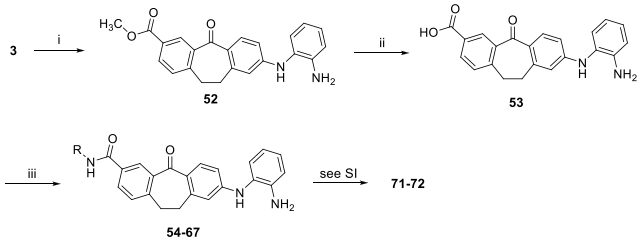
Scheme 3. Synthesis of 42–44[a]



[a]Reagents and conditions: (i) KOH, MeOH, reflux temperature.

Dibenzosuberones **54**–**72** having an *o*-phenylenediamine moiety at position C-8 were prepared using an analogous synthetic strategy, as in the case of compounds **6**–**51** (Schemes 4–7 and Scheme S7 (SI)). Buchwald–Hartwig coupling of ester **3** with *o*-phenylenediamine yielded diarylamine **52**, which was subsequently transferred into carboxylic acid **53** (Scheme 4). The amide moiety at position C-3 of the dibenzosuberone core was installed using CDI as a coupling reagent. The modification of dibenzosuberone **67** into compounds **71** and **72** is shown in Scheme S7 (SI).

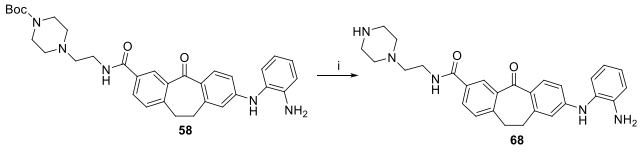
Scheme 4. Synthesis of Dibenzosuberones 54–67 (Skepinone-N Series)[a]



[a]Reagents and conditions: (i) *o*-phenylenediamine, Cs_2CO_3, XPhos-Pd second generation, 1,4-dioxane/*t*-BuOH (5:1, v/v), 60 °C. (ii) KOH, MeOH, reflux temperature. (iii) CDI, DMF, 50 °C. For the detailed structures of compounds **54**–**67** and **71**–**72**, see Tables 3 and 4 and Table S1, SI.

Dibenzosuberone **68** was obtained in excellent yield by treatment of Boc-protected derivative **58** with TFA at ambient temperature (Scheme 5).

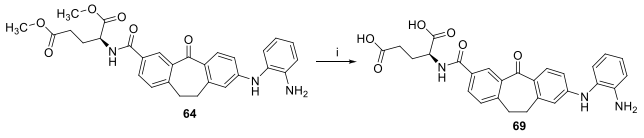
Scheme 5. Synthesis of 68[a]



[a]Reagents and conditions: (i) TFA, DCM, rt.

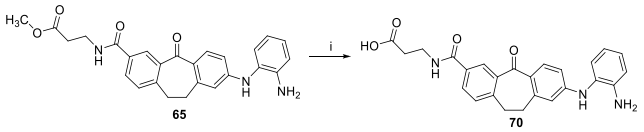
Dicarboxylic acid **69** and carboxylic acid **70** were synthesized from the corresponding diester **64** and ester **65**, respectively, using similar conditions as described for compounds **42**–**44** of the Skepinone-L series (Schemes 6 and 7).

Scheme 6. Synthesis of 69[a]



[a]Reagents and conditions: (i) KOH, MeOH, reflux temperature.

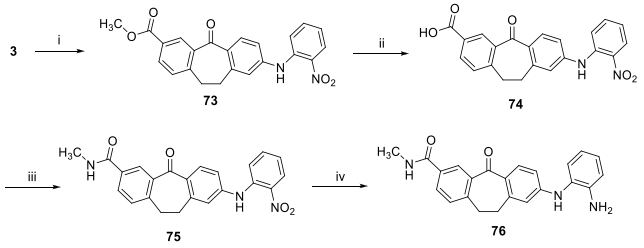
Scheme 7. Synthesis of 70[a]



[a]Reagents and conditions: (i) KOH, MeOH, reflux temperature.

Dibenzosuberone **76** bearing an *N*-methyl carboxamide moiety at position C-3 was synthesized using a slightly different synthetic strategy (Scheme 8). The aniline moiety targeting HR I was installed via the Buchwald–Hartwig coupling reaction of aryl chloride **3** with 2-nitroaniline. After the subsequent hydrolysis of ester **73**, the methyl amide moiety was introduced by treatment of carboxylic acid **74** with methylamine in the presence of CDI as a coupling reagent. Finally, compound **76** was obtained by reduction of the nitro group present in compound **75** with tin(II) chloride in ethanol.

Scheme 8. Synthesis of Dibenzosuberone 76 (Skepinone-N Series)[a]

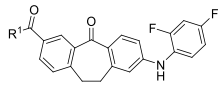


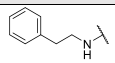
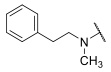
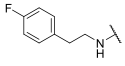
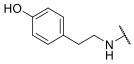
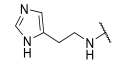
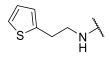
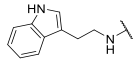
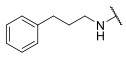
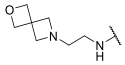
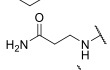
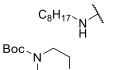
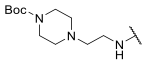
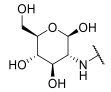
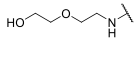
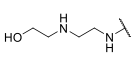
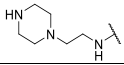
[a]Reagents and conditions: (i) 2-nitroaniline, Cs_2CO_3, XPhos, $Pd(OAc)_2$, 1,4-dioxane/*t*-BuOH (5:1, v/v), 110 °C. (ii) KOH, MeOH, reflux temperature. (iii) Methylamine (2 N THF), CDI, DMF, 50 °C. (iv) $SnCl_2$, EtOH, reflux temperature.

Biological Evaluation. *Kinase Activity Assay.* The dibenzosuberones **6**–**37**, **41**–**51**, **54**–**66**, **68**–**72**, and **76** were evaluated for their ability to inhibit p38α MAP kinase.[31] The nature of the amide was essential for the inhibitory activity. Secondary amide **7** (IC_{50} = 21 nM) showed an almost 20 times lower IC_{50} value compared with tertiary amide **8** (Table 1). Similarly, the tertiary amides **6** (IC_{50} = 95 nM) and **24** (IC_{50} = 120 nM) were less potent than the corresponding secondary *N*-methylamide (IC_{50} = 3 nM).[30] For hydrophobic moieties, the inhibitory activity decreased with chain lengths over two methylene groups: Compounds **7**, **16**, and **25** had lower IC_{50} values than compounds **14**, **21**, and **22**. The introduction of a fluorine atom at the para position of the benzene ring of inhibitor **7** resulted in an equipotent compound **9**. The introduction of a hydroxyl group in this position (compound **10**) or the bioisosteric replacement of the benzene ring by a thiophene ring (compound **12**) resulted in a

https://doi.org/10.1021/acs.jmedchem.0c01773
J. Med. Chem. XXXX, XXX, XXX–XXX

Table 1. Biological Activities of 3-Substituted 2-((2,4-Difluorophenyl)amino)-5-dibenzosuberones 6−30 and 41 (Skepinone-L Series) (n = 3)



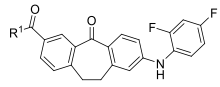
Cpd.	R¹	p38α [nM] IC_{50} ± SEM	Cpd.	R¹	p38α [nM] IC_{50} ± SEM
6		95 ± 10	7		21 ± 1
8		390 ± 60	9		21 ± 4
10		7.8 ± 0.9	11		5.6 ± 0.8
12		6.4 ± 2.6	13		28 ± 1
14		50 ± 4	15		5.9 ± 0.4
16		2.5 ± 0.0	17		22 ± 0
18		2.5 ± 0.4	19		6.2 ± 1.8
20		3.5 ± 0.1	21		390 ± 30
22		>1,000	23		2.4 ± 0.1
24		120 ± 20	25		2.4 ± 0.2
26		5.2 ± 0.2	27		3.0 ± 0.2
28		5.2 ± 0.5	29		10 ± 1
30		1.7 ± 0.3	41		1.3 ± 0.1

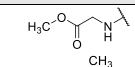
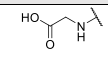
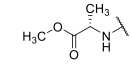
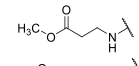
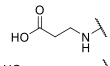
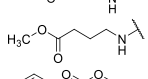
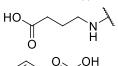
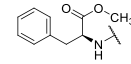
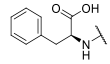
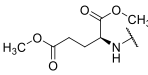
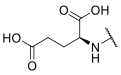
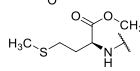
three times lower IC_{50} value. Compound **15** bearing a spirocyclic morpholine isostere was also a potent inhibitor (IC_{50} = 5.9 nM). Compound **17** having a branched moiety was intermediate in potency—the racemic mixture **17** (IC_{50} = 22 nM) was more potent than the (R)-enantiomer (IC_{50} 89 nM),[30] suggesting that the (S)-enantiomer is worthy of purification. Compounds **18** and **20** (IC_{50} = 2.5 and 3.5 nM, respectively) bearing an 2-aminoethyl and a 2,2,2-trifluoroethyl moiety at the dibenzosuberone-C3-carboxamide function, respectively, were likewise potent.

The hydroxamic acid derivative **26** (IC_{50} = 5.2 nM) and compounds having a (poly-)alcohol (**27** (IC_{50} = 3.0 nM), **29** (IC_{50} = 10 nM), **30** (IC_{50} = 1.7 nM)) or a glucosamine moiety (**28** (IC_{50} = 5.2 nM)) were also potent p38α MAP kinase inhibitors (Table 1).

The introduction of amino acid derivatives (**31**−**37** and **42**−**51**) provided consistently potent inhibitors (Table 2). Assuming that interaction with transporters for central nervous system (CNS) uptake required the physiological (S)-configuration, all amino acid derivatives were assayed in this form. The ester prodrugs were, in most cases, slightly more inhibitory than their corresponding carboxylic acids (compare **33** vs **43**, **34** vs **44**, **35** vs **45**, **36** vs **46**, **37** vs **47**, **48** vs **51**); however, in the case of ester **31** and carboxylic acid **42**, the reverse was apparent. Ester **33** (IC_{50} = 1.2 nM) was the most potent inhibitor of this series.

Table 2. Biological Activities of 3-Substituted 2-((2,4-Difluorophenyl)amino)-5-dibenzosuberones 31−37 and 42−51 (Ester Prodrugs and Corresponding Acids of Skepinone-L Series) (n = 3)



Ester			Corresponding acid		
Cpd.	R¹	p38α [nM] IC_{50} ± SEM	Cpd.	R¹	p38α [nM] IC_{50} ± SEM
31		12 ± 1	42		9.3 ± 0.7
32		3.0 ± 0.2			
33		1.2 ± 0.4	43		5.5 ± 0.1
34		3.4 ± 0.3	44		12 ± 1
35		15 ± 0	45		36 ± 4
36		1.2 ± 0.5	46		8.0 ± 1.3
37		6.9 ± 0.4	47		11 ± 0
48		2.1 ± 0.0	51		3.5 ± 0.3
49		5.4 ± 0.8			
50		2.3 ± 1.0			

As observed in the case of Skepinone-L (**2a**) and Skepinone-N (**2b**) (Figure 1), most dibenzosuberone derivatives of our study bearing an o-phenylenediamine substituent at position C-8 (Skepinone-N series) were less active than their corresponding Skepinone-L counterparts (compare **63** vs **18**, **65** vs **33**, **57** vs **11**, **64** vs **36**, **69** vs **46**, **68** vs **41**, **66** vs **37**, **71** vs **48**, **72** vs **51**, Tables 1−4). In the case of inhibitors **55**, **58**, and **70**, the Skepinone-N analog was, however, equal to or more potent than the corresponding Skepinone-L derivatives **7**, **23**, and **43**, respectively.

In the series presented in Table 3, the secondary amides **58**, **63**, and **76** having an N-Boc-protected piperazinoethyl, a 2-aminoethyl, or a methyl moiety at the carboxamide group, respectively, have an equal or a better inhibitory activity than primary amide **62**. Hydroxyethyl-substituted dibenzosuberone **59** as well as diols **60** and **61** have similar inhibitory activities in the low double-digit nanomolar range. Cleavage of the Boc-group present in compound **58** resulted in a nine-fold loss of inhibitory activity (compound **68**).

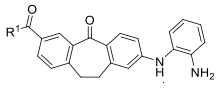
Esters **64** and **71** are approximately 1.5 times more active than their corresponding carboxylic acids **69** and **72**, respectively (Table 4). In the case of ester **65**, however, the corresponding acid **70** displays a 30 times lower IC_{50} value. Dibenzosuberone **70** represents the most potent p38α MAP kinase inhibitor of our study.

Metabolism. Inhibitors **11**, **13**, **15**, **19**, **26**, **31**−**33**, **41**−**44**, **46**, **49**, **50**, **54**, **56**, **59**, **66**, **70**, and **76** were further evaluated for their metabolic stability in human liver microsomes (HLMs) (Tables 5 and 6, Figures S1−S21, SI).

The ester prodrugs were converted into the free carboxylic acids. Simple esters such as **31** and **33** were cleaved within a

https://doi.org/10.1021/acs.jmedchem.0c01773
J. Med. Chem. XXXX, XXX, XXX−XXX

Table 3. Biological Activities of 3-Substituted 2-((2-Aminophenyl)amino)-5-dibenzosuberones 54−63, 68, and 76 (Skepinone-N Series) (n = 3)



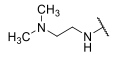
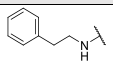
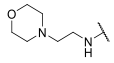
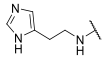
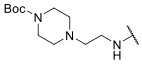
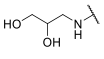
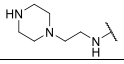
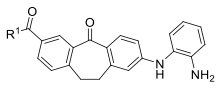
Cpd.	R¹	p38α [nM] IC_{50} ± SEM	Cpd.	R¹	p38α [nM] IC_{50} ± SEM
54		8.4 ± 0.2	55		20 ± 1
56		18 ± 1	57		26 ± 1
58		1.9 ± 0.8	59		14 ± 2
60		15 ± 1	61		15 ± 1
62		6.1 ± 0.8	63		5.3 ± 0.6
68		18 ± 1	76		5.7 ± 1.2

Table 4. Biological Activities of 3-Substituted 2-((2-Aminophenyl)amino)-5-dibenzosuberones 64−66 and 69−72 (Ester Prodrugs and Corresponding Acids of Skepinone-N Series) (n = 3)



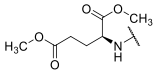
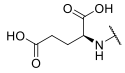
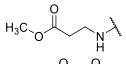
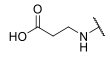
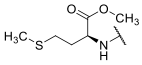
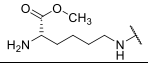
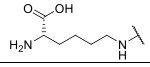
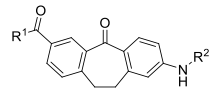
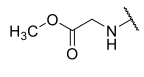
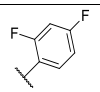
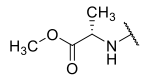
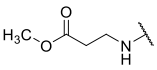
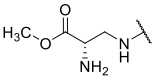
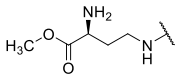
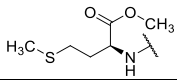
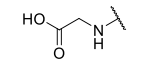
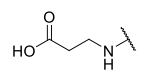
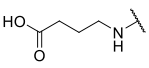
Ester			Corresponding acid		
Cpd.	R¹	p38α [nM] IC_{50} ± SEM	Cpd.	R¹	p38α [nM] IC_{50} ± SEM
64		17 ± 2	69		27 ± 3
65		29 ± 3	70		0.98 ± 0.7
66		13 ± 1			
71		12 ± 1	72		17 ± 2

Table 5. Metabolic Stability of Selected Dibenzosuberone Inhibitors after 120 min of Incubation with HLMs (% Parent Remaining)



Cpd.	R¹	R²	Metabolic stability [%]
esters			
31			0
32			93
33			0
49			12
50			6
66			0
carboxylic acids			
42			93
43			97
44			99
46			93
70			99

few minutes. Their resulting acids **42** and **43**, respectively, were metabolically stable. Carboxylic acids **44** and **70** also displayed excellent metabolic stability. Sterically hindered esters with terminal amino acids such as **49** and **50** were cleaved more slowly. Compound **32**, whose additional methyl group may shield the ester function from enzymatic hydrolysis, was degraded by <10% within 2 h. Compound **66** bearing a methionine methyl ester substituent formed the sulfoxide in addition to the carboxylic acid.

In both series, the aromatic moiety used to fill HR I and the dibenzosuberone core remained stable, whereas the moiety targeting HR II was labile.

Dibenzosuberone analog **13** bearing a tryptamine at the C-3 amide function underwent a slow oxidative biotransformation (Table 6). After an incubation time of 120 min, 71% of **13** was present. In contrast, compound **11** having a histamine moiety in the same position was stable to HLMs (5% degradation after 120 min).

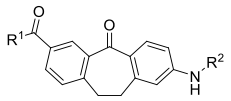
Morpholino derivative **56** (Skepinone-N series) had an *in vitro* half life in HLMs of <1 h. After an incubation time of 2 h, <38% of **56** remained. The supposedly metabolically more stable 2-oxa-6-azaspiro[3.3]heptan-6-yl derivative **15** (Skepinone-L series) had a half life of 113 min and was more stable than **56**, but more varied metabolites were formed during the incubation.

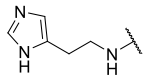
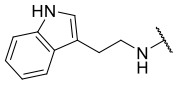
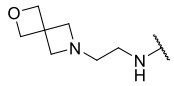
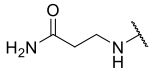
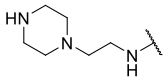
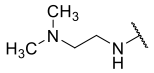
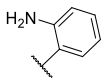
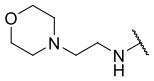
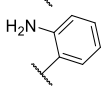
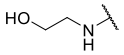
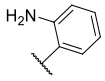
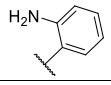
The aliphatic dimethylethylamino moiety present in compound **54** was transformed by HLMs to the *N*-demethylated and a hydroxylated metabolite. Compound **19** was stable (94% remaining). Hydroxamic acid **26** and *N*-methylamide **76** were rapidly metabolized in HLMs (*in vitro* half life of 22 and 12 min, respectively) to the corresponding acids: **5** (m/z 380.3 [M + H$^+$]) and **53** (m/z 359.3 [M + H$^+$]), respectively.

Pharmacokinetic and CNS Penetration Study in RjOrl:Swiss CD-1 Mice. To evaluate brain penetration and plasma stability, compounds **15**, **16**, **28**, **30**, **31**, **33**−**36**, **41**, **48**, **49**, **54**, **56**, and **57**, were tested in adult male RjOrl:Swiss CD-1 mice. The mice were treated with a single intravenous (i.v.) injection (10 mg/kg), and plasma samples were collected 10, 30, and 120 min after the treatment. After the last sample was

https://doi.org/10.1021/acs.jmedchem.0c01773
J. Med. Chem. XXXX, XXX, XXX−XXX

Table 6. Metabolic Stability of Selected Dibenzosuberone Inhibitors after 120 min of Incubation with HLMs (% Parent Remaining)



Cpd.	R^1	R^2	Metabolic stability [%]
11			95
13			71
15			47
19			95
26			13
41			93
54			50
56			38
59			99
76			3

obtained, the mice were sacrificed for sampling of the brains. Blood and brain samples were analyzed via liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS) after preparation. (See the SI.)

Consistent with the HLM study, esters **31** and **33** underwent a very fast metabolism and are no longer detectable 10 min after i.v. administration (Table S5, SI). Their active metabolites (corresponding acids **42** and **43**, respectively) were detected after 2 h at a concentration of 8.2 and 102.9 ng/g, which corresponds to a brain-to-plasma ratio of 0.13 and 1.25, respectively (Table 7 and Table S5, SI). A similar level of the carboxy product **44** was detected in the brain (98.2 ng/g), which corresponds to a brain-to-plasma ratio of 4.4. Because passive transport of the charged compounds **43** and **44** seems unlikely under physiological conditions, these inhibitors may have crossed the BBB via transporter systems.

Compared with **31** and **34**, esters **35** and **36** were hydrolyzed more slowly to their corresponding acids **45** and **46**. Only low levels of acid **45** (15.0 ng/g) were detected in the brain (Table S5, SI); however, glutamic acid derivative **46** did not penetrate into the brain (Table 7 and Table S6, SI).

Table 7. Brain-to-Plasma Ratio of i.v. Administered Prodrugs (Esters 31, 33−36, 48, and 49) and Their Corresponding Acids (42−46, 51, and S2) after 2 h

prodrug	**31**	**33**	**34**	**35**	**36**	**48**	**49**
brain-to-plasma	NA[a]	NA	NA	NA	NA	NA	NA
active metabolite	**42**	**43**	**44**	**45**	**46**	**51**	**S2**[b]
brain-to-plasma	0.13	1.25	4.38	0.31	NA	0.01	0.003

[a]NA, not applicable. [b]For the structure of **S2**, see the SI.

As observed in the *in vitro* metabolism study of **49** (Figure S14, SI), the esters of α-amino acids (compounds **48** and **49**) were not fully converted into their corresponding free α-amino acids **51** and **S2** after 2 h. Only traces of both acids were detected in the brain (Table S6, SI).

Concentrations of 68 and 49 ng/g of compounds **15** and **16** (Table S3, SI), respectively, were found in the brain after 2 h; this corresponds, in the case of **16**, to a brain-to-plasma ratio of 0.51 (Table 8). The spirocyclic moiety present in **15** as well as the lipophilic moiety of **16** are unlikely to be recognized by specific transporters. Therefore, these inhibitors are expected to cross the BBB only via passive diffusion.

Dibenzosuberones **28**, **30**, and **41** showed a fast degradation or elimination (Tables S3 and S4, SI). Inhibitor **28** bearing a glucosamine residue may be transported by the glucose transporter. However, only 5.9 ng/g of **28** was detected after 2 h, which correlates to a low brain-to-plasma ratio of 0.09. Brain-to-plasma ratios of 0.17 and 0.24 were observed for compounds **30** and **41**, respectively (Table 8).

Within the Skepinone-N series, only low amounts of morpholino-containing inhibitor **56** were detected in the brain. Compound **54**, having a tertiary amine in the amide chain, showed a four times higher concentration in the brain accompanied by a higher brain-to-plasma ratio.

Dibenzosuberone **57** bearing the biogenic histamine substituent was eliminated relatively quickly and is not able to cross the BBB (Table S4, SI).

In Vitro and in Vivo Study in Balb/c and C57BL/6 Mice. Effects in disease models can be influenced by partition to specific cell types and degrees of inhibition in these cells (i.e., due to acid trapping in myeloid cells vs lymphoid cells). To assess effects in different cells, selected compounds (**18**, **25**, **27**, **29**, **33**, **34**, **43**, **44**, **50**, **65**, **70**, **76**) were further evaluated in *in vitro* and *in vivo* studies.

First, the selected inhibitors were characterized in splenocytes isolated from female Balb/c mice. In total, three different assays were done in mouse splenocytes stimulated with 500 ng/mL lipopolysaccharide (LPS), and the release of pro-inflammatory cytokines IL-6 and TNF-α was quantified. The metabolic activity in these cells was measured via (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction to formazan to control for cytotoxic effects.

There was a trend for the tested compounds to increase the metabolic activity of splenocytes at a concentration of 100 nM (66−176% relative to LPS-stimulated splenocytes) (Figure S23, SI). At concentrations of 1 and 10 nM, there was no effect on the metabolic activity of splenocytes. No cytotoxicity was observed, whereas the anti-inflammatory activity of all investigated compounds was dose-dependent, with reductions in both IL-6 and TNF-α (Figure S24, SI). Inhibitor **70** increased IL-6 at concentrations of 1 and 10 nM (138 and 118%, respectively), then decreased it to 29% at 100 nM

https://doi.org/10.1021/acs.jmedchem.0c01773
J. Med. Chem. XXXX, XXX, XXX−XXX

Table 8. Brain-to-Plasma Ratio of i.v. Administered Inhibitors 15, 16, 28, 30, 41, 54, 56, and 57 after 2 h

inhibitor	15	16	28	30	41	54	56	57
brain-to-plasma	0.16	0.51	0.09	0.17	0.24	0.59	0.08	0.00

relative to PBS-treated LPS-stimulated splenocytes. Compound **25** caused moderate inhibition of LPS-stimulated IL-6 release (51% at 100 nM). Compound **50** caused the highest reductions in IL-6 (Figure S24a, SI). Compounds **18**, **25**, **27**, **29**, **43**, **50**, **70**, and **76** were potent inhibitors of the LPS-stimulated TNF-α release (Figure S24b, SI). Compounds **18**, **27** and **76** were most potent, whereas compounds **33**, **34**, **44**, and **65** were only moderate to poor inhibitors.

Heparinized whole blood was prepared from C57BL/6 mice (MWB) stimulated with 500 ng/mL LPS. As for mouse splenocytes, all tested compounds reduced the release of IL-6 and TNF-α dose-dependently (Figure S25, SI). Compounds **27**, **29**, and **50** caused the highest reductions in IL-6 release (Figure S25a, SI), whereas compounds **33**, **34**, **44**, and **65** were moderate (85, 64, 71, and 81%, respectively). Dibenzosuberones **18**, **25**, **27**, **29**, **43**, **50**, **70**, and **76** were potent inhibitors of LPS-stimulated TNF-α from MWB (range: 5−23% at 1 nM in comparison with LPS-stimulated MWB). Compounds **27**, **43**, and **76** (Figure S25b, SI) were most potent in this assay. Compounds **33**, **34**, **44**, and **65** had lower activity in this assay (~50% inhibition at 10 nM). Consistent with mouse splenocytes, compound **33** was the least active of the series in MWB.

Cellular compound uptake using human buffy coat (HBC) was also evaluated. Cells (1×10^6 HBC cells/mL) were treated with compounds at a final concentration of 100 μM, and cell culture supernatants were collected at different time points and analyzed for compound concentration (Tables S24 and S25, SI).

The effects of compounds on cytokine release were also assessed in LPS-stimulated HBC cells in the same format as mouse splenocytes and whole blood. All compounds had dose-dependent effects, with **50** being most active (Figure S26, SI) and **33** being the least active, suggesting broadly similar data in the human and murine systems.

Because most transgenic animal models of neurodegenerative disorders are on a C57BL/6 background, this strain was used for further pharmacokinetic studies including penetration of the BBB of a second set of inhibitors (**18**, **25**, **27**, **29**, **50**, **70**, and **76**) to augment data from CD1 mice. Blood samples were taken at 5, 15, 30, 60, 120, and 240 min. Animals were terminated for brain sampling at 120 and 240 min post substance application. The substance was quantified in peripheral plasma, heart-derived plasma, liver, kidney, brain right hemisphere, and brain left hemisphere by LC-MS/MS. Compounds were administered (0.4 mg/kg) in mixtures to reduce the number of animals used. (See the SI for details.) The rationale for the lower dose was to model the likely concentrations following oral application and thus to simulate the likely conditions for transport systems in long-term neurodegenerative models in C57BL/6.

The compounds from the Skepinone-L series (**18**, **25**, **27**, **29**, **47**, **50**) showed no or only low brain uptake after i.v. or p.o. application (Tables S9−S13 and S16−S20, SI). Compound **76** was BBB-penetrant after i.v. and p.o. application (~20 nM in brain at 2 h), exceeding the IC_{50} value for free p38α MAP kinase (Tables S15 and S22, SI). Compound **70** (~590 nM in brain, 2 h p.o.) had a brain-to-plasma ratio of 4.7 (Table S21, SI) and the highest levels in all organs after 4 h (heart ~120 nM, liver ~330 nM, kidney ~380 nM, and brain ~540 nM). When used in the context of tissue, "nM" is shorthand for nmol/kg tissue fresh weight and is used in this way to allow direct comparison to values for inhibition of the enzyme.

Human Whole Blood Assay. The human whole blood (HWB) assay is a means to evaluate the effectiveness of p38α MAP kinase inhibitors in modulating pro-inflammatory cytokine secretion in a primary cell system. In this *ex vivo* assay, the efficacy is estimated with *in vivo* relevant parameters like plasma protein binding and cellular permeability. Thus the IC_{50} values are typically higher than those for the direct enzyme inhibition assay. Ester **34** is a potent inhibitor of the LPS-stimulated TNF-α release displaying an IC_{50} value of 64 nM (Table S23, SI). Its active metabolite, the corresponding acid **44**, shows a two-fold decrease in inhibitory activity. The release of LPS-stimulated TNF-α from HWB was inhibited by compound **15** at concentrations in the double-digit nanomolar range.

CYP and hERG Assay. The p38α MAP kinase inhibitors **15**, **33**, **34**, **43**, **44**, and **70** were tested for the inhibition of hERG and relevant CYP isoforms (Table 9). At a test concentration of 10 μM, both esters **33** and **34** display a 59.9 and 49.7% inhibition of hERG and inhibit the tested CYP isoforms 2C9 and 2C19 by more than 98%. At the same concentration, their active metabolites **43** and **44** that are formed within a few minutes show no interaction with hERG and a reduced CYP inhibition. No affinity toward CYP2D6 (a significant CNS CYP) was observed in the case of **43** and **44**. Compound **70**, the Skepinone-N counterpart of **44**, had no or a limited effect on CYP and hERG activity. In contrast, compound **15** had both a higher inhibition of hERG (76.6%) and an elevated CYP inhibition profile for isoforms CYP2C9, CYP2C19, CYP2D6, and CYP3A4 compared with carboxylic acids **43**, **44**, and **70**. No inhibition of CYP1A2 was observed for **15**.

Table 9. *In vitro* CYP and hERG Inhibition Data of Inhibitors 15, 33, 43, 34, 44, and 70

	% inhibition @ 10 μM					
Cpd	hERG	CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP3A4
15	76.6	0	88.7	93.6	18.3	83.6
33	59.9	27.3	99.4	100	73.7	68.0
43	11.8	11.6	68.4	60.4	3.0	34.9
34	49.7	33.6	98.5	100	36.6	54.8
44	14.4	16.2	66.0	63.2	2.0	38.1
70	22.5	28.1	13.1	21.8	11.4	4.1

[a] $n = 2$.

Kinome Selectivity. Compounds **31**, **42**−**44**, and **57** were tested at 1 and 5 μM against a panel of 320 kinases to evaluate their selectivity (Tables S26 and S27, SI).

Ester **31** is a highly selective p38α MAP kinase inhibitor. At both concentrations, only the closely related kinases p38β MAP kinase, JNK2, and JNK3[32] are inhibited by more than 60% (Table S26, SI), resulting in an S_{60} selectivity score of 0.013. At 1 μM, the active metabolite of **31**, carboxylic acid **42**,

https://doi.org/10.1021/acs.jmedchem.0c01773
J. Med. Chem. XXXX, XXX, XXX−XXX

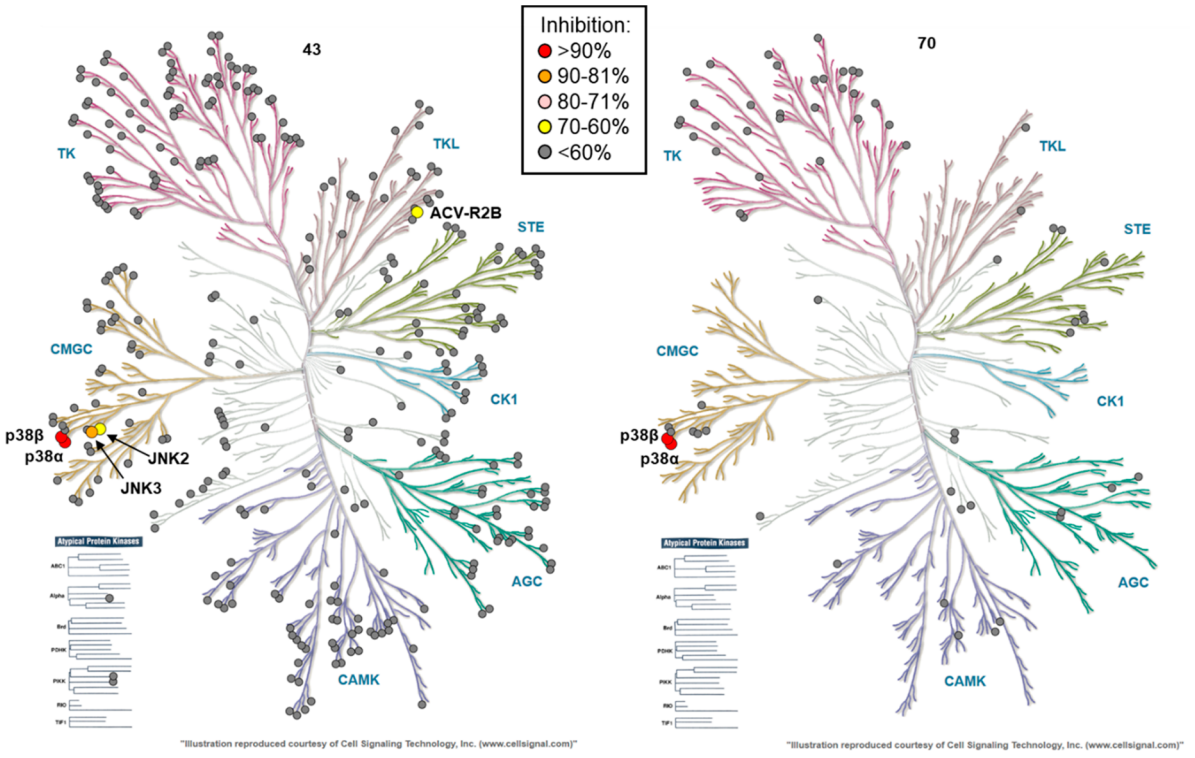


Figure 3. Selectivity profile of carboxylic acids **43** (left) and **70** (right). Tested kinases are highlighted with circles and colored according to their inhibition at 1 μM inhibitor concentration. This figure was generated with the help of the KinMap.[33]

had similar selectivity, interacting only with p38β MAP kinase, JNK3, and ACV-R2B (Table S26, SI). At a higher concentration (5 μM), CAMK2G, EGF-R, EIF2AK2, JNK2, and MYLK were additionally inhibited by more than 60% by **42**. Inhibitor **43**, having an additional methylene unit between the carboxylic group and the amide function compared with **42**, was similar to **42** (Table S26, SI). At 1 μM, the main target kinase p38α MAP kinase and the MAP kinase family members p38β MAP kinase, JNK2, and JNK3 as well as the tyrosine kinase-like kinase ACV-R2B were inhibited by **43** by more than 60% (Figure 3). At 5 μM (900 times its IC_{50}), **43** inhibited the tyrosine kinases EGF-R, VEGF-R2, and MYLC as well as the serine/threonine kinase JNK1. Carboxylic acid **44** interacted with p38β MAP kinase, JNK3, and TLK2) at 1 μM (Table S27, SI) but inhibited 14 kinases by more than 60% at 5 μM with an S_{60} selectivity score of 0.044. Compound **57** was similar to **31** with an S_{60} selectivity score of 0.013 at both concentrations (Table S27, SI).

Inhibitor **70** was tested at concentrations of 1 and 5 μM against a panel of 65 kinases consisting of all kinases that were inhibited in the previous screening by more than 40% by compounds **31**, **42**−**44**, and **57** and all members of the p38 MAP kinase family (Figure 3 and Table S28). Besides the target kinase p38α MAP kinase, only the closely related p38β MAP kinase was inhibited by more than 60% by **70** at test concentrations of 1 and 5 μM.

CONCLUSIONS

62 novel Skepinone-based p38α MAP kinase inhibitors with IC_{50} values down to the low single-digit nanomolar range were characterized. Various substituents (lipophilic and basic moieties, (amino) acid derivatives, biogenic amines, amino alcohols, and carbohydrates) at position C-3 of the dibenzosuberone core remained inhibitory. Ester prodrugs **33** and **34** were rapidly converted into their corresponding carboxylic acids **43** and **44**, respectively. The active metabolites **43** (p38α, IC_{50} = 6 nM) and **44** (p38α, IC_{50} = 12 nM) crossed the BBB (concentration in brain after 2 h, ∼100 ng/g, ∼220 nM) and displayed promising brain-to-plasma ratios of 1.4 and 4.4, respectively. Skepinone-L-derived inhibitors **43** and **44** were selective and metabolically stable and had low hERG affinity. Compound **70** (p38α, IC_{50} = 1 nM), the Skepinone-N counterpart of **43**, had the highest concentrations in the brain 2 h after p.o. application (580 nM) (brain-to-plasma ratio of 4.7).

Given its very good general attributes (selectivity, CYP, and hERG interaction), this very high partition to the CNS makes compound **70** a promising candidate for the study of the role of p38α MAP kinase in the development of CNS pathology in murine models of CNS degeneration. The combination of a high affinity for p38α MAP kinase and very high central exposure has not been obtained in either Neflamapimod (p38α, IC_{50} = 10 nM) or MW150 (p38α, IC_{50} = 230 nM). Reported brain exposure for Neflamapimod in the rat was approximately 130 to 360 nmol/kg at effective doses (1.5 to 4.5 mg/kg), corresponding to a 13- to 36-fold IC_{50} value for p38α MAP kinase. The data for MW150 were not directly reported (pharmacokinetics at 5 or 6 mg/kg in rat) but, as calculated in the Introduction, vary between two and three times the IC_{50} value for p38α MAP kinase at an effective dose of 2.5 mg/kg in the APP/PS1 mouse.

Compound **70** (1 nM) reached 580 times the IC_{50} value for p38α MAP kinase at 0.4 mg/kg in the C57BL/6 mouse brain, suggesting that it and its near analogs have adequate potency and physical properties to effectively modulate p38α MAP kinase activity in the brain.

https://doi.org/10.1021/acs.jmedchem.0c01773
J. Med. Chem. XXXX, XXX, XXX−XXX

EXPERIMENTAL PART

General. All reagents and solvents were commercially available and were used without further purification. ^{1}H and ^{13}C NMR spectra were obtained with a Bruker Avance 200 or Bruker Avance 400 apparatus. The spectra were obtained in the indicated solvent and calibrated against the residual proton peak of the deuterated solvent. Chemical shifts (δ) are reported in parts per million. Mass spectra were performed on an Advion Expression S electrospray ionization mass spectrometer (ESI-MS) with an Advion Plate Express (TLC interface) apparatus. TLC analysis was performed on fluorescent silica gel 60 F254 plates (Merck) and visualized under UV illumination at 254 and 366 nm. Column chromatography was performed using an Interchim PuriFlash 430 automated flash chromatography system. The purity of all compounds was, unless otherwise stated, >95% and was determined via reverse-phase high-performance liquid chromatography on a Hewlett-Packard HP 1090 series II LC equipped with a UV diode array detector (DAD, detection at 230 and 254 nm). The chromatographic separation was performed on a Phenomenex Luna 5u C8 column (150 mm × 4.6 mm, 5 μm) at a 35 °C oven temperature. The injection volume was 5 μL, and the gradient of the used method was as follows (flow, 1.5 mL/min), with 0.01 M KH_2PO_4, pH 2.3 (solvent A) and methanol (solvent B): from 40 to 85% B in 8 min, 85% B for 5 min, from 85 to 40% B in 1 min, 40% B for 2 min, stop time 16 min.

General Procedures. *General Procedure A.* The carboxylic ester was dissolved in methanol, and KOH (2.5 equiv) was added. The reaction mixture was heated to reflux temperature until completion of the reaction. After cooling to rt, the solvent was removed under reduced pressure, and the residue was taken up in ethyl acetate. The organic phase was washed with a diluted HCl solution and dried over Na_2SO_4, and the solvent was evaporated under reduced pressure, yielding a solid product. If the purity of the product was <95%, then the product was purified by flash chromatography (SiO_2, DCM/MeOH/formic acid 94:4:2).

General Procedure B. The carboxylic acid **5**, **53**, or **75** was dissolved in 5 mL of DMF under an argon atmosphere with gentle heating (50 °C). CDI (2 equiv) was added, and the reaction mixture was stirred for 2 h. The corresponding amine or alcohol was added to the reaction mixture, and it was stirred for 1 h at 50 °C. Then, the reaction was stopped by adding H_2O (10 mL) and extracted with either ethyl acetate or DCM (three times). The combined organic phases were dried over Na_2SO_4, filtered, and evaporated under reduced pressure.

General Procedure C. The Boc-protected compound was dissolved in dry DCM (5 mL), and TFA (1.5 mL) was added. The reaction mixture was stirred at rt until completion; then, a saturated aq. $NaHCO_3$ solution was carefully added. The reaction was extracted with DCM (three times). The organic extracts were collected, and the solvent was removed in vacuo. The product was purified by flash chromatography.

Methyl 8-((2,4-Difluorophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxylate (4). 2,4-Difluoroaniline (0.23 g, 1.79 mmol) was dissolved in 12 mL of 1,4-dioxane/*t*-BuOH (5:1, v/v) under an argon atmosphere. XPhos (0.025 equiv), Cs_2CO_3 (1.5 equiv), and $Pd(OAc)_2$ (0.05 equiv) followed by aryl chloride **3** (0.50 g, 1.79 mmol) were added, and the reaction mixture was heated for 1 h to 110 °C. The reaction was cooled to rt and filtered. The residue was washed several times with DCM, MeOH, and EtOAc. The combined organic extracts were evaporated, and the brown-black residue was purified by flash chromatography (SiO_2, petroleum ether/ethyl acetate 4:1) to yield **4** as a yellow solid (0.52 g, 89%).^{1}H NMR (400 MHz, DMSO-d_6) δ 8.66 (d, J = 1.8 Hz, 1H), 8.16 (d, J = 8.7 Hz, 1H), 8.06 (dd, J = 1.9 Hz, 8.0 Hz, 1H), 7.41−7.25 (m, 2H), 6.81−6.99 (m, 3H), 6.66 (d, J = 2.4 Hz, 1H), 6.00 (s, 1H), 3.92 (s, 3H), 3.23−3.06 (m, 4H). Analytical data are in agreement with the literature.[30]

8-((2,4-Difluorophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxylic Acid (5). Compound **5** was prepared according to General Procedure A starting from carboxylic ester **4** (1.00 g, 2.54 mmol). Yield: 0.96 g (99%), yellow solid. ^{1}H NMR (200 MHz, DMSO-d_6) 13.02 (br. s, 1H), 8.60 (s, 1H), 8.43 (d, J = 1.6 Hz, 1H), 7.95−8.03 (m, 2H), 7.30−7.49 (m, 3H), 7.04−7.16 (m, 1H), 6.75 (d, J = 7.5 Hz, 1H), 6.62 (s, 1H), 3.03−3.18 (m, 4H). Analytical data are in agreement with the literature.[30]

8-((2,4-Difluorophenyl)amino)-*N*,*N*-dimethyl-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (6). Compound **6** was prepared according to General Procedure B using **5** (0.050 g, 0.13 mmol) and dimethylamine hydrochloride (0.012 g, 0.14 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.041 g (77%), yellow solid. MS (ESI_{neg}) (m/z): 404.8 $[M - H]^-$. IR (ATR) [cm^{-1}] 3247, 2921, 2852, 1621, 1589, 1500, 1392, 1358, 1257, 1212, 1138, 965, 866, 601. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.61 (s, 1H), 8.00−7.97 (m, 1H), 7.86 (s, 1H), 7.51−7.50 (m, 1H), 7.48−7.34 (m, 3H), 7.13−7.09 (m, 1H), 6.75 (d, J = 8.7 Hz, 1H), 6.63 (s, 1H), 3.12−3.04 (m, 4H), 2.98−2.93 (m, 6H).

8-((2,4-Difluorophenyl)amino)-5-oxo-*N*-phenethyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (7). Compound **7** was prepared according to General Procedure B using **5** (0.075 g, 0.20 mmol) and 2-phenylethylamine (0.026 g, 0.22 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.043 g (45%), yellow solid. MS (ESI_{neg}) (m/z): 481.9 $[M - H]^-$. IR (ATR) [cm^{-1}] 3283, 2923, 1632, 1338, 1314, 1258, 1141, 965, 851, 599. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.69−8.64 (m, 1H), 8.58 (s, 1H), 8.33 (s, 1H), 8.00 (d, J = 9.1 Hz, 1H), 7.90 (d, J = 7.3 Hz, 1H), 7.41−7.36 (m, 2H), 7.29−7.18 (m, 6H), 7.15−7.06 (m, 2H), 6.76 (d, J = 8.4 Hz, 1H), 6.63 (s, 1H), 3.51−3.48 (m, 2H), 3.14−3.02 (m, 4H), 2.89−2.85 (m, 2H).

8-((2,4-Difluorophenyl)amino)-*N*-methyl-5-oxo-*N*-phenethyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (8). Compound **8** was prepared according to General Procedure B using **5** (0.040 g, 0.11 mmol) and *N*-methyl-2-phenylethylamine (0.029 g, 0.21 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.029 g (55%), yellow solid. MS (ESI_{neg}) (m/z): 495.1 $[M - H]^-$. IR (ATR) [cm^{-1}] 1602, 1579, 1500, 1397, 1354, 1258, 1214, 1139, 964, 842, 698. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60 (s, 1H), 8.00−7.97 (m, 1H), 7.43−7.36 (m, 3H), 7.27 (d, J = 22.0 Hz, 3H), 7.17−7.07 (m, 4H), 6.98−6.96 (m, 1H), 6.77−6.75 (m, 1H), 6.63 (s, 1H), 3.68−3.64 (m, 2H), 3.09−3.02 (m, 7H), 2.91−2.88 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.6, 170.5, 159.0 (dd, J = 11.2, 241.9 Hz), 156.2 (dd, J = 12.5, 246.7 Hz), 149.8, 145.7, 143.3, 139.9, 139.1, 135.3, 133.9, 129.2, 129.1, 128.8, 128.7, 127.7, 126.7, 126.6, 125.3 (dd, J = 3.3, 13.3 Hz), 114.0, 112.8, 112.3 (dd, J = 3.4, 21.8 Hz), 105.2 (dd, J = 26.0, 27.0 Hz), 52.7, 51.8, 48.9, 35.9, 34.3.

8-((2,4-Difluorophenyl)amino)-*N*-(4-fluorophenethyl)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (9). Compound **9** was prepared according to General Procedure B using **5** (0.075 g, 0.20 mmol) and 2-(4-fluorophenyl)-ethylamine (0.138 g, 0.99 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.037 g (39%), yellow solid. MS (ESI_{neg}) (m/z): 499.8 $[M - H]^-$. IR (ATR) [cm^{-1}] 3280, 2923, 2853, 1602, 1580, 1257, 1139, 1094, 964, 846, 783, 518. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.69−8.66 (m, 1H), 8.60 (s, 1H), 8.31 (s, 1H), 8.00 7.97 (m, 1H), 7.89 (d, J = 7.3 Hz, 1H), 7.43−7.30 (m, 4H), 7.13−7.00 (m, 4H), 6.76 (d, J = 8.3 Hz, 1H), 6.62 (s, 1H), 3.53−3.48 (m, 2H), 3.13−3.03 (m, 4H), 2.89−2.86 (m, 2H).

8-((2,4-Difluorophenyl)amino)-*N*-(4-hydroxyphenethyl)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (10). Compound **10** was prepared according to General Procedure B using **5** (0.050 g, 0.13 mmol) and tyramine hydrochloride (0.069 g, 0.40 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.018 g (28%), yellow solid. MS (ESI_{neg}) (m/z): 497.5 $[M - H]^-$. IR (ATR) [cm^{-1}] 3292, 2922, 2852, 1601, 1505, 1360, 1260, 1097, 967, 843, 540. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.20 (s, 1H), 8.66−8.62 (m, 1H), 8.60 (s, 1H), 8.32 (s, 1H), 8.01−7.98 (m, 1H), 7.90−7.88 (m, 1H), 7.44−7.33 (m, 3H), 7.12−7.10 (m, 1H), 7.02 (d, J = 7.7 Hz, 2H), 6.76 (d, J = 8.4 Hz, 1H), 6.69−6.66 (m, 2H), 6.62 (s, 1H), 3.53−3.48 (m, 2H, overlain by water peak), 3.12−3.03 (m, 4H), 2.74−2.70 (m, 2H).

https://doi.org/10.1021/acs.jmedchem.0c01773
J. Med. Chem. XXXX, XXX, XXX−XXX

***N*-(2-(1*H*-Imidazol-4-yl)ethyl)-8-((2,4-difluorophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (11).** Compound **11** was prepared according to General Procedure B using **5** (0.050 g, 0.13 mmol) and histamine dihydrochloride (0.049 g, 0.26 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.018 g (29%), yellow solid. MS (ESI_{neg}) (*m*/*z*): 471.5 $[M - H]^-$. IR (ATR) [cm^{-1}] 2921, 2851, 1603, 1579, 1505, 1354, 1258, 1094, 964, 801, 621. ^1H NMR (400 MHz, DMSO-d_6) δ 8.69−8.66 (m, 1H), 8.60 (s, 1H), 8.32 (s, 1H), 8.00−7.98 (m, 1H), 7.91−7.89 (m, 1H), 7.57 (s, 5H), 7.43−7.34 (m, 4H), 7.13−7.09 (m, 1H), 6.83 (s, 1H), 6.76 (d, *J* = 8.7 Hz, 1H), 6.62 (s, 1H), 3.49−3.46 (m, 2H, overlain by water peak), 3.12−3.03 (m, 4H), 2.78−2.74 (m, 2H).

8-((2,4-Difluorophenyl)amino)-5-oxo-*N*-(2-(thiophen-2-yl)ethyl)-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (12). Compound **12** was prepared according to General Procedure B using **5** (0.030 g, 0.08 mmol) and 2-(thiophen-2-)ethylamine (0.015 g, 0.12 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.021 g (53%), yellow solid. MS (ESI_{neg}) (*m*/*z*): 486.9 $[M - H]^-$. IR (ATR) [cm^{-1}] 1602, 1580, 1504, 1354, 1258, 1139, 964, 845, 696, 594. ^1H NMR (400 MHz, DMSO-d_6) δ 8.77−8.74 (m, 1H), 8.60 (s, 1H), 8.34 (d, *J* = 2.0 Hz, 1H), 8.00 (d, *J* = 8.8 Hz, 1H), 7.91 (dd, *J* = 7.8, 2.0 Hz, 1H), 7.42−7.32 (m, 4H), 7.13−7.09 (m, 1H), 6.96−6.91 (m, 2H), 6.77−6.74 (m, 1H), 6.62 (d, *J* = 1.4 Hz, 1H), 3.53−3.48 (m, 2H), 3.13−3.05 (m, 6H).

***N*-(2-(1*H*-Indol-3-yl)ethyl)-8-((2,4-difluorophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (13).** Compound **13** was prepared according to General Procedure B using **5** (0.050 g, 0.13 mmol) and tryptamine hydrochloride (0.106 g, 0.66 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.053 g (81%), yellow solid. MS (ESI_{neg}) (*m*/*z*): 520.7 $[M - H]^-$. IR (ATR) [cm^{-1}] 2916, 2850, 1633, 1567, 1486, 1259, 1092, 964, 741, 461. ^1H NMR (400 MHz, DMSO-d_6) δ 10.81 (s, 1H), 8.75−8.72 (m, 1H), 8.60 (s, 1H), 8.35 (s, 1H), 8.01−7.91 (m, 2H), 7.58 (d, *J* = 7.7 Hz, 1H), 7.46−7.31 (m, 4H), 7.17 (s, 1H), 7.13−7.03 (m, 2H), 7.00−6.96 (m, 1H), 6.76 (d, *J* = 8.5 Hz, 1H), 6.63 (s, 1H), 3.57−3.52 (m, 2H), 3.14−3.03 (m, 4H), 2.97−2.93 (m, 2H).

8-((2,4-Difluorophenyl)amino)-5-oxo-*N*-(3-phenylpropyl)-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (14). Compound **14** was prepared according to General Procedure B using **5** (0.075 g, 0.20 mmol) and 3-phenylpropylamine (0.134 g, 0.99 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.023 g (23%), yellow solid. MS (ESI_{neg}) (*m*/*z*): 495.9 $[M - H]^-$. IR (ATR) [cm^{-1}] 3315, 1581, 1603, 1527, 1504, 1262, 1141, 964, 854, 700, 631. ^1H NMR (400 MHz, DMSO-d_6) δ 8.59 (s, 2H), 8.34 (s, 1H), 8.02 (d, *J* = 8.5 Hz, 1H), 7.92 (d, *J* = 7.8 Hz, 1H), 7.45−7.35 (m, 3H), 7.30−7.21 (m, 4H), 7.19−7.15 (m, 1H), 7.13−7.09 (m, 1H), 6.76 (d, *J* = 8.7 Hz, 1H), 6.62 (s, 1H), 3.30−3.26 (m, 2H), 3.13−3.03 (m, 4H), 2.65−2.60 (m, 2H), 1.83 (quin, *J* = 7.1 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.5, 165.5, 149.3, 145.2, 144.5, 141.7, 138.9, 133.3, 133.0, 130.4, 129.1, 128.8, 128.3 (2× C), 128.2 (2× C), 127.2, 126.2 (dd, *J* = 2.5, 9.8 Hz), 125.7, 124.8 (dd, *J* = 2.9, 12.2 Hz), 113.5, 112.3, 111.8 (dd, *J* = 3.0, 21.8 Hz), 104.9 (dd, *J* = 24.0, 26.2 Hz), 38.9, 35.4, 33.7, 32.6, 30.8, (C2′-F and C4′-F not visible).

***N*-(2-(2-Oxa-6-azaspiro[3.3]heptan-6-yl)ethyl)-8-((2,4-difluorophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (15).** Compound **15** was prepared according to General Procedure B using **5** (0.060 g, 0.16 mmol) and 2-(2-oxa-6-azaspiro[3.3]heptan-6-yl)ethylamine (0.034 g, 0.24 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.063 g (79%), yellow solid. MS (ESI_{neg}) (*m*/*z*): 502.1 $[M - H]^-$. IR (ATR) [cm^{-1}] 3291, 2924, 2854, 1634, 1603, 1580, 1505, 1355, 1259, 1093, 963, 761, 520. ^1H NMR (400 MHz, $CDCl_3$) δ 8.31 (d, *J* = 1.3 Hz, 1H), 8.16 (d, *J* = 8.7 Hz, 1H), 7.97 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.39−7.32 (m, 1H), 7.29 (d, *J* = 7.9 Hz, 1H), 6.96−6.83 (m, 3H), 6.77−6.74 (m, 1H), 6.66−6.65 (m, 1H), 5.96 (s, 1H), 4.74 (s, 4H), 3.43−3.39 (m, 6H), 3.19−3.16 (m, 2H), 3.11−3.09 (m, 2H), 2.62 (t, *J* = 5.6 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.5, 166.7, 158.8 (dd, *J* = 11.5, 244.8 Hz), 155.3 (dd, *J* = 12.8, 248.3 Hz), 148.1, 145.3, 145.1, 138.9, 134.2, 133.0, 131.4, 129.4, 129.3, 128.6, 124.8 (dd, 3.9, 11.9 Hz), 123.9 (dd, *J* = 2.5, 9.3 Hz), 114.6, 113.3, 111.4 (dd, *J* = 3.7, 22.1 Hz), 104.8 (dd, *J* = 23.6, 26.2 Hz), 81.2 (2× C), 63.7 (2× C), 57.9, 39.2, 37.7, 35.8, 34.7.

8-((2,4-Difluorophenyl)amino)-5-oxo-*N*-(1-phenylcyclopropyl)-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (16). Compound **16** was prepared according to General Procedure B using **5** (0.030 g, 0.08 mmol) and 1-phenyl-1-cyclopropylamine (0.017 g, 0.10 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.017 g (44%), yellow solid. MS (ESI_{neg}) (*m*/*z*): 493.0 $[M - H]^-$. IR (ATR) [cm^{-1}] 3268, 2922, 2850, 1632, 1603, 1581, 1505, 1353, 1257, 1139, 964, 844, 598. ^1H NMR (400 MHz, $CDCl_3$) δ 8.34 (s, 1H), 8.11 (d, *J* = 8.6 Hz, 1H), 7.98 (d, *J* = 7.4 Hz, 1H), 7.36−7.22 (m, 6H + solvent peak), 7.16−7.14 (m, 2H), 6.93−6.87 (m, 2H), 6.79 (d, *J* = 8.5 Hz, 1H), 6.63 (s, 1H), 6.00 (s, 1H), 3.15−3.13 (m, 2H), 3.07−3.04 (m, 2H), 1.37−1.30 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.4, 166.6, 158.8 (dd, *J* = 10.1, 244.5 Hz), 155.4 (dd, *J* = 12.2, 246.3 Hz), 148.2, 145.4, 145.3, 142.3, 138.8, 134.2, 132.9, 131.6, 129.6, 129.2, 128.4 (2× C), 128.3, 126.4, 125.7 (2× C), 124.8 (dd, *J* = 3.8, 11.8 Hz), 124.0 (dd, *J* = 2.3 Hz, 9.4 Hz), 114.6, 113.3, 111.4 (dd, *J* = 3.9, 22.1 Hz), 104.8 (dd, *J* = 23.5, 26.1 Hz), 35.8, 35.4, 34.7, 17.8 (2× C).

***N*-(1-Cyclohexylethyl)-8-((2,4-difluorophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (17).** Compound **17** was prepared according to General Procedure B using **5** (0.030 g, 0.08 mmol) and 1-cyclohexylethylamine (0.012 g, 0.08 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.012 g (31%), yellow solid. MS (ESI_{neg}) (*m*/*z*): 487.1 $[M - H]^-$. IR (ATR) [cm^{-1}] 3268, 2922, 2850, 2361, 1633, 1603, 1581, 1505, 1257, 1139, 1094, 964, 598. ^1H NMR (400 MHz, $CDCl_3$) δ 8.24 (s, 1H), 8.11 (d, *J* = 8.2 Hz, 1H), 7.94 (d, *J* = 6.6 Hz, 1H), 7.32−7.24 (m, 2H), 6.92−6.79 (m, 3H), 6.62 (s, 1H), 6.13 (d, *J* = 8.1 Hz, 1H), 6.07 (s, 1H), 4.07−3.99 (m, 1H), 3.14−3.12 (m, 2H), 3.06−3.04 (m, 2H), 1.77−1.62 (m, 3H), 1.21−1.10 (m, 8H), 1.05−0.96 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.5, 165.9, 160.0 (dd, *J* = 10.9, 244.7 Hz), 155.4 (dd, *J* = 12.3, 246.5 Hz), 148.2, 145.4, 144.9, 138.9, 134.2, 133.5, 131.5, 129.4, 129.1, 128.0, 124.9 (dd, *J* = 3.9, 11.5 Hz), 124.0 (dd, *J* = 2.3 Hz, 9.4 Hz), 114.6, 113.2, 111.5 (dd, *J* = 3.7, 22.1 Hz), 104.8 (dd, *J* = 23.7, 26.0 Hz), 50.0, 43.3, 38.6, 35.9, 34.7, 29.3, 29.2, 26.4, 26.2, 18.0.

***N*-(2-Aminoethyl)-8-((2,4-difluorophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (18).** Compound **18** was prepared according to General Procedure B using **5** (0.040 g, 0.11 mmol) and ethylene-1,2-diamine (0.015 g, 0.26 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.027 g (61%), yellow solid. MS (ESI_{neg}) (*m*/*z*): 420.0 $[M - H]^-$. IR (ATR) [cm^{-1}] 1602, 1577, 1504, 1354, 1258, 1214, 1114, 1094, 964, 844, 516. ^1H NMR (400 MHz, DMSO-d_6) δ 8.61 (s, 1H), 8.56−8.53 (m, 1H), 8.34−8.33 (m, 1H), 8.00 (d, *J* = 8.8 Hz, 1H), 7.92 (dd, *J* = 1.5, 7.8 Hz, 1H), 7.45−7.34 (m, 3H), 7.13−7.09 (m, 1H), 6.76 (d, *J* = 8.5 Hz, 1H), 6.63 (s, 1H), 3.38−3.16 (m, 2H + 2H, overlain by water), 3.12−3.03 (m, 4H), 2.69 (t, *J* = 6.2 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.5, 165.7, 149.3, 145.2, 144.5, 138.8, 133.3, 132.9, 130.4, 129.2, 128.8, 127.2, 126.2 (dd, *J* = 2.6, 9.2 Hz), 124.8 (dd, *J* = 2.6, 11.5 Hz), 113.5, 112.3, 111.8 (dd, *J* = 3.7, 21.9 Hz), 104.9 (dd, *J* = 24.0, 26.2 Hz), 42.8, 41.1, 35.4, 33.8, (C2′-F and C4′-F not visible).

***N*-(3-Amino-3-oxopropyl)-8-((2,4-difluorophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (19).** Compound **19** was prepared according to General Procedure B using **5** (0.040 g, 0.11 mmol) and 3-aminopropanamide (0.035 g, 0.28 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.042 g (82%), yellow solid. $C_{25}H_{21}F_2N_3O_3$ (*M* = 449.16 g/mol). MS (ESI_{neg}) (*m*/*z*): 448.2 $[M - H]^-$. IR (ATR) [cm^{-1}] 1654, 1602, 1544, 1504, 1361, 1258, 1113, 968, 847, 569, 470. ^1H NMR (400 MHz, DMSO-d_6) δ 8.64−8.60 (m, 2H), 8.32 (s, 2H), 7.99 (d, *J* = 8.8 Hz, 1H), 7.90 (dd, *J* = 1.4, 7.7 Hz, 1H), 7.45−7.35 (m, 4H), 7.13−7.09 (t, *J* = 8.2 Hz, 1H), 6.84 (s, 1H), 6.76 (d, *J* = 8.7 Hz, 1H), 6.62 (s, 1H), 3.50−3.45 (m, 2H, overlain by water), 3.13−3.10 (m, 2H), 3.05−3.03 (m, 2H), 2.36 (t, *J* = 7.1 Hz,

https://doi.org/10.1021/acs.jmedchem.0c01773
J. Med. Chem. XXXX, XXX, XXX−XXX

2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.5, 172.6, 165.5, 149.3, 145.2, 144.6, 138.8, 133.3, 132.8, 130.3, 129.1, 128.9, 127.2, 126.2 (dd, J = 2.9, 10.0 Hz), 124.8 (dd, J = 2.5, 11.8 Hz), 113.5, 112.3, 111.8 (dd, J = 3.6, 22.1 Hz), 104.9 (dd, J = 24.1, 26.8 Hz), 36.0, 35.4, 34.9, 33.8 (C2′-F and C4′-F not visible).

8-((2,4-Difluorophenyl)amino)-5-oxo-*N*-(2,2,2-trifluoroethyl)-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (20). Compound **20** was prepared according to General Procedure B using **5** (0.075 g, 0.20 mmol) and 2,2,2-trifluoroethylamine (0.120 g, 0.99 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.057 g (63%), yellow solid. MS (ESI_{neg}) (m/z): 459.8 $[M - H]^-$. IR (ATR) [cm^{-1}] 1668, 1604, 1539, 1355, 1243, 1151, 964, 833, 729. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.22−9.20 (m, 1H), 8.60 (s, 1H), 8.39 (s, 1H), 8.01−7.96 (m, 2H), 7.46−7.34 (m, 3H), 7.12−7.08 (m, 1H), 6.78 (s, 1H), 6.63 (s, 1H), 4.14−4.04 (m, 2H), 3.15−3.12 (m, 2H), 3.06−3.04 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.6, 166.3, 159.8 (dd, J = 11.1, 243.6 Hz), 154.5 (dd, J = 12.6, 248.6 Hz), 149.4, 145.4, 145.3, 139.0, 133.4, 131.5, 130.6, 129.4, 129.1, 127.1, 126.3 (dd) 124.9 (dd), 123.4, 113.5, 112.3, 111.8 (dd, J = 3.6, 22.1 Hz), 104.9 (dd J = 24.4, 26.3 Hz), 40.2 (q, J = 33.7 Hz), 35.3, 33.8.

8-((2,4-Difluorophenyl)amino)-*N*-octyl-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (21). Compound **21** was prepared according to General Procedure B using **5** (0.075 g, 0.20 mmol) and octylamine (0.240 g, 1.98 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.024 g (25%), yellow solid. MS (ESI_{neg}) (m/z): 489.9 $[M - H]^-$. IR (ATR) [cm^{-1}] 3274, 2923, 2853, 1603, 1505, 1257, 1139, 1094, 964, 845, 597. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58−8.54 (m, 2H), 8.33 (s, 1H), 8.01−7.90 (m, 2H), 7.42−7. 32 (m, 3H), 7.11−7.07 (m, 1H), 6.76 (d, J = 8.0 Hz, 1H), 6.63 (s, 1H), 3.27−3.21 (m, 2H), 3.12−3.02 (m, 4H), 1.50 (br. s, 2H), 1.25−1.23 (s, 10H), 0.83 (br. s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.4, 165.4, 158.4 (dd, J = 11.3, 240.9 Hz), 155.7 (dd, J = 12.6, 246.6 Hz), 149.2, 145.2, 144.4, 138.9, 133.3, 130.3, 133.0, 129.1, 128.8, 127.2, 126.2 (dd, J = 2.7, 9.6 Hz), 124.8 (dd, J = 3.2, 12.1 Hz), 113.5, 112.3, 111.8 (dd, J = 3.4, 21.7 Hz), 105.0 (dd, J = 24.0, 25.9 Hz), 39.2, 35.4, 33.8, 31.1, 29.0, 28.7, 28.6, 26.4, 22.0, 13.8.

8-((2,4-Difluorophenyl)amino)-*N*-hexadecyl-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (22). Compound **22** was prepared according to General Procedure B using **5** (0.075 g, 0.20 mmol) and hexadecylamine (0.955 g, 3.95 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.055 g (46%), yellow solid. MS (ESI_{neg}) (m/z): 602.1 $[M - H]^-$. IR (ATR) [cm^{-1}] 3345, 2919, 2847, 1631, 1604, 1495, 1358, 1265, 963, 858, 624. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (br. s, 1H), 8.16 (d, J = 8.8 Hz, 1H), 8.01−7.97 (m, 1H), 7.40−7.30 (m, 2H), 6.98−6.85 (m, 3H), 6.69−6.68 (m, 1H), 6.30 (br. s, 1H), 5.81 (br. s, 1H), 3.48−3.43 (m, 2H), 3.21−3.18 (m, 2H), 3.13−3.11 (m, 2H), 1.65−1.58 (m, 2H), 1.37−1.26 (m, 26H), 0.88 (d, J = 6.9 Hz, 3H).

***tert*-Butyl 4-(2-(8-((2,4-Difluorophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamido)ethyl)-piperazine-1-carboxylate (23).** Compound **23** was prepared according to General Procedure B using **5** (0.125 g, 0.33 mmol) and *tert*-butyl 4-(2-aminoethyl)piperazine-1-carboxylate (0.378 g, 1.65 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.094 g (49%), yellow solid. MS (ESI_{neg}) (m/z): 598.8 $[M - H]^-$. IR (ATR) [cm^{-1}] 1634, 1581, 1505, 1354, 1259, 1165, 1002, 964, 845, 517. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60 (s, 1H), 8.54 (s, 1H), 8.32 (s, 1H), 8.00 (d, J = 8.7 Hz, 1H), 7.90 (d, J = 7.7 Hz, 1H), 7.49−7.31 (m, 3H), 7.11 (t, J = 7.8 Hz, 1H), 6.76 (d, J = 8.7 Hz, 1H), 6.62 (s, 1H), 3.42−3.40 (m, 2H), 3.31−3.28 (s, 4H), 3.13−3.10 (m, 2H), 3.05−3.03 (s, 2H), 2.50−2.48 (m, 2H), 2.40−2.37 (m, 4H), 1.39 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.4, 165.5, 153.8, 149.3, 145.2, 144.5, 138.9, 133.3, 132.8, 130.3, 129.1, 128.9, 127.2, 126.2 (J = 3.3, 9.6 Hz), 124.8 (dd, J = 3.8, 12.2 Hz), 113.5, 112.3, 111.8 (dd, J = 3.8, 22.0 Hz), 104.9 (dd, J = 24.3, 26.3 Hz), 78.7, 56.8, 52.4 (2× C), 36.7 (2× C), 35.4, 33.8, 28.0 (3× C), (C2′-F and C4′-F not visible).

2-((2,4-Difluorophenyl)amino)-7-(piperidine-1-carbonyl)-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulen-5-one (24). Compound **24** was prepared according to General Procedure B using **5** (0.011 g, 0.03 mmol) and piperidine (0.018 g, 0.07 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.007 g (40%), yellow solid. MS (ESI_{neg}) (m/z): 445.1 $[M - H]^-$. IR (ATR) [cm^{-1}] 2922, 2852, 2359, 2341, 1602, 1579, 1506, 1437, 1353, 1255, 1093, 799. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60 (s, 1H), 7.98 (d, J = 8.8 Hz, 1H), 7.82 (s, 1H), 7.47−7.35 (m, 4H), 7.12−7.09 (m, 1H), 6.75 (d, J = 8.5 Hz, 1H), 6.62 (s, 1H), 3.73−3.55 (m, 4H), 3.11−3.06 (m, 4H), 1.60−1.45 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.1, 168.3, 149.3, 145.3, 142.9, 138.6, 134.6, 133.5, 130.1, 129.0, 128.5, 127.1, 126.2 (J = 2.6, 9.8 Hz), 124.8 (dd, J = 3.3, 12.0 Hz), 113.5, 112.2, 111.8 (dd, J = 3.8, 21.7 Hz), 104.9 (dd, J = 24.2, 27.4 Hz), 48.0 (2× C), 35.4, 33.2, 25.9 (2× C), 24.0, (C2′-F and C4′-F not visible).

8-((2,4-Difluorophenyl)amino)-5-oxo-*N*-phenyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (25). Compound **25** was prepared according to General Procedure B using **5** (0.043 g, 0.11 mmol) and aniline (0.026 g, 0.28 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.050 g (97%), yellow solid. MS (ESI_{neg}) (m/z): 453.0 $[M - H]^-$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.35 (s, 1H), 8.62 (s, 1H), 8.44 (d, J = 1.6 Hz, 1H), 8.05−8.00 (m, 2H), 7.77 (d, J = 7.8 Hz, 2H), 7.49−7.33 (m, 5H), 7.14−7.09 (m, 2H), 6.77 (dd, J = 1.3, 8.8 Hz, 1H), 6.64 (s, 1H), 3.17−3.15 (m, 2H), 3.08−3.06 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.4, 164.8, 149.4, 145.3, 145.1, 139.1, 138.9, 133.4, 133.2, 130.8, 129.6, 129.0, 128.6 (2× C), 127.1, 126.3 (dd, J = 2.6, 10.0 Hz), 124.8 (dd, J = 3.3, 12.4 Hz), 123.7, 120.4 (2× C), 113.5, 112.3, 111.9 (dd, J = 3.7, 21.8 Hz), 105.0 (dd, J = 24.5, 26.3 Hz), 35.4, 33.8, (C2′-F and C4′-F not visible).

8-((2,4-Difluorophenyl)amino)-*N*-hydroxy-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (26). Compound **26** was prepared according to General Procedure B using **5** (0.040 g, 0.11 mmol) and hydroxylamine hydrochloride (0.009 g, 0.13 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.034 g (77%), yellow solid. MS (ESI_{neg}) (m/z): 392.5 $[M - H]^-$. IR (ATR) [cm^{-1}] 2920, 2851, 1602, 1505, 1354, 1258, 1093, 1028, 839, 596, 451. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.30 (s, 1H), 9.07 (s, 1H), 8.60 (s, 1H), 8.25 (s, 1H), 7.99 (d, J = 8.7 Hz, 1H), 7.82 (d, J = 7.7 Hz, 1H), 7.45−7.35 (m, 3H), 7.13−7.09 (m, 1H), 6.76 (d, J = 8.4 Hz, 1H), 6.62 (s, 1H), 3.12−3.02 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.7, 163.6, 160.4, 158.5 (dd, J = 11.0, 244.2 Hz), 155.7 (dd, J = 11.7, 248.0 Hz), 149.8, 145.2, 144.6, 138.9, 133.4, 131.4, 129.9, 129.0, 127.1, 126.1, 124.7, 113.5, 112.3, 111.8 (dd, J = 3.3, 21.9 Hz), 104.9 (dd, J = 22.1, 26.5 Hz), 35.3, 33.8.

8-((2,4-Difluorophenyl)amino)-*N*-(1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (27). Compound **27** was prepared according to General Procedure B using **5** (0.080 g, 0.21 mmol) and tris(hydroxymethyl)aminomethane hydrochloride (0.083 g, 0.53 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.023 g (23%), yellow solid. MS (ESI_{neg}) (m/z): 480.9 $[M - H]^-$. IR (ATR) [cm^{-1}] 3300, 1602, 1580, 1505, 1403, 1354, 1258, 1214, 1042, 846, 595. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60 (s, 1H), 8.27 (d, J = 1.5 Hz, 1H), 8.00 (d, J = 8.8 Hz, 1H), 7.88 (dd, J = 7.8, 1.6 Hz, 1H), 7.45−7.35 (m, 4H), 7.13−7.08 (m, 1H), 6.76 (d, J = 8.8 Hz, 1H), 6.62 (s, 1H), 4.77 (t, J = 5.7 Hz, 3H), 3.70 (d, J = 5.7 Hz, 6H), 3.13−3.11 (m, 2H), 3.05−3.03 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.4, 166.6, 158.5 (dd, J = 11.5, 241.8 Hz), 155.7 (dd, J = 12.2, 246.4 Hz), 149.3, 145.3, 144.6, 138.8, 133.5, 133.4, 130.6, 129.2, 128.7, 127.1, 126.2 (dd, J = 3.2, 9.6 Hz), 124.8 (dd, J = 3.1, 12.2 Hz), 113.5, 112.3, 111.8 (dd, J = 3.3, 21.9 Hz), 104.9 (dd, J = 24.3, 26.3 Hz), 62.7, 60.5, 35.4, 33.8.

8-((2,4-Difluorophenyl)amino)-5-oxo-*N*-((2*R*,3*R*,4*R*,5*S*,6*R*)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3-yl)-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (28). Compound **28** was prepared according to General Procedure B using **5** (0.060 g, 0.16 mmol) and D-glucosamine hydrochloride (0.038 g, 0.17 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.048 g (56%), yellow

https://doi.org/10.1021/acs.jmedchem.0c01773
J. Med. Chem. XXXX, XXX, XXX−XXX

solid. MS (ESI_{neg}) (m/z): 538.7 $[M - H]^-$. IR (ATR) [cm^{-1}] 3300, 1633, 1603, 1505, 1355, 1259, 1094, 1027, 965, 845, 516. ^1H NMR (400 MHz, DMSO-d_6) δ 8.59 (s,1H), 8.37−8.36 (m, 1H), 8.32 (d, J = 8.8 Hz, 0.3H), 8.20 (d, J = 7.1 Hz, 0.7H), 8.01−7.93 (m, 2H), 7.45−7.34 (m, 3H), 7.13−7.08 (m, 1H), 6.76 (d, J = 8.8 Hz, 1H), 6.63 (s, 1H), 6.58 (d, J = 6.2 Hz, 0.3H), 6.48 (d, J = 4.2 Hz, 0.7H), 5.10−5.08 (s, 0.7H), 4.99−4.97 (m, 1H), 4.93 (d, J = 5.1 Hz, 0.3H), 4.72 (d, J = 4.6 Hz, 0.7H), 4.64−4.56 (m, 0.6H), 4.50−4.47 (m, 0.7H), 3.81−3.63 (m, 3H), 3.54−3.48 (m, 1H), 3.22−3.17 (m, 1H), 3.12−3.10 (m, 2H), 3.05−3.03 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.6, 165.9, 158.5 (dd, J = 11.3, 241.9 Hz), 155.7 (dd, J = 12.6, 246.8 Hz), 149.3, 145.2, 144.5, 138.9, 133.3, 132.8, 130.7, 129.4, 128.7, 127.2, 126.2 (dd, J = 3.0, 9.7 Hz), 124.9 (dd, J = 3.6, 12.1 Hz), 113.6, 112.3, 111.8 (dd, J = 3.3, 21.9 Hz), 104.9 (dd, J = 24.1, 26.2 Hz), 90.5, 72.1, 71.1, 70.0, 61.2, 55.5, 35.5, 33.8.

8-((2,4-Difluorophenyl)amino)-*N*-(2-(2-hydroxyethoxy)-ethyl)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (29). Compound **29** was prepared according to General Procedure B using **5** (0.040 g, 0.11 mmol) and 2-(2-aminoethoxy)ethanol (0.027 g, 0.26 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.017 g (34%), yellow solid. MS (ESI_{neg}) (m/z): 464.9 $[M - H]^-$. IR (ATR) [cm^{-1}] 2950, 1651, 1601, 1578, 1506, 1348, 1261, 1211, 1133, 1095, 966, 844, 784. ^1H NMR (400 MHz, DMSO-d_6) δ 8.64−8.60 (m, 2H), 8.34 (d, J = 1.5 Hz, 1H), 8.00 (d, J = 8.8 Hz, 1H), 7.92 (dd, J = 7.9, 1.6 Hz, 1H), 7.45−7.34 (m, 3H), 7.13−7.09 (m, 1H), 6.76 (d, J = 1.3, 8.9 Hz, 1H), 6.63 (s, 1H), 4.63 (t, J = 5.3 Hz, 1H), 3.55−3.52 (m, 2H), 3.50−3.48 (m, 2H), 3.45−3.41 (m, 4H), 3.12−3.10 (m, 2H), 3.05−3.03 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.4, 165.7, 158.5 (dd, J = 11.3, 241.8 Hz), 156.7 (dd, J = 12.6, 245.4 Hz), 149.3, 145.3, 144.6, 138.9, 133.3, 132.7, 130.4, 129.2, 128.9, 127.1, 126.2 (dd, J = 3.0, 9.8 Hz), 124.8 (dd, J = 3.5, 12.2 Hz), 113.5, 112.3, 111.8 (dd, J = 3.4, 21.9 Hz), 104.9 (dd, J = 24.5, 26.6 Hz), 72.1, 68.8, 60.2, 38.2, 35.4, 33.8.

8-((2,4-Difluorophenyl)amino)-*N*-(2-((2-hydroxyethyl)-amino)ethyl)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]-annulene-3-carboxamide (30). Compound **30** was prepared according to General Procedure B using **5** (0.040 g, 0.11 mmol) and 2-((2-aminoethyl)amino)ethanol (0.027 g, 0.26 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.025 g (51%), yellow solid. MS (ESI_{neg}) (m/z): 463.9 $[M - H]^-$. IR (ATR) [cm^{-1}] 1601, 1557, 1524, 1439, 1354, 1257, 1140, 848, 754, 687. ^1H NMR (400 MHz, DMSO-d_6) δ 8.61 (s, 1H), 8.57 (t, J = 5.3 Hz, 1H), 8.34 (d, J = 1.3 Hz, 1H), 8.00 (d, J = 8.8 Hz, 1H), 7.92 (dd, J = 7.8, 1.7 Hz, 1H), 7.45−7.34 (m, 3H), 7.13−7.08 (m, 1H), 6.76 (dd, J = 8.8, 1.4 Hz, 1H), 6.63 (s, 1H), 4.55 (br. s, 1H), 3.46 (t, J = 5.7 Hz, 2H), 3.39−3.35 (m, 2H, overlain by water), 3.12−3.10 (m, 2H), 3.05−3.03 (m, 2H), 2.73 (t, J = 6.4 Hz, 2H), 2.64 (t, J = 5.7 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.5, 165.6, 158.5 (dd, J = 11.1, 241.9 Hz), 155.7 (dd, J = 12.5, 246.5 Hz), 149.3, 145.2, 144.5, 138.9, 133.3, 132.9, 130.4, 129.2, 128.8, 127.2, 126.2 (dd, J = 2.9, 9.6 Hz) 124.8 (dd, J = 3.6, 12.1 Hz), 113.5, 112.3, 111.8 (dd, J = 3.4, 22.1 Hz), 104.9 (dd, J = 24.4, 26.5 Hz), 60.1, 51.2, 48.3, 35.4, 33.8, 30.6.

Methyl (8-((2,4-Difluorophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carbonyl)glycinate (31). Compound **31** was prepared according to General Procedure B using **5** (0.060 g, 0.16 mmol) and glycine methyl ester hydrochloride (0.024 g, 0.19 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.041 g (58%), yellow solid. MS (ESI_{neg}) (m/z): 448.8 $[M - H]^-$. IR (ATR) [cm^{-1}] 3383, 3278, 1758, 1650, 1606, 1575, 1258, 1195, 962, 760, 512. ^1H NMR (400 MHz, $CDCl_3$) δ 8.32−8.30 (s, 1H), 8.05−8.03 (d, J = 7.5 Hz, 1H), 7.87−7.85 (m, 1H), 7.34−7.28 (m, 2H), 6.87−6.84 (m, 2H), 6.76−6.74 (m, 1H), 6.57 (s, 1H), 4.11 (s, 2H), 3.70 (s, 3H), 3.20 (s, 1H), 3.10−3.04 (m, 4H).

Methyl (8-((2,4-Difluorophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carbonyl)-L-alaninate (32). Compound **32** was prepared according to General Procedure B using **5** (0.050 g, 0.13 mmol) and L-alanine methyl ester hydrochloride (0.092 g, 0.66 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.023 g (38%), yellow solid. MS (ESI_{neg}) (m/z): 463.6 $[M - H]^-$. IR (ATR) [cm^{-1}] 3304, 2921, 2852, 1738, 1634, 1603, 1505, 1454, 1354, 1172, 1212, 1140, 845. ^1H NMR (400 MHz, DMSO-d_6) δ 8.94 (d, J = 6.7 Hz, 1H), 8.61 (s, 1H), 8.39 (s, 1H), 8.02 − −8.00 (m, 1H), 7.97−7.94 (m, 1H), 7.43−7.35 (m, 3H), 7.13−7.09 (m, 1H), 6.77 (d, J = 8.7 Hz, 1H), 6.63 (s, 1H), 4.53−4.46 (m, 1H), 3.65 (s, 3H), 3.14−3.12 (m, 2H), 3.06−3.04 (m, 2H), 1.41 (d, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.4, 173.1, 165.6, 149.3, 145.2, 144.9, 139.0, 133.3, 132.0, 130.6, 129.4, 128.9, 127.1, 126.2 (dd, J = 3.4, 9.4 Hz), 124.9 (dd, J = 3.2, 12.1 Hz), 113.5, 112.3, 111.8 (dd, J = 3.7, 22.1 Hz), 104.9 (dd, J = 24.7, 26.5 Hz), 51.8, 48.3, 35.4, 33.8, 16.7, (C2′-F and C4′-F not visible).

Methyl 3-(8-((2,4-Difluorophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamido)propanoate (33). Compound **33** was prepared according to General Procedure B using **5** (0.060 g, 0.16 mmol) and methyl 3-aminopropanoate hydrochloride (0.024 g, 0.17 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.075 g (95%), yellow solid. MS (ESI_{neg}) (m/z): 462.8 $[M - H]^-$. IR (ATR) [cm^{-1}] 1717, 1602, 1581, 1525, 1434, 1244, 1116, 915, 842, 718, 508. ^1H NMR (400 MHz, DMSO-d_6) δ 8.69−8.66 (s, 1H), 8.60 (s, 1H), 8.31 (s, 1H), 8.01−7.98 (m, 1H), 7.91−7.89 (m, 1H), 7.45−7.35 (m, 3H), 7.13−7.08 (m, 1H), 6.76 (d, J = 8.7 Hz, 1H), 6.62 (s, 1H), 3.61−3.60 (m, 3H), 3.51−3.46 (m, 2H), 3.12−3.10 (m, 2H), 3.05−3.03 (m, 2H), 2.62−2.55 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.4, 171.7, 165.6, 149.3, 145.2, 144.7, 138.8, 133.3, 132.6, 130.4, 129.1, 128.9, 127.1, 126.2 (dd, J = 3.0, 9.5 Hz), 124.8 (dd, J = 3.7, 12.0 Hz), 113.5, 112.3, 111.8 (dd, J = 3.6, 22.1 Hz), 104.9 (dd, J = 24.2, 26.7 Hz), 51.3, 35.5, 35.4, 33.8, 33.5, (C2′-F and C4′-F not visible).

Methyl 4-(8-((2,4-Difluorophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamido)butanoate (34). Compound **34** was prepared according to General Procedure B using **5** (0.040 g, 0.11 mmol) and methyl 4-aminobutanoate hydrochloride (0.027 g, 0.17 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.070 g (86%), yellow solid. MS (ESI_{neg}) (m/z): 476.8 $[M - H]^-$. IR (ATR) [cm^{-1}] 3290, 1728, 1601, 1505, 1437, 1357, 1261, 1097, 967, 850, 528. ^1H NMR (400 MHz, $CDCl_3$) δ 8.31 (d, J = 1.2 Hz, 1H), 8.10 (d, J = 8.7 Hz, 1H), 7.94−7.92 (m, 1H), 7.36−7.31 (m, 1H), 7.27−7.25 (m, 1H + solvent peak), 6.93−6.76 (m, 4H), 6.64 (s, 1H), 6.11 (br. s, 1H), 3.66 (s, 3H), 3.51−3.46 (m, 2H), 3.15−3.13 (m, 2H), 3.07−3.05 (m, 2H), 2.42 (t, J = 7.1 Hz, 2H), 1.95 (quin, J = 6.9 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.3, 173.9, 166.8, 158.8 (dd, J = 10.7, 244.5 Hz), 155.4 (dd, J = 11.5, 246.2 Hz), 148.1, 145.2, 145.1, 138.9, 134.2, 133.0, 131.2, 129.4, 129.2, 128.5, 124.9 (dd, J = 3.1, 11.4 Hz), 124.0 (dd, J = 2.3, 9.4 Hz), 114.6, 113.3, 111.d (dd, J = 3.7, 22.0 Hz), 104.8 (dd, J = 23.7, 26.2 Hz), 51.7, 39.6, 35.8, 34.7, 31.6, 24.6.

Methyl (8-((2,4-Difluorophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carbonyl)-L-phenylalaninate (35). Compound **35** was prepared according to General Procedure B using **5** (0.080 g, 0.21 mmol) and L-phenylalanine methyl ester hydrochloride (0.068 g, 0.32 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.103 g (90%), yellow solid. MS (ESI_{neg}) (m/z): 538.9 $[M - H]^-$. IR (ATR) [cm^{-1}] 2157, 2006, 1738, 1651, 1603, 1581, 1258, 1434, 1177, 1118, 1140, 699. ^1H NMR (400 MHz, DMSO-d_6) δ 9.00 (d, J = 7.5 Hz, 1H), 8.61 (s, 1H), 8.33 (s, 1H), 8.01 (d, J = 8.1 Hz, 1H), 7.88−7.86 (m, 1H), 7.45−7.34 (m, 3H), 7.30−7.17 (m, 5H), 7.13−7.09 (m, 1H), 6.76 (d, J = 8.7 Hz, 1H), 6.62 (s, 1H), 4.71−4.66 (m, 1H), 3.64 (s, 3H), 3.20−3.10 (m, 4H), 3.05−3.03 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.3, 172.1, 165.7, 158.5 (dd, J = 11.6, 243.5 Hz), 155.7 (dd, J = 12.6, 248.5 Hz), 149.3, 145.8, 145.0, 139.0, 137.6, 133.3, 131.9, 130.5, 129.3, 129.0 (2× C), 128.9, 128.2 (2× C), 127.1, 126.4, 126.2 (dd, J = 3.0, 9.6 Hz), 124.8 (dd, J = 3.1, 11.9 Hz), 113.5, 112.3, 111.8 (dd, J = 3.4, 22.1 Hz), 104.9 (dd, J = 24.4, 26.5 Hz), 54.2, 51.9, 36.2, 35.3, 34.7.

Dimethyl (8-((2,4-Difluorophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carbonyl)-L-glutamate (36). Compound **36** was prepared according to General Procedure B using **5** (0.080 g, 0.21 mmol) and dimethyl L-glutamate hydrochloride

https://doi.org/10.1021/acs.jmedchem.0c01773
J. Med. Chem. XXXX, XXX, XXX−XXX

(0.049 g, 0.23 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.110 g (97%), yellow solid. MS (ESI_{neg}) (m/z): 534.7 $[M - H]^-$. IR (ATR) [cm^{-1}] 1732, 1633, 1603, 1505, 1435, 1354, 1258, 1211, 1094, 964, 846. ^1H NMR (400 MHz, DMSO-d_6) δ 8.90 (d, J = 7.4 Hz, 1H), 8.61 (s, 1H), 8.37 (d, J = 1.8 Hz, 1H), 8.00 (d, J = 8.8 Hz, 1H), 7.96−7.93 (m, 1H), 7.47−7.36 (m, 3H), 7.13−7.09 (m, 1H), 6.76 (dd, J = 8.8, 1.6 Hz, 1H), 6.63 (d, J = 1.6 Hz, 1H), 4.50−4.45 (m, 1H), 3.64 (s, 3H), 3.58 (s, 3H), 3.14−3.12 (m, 2H), 3.06−3.04 (m, 2H), 2.45 (t, J = 7.5 Hz, 2H), 2.15−2.08 (m, 1H), 2.06−1.97 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) 190.5, 172.7, 172.2, 166.0, 149.4, 145.3, 145.0, 139.0, 133.4, 131.2, 130.7, 129.4, 128.9, 127.1, 126.2 (dd, J = 3.0, 9.7 Hz), 113.6, 112.3, 111.8 (dd, J = 3.7, 22.0 Hz), 105.0 (dd, J = 24.6, 26.9 Hz), 52.0, 52.0, 51.3, 35.4, 33.8, 30.0, 25.7, ($C^{1'}$, $C^{2'}$-F, and $C^{4'}$-F not visible).

Methyl (8-((2,4-Difluorophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carbonyl)-L-methioninate (37). Compound **37** was prepared according to General Procedure B using **5** (0.080 g, 0.21 mmol) and L-methionine methyl ester hydrochloride (0.105 g, 0.53 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.043 g (39%), yellow solid. MS (ESI_{neg}) (m/z): 523.1 $[M - H]^-$. IR (ATR) [cm^{-1}] 2921, 2852, 1737, 1635, 1602, 1505, 1435, 1354, 1257, 1213, 1094, 845. ^1H NMR (400 MHz, DMSO-d_6) δ 8.91 (d, J = 7.4 Hz, 1H), 8.60 (s, 1H), 8.38 (s, 1H), 8.00 (d, J = 8.8 Hz, 1H), 7.96 (d, J = 7.8 Hz, 1H), 7.44−7.35 (m, 3H), 7.13−7.08 (m, 1H), 6.76 (d, J = 8.8 Hz, 1H), 6.63 (s, 1H), 4.62−4.57 (m, 1H), 3.65 (s, 3H), 3.14−3.12 (m, 2H), 3.06−3.04 (m, 2H), 2.63−2.53 (m, 2H), 2.08−2.03 (m, 5H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.9, 172.9, 166.5, 149.9, 145.8, 145.5, 139.5, 133.8, 132.5, 131.2, 129.9, 129.4, 127.6, 126.7 (dd, J = 2.9, 9.7 Hz), 125.3 (dd, J = 3.2, 11.2 Hz), 114.0, 112.8, 112.3 (dd, J = 3.3, 22.0 Hz), 105.4 (dd, J = 23.8, 26.4 Hz), 52.4, 52.2, 35.9, 34.3, 30.6, 30.4, 15.0, (C2′-F and C4′-F not visible).

Methyl *N*2-(((9*H*-Fluoren-9-yl)methoxy)carbonyl)-*N*6-(8-((2,4-difluorophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carbonyl)-L-lysinate (38). Compound **38** was prepared according to General Procedure B using **5** (0.10 g, 0.26 mmol) and *N*-α-Fmoc-L-lysine methyl ester (**S1**) (0.221 g, 0.53 mmol). Product was used in the next step without further purification.

(*S*)-2-((*tert*-Butoxycarbonyl)amino)-3-(8-((2,4-difluorophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*]-[7]annulene-3-carboxamido)propanoic Acid (39). Compound **39** was prepared according to General Procedure B using **5** (0.066 g, 0.17 mmol) and (*S*)-3-amino-2-((*tert*-butoxycarbonyl)amino)-propanoic acid (0.053 g, 0.26 mmol). Product was used in the next step without further purification.

(*S*)-2-((*tert*-Butoxycarbonyl)amino)-4-(8-((2,4-difluorophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*]-[7]annulene-3-carboxamido)butanoic Acid (40). Compound **40** was prepared according to General Procedure B using **5** (0.066 g, 0.17 mmol) and (*S*)-4-amino-2-((*tert*-butoxycarbonyl)amino)butanoic acid (0.057 g, 0.26 mmol). Product was used in the next step without further purification.

8-((2,4-Difluorophenyl)amino)-5-oxo-*N*-(2-(piperazin-1-yl)-ethyl)-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (41). Compound **41** was prepared according to General Procedure C starting from **23** (0.113 g, 0.19 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.089 g (95%), yellow solid. MS (ESI_{pos}) (m/z): 491.9 $[M + H]^+$. IR (ATR) [cm^{-1}] 1632, 1603, 1577, 1584, 1354, 1258, 1139, 964, 844, 729. ^1H NMR (400 MHz, DMSO-d_6) δ 8.60 (s, 1H), 8.52−8.49 (m, 1H), 8.31 (s, 1H), 7.99 (t, J = 8.7 Hz, 1H), 7.90 (d, J = 7.5 Hz, 1H), 7.45−7.35 (m, 3H), 7.13−7.08 (m, 1H), 6.76 (d, J = 8.8 Hz, 1H), 6.62 (s, 1H), 3.16 (s, 1H), 3.12−3.10 (m, 2H), 3.05−3.03 (m, 2H), 2.82−2.76 (m, 2H), 2.45−2.40 (m, 10H).

(8-((2,4-Difluorophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carbonyl)glycine (42). Compound **42** was prepared according to General Procedure A starting from carboxylic ester **31** (0.020 g, 0.04 mmol). Yield: 0.012 g (62%), yellow solid. MS (ESI_{neg}) (m/z): 434.7 $[M - H]^-$. IR (ATR) [cm^{-1}] 3292, 1715, 1601, 1519, 1397, 1284, 1260, 1211, 1138, 964, 841. ^1H NMR (400 MHz, MeOD-d_4) δ 8.43 (d, J = 1.6 Hz, 1H), 8.07 (d, J = 8.8 Hz, 1H), 7.92 (dd, J = 7.8, 1.4 Hz, 1H), 7.42−7.36 (m, 2H), 7.10−7.04 (m, 1H), 7.00−6.95 (m, 1H), 6.80−6.77 (m, 1H), 6.65 (s, 1H), 4.10 (s, 2H), 3.18−3.16 (m, 2H), 3.10−3.08 (m, 2H). ^{13}C NMR (100 MHz, MeOD-d_4) δ 193.1, 173.2, 169.7, 160.7 (dd, J = 10.6, 243.6 Hz), 157.7 (dd, J = 12.0, 246.7 Hz), 151.4, 147.2, 147.1, 140.8, 134.9, 133.7, 131.8, 130.7, 130.3, 129.0, 127.1 (dd, J = 2.8, 9.4 Hz), 126.5 (dd, J = 3.6, 12.1 Hz), 115.0, 113.7, 112.4 (dd, J = 3.8, 22.2 Hz), 105.6 (dd, J = 24.2, 26.4 Hz), 42.4, 37.0, 35.6.

3-(8-((2,4-Difluorophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamido)propanoic Acid (43). Compound **43** was prepared according to General Procedure A starting from carboxylic ester **33** (0.043 g, 0.09 mmol). Yield: 0.029 g (68%), yellow solid. MS (ESI_{neg}) (m/z): 448.8 $[M - H]^-$. IR (ATR) [cm^{-1}] 1644, 1601, 1549, 1519, 1188, 1260, 968, 839, 804, 731, 604. ^1H NMR (400 MHz, DMSO-d_6) δ 12.24 (br. s, 1H), 8.67−8.64 (m, 1H), 8.60 (s, 1H), 8.32 (d, J = 1.7 Hz, 1H), 7.97 (d, J = 8.8 Hz, 1H), 7.90 (dd, J = 7.9, 1.8 Hz, 1H), 7.54−7.34 (m, 3H), 7.13−7.08 (m, 1H), 6.76 (dd, J = 8.8, 1.4 Hz, 1H), 6.62 (s, 1H), 3.48−3.43 (m, 2H), 3.12−3.10 (m, 2H), 3.05−3.03 (m, 2H), 2.55−2.51 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.7, 173.1, 165.8, 158.7 (dd, J = 11.5, 241.8 Hz), 155.9 (dd. J = 12.2, 246.5 Hz), 149.6, 145.5, 144.9, 139.1, 133.6, 132.9, 130.6, 129.4, 129.1, 127.4, 126.5 (dd, J = 3.2, 9.7 Hz), 125.0 (dd, J = 3.4, 12.2 Hz), 113.8, 112.5, 112.1 (dd, J = 3.6, 21.9 Hz), 105.1 (dd, J = 23.9, 26.4 Hz), 35.8, 35.6, 34.0, 33.9.

4-(8-((2,4-Difluorophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamido)butanoic Acid (44). Compound **44** was prepared according to General Procedure A starting from carboxylic ester **34** (0.026 g, 0.05 mmol). Yield: 0.010 g (41%), yellow solid. MS (ESI_{neg}) (m/z): 462.9 $[M - H]^-$. IR (ATR) [cm^{-1}] 3281, 1733, 1645, 1601, 1519, 1264, 1096, 969, 850, 571. ^1H NMR (400 MHz, $CDCl_3$) δ 8.45 (s, 1H), 8.16 (d, J = 8.7 Hz, 1H), 8.05−8.03 (m, 1H), 7.39−7.29 (m, 3H), 6.96−6.88 (m, 2H), 6.83 (d, J = 8.3 Hz, 1H), 6.65 (s, 1H), 5.97 (br. s, 1H), 3.56−3.51 (m, 2H), 3.17−3.15 (m, 2H), 3.10−3.08 (m, 2H), 2.50 (t, J = 6.7 Hz, 2H), 2.05−1.97 (m, 2H), (COOH not visible). ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.1, 176.2, 166.9, 148.4, 145.8, 145.3, 138.5, 134.6, 132.8, 132.0, 129.5, 128.8, 128.7, 124.1 (dd, J = 2.2, 9.4 Hz), 114.5, 113.3, 111.5 (dd, J = 3.5, 22.3 Hz), 104.8 (dd, J = 23.8, 26.2 Hz), 39.6, 35.9, 34.7, 31.4, 24.6, ($C^{1'}$, $C^{2'}$-F, and $C^{4'}$-F not visible).

Methyl 8-((2-Aminophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxylate (52). In a three-necked round-bottomed flask, *o*-phenylenediamine (0.194 g, 1.79 mmol), XPhos-Pd second generation (0.03 equiv), and aryl chloride **3** were dissolved in 12 mL of 1,4-dioxane/*t*-BuOH (5:1, v/v) by gentle heating. Then, Cs_2CO_3 (1.5 equiv) was added, and the reaction mixture was heated to 60 °C for 4 h. After cooling to rt, the reaction mixture was filtered, and the residue was washed several times with DCM, MeOH, and ethyl acetate. The combined organic extracts were evaporated under reduced pressure. The dark-brown residue was purified by flash chromatography (SiO_2, petroleum ether/ethyl acetate 3:1) to yield **52** as a yellow solid (0.53 g, 95%). MS (ESI_{neg}) (m/z): 370.9 $[M - H]^-$. IR (ATR) [cm^{-1}] 1714, 1602, 1577, 1498, 1353, 1242, 1116, 837, 743, 626. ^1H NMR (400 MHz, DMSO-d_6) δ 8.45 (d, J = 1.6 Hz, 1H), 8.06 (s, 1H), 8.00−7.96 (m, 2H), 7.43 (d, J = 7.9 Hz, 1H), 7.02 (d, J = 7.6 Hz, 1H), 6.97−6.93 (m, 1H), 6.79 (d, J = 7.9 Hz, 1H), 6.64 (dd, J = 8.9, 1.5 Hz, 1H), 6.61−6.57 (m, 1H), 6.47 (d, J = 1.3 Hz, 1H), 4.86 (s, 2H), 3.86 (s, 3H), 3.12−3.10 (m, 2H), 3.00−2.97 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 189.1, 165.7, 151.1, 146.9, 145.3, 143.7, 139.3, 133.7, 131.8, 131.3, 129.4, 127.9, 126.2, 125.9, 125.5, 124.7, 116.5, 115.5, 112.7, 111.9, 52.1, 35.4, 34.1.

8-((2-Aminophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxylic Acid (53). Compound **53** was prepared according to General Procedure A starting from **52** (0.26 g, 0.70 mmol). Yield: 0.24 g (96%). MS (ESI_{neg}) (m/z): 357.1 $[M - H]^-$. IR (ATR) [cm^{-1}] 1682, 1579, 1505, 1454, 1353, 1239, 1108, 831, 745, 622. ^1H NMR (400 MHz, DMSO-d_6) δ 13.04 (br. s, 1H), 9.70 (s, 1H), 8.44 (s, 1H), 8.26 (s, 1H), 8.03−7.98 (m, 3H),

https://doi.org/10.1021/acs.jmedchem.0c01773
J. Med. Chem. XXXX, XXX, XXX−XXX

7.44 (s, 1H), 7.36−7.30 (m, 1H), 7.18 (s, 1H), 7.03−6.93 (m, 1H), 6.79−6.47 (m, 3H), 3.14−3.12 (m, 2H), 3.04−3.01 (m, 2H).

8-((2-Aminophenyl)amino)-*N*-(2-(dimethylamino)ethyl)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (54). Compound **54** was prepared according to General Procedure B using **53** (0.025 g, 0.07 mmol) and 3-aminopropanamide (0.012 g, 0.14 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.018 g (60%), yellow solid. MS (ESI_{pos}) (*m*/*z*): 429.1 $[M + H]^+$. IR (ATR) [cm^{-1}] 2921, 2852, 1633, 1603, 1574, 1455, 1353, 1262, 1112, 852, 744, 543. ^1H NMR (400 MHz, $CDCl_3$) δ 8.37 (s, 1H), 8.13 (d, *J* = 8.7 Hz, 1H), 7.96 (d, *J* = 7.6 Hz, 1H), 7.26−7.24 (m, 1H), 7.15−7.08 (m, 2H), 6.84−6.76 (m, 2H), 6.65 (dd, *J* = 8.7, 2.0 Hz, 1H), 6.43 (d, *J* = 1.9 Hz, 1H), 5.71 (s, 1H), 3.62−3.58 (m, 2H), 3.14−3.12 (m, 2H), 3.06−3.04 (m, 2H), 2.67−2.65 (m, 2H), 2.37 (s, 6H), (NH, NH, NH_2 not visible). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.3, 166.9, 150.0, 145.5, 145.0, 142.8, 139.3, 134.3, 132.9, 131.0, 129.1, 128.9, 128.1, 127.3, 126.9, 125.8, 119.1, 116.4, 113.5, 112.7, 57.9, 44.9 (2× C), 37.0, 35.9, 34.7.

8-((2-Aminophenyl)amino)-5-oxo-*N*-phenethyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (55). Compound **55** was prepared according to General Procedure B using **53** (0.043 g, 0.12 mmol) and 2-phenylethylamine (0.013 g, 0.03 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.013 g (24%), yellow solid. MS (ESI_{neg}) (*m*/*z*): 460.1 $[M - H]^-$. IR (ATR) [cm^{-1}] 3313, 2923, 2854, 1358, 2341, 1633, 1575, 1354, 1261, 1112, 745, 698. ^1H NMR (400 MHz, $CDCl_3$) δ 8.28 (s, 1H), 8.06 (d, *J* = 8.5 Hz, 1H), 7.88 (d, *J* = 7.4 Hz, 1H), 7.23 (d, *J* = 7.6 Hz, 1H), 7.17−7.04 (m, 5H), 6.95−6.82 (m, 3H), 6.64 (d, *J* = 8.2 Hz, 1H), 6.44 (s, 1H), 6.08 (br. s, 1H), 3.72−3.69 (m, 2H), 3.09−3.07 (m, 2H), 3.00−2.98 (m, 2H), 2.65 (t, *J* = 5.7 Hz, 2H), (NH, NH, NH_2 not visible).

8-((2-Aminopheinyl)amino)-*N*-(2-morpholinoethyl)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (56). Compound **56** was prepared according to General Procedure B using **53** (0.032 g, 0.09 mmol) and 2-morpholinoethan-1-amine (0.040 g, 0.31 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.029 g (70%), yellow solid. MS (ESI_{pos}) (*m*/*z*): 471.2 $[M + H]^+$. IR (ATR) [cm^{-1}] 3304, 2921, 2852, 1633, 1602, 1574, 1498, 1353, 1264, 1112, 859, 745, 537. ^1H NMR (400 MHz, DMSO-d_6) δ 8.55−8.52 (m, 1H), 8.33 (s, 1H), 8.04−7.99 (m, 2H), 7.90 (d, *J* = 7.8 Hz, 1H), 7.40 (d, *J* = 7.8 Hz, 1H), 7.06 (d, *J* = 7.6 Hz, 1H), 6.98−6.94 (m, 1H), 6.80−6.78 (m, 1H), 6.66−6.58 (m, 2H), 6.48 (br. s, 1H), 4.88 (br. s, 2H), 3.57 (br. s, 4H), 3.40−3.37 (m, 2H), 3.11−3.09 (m, 2H), 3.01−2.99 (m, 2H), 2.46 (d, *J* = 6.9 Hz, 2H), 2.41 (br. s, 4H).

***N*-(2-(1*H*-Imidazol-5-yl)ethyl)-8-((2-aminophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (57).** Compound **57** was prepared according to General Procedure B using **53** (0.050 g, 0.14 mmol) and histamine dihydrochloride (0.039 g, 0.21 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.028 g (46%), yellow solid. MS (ESI_{neg}) (*m*/*z*): 450.1 $[M - H]^-$. IR (ATR) [cm^{-1}] 2920, 2851, 1602, 1557, 1495, 1353, 1263, 1112, 829, 746, 619. ^1H NMR (400 MHz, DMSO-d_6) δ 11.81 (s, 1H), 8.68 (t, *J* = 5.4 Hz, 1H), 8.32 (d, *J* = 1.6 Hz, 1H), 8.04 (s, 1H), 7.98 (d, *J* = 8.8 Hz, 1H), 7.89 (dd, *J* = 7.9, 1.7 Hz, 1H), 7.53 (s, 1H), 7.39 (d, *J* = 8.0 Hz, 1H), 7.02 (d, *J* = 7.6 Hz, 1H), 6.97−6.93 (m, 1H), 6.85−6.77 (m, H), 6.64 (dd, *J* = 8.9, 2.1 Hz, 1H), 6.60−6.57 (m, 1H), 6.47 (d, *J* = 1.9 Hz, 1H), 4.86 (s, 2H), 3.49−3.46 (m, 2H, overlain by water), 3.10−3.08 (m, 2H), 3.00−2.98 (m, 2H), 2.77−2.74 (m, 2H).

***tert*-Butyl 4-(2-(8-((2-Aminophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamido)ethyl)-piperazine-1-carboxylate (58).** Compound **58** was prepared according to General Procedure B using **53** (0.110 g, 0.31 mmol) and *tert*-butyl 4-(2-aminoethyl)piperazine-1-carboxylate (0.077 g, 0.34 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.081 g (46%), yellow solid. MS (ESI_{neg}) (*m*/*z*): 568.1 $[M - H]^-$. IR (ATR) [cm^{-1}] 3289, 2923, 1660, 1603, 1573, 1417, 1246, 1164, 1113, 860, 786. ^1H NMR (400 MHz, $CDCl_3$) δ 8.37 (s, 1H), 8.17 (d, *J* = 8.7 Hz, 1H), 7.98 (dd, *J* = 1.5, 7.8 Hz, 1H), 7.31 (d, *J* = 7.0 Hz, 1H), 7.17−7.11 (m, 3H), 6.86−6.79 (m, 3H), 6.68 (dd, *J* = 8.7, 2.1 Hz, 1H), 6.45 (d, *J* = 1.8 Hz, 1H), 5.66 (s, 1H), 3.65 (s, 2H), 3.55 (s, 4H), 3.18−3.14 (m, 2H), 3.11−3.08 (m, 2H), 2.75 (br. s, 2H), 2.60 (br. s, 4H), 1.48 (s, 9H).

8-((2-Aminophenyl)amino)-*N*-(2-hydroxyethyl)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (59). Compound **59** was prepared according to General Procedure B using **53** (0.050 g, 0.14 mmol) and 2-aminoethanol (0.026 g, 0.42 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.007 g (13%), yellow solid. MS (ESI_{neg}) (*m*/*z*): 400.1 $[M - H]^-$. IR (ATR) [cm^{-1}] 3305, 2923, 1603, 1557, 1498, 1354, 1266, 1216, 1113, 1065, 746, 451. ^1H NMR (400 MHz, DMSO-d_6) δ 8.54−8.51 (m, 1H), 8.33 (s, 1H), 8.01−7.97 (m, 2H), 7.92−7.98 (m, 1H), 7.38 (d, *J* = 7.9 Hz, 1H), 7.02 (d, *J* = 7.7 Hz, 1H), 6.97−6.93 (m, 1H), 6.78 (d, *J* = 8.0 Hz, 1H), 6.65−6.62 (m, 1H), 6.60−6.57 (m, 1H), 6.47 (s, 1H), 4.84 (s, 2H), 4.75 (br. s, 1H), 3.53−3.48 (m, 2H), 3.35−3.30 (m, 2H), 3.10−3.08 (m, 2H), 3.00−2.98 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.5, 166.2, 151.4, 145.8, 145.0, 144.1, 139.6, 134.0, 133.3, 130.7, 129.7, 129.2, 126.6, 126.4, 126.2, 125.3, 117.0, 116.0, 113.3, 112.3, 60.2, 42.7, 36.2, 34.3.

8-((2-Aminophenyl)amino)-*N*-(2,3-dihydroxypropyl)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (60). Compound **60** was prepared according to General Procedure B using **53** (0.040 g, 0.11 mmol) and 3-aminopropane-1,2-diol (0.011 g, 0.12 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.010 g (21%), yellow solid. MS (ESI_{neg}) (*m*/*z*): 429.7 $[M - H]^-$. IR (ATR) [cm^{-1}] 3306, 2920, 2851, 1603, 1574, 1557, 1455, 1354, 1263, 1111, 1036, 745. ^1H NMR (400 MHz, DMSO-d_6) δ 8.51−8.48 (m, 1H), 8.34−8.33 (m, 1H), 8.01−7.97 (m, 2H), 7.91 (dd, *J* = 7.8, 2.0 Hz, 1H), 7.39 (d, *J* = 7.8 Hz, 1H), 7.02 (dd, *J* = 7.8, 1.2 Hz, 1H), 6.97−6.93 (m, 1H), 6.78 (dd, *J* = 8.1, 1.5 Hz, 1H), 6.64 (dd, *J* = 8.9, 2.3 Hz, 1H), 6.60−6.56 (m, 1H), 6.47 (d, *J* = 2.4 Hz, 1H), 4.86 (s, 2H), 4.82−4.81 (m, 1H), 4.57 (t, *J* = 5.7 Hz, 1H), 3.67−3.60 (m, 1H), 3.41−3.34 (m, 3H), 3.22−3.16 (m, 1H), 3.11−3.07 (m, 2H), 3.02−2.98 (m, 2H).

8-((2-Aminophenyl)amino)-*N*-(1,3-dihydroxypropan-2-yl)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (61). Compound **61** was prepared according to General Procedure B using **53** (0.040 g, 0.11 mmol) and 2-aminopropane-1,3-diol (0.011 g, 0.12 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.011 g (22%), yellow solid. MS (ESI_{neg}) (*m*/*z*): 430.3 $[M - H]^-$. IR (ATR) [cm^{-1}] 3307, 2918, 2850, 1603, 1574, 1557, 1456, 1353, 1266, 1036, 746, 565. ^1H NMR (400 MHz, DMSO-d_6) δ 8.34 (d, *J* = 1.7 Hz, 1H), 8.08 (d, *J* = 8.1 Hz, 1H), 8.01−7.97 (m, 2H), 7.92 (dd, *J* = 7.8, 2.0 Hz, 1H), 7.39 (d, *J* = 7.8 Hz, 1H), 7.03−7.00 (m, 1H), 6.97−6.93 (m, 1H), 6.78 (dd, *J* = 7.9, 1.3 Hz, 1H), 6.64 (dd, *J* = 8.9, 2.3 Hz, 1H), 6.60−6.56 (m, 1H), 6.47 (d, *J* = 2.0 Hz, 1H), 4.86 (s, 2H), 4.68−4.64 (m, 2H), 4.00−3.94 (m, 1H), 3.52−3.49 (m, 4H), 3.11−3.07 (m, 2H), 3.02−2.98 (m, 2H).

8-((2-Aminophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (62). Compound **62** was prepared according to General Procedure B using **53** (0.050 g, 0.14 mmol) and aq. ammonia (0.019 g, 0.28 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH [7 N NH_3] 1:0 to 9:1). Yield: 0.031 g (62%), yellow solid. MS (ESI_{neg}) (*m*/*z*): 356.0 $[M - H]^-$. IR (ATR) [cm^{-1}] 3305, 2922, 2853, 1652, 1602, 1567, 1513, 1353, 1270, 1108, 830, 145, 524. ^1H NMR (400 MHz, DMSO-d_6) δ 8.34 (d, *J* = 1.4 Hz, 1H), 8.07 (s, 1H), 8.01 (s, 1H), 7.98 (d, *J* = 8.9 Hz, 1H), 7.92 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.38−7.36 (m, 2H), 7.02 (d, *J* = 7.6 Hz, 1H), 6.99−6.93 (m, 1H), 6.78 (d, *J* = 7.9 Hz, 1H), 6.63 (dd, *J* = 8.9, 2.0 Hz, 1H), 6.61−6.56 (m, 1H), 6.47 (d, *J* = 1.7 Hz, 1H), 4.84 (s, 2H), 3.10−3.08 (m, 2H), 3.01−2.98 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.5, 167.9, 151.4, 145.8, 145.1, 144.1, 139.6, 134.0, 133.0, 130.9, 130.1, 129.2, 126.6, 126.4, 126.2, 125.3, 117.0, 116.0, 113.3, 112.3, 36.2, 34.3.

***N*-(2-Aminoethyl)-8-((2-aminophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (63).** Compound **63** was prepared according to General Procedure B using **53** (0.050 g, 0.14 mmol) and ethylene-1,2-diamine (0.025 g, 0.42 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH [7 N NH_3] 1:0 to 9:1). Yield: 0.012 g (22%), yellow solid. MS

https://doi.org/10.1021/acs.jmedchem.0c01773
J. Med. Chem. XXXX, XXX, XXX−XXX

(ESI_{neg}) (m/z): 399.1 [M − H]$^-$. IR (ATR) [cm^{-1}] 3290, 2920, 2851, 1602, 1564, 1495, 1352, 1262, 1112, 831, 745, 598. ^{1}H NMR (400 MHz, DMSO-d_6) 8.53 (s, 1H), 8.36−8.31(m, 1H), 8.07 (s, 1H), 8.00−7.96 (m, 1H), 7.91 (d, J = 7.7 Hz, 1H), 7.38 (d, J = 8.1 Hz, 1H), 7.02 (d, J = 7.6 Hz, 1H), 6.97−6.95 (m, 1H), 6.78 (d, J = 7.9 Hz, 1H), 6.64 (dd, J = 1.8, 8.9 Hz, 1H), 6.60−6.57 (m, 1H), 6.47 (s, 1H), 4.87 (s, 2H), 3.50 (s, 2H), 3.27−3.23 (m, 2H), 3.10−3.08 (m, 2H), 3.00−2.97 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.5, 166.2, 151.4, 145.8, 144.9, 144.1, 139.6, 134.0, 133.4, 130.7, 129.7, 129.1, 126.6, 126.3, 126.2, 125.3, 116.9, 116.0, 113.3, 112.3, 79.6, 70.3, 36.2, 34.3.

Dimethyl (8-((2-Aminophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carbonyl)-L-glutamate (64). Compound **64** was prepared according to General Procedure B using **53** (0.070 g, 0.20 mmol) and dimethyl L-glutamate (0.045 g, 0.21 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.066 g (65%), yellow solid. MS (ESI_{neg}) (m/z): 514.1 [M − H]$^-$. IR (ATR) [cm^{-1}] 2921, 2852, 1732, 1606, 1580, 1505, 1447, 1356, 1308, 1208, 1105, 848, 744. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.89 (d, J = 7.4 Hz, 1H), 8.36 (d, J = 1.8 Hz, 1H), 8.02 (s, 1H), 7.98 (d, J = 8.9 Hz, 1H), 7.92 (dd, J = 7.9, 1.9 Hz, 1H), 7.41 (d, J = 8.0 Hz, 1H), 7.03−7.00 (m, 1H), 6.97−6.93 (m, 1H), 6.78 (dd, J = 8.0, 1.1 Hz, 1H), 6.64 (dd, J = 8.9, 2.2 Hz, 1H), 6.61−6.56 (m, 1H), 6.47 (d, J = 2.0 Hz, 1H), 4.84 (s, 2H), 4.50−4.44 (m, 1H), 3.64 (s, 3H), 3.58 (s, 3H), 3.12−3.09 (m, 2H), 3.00−2.98 (m, 2H), 2.44 (t, J = 7.5 Hz, 2H), 2.16−1.96 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 189.9, 172.7, 172.1, 166.1, 151.0, 145.3, 145.0, 143.6, 139.2, 133.5, 131.8, 130.5, 129.4, 128.7, 126.1, 125.9, 125.6, 124.7, 116.5, 115.5, 112.8, 111.8, 52.0, 51.9, 51.3, 35.7, 33.8, 29.9, 25.6.

Methyl 3-(8-((2-Aminophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamido)propanoate (65). Compound **65** was prepared according to General Procedure B using **53** (0.050 g, 0.14 mmol) and methyl 3-aminopropanoate (0.021 g, 0.15 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.061 g (99%), yellow solid. MS (ESI_{pos}) (m/z): 442.1 [M − H]$^-$. ^{1}H NMR (400 MHz, DMSO-d_6) 8.69−8. 67 (m, 1H), 8.32 (s, 1H), 8.03−7.98 (m, 2H), 7.90 (d, J = 7.8 Hz, 1H), 7.40 (d, J = 7.8 Hz, 1H), 7.03 (d, J = 7.8 Hz, 1H), 6.98−6.94 (m, 1H), 6.80 (d, J = 7.8 Hz, 1H), 6.66−6.58 (m, 2H), 6.48 (br. s, 1H), 4.88 (br. s, 2H), 3.61 (s, 3H), 3.53−3.47 (m, 2H), 3.11−3.08 (m, 2H), 3.03−2.98 (m, 2H), 2.61 (t, J = 6.8 Hz, 2H).

Methyl (8-((2-Aminophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carbonyl)-L-methioninate (66). Compound **66** was prepared according to General Procedure B using **53** (0.070 g, 0.20 mmol) and L-methionine methyl ester hydrochloride (0.043 g, 0.21 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.012 g (12%), yellow solid. MS (ESI_{neg}) (m/z): 502.1 [M − H]$^-$. IR (ATR) [cm^{-1}] 2920, 2852, 1737, 1604, 1575, 1504, 1444, 1353, 1256, 1209, 742. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (s, 1H), 7.98 (d, J = 8.7 Hz, 1H), 7.90 (d, J = 7.8 Hz, 1H), 7.45−7.21 (m, 3H + solvent peak), 7.16−7.05 (m, 3H), 7.01−6.97 (m 1H), 6.66−6.64 (m, 1H), 6.48 (s, 1H), 4.95−4.90 (m, 2H), 3.80−3.73 (m, 4H), 3.05−3.03 (m, 2H), 2.95−2.93 (m, 2H), 2.59 (t, J = 7.4 Hz, 2H), 2.32−2.22 (m, 1H), 2.18−2.00 (m, 4H). ^{13}C NMR (100 MHz$CDCl_3$) δ 191.3, 172.6, 166.5, 149.2, 145.5, 145.4, 143.6, 139.1, 138.0, 134.1, 132.2, 132.0, 131.7, 130.1, 129.3, 128.9, 128.1, 126.3, 126.1, 114.1, 113.0, 52.6, 52.1, 35.7, 34.6, 30.2, 29.7, 15.4.

Methyl *N*2-(((9*H*-Fluoren-9-yl)methoxy)carbonyl)-*N*6-(8-((2-aminophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*]-[7]annulene-3-carbonyl)-L-lysinate (67). Compound **67** was prepared according to General Procedure B using **53** (0.100 g, 0.28 mmol) and *N*-α-Fmoc-L-lysine methyl ester (**S1**) (0.234 g, 0.56 mmol). Product was used in the next step without further purification.

8-((2-Aminophenyl)amino)-5-oxo-*N*-(2-(piperazin-1-yl)-ethyl)-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (68). Compound **68** was prepared according to General Procedure C starting from **58** (0.010 g, 0.02 mmol). Purification: flash chromatography (SiO_2, DCM/MeOH [7 N NH_3] 1:0 to 9:1). Yield: 0.006 g (67%), yellow solid. MS (ESI_{pos}) (m/z): 470.2 [M + H]$^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (s, 1H), 8.12 (d, J = 8.8 Hz, 1H), 7.92 (d, J = 7.8 Hz, 1H), 7.29 (s, 1H), 7.16−7.06 (m, 2H), 6.84−6.75 (m, 2H), 6.64 (dd, J = 1.6, 8.6 Hz, 1H), 6.42 (d, J = 5.7 Hz, 1H), 3.55−3.51 (m, 2H), 3.16−3.06 (m, 8H), 2.68−2.64 (m, 6H), (NH, NH, NH, NH_2 not visible).

(8-((2-Aminophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carbonyl)-L-glutamic Acid (69). Compound **69** was prepared according to General Procedure A starting from carboxylic ester **64** (0.066 g, 0.13 mmol). Yield: 0.052 g (84%), yellow solid. MS (ESI_{neg}) (m/z): 486.1 [M − H]$^-$. IR (ATR) [cm^{-1}] 2922, 2852, 1714, 1603, 1519, 1451, 1394, 1266, 1101, 743. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.75 (d, J = 7.7 Hz, 1H), 8.38 (d, J = 1.8 Hz, 1H), 8.03 (s, 1H), 8.00−7.97 (m, 2H), 7.94 (dd, J = 7.9, 1.9 Hz, 1H), 7.73−7.71 (m, 1H), 7.57−7.53 (m, 1H), 7.43−7.40 (m, 2H), 7.04 (dd, J = 1.1, 7.7 Hz, 1H), 6.99−6.94 (m, 1H), 6.81 (dd, J = 7.9, 1.1 Hz, 1H), 6.66−6.59 (m, 2H), 6.48 (d, J = 2.0 Hz, 1H), 4.44−4.38 (m, 1H), 3.12−3.09 (m, 2H), 3.01−2.99 (m, 2H), 2.35 (t, J = 7.4 Hz, 2H), 2.14−2.05 (m, 1H), 2.00−1.92 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.0, 173.8, 173.3, 166.0, 150.9, 145.3, 144.8, 143.0, 139.2, 133.5, 132.2, 130.5, 129.4, 128.7, 126.1, 125.9, 125.7, 124.6, 116.9, 115.9, 112.9, 111.9, 52.0, 35.7, 33.8, 30.4, 25.8.

3-(8-((2-Aminophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamido)propanoic Acid (70). Compound **70** was prepared according to General Procedure A starting from carboxylic ester **65** (0.050 g, 0.12 mmol). Yield: 0.042 g (85%), yellow solid. MS (ESI_{pos}) (m/z): 428.4 [M − H]$^-$. ^{1}H NMR (400 MHz, DMSO-d_6) 12.20 (br. s, 1H), 8.66−8.64 (m, 1H), 8.32 (s, 1H), 8.02−7.98 (m, 2H), 7.90 (d, J = 7.3 Hz, 1H), 7.39 (d, J = 7.8 Hz, 1H), 7.03 (d, J = 7.3 Hz, 1H), 6.97−6.93 (m, 1H), 6.79 (d, J = 7.8 Hz, 1H), 6.66−6.57 (m, 2H), 6.48 (br. s, 1H), 4.93 (br. s, 2H), 3.51−3.45 (m, 2H), 3.11−3.09 (m, 2H), 3.00−2.98 (m, 2H), 2.54−2.50 (m, 2H + solvent peak).

Methyl 8-((2-nitrophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxylate (73). 2-Nitroaniline (0.247 g, 1.79 mmol) was dissolved in 12 mL of 1,4-dioxane/*t*-BuOH (5:1, v/v) under an argon atmosphere. XPhos (0.025 equiv), Cs_2CO_3 (1.5 equiv), and $Pd(OAc)_2$ (0.05 equiv) followed by aryl chloride **3** (0.50 g, 1.79 mmol) were added, and the reaction mixture was heated for 1 h to 110 °C. The reaction was cooled to rt and filtered. The residue was washed several times with DCM, MeOH, and EtOAc. The combined organic extracts were evaporated, and the brown-black residue was purified by flash chromatography (SiO_2, petroleum ether/ethyl acetate 3:1) to yield **73** as a yellow solid (0.48 g, 80%). MS (ESI_{neg}) (m/z): 401.1 [M − H]$^-$. IR (ATR) [cm^{-1}] 1722, 1601, 1563, 1506, 1435, 1348, 1240, 1156, 838, 761, 640, 539. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.46 (d, J = 1.7 Hz, 1H), 8.10 (dd, J = 0.9, 8.3 Hz, 1H), 8.04−8.01 (m, 2H), 7.64−7.60 (m, 1H), 7.58−7.56 (m, 1H), 7.49 (d, J = 8.0 Hz, 1H), 7.20 (dd, J = 8.7, 2.1 Hz, 1H), 7.14−7.09 (m, 2H), 3.87 (s, 3H), 3.21−3.19 (m, 2H), 3.14−3.12 (m, 2H), (NH not visible). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.8, 165.6, 147.1, 145.2, 144.8, 138.6, 137.7, 137.5, 135.3, 132.9, 132.2, 131.1, 130.6, 129.8, 128.1, 126.1, 121.2, 120.5, 118.9, 116.9, 52.0, 34.5, 33.9.

8-((2-Nitrophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxylic Acid (74). Compound **74** was prepared according to General Procedure A starting from carboxylic ester **73** (0.15 g, 2.37 mmol). Yield: 0.137 g (95%), orange solid. MS (ESI_{neg}) (m/z): 387.0 [M − H]$^-$. IR (ATR) [cm^{-1}] 1690, 1598, 1571, 1495, 1345, 1253, 1115, 882, 741, 507. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.31 (s, 1H), 8.44 (d, J = 1.8 Hz, 1H), 8.10 (d, J = 8.4, 1.3 Hz, 1H), 8.04−8.00 (m, 2H), 7.65−7.61 (m, 1 H), 7.57−7.49 (m, 2H), 7.17 (dd, J = 2.1, 8.7 Hz, 1H), 7.14−7.10 (m, 2H), 3.12−3.10 (m, 2H), 3.21−3.18 (m, 2H), (COOH not visible). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.9, 165.7, 147.2, 145.6, 144.8, 138.7, 137.8, 137.8, 135.5, 133.0, 132.4, 131.2, 130 6, 130.0, 128.2, 126.2, 121.5, 120.9, 119.0, 117.0, 34.6, 34.0.

***N*-Methyl-8-((2-nitrophenyl)amino)-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (75).** Compound **75** was prepared according to General Procedure B using **74** (0.050 g, 0.13 mmol) and methylamine (2 N THF) (0.010 g, 0.32 mmol).

https://doi.org/10.1021/acs.jmedchem.0c01773
J. Med. Chem. XXXX, XXX, XXX−XXX

Purification: flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1). Yield: 0.045 g (87%), yellow solid. M (ESI_{neg}) (m/z): 400.3 $[M - H]^-$. ^1H NMR (400 MHz, DMSO-d_6) δ 9.32 (s, 1H), 8.58−8.54 (m, 1H), 8.35 (s, 1H), 8.11 (d, J = 8.2 Hz, 1H), 8.02 (d, J = 8.6 Hz, 1H), 7.94 (d, J = 7.4 Hz, 1H), 7.65−7.61 (m, 1H), 7.57−7.55 (m, 1H), 7.44 (d, J = 7.9 Hz, 1H), 7.22−7.19 (m, 1H), 7.14−7.10 (m, 2H), 3.19−3.16 (m, 2H), 3.14−3.12 (m, 2H), 2.79 (d, J = 4.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 191.4, 165.8, 145.2, 144.7, 144.5, 138.4, 137.8, 137.6, 135.3, 132.9, 132.7, 130.9, 130.5, 129.2, 128.9, 126.1, 121.2, 120.6, 119.1, 117.0, 34.7, 33.7, 26.2.

8-((2-Aminophenyl)amino)-*N*-methyl-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene-3-carboxamide (76). The nitro compound **75** (0.030 g, 0.07 mmol) was dissolved in EtOH (40 mL) with heating (50 °C). $SnCl_2$ (3 equiv) was added in one portion, and the reaction was heated to reflux temperature. After completion of the reaction, the reaction was cooled to rt, and $NaHCO_3$ was carefully added and stirred for another 15 min. Then, EtOH was removed under reduced pressure, and the residue was extracted three times with EtOAc. The combined organic phases were dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by flash chromatography (SiO_2, DCM/MeOH 1:0 to 9:1) to yield **76** (0.020 mg, 69%) as a yellow solid. MS (ESI_{neg}) (m/z): 370.1 $[M - H]^-$. IR (ATR) [cm^{-1}] 1633, 1603, 1574, 1455, 1403, 1353, 1262, 1111, 853, 745. ^1H NMR (400 MHz, DMSO-d_6) δ 8.54−8.53 (m, 1H), 8.33 (s, 1H), 8.02 (s, 1H), 7.98 (d, J = 8.8 Hz, 1H), 7.92−7.84 (m, 1H), 7.38 (d, J = 7.9 Hz, 1H), 7.02 (d, J = 7.5 Hz, 1H), 6.95 (t, J = 7.4 Hz, 1H), 6.78 (d, J = 7.7 Hz, 1H), 6.64 (dd, J = 8.8, 1.6 Hz, 1H), 6.61−6.57 (m, 1H), 6.48−6.47 (m, 1H), 4.86 (s, 2H), 3.11−3.07 (m, 2H), 3.02−2.99 (m, 2H), 2.78 (d, J = 4.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 189.9, 166.0, 150.9, 145.3, 144.4, 143.7, 139.1, 133.5, 132.8, 130.0, 129.1, 128.7, 126.1, 125.8, 125.7, 124.8, 116.4, 115.5, 112.8, 111.8, 35.7, 33.8, 26.2.

p38α MAP Kinase Activity Assay. The inhibitory activity of the final compounds was tested using a protocol reported by Goettert et al.[31] In detail, 96-well plates were coated with 50 μL/well (10 μg/mL) in Tris-buffered saline (TBS) of ATF-2 and stored overnight at 4 °C. In the next morning, each plate was washed with water (three times), and the remaining binding sites were blocked with blocking buffer (0.05% Tween 20, 0.025% bovine serum albumin (BSA), and 0.02% NaN_3 in TBS) for 30 min at rt and washed again with water (three times). A 10 mM stock solution of the test compound in DMSO was further diluted in a kinase buffer (12 ng/50 μL activated p38α MAPK, 50 mM Tris of pH 7.5, 10 mM $MgCl_2$, 10 mM β-glycerophosphate, 100 μg/mL BSA, 1 mM dithiothreitol, 0.1 mM Na_3VO_4, and 100 μM ATP). 50 μL of each dilution was pipetted into the corresponding wells and incubated for 1 h at 37 °C. After washing with water (three times), blocking for 15 min, and washing with water (three times), a diluted monoclonal antiphospho-ATF-2 (Thyr69/71)-peroxidase-conjugated antibody (1:5000) in blocking buffer (50 μL) adjusted to a pH of 6.5 was added to each well. After incubation for 1 h at 37 °C, 3,3′,5,5′-tetramethylbenzidine (50 μL) was added to all wells, and the peroxide-labeled conjugates developed a definitive blue color that was measured photometrically at 650 nm.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.0c01773.

Detailed procedures for the preparation of compounds **45**−**51**, **70**−**72**, and **S1**, CNS penetration studies, in vitro metabolic stability study, CYP and hERG inhibition, the selectivity screening as well as selected HPLC traces and NMR spectra (PDF)

SMILES strings of tested compounds (CSV)

■ AUTHOR INFORMATION

Corresponding Author

Pierre Koch − *Department of Pharmaceutical and Medicinal Chemistry, Institute of Pharmaceutical Sciences, Eberhard Karls Universität Tübingen, 72076 Tübingen, Germany; Department of Pharmaceutical/Medicinal Chemistry II, Institute of Pharmacy, University of Regensburg, 93053 Regensburg, Germany;* orcid.org/0000-0003-4620-4650; Phone: +49 941 943 4827; Email: pierre.koch@uni-tuebingen.de, pierre.koch@ur.de

Authors

Niklas M. Tormählen − *Department of Pharmaceutical and Medicinal Chemistry, Institute of Pharmaceutical Sciences, Eberhard Karls Universität Tübingen, 72076 Tübingen, Germany*

Mariella Martorelli − *Synovo GmbH, 72076 Tübingen, Germany*

Annette Kuhn − *Department of Pharmaceutical and Medicinal Chemistry, Institute of Pharmaceutical Sciences, Eberhard Karls Universität Tübingen, 72076 Tübingen, Germany*

Florian Maier − *Synovo GmbH, 72076 Tübingen, Germany*

Jamil Guezguez − *Synovo GmbH, 72076 Tübingen, Germany*

Michael Burnet − *Synovo GmbH, 72076 Tübingen, Germany*

Wolfgang Albrecht − *Teva-ratiopharm, 89079 Ulm, Germany*

Stefan A. Laufer − *Department of Pharmaceutical and Medicinal Chemistry, Institute of Pharmaceutical Sciences, Eberhard Karls Universität Tübingen, 72076 Tübingen, Germany;* orcid.org/0000-0001-6952-1486

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jmedchem.0c01773

Author Contributions

⊥N.M.T. and M.M. contributed equally.

Notes

The authors declare the following competing financial interest(s): M.M., F.M., J.G., and M.B. are employees of Synovo GmbH, a pharmaceutical company that has an interest in the development of this class of compounds.

■ ACKNOWLEDGMENTS

We thank Jens Strobach and Katharina Bauer for their assistance in the p38α MAP kinase ELISA activity assay. Dr. Michael Forster and Dr. Stanislav Andreev are gratefully acknowledged for synthetic and analytical support. We thank Mark Kudolo for his support with metabolism data of compounds **44** and **70**. This study was supported by the Federal Ministry of Education and Research (BMBF) within the BioPharma-Neuroallianz consortium (Neuro-T8B project). We also thank the pharmacology staff of Synovo GmbH, Luciano De Oliveira, Thilo Weinstein, Christina Frech, Natascha Cloos, and Santiago Cruces, for their assistance.

■ ABBREVIATIONS USED

AD, Alzheimer's disease; BBB, blood−brain barrier; CDI, 1,1′-carbonyldiimidazole; CNS, central nervous system; HBC, human buffy coat; HLM, human liver microsome; HR, hydrophobic region; HWB, human whole blood; IL, interleukin; i.v., intravenous; LC-MS, liquid chromatography mass spectrometry; LPS, lipopolysaccharide; MWB, mouse

whole blood; MAP, mitogen-activated protein; TNF, tumor necrosis factor

REFERENCES

(1) Lee, J. C.; Laydon, J. T.; McDonnell, P. C.; Gallagher, T. F.; Kumar, S.; Green, D.; McNulty, D.; Blumenthal, M. J.; Keys, J. R.; Landvatter, S. W.; et al. A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. *Nature* **1994**, *372*, 739−746.

(2) Munoz, L.; Ammit, A. J. Targeting p38 MAPK pathway for the treatment of Alzheimer's disease. *Neuropharmacology* **2010**, *58*, 561−568.

(3) Munoz, L.; Ranaivo, H.; Roy, S. M.; Hu, W.; Craft, J. M.; McNamara, L. K.; Chico, L. W.; van Eldik, L. J.; Watterson, D. M. A novel p38 alpha MAPK inhibitor suppresses brain proinflammatory cytokine up-regulation and attenuates synaptic dysfunction and behavioral deficits in an Alzheimer's disease mouse model. *J. Neuroinflammation* **2007**, *4*, 21.

(4) Yasuda, S.; Sugiura, H.; Tanaka, H.; Takigami, S.; Yamagata, K. p38 MAP kinase inhibitors as potential therapeutic drugs for neural diseases. *Cent. Nerv. Syst. Agents Med. Chem.* **2011**, *11*, 45−59.

(5) Lee, J. K.; Kim, N. J. Recent advances in the inhibition of p38 MAPK as a potential strategy for the treatment of alzheimer's disease. *Molecules* **2017**, *22*, 1287.

(6) Bohush, A.; Niewiadomska, G.; Filipek, A. Role of mitogen activated protein kinase signaling in parkinson's disease. *Int. J. Mol. Sci.* **2018**, *19*, 2973.

(7) Asih, P. R.; Prikas, E.; Stefanoska, K.; Tan, A. R. P.; Ahel, H. I.; Ittner, A. Functions of p38 MAP kinases in the central nervous system. *Front. Mol. Neurosci.* **2020**, *13*, 570586.

(8) Germann, U. A.; Alam, J. J. P38alpha MAPK signaling-a robust therapeutic target for rab5-mediated neurodegenerative disease. *Int. J. Mol. Sci.* **2020**, *21*, 5485.

(9) Hensley, K.; Floyd, R. A.; Zheng, N.-Y.; Nael, R.; Robinson, K. A.; Nguyen, X.; Pye, Q. N.; Stewart, C. A.; Geddes, J.; Markesbery, W. R.; Patel, E.; Johnson, G. V. W.; Bing, G. p38 Kinase is activated in the alzheimer's disease brain. *J. Neurochem.* **1999**, *72*, 2053−2058.

(10) Sun, A.; Liu, M.; Nguyen, X. V.; Bing, G. P38 MAP kinase is activated at early stages in Alzheimer's disease brain. *Exp. Neurol.* **2003**, *183*, 394−405.

(11) Bachstetter, A. D.; Xing, B.; de Almeida, L.; Dimayuga, E. R.; Watterson, D. M.; van Eldik, L. J. Microglial p38alpha MAPK is a key regulator of proinflammatory cytokine up-regulation induced by toll-like receptor (TLR) ligands or beta-amyloid (Abeta). *J. Neuroinflammation* **2011**, *8*, 79.

(12) Correa, S. A.; Eales, K. L. The role of p38 MAPK and its substrates in neuronal plasticity and neurodegenerative disease. *J. Signal Transduction* **2012**, *2012*, 649079.

(13) Duffy, J. P.; Harrington, E. M.; Salituro, F. G.; Cochran, J. E.; Green, J.; Gao, H.; Bemis, G. W.; Evindar, G.; Galullo, V. P.; Ford, P. J.; Germann, U. A.; Wilson, K. P.; Bellon, S. F.; Chen, G.; Taslimi, P.; Jones, P.; Huang, C.; Pazhanisamy, S.; Wang, Y. M.; Murcko, M. A.; Su, M. S. The discovery of VX-745: a novel and selective p38alpha kinase inhibitor. *ACS Med. Chem. Lett.* **2011**, *2*, 758−63.

(14) Alam, J.; Blackburn, K.; Patrick, D. Neflamapimod: clinical phase 2b-ready oral small molecule inhibitor of p38alpha to reverse synaptic dysfunction in early alzheimer's disease. *J. Prev. Alzheimer's Dis.* **2017**, *4* (4), 273−278.

(15) Alam, J. J. Selective brain-targeted antagonism of p38 MAPKalpha reduces hippocampal IL-1beta levels and improves morris water maze performance in aged rats. *J. Alzheimer's Dis.* **2015**, *48*, 219−227.

(16) Roy, S. M.; Grum-Tokars, V. L.; Schavocky, J. P.; Saeed, F.; Staniszewski, A.; Teich, A. F.; Arancio, O.; Bachstetter, A. D.; Webster, S. J.; Van Eldik, L. J.; Minasov, G.; Anderson, W. F.; Pelletier, J. C.; Watterson, D. M. Targeting human central nervous system protein kinases: an isoform selective p38alphaMAPK inhibitor that attenuates disease progression in Alzheimer's disease mouse models. *ACS Chem. Neurosci.* **2015**, *6*, 666−680.

(17) Robson, M. J.; Quinlan, M. A.; Margolis, K. G.; Gajewski-Kurdziel, P. A.; Veenstra-VanderWeele, J.; Gershon, M. D.; Watterson, D. M.; Blakely, R. D. p38alpha MAPK signaling drives pharmacologically reversible brain and gastrointestinal phenotypes in the SERT Ala56 mouse. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115*, E10245−E10254.

(18) Zhou, Z.; Bachstetter, A. D.; Spani, C. B.; Roy, S. M.; Watterson, D. M.; Van Eldik, L. J. Retention of normal glia function by an isoform-selective protein kinase inhibitor drug candidate that modulates cytokine production and cognitive outcomes. *J. Neuroinflammation* **2017**, *14*, 75.

(19) Rutigliano, G.; Stazi, M.; Arancio, O.; Watterson, D. M.; Origlia, N. An isoform-selective p38alpha mitogen-activated protein kinase inhibitor rescues early entorhinal cortex dysfunctions in a mouse model of Alzheimer's disease. *Neurobiol. Aging* **2018**, *70*, 86−91.

(20) Roy, S. M.; Minasov, G.; Arancio, O.; Chico, L. W.; Van Eldik, L. J.; Anderson, W. F.; Pelletier, J. C.; Watterson, D. M. A selective and brain penetrant p38alphaMAPK inhibitor candidate for neurologic and neuropsychiatric disorders that attenuates neuroinflammation and cognitive dysfunction. *J. Med. Chem.* **2019**, *62*, 5298−5311.

(21) Koeberle, S. C.; Romir, J.; Fischer, S.; Koeberle, A.; Schattel, V.; Albrecht, W.; Grutter, C.; Werz, O.; Rauh, D.; Stehle, T.; Laufer, S. A. Skepinone-L is a selective p38 mitogen-activated protein kinase inhibitor. *Nat. Chem. Biol.* **2012**, *8*, 141−143.

(22) Koeberle, S. C.; Fischer, S.; Schollmeyer, D.; Schattel, V.; Grütter, C.; Rauh, D.; Laufer, S. A. Design, synthesis, and biological evaluation of novel disubstituted dibenzosuberones as highly potent and selective inhibitors of p38 mitogen activated protein kinase. *J. Med. Chem.* **2012**, *55*, 5868−5877.

(23) Paige, C.; Maruthy, G. B.; Mejia, G.; Dussor, G.; Price, T. Spinal inhibition of P2XR or p38 signaling disrupts hyperalgesic priming in male, but not female, mice. *Neuroscience* **2018**, *385*, 133−142.

(24) Guenthoer, P.; Fuchs, K.; Reischl, G.; Quintanilla-Martinez, L.; Gonzalez-Menendez, I.; Laufer, S.; Pichler, B. J.; Kneilling, M. Evaluation of the therapeutic potential of the selective p38 MAPK inhibitor Skepinone-L and the dual p38/JNK 3 inhibitor LN 950 in experimental K/BxN serum transfer arthritis. *Inflammopharmacology* **2019**, *27*, 1217−1227.

(25) Borst, O.; Walker, B.; Munzer, P.; Russo, A.; Schmid, E.; Faggio, C.; Bigalke, B.; Laufer, S.; Gawaz, M.; Lang, F. Skepinone-L, a novel potent and highly selective inhibitor of p38 MAP kinase, effectively impairs platelet activation and thrombus formation. *Cell. Physiol. Biochem.* **2013**, *31*, 914−924.

(26) Rudalska, R.; Dauch, D.; Longerich, T.; McJunkin, K.; Wuestefeld, T.; Kang, T. W.; Hohmeyer, A.; Pesic, M.; Leibold, J.; von Thun, A.; Schirmacher, P.; Zuber, J.; Weiss, K. H.; Powers, S.; Malek, N. P.; Eilers, M.; Sipos, B.; Lowe, S. W.; Geffers, R.; Laufer, S.; Zender, L. In vivo RNAi screening identifies a mechanism of sorafenib resistance in liver cancer. *Nat. Med.* **2014**, *20*, 1138−1146.

(27) Koeberle, A.; Pergola, C.; Shindou, H.; Koeberle, S. C.; Shimizu, T.; Laufer, S. A.; Werz, O. Role of p38 mitogen-activated protein kinase in linking stearoyl-CoA desaturase-1 activity with endoplasmic reticulum homeostasis. *FASEB J.* **2015**, *29*, 2439−2449.

(28) Walter, N. M.; Wentsch, H. K.; Bührmann, M.; Bauer, S. M.; Döring, E.; Mayer-Wrangowski, S.; Sievers-Engler, A.; Willemsen-Seegers, N.; Zaman, G.; Buijsman, R.; Lämmerhofer, M.; Rauh, D.; Laufer, S. A. Design, synthesis, and biological evaluation of novel type I(1)/2 p38alpha MAP kinase inhibitors with excellent selectivity, high potency, and prolonged target residence time by interfering with the R-spine. *J. Med. Chem.* **2017**, *60*, 8027−8054.

(29) Wentsch, H. K.; Walter, N. M.; Bührmann, M.; Mayer-Wrangowski, S.; Rauh, D.; Zaman, G. J. R.; Willemsen-Seegers, N.; Buijsman, R. C.; Henning, M.; Dauch, D.; Zender, L.; Laufer, S. Optimized target residence time: type I1/2 Inhibitors for p38alpha MAP kinase with improved binding kinetics through direct interaction with the R-spine. *Angew. Chem., Int. Ed.* **2017**, *56*, 5363−5367.

https://doi.org/10.1021/acs.jmedchem.0c01773
J. Med. Chem. XXXX, XXX, XXX−XXX

(30) Fischer, S.; Wentsch, H. K.; Mayer-Wrangowski, S. C.; Zimmermann, M.; Bauer, S. M.; Storch, K.; Niess, R.; Koeberle, S. C.; Grutter, C.; Boeckler, F. M.; Rauh, D.; Laufer, S. A. Dibenzosuberones as p38 mitogen-activated protein kinase inhibitors with low ATP competitiveness and outstanding whole blood activity. *J. Med. Chem.* **2013**, *56*, 241−253.
(31) Goettert, M.; Graeser, R.; Laufer, S. A. Optimization of a nonradioactive immunosorbent assay for p38alpha mitogen-activated protein kinase activity. *Anal. Biochem.* **2010**, *406*, 233−234.
(32) Koch, P.; Gehringer, M.; Laufer, S. A. Inhibitors of c-Jun N-terminal kinases: an update. *J. Med. Chem.* **2015**, *58*, 72−95.
(33) Eid, S.; Turk, S.; Volkamer, A.; Rippmann, F.; Fulle, S. KinMap: a web-based tool for interactive navigation through human kinome data. *BMC Bioinf.* **2017**, *18*, 16.

https://doi.org/10.1021/acs.jmedchem.0c01773
J. Med. Chem. XXXX, XXX, XXX−XXX