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Metabolically Stable Dibenzo[*b*,*e*]oxepin-11(6*H*)-ones as Highly Selective p38 MAP Kinase Inhibitors: Optimizing Anti-Cytokine Activity in Human Whole Blood

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Supporting Information

ABSTRACT: Five series of metabolically stable disubstituted dibenzo[*b*,*e*]oxepin-11(6*H*)-ones were synthesized and tested in a p38α enzyme assay for their inhibition of tumor necrosis factor-α (TNF-α) release in human whole blood. Compared to the monosubstituted dibenzo[*b*,*e*]oxepin-11(6*H*)-one derivatives, it has been shown that the additional introduction of hydrophilic residues at position 9 leads to a substantial improvement of the inhibitory potency and metabolic stability. Using protein X-ray crystallography, the binding mode of the disubstituted dibenzoxepinones and the induction of a glyince flip in the hinge region were confirmed. The most potent compound of this series, **32e**, shows an outstanding biological activity on isolated p38α, with an IC_{50} value of 1.6 nM, extraordinary selectivity (by a factor >1000, Kinase WholePanelProfiler), and low ATP competitiveness. The ability to inhibit the release of TNF-α from human whole blood was optimized down to an IC_{50} value of 125 nM. With the promising dibenzoxepinone inhibitor **3i**, a pharmacokinetic study in mice was conducted.

INTRODUCTION

The p38α MAP kinase is a member of the mitogen-activated protein (MAP) kinase family. It plays a central role in the regulation of cytokine production, including tumor necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β).[1] These cytokines promote the genesis of inflammatory diseases, such as rheumatoid arthritis (RA), psoriasis, and Crohn's disease.[2]

Biological substrates, targeting these cytokines or their related receptors, have been proven to be efficient in the treatment of RA and psoriasis.[3] Recent investigations indicated that p38 also plays an important role concerning other autoimmune diseases, such as type I diabetes and multiple sclerosis.[4]

Consequently, small molecule p38 MAP kinase inhibitors are valuable agents for the treatment of RA and other inflammatory diseases. The first inhibitors of p38 MAP kinase belong to the class of pyridinylimidazoles,[5] with SB203580 as a frequently cited prototype.[6,7]

In animal studies, these pyridinylimidazole-based p38 inhibitors showed high toxicity due to interaction of the imidazole heterocycle with CYP enzymes. Furthermore, they are characterized by a poor selectivity profile.[8,9]

In a recently published report, we described a novel class of p38α inhibitors with structurally rigid dibenzo[*b*,*e*]oxepin-11(6*H*)-one and 5*H*-dibenzo[*a*,*d*][7]annulen-5-one scaffolds. These compounds exhibited a good biological activity in a p38α MAP kinase assay with IC_{50} values down to 38 nM.[10] Nevertheless, they were only moderately active in a human whole blood TNF-α release assay (e.g., compound **7**, IC_{50} = 22 μM, Figure 1).

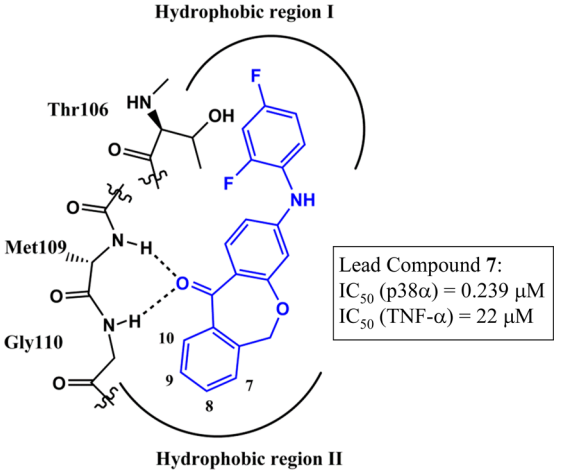

Figure 1. Proposed binding mode of compound **7** in the p38α MAP kinase.

In a subsequent study using 2-(2-amino)amino-substituted dibenzosuberone as starting compound, it has been possible to improve the biological activity in the p38α MAP kinase assay as well as in a human whole blood TNF-α release assay by introducing hydrophilic residues.[11] Protein X-ray crystallography studies of the resulting compound Skepinone-L in complex with p38α revealed that the compound induces a peptide flip: Gly110,

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located in the hinge region, is rotated about 180°. Due to this peptide flip, two hydrogen bonds can be formed between the central carbonyl oxygen of Skepinone-L and the NH of Met109 and Gly110.[11] Using this information, the following binding mode of compound 7, the lead compound of this work, was proposed (Figure 1).

While the central carbonyl group forms two hydrogen bonds toward the hinge region (Met109 and Gly110), the 2,4-difluorophenyl residue occupies the hydrophobic region I (selectivity pocket) of p38α. The hydrophobic region II remains unoccupied and could therefore represent an appropriate region to be further addressed. The introduction of hydrophilic moieties most likely leads to improved selectivity and increased bioavailability of the resulting compounds. As shown in former optimization studies of p38α inhibitors, the motifs to introduce could potentially be hydrophilic dihydroxy or ethylmorpholine groups.[11−15]

Dibenzosuberone inhibitors with high lipophilicity,[10] as exemplified by the compound **33** in Figure 2, are metabolized

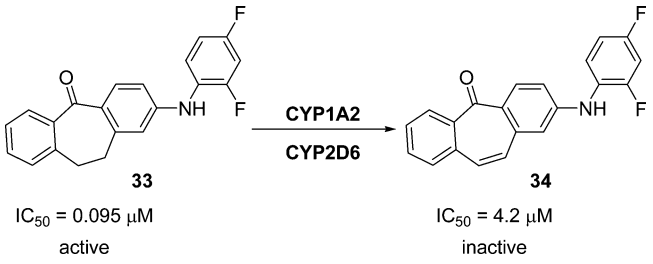


Figure 2. Metabolism of the dibenzosuberones.

by CYP1A2 and CYP2D6 in weakly active, unsaturated compounds. This is the origin of two problems. First of all, CYP2D6 is showing a significant polymorphism. Furthermore, metabolic conversion to the unsaturated compound might include toxic intermediates, such as diols or epoxides. In order to avoid these problems, we identified two different synthetic routes. Hydrophilization of the residue in the 9 position of the dibenzosuberone scaffold led to a total suppression of the metabolism.[11] The second option was blocking the metabolically active position by the introduction of a heteroatom in the main scaffold, resulting in dibenzoxepinone derivatives. These are characterized by metabolic stability, as will be later described in detail.

In the present work, the SARs of Skepinone-L[15] are transferred to the class of dibenzo[*b*,*e*]oxepin-11(6*H*)-ones: structural optimization of compound 7 by the introduction of hydrophilic residues at positions 7, 8, 9, and 10 (Scheme 1) should increase the biological activity in a human whole blood TNF-α release assay.

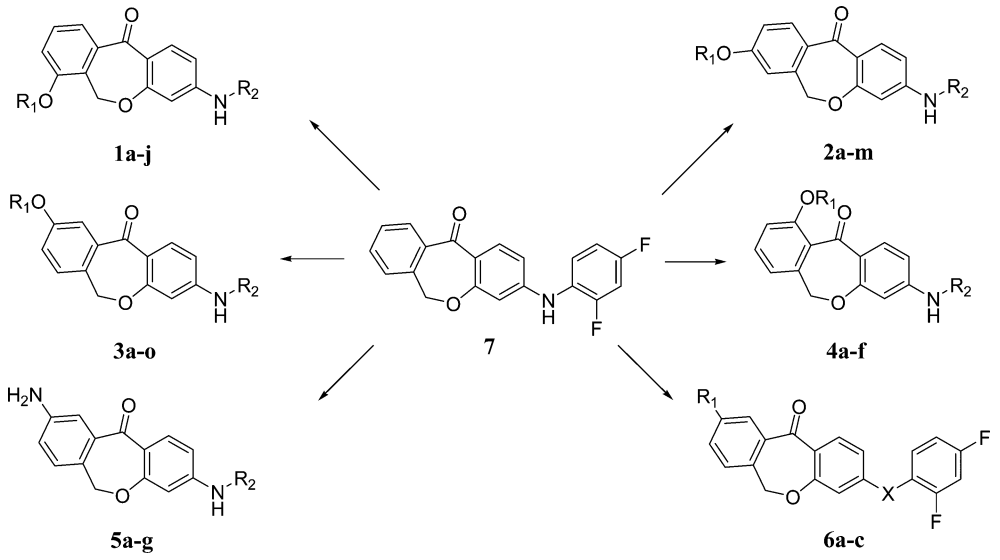
CHEMISTRY

The synthesis of the 7- and 8-substituted dibenzo[*b*,*e*]oxepin-11(6*H*)-one scaffolds **13a** and **13b** was performed, starting from the 3- or 4-methoxy-substituted 2-methylbenzoic acid derivatives **8a** or **8b** via esterification, followed by bromination of the benzyl group and etherification with 3-chlorophenole (Scheme 2).[10] The corresponding esters **11a** and **11b** were hydrolyzed and the resulting acids **12a** and **12b** were then activated with thionyl chloride and treated with aluminium chloride to obtain the disubstituted intermediates **13a** and **13b** by intramolecular Friedel−Crafts acylation.

The 9- and 10-methoxy substituted dibenzo[*b*,*e*]oxepin-11(6*H*)-one scaffolds **24a** and **24b** were obtained by a seven-step synthesis (Scheme 3).

At first, the nitro group of 5- and 6-nitro-2-methylbenzoic acids **17a** and **17b** was reduced to yield the respective amino compounds **18a** and **18b**. These were then treated with sodium nitrite to obtain the corresponding diazonium salt. Reaction with water under reflux conditions gave the desired phenols **19a** and **19b**. Reaction with iodomethane and potassium carbonate led to the methoxy-substituted methyl benzoates **20a** and **20b**.[16] The following reaction pathway, consisting of a bromination, etherification of both compounds **21a** and **21b**, and ester hydrolysis to form the respective acids **23a** and **23b**, was conducted similar to the above-mentioned synthetic route (Scheme 2), whereas the ring closure was achieved by using trifluoroacetic anhydride and boron trifluoride etherate.[17] The selective generation of the phenolic group from the methoxy group in the presence of the cyclic phenoxy group turned out to

Scheme 1. Synthesized Derivatives



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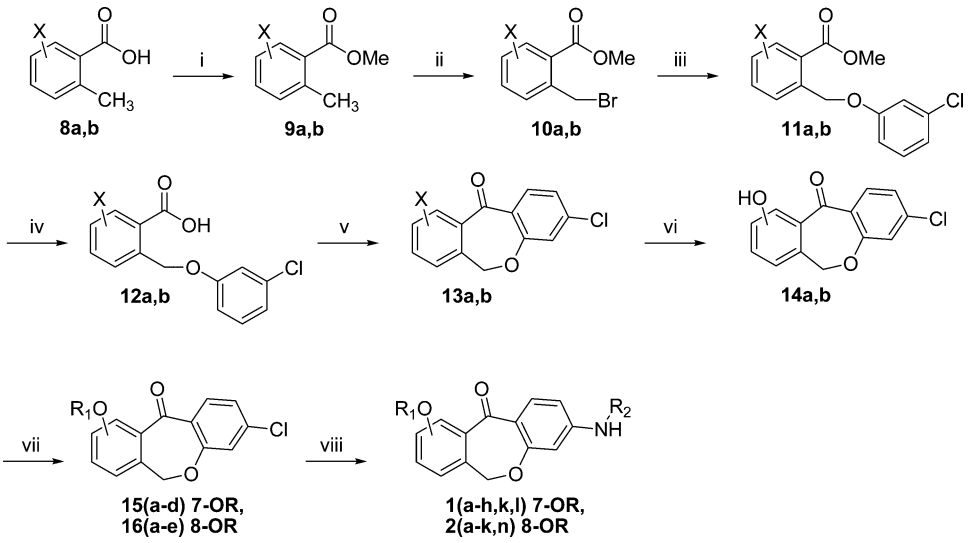
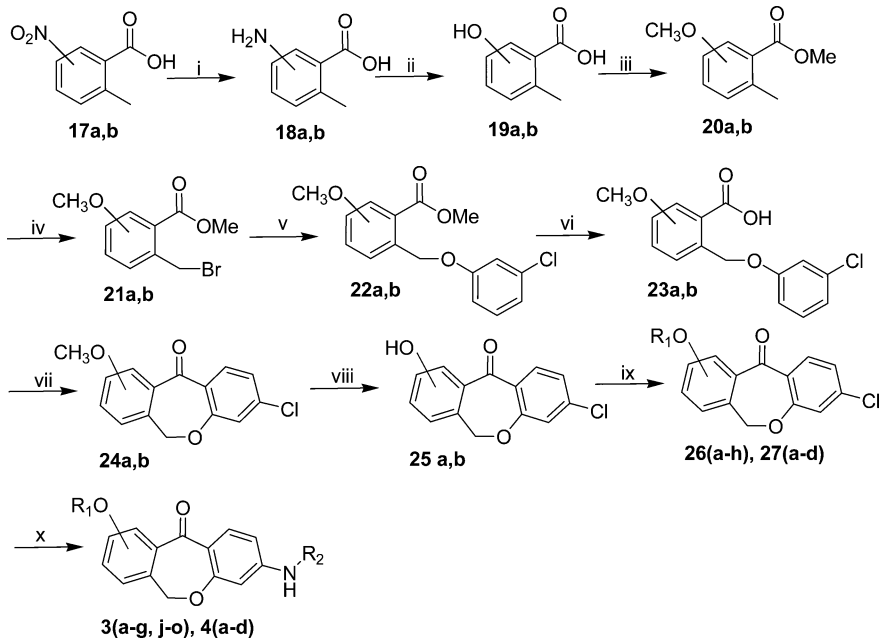
Scheme 2. Preparation of 7- and 8-Substituted 3-(Phenylamino)dibenzo[*b*,*e*]oxepin-11(6*H*)-ones 1a−h,k,l and 2a−k,n[a]



[a]Reagents and conditions: **a**, 3-OCH_3; **b**, 4-OCH_3. (i) H_2SO_4, MeOH, reflux; (ii) NBS, CCl_4, 72 °C; (iii) K_2CO_3, 3-chlorophenole, Me_2CO, reflux; (iv) KOH, MeOH/H_2O, 40 °C; (v) $SOCl_2$, $AlCl_3$, DCM, room temperature/**13a**, TFAA, BF_3−Et_2O, DCM, room temperature; (vi) BBr_3, DCM, −20°C; (vii) K_2CO_3, R_1−X, Me_2CO/DMF, reflux; (viii) substituted aniline derivative, Cs_2CO_3, X-Phos, $Pd(OAc)_2$, 1,4-dioxane, *t*-BuOH, reflux. For the nature of R_1 and R_2, see Tables 1 and 2.

Scheme 3. Preparation of 9- and 10-Substituted 3-(Phenylamino)dibenzo[*b*,*e*]oxepin-11(6*H*)-ones 3a−g,j−o and 4a−d[a]



[a]Reagents and conditions: **a**, 5-NO_2, **b**, 6-NO_2; (i) Pd/C, H_2, ethyl acetate, room temperature; (ii) $NaNO_2$, H_2SO_4, H_2O, −10 °C → reflux; (iii) K_2CO_3, CH_3I, DMF, 50 °C; (iv) NBS, CCl_4, 72 °C; (v) K_2CO_3, 3-chlorophenole, Me_2CO, reflux; (vi) KOH, MeOH/H_2O, 40 °C; (vii) TFAA, BF_3−Et_2O, DCM, rt/**24b**, $SOCl_2$, $AlCl_3$, DCM, rt; (viii) BBr_3, DCM, −20°C; (ix) K_2CO_3, R_1−X, Me_2CO/DMF, reflux; (x) substituted aniline, Cs_2CO_3, X-Phos, $Pd(OAc)_2$, 1,4-dioxane, *t*-BuOH, reflux. For the nature of R_1 and R_2, see Tables 5−9.

be a challenging step in this synthetic route. It was accomplished by the use of boron tribromide in dichloromethane at low temperatures. The obtained phenol derivatives **14a** and **14b** (Scheme 2) as well as **25a** and **25b** (Scheme 3) served as key intermediates for the preparation of the final test compounds **1a−h,k,l**, **2a−k,n**, **3a−g,j−o**, and **4a−d**. The hydrophilic residues were introduced via a typical nucleophilic substitution with the respective phenols of choice, halogen alkanes or tosyl-protected 2,2-dimethyl-1,3-dioxolanes under basic conditions (compounds **15a−d** and **16a−e** in Scheme 2 and compounds **26a−h** and **27a−d** in Scheme 3).[12,14]

Finally, the introduction of the substituted anilines and phenols, resulting in the formation of the substituted 3-(phenylamino)dibenzo[*b*,*e*]oxepin-11(6*H*)-one and 3-(phenoxy)dibenzo[*b*,*e*]oxepin-11(6*H*)-one derivatives **1a−h,k,l**, **2a−k,n**, **3a−g,j−o**, and **4a−d** proved to be the key step in the synthesis. For this purpose, a palladium-catalyzed amination reaction using dicyclohexyl(2′,4′,6′-triisopropyl-1,1′-

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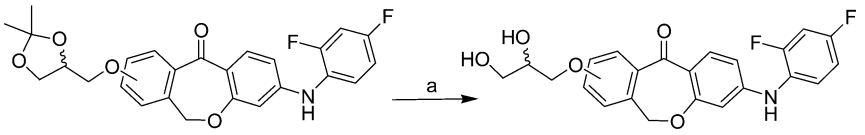
Scheme 4. Preparation of the Dihydroxy Substituted 3-(Phenylamino)dibenzo[*b*,*e*]oxepin-11(6*H*)-ones 1i,j, 2l,m, 3h,i, and 4e,f[a]

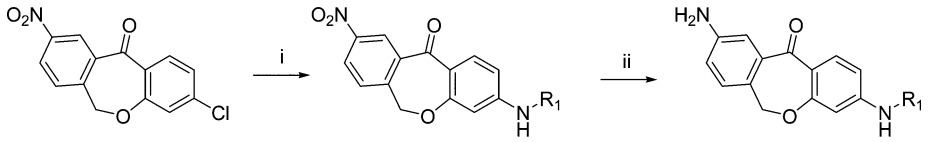


1g: 7-(4*R*)-4-ethyl-2,2-dimethyl-1,3-dioxolane
1h: 7-(4*S*)-4-ethyl-2,2-dimethyl-1,3-dioxolane
2j: 8-(4*R*)-4-ethyl-2,2-dimethyl-1,3-dioxolane
2k: 8-(4*R*)-2,2-dimethyl-4-propyl-1,3-dioxolane
3f: 9-(4*R*)-4-ethyl-2,2-dimethyl-1,3-dioxolane
3g: 9-(4*S*)-ethyl-2,2-dimethyl-1,3-dioxolane
4c: 10-(4*R*)-4-ethyl-2,2-dimethyl-1,3-dioxolane
4d: 10-(4*S*)-4-ethyl-2,2-dimethyl-1,3-dioxolane

1i: 7-(2*S*)-propane-1,2-diol
1j: 7-(2*R*)-propane-1,2-diol
2l: 8-(2*S*)-propane-1,2-diol
2m: 8-(2*R*)-butane-1,2-diol
3h: 9-(2*S*)-propane-1,2-diol
3i: 9-(2*R*)-propane-1,2-diol
4e: 10-(2*S*)-propane-1,2-diol
4f: 10-(2*R*)-propane-1,2-diol

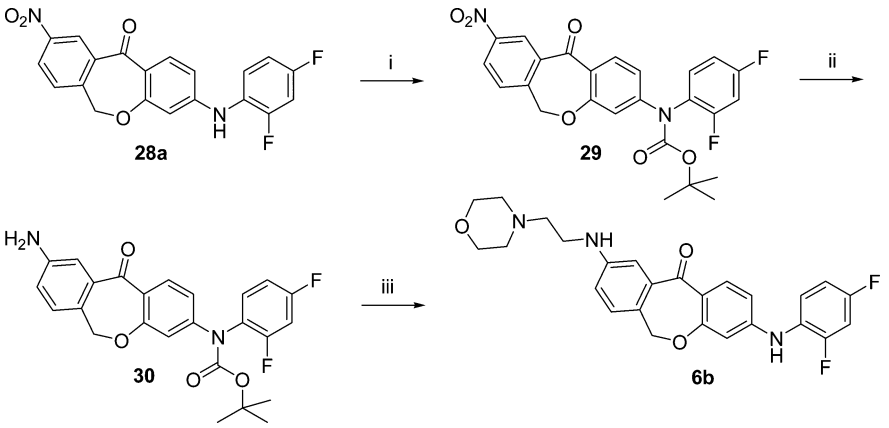
[a]Reagents and conditions: (a) TsOH, $MeOH/H_2O$, reflux.

Scheme 5. Preparation of the Substituted 9-Amino(phenylamino)dibenzo[*b*,*e*]oxepin-11(6*H*)-ones 5a−g[a]



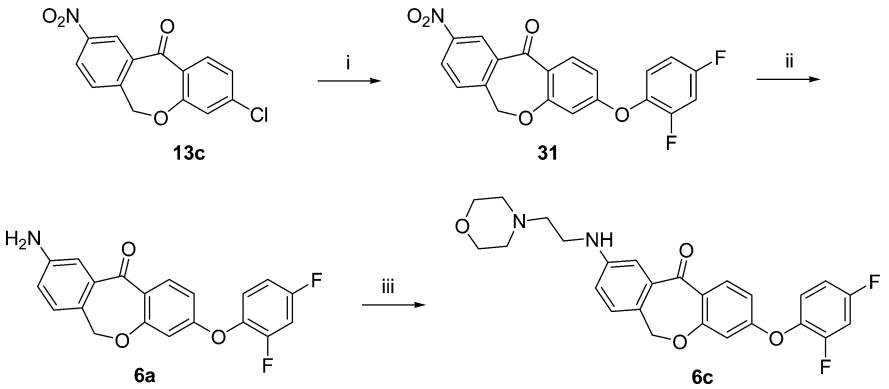
[a]Reagents and conditions: (i) substituted aniline derivative, Cs_2CO_3, X-Phos, $Pd(OAc)_2$, 1,4-dioxane, *t*-BuOH, reflux; (ii) Pd/C, H_2, ethyl acetate, rt. For the nature of R_1, see Tables 3 and 4.

Scheme 6. Preparation of Compound 6b[a]



[a]Reagents and conditions: (i) Boc_2O, DMAP, toluene, reflux; (ii) Pd/C, H_2, ethyl acetate, rt; (iii): K_2CO_3, 4-(2-chloroethyl)morpholine hydrochloride, KI, microwave, 200 W, 110 °C, MeCN.

Scheme 7. Preparation of Compounds 6a,c[a]



[a]Reagents and conditions: (i) 2,4-difluorophenole, Cs_2CO_3, *t*BuX-Phos, $Pd(OAc)_2$, 1,4-dioxane, reflux; (ii) Pd/C, H_2, ethyl acetate, rt; (iii) K_2CO_3, 4-(2-chloroethyl)morpholine hydrochloride, KI, microwave, 200 W, 110 °C, MeCN.

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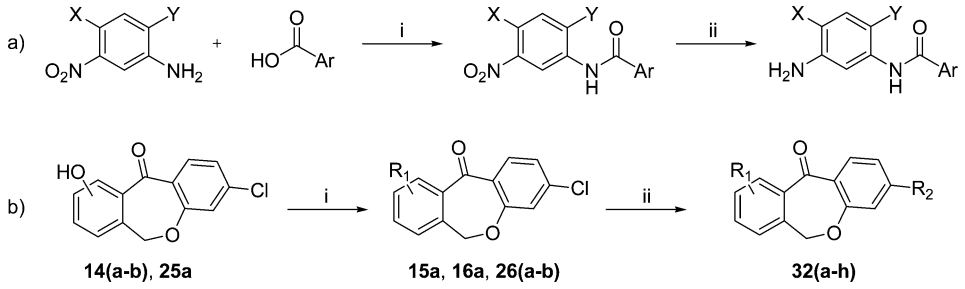
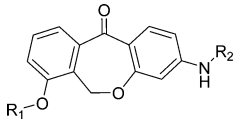
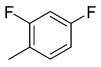
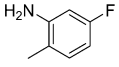
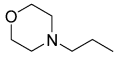
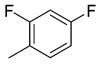
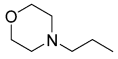
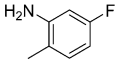
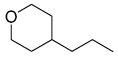
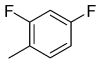
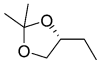
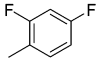
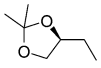
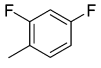
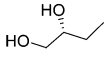
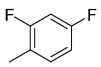
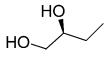
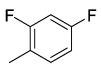
Scheme 8. Preparation of the Diarylamides (a) and of the Disubstituted Dibenzo[*b*,*e*]oxepin-11(6*H*)-ones 32a–h Targeting the Activation Loop (b)[a]



[a]Reagents and conditions: (path a) (i) $(COCl)_2$, Et_3N, $AlCl_3$, DCM, rt; (ii) Pd/C, H_2, EtOAc, rt; (path b) (i) K_2CO_3, R_1–X, Me_2CO/DMF, reflux; (ii) diarylamide, X-Phos, $Pd(OAc)_2$, Cs_2CO_3, 1,4-dioxane/*t*-BuOH (5:1), 110 °C. For the nature of X, Y, Ar, R_1, and R_2, see Table 9.

Table 1. Biological Activity of the 7- and 3-Substituted Dibenzo[*b*,*e*]oxepin-11(6*H*)-ones 1a–j[c]



Compound	R_1	R_2	IC_{50} ± SEM p38α[a] [μM]	IC_{50} ± SEM TNF-α[b] [μM]
1a			0.063 ± 0.005	44.7% @ 100 μM
1b			0.094 ± 0.012	n. d.
1c			0.087 ± 0.025	n. d.
1d			0.034 ± 0.002	32.2% @ 100 μM
1e			0.072 ± 0.004	38.8% @ 100 μM
1f			0.571 ± 0.016	n. d.
1g			0.563 ± 0.029	48.6% @ 100 μM (n = 2)
1h			0.481 ± 0.021	42.4% @ 100 μM
1i			0.025 ± 0.004	90.41 ± 13.47
1j			0.042 ± 0.003	48.5% @ 100 μM

[a]Results from three experiments. [b]Results from four experiments unless otherwise stated. n. d.: not determined. [c]Variation of the hydrophilic residue R_1 and the arylamino residue R_2.

biphenyl-2-yl)phosphine (X-Phos) as ligand was applied (Schemes 2 and 3).[18]

The conversion of the 2,2-dimethyl-1,3-dioxolane derivatives **1g,h**, **2j,k**, **3f,g**, and **4c,d** to the corresponding 2,3-dihydroxypropyl or 3,4-dihydroxybutyl derivatives **1i,j**, **2l,m**, **3h,i**, and **4e,f** was achieved by a *p*-toluenesulfonic acid catalyzed reaction in methanol and water (Scheme 4).[12]

With regard to the synthesis of the substituted 9-amino(phenylamino)dibenzo[*b*,*e*]oxepin-11(6*H*)-ones **5a–g** (Scheme 5), the compound 3-chloro-9-nitrodibenzo[*b*,*e*]oxepin-11(6*H*)-one **13c**, which was prepared in five steps starting from the commercially available 5-nitro-2-methylbenzoic acid in analogy to the above-mentioned route in Scheme 2, served as intermediate for the introduction of several substituted anilines

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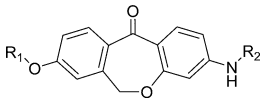
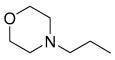
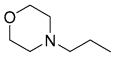
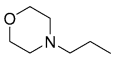
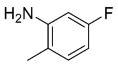
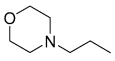
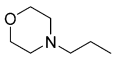
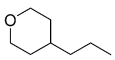
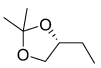
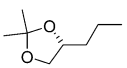
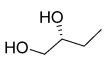
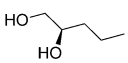
Table 2. Biological Activity of the 8- and 3-Substituted Dibenzo[*b*,*e*]oxepin-11(6*H*)-ones 2a−m[c]



Compound	R_1	R_2	IC_{50} ± SEM p38α[a] [μM]	IC_{50} ± SEM TNF-α[b] [μM]
2a			0.218 ± 0.042	n. d.
2b			0.132 ± 0.010	71.79 ± 3.05
2c			0.033 ± 0.002	71.79 ± 1.97
2d			0.147 ± 0.020	58.73 ± 3.81 (n=2)
2e			0.041 ± 0.004	94.80 ± 5.64
2f			0.085 ± 0.006	30.3% @ 100μM
2g			0.160 ± 0.040	n. d.
2h			0.557 ± 0.033	n. d.
2i			0.053 ± 0.003	38.08 ± 1.83
2j			0.665 ± 0.080	91.19 ± 0,032
2k			0.516 ± 0.030	n. d.
2l			0.027 ± 0.002	28.19 ± 6.39
2m			0.021 ± 0.005	37.94 ± 5.90

[a]Results from three experiments. [b]Results from four experiments unless otherwise stated. n. d.: not determined. [c]Variation of the hydrophilic residue R_1 and the phenylamino residue R_2.

via Buchwald−Hartwig coupling (compounds **28a−g**). Subsequent reduction of the nitro group with palladium on activated carbon led to the respective amino compounds **5a−g** (Scheme 5).

To examine the influence of the heteroatom at the C3-position in combination with different hydrophilic residues at position 9 on the biological activity of the dibenzo[*b*,*e*]oxepin-11(6*H*)-one derivatives against p38α MAP kinase, both amino-substituted scaffolds **30** (Scheme 6) and **6a** (Scheme 7) were synthesized in a similar way. In the case of intermediate **30**, the reduction of the nitro group of compound **29**, using Pd/C and H_2, is preceded by a necessary protection of the diaryl amine of compound **28a** with a *tert*-butyloxycarbonyl group (Scheme 6). In analogy to the synthesis of compound **28a**, the corresponding 2,4-difluorophenoxy derivative **31** was obtained by a modified Buchwald−Hartwig coupling with *t*BuX-Phos as ligand (Scheme 7). The introduction of a 2-morpholin-4-ylethyl residue and the simultaneous deprotection of the diaryl amine nitrogen of compound **30** to yield both test compounds **6b** and **6c** were achieved by utilizing a potassium iodide catalyzed microwave reaction at 110 °C in acetonitrile (Schemes 6 and 7).[19]

In the last step of the optimization study of p38α inhibitors, benzamide moieties targeting the activation loop of p38[21] were introduced in position 3 and connected to promising hydrophilic residues to yield the final test compounds **32a−h** (Scheme 8). The introduction of promising benzamide moieties to the phenyl ring, which occupies hydrophobic region I, emerges from our previous publications,[15,20−22] which report on the successful design of novel dibenzosuberone inhibitors of p38α MAPK, displaying subnanomolar IC_{50} values with respect to p38α MAPK and low nanomolar IC_{50} values with respect to TNF-α release in whole blood.[22] As shown in Scheme 8, the required

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diarylamides were prepared by activating the respective benzoic acids with oxalyl chloride, followed by the nucleophilic attack of the nitro-substituted anilines to form a stable amide bond. After reduction of the nitro group with Pd/C and H_2, the resulting benzamides were coupled with the modified main scaffolds **15a**, **16a**, and **26a,b** via Buchwald–Hartwig coupling to obtain the respective target compounds **32a–h** (Scheme 8).[21,22]

BIOLOGICAL RESULTS AND DISCUSSION

In order to investigate the inhibitory effect of the synthesized compounds on p38α, both an activity based assay system, using the isolated kinase domain, as well as a human whole blood TNF-α release assay were utilized.[23,24] The results of the whole blood assays may differ from the in vitro enzyme assay results by additionally factors, such as solubility, plasma protein binding, cell permeability, and ATP concentration, all influencing heavily the in vitro potency of the test compound. The serum albumins are the major protein transporters in blood and have the ability to bind to small molecules reversibly such as drugs and amino acids, leading to the target tissue. The lead compound **7** exhibited a moderate inhibitory activity in the p38α MAP kinase assay (IC_{50} = 0.239 μM) but, unfortunately, only a weak biological effect in the TNF-α whole blood assay (IC_{50} = 22 μM) (Figure 1). By introducing hydrophilic residues at positions 7, 8, 9, and 10, the biological effect with respect to the TNF-α release assay was expected to be increased.

7- And 8-Substituted 3-Phenylaminodibenzo[*b,e*]-oxepin-11(6*H*)-ones. The introduction of small moieties at position 7, e.g., methoxy (compound **1a**, IC_{50} = 63 nM, p38α kinase assay), leads to an improvement in inhibitory activity compared to **7** (Table 1). Optimization efforts concerning the arylamino residues by replacing fluorine for an amino group (compounds **1b** and **1c**) did not improve the biological activity. Therefore, the 2,4-difluorophenylamino part was used for further optimization. The 2-morpholin-4-ylethoxy substituted compounds **1d** and **1e** revealed excellent biological activity down to an IC_{50} value of 34 nM (compound **1d**). The replacement of the basic morpholine with a tetrahydro-2*H*-pyran in compound **1f** leads to a dramatic loss in biological activity (IC_{50} = 571 nM, Table 1). The (2*R*)- and (2*S*)-2,3-dihydroxypropyl compounds **1i** and **1j** illustrated the most significant inhibitory effect within this series, showing IC_{50} values of 25 nM (compound **1i**) and 42 nM (compound **1j**), whereas the corresponding (4*S*)- and (4*R*)-2,2-dimethyl-1,3-dioxolanes **1g** and **1h** are characterized by loss of biological activity (Table 1). The stereoisomers have almost the same biological activity. However, the activity of compounds with different groups (diol, 1,3-dioxolane) is very different.

In contrast to the optimized biological activity in the p38α enzyme assay, the compounds **1a–j** were only weak inhibitors of the TNF-α release. Solely, compound **1i** exhibited an IC_{50} value below 100 μM in the TNF-α whole blood assay (Table 1).

Substitutions on position 8 led to comparable inhibitory effects (Table 2), revealing a distinct structure–activity relationship. The methoxy-substituted 2-(aminophenyl)amino derivative **2b** (IC_{50} = 132 nM) showed an improved biological activity compared to the 2,4-diflourophenylamino compound **2a** (IC_{50} = 218 nM). In comparison with the reference compound **7**, the activity of compound **2b** was increased by less than a factor of 2, leading to an IC_{50} value of 132 nM (Table 2). It could be shown that within the morpholin-4-ylethoxy substituted series **2c–g**, the 2,4-difluorophenylamino compound **2c** demonstrated the best biological activity (IC_{50} = 33 nM) and was therefore used for further optimization. Notably, the replacement of the 2-amino group for a 2-methyl group to obtain compound **2f** leads to an IC_{50} value of 85 nM, which represents an improvement by a factor of 2 compared to compound **2d** (Table 2).

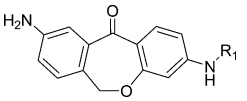
The exchange of the morpholin-4-ylethoxy nitrogen for carbon (compound **2h**) results in a loss of biological activity (IC_{50} = 557 nM), whereas the conversion of the morpholine to a dimethylaminoethoxy group (compound **2i**) did not decrease the activity (IC_{50} = 53 nM). The introduction of dihydroxypropyl (compound **2l**) and dihydroxybutyl (compound **2m**) residues is associated with the best inhibitory activity in this series (Table 2). This observation indicates that the length of the diol chain may not be crucial for the generated biological activity. Both compounds showed comparable activity with an IC_{50} value of 27 nM (compound **2l**) and 21 nM (compound **2m**), respectively, while the corresponding 2,2-dimethyl-1,3-dioxolanes **2j** and **2k** showed only weak biological activity (IC_{50} = 665 nM for compound **2j** and IC_{50} = 516 nM for compound **2k**; Table 2).

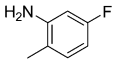
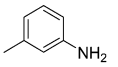
The results of the TNF-α whole blood assay demonstrated that the position-8-substituted compounds **2a–m** are weak inhibitors of TNF-α release (Table 2). Nevertheless, some IC_{50} values below 50 μM were determined, which represents an improvement compared to the position-7-substituted compounds **1a–j** (Table 1). The (2*S*)-2,3-dihydroxypropyl-substituted compound **2l** showed the best activity in the TNF-α release assay, with an IC_{50} value of 28 μM, which is comparable to that of compound **7**.

In summary, substitutions at positions 7 and 8 lead to an improvement of the biological activity in an activity-based p38α MAP kinase assay by more than factor 10. Surprisingly, the TNF-α release inhibition in the human whole blood assay could not be improved as evaluated.

9-Substituted 3-(Phenylamino)dibenzo[*b,e*]oxepin-11(6*H*)-ones. At first, we concentrated on the introduction of small hydrophilic residues like an amino group (compounds **5a–g**). Within this series, the phenylamino residue was further optimized (Tables 3 and 4).

Table 3. Biological Activity of the 3-Substituted 9-Aminodibenzo[*b,e*]oxepin-11(6*H*)-ones 5a–d[c]



Compound	R_1	IC_{50} ± SEM p38α[a] [μM]	IC_{50} ± SEM TNF-α[b] [μM]
5a		0.043 ± 0.007	1.944 ± 0.169
5b		0.151 ± 0.005	1.17 ± 0.28
5c		0.205 ± 0.011	0.683 ± 0.189
5d		0.651 ± 0.033	2.430 ± 0.488

[a]Results from three experiments. [b]Results from four experiments unless otherwise stated. [c]Variation of the arylamino residue R_1.

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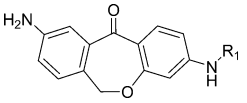
Table 4. Biological Activity of the 3-Substituted 9-Aminodibenzo[*b*,*e*]oxepin-11(6*H*)-ones 5a,e−g[c]



Compound	R_1	IC_{50} ± SEM p38α[a] [μM]	IC_{50} ± SEM TNF-α[b] [μM]
5a		0.043 ± 0.007	1.94 ± 0.169
5e		0.111 ± 0.008	4.54 ± 0.510
5f		0.103 ± 0.005	5.70 ± 0.350
5g		0.074 ± 0.009	7.81 ± 0.423

[a]Results from three experiments. [b]Results from four experiments unless otherwise stated. [c]Variation of the phenylamino residue R_1.

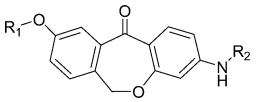
We investigated the biological activity of the 2,4-fluorophenylamino residue of compound **5a**, which showed an excellent inhibition of p38α with an IC_{50} value of 43 nM (Table 3). The replacement of the 2-fluoro group with an amino group to form compound **5b** is combined with a decrease in biological activity by more than a factor of 3. The absence of all fluorine groups leads to the 2- and 3-aminophenylamino compounds **5c** and **5d**, resulting in a total loss of activity. In particular, compound **5d** shows a significantly decreased activity (IC_{50} = 651 nM, Table 3).

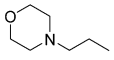
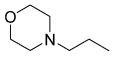
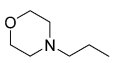
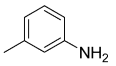
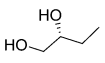
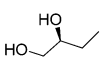
In the following step of optimization, we studied the influence of the fluorine groups of the phenylamino residue on the biological activity (Table 4).

We synthesized the 2- and 4-fluorophenylamino derivatives **5e** and **5f**. Both compounds showed a similar activity (IC_{50} = 111 nM for compound **5e** and IC_{50} = 103 nM for compound **5f**). In order to investigate the influence of the number of fluorine groups on the biological activity, we tested the 2,4,5-trifluorophenylamino compound **5g** (IC_{50} = 74 nM). We conclude that the 2,4-difluorophenylamino residue in compound **5a** is the optimal motif to occupy the hydrophobic region I and it is therefore used as standard for further optimization (Table 5).

The combination of 2,4-difluorophenylamino and 2-morpholin-4-yl-ethoxy groups (compound **3c**) leads to an excellent biological activity (IC_{50} = 21 nM). The corresponding 2- and 3-aminophenylamino residues (compounds **3d** and **3e**) displayed only moderate inhibitory activity (IC_{50} = 107 nM for compound **3d** and IC_{50} = 146 nM for compound **3e**).

Table 5. Biological Activity of the 9- and 3-Substituted Dibenzo[*b*,*e*]oxepin-11(6*H*)-ones 3a−i[c]



Compound	R_1	R_2	IC_{50} ± SEM p38α[a] [μM]	IC_{50} ± SEM TNF-α[b] [μM]
3a			0.115 ± 0.018	4.71 ± 1.33
3b			0.122 ± 0.002	1.85 ± 0.021
3c			0.021 ± 0.0006	0.429 ± 0.016
3d			0.107 ± 0.017	0.490 ± 0.021
3e			0.146 ± 0.007	0.684 ± 0.017
3f			0.207 ± 0.022	1.44 ± 0.369
3g			0.048 ± 0.005	1.30 ± 0.183
3h			0.061 ± 0.002	0.591 ± 0.194
3i			0.014 ± 0.001	0.233 ± 0.024

[a]Results from three experiments. [b]Results from four experiments unless otherwise stated. [c]Variation of the hydrophilic residue R_1 and the arylamino residue R_2.

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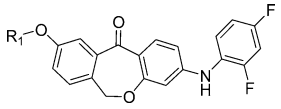
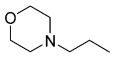
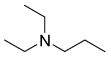
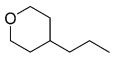
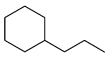
In the series of the 3- and 9-substituted dibenzo[*b*,*e*]oxepin-11(6*H*)-ones **3a**–**i** (Table 5), the (2*R*)- and (2*S*)-2,2-dimethyl-1,3-dioxolane-substituted derivatives **3f** and **3g** displayed a good inhibition of p38α down to an IC_{50} value of 48 nM (compound **3g**) and 207 nM (compound **3f**).

The corresponding (2*R*)- and (2*S*)-dihydroxypropoxy derivatives **3h** and **3i** showed an excellent activity down to an IC_{50} value of 14 nM (compound **3i**), which is an improvement by a factor of about 17 with regard to compound **7**. Interestingly, the stereochemistry appears to play a role in this case. The inhibitory effect of the (2*R*)- and (2*S*)-dihydroxypropoxy derivatives **3h** and **3i** differs by more than factor 4 (IC_{50} = 61 nM for compound **3h** and IC_{50} = 14 nM for compound **3i**, Table 5).

In the next step, we studied the influence of the heteroatoms in the 2-morpholin-4-yl-ethoxy group (Table 6). The elimination of the oxygen bridge of compound **3c** leads to the diethylaminoethoxy compound **3j** (IC_{50} = 101 nM), which showed a 5-fold reduced biological activity. The replacement of the nitrogen for a carbon also decreases the biological activity (compound **3k**, IC_{50} = 79 nM). In the case of complete absence of heteroatoms, as for 2-cyclohexylethoxy compound **3l**, a major loss of activity in the p38α enzyme assay was observed (IC_{50} > 10 μM, Table 6).

Table 6. Biological Activity of the 9-Substituted 3-(Difluorophenylamino)dibenzo[*b*,*e*]oxepin-11(6*H*)-ones 3c,j–l[c]



Compound	R1	IC_{50} ± SEM p38α[a] [μM]	IC_{50} ± SEM TNF-α[b] [μM]
3c		0.021 ± 0.001	0.429 ± 0.016
3j		0.101 ± 0.009	1.06 ± 0.090
3k		0.079 ± 0.001	0.364 ± 0.092
3l		41.3% @ 10 μM	18.57 ± 1.24

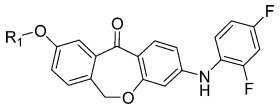
[a]Results from three experiments. [b]Results from four experiments unless otherwise stated. [c]Variation of the hydrophilic residue R_1.

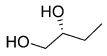
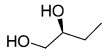
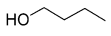
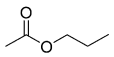
Furthermore, the linker between the 2,4-difluorophenyl residue and the tricyclic ring system is modified (Table 8).

At first, the diarylamine of compound **5a** (IC_{50} = 43 nM) was changed to a phenoxy ether (compound **6a**), resulting in a loss of inhibitory activity by a factor of approximately 17 (IC_{50} = 763 nM for compound **6a**).

Subsequently, the oxygen atom of the morpholin-4-yl-ethoxy residue at position 9 of compound **3c** was substituted for a nitrogen atom during formation of compound **6b**. The observed IC_{50} value of 28 nM was comparable to the oxygen derivative **3c**

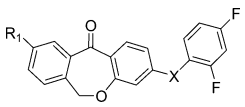
Table 7. Biological Activity of the 9-Substituted (2,4-Difluorophenylamino)dibenzo[*b*,*e*]oxepin-11(6*H*)-ones 3h–i, 3m–o[c]

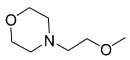
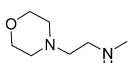
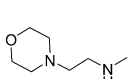


Compound	R_1	IC_{50} ± SEM p38α[a] [μM]	IC_{50} ± SEM TNF-α[b] [μM]
3h		0.061 ± 0.002	0.591 ± 0.194
3i		0.014 ± 0.001	0.233 ± 0.024
3m		0.020 ± 0.002	0.588 ± 0.051
3n		0.008 ± 0.001	0.631 ± 0.091
3o		0.022 ± 0.002	n. d.

[a]Results from three experiments. [b]Results from four experiments except unless stated. n. d.: not determined. [c]Variation of the hydrophilic residue R_1.

Table 8. Biological Activity of the 9- and 3-Substituted Dibenzo[*b*,*e*]oxepin-11(6*H*)-ones 3c, 5a, 6a–c[c]



Compound	R_1	X	IC_{50} ± SEM p38α[a] [μM]	IC_{50} ± SEM TNF-α[b] [μM]
5a		NH	0.043 ± 0.007	1.94 ± 0.169
6a		O	0.763 ± 0.075	n. d.
3c		NH	0.021 ± 0.0006	0.429 ± 0.016
6b		NH	0.028 ± 0.001	1.10 ± 0.070
6c		O	0.819 ± 0.072	n. d.

[a]Results from three experiments. [b]Results from four experiments unless otherwise stated. n. d.: not determined. [c]Variation of the hydrophilic residue R_1 and the heteroatom X.

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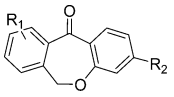
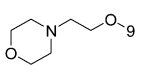
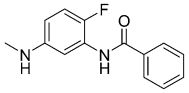
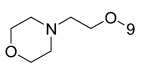
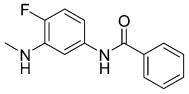
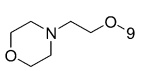
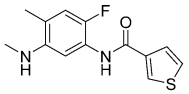
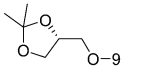
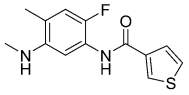
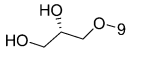
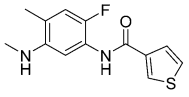
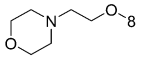
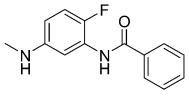
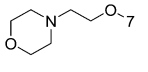
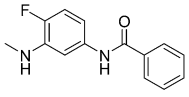
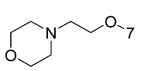
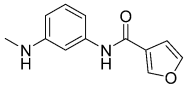
Table 9. Biological Activity of the Disubstituted Dibenzo[*b*,*e*]oxepin-11(6*H*)-ones 32a–h Targeting the Activation Loop[c]



Compound	R_1	R_2	IC_{50} ± SEM p38α[a] [μM]	IC_{50} ± SEM TNF-α[b] [μM]
32a			0.001 ± 0.0002	34% @ 100μM
32b			0.003 ± 0.0006	1.56 ± 0.235
32c			0.0017 ± 0.0001	0.148 ± 0.011
32d			0.0018 ± 0.0001	0.287 ± 0.070
32e			0.0016 ± 0.0001	0.125 ± 0.010
32f			0.017 ± 0.005	36% @ 100μM
32g			0.010 ± 0.001	39% @ 100μM
32h			0.025 ± 0.002	51.38 ± 1.03

[a]Results from three experiments. [b]Results from four experiments unless otherwise stated. [c]Variation of the hydrophilic residue R_1 and the benzamide moiety R_2.

(IC_{50} = 21 nM). Therefore, an exchange of the heteroatom at position 9 has no major influence on the biological activity.

The combination of both substitution patterns (compound **6c**) leads to a considerable decrease of the biological activity up to an IC_{50} value of 819 nM (Table 8).

It was demonstrated that the 9-substituted compounds reveal a highly improved activity in the human whole blood TNF-α assay compared to compound **7**. Even small hydrophilic moieties like an amino group in compound **5a** (IC_{50} = 1.94 μM, Table 3) or a hydroxyl group in compound **3b** (IC_{50} = 1.85 μM, Table 5) lead to an improvement by a factor of 11 with respect to compound **7**.

The introduction of the morpholin-4-ylethoxy residue (compound **3c**) resulted in an IC_{50} value of 429 nM in the TNF-α release assay, which is an improvement by a factor of 50 related to the reference compound **7** (Table 5). In the series of position-9-substituted compounds, the (2*R*)-dihydroxypropoxy-decorated molecule **3i** showed a nearly 100-fold higher activity than compound **7** in the TNF-α release assay with an IC_{50} value of 233 nM (Table 5).

10-Substituted 3-(Phenylamino)dibenzo[*b*,*e*]oxepin-11(6*H*)-ones. In the next step of this work, hydrophilic residues were introduced at position 10 (Table S1 in the Supporting Information).

It could be shown that only small hydrophilic moieties like the methoxy group in compound **4a** are tolerated (IC_{50} = 0.280 μM). Larger moieties like ethoxymorpholine (compound **4b**) or (2*R*)- and (2*S*)-dihydroxypropoxy (compounds **4e** and **4f**) lead to a substantial loss of activity (IC_{50} values above 10 μM).

Disubstituted Dibenzo[*b*,*e*]oxepin-11(6*H*)-ones Targeting the Activation Loop. According to our previous publications,[15,21,22] the presence of benzamide compounds **32a–h** shows IC_{50} values in the low nanomolar range (Table 9) in an activity-based p38α assay. However, derivatives with hydrophilic residues in position 9 are characterized by a slightly higher activity. The most convincing compound of this series (**32a**) showed an IC_{50} value of 1 nM.

In the human whole blood assay, compounds **32a–h** vary in their activity from 125 nM (compound **32e**) to 34% inhibition at 100 μM (compound **32a**). Surprisingly, the substitution position of the hydrophilic residue is most likely not essential with respect to the TNF-α release inhibition. Compound **32e** shows the

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strongest activity regarding TNF-α release in the series of the disubstituted dibenzo[b,e]oxepin-11(6H)-ones so far.

Complex Crystal Structure of Compound 32a in p38α. Using protein X-ray crystallography, we investigated the binding mode of compound **32a** in complex with p38α MAP kinase to confirm the proposed general binding mode (Figure 3). The

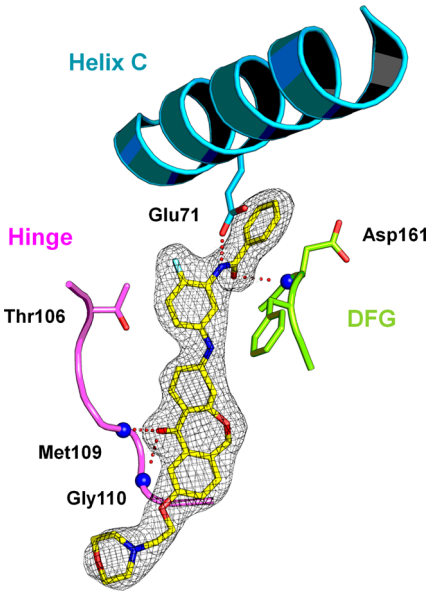


Figure 3. Crystal structure of p38α in complex with **32a** at 2.1 Å resolution. The inhibitor binds to the ATP pocket of p38α by forming hydrogen bonds (red dotted lines) to the peptide backbone of the hinge region (pink) (Met109, Gly110). In addition, the allosteric back pocket is occupied by the phenylamide moiety and further hydrogen bonds are formed to Glu71 from helix C (cyan) and Asp168 in the DFG-motif (green). The DFG-motif adopts an in-between conformation, in which Phe169 is directed toward the glycine-rich loop. Electron density map ($2F_o - F_c$) is contoured at 1σ (PDB ID: 4L8M).

inhibitor binds in a type II manner, accessing both allosteric and ATP-binding pocket. Thereby, the kinase is locked in its inactive conformation with the DFG-motif adopting an *in-between* state, which is characterized by the orientation of Phe169 toward the glycine-rich loop. Furthermore, the dibenzoxepinone derivative **32a** forms hinge region contacts with the peptide backbone NH's of Met109 and Gly110, which induce the characteristic glycine-flip as previously reported for this compound class. This specific interaction was shown to ensure selectivity for p38α over other kinases, bearing less flexible amino acid residues at this position.[11] Furthermore, the inhibitor's amide moiety, embedded between DFG-motif and helix C, addresses Glu71 as well as Asp168 with hydrogen bonds.

Selectivity Screen. To confirm that the modifications in comparison to the selective dibenzosuberone compounds[11,22] were not detrimental with respect to selectivity, compound **32e** was screened against 333 kinases[26] (Kinase WholePanelProfiler, Proqinase; see Supporting Information for further information). At a concentration of 10 μM, the compound **32e** inhibits—besides p38α and p38β—only two other kinases >50%: SLK and CAMK2D. However, only p38α and β were inhibited substantially. Therefore, compound **32e** can be considered as extremely selective.

ATP Competitiveness.[22] X-ray crystallographic analysis exhibited that the inhibitor stabilizes the DFG-out conformation of p38 to some extent, but does not clearly address its deep pocket. To further characterize the mode of inhibition of our compound, we investigated its ATP competitiveness. In order to differentiate between DFG-in and DFG-out binders, we used a modified enzyme assay using increased ATP concentration: loss of inhibitory activity will be less for the latter, while the classical in-binders will more affected by high ATP concentration. Even at high concentrations of ATP (300 μM, Table 10), compound **32e**

Table 10. Correlation of Inhibitory Activity and Concentration of ATP

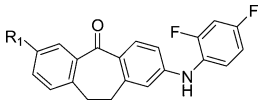
	IC_{50} ± SEM p38α[a]	
compd	100 μM ATP	300 μM ATP
32e	0.0016 ± 0.0001	0.004 ± 0.0001
SB203580	0.048 ± 0.004	0.723 ± 0.019

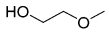
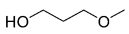
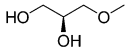
[a]Results from three experiments.

is extremely potent. In contrast, the traditional DFG-in binder SB203580 shows only a moderate inhibition at 300 μM ATP (IC_{50} = 723 nM). All in all, this experimental setup confirmed a low ATP competitiveness for the compound **32e**.

Metabolic Stability in Microsomes. In order to investigate the metabolic stability of a series of dibenzosuberone inhibitors **33** and **33a**–**e** as well as of the dibenzo[b,e]oxepin-11(6H)-one derivative **3i** in vitro (Table 11), the biotransformation with liver

Table 11. Metabolic Stability of a Series of Dibenzosuberone Inhibitors after 180 min Incubation



Compound	R_1	Metabolic stability	IC_{50} ± SEM p38α[a]	IC_{50} ± SEM TNF-α[b]
		[%]	[μM]	[μM]
33	H—	43	0.114 ± 0.001	1.907 ± 0.123
33a	HO—	70	0.049 ± 0.005	1.247 ± 0.147
33b		85	0.044 ± 0.014	1.647 ± 0.172
33c		86	0.019 ± 0.003	0.583 ± 0.019
33d		90	0.013 ± 0.000	0.191 ± 0.008
33e		100	0.017 ± 0.003	0.052 ± 0.003

[a]Results from three experiments. [b]Results from four experiments except unless stated.

microsomes from rats and humans was examined. To compare the rates of substrate degradation, the microsomal protein content was standardized (1 mg/mL). Immediately after initiation of the reaction as well as after 10, 20, 40, 60, 120, and 180 min, aliquots were withdrawn and prepared for LC–MS analysis. The metabolite, as demonstrated with the example of compound **33** in Figure 2, could be found in different amounts in liver microsomes of all examined species (Table 11). By

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introducing a hydroxy group in position 2 of the 3-hydroxypropoxy residue of the compound **33d** to obtain the corresponding derivative **33e**, the metabolite formation gets suppressed (Table 11). In addition, the dibenzo[*b*,*e*]oxepin-11(6*H*)-one derivative **3i** did not show any metabolite formation in vitro.

In Vitro Metabolism in Female and Male Rat and Human Liver Microsomes. To examine gender-specific differences, incubations of male and female pooled rat liver microsomes were compared with male and female pooled human liver microsomes. The time profile of the rat liver micrsomes showed clear-cut differences in the metabolite formation rates (Figure 4). Male rat liver microsomes produced the metabolite

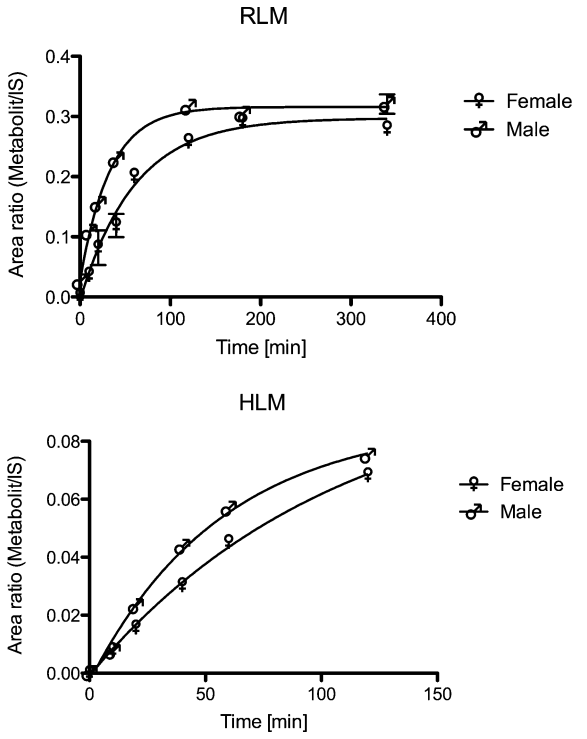

Figure 4. Time−concentration profiles for the biotransformation of compound **33** in rat liver microsomes (RLM) and human liver microsomes (HLM). Values represent the mean ± SEM of an experiment, performed in triplicates.

more quickly compared with female rat liver microsomes. In human liver microsomes, gender differences were much less distinct, especially when considering the higher P450 enzyme activity of the female microsomes and the genetic CYP2D6 polymorphism.

In Vitro Metabolism in Baculovirus-Expressed Human P450 Isoenzymes. To identify the enzymes that are involved in the biotransformation of a series of dibenzosuberone inhibitors **33** and **33a**−**e** (Table 11), incubations with 10 cytochrome P450 isoenzymes (CYP1A2, CYP2A6, CYP2B6, CYP3A4, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2J2 and CYP4F12) were performed. The dehydrogenation of all compounds (Figure 2) was catalyzed by CYP1A2 and CYP2D6, which was confirmed by incubations with the isolated P450 isoform. Incubations without NADPH and with cytochrome P450 reductase/b5 without P450 enzymes served to exclude nonenzymatic metabolite formation. CYP2D6 was identified to be the main driver of the conversion (Figure 5).

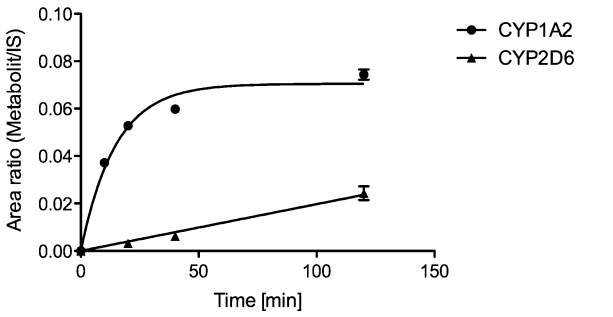

Figure 5. Time−concentration profiles for the biotransformation of compound **33** in isolated isoenzymes. Values represent the mean ± SEM of an experiment, performed in triplicates.

Pharmacokinetics of the Dibenzo[*b*,*e*]oxepin-11(6*H*)-one Derivative 3i in Mice. To investigate the rate and extent of systemic exposure of the parent drug **3i** in vivo, a dose of 10 mg/kg was orally administered to male Black 6 C57 mice. A dose formulation, suitable for intravenous administration, was not available. The plasma concentrations of compound **3i** were determined by LC−MS/MS after derivatization with 4-pyridinylboronic acid (Scheme 9). The pharmacokinetic parameters were derived by noncompartmental analysis of mean plasma concentration−time profiles (Figure 6). The individual and derived mean concentration−time profiles reflect a rapid absorption of the parent drug **3i**. A monophasic elimination was observed. In all animals, a concentration−time profile could be followed for 72 h postdose. Calculated pharmacokinetic parameters for compound **3i**, based on mean concentration−time profiles, are given in Table 12.

Identification of Phase II Metabolites of Compound 3i in Black 6 C57 Mice. Although there were no phase I metabolites found in 72 h blood, several phase II metabolites could be identified. Four dominant peaks could be detected in plasma samples with protonated molecule peaks at 603 *m*/*z* (M − 603), 605 *m*/*z* (M − 605), 732 *m*/*z* (M − 732), and 734 *m*/*z* (M − 734), respectively. The structures and characteristics of all identified in vivo metabolites are given in Table 13.

CONCLUSION

We could prove that the introduction of hydrophilic moieties at positions 7 and 8 in dibenzoxepinones leads to a substantial improvement of the biological activity in the p38α assay (Tables 1 and 2). The most potent compounds of this series, **1i** (IC_{50} = 25 nM) and **2m** (IC_{50} = 21 nM), improved the biological activity in a p38α assay by a factor of 10 compared to compound **7**, whereas the biological activity in the TNF-α release assay remains unsatisfying.

At position 10, only small moieties are tolerated, whereas bigger moieties resulted in loss of biological activity.

The compounds with large hydrophilic residues in position 9 showed the highest enhancement of the biological activity so far. The introduction of a propoxy group (compound **3n**) leads to an IC_{50} value of 8 nM, which is an improvement by a factor of 30 with regard to compound **7**.

The introduction of hydrophilic substituents at position 9 resulted in a highly improved biological activity in the human whole blood TNF-α release assay. The introduction of a (2*R*)-dihydroxypropoxy group in position 9 and of a 2,4-difluorophenylamino residue in position 3 (compound **3i**) leads to the best biological activity in the TNF-α release assay in this series, with an IC_{50} value of 233 nM. This is an improvement

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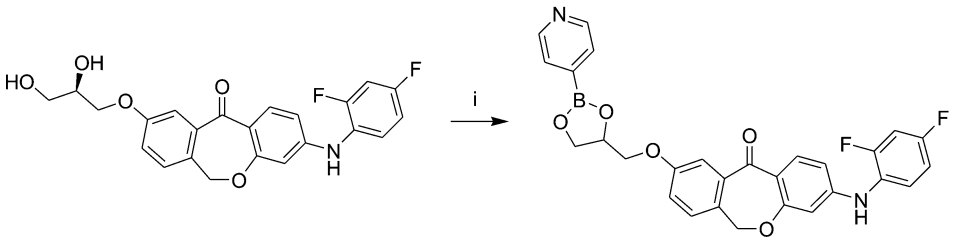
Scheme 9. Preparation of the Derivative of Compound 3i[a]



3i

[a]Reagent and conditions: (i) 4-pyridinylboronic acid, 70 °C, 40 min.

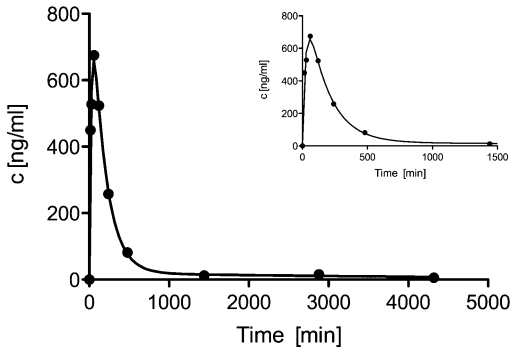


Figure 6. Mean plasma concentration−time profiles of compound **3i** in male Black 6 C57 mice after oral administration of 10 mg/kg substance. Values represent the means with error bars (±SEM).

Table 12. Calculated Pharmacokinetic Parameters of Compound 3i in Black 6 C57 Mice, Derived by Noncompartmental Analysis of Mean Concentration−Time Profile after Oral Dosage[a]

c_{max} (ng/mL)	t_{max} (h)	λ_z (1/h)	$t_{1/2}$ (h)	AUC_{0-t} (ng h/mL)	$AUC_{0-\infty}$ (ng h/mL)
626.81	1	0.0243	3.95	6594.24	6990.57

[a]All results are from three experiments.

Table 13. Compilation of the Identified Metabolites of the Compound 3i in 72 h Blood

phase II metabolite	$[M + H]^+$	proposed structural element	major responsible transferases
M − 603	603.1	glucuronic acid	UGT
M − 605	605.1	cysteine-glycine	cysteinyl glycinase
M − 732	732.1	gluthation	GST
M − 734	734.1	gluthation	GST

in activity by a factor of 100 compared to the starting compound **7**.

By combining hydrophilic substituents with benzamide moieties targeting the activation loop, the most active compound of the complete series was synthesized: Compound **32e** shows an outstanding activity in the p38 assay with an IC_{50} value as low as 1.6 nM. In addition, compound **32e** inhibits the TNF-α release with an IC_{50} value of 125 nM. Furthermore, compound **32e** is characterized by an extraordinarily low ATP competitiveness, high selectivity, and metabolic stability.

EXPERIMENTAL SECTION

General. All commercially available reagents and solvents were used without further purification. NMR data were recorded at ambient temperature either on a Bruker Avance 200 at 200 and 50 MHz or a Bruker Avance 400 at 400 and 100 MHz. Chemical shifts (δ) are reported in ppm relative to the solvent resonance. IR data were determined on a Perkin-Elmer Spectrum One spectrometer (ATR technique). High-resolution mass spectral analyses were performed on a Finnigan Sektorfield mass spectrometer using electron impact (EI) or on a Bruker APEX II with electron spray ionization (ESI). Flash chromatography was performed using a LaFlash system (VWR) with Merck silica gel (PharmPrep 60 cc 25−40 μm). Reaction progress was determined by thin-layer chromatography (TLC) using Merck silica gel 60 F_{254} plates. The purity of the final compounds was determined by HPLC on a Hewlett-Packard HP1090 series II liquid chromatograph with a Phenomenex Luna C8 (150 × 4.60 mm) column at 254 nm using a gradient of 0.01 M KH_2PO_4 (pH 2.3) and methanol with a flow rate of 1.5 mL/min. All final products were >95% pure. Metabolic and pharmacokinetic experiments were analyzed using a Micromass Quattro micro triple quadrupole mass spectrometer coupled to a JASCO HPLC system.

Synthesis of the Disubstituted Dibenzo[*b*,*e*]oxepin-11(6*H*)-ones (General Procedure A). The reaction mixture, composed of the respective dibenzo[*b*,*e*]oxepin-11(6*H*)-one, the respective aniline, Cs_2CO_3, X-Phos, and $Pd(OAc)_2$, was suspended in 1,4-dioxane (10 mL) and *t*-BuOH (2 mL) under an argon atmosphere and heated to a temperature of 110 °C. The reaction progress was monitored until TLC indicated complete conversion. The mixture was cooled to room temperature and filtered over silica gel. The organic phase was evaporated as far as possible and the resulting dark oil was then purified via flash chromatography.

3-[(2,4-Difluoroaminophenyl)amino]-7-methoxydibenzo-[*b*,*e*]oxepin-11(6*H*)-one (1a). Compound **1a** was prepared according to general procedure A using **13a** (200 mg, 0.72 mmol), 2,4-difluoroaniline (200 mg, 1.56 mmol), Cs_2CO_3 (700 mg, 2.14 mmol), X-Phos (50 mg, 0.11 mmol), and $Pd(OAc)_2$ (20 mg, 0.09 mmol). The reaction mixture was refluxed for 3 h and purified by flash chromatography (SiO_2, hexane/ethyl acetate 2:1). Yield: 70 mg (27.7%), yellow oil. HPLC: t_R = 8.72 min, purity 100%. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) = 3.87 (m, 3 H, $-OCH_3$) 5.23 (m, 2 H, C^6H_2) 6.22 (s, 1 H, C^4H) 6.59 (dd, J = 8.91, 2.21 Hz, 1 H, C^2H) 7.10 (m, 1 H, $C^{3'}H$) 7.36 (m, 5 H, $C^{5'}H$, $C^{6'}H$, $C^{10}H$, C^8H, C^9H) 7.92 (d, J = 8.97 Hz, 1 H, C^1H), 8.69 (s, 1 H, NH). HRMS-EI, m/z ($C_{21}H_{15}F_2NO_3$): calcd 367.1020, found 367.1024.

3-[(2-Aminophenyl)amino]-7-methoxydibenzo[*b*,*e*]oxepin-11(6*H*)-one (1b). Compound **1b** was prepared according to general procedure A using **13a** (500 mg, 1.82 mmol), 2-aminoaniline (300 mg, 2.80 mmol), Cs_2CO_3 (700 mg, 2.14 mmol), X-Phos (100 mg, 0.21 mmol), and $Pd(OAc)_2$ (20 mg, 0.09 mmol). The reaction mixture was heated for 2.5 h at reflux temperature and purified by flash chromatography (SiO_2, hexane/ethyl acetate 1:1). Yield: 135 mg (26.0%), yellow solid. HPLC: t_R = 7.32 min, purity 96.7%. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) = 3.86 (s, 3 H, $-OCH_3$), 4.84 (s, 2 H, $-NH_2$), 5.20 (s, C^6H_2), 6.05 (d, J = 2.02 Hz, 1 H, C^4H), 6.53 (m, 2 H, C^2H, $C^{3'}H$), 6.77 (m, 1 H, $C^{6'}H$), 6.97 (m, 2 H, $C^{4'}H$, $C^{5'}H$), 7.27 (m, 2 H, C^8H, C^9H), 7.42 (m, 1 H, $C^{10}H$), 7.89 (d, J = 8.84 Hz, 1 H, C^1H), 8.12 (s, 1 H, NH). HRMS-EI, m/z ($C_{21}H_{18}N_2O_3$): calcd 346.1332, found 346.1317.

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3-[(2,4-Difluorophenyl)amino]-7-(2-morpholin-4-ylethoxy)-dibenzo[*b*,*e*]oxepin-11(6*H*)-one (1d). Compound **1d** was prepared according to general procedure A using **15a** (300 mg, 0.80 mmol), 2,4-difluoroaniline (150 mg, 1.16 mmol), Cs_2CO_3 (700 mg, 2,14 mmol), X-Phos (50 mg, 0.11 mmol), and $Pd(OAc)_2$ (20 mg, 0.09 mmol). The reaction mixture was heated for 1.5 h at reflux temperature and purified by flash chromatography (SiO_2, dichloromethane/ethanol 95:5). Yield: 126 mg (33.7%), yellow solid. HPLC: t_R = 5.01 min, purity 100%. Mp = 63.9 °C (dec). ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) = 2.59 (t, J = 4,30 Hz, 4 H, $CH_2{}^{3/5}{}_{morpholinyl}$), 2.86 (t, J = 5.62 Hz, 2 H, $C^2{}_{ethoxy}H_2$), 3.74 (t, J = 4,41 Hz, 4 H, $C^{2/6}{}_{morpholinyl}CH_2$), 4.19 (t, J = 5.56 Hz, 2 H, $C^1{}_{ethoxy}H_2$), 5.33 (s, 2 H, C^6H_2), 5.97 (s, 1 H, NH), 6.45 (d, J = 2.27 Hz, 1 H, C^4), 6.61 (dd, J = 8.78, 2.34 Hz, 1 H, C^2), 6.88 (m, 2 H, $C^{3'}$H,$C^{6'}$H), 7.08 (d, J = 7.96 Hz, 1 H, C^8), 7.40 (m, 3 H, $C^{5'}$H, C^9H, C^{10}H), 8.14 (d, J = 8.84 Hz, 1 H, C^1). HRMS-EI, m/z ($C_{26}H_{24}F_2N_2O_4$): calcd 466.1704, found 466.1694.

3-[(2,4-Difluorophenyl)amino]-7-(2-tetrahydro-2*H*-pyran-4-ylethoxy)dibenzo[*b*,*e*]oxepin-11(6*H*)-one (1f). Compound **1f** was prepared according to general procedure A using **15b** (200 mg, 0.54 mmol), 2,4-difluoroaniline (230 mg, 1.78 mmol), Cs_2CO_3 (700 mg, 2,14 mmol), X-Phos (50 mg, 0.11 mmol), and $Pd(OAc)_2$ (10 mg, 0.05 mmol). The reaction mixture was heated for 3 h at reflux temperature and purified by flash chromatography (SiO_2, dichloromethane/ethanol 95:5). Yield: 80 mg (31.8%), yellow solid. HPLC: t_R = 9.26 min, purity 100%. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) = 1.41 (m, 2 H, $C^3{}_{tetrahydropyran}$H, $C^5{}_{tetrahydropyran}$H), 1.75 (m, 5H, $C^3{}_{tetrahydropyran}$H, $C^2{}_{Ethoxy}H_2$, $C^4{}_{tetrahydropyran}$H), 3.42 (m, 2 H, $C^2{}_{tetrahydropyran}$H, $C^6{}_{tetrahydropyran}$H), 3.97 (m, 2 H, $C^2{}_{tetrahydropyran}$H, $C^6{}_{tetrahydropyran}$H), 4.09 (t, J = 6.00 Hz, 2 H, $C^1{}_{Ethoxy}H_2$), 5.33 (s, 2 H, C^6H_2), 5.91 (s, 1 H, NH), 6.47 (d, J = 2.40 Hz, 1 H, C^4H), 6.62 (dd, J = 8.91, 2.46 Hz, 1 H, C^2H), 6.91 (m, 2 H, $C^{3'}$H, $C^{6'}$H), 7.07 (m, 1 H, $C^{5'}$H), 7.39 (m, 3 H, C^8H, C^9H, C^{10}H), 8.15 (d, J = 8.84 Hz, 1 H, C^1H). HRMS-EI, m/z ($C_{27}H_{24}F_2NO_4$): calcd 465.1751, found 465.1751.

3-[(2,4-Difluorophenyl)amino]-7-{[(4*R*)-2,2-dimethyl-1,3-dioxolan-4-yl]methoxy}dibenzo[*b*,*e*]oxepin-11(6*H*)-one (1g). Compound **1g** was prepared according to general procedure A using **15c** (200 mg, 0.53 mmol), 2,4-difluoroaniline (200 mg, 1.55 mmol), Cs_2CO_3 (700 mg, 2.14 mmol), X-Phos (50 mg, 0.11 mmol), and $Pd(OAc)_2$ (20 mg, 0.09 mmol). The reaction mixture was heated for 4 h at reflux temperature and purified by flash chromatography (SiO_2, dichloromethane/ethyl acetate 95:5). Yield: 90 mg (27.5%), yellow solid. HPLC: t_R = 8.87 min, purity 100%. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) = 1.42 (s, 3 H, $-CH_3$), 1.48 (s, 3 H, $-CH_3$), 4.09 (m, 4 H, $C^3{}_{propoxy}H_2$, $C^1{}_{propoxy}H_2$), 4.53 (m, 1 H, $C^2{}_{propoxy}$H), 5.33 (s, 2 H, C^6H_2), 5.91 (s, 1 H, NH), 6.46 (d, J = 2.27 Hz, 1 H, C^4H), 6.62 (dd, J = 8.91, 2.34 Hz, 1 H, C^2H), 6.91 (m, 2 H, $C^{6'}$H, $C^{3'}$H), 7.09 (m, 1 H, $C^{5'}$H), 7.40 (m, 3 H, C^8H, C^9H, C^{10}H), 8.14 (d, J = 8.84 Hz, 1 H, C^1H). HRMS-EI, m/z ($C_{26}H_{23}F_2NO_5$): calcd 467.1544, found 467.1561.

3-[(2,4-Difluorophenyl)amino]-7-{[(4*S*)-2,2-dimethyl-1,3-dioxolan-4-yl]methoxy}dibenzo[*b*,*e*]oxepin-11(6*H*)-one (1h). Compound **1h** was prepared according to general procedure A using **15d** (200 mg, 0.53 mmol), 2,4-difluoroaniline (200 mg, 1.55 mmol), Cs_2CO_3 (700 mg, 2.14 mmol), X-Phos (50 mg, 0.11 mmol), and $Pd(OAc)_2$ (20 mg, 0.09 mmol). The reaction mixture was heated for 4 h at reflux temperature and purified by flash chromatograph (SiO_2, hexane/ethyl acetate 2:1).Yield: 110 mg (44.2%), yellow oil. HPLC: t_R = 8.89 min, purity 100%. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) = 1.40 (s, 3 H, $-CH_3$), 1.47 (s, 3 H, $-CH_3$), 4.08 (m, 4 H, $C^3{}_{propoxy}H_2$, $C^1{}_{propoxy}H_2$), 4.51 (m, 1 H, $C^2{}_{propoxy}$H), 5.31 (s, 2 H, C^6H_2), 5.89 (s, 1 H, NH), 6.44 (d, J = 2.27 Hz, 1 H, C^4H), 6.60 (dd, J = 8.84, 2.40 Hz, 1 H, C^2H), 6.88 (m, 2 H, $C^{6'}$H, $C^{3'}$H), 7.07 (m, 1 H, $C^{5'}$H), 7.38 (m, 3 H, C^8H, C9H, C^{10}H), 8.12 (d, J = 8.84 Hz, 1 H, C^1H). HRMS-EI, m/z ($C_{26}H_{23}F_2NO_5$): calcd 467.1544, found 467.1559.

3-[(2,4-Difluorophenyl)amino]-8-methoxydibenzo[*b*,*e*]-oxepin-11(6*H*)-one (2a). Compound **2a** was prepared according to general procedure A using **13b** (300 mg, 1.09 mmol), 2,4-difluoroaniline (200 mg, 1.56 mmol), Cs_2CO_3 (700 mg, 2.14 mmol), X-Phos (50 mg, 0.11 mmol), and $Pd(OAc)_2$ (20 mg, 0.09 mmol). The reaction mixture was heated for 2.5 h at reflux temperature and purified by flash chromatography (SiO_2, hexane/ethyl acetate 1:1). Yield: 135 mg (26.0%), yellow solid. HPLC: t_R = 8.44 min, purity 100%. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) = 3.86 (s, 3 H, $-OCH_3$), 5.16 (s, 2 H, C^6H_2), 6.27 (s, 1 H, C^4H), 6.63 (d, J = 8.84 Hz, 1 H, C^2H), 7.08 (m, 3 H, $C^{3'}$H, $C^{5'}$H, $C^{6'}$H), 7.40 (m, 2 H, C^{10}H, C^7H), 7.84 (d, J = 8.59 Hz, 1 H, C^9H), 8.04 (d, J = 9.10 Hz, 1 H, C^1H), 8.67 (s, 1 H, NH). HRMS-EI, m/z ($C_{21}H_{15}F_2NO_3$): calcd 367.1020, found 367.1026.

3-[(2-Aminophenyl)amino]-8-methoxydibenzo[*b*,*e*]oxepin-11(6*H*)-one (2b). Compound **2b** was prepared according to general procedure A using **13b** (400 mg, 1.45 mmol), 2-aminoaniline (200 mg, 1.55 mmol), Cs_2CO_3 (700 mg, 2.14 mmol), X-Phos (100 mg, 0.21 mmol), and $Pd(OAc)_2$ (20 mg, 0.09 mmol). The reaction mixture was heated for 3 h at reflux temperature and purified by flash chromatography (SiO_2, hexane/ethyl acetate 2:1). Yield: 130 mg (25.8%), yellow solid. HPLC: t_R = 7.03 min, purity 96.7%. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) = 3.84 (s, 3 H, $-OCH_3$), 4.84 (s, 2 H, NH_2), 5.11 (s, 2 H, C^6H_2), 6.08 (m, 1 H, C^4H), 6.55 (m, 2 H, C^2H, $C^{3'}$H), 6.78 (m, 1 H, $C^{6'}$H), 7.00 (m, 4 H, $C^{4'}$H, $C^{5'}$H, C^7H, C^9H), 7.83 (d, J = 8.59 Hz, 1 H, C^1H), 7.98 (d, J = 9.10 Hz, 1 H, C^{10}H), 8.09 (s, NH, 1 H). HRMS-EI, m/z ($C_{21}H_{18}N_2O_3$): calcd 346.1317, found 346.1302.

3-[(2,4-Difluorophenyl)amino]-8-(2-morpholin-4-ylethoxy)-dibenzo[*b*,*e*]oxepin-11(6*H*)-one (2c). Compound **2c** was prepared according to general procedure A using **16a** (250 mg, 0.67 mmol), 2,4-difluoroaniline (150 mg, 1.16 mmol), Cs_2CO_3 (700 mg, 2,14 mmol), X-Phos (50 mg, 0.11 mmol), and $Pd(OAc)_2$ (10 mg, 0.05 mmol). The reaction mixture was heated for 6 h at reflux temperature and purified by flash chromatography (SiO_2, dichloromethane/ethanol 95:5). Yield: 80 mg (25.6%), yellow solid. HPLC: t_R = 4.80 min, purity 98.2%. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) = 2.54 (m, 4 H, $C^{2/6}{}_{morpholinyl}H_2$), 2.80 (t, J = 5.62 Hz, 2 H, $C^2{}_{ethoxy}H_2$), 3.70 (m, 4 H, $C^{3/5}{}_{morpholinyl}H_2$), 4.16 (t, J = 5.56 Hz, 2 H, $C^1{}_{ethoxy}H_2$), 5.07 (s, 2 H, C^6H_2), 6.27 (s, 1 H, NH), 6.43 (d, J = 2.15 Hz, 1 H, C^4H), 6.62 (dd, J = 8.97, 2.27 Hz, 1 H, C^2H), 6.87 (m, 4 H, $C^{3'}$H, $C^{6'}$H, $C^{4'}$H, C^7H), 7.34 (m, 1 H, C^9H)), 7.96 (d, J = 8.72 Hz, 1 H, C^1H), 8.20 (d, J = 8.84 Hz, 1 H, C^{10}H). HRMS-EI, m/z ($C_{26}H_{24}F_2N_2O_4$): calcd 466.1704, found 477.1696.

3-[(2-Aminophenyl)amino]-8-(2-morpholin-4-ylethoxy)-dibenzo[*b*,*e*]oxepin-11(6*H*)-one (2d). Compound **2d** was prepared according to general procedure A using **16a** (250 mg, 0.67 mmol), 2-aminoaniline (200 mg, 1.85 mmol), Cs_2CO_3 (350 mg, 1.07 mmol), X-Phos (50 mg, 0.11 mmol), and $Pd(OAc)_2$ (10 mg, 0.05 mmol). The reaction mixture was heated for 6 h at reflux temperature and purified by flash chromatography (SiO_2, dichloromethane/ethanol 95:5). Yield: 80 mg (26.8%), yellow solid. HPLC: t_R = 4.80 min, purity 98.2%. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) = 2.59 (m, 4 H, $C^{2/6}{}_{morpholinyl}H_2$), 2.83 (t, J = 5.56 Hz, 2 H, $C^2{}_{ethoxy}H_2$), 3.74 (m, 4 H, $C^{3/5}{}_{morpholinyl}H_2$), 4.19 (t, J = 5.68 Hz, 2 H, $C^1{}_{ethoxy}H_2$), 5.08 (m, 2 H, C^6H_2), 5.67 (s, 1 H, NH), 6.21 (d, J = 2.27 Hz, 1 H, C^4H), 6.51 (dd, J = 8.91, 2.21 Hz, 1 H, C^2H), 6.82 (m, 2 H, $C^{3'}$H, $C^{5'}$H), 6.96 (dd, J = 8.72, 2.27 Hz, 1 H, $C^{6'}$H), 7.11 (m, 2 H, C^7H, C^9H), 7.99 (d, J = 8.72 Hz, 1 H, C^1H), 8.22 (d, J = 8.84 Hz, 1 H, C^{10}H). HRMS-EI, m/z ($C_{26}H_{27}N_3O_4$): calcd 445.2001, found 445.2008.

3-[(2-Methylphenyl)amino]-8-(2-morpholin-4-ylethoxy)-dibenzo[*b*,*e*]oxepin-11(6*H*)-one (2f). Compound **2f** was prepared according to general procedure A using **16a** (200 mg, 0.53 mmol), 2-methylaniline (200 mg, 1.86 mmol), Cs_2CO_3 (700 mg, 2.14 mmol), X-Phos (50 mg, 0.11 mmol), and $Pd(OAc)_2$ (10 mg, 0.05 mmol). The reaction mixture was heated for 3 h at reflux temperature and purified by flash chromatography (SiO_2, dichloromethane/ethanol 95:5). Yield: 80 mg (25.6%), yellow solid. HPLC: t_R = 5.11 min, purity 100%. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) = 2.24 (s, 3H, $-CH_3$), 2.57 (m, 4 H, $C^{2/6}{}_{morpholinyl}H_2$), 2.82 (t, J = 5.68 Hz, 2 H, $C^2{}_{ethoxy}H_2$), 3.72 (m, 4 H, $C^{3/5}{}_{morpholinyl}H_2$), 4.18 (t, J = 5.62 Hz, 2 H, $C^1{}_{ethoxy}H_2$), 5.07 (s, 2 H, C^6H_2), 5.92 (s, 1 H, C^4H), 6.57 (dd, J = 8.91, 2.34 Hz, 1 H, C^2H), 6.80 (d, J = 2.53 Hz, 1 H, C^7H), 6.95 (dd, J = 8.59, 2.53 Hz, 1 H, $C^{4'}$H), 7.16 (m, 3 H, C^9H, $C^{3'}$H, $C^{5'}$H), 7.99 (d, J = 8.59 Hz, 1 H, C^1H), 8.22 (d, J = 8.84 Hz, 1 H, C^{10}H). HRMS-EI, m/z ($C_{27}H_{28}N_2O_4$): calcd 444.2048, found 444.2040.

3-[(4-Fluoro-2-methylphenyl)amino]-8-(2-morpholin-4-ylethoxy)dibenzo[*b*,*e*]oxepin-11(6*H*)-one (2g). Compound **2g** was prepared according to general procedure A using **16a** (200 mg, 0.53 mmol), 4-fluoro-2-methylaniline (200 mg, 1.60 mmol), Cs_2CO_3 (700 mg, 2.14 mmol), X-Phos (50 mg, 0.11 mmol), and $Pd(OAc)_2$ (10 mg,

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0.05 mmol). The reaction mixture was heated for 4 h at reflux temperature and purified by flash chromatograph (SiO_2, dichloromethane/ethanol 95:5). Yield: 80 mg (25.6%), yellow solid. HPLC: t_R = 5.30 min, purity 100%. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) = 2.22 (s, 3 H, $-CH_3$), 2.57 (m, 4 H, $C^{2/6}{}_{morpholinyl}H_2$), 2.82 (t, J = 5.68 Hz, 2 H, $C^2{}_{ethoxy}H_2$), 3.73 (m, 4 H, $C^{3/5}{}_{morpholinyl}H_2$), 4.18 (t, J = 5.62 Hz, 2 H, $C^1{}_{ethoxy}H_2$), 5.06 (s, 2 H, C^6H_2), 5.84 (s, 1 H, NH), 6.16 (d, J = 2.27 Hz, 1 H, C^4H), 6.47 (dd, J = 8.91, 2.34 Hz, 1 H, C^2H), 6.80 (m, 1 H, $C^{3'}H$), 6.95 (m, 3 H, $C^{6'}H$, $C^{5'}H$, C^7H), 7.19 (m, 1 H, C^9H), 7.98 (d, J = 8.59 Hz, 1 H, C^1H), 8.20 (d, J = 8.97 Hz, 1 H, $C^{10}H$). HRMS-EI, m/z ($C_{27}H_{27}FN_2O_4$): calcd 462.1946, found 462.1941.

3-[(2,4-Difluorophenyl)amino]-8-(2-tetrahydro-2*H*-pyran-4-ylethoxy)dibenzo[*b*,*e*]oxepin-11(6*H*)-one (2h). Compound **2h** was prepared according to general procedure A using **16b** (100 mg, 0.27 mmol), 2,4-difluoroaniline (120 mg, 0.80 mmol), Cs_2CO_3 (350 mg, 1.07 mmol), X-Phos (50 mg, 0.11 mmol), and $Pd(OAc)_2$ (10 mg, 0.05 mmol). The reaction mixture was heated for 3 h at reflux temperature and purified by flash chromatography (SiO_2, dichloromethane/ethanol 95:5). Yield: 20 mg (15.9%), white solid. HPLC: t_R = 9.11 min, purity 100%. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) = 1.55 (m, 7 H, $C^3{}_{tetrahydropyran}H_2$, $C^5{}_{tetrahydropyran}H_2$, $C^2{}_{ethoxy}H_2$, $C^4{}_{tetrahydropyran}H$), 3.40 (m, 2 H, $C^6{}_{tetrahydropyran}H_2$), 4.03 (m, 4 H, $C^2{}_{ethoxy}H_2$, $C^1{}_{ethoxy}H_2$), 5.10 (s, 2 H, C^6H_2), 6.01 (s, 1 H, NH), 6.47 (m, 1 H, C^4H), 6.65 (m, 1 H, C^2H), 6.90 (m, 4 H, C $^{3'}$H, $C^{6'}H$, $C^{5'}H$, C^7H), 7.38 (m, 1 H, C^9H), 7.99 (d, J = 8.72 Hz, 1 H, C^1H), 8.24 (d, J = 8.97 Hz, 1 H, $C^{10}H$). HRMS-EI, m/z ($C_{27}H_{25}F_2NO_4$): calcd 465.1751, found 465.1755.

3-[(2,4-Difluorophenyl)amino]-8-[2-(dimethylamino)-ethoxy]dibenzo[*b*,*e*]oxepin-11(6*H*)-one (2i). Compound **2i** was prepared according to general procedure A using **16c** (200 mg, 0.60 mmol), 2,4-difluoroaniline (200 mg, 1.55 mmol), Cs_2CO_3 (350 mg, 1.07 mmol), X-Phos (50 mg, 0.11 mmol), and $Pd(OAc)_2$ (10 mg, 0.05 mmol). The reaction mixture was heated for 4 h at reflux temperature and purified by flash chromatography (SiO_2, dichloromethane/ethanol 95:5). Yield: 40 mg (15.6%), yellow solid. HPLC: t_R = 4.74 min, purity 100%. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) = 2.22 (s, 6 H, $N(CH_3)_2$), 2.65 (m, 2 H, $C^2{}_{ethoxy}H_2$), 4.15 (m, 2 H, $C^1{}_{ethoxy}H_2$), 5.13 (s, 2 H, C^6H_2), 6.25 (m, 1 H, C^4H), 6.61 (m, 1 H, C^2H), 7.09 (m, 3 H, $C^{3'}H$, $C^{6'}H$, $C^{5'}H$), 7.40 (m, 2 H, C^7H, C^9H), 7.81 (d, J = 8.72 Hz, 1 H, C^1H), 8.01 (d, J = 7.96 Hz, 1 H, $C^{10}H$), 8.66 (s, 1 H, NH). HRMS-EI, m/z ($C_{24}H_{22}F_2N_2O_3$): calcd 424.1598, found 424.1583.

3-[(2,4-Difluorophenyl)amino]-8-{[(4*R*)-2,2-dimethyl-1,3-dioxolan-4-yl]methoxy}dibenzo[*b*,*e*]oxepin-11(6*H*)-one (2j). Compound **2j** was prepared according to general procedure A using **16d** (240 mg, 0.63 mmol), 2,4-difluoroaniline (200 mg, 1.55 mmol), Cs_2CO_3 (1.30 g, 3.99 mmol), X-Phos (100 mg, 0.21 mmol), and $Pd(OAc)_2$ (20 mg, 0.09 mmol). The reaction mixture was heated for 5 h at reflux temperature and purified by flash chromatography (SiO_2, dichloromethane/ethanol 95:5). Yield: 110 mg (37.4%), yellow solid. HPLC: t_R = 8.70 min, purity 100%. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) = 1.30 (s, 3 H, $-CH_3$), 1.35 (s, 3 H, $-CH_3$), 3.76 (m, 1 H, $C^3{}_{propoxy}H$) 4.11 (m, 3 H, $C^3{}_{propoxy}H$, $C^1{}_{propoxy}H_2$), 4.43 (m, 1 H, $C^2{}_{propoxy}H$), 5.14 (s, 2 H, C^6H_2), 6.25 (m, 1 H, C^4H), 6.61 (dd, J = 9.22, 1.77 Hz, 1 H, C^2H), 7.09 (m, 3 H, C^7H, C^9H, $C^{3'}H$), 7.39 (m, 2 H, $C^{5'}H$, $C^{6'}H$), 7.82 (d, J = 8.46 Hz, 1 H, $C^{10}H$), 8.02 (d, J = 8.97 Hz, 1 H, C^1H), 8.68 (s, $-$NH, 1 H). HRMS-EI, m/z ($C_{26}H_{23}F_2NO_5$): calcd 467.1544, found 467.1569.

3-[(2,4-Difluorophenyl)amino]-8-{2-[(4*R*)-2,2-dimethyl-1,3-dioxolan-4-yl]ethoxy}dibenzo[*b*,*e*] oxepin-11(6*H*)-one (2k). Compound **2k** was prepared according to general procedure A using **16e** (200 mg, 0.51 mmol), 2,4-difluoroaniline (150 mg, 1.16 mmol), Cs_2CO_3 (700 mg, 2.14 mmol), X-Phos (50 mg, 0.11 mmol), and $Pd(OAc)_2$ (20 mg, 0.09 mmol). The reaction mixture was heated for 8 h at reflux temperature and purified by flash chromatography (SiO_2, hexane/ethyl acetate 1:1).Yield: 80 mg (32.6%), yellow solid. HPLC: t_R = 9.08 min, purity 97.5%. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) = 1.26 (s, 3 H, $-CH_3$),1.32 (s, 3 H, $-CH_3$), 1.97 (m, 2 H, $C^2{}_{butoxy}H_2$), 3.56 (m, 1 H, $C^3{}_{butoxy}H$), 4.13 (m, 4 H, $C^1{}_{butoxy}H_2$, $C^4{}_{butoxy}H_2$), 5.14 (s, 2 H, C^6H_2), 6.26 (d, J = 0.76 Hz, 1 H, C^4H), 6.62 (dd, J = 8.91, 1.83 Hz, 1 H, C^2H), 7.07 (m, 3 H, C^7H, C^9H, $C^{3'}H$), 7.37 (m, 2 H, $C^{5'}H$, $C^{6'}H$), 7.82 (d, J = 8.46 Hz, 1 H, $C^{10}H$), 8.03 (d, J = 8.84 Hz, 1 H, C^1H), 8.67 (s, NH, 1 H). HRMS-EI, m/z ($C_{27}H_{25}F_2NO_5$): calcd 481.1700, found 481.1682

3-[(2,4-Difluorophenyl)amino]-9-methoxydibenzo[*b*,*e*]-oxepin-11(6*H*)-one (3a). Compound **3a** was prepared according to general procedure A using **24a** (150 mg, 0.54 mmol), 2,4-difluoroaniline (150 mg, 1.16 mmol), Cs_2CO_3 (350 mg, 1.07 mmol), X-Phos (50 mg, 0.11 mmol), and $Pd(OAc)_2$ (10 mg, 0.05 mmol). The reaction mixture was heated for 1.5 h at reflux temperature and purified by flash chromatography (SiO_2, hexane/ethyl acetate 2:1). Yield: 50 mg (25.2%), yellow solid. HPLC: t_R = 8.64 min, purity 98.3%. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) = 3.80 (s, 3 H, $-OCH_3$), 5.12 (s, 2 H, C^6H_2), 6.23 (m, 1 H, C^4H), 6.60 (dd, J = 8.97, 1.64 Hz, 1 H, C^2H), 7.11 (m, 2 H, $C^{6'}H$, $C^{3'}H$), 7.37 (m, 4 H, $C^{5'}H$, C^8H, $C^{10}H$, C^7H), 7.98 (d, J = 8.97 Hz, 1 H, C^1H), 8.70 (s, NH, 1 H). HRMS-EI, m/z ($C_{21}H_{15}F_2NO_3$): calcd 367.1020, found 367.1028.

3-[(2,4-Difluorophenyl)amino]-9-hydroxydibenzo[*b*,*e*]-oxepin-11(6*H*)-one (3b). Compound **3b** was prepared according to general procedure A using **25a** (200 mg, 0.78 mmol), 2,4-difluoroaniline (200 mg, 1.55 mmol), Cs_2CO_3 (700 mg, 2.14 mmol), X-Phos (100 mg, 0.21 mmol), and $Pd(OAc)_2$ (20 mg, 0.09 mmol). The reaction mixture was heated for 1.5 h at reflux temperature and purified by flash chromatography (SiO_2, hexane/ethyl acetate 2:1). Yield: 15 mg (5.4%), yellow solid. HPLC: t_R = 7.53 min, purity 100%. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 5.08 (s, 2 H, C^6H_2), 6.23 (s, 1 H, C^4H), 6.60 (d, J = 9.10 Hz, 1 H, C^2H), 6.96 (m, 1 H, $C^{3'}H$), 7.09 (m, 1 H, $C^{5'}H$), 7.20 (m, 1 H, $C^{6'}H$), 7.31 (d, J = 8.08 Hz, 1 H, C^7H), 7.40 (m, 2 H, C^8H, C^1H), 7.98 (d, J = 8.84 Hz, 1 H, $C^{10}H$), 8.68 (s, NH, 1 H) 9.86 (s, OH, 1 H). HRMS-EI, m/z ($C_{20}H_{13}F_2NO_3$): calcd $[M + H]^+$ 354.0936, found 354.0937.

3-[(2,4-Difluorophenyl)amino]-9-(2-morpholin-4-ylethoxy)-dibenzo[*b*,*e*]oxepin-11(6*H*)-one (3c). Compound **3c** was prepared according to general procedure A using **26a** (200 mg, 0.53 mmol), 2,4-difluoroaniline (200 mg, 1.54 mmol), Cs_2CO_3 (700 mg, 2,14 mmol), X-Phos (50 mg, 0.11 mmol), and $Pd(OAc)_2$ (10 mg, 0.05 mmol). The reaction mixture was heated for 5 h at reflux temperature and purified by flash chromatography (SiO_2, dichloromethane/ethanol 95:5). Yield: 65 mg (26.2%), yellow solid. HPLC: t_R = 4.69 min, purity 100%. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) = 2.64 (m, 4 H, $C^{2/6}{}_{morpholinyl}H_2$), 2.86 (t, J = 5.37 Hz, 2 H, $C^2{}_{ethoxy}H_2$), 3.77 (m, 4 H, $C^{3/5}{}_{morpholinyl}H_2$), 4.20 (t, J = 4.74 Hz, 2 H, $C^1{}_{ethoxy}H_2$), 5.10 (s, 2 H, C^6H_2), 6.01 (s, 1 H, NH), 6.44 (d, J = 2.15 Hz, 1 H, C^4H), 6.63 (dd, J = 8.97, 2.27 Hz, 1 H, C^2H), 6.95 (m, 4 H, $C^{3'}H$, $C^{5'}H$, $C^{10}H$, C^8H), 7.39 (m, 2 H, $C^{6'}H$ C^7H), 8.18 (d, J = 9.10 Hz, 1 H, C^1H). HRMS-ESI, m/z ($C_{26}H_{24}F_2N_2O_4$): calcd $[M + H]^+$ 467.1776, found 467.1773

3-[(2-Aminophenyl)amino]-9-(2-morpholin-4-ylethoxy)-dibenzo[*b*,*e*]oxepin-11(6*H*)-one (3d). Compound **3d** was prepared according to general procedure A using **26a** (200 mg, 0.53 mmol), 2-aminoaniline (200 mg, 1.85 mmol), Cs_2CO_3 (350 mg, 1.07 mmol), X-Phos (50 mg, 0.11 mmol), and $Pd(OAc)_2$ (10 mg, 0.05 mmol). The reaction mixture was heated for 6 h at reflux temperature and purified by flash chromatography (SiO_2, dichloromethane/ethanol 95:5). Yield: 50 mg (21.2%), yellow solid. HPLC: t_R = 3.42 min, purity 100%. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) = 2.64 (m, 4 H, $C^{2/6}{}_{morpholinyl}H_2$), 2.87 (t, J = 4.93 Hz, 2 H, $C^2{}_{ethoxy}H_2$), 3.77 (m, 4 H, $C^{3/5}{}_{morpholinyl}H_2$), 4.21 (t, J = 5.43 Hz, 2 H, $C^1{}_{ethoxy}H_2$), 5.07 (s, 2H, C^6H_2), 5.74 (s, 1 H, NH), 6.18 (d, J = 2.02 Hz, 1 H, C^4H), 6.50 (dd, J = 8.84, 2.15 Hz, 1 H, C^2H), 6.79 (m, 2 H, $C^{3'}H$, $C^{6'}H$), 7.13 (m, 4 H, $C^{4'}H$, $C^{5'}H$, C^8H, C^7H), 7.45 (d, J = 2.27 Hz, 1 H, $C^{10}H$), 8.16 (d, J = 8.97 Hz, 1 H, C^1H). HRMS-EI, m/z ($C_{26}H_{27}N_3O_4$): calcd 445.2001, found: 445.2004.

3-[(3-Aminophenyl)amino]-9-(2-morpholin-4-ylethoxy)-dibenzo[*b*,*e*]oxepin-11(6*H*)-one (3e). Compound **3e** was prepared according to general procedure A using **26a** (200 mg, 0.53 mmol), 2-aminoaniline (200 mg, 1.85 mmol), Cs_2CO_3 (350 mg, 1.07 mmol), X-Phos (50 mg, 0.11 mmol), and $Pd(OAc)_2$ (10 mg, 0.05 mmol). The reaction mixture was heated for 6 h at reflux temperature and purified by flash chromatography (SiO_2, dichloromethane/ethanol 95:5). Yield: 25 mg (10.6%), yellow oil. HPLC: t_R = 2.39 min, purity 100%. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) = 2.44 (m, 4 H, $C^{2/6}{}_{morpholinyl}H_2$), 2.68 (t, J = 5.75 Hz, 2 H, $C^2{}_{ethoxy}H_2$), 3.56 (m, 4 H, $C^{3/5}{}_{morpholinyl}H_2$), 4.13 (t, J = 5.62 Hz, 2 H, $C^1{}_{ethoxy}H_2$), 5.11 (m, 4 H, C^6H_2, $-NH_2$), 6.28 (m, 2 H,

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$C^{2'}H$, $C^{4'}H$), 6.43 (m, 1 H, $C^{6'}H$), 6.51 (d, J = 2.02 Hz, 1 H, C^4H), 6.73 (dd, J = 8.97, 2.27 Hz, 1 H, C^2H), 6.95 (m, 1 H, $C^{10}H$), 7.15 (m, 1 H, C^8H), 7.28 (m, 1 H, $C^{5'}H$), 7.42 (d, J = 8.21 Hz, 1 H, C^7H), 7.96 (d, J = 8.97 Hz, 1 H, C^1H), 8.71 (m, 1 H, $C^{10}H$). HRMS-EI, m/z ($C_{26}H_{27}N_3O_4$): calcd $[M + H]^+$ 446.2074, found 446.2070.

3-[(2,4-Difluorophenyl)amino]-9-{[(4*R*)-2,2-dimethyl-1,3-dioxolan-4-yl]methoxy}dibenzo[*b*,*e*]oxepin-11(6*H*)-one (3f). Compound **3f** was prepared according to General Procedure A using **26b** (200 mg, 0.53 mmol), 2,4-difluoroaniline (200 mg, 1.55 mmol), Cs_2CO_3 (1.30 g, 3.99 mmol), X-Phos (100 mg, 0.21 mmol) and $Pd(OAc)_2$ (20 mg, 0.09 mmol). The reaction mixture was heated for 4 h at reflux temperature and purified by flash chromatography (SiO_2, dichloromethane/ethanol 95:5). Yield: 90 mg (36.3%), yellow oil. HPLC: t_R = 8.90 min, purity 95.7%. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) = 1.29 (s, 3 H, $-CH_3$), 1.34 (s, 3 H, $-CH_3$), 3.76 (m, 1 H, $C^3_{propoxy}H$), 4.06 (m, 3 H, $C^3_{propoxy}H$, $C^1_{propoxy}H_2$), 4.40 (m, 1 H, $C^2_{propoxy}H$), 5.12 (s, 2 H, C^6H_2), 6.22 (m, 1 H, C^4H), 6.60 (dd, J = 8.65, 2.08 Hz, 1 H, C^2H), 7.15 (m, 2 H, $C^{6'}H$, $C^{3'}H$), 7.29 (d, J = 2.53 Hz, 1 H, $C^{5'}$), 7.38 (m, 1 H, C^8H), 7.44 (m, 2 H, $C^{10}H$, C^7H), 7.98 (d, J = 8.84 Hz, 1 H, C^1H), 8.70 (s, 1 H, NH). HRMS-EI, m/z ($C_{26}H_{23}F_2NO_5$): calcd 467.1543, found 467.1563.

3-[(2,4-Difluorophenyl)amino]-9-{[(4*S*)-2,2-dimethyl-1,3-dioxolan-4-yl]methoxy}dibenzo [*b*,*e*]oxepin-11(6*H*)-one (3g). Compound **3g** was prepared according to general procedure A using **26c** (300 mg, 0.79 mmol), 2,4-difluoroaniline (200 mg, 1.55 mmol), Cs_2CO_3 (1.30 g, 3.99 mmol), X-Phos (100 mg, 0.21 mmol), and $Pd(OAc)_2$ (20 mg, 0.09 mmol). The reaction mixture was heated for 4 h at reflux temperature and purified by flash chromatography (SiO_2, dichloromethane/ethanol 95:5). Yield: 120 mg (32.5%), yellow solid. HPLC: t_R = 8.85 min, purity 99.2%. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) = 1.29 (s, 3 H, $-CH_3$), 1.34 (s, 3 H, $-CH_3$), 3.76 (m, 1 H, $C^3_{propoxy}H$), 4.05 (m, 3 H, $C^3_{propoxy}H$, $C^1_{Propoxy}H_2$), 4.41 (m, 1 H, $C^2_{propoxy}H$), 5.12 (s, 2 H, C^6H_2), 6.24 (m, 1 H, C^4H), 6.61 (dd, J = 9.09, 1.52 Hz, 1 H, C^2H), 7.12 (m, 2 H, $C^{6'}H$, $C^{3'}H$), 7.38 (m, 4 H $C^{5'}H$, C^8H, $C^{10}H$, C^7H), 7.98 (d, J = 8.97 Hz, 1 H, C^1H), 8.71 (s, 1 H, NH). HRMS-EI, m/z ($C_{26}H_{23}F_2NO_5$): calcd 467.1544, found 467.1564.

9-[2-(Diethylamino)ethoxy]3-[(2,4-difluorophenyl)amino]-dibenzo[*b*,*e*]oxepin-11(6*H*)-one (3j). Compound **3j** was prepared according to general procedure A using **26d** (250 mg, 0.69 mmol), 2,4-difluoroaniline (200 mg, 1.55 mmol), Cs_2CO_3 (700 mg, 2.14 mmol), X-Phos (100 mg, 0.21 mmol), and $Pd(OAc)_2$ (20 mg, 0.09 mmol). The reaction mixture was heated for 3 h at reflux temperature and purified by flash chromatography (SiO_2, dichloromethane/ethanol 95:5). Yield: 80 mg (25.6%), yellow oil. HPLC: t_R = 5.35 min, purity 97.3%. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.04 (t, J = 7.07 Hz, 6 H, $2*C^2_{ethylamino}H_3$), 2.64 (m, 4 H, $2*C^1_{ethylamino}H_2$), 2.88 (m, 2 H, $C^2_{ethoxy}H_2$), 4.08 (t, J = 5.56 Hz, 2 H, $C^1_{ethoxy}H_2$), 5.02 (s, 2 H, C^6H_2), 5.86 (s, 1 H, NH), 6.38 (s, 1 H, C^4H), 6.55 (d, J = 8.84 Hz, 1 H, C^2H), 6.82 (m, 2 H, $C^{3'}H$, $C^{5'}H$), 6.98 (m, 1 H, $C^{6'}H$), 7.17 (d, J = 8.08 Hz, 1 H, C^7H), 7.28 (m, 1 H, C^8H), 7.37 (s, 1 H, $C^{10}H$), 8.12 (d, J = 8.84 Hz, 1 H, C^1H). HRMS-EI, m/z ($C_{26}H_{26}F_2N_2O_3$): calcd 452.1911, found 452.1894.

3-[(2,4-Difluorophenyl)amino]-9-(2-tetrahydro-2*H*-pyran-4-ylethoxy)dibenzo[*b*,*e*]oxepin-11(6*H*)-one (3k). Compound **3k** was prepared according to general procedure A using **26e** (200 mg, 0.54 mmol), 2,4-difluoroaniline (200 mg, 1.54 mmol), Cs_2CO_3 (350 mg, 1.07 mmol), X-Phos (50 mg, 0.11 mmol), and $Pd(OAc)_2$ (10 mg, 0.05 mmol). The reaction mixture was heated for 6 h at reflux temperature and purified by flash chromatography (SiO_2, dichloromethane/ethanol 95:5). Yield: 75 mg (29.9%), yellow solid. HPLC: t_R = 9.27 min, purity 100%. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 1.21 (m, 2 H, $C^2_{ethoxy}H_2$), 1.65 (m, 5 H, $C^{3/5}_{tetrahydropyran}H_2$), $C^4_{tetrahydropyran}H$), 3.26 (m, 2 H, $C^2_{tetrahydropyran}H_2$), 3.81 (m, 2 H, $C^5_{tetrahydropyran}H_2$), 4.06 (t, J = 5.94 Hz, 2 H, $C^1_{ethoxy}H_2$), 5.12 (s, 2 H, C^6H_2), 6.24 (s, 1 H, C^4H), 6.62 (d, J = 9.10 Hz, 1 H, C^2H), 7.11 (m, 2 H, $C^{3'}H$, $C^{5'}H$), 7.29 (m, 1 H, $C^{6'}H$), 7.39 (m, 3 H, C^8H, C^7H, C^1H), 7.99 (d, J = 9.10 Hz, 1 H, $C^{10}H$), 8.70 (s, 1 H, NH). HRMS-EI, m/z ($C_{27}H_{25}F_2NO_4$): calcd 465.1751, found 465.1755.

9-[2-Cyclohexylethoxy]3-[(2,4-difluorophenyl)amino]-dibenzo[*b*,*e*]oxepin-11(6*H*)-one (3l). Compound **3l** was prepared according to general procedure A using **26f** (250 mg, 0.67 mmol), 2,4-difluoroaniline (200 mg, 1.54 mmol), Cs_2CO_3 (350 mg, 1.07 mmol), X-Phos (50 mg, 0.11 mmol), and $Pd(OAc)_2$ (20 mg, 0.09 mmol). The reaction mixture was heated for 6 h at reflux temperature and purified by flash chromatography (SiO_2, dichloromethane/ethanol 95:5). Yield: 100 mg (31.9%), colorless oil. HPLC: t_R = 12.4 min, purity 100%. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 1.41 (m, 13 H, $C^{1-6}_{cyclohexyl}$, $C^2_{ethoxy}H_2$), 4.05 (t, J = 6.44 Hz, 2 H, $C^1_{ethoxy}H_2$), 5.12 (s, 2 H, C^6H_2), 6.23 (s, 1 H, C^4H), 6.62 (d, J = 8.84 Hz, 1 H, C^2H), 7.12 (m, 2 H, $C^{3'}H$, $C^{6'}H$), 7.27 (s, 1 H, $C^{10}H$), 7.40 (m, 3 H, $C^{4'}H$, C^7H, C^8H), 7.98 (d, J = 9.10 Hz, 1 H, C^1H), 8.70 (s, 1 H, NH). HRMS-EI, m/z ($C_{26}H_{26}F_2N_2O_3$): calcd 463.1958, found 463.1940.

3-[(2,4-Difluorophenyl)amino]-9-(2-hydroxyethoxy)-dibenzo[*b*,*e*]oxepin-11(6*H*)-one (3m). Compound **3m** was prepared according to general procedure A using **26g** (250 mg, 0.72 mmol), 2,4-difluoroaniline (200 mg, 1.54 mmol), Cs_2CO_3 (700 mg, 2.14 mmol), X-Phos (100 mg, 0.21 mmol), and $Pd(OAc)_2$ (20 mg, 0.09 mmol). The reaction mixture was heated for 6 h at reflux temperature and purified by flash chromatography (SiO_2, dichloromethane/ethanol 95:5). Yield: 55 mg (19.2%), yellow oil. HPLC: t_R = 7.40 min, purity 100%. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 3.73 (m, 2 H, $C^1_{ethoxy}H_2$), 4.05 (t, J = 4.67 Hz, 2 H, $C^2_{ethoxy}H_2$), 4.88 (t, J = 5.56 Hz, 1 H, OH), 5.13 (s, 2 H, C^6H_2), 6.24 (s, 1 H, C^4H), 6.62 (d, J = 8.84 Hz, 1 H, C^2H), 7.13 (m, 2 H, $C^{3'}H$, $C^{5'}H$), 7.30 (s, 1 H, $C^{6'}H$), 7.40 (m, 3 H, C^8H, C^7H, $C^{10}H$), 8.00 (d, J = 8.84 Hz, 1 H, C^1H), 8.70 (s, 1 H, NH). HRMS-EI, m/z ($C_{22}H_{17}F_2NO_4$): calcd 397.1125, found 397.1106.

3-[(2,4-Difluorophenyl)amino]-9-(2-hydroxypropoxy)-dibenzo[*b*,*e*]oxepin-11(6*H*)-one (3n). Compound **3n** was prepared according to general procedure A using **26h** (250 mg, 0.69 mmol), 2,4-difluoroaniline (200 mg, 1.54 mmol), Cs_2CO_3 (700 mg, 2.14 mmol), X-Phos (100 mg, 0.21 mmol), and $Pd(OAc)_2$ (20 mg, 0.09 mmol). The reaction mixture was heated for 3 h at reflux temperature and purified by flash chromatography (SiO_2, dichloromethane/ethanol 95:5). Yield: 45 mg (15.9%), yellow oil. HPLC: t_R = 7.92 min, purity 98.6%. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 2.07 (m, 2 H, $C^2_{propoxy}H_2$), 3.87 (t, J = 5.81 Hz, 2 H, $C^3_{propoxy}H_2$), 4.19 (t, J = 5.94 Hz, 2 H, $C^1_{propoxy}H_2$), 5.10 (s, 2 H, C^6H_2), 6.02 (s, 1 H, $-$NH), 6.46 (s, 1 H, C^4H), 6.63 (d, J = 8.84 Hz, 1 H, C^2H), 6.91 (m, 2 H, $C^{3'}H$, $C^{5'}H$), 7.06 (m, 1 H, $C^{6'}H$), 7.24 (m, 1 H, C^8H), 7.36 (m, 1 H, C^7H), 7.47 (s, 1 H, $C^{10}H$), 8.19 (d, J = 8.84 Hz, 1 H, C^1H). HRMS-EI, m/z ($C_{23}H_{19}F_2NO_4$): calcd 411.1281, found 411.1289.

2-({3-[(2,4-Difluorophenyl)amino]-11-oxo-6,11-dihydrodibenzo[*b*,*e*]oxepin-9-yl}oxy)ethyl Acetate (3o). Compound **3o** was prepared according to general procedure A using **26g** (250 mg, 0.72 mmol), 2,4-difluoroaniline (200 mg, 1.54 mmol), Cs_2CO_3 (700 mg, 2.14 mmol), X-Phos (100 mg, 0.21 mmol), and $Pd(OAc)_2$ (20 mg, 0.09 mmol). The reaction mixture was heated for 6 h at reflux temperature and purified by flash chromatography (SiO_2, dichloromethane/ethanol 95:5). Yield: 15 mg (4.7%), yellow oil. HPLC: t_R = 8.18 min, purity 100%. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 2.11 (s, 3 H, $-CH_3$), 4.23 (m, 2 H, $C^1_{ethoxy}H_2$), 4.43 (m, 2 H, $C^2_{ethoxy}H_2$), 5.10 (s, 2 H, C^6H_2), 5.96 (s, 1 H, $-$NH), 6.45 (d, J = 2.27 Hz, 1 H, C^4H), 6.63 (dd, J = 8.91, 2.34 Hz, 1 H, C^2H), 6.92 (m, 2 H, $C^{3'}H$, $C^{5'}H$), 7.07 (dd, J = 8.21, 2.78 Hz, 1 H, C^8H), 7.29 (m, 1 H, $C^{6'}H$), 7.38 (m, 1 H, C^7H), 7.46 (d, J = 2.78 Hz, 1 H, $C^{10}H$), 8.19 (d, J = 8.97 Hz, 1 H, C^1H). HRMS-EI, m/z ($C_{24}H_{19}F_2NO_6$): calcd 439.1230, found 439.1222.

3-[(2,4-Difluorophenyl)amino]-10-(methoxy)dibenzo[*b*,*e*]-oxepin-11(6*H*)-one (4a). Compound **4a** was prepared according to general procedure A using **24b** (200 mg, 0.73 mmol), 2,4-difluoroaniline (200 mg, 1.55 mmol), Cs_2CO_3 (700 mg, 2.14 mmol), X-Phos (100 mg, 0.21 mmol), and $Pd(OAc)_2$ (20 mg, 0.09 mmol). The reaction mixture was heated for 3 h at reflux temperature and purified by flash chromatography (SiO_2, hexane/ethyl acetate 2:1). Yield: 70 mg (26.1%), yellow solid. HPLC: t_R = 7.88 min, purity 100%. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) = 3.74 (s, 3 H, $-OCH_3$), 5.07 (s, 2 H, C^6H_2), 6.19 (m, 1 H, C^4H), 6.56 (dd, J = 8.91, 1.96 Hz, 1 H, C^2H), 7.08 (m, 3 H, $C^{3'}H$, $C^{5'}H$, C^9H), 7.39 (m, 3 H, C^7H, $C^{6'}H$, C^1H), 7.69 (d, J = 8.84 Hz, 1 H, C^8H), 8.55 (s, 1 H, NH). HRMS-EI, m/z ($C_{21}H_{15}F_2NO_3$): calcd 367.1020, found 367.1033.

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3-[(2,4-Difluorophenyl)amino]-10-(2-morpholin-4-ylethoxy)-dibenzo[*b*,*e*]oxepin-11(6*H*)-one (4b). Compound **4b** was prepared according to general procedure A using **27a** (250 mg, 0.67 mmol), 2,4-difluoroaniline (200 mg, 1.54 mmol), Cs_2CO_3 (350 mg, 1.07 mmol), X-Phos (50 mg, 0.11 mmol), and $Pd(OAc)_2$ (20 mg, 0.09 mmol). The reaction mixture was heated for 6 h at reflux temperature and purified by flash chromatography (SiO_2, dichloromethane/ethanol 95:5). Yield: 55 mg (17.6%), yellow solid. HPLC: t_R = 5.38 min, purity 100%. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) = 2.61 (m, 4 H, $C^{2/6}_{morpholinyl}H_2$), 2.85 (t, J = 5.43 Hz, 2 H, $C^{2}_{ethoxy}H_2$), 3.70 (m, 4 H, $C^{3/5}_{morpholinyl}H_2$), 4.19 (t, J = 5.43 Hz, 2 H, $C^{1}_{ethoxy}H_2$), 5.07 (s, 2 H, C^6H_2), 5.97 (s, 1 H, NH), 6.40 (d, J = 2.15 Hz, 1 H, C^2H), 6.58 (dd, J = 8.84, 2.27 Hz, 1 H, C^4H), 6.90 (m, 4 H, $C^{3'}H$, $C^{5'}H$, C^9H), 7.33 (m, 2 H, C^7H, C^8H), 7.86 (d, J = 8.72 Hz, 1 H, C^1H). HRMS-ESI, m/z ($C_{26}H_{24}F_2N_2O_4$): calcd $[M + H]^+$ 467.1776, found 467.1773

3-[(2,4-Difluorophenyl)amino]-10-{[(4*R*)-2,2-dimethyl-1,3-dioxolan-4-yl]methoxy}dibenzo[*b*,*e*]oxepin-11(6*H*)-one (4c). Compound **4c** was prepared according to general procedure A using **27b** (250 mg, 0.66 mmol), 2,4-difluoroaniline (200 mg, 1.55 mmol), Cs_2CO_3 (1.30 g, 3.99 mmol), X-Phos (100 mg, 0.21 mmol), and $Pd(OAc)_2$ (20 mg, 0.09 mmol). The reaction mixture was heated for 4 h at reflux temperature and purified by flash chromatography (SiO_2, hexane/ethyl acetate 1:1). Yield: 100 mg (32.4%), yellow solid. HPLC: t_R = 8.50 min, purity 98.9%. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 1.29 (s, 3 H, $-CH_3$), 1.31 (s, 3 H, $-CH_3$), 3.85 (m, 1 H, $C^{3}_{propoxy}H$), 4.04 (m, 3 H, $C^{3}_{propoxy}H$, $C^{1}_{propoxy}H_2$), 4.30 (m, 1 H, $C^{2}_{propoxy}H$), 5.08 (s, 2 H, C^6H_2), 6.20 (s, 1 H, C^4H) 6.57 (d, J = 8.59 Hz, 1 H, C^2H), 7.08 (m, 2 H, $C^{3'}H$, $C^{6'}H$), 7.17 (d, J = 8.34 Hz, 1 H, C^9H), 7.41 (m, 3 H, $C^{6'}H$, C^7H, C^8H), 7.67 (d, J = 8.59 Hz, 1 H, C^1H), 8.55 (s, 1 H, NH). HRMS-EI, m/z ($C_{26}H_{23}F_2NO_5$): calcd 467.1544, found 467.1540.

3-[(2,4-Difluorophenyl)amino]-10-{[(4*S*)-2,2-dimethyl-1,3-dioxolan-4-yl]methoxy}dibenzo[*b*,*e*]oxepin-11(6*H*)-one (4d). Compound **4d** was prepared according to general procedure A using **27c** (250 mg, 0.66 mmol), 2,4-difluoroaniline (200 mg, 1.55 mmol), Cs_2CO_3 (1.30 g, 3.99 mmol), X-Phos (100 mg, 0.21 mmol), and $Pd(OAc)_2$ (20 mg, 0.09 mmol). The reaction mixture was heated for 4 h at reflux temperature and purified by flash chromatography (SiO_2, hexane/ethyl acetate 1:1). Yield: 90 mg (29.2%), yellow solid. HPLC: t_R = 8.41 min, purity 95.1%. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 1.29 (s, 3 H, $-CH_3$), 1.31 (s, 3 H, $-CH_3$), 3.85 (m, 1 H, $C^{3}_{propoxy}H$), 4.04 (m, 3 H, $C^{3}_{propoxy}H$, $C^{1}_{propoxy}H_2$), 4.30 (m, 1 H, $C^{2}_{propoxy}H$), 5.08 (s, 2 H, C^6H_2), 6.20 (s, 1 H, C^4H), 6.57 (d, J = 8.59 Hz, 1 H, C^2H), 7.09 (m, 2 H, $C^{3'}H$, $C^{5'}H$), 7.17 (d, J = 8.34 Hz, 1 H, C^9H), 7.41 (m, 3 H, C^7H, $C^{6'}H$, C^8H), 7.67 (d, J = 8.59 Hz, 1 H, C^1H), 8.55 (s, 1 H, NH). HRMS-EI, m/z ($C_{26}H_{23}F_2NO_5$): calcd 467.1544, found 467.1536.

Biological Testing. The effect of the synthesized compounds on p38α inhibition was tested in an isolated p38α MAP kinase assay, in which the tested compound competes with adenosine triphosphate (ATP) for the ATP binding site in the p38α catalytic domain. The phosphorylation of activating transcription factor-2 (ATF-2) is determined with an anti-phospho-ATF-2 antibody. The degree of phosphorylation inversely correlates with the inhibitory activity of the tested compounds.[23]

Test compounds that showed a high biological activity in the p38α assay were tested in a human whole blood TNF-α release assay. The whole blood was stimulated with lipopolysaccharide (LPS), and the release of TNF-α was measured using an enzyme-linked immunosorbent assay (ELISA).[24]

Crystallization and Structure Determination of p38α−32a. Inactive (nonphosphorylated) human p38α MAP kinase was expressed and purified as described previously.[27] The purified protein was concentrated to 14 mg/mL and flash frozen in liquid nitrogen. Protein−inhibitor complex crystals were generated by initial preincubation of 0.4 μL of inhibitor (100 mM in DMSO) along with 40 μL of wild-type p38α (stored in 20 mM HEPES pH 7.1, 50 mM NaCl, 10 mM DTT, 100 mg/L methionine) for 1 h on ice to form the enzyme−inhibitor complex prior to crystallization. Samples were centrifuged at 13 000 rpm for 5 min to remove precipitate. Crystals were grown using the hanging drop method at 20 °C after mixing 1.6 μL of protein−inhibitor solution with 0.5 μL of reservoir solution [100 mM MES (pH 5.8−6.1), 22−27% PEG4000, 50 mM *n*-octyl-β-D-glucopyranoside]. Crystals were flash frozen using a cryogenic solution of reservoir containing 25% (v/v) PEG400. Diffraction data of the p38α−**32a** complex crystal were collected at the PX10SA beamline of the Swiss Light Source (PSI, Villingen, Switzerland) to a resolution of 2.1 Å, using wavelengths close to 1 Å. Images were processed with XDS[28] and scaled using XSCALE.[27]

Structure Determination and Refinement of p38α−32a. The complex structure was solved by molecular replacement with PHASER[29] using the published p38α structure 3QUD as template. The p38α molecule in the asymmetric unit was manually modified using the program COOT.[30] Initially, the model was refined with CNS[31] using simulated annealing to remove model bias. Final refinement was performed with REFMAC5.[32] Inhibitor topology files were generated using the Dundee PRODRG2 server.[33] Refined structures were validated with PROCHECK.[34] Data collection, structure refinement statistics, PDB ID codes, and further details for the data collection as well as Ramachandran plot results are shown in Table S2 (Supporting Information). PyMOL[35] was used to generate the figures.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental and analytical data, including HPLC purity and HRMS data as well as the crystal X-ray structure statistics of the compound **32a** in complex with p38α. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

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Notes

The authors declare no competing financial interest.

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