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Design, Synthesis, and Biological Evaluation of Novel Disubstituted Dibenzosuberones as Highly Potent and Selective Inhibitors of p38 Mitogen Activated Protein Kinase

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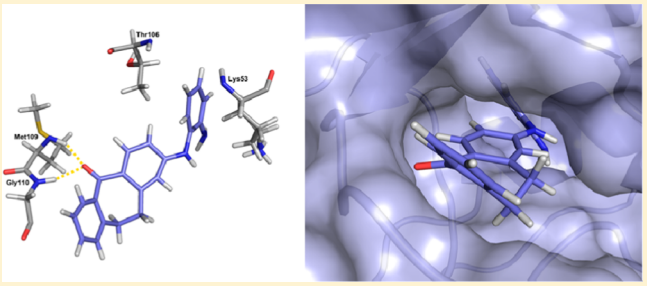
Supporting Information

ABSTRACT: Synthesis, biological testing, structure−activity relationships (SARs), and selectivity of novel disubstituted dibenzosuberone derivatives as p38 MAP kinase inhibitors are described. Hydrophilic moieties were introduced at the 7-, 8-, and 9-position of the 2-phenylamino-dibenzosuberones, improving physicochemical properties as well as potency. Extremely potent inhibitors were obtained, with half-maximal inhibitory concentration (IC_{50}) values in the low nM range in a whole blood assay measuring the inhibition of cytokine release. The high potency of the target compounds together with the outstanding selectivity of this novel class of compounds toward p38 mitogen activated protein (MAP) kinase as compared to other kinases indicate them to be most applicable as tools in pharmacological research and eventually they may foster a new generation of anti-inflammatory drugs.



INTRODUCTION

Therapy for chronic inflammatory diseases like rheumatoid arthritis (RA) and inflammatory bowel diseases (IBD) has progressed greatly in recent years. Introduction of biological agents, particularly monoclonal antibodies which neutralize TNF-α (e.g., etanercept, infliximab, and adalimumab) or IL-1β (e.g., anakinra), have afforded many advances in treatment of these disorders. The main drawbacks of these drugs are their lack of oral bioavailability and their high cost of production.

Several global players have invested considerable resources to discover low molecular weight drugs with the same efficacy as biologic agents in therapy of inflammatory diseases.[1−3] One main target is p38α mitogen activated protein (MAP) kinase, a key enzyme in the regulation of the pro-inflammatory cytokines TNF-α and IL-1β.[4−6]

Until now, no p38α MAP kinase inhibitor has been approved for therapy of inflammatory diseases.[1−3] Since the cloning of p38α MAP[7] was first described and the corresponding inhibitors were developed, the p38α MAP kinase community has strived to produce a commercial drug. A serious problem in the development of p38α MAP kinase inhibitors is the lack of selectivity. Novel and fast screening methods revealed that even VX-745 (Figure 1), purported to be a highly selective inhibitor against p38α MAP kinase, exhibited considerable off-target activity.[8,9]

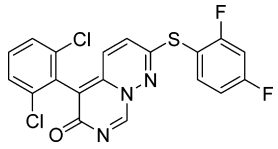


Figure 1. VX-745.

The ATP binding site, the locus where all inhibitors of p38α MAP kinase at least partially bind, is highly conserved throughout all kinases. An inhibitor which binds only to this site will certainly inhibit several other kinases as well. Therefore, the successful design of p38α MAP kinase inhibitors must exploit characteristics that are unique to p38α MAP kinase such as the hydrophobic region I and the glycine flip.[2,10−12] The hydrophobic region I, which is adjacent to the ATP binding site, is accessible in p38α MAP kinase because the opening of this area is occupied by the small gatekeeper amino acid Thr106.[10,11] In other types of kinases, the entrance to this region is blocked by a bulky methionine residue. Inhibitors occupying hydrophobic region I should therefore have only weak binding affinities to kinases with large gatekeeper amino acids. Another p38α MAP kinase structural feature is

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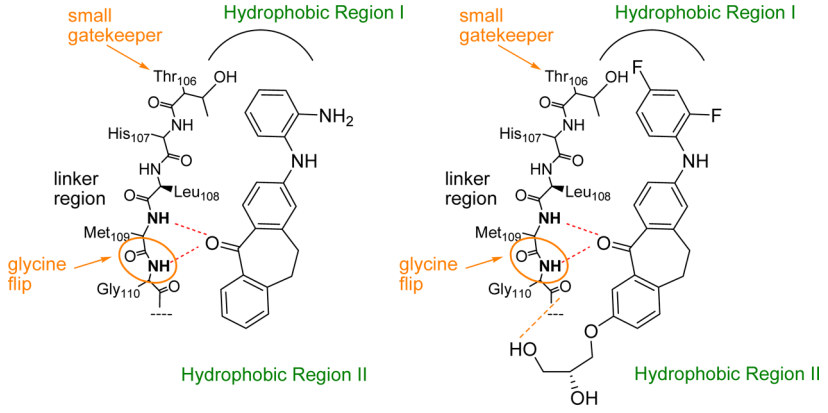


Figure 2. Binding modes of 2-(2-amino-phenylamino)-10,11-dihydro-dibenzo[*a*,*d*]cyclohepten-5-one (**1**) and Skepinone-L (**2**). Left: Schematic of the binding mode of 2-(2-amino-phenylamino)-10,11-dihydro-dibenzo[*a*,*d*]cyclohepten-5-one (**1**, PDB ID 3ZYA) in complex with p38α MAP kinase; Right: Schematic of the binding mode of 2-(2,4-difluoro-phenylamino)-7-(2*R*,3-dihydroxy-propoxy)-10,11-dihydro-dibenzo[*a*,*d*]cyclohepten-5-one (**2**, Skepinone-L, PDB ID 3QUE) with p38α MAP kinase.[13]

exemplified by Gly110 in the hinge region. This small amino acid at this position enables the enzyme to adjust its conformation (i.e., to undergo the glycine flip)[12] in order to form a second hydrogen bond with the inhibitor. A carbonyl oxygen in the inhibitor is most commonly used to induce the glycine flip. The peptide bond between Met109 and Gly110 rotates and places the NH of glycine at a distance favorable to the inhibitor (Figure 2). Kinases which feature amino acids with longer side chains at this position are not able to perform the glycine flip.

Despite taking into account the occupation of the hydrophobic region I and the induction of the glycine flip in the design of VX-745, there was still off-target activity. Therefore, other selectivity features have been sought in order to achieve selective inhibitors of p38α MAP kinase. The flexible structures of kinases permit them to undergo conformational changes during activation. In conjunction with flexible inhibitors, these conformational changes might provoke undesirable induced fits that may cause nonspecific effects. Therefore, it was our aim to design very rigid inhibitors. We started out from monosubstituted dibenzosuberones structures, compounds which should possess the required rigidity. They proved to be quite potent in the isolated p38α MAP kinase enzyme assay (compound **1**, IC_{50} = 104 nM) and were demonstrated to occupy hydrophobic region I and to induce the glycine flip (Figure 2).[13,14] Unfortunately, they were highly lipophilic, which probably accounted for their low activity in whole blood. In an effort to improve hydrophilicity of the dibenzosuberone scaffold, we synthesized aza-analogue structures, but these compounds lost potency in the p38α MAP kinase enzyme assay.[15]

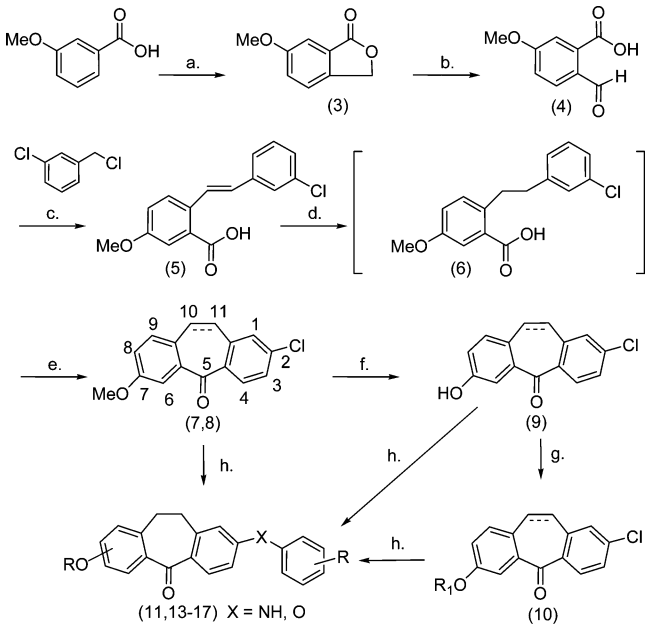
Docking studies suggested that hydrophilic moieties at positions 7, 8, and 9 should be well tolerated. This approach eventually led to Skepinone-L (**2**, Figure 2, PDB ID 3QUE), which is highly potent in vivo and, to the best of our knowledge, is the most selective p38α MAP kinase inhibitor described in literature.[13] Herein, we will discuss optimization and structure–activity relationships (SARs) of scaffolds occupying hydrophobic regions I and II, which were synthesized during the design of Skepinone-L (**2**).

CHEMISTRY

Synthesis of the dibenzosuberone derivatives in this study required dibenzosuberone scaffolds with two functionalities. As the 2-phenylamino substituent was assembled by Buchwald–Hartwig reaction,[16] a chloro group was introduced in the 2-position. Oxygen-linked hydrophilic moieties in the 7/8/9-positions can be accessed via the corresponding phenols. Thus, 2-chloro-7/8/9-hydroxydibenzosuberones were synthesized. Schemes 1–3 outline a synthetic strategy for the assembly of the target compounds.

Starting from 3-methoxy benzoic acid, 6-methoxyphthalide **3** was obtained by treatment with formaldehyde/acetic acid.[17]

Scheme 1. Synthesis of 2-Chloro-7-hydroxydibenzosuberone and Corresponding Derivatives[a]



[a]Reagents and conditions: (a) formaldehyde (37%), acetic acid, 90 °C, 64%; (b) (1) NBS, AIBN, chlorobenzene, 90 °C, (2) NaOH, 87%; (c) (1) PPh_3, MeOH, reflux, (2) NaOMe (30%), 0 °C, 40%; (d) H_2/Pd/C, ethyl acetate/acetonitrile, room temperature, 95%; (e) (1) $SOCl_2$, DCM, (2) $AlCl_3$, 58–93%; (f) HBr (48%), acetic acid, reflux, 65–95%; (g) K_2CO_3, acetonitrile, reflux or K_2CO_3, DMF, reflux, 78–91%. (h) Compounds **11**, **13**–**16**: $Pd(OAc)_2$, X-Phos, NaO-*tert*-Bu, toluol, *tert*-BuOH, 90 °C, 32–87%. Compounds **17**: $Pd(OAc)_2$, di-*tert*-butyl-X-Phos, K_3PO_4, toluol, 90 °C, 27–48%.

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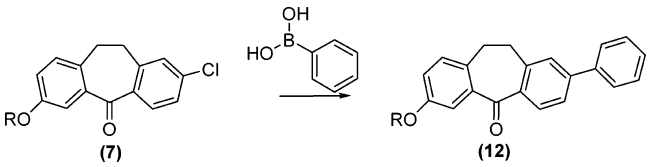
Oxidative ring-opening with *N*-bromo-succinimide/azoisobutyronitrile (NBS/AIBN) yielded the desired aldehyde **4**. The central step of the synthesis is the Wittig reaction, yielding the respective stilbeno-2-carboxylic acid **5**. Reduction of the etheno-linker using H_2 and Pd/carbon provided the corresponding saturated acid (**6**), which was cyclized by an intramolecular Friedel–Crafts reaction with $AlCl_3$. Friedel–Crafts acylations are possible in both the ortho- and para-positions. We supposed that the acylation in the para-position would be preferred because the large volume of the chlorine may hinder the acylation in ortho-position. We detected two peaks (one very large peak and a very small one) in GC-MS analysis. The fraction from the large peak was collected during flash chromatography purification, and crystal structure confirmed that the isolated product was the desired 2-chloro-methoxydibenzosuberone (Supporting Information Figure S1). Treatment of the respective 2-chloro-methoxydibenzosuberones (**7**, **8**) with HBr/acetic acid[18] proceeded in high yields to give the corresponding 2-chloro-7-hydroxy-dibenzosuberone (**9**).

Hydrophilic moieties were introduced either by Williamson's ether synthesis with alkyl halogenides or with activated alcohols (*R*- or *S*-*O*-isopropylidene glycerol tosylate). Buchwald–Hartwig reaction was performed with different anilino-derivatives, providing the 2-substituted dibenzosuberone derivatives (**11**, **13**–**16**) in very good yields. We employed di-*tert*-butyl-X-Phos for coupling phenoxy residues (**17**). Chiral dihydroxypropoxy derivatives were accomplished by deprotection of the acetal with *p*-toluenesulfonic acid.[19] Analogue **12** was prepared by Suzuki coupling[20] of **7** with boronic acid as described in Scheme 2.

Scheme 2. Suzuki Coupling[a]



[a]Reagents and conditions: $Pd(OAc)_2$, K_2CO_3, PEG-400, 90 °C, 32%.

Starting from the commercially available methoxy-methyl-benzoic acids **18a** and **18b**, the bromomethyl derivatives **19a** and **19b** can be readily formed by treatment with *N*-bromosuccinimide (NBS)/azo-isobutyronitrile (AIBN). Wittig reaction provided the analogues **20a** and **20b**, and subsequent hydrogenation of the unsaturated etheno-linker, using H_2 and either catalytic palladium on carbon (**20a**) or palladium on $BaSO_4$ (**20b**), afforded derivatives **21a** and **21b**. Ring closure was performed using $SOCl_2/AlCl_3$, resulting in 8-methoxy-dibenzosuberone **22a** or 9-methoxy-dibenzosuberone **22b**. Treatment of the respective 2-chloro-methoxydibenzosuberones (**22a**, **22b**) with HBr/acetic acid[18] proceeded in high yields to give the corresponding 2-chloro-hydroxy-dibenzosuberones (**23**, **24**).

Introduction of hydrophilic moieties (**25**, **26**) and Buchwald–Hartwig (**27**–**30**) reaction were performed as described in Scheme 3.

Lead Compound Optimization and Biological Testing. We started from substituted benzophenones as initial lead structures which had been reported as inhibitors of p38α MAP kinase in 2003 (Figure 3).[21] We supposed that rigid inhibitors may have enhanced selectivity compared to their more flexible counterparts as well as increased potency. This hypothesis is based on the fact that the kinase may also induce conformational changes at a flexible inhibitor which is not possible with a rigid compound.[22,23] Unlike what was suggested by Ottosen et al.[21] (Figure 3, left), our docking studies with both compounds suggested that the amino-linked phenyl residue occupies the hydrophobic pocket one (Figure 3, middle). We crystallized the 2-amino-phenyl-amino-benzophenone in complex with p38α MAP kinase (PDB ID 3QUD, Figure 3) as well as the 2-aminophenylaminodibenzosuberon to gain insights into its binding mode. In contrast to the binding mode suggested by Ottosen et al.,[21] the aminophenylamino residue of 2-amino-phenyl-amino-benzophenone occupies the hydrophic region I (Figure 3, right). The rigidized compound **1** confirmed a binding mode isostructural to that of the benzophenone and has been previously published (Figure 2, PDB ID 3ZYA).[13,14]

A first optimization step was performed with the phenyl-amino moieties which occupy the hydrophobic region I (see Figures 2 and 3), and all compounds were primarily screened in a p38α MAP kinase activity assay.[24]

Because of the small dimensions of this pocket, small substituents like NH_2, F, or CH_3 at the phenyl-amino moiety are better tolerated than larger residues like CF_3. Among the monosubstituted derivatives, the 2-amino moiety (**11a**) was quite efficient, with an IC_{50} value of 40 nM in the kinase assay (Table 1). The small 2-fluoro moiety (**11g**) was moderately active, with an IC_{50} value of 70 nM in the kinase assay. Larger substituents like methoxy- (**11d**), chloro- (**11e**), or trifluoromethyl (**11f**) led to less active compounds. The unsubstituted analogue **11h** was moderately active, resulting in a 2-fold lower potency compared to compound **11a**. 2,4-Difluorophenylamino derivative **11b** was slightly superior to all target compounds, and target compounds with 2-chloro-4-fluoro (**11i**) and 2-methyl-4-fluoro-(**11j**)-substituents were good inhibitors of p38α MAP kinase. Larger substituents in the para-position were not tolerated very well, thus resulting in less active compounds (for example, 2-amino-4-trifluoromethyl, **11k**). Furthermore, 3-methyl-4-fluoro- (**11l**) and 2-,3-,5-trifluoro-substituents (**11m**) could be introduced without loss of activity, whereas the 2-,3-,4-trifluoro- substitution (**11n**) led to a 50-fold loss of activity as compared to **11m**. Because the gatekeeper Thr106 refuses access to the direct-linked phenyl-substituent to the hydrophobic region I, replacement of the 2-phenylamino-substituent with a phenyl-substituent (compound **12**) resulted in an almost complete loss of activity (32% inhibition at 10 μM). The target compound with an etheno-linker (**13**) also resulted in a complete loss of activity.

To test the compounds under physiologically more relevant conditions in the presence of serum albumin and other proteins, inhibition of cytokine release (TNF-α) from immune cells was observed using diluted fresh human whole blood.[25]

Compounds **11a**, **11b**, **11c**, and **11l** exhibited the best potency in blocking the release of TNF-α. Most of the other compounds (**11d**, **11e**, **11h**, **11i**, **11j**, **11m**) were less potent in this assay, with IC_{50} values of 3.2–7.6 μM. The lipophilic compounds **11f**, **11k**, and **11n**, which showed low activity in the p38 MAP kinase activity assay, exhibited no activity in the whole blood assay, with IC_{50} values in the double-digit μM range. Cleavage of the methoxy-group resulted in more hydrophilic 7-hydroxydibenzosuberones **14** (Table 1). The IC_{50} values in the p38 MAP kinase activity assay were comparable to the methoxy compounds. In the whole blood

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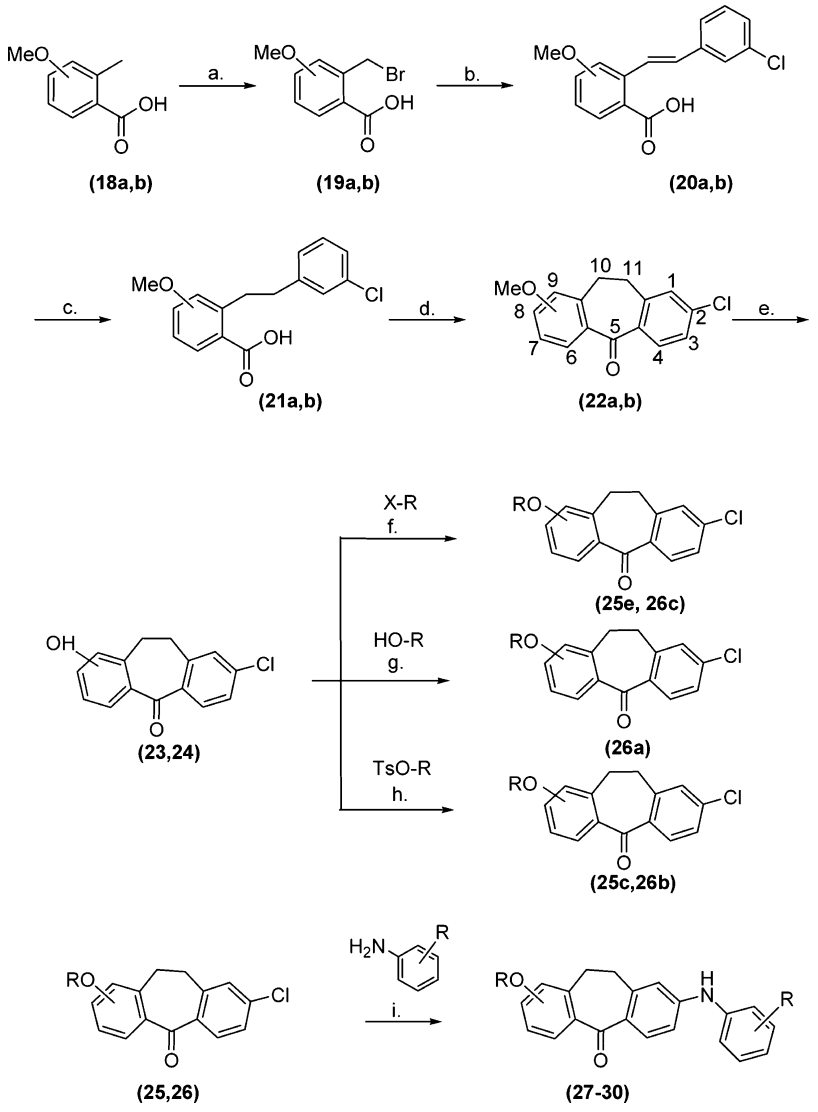
Scheme 3. Synthesis of 2-Chloro-8/9-hydroxydibenzosuberones[a]



[a]Reagents and conditions: (a) NBS, AIBN, chlorobenzene, 90−120 °C, 25−55%; (b) (1) $P(Ph)_3$, acetone, reflux, (2) NaOMe (30%), MeOH, 3-chlorobenzaldehyde, reflux, 28−48%; (c) H_2/Pd/C, ethyl acetate, acetonitrile, MeOH room temperature, 83% (**21a**) or H_2/Pd, acetonitril/ethyl acetate/$BaSO_4$, MeOH, HCl, room temperature, 87% (**21b**); (d) (1) $SOCl_2$, DCM, (2) $AlCl_3$, DCM, 47−90%; (e) HBr (48%), acetic acid, 91−95%; (f) K_2CO_3, DMF, reflux, 94−98%; (g) PPh_3, azodiisopropylcarboxylate, DCM, 0 °C, 45%; (h) K_2CO_3, DMF, 90 °C, 53−97%; (i) $Pd(OAc)_2$, X-Phos, NaO-*tert*-Bu, toluol, *tert*-BuOH, 90 °C, 20−62%.

assay, however, potency was improved by ∼1.7-fold over that with the corresponding 7-methoxy variants, probably due to lower lipophilicity. Because the higher hydrophilicity of the 2-aminophenylamino-derivative (**11a**) resulted in activity in the whole blood assay comparable to that of compound **11b**, both were selected for further optimization of the hydrophilic moieties at the 7-position.

To optimize the physiological properties of the compounds, docking studies were performed using the crystal structure of the 2-aminophenyl-aminodibenzosuberone (PBD ID 3ZYA)[13] as a template (Figure 4). The results from our docking studies indicated that introduction of substituents in 7-, 8-, and 9-position should be tolerated by the protein. In addition, these moieties can be used to improve potency by providing additional interaction possibilities to the protein backbone proximal to the hydrophobic region II or to its surrounding solvent-exposed area. Introduction of polar substituents should improve water solubility as well as potency by providing additional interaction possibilities. Orientation of the OH-groups toward the surrounding solvent-exposed area may favor the formation of additional hydrogen bonds and result in more potent compounds. The hydrophobic region II is mostly defined by the amino acids Val30, Gly110, Ala111, Arg49, and Leu108. Except for Arg49, all side chains are hydrophobic (see PBD 3ZYA). Therefore, compounds which are able to generate hydrophobic interactions in this region were also synthesized to evaluate whether this approach is valid to increase potency.

As small alkyl-moieties can be well incorporated into the hydrophobic region II and form additional interactions, flexible, linear hydroxyl-alkoxy moieties were first introduced. The 2,3-dihydroxypropoxy moiety increased potency 2-fold in the enzyme assay compared to the methoxy residue (**11a**). Potency was increased ∼20-fold (**15b** vs **11a**) in the whole blood assay, indicating that these compounds are highly potent under

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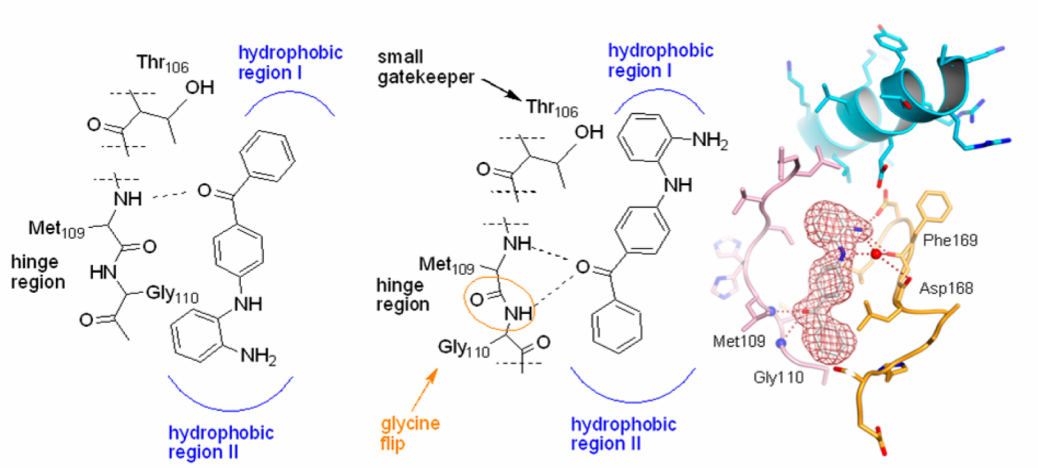

Figure 3. Binding mode of 2-amino-phenyl-amino-benzophenone. Left: Schematic binding mode according to Ottosen et al.[21] Middle: Schematic binding mode suggested by our group. Right: Crystal structure of 2-amino-phenyl-amino-benzophenone in complex with p38α MAP kinase (PDB ID 3QUD), confirming the binding mode suggested by our group.

physiological conditions. Acetal analogues with increased spatial dimension were synthesized in order to obtain additional hydrophobic interactions in the hydrophobic region II. Furthermore, the acetal analogues (**15a**, **15c**) provided only hydrogen bond acceptors, whereas the dihydroxypropoxy compounds could act as hydrogen bond donors as well as acceptors. Although both compounds with acetal residues (**15a**, **15c**) were comparable to the methoxy derivative (**11a**) in respect to potency in the enzyme assay, they showed ~8-fold higher potency in whole blood. Morpholino-ethoxy- (**15e**) provided a hydrogen bond donor (protonated nitrogen) and a hydrogen bond acceptor (oxygen). The saturated ring offered the possibility of additional hydrophobic interactions similar to the acetals. The morpholino derivative was very potent both in enzyme and whole blood assays with IC_{50} values in the low nM range, exhibiting similar inhibitory efficiency as the 2,3-dihydroxypropoxy target compounds.

Taken together, particularly the very hydrophilic derivatives (dihydroxypropoxy and (**15b**, **15d**)), were very potent, with IC_{50} values in the double-digit nM range, suggesting them to be promising drug candidates. Interestingly, the more lipophilic acetals (**15a**, **15c**) did not show improved potency in the enzyme assay but displayed superior activity in whole blood when compared to the methoxy derivatives.

Encouraged by these findings, we conducted a similar optimization strategy for the 2,4-difluorophenylamino derivative **11b**. We tested an even larger panel of candidate compounds. To optimize the spacer length for the hydroxyl-group, hydroxyethoxy- (**16a**) and hydroxypropoxy- (**16b**) derivatives were synthesized. The potencies of both these derivatives increased by 2−2.5-fold compared to the methoxy derivative **11b**. The spacer was made rigid by introducing a tetrahydropyran-4-yl-moiety (**16c**); however, this change abolished the increase in potency. This result indicated the need for either a flexible, adaptable substituent or a hydrogen bond donor functionality (which was lost in the tetrahydropyran-4-yl ring). Both the 3-hydroxypropoxy and 2-hydroxyethoxy moieties were beneficial for potency; therefore, we introduced a 2,3-dihydroxypropoxy moiety that combined both structural features. 2,3-Dihydroxypropoxy-derivatives (**2** = Skepinone-L, **16f**) showed potencies similar to the monohydroxyalkoxy compounds. The two configurations (**2** vs **16f**) were not substantially different in potency. The crystal structure of Skepinone-L (**2**) in complex with p38α MAP kinase revealed that an additional hydrogen bond was formed between the dihydroxypropoxy moiety and the backbone carbonyl of Gly110.[13] This additional interaction could explain the superior binding affinity of the dihydroxyalkoxy-substituted dibenzosuberones (Skepinone-L, **2**) compared to the methoxy derivative (**11b**). The dihydroxybutanoxy derivative **16h** was also extremely potent, with an IC_{50} of 10 nM in the in vitro enzyme assay. In contrast to **16c**, all compounds with acetal residues (**16d**, **16e**, **16g**) had potencies that were comparable to that of **11b**. The potencies increased according to the sequence: **16d**, **16e** < **16g**, which corresponded to increasing compound flexibility. As observed in the diols (Skepinone-L, **2**), the configuration did not affected potency (**16d** vs **16e**). Morpholino-ethoxy- (**16i**) and morpholino-propoxy- (**16j**) derivatives were very potent, with IC_{50} values in the low nM range; the inhibitory efficiency was similar to that observed for the diol target compounds (Skepinone-L, **16f**, **16h**). Because the hydrogen bond donor function in **16i** and **16j** was located at the same distance as the OH functions of the alcohol moieties, the arrangements of these residues were expected to be broadly similar to the arrangements observed in the alcohols and to form a hydrogen bond with Gly110, like the interaction revealed in the X-ray structure of Skepinone-L (**2**, Figure 1).[13] In addition to the gain in potency offered by these additional interactions, we reasoned that disubstituted compounds might also benefit from higher hydrophilicity. Indeed, in the whole blood assay, the potency of the disubstituted compounds (Skepinone-L, **16f**, **16i**) showed an overall improvement of ~15−40-fold compared to compound **11b**. The potencies of these compounds decreased in the order from monohydroxyalkoxy compounds **16a** and **16b** to acetals and tetrahydropyranyl species (**16c**, **16d**, **16e**) according to their increases in lipophilicity.

Both series of compounds (2-aminophenylamino-derivatives, Table 2 and 2,4-difluorophenylamino-derivatives, Table 3) showed comparable results. Small flexible substituents with polar groups were more advantageous than lipophilic moieties, especially if they were conformational hindered. Generally,

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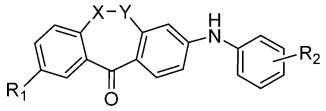
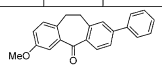
Table 1. Biological Activity of Different Phenylamino-Substituted 7-Methoxy- and 7-Hydroxy-dibenzosuberones



#	-X-Y-	R1	R2	p38 IC_{50} ± SEM [μM]	TNF-α IC_{50} ± SEM [μM]
11a	-CH_2-CH_2-	OMe	2-NH_2	0.04±0.01 (n = 3)	1.5±0.6 (n = 2)
11b	-CH_2-CH_2-	OMe	2, 4-F	0.02±0.01 (n =6)	1.2±0.4 (n = 2)
11c	-CH_2-CH_2-	OMe	2- NH_2, 4-F	0.04±0.00 (n = 3)	1.9±0.6 (n = 2)
11d	-CH_2-CH_2-	OMe	2-OMe	0.38±0.02 (n = 3)	7.6±1.8 (n = 2)
11e	-CH_2-CH_2-	OMe	2-Cl	0.56±0.01 (n = 3)	4.6±0.1 (n = 2)
11f	-CH_2-CH_2-	OMe	2-CF_3	0.40±0.04 (n = 3)	33.6±5.6 (n = 2)
11g	-CH_2-CH_2-	OMe	2-F	0.07±0.01 (n = 3)	4.9±1.5 (n = 2)
11h	-CH_2-CH_2-	OMe	-	0.07±0.01 (n = 3)	3.2±0.1 (n = 2)
11i	-CH_2-CH_2-	OMe	2-Cl, 4-F	0.04±0.01 (n = 3)	5.4±0.2 (n = 2)
11j	-CH_2-CH_2-	OMe	2-CH_3, 4-F	0.06±0.01 (n = 3)	3.9±0.5 (n = 2)
11k	-CH_2-CH_2-	OMe	2-NH_2, 4-CF_3	0.90±0.05 (n = 3)	25.5±3.1 (n = 2)
11l	-CH_2-CH_2-	OMe	3-CH_3, 4-F	0.04±0.00 (n = 3)	1.9±0.5 (n = 2)
11m	-CH_2-CH_2-	OMe	2-, 3-, 5-F	0.05±0.00 (n = 3)	4.3±1.3 (n = 2)
11n	-CH_2-CH_2-	OMe	2-, 3-, 4-F	3.53±0.8 (n = 3)	81.0±12.6 (n = 2)
12				32% at 10 μM (n = 3)	n.t.
13	-CH=CH-	OMe	2-, 4-F	21% at 10 μM (n = 3)	n.t.
14a	-CH_2-CH_2-	OH	2, 4-F	0.02±0.01 (n = 3)	0.7±0.1 (n = 2)
14b	-CH_2-CH_2-	OH	2-Cl	0.14±0.01 (n = 3)	7.5±1.3 (n = 2)
14c	-CH_2-CH_2-	OH	2-Cl, 4-F	0.56±0.02 (n = 3)	3.8±0.1 (n = 2)
14d	-CH_2-CH_2-	OH	-	0.06±0.01 (n = 3)	2.2±0.0 (n = 2)
14e	-CH=CH-	OH	2-,4-F	0.30±0.01 (n = 3)	26.7±5.3 (n = 2)
reference SB 203580				0.04±0.00 (n = 33)	2.0±0.3 (n = 16)

compounds of the 2,4-difluorophenylamino series are more potent than the corresponding inhibitors of the 2-amino-phenylamino series (**15e** vs **16i**) in the enzyme activity assay but are less active in whole blood, probably due to the higher hydrophilicity of the amino residue. These differences are only minor as the activities of both compounds are very high.

In addition to the series shown in Table 1 and Table 2, several phenoxy-substituted dibenzosuberones (Table 3) were also synthesized. All of them were less active than their corresponding phenylamino derivatives (**17a** vs **11b**, **17c** vs **16f**, **17d** vs **16i**) . We ascribe this decrease in activity to the loss of a hydrogen bond. The crystal structure of Skepinone-L (**2**)[13] suggests that the phenylamino nitrogen might form a (water-mediated) hydrogen bond to Asp169, which is located in an allosteric pocket of the enzyme. This interaction could be lost due to the exchange of the nitrogen atom for oxygen. Therefore compounds **17a**, **17c**, and **17d** support this mode of binding (Table 4). As expected, potency in whole blood likewise decreased. We attributed this loss of potency in the ex vivo assay to the decreased hydrophilicity.

Dibenzosuberones with moieties in the 8- and 9- positions (Table 5) were slightly less active in the isolated p38α MAP kinase assay than the corresponding derivatives with residues at position 7. The SARs of the different residues were similar to the ones of the 7-position. In contrast to this, potency in the whole blood assay dramatically decreased. There are several possible explanations for this phenomenon. The position of the residues might alter the binding to plasma proteins or may prevent the inhibitor from crossing through the cell membranes. The participation of a specific membrane transporter could also play a role in this process.

Kinase Selectivity Profiling. After we improved potency in the ex vivo whole blood assay, we had to confirm that introduction of residues at position 7/8/9 of the dibenzosuberone scaffold was not detrimental to the selectivity of the compounds. Therefore selectivity profiling of 32 compounds against a panel of 16 different kinases (Table 6) was performed by an independent laboratory.[27] All compounds tested revealed no significant inhibition of the 16 tested kinases, even at a concentration as high as 10 μM. As this result suggested high selectivity of the whole structural class, we tested Skepinone-L (**2**) against a panel of 402 kinases.[13] Only p38α and p38β MAP kinase were inhibited in a significant manner.

CONCLUSION

In summary, we have synthesized a large number of disubstituted phenylamino-dibenzosuberones. The recently published cocrystal structure of 2-(2-aminophenylamino)-dibenzosuberone with p38 MAP kinase[13] guided our efforts to improve potency as well as to select the physicochemical properties of our initial lead compound. Specifically, hydrophilic moieties at the 7-position resulted in highly potent compounds both in the p38 MAP kinase enzyme assay and in the whole blood assay with IC_{50} values in the single-digit (enzyme assay) and double-digit (whole blood assay) nM range. The use of the dihydroxypropoxy and the morpholin-4-yl-ethoxy moiety resulted in molecules with excellent drug-like properties while increasing potency toward p38α MAP kinase. Kinase selectivity profiling of 32 compounds against a panel of 16 kinases suggested the whole structural class to be highly selective. Our efforts finally yielded Skepinone-L (**2**), which is highly potent and shows an outstanding selectivity, both in vitro and in vivo.[13] Therefore it is an excellent tool which should lead to a better understanding of p38α MAP kinase and may foster the next generation of anti-inflammatory drugs.

EXPERIMENTAL SECTION

General. All commercially available reagents and solvents were used without further purification. Flash chromatography was performed with a LaFlash system (VWR) and Merck silica gel (PharmPrep 60 CC 25−40 μm). Melting points were determined with a Büchi Melting Point B-545 melting point apparatus, and they were

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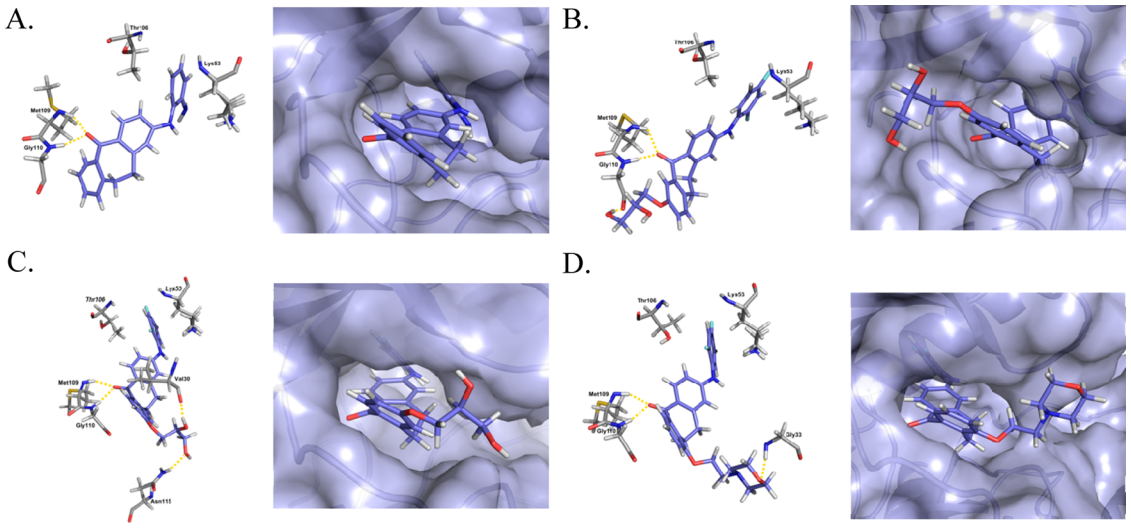
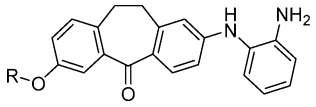


Figure 4. Suggested binding mode of selected dibenzosuberones. The molecules have been docked in the p38 MAP kinase active center by using the Induced Fit docking tool.[26] As a protein model, the X-ray structure of the 2-aminophenylaminodibenzosuberone (**1**) has been used. (A) Binding mode of compound **1**. (B) Binding mode of compound **2**. (C) Binding mode of compound **27g**. (D) Binding mode of compound **29f**.

Table 2. Biological Activity of 2-Aminophenylamino-Substituted Dibenzosuberones with Hydrophilic Moieties in the 7-Position



#	R	p38α IC_{50} ± SEM [μM]	TNF-α IC_{50} ±SEM [μM]
11a	OMe	0.04±0.01 (n = 3)	1.5±0.6 (n = 2)
15a		0.03 ±0.00 (n = 3)	0.19 ±0.02 (n = 4)
15b		0.02 ±0.00 (n = 3)	0.08 ±0.02 (n = 4)
15c		0.06 ±0.00 (n = 3)	0.19 ±0.06 (n = 4)
15d		0.008 ±0.003 (n = 3)	0.17 ±0.07 (n = 2)
15e		0.03 ±0.00 (n = 3)	0.11 ±0.05 (n = 4)
reference SB 203580		0.04 ±0.00 (n = 33)	2.0 ±0.3 (n = 16)

thermodynamically corrected. IR data were recorded with a Perkin-Elmer Spectrum One (ATR technique) spectrometer. Mass spectra were acquired on a Hewlett-Packard HP 6890 Series GC system equipped with a HP-5MS capillary column (0.25 μm film thickness, 30 m × 0.25 mm i.d.) (Hewlett-Packard) and a HP 5973 mass selective detector (70 eV) (Hewlett-Packard). High-resolution measurements (Fourier transform ion cyclotron resonance) were obtained on a Bruker APEX II with electron spray ionization. ^{1}H NMR (200 MHz) and ^{13}C NMR (50 MHz) spectroscopy was performed on a Bruker Advance 200 MHz instrument using TMS as internal standard. The chemical shifts were reported in ppm. The purity of the final compounds was determined by HPLC on a (method 1) Merck HPLC instrument (autosampler AS-2000, interface module D-6000, pump L-6200, detector L-4250) equipped with a LiChrospher RP18 column (5 μm) (Merck) or (method 2) on a LaChrome Ultra HPLC instrument (autosampler, interface module, oven L2300, pump L-216OU, detector diode array C-2455U) equipped with a Symmetry column (150 mm × 4.6 mm; 5 μm) (Waters) employing a gradient of 0.01 M KH_2PO_4 (pH 2.3) and methanol as the solvent system with a flow rate of 1.0 mL/min (method 1) or 1.5 mL/min (method 2). All final compounds have a purity of >95% (purity **17d** = 94.1).

General Procedure E. A mixture of the corresponding dibenzsouberone, aniline deriviative, $Pd(OAc)_2$, 2-(dicyclohexylphosphino)-2′-,4′-,6′-triisopropyl-biphenyl, KOtert-Bu/NaOtert-Bu, toluene, and *tert*-BuOH was heated at 90 °C under argon. After stirring at this temperature for 2 h, the reaction mixture was poured into water and extracted with 3 × 200 mL ethyl acetate. The residue was purified by flash chromatography.

General Procedure F. Water and *p*-toluenesulfonic acid monohydrate were added to a solution of the acetal in methanol. The solution was warmed to 50 °C under an argon atmosphere. After 6 h, the reaction mixture was cooled to room temperature and concentrated in vacuo to a yellow oil. This oil was redissolved in ethyl acetate and 5% sodium bicarbonate solution (50 mL). The organic layer was separated, dried over magnesium sulfate, and concentrated in vacuo. Recrystallization from dichloromethane/hexane gave the pure diol.

General Procedure H. A mixture of the corresponding dibenzsouberone, aniline deriviative, $Pd(OAc)_2$, 2-di-*tert*-butylphosphino-2′,4′,6′-triisopropylbiphenyl, K_3PO_4, and toluene was heated at 90 °C under argon. After stirring at this temperature for 30 min, the reaction mixture was poured into water and extracted with 3 × 200 mL ethyl acetate. The residue was purified by flash chromatography.

2-(2-Aminophenylamino)-7-methoxy-10,11-dihydrodibenzo[*a*,*d*]-cyclohepten-5-one (11a). **11a** was synthesized according to general procedure E using 0.52 g (1.9 mmol) of 7, 1.00 g (9.2 mmol) of 1,2-phenylenediamine, 0.05 g of $Pd(OAc)_2$, 0.15 g of 2-(dicyclohexylphosphino)-2′-,4′-,6′-triisopropyl-biphenyl, and 1.60 g (17.0 mmol) of NaO*tert*-Bu in 10 mL of toluene and 2 mL of *tert*-BuOH. The crude product was purified by flash chromatography (SiO_2; hexane/ethyl acetate 8 + 2). Yield 78%; purity ≥95%; mp 106,5 °C. IR (cm^{-1}) 3418−3343 $(C-H)_{arom}$, 3320 (N−H), 2976−2939 (C−H), 1546, 1516, 1494, 1323, 1284, 1147, 1026, 828, 751, 740. ^{1}H NMR (DMSO-d_6) δ in ppm: 2.96 (s, 4 H, $-CH_2-CH_2-$),

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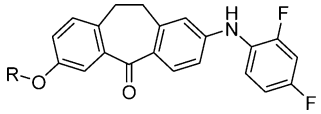
Table 3. Biological Activity of 2,4-Difluorphenylamino-Substituted Dibenzosuberones with Hydrophilic Moieties in the 7-Position

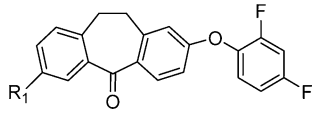


#	R	p38α IC_{50} ± SEM [μM]	TNF-α IC_{50} ±SEM [μM]
11b	OMe	0.02±0.01 (n =6)	1.2±0.4 (n = 2)
16a		0.01±0.00 (n = 3)	0.48±0.11 (n = 2)
16b		0.008±0.000 (n = 3)	0.19±0.00 (n = 2)
16c		0.06±0.00 (n = 3)	0.53±0.06 (n = 2)
16d		0.02±0.00 (n = 3)	0.23±0.00 (n = 2)
2		0.005±0.002 (n = 6)	0.04±0.01 (n = 6)
16e		0.04±0.02 (n = 3)	0.23±0.05 (n = 4)
16f		0.008±0.000 (n = 3)	0.06±0.02 (n = 6)
16g		0.01±0.01 (n = 3)	0.32±0.02 (n = 2)
16h		0.01±0.01 (n = 3)	0.08±0.01 (n = 4)
16i		0.01±0.00 (n = 3)	0.08±0.02 (n = 4)
16j		0.008±0.000 (n = 3)	0.08±0.01 (n = 4)
reference SB 203580		0.04±0.00 (n = 33)	2.0±0.3 (n = 16)

3.76 (s, 3 H, $-OCH_3$), 4.84 (s, 2 H, $-NH_2$), 6.46 (s, 1 H, C^1–H), 6.54–6.64 (m, 2 H, $C^{4\prime}$–H, $C^{5\prime}$–H), 4.78 (d, 1 H, J = 7.8 Hz, C^3–H), 6.91–7.04 (m, 3 H, C^8–H, $C^{3\prime}$–H, $C^{6\prime}$–H), 7.20 (d, 1 H, J = 8.3 Hz, C^9–H), 7.39 (s, 1 H, C^6–H), 7.92–7.97 (m, 2 H, C^4–H, –NH–). ^{13}C NMR (DMSO-d_6) δ in ppm: 33.6 (C^{10}), 36.6 (C^{11}), 55.5 ($-OCH_3$), 112.1 (C^6), 113.1 (C^3), 114.8 (C^1), 115.8 (C^3), 116.8 ($C^{4\prime}$), 118.3 (C^8), 125.2 ($C^{5\prime}$), 126.2 ($C^{6\prime}$), 126.3 ($C^{1\prime}$), 126.4 (C^{4a}), 130.4 (C^9), 133.9 (C^{5a}), 134.5 (C^4), 140.3 (C^{9a}), 144.0 ($C^{2\prime}$), 145.7 (C^{11a}), 151.1 (C^2). 158.0 (C^7), 190.3 (C^5).

2-(2,4-Difluorophenylamino)-7-(*S*-1,2-isopropylidenglycer-3-yl)-10,11-dihydro-dibenzo-[*a*,*d*]-cyclohepten-5-one (16d). **16d** was synthesized according to general procedure E using 0.67 g (1.8 mmol) of **10d**, 0.25 g (1.9 mmol) of 2,4-difluoroaniline, 0.05 g (0.22 mmol) of $Pd(OAc)_2$, 0.10 g (0.21 mmol) of 2-(dicyclohexylphosphino)-2′-,4′-,6′-triisopropyl-biphenyl, and 0.60 g (6.2 mmol) of NaO*tert*-Bu in 10 mL of toluene and 2 mL of *tert*-BuOH. The crude product is purified by flash chromatography (SiO_2; hexane/ethyl acetate 7 + 3). Yield 0.57 g (68%); purity ≥95%; mp 114.6 °C. IR (ATR) (cm^{-1}): 3352 (N–H), 2997–2944 (C–H), 1560, 1509, 1289, 1262, 1140, 843, 814, 802. ^1H NMR (DMSO-d_6) δ in ppm: 1.29 (s, 3 H, $-CH_3$), 1.34 (s, 3 H, $-CH_3$), 3.31 (s, 4 H, $-CH_2-CH_2-$), 3.72–4.45 (m, 5 H, $C^{1/3}{}_{glyceryl}$–H, $C^2{}_{isopropyliden}$–H), 6.60 (s, 1 H, C^1–H), 6.73 (d, 1 H, J = 9.3 Hz, C^3–H), 7.00–7.47 (m, 6 H, C^6–H, C^8–H, C^9–H, $C^{3\prime}$–H, $C^{5\prime}$–H, $C^{6\prime}$–H), 7.94 (d, 1 H, J = 8.6 Hz, C^4–H), 8.54 (s, 1 H, –NH–). ^{13}C NMR (DMSO-d_6) δ in ppm: 25.8 ($-CH_3$), 26.9 ($-CH_3$), 33.5 (C^{11}), 36.2 (C^{10}), 66.0 ($C^3{}_{glyceryl}$), 69.2 ($C^1{}_{glyceryl}$), 74.1 ($C^2{}_{glyceryl}$), 105.3 (dd, 1 C, J_1 = 24.2 Hz, J_2 = 26.6 Hz, $C^{3\prime}$), 109.2 ($C^2{}_{isopropyliden}$), 112.2 (dd, 1 C, J_1 = 3.9 Hz, J_2 = 22.0 Hz, $C^{5\prime}$), 112.6 (C^6), 113.9 (C^3), 115.4 (C^1), 119.1 (C^8), 125.2 (dd, 1 C, J_1 = 3.5 Hz, J_2 = 12.1 Hz, $C^{1\prime}$), 126.5 (dd, 1 C, J_1 = 3.5 Hz, J_2 = 9.8 Hz, $C^{6\prime}$), 127.7 C^{4a}), 130.6 (C^9), 133.8 (C^{5a}), 134.9 (C^4) 140.1 (C^{9a}), 145.7 (C^{11a}), 149.5 (C^2), 156.0 (dd, J_1 = 12.6 Hz, J_2 = 247.9 Hz, $C^{4\prime}$), 157.2 (C^7), 158.8 (dd, J_1 = 11.5 Hz, J_2 = 243.0 Hz, $C^{2\prime}$), 190.7 (C^5).

Table 4. Biological Activity of Phenoxy-Substituted Dibenzosuberones with Hydrophilic Moieties in the 7-Position



#	R1	p38 IC_{50} ± SEM [μM]	TNF-α IC_{50} ± SEM [μM]
17a	OMe	0.1±0.03 (n = 3)	-
17c		0.04±0.001 (n =6)	6.7±9.8 (n = 2)
17d		0.08±0.01 (n = 3)	4.5±1.9 (n = 2)
reference SB 203580		0.04 ±0.00 (n = 33)	2.0 ±0.3 (n = 16)

2-(2,4-Difluorophenylamino)-7-[2*R*-,3-dihydroxypropoxy]-10,11-dihydro-dibenzo[*a*,*d*]-cyclohepten-5-one (2). **2** was synthesized according to general procedure F using 1.0 g (2.1 mmol) of **16d**, 0.25 g (1.3 mmol) of *p*-toluensulfonic acid monohydrate, 40 mL of methanol, and 10 mL of H_2O. Yield 0.85 g (95%); purity ≥95%; mp 130.3 °C. IR (ATR) (cm^{-1}): 3305 (N–H), 2934 (C–H), 1629, 1607, 1569, 1510, 1327, 1278, 1218, 1097, 847, 781. ^1H NMR (DMSO-d_6) δ in ppm: 3.32 (s, 4 H, $-CH_2-CH_2-$), 3.40–3.46 (m, 2 H, $-C^{1/3}{}_{propoxy}$–H), 3.77–4.05 (m, 3 H, $C^{1//2/3}{}_{propoxy}$–H), 4.66 (t, 1 H, J = 5.7 Hz, –OH), 4.94 (d, 1 H, J = 4.9 Hz, –OH), 6.60 (s, 1 H, C^1–H), 6.73 (d, 1 H, J = 8.9 Hz, C^3–H), 7.01–7.10 (m, 2 H, $C^{3\prime}$–H, $C^{6\prime}$–H), 7.22 (d, 1 H, J = 8.3 Hz, $C^{4\prime}$–H), 7.30–7.47 (m, 3 H, C^6–H, C^8–H, C^9–H), 7.94 (d, 1 H, J = 8.8 Hz, C^4–H), 8.54 (s, 1 H, –NH–). ^{13}C NMR (DMSO-d_6) δ in ppm: 33.6 (C^{11}), 36.3 (C^{10}), 63.1 ($C^3{}_{propoxy}$), 70.2 ($C^1{}_{propoxy}$), 70.3 ($C^2{}_{propoxy}$), 105.3 (dd, 1 C, J_1 = 24.1 Hz, J_2 = 27.0 Hz, $C^{3\prime}$), 112.2 (dd, 1 C, J_1 = 3.8 Hz, J_2 = 22.5 Hz, $C^{5\prime}$), 112.6 (C^6), 113.8 (C^3), 115.4 (C^1), 119.1 (C^8), 125.3 (dd, 1 C, J_1 = 3.4 Hz, J_2 = 11.9 Hz, $C^{1\prime}$), 126.4 (dd, 1 C, J_1 = 3.1 Hz, J_2 = 9.8 Hz, $C^{6\prime}$), 127.7 (C^{4a}), 130.6 (C^9), 133.8 (C^{5a}), 134.6 (C^4), 139.9 (C^{9a}), 145.7 (C^{11a}), 149.4 (C^2), 156.0 (dd, 1 C, J_1 = 12.6 Hz, J_2 = 248.5 Hz, $C^{4\prime}$), 157.6 (C^7), 158.8 (dd, 1 C, J_1 = 12.3 Hz, J_2 = 243.8 Hz, $C^{2\prime}$), 190.8 (C^5).

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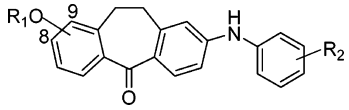
Table 5. Biological Activity of Different Phenylamino-Substituted Dibenzosuberones with Hydrophilic Moieties at the 8-Position and 9-Position



#	Position	R1	R2	p38 IC_{50} ±	TNF-α IC_{50} ±
27a	8-	H_3C–	2,4-F	0.02±0.00	10.2±1.6 (n = 2)
27b	8-	HO–	2,4-F	0.02±0.00	4.7±0.3 (n = 2)
27c	8-	HO–	2,4-F	0.03±0.00	19.5±1.2 (n = 2)
27d	8-		2,4-F	0.02±0.01	170±1.5 (n = 2)
27e	8-	HO, OH	2,4-F	0.03±0.00	10.4±2.4 (n = 2)
27f	8-		2,4-F	0.36±0.05	123±0.3 (n = 2)
27g	8-	HO, OH	2,4-F	0.005±0.001	5.2±0.0 (n = 2)
27h	8-		2,4-F	0.02±0.00	40.0±13.2 (n = 2)
28a	8-	H_3C–	2-NH_2	0.07±0.01	12.3±0.5 (n = 2)
28b	8-		2-NH_2	0.08±0.00	93.6±21.7 (n = 2)
28c	8-	HO, OH	2-NH_2	0.03±0.00	6.0±0.3 (n = 2)
29a	9-	H_3C–	2,4-F	0.08±0.00	78.7±11.9 (n = 2)
29b	9-	H–	2,4-F	0.02±0.00	37.3±3.9 (n = 4)
29c	9-		2,4-F	0.29±0.01	87.2 (n = 1)
29d	9-		2,4-F	0.44±0.01	40% @ 100 μM
29e	9-	HO, OH	2,4-F	0.02±0.00	100±5.2 (n = 2)
29f	9-		2,4-F	0.03±0.00	200 (n = 1)
30a	9-	H_3C–	2-NH_2	0.08±0.00	52.1±3.2 (n = 2)
30b	9-		2-NH_2	1.04±0.02	158 (n = 1)
30c	9-	HO, OH	2-NH_2	0.04±0.00	78.8±14.1 (n = 2)
reference SB 203580				0.04±0.00	2.0± (n = 16)

2-(2,4-Difluoro-phenoxy)-7-methoxy-10,11-dihydro-dibenzo[*a*,*d*]cyclohepten-5-one (17a). **17a** was synthesized according to general procedure H using 0.5 g (1.8 mmol) of 2-chloro-7-methoxy-10,11-dihydro-dibenzo[*a*,*d*]cyclohepten-5-one (**7**), 0.28 g (2.2 mmol) of 2,4-difluorophenol, 0.013 g (0.058 mmol) of $Pd(OAc)_2$, 0,024 g (0.057 mmol) of 2-di-*tert*-butylphosphino-2′,4′,6′-triisopropylbiphenyl, 0.78 g (3.7 mmol) of K_3PO_4, and 10 mL of toluene. The crude product is purified by flash chromatography (SiO_2; hexane/ethyl acetate 7 + 3). Yield (39%); purity ≥95%; mp 113 °C. IR (ATR) (cm^{-1}): 3054, 2906, 1501, 1423, 1293, 1248, 1236, 1186, 1136, 1090, 961, 890, 834, 786. ^1H NMR (DMSO-d_6) δ in ppm: 2.98−3.13 (m, 4 H, $-CH_2-CH_2-$), 3.77 (s, 3 H, $-OCH_3$), 6.84−6.93 (m, 2 H, $C^{3'/5'}-H$), 7.08 (dd, 1 H, J_1 = 2.9 Hz, J_2 = 8.3 Hz, C^3-H), 7.12−7.29 (m, 2 H, $C^{1/6'}-H$), 7.34−7.59 (m, 3 H, $C^{8/6/9}-H$), 7.95 (d, 1 H, J = 9.5 Hz, C^4-H). ^{13}C NMR (DMSO-d_6) δ in ppm: 33.4 (C^{10}), 35.1 (C^{11}), 55.6 ($-OCH_3$), 106.3 (dd, J_1 = 22.5 Hz, J_2 = 27.4 Hz, $C^{3'}$), 112.8 (dd, J_1 = 3,8 Hz, J_2 = 22.9 Hz, $C^{5'}$), 114.2 (C^3), 114.3 (C^6), 116.4 (C^1), 119.4 (C^8), 124.8 (dd, J_1 = 1.9 Hz, J_2 = 9.9 Hz, $C^{6'}$), 131.2 (C^9), 132.8 (C^{4a}), 133.8 (C^4), 134.7 (C^{5a}), 138.2 (dd, J_1 = 3,6 Hz, J_2 = 12.0 Hz, $C^{1'}$) 139.1 (C^{9a}), 145.8 (C^{11a}), 154.2 (dd, J_1 = 13.0 Hz, J_2= 248.0 Hz, $C^{2'}$), 158.1 (C^7), 159.3 (dd, J_1 = 10.7 Hz, J_2 = 242.7 Hz, $C^{4'}$), 160.7 (C^2), 192.4 (C^5).

2-(2,4-Difluorophenylamino)-8-methoxy-10,11-dihydro-dibenzo[*a*,*d*]cyclohepten-5-one (27a). **27a** is synthesized according to general procedure E using 0.52 g (1.9 mmol) of **22a**, 0.30 g (2.3 mmol) of 2,4-difluoroaniline, 0.04 g of $Pd(OAc)_2$, 0.16 g of 2-(dicyclohexylphosphino)-2′-,4′-,6′-triisopropyl-biphenyl, and 0.86 g (8.9 mmol) of NaO*tert*-Bu in 10 mL of toluene and 2 mL of *tert*-BuOH. The crude product is purified by flash chromatography (SiO_2; hexane/ethyl acetate 9 + 1); mp 157 °C. ^1H NMR (DMSO-d_6) δ in ppm: 3.02 (dd, 4H, J_1 = 13.73 Hz, J_2 = 8.96 Hz, $-CH_2-CH_2-$), 3.82 (s, 3H, $-OCH_3$), 6.61 (s, 1H, C^1-H), 6.73 (d, 1H, J = 8.64 Hz, C^3-H), 6.84−6.92 (m, 2H, $C^{3'}-/C^{6'}-H$), 7.04−7.13 (m, 1H, $C^{5'}-H$), 7.34−7.42 (m, 2H, C^7-/C^9-H), 7.96 (d, 2H, J = 8.64 Hz, C^4-/C^6-H), 8.49 (s, 1H, $-NH-$). ^{13}C NMR (DMSO-d_6) δ in ppm: 35.2 (C^{11}), 35.8 (C^{10}), 55.7 ($-OCH_3$), 105.3 (dd, J_1 = 26.5 Hz, J_2 = 24.0 Hz, $C^{3'}$), 112.1 (dd, J_1 = 21.5 Hz, J_2 = 3.7 Hz, $C^{5'}$), 112.5 (C^7), 112.7 (C^9), 113.6 (C^3), 113.9 (C^1), 125.4 (dd, J_1 = 12.1 Hz, J_2 = 3.6 Hz, $C^{1'}$), 126.3 (dd, J_1 = 9.1 Hz, J_2 = 3.3 Hz, $C^{6'}$), 128.2 (C^{4a}), 131.3 (C^{5a}), 133.7 (C^4), 133.8 (C^6), 145.1 (C^2), 145.3 (C^{11a}), 149.1 (C^{9a}), 155.9 (dd, J_1 = 246.4 Hz, J_2 = 12.5 Hz, $C^{4'}$), 158.7 (dd, J_1 = 241.6 Hz, J_2 = 11.4 Hz, $C^{2'}$), 162.4 ($C^{8'}$), 189.1 (C^5).

■ ASSOCIATED CONTENT

Supporting Information

Crystal structure of compound **7**, synthetic procedures, ^1H NMR, ^{13}C NMR, HPLC, HRMS, IR, melting points molecular modeling, eExpression and purification of p38α, crystallization and structure determination of p38α in complex with 2-amino-phenylamino-benzophenone. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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Table 6. Kinases and Target Compounds Tested for Selectivity by an Independent Laboratory

kinase no.	AKT1 SAK	ARK5 TRK-B	Aurora-B SRC	B-RAF VE VEGF-R2	INS-R IGF1-R	MET FAK	PLK1 AXL	PRK1 CK2-alpha1
11e	**11f**	**11i**	**11j**	**11k**	**11l**	**11m**	**12**	**13**
15a	**15b**	**15e**	**16a**	**16b**	**16c**	**16d**	**16f**	**16g**
16j	**27d**	**27e**	**27f**	**27g**	**29a**	**29b**	**29c**	**29d**
29e	**29f**	**30a**	**30b**	**30c**				

■ ABBREVIATIONS USED

AIBN, azo-isobutyronitrile; IBD, inflammatory bowel disease; IC_{50}, half-maximal inhibitory concentration; IL, interleukin; MAP, mitogen activated protein; NBS, *N*-bromosuccinimide; RA, rheumatoid arthritis; SARs, structure−activity relationships; TNF, tumor necrosis factor

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