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Design, synthesis and SAR of phenylamino-substituted 5,11-dihydro-dibenzo[*a*,*d*]cyclohepten-10-ones and 11*H*-dibenzo[*b*,*f*]oxepin-10-ones as p38 MAP kinase inhibitors

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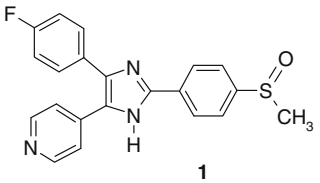
ABSTRACT

The p38 MAP kinase is a key enzyme in inflammatory diseases as it is involved in the biosynthesis of pro-inflammatory cytokines such as TNF-α and IL-1β. Small molecule p38 inhibitors suppress the production of these cytokines and therefore p38 is a promising drug target for novel anti-inflammatory therapeutics. In this study, we report the design, synthesis, and SAR of novel *N*-substituted 11*H*-dibenzo[*b*,*f*]oxepin-10-ones and 5,11-dihydro-dibenzo[*a*,*d*]cyclohepten-10-ones as p38 inhibitors.

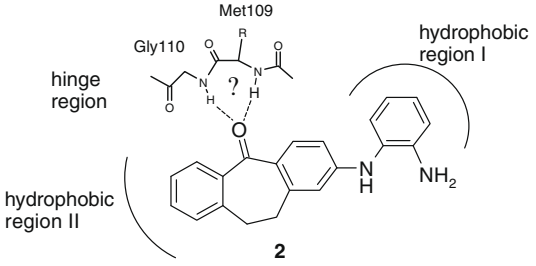
The central role of p38 mitogen activated protein (MAP) kinase in inflammatory cell signaling accounts for the continuing interest in the development of novel p38 MAPK inhibitors. p38 MAPK belongs to the class of serine-threonine MAP kinases and regulates the biosynthesis of inflammatory cytokines such as TNF-α and IL-1β at the translational and transcriptional levels.[1–3] Elevation of the levels of these mediators beyond normal physiological limits is involved in the pathogenesis of chronic inflammatory diseases including rheumatoid arthritis, psoriasis, and Crohn's disease. Small molecule p38 MAPK inhibitors suppress the production of these cytokines and therefore p38 MAPK is an attractive and promising drug target for novel anti-inflammatory therapeutics.[4,5]

Several companies have reported preliminary human clinical results for p38 MAPK inhibitors, but until now no drug has reached the market. Problems seem to be structure related, for example, a well investigated lead compound, the pyridinylimidazole SB203580 (Fig. 1), causes liver toxicity. As the toxicity of p38 MAPK inhibitors is considered to be structural related rather than mechanism based, there is a continuous need for novel chemotypes. Recently, researchers from our group designed and synthesized a novel series of p38 MAPK inhibitors based on the benzophenones, resulting in dibenzosuberones and dibenzo-oxepinones.[6,7] The ATP competitive inhibitor **2** (Fig. 2) binds in the ATP binding site located in the cleft between the N- and the C-terminal domains of p38α MAP kinase. Two hydrogen bonds, one between the carbonyl oxygen of **2** and the backbone NH of Met109 and the other between the carbonyl oxygen of **2** and the backbone NH of Gly110, could be formed if the glycine-flip in the kinase was induced. Without a flip just the hydrogen bond to the Met109 is possible. The carbonyl functionality is essential for any inhibitory activity. The linked aniline moiety occupies the hydrophobic region I (also called selectivity pocket) of the kinase.



1
SB203580

Figure 1. Inhibitor of p38 MAP kinase: SB203580 (**1**).



2

Figure 2. Schematic drawing of important interactions between the competitive inhibitor **2** and the ATP binding site of p38α MAPK.

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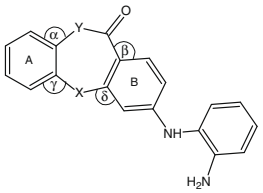
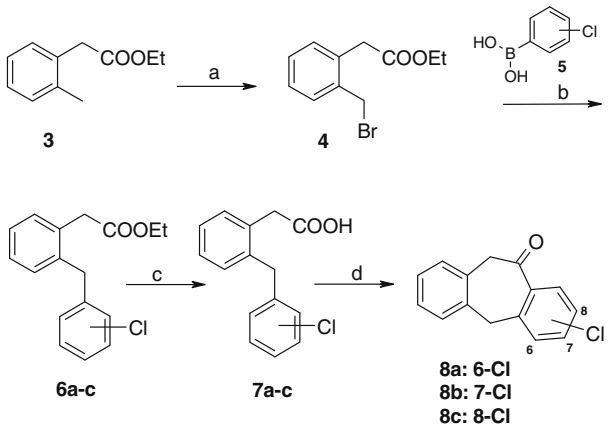
Table 1
Molecular geometries of the tricyclic scaffolds **16a**, **21a** and **24a**[a]



Compd	**16a**	**21a**	**24a**
X, Y	CH_2, CH_2	O, CH_2	O, NH
Dihedral angle[b]	58.3	57.5	56.0
α	120.7	121.7	118.1
β	116.1	117.4	119.1
γ	120.9	121.0	119.2
δ	116.4	116.3	118.3

[a] Calculation based on computationally minimized structures.[9]
[b] Angle between the two phenyl rings A and B.



Scheme 1. Synthesis of **8a–c**. Reagents and conditions: (a) NBS, AIBN, CCl_4, reflux, 4 h, 83%; (b) $Pd(PPh_3)_4$ (10 mol %), DME, Na_2CO_3 (aq), 50 °C to reflux temperature, 3 h, 52–57%; (c) NaOH, EtOH, H_2O, reflux, 6 h, 62–73%; (d) (i) $(COCl)_2$, CH_2Cl_2; 1.5 h; (ii) $AlCl_3$, CH_2Cl_2, rt, 2.5 h, 82–90%.

Docking studies conducted with docking tools from Schroedinger[8] predicted alternative positions that the carbonyl oxygen of the inhibitor might occupy. Within these inhibitor structures, the carbonyl functionality was moved on the bridge side and the linker atoms X and Y were varied to obtain distinctive molecular geometries and to obtain additional interaction opportunities (Table 1 and Fig. 3).

Our aim was to retain the key interaction: the bidentate hydrogen bond of the carbonyl oxygen of the inhibitor to the backbone NH of Met109 and the backbone NH of Gly110. The docking studies predict binding modes with and without a glycine-flip, depending on the X-ray structure used. But based on existing X-ray structures with similar ligands we suppose that our compounds induce a Gly110-flip in the p38α. Compounds that induce the glycine-flip exhibit selectivity for p38α over other kinases with less flexible, non-glycine residues at this position.[10] The possible binding modes of compounds **16a**, **21a** and **24a** in p38α are illustrated in Figure 3.

The phenylamino moiety occupies the selectivity pocket of the kinase. As the tricyclic unit is asymmetric, positions for the phenylamino moiety at both aromatic rings were chosen. Furthermore, positions adjacent to the para position were also investigated, because the molecular geometry and the orientation of the carbonyl group differ from the lead structure.

A series of dibenzosuberones was prepared by two different methods. In this paper we disclose a new, 4-step synthesis of **8** (Scheme 1) that gives access to *N*-substituted 5,11-dihydrodibenzo[*a*,*d*]cyclohepten-10-ones. The first step of the novel synthesis route to **8** involves the bromination of the methyl group of compound **3** by using *N*-bromosuccinimide and AIBN. The benzyl bromide **4** underwent a palladium-catalyzed Suzuki cross coupling reaction with the corresponding boronic acid **5**. The general syntheses for the diarylmethanes via modification of Suzuki reaction conditions have been reported earlier.[11,12] Subsequent hydrolysis of the ethylester **6** followed by intramolecular ring closure yielded **8**.

Dibenzosuberones with a chloro-substituent in the 2- and 3-positions (**8d** and **8e**) were derived by a modified synthetic route according to Nizamuddin,[13] since the starting material corresponding to **3** was not available for this synthesis (as shown in Scheme 2).

The dibenz[*b*,*f*]oxepinone scaffolds **14**, the key intermediates for the synthesis of **21**–**23**, were prepared by a modified synthetic pathway according to Ong et al.[14,15] (Scheme 3). The initial step to **14a–c** was the reduction of the phenoxybenzoic acids **9a–c** to the corresponding alcohols with $LiAlH_4$, followed by treatment with thionyl chloride to yield the chlorides which were converted to the nitriles **12a–c** with potassium cyanide. The phenoxyacetonitriles were hydrolyzed with potassium hydroxide to give **13a–c**, and the subsequent ring closure to the ketones **14a–c** was best achieved by the intramolecular Friedel–Crafts reaction under standard conditions.

Preparation of the scaffolds containing an oxazepinone structure **24a**,**b** was derived from a modified synthesis according to Smits et al.[16] The synthesis involves amide formation with activated 4-nitro-2-chloro benzoic acid and 2-aminophenole. The fol-

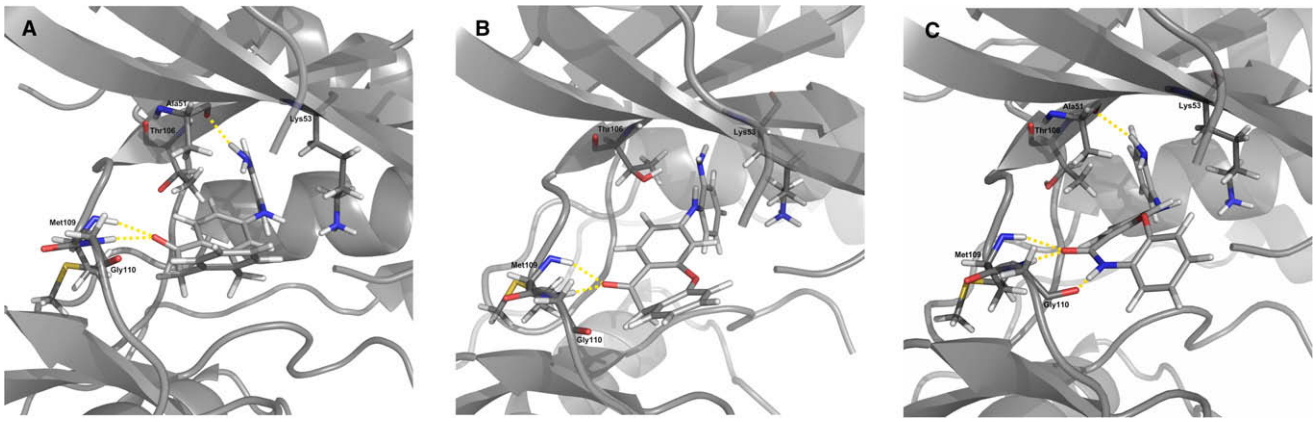
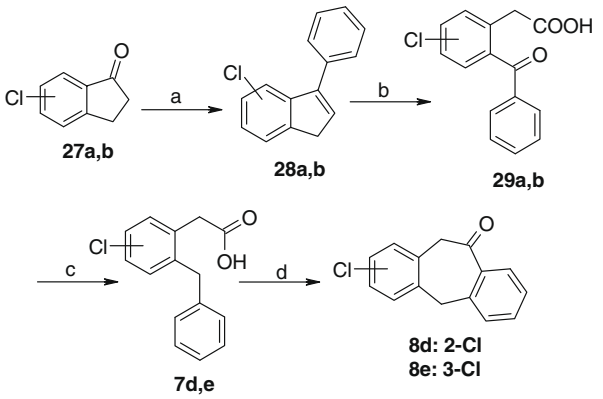
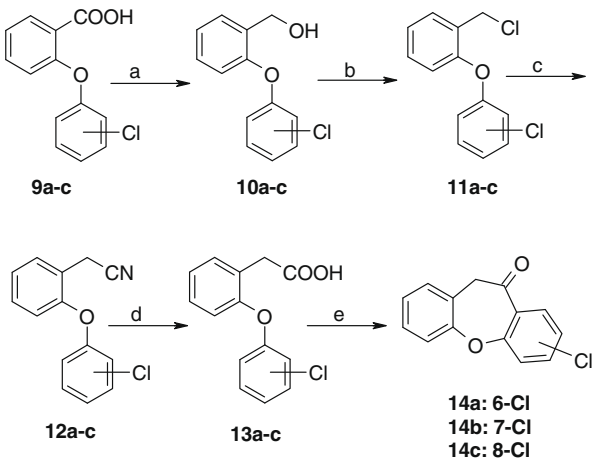


Figure 3. (A) Docking result for compound **16a**; (B) Docking result for compound **21a**; (C) Docking result for compound **24a**. The hydrogen bonds are indicated as yellow dotted lines. For these docking results the X-ray structure 2QD9 from the PDB database was used.



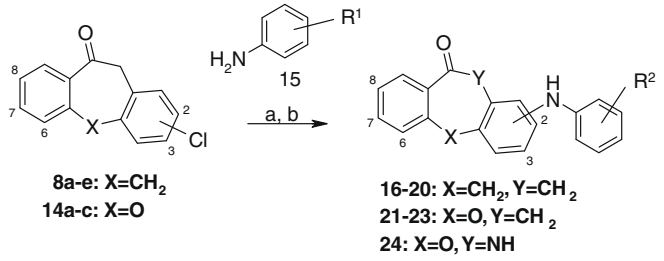
Scheme 2. Synthesis of chloro-substituted 5,11-dihydrodibenzo[*a*,*d*]cyclohepten-10-ones (**8d**,**e**).[12] Reagents and conditions: (a) (i) C_6H_4Br, Mg, diethylether; (ii) HCl, H_2O, rt, 95–99%; (b) $NaIO_4$, $RuCl_3{\cdot}H_2O$, hexane, CH_3CN, H_2O, rt, 43–47%; (c) N_2H_4, NaOH, DEG, 120 °C then 200 °C, 62–69%; (d) (i) $(COCl)_2$, CH_2Cl_2; (ii) $AlCl_3$, CH_2Cl_2, rt,82–90%.



Scheme 3. Synthesis of **14a**–**c**. Reagents and conditions: (a) $LiAlH_4$, THF (abs), 0 °C, then reflux temperature; 4 h, 60–63%; (b) $SOCl_2$, toluene, 80 °C, 1.5 h, 93–99%; (c) KCN, H_2O, EtOH, reflux temperature, 5 h, 91–95%; (d) KOH, H_2O, EtOH, reflux temperature, 4 h, 64–66%; (e) (i) $(COCl)_2$, CH_2Cl_2, 1.5 h; (ii) $AlCl_3$, CH_2Cl_2, rt, 2.5 h, 83–91%.

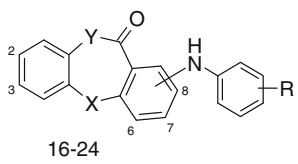
lowing intramolecular ring closure was performed with NaOH in DMF. Nitro reduction over 10% Pd/C provided **24**.

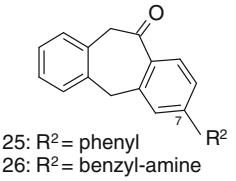
The target compounds **16**–**24** were prepared via Buchwald Hartwig reaction of the tricyclic scaffold with respective phenylamino moieties (as shown in Scheme 4). If the phenylamino moiety bears a nitro functionality a subsequent reduction step was required to



Scheme 4. Synthesis of **16**–**24**. Reagents and conditions: (a) $Pd(OAc)_2$, 2-(dicyclohexyl-phosphino)-2′-, 4′-, 6′-triisopropyl-biphenyl, KO*tert*-Bu or Cs_2CO_3, toluene, *tert*-BuOH, 3–5 h, 7–42%; (b) this step is only for $R^1 = NO_2$ required: $SnCl_2$, ethanol, 70 °C, 4.5 h, 44–68%. R^1 = H; 2-NO_2; 2-NO_2, 4-F or 2,4-difluoro. For the nature of R^2 (compd **16**–**24**) see Table 1.

Table 2
Evaluation of p38 MAP kinase activity based on the rate of phosphorylation of ATF-2 (activation transcription factor 2) in a previously described assay[17]





Compd	X	Y	Position	R[c]	Inhibition IC_{50}[a] (μM)
2					0.05 ± 0.02
16a	CH_2	CH_2	7	2-NH_2	0.99 ± 0.03
16b	CH_2	CH_2	7	2,4-Difluoro	1.51 ± 0.21
16c	CH_2	CH_2	7	2-NH_2, 4-F	1.72 ± 0.11
16d	CH_2	CH_2	7	H	1.75 ± 0.27
16e	CH_2	CH_2	7	4-NH_2	15%[b]
17a	CH_2	CH_2	8	2-NH_2	4.01 ± 1.02
17b	CH_2	CH_2	8	2,4-Difluoro	44%[b]
18a	CH_2	CH_2	6	2,4-Difluoro	6%[b]
18b	CH_2	CH_2	6	H	47%[b]
19a	CH_2	CH_2	3	2-NH_2	1.93 ± 0.40
19b	CH_2	CH_2	3	2-NH_2, 4-F	3.82 ± 0.90
19c	CH_2	CH_2	3	H	3.21 ± 0.49
20a	CH_2	CH_2	2	2-NH_2	2.10 ± 0.83
20b	CH_2	CH_2	2	2-NH_2, 4-F	0.98 ± 0.58
20c	CH_2	CH_2	2	H	47%[b]
21a	O	CH_2	7	2-NH_2	1.20 ± 0.31
21b	O	CH_2	7	2-NH_2, 4-F	1.63 ± 0.71
21c	O	CH_2	7	H	2.92 ± 1.19
22a	O	CH_2	8	2-NH_2	36%[b]
22b	O	CH_2	8	2-NH_2, 4-F	46%[b]
22c	O	CH_2	8	H	25%[b]
23a	O	CH_2	6	2-NH_2	37%[b]
23b	O	CH_2	6	2,4-Difluoro	12%[b]
23c	O	CH_2	6	H	32%[b]
24a	O	NH	7	2-NH_2	2.70 ± 0.62
24b	O	NH	7	2-NH_2, 4-F	2.92 ± 1.19
25					43%[b]
26					9%[b]

IC_{50} of SB203580 = 0.033 μM.
[a] Mean ± S.D. of three trials.
[b] % Inhibition at 10 μM.
[c] Substituents are numbered as shown for presentational uniformity; see Experimental Section for correct numbering of substituents.

obtain the target compounds. For the phenylamino moieties we chose the unsubstituted one and also favorable substituents from our earlier reported dibenzosuberone series,[6] like 2-NH_2, 2,4-difluoro and 2-NH_2–4-F.

All compounds were screened in an immunosorbent non-radioactive p38 enzyme assay,[17] at concentrations ranging from 10^{-5} to 10^{-8} M. The ability of test compounds to compete with ATP for the kinase's ATP binding site correlates with the capacity of p38 MAPK to phosphorylate its natural substrate, activating transcription factor 2 (ATF-2), when incubated with ATP and the test compound. SB203580 was used as reference and the optimized ATP concentration at which the test was performed was 100 μM. The results of p38 MAP kinase enzyme assays are summarized in Table 2.

In the dibenzosuberone series (X and Y = CH_2), analysis of the SAR revealed that introduction of phenylamino moieties at the 7 position resulted in a moderate inhibition of p38 MAP kinase. Compound **16a** showed an IC_{50} of 0.99 μM and therefore was 20-fold weaker than reference **2**. The substitution pattern of the phenylamino moiety had no clear effect on the inhibitory activity (compounds **16a**–**d**), only the 4-amino substituent (compound **16e**) was not tolerated. Compounds with phenylamino residues in 8-position were less tolerated, compound **17a** showed an IC_{50} of 4.01 μM. Compounds with phenylamino residues in 6-position

were also less tolerated, compound **18b** was only weakly active and compound **18a** was inactive against p38 MAP kinase.

Compounds with phenylamino substituent in position 7, 3 and 2 were equally potent (compare compounds **16a–d** with **19a–c** and **20a**,**b**). Replacement of the carbon linker with an oxygen linker (X = O, compounds **21–23**) altered the angle between the phenyl rings. Only compounds with substituents at the 7 position were tolerated, while modifications at positions 8 and 6 resulted in weakly active compounds.

Comparison of **16a** and **21a** reveals that the corresponding suberones and oxepinones are equally potent. Benzoxazepinones like compounds **24a** and **24b** were compared to their corresponding suberones and oxepinones equally potent. Interestingly, the introduction of an additional hydrogen bond acceptor (Y = NH) did not result in improved activity.

Based on the favorable results with compound **16d**, we varied the distance between the two aromatic scaffolds to determine if this extension would result in a better fit with the selectivity pocket. Therefore, compounds **25** and **26** were synthesized (for the synthesis see Supplementary data) and compared to compound **16d**. Compound **25** was only weakly active and compound **26** was inactive against p38 MAPK.

Compounds **16a–e**, **17a**, **19a–c**, **20a–c**, **24a–b**, **25** and **26** were screened in a kinase assay by ProQuinase[18] in 16 kinases including AKT1, ARK5, Aurora-B, AXL, B-RAF VE, CK2-α1, FAK, IGF1-R, INS-R, MET, PLK1, PRK1, SAK, SRC, TRK-B and VEGFR-2. None of the tested compounds showed a relevant inhibitory effect.

In summary, a new, short route for the preparation of **8a–c** as key intermediates has been developed using synthesis of **6a–c** via Suzuki coupling. Initial investigations of the inhibitory activities and structure–activity relationships of phenylamino-substituted dibenzo[*b*,*f*]oxepin-10(11*H*)-ones, 5,11-dihydro-(10*H*)-dibenzo[*a*,*d*]cyclohepten-10-ones and dibenzo[*b*,*f*][1,4] oxazepin-11(10*H*)-ones inhibitors for p38 MAP kinase were accomplished. The promising structural variations suggested by our docking experiments did not result in any novel compounds as active as the previously defined compound **2** (IC_{50} = 0.05 μM for R = NH_2). While our initial hypothesis that substitution of a tricyclic scaffold would result in a favorable position for the carbonyl functionality in the inhibitor was not supported by our data, we did identify some structural determinants that may be useful for development of future p38 MAPK inhibitors.

Acknowledgments

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Supplementary data

Supplementary data (general synthetic procedures, spectral and analytical data, HPLC purity and HRMS data of test compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.03.107.

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18. http://www.proqinase.com/ (the assay was performed at 10 μM inhibitor-concentration; values <20% are considered as no relevant inhibition).