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Simplified staurosporine analogs as potent JAK3 inhibitors

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Abstract—Simplification of bottom ring and regioselective functionalization of the indolocarbazole unit of staurosporine (**2**) are described. The modification led to a new series of simplified staurosporine analogs, which exhibited significant inhibitory activity against Janus kinase 3 (JAK3). The structure–activity relationships (SAR) are discussed and a proposed binding model is also highlighted.

Janus kinases (JAK), including JAK1, JAK2, Tyk2, and JAK3, are cytoplasmic protein tyrosine kinases that play pivotal roles in cytokine mediated biological responses by activating the cytoplasmic latent forms of STAT proteins.[1] Among the four members of the JAK family, JAK3 is abundantly expressed in lymphoid cells and plays a crucial role in normal lymphocyte development and function, as evidenced by qualitative and quantitative deficiencies in the B-cell as well as T-cell compartments of the immune system of JAK3-deficient mice[2] and development of severe combined immunodeficiency in JAK3-deficient patients.[3] Furthermore, it has been demonstrated that JAK3 plays a key role in the regulation of mast cell mediated allergic and asthmatic responses.[4] Therefore, a potent and specific chemical inhibitor of JAK3 may be useful for the treatment of immune-mediated diseases including rejection of organ transplant, atopic dermatitis, allergy, and asthma.[4,5]

Several potent JAK3 inhibitors have been recently identified,[6] including naphthyl ketones,[6d] oxindole derivatives,[6e] pyridine-containing tetracycles,[6f] and CP-690550 (**1**,[6g] Fig. 1). In particular, **1** has shown selectivity for JAK3 over JAK1 and JAK2 (100- and 20-fold, respectively) and has demonstrated efficacy in animal organ transplant models.

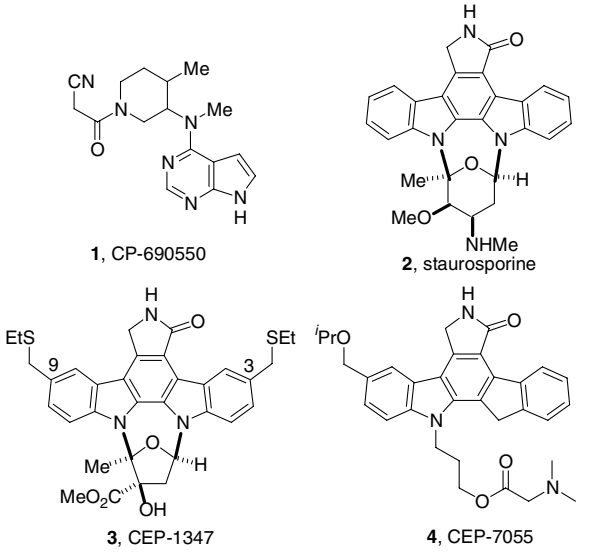

Figure 1. Chemical structures of CP-690550 (**1**), staurosporine (**2**), CEP-1347 (**3**), and CEP-7055 (**4**).

Staurosporine (**2**) is a potent inhibitor of several kinases.[7] Despite the nonselective kinase activities, staurosporine's ability to inhibit phosphorylation and to modulate expression of JAK proteins has been demonstrated.[8] In addition, introduction of a suitable substituent at the C-3 and/or C-9 positions of the indolocarbazole unit of staurosporine provided potent kinase inhibitors CEP-1347 (**3**, for ChAT, JNK, and MLKs)[9] and CEP-7055 (**4**, for VEGF-R)[10] that entered

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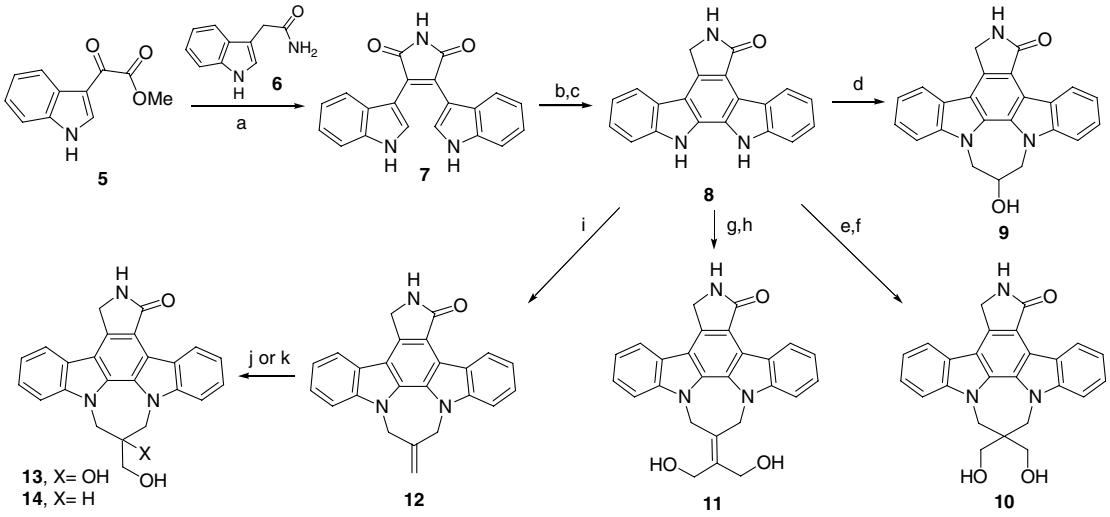


Scheme 1. Reagents and conditions: (a) *t*-BuOK, THF, 0 °C to rt, 4 h, then HCl, 89%; (b) $PdCl_2$, DMF, 90 °C, 3 h, 80%; (c) Sn, HCl, HOAc, 110 °C, 5 h, 96%; (d) epibromohydrin, Cs_2CO_3, DMF, rt, 24 h, 74%; (e) 5,5-dibromomethyl-2,2-dimethyl-1,3-dioxane, Cs_2CO_3, MeCN, microwave, 160 °C, 90 min, 35%; (f) THF, MeOH, cat. *p*-TSA, 45 °C, 3 h, 55%; (g) 1,1-bis(*tert*-butyldiphenylsiloxy)-2,2-bis(chloromethyl)ethane, Cs_2CO_3, DMF, 50 °C, 24 h, 50%; (h) TBAF, THF, rt, 15 min, 78%; (i) 3-chloro-2-chloromethyl-1-propene, Cs_2CO_3, DMF, rt, 24 h, 80%; (j) $OsCl_3$, Me_3NO, THF, $CHCl_3$, rt, 48 h, **13** (83%); (k) 9-BBN, THF, 65 °C, 3 h, then H_2O_2, 10% $NaOH_{(aq)}$, 65 °C, **14** (81%).

clinical trials. Herein we describe the discovery of novel JAK3 inhibitors related to staurosporine. Our synthetic efforts focused on the simplifying the bottom ring and introducing regioselective functionality on the indole rings. This led to a new series of potent JAK3 inhibitors.

The initial focus was to reduce the synthetic complexity of the bottom piece of **2**, by replacing the amino-sugar ring with a medium size alkyl ring. The hydroxyl group was appended to further mimic the H-bonding interaction of the methylamino group of AFN941 with Arg953 of the JAK3 backbone.[11] The synthesis of the key intermediate **8** started with the condensation of **5** and **6** under *t*-BuOK conditions to form the bis-indole **7** (Scheme 1).[12] Subsequent oxidative cyclization mediated by $PdCl_2$ followed by monoreduction of the biscarbonyl using tin metal afforded **8** in excellent yield. With the intermediate **8** in hand, subsequent alkylation with suitable electrophiles rapidly provided diverse hydroxyl compounds with a simplified bottom ring. For example, double alkylation of **8** with dibromide or dichloride followed by a suitable deprotective process gave diols **10** and **11**. Furthermore, alkylation with epibromohydrin accompanied by a ring opening of the epoxide afforded **9**. Dialkylation with 3-chloro-2-chloromethyl-1-propene gave alkene **12** that could be further converted to **14** or diol **13** by hydroboration or dihydroxylation, respectively.

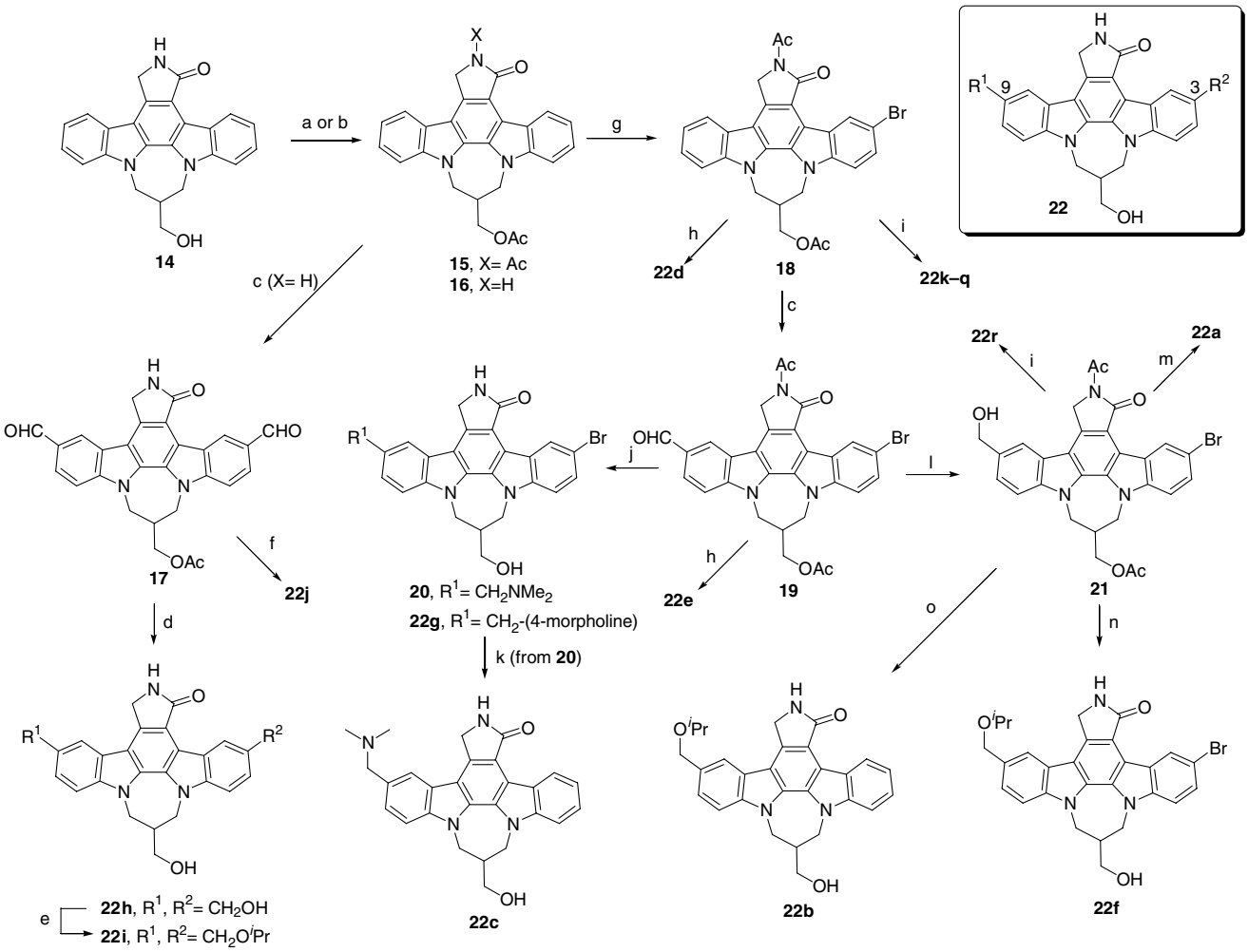
The JAK3 inhibitory activities (IC_{50}) for the hydroxyl compounds **9**–**14** compared to **2** are shown in Table 1.[13] These results, in general, revealed that these simplified staurosporine analogs with hydroxyl groups in the bottom ring exhibited good activity with IC_{50}'s below 40 nM with the exception of **9** (142 nM). Extension of the OH group by one methylene (**9** vs **14**) resulted in an 8-fold increase in potency. Moreover, comparing **9** and **13** clearly shows the importance of the hydroxymethyl group. This modification enhanced activity, presumably by providing a crucial H-bonding interaction with the Arg953 residue. Furthermore, the introduction of a second hydroxymethyl group (**10**) gave a slight improvement in potency. Compound **10** was only 2-fold less potent than staurosporine (**2**). The slight decrease in potency of the diol **11** (IC_{50} of 36 nM) could be due to an unfavorable positioning of the hydroxyl group by the planar alkene configuration. Compound **12**, which was devoid of the hydroxyl group, still gave good activity at 72 nM.

Table 1. IC_{50} of **9**–**14** against JAK3

Compound	IC_{50}[a] (nM)
9	142[b]
10	13.5 (±1.5)
11	36 (±1)
12	72 (±10)
13	27 (±2)
14	17.5 (±4.5)
2	6 (±3)

[a] Values are means of 3–5 experiments, standard deviation is given in parentheses.
[b] Single experiment.

After completing the investigation of the simplified bottom pieces, the next effort was focused on regioselective functionalization at the C-3 and/or C-9 position of the indolecarbazole unit to explore the SAR of the new lead structure **14**. Synthesis was initiated with the protection of **14** to afford acetyl-protected **15** or **16** depending on reaction temperature (Scheme 2). Subsequent Lewis acid promoted bisformylation[9c] with dichloromethyl methyl ether gave **17**, which could be further manipulated to provide bis-substituted analogs **22j** and **22h**. Further conversion of **22h** in isopropanol

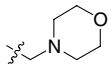
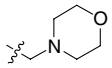
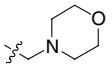
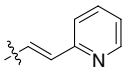
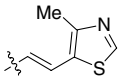
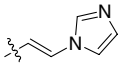
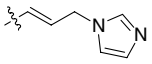
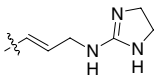
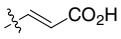
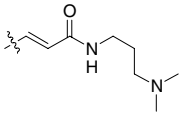
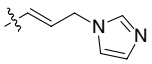


Scheme 2. Reagents and conditions: (a) Ac_2O, DMAP, THF, 50 °C, **15** (82%); (b) Ac_2O, DMAP, THF, rt, **16** (47%); (c) Cl_2CHOMe, $TiCl_4$, CH_2Cl_2, rt, 24 h, **17** (83%), **19** (83%); (d) $NaBH_4$, THF, rt, then MeOH, 82%; (e) ^{i}PrOH, camphorsulfonic acid (CSA), rt, 3 days, 17%; (f) i—morpholine, $NaBH(OAc)_3$, AcOH, THF, CH_2Cl_2; ii—NaOMe, MeOH, 19%; (g) NBS, $CHCl_3$, MeOH, rt, 1 h, 81%; (h) NaOMe, MeOH, rt, 1 h, **22d** (80%), **22e** (75%); (i) alkene, $Pd(OAc)_2$ (15 mol %), (*o*-tolyl)$_3$P (30 mol %), H_2O/DMF (1:19), microwave, 150 °C, 30 min, then NaOMe, MeOH, ca. 20–40% (two steps); (j) morpholine or dimethyl amine, $NaBH(OAc)_3$, THF, rt, 4 h, then NaOMe, MeOH, **22g** (80%), **20** (72%); (k) *N*,*N*,*N*′,*N*′-tetramethylethylenediamine, $NaBH_4$, $PdCl_2$(dppf) (10 mol%), DMF, rt to 45 °C, 6 h, 35%; (l) $NaBH_4$, THF, rt, 1 h, 72%; (m) $PdCl_2$(dppf) (10 mol %), HCO_2Na, DMF, 100 °C, 1.5 h, then NaOMe, MeOH, 58%; (n) i—$(CF_3CO)_2O$, Et_3N, CH_2Cl_2, 0 °C to rt, 1 h, 81%; ii—^{i}PrOH, CSA, microwave, 150 °C, 1 h, then NaOMe, MeOH, 75%; (o) i—$PdCl_2$(dppf) (10 mol %), HCO_2Na, DMF, 100 °C, 1.5 h, 75%; ii—^{i}PrOH, CSA, 70 °C, 36 h, then NaOMe, MeOH, 29%.

in the presence of camphorsulfonic acid[9c] gave **22i**. Regioselective bromination at the C-3 position of the more electron rich indole ring was achieved to afford intermediate **18** and **22d** after deprotection. The bromine substituent, in fact, offered more options as a protecting group or for further installation of a C-3 substituent. For example, the Heck coupling of **18** with different substituted alkenes afforded **22k**–**q**.[14] Formylation of **18** gave **19** and **22e** after typical deprotection. Further elaboration of **19** by reductive amination gave **22g** and **20**, which could undergo debromination to afford **22c** with a dimethylaminomethyl group at the C-9 position.[15a] Moreover, the hydroxyl compound **21**, formed by reduction of **19**, was converted to **22a** by debromination with $PdCl_2$(dppf) and sodium formate,[15b] and **22r** by a Heck coupling reaction. Finally, compounds **22b** and **22f** were obtained in a similar manner from intermediate **21**.

The effect of these compounds was tested in vitro against JAK3 enzyme and compared to that of the parent hydroxyl compound **14** (Table 2). The IC_{50} fluctuated considerably depending on the position and pattern of the C-3 and C-9 substituents. For instance, introduction of a hydroxymethyl at C-9 position (R^1, **22a**) resulted in a marked improvement in potency (ca. 6-fold) compared to the parent **14**. However, a bulkier substituent such as an isopropoxymethyl (**22b**) or dimethylaminomethyl (**22c**) caused a 27- and 83-fold loss of potency, respectively. This observation differed slightly from that reported for compound **4** (Fig. 1) for which the isopropoxide group was well tolerated.[10] Moreover, the activity dropped dramatically to the micromolar range when the morpholinylmethyl group was attached (**22g**). The steric influence of R^1 was consistent and could also be observed in compounds **22d**–**g**, suggesting the existence of a small pocket around the C-9 position.

Table 2. Substituent effect and SAR of **22**

Compound	R^1 (C-9)	R^2 (C-3)	IC_{50}[a] (nM)
14	–(H)	–(H)	17.5 (±4.5)
22a	CH_2OH	H	3 (±0.5)
22b	CH_2O^iPr	H	80 (±27)
22c	CH_2NMe_2	H	250 (±10)
22d	H	Br	23.5 (±1.5)
22e	CHO	Br	15 (±3)
22f	CH_2O^iPr	Br	70 (±9)
22g		Br	3.13[b] μM
22h	CH_2OH	CH_2OH	14 (±6)
22i	CH_2O^iPr	CH_2O^iPr	1.05[b] μM
22j			>5[b] μM
22k	H		354 (±40)
22l	H		592[b]
22m	H		109 (±19)
22n	H		11 (±3)
22o	H		31 (±1)
22p	H		686[b]
22q	H		237[b]
22r	CH_2OH		3 (±0.5)

[a] Values are means of 3–5 experiments, standard deviation is given in parentheses.
[b] Single experiment.

A comparison between **14** and **22d** as well as **22a** and **22h** shows how well a small substituent (R^2), such as bromine or hydroxylmethyl, could be tolerated at the C-3 position without significantly altering potency. Increasing the size of the hydrophobic substituent at C-3, for example isopropoxymethyl (**22i**), was unfavorable, causing a 13-fold decrease in potency. The loss of potency of compound **22j** (IC_{50} > 5 μM) might be attributed to the morpholinomethyl substitution at C-9. Heck coupling of the intermediate bromide, as described above, provided several C-3 substituted compounds that gave moderate to good potency depending on the nature of the aromatic ring and the length of the linker. For example, the activity of the pyridine or thiazole substituted alkenes (**22k**–**l**) was greater than 300 nM. However, the imidazole substituted alkene (**22m**) provided a compound with an IC_{50} of 109 nM. Notably, an elongated linker, such as allyl (**22n**), increased the activity to 11 nM, which was a slight improvement over the parent compound **14** and ca. 10- and 54-fold better than **22m** and **22l**, respectively. Moreover, the allyl guanidine substituted compound **22o** also provided an IC_{50} value in the double-digit range (31 nM). These results suggest that the imidazole ring or guanidine unit can be tethered with a suitable linker to provide a crucial interaction with the JAK3 enzyme. Further introduction of a carboxylic acid (**22p**) or amide with a longer solubilizing group (**22q**) gave no improvement. Finally, the combination of both optimized R^1 and R^2 substituents, namely the hydroxymethyl at C-9 and the allyl imidazole at C-3, led to compound **22r** having an IC_{50} value of 3 nM, which is more potent than staurosporine.

The possible binding mode of **22r** in the ATP-binding site of JAK3 was investigated by molecular docking,[16] based on the newly published JAK3 crystal structure[11]

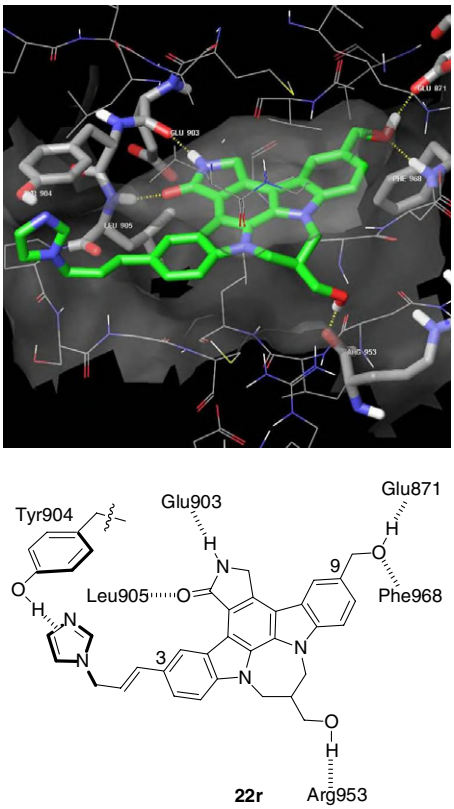


Figure 2. Docking study of **22r** into ATP-binding site of JAK3.

(Fig. 2). The docking result indicated that **22r** may adopt a typical bidentate hydrogen-bonding mode with the JAK3 backbone at the hinge region the lactam nitrogen with the carbonyl oxygen of Glu903 and the carbonyl oxygen with the amide nitrogen of Leu905. The hydroxymethyl on the bottom ring may form a hydrogen bond with the side chain of Arg953. The hydroxymethyl side chain at the C-9 position may point to the inside of a small binding pocket and form hydrogen bonds with Glu871 and Phe968 explaining the large contribution of the hydroxyl group to the potency. The imidazole side chain at the C-3 position extends into the solvent. Interestingly, the OH of the phenol side chain of the Tyr904 residue is perpendicular to the imidazole ring, which could potentially act as a hydrogen bond acceptor.[17] This may explain the observed activities.

In summary, a series of simplified staurosporine analogs with different substituted groups attached at C-3 and/or C-9 position have been synthesized. These compounds exhibited excellent inhibitory activity against JAK3. The SAR indicated that a small group with H-bonding capability, such as a hydroxymethyl, at the C-9 position is preferred to lower the IC_{50} to a single-digit nanomolar range. Moreover, an allyl linker with an imidazole unit was well tolerated at the C-3 position. Unfortunately, **22n** and **22r** exhibited poor solubility precluding them from further development. However **22o** had moderate solubility at pH 2.0 (0.03 mg/mL). Introduction of a suitable group at the C-3 position may provide a compound with improved solubility.

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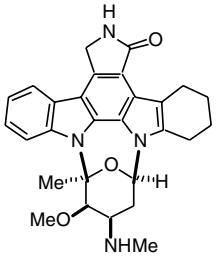
11. Boggon, T. J.; Li, Y.; Manley, P. W.; Eck, M. J. *Blood* **2005**, *106*, 996, The structure of AFN941 is presented below



AFN941

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13. JAK3 enzyme was purified from Sf21 cells (San Diego, CA) infected with a baculovirus expression vector for JAK3 (JH1 and JH2 domain). Enzyme activity of JAK3 was determined in terms of enzyme phosphorylation by the following method. JAK3 enzyme solution (48 μL) in 1.25× TK buffer (62.5 mM HEPES, pH 7.5, 12.5 mM $MgCl_2$) containing 42 mM DTT (Sigma, St. Louis, MO) was added into the each well of a polypropylene 96-well plate. Next, 5 μL of diluted compounds in DMSO and 48 μL of biotinylated peptide enzyme substrate (5 μg diluted in TK buffer containing 10 μM ATP) were added to each well. Control wells received equal volume of vehicle. The contents of the wells were mixed and the reaction mixture was incubated for 1 h at room temperature. Post incubation, 90 μL of reaction mixture was transferred into a washed Neutravidin-coated plate (Pierce Neutravidin Biotin-binding Protein 31000; 1 mg/mL 1:100). The plate was then incubated for 15 min at room temperature and washed three times with PBS-T. PY99 anti-phosphotyrosine antibody (Santa Cruz #sc-7020HRP) was added into each well and the plate was incubated for 40 min at room temperature. After incubations, the plate was washed three times and 100 μL TMB (Sigma, St. Louis, MO) was added to each well. The plate was incubated for another 40 min at room temperature in the dark. The reaction was stopped by the addition of 1 M H_2SO_4 (50 μL/well) and the optical density was read at 450/650 nm.
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16. The compound **22r** was docked, using the docking program Glide (Glide, Schrodinger, 1500 S. W. First Avenue, Suite 1180, Portland, OR 97201). The molecular mechanism calculations were done with MacroModel (MacroModel, Schrodinger, 1500 S. W. First Avenue, Suite 1180, Portland, OR 97201), using OPLS2001 force field. The effect of aqueous solution was treated by GB/SA model. Polak–Ribiere conjugate gradient method was used for energy minimization, and the derivative convergence criterion was set at 0.05 KJ/Å-Mol.
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