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Design, synthesis, and studies of small molecule STAT3 inhibitors

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Abstract—A series of small molecule STAT3 inhibitors originally derived from our lead compound **STA 21** were synthesized and evaluated. The most potent compound in this series, compound **1**, exhibited the same anti-proliferative activities as **STA 21** against prostate cancer cell lines that express constitutively active STAT3. Molecular docking showed compound **1** bound to the STAT3β SH2 domain in a similar manner as **STA 21**.

Signal transducers and activators of transcription 3 (STAT3) is one of the downstream signaling proteins for cytokine and growth factor receptors.[1,2] Activation of the receptors induces the phosphorylation of STAT3 at tyrosine residue 705, which leads to dimerization of two STAT3 monomers through SH2 domains of the proteins.[3,4] The activated STAT3 dimers then translocate into the nucleus and activate the transcription of genes that control cell proliferation, apoptosis, angiogenesis, and other cell functions.[5,6]

Since STAT3 serves a pivotal role in cell proliferation and survival, it is recognized as one of the significant oncogenic signaling pathways. Constitutive activation of STAT3 was first reported in head and neck and multiple myeloma.[7,8] Subsequently, the overexpression of the transcription factor was also reported in different kinds of leukemias and lymphomas and in solid tumors such as melanoma, breast, ovarian, lung, pancreatic, and prostate cancers.[9–12]

Constitutive activation of STAT3 was reported in prostate cancer. Studies showed that the elevated levels of constitutively active STAT3 in prostate tumor samples ranged from 82% to 100%.[13,14] In addition, the elevated STAT3 activity appeared to localize mainly in the tumor cells but not in the surrounding normal tissues.[13,14] Constitutively active STAT3 in prostate cancer played an important role in enhancing its development and progression through stimulating the cancer cell proliferation as well as inhibiting apoptosis. In prostate cancer cell lines, STAT3 activity was higher in androgen-independent as compared to androgen-dependent cells.[13,15] In addition, the overexpression of STAT3 in androgen-dependent cells stimulated the growth of the cells in an androgen-independent manner.[16] Reports showed that inhibiting STAT3 activation in human prostate cancer cells suppressed proliferation; induced apoptosis in vitro and tumorigenicity in vivo.[13,14,17,18] Thus, STAT3 has emerged as one of the promising molecular targets for the treatment of prostate cancer.

Several strategies were used in inhibiting STAT3 functions. One of the approaches was to inhibit the upstream signals of STAT3 such as Jak and Src kinases.[19–21] The kinases activated other signaling pathways in addition to STAT3 and inhibitors of Jak and Src kinases might inhibit other downstream targets, thus potentially causing undesirable side effects.[22] Another approach for inhibiting STAT3 activation was through RNA interference. Strategies such as double negative STAT3 mutants, antisense STAT3 oligonucleotides, and a decoy oligonucleotide all inhibited STAT3.[17,18,23,24]

Since STAT3 is activated by multiple upstream receptor tyrosine kinases, the best approach is to inhibit STAT3

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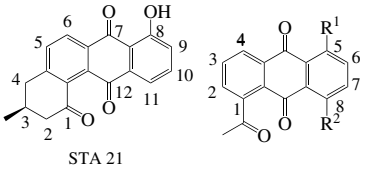
directly. Several peptide aptamers and a series of tripeptides and peptidomimetics containing a phosphotyrosine residue were reported to inhibit STAT3 dimerization, cell proliferation, and induction of apoptosis in Src-transformed fibroblasts with constitutive expression of STAT3.[25,26] However, peptide-based inhibitors usually suffer from poor cell permeability and in vivo stability.[25]

In order for STAT3 to be activated, STAT3 must first form dimers through their SH2 domains and translocate to the nucleus to activate targeted genes that promote cell growth and survival.[27,28] Recently, we identified a small molecule (**STA 21**—8-hydroxy-3-methyl-3,4-dihydro-2*H*-benzo[*a*]anthracene-1,7,12-trione) with STAT3 inhibitory activity through structure-based virtual screening (Fig. 1).[29] **STA 21** was hypothesized to bind to the SH2 domain of STAT3 and subsequently block the dimerization of STAT3. Studies showed that **STA 21** inhibited STAT3 DNA binding, dimerization, and STAT3-dependent luciferase activity. In addition, **STA 21** exhibited selective anti-proliferative activity against breast cancer cell lines (MDA-MB-435, MDA-MB-468, and MDA-MB-231) with constitutive STAT3 expression but with no effect on cells without STAT3 overexpression (MCF-7 and MDA-MB-453).[29]

Synthetically it is difficult to generate analogs of **STA 21** for structure–activity-relationship studies because of its structural complexity. Our first approach was to simplify **STA 21** by retaining the anthracene moiety and the functional groups that are critical for binding to the SH2 domain of STAT3 to form compound **1** (Fig. 1). The predicted binding model of **STA 21** to the STAT3β SH2 binding domain is shown in Figure 2. The model predicts that **STA 21** binds and forms a number of hydrogen bonds at the SH2 domain with nearby residues, including Arg 595, Arg 609, and Ile 634 (Fig. 2). Molecular docking revealed that compound **1** retains the hydrogen-bonding characteristic similar to **STA 21** at the SH2 domain of STAT3 (Fig. 2).

As shown in Figure 2, the OH group in **STA 21** serves as both hydrogen bond donor (Ile 634) and acceptor (Arg 595) at the SH2 domain. Compounds **2**, **3**, and **4** were designed to define the importance of the hydrogen-bonding interaction at the SH2 domain. In addition, they also serve to validate the binding model predicted by the program Autodock (v 4.0).

The syntheses of compounds **1**–**4** are shown in Figure 3 according to the reported procedure.[30] In brief, the syntheses began with the oxidation of **5** to yield the naphthoquinone **6** with chromium (VI) oxide. Diels–Alder reaction of **6** with 1-methoxy-cyclohexa-1,3-diene yielded the mixture of **7** and **8** which were transformed to hydroquinones **9** and **10**. The ethylene bridges in compounds **9** and **10** were cleaved through retrodiene elimination to yield the mixture of compounds **3** and **4**. The compounds were easily separated by preparative TLC (EtOAc/Hex, 2:1). The structures of the compounds were assigned using 2D-NOSY NMR (data not shown). Demethylation of compounds **3** and **4** with hydrobromic acid afforded compounds **1** and **2**, respectively.



Compound	R^1	R^2
1	OH	H
2	H	OH
3	OMe	H
4	H	OMe

Figure 1. Structures of **STA 21** and proposed compounds **1**–**4**.

Compounds **1** and **2** were also obtained through Diels–Alder reaction of compound **6** with 3-hydroxy-2-pyrone in refluxing xylene for 48 h.[30] However, the yield was low (26%) and the separation of the compounds was difficult. Recently, Komiyama et al. obtained a series of 5-hydroxy-1,4-naphthoquinones through base-catalyzed Diels–Alder reaction between 3-hydroxy-2-pyrone and 1,4-benzoquinones in the presence of triethylamine at low temperature (−15 °C).[31] Similar reaction conditions were then employed for the synthesis of compounds **1** and **2** by reacting compound **6** with 3-hydroxy-2-pyrone (Fig. 4). The reaction was completed in 30 min with an overall yield of 92% and in a ratio of 5:1 for compound **1**/compound **2**, which were separated by silica gel column chromatography.

Compounds **1**–**4** and **STA 21** were examined for their anti-proliferative activities against three prostate cancer cell lines, DU145, PC3, and LNCaP. All three cell lines were reported to exhibit constitutive activation of STAT3 with LNCaP and DU145 cells possessing the lowest and highest levels of expression, respectively.[13] MCF-7 breast cancer cells, a cell line with no constitutive expression of STAT3, were used as a negative control.[29] Cells were treated with test compounds for 72 h and cell viability was determined by the MTS assay. As shown in Table 1, **STA 21**, a small molecule that inhibits STAT3 dimerization, exhibited good anti-proliferative activity in DU145 and PC3 androgen-independent prostate cancer cell lines with IC_{50} values of 12.2 and 18.7 μM, respectively. However, **STA 21** showed weak inhibitory activity (IC_{50} = 124 μM) toward MCF-7 cells that have no constitutive STAT3 expression.

The benzo[*a*]anthracene-1,7,12-trione moiety in **STA 21** renders it difficult to generate analogs for structure–activity-relationship studies. Our approach was to convert the benzo[*a*]anthracene-1,7,12-trione moiety in **STA 21** to anthraquinone to generate compound **1**. Molecular modeling studies of **STA 21** bound to STAT3β indicated that the 8-OH of **STA 21** formed H-bonds with Ile 634 and Arg 595 (Fig. 2). In addition, the 1-keto group in **STA 21** served as a H-bond acceptor and interacted with Arg 609 and Ser 636 (Fig. 2). In compound **1**, the 1-acetyl and the 5-OH groups structurally correspond to the 1-keto and 8-OH groups of **STA 21**, respectively. Molecular docking showed that compound **1** interacts similarly with STAT3β at the SH2 domain with H-bonds formed between 5-OH—Ile 634 and 1-acetyl—Arg 609. The anti-proliferative activities of

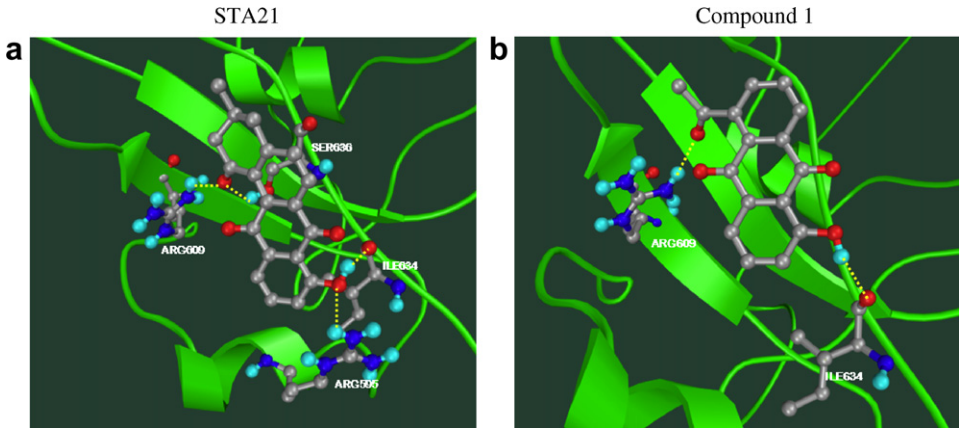


Figure 2. The predicted binding model of **STA 21** (a) and compound **1** (b) to the STAT3β. The models were predicted by Autodock (v 4.0). Only the residues that form hydrogen bonds with the compounds are shown.

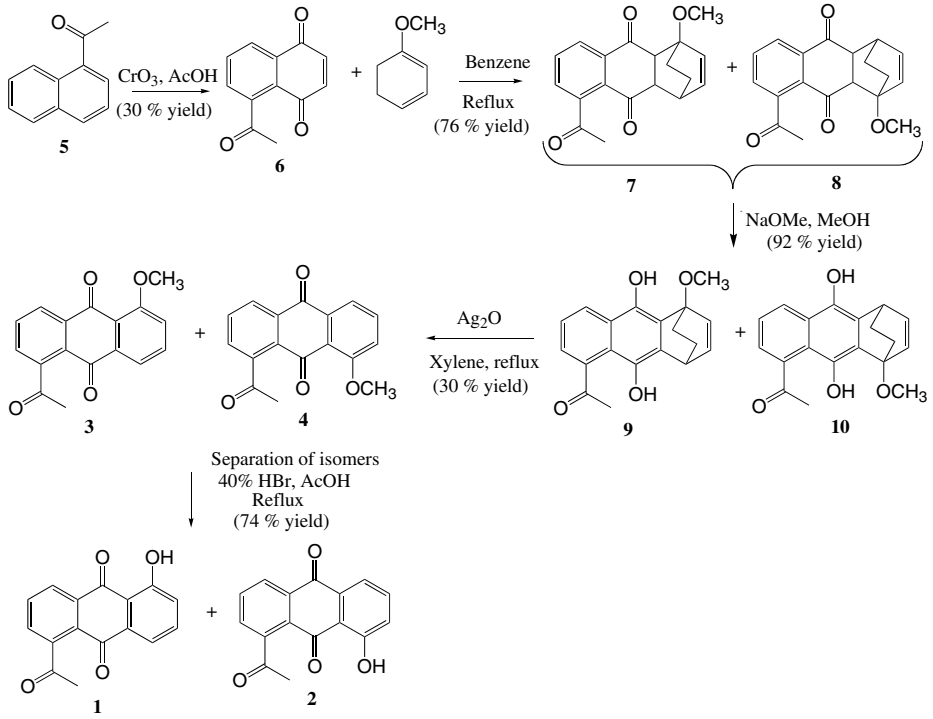


Figure 3. Synthesis of compounds **1**–**4**.

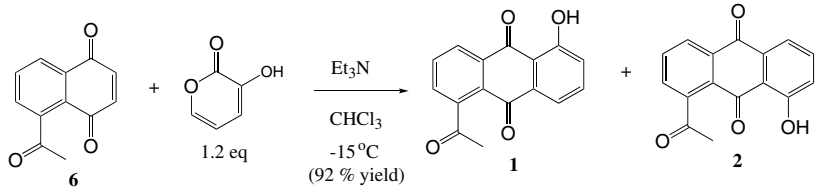


Figure 4. Improved synthesis of compounds **1** and **2**.

compound **1** on DU145, PC3, and LNCaP were similar to **STA 21** with IC_{50} values of 16.2, 13.4, and 34.1 μM, respectively (Table 1). In addition, the anti-proliferative activities were directly proportional to the level of constitutively active STAT3 expression (Table 1). Similar to **STA 21**, compound **1** showed weak anti-proliferative activity toward MCF-7 cells (IC_{50} = 84 μM—Table 1). Compounds **3** and **4** lacked the 5-OH groups on the anthracene moieties and would not form hydrogen bonds with Ile 634 at the SH2 domain. As predicted, both compounds were inactive in all three cell lines (Table 1). Surprisingly, compound **2** showed significant

Table 1. Anti-proliferative activity of **STA 21** and compounds **1**–**4** on prostate cancer cell lines DU145, PC3, LNCaP, and breast cancer cell line MCF-7

Drug	DU145 IC_{50} (μM)	PC3 IC_{50} (μM)	LNCaP IC_{50} (μM)	MCF-7 IC_{50} (μM)
STA 21	12.2	18.7	Not tested	124
1	16.2	13.4	34.1	88.5
2	31.5	32.4	31.5	Not tested
3	>100	>100	>100	Not tested
4	>100	>100	>100	Not tested

Cells (2000 cells/well) were treated with varying concentrations of the compounds and cell associated protein was determined using MTS assay. The IC_{50} values represent means of two experiments in triplicate. Values are the average of two separate experiments.

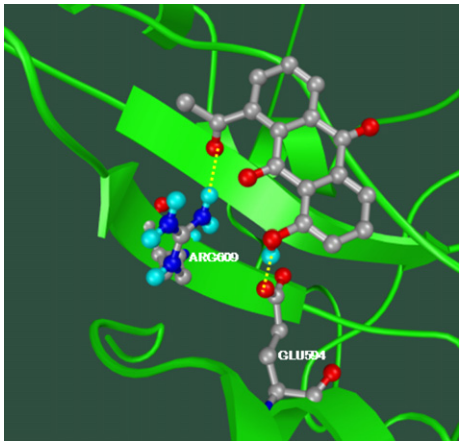


Figure 5. Predicted binding model of compound **2** to STAT3β. The model was predicted by Autodock (v 4.0). Only the residues that form hydrogen bonds with the compound are shown.

anti-proliferative activities against all three cell lines (DU145, PC3, and LNCaP). Compound **2** had the 8-OH group instead of the 5-OH on the anthracene moiety and could not form a hydrogen bond with Ile 634 at the SH-2 domain. However, molecular docking revealed that the 8-OH group of compound **2** was H-bonded to Glu 594 at the SH2 domain (Fig. 5). This may explain the anti-proliferative activities of the compound.

In conclusion, we have successfully modified our small molecule STAT3 inhibitor **STA 21** to generate a structurally simpler molecule (compound **1**). Molecular docking showed that compound **1** bound to the STAT3β SH2 domain in a similar manner as **STA 21**. Compound **1** also exhibited the same anti-proliferative activities as **STA 21** against prostate cancer cell lines that express constitutively active STAT3. Thus, compound **1** serves as a lead compound for the design of more potent and selective STAT3 inhibitors.

Acknowledgments

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