

Docking and Synthetic Studies of Some Salicylic and Indomethacin Derivatives as Potential Inhibitors of Cyclin-Dependent Kinase 2

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Abstract

The anti-inflammatory drug aspirin and its metabolite salicylic acid are reported to decrease the levels of cyclin A2 and CDK2 (cyclin-dependent kinase 2). Our objective in the present study is to design, synthesize and investigate salicylic acid derivatives (analogs and isosters) and indomethacin analogs which have the potential to inhibit CDK2 so that colon cancer cell growth can be arrested. The ability of several compounds to dock with CDK2 utilizing OpenEye software program was performed. Docking study showed that anthranilic acid interacts with PDB 1PYE through HB with Asp 145: A and Val 64: A. Different synthetic studies by using palladium catalyzed chemistry were adopted to prepare the starting materials for generation aza-indomethacin.

Keywords: Aspirin; Indomethacin; Stille coupling; OpenEye docking.

Introduction

Anticancer drugs reduce the body's white blood cell count and block cell growth and replication, which halts cancer cells from growing [1-22]. Patients receiving chemotherapy, however, become more prone to infections and illnesses with a lowered immune system. So, the obvious next step in chemotherapy drugs would be to create some that make the immune system fight the cancer cells as well. Extensive studies determined that NSAID as indomethacin has growth inhibitory effect on different cancer cell lines^{1, 2, 3, 4}. Epidemiological studies and *in vitro* experiments reported in the last 10 years suggest that aspirin (acetyl salicylic acid) is an effective chemo preventive agent and reduces the risks of various types of cancer including colon cancer^{4, 1}. Studies have shown that doses ranging from 81 mg-325 mg aspirin taken over prolonged periods of time decrease the incidence and mortality associated with colorectal cancer^{5, 6, 7}. Once taken into the body, aspirin is

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rapidly metabolized to salicylic acid. It is hypothesized that both functional groups of aspirin (the acetyl and salicylate group) contribute to its anti-cancer effects. Currently, researchers are interested in discovering novel pathways by which aspirin exerts anti-cancer effects in human epithelial tissues, and identifying novel compounds that are capable of inhibiting cancer cell growth. 1, 8 Literatures finding showed that aspirin and its primary metabolite, salicylic acid, decrease the levels of cyclin A2 and CDK2 in a diverse panel of human cancer cells. 9 Salicylic acid mimicked all the growth inhibitory actions of aspirin suggesting that the anti-cancer effects of aspirin probably occurs through formation of salicylic acid within the cells. Cyclins control the progression of cells through the cell cycle by physically interacting and activating cyclin-dependent kinase (CDK) enzymes¹⁰. Molecular docking studies revealed that salicylic acid interacts with CDK2 through Asp 145: A and Lys33: A. Interestingly, in many cancers, CDK2 activity is significantly up-regulated. Therefore, attention is increasingly being focused on anti-inflammatory drugs, as indomethacin 2, 3, or salicylates as CDK2 inhibitors as a potential target for cancer therapeutic intervention. In this frame, we envisioned to use some anti-inflammatory drugs or salicylates derivatives or analogues from natural or synthetic sources. Our departure point started from docking study for our designed compounds with CDK2 protein using OpenEye software. Moreover, our objective directed to prepare indomethacin analogues, as CDK2 inhibitors FIG. 1.

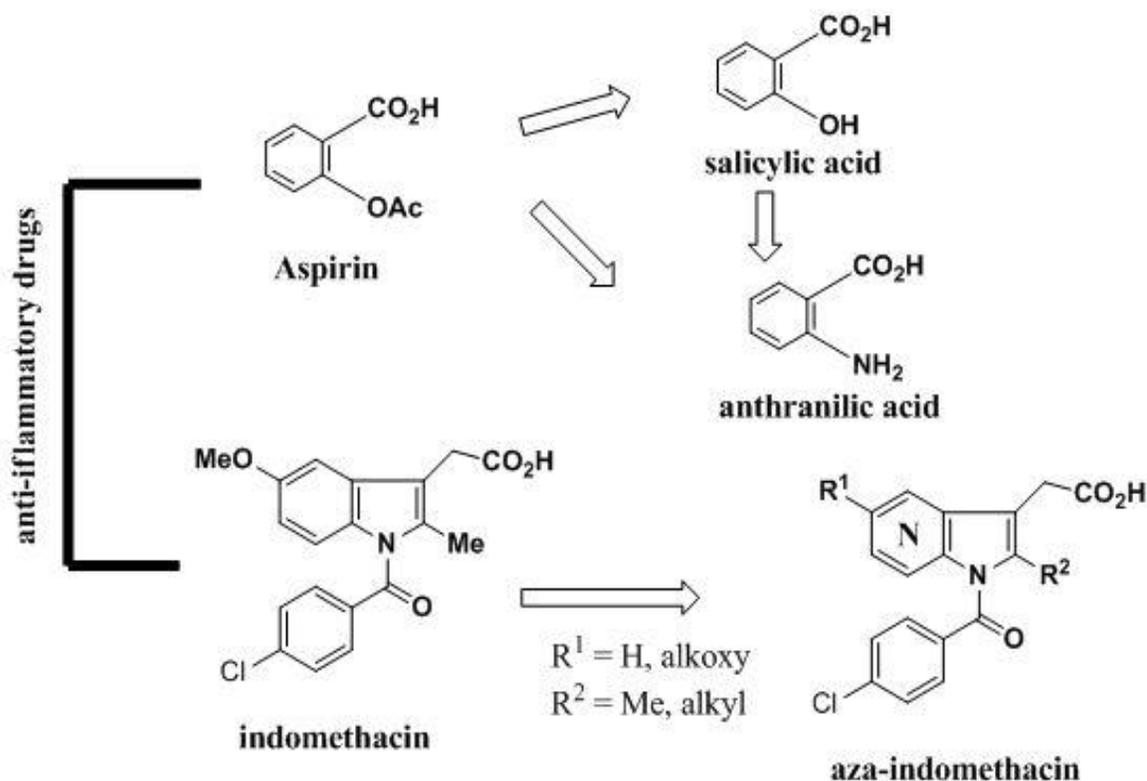


FIG. 1. Chemical structure of Aspirin and Indomethacin and their derivatives.

Experimental Methods

General chemistry

Melting points are uncorrected. IR spectra were measured in CHCl₃. ¹H NMR spectra were taken in CDCl₃ unless otherwise indicated. CHCl₃ (7.26 ppm) for silyl compounds and tetramethylsilane (0.00 ppm) for compounds without a silyl group were used as internal standards. ¹³C NMR spectra were recorded in CDCl₃ with CDCl₃ (77.00 ppm) as an internal standard. All reactions were carried out under a nitrogen atmosphere. Silica gel (silica gel 60, 230-400 mesh) was used for chromatography. Organic extracts were dried over anhydrous Na₂SO₄.

Synthesis of 5-((tert-butyldimethylsilyl) oxy)-1-((4-methoxybenzyl) oxy) pent-3-yn-2-yl methyl carbonate (5)

To a solution of compound 4 (380 mg, 0.97 mmol) in CH₂Cl₂ (5 mL) and pyridine (0.55 mL, 6.76 mmol) was added methyl chloroformate (0.22 mL, 2.90 mmol) at 0°C. The reaction mixture was allowed to warm up room temperature with stirring for 12 h. The reaction mixture was concentrated. The residue was chromatographed directly by hexane: Et₂O (9:1) to give 5 as colorless oil (351 mg, 78%). IR 1749 cm⁻¹; ¹H NMR δ 7.27-7.18 (m, 5H), 5.21 (t, J=6.6 Hz, 1H), 4.50 (s, 2H), 4.12 (d, J=1.3 Hz, 2H), 3.81 (s, 3H), 3.72 (s, 3H), 3.54 (t, J=5.7 Hz, 2H), 1.80-1.75 (m, 2 H), 1.48-1.46 (m, 4H), 0.81 (s, 9H), -0.03 (s, 6H); ¹³C NMR δ 159.1, 154.8, 130.7, 129.3, 113.7, 84.9, 82.3, 72.5, 69.5, 68.0, 55.2, 55.21, 55.00, 50.8, 34.3.

Synthesis of methyl 2-((benzyloxy) methyl)-5-((tert-butyldimethylsilyl) oxy) penta-2, 3-dienoate compound with methane (6)

To a stirred solution of 5 (75 mg, 0.18 mmol) in MeOH (1.8 mL) was added Pd (PPh₃)₄ (10 mg, 8.9 × 10⁻³ mmol) at room temperature. The reaction mixture was warmed to 40°C under CO (1 atm) and stirred for 1 h. MeOH was evaporated off, and the residue was chromatographed with hexane: Et₂O (19:1) to afford 6 (16 mg, 67%) as a colorless oil; IR 1959, 1708 cm⁻¹; ¹H NMR δ 7.27-7.19 (m, 5H), 5.58 (t, J=5.9 Hz, 1H), 4.49 (s, 2H), 4.17 (dd, J=9.6, 10 Hz, 2H), 3.68 (s, 3H), 3.53 (t, J=6.0 Hz, 2H), 2.11 (dd, J=7.2, 7.2 Hz, 2H), 1.53-1.43 (m, 4H), 0.82 (s, 9H), -0.03 (s, 6H); ¹³C NMR δ 155.2, 85.8, 82.0, 68.2, 62.8, 54.8, 51.6, 34.5, 32.2, 25.9, 25.8, 21.3, -5.2, -5.3; ¹³C NMR δ 211.0, 166.8, 138.1, 128.3, 127.7, 127.6, 127.5, 98.00, 95.6, 72.2, 67.4, 62.7, 52.1, 32.0, 27.7, 25.9, 25.2, 18.5, 18.3, -5.3, -5.5.

Synthesis of 2-azaindole derivative 3 and 4

KOH (22 mg, 0.40 mmol) was added to a solution of 2 (0.10 mmol) in MeOH (1 mL). After heating for 0.25 h-10 h at room temperature, the reaction mixture was concentrated. The residue was chromatographed with hexane/AcOEt (9:1) to afford compound 3 and 4 (2:1).

2, 2-dimethyl-1-(2-(prop-1-en-2-yl)-1H-pyrrolo [2, 3-b] pyridin-1-yl) propan-1-one (3)

Colorless oil; IR 1728 cm⁻¹; ¹H NMR δ 8.08 (d, J =7.9 Hz, 1H), 7.49 (d, J=7.6 Hz, 1H), 7.28-7.25 (m, 1H), 7.22-7.19 (m, 2H), 6.44 (s, 1H), 2.09 (s, 3H), 1.21 (s, 9H);

1-(2-(1-((tert-butyldimethylsilyl) oxy)-2-hydroxypropan-2-yl)-1H-pyrrolo [2, 3-b] pyridin-1-yl)-2, 2-dimethylpropan-1-one (4)

Colorless oil; IR 3468, 1740 cm^{-1} ; ^1H NMR δ 8.26-8.29 (m, 2H), 7.55 (s, 1H), 6.31 (s, 1H), 4.25 (dd, $J=10.5, 6.9$ Hz, 1H), 4.19 (dd, $J=10.5, 6.9$ Hz, 1H), 3.30 (brs, 1H), 1.48 (s, 9H), 1.42 (s, 3H), 1.22 (s, 9H).

Molecular docking study

This was done using OpenEye molecular modeling software. A virtual library of structurally modified salicylates and some anti-inflammatory drugs as indomethacin derivatives were energy minimized using MMFF94 force field, followed by generation of multi-conformers using OMEGA application. The whole energy minimized library will be docked along with the prepared receptor Cyclin A, CyclinA/CDK and CDK using FRED application to generate a physical property (ΔG) reflecting the predicted energy profile of ligand-receptor complex. Veda application can be employed as a visualization tool to show the potential binding interactions of the ligands to the receptor of interest.

Total cell lysate preparation, immunoprecipitation and Western blotting.

Cells were treated with anthranilic acid at different concentrations for the indicated time and washed with PBS. Cells were scraped in lysis buffer (10 mmol/L Tris-Cl, pH 7.4, 150 mmol/L NaCl, 15% glycerol, 1% Triton X-100 with protease inhibitors). Samples containing 50 mg of proteins were separated by an 8% or 10% PAGE and immunoblotted with respective antibodies. For immunoprecipitations, 500 mg of the total proteins isolated from cells, or 300 mg of the recombinant CDK2 protein, were diluted to 1 mL of lysis buffer, immunoprecipitated with anti-CDK2 antibodies overnight at 4°C , the immune complex was captured by adding 35 mL of protein G agarose for 3 h. The immunocomplexes were washed 3 times with PBS and dissolved in SDS-sample buffer. The samples were analyzed on a SDS-PAGE, immunoblotted with either anti-cyclin A2 antibody or anti-CDK2 antibody. Immunoreactive bands were detected using chemiluminescence reagents. The intensities of bands were determined using NIH ImageJ software.

Results and Discussion

A virtual library of salicylic analogs like anthranilic acid, propionyl salicylic acid (isosters of aspirin), anti-inflammatory drugs and their analogues were designed and energy minimized using MMFF94 force field calculations. The target proteins were downloaded from protein data bank website (<https://www.rcsb.org/pdb/home/home.do>). The catalytic domains of CDK2 protein receptors were chosen (PDB code: 1PYE), CyclinA (PDB code: 1FIN), and CyclinA/CDK (PDB code: 5NEV) were prepared for docking using OpenEye® software 11, 12. Open Eye Omega application was used to generate different conformations of each ligand. Docking was conducted using Fred and the data was visualized by Veda application. This software package generates consensus scoring which is a filtering processes to obtain virtual binding affinity, the lower consensus score, and the better binding affinity of the ligands towards the receptor. This study revealed that compounds isosters to salicylic acid, showed good score and good interaction with receptor of CDK2. The NSIDs Sulindac¹³ and indomethacin³ showed the best binding score in this library in comparison to other compounds. 5-Amino salicylic acid, 2-propionyl salicylic acid (aspirin analog), anthranilic acid showed consensus score better than aspirin which in turn better than salicylic acid. TABLE 1 illustrated the compounds sorted based on consensus score. Anthranilic acid showed HB interaction with Val 64: A and Phe 146: A (FIG. 2). This docking mode is similar to docking mode of salicylic acid. This kind of similarity between salicylic acid and anthranilic acid with CDK ligand emphasizes our hypothesis for chosen anthranilic acid as salicylic analogues. In this regard, anthranilic acid will be subjected to Western blot analyses. In continue to our theme for

design a new derivative of anti-inflammatory drugs as anticancer through CDK inhibitory activity, our study includes docking with some reported anti-inflammatory drugs like sulindac and indimthacin as CDK inhibitors. Indomthacin showed two HB interactions with Asp 145 and Luec 83 (FIG. 3). Based on this finding, we envisioned to synthesis indomethacin analogs (aza indomethacin) in order to study the effect of nitrogen atom instead of carbon in phenyl ring.

TABLE 1. Consensus score for selected compound.

Compound	Consensus score		
	CyclinA (ID: 1FIN)	CDK2 (1A1Q)	CyclinA/CDK (ID: 5NEV)
sulindac	4	1	2
indomethacin	0	3	2
5-Amino Salicylic acid	27	23	44
Propionyl salicylic acid	31	29	16
Anthranilic acid	69	35	67
aspirin	44	37	19
Salicylic acid	55	47	54

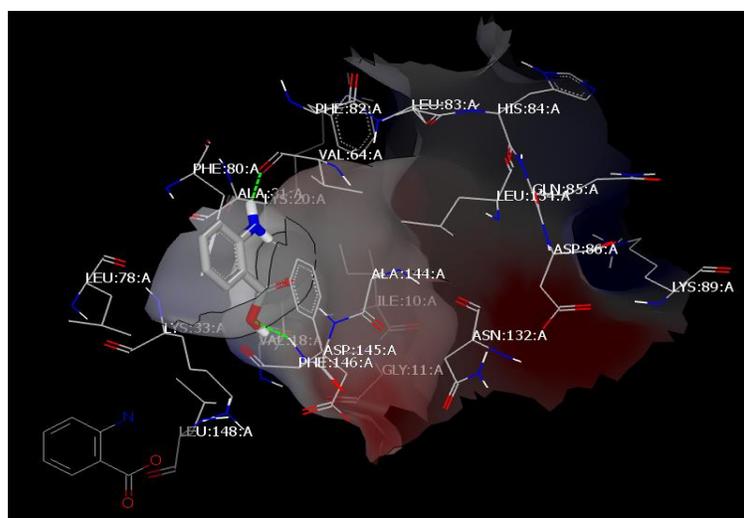


FIG. 2. Visual representation of anthranilic in the 1A1Q binding site of CDK2. HB with Asp 145 and Val 64 (dotted green line).

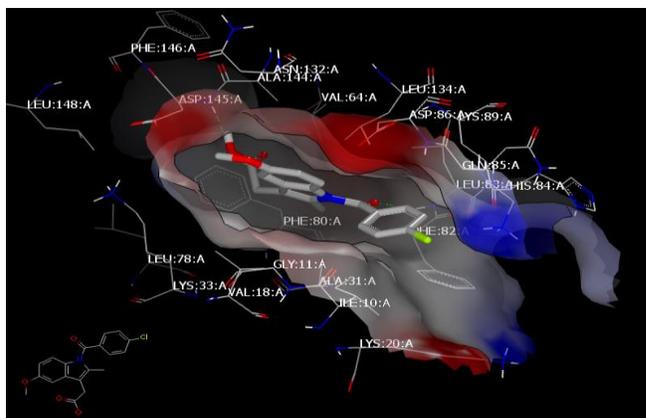
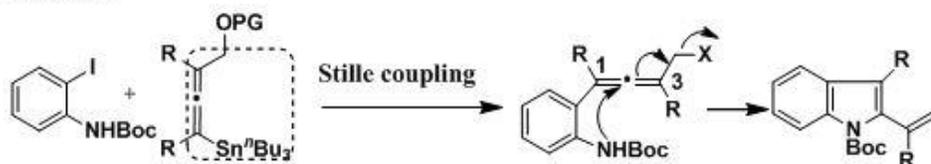


FIG. 3. Visual representation of indomethacin in the 1A1Q binding site of CDK2. HB with Asp 145 and Leu 83 (dotted green line).

Chemistry

In order to obtain the desired azaindomethacin, our strategy was directed to prepare azaindole derivatives by advances in carbocyclizations in organic synthesis catalyzed by transition metals and their complexes^{14, 15}. Utilizing our previous reported method¹⁶ SCHEME 1, our attempt was commenced to start by Stille coupling of specific design allenylstannane^{17, 18} bearing carboxylate ester functionality as depicted in SCHEME 1.

Our previous work



Current objective

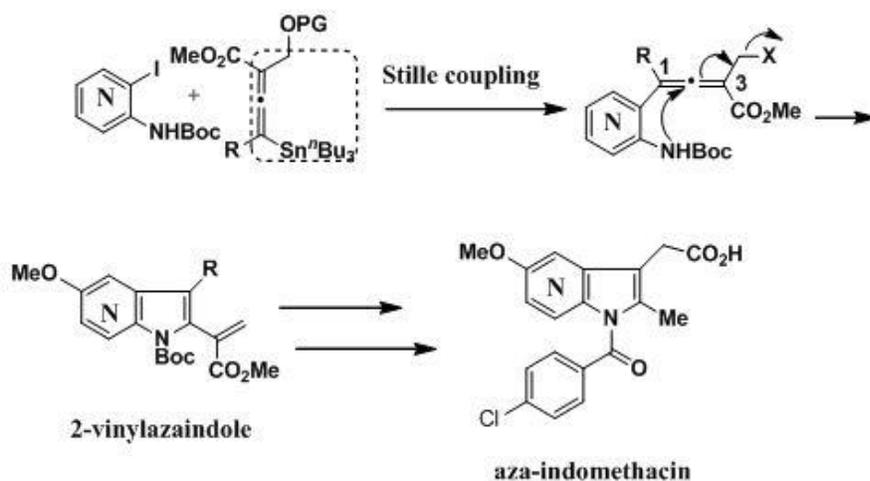
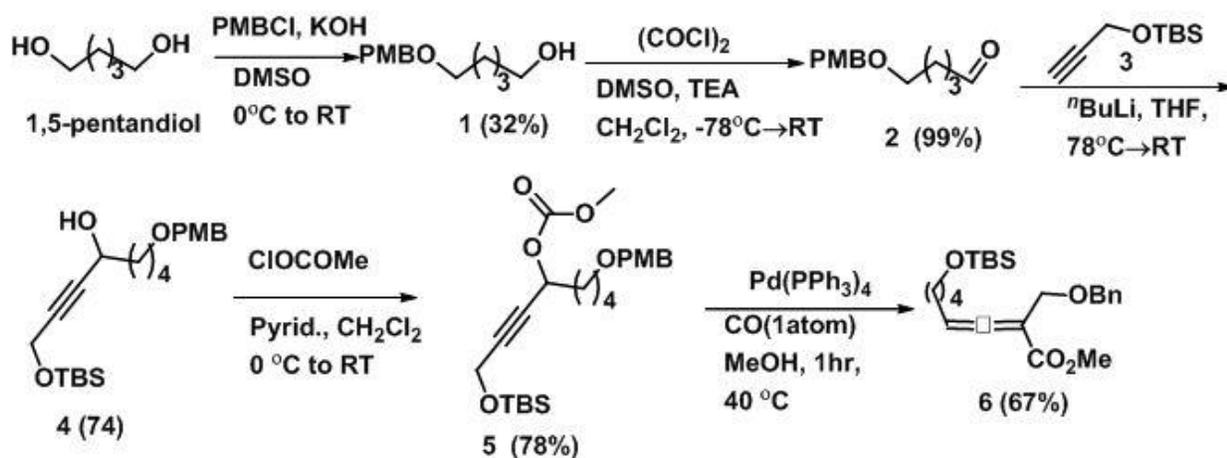


FIG. 2. Still coupling strategy for synthesis of azaindole derivatives.

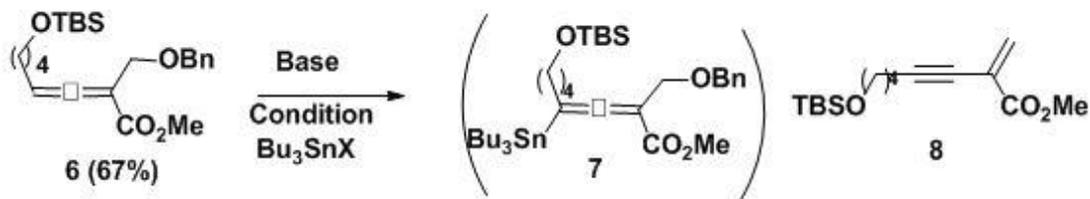
Based on this theme, the first step in this study was directed to prepare the target allenyl stannane containing carboxylate group in position 3. The aldehyde 219 was obtained through oxidation of alcohol 119 by the action of oxalyl chloride in presence of TEA. Mono protection of 1, 5-pentandiol was run by the effect of PMBCl in DMSO in presence of KOH. The target propargyl alcohol 4 was separated from the reaction of aldehyde 219 with the lithiated propargyl alcohol 320 in 74% and it was converted to its corresponding carbonate in high yield. However, compound 4 is unstable. It was carbonylated rapidly to the corresponding carbonate 5 by using pyridine as base. Having in hand the desired carbonate 5, the attention was directly run to allenation step. Upon stirring 5 with Pd (PPh₃)₄ under CO atmosphere in thermal conditions, the desired allene ester 6 was separated in good yield.

SCHEME 1

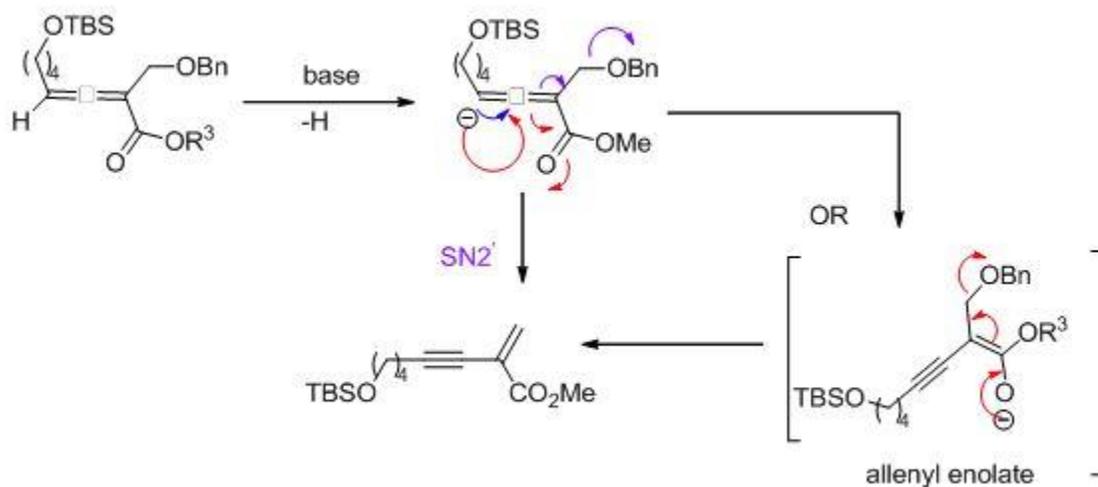


Then, our interest was focused on preparing the desired 4-substituted allenyl stannane (stannation step). Extensive studies especially using different bases as NaOH, NaH, nBuLi, DBU and TBAF, different solvents as THF, benzene and DMF, variation in equivalent of reagents and changing the temperature from -80 to reflux were used to get the target allenylstanne 7. Certainly, the desired compound did not obtain and major product was enyne 8. Separated product showed spectra data indicated the disappearance of allene functionality and presence of enyne part. For example, IR spectrum for this product showed peak at 3330 cm^{-1} and no peak was detected in allene region. Judging from all these results, such type of allene ester under basic conditions doesn't prefer SN2 reactions and prefers others reactions like SN2' (formation enyne 5).

SCHEME 2

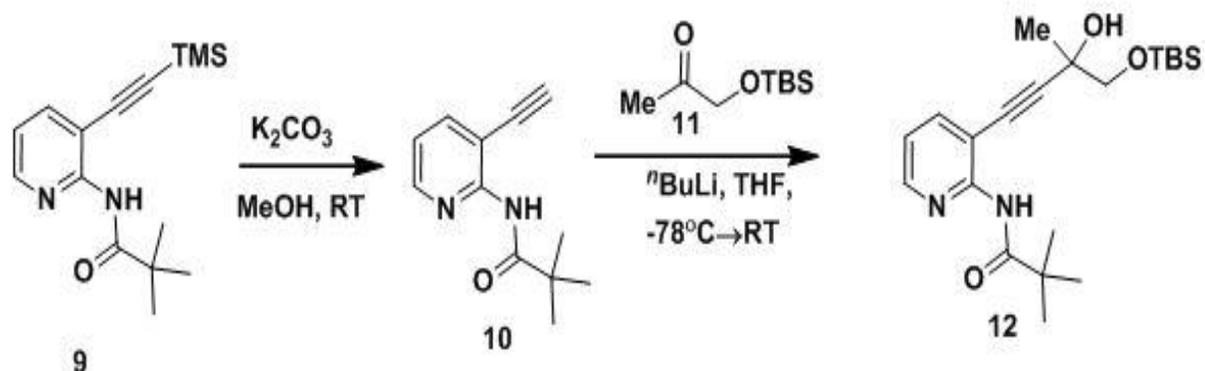


The suggested mechanism for getting the enyne 8 as follows:

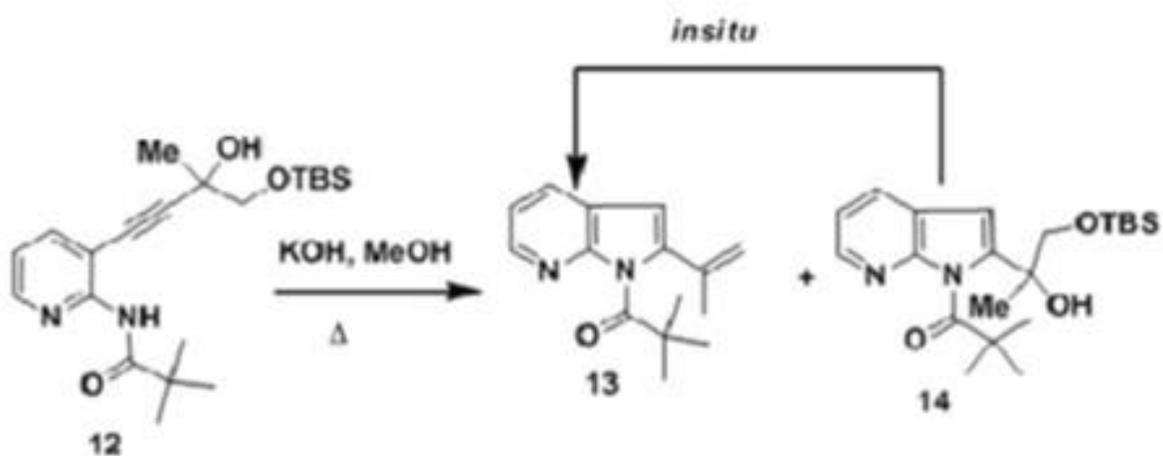


Based on these findings, we turned our attention to utilize Alkyne chemistry coupling, as Sonogashira coupling 21, instead of previous allene chemistry. The desired propargyl alcohol (12) was generated from commercially available alkynyl pyridine (9). Compound 10 was confirmed by disappearance of TMS group and presence of peak in IR at 3030 cm⁻¹. Lithiation of alkyne (10) with protected hydroxyacetone (11) 22 (16) generates the target propargyl alcohol (4), (Scheme 1). Having in hand the propargyl alcohol (12) in hand, the next step was directed to synthesis azaindole derivatives, scheme 2. Different conditions were performed to cyclize the propargyl pyridine in to desired 2-azindole (14). The most acceptable condition for cyclization was through heating compound (14) with KOH in methanol. The 2-vinylazindole (13) was detected during reaction course. Follow-up the reaction indicated that 2-azindole alcohol (4) was eliminated *in situ* to produce 2-vinylazindole (13). 2-Azindole alcohol (14) will be the main precursor to prepare the target aza-indomethacine which will be published in the future work.

SCHEME 3



SCHEME 4



Cell lysate preparation and Western blotting

After treatment with anthranilic acid at the indicated concentrations, cells were prepared as described previously. Samples containing 50 μg of proteins were separated by 8% polyacrylamide gel electrophoresis (PAGE) and immune blotted with anti-CDK2 antibodies. Intensities of the bands were quantified using NIH ImageJ software, FIG. 4.

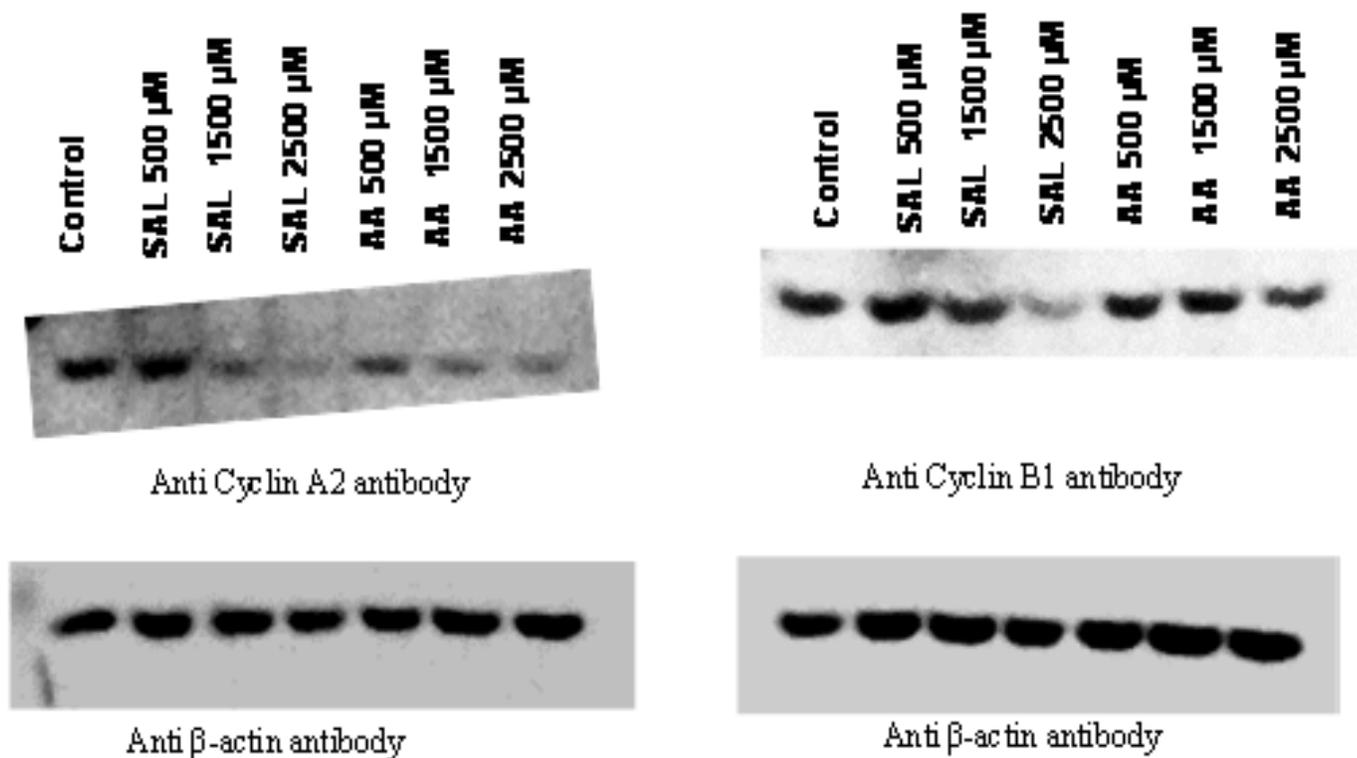


FIG. 4. *In vitro* kinase assays showed that lysates from cells treated with anthranilic acid had lower levels of Cyclin A activity

Conclusion

By the advantage of computer aided drug design in understanding the interaction between aspirin, salicylates, and other anti-inflammatory drugs, we can design and synthesis a new series of compounds that has unique anticancer activity. In addition to this hypothesis, we can examine the combination therapy between salicylates and anticancer drugs. From this virtual study, we can find common interactions between target proteins and known salicylates. This step will help us to understand the kind of interaction and gives clear view of receptor and active sites.

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