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Synthesis of Derivatives of 3-Aminoquinazolinone and 2'-Deoxyguanosine as Potential Protein Tyrosine Kinase Inhibitors

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ABSTRACT Protein kinases play an important role in post-translational cellular signaling by regulating cell growth, differentiation and apoptosis, among other cellular activities. Furthermore, protein kinase (PK) deregulation has been implicated in many diseases, including cancer. For this reason, there has been considerable interest in the development of PK inhibitors, which could lead to the discovery of new cancer-treating drugs. Derivatives of 3-aminoquinazolinone and 2'-deoxyguanosine were synthesized as potential ATP-competitive inhibitors of the Src and FAK protein tyrosine kinases, and the effectiveness of the synthesized derivatives as protein tyrosine kinase inhibitors was quantified using [γ-^{32}P]ATP radioisotope assays. The results of the assays indicated that none of the compounds synthesized were as effective as other previously discovered inhibitors.

INTRODUCTION

Protein kinases (*PKs)* play an important role in post-translational cellular signaling by regulating cell growth, differentiation and apoptosis; among other cellular activities. Protein tyrosine kinases (*PTK*s) are a class of protein kinases which catalyze the transfer of a phosphate group from ATP to a tyrosine residue. PK deregulation has been implicated in numerous diseases, with the most notable being cancer.[1] Given the association between PK deregulation and disease, it is no surprise that PKs have become some of the most extensively studied biological targets in drug discovery research, accounting for an estimated

20-30% of all drug discovery projects in the pharmaceutical industry. One major focus of these research efforts is the development of PK inhibitors which has received much attention after the FDA approval in 2001 of the drug Gleevec—a PK inhibitor—for the treatment of certain forms of cancer. As a result of these efforts, numerous protein kinase inhibitors are currently in human clinical trials as cancer treatments.[2]

Focal adhesion kinase (FAK) is a protein tyrosine kinase that was discovered less than twenty years ago, making its discovery relatively recent. Despite extensive efforts to characterize FAK and to understand its functions in cellular signaling, many questions regarding the precise role of FAK in the regulation of vital cellular functions remain unanswered. FAK is thought to play a role in the regulation of cellular activities such as cell growth, cell division, cell migration, and apoptosis.[3] Further research has shown that human tumor cells displayed increased levels of FAK, as compared to those levels in non-neoplastic cells from the same subject.[4] Moreover, other studies have shown that FAK inhibition has led to apoptosis in human cancer cells, and that combining this inhibition with the inhibition of another PK such as EGFR or c-Src, has had the effect of further enhancing apoptosis.[5,6] The Src family of PKs has also been shown to regulate a variety of cellular activities such as cell division, motility and apoptosis.[7] The activity of the Src family of kinases has been shown to be elevated in human breast and colon cancers, and their inhibition has been shown to cause mitotic arrest in human cancer cells.[8] Given that Src and FAK have been shown to be deregulated in several types of human tumors, the development of selective inhibitors of these *PK*s has emerged to be a promising area of cancer research.

Previous work in our laboratory resulted in the discovery of a 3-aminoquinazolinone derivative and a 2'-deoxyadenosine derivative which were found to be effective inhibitors of the Src and FAK kinases.[9] Encouraged by these results, we decided to synthesize other derivatives of 3-aminoquinazolinone and 2'-deoxyguanosine and test them as potential inhibitors of the FAK and Src *PTK*s. Herein, the results of the synthesis and inhibition studies involving various derivatives of 3-aminoquinazolinone and 2'-deoxyguanosine are reported.

RESULTS AND DISCUSSION

Imine and amide derivatives of 3-aminoquinazolinone are easy to prepare, and given the availability of a vast number of aldehydes and carboxylic acids (with which to react the parent compound) in our laboratory, we decided that preparing imine and amide derivatives would provide us with the greatest diversity of 3-aminoquinazolinone derivatives. Furthermore, as previous work in our laboratory resulted in the discovery of a 2'-deoxyadenosine derivative which was found to be an effective PTK inhibitor, we decided to pursue the synthesis of a broad array of 2'-deoxyguanosine (dG) derivatives given the structural similarity between 2'-deoxyadenosine and 2'-deoxyguanosine.

Synthesis of 3-Aminoquinazolinone Derivatives

3-Aminoquinazolinone was prepared according to a method reported in the literature, and subsequently reacted with either aldehydes to give imine derivatives or with acid chlorides to give amide derivatives (Scheme 1).[10] Reacting 3-aminoquinazolinone (**1**) with the appropriate acid chloride in dry THF, in the presence of pyridine resulted in the formation of the amide derivatives **3a-c**. Similarly, in a single step, the reaction of 3-aminoquinazolinone (**1**) with the appropriate aldehyde in the presence of a catalytic amount of acetic acid resulted in the formation of imine derivatives **2a-h**. Examples of imine and amide derivatives synthesized are shown in Figure 1.

Scheme 1. Preparation of imine and amide derivatives of 3-aminoquinazolinone

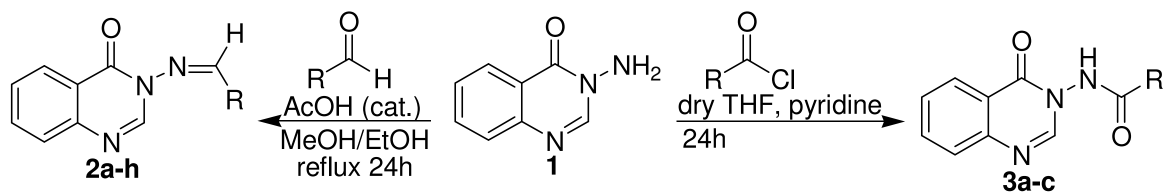
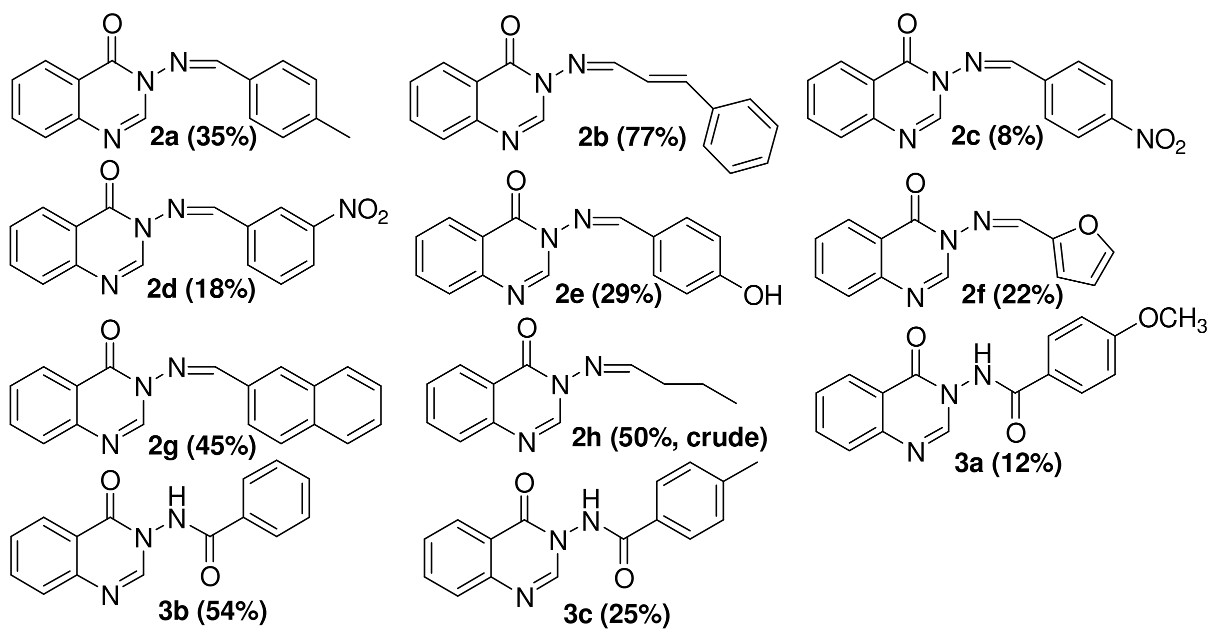


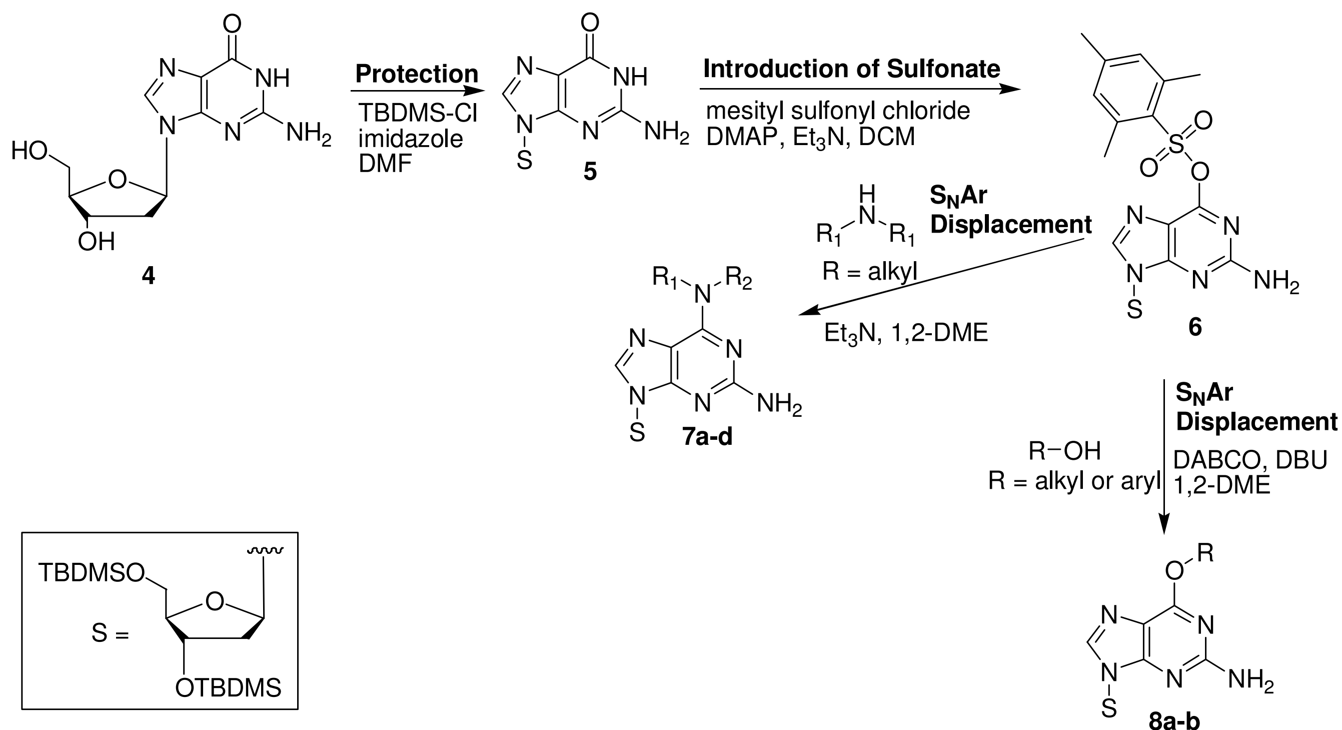
Figure 1. List of 3-aminoquinazolinone derivatives prepared



Synthesis of 2'-Deoxyguanosine Derivatives

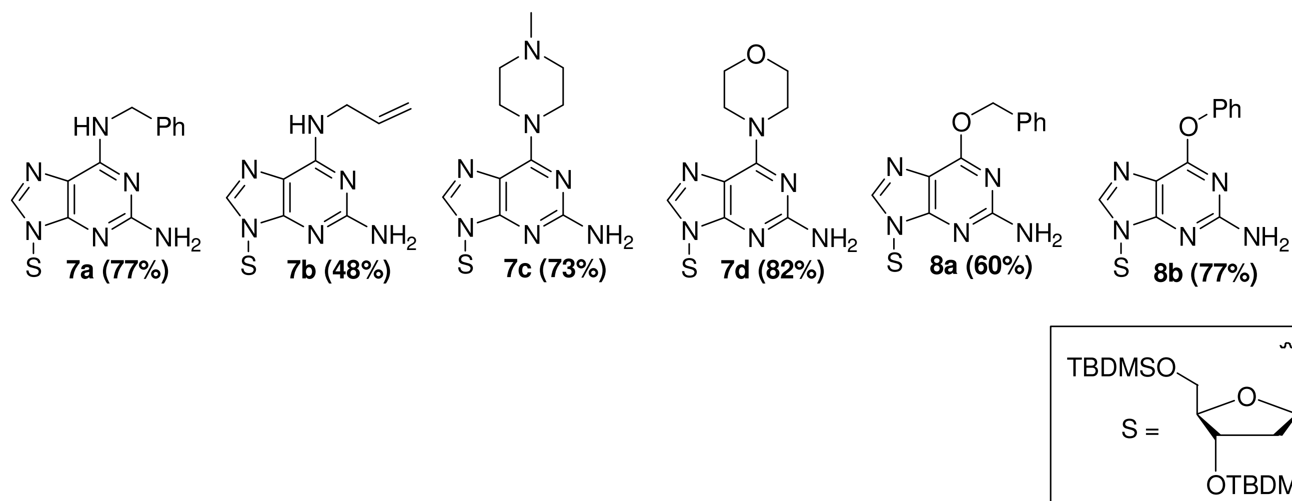
Derivatives of dG could not be synthesized directly from commercially-available dG. Instead, the sugar hydroxyl groups first had to be protected, and a sulfonate group introduced at O^6 to facilitate S_NAr displacement by alcohol and amine nucleophiles, as reported in the literature (Scheme 2).[11]

Scheme 2. Preparation of O^6-alkyl–, O^6-aryl– and N^6-alkyl– derivatives of 2'-deoxyguanosine



Nucleophilic alcohols, amines and phenols promoted facile displacement of the sulfonate group resulting in moderate to good yields of the desired substituted products (Figure 2).

Figure 2. List of 2'-deoxyguanosine derivatives prepared

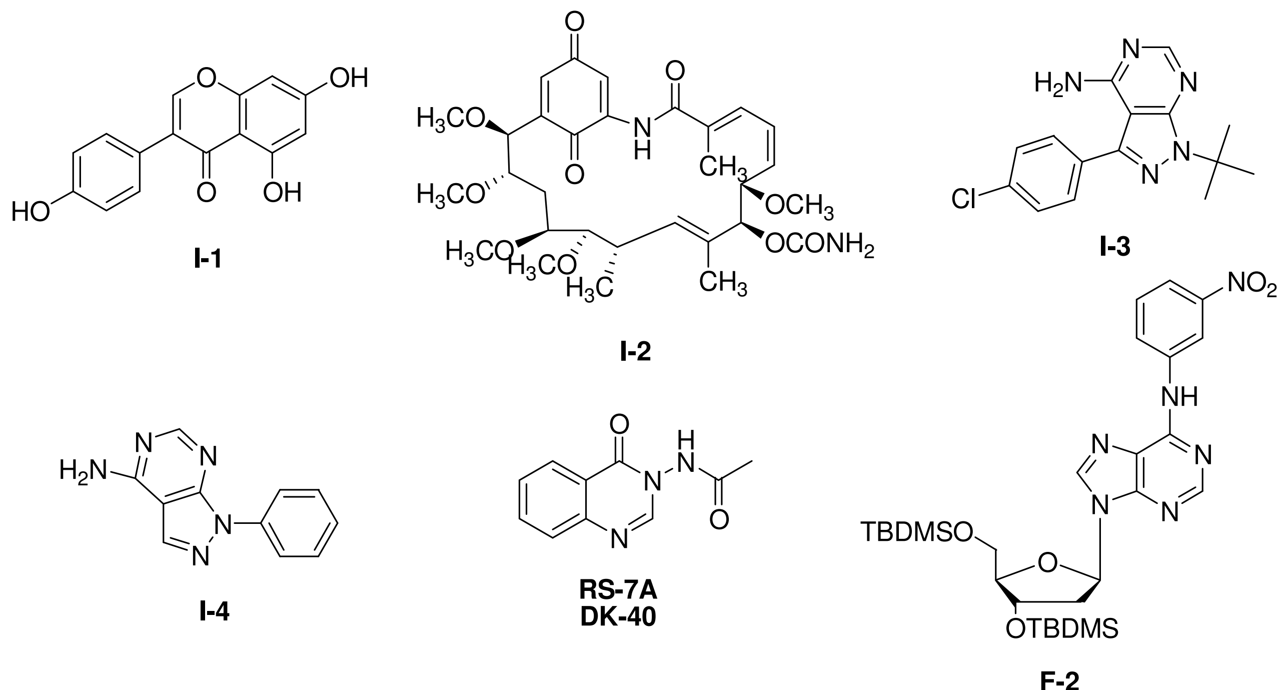


[γ-^{32}P]ATP Radiolabel Assay

The activity of FAK and Src in the presence of 3-aminoquinazolinone derivatives and dG derivatives was quantified using a [γ-^{32}P]ATP radioisotope labeling assay. As points of reference, the compounds

were tested alongside several commercially-available inhibitors (**I-1**, **I-2**, **I-3**, **I-4**, Figure 3) as well as the two lead compounds that generated this research project (**RS-7A** and **DK-40** both refer to two different samples of the same 3-aminoquinazolinone lead compound; **F-2** was the 2'-deoxyadenosine lead compound). In the graphs that follow, the activity of the kinase is shown in the presence of each compound. A low percent activity indicates that the compound is effective at inhibiting the kinase.

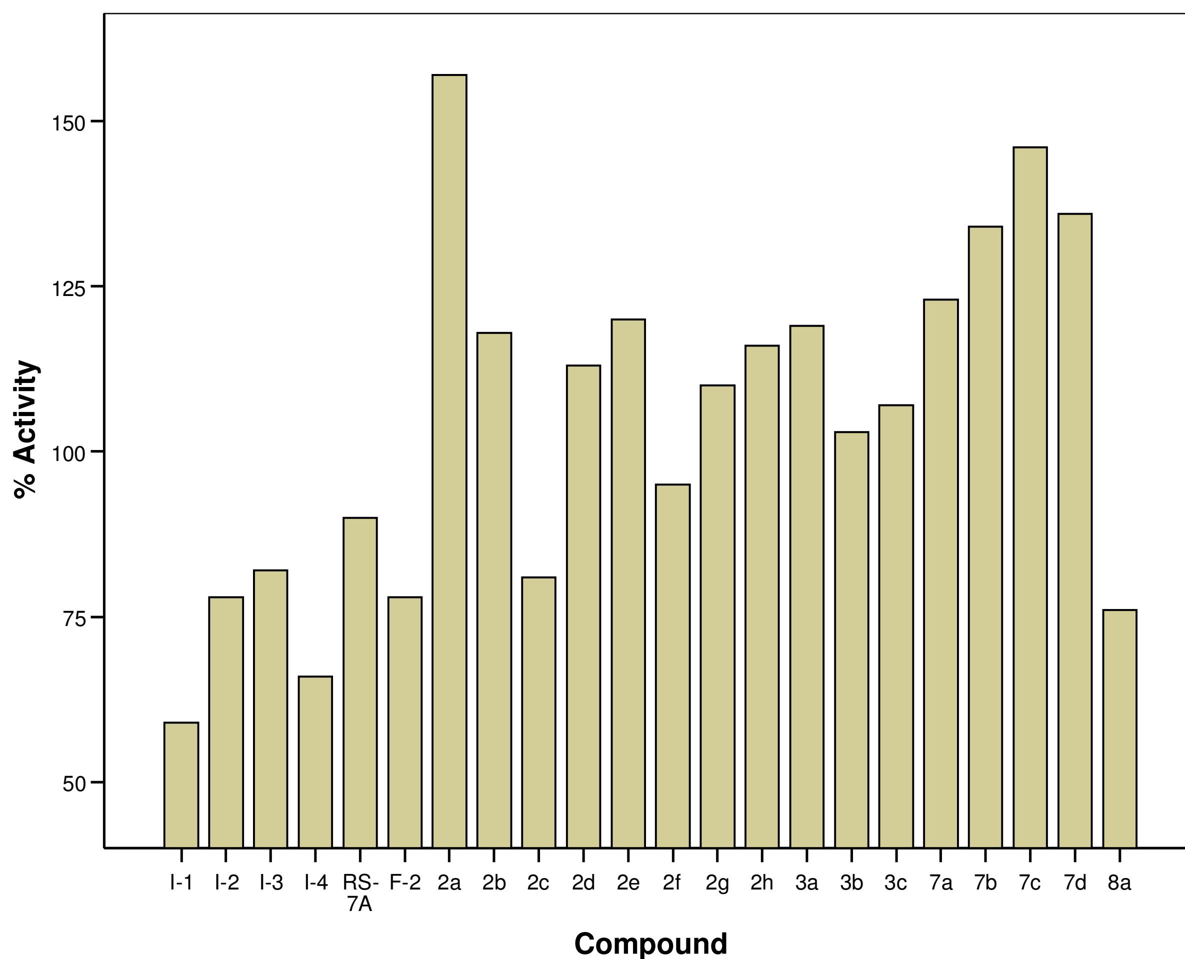
Figure 3. Structures of known inhibitors



FAK Activity in the Presence of Inhibitors

The activity of FAK in the presence of each of the inhibitors was quantified. A 10mM solution of each inhibitor (to give a concentration of 1mM when tested) was prepared for the assays; however, several of the inhibitors were not fully soluble in DMSO at 10mM, and therefore, the assays do not all reflect inhibition at a uniform concentration of the inhibitor. Nevertheless, these data were used to screen for any initial "hits" which could then be further investigated. Compound **8b** was not tested here because the supply of kinase ran out during the assay.

Figure 4. Activity of FAK in the presence of inhibitors

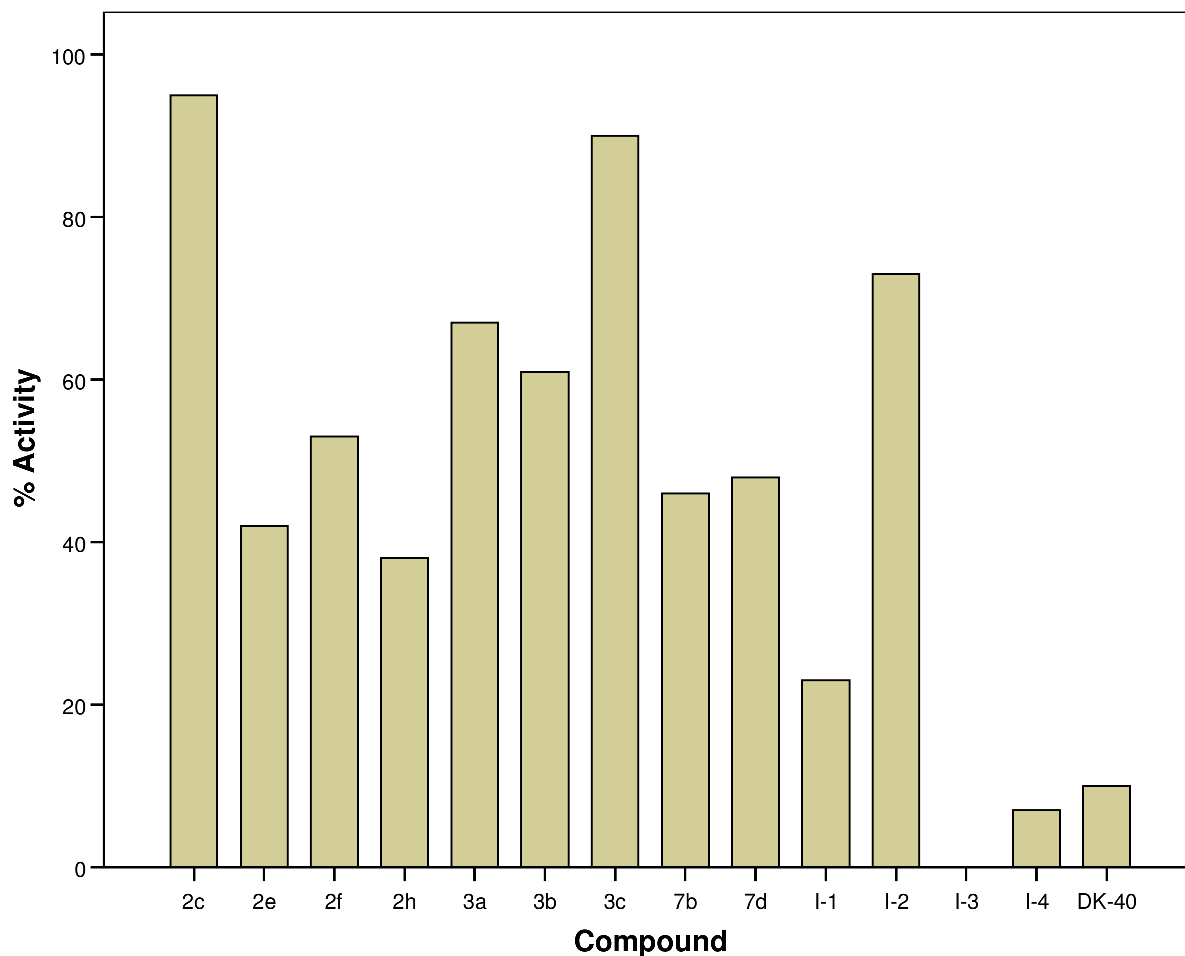


Several activities were found to be in excess of 100%, and we suspect that this may be due to improperly calibrated pipettors. Not withstanding, the effectiveness of commercially-available inhibitors **I-1** and **I-4** is apparent. Lead compounds **RS-7A** and **F-2** are also shown to exhibit some inhibition of the kinase, confirming previous results; however none of the novel inhibitors were shown to be more effective (within experimental error) than the lead compounds or the best commercially-available inhibitors. A second screening of compound **2c**—the most promising of the inhibitors from the initial screening—alongside lead compound **RS-7A** showed that **2c** displayed 74% activity, while the lead compound displayed 56% activity. Given these results, we concluded that none of the compounds prepared were effective inhibitors of FAK.

Src Activity in the Presence of Inhibitors

Given the solubility issues, only those compounds that were soluble in DMSO at 10mM were tested in the initial screening with Src.

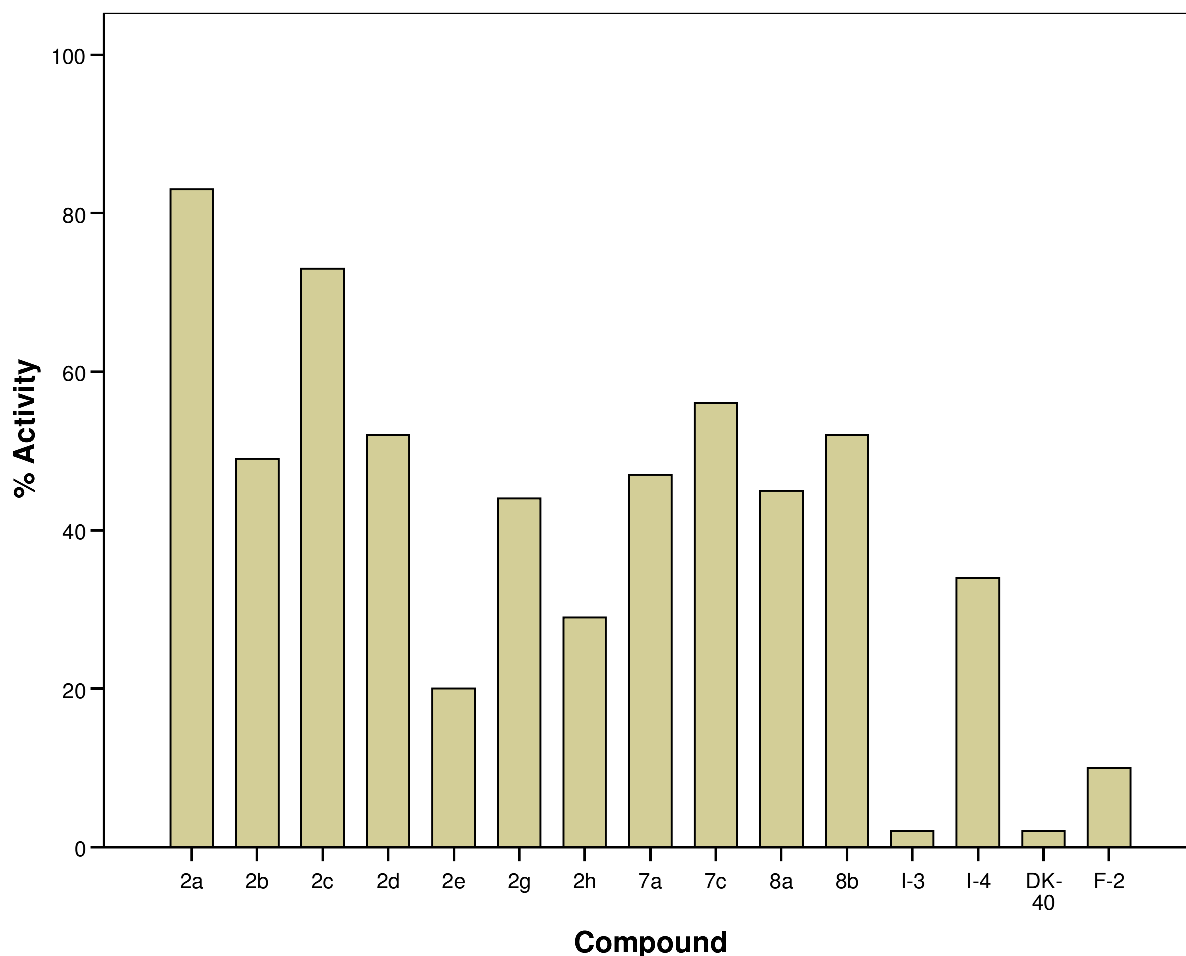
Figure 5. Activity of Src in the presence of inhibitors



From this screening, the most promising of the novel inhibitors were **2e** and **2h**; however, neither was as effective as commercially-available inhibitors **I-1**, **I-3**, **I-4** or lead compound **DK-40**.

All of the novel inhibitors that were tested with Src in the next screening were tested at 0.167mM (except for **2h** and **2e** which were tested at 1mM).

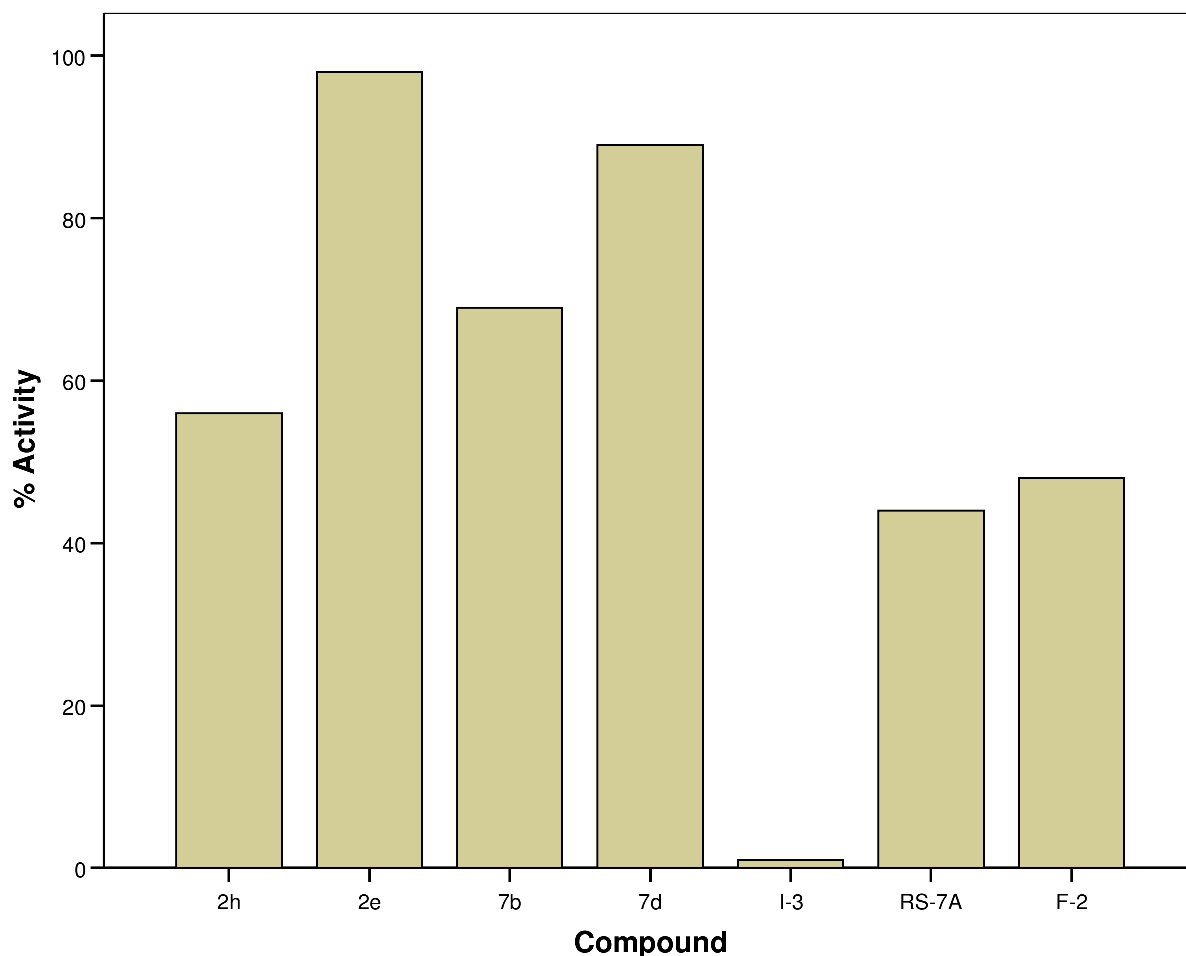
Figure 6. Activity of Src in the presence of inhibitors



Compounds **2e** and **2h** were shown to effectively inhibit Src; however, these were tested at 1mM, and all other inhibitors had been tested at 0.167mM.

Since all of the inhibitors have to be tested at a uniform concentration in order to compare them side-by-side, another screen needed to be done to compare all of the inhibitors at the same concentration. Thus, in the next screen, **2e** and **2h** were tested alongside two other promising novel inhibitors of Src (**7b** and **7d**), a commercially-available inhibitor and the two lead compounds at 0.1mM.

Figure 7. Activity of Src in the presence of inhibitors



These data indicate that the best of the novel Src inhibitors was **2h**; however, it was tested as a crude product, and thus an assay with a pure product would be necessary to definitively identify the compound as an effective inhibitor. Compound **2e** was found in other screens to be an effective inhibitor of Src; however, the results were not reproducible in this screen. Despite the fact that these inhibitors were somewhat effective, they were not as effective as commercially-available inhibitor **I-3** or the two lead compounds **RS-7A** and **F-2**.

EXPERIMENTAL

Synthesis of 3-Aminoquinazolinone Derivatives

General Procedure for the Synthesis of Imine Derivatives of 3-Aminoquinazolinone (2a-h)

Imine derivatives of 3-aminoquinazolinone were generally prepared by stirring the parent compound (1 eq), the appropriate aldehyde (1 eq) and a few drops of AcOH in MeOH or EtOH at reflux for 24 hours under an inert nitrogen atmosphere. The reaction mixture was then slowly cooled to room temperature and chilled on ice to precipitate the product, which was isolated via vacuum filtration and rinsed with cold alcohol.

General Procedure for the Synthesis of Amide Derivatives of 3-Aminoquinazolinone (3a-c)

Amide derivatives of 3-aminoquinazolinone were generally prepared by stirring the parent compound (1 eq), the appropriate acid chloride (1.2 eq) and pyridine (1.5 eq) in dry THF at 0°C, then slowly bringing the reaction mixture to room temperature and stirring at room temperature for 24 hours under an inert nitrogen atmosphere. The reaction was quenched with saturated $NaHCO_3$ and the reaction mixture was diluted with EtOAc, followed by sequential extractions with saturated $NaHCO_3$ and saturated NaCl. The organic layer was dried over anhydrous Na_2SO_4. Evaporation of the organic solvents gave the crude product, which was recrystallized in methanol to give the final product.

3-amino-4(3H)quinazolinone 1. The 3-aminoquinazolinone parent compound was prepared by stirring a mixture of 2-aminobenzhydrazide (1 eq) and triethyl orthoformate (1.08 eq) in ethanol (0.5 M) at reflux for 5.5 hours under an inert nitrogen atmosphere. More triethyl orthoformate (0.1 eq) was added to drive the reaction to completeness, and the reaction mixture was stirred o.n. at r.t. Reaction mixture was then stirred for 1 hour at reflux, then cooled to r.t. on benchtop and subsequently chilled on ice to precipitate all of the product, which was filtered under vacuum and rinsed with cold ethanol. Product was dried under vacuum. Second crop was obtained via filtration, and third crop via trituration to give 2.055g (60%) yield of a tan solid. M.P.(°C): 204-209, ^{1}HNMR (400 MHz, DMSO-d_6, δ): 8.33 (s, 1H), 8.14 (d, 1H), 7.79 (t, 1H), 7.65 (d, 1H), 7.53 (t, 1H), 5.82 (s, 2H).

3-(4-Methylbenzylideneamino)quinazolin-4(3H)-one 2a. Methanol was used as the solvent, and the product (white solid) was oven-dried at 90-100°C for 1 hr to remove solvent impurities and give the final product with 55mg (35%) yield. M.P.(°C): 153-156, ^{1}HNMR (300 MHz, DMSO-d_6, δ): 9.2 (s, 1H), 8.6 (s, 1H), 8.2 (d, 1H), 7.8 (overlapping, 3H), 7.7 (d, 1H), 7.6 (t, 1H), 7.3 (d, 2H), 2.4 (s, 3H)

3-((E)-3-phenylallylidene)aminoquinazolin-4(3H)-one 2b. Methanol was used as the solvent and the product (white solid) was isolated with 132mg (77%) yield. M.P.(°C): 113-115, ^{1}HNMR (400 MHz, DMSO-d_6, δ): 9.0 (d, 1H), 8.4 (s, 1H), 8.2 (d, 1H), 7.8 (t, 1H), 7.7 (d overlapping 3H), 7.6 (t, 1H), 7.4 (overlapping, 4H), 7.2 (dd, 1H).

3-(4-Nitrobenzylideneamino)quinazolin-4(3H)-one 2c. Methanol was used as the solvent, and 3Å molecular sieves were added to the reaction mixture. TLC indicated no reaction progress after several hours, so more MeOH and one additional drop of AcOH was added, and the reaction was stirred at reflux overnight. TLC the next morning showed that the reaction was still incomplete. MeOH was evaporated on rotary evaporator, and the residual solid was dissolved in a minimal amount of hot DMF. The solution was cloudy-tan (presumably due to molecular sieves being ground up), so the solution was hot-filtered to give a clear/tan solution. DMF was then evaporated to give a solid, which was dissolved in hot EtOH, and two drops AcOH were added. The reaction mixture was stirred at reflux overnight, and the reaction mixture cooled to r.t. on benchtop, then chilled in an icebath. The precipitate (yellow solid) was isolated by vacuum filtration to give only 14mg (8%) yield. M.P.(°C): 231-237, ^{1}HNMR (300 MHz, $CDCl_3$, δ): 10.1 (s, 1H), 8.4 (s, 1H), 8.4 (overlapping, 3H), 8.0 (d, 2H), 7.8 (overlapping, 2H), 7.6 (t, 1H).

3-(3-Nitrobenzylideneamino)quinazolin-4(3H)-one 2d. Methanol was used as the solvent, and 3Å molecular sieves were added to the reaction mixture. TLC indicated no reaction progress after several hours, so more MeOH and one additional drop of AcOH was added, and the reaction was stirred at reflux overnight. TLC the next morning showed that the reaction was still incomplete. MeOH was evaporated on rotary evaporator, and the residual solid was dissolved in a minimal amount of hot DMF.

The solution was cloudy-tan (presumably due to molecular sieves being ground up), so the solution was hot-filtered to give a clear/tan solution. DMF was then evaporated to give a solid, which was dissolved in hot EtOH, and two drops AcOH were added. The reaction mixture was stirred at reflux overnight, and the reaction mixture cooled to r.t. on benchtop, then chilled in an icebath. The precipitate (yellow solid) was isolated by vacuum filtration to give only 32mg (18%) yield. M.P.(°C): 209-211, ^{1}HNMR (300 MHz, DMSO-d_6, δ): 10.1 (s, 1H), 8.8 (s, 1H), 8.4 (s, 1H), 8.4 (d, 2H), 8.2 (d, 1H), 7.8 (overlapping, 2H), 7.7 (t, 1H), 7.6 (t, 1H).

3-(4-Hydroxybenzylideneamino)quinazolin-4(3H)-one 2e. Methanol was used as the solvent, and the product (brown/tan solid) was isolated with 47mg (29%) yield. M.P.(°C): 196-197, ^{1}HNMR (400 MHz, DMSO-d_6, δ): 10.3 (br s, 1H), 9.0 (s, 1H), 8.5 (s, 1H), 8.2 (d, 1H), 7.8 (t, 1H), 7.7 (overlapping, 3H), 7.5 (t, 1H), 6.9 (d, 2H).

3-(Furan-2-ylmethyleneamino)quinazolin-4(3H)-one 2f. Methanol was used as the solvent, and the product (brown needles) was isolated with 32mg (22%) yield. M.P.(°C): 135-138, ^{1}HNMR (400 MHz, $CDCl_3$, δ): 9.6 (s, 1H), 8.4 (s,1H), 8.3 (d, 1H), 7.8 (m, 2H), 7.6 (s, 1H), 7.5 (t, 1H), 7.0 (d, 1H), 6.6 (q, 1H).

3-(Naphthalen-2-ylmethyleneamino)quinazolin-4(3H)-one 2g. Methanol was used as the solvent, and the product (white powder) was isolated with 82mg (45%) yield. M.P.(°C): 169-172, ^{1}HNMR (400 MHz, $CDCl_3$, δ): 9.8 (s, 1H), 8.4 (s, 1H), 8.4 (d, 1H), 8.2 (s, 1H), 8.1 (d, 1H), 7.9 (d, 2H), 7.9 (d, 1H), 7.8 (overlapping, 2H), 7.6 (overlapping, 3H).

3-(Butylideneamino)quinazolin-4(3H)-one 2h. Butanal (1eq) was dissolved in toluene and five drops of AcOH were added. The mixture was stirred for five minutes, and then 3-aminoquinazolinone (1 eq) was added. The flask was connected to a Dean-Stark apparatus filled with toluene and stirred at reflux overnight. The reaction mixture was cooled to r.t. on benchtop, then chilled on ice. No solid precipitated. Toluene was evaporated, and the residual oil was dissolved in hot EtOH, cooled to r.t., and chilled on ice. No solid precipitated. Upon addition of water, a milky-white emulsion formed. Water and ethanol

were evaporated *in vacuo* to give a tan/light brown solid. Trituration in DCM was attempted to purify the solid; however, TLC and ^{1}HNMR indicated that impurities were still present in the white solid that was obtained. 66mg (50%) of the crude product was obtained, and the crude product was used for the assay. M.P.(°C): 133-137, 165-185, ^{1}HNMR (300 MHz, $CDCl_3$, δ): 8.4 (t, 1H), 8.3 (d, 1H) 8.1 (app. t, 1H), 7.8 (q, 1H), 7.6 (t, 1H), 7.4 (q, 1H), 2.5 (q, 2H), 1.7 (m, 2H), 1.0 (t, 3H).

4-Methoxy-N-(4-oxoquinazolin-3(4H)-yl)benzamide 3a. Due to a mishap, toluene was added to the reaction mixture after 24 hours of mixing, but before the work-up was conducted. The crude product (light tan solid) was recrystallized in methanol, and the resulting solid was oven-dried at 90°C-100°C for 1 hr to remove solvent impurities apparent in ^{1}HNMR. The dried solid was then triturated in 75:25 EtOAc:Hexanes to give the final product (white solid) with 22mg (12%) yield. M.P.(°C): 168-169, ^{1}HNMR (400 MHz, DMSO-d_6, δ): 11.7 (s, 1H), 8.4 (s, 1H), 8.2 (dd, 1H), 8.0 (overlapping, 2H), 7.9 (d, 1H), 7.8 (d, 1H), 7.6 (t, 1H), 7.1 (d, 2H), 3.9 (s, 3H).

N-(4-Oxoquinazolin-3(4H)-yl)benzamide 3b. The crude product was oven-dried at 90°C-100°C for 1 hr to remove solvent impurities and give the final product (tan solid) with 88mg (54%) yield. M.P.(°C): 186-188, ^{1}HNMR (300 MHz, DMSO-d_6, δ): 11.9 (s, 1H), 8.5 (s, 1H), 8.2 (d, 1H), 8.0 (overlapping, 3H), 7.9 (t, 1H), 7.8 (d, 1H) 7.6 (broad overlapping, 3H).

4-Methyl-N-(4-oxoquinazolin-3(4H)-yl)benzamide 3c. The same procedure was followed as in the other amide syntheses, except that the acid chloride was formed *in situ* by stirring *p*-toluic acid (1.2 eq) and oxalyl chloride (1.4 eq) in THF (5 mL) and DMF (two drops) for one hour. The reaction was quenched by the addition of pyridine (1 eq). After the reaction, the crude product was recrystallized in MeOH to give the final product (tan solid) with 44mg (25%) yield. M.P.(°C): 202, ^{1}HNMR (300 MHz, DMSO-d_6, δ): 11.8 (s, 1H), 8.4 (s, 1H), 8.2 (d, 1H), 7.9 (overlapping, 3H), 7.8 (d, 1H), 7.6 (t, 1H), 7.4 (d, 2H), 2.4 (s, 3H).

Synthesis of 2'-Deoxyguanosine Derivatives

General Materials and Methods

NMR spectra of **7a-3** and **8a-b** were recorded at 300 MHz in deacidified $CDCl_3$. Deacidification was performed by percolating the solvent through solid $NaHCO_3$ and basic alumina. Syntheses of **7a-3** and **8a-b** were performed in oven-dried screw-capped vials equipped with oven-dried stirbars and purged with nitrogen.

General Procedure for the Preparation of N^6-alkyl– Derivatives of dG

N^6-alkyl– derivatives of dG were prepared by stirring a mixture of sulfonated TBDMS-dG (1 eq), Et_3N (5 eq) and the appropriate amine (5 eq) in 1,2-DME with 4Å molecular sieves at 50°C for 23 hours under an inert atmosphere. The reaction mixture was then decanted into a separatory funnel and diluted with EtOAc. The organic layer was extracted with 10% (w/v) citric acid and saturated $NaHCO_3$. The organic layer was then dried over anhydrous Na_2SO_4. Evaporation of organic solvents gave the crude product, which was purified by column chromatography generally using DCM to load the compound and 2% MeOH in DCM to elute the product. Alike fractions were collected and the organic solvents were evaporated to leave behind the product, which was dried overnight under vacuum.

General Procedure for the Preparation of O^6-alkyl–, and O^6-aryl– Derivatives of dG

O^6-alkyl–, and O^6-aryl– derivatives of dG were prepared by stirring a mixture of sulfonated TBDMS-dG (1 eq), DABCO (2 eq) and the appropriate alcohol (5 eq) in 1,2-DME with 4Å molecular sieves at room temperature under an inert atmosphere. After 30 minutes of stirring, DBU was added and the reaction mixture was stirred at room temperature for 24 hours under an inert atmosphere. The reaction mixture was then decanted into a separatory funnel and diluted with EtOAc, followed by sequential extractions with 1N NaOH and H_2O. The organic layer was dried over anhydrous Na_2SO_4, and the solvents were evaporated to give the crude product, which was purified via column chromatography using either a combination of DCM to load the compound and 2% MeOH in DCM to elute the product or a 4% MeOH in DCM to load the compound and elute the product, depending on the polarity of the

product. Alike fractions were collected and the organic solvents were evaporated to leave behind the product, which was dried overnight under vacuum.

2-Amino-6-(benzylamino)-9-[2-deoxy-3,5-bis-O-(*tert*-butyldimethylsilyl)-β-D-erythro-pento-furanosyl]purine 7a. Due to a mishap, the vial was not purged with nitrogen until fifteen minutes after reaction start. This did not appear to have any adverse effect on the outcome of the experiment. The reaction mixture was stirred for 24 hours and the product (tan/white solid) was obtained with 66.4mg (77%) yield. R_f(silica/2% MeOH in DCM) = 0.41, ^{1}HNMR (300 MHz, deacidified $CDCl_3$, δ): 7.74 (s, $1H_8$), 7.3 (m, 5H, ArH), 6.32 (app. t, $1H_1$), 5.88 (s, 1H, NH), 4.78 (s, 2H, NH_2), 4.72 (s, 2H, NCH_2), 4.54 (app. m, $1H_3$), 4.00 (app. q, $1H_4$), 3.78 (app. dd, $2H_5$), 2.58 (app. m, $1H_2$), 2.32 (app. ddd, $1H_2$), 1.73, 1.26, 0.89 (s, 18H, *t*-butyl), 0.08 (s, 12H, $SiCH_3$).

2-Amino-6-(allylamino)-9-[2-deoxy-3,5-bis-O-(*tert*-butyldimethylsilyl)-β-D-*erythro*-pento-furanosyl]purine 7b. The reaction mixture was stirred for 23 hours, and after the first chromatographic separation using DCM to load the compound and 2% MeOH in DCM to elute the product, the product was not clean as judged by ^{1}HNMR and TLC. The crude product was rechromatographed using the same solvent system, but this second separation was not successful either. Trituration in cold hexane was attempted; however, it did not result in any further purification. Finally, the crude product was rechromatographed using 1:1 hexane:ethyl acetate to load the compound and elute the product. Alike fractions were collected and the organic solvents were evaporated to leave behind the clean product, which was dried overnight under vacuum. The product (white solid) was obtained with 38.1mg (48%) yield. R_f(silica/2% MeOH in DCM) = 0.36, ^{1}HNMR (300 MHz, deacidified $CDCl_3$, δ): 7.74 (s, $1H_8$), 6.27 (t, $1H_1$), 5.94 (m, 1H, =CH), 5.58 (br s, 1H, NH), 5.27 (dd, 1H, $=CH_{trans}$), 5.13 (dd, 1H, $=CH_{cis}$), 4.72 (br s, 2H, NCH_2), 4.56 (m, $1H_3$), 4.20 (br s, 2H, NH_2) 3.94 (m, $1H_4$), 3.76 (m, $2H_5$), 2.56 (quint, $1H_2$), 2.31 (m, $1H_2$), 2.13 (br s, residual H_2O), 0.89 (s, 18H, *t*-butyl), 0.09 (s, 12H, $SiCH_3$).

2-Amino-6-(4-methylpiperazino)-9-[2-deoxy-3,5-bis-O-(*tert*-butyldimethylsilyl)-β-D-*erythro*-pento-furanosyl]purine 7c. The reaction mixture was stirred for 25 hours, and the solvents used for the

purification were DCM to load the compound and 10% MeOH in DCM to elute the product, which was isolated as a yellow oil in 62.2mg (73%) yield. R_f(silica/2% MeOH in DCM) = 0.02, ^{1}HNMR (300 MHz, deacidified $CDCl_3$, δ): 7.68 (s, $1H_8$), 6.29 (t, $1H_1$), 4.64 (br s, 2H, NH_2), 4.53 (m, $1H_3$), 4.21 (br s, 4H, N-C-N-CH_2), 3.94 (q, $1H_4$), 3.73 (m $2H_5$), 2.48 (t, m overlapping, 5H, 4CH_3-N-CH_2, $1H_2$), 2.31 (s, m overlapping, 4H, 3N-CH_3, $1H_2$), 0.88 (s, 18H, *t*-butyl), 0.04 (s, 12H, $SiCH_3$).

2-Amino-6-morpholino-9-[2-deoxy-3,5-bis-O-(*tert*-butyldimethylsilyl)-β-D-*erythro*-pento-furanosyl]purine 7d. The reaction mixture was stirred for 25 hours, and the solvents for the purification were DCM to load the compound and 5% MeOH in DCM to elute the product, which was isolated as a yellow oil with 64.8mg (82%) yield. R_f(silica/2% MeOH in DCM) = 0.32, ^{1}HNMR (300 MHz, deacidified $CDCl_3$, δ): 7.70 (s, $1H_8$), 6.30 (t, $1H_1$), 4.65 (br s, 2H, NH_2), 4.55 (m, $1H_3$), 4.20 (app. t, 4H, CH_2-N-CH_2), 3.94 (m, $1H_4$), 3.75 (overlapping m, 6H, 4CH_2-O-CH_2, $2H_5$), 2.52 (app. quint, $1H_2$), 2.31 (m, $1H_2$), 0.89 (s, 18H, *t*-butyl), 0.08 (s, 12H, $SiCH_3$).

O^6-Benzyl-3'-5'-bis-*O*-tert-butyldimethylsilyl-2'-deoxyguanosine 8a. The reaction mixture was stirred for 24 hours. The solvent system for the purification was DCM to load the compound and 2% MeOH in DCM to elute the product (yellow oil with crystal formation evident) with 51.7mg (60%) yield.. R_f(silica/2% MeOH in DCM) = 0.66, ^{1}HNMR (300 MHz, deacidified $CDCl_3$, δ)[1]: 7.89 (s, $1H_8$), 7.48 (d, 2H, ArH), 7.36 (overlapping, 2H, ArH), 7.31 (overlapping, 1H, ArH), 6.31 (t, $1H_1$), 5.5 (s, 2H, OCH_2), 4.84 (br s, 2H, NH_2), 4.56 (m, $1H_3$), 3.98 (m, $1H_4$), 3.77 (overlapping dd, $2H_5$), 2.53 (quint, $1H_2$), 2.35 (quint, $1H_2$), 0.90 (s, 18H, *t*-butyl), 0.08 (s, 12H, $SiCH_3$).

O^6-Phenyl-3'-5'-bis-*O*-tert-butyldimethylsilyl-2'-deoxyguanosine 8b. The reaction mixture was stirred for 24 hours. The solvent system for the purification was 4% MeOH in DCM to load the column and elute the product (slightly oily yellow/white solid) with 64.8mg (77%) yield. R_f(silica/2% MeOH in DCM) = 0.63, ^{1}HNMR (300 MHz, deacidified $CDCl_3$, δ): 7.99 (s, 1H), 7.38 (m, 2H, ArH), 7.20

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[1] ^{1}HNMR showed minor uncharacterized impurities

(overlap, 3H, ArH), 6.33 (t, $1H_1$), 4.80 (br s, 2H, NH_2), 4.58 (m, $1H_3$), 3.98 (m, $1H_4$), 3.79 (qd, $2H_5$), 2.57 (app quint, $1H_2$), 2.37 (m, $1H_2$), 0.91 (s, 18H, *t*-butyl), 0.08 (s, 12H, $SiCH_3$).

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