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Development of New Fluorescent Xanthines as Kinase Inhibitors

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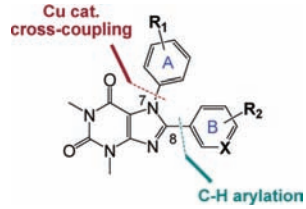
ABSTRACT



An efficient and versatile synthetic approach for the preparation of highly substituted xanthine derivatives has been developed by a combination of direct N7- and C8-arylation. With this method, diverse xanthine analogues were prepared and potent kinase inhibitors could be identified. For example, compound 8a inhibits PI3Ks and proliferation in T47D tumor cells. In addition, these xanthine-based kinase inhibitors exhibited significant fluorescence emission in a concentration-dependent response.

The identification of small molecules capable of detecting specific targets is of extreme importance for fluorescent sensing and probing the properties of targets.[1] Since the conjugation of a target-specific ligand with a fluorescent moiety[2] often results in an undesired effect on cell permeability, binding affinity, and toxicity, an emissive ligand can be considered as an ideal fluorescent probe.

The importance of xanthine scaffolds as fluorophores was disclosed by recent work in the You group.[3] In this literature, a series of C8-aryl-substituted xanthine derivatives exhibit significant photonic luminescence. Xanthines are also known as important biologically active alkaloids, constituting a major class of adenosine receptor antagonists.[4] For example, by virtue of xanthine's purine structure (Figure 1), caffeine binds to adenosine receptors in the brain blunting the effects of adenosine.[5] Since adenosine-5′-triphosphate (ATP) consists of adenosine and triphosphate (Figure 1), we hypothesized that adenosine receptor antagonists might fit into the ATP binding site of kinases in a manner similar to binding to adenosine receptor. Our research was

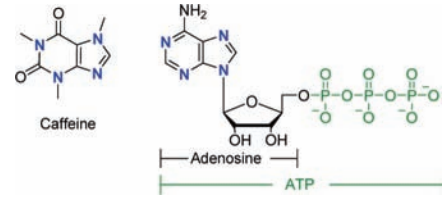


Figure 1. Structures of N7-methyl xanthine (caffeine), adenosine, and ATP, presented side by side for comparison.

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inspired by these unique features of xanthine to design and identify fluorescent xanthine-based kinase inhibitors.

The classical condensation route appeared unsuitable for the synthesis of diverse derivatives because the preparation requires many steps and often provides low yields.[6] To facilitate the exploration of N7- and C8-substituents, we identified theophylline (ca. $75/500 g, TCI) as a convenient starting point. Our synthetic strategy toward the target compounds was based on a versatile combination of regioselective N7-arylation and/or direct C8-arylation (Figure 2).

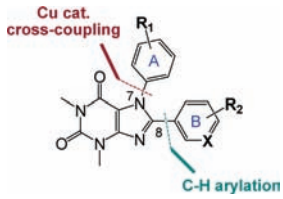
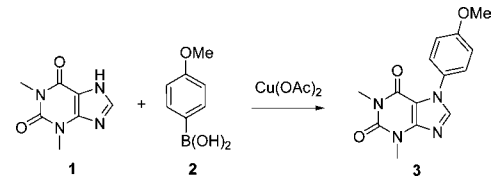


Figure 2. Strategy to xanthine derivatives from arrays of A and B rings.

Copper-mediated C−N coupling reactions of NH functions with arylboronic acids have been developed as powerful synthetic methodology.[7] On the basis of published procedures for the N-arylation of general nucleobases,[8] theophylline could be arylated at the N7 position selectively but in low yield (Table 1, entries 1−6). These disappointing results prompted us to search for other reaction conditions. Fortunately, when pyridine or 2,6-lutidine was used as the base, the yield of the product increased significantly. After screening a variety of bases, solvents, and temperatures (Table 2, entries 1−13), the best result was obtained in CH_2Cl_2 at 40 °C for 24 h with 2 equiv of pyridine as the base in the presence of copper(II) acetate (Table 1, entry 12). Both electron-donating and electron-withdrawing substituents on the aryl boronic acids were well tolerated.

Table 1. Optimization of the Coupling of Theophylline (**1**) with 4-Methoxyphenylboronic Acid (**2**)[a]



1 2 3

entry	base (equiv)	solvent	temp, °C	yield,[b] %
1	TMEDA (2)	$MeOH/H_2O$	rt	3
2	TMEDA (2)	MeOH	rt	trace
3	TMEDA (2)	$MeOH/H_2O$	70	10
4	Et_3N (2)	DMF	rt	10
5	Et_3N (2)	$MeOH/H_2O$	rt	15
6	K_2CO_3 (2)	DMF	rt	trace
7	−	MeOH	rt	9
8[c]	pyridine (2)	CH_2Cl_2	rt	23
9	pyridine (2)	DMF	rt	15
10	pyridine (2)	CH_2Cl_2	rt	22
11	pyridine (2)	CH_2Cl_2	40	38
12[d]	pyridine (2)	CH_2Cl_2	40	60
13[d]	lutidine (2)	CH_2Cl_2	40	35

[a] Reactions were carried out with $Cu(OAc)_2$ (2 equiv), theophylline (1 equiv), and 4-methoxyphenylboronic acid (1.5 equiv) for 24 h. [b] Yield of isolated product. [c] Molecular sieves (4 Å) added. Reaction was carried out for 3 days. [d] Theophylline (2 equiv) and 4-methoxyphenylboronic acid (1 equiv). Yield of isolated products was calculated from the amount of boronic acid. DMF = *N*,*N*-dimethylformamide, TMEDA = *N*,*N*,*N*′,*N*′-tetramethylethylenediamine.

Having successfully installed the N7-aryl ring onto the theophylline, we next turned to direct C8 arylation. We intended to build the C8-(hetero)aryl ring by means of the copper-catalyzed C−H bond activation path. Whereas the coupling of N7-methyl or benzyl xanthine with aryl bromide under comparable reaction conditions yielded the desired products in high yields, attempts to couple the N7-aryl xanthine such as **3** with aryl bromide **4** were unsuccessful, affording only unreacted materials. It seemed that the replacement of the N7 methyl with an aryl group influences the electronics of the xanthine core leading to a change in the reactivity for C−H arylation. To circumvent these synthetic challenges, we investigated more effective reaction conditions for the direct C−H arylation.[9]

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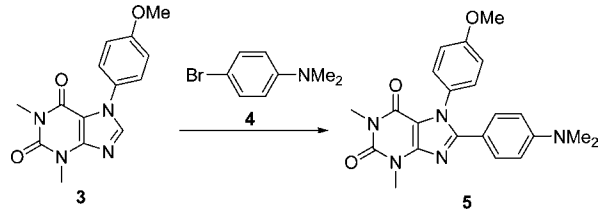
Table 2. Optimization of the Coupling of N-7 Aryl Theophylline **3** with 4-Bromo-*N*,*N*-dimethylaniline (**4**)[a]



entry	catalyst system (equiv)	solvent (ratio)	additive (equiv)	yield,[b] %
1	CuI (0.2)	DMF (1)/xylene (1)	K_3PO_4 (2); Phen (0.2)	trace
2	CuI (0.2)	DMF (1)/xylene (1)	LiO^{t}Bu (2); Phen (0.2)	–
3	CuI (0.2)	DMF	K_2CO_3 (2); ^{t}BuCO$_2$H (0.2)	–
4	$Pd(OAc)_2$ (0.1)	DMF	K_2CO_3 (2); ^{t}BuCO$_2$H (0.2)	–
5	$Pd(OAc)_2$ (0.1); CuI (2)	DMF	Cs_2CO_3 (2.5)	61
6	$Pd(OAc)_2$ (0.1); CuI (3)	DMF	Cs_2CO_3 (2.5)	78

[a] Reactions were carried out with base, N7-aryl xanthine **3** (1 equiv), and aryl bromide **4** (1.5 equiv) at 140 °C for 40 h in a sealed tube. [b] Yield of isolated product. DMF = *N*,*N*-dimethylformamide, Phen = 1,10-phenanthroline.

Later, it was found that when the coupling was conducted with $Pd(OAc)_2$, CuI, and Cs_2CO_3 in DMF at 140 °C for 40 h, the desired product could be isolated in 78% yield (Table 2, entry 6). It should be noted that no improvement of the yield was obtained with aryl iodide in place of aryl bromide **4**. We applied these reaction conditions to install C8-aryl rings with various functional groups such as acetamide, sulfonamide, and carbamate groups, and the desired products were successfully prepared (Figure 3).

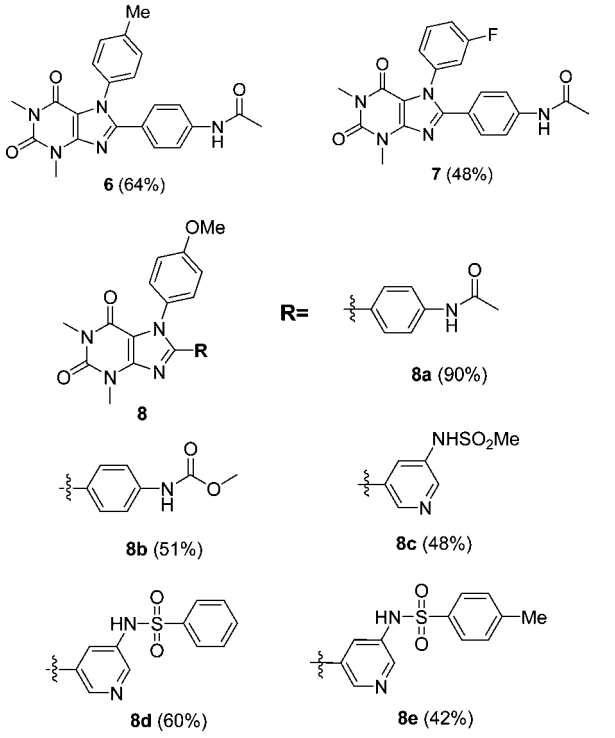


Figure 3. Examples of xanthine derivatives from arrays of A and B rings. Reactions were carried out under the conditions of entry 6 in Table 2.

With the above practical methodology, a series of xanthine analogues were prepared and their antiproliferative effects were screened over tumor cells. To measure the inhibitory effect of compounds on T47D cell growth, cell viability was tested by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. To our delight, acetamide **8a** shows good antiproliferative activity and growth of T47D cells was inhibited in a dose-dependent manner (IC_{50} = 3.7 μM, Figure S3 in the Supporting Information). Replacement of the acetamide group of **8a** with a simple alkylamine group led to a large loss in activity. For example, dimethylaniline **5** was nearly 6-fold less active (IC_{50} = ~20 μM) than acetamide **8a**.

Since we originally surmised that the antiproliferative activity of compound **8a** is associated with inhibitory effects of certain kinases, it seemed necessary to evaluate the kinase inhibitory activity to probe the kinase inhibition and confirm our hypothesis. To this end, compounds **8a** and **8c** were tested at 10 μM concentration in a high-throughput binding assay (KINOMEscan, Ambit Biosciences)[10] against a panel of 96 kinases measuring binding with phage display technology. Indeed, compounds **8a** and **8c** were discovered as potent inhibitors over several kinases [POC (percent of control) values <50] shown in Table 3.[11] The highest affinity binding at this concentration was to PI3Kα and PI3Kγ for both compounds. Compound **8c** also binds with high affinity to AKT1 and c-KIT (D816 V). These results support our prediction described earlier that properly modified adenosine receptor antagonists could bind to the ATP binding site of kinases.[12]

To obtain a detailed picture of the inhibitory effect on PI3Kα, we determined the full IC_{50} value of compound **8a**

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(11) For full POC values of the 96 kinase panel, see Table S1 in the Supporting Information.

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Table 3. Kinase Affinity of Compounds **8a** and **8c**[c]

kinase[a]	POC of **8a**	POC of **8c**
AKT1	(100)	9.7
CDK7	49	50
JUK	(95)	48
c-KIT(D816 V)	(98)	29
PI3Kα	44 (IC_{50} = 0.15 μM)[b]	47
PI3Kγ	5.8	2
PIM2	50	44

[a] Kinases with POC < 50 at 10 μM concentration are shown. Lower numbers of POC (percent of control) indicate stronger hits. Values show an average of duplicate measurements. [b] IC_{50} values against PI3Kα determined with PI3-Kinase ELISA kit (Echelon-inc). [c] A panel of 96 kinases were tested at 10 μM in a high-throughput binding assay (Ambit Bioscience).

using PI3-Kinase ELISA kit (Echelon-inc) and compared with the inhibitory activity of LY294002 (a quercetin analogue). The flavonoid-related compound LY294002 is one of the well-known PI3K inhibitors with an IC_{50} of 0.63 μM and has been widely used to elucidate the functional role of PI3K.[13] Compound **8a** was found to be a more potent PI3Kα inhibitor (IC_{50} = 0.15 μM) than LY294002 (Figure S1 in the Supporting Information).

Compound **8a** was further profiled in Western blot assays monitoring inhibition of the phosphorylation of AKT (Ser^{473}) in T47D. Besides p-AKT (Ser^{473}) being inhibited, the phosphorylation of m-TOR was also inhibited in a dose-dependent manner (Figure S2 in the Supporting Information). These data suggest that antiproliferative effect of compound **8a** might be associated with the inhibition of the intracellular PI3K/AKT/m-TOR pathway. Recent studies describe that numerous components of the PI3K, AKT, and m-TOR pathway are targeted by amplification, mutation, and translocation more frequently than any other pathway in cancer patients, resulting in activation of the pathway.[14] Since deregulation of this pathway plays a role in various cancers, PI3K, AKT, and m-TOR may be good targets for anticancer drug discovery.[15] Further optimization of the xanthine series to identify more potent inhibitors is currently ongoing.

Next, to determine the scope as a potential fluorescent probe, the photonic properties were studied. Remarkably, this type of derivative exhibited significant photonic luminescence: **5** (λ_{max} = 348 nm, λ_{em} = 428 nm, ϕ_F = 0.034) and **8a** (λ_{max} = 322 nm, λ_{em} = 410 nm, ϕ_F = 0.013). With potent kinase inhibitor **8a**, a concentration-dependent fluorescence response was observed in the 1−10 μM range without shifts in emission or excitation wavelengths (Figure 4). More

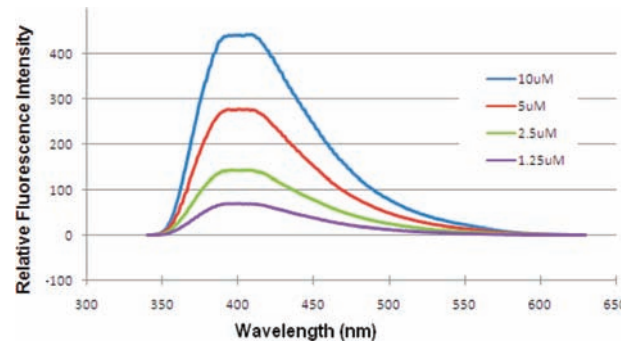

Figure 4. Photonic luminescence of potent kinase inhibitor **8a** in CH_2Cl_2 (λ_{ex} = 322 nm).

detailed studies regarding photonic properties and applications are currently under investigation in our group.

In summary, novel fluororescent kinase inhibitors based on the xanthine scaffold were discovered by using our efficient synthetic route and cell-based screening. Compound **8a** is a potent PI3Kα inhibitor and is cell permeable. It shows good antiproliferative activity in T47D, and exhibits a significant fluorescence response. Our findings provide valuable insight in the design of fluorescent kinase inhibitors derived from xanthine scaffolds.

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Supporting Information Available: Experimental procedures and characterization of all new compounds and biological evaluation data. This material is available free of charge via the Internet at http://pubs.acs.org.

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