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New class of 8-aryl-7-deazaguanine cell permeable fluorescent probes

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Abstract

A one step synthesis of fluorescent 8-aryl-(7-deazaguanines) has been accomplished. Probes exhibit blue to green high quantum yield fluorescence in a variety of organic and aqueous solutions, high extinction coefficients, and large Stokes shifts often above 100 nm. The probes are highly cell permeable, and exhibit stable bright fluorescence once intracellular; therefore are suited to the design of biosensors.

Graphical Abstract

Keywords

8-aryl-7-deazaguanine; Fluorescent probes; 2,4-diamino-6-hydroxypyrimidine; Phenacyl bromides; Cellular permeability

7-deazaguanine, nomenclature name 2-amino-3,7-dihydro-4H-pyrrolo[2,3-d]pyrimidin-4-one, is an important modification of nucleotide base guanine where N7 is replaced by carbon.¹ Naturally occurring 7-deazaguanines such as nucleotide Q, preQ, preQ₀, and archaeosine serve as substrates of TGT enzymes that mediate their introduction into select

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Supplementary Material

Supplementary data associated with this work, including synthetic procedures, NMR, HRMS, absorbance and fluorescence spectra can be found in the online version.

tRNA of both eukaryotic and prokaryotic cells in place of guanine.² This tRNA modification is thought to have significant impact on cellular proliferation, aging, and tumor progression. Applications of synthetic 7-deazaguanines in nucleic acid hybridization probes are important and well documented.³⁻⁵ Prominently chemotherapeutic drugs based on 7-deazaguanine are capable of a multitude of interactions with intracellular enzymes of the folic acid cycle that are indispensable to cellular proliferation such as TS, DHFR, GARF, and cellular membrane transporters such as folate receptors (FR α , FR β , RFC, PCFT).⁶⁻⁸ In light of the importance of this motif in the biochemistry of living cells, we investigated the design of fluorescent probes around 7-deazaguanine. In terms of precedent, fluorescent 8-aryl-7-deazaguanines (⁸aryl7DGs) were not described in literature. However, extension of the π -system of guanine itself at the 8-position with various aromatic motifs results in highly fluorescent probes.⁹ Therefore, we hypothesized that 7-deazaguanine analogs would exhibit similar fluorescence emission.¹⁰⁻¹⁴ Data presented here far exceeded our expectations. Specific experimental procedures for the synthesis of ⁸aryl7DGs were not published. Often 7-deazaguanines are obtained using the addition-condensation reaction by Secríst III (Scheme 1).¹⁵ We implemented the above protocol with minor modifications. ⁸aryl7DGs were obtained as single products in high yields by reacting equivalent molar amounts of commercially available 2,4-diamino-6-hydroxypyrimidine **2** and substituted phenacyl bromides **1(a-l)**. We found that using Et₃N in 1,4-dioxane instead of the usual sodium acetate in THF-water mixture led to simple and efficient purification by precipitation in water. Due to mild reaction conditions, a wide range of substrates is tolerated.

The S_N2 reaction between nucleophilic C5 of pyrimidine **2** and phenacyl bromides **1** determines the regiochemistry of the final products, of which there was no ambiguity. Our spectral data were compared, and are substantially different from, published data on ⁷aryl7DG regioisomers.¹⁶ This synthetic work adds to an already rich literature on 7DG. There is interest in efficient syntheses of ⁸aryl7DG as intermediates of pharmacologically active lead compounds.¹⁷ A similar synthesis of ⁸aryl7DGs was recently reported in literature without any experimental or spectroscopic details.¹⁸ Our protocol was developed independently prior to this publication.¹⁹

Three other ⁸aryl7DGs were produced from **3c**, according to literature protocol, as shown in scheme 2.¹⁶

⁸aryl7DGs are freely soluble in DMSO; therefore, samples were prepared from DMSO stock solutions diluted in the appropriate solvent to less than 2% DMSO. Fluorescence emission and absorption spectra were measured in DMSO, diluted aqueous solutions, and 10 % fetal bovine serum (FBS) in phosphate buffer, which are most likely to be used in biological applications. Optical properties are summarized Tables 1-3. Detailed spectra in various organic and aqueous solvents for each probe are included in SI. With the exception of nitro substituted, all ⁸aryl7DGs exhibit above average to excellent quantum yields in DMSO. All scenarios of fluorescence modulation are observed: moderate changes in fluorescence in all mediums (**3b**, **3h**, **3j**), several fold increase in fluorescence intensity from DMSO to bovine serum solutions (**3k**, **3l**), as well as the opposite (**3a**, **3c**, **3e**). The probes can be thought of as refinements of core ⁸Ph7DG (**3a**). Introducing methoxy group (**3e**, **3h**) leads to high quantum yield blue fluorescence. Introducing bromine leads to poor fluorescence (**3d**). No change in

λ_{em} ~400nm is observed compared to 3a. Stokes shifts measure ~ 80nm (fig.1A). One can fine-tune fluorescence properties compared to 3a to higher λ_{em} ~450-470 nm by extending the aryl system (3b, 3j) or introducing groups such as CN (3c), CO₂H (3m), and amide (3n). These probes exhibit excellent green fluorescence, and large Stokes shift often >100nm (fig. 1B). Attaching two known hydrocarbon fluorophores such as pyrene and anthracene to 7DG results in probes 3k, 3l with extraordinary large Stokes shifts in DMSO, 146nm and 202nm, respectively (Table 1), and maximum λ_{em} ~530nm (fig.1C). All probes exhibit equal or higher fluorescence emission intensities in organic solvents such as DMF, CHCl₃, acetonitrile, 1,4-dioxane compared to DMSO (see SI, pg8). The highly hydrophobic 3k (^{8pyr}7DG) and 3l (^{8anthra}7DG) exhibit a 2-4 fold increase in fluorescence emission intensity in benzene, chloroform and 1,4-dioxane compared to DMSO (figs.SI-25, SI-28). As expected, ^{8aryl}7DGs experience various degrees of fluorescence quenching in methanol and aqueous solutions (SI, pg8). However, most probes exhibit analytically viable quantum yields Φ_F >0.1 in 10% FBS solutions, and often above average Φ_F ~0.2-0.5 (Table 2). Impressively, six out of 13 ^{8aryl}7DGs retain high quantum yield fluorescence either in pure water, or basic aqueous solutions, often in higher values compared to serum (3a, 3b, and 3h). Fluorescence emission of all probes is quenched in acidic solutions, except for probe 3b.

Next we proceeded to test the cellular permeability of ^{8aryl}7DGs. Two human cell lines were used: nasopharyngeal (KB) cancer cells and A549 lung cancer cells. The toxicity of the probes on both cell lines is minimal at physiological levels of use. In repeated measurements, we were unable to find an IC₅₀ using the XTT assay in concentrations up to 20 μ M. Probes excited optimally at λ_{ex} ~350nm were imaged using a low resolution optical microscope equipped with a DAPI fluorescence filter. Probe 3k (^{8pyr}7DG) that is excited optimally at λ_{ex} =405nm was imaged using a high resolution confocal microscope. KB and A549 cells were stained at 37°C, 5% CO₂, 95 % air, in RPMI 1640 growth medium containing probes at 1-20 μ M concentrations. No cellular uptake was observed for hydrophilic probes 3c, 3m and 3n at 5 μ M and 20 μ M concentration. This result was expected based on sequestration by serum proteins of probes, which are anionic under incubating conditions. On the other hand, hydrophobic 3b, 3j, 3k, and 3l exhibited high cellular uptake and stability inside cells. Perhaps most informative are confocal microscopy images of cells stained with 1 μ M solution of probe 3k (fig. 2). Within minutes, intracellular fluorescence is observed. Images were taken successively for 2 hours. The complete set of low and high resolution images is included in SI. Note that probes remain largely cytosolic upon prolonged exposure with little to no fluorescence observed in the nuclear compartments in low and high resolution microscopy. This suggests receptor mediated uptake as efflux mechanisms might be working in tandem with free diffusion cellular uptake.

In conclusion, the diversity of the library examined suggests any 8-aryl-7-deazaguanine could be an above average fluorophore. Bright fluorescence in the blue to green region and large Stokes shifts are some of the qualities observed. It is important for future biological applications that fluorescence properties are preserved in aqueous solutions, and in some instances enhanced in 10 % FBS in phosphate buffer. Functional groups introduced into the aryl frame of ^{8Ph}7DG (3a) have defined effects on fluorescence properties causing ~80nm redshift to λ_{em} ~465-480nm for phenyl, CN, CO₂H and amide groups, and λ_{em} ~530nm for larger aryl groups such as pyrene or anthracene. Diversity-oriented synthesis of larger

fluorophore libraries is possible because ⁸aryl⁷DG core is assembled in one step from readily available starting materials. This process will facilitate identification of probes for specific future applications. Few fluorescent C8-arylguanine designs are used to study the reaction of phenoxyl radical with DNA strands.^{11,20} C8-arylguanines are reported to stabilize GG mismatches and to stabilize the transition from B-DNA to Z-DNA as well.^{21,22} Therefore, ⁸aryl⁷DGs being isosteric might find immediate applications. It is well known that groups appended to the 7-position of 7-deazaguanine are known to accommodate better within the major groove of the DNA duplexes.³ Thus, designing an analogous fluorescent ⁷aryl⁷DG series to complement the available ⁸aryl⁷DG series is a priority. Importantly, there is room for improving fluorescence properties. Introducing polyene bridges into 7-deazaguanine, and/or heterocyclic chromophores in designs which are similar to cyanine dyes could lead to near infrared probes with $\lambda_{em} > 600\text{nm}$ which are better suited for optical spectroscopy and imaging applications, in vivo. Hydrophobic ⁸aryl⁷DGs exhibited excellent cellular permeability in low concentrations and no apparent toxicity; therefore, are suited to the design of biosensors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References and notes

- (1). Bruckl T, Klepper F, Gutsmedl K, Carell T. *Org Biomol Chem*. 2007; 5:3821. [PubMed: 18004462]
- (2). Morris RC, Elliott MS. *Mol Genet Metab*. 2001; 74:147. [PubMed: 11592812]
- (3). Ingale SA, Pujari SS, Sirivolu VR, Ding P, Xiong H, Mei H, Seela F. *J Org Chem*. 2012; 77:188. [PubMed: 22129276]
- (4). Guo J, Yu L, Turro NJ, Ju J. *Acc Chem Res*. 2010; 43:551. [PubMed: 20121268]
- (5). Meng QL, Kim DH, Bai XP, Bi LR, Turro NJ, Ju JY. *J Org Chem*. 2006; 71:3248. [PubMed: 16599623]
- (6). Chattopadhyay S, Moran RG, Goldman ID. *Mol Cancer Ther*. 2007; 6:404. [PubMed: 17308042]
- (7). Cherian C, Wang YQ, Mitchell-Ryan S, Orr S, Hou ZJ, Gangjee A, Matherly LH. *Cancer Res*. 2013; 73
- (8). Mitchell-Ryan S, Wang YQ, Raghavan S, Ravindra MP, Hales E, Orr S, Cherian C, Hou ZJ, Matherly LH, Gangjee A. *J Med Chem*. 2013; 56:10016. [PubMed: 24256410]
- (9). Sinkeldam RW, Greco NJ, Tor Y. *Chem Rev*. 2010; 110:2579. [PubMed: 20205430]
- (10). Zilbershtein L, Silberman A, Fischer B. *Organic & Biomolecular Chemistry*. 2011; 9:7763. [PubMed: 21960279]
- (11). Manderville RA, Omumi A, Rankin KM, Wilson KA, Millen AL, Wetmore SD. *Chem Res Toxicol*. 2012; 25:1271. [PubMed: 22667322]
- (12). Matsumoto K, Takahashi N, Suzuki A, Morii T, Saito Y, Saito I. *Bioorg Med Chem Lett*. 2011; 21:1275. [PubMed: 21273064]
- (13). Greco NJ, Tor Y. *Tetrahedron*. 2007; 63:3515. [PubMed: 18431439]

- (14). Wanninger-Weiss C, Valis L, Wagenknecht HA. Bioorganic & medicinal chemistry. 2008; 16:100. [PubMed: 17509886]
- (15). Secrist JAL III, P. S. J. Org. Chem. 1978; 43:3937.
- (16). Taylor EC, Liu B. J Org Chem. 2003; 68:9938. [PubMed: 14682686]
- (17). Khalaf AI, Huggan JK, Suckling CJ, Gibson CL, Stewart K, Giordani F, Barrett MP, Wong PE, Barrack KL, Hunter WN. J Med Chem. 2014; 57:6479. [PubMed: 25007262]
- (18). Gao LJ, Schwed JS, Weizel L, De Jonghe S, Stark H, Herdewijn P. Bioorg Med Chem Lett. 2013; 23:132. [PubMed: 23218604]
- (19). Dhimitruka, I. Wayne State University, PhD thesis. 2004.
- (20). Omumi A, Beach DG, Baker M, Gabryelski W, Manderville RA. Journal of the American Chemical Society. 2011; 133:42. [PubMed: 21067186]
- (21). Omumi A, Millen AL, Wetmore SD, Manderville RA. Chem Res Toxicol. 2011; 24:1694. [PubMed: 21905681]
- (22). Gannett PM, Heavner S, Daft JR, Shaughnessy KH, Epperson JD, Greenbaum NL. Chem Res Toxicol. 2003; 16:1385. [PubMed: 14565779]

Figure 1.
A, B, C. Normalized fluorescence excitation (dashed lines) and emission spectra (solid lines) in DMSO.

Figure 2.

Confocal microscopy single slice images of A549 and KB cells stained with 1 μ M solution of 3k for 15 min.

Scheme 1. One step syntheses of hydrophobic ⁸ary17DG

Scheme 2.
Syntheses of hydrophilic ⁸aryl⁷DG

Table 1Fluorescence properties, ε ($M^{-1}cm^{-1}$), λ (nm), measured in DMSO

	3a	3b	3c	3d	3e	3g	3h	3j	3k	3l	3m	3n	3o
λ_{abs}	328	355	382	340	319	350	329	342	384	344	365	358	359
λ_{em}	401	470	474	403	402	432	399	474	530	546	410	468	467
λ	73	115	92	63	83	82	70	132	146	202	45	100	108
ε	20,634	22,772	21,227	18,243	20,615	11,640	15,204	21,188	11,906	29,422	18,224	13,231	12,503
Φ	0.96	0.70	0.84	0.04	0.55	0.78	0.80	0.69	0.09	0.08	0.52	0.85	0.13

Table 2
Fluorescence properties, ε ($\text{M}^{-1}\text{cm}^{-1}$), λ (nm), measured in 10% FBS in phosphate buffer.

	3a	3b	3c	3d	3e	3g	3h	3j	3k	3l	3m	3n	3o
λ_{abs}	318	343	354	326	310	336	316	337	378	339	341	343	344
λ_{em}	385	431	466	405	381	413	390	436	496	504	455	487	484
λ	77	88	112	79	71	77	86	99	118	165	114	144	140
ε	16,696	18,940	19,093	15,764	19,592	9,892	13,823	17,330	10,290	14,320	18,958	10,485	11,263
Φ	0.12	0.40	0.19	0.03	0.017	0.50	0.14	0.38	0.27	0.30	0.23	0.10	0.057

Table 3

Fluorescence properties, ε ($\text{M}^{-1}\text{cm}^{-1}$), λ (nm), measured in distilled water.

	3a ⁽¹⁾	3b	3c	3d	3e	3g	3h ⁽¹⁾	3j	3k	3l	3m	3n	3o
λ_{abs}	322	331	351	318	306	327	325	329	-	-	336	342	340
λ_{em}	410	461	476	400	403	416	415	482	-	-	466	491	509
λ	88	129	125	82	97	89	80	153	-	-	110	149	169
ε	22,051	16,229	23,120	17,743	19,403	10,842	14,278	17,525	-	-	19,110	12,829	11,405
Φ	0.27	0.68	0.05	0.0015	0.006	0.32	0.55	0.21	-	-	0.18	0.05	0.006

⁽¹⁾ Probes dissolved in 10mM NaOH in distilled water.