Bioorganic & Medicinal Chemistry Letters 25 (2015) 4593–4596

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

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

Ilirian Dhimitruka [a,*], Timothy D. Eubank [b], Amy C. Gross [a], Valery V. Khramtsov [a]

[a] Dorothy M. Davis Heart & Lung Research Institute, and Department of Internal Medicine, The Ohio State University, Columbus, OH 43210, United States
[b] Department of Microbiology, Immunology, and Cell Biology, West Virginia University Health Sciences, Morgantown, VW 26506, United States

ARTICLE INFO

Article history:
Received 27 July 2015
Accepted 20 August 2015
Available online 21 August 2015

Keywords:
8-Aryl-7-deazaguanine
Fluorescent probes
2,4-Diamino-6-hydroxypyrimidine
Phenacyl bromides
Cellular permeability

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.

7-Deazaguanine, nomenclature name 2-amino-3,7-dihydro-4*H*-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, preQ$_0$, and archaeosine serve as substrates of TGT enzymes that mediate their introduction into select 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 (FRα, FRβ, 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 (^{8aryl}7DGs) 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 ^{8aryl}7DGs were not published. Often 7-deazaguanines are obtained using the addition–condensation reaction by Secrist III (Scheme 1).[15] We implemented the above protocol with minor modifications. ^{8aryl}7DGs 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 Et_3N 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.

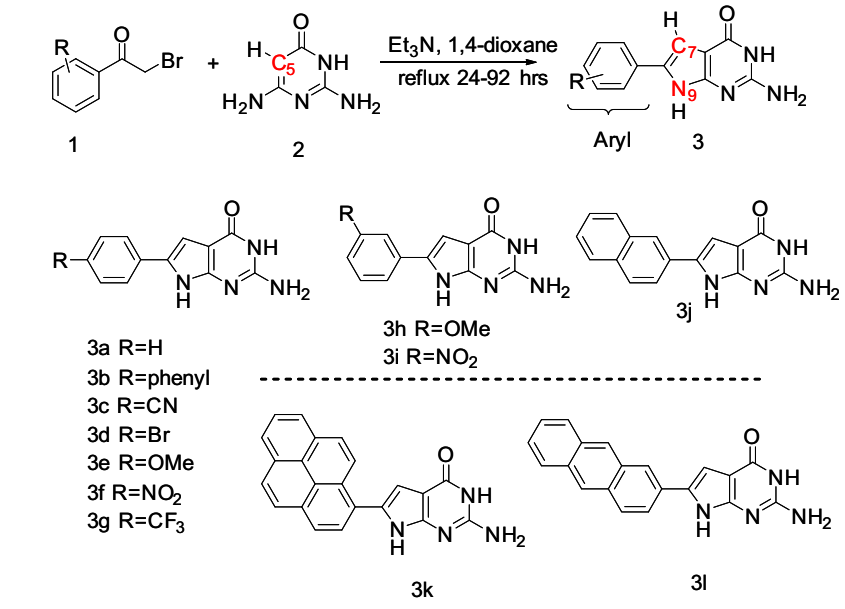
The SN_2 reaction between nucleophilic C5 of pyrimidine **2** and phenacyl bromides **1** determines the regiochemistry of the final products, of which there was no ambiguity. Our spectral data were compared, and are substantially different from, published data on ^{7aryl}7DG regioisomers.[16] This synthetic work adds to an already rich literature on 7DG. There is interest in efficient syntheses of ^{8aryl}7DG as intermediates of pharmacologically active lead compounds.[17] A similar synthesis of ^{8aryl}7DGs was recently reported in literature without any experimental or spectroscopic details.[18] Our protocol was developed independently prior to this publication.[19]

Three other ^{8aryl}7DGs were produced from **3c**, according to literature protocol, as shown in Scheme 2.[16]

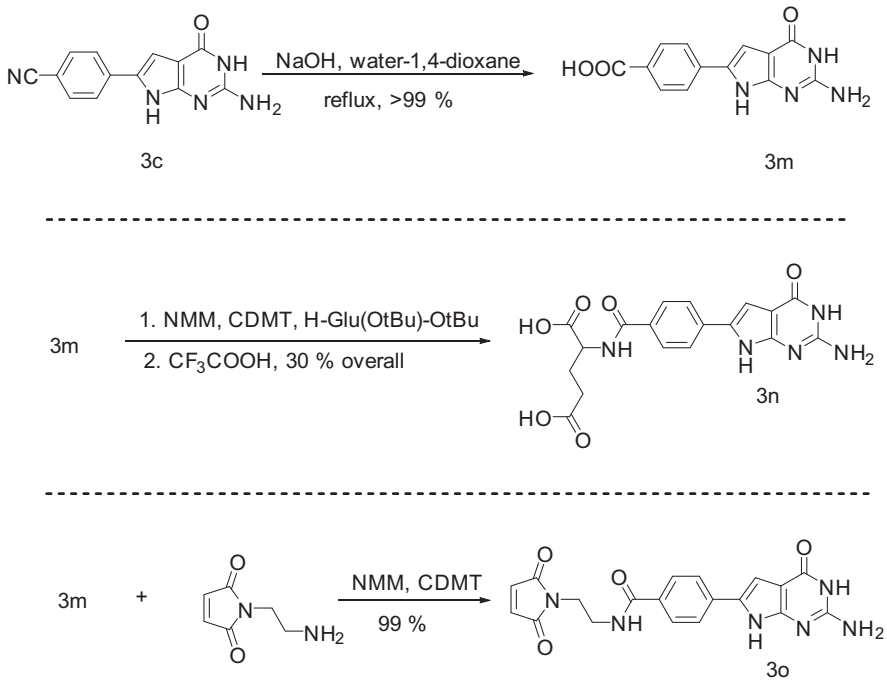
^{8aryl}7DGs are freely soluble in DMSO; therefore, samples were prepared from DMSO stock solutions diluted in the appropriate solvent to less than 2% DMSO. Fluorescence emission and absorption spectra were measured in DMSO, diluted aqueous solutions, and 10% fetal bovine serum (FBS) in phosphate buffer, which are most likely to be used in biological applications. Optical properties are summarized Tables 1–3. Detailed spectra in various organic

* Corresponding author.

http://dx.doi.org/10.1016/j.bmcl.2015.08.054
0960-894X/



Scheme 1. One step syntheses of hydrophobic ^{8aryl}7DG.



Scheme 2. Syntheses of hydrophilic ^{8aryl}7DG.

Table 1
Fluorescence 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
$\Delta\lambda$	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

and aqueous solvents for each probe are included in SI. With the exception of nitro substituted, all ^{8aryl}7DGs 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 oppo-

Table 2
Fluorescence properties, ε (M^{-1} 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
$\Delta\lambda$	77	88	112	79	71	77	86	99	118	165	114	144	140
ε	16,696	18,940	19,093	15,764	19,592	9892	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 (M^{-1} cm^{-1}), λ (nm), measured in distilled water

	3a[a]	3b	3c	3d	3e	3g	3h[a]	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
$\Delta\lambda$	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

[a] Probes dissolved in 10 mM NaOH in distilled water.

site (**3a**, **3c**, **3e**). The probes can be thought of as refinements of core ^{8Ph}7DG (**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} ~400 nm is observed compared to **3a**. Stokes shifts measure ~80 nm (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**), CO_2H (**3m**), and amide (**3n**). These probes exhibit excellent green fluorescence, and large Stokes shift often >100 nm (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, 146 nm and 202 nm, respectively (Table 1), and maximum λ_{em} ~530 nm (Fig. 1C). All probes exhibit equal or higher fluorescence emission intensities in organic solvents such as DMF, $CHCl_3$, 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 and 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 IC_{50} using the XTT assay in concentrations up to 20 μM. Probes exited optimally at λ_{ex} ~350 nm were imaged using a low resolution optical microscope equipped with a DAPI fluorescence filter. Probe **3k** (^{8pyr}7DG) that is exited optimally at λ_{ex} = 405 nm was imaged using a high resolution confocal microscope. KB and A549 cells were stained at 37 °C, 5% CO_2, 95% air, in RPMI 1640 growth medium containing probes at 1–20 μM concentrations. No cellular uptake was observed for hydrophilic probes **3c**, **3m** and **3n** at 5 μM and 20 μM 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 μM solution of probe **3k** (Fig. 2). Within minutes, intracellular fluorescence is observed. Images were taken successively for 2 h. 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

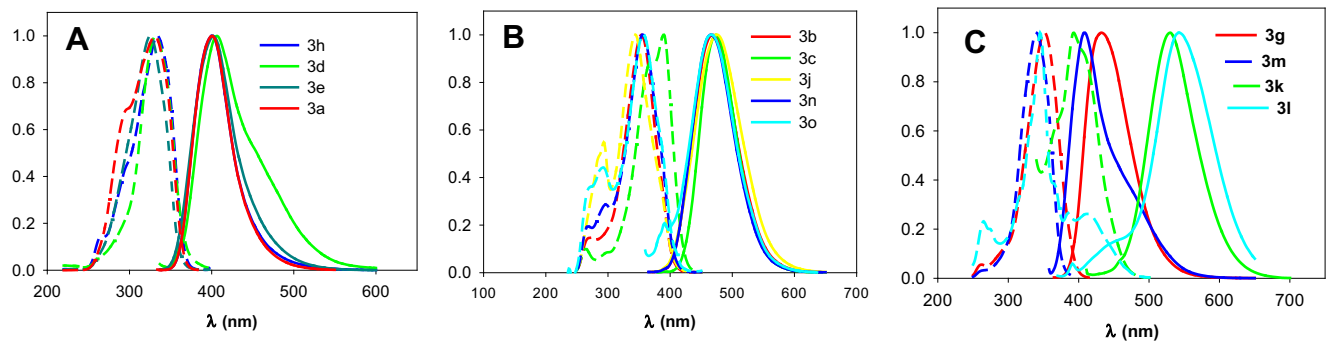


Figure 1. (A–C) Normalized fluorescence excitation (dashed lines) and emission spectra (solid lines) in DMSO.

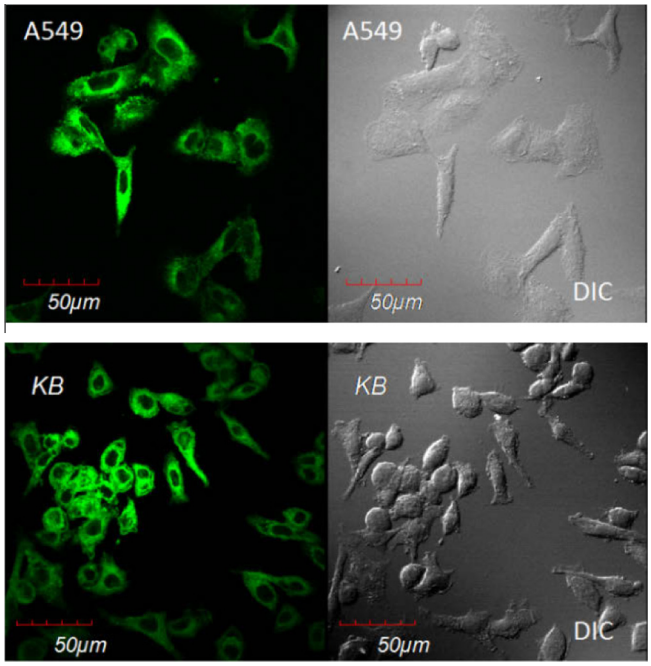


Figure 2. Confocal microscopy single slice images of A549 and KB cells stained with 1 μM solution of **3k** for 15 min.

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 ~80 nm redshift to λ_{em} ~465–480 nm for phenyl, CN, CO_2H and amide groups, and λ_{em} ~530 nm for larger aryl groups such as pyrene or anthracene. Diversity-oriented synthesis of larger fluorophore libraries is possible because ^{8aryl}7DG 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, ^{8aryl}7DGs 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 ^{7aryl}7DG series to complement the available ^{8aryl}7DG 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 λ_{em} >600 nm which are better suited for optical spectroscopy and imaging applications, in vivo. Hydrophobic ^{8aryl}7DGs exhibited excellent cellular permeability in low concentrations and no apparent toxicity; therefore, are suited to the design of biosensors.

Acknowledgments

This work was supported by the Pulmonary Division of the Department of Internal Medicine, the Ohio State University Medical College, and NIH Grant R03 EB016859. The authors thank Dr. Sara Cole, assistant director of Campus Microscopy & Imaging Facility (CMIF) of the Ohio State University.

Supplementary data

Supplementary data (synthetic procedures, NMR, HRMS, absorbance and fluorescence spectra) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.08.054.

References and notes

1. Bruckl, T.; Klepper, F.; Gutsmiedl, K.; Carell, T. *Org. Biomol. Chem.* **2007**, *5*, 3821.
2. Morris, R. C.; Elliott, M. S. *Mol. Genet. Metab.* **2001**, *74*, 147.
3. Ingale, S. A.; Pujari, S. S.; Sirivolu, V. R.; Ding, P.; Xiong, H.; Mei, H.; Seela, F. *J. Org. Chem.* **2012**, *77*, 188.
4. Guo, J.; Yu, L.; Turro, N. J.; Ju, J. *Acc. Chem. Res.* **2010**, *43*, 551.
5. Meng, Q. L.; Kim, D. H.; Bai, X. P.; Bi, L. R.; Turro, N. J.; Ju, J. Y. *J. Org. Chem.* **2006**, *71*, 3248.
6. Chattopadhyay, S.; Moran, R. G.; Goldman, I. D. *Mol. Cancer Ther.* **2007**, *6*, 404.
7. Cherian, C.; Wang, Y. Q.; Mitchell-Ryan, S.; Orr, S.; Hou, Z. J.; Gangjee, A.; Matherly, L. H. *Cancer Res.* **2013**, *73*.
8. Mitchell-Ryan, S.; Wang, Y. Q.; Raghavan, S.; Ravindra, M. P.; Hales, E.; Orr, S.; Cherian, C.; Hou, Z. J.; Matherly, L. H.; Gangjee, A. *J. Med. Chem.* **2013**, *56*, 10016.
9. Sinkeldam, R. W.; Greco, N. J.; Tor, Y. *Chem. Rev.* **2010**, *110*, 2579.
10. Zilbershtein, L.; Silberman, A.; Fischer, B. *Org. Biomol. Chem.* **2011**, *9*, 7763.
11. Manderville, R. A.; Omumi, A.; Rankin, K. M.; Wilson, K. A.; Millen, A. L.; Wetmore, S. D. *Chem. Res. Toxicol.* **2012**, *25*, 1271.
12. Matsumoto, K.; Takahashi, N.; Suzuki, A.; Morii, T.; Saito, Y.; Saito, I. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1275.
13. Greco, N. J.; Tor, Y. *Tetrahedron* **2007**, *63*, 3515.
14. Wanninger-Weiss, C.; Valis, L.; Wagenknecht, H. A. *Bioorg. Med. Chem.* **2008**, *16*, 100.
15. Secrist, J. A., III; Liu, P. S. *J. Org. Chem.* **1978**, *43*, 3937.
16. Taylor, E. C.; Liu, B. *J. Org. Chem.* **2003**, *68*, 9938.
17. Khalaf, A. I.; Huggan, J. K.; Suckling, C. J.; Gibson, C. L.; Stewart, K.; Giordani, F.; Barrett, M. P.; Wong, P. E.; Barrack, K. L.; Hunter, W. N. *J. Med. Chem.* **2014**, *57*, 6479.
18. Gao, L. J.; Schwed, J. S.; Weizel, L.; De Jonghe, S.; Stark, H.; Herdewijn, P. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 132.
19. Dhimitruka, I. Ph.D. Thesis, Wayne State University, 2004.
20. Omumi, A.; Beach, D. G.; Baker, M.; Gabryelski, W.; Manderville, R. A. *J. Am. Chem. Soc.* **2011**, *133*, 42.
21. Omumi, A.; Millen, A. L.; Wetmore, S. D.; Manderville, R. A. *Chem. Res. Toxicol.* **2011**, *24*, 1694.
22. Gannett, P. M.; Heavner, S.; Daft, J. R.; Shaughnessy, K. H.; Epperson, J. D.; Greenbaum, N. L. *Chem. Res. Toxicol.* **2003**, *16*, 1385.