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1. NMR Spectra



Figure S1. ¹H NMR spectrum of 2-amino-9-(4-fluorophenyl)-9*H*-purine-6-carbonitrile (2a).



Figure S2. ¹³C NMR spectrum of 2-amino-9-(4-fluorophenyl)-9H-purine-6-carbonitrile (2a).



Figure S3. ¹H NMR spectrum of 2-amino-9-(4-chlorophenyl)-9*H*-purine-6-carbonitrile (2b).



Figure S4. ¹³C NMR spectrum of 2-amino-9-(4-chlorophenyl)-9*H*-purine-6-carbonitrile (2b).



Figure S5. ¹H NMR spectrum of 2-amino-9-(4-bromophenyl)-9H-purine-6-carbonitrile (2c).



Figure S6.¹³C NMR spectrum of 2-amino-9-(4-bromophenyl)-9H-purine-6-carbonitrile (2c).



Figure S7. ¹H NMR spectrum of 2-amino-9-(3-bromophenyl)-9*H*-purine-6-carbonitrile (2d).



Figure S8. ¹³C NMR spectrum of 2-amino-9-(3-bromophenyl)-9*H*-purine-6-carbonitrile (2d).



Figure S9. ¹H NMR spectrum of 2-amino-9-phenyl-9*H*-purine-6-carbonitrile (2e).



Figure S10. ¹³C NMR spectrum of 2-amino-9-phenyl-9*H*-purine-6-carbonitrile (2e).



Figure S11. ¹H NMR spectrum of 2-amino-9-(*p*-tolyl)-9*H*-purine-6-carbonitrile (2f).



Figure S12. ¹³C NMR spectrum of 2-amino-9-(p-tolyl)-9*H*-purine-6-carbonitrile (2f).



Figure S13. ¹H NMR spectrum of 2,5-diamino-6-cyano-4-((4-fluorophenyl)amino)pyrimidinium chloride (3a).



Figure S14. ¹³C NMR spectrum of 2,5-diamino-6-cyano-4-((4-fluorophenyl)amino)pyrimidinium chloride (3a).



Figure S15. ¹H NMR spectrum of 2,5-diamino-6-cyano-4-((4-chlorophenyl)amino)pyrimidinium chloride (3b).



Figure S16. ¹³C NMR spectrum of 2,5-diamino-6-cyano-4-((4-chlorophenyl)amino)pyrimidinium chloride (3b).



Figure S17. ¹H NMR spectrum of 2,5-diamino-6-cyano-4-((4-bromophenyl)amino)pyrimidinium chloride (3c).



Figure S18. ¹³C NMR spectrum of 2,5-diamino-6-cyano-4-((4-bromophenyl)amino)pyrimidinium chloride (3c).



Figure S19. ¹H NMR spectrum of 2,5-diamino-6-cyano-4-((3-bromophenyl)amino)pyrimidinium chloride (3d).



Figure S20. ¹³C NMR spectrum of 2,5-diamino-6-cyano-4-((3-bromophenyl)amino)pyrimidinium chloride (3d).



Figure S21. ¹H NMR spectrum of 2,5-diamino-6-cyano-4-((phenylamino)pyrimidinium chloride (3e).



Figure S22. ¹³C NMR spectrum of 2,5-diamino-6-cyano-4-((phenylamino)pyrimidinium chloride (3e).



Figure S23. ¹H NMR spectrum of 2,5-diamino-6-cyano-4-((*p*-tolylamino)pyrimidinium chloride (3f).



Figure S24. ¹³C NMR spectrum of 2,5-diamino-6-cyano-4-(*p*-tolylamino)pyrimidinium chloride (3f).



Figure S25. ¹H NMR spectrum of 2,5-diamino-6-cyano-4-((4-fluorophenyl)amino)pyrimidine (4a).



Figure S26. ¹³C NMR spectrum of 2,5-diamino-6-cyano-4-(4-fluorophenyl)amino)pyrimidine (4a).



Figure S27. ¹H NMR spectrum of 2,5-diamino-6-cyano-4-((4-chlorophenyl)amino)pyrimidine (4b).



Figure S28. ¹³C NMR spectrum of 2,5-diamino-6-cyano-4-((4-chlorophenyl)amino)pyrimidine (4b).



Figure S29. ¹H NMR spectrum of 2,5-diamino-6-cyano-4-((4-bromophenyl)amino)pyrimidine (4c).



Figure S30. ¹³C NMR spectrum of 2,5-diamino-6-cyano-4-((4-bromophenyl)amino)pyrimidine (4c).



Figure S31. ¹H NMR spectrum of 2,5-diamino-6-cyano-4-((3-bromophenyl)amino)pyrimidine (4d).



Figure S32. ¹³C NMR spectrum of 2,5-diamino-6-cyano-4-((3-bromophenyl)amino)pyrimidine (4d).



Figure S33. ¹H NMR spectrum of 2,5-diamino-6-cyano-4-(phenylamino)pyrimidine (4e).



Figure S34 ¹³C NMR spectrum of 2,5-diamino-6-cyano-4-(phenylamino)pyrimidine (4e).



Figure S35. ¹H NMR spectrum of 2,5-diamino-6-cyano-4-(*p*-tolylamino)pyrimidine (4f).



Figure S36. ¹³C NMR spectrum of 2,5-diamino-6-cyano-4-(*p*-tolylamino)pyrimidine (4f).

2. HMBC Spectra

2.1. 2-amino-9-(4-fluorophenyl)-9H-purine-6-carbonitrile (2a)



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2.2. 2,5-diamine-6-(4-fluorophenyl)-amino-pyrimidine-4-carbonitrile chloride (3a)

HMBC spectra compound 3a (expansion)

HMBC spectra compound 3a



2.3. 2,5-diamino-6-cyano-4-((4-fluorophenyl)amino)pyrimidine (4a)

3. Mass Spectra

Mass spectrometry was measured in Vigo, Spain (Centro de Apoio Científico-Tecnolóxico á Investigación), using a Bruker SolarisX XR through an Electrospray Ionization (ESI) analysis.



3.1. 2,5-diamino-6-cyano-4-((4-fluorophenyl)amino)pyrimidine (4a)

3.2. 2,5-diamino-6-cyano-4-((4-chlorophenyl)amino)pyrimidine (4b)

3.3. 2,5-diamino-6-cyano-4-((4-bromophenyl)amino)pyrimidine (4c)

3.4. 2,5-diamino-6-cyano-4-((3-bromophenyl)amino)pyrimidine (4d)

3.5. 2,5-diamino-6-cyano-4-(phenylamino)pyrimidine (4e)

3.6. 2,5-diamino-6-cyano-4-(p-tolylamino)pyrimidine (4f)

4. Photophysical properties

The photophysical properties of all compounds were studied in the solvents dioxane and ethanol, while some representative molecules (with PhF and (m)Br substituents) were chosen for the study in a wider variety of solvents, including aqueous buffers of neutral (pH=7.0) and slight acidic pH (pH=5.5).

The maximum absorption wavelengths of the synthesized compounds in several solvents are presented in **Table S1**. Molar absorption coefficients in ethanol are indicated, as examples.

Table S1. Maximum absorption (λ_{abs}) wavelengths for compounds 3a-3f and 4a-4f in several solvents. Molar absorption coefficients in ethanol are also indicated.

| Compound | | Dichloromethane (DCM) | loromethane Dioxane A (DCM) | | Acetonitrile Ethanol | | | buffer solution pH=5.5 |
|------------|-----------------------------------|--------------------------|--------------------------------|-----------------------|-----------------------|----------------------|-----------------------|---------------------------|
| | R | λ _{abs} (nm) | λ _{abs} (nm) | λ _{abs} (nm) | λ _{abs} (nm) | ε (M⁻¹ cm⁻¹) | λ _{abs} (nm) | λ _{abs} (nm) |
| 3a | 4-FC ₆ H ₄ | 352 | 363 | 365 | 370 | 1.22×10 ⁴ | 351 | 357 |
| 3b | 4-CIC ₆ H ₄ | | 358 | | 370 | 1.24×10 ⁴ | | |
| 3c | 4-BrC ₆ H ₄ | | 359 | | 372 | 1.31×10 ⁴ | | |
| 3d | 3-BrC ₆ H ₄ | 353 | 353 | 360 | 371 | 1.09×10 ⁴ | 361 | 362 |
| 3e | C_6H_5 | | 359 | | 370 | 1.55×10 ⁴ | | |
| 3f | 4-MeC ₆ H ₄ | | 356 | | 372 | 9.43×10 ³ | | |
| 4 a | $4-FC_6H_4$ | 354 | 357 | 367 | 370 | 1.08×10 ⁴ | 355 | 357 |
| 4b | 4-CIC ₆ H ₄ | | 361 | | 371 | 1.20×10 ⁴ | | |
| 4c | 4-BrC ₆ H ₄ | | 364 | | 367 | 2.14×10 ⁴ | | |
| 4d | 3-BrC ₆ H ₄ | 350 | 365 | 365 | 363 | 2.46×10 ⁴ | 363 | 365 |
| 4e | C ₆ H ₅ | | 364 | | 367 | 1.26×10 ⁴ | | |
| 4f | 4-MeC ₆ H ₄ | | 361 | | 363 | 1.34×10 ⁴ | | |

The maximum emission wavelengths of the synthesized compounds in several solvents and in aqueous buffers (pH=5.5 and 7.0) are presented in **Table S2**. Examples of fluorescence emission spectra are displayed in **Figure S37** to **Figure S39**.

| Co | ompound | Dichloromethane (DCM) | Dioxane Acetonitrile | | Ethanol | buffer solution pH=7.0 | buffer solution pH=5.5 |
|-----------|-----------------------------------|--------------------------|----------------------|----------------------|----------------------|---------------------------|---------------------------|
| | R | λ _{em} (nm) | λ _{em} (nm) | λ _{em} (nm) | λ _{em} (nm) | λ _{em} (nm) | λ _{em} (nm) |
| 3a | 4-FC ₆ H ₄ | 441 | 465 | 448 | 459 | 449 | 458 |
| 3b | 4-CIC ₆ H ₄ | | 466 | | 466 | | |
| 3c | 4-BrC ₆ H ₄ | | 465 | | 442 | | |
| 3d | 3-BrC ₆ H ₄ | 459 | 422 | 464 | 474 | 458 | 452 |
| 3e | C ₆ H ₅ | | 464 | | 457 | | |
| 3f | 4-MeC ₆ H ₄ | | 463 | | 443 | | |
| 4a | 4-FC ₆ H ₄ | 452 | 464 | 442 | 445 | 449 | 448 |
| 4b | 4-CIC ₆ H ₄ | | 466 | | 469 | | |
| 4c | 4-BrC ₆ H ₄ | | 466 | | 465 | | |
| 4d | 3-BrC ₆ H ₄ | 459 | 465 | 464 | 474 | 449 | 451 |
| 4e | C_6H_5 | | 463 | | 452 | | |
| 4f | 4-MeC ₆ H ₄ | | 460 | | 436 | | |

Table S2. Maximum emission (λ_{em}) wavelengths for compounds 3a-3f and 4a-4f in several solvents.

Figure S37. Normalized fluorescence spectra of 2.5×10⁻⁶ M solutions of compound **3a** in several solvents and aqueous buffer solutions.

Figure S38. Normalized fluorescence spectra of 2.5×10⁻⁶ M solutions of compound **4a** in several solvents and aqueous buffer solutions.

Figure S39. Normalized fluorescence spectra of 2.5×10⁻⁶ M solutions of compound **3d** in several solvents and aqueous buffer solutions.

Figure S40. Normalized fluorescence spectra of 2.5×10⁻⁶ M solutions of compound **4d** in several solvents and aqueous buffer solutions.

The Stokes Shifts of the synthesized compounds in several solvents of different polarities and, in some cases, in aqueous buffers (pH=5.5 and 7.0) are presented in **Table S3**.

| Compound | | Dichloromethane (DCM) | Dioxane Acetonit | Acetonitrile | Ethanol | Aqueous buffer pH=7.0 | Aqueous buffer pH=5.5 |
|-----------|-----------------------------------|--------------------------|------------------|--------------|---------|--------------------------|--------------------------|
| | R | S (nm) | S (nm) | S (nm) | S (nm) | S (nm) | S (nm) |
| 3a | 4-FC ₆ H ₄ | 89 | 102 | 83 | 89 | 98 | 101 |
| 3b | 4-CIC ₆ H ₄ | | 108 | | 96 | | |
| 3c | 4-BrC ₆ H ₄ | | 106 | | 70 | | |
| 3d | 3-BrC ₆ H ₄ | 106 | 69 | 104 | 103 | 97 | 90 |
| 3e | C_6H_5 | | 105 | | 87 | | |
| 3f | 4-MeC ₆ H ₄ | | 107 | | 71 | | |
| 4a | 4-FC ₆ H ₄ | 98 | 107 | 75 | 75 | 94 | 91 |
| 4b | 4-CIC ₆ H ₄ | | 105 | | 98 | | |
| 4c | 4-BrC ₆ H ₄ | | 102 | | 98 | | |
| 4d | 3-BrC ₆ H ₄ | 109 | 100 | 99 | 111 | 86 | 86 |
| 4e | C_6H_5 | | 99 | | 85 | | |
| 4f | 4-MeC ₆ H ₄ | | 99 | | 73 | | |

Table S3. Stokes shift (S) for compounds 3a-3f and 4a-4f in several solvents.

The fluorescence quantum yields of the synthesized compounds in several solvents of different polarities and, in some cases, in aqueous buffers (pH=5.5 and 7.0) are presented in **Table S4**.

| Compound | | Dichloromethane (DCM) | Dioxane | Acetonitrile | Ethanol | Aqueous buffer pH=7.0 | Aqueous buffer pH=5.5 |
|------------|-----------------------------------|--------------------------|-------------------------|---------------------|-----------------|--------------------------|--------------------------|
| | R | Φ _F (nm) | $\Phi_{	extsf{F}}$ (nm) | $\Phi_{\sf F}$ (nm) | Φ_{F} (nm) | Φ _F (nm) | Φ _F (nm) |
| 3a | 4-FC ₆ H ₄ | 0.13 | 0.37 | 0.10 | 0.03 | 0.04 | 0.03 |
| 3b | 4-CIC ₆ H ₄ | | 0.32 | | 0.04 | | |
| 3c | 4-BrC ₆ H ₄ | | 0.35 | | 0.01 | | |
| 3d | 3-BrC ₆ H ₄ | 0.67 | 0.34 | 0.54 | 0.10 | 0.01 | 0.03 |
| 3e | C_6H_5 | | 0.34 | | 0.10 | | |
| 3f | 4-MeC ₆ H ₄ | | 0.20 | | 0.10 | | |
| 4a | 4-FC ₆ H ₄ | 0.39 | 0.61 | 0.01 | 0.10 | 0.02 | 0.03 |
| 4 b | 4-CIC ₆ H ₄ | | 0.62 | | 0.04 | | |
| 4c | 4-BrC ₆ H ₄ | | 0.60 | | 0.04 | | |
| 4d | 3-BrC ₆ H ₄ | 0.67 | 0.57 | 0.53 | 0.10 | 0.01 | 0.01 |
| 4e | C_6H_5 | | 0.56 | | 0.10 | | |
| 4f | 4-MeC ₆ H ₄ | | 0.36 | | 0.20 | | |

Table S4. Fluorescence quantum yields (Φ_F) for compounds **3a-3f** and **4a-4f** in several solvents. Reference: Quinine sulfate in sulfuric acid dilute solution (0.05 M) [1], $\Phi_F = 0.546$ at 25 °C [2]. Error about 10%.

5. Interaction with DNA

The binding constant and binding site size were determined by the modified Scatchard equation, given by McGhee and von Hippel [3]:

$$\frac{r}{c_{\rm f}} = K_{\rm i} \left(1 - nr\right) \left[(1 - nr) / [1 - (n-1)r] \right]^{n-1}$$

where K_i is the intrinsic binding constant, n is the binding site size, r is the ratio $c_b/[DNA]$, c_b and c_f are the concentrations of bound and free compound, respectively, calculated by

$$\mathbf{c}_{b} = \frac{I_{F,0} - I_{F}}{I_{F,0} - I_{F,b}} \times \mathbf{c}_{total} \quad ; \quad \mathbf{c}_{total} = \mathbf{c}_{f} + \mathbf{c}_{b}$$

being $I_{F,0}$ the fluorescence intensity of the free compound and $I_{F,b}$ the fluorescence intensity of the bound compound at total binding.

The quenching data were first plotted according to the Stern-Volmer relation [5]:

$$\frac{I_0}{I} = 1 + K_{\rm SV} [Q]$$

where I_0 and I are, respectively, the fluorescence intensities in the absence and in the presence of quencher (I⁻), K_{SV} is the Stern-Volmer constant and [Q] is the quencher concentration. When some compound molecules are accessible to the quencher and other molecules are not accessible and the Stern-Volmer equation must be modified [6]:

$$\frac{I_0}{\Delta I} = \frac{1}{f_a} + \frac{1}{f_a K_{\rm SV}[Q]}$$

where $\Delta I = I_0 - I$ and f_a is the accessibility to the quencher.

6. Fluorescence microscopy

Figure S43. Dead *Saccharomyces cerevisiae* BY4741 cells stained with **3b**, **3c**, and **3e** at 250 μ M; 15 min incubation at RT in PBS pH 7.2, protected from light). The death of cells was achieved by heat treating the cells at 80 °C for 2 min in a water bath. Scale bar = 20 μ m.

7. Docking results

4d protonated form in 3ft6

4d neutral form in 3ft6

4d protonated form in 7kwk

Figure S44. Docking poses of compound 4d in DNA sequences 1hq7, 3ft6 and 7kwk

8. *Ab initio* calculation results

Figure S45: Equilibrium geometries of ground state and first excited state of compound 4d.

| | S ₀ equilibrium geometry | S ₁ equilibrium | | So | S1 |
|--------|--|----------------------------|-------------|-------------|-------------|
| Normal | | | Normal mode | equilibrium | equilibrium |
| mode | | geometry | | geometry | geometry |
| 1 | 21.61328 | 18.14505 | 40 | 961.79437 | 956.94405 |
| 2 | 38.09624 | 39.73726 | 41 | 976.19751 | 975.25884 |
| 3 | 60.08875 | 62.85013 | 42 | 990.83217 | 978.08551 |
| 4 | 64.37417 | 67.34663 | 43 | 1008.28113 | 995.35478 |
| 5 | 100.11089 | 90.09156 | 44 | 1014.36829 | 997.10437 |
| 6 | 119.24364 | 117.99973 | 45 | 1079.51911 | 1062.29310 |
| 7 | 140.35813 | 124.69518 | 46 | 1089.26652 | 1068.35176 |
| 8 | 145.43535 | 133.76455 | 47 | 1104.05001 | 1096.38080 |
| 9 | 195.08116 | 140.28367 | 48 | 1127.95870 | 1107.76724 |
| 10 | 205.65276 | 162.77921 | 49 | 1157.21311 | 1135.00830 |
| 11 | 223.84479 | 192.46998 | 50 | 1194.24809 | 1188.80115 |
| 12 | 235.26691 | 220.06952 | 51 | 1223.73312 | 1213.43949 |
| 13 | 294.54622 | 233.14663 | 52 | 1261.16515 | 1269.43778 |
| 14 | 296.25468 | 272.93875 | 53 | 1307.81690 | 1305.82245 |
| 15 | 319.84436 | 290.25116 | 54 | 1314.52488 | 1320.69079 |
| 16 | 334.17333 | 304.51773 | 55 | 1327.62847 | 1389.57942 |
| 17 | 347.59422 | 319.88814 | 56 | 1389.64644 | 1404.59362 |
| 18 | 363.14991 | 343.10500 | 57 | 1460.83871 | 1437.55361 |
| 19 | 380.17355 | 353.45919 | 58 | 1469.83154 | 1460.20533 |
| 20 | 399.54138 | 393.17455 | 59 | 1481.38522 | 1471.25407 |
| 21 | 456.47311 | 403.29820 | 60 | 1517.49703 | 1476.49551 |
| 22 | 464.28750 | 448.76108 | 61 | 1546.40140 | 1500.36408 |
| 23 | 494.23799 | 476.46984 | 62 | 1597.92488 | 1519.58439 |
| 24 | 506.67463 | 487.76570 | 63 | 1611.11649 | 1563.90971 |
| 25 | 541.88658 | 495.26412 | 64 | 1632.40952 | 1592.71948 |
| 26 | 559.06123 | 539.06776 | 65 | 1649.43277 | 1634.31191 |
| 27 | 588.43240 | 541.93998 | 66 | 1670.67730 | 1648.41369 |
| 28 | 646.43956 | 582.56638 | 67 | 1692.64781 | 1676.84841 |
| 29 | 654.58867 | 639.73706 | 68 | 1791.72398 | 1781.08738 |
| 30 | 698.26872 | 640.04487 | 69 | 2328.36735 | 2228.48298 |
| 31 | 730.03569 | 692.39000 | 70 | 2802.68543 | 2609.23835 |
| 32 | 733.53357 | 718.06894 | 71 | 3056.76668 | 2981.10700 |
| 33 | 742.59646 | 725.46625 | 72 | 3219.08982 | 3218.00844 |
| 34 | 771.04074 | 728.67305 | 73 | 3232.58433 | 3231.65980 |
| 35 | 775.39670 | 757.08230 | 74 | 3256.84808 | 3258.42195 |
| 36 | 792.95097 | 824.92870 | 75 | 3304.03777 | 3294.51124 |
| 37 | 839.46001 | 833.17156 | 76 | 3623.24404 | 3653.78288 |
| 38 | 842.75952 | 881.74369 | 77 | 3683.83933 | 3655.56966 |
| 39 | 890.62430 | 890.94490 | 78 | 3743.53820 | 3799.64917 |

Table S5. Frequencies of normal vibrational modes (cm⁻¹)

9. References

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