Supplementary data

Water solubility enhancement of pyrazolo[3,4-*d*]pyrimidine derivatives via miniaturised polymer-drug microarray.

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General information

Polyvinylpyrrolidone-vinyl acetate copolymer (PVPVA), Polyvinylpyrrolidone (PVP), (hydroxypropyl)-methyl cellulose (HPMC), polyethylene glycol 8000 and 20000 (PEG), Tween 80, Pluronic F-68 and dimethyl sulfoxide (DMSO) were purchased from SIGMA Aldrich and the latter used as a common solvent to dissolve all the printable materials.

Starting materials for the chemical synthesis of pyrazolo[3,4-*d*]pyrimidine derivatives were purchased from Aldrich-Italia (Milan, Italy). Melting points were determined with a Büchi 530 apparatus and are uncorrected. IR spectra were measured in KBr with a PerkinElmer 398 spectrophotometer. ^{1}H NMR spectra were recorded in $CDCl_3$ by using a Varian Gemini 200 (200 MHz) instrument. Chemical shifts are reported as δ (ppm) relative to TMS as the internal standard, *J* in Hz. ^{1}H patterns are described using the following abbreviations: s = singlet, d = doublet, q = quartet, m = multiplet, and br s = broad singlet. Chromatographic purifications were performed by columns packed with Merk 60 silica gel, 23-400 mesh, for flash technique. Mass spectra (MS) data were obtained using an Agilent 1100 LC/MSD VL system (G1946C) with a 0.4 mL/min flow rate using a binary solvent system of 95:5 methanol/water. MS were acquired in positive and negative modes, scanning over the mass range 50-1500. The following ion source parameters were used: drying gas flow, 9 mL/min; nebulizer pressure, 40 psig; drying gas temperature, 350 °C. All target compounds possessed a purity of $\geq$ 95% as verified by elemental analyses by comparison with the theoretical values. Analyses for C, H, N, and S were within ±0.3% of the theoretical value.

Printing

Prior to dispensing the drug solution into a 96-well plate, the target had to be programmatically defined. The probe substrate consists of the well plate, drug solutions were dispensed via a piezoelectric inkjet printer (Sciflexarray S5, Scienion) using a 90 µm orifice nozzle. The droplet size was controlled by the values of the voltage and electrical pulse. A fixed amount of drug (6 µg) was dispensed for each well, by adjusting the number of drops. The number of drops per spot were selected in such way that the volume aspired delivered by the nozzle (max 10 µL) at the beginning of a run was sufficient to print the whole print pattern. In a routine experiment DMSO solution (10 mg/mL) droplets with nominal volumes ranging from 250-280 pL, were dispensed at a 300 Hz jetting frequency by adjusting the voltage and pulse between 98-105 Volt (Voltage) and 45-55 µs (Pulse) respectively. The nozzle was washed with DMF, in between each printing cycle, as part of the automated printing-washing loop. DMSO was chosen due to both its high evaporation point that avoids nozzle blockage and its ability to dissolve all the selected drugs.

The initial drugs and polymers solutions were prepared by dissolving the right amount of drugs in DMSO and, separately, the polymers in deionized (DI) water, in order to reach a final concentration of 10 mg/mL and 1 mg/mL, respectively.

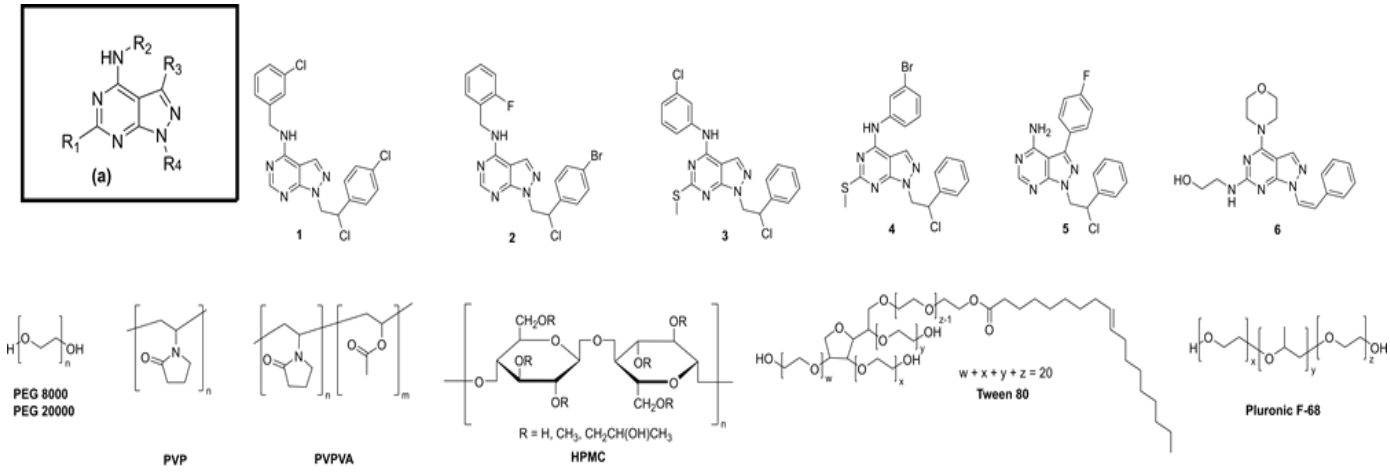


Figure 1SI.Structures of drugs 1-6 and selected hydrophilic polymers.

UV screening

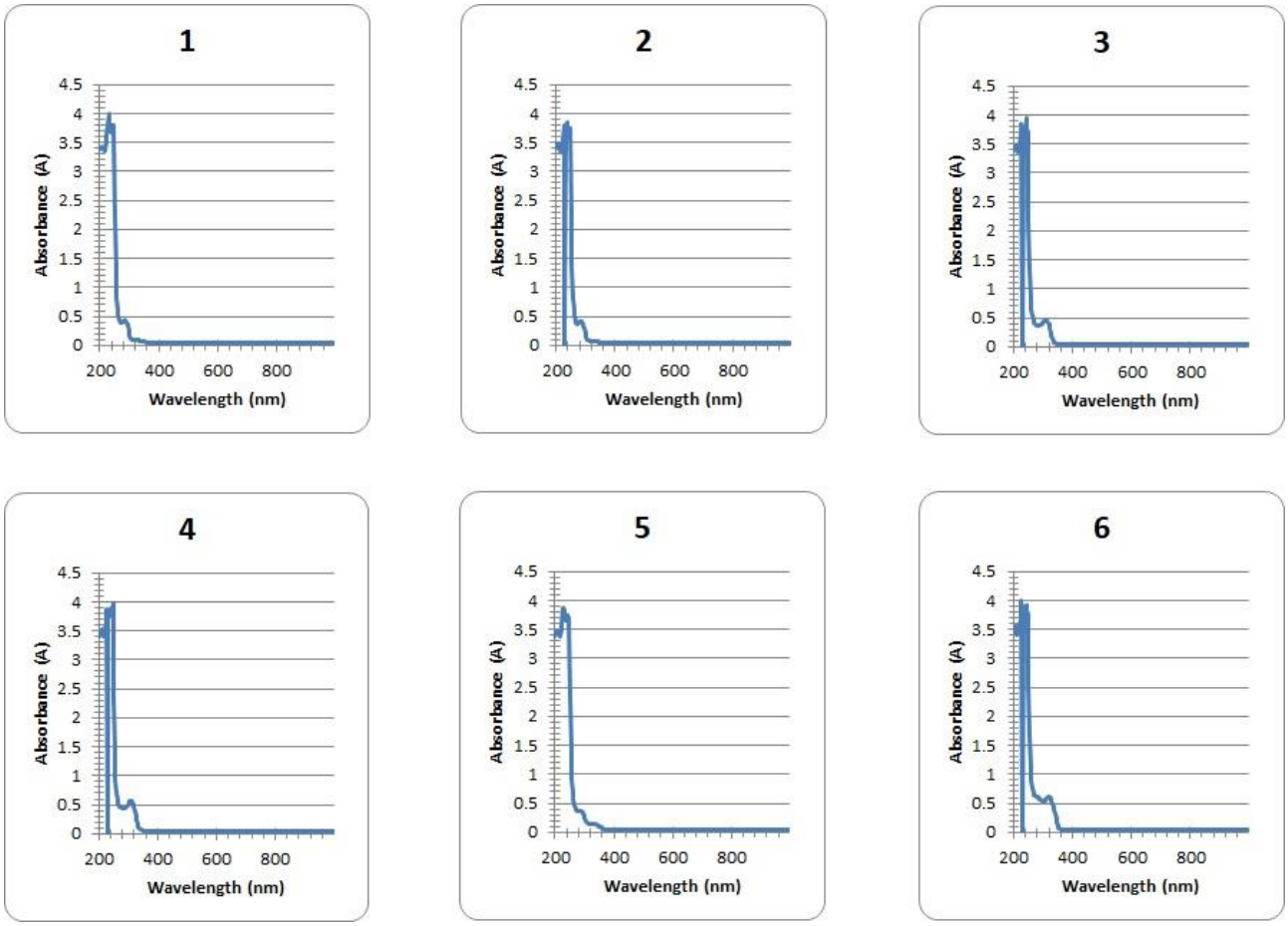


Figure 2SI. UV-vis spectra of drugs **1-6** in DMSO (30 µg/ml).

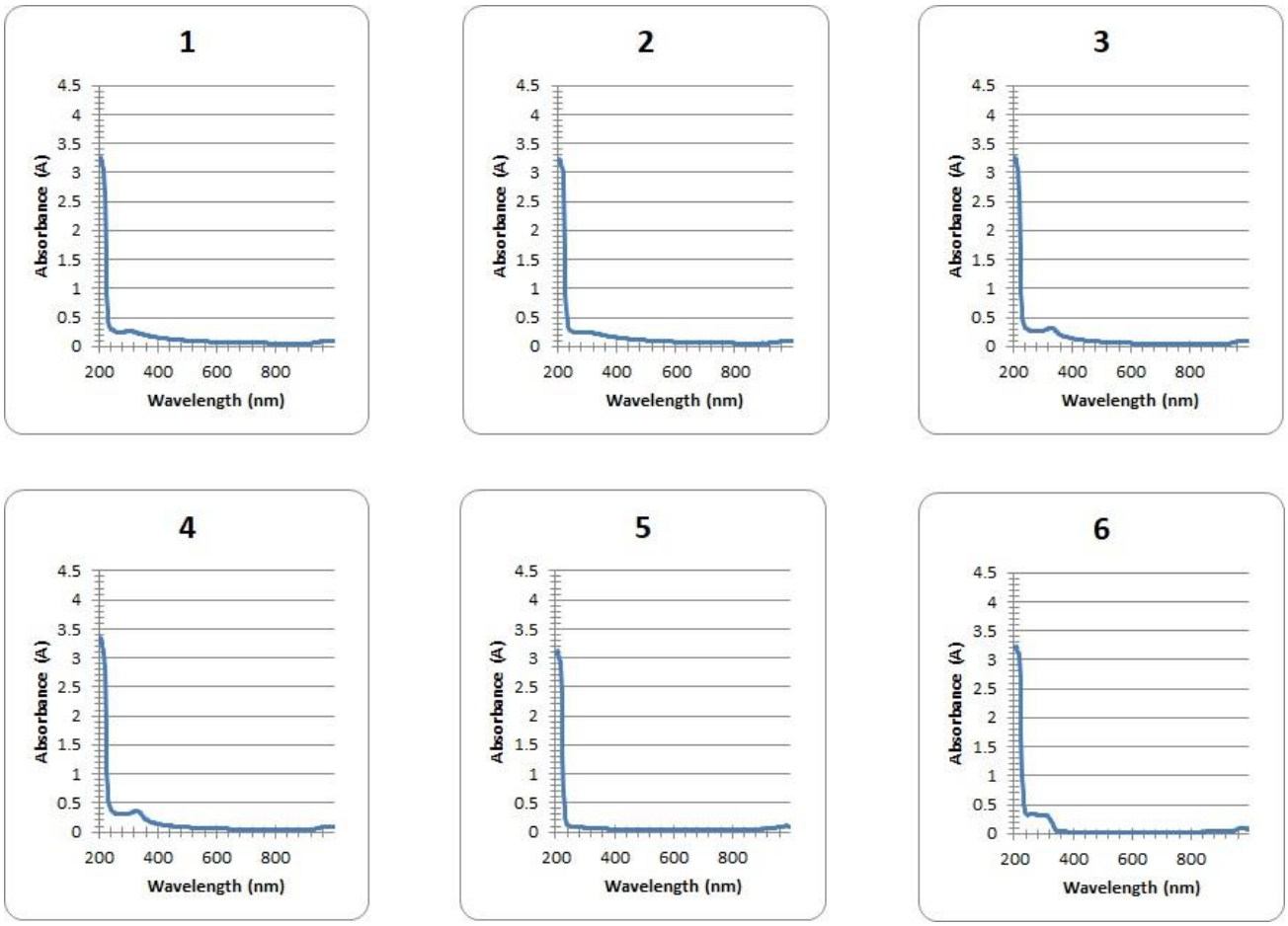


Figure 3SI. UV-vis spectra of drugs **1-6** in water (30 µg/ml).

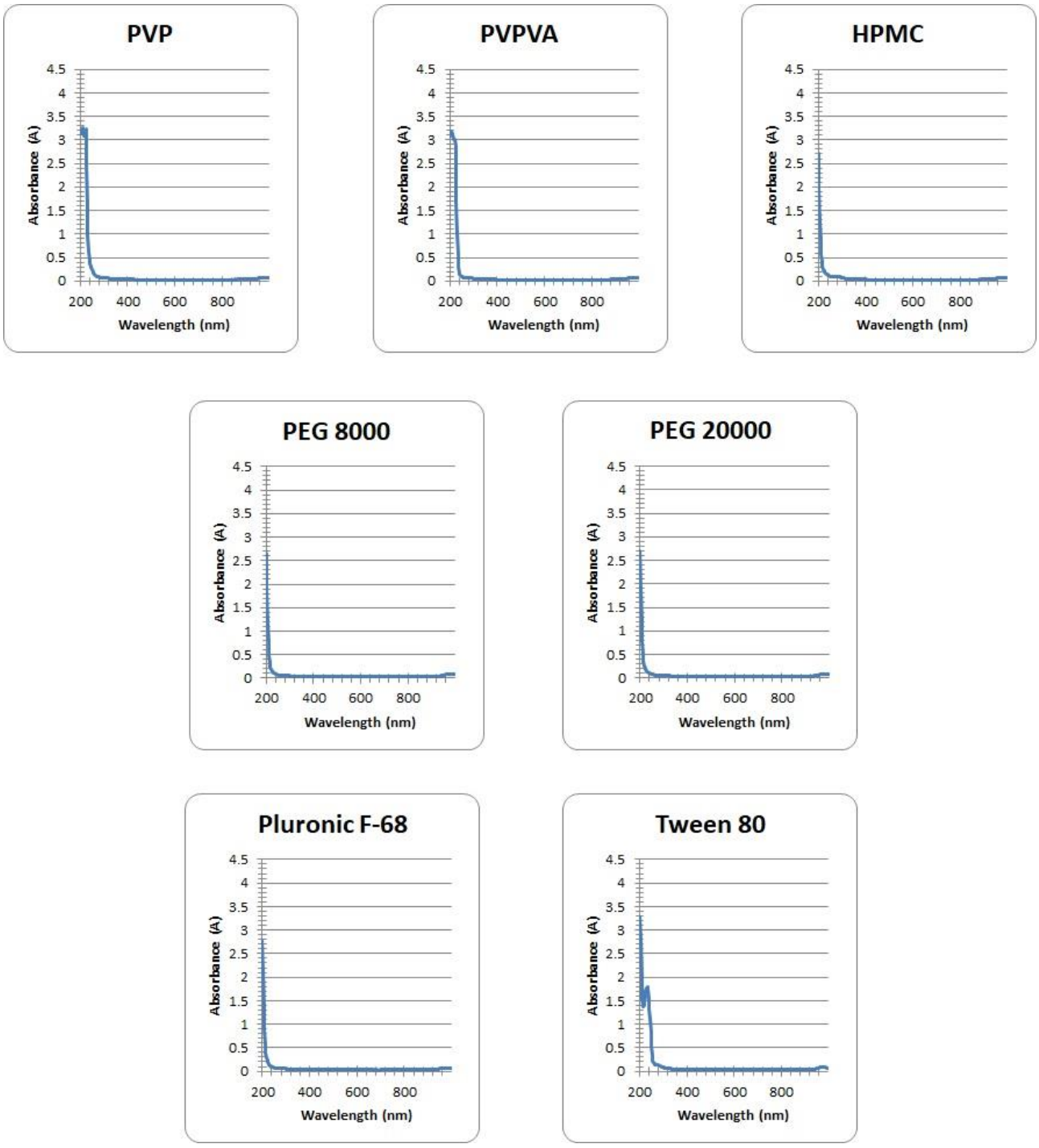


Figure 4SI. UV-vis spectra of polymers in water (200 µg/ml).

Polymers	Drugs						
		1	**2**	**3**	**4**	**5**	**6**
	PVP	120.395	72.489	185.028	346.216	126.803	20.949
	PVPVA	217.239	201.479	420.386	540.752	160.331	148.142
	HPMC	126.535	108.380	207.062	305.714	200.390	88.802
	PEG 8000	33.195	-5.453	244.021	499.348	101.170	18.594
	PEG 20000	25.598	-7.794	314.783	364.962	32.797	3.558
	Pluronic F-68	126.466	125.139	337.477	472.381	216.179	85.802
	Tween 80	294.381	242.083	510.122	577.093	260.429	181.284

Table 1SI. ΔA% of drugs **1-6** and polymers.

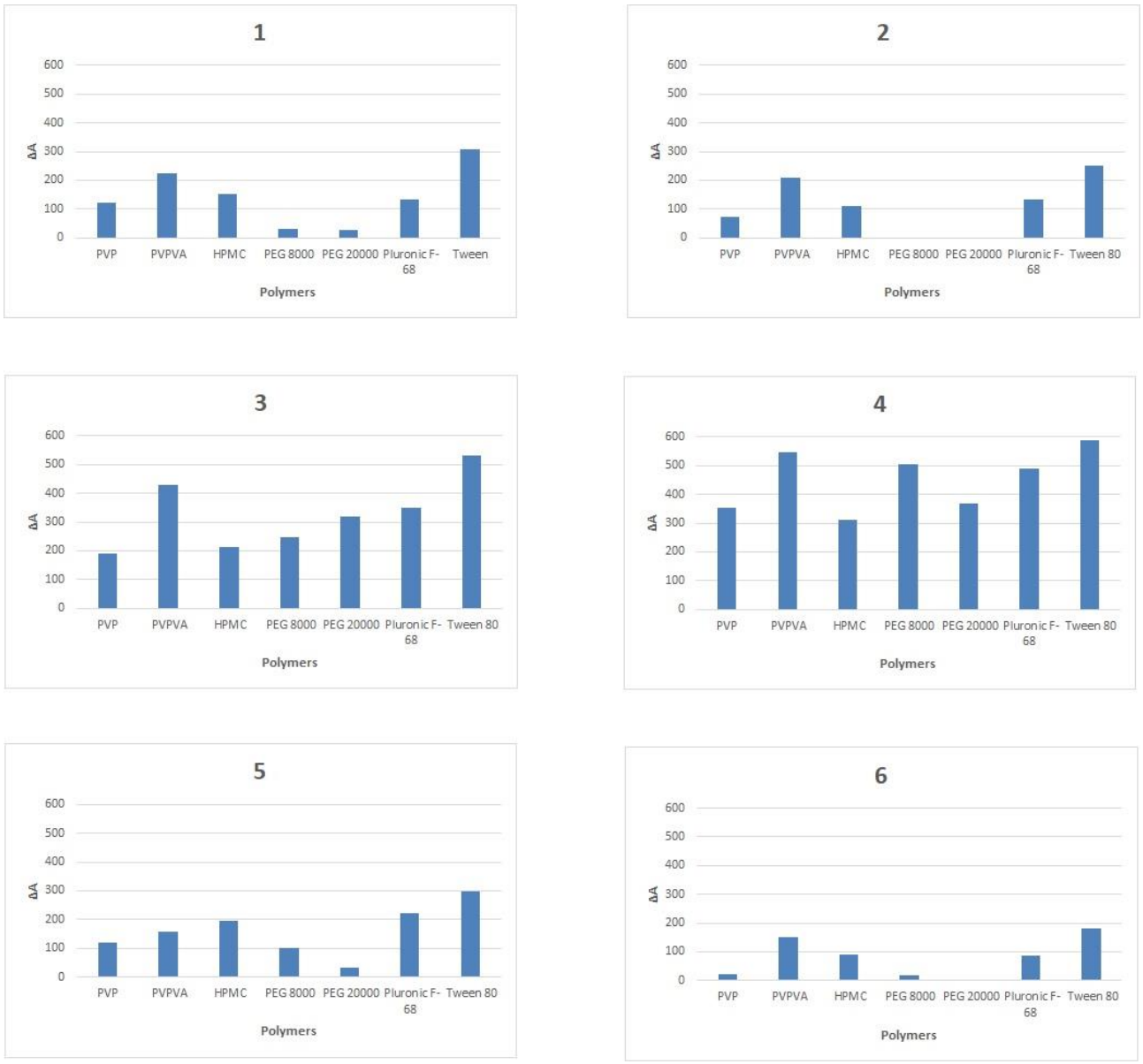
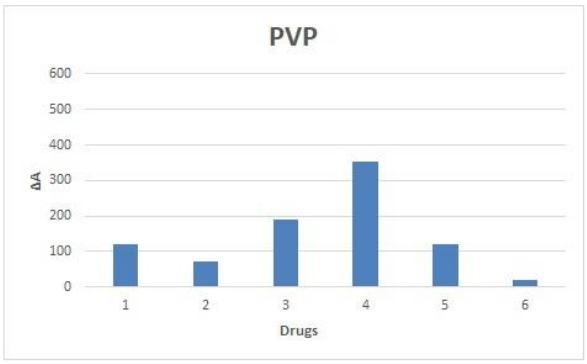
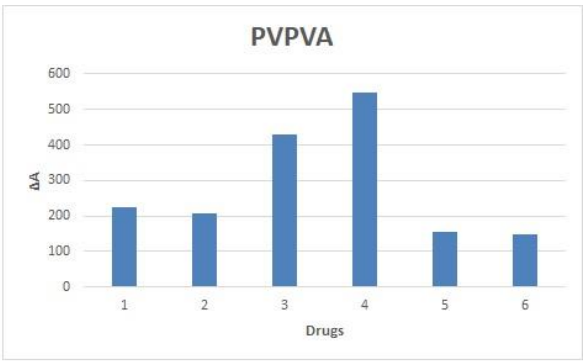
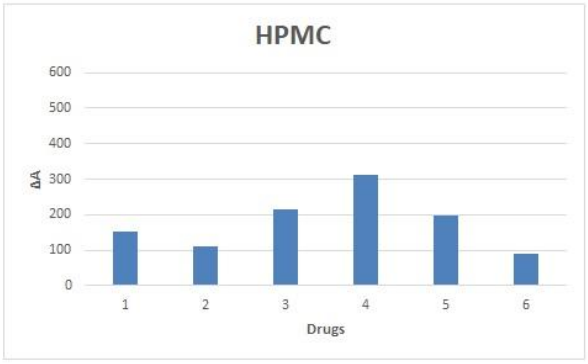
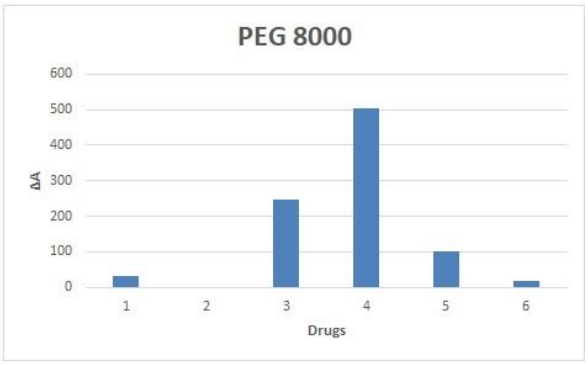


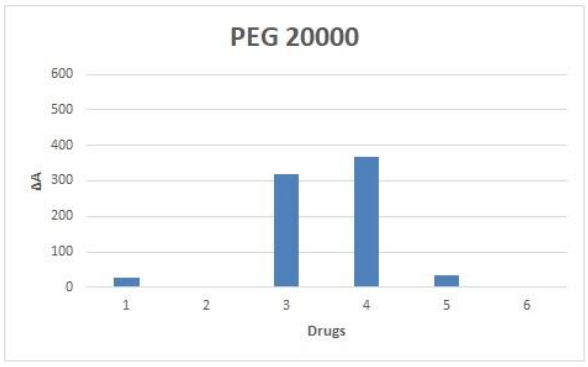
Figure 5SI. ΔA% values of each drug vs the panel of polymers.

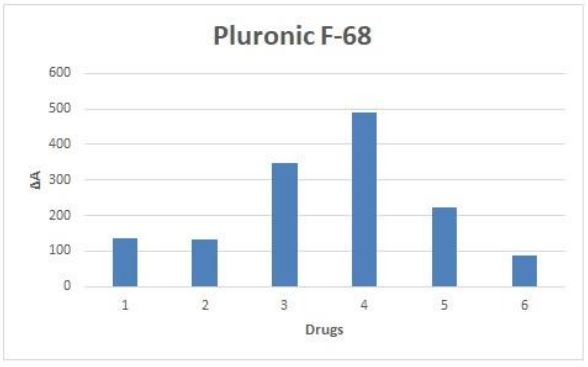












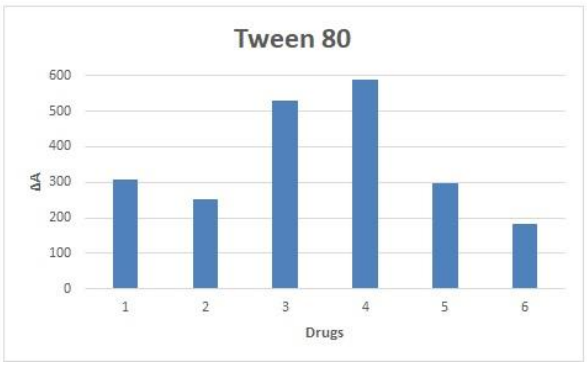


Figure 6SI. ΔA% values of polymers against the panel of drugs.

Dynamic Light Scattering (DLS)

Particle size analyses were performed by DLS employing a Zetasizer Nano spectrometer (Malvern Instruments Ltd) equipped with a 633 nm laser at fixed angle of 173 °. All experiments were carried out in triplicate on the same sample. Concentrations of each sample were in the range of 0.5 mg/ml in drug (where the drug/polymer ratio was kept constant at 10/90 % w/w), for instrument detection limits.

Cellular assays

A549 human lung adenocarcinoma cells were obtained from the American Type Culture Collection, cultured at 37 °C in a humidified atmosphere containing 5% CO_2 and grown continuously in DMEM supplemented with 10% FBS.

Cells (1 x 10^4) were placed in 96-well plates and cultured in 200 µL of cell medium with FBS at 10%. After 24 h, cells were treated with the different NPs suspensions dispersed into the cell media, thus reaching a final concentration of 50 µg/mL. Cells treated with 0.1% (v/v) Triton-X 100 and fresh media were used as a positive and a negative control, respectively. After 24 h of incubation, cells were treated with methylthiazolyldiphenyl-tetrazolium bromide (MTT), reagent (Sigma) (25 µL of 5mg/mL in PBS per well). After further incubation (3 h), the cells were washed with PBS, then 150 µL of DMSO was added and the absorbance was read at 570 nm in a microplate reader (Tecan Spark Platereader, UK) and the percentage of metabolic activity (%) calculated.

$$\text{Metabolic activity (\%)} = S-T / C-T * 100$$

Where, S is the absorbance obtained with the tested samples, T is the absorbance observed with Triton, and C is the absorbance observed with control.

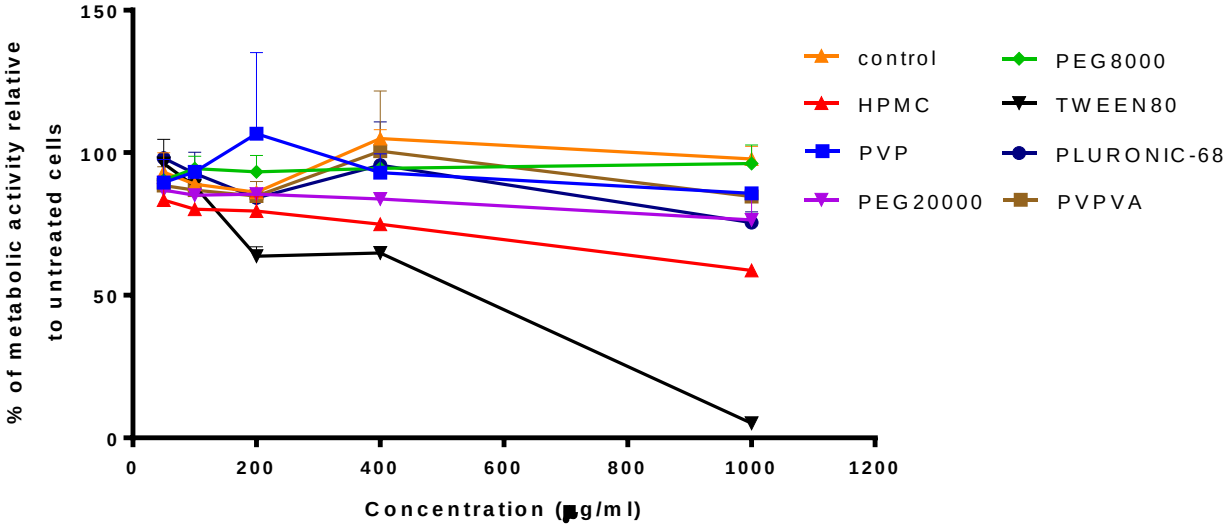


Figure 7SI. Polymer cytotoxicity screening at different concentrations ranging from 10 to 1000 µg/mL.

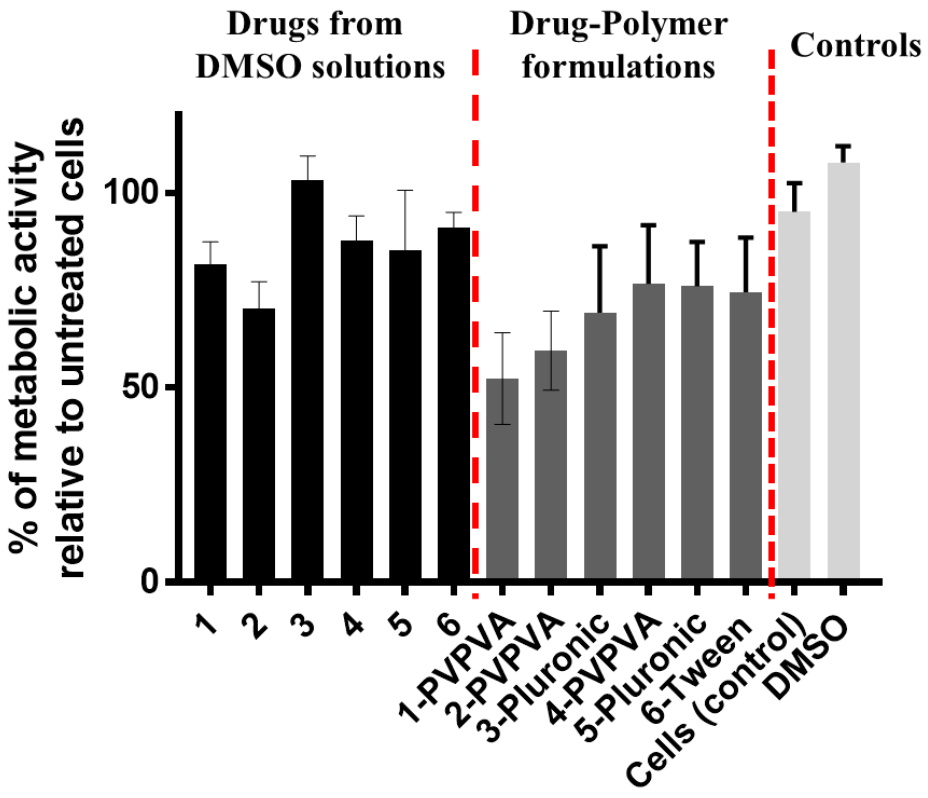


Figure 8SI. Cytotoxicity of each DMSO (final concentration was around 0.2 % vol/vol) drug solutions (30 µg/mL) against the best selected drug-polymer formulations. DMSO and cells alone were used as final double controls.

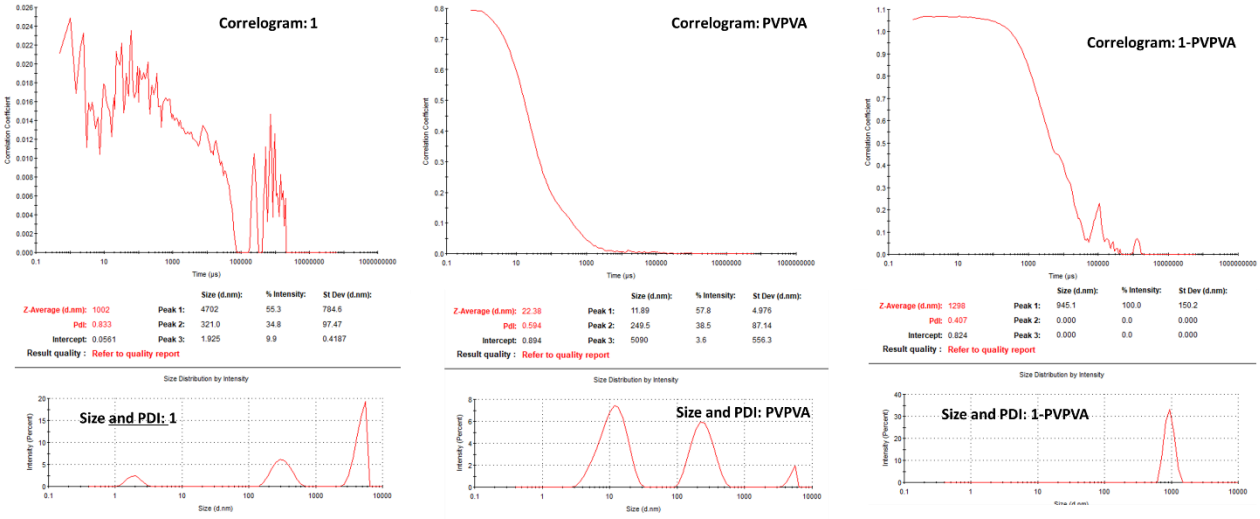
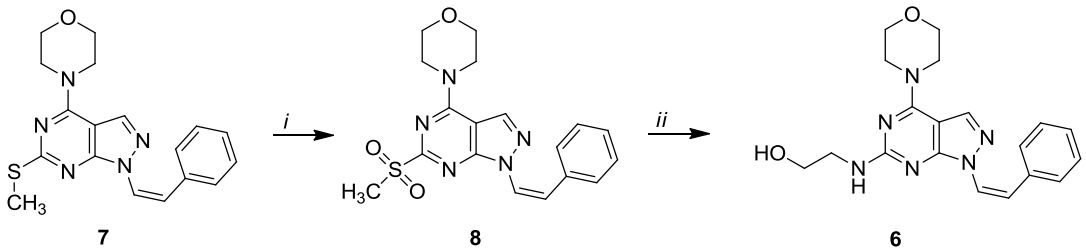


Figure 9SI. DLS correlograms, sizes and PDIs of free **1**, PVPVA and **1**-PVPVA formulation.

Chemical synthesis



Reagents and conditions: (*i*) *m*CPBA, $CHCl_3$, 0 °C, then rt, 6 h; (*ii*) ethanolamine, 1-butanol, DMSO, 90 °C, 12 h.

6-(Methylthio)-4-morpholin-4-yl-1-(2-phenylvinyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (7).

Previously synthesized according to procedure described in *J. Med. Chem.* **2006**, *49*, 1549–1561.

6-(Methylsulfonyl)-4-morpholin-4-yl-1-(2-phenylvinyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (8).

3-Chloroperoxybenzoic acid (2 mmol of 77% suspension in mineral oil) was added portionwise to a solution of **7** (1 mmol) in $CHCl_3$ (10 mL) at 0 °C. The mixture was stirred at room temperature for 6 h; the organic phase was washed with saturated $NaHCO_3$ solution (2 x 20 mL), then with water (20 mL), dried ($MgSO_4$),

and evaporated under reduced pressure. The crude was crystallized by adding petroleum ether (b.p. 40-60 °C).

Yield 87%, mp 215-216 °C (dec). ^{1}H-NMR: δ 3.41 (s, 3H, CH_3), 3.86-3.97 and 4.02-4.16 (2m, 8H, $4CH_2$ morph), 7.27-7.59 (m, 6H, 5Ar + CH=), 8.04 (d, J_{trans} = 14.4, 1H, CH=), 8.16 (s, 1H, H-3). IR (cm^{-1}): 1658 (C=C), 1315 and 1128 (SO_2). Anal. calcd for $C_{18}H_{19}N_5O_3S$, C 56.09, H 4.97, N 18.17, S 8.32, found C 56.18, H 5.07, N 17.86, S 8.64. MS: *m/z* calcd 385, found 385 $[M+1]^+$.

2-{[4-Morpholin-4-yl-1-(2-phenylvinyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-yl]amino}ethanol (6).

Ethanolamine (3 mmol) was added to a suspension of **15** (1 mmol) in 1-butanol (16 mL) and DMSO (4 mL), and the mixture was heated at 90 °C for 12 h. After cooling, 1-butanol was removed under reduced pressure; then water was added, and the solution was extracted with ethyl acetate (2 x 20 mL); the organic phase was washed with water (20 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The white solid was filtered and recrystallized from absolute ethanol.

Yield 68%, mp 177-178 °C. ^{1}H NMR: δ 3.70 (q, *J* = 4.0, 2H, $\underline{CH_2}$NH), 3.81-3.96 (m, 10H, $4CH_2$ morph. + $\underline{CH_2}$OH), 4.06 (br s, 1H, disappears with D_2O), 5.52 (br s, 1H, disappears with D_2O), 7.19-7.52 (m, 6H, 5H Ar + CH=), 7.87 (s, 1H, H-3), 7.88 (d, J_{trans} = 14.4, 1H, CH=). IR (cm^{-1}): 3250-3150 (OH + NH), 1656 (C=C). MS: *m/z* 366 $[M+1]^+$. Anal. calcd for $C_{19}H_{22}N_6O_2$, C 62.28, H 6.05, N 22.94, found C 62.23, H 6.19, N 23.25. MS: *m/z* calcd 366, found 366 $[M+1]^+$.