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Research Article

Radiotracer-based method for determining water solubility of highly insoluble compounds

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Summary

Ascertaining the aqueous solubility of compounds is important in the selection of drug candidates. We describe a radiotracer method for estimating water solubility of compounds that are soluble in dimethyl sulfoxide (DMSO). Various volumes of DMSO, saturated with ^{127}I-labeled compound and spiked with the corresponding ^{125}I-labeled derivative, are mixed in de-ionized water and the tubes centrifuged to remove insoluble material. Since (i) the iodine-127 and iodine-125 compounds have the same solubilities and are equally distributed in the DMSO–water solution, and (ii) the nonradioactive compound is accurately weighed, dissolved in a known volume of DMSO, and then further diluted as required, the concentration of compound in solution can be calculated and plotted as a function of the DMSO-to-water ratio. Water solubility of the compound is then determined by extrapolation of the linear fit of data points to zero DMSO. As proof of the methodology, 5-iodo-2′-deoxyuridine (IUdR) and 2-iodo-8-methyl-8*H*-quino[4,3,2-*kl*]acridine (IMAc), water-soluble compounds, were assessed using ^{125}IUdR and ^{125}IMAc, respectively. The solubility values obtained by the radiotracer method were similar to those acquired by spectrophotometry. Values calculated for several water-insoluble compounds indicate that the radiotracer method can accurately quantify the solubility of low-molecular-weight compounds (1000–2000 Da) in the pg–ng/ml range. Copyright © 2006 John Wiley & Sons, Ltd.

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Key Words: dimethyl sulfoxide; water solubility; radiotracer

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Introduction

Water solubility is a key property of administered drugs.[1–3] Both insoluble and soluble compounds can be candidates for drug development depending on the treatment goals. Traditionally, 'equilibrium' solubility is determined by shaking the compound in the selected solvent until no more dissolves over a 24-h period. The solution is then filtered and the concentration of dissolved compound assessed by a suitable analytic method. Such an approach, however, is not appropriate for the submicromolar level at which most radiopharmaceutical research is performed. As part of our program to synthesize and characterize radiolabeled drugs for tumor imaging and therapy, we have developed a highly sensitive method that utilizes measurement of radioactivity to determine the solubility of molecules not readily dissolved in water. The procedure can be used for any compound that can be radiolabeled with an isotope of one of its constituent atoms (in this case, iodine). The approach is simple, rapid, and reliable and can ascertain the aqueous solubility ($= 10^{-14}$ mol/ml) of highly water-insoluble, low-molecular-weight compounds. The method was initially validated by comparing radioactive tracer and traditional 'equilibrium' solubility values for two water-soluble radiopharmaceuticals, 5-iodo-2′-deoxyuridine (IUdR) and 2-iodo-8-methyl-8*H*-quino[4,3,2-*kl*]acridine (IMAc) (Figure 1). The physical properties of the ^{127}I-containing and the ^{125}I-labeled compounds are identical. The solubility

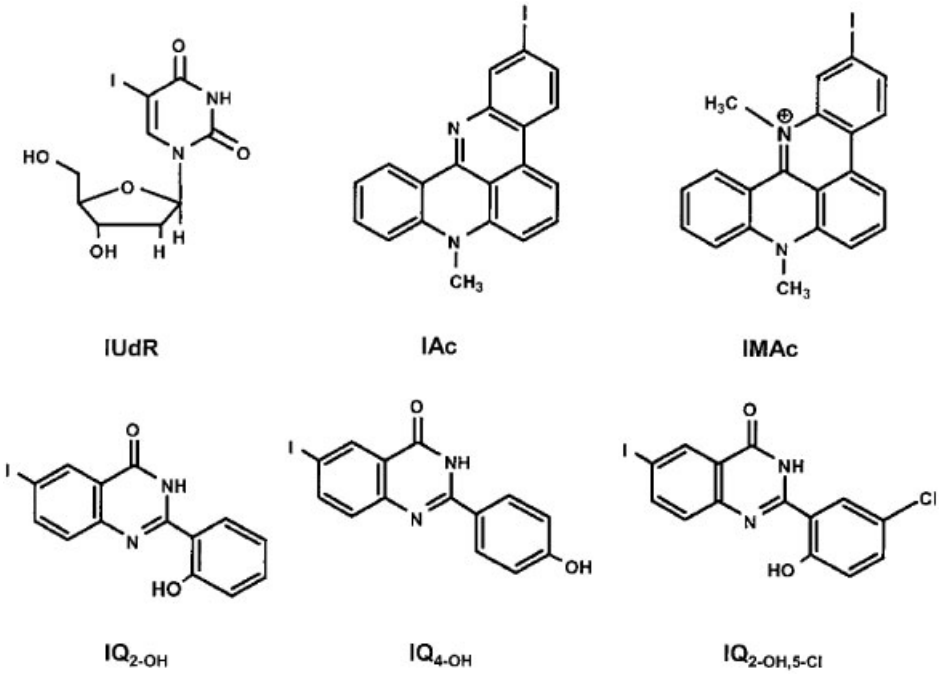

Figure 1. Structures of compounds whose aqueous solubility was assessed

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values for four highly water-insoluble iodinated compounds (Figure 1) were then obtained by the radiotracer method. All compounds in our studies are stable under the experimental conditions used.

Results and discussion

Proof of principle

A comparison of the solubility data from the radiotracer (^{125}IUdR) and standard spectrophotometry methods for 5-iodo-2′-deoxyuridine (IUdR) can be found in Table 1 (all values determined in triplicate). Iododeoxyuridine is a thymidine analog that has been used for over 40 years as an indicator of cell proliferation, a radiosensitizer, and a tumor imaging agent (when radioiodinated). This compound is highly water-soluble and its radioiodination (^{125}IUdR) is facile.[4] When the amount of DMSO in the total aqueous solution is increased, the amount of IUdR needed to saturate the solution increases proportionally in a linear manner (Table 2, Figure 2). Consequently, the solubility of IUdR is a function of the DMSO-to-water ratio and its aqueous solubility can be obtained at the point where the DMSO concentration is zero. For this curve (Figure 2), $y = 2.34 + 0.119x$, and the IUdR solubility in water, assessed by this method, is 2.34 ± 0.06 mg/ml. To obtain the solubility by UV spectrophotometery, a solution saturated with IUdR was prepared (see Methods), diluted 80-fold and then serially so that its concentrations would fall within the range used in preparing the standard curve (Figure 3), and its UV absorption was measured at 254 nm. The value obtained (0.57 ± 0.01) corresponds to 28.30 ± 0.45 μg/ml when the linear fit equation for Figure 3 ($y = 0.02011x$) is used. Consequently, the solubility of IUdR assessed by this UV method is 2.26 ± 0.72 mg/ml (28.30 μg/ml × 80), a value comparable with that acquired by the new radiotracer method (2.34 ± 0.06 mg/ml) (Figure 2). The IUdR solubility data obtained by both the radiotracer and the UV methods are similar (<4% difference), thus validating the accuracy of the radiotracer determination.

Our laboratory has had a long-standing interest in the development of radiopharmaceuticals labeled with Auger electron emitters (e.g. ^{123}I, ^{125}I) as potential radiotherapeutic agents. Since telomerase has emerged as a promising molecular target in the search for more selective antitumor agents

Table 1. Validation of radiotracer method

Compound	Absorbance method	Radiotracer method
IUdR	2.26 ± 0.72 mg/ml	2.34 ± 0.06 mg/ml
IMAc	42.0 ± 0.53 μg/ml	47.3 ± 3.3 μg/ml

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Table 2. Experimental conditions and solubility values

Compound	V_1 (μl)[a]	V_2 (ml)[b]	V_1/V_2 (× E-3)	μCi added	Amount (mg) of compound added	% of compound in solution	Concentration of compound per ml
IUdR	45	5	9	1.8	27.5	62.0 ± 0.5	3.41 ± 0.03 mg
	55	5	11	2.2	33.6	54.8 ± 0.7	3.68 ± 0.05 mg
	65	5	13	2.6	39.7	49.1 ± 0.5	3.89 ± 0.04 mg
	75	5	15	3.0	45.8	45.3 ± 0.9	4.18 ± 0.08 mg
	85	5	17	3.4	51.9	41.6 ± 0.9	4.31 ± 0.09 mg
	95	5	19	3.8	58.00	39.7 ± 0.3	4.61 ± 0.04 mg
IMAc	10	1	10	0.1	0.13	51.7 ± 2.1	68.0 ± 2.8 μg
	20	1	20	0.2	0.26	32.6 ± 2.9	85.8 ± 7.5 μg
	30	1	30	0.3	0.40	28.2 ± 1.7	111.4 ± 6.6 μg
	40	1	40	0.4	0.53	24.8 ± 0.8	130.3 ± 4.3 μg
	50	1	50	0.5	0.66	23.2 ± 0.9	152.5 ± 5.2 μg
	60	1	60	0.6	0.79	21.6 ± 0.5	170.6 ± 4.2 μg
$IQ_{4\text{-}OH}$	0.5	50	0.01	0.5	0.17E-1	42.3 ± 1.3	0.15 ± 0.043 μg
	1	50	0.02	1.0	0.36E-1	36.1 ± 1.3	0.26 ± 0.027 μg
	2	50	0.04	2.0	0.71E-1	34.4 ± 3.2	0.47 ± 0.045 μg
	3	50	0.06	3.0	0.11	29.3 ± 1.7	0.62 ± 0.037 μg
	4	50	0.08	4.0	0.14	27.8 ± 1.5	0.79 ± 0.041 μg
	5	50	0.10	5.0	0.18	25.8 ± 1.6	0.94 ± 0.058 μg
$IQ_{2\text{-}OH}$	10	1	10	0.04	2.0E-5	84.6 ± 0.5	16.9 ± 0.1 ng
	15	1	15	0.06	3.0E-5	83.9 ± 2.3	24.7 ± 0.7 ng
	20	1	20	0.08	4.0E-5	82.5 ± 0.9	30.0 ± 0.4 ng
	25	1	25	0.10	5.0E-5	78.8 ± 0.7	39.4 ± 0.4 ng
	30	1	30	0.12	6.0E-5	75.5 ± 1.1	45.3 ± 0.7 ng
	35	1	35	0.14	7.0E-5	76.2 ± 3.8	53.3 ± 2.7 ng
$IQ_{2\text{-}OH,\,5\text{-}Cl}$	10	1	10	0.04	2.0E-5	68.2 ± 1.5	14.4 ± 0.3 ng
	15	1	15	0.06	3.0E-5	72.6 ± 1.0	21.8 ± 0.3 ng
	20	1	20	0.08	4.0E-5	68.7 ± 2.5	27.5 ± 1.0 ng
	25	1	25	0.10	5.0E-5	68.4 ± 1.2	34.2 ± 0.6 ng
	30	1	30	0.12	6.0E-5	69.0 ± 0.2	41.4 ± 0.1 ng
	35	1	35	0.14	7.0E-5	69.8 ± 1.2	48.9 ± 0.8 ng
IAc	5	1	5	0.02	4.0E-8	57.3 ± 4.3	22.9 ± 1.7 pg
	10	1	10	0.04	8.0E-8	52.3 ± 1.6	41.8 ± 1.3 pg
	15	1	15	0.06	12.0E-8	47.9 ± 2.4	57.5 ± 2.9 pg
	20	1	20	0.08	16.0E-8	49.5 ± 1.1	79.2 ± 1.8 pg
	25	1	25	0.10	20.0E-8	48.5 ± 0.8	97.1 ± 1.6 pg
	30	1	30	0.12	24.0E-8	47.2 ± 1.1	113.2 ± 2.6 pg

[a]DMSO; [b]Water.

and polycyclic iodoacridines are potent telomerase inhibitors,[5] in collaboration with C. A. Laughton (University of Nottingham, UK), we have initiated studies to examine DNA fragmentation following the decay of ^{125}I-labeled acridines such as 2-iodo-8-methyl-8*H*-quino[4,3,2-*kl*]acridine (IMAc). Towards this end, we have radiolabeled IMAc by replacing the iodine atom with ^{125}I

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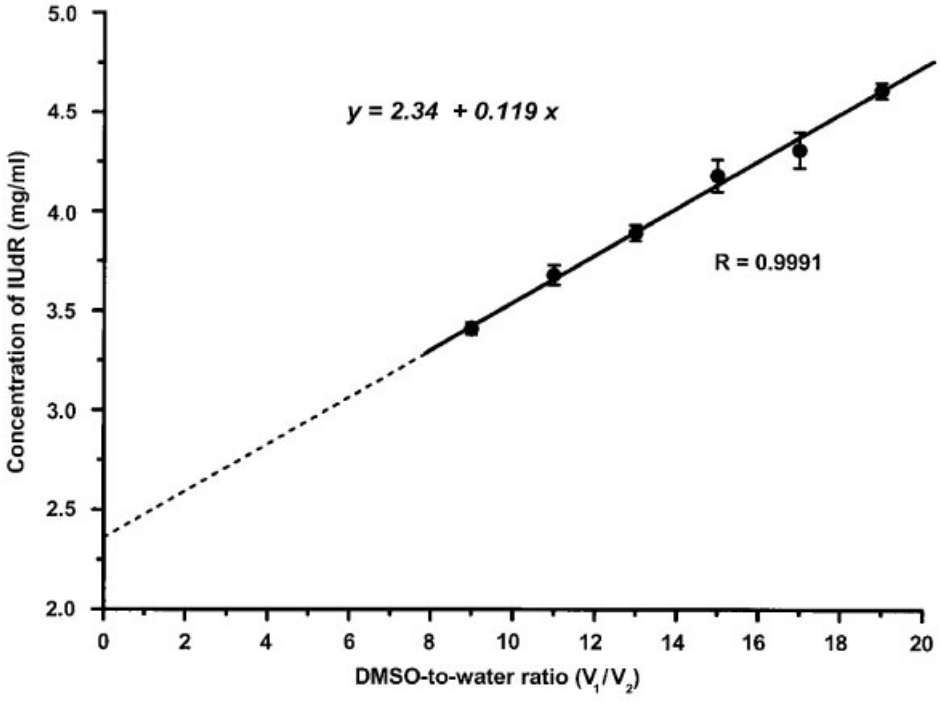

Figure 2. Solubility of IUdR (mg/ml) versus ratio of DMSO-to-water

(^{125}IMAc) and have assessed its aqueous solubility. Figure 4, which correlates IMAc solubility and DMSO-to-water ratio ($y = 47.3 + 2.073x$), shows that, in the absence of DMSO, the aqueous solubility of IMAc is $47.3 \pm 3.3\,\mu g/ml$. The comparable solubility of this compound obtained using the standard UV method ($42.0 \pm 0.53\,\mu g/ml$, $\sim 10\%$ difference) further validates the accuracy of the radiotracer method (Table 1).

Radiotracer method determinations using compounds with low aqueous solubilities

Our group has also been developing a novel technology that aims to entrap radioiodinated compounds within solid tumors for noninvasive tumor detection and therapy.[6,7] In this approach, which is called Enzyme-Mediated Cancer Imaging and Therapy (EMCIT), a water-soluble, radioiodinated molecule is hydrolyzed *in vivo* to a highly water-insoluble compound by an enzyme overexpressed extracellularly by tumor cells. For this project, we have identified and synthesized three iodoquinazolinone derivatives and have assessed their water solubility by the radiotracer method (Table 3). As expected, the data indicate that all three compounds are highly insoluble

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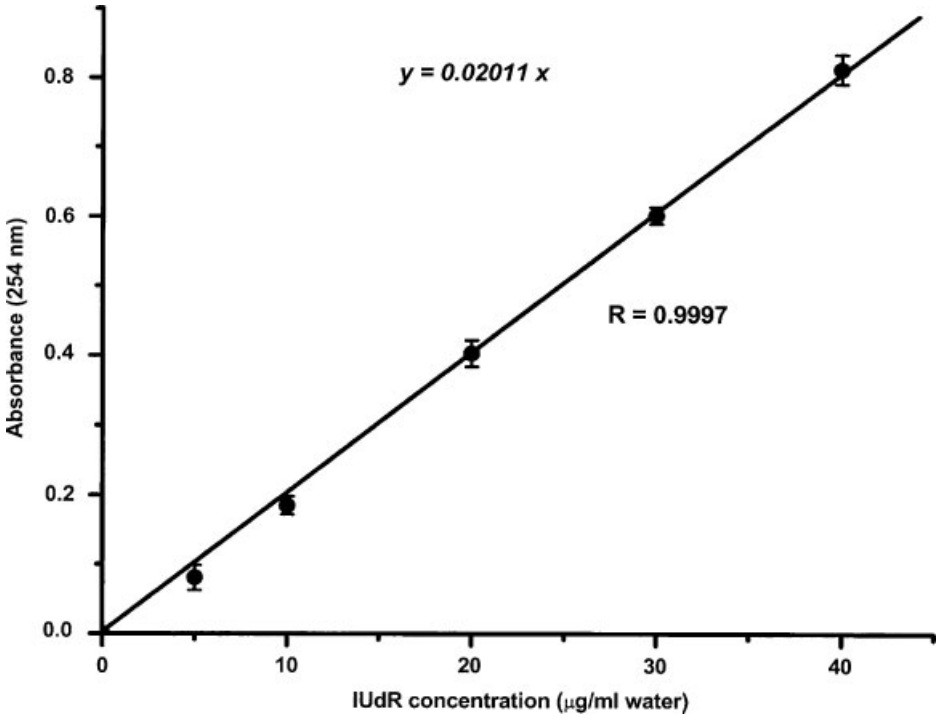

Figure 3. Absorbance (measured at 254 nm) of IUdR versus IUdR concentration (μg/ml) in water

(Figures 6–8, Table 3) and that the detection limits of the radiotracer method described herein extend to a few ng/ml.

Finally, we have also examined the solubility of 2-iodo-8*H*-quino[4,3,2-*kl*]acridine (IAc), an analog of IMAc, and have observed it to have the lowest solubility (Figure 9, 4.86 ± 1.56 pg/ml) among all the compounds tested (Table 3). This finding illustrates that the radiotracer method can determine water solubilities that are within a few pg/ml.

Experimental

Materials

Alkaline phosphatase and all other chemicals were obtained from Sigma Aldrich Chemical Company, and carrier-free sodium [^{125}I]iodide was purchased from GE Healthcare.

General

Vortex mixing was performed with a Vortex-Genie 2 (Scientific Industries, Incorporated), which mixes samples in a hands-free operation. HPLC

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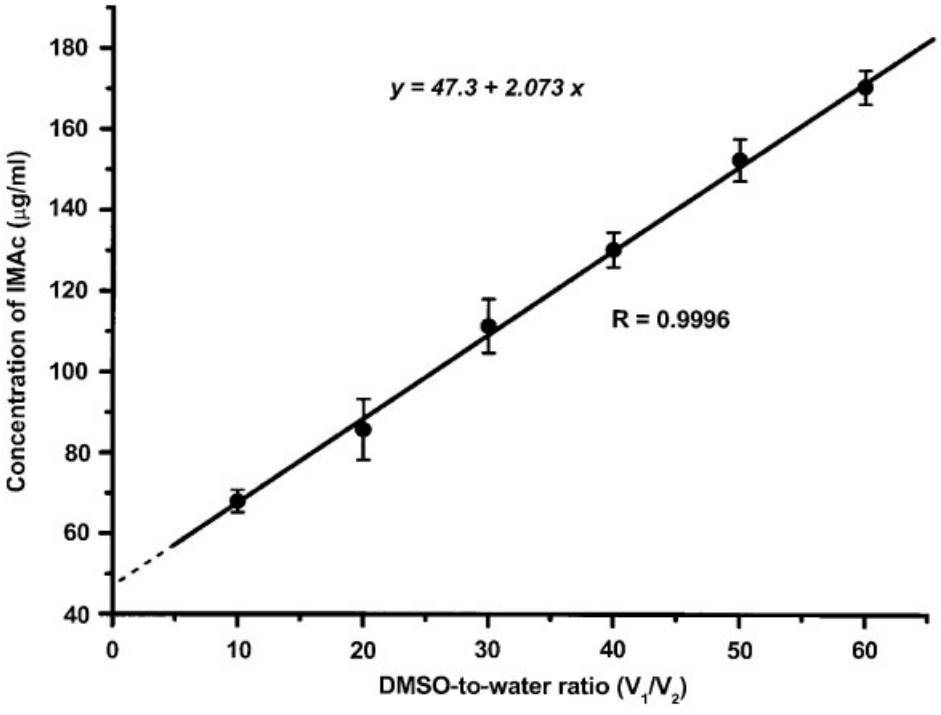

Figure 4. Solubility of IMAc (μg/ml) versus ratio of DMSO-to-water

(Waters) separation of radioactive products was performed on a reversed-phase Zorbax SB-C_{18} column, 9.4×250 mm (Agilent Technology) with a linear gradient from 10% 0.05 M disodium hydrogen phosphate, pH 2, to 100% methanol at a flow rate of 3 ml/min for 6 min, with UV absorption at 280 nm (Waters 486 detector) and γ-ray detection (gamma-ram, IN/US Systems) used to analyze the eluates. All products were collected in pure methanol which was then removed by purging with argon; the residue was dissolved in water. Spectrophotometry was performed with a UV/VIS model LS50B luminescence spectrometer (PerkinElmer), and radiotracer activity was determined in an automatic gamma counter (WIZARD 1480, PerkinElmer). All compounds have been characterized by ^{1}H NMR and ESI–HRMS.

Synthesis of ^{125}I-labeled 5-iodo-2′-deoxyuridine (^{125}IUdR) (Figure 1)

The method of Foulon *et al.*[8] with some modification was used for the synthesis of ^{125}I-labeled 5-iodo-2′-deoxyuridine (^{125}IUdR). To a stirred solution of ^{127}IUdR (1 g, 2.82 mmol) in dioxane (20 ml), bis(tributyltin) (3.6 g, 5.9 mmol) was added, followed by tetrakis(triphenylphosphine)palladium (TTPP, 47 mg) as catalyst. The reaction mixture was refluxed for approximately 2.5 h, and the progress of the reaction was monitored by thin

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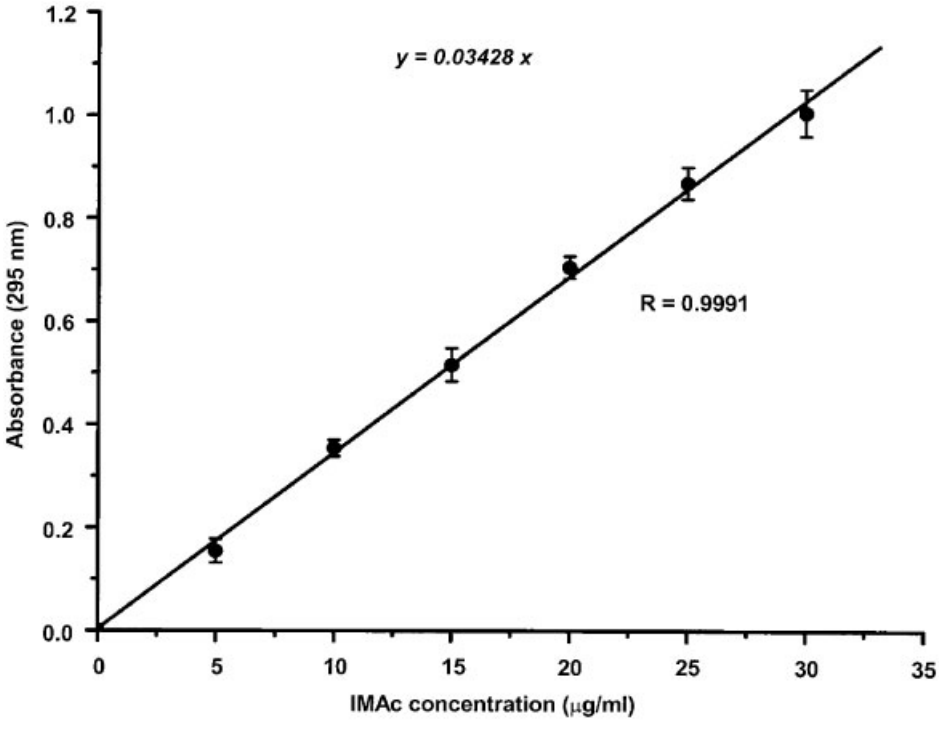

Figure 5. Absorbance (measured at 295 nm) of IMAc versus IMAc concentration (μg/ml) in water

Table 3. Water-solubility of compounds determined by radiotracer method

Compound	Water solubility
$IQ_{4\text{-OH}}$	84 ± 28 ng/ml
$IQ_{2\text{-OH}}$	1.96 ± 0.25 ng/ml
$IQ_{2\text{-OH, 5-Cl}}$	1.28 ± 0.36 ng/ml
IAc	4.86 ± 1.56 pg/ml

layer chromatography (silica gel–ethyl acetate as eluent) to test for formation of the 5-tributylstannyl-2′-deoxyuridine (SnUdR) product ($R_f = 0.5$). The solvent was evaporated, and the crude solid was purified on a silica gel column eluted with a stepwise gradient of hexane, followed by dichloromethane, and then by ethyl acetate. The solvent was removed to give a pure greasy product SnUdR (0.523 g, 36%).

1,3,4,6-Tetrachloro-3α,6α-diphenylglycouril (Iodogen, 10 μg) was dissolved in dichloromethane, placed in a reaction vial, and the solvent was evaporated (Iodogen adheres to the glass). To the vial was added phosphate buffer (10 ml, 0.01 M, pH 7.4), SnUdR (1 ml, 1.0 μg/ml DMSO solution), and $Na^{125}I$ (1 μl,

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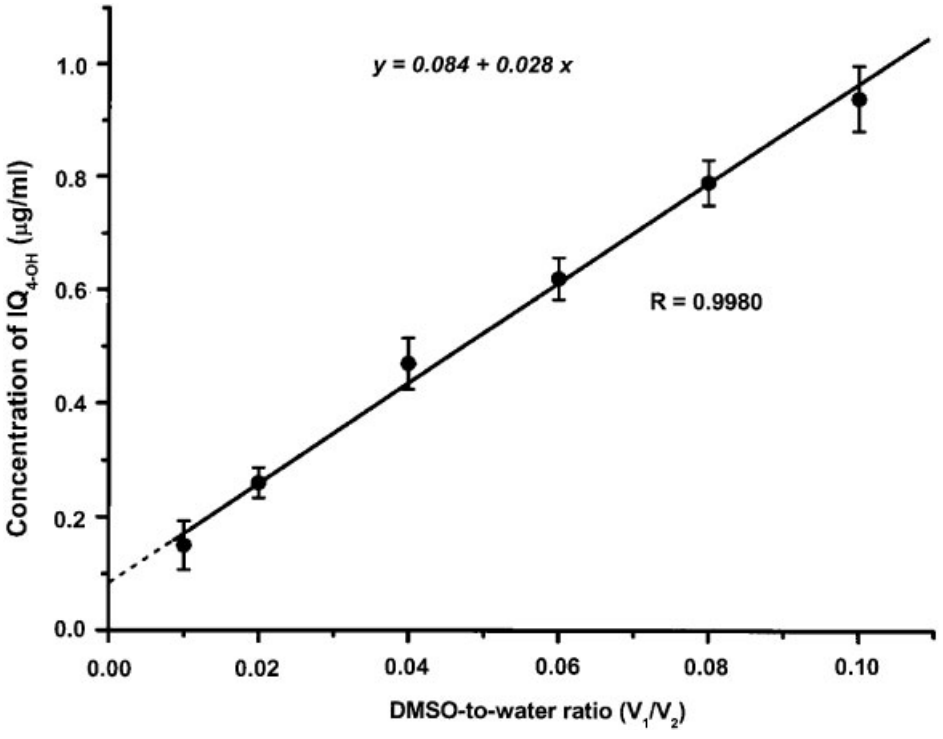

Figure 6. Solubility of $IQ_{4\text{-}OH}$ (µg/ml) versus ratio of DMSO-to-water

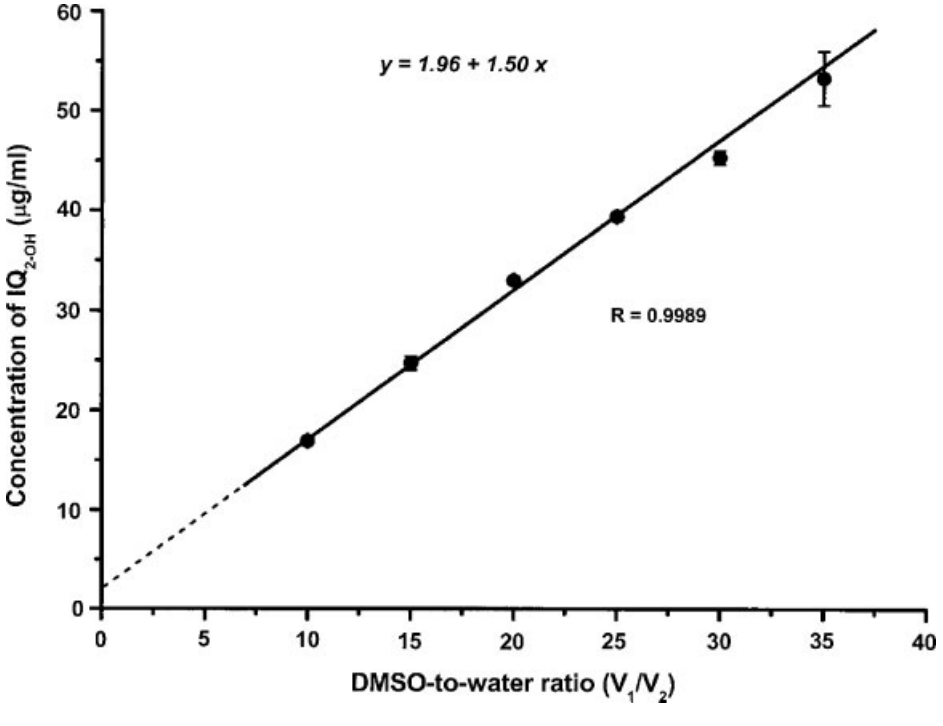

Figure 7. Solubility of $IQ_{2\text{-}OH}$ (µg/ml) versus ratio of DMSO-to-water

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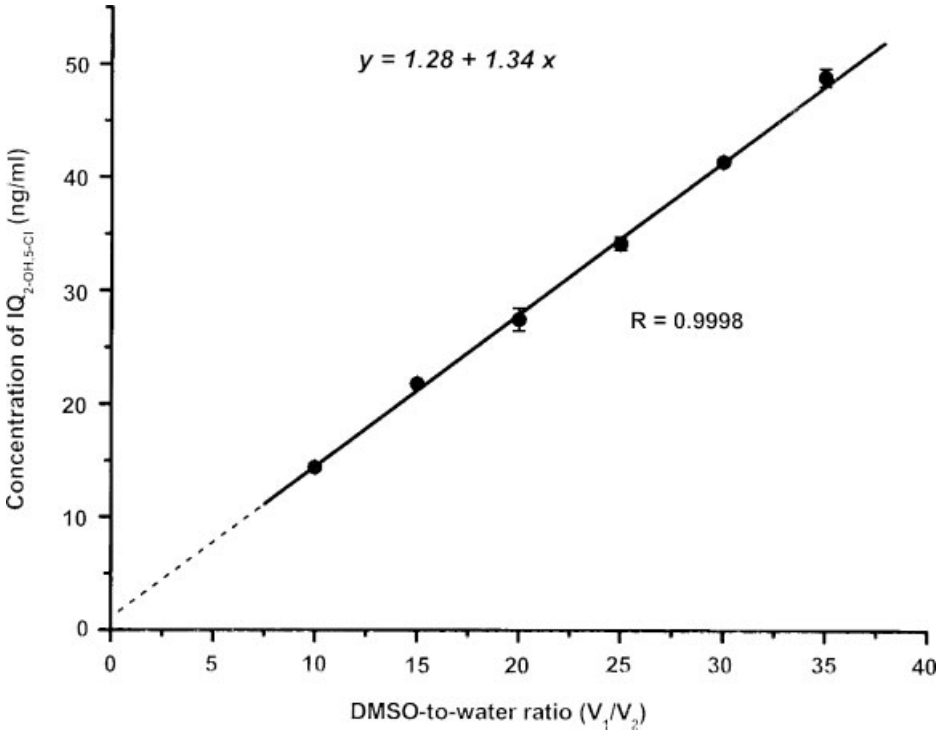

Figure 8. Solubility of $IQ_{2\text{-}OH,5\text{-}Cl}$ (ng/ml) versus ratio of DMSO-to-water

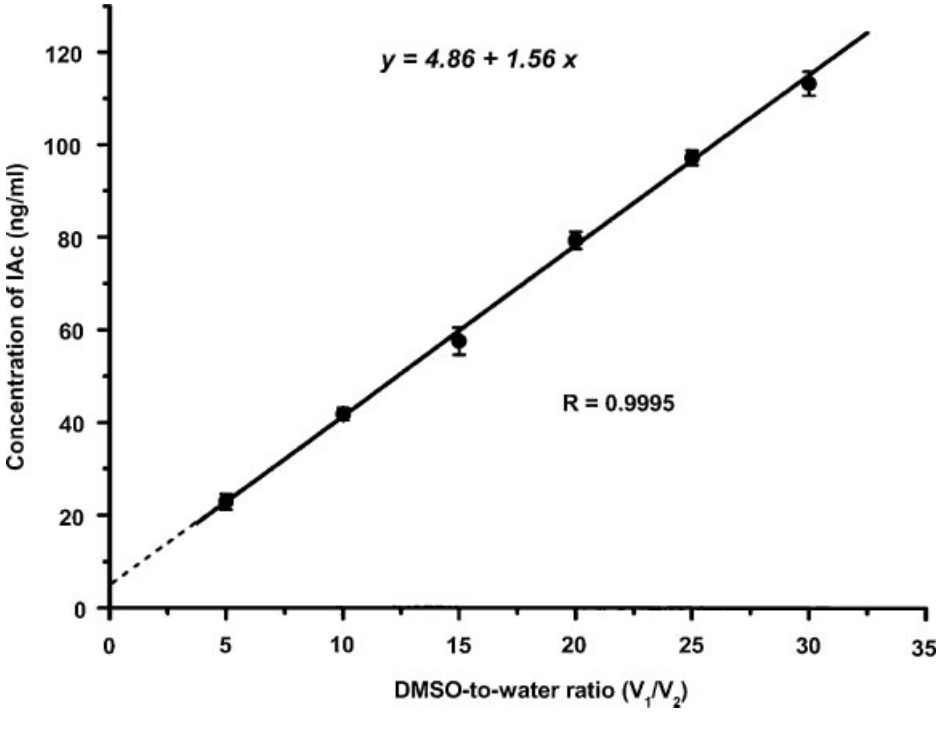

Figure 9. Solubility of IAc (ng/ml) versus ratio of DMSO-to-water

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10 mCi/22 µl, pH 7–9). After vortex mixing at ambient temperature for 2 min, the solution was analyzed by HPLC (C_{18} column with 10% acetonitrile in water as eluent, 3 ml/min) and the reaction was determined to be > 99% complete. The retention time (t_R) of ^{125}IUdR (9.13 min) was consistent with that of authentic (^{127}I-labeled) IUdR (9.05 min). The reaction solution was purified on a C_{18} cartridge which also removed unreacted ^{125}I giving ^{125}IUdR in 70% yield.

Synthesis of ^{125}I-labeled 2-iodo-8-methyl-8H-quino[4,3,2-kl]acridine (^{125}IMAc) (Figure 1)

To a reaction vial containing phosphate buffer (10 µl, 0.1 M, pH 5.0), 2-tributylstannyl-8-methyl-8*H*-quino[4,3,2-*kl*]acridine (kindly provided by C. A. Laughton, University of Nottingham, UK[9]) (3 µl, 1 µg/µl DMSO) was added, followed by Na^{125}I (2.0 µl, 10 mCi/22 µl) and chloramine-T (2 µl, 5 µg/µl water). After vortex mixing at ambient temperature for 5 min, the reaction mixture was analyzed by C_{18} HPLC with methanol–water as eluent. 2-[^{125}I]iodo-8-methyl-8*H*-quino[4,3,2-*kl*]acridine (^{125}IMAc, $t_R = 8.41$ min; ^{127}IMAc, $t_R = 8.35$ min) was obtained in 98% yield. ^{127}IMAc was also a gift from C. A. Laughton.

Synthesis of ^{127}I-labeled and ^{125}I-labeled 2-(4′-hydroxyphenyl)-6-iodo-4(3H)-quinazolinone ($^{127}IQ_{4\text{-}OH}$ and $^{125}IQ_{4\text{-}OH}$) (Figure 1)

Iodoanthranilamide[6] (4.0 g, 15.3 mmol) and 4-hydroxybenzaldehyde (2.1 g, 16.8 mmol) were suspended in methanol (50 ml) and refluxed in the presence of *p*-toluenesulfonic acid (0.8 g, 20%) for 2 h. The suspension was filtered, and the solid obtained was washed with cold methanol. It was then oxidized by the addition of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (4.0 g) in methanol (80 ml) with refluxing for 2 h. The deep-orange solid acquired by filtration was washed with cold methanol until color no longer appeared in the washing solvent. 2-(4′-Hydroxyphenyl)-6-iodo-4(3*H*)-quinazolinone ($^{127}IQ_{4\text{-}OH}$) was obtained as a yellow solid (3.5 g, 9.6 mmol). ESI–HRMS calculated for $C_{14}H_9IN_2O_2$ $[M + H]^+$: 364.9781; found: 364.9776.

To a stirred solution of $^{127}IQ_{4\text{-}OH}$ (5 g/100 ml dioxane, 13.7 mmol), bis(tributyltin) (10 ml) was added, followed by TTPP (500 mg) as catalyst. The reaction mixture was refluxed for 3 h. The solvent was evaporated, and the crude dark solid was purified on a silica gel column eluted with a stepwise gradient of hexane followed by hexane:dichloromethane (1:2). The solvent was removed to give a light-yellow solid, 2-(4′-hydroxyphenyl)-6-tributylstannyl-4(3*H*)-quinazolinone ($SnQ_{4\text{-}OH}$) ($t_R = 15.3$ min). ESI–HRMS calculated for $C_{26}H_{36}N_2O_2Sn$ $[M + H]^+$: 529.1872; found: 529.1883.

Phosphorus oxychloride (5 µl, 53.6 µmol/0.5 ml dry pyridine) was added dropwise to a cooled (0°C), stirred solution of $SnQ_{4\text{-}OH}$ (25 mg/0.4 ml dry

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pyridine, 47.5 μmol). The solution turned yellow. The mixture was stirred for 1 h at 0°C and then the reaction was quenched by adding ammonium hydroxide (15 μl, 28% in 1 ml de-ionized water, approximately pH 7). The solvent was evaporated under reduced pressure and a light-yellow solid, ammonium 2-(4′-phosphoryloxyphenyl)-6-tributylstannyl-4(3*H*)-quinazolinone ($SnQ_{4\text{-}P}$), was obtained.

To a reaction vial coated with Iodogen (10 μg), phosphate buffer (10 μl, 0.1 M, pH 7.4), $SnQ_{4\text{-}P}$ (1 μl, 5 μg/μl DMSO solution), and $Na^{125}I$ (2.0 μl, 10 mCi/22 μl) were added. The solution was vortexed at ambient temperature for 5 min. Analysis by C_{18} HPLC indicated a radioiodination yield for $^{125}IQ_{4\text{-}P}$ of 99% ($^{125}IQ_{4\text{-}P}$, $t_R = 9.14$ min; $^{127}IQ_{4\text{-}P}$, $t_R = 9.06$ min). The reaction solution was then transferred to a vial along with alkaline phosphatase (10 Units/2.0 μl), and the mixture was incubated at 37°C for 5 min. HPLC performed with an internal standard ($^{127}IQ_{4\text{-}OH}$; $t_R = 10.03$ min) indicated the complete dephosphorylation of $^{125}IQ_{4\text{-}P}$ and the formation of $^{125}IQ_{4\text{-}OH}$ ($t_R = 10.10$ min).

Synthesis of ^{127}I-labeled and ^{125}I-labeled 2-(2′-hydroxyphenyl)-6-iodo-4(3H)-quinazolinone ($^{127}IQ_{2\text{-}OH}$ and $^{125}IQ_{2\text{-}OH}$) (Figure 1)

2-(2′-Hydroxyphenyl)-6-iodo-4(3*H*)-quinazolinone ($^{127}IQ_{2\text{-}OH}$) was synthesized as described by Ho *et al.*[6] To a stirred solution of $^{127}IQ_{2\text{-}OH}$ (2 g, 3.8 mmol) in dioxane (40 ml), bis(tributyltin) (3.8 ml) was added, followed by TTPP (200 mg) as catalyst. The mixture was refluxed for 3 h, the solvent was evaporated, and the crude dark solid was purified on a silica gel column eluted with a stepwise gradient of hexane followed by hexane:dichloromethane (1:1). The solvent was removed to give a light-yellow fluorescent solid, 2-(2′-hydroxyphenyl)-6-tributylstannyl-4(3*H*)-quinazolinone ($SnQ_{2\text{-}OH}$).

Phosphorus oxychloride (5 μl, 5.36 μmol/0.5 ml dry pyridine) was added dropwise to a cooled (0°C), stirred solution of $SnQ_{2\text{-}OH}$ (25 mg/0.5 ml dry pyridine, 47.5 μmol). The solution turned yellow in color. The mixture was stirred for 1 h at 0°C after which the reaction was quenched by adding ammonium hydroxide (15 μl, 28% in 1 ml de-ionized water, pH 7.0). The solvent was evaporated under reduced pressure (rotary evaporator) and a light-yellow solid, ammonium 2-(2′-phosphoryloxyphenyl)-6-tributylstannyl-4(3*H*)-quinazolinone ($SnQ_{2\text{-}P}$), was obtained. TLC and HPLC indicated purity greater than 90%.

$SnQ_{2\text{-}P}$ (0.5 mg) was then dissolved in DMSO (100 μl) and kept overnight. To a reaction vial coated with Iodogen (10 μg), phosphate buffer (10 μl, 0.01 M, pH 7.4), $SnQ_{2\text{-}P}$ (1 μl, 5 μg/μl DMSO), and $Na^{125}I$ (2.0 μl, 10 mCi/22 μl) were added. The solution was vortexed at ambient temperature for 2 min. Analysis by C_{18} HPLC indicated the disappearance of free ^{125}I and the appearance of $^{125}IQ_{2\text{-}P}$ (radioiodination reaction yield 99%) ($^{125}IQ_{2\text{-}P}$,

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$t_R = 9.46$ min; $^{127}IQ_{2\text{-}P}$, $t_R = 9.37$ min). The reaction solution containing $^{125}IQ_{2\text{-}P}$ was transferred to a vial, alkaline phosphatase (10 Units/2 µl) was added, and the solution was kept for 3 min at 37°C. HPLC performed with an internal standard ($^{127}IQ_{2\text{-}OH}$) indicated the complete dephosphorylation of $^{125}IQ_{2\text{-}P}$ and the formation of $^{125}IQ_{2\text{-}OH}$. The t_R of $^{125}IQ_{2\text{-}OH}$ (11.26 min) was consistent with that of $^{127}IQ_{2\text{-}OH}$ (11.18 min).

Synthesis of ^{127}I-labeled and ^{125}I-labeled 2-(2′-hydroxy-5-chlorophenyl)-6-iodo-4(3H)-quinazolinone ($^{127}IQ_{2\text{-}OH,5\text{-}Cl}$ and $^{125}IQ_{2\text{-}OH,5\text{-}Cl}$) (Figure 1)

Iodoanthranilamide[6] (5.0 g, 32.4 mmol) and 5-chlorosalicylaldehyde (5.6 g, 21.0 mmol) were suspended in methanol (70 ml) and refluxed in the presence of *p*-toluenesulfonic acid (1.0 g, 20%) for 2 h. The mixture was then filtered, and the solid was washed with cold methanol and oxidized by the addition of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (5.0 g) in methanol (100 ml). The suspension was refluxed for 2 h and turned a deep-orange color. After filtration, the solid acquired was washed with cold methanol until color no longer appeared in the solvent. 2-(2′-Hydroxy-5-chlorophenyl)-6-iodo-4(3*H*)-quinazolinone ($^{127}IQ_{2\text{-}OH,5\text{-}Cl}$) was obtained as a yellow solid (3.6 g, 9.1 mmol). ESI–HRMS calculated for $C_{14}H_8IN_2O_2Cl$ $[M + H]^+$: 396.9241; found: 396.9235.

To a stirred solution of $^{127}IQ_{2\text{-}OH,5\text{-}Cl}$ (3 g, 7.56 mmol/60 ml dioxane), bis(tributyltin) (8.77 g/5.5 ml, 15.1 mmol) was added, followed by TTPP (150 mg) as catalyst. As the reaction mixture was refluxed for approximately 2 h, it became dark. Refluxing was continued for 3 h until the solution no longer deepened in color. The solvent was evaporated, and the crude solid was purified on a silica gel column eluted with a stepwise gradient, starting with hexane followed by dichloromethane. The solvent was removed to give a pure, light-green product, 2-(2′-hydroxy-5-chlorophenyl)-6-tributylstannyl-4(3*H*)-quinazolinone ($SnQ_{2\text{-}OH,5\text{-}Cl}$) (1.95 g, $t_R = 26.4$ min). ESI–HRMS calculated for $C_{26}H_{35}N_2O_2ClSn$ $[M + H]^+$: 563.1487; found: 563.1497.

Phosphorus oxychloride (5 µl, 53.6 µmol/0.3 ml dry pyridine) was added dropwise to a cooled (0°C), stirred solution of $SnQ_{2\text{-}OH,5\text{-}Cl}$ (25 mg, 44.4 µmol/0.4 ml dry pyridine). The solution turned yellow in color. The reaction mixture was stirred for 1 h at 0°C and the reaction was quenched by adding ammonium hydroxide (15 µl, 28% in 1 ml de-ionized water, approximately pH 7). The solvent was evaporated under reduced pressure and a light-yellow solid, ammonium 2-(2′-phosphoryloxy-5-chlorophenyl)-6-tributylstannyl-4(3*H*)-quinazolinone ($SnQ_{2\text{-}P,5\text{-}Cl}$), was obtained.

$SnQ_{2\text{-}P,5\text{-}Cl}$ (0.3 mg) was then dissolved in DMSO (100 µl) and kept overnight. To a reaction vial coated with Iodogen (10 µg), phosphate buffer (10 µl, 0.01 M, pH 7.4), $SnQ_{2\text{-}P,5\text{-}Cl}$ (1 µl, 3 µg/µl DMSO), and $Na^{125}I$ (2.0 µl, 10 mCi/22 µl) were added. The solution was vortexed at ambient temperature

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for 5 min. Analysis by C_{18} HPLC indicated the product $^{125}IQ_{2\text{-}P,5\text{-}Cl}$ ($t_R = 9.58$ min; $^{127}IQ_{2\text{-}P,5\text{-}Cl}$, $t_R = 9.51$ min) was obtained in 99% yield. $^{125}IQ_{2\text{-}P,5\text{-}Cl}$ (10 µl) was transferred to a vial along with alkaline phosphatase (10 Units/2 µl), and the solution was kept for 5 min at 37°C. HPLC of the solution with an internal standard indicated complete dephosphorylation of $^{125}IQ_{2\text{-}P,5\text{-}Cl}$ and formation of $^{125}IQ_{2\text{-}OH,5\text{-}Cl}$. The t_R of $^{125}IQ_{2\text{-}P,5\text{-}Cl}$ (12.35 min) was consistent with that of $^{127}IQ_{2\text{-}P,5\text{-}Cl.}$ (12.29 min).

Synthesis of ^{125}I-labeled 2-iodo-8H-quino[4,3,2-kl]acridine (^{125}IAc)

To a reaction vial coated with Iodogen (10 µg), phosphate buffer (8 µl, 0.01 M, pH 7.4) and 2-tributylstannyl-8*H*-quino[4,3,2-*kl*]acridine (kindly provided by C. A. Laughton, University of Nottingham, UK) (3 µl, 1 µg/µl DMSO solution) were added followed by $Na^{125}I$ (2.0 µl, 10 mCi/22 µl). The solution was vortexed at ambient temperature for 5 min. Analysis of the reaction mixture by C_{18} HPLC indicated that the product ^{125}IAc ($t_R = 15.31$ min; ^{127}IAc, $t_R = 15.23$ min) was obtained in 99% yield. The solvent was removed by bubbling argon through the solution. ^{127}IAc was also a gift from C. A. Laughton).

Determination of solubility by the radiotracer method

In this approach, several milligram of test compound (an amount that is accurately weighed) was dissolved in DMSO (the volume adjusted until the solution was visibly clear). The radiotracer was then added (^{125}IUdR/^{127}IUdR: 40 µCi ^{125}IUdR + 611 mg IUdR/ml; ^{125}IMAc/^{127}IMAc: 10 µCi ^{125}IMAc + 13 mg ^{127}IMAc/ml; $^{125}IQ_{4\text{-}OH}$/$^{127}IQ_{4\text{-}OH}$: 1 mCi $^{125}IQ_{4\text{-}OH}$ + 34 mg $^{127}IQ_{4\text{-}OH}$/ml; $^{125}IQ_{2\text{-}OH}$/$^{127}IQ_{2\text{-}OH}$: 4 µCi $^{125}IQ_{2\text{-}OH}$ + 2 µg $^{127}IQ_{2\text{-}OH}$/ml; $^{125}IQ_{2\text{-}OH,5\text{-}Cl}$/$^{127}IQ_{2\text{-}OH,5\text{-}Cl}$: 4 µCi $^{125}IQ_{2\text{-}OH,5\text{-}Cl}$ + 2 µg $^{127}IQ_{2\text{-}OH,5\text{-}Cl}$/ml; ^{125}IAc/^{127}IAc: 4 µCi ^{125}IAc + 8 ng ^{127}IAc/ml). The samples were vortexed and various volumes (V_1) of each of the radiotracer–DMSO solutions (Table 2, column 2) were placed in centrifuge tubes containing de-ionized water and sufficient water was added to bring the volumes to V_2 (Table 2, column 3). Because DMSO increases the water solubility of the compound, the amount of compound remaining in solution was not entirely a function of aqueous solubility. Additionally, the amount of sample dissolved under these saturating conditions is proportional to the amount of DMSO present. After vortex-mixing for 15 min at room temperature, the tubes were centrifuged for 30 min at 3000 rpm to remove any material that had precipitated. Since (i) the iodine-125 and iodine-127 compounds have the same solubilities and are thus equally distributed in the DMSO–water solution, and (ii) the specific activity of the test material (added in the DMSO solution) is known, the concentration of the compound in solution can be calculated (Table 2, column 8) and plotted

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as a function of the DMSO-to-water ratio (V_1/V_2, Table 2, column 4). Each point was determined by three measurements and the value is the mean $\pm$ the standard error. The water solubility of the compound was then determined by extrapolation of the linear fit of the data points to zero DMSO, i.e. the y-axis intercept when $x = 0$ (Origin 7.0, OriginLab Corporation, Northampton, MA).

Determination of solubility by UV spectrophotometry

The validity of the radiotracer method was ascertained by comparing the data obtained for two compounds (^{125}IUdR and ^{125}IMAc) with those from traditional 'equilibrium' solubility determinations. In this latter approach, an aqueous solution of each test compound (0.10 mg/ml IUdR, 30 μg/ml IMAc) was prepared and serially diluted. A standard curve of UV absorption (measured at 254 nm for IUdR, 295 nm for IMAc) versus concentration was constructed (0–40 μg/ml IUdR, 0–30 μg/ml IMAc), and a linear fit of the data was generated (Origin 7). To determine the solubility of these two compounds in water, saturated solutions were made by stirring solute (55 mg IUdR, 1.5 mg IMAc) in 10 ml water for 48 h at room temperature. The solutions were kept overnight at room temperature and then centrifuged at 3000 rpm for 30 min. The UV absorption of various dilutions of the supernatants (3-ml aliquots) was measured, and the concentrations were calculated using the linear fit equations of the standard curves.

Conclusions

The determination of aqueous solubility by the radiotracer method is consistent and dependable as demonstrated by the correspondence of the solubility values obtained by this method with those acquired by UV spectrophotometry. The method is rapid and simple and, because of the sensitivity of radioactivity measurements, can accurately quantify the solubility of low-molecular-weight compounds (1000–2000 Da) in the pg–ng/ml range.

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