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Targeting Epidermal Growth Factor Receptor with Ferrocene-Based Kinase Inhibitors

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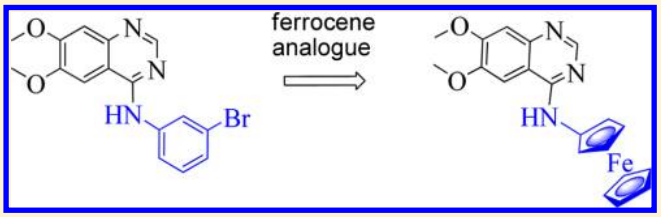
Supporting Information

ABSTRACT: A series of ferrocene analogues based on a 6,7-dimethoxy-*N*-phenylquinazolin-4-amine template has been synthesized, and two compounds were characterized in the solid state by X-ray crystallography. The compounds have been tested for in vitro anticancer activity, against epidermal growth receptor (EGFR), and submicromolar IC_{50} values have been determined.



INTRODUCTION

Protein kinase inhibitors (PKIs) are used clinically for the treatment of a number of cancers.[1] Compounds **1** and **2** act as epidermal growth factor receptor (EGFR) inhibitors, combining a quinazoline unit, mimicking the adenosine in ATP, a solvent-exposed region composed of a solubilizing ether or amine side chain, and a substituted anilide function (in red, Figure 1), serving as a hydrophobic pocket binding group, which imparts selectivity to the PKI.[1]

We have successfully employed ferrocenes as aryl bioisosteres ("bioequivalents") in a number of applications ranging from histone deacetylase inhibitors (HDACis, where the ferrocene leads to changes in enzyme isoform selectivity in comparison to its phenyl congener) to kinase inhibitors (where a loss of kinase activity is observed, attributed to steric effects, in comparison with its aryl congeners).[2] Others have explored ferrocene analogues of tamoxifen[3a] (where the ferrocene can significantly enhance the activity of the drug) as well as chloroquine derivatives, where drug resistance is significantly diminished.[3b]

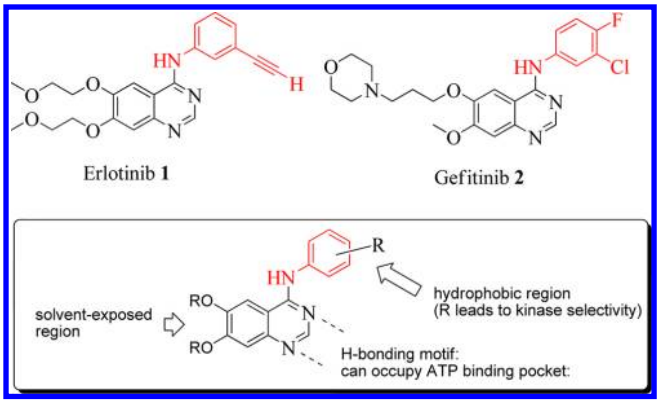


Figure 1. Clinically approved PKIs (general pharmacophore shown in box).

We now report a synthesis of simplified ferrocene analogues of **1** and **2**. The reasons for this are numerous:

(i) The introduction of a ferrocene unit can have beneficial or detrimental consequences in terms of biological activity in comparison with its organic congener, as noted above.

(ii) An organometallic unit, by virtue of its heavy-metal atom, can be used as a biological probe for cocrystal studies for protein structure determination.[4]

(iii) Compounds **1** and **2** contain elaborated side chains and require a multistep synthesis to introduce these solubility-enhancing groups. As a direct comparative study, simpler methoxy-substituted quinazolines were readily accessible (**6d**,**e**; see below).[5] Moreover, the latter are easier to synthesize yet still display excellent biological activity.

We have now applied this principle to the design of compounds **6**, whereby the aniline moiety has been replaced by an aminoferrocene bioisostere. In standing with our aims of synthesizing molecules rapidly with minimum workup bottlenecks, following standard conversion of 6,7-dimethoxyquinazolin-4(3*H*)-one **3** to the chloroquinazoline **4**, a microwave-mediated[6] nucleophilic displacement of **4** with aminoferrocene **5a** or anilinoferrocenes **5b**,**c** afforded the corresponding products **6a**−**c** (Scheme 1). The last species were characterized

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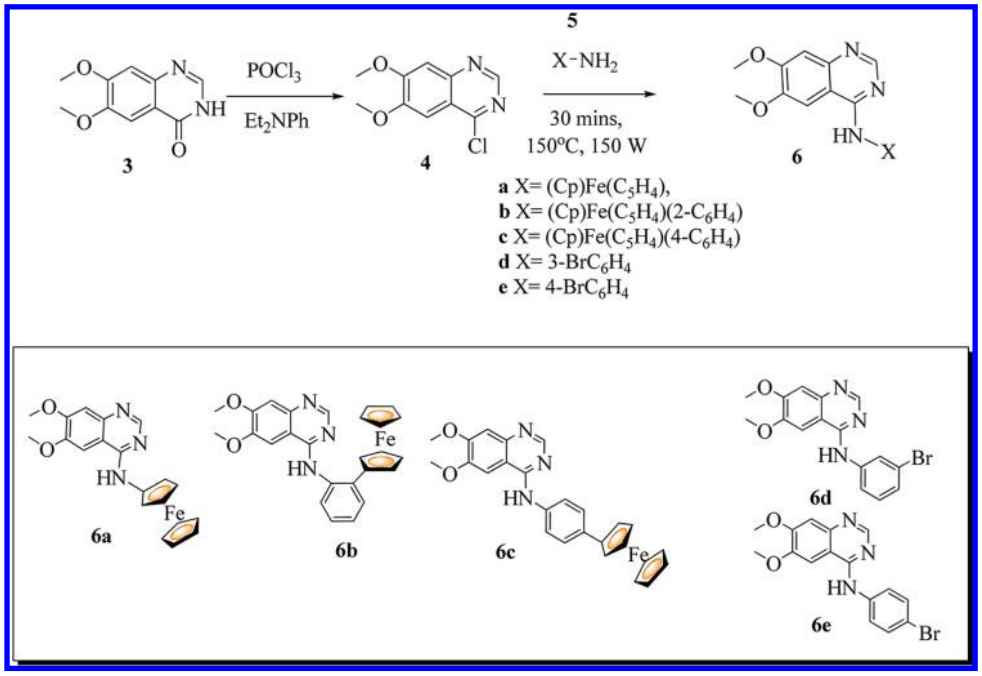
Scheme 1. General Method of Synthesis of 6



by NMR spectroscopy and elemental analysis. The two non-ferrocene products **6d,e** were also synthesized as positive controls for comparison of EGFR inhibition.[5]

The two ferrocene analogues **6a,c** were studied in the solid state by X-ray crystallography (Figures 2 and 3, respectively). **6a** was found to crystallize in the monoclinic crystal system, space group $P2_1/n$, with two quinazoline molecules and one molecule of dichloromethane in the asymmetric unit. The two quinazoline molecules display a negligible difference in conformation, as shown by the results in Table S1 (Supporting Information) and the RMS deviation of 0.1720 when the molecules are overlaid. The crystal structure consists of stacked 1-D "cross-hatch tapes" comprising H-bonded quinazoline molecules (N101···N203 = 2.989(7) Å; ∠D−H···A = 158.4°; N201···N103 = 2.942(7) Å; ∠D−H···A = 156.0°) propagating along the crystallographic *b* axis. The solvent dichloromethane molecules reside in the voids created as these stacks pack along the *c* axis. The structure of **6c** (determined using a synchrotron source due to its poor diffraction using our standard equipment) was found to be monoclinic, space group $P2_1/c$, and contained a water of crystallization. The crystal structure consists of 1-D "corrugated tapes" of water-mediated H-bonded quinazoline molecules (N1···O1W = 2.967(7) Å, ∠D−H···A = 165.1°; O1W···N3 = 2.752(2) Å, ∠D−H···A = 174(3)°) propagating along the crystallographic *c* axis. These tapes stack along the *b* axis and are interleaved with an inverted stack such that weak H bonds exist between the water molecules of one layer and the amine N atoms of the adjacent inverted layer (O1W···N1 = 3.232(2) Å, ∠D−H···A = 160(3)°). Tables S1 and S2 (Supporting Information) summarize important bond lengths and angles as well as structural parameters for both compounds.

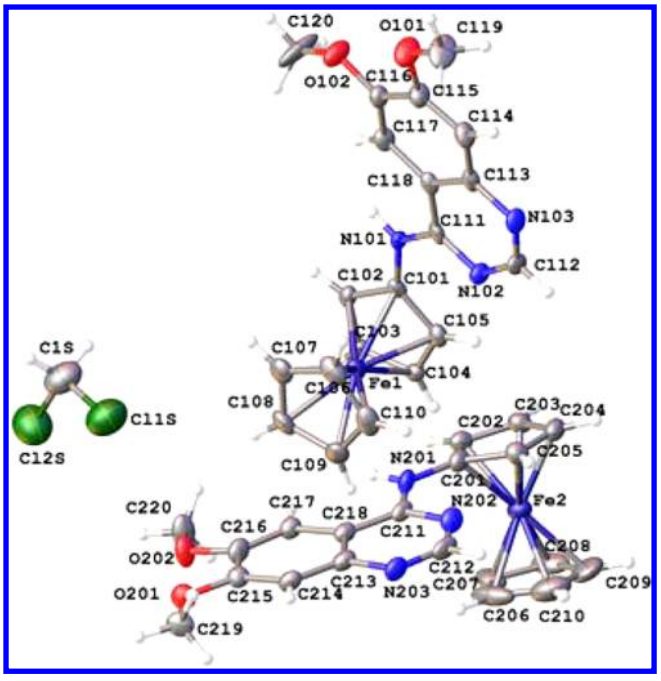


Figure 2. ADP plot for complex **6a** with ADP ellipsoids drawn at the 50% probability level.

Next, the complexes were evaluated for biological activity in in vitro assays against EGFR, since this is the target of the inhibitors **1**, **2**, and **6d,e** (Table 1).[5] Complex **6b** exhibited an impressive submicromolar activity against EGFR, whereas **6a,c** gave essentially micromolar inhibition. However, EGFR inhibitory activity was substantially weaker than the controls **6d,e**, which displayed very low nM affinities. Indeed, compound **6d** (termed PD 153035) is reported to have an IC_{50} value of 0.025 nM.[5b]

Next, the compounds were tested for in vitro activity in K562 cells. Neither the organometallic complexes **6a,b** nor the organic entities **6d,e** showed any appreciable activity, with IC_{50} values >20 μM. It has been reported that EGFR inhibition is dependent on the number of EGF receptors expressed as well as the type of cell line used, many of which show IC_{50} values of the same magnitude.[9a] Moreover, EGFR is not expressed in K562 cells.[9b] Only complex **6c** showed cellular activity with

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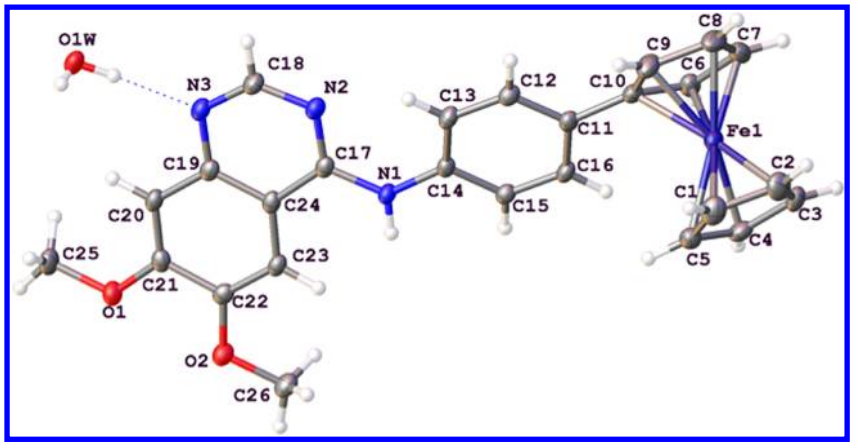

Figure 3. ADP plot for **6c** with ADP ellipsoids drawn at the 50% probability level.

Table 1. Biological Evaluation of 6

compd	IC_{50} (nM) vs EGFR[a]	in vitro IC_{50} (μM)[b]
6a	1800	>20
6b	133	>20
6c	905	16
6d	<5	>20
6e	4.6	>20

[a]In vitro assay (Reaction Biology Corp. US),[7] average of two runs. [b]In K562 cells,[8] average of two runs.

IC_{50} = 16 μM, which is highly likely to be due to off-target effects.[7b]

In order to rationalize the biological activity of these compounds, they were studied by molecular modeling. Compound **6e** can be docked in a conformation (Figure 4a) with its core close to that of the reported cocrystallized ligand (erlontinib (pdb code: 4hjo)). A nitrogen atom in the quinazoline forms an expected hydrogen bond with a backbone NH of a methionine in the kinase receptor. The two rings (quinazoline and phenyl) both fit well in the hydrophobic region. Of the organometallic complexes, only **6b** can be docked with its core in a close position to that of **6e** (Figure 4b). It can thus form the H bond and places the (aniline-substituted) phenyl group in the correct orientation, hence accounting for **6b** having the best activity among the ferrocenes studied. However, **6a,c** can only be docked with a shifted core, leading to binding penalties (Figure 4c,d). This is probably because these particular ferrocene substitution patterns hinder binding to the pocket.

CONCLUSION

Three ferrocene analogues containing a quinazoline skeleton have been synthesized. Two have been further characterized in the solid state. Biological evaluation of the complexes showed them to be effective EGFR inhibitors with micromolar or submicromolar potencies, which are significantly weaker than those of their related organic prototypes. Docking studies have accounted for the good in vitro EGFR inhibition observed for the ferrocene **6b**. Bioorganometallic complexes remain important as tool compounds in the study of kinases, and we are actively pursuing this area of research.[10]

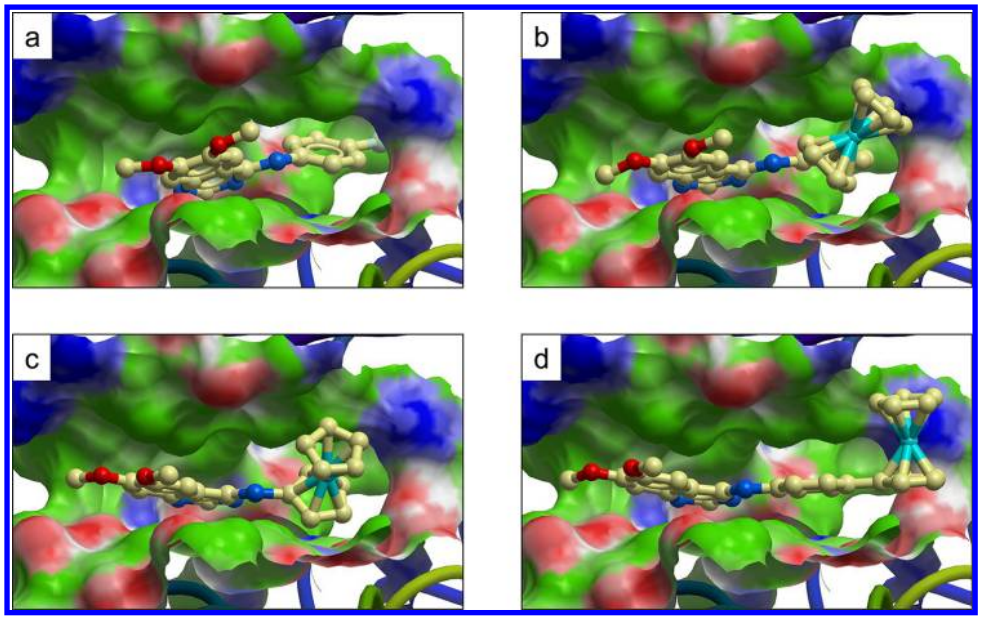

Figure 4. Docking pose of compounds in the ATP pocket of EGFR (pdb code: 4hjo): (a) **6e**; (b) **6b**; (c) **6a**; (d) **6c**. Color scheme for the atom types: carbon, yellow; nitrogen, blue; oxygen, red; iron, cyan. Methods have been previously reported.[2b]

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EXPERIMENTAL PROCEDURES

Experimental and spectroscopic methods[2] as well as biological experiments (K562, MTT assay)[11] have been outlined in previous papers.

Reactions were carried out under argon using reagent grade solvents and reagents. Compounds **6d** (PD 153035) and **6e** were made by literature routes.[5]

Synthesis of 4-Chloro-6,7-dimethoxyquinazoline 4. In an oven-dried three-necked round-bottom flask (250 mL) equipped with a stirrer and reflux condenser 6,7-dimethoxy-3*H*-quinazoline-4-one (**3**; 1.00 g, 4.85 mmol) was added followed by phosphoryl chloride (2.70 mL, 29.1 mmol) and *N*,*N*′-diethylaniline (0.51 mL, 3.20 mmol). With vigorous stirring the reaction mixture was heated to reflux for 5 h. After this time had elapsed, the hot reaction mixture was carefully poured into a slurry of ice/water (100 mL), ensuring no precipitate formed at this stage. The cooled crude mixture was extracted using methylene chloride (30 mL) and washed with brine (30 mL). The organic layer was dried using magnesium sulfate and then filtered through fluted filter paper. The solvent was removed under reduced pressure. The crude mixture was purified using silica gel column chromatography with 1/99 methanol/methylene chloride as eluent to give the white solid **4** (0.31 g, 28%). ^{1}H NMR (δ, 270.0 MHz, $CDCl_3$): 4.08 (6H, s, 2 × CH_3), 7.35 (1H, s, ArC*H*), 7.40 (1H, s, ArC*H*), 8.86 (1H, s, ArC*H*). ^{13}C NMR (δ, 67.0 MHz, $CDCl_3$): 56.5, 56.6, 102.7, 107.0, 119.6, 149.2, 151.5, 152.6, 156.8, 159.1.

General Procedure for the Synthesis of 6. In an oven-dried microwave tube (35 mL) equipped with a stirrer, **4** (0.07 g, 0.31 mmol) was dissolved in anhydrous 1,4-dioxane (1 mL). A portion of HCl (in 1,4-dioxane) (4 M, 0.25 mL) was then added dropwise at room temperature, and the mixture was stirred for 15 min before the addition of ferrocenylamine (**5a**), (2-aminophenyl)ferrocene (**5b**), or (4-aminophenyl)ferrocene (**5c**); 3-bromoaniline (**5d**) and 4-bromoaniline (**5e**) were heated in *tert*-butyl alcohol (0.31 mmol scale). The reaction mixture was heated (ramped) to 150 °C at 150 W and held at this temperature for 30 min with microwave moderation of power. The reaction mixture was then quenched with saturated sodium hydrogen carbonate (10 mL). The crude reaction mixture was then extracted using methylene chloride (10 mL), and the aqueous layer was sonicated before being extracted with methylene chloride (10 mL). The organic layer wa washed with saturated brine (10 mL) before being dried using magnesium sulfate and filtered. The filtrate was removed under reduced pressure. The following purification protocols were employed with final yields for the compounds indicated.

Compound ***6a***. Silica gel column chromatography (1:99 MeOH/DCM) afforded **6a** (0.06 g, 50%) as an orange solid. Mp: 240−242 °C dec. IR (Nujol mull): ν_{max} 1025 cm^{-1} (C═N stretch). ^{1}H NMR (δ, 270.0 MHz, $CDCl_3$); 3.97 (3H, s, OCH_3), 4.01 (3H, s, OCH_3), 4.14 (2H, s, Fc), 4.20 (5H, s, C$_5H_5$), 4.78 (2H, s, Fc), 6.57 (1H, brs, N*H*), 6.91 (1H, s, ArC*H*), 7.24 (1H, s, ArC*H*), 8.67 (1H, s, ArC*H*). ^{13}C NMR (δ, 67.0 MHz, $CDCl_3$): 56.2 (2*C*), 63.4 (2*C*), 65.3 (2*C*), 69.5 (5*C*), *ipso*-Fc not observed, 99.7, 107.8, 108.8, 147.2, 149.0, 153.8, 154.4, 157.0. HRMS (*m*/*z*, HNESP): [M + H]$^+$ for $C_{20}H_{20}FeN_3O_2$ calcd 390.0891, observed 390.0890. Anal. Calcd for $C_{20}H_{19}FeN_3O_2$: C, 61.7; H, 4.9; N, 10.8. Found, C, 61.7; H, 5.0; N, 10.6.

Compound ***6b***. Crystallization using 1/19 ethyl acetate/hexane afforded **6b** (0.06 g, 44%) as bright orange needles. Mp: 198−200 °C. IR (thin film): ν_{max} 1023 cm^{-1} (C═N stretch).; ^{1}H NMR (δ, 270.0 MHz, DMSO-d_6); 3.90 (3H, s, OCH_3), 4.01 (3H, s, OCH_3), 4.23 (5H, s, C$_5H_5$), 4.39 (2H, t, J = 2.7 Hz, Fc), 4.53 (2H, t, J = 2.7 Hz, Fc), 6.81 (1H, s, ArC*H*), 7.13 (1H, dt, J = 8.1, 1.2 Hz, ArC*H*), 7.23 (1H, s, ArC*H*), 7.36 (1H, dt, J = 8.1, 1.8 Hz, ArC*H*), 7.77−7.80 (2H, m, 2 × ArC*H*), 8.50 (1H, dd, J = 10.8, 1.2 Hz, ArC*H*), 8.68 (1H, s, N*H*). ^{13}C NMR (δ, 67.0 MHz, DMSO-d_6): 56.1, 56.2, 68.7 (2*C*), 69.5 (2*C*), 69.7 (5*C*), 84.9 (*ipso*-Fc), 99.4, 108.0, 109.4, 121.8, 123.3, 127.4, 128.4, 130.9, 136.7, 147.4, 149.3, 153.7, 154.5, 156.0. HRMS (*m*/*z*, HNESP): [M + H]$^+$ for $C_{26}H_{24}FeN_3O_2$ calcd 466.1212, observed 466.1205. Anal. Calcd. for $C_{26}H_{23}N_3O_2Fe{\cdot}0.2CH_2Cl_2$; C, 64.8; H, 4.9; N, 8.6. Found, C, 64.8; H, 4.9, N; 8.5.

Compound ***6c***. Crystallization using 1/19 ethyl acetate/hexane afforded **6c** (0.12 g, 87%) as a bright orange solid. Mp: 200−202 °C. IR (thin film): ν_{max} 1030 cm^{-1} (C═N stretch). ^{1}H NMR (δ, 270.0 MHz, $CDCl_3$): 4.03 (3H, s, OCH_3), 4.04 (3H, s, OCH_3), 4.06 (5H, s, C$_5H_5$), 4.31 (2H, t, J = 2.7 Hz, Fc), 4.63 (2H, t, J = 2.7 Hz, Fc), 7.06 (1H, s, ArC*H*), 7.27 (2H, m, ArC*H*), 7.50 (2H, d, J = 5.4 Hz, ArC*H*), 7.62 (2H, d, J = 5.4 Hz, ArC*H*), 8.68 (1H, s, N*H*). ^{13}C NMR (δ, 100.5 MHz, $CDCl_3$); 56.3, 56.4, 66.3 (2*C*), 68.9 (2*C*), 69.6 (5*C*), 85.1 (*ipso*-Fc), 99.5, 107.7, 109.0, 121.9 (2*C*), 126.6 (2*C*), 135.4, 136.4, 147.2, 149.5, 153.5, 154.7, 156.3. HRMS (*m*/*z*, HNESP): [M + H]$^+$ for $C_{26}H_{24}FeN_3O_2$ calcd 466.1212, observed 466.1255; Anal. Calcd for $C_{26}H_{23}N_3O_2Fe{\cdot}0.2CH_2Cl_2$; C, 65.2; H, 4.9; N, 8.7. Found, C, 65.2; H, 5.2; N, 8.6.

X-ray Crystallography. Single-crystal X-ray diffraction analyses were performed using either a Bruker APEXII CCD diffractometer mounted at the window of a Bruker FR591 rotating anode ($\lambda_{Mo\ K\alpha}$ = 0.710 73 Å) and equipped with an Oxford Cryosystems cryostream device (**6a**) or at Station I19 of the Diamond Light Source (λ = 0.6889 Å), using a Crystal Logics κ-geometry diffractometer and a Rigaku Saturn 724+ CCD detector with a Cryostream cooler (at 120 K) and CrystalClear-SM Expert 2.0 r7 used to record images[12] (**6c**). Data were processed using either the Collect package[13] (**6a**) or CrystalClear-SM Expert 2.0 r7 (**6c**), and unit cell parameters were refined against all data. An empirical absorption correction was carried out using SADABS[14] (**6a**) or CrystalClear-SM Expert 2.0 r7 (**6c**). The structures were solved by direct methods using SHELXS-97 and refined on F_o^2 by full-matrix least-squares refinements using SHELXL-97.[15] All non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were added at calculated positions, except those of the water molecule in **6c**, which were located and restrained to a reasonable geometry. All hydrogen atoms were refined using a riding model with isotropic displacement parameters based on the equivalent isotropic displacement parameter (U_{eq}) of the parent atom. Figures were produced using OLEX2.[16] The CIF files for the crystal structures of **6a**,**c** have been deposited with the CCDC and have been given the deposition numbers 906133 and 906134, respectively.

ASSOCIATED CONTENT

Supporting Information

Tables and CIF files giving crystallographic data for **6a**,**c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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