Luminescent rhenium(I) diimine indole conjugates – photophysical, electrochemical and protein-binding properties

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Electronic Supplementary Information

1. Synthesis and characterisation data

(i) *N*-(3-Pyridoyl)tryptamine

A mixture of nicotinic acid succinimidyl ester (128 mg, 0.58 mmol) and tryptamine (93 mg, 0.58 mmol) in 20 ml CH_2Cl_2 was stirred under nitrogen for 12 h. The white precipitate formed was removed by filtration. The filtrate was then evaporated to dryness to yield a yellow solid, which was purified by column chromatography on silica gel using acetone as the eluent. Recrystallisation of the product from acetone–diethyl ether afforded *N*-(3-pyridoyl)tryptamine as pale yellow crystals in 98 mg yield (64%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 10.03 (s, 1H; NH of indole ring), 9.02 (s, 1H; H2 of pyridine), 8.66 (d, 1H, J = 4.7 Hz; H6 of pyridine), 8.18 (d, 1H, J = 7.9 Hz; H4 of pyridine), 8.04 (s, 1H; py-CON*H*), 7.63 (d, 1H, J = 7.9 Hz; H4 of indole ring), 7.44 (dd, 1H, J = 7.9 and 4.7 Hz; H5 of pyridine), 7.37 (d, 1H, J = 7.6 Hz; H7 of indole ring), 7.20 (s, 1H; H2 of indole ring), 7.09 (t, 1H, J = 7.5 Hz; H6 of indole ring), 7.00 (t, 1H, J = 7.3 Hz; H5 of indole ring), 3.75 – 3.69 (m, 2H; NHCH_2CH$_2$), 3.08 ppm (t, 2H, J = 7.2 Hz; NHCH$_2$CH_2). IR (KBr): ν = 3278 (m, NH), 3053 (m, NH), 1650 (s, C=O), 1537 cm^{-1} (s, NH). Positive-ion ESI-MS: m/z = 266 $[M + H^+]^+$.

(ii) *N*-(3-Pyridoyl)-6-aminohexanoic acid

Nicotinic acid succinimidyl ester (1 g, 4.70 mmol) in 10 ml DMF was added to a

mixture of 6-aminohexanoic acid (613 mg, 4.70 mmol) and triethylamine (2 ml, 14.0 mmol) in 10 ml DMF. The mixture was stirred under nitrogen at room temperature overnight. After which, the mixture was evaporated to dryness to give a white solid. Recrystallisation of the solid from acetone–diethyl ether afforded *N*-(3-pyridoyl)-6-aminohexanoic acid as white crystals in 1 g yield (95%). IR (KBr): ν = 3353 (m, NH), 1711 (s, C=O), 1632 (s, C=O), 1540 cm^{-1} (s, NH). Positive-ion ESI-MS: m/z = 237 $[M + H^+]^+$.

(iii) *N*-(3-Pyridoyl)-6-aminohexanoic acid succinimidyl ester

N-(3-Pyridoyl)-6-aminohexanoic acid (102 mg, 0.43 mmol) was dissolved in 5 ml hot DMF. After the solution was cooled to room temperature, a mixture of *N*-hydroxysuccinimide (76 mg, 0.66 mmol) and *N,N'*-dicyclohexylcarbodiimide (112 mg, 0.54 mmol) in 2 ml DMF was added with stirring. A white solid (dicyclohexylurea) appeared immediately. The suspension was stirred under nitrogen at room temperature overnight. The dicyclohexylurea was then removed by filtration and the filtrate was evaporated to dryness to yield a while solid. Recrystallisation of the solid from acetone–diethyl ether afforded *N*-(3-pyridoyl)-6-aminohexanoic acid succinimidyl ester as white crystals in 106 mg yield (73%). IR (KBr): ν = 3327 (m, NH), 1735 (s, C=O), 1627 (s, C=O), 1577 (s, C=O), 1534 cm^{-1} (m, NH). Positive-ion ESI-MS: m/z = 334 $[M + H^+]^+$.

(iv) *N*-[*N*-(3-Pyridoyl)-6-aminohexanoyl]tryptamine

N-(3-Pyridoyl)-6-aminohexanoic acid succinimidyl ester (1.2 g, 3.80 mmol) was dissolved in 8 ml hot DMF. After the solution was cooled to room temperature, a mixture of tryptamine (611 mg, 3.80 mmol) and triethylamine (1.6 ml, 11 mmol) in 9 ml DMF was added. The mixture was stirred under nitrogen at room temperature overnight. After which, the mixture was evaporated to dryness. The pale yellow solid was purified by column chromatography on silica gel using acetone as the eluent. Recrystallisation of the product from acetone–diethyl ether afforded *N*-[*N*-(3-pyridoyl)-6-aminohexanoyl]tryptamine as pale yellow crystals in 855 mg yield (60%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 10.03 (s, 1H; NH of indole ring), 9.04 (s, 1H; H2 of pyridine), 8.65 (d, 1H, J = 4.7 Hz; H6 of pyridine), 8.20 (d, 1H, J = 7.9 Hz; H4 of pyridine), 7.97 (s, 1H; py-CON*H*), 7.57 (d, 1H, J = 7.6 Hz; H4 of indole ring), 7.42 (dd, 1H, J = 7.9 and 4.7 Hz; H4 of pyridine), 7.35 (d, 1H, J = 7.3 Hz; H7 of indole ring), 7.13 – 6.96 (m, 4H; CON*H*-CH_2-CH_2-indole and H2, H5 and H6 of indole ring), 3.52 – 3.37 (m, 4H; py-CONH-C*H*$_2$-C_4H_8-CONH-C*H*$_2$-CH_2-indole), 2.92 (t, 2H, J = 7.3 Hz; CONH-CH_2-C*H*$_2$-indole), 2.16 (t, 2H, J = 7.2 Hz; py-CONH-C_4H_8-C*H*$_2$), 1.67 – 1.29 ppm (m, 6H; py-CONH-CH_2-C_3H_6-CH_2). IR (KBr): ν = 3393 (m, NH), 3334 (m, NH), 3247 (m, NH), 1633 (s, C=O), 1534 cm^{-1} (s, NH). Positive-ion ESI-MS: m/z =

379 $[M + H^+]^+$.

(v) *N*-Ethyl-(3-pyridyl)formamide, py-CONH-Et

Ethylamine (0.19 ml, 3.32 mmol) was added to nicotinic acid succinimidyl ester (514 mg, 2.34 mmol) dissolved in 10 ml acetonitrile. The mixture was stirred under nitrogen for 12 h. After which, the mixture was evaporated to dryness under vacuum. The crude product was then purified by column chromatography on silica gel using CH_2Cl_2/acetone (3:1 v/v) as the eluent. Recrystallisation of the product from acetone–diethyl ether afforded py-CONH-Et as white crystals in 270 mg yield (77%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 9.02 (s, 1H; H2 of pyridine), 8.66 (d, 1H, J = 4.7 Hz; H6 of pyridine), 8.19 (d, 1H, J = 7.9 Hz; H4 of pyridine), 7.92 (s, 1H; py-CON*H*), 7.45 (dd, 1H; J = 7.9 and 4.7 Hz; H5 of pyridine), 3.48 – 3.38 (m, 2H; py-CONH-C*H*$_2$-CH$_3$), 1.20 ppm (t, 3H; J = 7.3 Hz; py-CONH-CH$_2$-C*H*$_3$). IR (KBr): ν = 3392 (m, NH), 1711 (s, C=O), 1639 (s, C=O), 1551 cm^{-1} (m, NH). Positive-ion ESI-MS: m/z = 151 $[M + H^+]^+$.

(vi) Complex **1**

A mixture of [Re(Me$_4$-phen)(CO)$_3$(CH$_3$CN)](CF$_3$SO$_3$) (174 mg, 0.25 mmol) and *N*-(3-pyridoyl)tryptamine (66 mg, 0.25 mmol) in 20 ml anhydrous THF was refluxed under nitrogen for 12 h. The mixture was then evaporated to dryness to give a pale yellow solid. Subsequent recrystallisation of the solid from acetone–diethyl ether

afforded **1** as pale yellow crystals in 156 mg yield (69%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 10.03 (s, 1H; NH of indole ring), 9.65 (s, 2H; H2 and H9 of Me_4-phen), 8.89 (d, 1H, J = 2.1 Hz; H2 of pyridine), 8.69 (d, 1H, J = 5.0 Hz; H6 of pyridine), 8.44 (s, 2H; H5 and H6 of Me_4-phen), 8.25 – 8.21 (m, 1H; H4 of pyridine), 8.11 (s, 1H; py-CON*H*), 7.53 (d, 1H, J = 7.9 Hz; H4 of indole ring), 7.41 – 7.34 (m, 2H; H5 of pyridine and H7 of indole ring), 7.12 (d, 1H, J = 2.3 Hz; H2 of indole ring), 7.07 (t, 1H, J = 7.6 Hz; H6 of indole ring), 6.95 (t, 1H, J = 7.5 Hz; H5 of indole ring), 3.61 – 3.54 (m, 2H; NHCH_2CH$_2$), 2.95 (t, 2H, J = 7.3 Hz; NHCH$_2$CH_2), 2.92 (s, 6H; Me at C4 and C7 of Me_4-phen), 2.79 ppm (s, 6H; Me at C3 and C8 of Me_4-phen). IR (KBr): ν = 3309 (m, NH), 2028 (s, C=O), 1936 (s, C=O), 1906 (s, C=O), 1654 (m, C=O), 1163 (m, $CF_3SO_3^-$), 1030 cm^{-1} (m, $CF_3SO_3^-$). Positive-ion ESI-MS: m/z = 771 [M – $CF_3SO_3^-$]$^+$, 506 [M – L – $CF_3SO_3^-$]$^+$. Anal Calcd (%) for $ReC_{36}H_{31}N_5O_7SF_3$: C, 46.95; H, 3.39; N, 7.60. Found: C, 46.73; H, 3.54; N, 7.82. UV/Vis (CH_3CN): λ_{abs}/nm (ε/dm^{3}mol^{-1}cm^{-1}): 250 sh (33,540), 280 (39,525), 290 sh (32,110), 308 sh (16,515), 370 sh (3,375).

(vii) Complex **2**

The preparation of **2** was similar to that of **1** except that *N*-[*N*-(3-pyridoyl)-6-aminohexanoyl]tryptamine (95 mg, 0.25 mmol) was used instead of *N*-(3-pyridoyl)tryptamine. Complex **2** was isolated as yellow crystals in 140 mg

yield (66%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 10.00 (s, 1H; NH of indole ring), 9.64 (s, 2H; H2 and H9 of Me_4-phen), 8.84 (s, 1H; H2 of pyridine), 8.72 (d, 1H, J = 5.6 Hz; H6 of pyridine), 8.44 (s, 2H; H5 and H6 of Me_4-phen), 8.28 (d, 1H, J = 8.2 Hz; H4 of pyridine), 8.02 (s, 1H; py-CON*H*), 7.50 (d, 1H, J = 7.9 Hz; H4 of indole ring), 7.39 – 7.33 (m, 2H; H5 of pyridine and H7 of indole ring), 7.09 – 6.91 (m, 4H; CON*H*-CH_2-CH_2-indole and H2, H5 and H6 of indole ring), 3.44 – 3.24 (m, 4H; py-CONH-C*H*$_2$-C_4H_8-CONH-C*H*$_2$-CH_2), 2.90 (m, 8H; Me at C4 and C7 of Me_4-phen and CONH-CH_2-C*H*$_2$-indole), 2.79 (s, 6H; Me at C3 and C8 of Me_4-phen), 2.12 (t, 2H, J = 7.0 Hz; py-CONH-C_4H_8-C*H*$_2$), 1.59 – 1.23 ppm (m, 6H; py-CONH-CH_2-C_3H_6-CH_2). IR (KBr): ν = 3329 (m, NH), 2029 (s, C=O), 1931 (s, C=O), 1649 (m, C=O), 1158 (m, $CF_3SO_3^-$), 1030 cm^{-1} (m, $CF_3SO_3^-$). Positive-ion ESI-MS: m/z = 885 [M – $CF_3SO_3^-$]$^+$, 506 [M – L – $CF_3SO_3^-$]$^+$. Anal Calcd (%) for $ReC_{42}H_{42}N_6O_8SF_3$: C, 48.78; H, 4.09; N, 8.12. Found: C, 48.64; H, 4.27; N, 7.95. UV/Vis (CH_3CN): λ_{abs}/nm (ε/dm^{3}mol^{-1}cm^{-1}): 247 sh (31,295), 281 (37,640), 292 sh (27,870), 323 sh (11,245), 368 sh (3,370).

(viii) Complex **3**

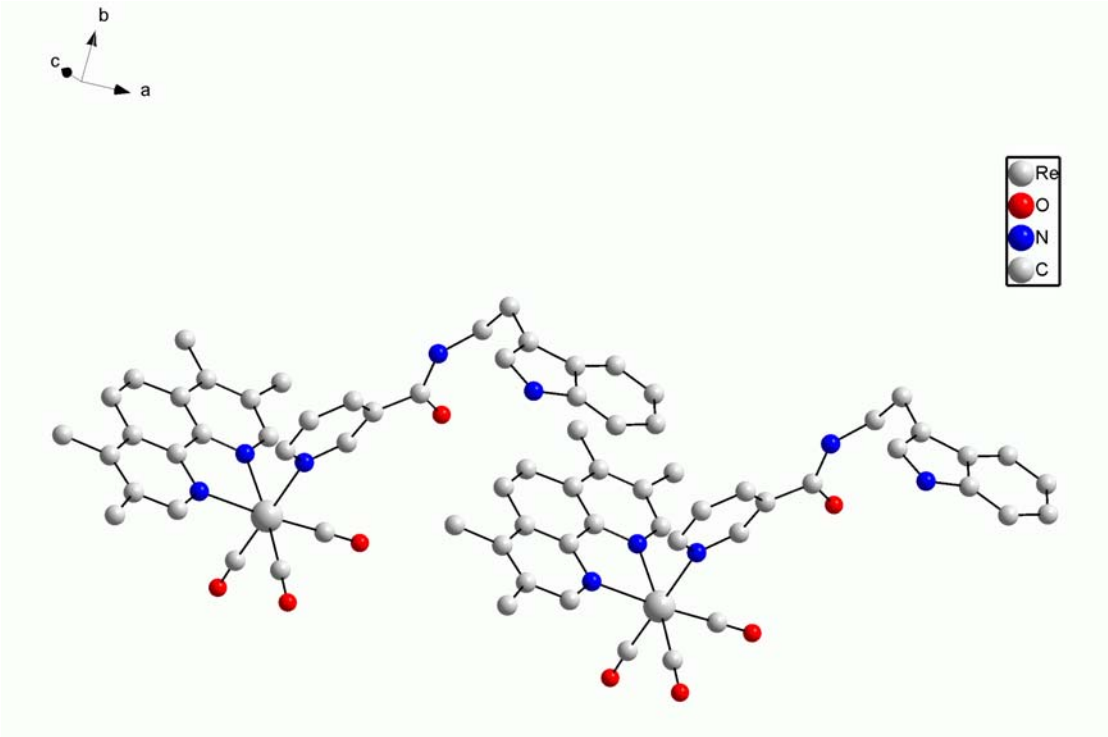
The preparation of **3** was similar to that of **1** except that py-CONH-Et (38 mg, 0.25 mmol) was used instead of *N*-(3-pyridoyl)tryptamine. The crude product was purified by column chromatography on alumina using methanol as the eluent.

Subsequent recrystallisation of the product from acetone–diethyl ether afforded **3** as yellow crystals in 108 mg yield (63%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 9.66 (s, 2H; H2 and H9 of Me-$_4$-phen), 8.85 (s, 1H; H2 of pyridine), 8.71 (d, 1H, J = 5.9 Hz; H6 of pyridine), 8.45 (s, 2H; H5 and H6 of Me$_4$-phen), 8.25 (d, 1H, J = 7.9 Hz; H4 of pyridine), 7.99 (s, 1H; py-CON*H*), 7.40 (dd, 1H; J = 5.6 and 7.9 Hz; H5 of pyridine), 3.36 – 3.25 (m, 2H; py-CONH-C*H*$_2$-CH$_3$), 2.93 (s, 6H; Me at C4 and C7 of Me$_4$-phen), 2.79 (s, 6H; Me at C3 and C8 of Me$_4$-phen), 1.08 ppm (t, 3H; J = 7.3 Hz ; py-CONH-CH$_2$-C*H*$_3$). IR (KBr): ν = 2033 (s, C=O), 1931 (s, C=O), 1900 (s, C=O), 1152 (m, $CF_3SO_3^-$), 1024 cm^{-1} (m, $CF_3SO_3^-$). Positive-ion ESI-MS: m/z = 657 [M – $CF_3SO_3^-$]$^+$, 507 [M – py-CONH-Et – $CF_3SO_3^-$]$^+$. Anal Calcd (%) for $ReC_{28}H_{26}N_4O_7SF_3$: C, 41.74; H, 3.25; N, 6.95. Found: C, 41.54; H, 3.29; N, 6.70. UV/Vis (CH_3CN): λ_{abs}/nm (ε/dm^{3}mol^{-1}cm^{-1}): 249 sh (32,570), 281 (34,870), 316 sh (14,130), 371 sh (3,540).

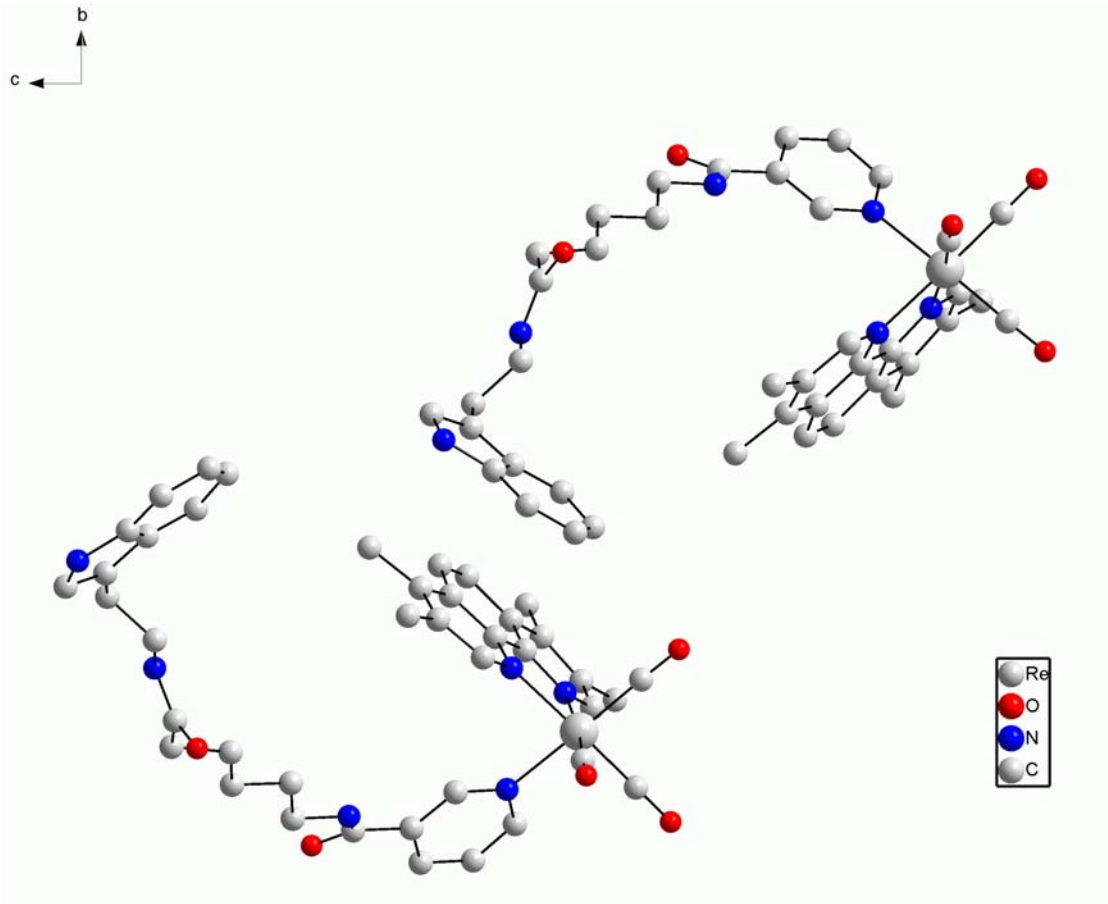
2. Additional information on the crystal structure solution of complex 2

The oxygen atoms of the $CF_3SO_3^-$ anion were disordered into two sets of positions. One acetone solvent molecule was also located with disorder by sharing the central C atom. Restrains were applied to the disordered acetone molecule, assuming flatness of the molecule and similar 1,2- and 1,3-bond lengths or distances (i.e. C43–C44 and C43–C45; C43–C44' and C43–C45'), respectively.

3. Perspective views of two neighbouring cations of complex 1 showing intermolecular stacking interactions

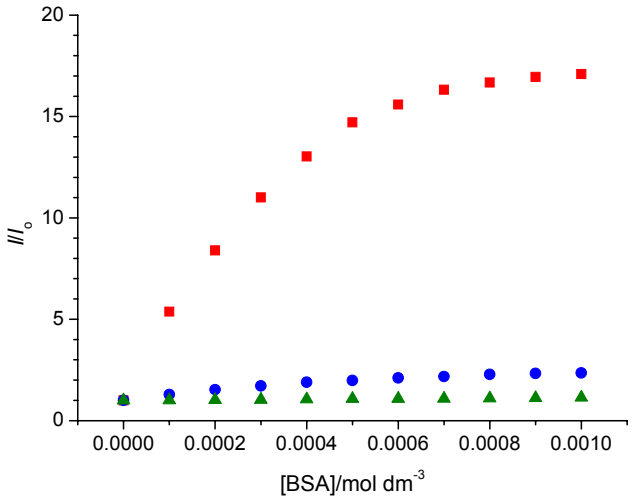


4. Perspective views of two neighbouring cations of complex 2 showing intermolecular stacking interactions



5. Cumulative emission titrations of complexes 1 – 3 with bovine serum albumin (BSA)

Complex **1**, **2** or **3** (0.12 μmol) dissolved in 50 μl MeOH/phosphate buffer solution (1:1 v/v) was added to BSA dissolved in 450 μl 50 mM potassium phosphate buffer pH 7.4. The concentration of BSA in the solutions ranged from 1×10^{-3} to 1×10^{-4} M. The solutions were gently stirred in the dark at room temperature for 12 h. The emission spectra of the solutions were then measured and compared to that of the control solutions in which BSA was absent.



Results of emission titrations of **1** (●), **2** (■) and **3** (▲) with BSA. I and I_o are the emission intensity of the solutions in the presence and absence of BSA, respectively.

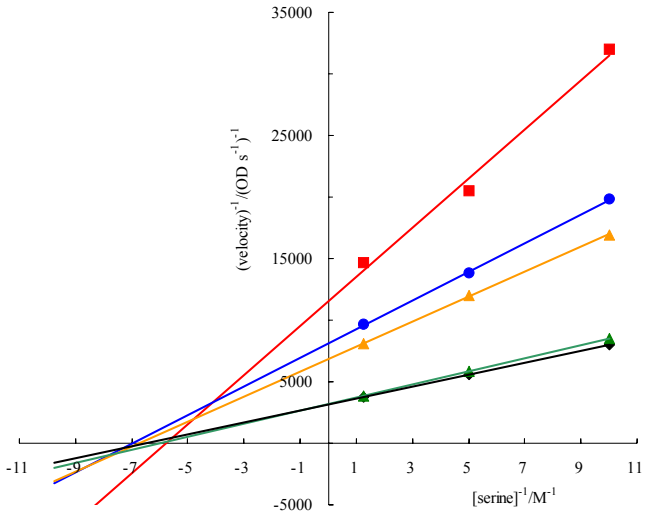
Binding constants of **1** and **2** with BSA were estimated to be ca. 1.0×10^4 and 1.7×10^4 M^{-1}, respectively, from the Scatchard plots ($\nu/[Re]_{free}$ vs. ν) where $\nu = [Re]_{bound}/[BSA]_{total}$. The binding stoichiometry, n, was determined to be ca. 0.6 and 0.7 for **1** and **2**, respectively.

6. Conjugation of Malachite Green isothiocyanate to lysozyme

Malachite Green isothiocyanate (Molecular Probes) (0.20 mg) in 100 μl anhydrous DMSO was added to lysozyme (4.0 mg) dissolved in 1 ml 50 mM carbonate buffer pH 9.0. The solution was stirred gently in the dark at room temperature for 4 h. Then the solution was loaded onto a PD-10 column (Pharmacia) that had been equilibrated with 50 mM potassium phosphate buffer pH 7.4. The first green band that came out from the column was collected and the solution was extensively dialysed against 50 mM potassium phosphate buffer pH 7.4. The solution was then concentrated by centrifugation with a YM-10 centricon (Amicon). The dye-to-protein ratio of the conjugate lysozyme–MG was determined to be ca. 0.3.

7. Tryptophanase inhibition assays

Complex **1**, **2**, **3** or free indole (0.10 μmol) dissolved in 100 μl MeOH/phosphate buffer solution (2:1 v/v) was added to a mixture of L-serine, pyridoxal phosphate (0.125 μmol), lactate dehydrogenase (8.225 U) and NADH (0.70 μmol) in 1.57 ml 0.15 M potassium phosphate buffer pH 8.1 in an absorption cuvette. The temperature of the solution was then thermostated at 37 ^{o}C for 5 min. The conversion of L-serine to pyruvate was then initiated by adding tryptophanase (5 U in 830 μl phosphate buffer) to the mixture. The concentration of L-serine in the cuvette varied from 100 to 800 mM. The formation of pyruvate is coupled to the lactate dehydrogenase/NADH system and is therefore associated with a decrease of absorbance at 340 nm due to the consumption of NADH. The percentage of inhibition was determined by comparing the decrease of absorbance of the solution to that of the control in which the rhenium(I) complexes or indole were absent.



The above figure is a plot of (velocity)$^{-1}$ vs. [serine]$^{-1}$ for the tryptophanase inhibition assays, in which inhibitors **1** (●), **2** (■), **3** (▲) and indole (▲) were employed, or when no inhibitor (♦) was used. From the *x*-intercepts, K_m values were determined to be ca. 143, 172, 166 and 149 mM for **1**, **2**, **3** and indole, respectively. When no inhibitor was used, L-serine showed a K_m of 154 mM. The plot shows that **1** and **2** inhibited the tryptophanase-catalysed conversion of L-serine to pyruvate in a similar fashion as observed for indole.