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Inhibitors of the serotonin transporter protein (SERT): The design and synthesis of biotinylated derivatives of 3-(1,2,3,6-tetrahydro-pyridin-4-yl)-1*H*-indoles. High-affinity serotonergic ligands for conjugation with quantum dots

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Abstract—There is a growing demand for compounds with specificity for the serotonin transporter protein (SERT) that can be conjugated to cadmium selenide/zinc sulfide core shell nanocrystals. This letter describes the design and synthesis of two different biotinylated SERT antagonists that can be attached to streptavidin-coated cadmium selenide/zinc sulfide core shell nanocrystals.

Cadmium selenide/zinc sulfide core shell nanocrystals or 'quantum dots' are novel semiconducting crystals that have unique physical properties that differ significantly from those of bulk material.[1] They have fluorescence line widths in the visible region of the electromagnetic spectra and do not rapidly photobleach. Our research efforts are based on applications of quantum dots as fluorescent tags for receptors and transporter proteins: Quantum dots have several distinct advantages over conventional fluorescent dyes, such as narrower emission spectra and increased brightness.[2–4] Several groups have reported attaching antibodies, nucleic acids, and proteins to quantum dots.[5–9]

Our approach has been to attach neurotransmitter and drug derivatives to nanocrystals.[10–12] We hope to be able to use such conjugates to image biological systems in vitro.[10,13] The emission spectra of quantum dots are size-tunable thus enabling the possibility of experiments where several biological targets are studied simultaneously and their increased photostability should enable experiments that can be run over significantly longer periods of time than is currently achievable with conventional dyes.

Specifically, we are developing systems to study the serotonin transporter protein based on derivatives of known compounds that can be attached to quantum dots. We hope to use such conjugates to gain greater insights into the biological mode of action of selective serotonin reuptake inhibitors. Also by dynamic and static fluorescent imaging, we hope to gain more knowledge about the distribution and localization of the serotonin reuptake protein within neuronal cell cultures.

In our early studies, we synthesized a derivative of serotonin (**1**).[14] This ligand was attached directly to the surface of a quantum dot via an acid–base interaction between a thiol in the ligand and the zinc on the surface of the nanocrystal. This ligand was shown to be biologically active and had an IC_{50} of 115 μM, when assayed against HeLa cells transiently transfected with human serotonin transporters (hSERT) (see Fig. 1).

Unfortunately, (**1**) lacked high affinity and specificity for hSERT. For our studies we required ligands with biological activities in the nanomolar region and specificity for SERT. Therefore, we designed and synthesized new ligands with high affinity and specificity for SERT that could be attached to quantum dots.

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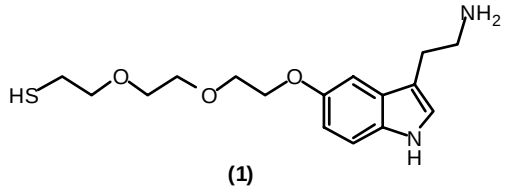


Figure 1. A pegylated derivative of serotonin.

A review of the literature showed that the indole[15] derivative 3-(1,2,3,6-tetrahydropyrindin-4-yl)-1*H*-indole (**2**) can be derivatized giving a biologically active compound **3**.[16–18] From these results, we reasoned that the pyridinyl nitrogen in 3-(1,2,3,6-tetrahydropyrindin-4-yl)-1*H*-indole (**2**) occupies a region of steric tolerance. We decided to attach linker arms to the nitrogen atom in the tetrahydropyridinyl indole derivative (**2**) resulting in **4**. X in compound **4** is a functionality that can be attached to the quantum dot (see Fig. 2).

We have observed that the fluorescence of quantum dots decreases (quenches) when thiols are attached directly to the surface of the quantum dot. We hoped that a ligand that contained another functionality may not quench the fluorescent emission of the dot. For this reason, we decided to attach biotin to the end of our linker arm as the point of attachment to quantum dots.

Our first biotinylated ligand was *N*-(2-(4-(1*H*-indol-3-yl)-5,6-dihydropyridin-1(2*H*)-yl)ethyl-5-(2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamide (**5**) and this was synthesized as shown in Scheme 1. Initially, indole was reacted with 4-piperidone monohydrate monohydrochloride in refluxing methanolic KOH for 6 h to give **2** in a 81% yield. The tetrahydropyridinyl nitrogen was alkylated by refluxing **2** in acetone with *N*-(2-bromoethyl)phthalimide in the presence of potassium carbonate for 18 h resulting in 2-(2-(4-(1*H*-indol-3-yl)-5,6-dihydropyridin-1(2*H*)-yl)ethyl)isoindoline-1,3-dione (**6**) in a 20% yield. The phthalimide group was removed using hydrazine monohydrate in ethanol at room temperature giving (**7**) in a 40% yield and this was coupled to biotin using DCC and NHS in

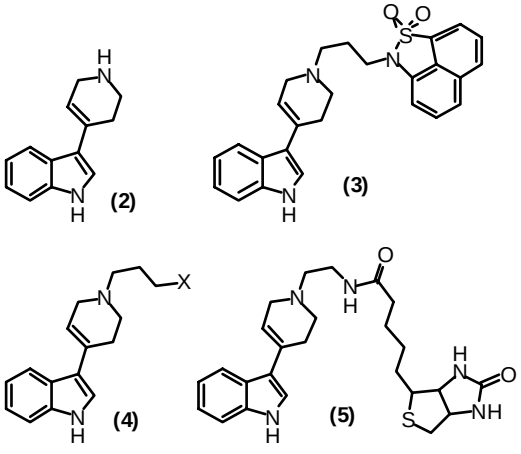
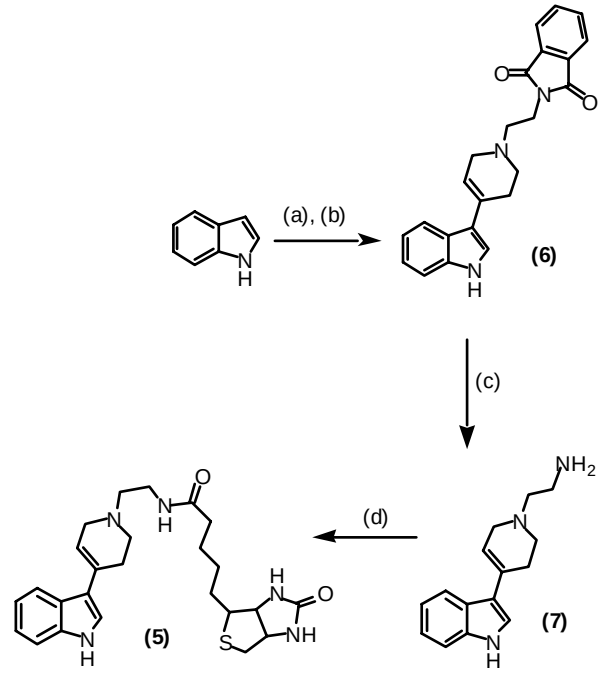


Figure 2. SERT selective ligands.

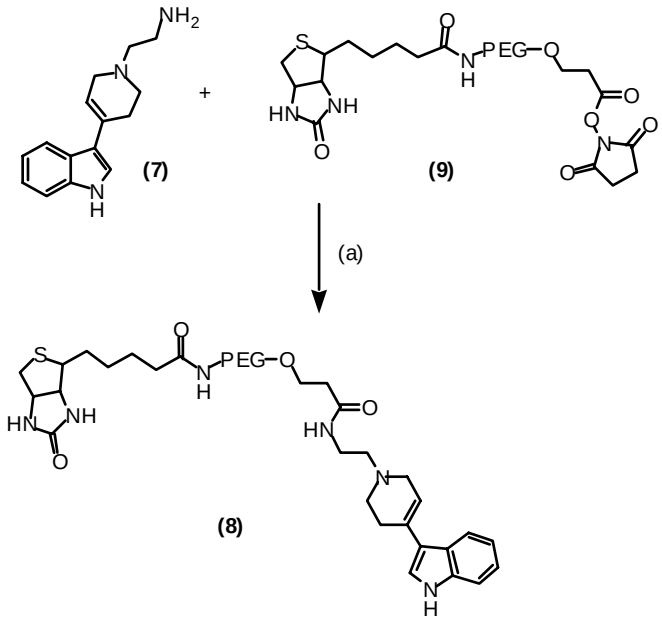


Scheme 1. Reagents: (a) 4-piperidone monohydrate monohydrochloride, KOH, MeOH; (b) *N*-(2-bromoethylene)phthalimide; (c) H_2NNH_2; (d) biotin, DCC, NHS.

methylene chloride by stirring for two days at room temperature, resulting in **5** in a 21% yield.

We thought that the short linker arm in (**5**) may reduce the biological activity of the compound when it was bound to streptavidin. There may be considerable steric interactions between the surface of the dot and the SERT transporter protein. In order to determine if this was happening, we synthesized **8**. The synthetic route of (**8**) is outlined in Scheme 2. In this route, 2-(4-(1*H*-indol-3-yl)-5,6-dihydropyridin-1-(2*H*)-yl)ethanamine (**7**) was dissolved in methylene chloride and stirred at room temperature for 18 h with a NHS-biotinylated PEG derivative (**9**) that was purchased from the Nektar Therapeutics Corporation of HuntsvilleAlabama. The polyethylene glycol in this linker arm was large and the molecular mass of **9** was 3400 g wt. The methylene chloride was removed under reduced pressure and the crude biotinylated material was attached to quantum dots.

Streptavidin-conjugated quantum dots were obtained from the Quantum Dot Corporation of Haywood California, and compounds **5** and **8** were attached to these dots. Compound (**5**) was dissolved in DMSO and added to a borate buffer solution containing quantum dots at a known concentration. This mixture was stirred overnight at room temperature and purified via column chromatography on a Sephadex column. The fluorescent band was collected and assayed against HEK293 cells transfected with hSERT. The concentration of the dots was measured using a UV–vis spectrophotometer[19] and the IC_{50} value for the conjugate is based upon the concentration of dots. The conjugate of **8** was prepared in a similar manner to the conjugate of **5**; however, the DMF



Scheme 2. Reagent and conditions: (a) CH_2Cl_2, rt, 24 h.

was replaced with borate. Where appropriate the IC_{50} of the free ligand was also determined.

Table 1 shows the biological activities of the conjugated ligands **5** and **8** as well as free ligand **5**. IC_{50} values for the parent drug (**2**) and our initial compound (**1**) in free solution and conjugated to dots are also shown. When ligands were conjugated to the streptavidin conjugated dots, there was no observable decrease in the fluorescent intensity of the dots. However, when the ligand **1** was attached to quantum dots, there was a significant reduction in fluorescence that could be observed by the naked eye. These observations indicated that the thiol in **1** may be quenching the dots and consequently ligands with a thiol attached to a linker arm should be avoided whenever possible. Also, the proximity of the ligand to quantum dots does not appear to have a detrimental effect on the biological activity of the conjugate.

In conclusion, we have prepared two biotinylated ligands (**5** and **8**) that may be attached to streptavidin-conjugated quantum dots. These ligands have high affinity for hSERT when in solution and bound to dots, and the length of the linker arm between the ligand and the dot doesnot appear to significantly affect the biological activity of the conjugate. These ligands have higher affinities for hSERT than our first ligand and we observed no quenching when the ligand was bound to the dot. We hope to be able to use these conjugates in fluorescent imaging assays in future studies.

Table 1. IC_{50} values for free ligands and ligands bound to quantum dots against the SERT transporter protein

Compounds	Free ligand inhibition IC_{50} (nM)	Bound ligand inhibition IC_{50} (nM)
1	115,000[a]	99,000[a]
2	80[b]	na
5	2	30
8	1[c]	100

[a] Values were obtained using HeLa cells.
[b] Literature value (na = not applicable).
[c] IC_{50} value of crude ligand.

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