SYNTHESIS OF N_2S_2 CONJUGATES OF THE HIGHLY SPECIFIC MITOCHONDRIAL DIAZAPAM BINDING INHIBITOR (DBI) RECEPTOR COMPLEXES

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Abstract- A novel approach for the synthesis of N_2S_2 ligands conjugated with 2-phenylindole analogs known as mitochondrial diazapam binding inhibitor receptor complexes (previously known as peripheral-type benzodiazepine receptor), as possible target specific Tc-99m imaging agents is described.

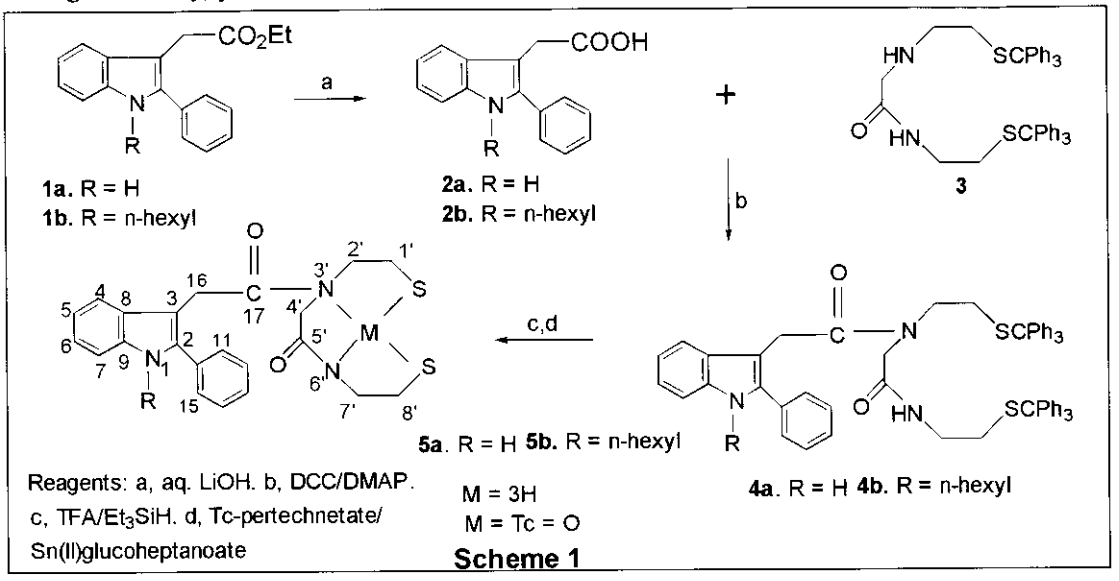
INTRODUCTION

For the last decade, conventional nuclear imaging has successfully visualized the brain with chemicals that can be radiolabeled (usually with Tc-99m). After peripheral intravenous injection, they penetrate the normal, intact blood-brain barrier and localize in brain parenchyma. Because their localization is virtually proportional to local brain blood flow, the images are "functional", reflecting an extremely wide variety of disease and even psychological states, varying from Alzheimer's to Attention Deficit Disorder. Despite the unquestioned value of nuclear imaging for this type of study, there is enormous room for improvement. Even the best of the current crop of nuclear neuroimaging compounds, [ethylcystein dimer (ECD) and hydroxymethylpropyleneamineoxime (HMPAO)], accumulate in the brain at only 4-8% of the injected dose. Thus, various nuclear medicinal scientists have focused their attention in developing target specific brain-avid imaging agents.[1]

RESULTS AND DISCUSSION

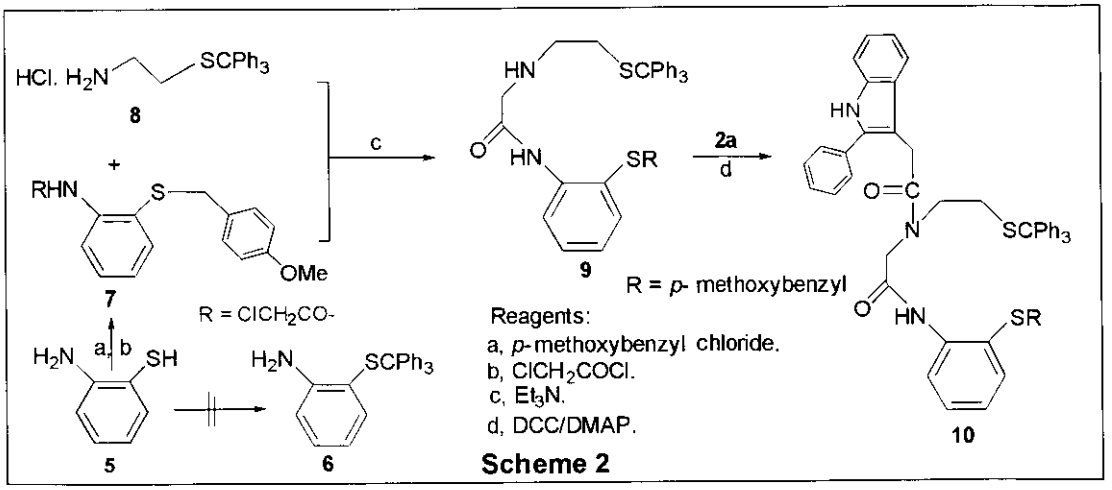
Recently, Kozikowski *et al.*[2] reported a novel class of 2-aryl-3-indolacetamides as potent and highly specific mDRC ligands, previously called peripheral benzodiazepine receptors (PBR). PBRs occur in particularly high density in steroid-producing tissues, such as adrenal, testes, ovary, placenta and brain. These authors also reported the synthesis of an indole derivative, namely the 7-nitrobenzofuran derivative

of 2-aryl-3-indoleacetamide as a fluorescent probe. The comparative binding studies of this compound with radiolabeled PK 11195 indicate that it might be a useful material to quantify the localization and function of PBR in different tissues, including the brain. It has been assumed that mDRC ligands may indirectly modulate GAB Aergic and glutamatergic transmission by virtue of their effects on glial cell steroid biosynthesis. Therefore, recent research on mRDC ligands has been carried out in order to provide drugs that can be used to modify neurosteroid production and to rectify neuropsychiatric abnormalities (pathological anxiety, panic and depression).



Scheme 1

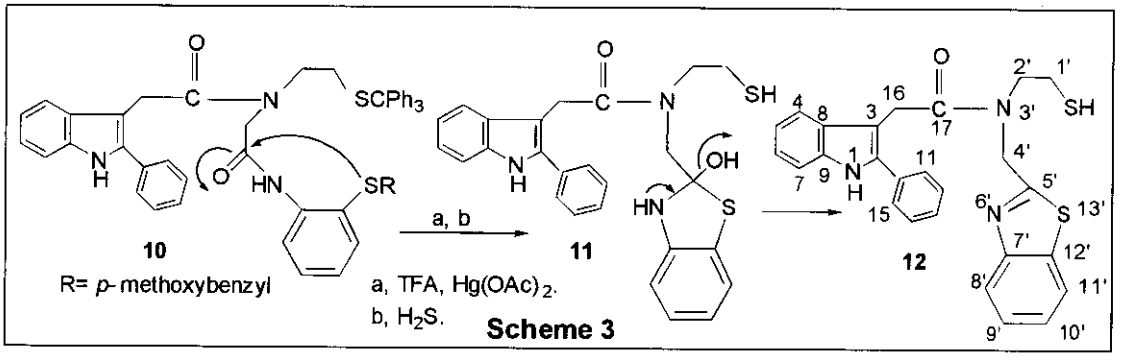
In our attempts to prepare target specific Tc-99m imaging agents, two series of compounds were synthesized from 2- aryl - 3-ethoxycarbonylmethyl indole (**1a**).[3] In our first approach, the ethyl ester



Scheme 2

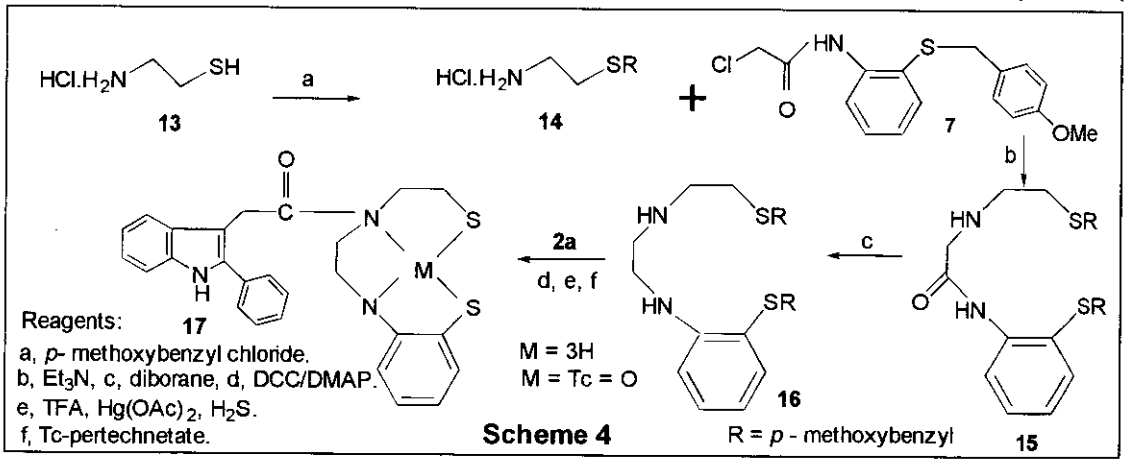
functionality in indole (**1a**) was converted into the corresponding carboxylic acid (**2a**) by reacting with aqueous lithium hydroxide. Further reaction of **2a** with thiol-protected bis-aminoethanethiol ligand

(**3**)[4] and subsequent deprotection with triethylsilane/TFA afforded the desired bisaminoethanethiol conjugate (**5a**) in >90% yield. In order to determine the effect of lipophilicity in drug uptake, the indole **1a** was converted to the corresponding *N*-hexyl analog (**1b**) which upon a sequence of reactions was converted into (**5b**) (Scheme 1). Under similar conditions, reaction of indolecarboxylic acid (**2a**) with an unsymmetrical ligand (**9**) (see Scheme 2) gave the thiol-protected intermediate (**10**) in a good yield. However, at the final step of the synthesis the removal of the S-protecting group under acidic conditions did not produce the desired thiol; instead a benzothiazole (**12**) was obtained *via* acid cyclization (see



Scheme 3

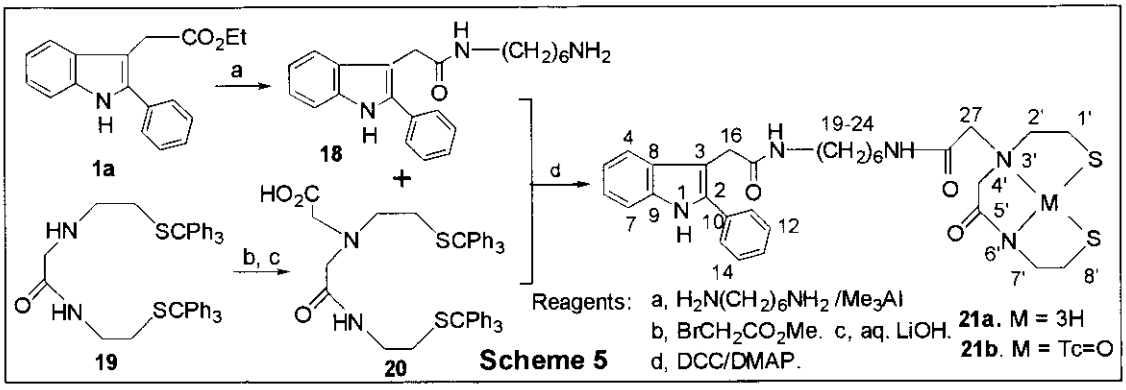
Scheme 2). Thus, in our attempts to achieve the desired indole conjugate, we followed the reaction sequence as shown in Scheme 4. In brief, the thiol group of amonoethanethiol (**13**) was first protected by reacting with *p*-methoxybenzylchloride before condensing with **7** to afford the protected thiol ligand (**15**) in 75% yield. Diborane reduction of **15** and further reaction with indole carboxylic acid (**2a**)



Scheme 4

after deprotection generated the indole conjugate (**17**) as a main product (Scheme 4). In another approach, to understand the effect of the length of carbon chain between the indole molecule and the ligand in localization of the drug, a spacer group containing six carbon units was attached to indole moiety by reacting **1a** with trimethylaluminum and hexamethyllenediamine. With slight modification in the

published reaction conditions and the purification procedure, the desired amine (**18**) was obtained in 60 % yield as cream colored foamy solid. It was then condensed with ligand (**20**) [for the preparation of the ligand, see Scheme 5] bearing an acetic acid side chain to afford the the thiol protected conjugate. The deprotection of the thiol groups was achieved by reacting the conjugate with TFA/Et_3SiH and the desired bis-thiol (**21a**) was isolated in quantitative yield. The structures of the newly synthesized compounds were confirmed by NMR and MS spectrometry analyses.



Scheme 5

EXPERIMENTAL[5]

The NMR spectra were recorded on Bruker Am-400 spectrometer and $CDCl_3$ was used as solvent with TMS as internal standard. FAB-MS was performed on MAT-90 spectrometer. All organic reagents were obtained from Aldrich Chemical Corporation except that [^{99m}Tc] pertechnetate, which was obtained from Department of Nuclear Medicine of Roswell Park Cancer Institute. Silica gel 60 F_{254} (Whatman Ltd) plates of 0.25 mm thickness were used for analytical thin-layer chromatography (TLC). Preparative TLC was performed on 20×20 cm TLC plates (Analtech, Inc.).

Compound (4a): 100 mg (0.4 mmol) of **2a** and 270 mg (0.4 mmol) of **3** were dissolved in 30 mL of dry CH_2Cl_2. 90 mg (0.44 mmol) of DCC and 54 mg (0.44 mmol) of DMAP were added successively. The reaction mixture was stirred at rt for 20 h. The solvent was removed, the resulting residue was subjected to column (silica gel) and eluted with CH_2Cl_2/acetone (20:1) to give 353 mg of **4a** as white gum, the yield was 97%. ^{1}H NMR ($CDCL_3$, δ ppm): Two sets of resonances in the ratio of 3:1 were observed. δ 8.05 (1H, s, H-1), 7.70-6.90 (39H, m, aromatic H), 5.99 (1H, t, J=5.7 Hz, H-6'), 3.69 (2H, s, H-16 or 4'), 3.62 (2H, s, H-16 or 4'), 3.02 (2H, t, J=7.5 Hz, H-2' or 7'), 2.80 (2H, t, J=6.1 Hz, H-2' or 7'), 2.23 (2H, t, J=7.4 Hz, H-1' or 8'), 2.19 (2H, t, J=7.3 Hz, H-1' or 8'). Another set: 7.97 (1H, s, H-1), 7.70-6.90 (39H, m, aromatic H), 5.99 (1H, t, J=5.7 Hz, H-6'), 3.79 (2H, s, H-16 or 4'), 3.28 (2H, s, H-16 or 4'), 2.88 (2H, t, J=7.5 Hz, H-2' or 7'), 2.51 (2H, t, J=6.4 Hz, H-2' or 7'), 2.37 (2H, t, J=5.5 Hz, H-1' or 8'), 1.93 (2H, t, J=6.5 Hz, H-1' or 8'). MS (FAB) calculated for $C_{60}H_{53}N_3O_2S_2$: 911. Found: m/z 912 $[M+1]^+$.

Compound (12): 100 mg (0.12 mmol) of **10** was dissolved in 2 mL of TFA and 0.1 mL of anisole at 0 °C, then 84 mg (0.26 mmol) of $Hg(OAc)_2$ was added. The mixture was stirred at 0 °C for half hour. TFA was removed with high vacuum at rt to give a brown oil, n-hexane was added to above oil, the resulting dark brown crystals were filtered and washed with n-hexane, dried under high vacuum in the presence of NaOH for 15 min. The solid was then suspended in 9 mL of EtOAc/EtOH (2:1), H_2S gas was bubbled into the solution for 10 min, and the reaction mixture was filtered, the filtrate was evaporated, the residue was purified by preparative TLC with 3% $MeOH/CH_2Cl_2$ as eluant to give 27 mg of **12** as yellow oil in 49% yield. ^1H NMR: Two sets of resonances in the ratio of 3 to 1 were observed. δ 8.40-7.10 (13H, m, aromatic H), 4.89 (2H , s, H-4'), 4.09 (2H , s, H-16), 3.43 (2H, t, J=7.4 Hz, H-2'), 2.36 (2H, dd, J=7.4 Hz, H-1'). Another set: δ 8.40-7.10 (13H, m, aromatic H), 4.78 (2H, s, H-4'), 4.08 (2H, s, H-16), 3.58 (2H, t, J=7.0 Hz, H-2'), 2.65 (2H, dd, J=7.0 Hz, H-1'). MS (FAB) calculated for $C_{26}H_{33}N_3OS_2$: 457. Found: m/z 458 $[M+1]^+$.

Compound (17) (M=3H): Following the procedure discussed above, the title compound was obtained in 53% yield from **2a.** NMR ($CDCl_3$, δ ppm): Two sets of resonances in the ratio of 3 to 2 were observed: 8.20-6.20 (13H, m, aromatic H), 4.04 (2H, s, H-16), 3.58 (2H, t, J=6.0 Hz, H-4'), 3.30 (2H, t, J=6.0 Hz, H-5'), 3.25 (2H, t, J=7.6 Hz, H-2'), 2.28 (2H, dd, J= 7.6 Hz, H-1'). Another set: 8.20-6.20 (13H, m, aromatic H), 3.98 (2H, s, H-16), 3.48 (2H, t, J=7.2 Hz, H-2' or H-4'), 3.42 (2H, t, J=6.4 Hz, H-2' or H-4'), 3.02(2H, br, H-5'), 2.65 (2H, dd, J= 7.6 Hz, H-1'). MS (FAB) calculated for $C_{26}H_{27}N_3OS_2$: 461. Found: m/z 461$[M^+]$.

Compound (21a): The indole amine (**18**) was converted into **21a** in 46 % yield by following the method as discussed for the preparation of **4a**. NMR ($CDCl_3$, δ ppm): 8.71 (1H, t, J=3.2 Hz, N-H), 7.60-7.18 (9H, m, aromatic H), 6.94 (1H, t, J=6.0 Hz, N-H), 5.81 (1H, t, J=5.2 Hz, N-H), 3.83 (2H, s, H-16), 3.45 (2H, dd, J=6.8 Hz, H-19 or H-24), 3.21 (2H, dd, J=6.0 Hz, H-19 or H-24), 3.20 (2H, s, H-4' or H- 27), 3.18 (2H, s, H-4' or H- 27), 3.13 (2H, dd, J=6.4 Hz, H-7'), 2.79 (2H, t, J=6.4 Hz, H-2'), 2.65 (4H, m, H-1' and 8'), 1.60-1.10 (8H,m, H-20, 21, 22 and 23). MS (FAB) calculated for $C_{30}H_{14}N_5O_3S_2$: 583. Found: m/z 584$[M+1]^+$.

Radiolabeling Procedure

The Tc-99m complex of each ligand was prepared by ligand-exchange reaction with ^{99m}Tc pertechnetate reduced by Sn(II)glucoheptonate by following the methodology of Kung and coworkers and is also discussed briefly in our preceding paper The radiolabeling yield was > 80%. The purity of each Tc-99m complex was >95%, determined by HPLC. The biological studies with these compounds are currently in progress.

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REFERENCES

1. (a) G.B. Saha, Fundamentals of Nuclear Pharmacy, *Springer-Verlag,* 1992. (b) Z. D. Grossman and S. F. Rosebrough Clinical Radioimmunoimaging, *Grune & Stratton, Inc.*, 1988.
2. A. P. Kozikowaski, M. Kotoula, D. Ma, M. Boujard, W. Tuckmantel, and V. Papadopoulos, *J. Med. Chem.*, 1997, **40**, 2435.
3. A. P. Kozikowaski, D. Ma, D. J. Breuer, S. Sun, E. Costa, E. Romeo, and A. Guidotti, *J. Med.Chem.*, 1993, **36**, 2908.
4. G. Li, G. Q. Ma, B. Ma, D. Grossman, and R. K. Pandey, the preceding paper.
5. The experimental details of only the key products are discussed. The synthetic procedure for the preparation of other compounds with detailed biological studies is in progress and will be reported in our full paper.
6. S. K. Meegalla, K. Plossl, M-P. Kung. S. Chumpradit, D. A. Stevenson, D., S. A. Kushner, W. T. McElgin, P. D. Mozley, and H. F. Kung, *J. Med. Chem., 1997*, **40**, 9.

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