

**DEVELOPMENT OF NOVEL LIGANDS FOR
TARGETED MOLECULAR IMAGING: SYNTHESSES
AND THEIR APPLICATIONS**

KANCHAN CHAUHAN



**DEPARTMENT OF CHEMISTRY
INDIAN INSTITUTE OF TECHNOLOGY DELHI
JANUARY 2016**

**DEVELOPMENT OF NOVEL LIGANDS FOR
TARGETED MOLECULAR IMAGING: SYNTHESSES
AND THEIR APPLICATIONS**

by

KANCHAN CHAUHAN

Department of Chemistry

Submitted

in fulfillment of the requirements of the degree of

Doctor of Philosophy

to the



Indian Institute of Technology Delhi

January 2016

Dedicated to my parents...

CERTIFICATE

This is to certify that the thesis entitled, **“DEVELOPMENT OF NOVEL LIGANDS FOR TARGETED MOLECULAR IMAGING: SYNTHESSES AND THEIR APPLICATIONS”** being submitted by **Ms. KANCHAN CHAUHAN** to the Indian Institute of Technology Delhi for the award of the degree of **Doctor of Philosophy** in Chemistry, is an authentic record of research work carried out by her. She has worked under the guidance and supervision of Prof. Ajai K. Singh and Dr. Anupama Datta. The work has not been submitted, in part or in full, to any other university or institute for award of any degree or diploma. Ms. Kanchan has fulfilled the requirements for the submission of this thesis, which to my knowledge has reached the requisite standard.

Dr. AJAI K. SINGH

Professor

Department of Chemistry

Indian Institute of Technology Delhi

New Delhi-110 016

Dr. ANUPAMA DATTA

Scientist ‘D’

Institute of Nuclear Medicine and

Allied Sciences, DRDO

Delhi-110 054

Date:

Place: New Delhi

ACKNOWLEDGEMENTS

The attainment of doctoral degree was a far flanged but a greatly yearned dream as I started to pursue my graduation in Chemistry (Honors) course in 2005. My dream of getting doctoral degree became a reality when I joined as a Ph.D scholar at INMAS under the guidance of my enthusiastic PhD co-supervisor, Dr. Anupama Datta. This work would not have been possible without her guidance, support and constant encouragement. She is a mentor who I have always been able to count on, whether it was troublesome time in laboratory work or any other advice or support I needed. I thank her for valuable suggestions on both research as well as on my career.

Similar, profound gratitude goes to my Ph.D supervisor, Prof. A K Singh, for his guidance, advice, unconditional support and valuable time. His flexibility in scheduling, gentle encouragement and relaxed demeanor made for a good working relationship and the impetus for me to finish.

My most sincere gratitude to Dr. A. K. Mishra, Head, Division of Cyclotron and Radiopharmaceutical Sciences, INMAS, DRDO, Dr. R. P. Tripathi, Director, INMAS, DRDO, and Head, Department of Chemistry, IIT-Delhi for providing me a chance to work as a research scholar in their departments and avail the facilities therein. I acknowledge the help extended by all faculty members during this work.

I would also like to thank my SRC committee members, Prof. S.K. Khare, Prof. Manjeet Jassal and Dr. S. Nagendran for their insightful comments and discussion.

I gratefully acknowledge Defence Research and Development Organization (DRDO) for providing financial support during my research work.

Special mention goes to Dr. A.K. Tiwari, Dr. Shubhra Chaturvedi and Mrs. Krishna Chuttani for their selfless support. I wish to express my deepest reverence to Mr. Ashish Gaur, Dr. Puja Panwar, Dr. Raunak, Dr. Rashi Mathur, Dr. Aruna Kaushik, Dr. Shweta, Dr. Dipti Kakkar, Mrs Pooja Srivastav, Mrs. Ankur Kaul and Mrs. Mita Dutta for their guidance, support and providing a friendly work environment.

I am thankful to all the technical and administrative staff members of INMAS and Department of Chemistry, IIT for their help and cooperation.

A special mention also goes to Mr. Ajay Kumar, who believed in my caliber and introduced me to the wonderful group of INMAS.

The lab members of DCRS group have contributed immensely to my personal and professional time at INMAS. The group has been a source of friendships as well as

good advice and support. I am especially grateful for the fun group of members: Sandhya, Ganesh and Anupriya who not only gave great memories while working in lab but also supported me through all struggles of life. I reserve a special token of thanks to my friends: Swati, Pooja and Vikas for their love, affection, immense assistance and persistent encouragement. I have had the great pleasure to work with my past and present lab members Dr. Jyoti Tanwar, Harleen Khurana, Narmada Bag, Surbhi, Nidhi, Ambika, Swarndeeep, Shivani, Shweta, Swastika, Neelam, Virendra, and Preeti.

Thanks and appreciation to my worthy labmates in IIT, Delhi for their help and support. Especially, Satyendra Kumar, Renu Bhaskar and Pooja Dubey who have been a tremendous support throughout my time in lab. I also thank my friends Meenal Goel and Anuj Thakur for rendering their support and help whenever needed.

My family members deserve special attention for their support and persistent confidence in me. I offer deep gratitude to my fiancé, Prakhar Sengar and his parents for being loving, supportive, encouraging, and patient during the final stages of this PhD.

Words cannot express how grateful I am to my parents who raised me with a love of science and supported me in all my pursuits. I am extraordinarily fortunate to have the blessings of my grandmother. I am grateful for the presence of my brother Rishi in my life who has been the pillar of my strength. I am indebted to them for all their love, encouragement and sacrifices made for sake of my education and career. I dedicate this thesis to my family.

My prayer and thanks to almighty for everything.

(Kanchan Chauhan)

ABSTRACT

The term molecular imaging represents the branch of medical imaging science intended to detect, localize and monitor the key physiological functions in cells, tissue and living systems at cellular and molecular level using sensitive instrumentation and contrast mechanisms. Aiming for the development of probes for early detection of life threatening diseases, tracers labeled with ^{11}C , ^{68}Ga and $^{99\text{m}}\text{Tc}$ have been synthesized. These radiopharmaceuticals, once administered to the patient, can localize to specific organs or cellular receptors. Important factors including half-life of the radioactive nuclide are its mode of decay, cost and availability. The subject matter of the thesis has been divided into five chapters. **Chapter 1** includes a detailed description about various imaging techniques, probes used in molecular imaging and molecular targets. **Chapter 2** describes the materials and methods required in the synthesis and application of bioconjugates in molecular imaging.

Chapter 3 discusses the implications of chalcone based bivalent ligands in PET imaging of Alzheimer's disease and traumatic brain injury by targeting one of the pathological hallmarks, beta amyloid plaques ($\text{A}\beta$). The chapter is divided in two sub-sections where **Section I** focuses on the utilization of ^{11}C radionuclide for PET imaging of beta amyloids. In this respect, **(Chal)₂DEA-Me** has been synthesized and bioevaluated after labeling with ^{11}C . The lead compound has shown the higher binding affinity, thermodynamic stability in comparison to the monomer, **Chal-Me** and classical ligands through computational and *in vitro* studies. It also demonstrated higher brain uptake with suitable brain pharmacokinetics. Taken together, the data suggests that the bivalent approach may be useful to achieve higher biological response towards TBI and AD imaging. **Section II** details the construction and evaluation of homodimeric ligand, **DT(Ch)₂**, targeting $\text{A}\beta$ aggregates. The developed

ligand allows simple synthesis and single-step incorporation of generator based metal radioisotope ^{68}Ga . The complex exhibited good *in vitro* binding affinity towards A β 42 aggregates with K_i in a nanomolar range and high brain uptake with desirable brain pharmacokinetics in healthy brain. These preliminary studies thus indicate that the radiocomplex, $^{68}\text{Ga-DT(Ch)}_2$ may offer a new perspective in generator produced PET imaging probes for A β imaging.

Chapter 4 describes the synthesis of estradiol based bivalent ligands for imaging breast cancer by targeting estrogen receptors. Two variants viz. **(EST) $_2$ DEA-Me** and **(EST) $_2$ DT** with different alkyl spacer arm ($n=2, 4$) have been developed for targeting ER. Both the ligands have been synthesized in three simple and straightforward steps using click chemistry. Synthesis was confirmed by ^1H NMR, ^{13}C NMR and ESI-MS spectrometry. The developed bivalent ligands possessed high affinity towards ER (RBA > 4%). The structure of (EST) $_2$ DEA-Me is proposed for ^{11}C -labeling in order to extend its role in PET imaging of ER(+) tumors. While, (EST) $_2$ DT was further evaluated for its potential role in diagnosis and therapy of ER(+) tumors. Early tumor localization of $^{99\text{m}}\text{Tc}-(\text{EST})_2\text{DT}$ was viable and feasible along with the improved tumor-targeting performance by the bivalent approach. The non-radiolabeled ligand further exhibited anticancer activity against ER(+) cells with IC_{50} comparable to tamoxifen. The underlying mechanism for programmed cell death was the over-expression of ER β and suppression of ER α levels in gene transcription leading to the ROS-mediated mitochondrial apoptosis (intrinsic pathway). The results are sufficiently encouraging for further evaluation of the ligand or the related compounds in possible imaging/therapy of breast cancer.

The last **Chapter 5** focuses on utilizing bismacrocylic agent, **DO3P-AME-DO3P**, a phosphonic acid derivative of DOTA in PET imaging of bone metastasis

after labeling with ^{68}Ga . As generator produced ^{68}Ga can be a new labeling approach for PET imaging, its use with DO3P-AME-DO3P has been evaluated at preclinical level. The ligand was radiolabeled with ^{68}Ga within 10 min in ~93% yield with high kinetic inertness. The first analysis on the synthetic hydroxyapatite depicted high binding of the ligand to the bone structure. Further, *in vivo* PET imaging revealed the high bone uptake. These preliminary analyses demonstrate the potential of the ligand in cost effective bone PET imaging.

TABLE OF CONTENTS

<i>Certificate</i>	i
<i>Acknowledgements</i>	ii
<i>Abstract</i>	iv
<i>Table of Contents</i>	vii
<i>List of Figures</i>	xvi
<i>List of Tables</i>	xxi
<i>Glossary of Symbols and Abbreviations</i>	xxii
 Chapter 1 Introduction	
1.1 Molecular Imaging	1
1.2 Approaches in Molecular Imaging	3
1.2.1 Anatomical Imaging	4
1.2.2 Optical Imaging	5
1.2.3 Nuclear Imaging	6
1.3 Molecular Imaging with Positron Emission Tomography (PET)	7
1.4 Molecular Imaging with Single Photon Emission Computed Tomography (SPECT)	10
1.5 Molecular Imaging Probes	12
1.5.1 Design of Molecular Imaging Probe	13
1.5.2 Computer-Aided Drug Designing for Molecular Imaging	14
1.6 Radiopharmaceuticals	15
1.6.1 Radiopharmaceutical Design	16
1.6.1a Direct Method	17
1.6.1b Chelation	21
1.7 Acyclic Chelating Agents	24
1.7.1 EDTA	24
1.7.2 DTPA	25
1.7.3 Aazta and Derivatives	28
1.7.4 Picolinic Acid based Chelators	29
1.7.5 DFO	31

1.8	Macrocyclic Chelators	32
1.8.1	DOTA	32
1.8.2	NOTA	33
1.8.3	TETA	34
1.9	Suitable Radionuclides for Diagnosis	35
1.9.1	PET Radionuclides	36
1.9.1a	Carbon-11	38
1.9.1b	Gallium-68	41
1.9.2	SPECT Radionuclides	48
1.9.2a	Technitium-99m	48
1.10	Molecular Targets	55
1.10.1	Receptors	55
1.10.1a	Internal Receptors	56
1.10.1b	External Receptors	57
1.11	Neuroscience	57
1.11.1	Neurodegeneration	58
1.12	Pathology of Alzheimer's Disease	60
1.12.1	Protein Aggregation and Amyloid Formation	61
1.12.2	Structural Morphology of A β Plaques	64
1.13	Cancers	65
1.13.1	Pathology of Breast Cancers	67
1.13.2	Estrogen Receptor	67
1.13.3	Estradiol Interaction with Estrogen Receptor	68
1.4	Metastasis	70
1.14.1	Pathology of Bone Metastasis	71
1.14.1a	Cancer Growth in Bones	71
1.14.1b	Mechanism of Bone Metastasis	72
1.15	Radiation Safety	73
1.16	Outline and Scope of Thesis	74
1.17	References	78

Chapter 2 Materials and Methods

2.1	Reagents and Solutions	90
2.1.1	Column Preparation	90

2.1.2	Standard Solutions of Metal Ions	91
2.2	Preparation of Buffer Solutions	91
2.2.1	Phosphate Buffer Saline (PBS)	91
2.2.2	Tris-Buffered Saline (TBS)	91
2.2.3	4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid, N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES)	92
2.2.4	Dulbecco's Modified Eagle Medium (DMEM)	92
2.2.5	Roswell Park Memorial Institute 1640 Medium (RPMI 1640)	92
2.2.6	Phenol Free α -Minimum Essential Medium (α -MEM)	92
2.2.7	Completing Media with FBS	93
2.2.8	10% Fetal Bovine Serum (10% FBS)	93
2.4	Cell Culture	93
2.5	Animal Models	94
2.6	Characterization Techniques	94
2.6.1	Elemental Analysis	94
2.6.2	Nuclear Magnetic Resonance Spectroscopy	94
2.6.3	Mass Spectrometry	95
2.6.4	Infrared Spectrometer	95
2.6.5	High Performance Liquid Chromatography (HPLC)	95
2.6.6	UV-Vis Studies	95
2.6.7	Potentiometric Studies	96
2.6.8	Radiolabeling Studies	96
2.6.9	γ -Scintigraphic Studies	97
2.7	Miscellaneous	97
2.8	Data Analysis	97
2.9	References	98

Chapter 3 Chalcone based Bivalent Ligands as Molecular Imaging Agents for Amyloid Plaques

3.1	Introduction	100
3.2	Prerequisites for A β Imaging Probes	101
3.2.1	High Selectivity/Affinity towards A β Aggregates	101
3.2.2	Specific Radioactivity	101

3.2.3	Metabolism and Position of Label	102
3.2.4	Blood-Brain Barrier Permeability	103
3.3	A β Specific Probes in Nuclear Imaging	103
3.4	Chalcone and its Analogues	107
3.5	Objectives	113
3.6	Experimental Section	114
3.6.1	Computational Methodology	114
3.6.2	Synthesis of <i>tert</i> -Butyl(2-bromoethyl)carbamate (1)	115
3.6.3	Synthesis of (<i>E</i>)-1-(4-Hydroxyphenyl)-3-(4-isopropylphenyl)prop-2-en-1-one (2).	115
3.6.4	Synthesis of (<i>E</i>)-3-(4-(Dimethylamino)phenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (3)	116
3.6.5	Synthesis of (<i>E</i>)- <i>tert</i> -Butyl-2-(4-(3-(4-(dimethylamino)phenyl)acryloyl)-phenoxy)ethylcarbamate (4).	117
3.6.6	Synthesis of (<i>E</i>)-1-(4-(2-Aminoethoxy)phenyl)-3-(4-(dimethylamino)phenyl)-prop-2-en-1-one (5)	117
3.6.7	Synthesis of (<i>E</i>)-1-(4-(2-Bromoethoxy)phenyl)-3-(4-(dimethylamino)phenyl)prop-2-en-1-one (6)	118
3.6.8	Synthesis of (2 <i>E</i> ,2' <i>E</i>)-1,1'-(((Azanediylbis(ethane-2,1-diyl))bis(oxy))bis(4,1-phenylene))bis(3-(4-(dimethylamino)phenyl)prop-2-en-1-one) (7)	118
3.6.9	Synthesis of (2 <i>E</i> ,2' <i>E</i>)-1,1'-((((Methylazanediy)bis(ethane-2,1-diyl))bis(oxy))bis(4,1-phenylene))bis(3-(4-(dimethylamino)phenyl)prop-2-en-1-one) (8)	119
3.6.10	Production of ¹¹ C-Iodomethane	119
3.6.11	Radiolabeling of Chal (2) with ¹¹ C	120
3.6.12	Radiolabeling of (Chal) ₂ DEA (7) with ¹¹ C	121
3.6.13	<i>In Vitro</i> Binding Studies with A β Aggregates	121
3.6.14	PET Imaging	122
3.6.15	Image Analysis	122
3.6.16	General Method for Metabolite Analysis	123
3.6.17	Biodistribution	123

3.6.18	UV-Vis Spectroscopic Measurements using A β Aggregates	123
3.7	Results and Discussion	124
3.7.1	Molecular Modeling	125
3.7.2	Ligand Synthesis and Radiolabeling	129
3.7.3	<i>In Vitro</i> Binding Study with A β 42 Aggregates	133
3.7.4	Small Animal PET Imaging and Biodistribution	135
3.7.5	Metabolite Study	137
3.7.6	UV-Vis Spectroscopic Measurements using A β Aggregates	138
3.8	Conclusions	140
3.9	Experimental Section	142
3.9.1	Synthesis of 5,8-Bis(carboxymethyl)-13-(4-((<i>E</i>)-3-(4-(dimethylamino)phenyl)-acryloyl)phenoxy)-2-(2-(2-(4-((<i>E</i>)-3-(4-(dimethylamino)phenyl)-acryloyl)phenoxy)ethylamino)-2-oxoethyl)-10-oxo-2,5,8,11-tetraazatridecane-1-carboxylic acid (9)	142
3.9.2	Synthesis of ^{69/71} Gallium Complex of DT(Ch ₂) (10)	143
3.9.3	Determination of Thermodynamic Stabilities	143
3.9.4	Radiolabeling	144
3.9.5	Stability Studies	145
3.9.6	Protein Binding Assay	145
3.9.7	Binding Assay using Aggregated A β Peptide	145
3.9.8	Partition Coefficient Determination	146
3.9.9	Blood Kinetics Evaluation	147
3.9.10	PET Acquisition	147
3.9.11	Biodistribution Studies	147
3.9.12	Fluorescence Studies using A β 42 Aggregates	148
3.10	Results and Discussion	148
3.10.1	Ligand Synthesis	149
3.10.2	Thermodynamic Studies	150
3.10.3	Radiochemistry	152
3.10.4	Log P Determination	152
3.10.5	Human Serum Stability and Protein Binding Assay	153
3.10.6	<i>In Vitro</i> Binding Assay	154

3.10.7	Blood Kinetics Evaluation	156
3.10.8	<i>In Vivo</i> PET Imaging and Biodistribution Studies	157
3.10.9	Fluorescence Studies using A β 42 Aggregates	159
3.11	Conclusions	160
3.12	References	161

Chapter 4 Synthesis and Bioevaluation of Ligands and Complexes Specific for ER(+) Tumors

4.1	Introduction	170
4.2	Influence of Structural Modifications in Estradiol on ER Binding	171
4.3	Bivalent Ligand Approach for Targeting ER	174
4.4	Click Chemistry	178
4.4.1	Mechanism of Copper-Catalyzed 1,3-Dipolar Cycloaddition	179
4.4.2	Properties of Triazoles	181
4.5	Objectives	181
4.6	Experimental Section	182
4.6.1	Synthesis of Bis(2-azidoethyl)amine (1)	182
4.6.2	Synthesis of 15-(1-(2-((2-(4-(3,17-Dihydroxy-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6 <i>H</i> -cyclopenta[α]phenanthren-17-yl)-1 <i>H</i> -1,2,3-triazol-1-yl)ethyl)amino)ethyl)-1 <i>H</i> -1,2,3-triazol-4-yl)-14-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6 <i>H</i> -cyclopenta[α]phenanthrene-2,15-diol (2)	182
4.6.3	Synthesis of 15-(1-(2-((2-(4-(17-Hydroxy-3-methoxy-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6 <i>H</i> -cyclopenta[α]phenanthren-17-yl)-1 <i>H</i> -1,2,3-triazol-1-yl)ethyl)amino)ethyl)-1 <i>H</i> -1,2,3-triazol-4-yl)-2-methoxy-14-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6 <i>H</i> -cyclopenta[α]phenanthren-15-ol (3)	183
4.6.4	Synthesis of 2-Azidoethanamine (4)	184
4.6.5	Synthesis of 17-(1-(2-Aminoethyl)-1 <i>H</i> -1,2,3-triazol-4-yl)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6 <i>H</i> -	184

	cyclopenta[α]phenanthrene-3,17-diol (5)	
4.6.6	Synthesis of 5,8-Bis(carboxymethyl)-13-(4-(3,17-dihydroxy-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6 <i>H</i> -cyclopenta[α]phenanthren-17-yl)-1 <i>H</i> -1,2,3-triazol-1-yl)-2-(2-(2-(4-(3,17-dihydroxy-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6 <i>H</i> -cyclopenta[α]phenanthren-17-yl)-1 <i>H</i> -1,2,3-triazol-1-yl)ethylamino)-2-oxoethyl)-10-oxo-2,5,8,11-tetraazatridecane-1-carboxylic acid, (6)	185
4.6.7	Synthesis of 2,2'-(1-Carboxy-2-(carboxymethyl)-13-(4-(3,17-dihydroxy-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6 <i>H</i> -cyclopenta[α]phenanthren-17-yl)-1 <i>H</i> -1,2,3-triazol-1-yl)-10-oxo-2,5,8,11-tetraazatridecane-5,8-diyl)diacetic acid (7)	186
4.6.8	Relative Binding Affinity Assay	186
4.6.9	Radiolabeling with ^{99m}Tc	187
4.6.10	Human Serum Stability	187
4.6.11	Cysteine and Histidine Challenge	187
4.6.12	Cellular Uptake Studies	188
4.6.13	<i>In Vivo</i> Scintigraphy	188
4.6.14	Biodistribution Study	188
4.6.15	MTT Assay	189
4.6.16	Cell Cycle Analysis	189
4.6.17	Morphological Analysis of Cells	190
4.6.18	Evaluation of Apoptosis with Flow Cytometry	190
4.6.19	Cellular ROS Assay	191
4.6.20	Western Blotting	191
4.6.21	ER α and ER β Transcriptional Activation Assay	191
4.6.22	Thermodynamic Studies	192
4.7	Results and Discussion	192
4.7.1	Synthesis of (EST) $_2$ DEA-Me for PET Application	194
4.7.2	Relative Binding Affinity	195
4.7.3	Synthesis of (EST) $_2$ DT for SPECT Application	196

4.7.4	Relative Binding Affinity	198
4.7.5	Radiolabeling and Stability of $^{99m}\text{Tc}-(\text{EST})_2\text{DT}$	198
4.7.6	Cellular Uptake Studies	199
4.7.7	<i>In Vivo</i> Scintigraphy and Biodistribution Studies	201
4.7.8	<i>In Vitro</i> Anti-Cancer Activity	203
4.7.9	Cell Cycle Analysis	205
4.7.10	Apoptosis Assay	205
4.7.11	Cellular ROS Assay	207
4.7.12	Gene Transcriptional Activation Assay and Protein Expression Study	210
4.7.13	Thermodynamic Studies	212
4.8	Conclusions	213
4.9	References	214

Chapter 5 Synthesis and Preclinical Studies of Polyazamacrocyclic Methylene Phosphonate for PET Imaging of Bone Metastases

5.1	Introduction	221
5.2	Objectives	227
5.3.1	Experimental Section	228
5.3.1	Radiolabeling with ^{68}Ga	228
5.3.2	<i>In Vitro</i> Stability in the Presence of Endogenous Metal Ions	229
5.3.3	Human Serum Stability	229
5.3.4	<i>In Vitro</i> Binding with Hydroxyapatite	229
5.3.5	Blood Kinetics	230
5.3.6	PET Imaging	230
5.4	Results and Discussion	230
5.4.1	Radiolabeling with ^{68}Ga	231
5.4.2	<i>In Vitro</i> Stability and Serum Protein Binding Assay	233
5.4.3	Binding with Hydroxyapatite	235
5.4.4	Blood Kinetics	236
5.4.5	<i>In Vivo</i> PET Imaging	237
5.5	Conclusions	237
5.6	References	238

Bio-Data

Annexure I Supporting Information in CD

LIST OF FIGURES

Figure 1.1	Key steps involved in the molecular imaging studies	2
Figure 1.2	(A) Basic principle of PET: comprehensive detection via fixed camera, (B) PET scanner	8
Figure 1.3	Bone scan images of a mouse with ^{18}F sodium fluoride	9
Figure 1.4	(A) SPECT: direction-selected detection via rotating camera, (B) SPECT scanner	11
Figure 1.5	Bone scan images of a mouse after $^{99\text{m}}\text{Tc}$ -MDP injection	11
Figure 1.6	Schematic of a molecular imaging probe	13
Figure 1.7	Schematic of approaches in molecular imaging probes designing	14
Figure 1.8	Labeling strategies for incorporation of radionuclides to targeting entity	17
Figure 1.9	Periodic table depicting suitable radionuclides for diagnostic imaging	36
Figure 1.10	Decay of ^{11}C to stable nuclei ^{11}B	38
Figure 1.11	Schematic representation of ^{68}Ge to ^{68}Zn	43
Figure 1.12	Schematic representation of the decay of ^{99}Mo to ^{99}Ru	49
Figure 1.13	Schematic representation of $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generator	50
Figure 1.14	(A) Examples of steroid hormones (B) interaction of hydrophobic ligand with the intracellular receptor inducing transcriptional changes	56
Figure 1.15	Underlying mechanism for (A) Ion channel; (B) Enzyme linked; (C) G-protein coupled receptors	57
Figure 1.16	Pathology of Alzheimer's disease in the brain	61
Figure 1.17	Mechanism for β -amyloid generation from APP	62
Figure 1.18	(A) Sequence of A β 42 that is derived from human APP (B) Structural constraints in A β 40 and A β 42 fibrils (C) ordered cross beta structure of A β fibrils	63
Figure 1.19	Pictorial representation of A β self assembly	64
Figure 1.20	Human brain sections showing different morphological	65

	states of A β after staining with histological dye	
Figure 1.21	Schematic diagram for ES binding with ER. A-F is the six regions of the ER	70
Figure 1.22	Steps involved in tumor cell metastasis from a primary site to the skeleton	73
Figure 3.1	The binding mode depicted by 3D docking posse and 2D view of ligand interaction diagram of (A) Chal-Me and (B) (Chal) ₂ DEA-Me associated with amyloid beta fibril (2BEG) respectively	127
Figure 3.2	3D docking posse depicting the binding and 2D view of ligand interaction diagram of: (A) ¹²⁵ I-DMIC; (B) ¹⁸ F-AV-45; (C) ¹¹ C-PIB respectively with amyloid beta fibril (2BEG) respectively	129
Figure 3.3	HPLC chromatograms of the precursor 2 and cold methylated product 3	131
Figure 3.4	HPLC chromatograms of the precursor 7 and cold methylated product 8	131
Figure 3.5	Radio HPLC of ¹¹ C-Chal-Me	132
Figure 3.6	Radio-HPLC showing hot synthesis of ¹¹ C-(Chal) ₂ DEA-Me	133
Figure 3.7	(A) Comparative binding assay and (B) Blocking assay of the radiolabeled ligands with A β 42 aggregates	134
Figure 3.8	PET images and time activity curves for ¹¹ C-(Chal) ₂ DEA-Me and ¹¹ C-Chal-Me	136
Figure 3.9	Biodistribution of ¹¹ C-Chal-Me in normal ddy mice	137
Figure 3.10	Biodistribution of ¹¹ C-(Chal) ₂ DEA-Me in normal ddy mice	137
Figure 3.11	Absorbance and fluorescence of Chal-Me and (Chal) ₂ DEA-Me	139
Figure 3.12	Emission spectra of Chal-Me, (Chal) ₂ DEA-Me before and after mixing with A β 42 aggregates	139
Figure 3.13	Species distribution curves calculated for the 1:1 (M:L) complexes of Ga ³⁺ with the ligand DT(Ch) ₂	151

Figure 3.14	Radio TLC of ^{68}Ga -DT(Ch) $_2$ run in 1:1 ammonium acetate and methanol	152
Figure 3.15	<i>In vitro</i> serum stability of ^{68}Ga -DT(Ch) $_2$ at 37 °C	154
Figure 3.16	Scatchard plot of ^{68}Ga -DT(Ch) $_2$ binding to A β aggregates with $K_d = 3.46 \pm 0.41$ nM	155
Figure 3.17	Binding and blocking assay of ^{68}Ga -L {L = DT(Ch) $_2$ } with preaggregated A β -peptide (n = 5). The binding is expressed as % radioactivity bound to A β 42 aggregates	156
Figure 3.18	Blood kinetics graph of ^{68}Ga -DT(Ch) $_2$ administered through ear vein in normal rabbit	157
Figure 3.19	Dynamic lateral planar PET images of normal BALB/c mice shown upto 25 min after i.v. injection of ^{68}Ga -DT(Ch) $_2$	157
Figure 3.20	Fluorescence emission of DT(Ch) $_2$ before (dash line) and after (solid line) mixing with A β aggregates	160
Figure 4.1	Representation of the shortest distance between the 17 α carbons of two estradiol units (space filling: grey = carbon, red = oxygen) within the ER α dimer structure (teal ribbon) (RCSB-PDB file names: 1ERE for ES)	176
Figure 4.2	(A) Structure of a dimeric ER ligand binding domain (LBD) where each ER α monomer LBD has one LBP and one subsite on the ER α LBD surface. (B–E) Bivalent ligands exhibiting four different binding mode with ER α LBD to reveal the progress from intramolecular to intermolecular bivalent binding	177
Figure 4.3	Human serum stability curve ^{99m}Tc -(EST) $_2$ DT and ^{99m}Tc -ESTDT	199
Figure 4.4	Binding assay of ^{99m}Tc -(EST) $_2$ DT with MCF-7 and MDA-MB-231 cells	200
Figure 4.5	Blocking assay of ^{99m}Tc -(EST) $_2$ DT and ^{99m}Tc -ESTDT with MCF-7 cells	201
Figure 4.6	Scintigraphy studies of ^{99m}Tc -(EST) $_2$ DT in orthotopic tumor-bearing nude mice at 0.5 and 2 h	201

Figure 4.7	Biodistribution of $^{99m}\text{Tc}-(\text{EST})_2\text{DT}$ after injection in tumor bearing nude mice	202
Figure 4.8	Effect of $(\text{EST})_2\text{DT}$ treatment on cell cycle of MCF-7 cells	205
Figure 4.9	Morphological alteration of MCF-7 cells by $(\text{EST})_2\text{DT}$ treatment	206
Figure 4.10	Effect of $(\text{EST})_2\text{DT}$ treatment on apoptosis and necrosis of MCF-7 determined by Annexin-V and PI staining	207
Figure 4.11	Effect of $(\text{EST})_2\text{DT}$ treatment on cellular ROS level of MCF-7 cells	208
Figure 4.12	Flow cytometric evaluation ROS dependency for apoptosis of MCF-7 cells induced by $(\text{EST})_2\text{DT}$ treatment	209
Figure 4.13	Effect of $(\text{EST})_2\text{DT}$ treatment on MMP of MCF-7 cells	210
Figure 4.14	Effect of $(\text{EST})_2\text{DT}$ treatment on $\text{ER}\alpha$ and $\text{ER}\beta$ protein	210
Figure 4.15	Flow cytometric evaluation of cellular ROS generation in $(\text{EST})_2\text{DT}$ -treated MCF-7 cells dependency on the estrogen receptor	212
Figure 5.1	Structure of ^{99m}Tc -MDP with the tridentate binding with Ca^{2+} and bone scans with ^{99m}Tc -MDP	224
Figure 5.2	Time course of ^{68}Ga -DO3P-AME-DO3P at (A) room temperature and (B) 90°C for varied concentration of the ligand	233
Figure 5.3	Effect of pH on radiochemical yield of ^{68}Ga -DO3P-AME-DO3P	233
Figure 5.4	Radio-TLC of ^{68}Ga -DO3P-AME-DO3P	233
Figure 5.5	The stability of ^{68}Ga -DO3P-AME-DO3P in (A) the presence of excess concentration of endogenous metal ions and (B) human serum in different time intervals	235
Figure 5.6	<i>In vitro</i> binding and blocking assay of ^{68}Ga -DO3P-AME-DO3P with synthetic hydroxyapatite	235
Figure 5.7	Blood kinetics graph of ^{68}Ga -DO3P-AME-DO3P in healthy rabbit	236

Figure 5.8 PET scan (coronal view) of normal rabbit after 2 h p.i. 237
of ^{68}Ga -DO3P-AME-DO3P

LIST OF TABLES

Table 1.1	Resolution and sensitivity of molecular imaging modalities	4
Table 1.2	Chemical structures of DTPA based derivatives and their labeling conditions	27
Table 1.3	Selected PET radionuclides and their physical properties	38
Table 1.4	Formation constants of selected chelators with Ga^{3+} cation	45
Table 1.5	Common γ -radionuclides utilised in SPECT imaging	48
Table 1.6	Selected $^{99\text{m}}\text{Tc}$ based radiopharmaceuticals used in clinical imaging	55
Table 1.7	Bone metastasis incidences in cancer	71
Table 3.1	Summary of thermodynamic parameters of IFD docking	126
Table 3.2	Inhibition constants (K_i) for ligands binding to $\text{A}\beta 42$ aggregates	135
Table 3.3	Percentage of intact tracer in plasma and brain homogenates of ddY mice	138
Table 3.4	Protonation constants ($\log K$) of ligands	151
Table 3.5	Inhibition constants (K_i) for ligands binding to $\text{A}\beta 42$ aggregates	155
Table 3.6	Biodistribution of ^{68}Ga -DT(Ch) $_2$ after tracer injection in normal mice	158
Table 3.7	Fluorescence profile of DT(Ch) $_2$ with $\text{A}\beta 42$ aggregates	160
Table 4.1	RBA value of developed ligands for ER binding affinity	196
Table 4.2	Anti-proliferative activity of (EST) $_2$ DT in terms of IC_{50}	204
Table 4.3	Stability constant values of metal complexes	213

GLOSSARY OF SYMBOLS AND ABBREVIATIONS

Å	Angstrom
cm	Centimeter
3D	Three dimensional
Da	Dalton
g	Gram
GBq	Gegabecquerel
h	Hour
H	Planck's constant
ΔH	Enthalpy
Hz	Hertz
I	Nuclear spin quantum number
%ID	Percentage of injected dose
KeV	Kiloelectron volt
L	Litre
MBq	Mega becquerel
MeV	Megaelectron volt
MHz	Megahertz
Min	Minute
ml	Millilitre
ms	Millisecond
mM	Millimole
nm	Nanogram
nm	Nanometre
nM	Nanomole
pM	Picomole
ppm	Parts per million
m/z	Mass/charge
M^+	Molecular ion
q	Hydration number
s	Second
ΔS	Entropy
$t_{1/2}$	Half-life

t_R	Retention time
μm	Micromole
μl	Microlitre
α	Alpha
β	Beta
δ	Delta
γ	Gamma
%	Percent
δ	Chemical shift
ν	Frequency
$^{\circ}\text{C}$	Degree centigrade
A β	Beta amyloid
ACN	Acetonitrile
ACSF	Artificial cerebrospinal fluid
AD	Alzheimer's disease
AECM	Artificial extra cellular matrix
APP	Amyloid precursor protein
ATP	Adenosine triphosphate
BAPTA	1,2-Bis(o-aminophenoxy)ethane-N,N,N',N'- tetraacetic acid
BBB	Blood brain barrier
BFC	Bifunctional chelating agents
BM	Biomolecule
BPs	Bisphosphonates
BSA	Bovine serum albumin
CaCO_3	Calcium carbonate
CDCl_3	Deuterated chloroform
CNS	Central nervous system
CT	Computed tomography
DAG	Diacylglycerol
D_2O	Deuterium oxide
DIPEA	N,N-Diisopropyl ethylamine

DO3A	2,2',2''-(1,4,7,10-Tetraazacyclododecane-1,4,7-triyl)triacetic acid
DOTA	2,2',2'',2'''-(1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetrayl)tetraacetic acid
DMEM	Dubecco's modified eagle medium
DMSA	Dimercaptosuccinic acid
DTPA	Diethylenetriaminepentaacetic acid
E ₂	Estradiol
EDTA	Ethylenediaminetetracetic acid
EGFR	Epidermal growth factor receptor
ER	Estrogen receptor
ES	17 α -Ethinylestradiol
ESI	Electron spray ionization
FBS	Fetal bovine serum
FDA	Food and drug association
FDG	Fluorodeoxyglucose
FES	16 α -Flouro-17 β -estradiol
FET	O-(2-[¹⁸ F-fluoroethyl)-l-tyrosine
HA	Hydroxyapatite
HEK cells	Human embryonic kidney cells
HEPES	(4-(2-Hydroxyethyl)-1-piprazineethanesulfonic acid
HPLC	High performance liquid chromatography
HR-MS	High resolution mass spectra
IFD	Induced fit docking
ITLC-SG	Instant thin layer chromatography-silica gel
i.v.	Intravenous
K ₂ CO ₃	Potassium carbonate
KOH	Potassium hydroxide
MDP	Methylene diphosphonate
MeOH	Methanol
MEM	Minimum essential medium
Me ₄ Si	Tetramethyl silane
MPP	Mitochondrial membrane potential

MRI	Magnetic resonance imaging
MTT	3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromine
NaI	Sodium iodide
NIR	Near infra-red
NaOH	Sodium hydroxide
NMR	Nuclear magnetic resonance
NOTA	1,4,7-Triazacyclononane-1,4,7-triacetic acid
PBS	Phosphate buffered saline
PDB	Protein data bank
PET	Positron emission tomography
PI	Propidium iodide
ROI	Region of interest
ROS	Reactive oxygen species
SPECT	Single photon emission computed tomography
THF	Tetrahydrofuron
TLC	Thin layer chromatography
TMS	Tetrametylsilane
TRIS	Tris(hydroxymethyl)aminomethane