Nuclear Medicine

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In-house Developed Synthetic Strategies for PSMA-617 & PSMA-11: Affordable Organic Ligands for Prostate Cancer

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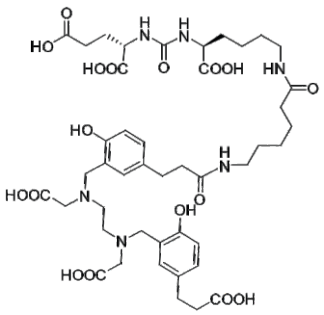

PSMA-11

ABSTRACT

Development of affordable medicines is a promising strategy to make a treatment method accessible to the cancer patients in our country. Organic chelator based radio-ligand treatment method, a branch of medicine generally called as nuclear medicine, is coming up as a primary treatment mode in addressing various cancer and related disorders. A collaborative research program between, Bio-Organic Division (BOD), Board of Research in Isotope Technology (BRIT, Vashi), and Radiopharmaceutical Division (RPhD), was directed towards the development of radiopharmaceuticals, [^{68}Ga]Ga-PSMA-11 and [^{177}Lu]Lu-PSMA-617 for prostate cancer patient treatment in India. In this program, main breakthrough was the in-house synthesis of organic ligands, PSMA-11 and PSMA-617, in cost effective manner, using indigenous synthetic method. Here in, we demonstrate the synthetic challenges that we had to surmount, while pursuing different synthetic routes, to accomplish the goal of making prostate cancer management equitable in India, using radioligand method.

KEYWORDS: *Ligands, Cancer, Prostate, PSMA, Therapy, Diagnosis*

Introduction

Prostate cancer (PC) is commonly encountered in men and it accounts for more than 15% of the total cancer cases. It is estimated that the total number of PC cases globally will be more than 2.9 million in a span of fifteen odd years [1-3], which is more than double that was observed in 2020. Hence, there is urgent need to popularize the most efficient treatment methods, currently available, as well as discovery of new treatment options. PC affects prostate gland in men and can be malignant, which makes it a perilous disease to cure. Most importantly, this disorder is the second major cause of cancer related death in men. Identification of new techniques based on tools that drive on molecular level diagnosis or therapy of PC can be rewarding. Such developments possess great relevance as there is large increase in the number of cancer incidences and related deaths in our country [4] and India is currently considered as the cancer capital of the world.

Staging of prostate cancer using non-invasive techniques like computed tomography (CT) or magnetic resonance imaging (MRI) are less sensitive to disclose the definite condition of the patient and hence are often less successful [5]. Therefore, the requirement for a sensitive technique like radioligand diagnosis, that could stage the disease and estimate the treatment progress after cancer therapy, at molecular level, is essential. In this approach, the identification of biomarker present in the cancer cell and targeting it with appropriate radionuclides [6] or that chelated to organic ligands is crucial [7]. This methodology is well appreciated by physicians and is being successfully utilized in the treatment of various malignant cancers, including PC. The success of this method in the treatment of PC may be credited to the identification and study of prostate specific membrane antigen (PSMA) [8], a type II transmembrane glycoprotein over-expressed on prostate cell surface. Significant expression of PSMA on the prostate cancer cells, compared to normal cells, make it an interesting molecular target. With respect to PC, few such molecules namely; PSMA-11 and PSMA 617 (collectively called as active pharmaceutical ingredients (APIs)), that target PSMA, has been identified and is providing breakthroughs in the designing of new treatment protocols [9,10].

Surgery, often a preferred treatment method for cancer, is not a desired option in PC as it is linked to a vital organ [11-13]. In such circumstances radioligand therapy (RLT) is a genuine option. But for RLT, most of the clinically approved [14] APIs are exorbitantly costly, which makes the treatment expensive. Especially, in countries with high population density and limited infrastructure, the non-availability of these medicines at an affordable cost can hamper the nations progress and most importantly, limits its availability to the needy patients. Considering these aspects, we have taken up the challenge to develop synthetic strategies to achieve these special molecules in affordable mode. Consequently, the past one decade of research resulted in the development of many established organic ligands in economical way. We were successful in achieving three important precursors (1a-c, Fig.1) for [^{18}F]-FLT **1**, [15] a positron emission tomography (PET) based brain cancer imaging agent, and more recently two highly important ligands, PSMA-617 **2** and PSMA-11 **3**, were also realized in affordable manner, using in-house developed synthetic strategies [16-19]. These developments may make the targeted treatment modality affordable to the people of our

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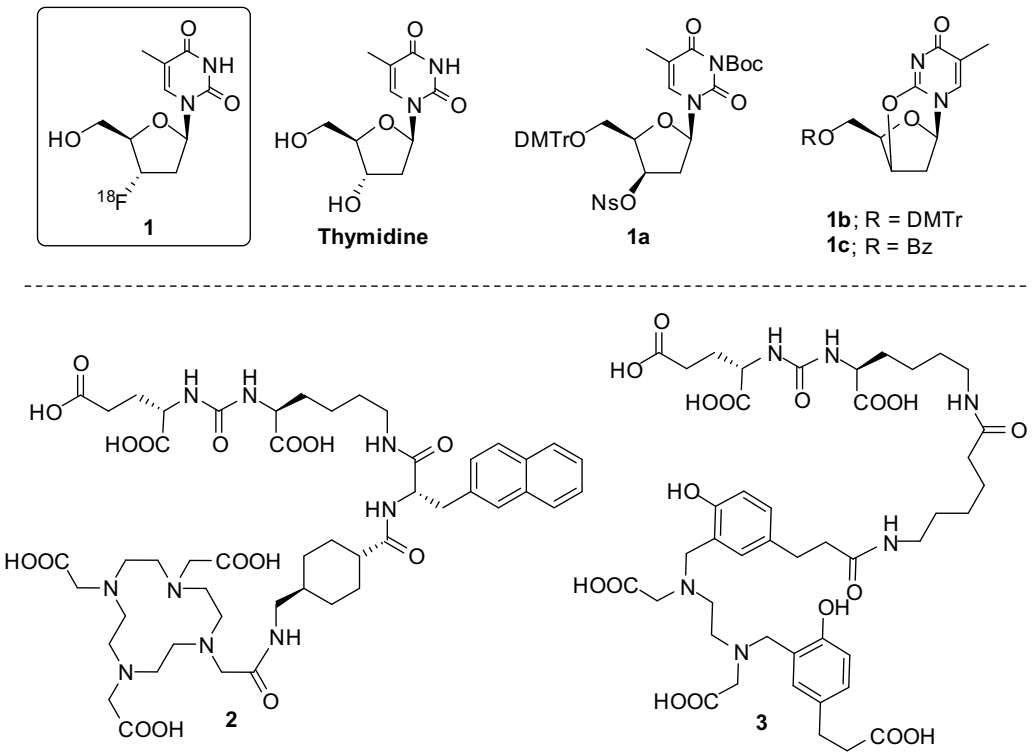


Fig.1: In-house developed organic ligands/precursors: [18F]-FLT (1), precursors for [18F]-FLT (1a-c) synthesized from Thymidine, PSMA-617 (2) and PSMA-11 (3).

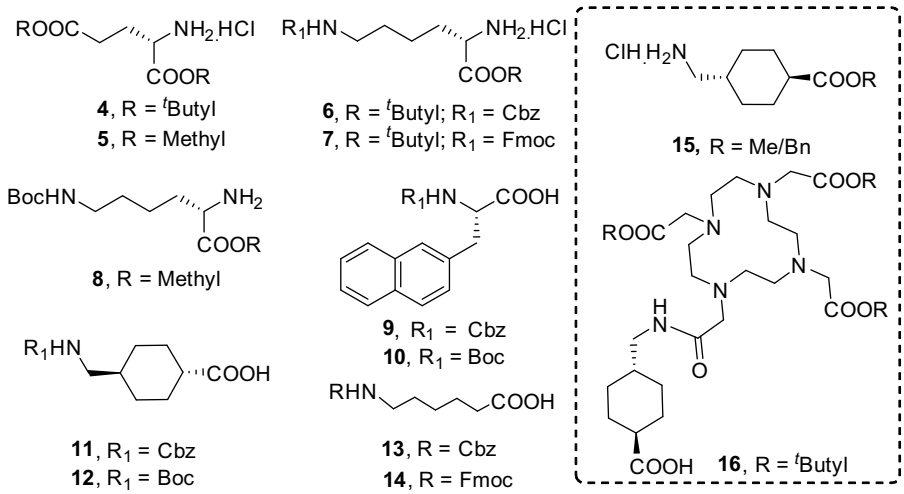


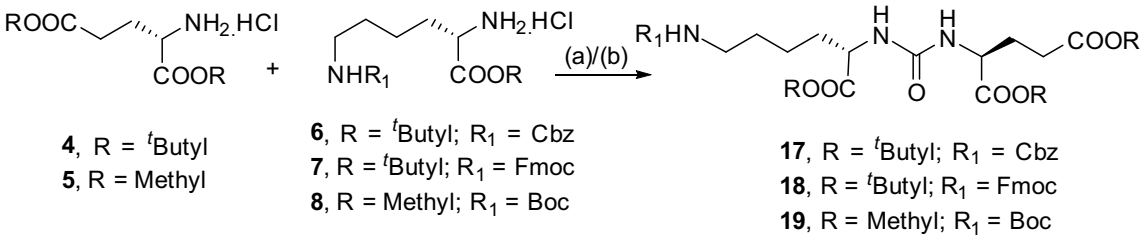
Fig.2: Amino acid templates/fragment for synthesizing PSMA-617 and PSMA-11.

country. In this account, we demonstrated the synthetic challenges that we encountered while employing different synthetic routes for achieving organic ligands, PSMA-617 and PSMA-11, in cost-effective way. After successful synthesis of these ligands, BRIT (Vashi) adopted these ligands as import substitutes, thereby significantly reducing the cost of radiopharmaceuticals, [^{68}Ga]Ga-PSMA-11 and [^{177}Lu]Lu-PSMA-617.

Results and Discussion

For the synthesis of PSMA-11 **2** and PSMA-617 **3** we opted for solution phase method which bestows the flexibility to adopt multiple approaches to the target molecule in the pursuit of a viable method. Inspection of the molecular structure of PSMA-11 **2** and PSMA-617 **3** reveals that it has one part in common, i.e. the dipeptide of glutamic acid and lysine; linked through a carbonyl moiety, which serves as the moiety that binds to the PSMA-protein. Consequently, to achieve the synthesis of this vital part of the target molecule, one can visualize it through the appropriate selection and assembly of differently protected hydrochloride salts (Fig.2) of glutamic acid **4**, and **5** and lysine **6, 7** and **8** [16-19]. Not to mention this would naturally leads towards different synthetic strategies for PSMA-617 and PSMA-11.

Among the two PSMA ligands, we first aimed for the synthesis of PSMA-617, most difficult of the two targets, primarily because of its potential to use as endotherapeutic agent. Nevertheless, due to structural similarity, parallel research efforts were in place for the synthesis of PSMA-11, using Fmoc-strategy. For the synthesis of PSMA-617, apart from the aforementioned hydrochloride salts, appropriately protected two unnatural amino acids, 3-(2-napthyl)-L-alanine derivatives, **9** and **10** and tranexamic acid derivatives, **11** and **12**, were also accomplished in efficient manner from their unprotected commercial equivalents [16-19].

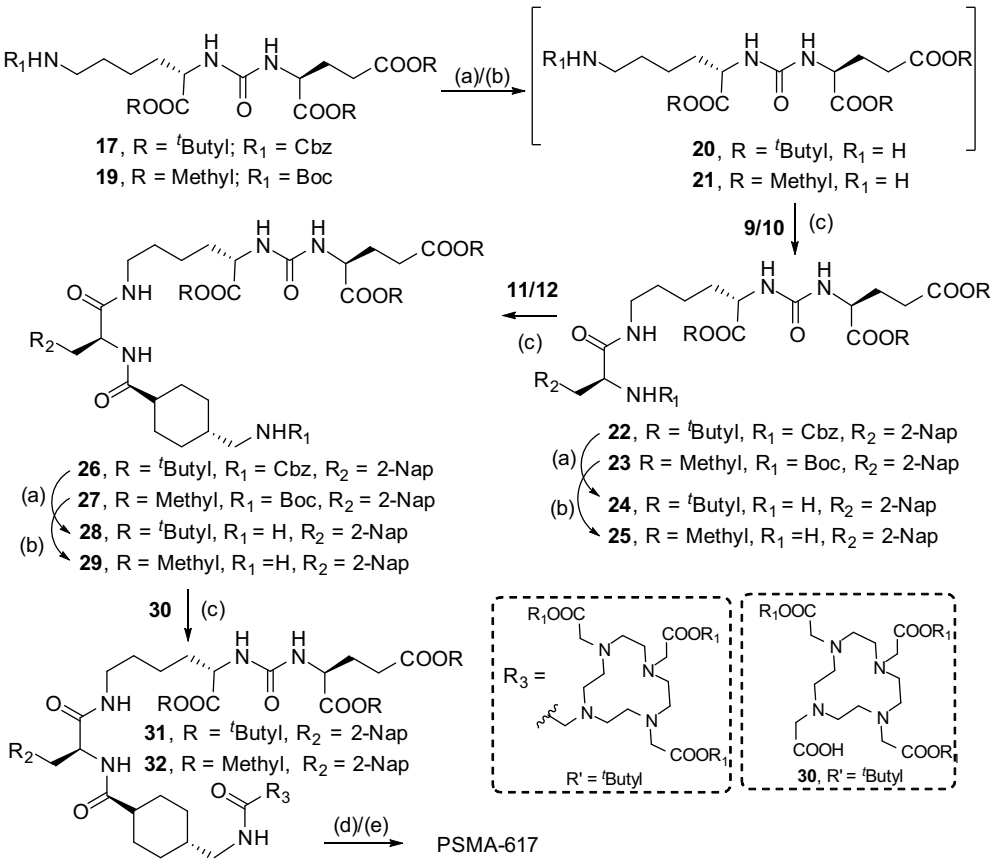


Scheme 1: Synthesis of urea templates (17/18/19): (a) Disuccinimidyl carbonate, DIEA, CH_2Cl_2; (b) $CO(OCCl_3)_2$, DIEA.

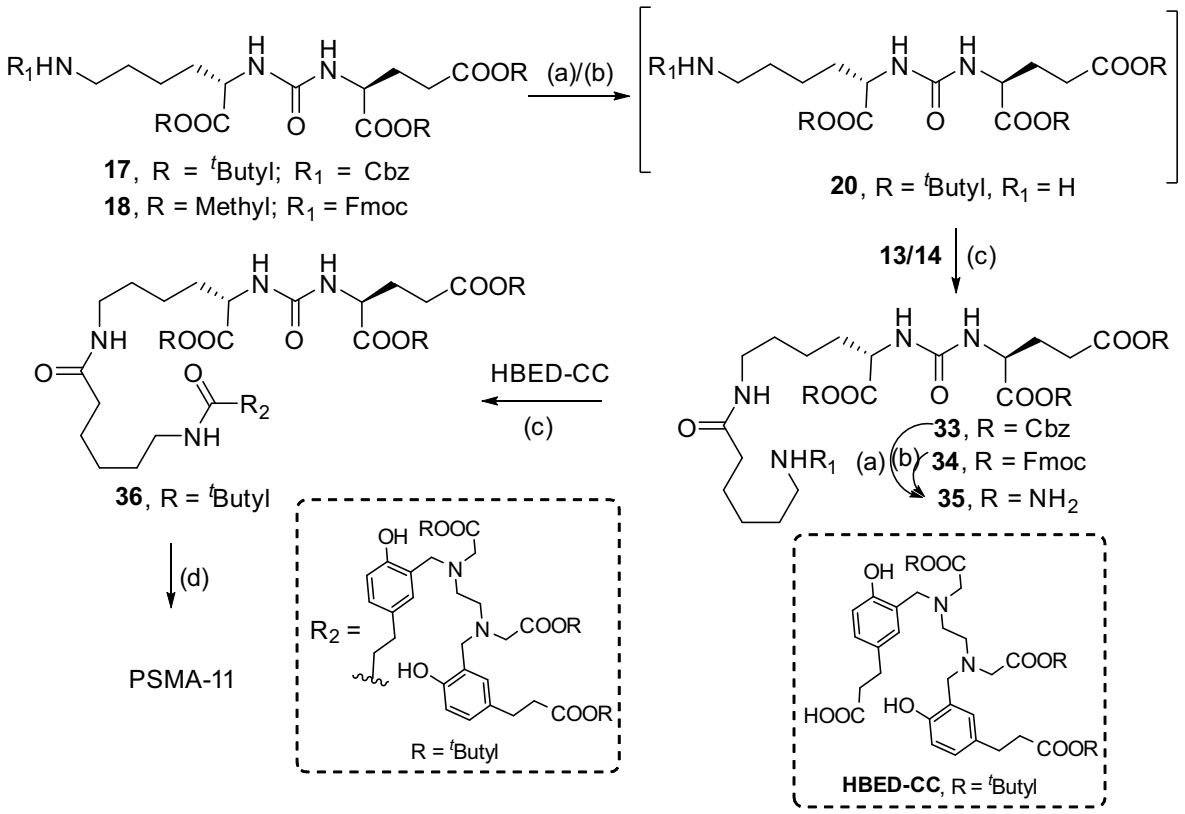
With the required amino acid precursors **4/5** and **6/7/8** in hand, we initiated the synthesis with the construction of three orthogonally protected urea templates **17-19** from the appropriately protected hydrochloride salts of glutamic acid and lysine as shown in Scheme 1. Hence, three urea templates, **17, 18** and **19** were obtained by chemical conjugation of appropriate amino acid residues using disuccinimidyl carbonate or triphosgene as the ligating reagent in the presence of an organic base. Even though the isolated yield of the products **17** and **19** were satisfactory [17,18], **18** was isolable in comparatively less yield [19]. Hence, it was decided to proceed with the synthesis of PSMA-617 using templates **17** and **19**.

Using the template **17**, the Cbz-strategy, wherein the Cbz group in the N-terminal of the synthetic sequence will be unmasked using metal catalysed nonhomogeneous reduction pathway. Therefore, in this synthetic strategy amino acids **9** and **11** would serve as the building blocks prior to the conjugation of DOTA chelator **30**. As a first step of linear Cbz strategy, protecting group in the side chain amine of **17** was deprotected using hydrogenolysis using flow reactor method in the presence of 10% Pd/C to generate corresponding amine **20** (Scheme 2). Subsequent reaction of amine with amino acid residue 9 in the presence of dicyclohexyl carbodiimide (DCC) as coupling agent furnished compound **22**. Repeating the hydrogenation step on **22** yielded amino derivative **24**. Reaction of **24** with tranexamic acid derivative **11** yielded the adduct **26**. Iteration of hydrogenation process on **26** furnished an amine **28** that on coupling with commercially available DOTA derivative **30** yielded fully protected PSMA-617 derivative **30**. Demasking of protecting group in **31** and subsequent purification yielded PSMA-617 **2** as a white foamy solid [17]. Purity and structural integrity of the synthesized compound was confirmed by HPLC, HRMS, NMR analysis and comparative studies with commercial equivalent. Alternatively, synthesis of PSMA-617 through convergent method, which theoretically provides better yield, was also exploited through the coupling of fragment **24** with **16**, (Fig.2) generated by the coupling of **15** with DOTA **30**, to yield **31**. Acidic hydrolysis of **31** furnished PSMA-617, in overall yield, almost similar to that of linear method [17].

Commercially, palladium and palladium based reagents are getting expensive. This prompted us further to search for an alternate method for making PSMA-617. In this regard, we employed Boc-strategy (Scheme 2), wherein amino protection in template **19**, prepared from amino acid constituents **5** and **8**



Scheme 2: Strategies for synthesis of PSMA-617 (2): (a) 10% Pd/C, H_2(15Psi), MeOH; (b) HCl (g), Ethyl acetate, 0 °C to rt; (c) DCC, DMF, 0 °C to rt; (d) i) LiOH, THF, ii) TFA-H_2O-PhSH; (e) TFA-H_2O-PhSH.



Scheme 3: Strategies for PSMA-11 (3): (a) 10% Pd/C, H_2(15Psi), MeOH; (b) Piperidine, CH_2Cl_2; (c) DCC, DMF; (d) (e) HBED-CC, DCC, DMF; (f) TFA-H_2O-PhSH.

(Scheme 1), was selectively made free, by reaction with HCl (g) solution in ethyl acetate, to yield **21**. This reaction was found to undergo without any side products and most importantly the resultant product after drying can be directly used for coupling with subsequent amino acid residues. Similar to Cbz strategy, subsequent coupling of **21** with amino acids **10** yielded **23**; removal of Boc-group in **23** furnished amino form **25**, which on coupling with **12** generated **27**. Deprotection of Boc-group in **27** followed by coupling with chelator **30** afforded **32**. Up to this stage the synthesis was highly efficient and there were only limited purification steps involved which made it a user-friendly process. However, the final deprotection step was not highly successful [19] due to the incomplete deprotection of three-methyl ester groups under saponification condition. Nevertheless, the strategy has the potential to further fine-tuning, employing an alternative protecting group at the carboxylic acid groups present in the binding motif.

For the synthesis of PSMA-11, we initially used Fmoc-strategy (Scheme 3) because Fmoc-template **18** could furnish **36**, precursor for PSMA-11, in few synthetic steps. Thus, deprotection of Fmoc group in **18** using piperidine, and subsequent coupling with amino acid template **14**, using DCC yielded compound **34** which was subjected to piperidine treatment to yield amino compound **35**. The conjugation of **35** with HBED-CC yielded fully protected PSMA-11 **36**. Subjecting **36** for acid hydrolysis yielded a crude mass which on purification using HPLC generated PSMA-11 **3** as a hygroscopic foamy off-white solid [19]. The structural integrity and the purity of the isolated product was confirmed by NMR, HPLC and HRMS analysis. Similarly, in Cbz-strategy, following analogous coupling sequence on **20**, obtained after the Cbz deprotection in **17** was subjected for coupling with amino acid **13** to furnish conjugate adduct **33**. The amine **35** obtained after the deprotection of Cbz group in **33** was ligated to commercially available chelator HBED-CC to furnish fully masked adduct **36** [16], which on acid hydrolysis furnished PSMA-11. Among the two approaches the isolated yield of PSMA-11 using Fmoc-strategy was understandably inferior [19] to the Cbz-strategy. From the lessons learned during the synthesis of PSMA-617, the use of Boc-strategy towards the synthesis of PSMA-11 was not explored.

After achieving the total synthesis of PSMA-617 and PSMA-11, both were tested for radiolabelling studies using radionuclides, ^{177}Lu and ^{68}Ga, respectively, at BRIT, Vashi. This study revealed the formation of radiolabelled products, [^{177}Lu]Lu-PSMA-617 and [^{68}Ga]Ga-PSMA-11 in purity >98% adequate for direct human applications. Successful labelling studies were followed with a series of *in-vitro* and *in-vivo* studies (BRIT, Vashi), and subsequent clinical studies conducted at TMH, (Parel), showed that the performance of fully indigenous radiolabelled products were comparable to the corresponding products made from commercial equivalents. To validate the use of in-house synthesized ligands, PSMA-617 and PSMA-11, for prostate cancer management, BRIT and RPhD too played prominent role in developing and getting regulatory approval (DAE-Radiopharmaceutical Committee; DAE-RPC) [20,21] for [^{177}Lu]Lu-PSMA-617 and [^{68}Ga]Ga-PSMA-11 and their supply to nuclear medicine centres in India. To date, using the fully indigenous [^{177}Lu]Lu-PSMA-617, >2500 Indian patients were treated.

In short, our collaboration with BRIT, RPhD, and RMC (Radiation Medicine Centre) were successful in the indigenization of three [^{18}F]-FLT precursors and two organic ligands, PSMA-617 and PSMA-11, in affordable manner, so that this treatment modality is made available to all needy patients. The work presented here demonstrates past one decade of our research efforts to decode the availability of these important API's in every nuclear medicine niches in our country.

Conclusion

Among the three solution phase strategies pursued namely; Fmoc-, Cbz-, and Boc-strategies; Cbz-strategy generated organic ligands PSMA-617 and PSMA-11 in affordable manner. Radiolabelling studies of the in-house synthesized ligands, PSMA-617 and PSMA-11, carried out at BRIT (Vashi), afforded corresponding labelled products in purity >98%. Clinical studies conducted at TMH (Parel), using the fully indigenous nuclear medicines, [^{177}Lu]Lu-PSMA-617 and [^{68}Ga]Ga-PSMA-11, showed results comparable to the commercial equivalents. Through the in-house developed strategy, we are currently capable of synthesizing these valuable import substitutes in purity >99.9%. Along with our endeavour to make these interesting ligands, complemented with the prompt supply of radionuclides by RPhD, is helping uninterrupted supply of refined radiolabelled product, [^{177}Lu]Lu-PSMA-617, by BRIT (Vashi), to nuclear medicine centres across India. BRIT (Vashi) is currently examining the possibility of local supply of [^{68}Ga]Ga-PSMA-11. Development of similar API's useful for diagnostic and therapeutic applications are in various stages of development.

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References

[1] N. D. James, I. Tannock, J. N'Dow, et. al The Lancet Commission on prostate cancer: planning for the surge in cases. The Lancet Commissions, (2024) https://doi.org/10.1016/S0140-6736(24)00651-2.

[2] J. Ferlay, E. Steliarova-Foucher, J. Lortet-Tieulent, S. Rosso, J. W. Coebergh, H. Comber, D. Forman, F. Bray, Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. Eur. J. Cancer. 49(2013) 1374-1403.

[3] R. L. Siegel, K. D. Miller, A. Jemal Cancer statsitics, 2015, CA Cancer J. Clin. 65 (2015) 5-29.

[4] R. Sharma, H. Abbastabar, D. M. Abdulah, et.al. Temporal patterns of cancer burden in Asia, 1990–2019: a systematic examination for the Global Burden of Disease 2019 study, The Lancet Regional Health - Southeast Asia, (2024) 100333.

[5] A. M. Hövels, R. A.Heesakkers, E. M. Adang, G. J. Jager, S. Strum, Y. L. Hoogeveen, J. L. Severens, J. O. Barentsz, The diagnostic accuracy of CT and MRI in the staging of pelvic lymph nodes in patients with prostate cancer: a meta-analysis. Clin. Radiol. 63 (2008) 387-395.

[6] Qaim, S. M. The present and future of medical radionuclide production. Radiochim. Acta 100 (2012) 635-651.

[7] G. Sgouros, L.Bodei, M. R. McDevitt, J. R. Nedrow, Radiopharmaceutical therapy in cancer: clinical advances and challenges. Nat. Rev. 19 (2020) 589-608.

[8] R. A. Clarke, H. J. Schirra, J. W. Catto, M. F. Lavin, R. A. Gardiner, Markers for detection of prostate cancer. Cancers 2 (2010) 1125.

[9] L. Maffioli, L. Florimonte, D. C. Costa, C. J. Correia, C. Grana, M. Luster, L. Bodei, M. Chinol, Reviews diagnostic And Therapeutic Management Of Locally Advanced And Advanced Prostate Cancer. J. Nucl. Med. Mol. Imaging 59 (2015) 420-438.

[10] M. Santoni, M. Scarpelli, R. Mazzucchelli, A. Lopez-Beltran, L. Cheng, S. Cascinu, R. Montironi, Targeting prostate-specific membrane antigen for personalized therapies in prostate cancer: morphologic and molecular backgrounds and future promises. J. Biol. Regul. Homeost. Agents 28 (2014) 555-563.

[11] M. Theiss, M. P. Wirth, A. Manseck, H. G. Frohmuller, Prognostic significance of capsular invasion and capsular penetration in patients with clinically localized prostate cancer undergoing radical prostatectomy. Prostate, 27 (1995) 13–17.

[12] G. P. Swanson, S. P. Lerner, Positive margins after radical prostatectomy: implications for failure and role of adjuvant treatment. UrolOncol. 31 (2013) 531-541.

[13] J. L. Wright, B. L. Dalkin, L. D. True, W. J. Ellis, J. L. Stanford, P. H. Lange, D. W. Lin, Positive surgical margins at radical prostatectomy predict prostate cancer specific mortality. J. Urol. 183 (2010) 2213-2218.

[14] J. A. Barrett, R. E. Coleman, S. J. Goldsmith, S. Vallabhajosula, N. A. Petry, S. Cho, T. Armor, J. B. Stubbs, K. P. Maresca1, M. G. Stabin, J. L. Joyal, W. C. Eckelman, J. W. Babich, First-in-Man Evaluation of 2 High-Affinity PSMA-Avid Small Molecules for Imaging Prostate Cancer. J. Nucl. Med. 54 (2013) 380-387.

[15] K. S. A. Kumar, V. P. Venugopalan, S. K. Ghosh, BARC NEWSLETTER, November-December-2020.

[16] K. S. A. Kumar, A. Mathur. Total chemical synthesis of PSMA-11: API for 68Ga-PSMA-11 used for prostate cancer diagnosis. Eur. J. Med. Chem. Rep. 3 (2021) 100014.

[17] K. S. A. Kumar, A. Mathur. A convenient total synthesis of PSMA-617: A prostate specific membrane antigen (PSMA) ligand for prostate cancer endotherapeutic applications. Eur. J. Med. Chem. Rep. 6 (2022) 100084.

[18] K. S. A. Kumar, A. Mathur. A total chemical synthesis of PSMA-617: A ligand for prostate cancer endotherapeutic applications. Radiochimica Acta, (2024) https//doi.org/10.1515/ract-2023-0205.

[19] K. S. A. Kumar, A. Mathur. Challenges in the solution phase synthesis of PSMA-11 and PSMA-617: Organic ligands for radiopharmaceutical preparations in prostate cancer medication. Radiochimica Acta, (2024) https//doi.org/10.1515/ract-2024-0280.

[20] K. S. A. Kumar, P. C. Vrinda, N. Sakhare, B. Karkhanis, C. Arjun, S. Das, R. K. Mohan, S. Chakraborty, A. Mathur, 177Lu-PSMA-617 'Ready to use' Injectable Formulation, September, (2019).

[21] K. S. A. Kumar, D. Kumar, S. Mirapurkar, R. K. Mohan, S. Das, A. Mathur, U. Pandey, In-house synthesized PSMA-11 as an API for manufacture of 68Ga-PSMA-11 radiopharmaceutical for PET imaging of prostate cancer, May, (2023).