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Design and synthesis of nitrogen containing heterocyclic fragments via isocyanide chemistry and their biological evaluation as potential inhibitors of HIV-1 replication

Rashamuse, Thompho Jason

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List of corrections from examiner 2

N.B: Chapter 7 was completely revised to include the final comparison of the libraries for the best AlphaScreen, best IC50, best ADME parameter and best binding energy as per suggested by examiner

- All the typo and grammatical error indicated in copy of the thesis as suggested by examiner 2 were corrected
- Page iii Sentence 3 TosMIC was abbreviated first as ***"para*-toluenesulfonylmethyl isocyanide**" between
- Page vi-vii list of abbreviations was arranged in alphabetical order and words " **and formulae**" was deleted" and also more abbreviation were added as per suggested
- Page vii **retardation** was change to **retention**
- Page 1 paragraph 3 line 3 "**exploit**" was change to "**explores**".
- Page 2 Section 1.1 Sentence 3 African was replaced by Africa.
- Page 11 Section 1.2 Sentence 1 to **drug discovery** were added between approach and has.
- Page 19 Paragraph 2 Sentence 3 abbreviation of van Leusen reaction "**3cvL**" was change to to **3-CvL**.
- Page 22 Section 1.3.3.2. Sentence 1 **heterocyclic** was change to **heterocycle** between membered and contained.
- Page 25 scheme 1.19 **haI on structure 46** was change to **X** to represent halide
- Page 26 section 1.3.4 sentence 1 "**the**" next to Baylis-Hillman was deleted
- Page 26 error bookmark not defined at the end of sentence 1 was removed
- Page 27 paragraph 1 sentence 2 "**1-butyl-3-methylimidazolium hydrogen sulfate**" was given as full name of {[Hmim]HSO_4} and subscript was sorted.
- Page 28 Section 1.4. **s** was replaced by **blocks** between building and reported
- Page 30 Section 1.4.2. Sentence 2 **Compounds containing the isoxazole moiety have** were added at the beginning
- Page 31 paragraph 1 Scheme 1.29 indeed the reaction does not involve Suzuki-coupling and the mistake was rectified by changing the paragraph to "**Crossley and Browne**Error! Bookmark not defined. **described a regio-selective synthesis of iodoisoxazoles 70 in excellent yields through cycloaddition of alkynyliodide 68 to nitrile oxides 69 in the presence of 0.25 M aq. Na_2CO_3 using DME as solvent under heating for 24 hours as shown in *Scheme 1.29.*** and the Scheme

1.29 was changed to **Synthesis of iodoisoxazoles using an alkynyliodide cycloaddition strategy**.

- Page 32 comment regarding aromatic character. The statement in the thesis indicates that aromatic character contributes towards activity, not that it is a complete answer. Depending on other substituents on the molecule, activity will differ. Phenyl rings are also present in many biologically active molecules.
- Page 33-34 starting from Sentence 4 the role of virtual screening was defined by adding the following two sentences: **Virtual screening is a useful tool to identify promising scaffolds as the starting point for designing and synthesizing potentially active compounds for a specific target. The huge number of possible compounds is reduced using a computerized system to a more convenient number of compounds for synthesis and screening in a biochemical assay against the relevant biological targets, which could lead to possible drug contenders.**
- Page 34 Paragraph 1 Sentence 2 **prelimiary** was replaced by **preliminary**
- Page 34 Paragraph 2 Sentence 1 **them** was deleted between employing and in.

- Page 39 Section 2.2.2.1.1 Sentence 2 **a series**" was added instead of "**mixtures**" at the begin of sentence.
- Page 40 Scheme 2.2 **R"** was change to **R'** next to structure 78
- Page 41 **Table 2.2** was fixed allowing the last line of the table to fit on the same page
- Page 42 Paragraph 1 sentence 2 *in situ* was deleted between the and addition.
- Page 42 Paragraph 2 sentence 1 **in a two-step one pot reaction** were added between prepare and by to address the suggested made.
- Page 42 Sentence 4 **7e** was change to **74e**
- Page 42 last sentence 5 was revised to **"This can be attributed to the fact that the two electron-withdrawing halides cause a decrease in the nucleophilicity of the aniline NH_2** to address comment made by examiner.
- Page 43 Section 2.2.2.2.2 sentence 2 **in order to assess the effect of solvent on the reaction yields, as per the conditions illustrated in *Scheme 2.3*.** was added
- Page 45 paragraph 2 line 1 words "**which is similar to the one proposed by van Leusen and co-workers, was inserted give credit to the person who proposed the mechanism.**
- Page 46 Section 2.2.2.4. Sentence 2 **bands** was change to **band** to sort grammar between stretching and appears.

- Page 46 Section 2.2.2.4. Sentence 3 singlets was change to singlet between two and signals.
- Page 47 Comments Table 2.4 **inclusion of the HRMS results throughout the thesis was necessary because it highlights the importance of assigning the formula with relate to mSigma values, and more importantly the HRMS experiments were conducted by myself**.
- Page 49 Table 2.5 positive and negative controls were included in the table while 83a superscript issue was resolved. Also the headings were repeated on the page 50 as per suggested.
- Page 51 Paragraph 1 Sentence 3 **2** in front of **74f-h** was deleted.
- Page 51 Figure 2.2 **with the cell based MT-4 assay data included** were inserted in the Figure tittle as per suggested
- Page 52 the headings were repeated on the page 53 as per suggested.
- Page 58 Sentence 1 **in spite of the instability** were added.
- Page 59 spacing between minus sign and 78 was addressed.
- Page 64 Sentence 2 **the hydrolyzed product 87a-e were"** was replaced by "**Complete hydrolysis to yield products 87a-e** at the beginning of sentence while **hydroxylic group** was replaced by **acid proton** between the and in.
- Page 65 Section 2.3.3.1. Sentence 2 word **kit** next to AlphaScreen was deleted.
- Page 65 Table 2.14 positive and negative controls were added. .
- Page 66 Paragraph 3 sentence 1 significant figures were checked all reported the same.
- Page 66 Paragraph 3 sentence 2 **83a** was added after compound**.**
- Page 66 Paragraph 3 Sentence 3 was modified to "**The IC_{50} values of compounds 87a-e appeared to be high, but these values may not be reliable because the compounds were found to have poor aqueous solubility.**
- Page 67 in section 2.3.3.2 one full stop was deleted between 3 and 2.
- Page 67 Table 2.15 6 superscript was addressed after **CYP2D6**
- Page 67 Table 2.15 was also modified to make it more obvious.
- Page 68 Sentence 1 **at the** was deleted between the and IN**.**
- Page 69 Sentence 3 words **"The lower the binding energy the tighter the compound binds to the target** "were inserted to answer the comments on Table 2.17
- Page 70 Table 2.17 divisions on the table was were cleared.
- Page 74 Comment: Overlaying of the docked compounds was conducted and is discussed in chapter 7.

- Page 80 paragraph 2 sentence 3 **used** was inserted between been and for.
- Page 81 section 3.2 the sentences 3, 4 and 5 were added to give originality to the background as per suggested. **In many instances, microwave irradiation has been shown to decrease significantly reaction time and increase reaction yields. In chapter 2, application of microwave irradiation to the van Leusen imidazole reaction was indeed shown to reduce the reaction time. With this in mind, and considering our interest in finding a more convenient preparation to build heterocyclic rings, we then focused our synthetic efforts on exploring the use of TosMIC 1 as a starting material under microwave irradiation to produce oxazoles in methanol or acetonitrile as solvents. Reaction progress was monitored by Thin Layer Chromatography (TLC).**

- Page 82 Scheme 3.1 the numbering is **7f, h-**j because the **7g** has already been reported in chapter 2 but not used in the chapter 3.
- Page 82 Table 3.1 words **by route 1** were added
- Page 83 sentence 3 was modified as **We assume the geometry of these products to be *trans*, corresponding to the (4*R*, 5*R*) / (4*S*, 5*S*)-isomers, which matches the NMR spectroscopic data, including coupling constants, reported in the literature**, to answer the question raised by examiner
- Page 84 yields on the Table 3.3 were added for comparison.
- Page 84 section 3.2.3 Mechanism proposed is the reported one and the mechanistic study was conducted hence the suggestion could not be accomplished and will form part of future work to investigate such mechanism
- Page 85 sentence 1 word **an example** was replace with **examples** between as and representing.
- Page 87 Table 3.5 the positive and negative controls were added.
- Page 90 Section 3.3.1 was modified to **The retrosynthetic analysis of the target *N*,5-diaryl-1,3-oxazole-4-carboxamides 100 is shown in *Scheme 3.4*. It was envisaged that the 1,3-oxazole ring system of the target scaffold 100 could be constructed through cycloaddition of commercially available benzoyl chloride 92 to 2-isocyano-*N*-arylacetamide 101. The key intermediate 101 in turn could obtained through a well-established protocol which could involve the dehydration of *N*-aryl-2-formamidoacetamide precursor 102, which could be derived by coupling of the aniline scaffold 74 with hydrazinyl formamide 103. The precursor 103 could be the obtained from previously reported literature *via* a nucleophilic substitution**

reaction of *N*-formyl glycine methyl ester 104 with hydrazine monohydrate. The scaffold 104 could be obtained from two possible commercially available synthons; amino acid ester hydrochloride salts 105 and methyl formate 106. to respond to the comment suggested by examiner

- Page 93 sentence 2 "**a secondary amine adjacent to the carbonyl group**" was replaced by **an amide group** of and which.
- Page 93 paragraph 1 sentence 3 was rephrased as "**A possible explanation for lack of reactivity of isocyanoacetamides might be the presence of an amide group, which tends to lower the acidity of the α-methylene protons adjacent to the isocyano group. Giustiniano and co-workers reported the lack of reactivity between acyl chloride and α-isocyanoacetamide containing a secondary amide, which is in agreement with the findings of this study** to answer the comment suggested
- Page 97 section 3.3.2.1. last sentence in the paragraph 1 "**amine**" was change to **amide**.
- Page 98 Scheme 3.11 Mechanistic pathway resembled the mechanism reported by schollkopf.
- Page 98 paragraph 2 sentence 3 next to corresponding to words "**a hydroxylic"** were replace by **the** between to and proton
- Page 99 section 3.3.2.3. Paragraph 2, sentence 2 contradiction was sorted by deleting **after crystallization** between precipitates and from.
- Page 100 section 3.3.2.5. Sentence 2 **amine** was replaced by **amide** between secondary and group.
- Page 106 table 3.10 **107b** and **109a** negative inhibitions were address by changing to 0 indicating that they are non-active.
- Page 106 positive and negative controls were included.
- Page 107 paragraph 2 sentence 3 4 was change to 3 between of and comma.
- Page 107-108 section 3.3.5 LibDock methods used was referred back to Section 2.3.4.
- Page 109 Figure 3.4 Picture numbering **D** and **E** were change to **C** and **D**
- Page 109 to 110 the overlaying of models was addressed.

- Page 114 section 4.2.1. Paragraph 2 sentence 3 **the** was replace by **a** next to HCl/EtOH system
- Page 116 section 4.2.2. the paragraph was revised to answer the comment on the same page and in page 117 "**Walton *et al.* described the reaction of various aldehydes with esterified amino acid salts using Et_3N as a base in the presence of $MgSO_4$ in DCM at room temperature to afford the corresponding ethyl (*E*)-2-{(arylidene)amino}acetates in quantitative yields.**

Inspired by this protocol, our free amino ethyl esters 115a-c were then exposed to the condensation reaction conditions by reacting with various benzaldehydes 7a-h in the presence of $MgSO_4$ using DCM as solvent at room temperature for 16 hours (*see Table 4.3*). Work up furnished the desired ethyl 2- (arylideneamino)acyl intermediates (116a-h, 117a-d and 118a-c) in yields of up to 96%. The yields of the products obtained (96% for 116h and 92% for 117d) were considerable improvements over those reported by López-Pérez *et al.* (81% for 116h and 87% for 117d). Compounds 116a-h, 117a-d and 118a-c were used in the next stage without further purification. This modification also addresses also comment in page 117 sentence 2.

- Page 120 Paragraph 2 sentence 1 was words "**Inspired by our experience with the oxazole synthesis in section 3.2.2 and a reported protocol where the elimination of the *para*-toluenesulfonyl group was accomplished in toluene, we explored removal of this group in toluene under microwave irradiation**," were inserted as per suggested while the protocol was cross reference to 230
- Page 124 paragraph 2 sentence 2 words **formula for** were inserted to answer question asked by examiner.
- Page 125 section 4.2.4.2.1 comment was addressed
- Page 130 sentence 2 following words were added **"This was inspired by the fact that microwaves can enable to make water to act like a pseudo-organic solvent, thus speed up the reactions in an aqueous medium**" to answer question asked by examiner 2 in page 129 scheme 4.6.
- Page 130 sentence 5 following words were added "**This method was better than procedure described by Zhou *et al.*, because no removal of the acid or base catalyst at the end of the reaction**." to answer question in scheme 4.6
- Page 131 The sentence 2-4 was modified by inserting the words "the appearance of a quaternary ammonium proton due to zwitterionic form (see ***Scheme 4.7***) as a broad signal at 6.01 ppm for compound **123d**"
- Page 131 the assignment of the proton signal in sentence one was resolved while sentence 2 was revised to "**The FTIR spectra of 2-(5-aryl-1*H*-imidazol-1-yl) acetic acids 123a and 123c-h showed absorption band in the region of 2434 to 2604 cm^{-1}attributed to NH^+ of the zwitterionic form which agree with the observed ^1H NMR spectroscopy**." to explain the correlation observed between NMR and FTIR

- Page 134 Figure 4.6. **NH_2** was change to **NH** in the NMR spectrum
- Page 134 paragraph 2 was sentence was revised to "**Extra signals were observed on NMR spectroscopic analysis which could be the result of rotamers. Rotamers arise due to hindered rotation around the amide C-N bond caused by delocalization (*Scheme 4.9*).**
- to add the missing part as per suggested
- Page 134 scheme 4.9 the mixing signed was inserted on the structure 126.
- Page 136 section 4.2.5.1. Paragraph 3 sentence 3 between group and derivatives **resulting in** was replaced by **gave.**
- Page 138 Table 4.11 **120 R =H** and **121 R= Me** were inserted in the table and the positive and negative control were inserted.
- Page 139 pargarph2 sentence 3 Interestly was omitted at beginning of sentence.
- Page 141 paragraph 2 sentence 2 was revised to "**These acetic acid scaffolds exhibited minimal inhibition activity (2-30%) with the exception of compounds 123g and 123h which were completely inactive**."
- Page 141 beginning of sentence 4 **however** was replace by **By contrast**.
- Page 142 paragraph 2 at the end sentence 2 **at a single dose of 100 μM** was replace by **in a dose response assay**.
- Page 142 paragraph 2 sentence 3 section 4.2.6. **Furthermore** was replaced by **By contrast**
- Page 143 Table 4.13 column 4 all the **0** were change to **false**
- Page 147 table 4.14 entry 127n **^{t}BU** was change to **^{t}Bu**.
- Page 149 section 4.3.2. sentence 2 **"Due to time constraints, only"** were inserted at the to answer reason why compound 120a was only used
- Page 149 Scheme 4.11 **128a** and **128b** were added.
- Page 150 table 4.16 the positive and negative control were added.
- Page 151 comment. The Lipinski's properties for the class of compound discussed in section 4.3.4. were included because of MW are range of recommended by Lipinski rule while other where compounds were still fragments.
- Page 151 end of sentence 6 one full stop was deleted.
- Page 151 sentence 7 was revised to "**Other hydrazine scaffolds exhibited minimal inhibition activity (6-37%) with the exception of compounds 127a and 127f which were entirely inactive**."

- Page 152 Table 4.17 Notes: an ideal ranges for drugability i.e.Mw <500; H donor <5; H acceptor between 5-7; PSA <140." were added under the table as per suggested.
- Page 152 section 4.3.4 the sentence was rephrase in order to eextracting meaning from the data. **ADME properties of the best three compounds 127j and 127n-o were also performed by means of Discovery Studio™ to determine their BBB penetration, aqueous solubility and human intestine absorption, and cytochrome p450 2D6 protein binding (*Table 4.18*). The three compounds were predicted to have low solubility (level 2) which implies that the compounds slightly dissolve in water, and all were projected to have good absorption levels, which indicate that they can be absorbed very well by human intestine after oral dosage. Compounds 127j and 127o were predicted to be high BBB penetrators indicating that they might cause the central nervous system side effects, whilst compound 127n was a medium penetrator which suggests that it might have low chances of cause side effects to central nervous system. Furthermore, compound 127n was predicted to be a non-inhibitor of CYP2D6 binding protein which indicate that the compound does not inhibit CYP2D6 enzyme after metabolization through cytochrome p450 (CYP) enzymes.**
- Page 153 section 4.3.5 value -53.8902 was matched with the value in page 154 table 4.19 entry 127j.
- Page 156 Figure 4.12 **120o** was change to **120n** and **120b** was change to **120o**.
- Page 156 paragraph 1 sentence 1 **best** was omitted between the and three compounds
- Page 156 paragraph 1 sentence 2 **intact** was replaced by **interact** between to and with.
- Page 156 paragraph 1 sentence 3 after furthermore, **the best** was replaced by **these**
- Page 159 Figure 4.13 the overlaying was discussed in chapter 7.

- Page 160 section 5.2 **The synthesis of these** was added at the beginning of sentence 2.
- Page 160 section 5.2 sentence 3 was split from sentence 2 and written as "**This chapter will discuss the synthetic challenges encountered in the preparation of isoxazoles, as well as characterization of compounds, reaction mechanisms and biological data of the isoxazole products."**
- Page 162 paragraph 2 sentence 2 "**obtained as racemates**" were inserted at the end.
- Page 163 sentence 4 and 5 were rephrased as **"). In our hands the yields of the products 133c and 133d obtained were significantly lower than yields previously reported by Ram and co-workers (i.e 96% for 133c and 90% for 133d). Compound 133a and 133b were not reported in**

the study conducted by Ram *et al.*, but they have been previously prepared using different starting materials. to answer question for table 5.3

- Page 165 section 5.2.1.3 was rephrased "**Zhang *et al.* had earlier reported a synthetic procedure for 2-chloro-1-phenylethanone oxime. To explore this route to 2-chloro-1-aryl-ethanone oxime intermediates, compounds 133a-d were subjected to condensation reaction conditions by reacting with hydroxylamine hydrochloride in the presence of water and MeOH as solvent at room temperature for 16 hours as indicated in *Scheme 5.7*. Work up by addition of more water, furnished the key 2-chloro-1-aryl-ethanone oxime intermediates 129a-d as precipitates in excellent yields (*Table 5.5*). The yield of the product 129c obtained was as good as that reported by Zhang (i.e. 83%)."** to accommodate the question asked.
- Page 167 Table 5.7 R was replaced with R' in column 2 .
- Page 167 section 5.2.2 sentence language was corrected by rephrasing the sentence to "**Thus, a series of aniline derivatives 74e-f, i and j were microwave irradiated neat with formic acid at a set temperature of 110°C for 1 hour (as monitored by TLC), as illustrated in *Scheme 5.8*. Work up afforded the corresponding *N*-aryl-formamide products 139a-d in yields similar to those reported under ultrasound irradiation and acetic formic anhydride methods (*Table 5.7*).**
- Page 169 section 5.2.3. Sentence 2 routinely was deleted between been and reported.
- Page 174 the heading 5.2.5. was bolded.
- Page 175 paragraph 1 sentence 2 **study** was added at the end
- Page 176 section 5.2.1 was change to section 5.3.1.
- Page 181 sentence 2 **led to** was replaced by **as in** between group and compound.
- Page 181 sentence 4 **resulting in** was replace by **gave**
- Page 204 abbreviations for **TBS** was defined fas **tris-buffered saline**
- Page 204 abbreviations of **TBST** was defined as "**a mixture of tris-buffer saline and Tween 20**
- **Note:** experimental procedure for **section 8.1.4.3** (page **204), 8.1.4.4 (page 204)** and **8.1.4.5** (**page 205)** were rephrasing as per suggested by examiner 3, hence the correction suggested by examiner 2 is invalid