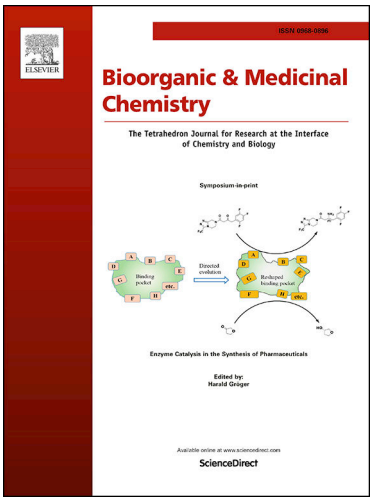
Journal Pre-proofs



Design, synthesis and biological evaluation of imidazole and oxazole fragments as HIV-1 integrase-LEDGF/p75 disruptors and inhibitors of microbial pathogens

Thompho J. Rashamuse, Angela T. Harrison, Salerwe Mosebi, Sandy van Vuuren, E. Mabel Coyanis, Moira L. Bode

PII: S0968-0896(19)31034-X
DOI: https://doi.org/10.1016/j.bmc.2019.115210
Reference: BMC 115210

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 22 June 2019
Revised Date: 23 October 2019
Accepted Date: 5 November 2019

Please cite this article as: T.J. Rashamuse, A.T. Harrison, S. Mosebi, S. van Vuuren, E. Mabel Coyanis, M.L. Bode, Design, synthesis and biological evaluation of imidazole and oxazole fragments as HIV-1 integrase-LEDGF/p75 disruptors and inhibitors of microbial pathogens, *Bioorganic & Medicinal Chemistry* (2019), doi: https://doi.org/10.1016/j.bmc.2019.115210

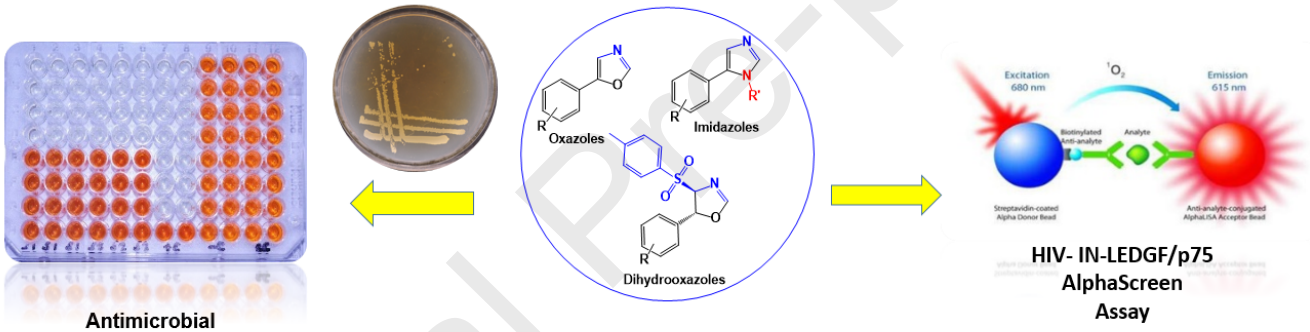
Graphical Abstract

To create your abstract, type over the instructions in the template box below.
Fonts or abstract dimensions should not be changed or altered.

Leave this area blank for abstract info.

Design, synthesis and biological evaluation of imidazole and oxazole fragments as HIV-1 integrase-LEDGF/p75 disruptors and inhibitors of microbial pathogens

Thompho J. Rashamuse, Angela T. Harrison, Salerwe Mosebi, Sandy van Vuuren, E. Mabel Coyanis, and Moira L. Bode



ELSEVIER

Bioorganic & Medicinal Chemistry
journal homepage: www.elsevier.com

Design, synthesis and biological evaluation of imidazole and oxazole fragments as HIV-1 integrase-LEDGF/p75 disruptors and inhibitors of microbial pathogens

Thompho J. Rashamuse[a,b], Angela T. Harrison[a], Salerwe Mosebi[a], Sandy van Vuuren[c], E. Mabel Coyanis[a],* and Moira L. Bode[b]*

[a] *Advanced Materials Division, Mintek, 200 Malibongwe Drive, Randburg, 2125, South Africa*
[b] *Molecular Sciences Institute, School of Chemistry, University of the Witwatersrand, Private Bag 3, PO WITS, 2050, South Africa*
[c] *Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown, 2193, South Africa*

ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
TosMIC
1-substituted-5-aryl-1*H*-imidazoles
5-aryl-1,3-oxazoles
HIV-1 integrase
LEDGF/p75
Antibacterial

ABSTRACT

We describe here the synthesis of libraries of novel 1-subtituted-5-aryl-1*H*-imidazole, 5-aryl-4-tosyl-4,5-dihydro-1,3-oxazole and 5-aryl-1,3-oxazole fragments *via* microwave (MW)-assisted cycloaddition of *para*-toluenesulfonylmethyl isocyanide (TosMIC) to imines and aldehydes. The compounds obtained were biologically evaluated in an AlphaScreen HIV-1 IN-LEDGF/p75 inhibition assay with six imidazole-based compounds (**16c**, **16f**, **17c**, **17f**, **20a** and **20d**) displaying more than 50% inhibition at 10 µM, with IC_{50} values ranging from 7.0 to 30.4 µM. Additionally the hypothesis model developed predicts all active scaffolds except **20d** to occupy similar areas as the *N*-heterocyclic (A) moiety and two aromatic rings (B and C) of previously identified inhibitor **5**. These results indicate that the identified compounds represent a viable starting points for their use as templates in the design of a next generation of inhibitors targeting the HIV-1 IN and LEDGF/p75 protein-protein interaction. In addition, the *in vitro* antimicrobial properties of these fragments were tested by minimum inhibitory concentration (MIC) assays showing that compound **16f** exhibited a MIC value of 15.6 µg/ml against *S. aureus*, while **17f** displayed a similar MIC value against *B. cereus*, suggesting that these compounds could be further developed to specifically target those microbial pathogens.

* Corresponding author. *E-mail*: MabelC@mintek.co.za (E. M Coyanis).
* Corresponding author. *E-mail*: Moira.Bode@wits.ac.za (M. L. Bode).

1. Introduction

Infectious diseases triggered by viral and bacterial pathogens continue to be a major threat to global health. The emergence of multi-drug resistance to current therapeutics due to the evolution of existing strains has hampered the treatment of both viral and bacterial pathogens, resulting in higher morbidity and mortality rates.[1-4] Thus, the search for new effective inhibitors to augment existing treatments remains a key objective in many drug discovery programs.

The human immunodeficiency virus type 1 integrase enzyme (HIV-1 IN) has been identified as an attractive target for the development of the next generation antiretroviral drugs.[5,6] The major role of this catalytic enzyme lies in facilitating the insertion of the reverse-transcribed viral DNA into the host DNA.[7-9] To date there are four FDA approved HIV-1 IN drugs (referred to as IN strand transfer inhibitors or INSTIs) that tightly bind the active site of the enzyme: Elvitegravir (**1**), Dolutegravir (**2**) Raltegravir (**3**) and Bictegravir (**4**) (Fig. 1).[10-14] Although these inhibitors manage to suppress HIV-1 as part of highly active antiretroviral therapy (HAART), cross resistance and poor tolerability has restricted the long-term use of these inhibitors.[2-4,15]

During the last decade, several small molecules have been identified as disruptors of the HIV-1 IN - host Lens Epithelium-Derived Growth Factor (LEDGF/p75) interaction, by binding to the IN catalytic core domain (CCD) dimer interface in the LEDGF/p75 binding pocket.[16-19] These include BI-1001 (**5**), CX05168 (**6**), BI-224436 (**7**) and CX14442 (**8**) (Fig. 1).[18-22] These allosteric compounds inhibit binding of IN with its cofactor LEDGF/p75 protein and promote aberrant IN multimerization, ultimately resulting in defective virions.[18,23-25] Although these compounds are not yet clinically approved, they provide proof-of-concept for the disruption of the HIV-1 IN-LEDGF/p75 interaction as a genuine target for the development of the next generation of anti-HIV treatment.

Fragment-based drug discovery (FBDD) has emerged as a reliable approach to identify small compounds that can be elaborated into good inhibitors.[26-30] Collections of these low molecular weight and low chemical complexity compounds (commercially obtained or chemically synthesized) are screened (usually by biophysical methods) for binding affinity to the target. One of the advantages of using in-house produced fragments is that the structure activity relationships (SAR) of a family of related compounds can be exploited at an early stage.[29] FBDD can provide good starting points for binding fragments to be grown (or linked) into larger drug-like molecules with greater target affinities.[30]

Some five-membered nitrogen-containing heterocycles are found as the central core of compounds inhibiting various stages of the HIV-1 replication cycle.[31-33] Reagents such as *para*-toluenesulfonylmethyl isocyanide (TosMIC) (**9**) can serve as the starting point for the synthesis of various nitrogen containing 5-membered rings, by varying the reaction conditions. Unlike most of the isocyanide family members, TosMIC is stable, non-volatile and odourless at room temperature.[34] This α-acidic isocyanide is a versatile building block which has been used to access biologically relevant heterocycles such as imidazoles,[34-36] oxazoles[37,38] and pyrroles.[39,40]

The small molecules **5**, **7** and **8** that interrupt the HIV-1 IN-LEDGF/p75 interaction can be disconnected into four chemical features: the oxoacetic acid moiety, the central *N*-heterocycle core (A), and the two aromatic rings (B and C). As a starting point, we set out to design possible compounds with the minimal structural features (i.e. one or two aromatic moieties linked to a central *N*-heterocyclic core) that could be synthesised and biologically evaluated to identify possible hit(s) that could later be improved through a structure-activity relationship (SAR) strategy.

We identified versatile isocyanide chemistry[41] as key to the synthesis of suitable small fragment-based libraries of 1-substituted-5-aryl-1*H*-imidazoles, 5-aryl-1,3-oxazoles and (4*R*,5*R*)/(4*S*,5*S*)-5-aryl-4-tosyl-2,4-dihydro-1,3-oxazoles which were subsequently evaluated for their ability to disrupt the interactions between HIV-1 IN and the host LEDGF/p75 proteins. The serious problem of opportunistic infections in HIV patients also led us to evaluate the compounds for their ability to inhibit infections which are prominent in these patients. Pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus,* amongst others, have demonstrated multidrug resistance and have become challenging in the clinical setting and are of great concern in hospital infections.

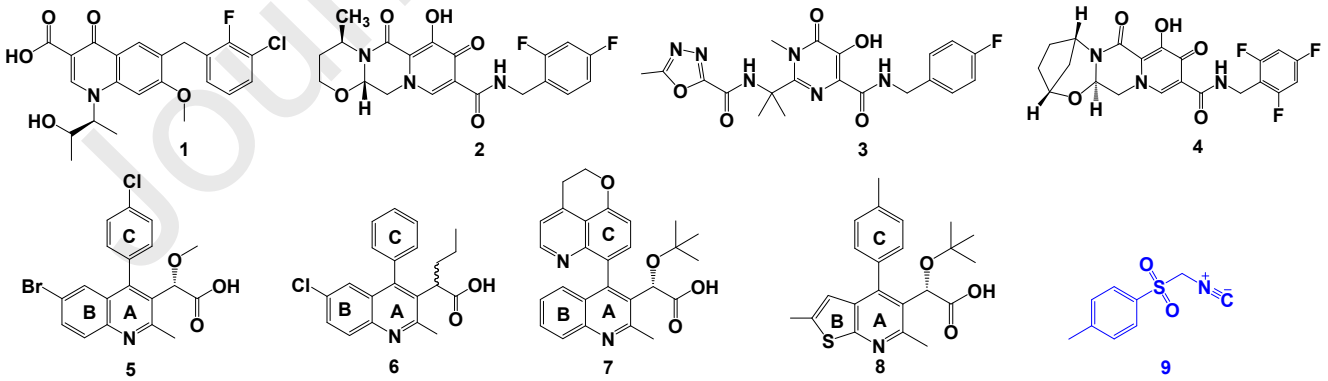


Fig. 1: Structures of different classes of HIV-1 IN inhibitors (**1-8**) and TosMIC (**9**).

2. Results and Discussion

2.1. Chemistry

As intermediates for the isocyanide-based synthesis of imidazoles, *N*-benzylidene alkylamines were prepared by

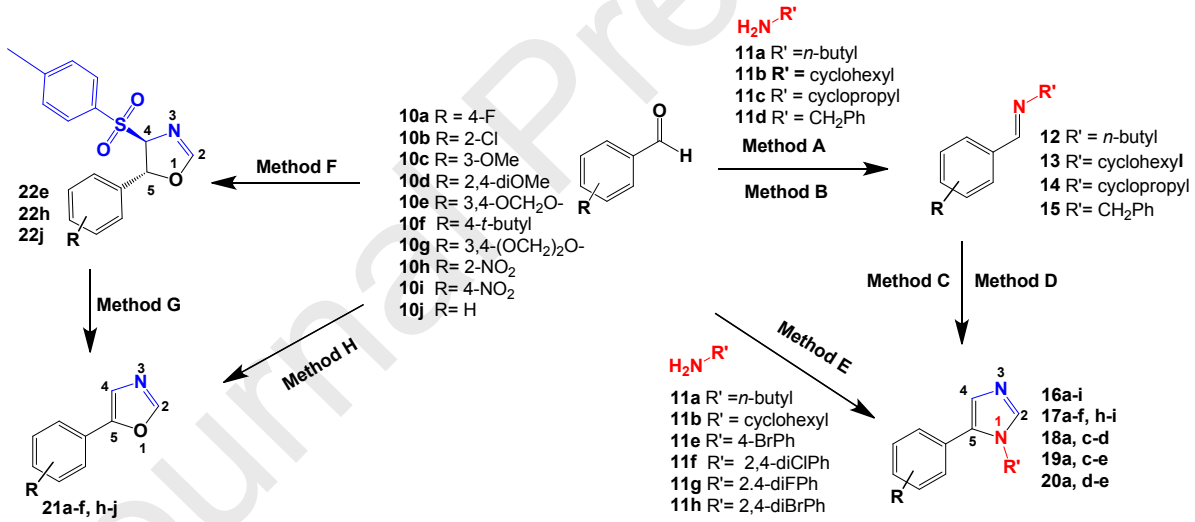
condensation of commercially available aldehydes (**10a-i)** with primary amines (**11a-d**), in DCM at room temperature for 16 h, as outlined in **Scheme 1** (Method A).[42] Although the reaction afforded *N*-benzylidene alkylamines **12-15** in good to excellent yields (71-96%,), reaction times could be shortened to just 4 min in excellent yields by neat microwave irradiation at a set temperature of 60°C (Method B).

The cycloaddition of TosMIC **9** to the imines (**12a-i**, **13a-f**, **h-i**, **14a**, **c-d**, and **15a**, **c-e**) was then carried out initially using K_2CO_3 in refluxing MeCN for 72 h to produce 1-substituted-5-aryl-1*H*-imidazoles (**16a-b**, **17d-g**, **18a-f**) (**Scheme 1**, Method C). Imidazoles (**16a-f**, **16h-i**; **17a-f**, **17h-i**; **18a**, **18c-d**; **19a**, **19c-e**) were also obtained by microwave irradiation in significantly shorter times (from 3 days by conventional heating to just 7 h) in slightly improved yields (**Scheme 1**, Method D, Table 1).

Imidazoles **16d-e** and **17d-e** were also obtained in a two-step-one-pot reaction in comparable yields to those obtained in the stepwise manner, by neat microwave irradiation of the aldehydes **10d-e** and aliphatic amines **11a-b** for 4 min at 60°C, followed by the addition of TosMIC **9**, K_2CO_3 and MeCN, and then subjecting the reaction mixture to the microwave conditions described in Method C (**Scheme 1**, Method E). 1,5-Diaryl-1*H*-imidazoles **20a**, **d-e** derived from aryl amines were also prepared in a two-step one pot reaction by neat irradiation of substituted benzaldehydes **10a**, **d-e** and 4-bromoaniline **11e**, followed by addition of TosMIC **9**, K_2CO_3 and MeCN (**Scheme 1**, Method E). However, when dihalogen-substituted anilines **11f-h** were used the desired products could not be isolated.

We extended the microwave-assisted cycloaddition of TosMIC to the preparation of a small library of 5-aryl-1,3-oxazoles **21** from commercially available aldehydes **10**. Initially we selected aldehydes **10e**, **10h** and **10j** as model substrates to examine the reaction. Thus, a mixture of these aldehydes, TosMIC **9** and K_2CO_3 in MeCN were microwave-irradiated in a pressurized vessel under inert conditions at a set temperature of 75°C and 120 Watts (**Scheme 1**, Method F). After 12 min the *trans*-5-aryl-4-tosyl-2,4-dihydro-1,3-oxazole intermediates **22e**, **22h**, and **22i** were isolated instead, as racemic mixtures, in yields ranging from 65-80% (Table 2).[43] These dihydro-1,3-oxazole intermediates were converted to their corresponding 5-aryl-1,3-oxazoles **21e**, **21h** and **21i** by refluxing in toluene (Method G) for 1 h as per Companyo *et al*.[44]

In order to investigate the effect of other solvents on this reaction, aldehydes **10a-j** were reacted under the same microwave conditions using anhydrous MeOH as solvent as shown in **Scheme 1** (Method H). Methanol proved to be a far superior solvent for the reaction, with the corresponding 5-aryl-1,3-oxazoles **21a-f**, **21h-j** being obtained in just 7 min in yields ranging from 43 % to 84% (Table 2)



Scheme 1: Synthesis of 1-substituted-5-aryl-1*H*-imidazoles **16-20**, 5-aryl-1,3-oxazoles **21** and (4*R*,5*R*)/(4*S*,5*S*)-5-aryl-4-tosyl-2,4-dihydro-1,3-oxazoles **22**: *Reaction conditions*: **Method A**: $MgSO_4$, DCM, r.t., 16 h; **Method B**: MW, 60°C, 4 min; **Method C**: **9**, K_2CO_3, anhydrous MeCN, reflux, 72 h.; **Method D**: MW: **9**, K_2CO_3, anhydrous MeCN, 120W, 90°C, 7 h.; **Method E**: (i) MW: 60°C, 1 min-1 h; (ii) **9**, K_2CO_3, anhydrous MeCN, 120W, 90°C, 7 h.; **Method F:** MW: **9**, K_2CO_3, anhydrous MeCN, 120W, 90°C, 12 min.; **Method G**: toluene, reflux, 1 h; **Method H**: MW: **9**, K_2CO_3, anhydrous MeOH, 120W, 75°C, 7 min.

Table 1: Isolated yields of 1-substituted-5-aryl-1*H*-imidazoles **16-20**

Compds	R	R'	Yields	Structure
16a	4-F	*n*-butyl	**C**: 38% **D**: 55%	
16b	2-Cl	*n*-butyl	**C**: 20% **D**: 26%	
16c	3-OMe	*n*-butyl	**D**: 60%	
16d	2,4-diOMe	*n*-butyl	**C**: 80% **D**: 87% **E**: 81%	
16e	3,4-OCH_2O-	*n*-butyl	**C**: 43% **D**: 48% **E**: 55%	
16f	4-*t*-buty	*n*-butyl	**C**: 44% **D**: 52%	
16g	3,4-$O(CH_2)_2O$-	*n*-butyl	**C**: 54%	
16h	2-NO_2	*n*-butyl	**D**: 15%	
16i	4-NO_2	*n*-butyl	**D**: 13%	
17a	4-F	cyclohexyl	**C**: 46% **D**: 51%	
17b	2-Cl	cyclohexyl	**C**: 23% **D**: 29%	
17c	3-OMe	cyclohexyl	**C**: 47% **D**: 53%	
17d	2,4-diOMe	cyclohexyl	**C**: 64% **D**: 74% **E**: 73%	
17e	3,4-OCH_2O-	cyclohexyl	**C**: 35% **D**: 54% **E**: 50%	
17f	4-*t*-butyl	cyclohexyl	**C**: 29% **D**:50%	
17h	2-NO_2	cyclohexyl	**D**: 12%	
17i	4-NO_2	cyclohexyl	**D**: 10%	
18a	4-F	cyclopropyl	**D**: 23%	
18c	3-OMe	cyclopropyl	**D**: 40%	
18d	2,4-diOMe	cyclopropyl	**C**: 68%	
19a	4-F	Benzyl	**D**: 26%	
19c	3-OMe	Benzyl	**D**: 24%	
19d	2,4-diOMe	Benzyl	**D**: 74%	
19e	3,4-OCH_2O-	Benzyl	**C**: 22%	
20a	4-F	4-BrPh	**E**: 68%	
20d	2,4-diOMe	4-BrPh	**E**: 38%	
20e	3,4-OCH_2O-	4-BrPh	**E**: 62%	

2.2. Biological evaluation

2.2.1. HIV-1 IN - LEDGF AlphaScreen™ assays

The synthesized libraries of 1-substituted-5-aryl-1*H*-imidazoles **16-20**, 5-aryl-1,3-oxazoles **21** and (4*R*,5*R*)/(4*S*,5*S*)-5-aryl-4-tosyl-2,4-dihydro-1,3-oxazoles **22**, of average molecular weight 359, were assessed in the AlphaScreen™ assay[45] to determine the percentage inhibition for the disruption of HIV-1 IN-LEDGF/p75 interaction at a concentration of 10 µM. Out of thirty seven compounds screened, six (**16c**, **16f**, **17c**, **17f**, **20a**, and **20d**) showed more than our pre-defined 50% inhibition benchmark (Table 3). Amongst them compound **17f**, containing a cyclohexyl moiety, showed the highest percentage inhibition (62%) and produced an IC_{50} value of 15 µM in a dose response assay. Compound **16f**, with an *n*-butyl moiety instead of the cyclohexyl ring gave an IC_{50} value of 22 µM. Both **16f** and **17f** contain a *tert*-butyl substituent at the *para*-position of the phenyl ring attached to the imidazole motif at the 5-position.

Compound **17c**, an analogue of **17f**, containing a methoxy substituent at the *meta*-position inhibited the protein-protein interaction by 55% with an IC_{50} value of 22 µM, while compound **16c**, also with a *meta*-methoxy substituent, showed an IC_{50} value of 30 µM. Two compounds, **20a** and **20d**, both of which possess a *para*-bromophenyl group at the 1-postion of the imidazole moiety, showed 52% inhibition of the IN-LEDGF/p75 interaction.

Table 2: Isolated yields of 5-aryl-1,3-oxazoles **21** and (4*R*,5*R*)/(4*S*,5*S*)-5-aryl-4-tosyl-2,4-dihydro-1,3-oxazoles **22**

Compds	R	Yields	Structure
21a	4-F	**H**: 84%	
21b	2-Cl	**H**: 74%	
21c	3-OMe	**H**: 60%	
21d	2,4-diOMe	**H**: 44%	
21e	3,4-OCH_2O-	**H**: 43% **G**: 69%	
21f	4-*t*-butyl	**H**: 59%	
21h	2-NO_2	**H**: 73%	
21i	4-NO_2	**H**: 68% **G**: 63%	
21j	H	**G** 68%	
Compds	**R**	**Yields**	**Structure**
22e	3,4-OCH_2O-	**F**: 80%	
22h	2-NO_2	**F**: 65%	
22j	H	**F**: 76%	

Table 3: The HIV-1 IN-LEDGF/p75 inhibition activities of the 5-aryl-1*H*-imidazoles **16-20**, 5-aryl-1,3-oxazoles **21** and 4,5-dihydro-1,3-oxazoles **22**.

Entry	Biochemical Target assays		Cell-based assay (MT-4 cells)	
	%Inhibition (@10 µM)	IC_{50} (µM)	Cytotoxicity CC_{50} (µM)	Antiviral EC_{50} (µM)
16a	30			
16b	37			
16c	51	30.5 ±4.92	29.6±11.00	>10
16d	26			
16e	35			
16f	56	22.4 ±1.46	23.9± 2.36	>10
16g	21			
16h	30			
16i	27			
17a	45			
17b	31			
17c	55	21.9 ±0.49	27.7 ± 5.44	>10
17d	40			
17e	40			
17f	62	14.6± 0.24	21.8 ± 2.84	>10
18a	31			
18c	40			
18d	28			
19a	25			
19c	21			
19d	17			
19e	43			
20a	52	25.1±2.47	48.0±13.00	>10
20d	52	7.0 ±1.49	55.5±16.25	>10
20e	48			
21a	23			
21b	33			
21c	33			
21d	42			
21e	35			
21f	35			
21h	30			
21i	33			
21j	31			
22e	33			
22h	38			
22j	34			
CX05168	98			

* IC_{50} - concentration of compound required to inhibit 50% of the specific biological process.

*CC_{50} – concentration of compounds that causes 50% reduction of cell growth

Compound **20d** with methoxy substituents at the *ortho*- and *para*-positions of the 5-aryl ring displayed the best IC_{50} value in the series (7 μM), while its counterpart with a fluorine atom at the *para*-position **20a** produced an IC_{50} value of 25 μM. The oxygen atoms present on the methoxy substituent groups could provide the possibility of hydrogen-bonding interactions with some of the amino acid residues within the binding site, leading to higher binding affinity. The IC_{50} data suggest that the presence of the *para*-bromophenyl moiety at the 1-position of the imidazole motif might be of significance for the binding ability of these scaffolds. It is interesting to note that two previously identified allosteric inhibitors, **5** and **6**, both also have aryl halide moieties within their structures.

These best six compounds (**16c**, **16f**, **17c**, **17f**, **20a** and **20d**) were further tested in the AlphaScreen TruHits™ Kit counter assay[46] to identify false positive results (which may arise from compound signal interfering with the AlphaScreen™ assay) and all six compounds were confirmed as true inhibitors of the interaction between the HIV-1 integrase and LEDGF/p75 proteins.

2.2.2. Cytotoxicity and antiviral studies

The newly synthesized 1-substituted-5-aryl-1*H*-imidazoles **16-20**, 5-aryl-1,3-oxazoles **21** and (4*R*,5*R*)/(4*S*,5*S*)-5-aryl-4-tosyl-2,4-dihydro-1,3-oxazoles **22** were evaluated for cytotoxicity in the metallothionein type 4 (MT-4) cell line. At an initial concentration of 10 μM, none of the compounds tested reduced the viability of the cells in culture as detected by MTS [(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium)].[18,47-48] The CC_{50} data obtained for the six compounds found to be most active in the AlphaScreen™ assay (**16c**, **16f**, **17c**, **17f**, **20a** and **20d**) showed that these compounds were not overtly toxic and produced CC_{50} values ranging from 55.5 to 21.8 μM (Table 3). In general, however, the IC_{50} values of compounds **16c**, **16f**, **17c, 17f** and **20a** were close to their cytotoxicity values. Compound **20d** was more potent (IC_{50} = 7.0 μM) and was not overly toxic (CC_{50} = 55.5 μM), suggesting a smaller adverse effect on host cells, and thus representing a good starting point for improvement of the biological activity. Finally, the six compounds were tested in a cell-based antiviral assay at 10 μM within the MT-4 cell line.[18,48] Unfortunately, the compounds did not reach potency levels to allow for antiviral activity at this relatively low compound concentration for fragment evaluation, which was chosen to avoid any direct toxic effect on the cells.

2.2.3. Antimicrobial activities

The synthesized fragments (**16-20**, **21** and **22**) were also evaluated for their *in vitro* antimicrobial efficacy towards two Gram-positive bacterial species (*Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 11779), two Gram-negative bacterial species (*Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 27858) and one yeast (*Candida tropicalis* ATCC 750) using the Minimum Inhibitory Concentration (MIC) assay.[49-51] Ciprofloxacin and nystatin were used as positive controls for bacteria and yeast, respectively, while 10% dimethyl sulfoxide (DMSO) in water and 100% acetone were used as the negative controls. All negative controls demonstrated that dissolution solvents on further serial dilution (resulting in a starting concentrations of 2.5%) had no effect on the antimicrobial activity. Selected antimicrobial activity results of the synthesized compounds are shown in Table 4.

Compounds which exhibited an MIC value of 15.6 μg/ml were considered to have good antibacterial activity, while MIC values of 31.3 and 62.5 μg/ml were considered to represent moderate and marginal activities, respectively. Compounds with antimicrobial activities of less than 100 μg/ml are noted to have clinical relevance.[52] The analysis of the antimicrobial results revealed that compound **16f** exhibited good MIC values of 15.6 μg/ml against *S. aureus* and moderate MIC values of 31.3 μg/ml against *B. cereus* and *C. tropicalis*. Compound **17f**, with a cyclohexyl group instead of the *n*-butyl group of **16f**, but with the same *tert*-butyl group at the *para*-position of the 5-aryl ring, was shown to be potent against *B. cereus* by exhibiting an MIC value of 15.6 μg/ml while against *C. tropicalis* it demonstrated a comparable MIC value to that of compound **16f**.Both compounds **16f** and **17f** were less active against the two Gram-negative bacterial strains. Compound **16e** was the only other compound showing noteworthy activity, with an MIC of 31.3 μg/ml against *B. cereus*. It was interesting to note that only the imidazoles showed activity, while the oxazoles and dihydrooxazoles were completely inactive. MIC values obtained suggest that the imidazole-based scaffolds could serve as a framework for further development through synthetic modification to obtain more potent biologically active compounds.

Table 4: Selected MIC (μg/ml) results for synthesized compounds

Compounds	Gram-positive bacteria		Gram-negative bacteria		Yeast
	S. aureus ATCC 2592	*B. cereus* ATCC 11779	*E. coli* ATCC 8739	*P. aeruginosa* ATCC 27858	*C. tropicalis* ATCC 750
16c	> 250	62.5	125	>250	62.5
16e	-	31.3	>250	>250	-
16f	15.6	31.3	250	250	31.3
17c	62.5	62.5	>250	>250	62.5
17d	62.5	250	>250	>250	125
17f	62.5	15.6	>250	>250	31.3
19a	62.5	125	>250	>250	>250
19d	62.5	125	>250	>250	250
Cipro-floxacin	0.156	0.156	0.313	0.313	-
Nysta-tin	-	-	-	-	1.25

3. Receptor-ligand pharmacophore model and screening

A pharmacophore hypothesis was developed manually using the receptor-ligand complex protocol of Maestro (Schrodinger software) in order to understand the binding similarity between the six identified hits with compounds known to induce multimerization of HIV-1 IN. The hypothesis was created based on the representative structure of ligand **5** binding at the LEDGF/p75 binding pocket in HIV-1 IN.[54-55] A 9-point hypothesis pharmacophore was generated as illustrated by the flow chart in Fig. 2 and is comprised of the following chemical features: A1 and A2 acceptor sites (pink); H3, H4 and H5 donor sites (green); the N6 negatively charged site (red) and R7, R8 and R9 hydrophobic sites (orange) with distance matching tolerance of 2.0 and excluded volume. The 3D hypothesis pharmacophore model was then used as a query to assess the best six hits **16c**, **16f**, **17c**, **17f**, **20a** and **20d**. The phase screen data showed that ligand **5** used as a reference mapped well with all features with a fitness score of 2.713. Compound **20a** shows a relatively higher fitness score of 1.824 than the other five compounds (i.e. **16f**, **17f**,

16c, **17c**, and **20d**) which displayed fitness scores of 1.776, 1.756, 1.689, 1.646 & 1.392, respectively. Furthermore, compound **20a** mapped well into five essential features on the hypothesis model, specifically, the two aromatic rings and the imidazole moiety predicted to occupy the R7, R8 and R9 hydrophobic spheres (orange) while the halide substituents were projected onto the H4 and H5 donor sites (green) as shown in Fig. 3A. The overlaying of **20a** with **5** displays the similar chemical features exhibited by the two aromatic rings (B and C) and *N*-heterocyclic moiety (A) of ligand **5** (Fig. 3B). Moreover, scaffolds **16c**, **16f**, **17c** and **17f** are predicted to have similar binding models and were projected to fit onto five chemical features on the hypothesis model. The *n*-butyl (for **16c** and **16f**) and cyclohexyl moieties (for **17c** and **17f**) were projected through R7 towards an H5 donor site (green) while their substituted aromatic rings pointed towards an R9 hydrophobic (orange) and an H4 donor site (green) as shown in see Fig 3C to 3J. Moreover, the imidazole moiety (for **16c**, **16f**, **17c** and **17f**) projected towards an R8 hydrophobic site (orange) while a nitrogen atom of the same imidazole motif projected towards the A1 acceptor point (pink). The superimposed models of **16c**, **16f**, **17c** and **17f** with **5** projected that the aliphatic and the aryl motifs lie in the same area occupied by the two aromatic rings (B and C) of **5** while the imidazole motif settled in the same area occupied by the *N*-heterocycle (A) of **5**. In contrast, the model of scaffold **20d** is predicted to have a different binding model in comparison to the other five hits (see. Fig 3K and 3L). The 2,4-dimethoxyphenyl group of **20d** is projected to occupy the A1 and A2 acceptor (pink) and the R8 hydrophobic sites (orange) while the *para*-bromophenyl group is shown to be pointing towards the R9 hydrophobic site (orange) and the H4 donor site (green). The imidazole motif of **20d** was predicted to be on the R7 hydrophobic point (orange).

4. Conclusion

In the present study, we have established the use of microwave-assisted cycloaddition of TosMIC to imines and aldehyde as an efficient approach for directly preparing small libraries of 1-substituted-5-aryl-1*H*-imidazoles, 5-aryl-1,3-oxazoles and *trans*-5-aryl-4-tosyl-4,5-dihydro-1,3-oxazoles with low molecular weight. Evaluation of the synthesized compounds in a direct HIV1 IN-LEDGF/p75 AlphaScreen™ assay at a single dose of 10 μM resulted in identification of six novel 1-substituted-5-aryl-1*H*-imidazoles (**16c**, **16f**, **17c**, **16f**, **20a**, and **20d**) able to disrupt the HIV-1 IN- LEDGF/p75 protein-protein interaction. To understand the structural features responsible for the biological activity of these compounds, a receptor-ligand pharmacophore hypothesis model was generated, built on previously identified inhibitor **5** at the LEDGF/p75 binding pocket in HIV-1 IN. The models superimposing **5** with the active compounds **16c**, **16f**, **17c**, **17f**, **20a** and **20d** showed that all the compounds besides **20d** settled in the same region occupied by the two aromatic rings (B and C) and the *N*-heterocyclic moiety (A) of compound **5**. Based on the hypothesis model, it is likely that the aromatic rings of these arylimidazoles occupy the hydrophobic pockets formed by helices at the interface made by two integrase monomers, as previously proposed for the quinoline and chlorophenyl rings of ligand **5**.[53-54] Despite having inhibitory activity within the biochemical assay, none of the best six active compounds inhibited viral replication within a cell based assay.

In an *in vitro* antimicrobial assay by the MIC method, compounds **16e**, **16f** and **17f** displayed activity against Gram-positive bacterial and yeast pathogens. Interestingly, compounds **16f** and **17f** both exhibited activity in inhibiting the HIV-1 IN-LEDGF/p75 interaction and antimicrobial activity and represent promising starting candidates for synthetic modifications to increase their potency. Once again, the versatility of the privileged imidazole moiety, which exhibits a variety of biological activities,[33,57-58] has been demonstrated. To the best of our knowledge, these compounds are novel and have not been reported previously in the literature as HIV-1 IN disruptors or inhibitors of bacterial pathogens. The success in the generation of diverse five membered nitrogen-containing heterocycle collections in the present study was made possible by the versatility of the TosMIC synthon. The viable hits identified provide a good starting point for synthetic variations that could result in compounds with improved potency, that are currently under investigation in our laboratory.

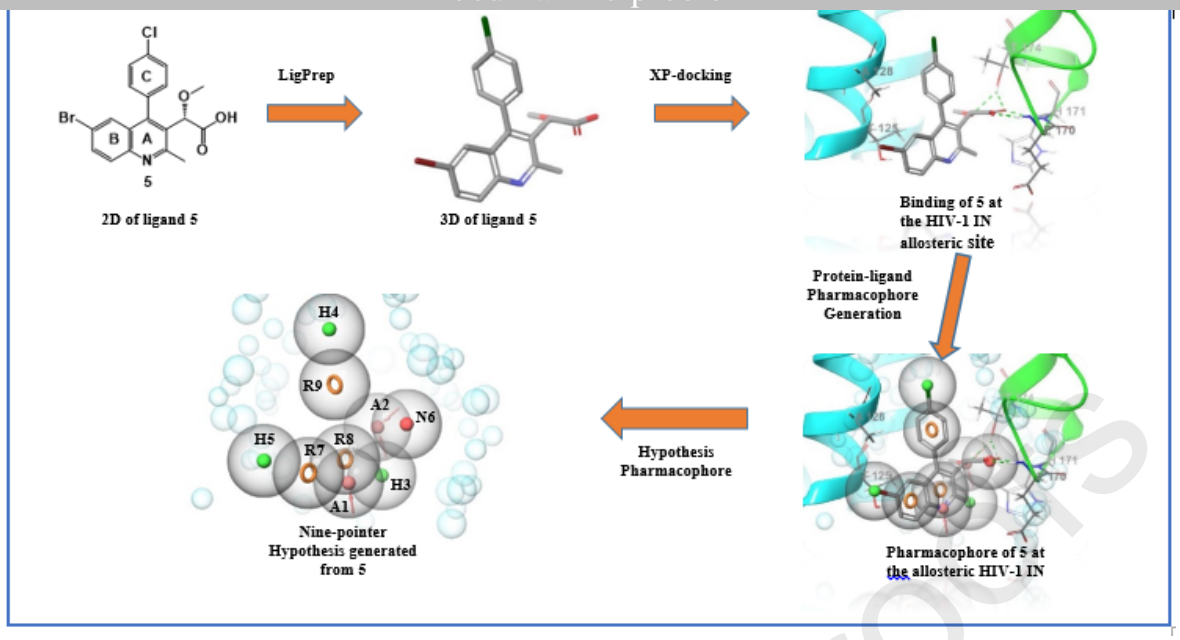


Fig 2: Overall flowchart of pharmacophore derived from **5** binding at the LEDGF/p75 binding pocket in HIV-1 IN.

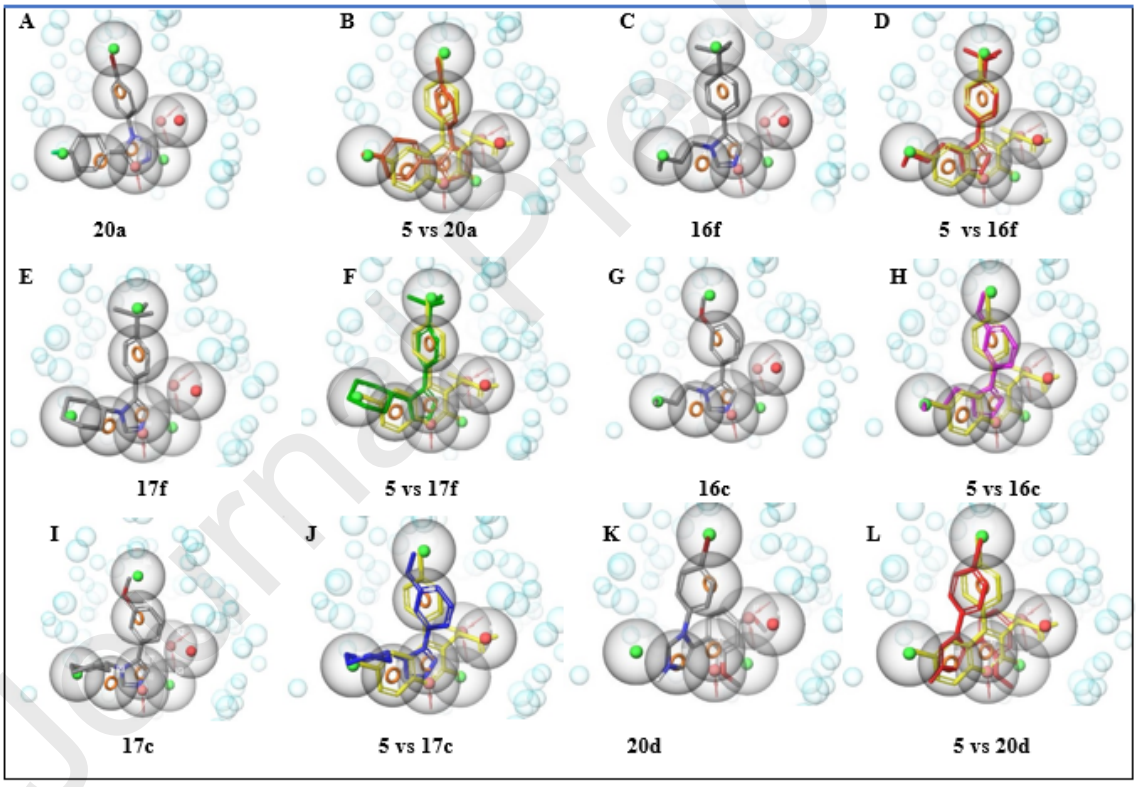


Fig. 3: mapping of: [A] **20a**; [C] **16f**; [E] **17f**; [G] **16c**; [I] **17c** and [K] **20d** onto the hypothesis model. Superposition mapping of **5** (yellow) with: [**B**] **20a** (orange); [**D**] **16f** (red); [**F**] **17f** (green); [**H**] **16c** (purple); [**J**] **17c** (blue) and [**L**] **20d** (red) onto the hypothesis model.

5.1. General methods and materials

All commercially available reagents were purchased from Sigma Aldrich. Dry solvents were used from an LC-Tech SP-1 Solvent Purification System stored under argon. Microwave-assisted reactions were performed in a CEM Discover reactor (Dynamic Temperature and Power set). Column chromatography was performed using Merck Silica gel 60 [particle size 0.040-0.063 mm (230-400 mesh)]. TLC was performed on pre-coated Merck silica gel F254 plates and viewed under UV light (254 nm) or following exposure to iodine. NMR spectra were recorded on a Bruker 400 Avance III Spectrometer at 298 K equipped with a 5 mm BBI probe Chemical shifts (δ) are given in parts per million and referenced by the solvent residual peak [all spectra were run in $CDCl_3$ δ: 7.26 ppm for ^{1}H NMR (400 MHz), and 77.0 ppm for ^{13}C NMR (101 MHz)]. High-resolution mass spectra were recorded on an LC-MS system consisting of a Dionex Ultimate 3000 Rapid Separation LC system equipped with a C-18 pre-coated column and coupled to a MicrOTOF QII Bruker mass spectrometer fitted with an electrospray source and operating in positive mode. FTIR spectra were recorded using a Thermo Nicolet 5700 spectrometer and samples were prepared as KBr mixtures. All melting points were obtained using a Stuart SMP10 melting point apparatus and are uncorrected.

5.2. Synthesis of *N*-benzylidene alkylamines (12-15)

5.2.1. Synthetic Method A: Conventional Method A

To a solution of aldehyde (2.72 mmol) in DCM (5 ml) was added primary amine (4.08 mmol) and $MgSO_4$ (5.44mmol). The resulting reaction mixture was then stirred at room temperature for 16h. The solution was filtered and the collected solid was washed with DCM (2 x 5 ml). The combined DCM filtrates were concentrated in *vacuo* and dried under high vacuum to give pure products.

5.2.2. Synthetic Method B: Microwave Irradiation

A mixture of aldehyde (4.80 mmol) and primary amine (5.76 mmol) in a 10 ml microwave vessel was irradiated neat at 60°C for 4 min (power applied controlled by the instrument). At the end of the reaction time, DCM (15 ml) was added and reaction mixture was transferred into round bottom flask and the vessel was then rinsed with DCM (2 x 5 ml). The combined DCM fractions were then concentrated in *vacuo* to give pure *N*-arylidene alkylamine.

5.2.3. (*E*)-*N*-(4-Fluorobenzylidene)butan-1-amine (***12a***). Yield: 87% (**Method A**), 98% (**Method B**) as pale brown oil; ^{1}H NMR: δ = 8.21 (1H, s, N=CH), 7.12 (2H, m, Hz, ArH), 7.02 (2H, t, J = 8.8 Hz, Ar**H**), 3.57 (2H, t, J = 7.0 Hz, N=CHCH_2), 1.63-1.70 (2H, m, N=CHCH_2CH_2), 1.33-1.42 (2H, m, N=CHCH_2CH_2CH_2), 0.92 (3H t, J = 7.4 Hz, N=CHCH_2CH_2CH_2CH_3); ^{13}C NMR: δ 164.1 (d, $^1J_{CF}$ = 250 Hz, *para*-CF), 159.1 (6-C) 132.6 (d, $^4J_{CF}$ = 3 Hz, 1'-C), 129.8 (d, $^3J_{CF}$ = 9 Hz, *ortho*-C), 115.5 (d, $^2J_{CF}$ = 22 Hz, *meta*-C), 61.3 (N=CHCH_2), 32.9 (N=CHCH_2CH2), 20.4 (N=CHCH_2CH_2CH_2), 13.8 (N=CHCH_2CH_2CH_2CH_3).

5.2.4. (E)-N-(2-Chlorobenzylidene)butan-1-amine (***12b***). Yield: 94% (**Method A**), 99% (**Method B**) as colourless oil; ^{1}H NMR: δ 8.61 (1H, s, N=C*H*), 7.92 (1H, d, J = 8.4 Hz, ArH), 7.17-7.28 (3H, m, ArH), 3.56 (2H, t, J = 7.0 Hz N=CHCH_2), 1.58-1.65 (2H, m, N=CHCH_2CH_2), 1.29-1.34 (2H, m, N=CHCH_2CH_2CH_2), 0.85 (3H, t, J = 7.4 Hz, N=CHCH_2CH_2CH_2CH_3); ^{13}C NMR δ 157.4 (N=*C*H), 134.9, 133.3, 131.2, 129.7, 128.2, 126.9 (Ar*C*), 61.5 (N=CHCH_2CH_2CH_2CH_3).

5.2.5. (E)-N-(3-Methoxybenzylidene)butan-1-amine (***12c***).Yield: 97% (**Method B**) as colourless oil. ^{1}H NMR: δ 8.26 (1H, s, N=C*H*), 7.28-7.37 (3H, m, Ar*H*), 6.98 (1H, d, J = 8.4 Hz, Ar*H*), 3.87 (3H, s, ArOCH_3), 3.62 (2H, t, J = 7.0 Hz, N=CHCH_2), 1.70-1.75 (2H, m, N=CHCH_2CH_2), 1.39-1.50 (2H, m, N=CHCH_2CH_2CH_2), 0.96 (3H, t, J = 7.4 Hz, N=CHCH_2CH_2CH_2CH_3); ^{13}C NMR: δ 160.5 (Ar*C*OCH$_3$), 159.8 (N=*C*H), 137.8, 129.4, 121.2, 117.1, 111.4 (Ar*C*), 61.3 (N=CH*C*H$_2$), 55.2 (ArCO*C*H$_3$), 32.9 (N=CHCH$_2$*C*H$_2$), 20.4 (N=CHCH$_2$CH$_2$*C*H$_2$), 13.8 (N=CHCH$_2$CH$_2$CH$_2$*C*H$_3$).

5.2.6. (E)-N-(2,4-Dimethoxybenzylidene)butan-1-amine (***12d***). Yields: 84% (**Method A**), 98% (**Method B**) as light brown oil; ^{1}H NMR: δ 8.48 (1H, s, N=C*H*), 7.77 (1H, d, J = 8.8 Hz, *ArH*), 6.37 (1H, d, J = 8.8 Hz, Ar*H*), 6.28 (1H, s, Ar*H*), 3.66 and 3.68 (6H, 2 x s, 2 x ArOCH_3), 3.44 (2H, t, J = 7.0 Hz, N=CHCH_2), 1.51-1.57 (2H, m, N=CHCH_2CH_2), 1.24-1.30 (2H, m, N=CHCH_2CH_2CH_2), 0.81 (3H, t, J = 7.4 Hz, N=CHCH_2CH_2CH_2CH_3); ^{13}C NMR: δ 162.5 and 159.6 (2 x Ar*C*OCH$_3$), 155.8 (N=*C*H), 128.1, 117.9, 105.0, 97.6 (Ar*C*), 61.4 (N=CH*C*H$_2$), 55.0 and 54.9 (2 x ArCO*C*H$_3$), 33.0 (N=CHCH$_2$*C*H$_2$), 20.2 (N=CHCH$_2$CH$_2$*C*H$_2$), 13.7 (N=CHCH$_2$CH$_2$CH$_2$*C*H$_3$).

5.2.7. (E)-N-(Benzo[d][1,3]dioxol-5-ylmethylene)butan-1-amine (***12e***). Yield: 96% (**Method A**), 100% (**Method B**) as brown oil; ^{1}H NMR: δ 8.11 (1H, s, N=C*H*), 7.32 (1H, s, Ar*H*), 7.05 (1H, d, J = 7.6 Hz, Ar*H*), 6.77 (1H, d, J = 8.0 Hz, Ar*H*), 5.94 (2H, s, ArOCH_2O-), 3.52 (2H, t, J = 7.0 Hz, N=CHCH_2), 1.60-1.67 (2H, m, N=CHCH_2CH_2), 1.33-1.39 (2H, m, N=CHCH_2CH_2CH_2), 0.90 (3H, t, J = 7.4 Hz, N=CHCH_2CH_2CH_2CH_3); ^{13}C NMR: δ 159.6 (N=*C*H), 149.5, 148.1, 131.1, 123.9, 107.8, 106.4 (Ar*C*), 101.2 (ArO*C*H$_2$O), 61.0 (N=CH*C*H$_2$), 33.0 (N=CHCH$_2$*C*H$_2$), 20.3 (N=CHCH$_2$CH$_2$*C*H$_2$), 13.8 (N=CHCH$_2$CH$_2$CH$_2$*C*H$_3$).

5.2.8. (*E)-N-(4-tert-butylbenzylidene)butan-1-amine* (***12f***). Yield: 84% (**Method A**), 93% (**Method B**) as pale brown oil; ^{1}H NMR: δ 8.30 (1H, s, N=C*H*), 7.65-7.68 (2H, m, Ar*H*), 7.41-7.43 (2H, m, Ar*H*), 3.15 (2H, t, J = 7.0 Hz, N=CHCH_2), 1.60-1.77 (2H, m, N=CHCH_2CH_2), 1.31-1.33 [11H, m, N=CHCH_2CH_2C$H_2$and ArC(CH_3)$_3$], 0.98 (3H, t, J = 7.4 Hz, N=CHCH_2CH_2CH_2CH_3); ^{13}C NMR: δ 158.4 (N=*C*H), 153.7, 134.0, 127.9 , 125.5 (Ar*C*), 70.1 (N=CH*C*H$_2$), 34.9 [ArC*C*(CH$_3$)], 33.0 (N=CHCH$_2$*C*H$_2$), 20.3 (N=CHCH$_2$CH$_2$*C*H$_2$), 13.8 (N=CHCH$_2$CH$_2$CH$_2$*C*H$_3$), 25.4 [ArCC(*C*H$_3$)$_3$].

5.2.9. (E)-N-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)methylene)-butan-1-amine (***12g***). Yield: 84% (**Method A**) as pale brown oil; ^{1}H NMR: δ 8.03 (1H, s, N=C*H*), 7.18 (1H, s, Ar*H*) 7.10 (1H, d, J = 8.4 Hz, Ar*H*), 6.77 (1H, d, J = 8.0 Hz, Ar*H*), 4.16 (4H, s, ArOCH_2CH_2O), 3.45-3.49 (2H, t, J = 7.0 Hz, N=CHCH_2), 1.55-159 (2H, m, N=CHCH_2CH_2), 1.26-1.31 (2H, m, N=CHCH_2CH_2CH_2), 0.83 (3H, t, J = 7.4 Hz, N=CHCH_2CH_2CH_2CH_3); ^{13}C NMR: δ 159.7 (N=*C*H), 145.5 and 143.5 (Ar*C*OCH$_2$CH$_2$O), 130.1, 121.5, 117.2, 116.5 (Ar*C*), 64.4 and 64.0 (ArCO*C*H$_2$*C*H$_2$O-), 61.3 (N=CH*C*H$_2$), 32.9 (N=CHCH_2*C*H$_2$), 20.3 (N=CHCH$_2$CH$_2$*C*H$_2$), 13.8 (N=CHCH$_2$CH$_2$CH$_2$*C*H$_3$).

5.2.10. (E)-N-(2-Nitrobenzylidene)butan-1-amine (***12h***). Yield: 73% (**Method B)** as yellow oil; ^{1}H NMR: δ 8.66 (1H, s, N=C*H*), 7.97-8.03 (2H, m, Ar*H*), 7.51-7.66 (2H, m, Ar*H*), 3.65 (2H, t, J = 7.0 Hz, N=CHCH_2), 1.66-1.73 (2H, m, N=CHCH_2CH_2), 1.35-1.44 (2H, m, N=CHCH_2CH_2CH_2), 0.92 (3H, t, J = 7.4 Hz,

N=C...H_2), 133.4, 131.3, 130.4, 129.6, 124.2 (Ar*C*), 61.4 (N=CH*C*H_2), 32.6 (N=CH*C*H_2*C*H_2), 20.3(N=CH*C*H_2*C*H_2*C*H_2), 13.8 (N=CH*C*H_2*C*H_2*C*H_2*C*H_3).

5.2.11. (E)-N-(4-Nitrobenzylidene)butan-1-amine (***12i***). Yield: 91% (**Method B**) as yellow oil; ^{1}H NMR: δ 8.34 (1H, s, N=C*H*), 8.22-8.25 (2H, m, Ar*H*), 7.86-7.90 (2H, m, Ar*H*), 3.64 (2H, t, *J* = 7.0 Hz, N=CHCH_2), 1.65-1.73 (2H, m N=CHCH$_2$CH_2), 1.38-1.43 (2H, m, N=CHCH$_2$CH$_2$CH_2), 0.92-0.96 (3H, t, *J* = 7.4 Hz, N=CHCH$_2$CH$_2$CH$_2$CH_3); ^{13}C NMR: δ 158.3 (N=*C*H), 148.8, 141.8, 128.6, 123.8 (Ar*C*), 61.6 (N=CH*C*H_2), 32.7 (N=CH*C*H_2*C*H_2), 20.4 (N=CHCH$_2$CH$_2$*C*H_2), 13.8 (N=CHCH$_2$CH$_2$CH$_2$*C*H_3).

5.2.12. (E)-N-(4-Fluorobenzylidene)cyclohexanamine (***13a***). Yield: 85% (**Method A**), 88% (**Method B**) as pale yellow oil; ^{1}H NMR: δ 8.26 (1H, s, N=C*H*), 7.69 (2H, m, Ar*H*), 7.04 (2H, t, *J* = 8.8 Hz, Ar*H*), 3.14 (1H, m, NC*H*CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$), 1.38-1.85 (10H, m, NCHCH_2CH_2CH_2CH_2CH_2); ^{13}C NMR: δ 164.0 (d, $^1J_{C,F}$ =249.6 Hz, *para*-*C*F), 157.0 (8-*C*), 132.9 (d, $^4J_{C,F}$ =3.0 Hz 1'-C), 129.8 (d, $^3J_{C,F}$ = 8.1 Hz, *ortho*-*C*), 115.6 (d, $^2J_{C,F}$ = 22.0 Hz, *meta*-*C*), 69.8 (N*C*HCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$), 34.3 (NCH*C*H$_2$CH$_2$CH$_2$CH$_2$*C*H_2), 25.6 (NCHCH$_2$*C*H$_2$CH$_2$*C*H$_2$CH_2), 24.7 (NCHCH$_2$CH$_2$*C*H$_2$CH$_2$CH_2).

5.2.13. (E)-N-(2-Chlorobenzylidene)cyclohexanamine (***13b***). Yield: 86% (**Method A**), 89% (**Method B**) as pale yellow solid; ^{1}H NMR: δ 8.71 (1H, s, N=C*H*), 7.99 (1H, d, *J* = 7.6 Hz, Ar*H*), 7.23-7.34 (3H, m, Ar*H*), 3.22-3.28 (1H, m, NC*H*CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$), 1.38-1.83 (10H, m, NCHCH_2CH_2CH_2CH_2CH_2); ^{13}C NMR: δ 155.4 (N=*C*H), 134.9, 133.6, 131.1, 129.6, 128.4, 126.9 (Ar*C*), 70.0 (N*C*HCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$), 34.3 (NCH*C*H$_2$CH$_2$CH$_2$CH$_2$*C*H_2), 25.6 (NCHCH$_2$*C*H$_2$CH$_2$*C*H$_2$CH_2), 24.7 (NCHCH$_2$CH$_2$*C*H$_2$CH$_2$CH_2).

5.2.14. (E)-N-(3-Methoxybenzylidene)cyclohexanamine (***13c***). Yield: 72% (**Method A**), 88% (**Method B**) as white solid; Mp: 58-61^{o}C; ^{1}H NMR: δ 8.30 (1H, s, N=C*H*), 7.28-7.34 (3H, m, Ar*H*), 6.95 (1H, dd, *J* = 8.0 Hz, 1.2 Hz, Ar*H*), 3.86 (3H, s, ArOCH_3), 3.17-3.25 (1H, m, NC*H*CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$), 1.38-1.88 (10H, m, NCHCH_2CH_2CH_2CH_2CH_2); ^{13}C NMR: δ 159.8 (Ar*C*OCH$_3$), 158.4 (N=*C*H), 138.1, 129.4, 121.2, 116.9, 111.7 (Ar*C*), 69.9 (N*C*HCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$), 55.3 (ArCO*C*H$_3$), 34.2 (NCH*C*H$_2$CH$_2$CH$_2$CH$_2$*C*H_2), 25.6 (4 NCHCH$_2$*C*H$_2$CH$_2$*C*H$_2$CH_2), 24.8 (NCHCH$_2$CH$_2$*C*H$_2$CH$_2$CH_2).

5.2.15. (E)-N-(2,4-Dimethoxybenzylidene)cyclohexanamine (***13d***). Yield: 81% (**Method A**), 99% (**Method B**) as a pale yellow solid; Mp: 55-58^{o}C; ^{1}H NMR: δ 8.63 (1H, s, N=C*H*), 7.87 (1H, d, *J*= 8.8 Hz, Ar*H*), 6.47 (1H, d, *J* = 6.8 Hz, Ar*H*), 6.40 (1H, s, ArH), 3.81 (6H 2 x s, 2 x ArOCH_3), 3.11-3.80 (1H, m, NC*H*CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$), 1.20-1.82 (10H, m, NCHCH_2CH_2CH_2CH_2CH_2); ^{13}C NMR: δ 162.7 and 159.8 (2 x Ar*C*OCH$_3$), 154.1 (N=*C*H), 127.5, 118.4, 105.2, 98.0 (Ar*C*), 70.2 (N*C*HCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$), 55.4 and 55.3 (2 x ArCO*C*H$_3$), 34.6 (NCH*C*H$_2$CH$_2$CH$_2$CH$_2$*C*H_2), 25.7 (NCHCH$_2$*C*H$_2$CH$_2$*C*H$_2$CH_2), 25.0 (NCHCH$_2$CH$_2$*C*H$_2$CH$_2$CH_2).

5.2.16. (E)-N-(Benzo[d][1,3]dioxol-5-ylmethylene)cyclohexanamine (***13e***). Yield: 90% (**Method A**), 97% (**Method B**) as a pale yellow solid; Mp: 64-67^{o}C (lit. [58]); ^{1}H NMR: δ 8.18 (1H, s, N=C*H*), 7.34 (1H, s, Ar*H*) 7.07 (1H, d, *J* = 6.8 Hz, Ar*H*), 6.79 (1H, d, *J* = 8.0 Hz, ArH), 5.96 (2H, s, ArOCH_2O), 3.10-3.16 (1H, m, NC*H*CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$), 1.36-1.84 (10H, m, NCHCH_2CH_2CH_2CH_2CH_2); ^{13}C NMR: δ 157.6 (N=*C*H), 149.4 and 148.1 (Ar*C*OCH$_2$O), 131.4, 123.9, 107.9 (Ar*C*), 101.3 (ArCO*C*H$_2$O), 61.0 (N*C*HCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$), 34.3 (NCHCH$_2$CH$_2$*C*H$_2$CH$_2$CH_2), 24.8 (NCHCH$_2$CH$_2$*C*H$_2$CH$_2$CH_2).

5.2.17. (E)-N-(4-tert-Butylbenzylidene)cyclohexanamine (***13f***). Yield: 93% (**Method A**), 99% (**Method B**) as a white solid; Mp.: 45-48^{o}C; ^{1}H NMR: δ 8.30 (1H, s, N=C*H*), 7.65 (2H, d, *J* = 8.4 Hz, Ar*H*), 7.41 (2H, d, *J* =8.4 Hz, Ar*H*), 3.15.-3.20 (1H, m, NC*H*CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$), 1.33-1.86 [19H, m, NCHCH_2CH_2CH_2CH_2CH_2 and ArC(CH_3)]; ^{13}C NMR: δ 158.4 (N=*C*H), 153.7 [Ar*C*C(CH$_3$)$_3$], 134.0, 127.9, 125.5 (Ar*C*), 70.1 (N*C*HCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$), 34.9 [ArC*C*(CH_3)$_3$], 34.4 (NCH*C*H$_2$CH$_2$CH$_2$CH$_2$*C*H_2), 31.3 [ArCC(*C*H$_3$)$_3$], 25.7 (NCHCH$_2$*C*H$_2$CH$_2$*C*H$_2$CH_2) 24.9 (NCHCH$_2$CH$_2$*C*H$_2$CH$_2$CH_2).

5.2.18. (E)-N-(2-Nitrobenzylidene)cyclohexanamine (***13h***). Yield: 95% (**Method B**) as a yellow oil; ^{1}H NMR: δ 8.70 (1H, s, N=C*H*), 7.97-8.03 (2H, m, Ar*H*), 7.61-7.65 (1H, m, Ar*H*), 7.49 (1H, m, Ar*H*), 3.28-3.33 (1H, m, NC*H*CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$), 1.23-1.85 (10H, m, NCHCH_2CH_2CH_2CH_2CH_2); ^{13}C NMR: δ 154.6 (N=*C*H), 148.7 (Ar*C*NO$_2$), 133.4, 131.6, 130.3, 12.7, 124.1 (Ar*C*), 70.0 (N*C*HCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$), 34.0 (NCH*C*H$_2$CH$_2$CH$_2$CH$_2$*C*H_2), 25.5 (NCHCH$_2$*C*H$_2$CH$_2$*C*H$_2$CH_2), 24.5 (NCHCH$_2$CH$_2$*C*H$_2$CH$_2$CH_2).

5.2.19. (E)-N-(4-Nitrobenzylidene)cyclohexanamine (***13i***). Yield: 91% (**Method B**) as a yellow oil; ^{1}H NMR: δ 8.38 (1H, s, N=C*H*), 8.23-8.25 (2H, m, Ar*H*), 7.86-7.89 (2H, m, Ar*H*), 3.25-3.30 (1H, m, NC*H*CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$), 1.25-1.87 (10H, m, NCHCH_2CH_2CH_2CH_2CH_2); ^{13}C NMR: δ 156.2 (N=*C*H), 148.8 (Ar*C*NO$_2$), 142.1, 128.7, 128.8 (Ar*C*), 70.1 (N*C*HCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$), 34.1 (NCH*C*H$_2$CH$_2$CH$_2$CH$_2$*C*H_2), 25.5 (NCHCH$_2$*C*H$_2$CH$_2$*C*H$_2$CH_2), 24.6 (NCHCH$_2$CH$_2$*C*H$_2$CH$_2$CH_2).

5.2.20. (E)-N-(4-Fluorobenzylidene)cyclopropaneamine (***14a***). Yield: 77% (**Method A**) as a brown oil; ^{1}H NMR: δ 8.40 (1H, s, N=C*H*), 7.64 (2H, d, *J* = 8.6 Hz, Ar*H*), 7.04 (2H, t, *J* = 8.6 Hz, Ar*H*), 2.98-3.02 (1H, m, NC*H*CH$_2$CH$_2$), 0.89-0.99 (4H, m, 3- & 4-CH_2); ^{13}C NMR: δ 163.9 (d, $^1J_{F,C}$ = 249.6 Hz, *para*-*C*F), 156.9 (N=*C*H), 132.8 (d, $^4J_{F,C}$ = 3.0 Hz, 1'-C), 129.8 (d, $^3J_{F,C}$ = 8.1 Hz, *ortho*-*C*), 115.5 (d, $^2J_{F,C}$ = 21.1 Hz, *meta*-*C*), 41.8 (N*C*HCH$_2$CH$_2$), 8.70 (NCH*C*H$_2$*C*H$_2$).

5.2.21. (*E*)-*N*-(3-Methoxybenzylidene)cyclopropanamine (***14c***). Yield: 92% (**Method A**) as a brown oil; ^{1}H NMR: δ 8.47 (1H, s, N=C*H*), 7.28-7.37 (3H, m, Ar*H*), 6.98 (1H, dd, *J* = 3.5 Hz, Ar*H*), 3.89 (3H, s, ArOCH_3), 3.07-3.10 (1H, m, NC*H*CH$_2$CH$_2$), 0.98-1.05 (4H, m, NCHCH_2CH_2); ^{13}C NMR: δ 159.8 (Ar*C*OCH$_3$), 158.2 (N=*C*H), 137.9, 129.5, 120.7, 116.6, 111.2 (Ar*C*), 55.3 (ArCO*C*H$_3$), 41.8 (N*C*HCH$_2$CH$_2$), 8.77 (NCH*C*H$_2$*C*H$_2$).

5.2.22. (E)-N-(2,4-Dimethoxybenzylidene)cyclopropanamine (***14d***). Yield: 87% (**Method A**) as a pale yellow solid; ^{1}H NMR: δ 8.77 (1H, s, N=C*H*), 7.79 (1H, d, *J*= 8.4 Hz, Ar*H*), 6.47 (1H, d, *J* = 8.4 Hz, Ar*H*), 6.42 (1H, s, Ar*H*), 3.81 and 3.84 (6H 2 x s, 2 x ArOCH_3), 2.99-3.02 (1H, m, NC*H*CH$_2$CH$_2$), 0.85-0.93 (4H, m, NCHCH_2CH_2); ^{13}C NMR: δ 162.7 and 159.8 (2 x Ar*C*OCH$_3$), 158.6 (N=*C*H), 129.5, 116.6, 111.2, 98.0 (Ar*C*), 55.4 and 55.3 (2 x ArCO*C*H$_3$), 41.8 (N*C*HCH$_2$CH$_2$), 8.77 (NCH*C*H$_2$*C*H$_2$).

5.2.23. (E)-N-(4-Fluorobenzylidene)-1-phenylmethanamine (1***5a***). Yield: 92% (**Method B)** as an orange solid; ^{1}H NMR: δ 8.37 (1H, s, C*H*=N), 7.78-7.82 (2H, m, Ar*H*) 7.05 (7H, m, ArH), 4.83 (2H, s, CH=NCH_2); ^{13}C NMR: δ 165.5 (d, $^1J_{C,F}$ = 250 Hz, *para*-*C*F), 160.4 (*C*H=N), 139.1 (Ar*C*), 132.4 (d, $^1J_{C,F}$ = 3 Hz, 1*'-C*), 130.1 , 130.0, 128.5 (d, $^3J_{C,F}$ = 8 Hz, *ortho*-*C*) 127.9 (Ar*C*), 115.6 (d, $^1J_{C,F}$ = 22 Hz, *meta*-*C*), 64.9 (CH=NCH_2).

5.2.24. (E)-N-(2-Chlorobenzylidene)-1-phenylmethanamine (***15b***). Yield: 93% (**Method B**) as a pale yellow oil; ^{1}H NMR: δ

8.86 (8H, m, Ar*H*), 4.89 (2H, s, CH=NCH_2); ^{13}C NMR: δ 158.7 (*C*H=N), 139.0, 135.2, 133.1, 131.6, 129.7, 128.6, 128.3, 128.0, 127.1, 127.0 (Ar*C*); 65.3 (CH=N*C*H_2).

5.2.25. (E)-N-(3-Methoxybenzylidene)-1-phenylmethanamine (***15c***). Yield: 98% (**Method B**) as a pale yellow oil;^{1}H NMR: δ 8.29 (1H, s, C*H*=N), 7.31 (1H, d, *J* = 8.4 Hz, Ar*H*), 6.91-7.29 (8H, m, Ar*H*), 4.72 (2H, s, CH=NCH_2), 3.76 (3H, s, ArOCH_3); ^{13}C NMR: δ 161.9 (*C*H=N), 159.8 (Ar*C*OCH$_3$), 139.1, 137.5, 129.5, 128.4, 127.9, 126.9, 121.9, 117.5, 115.6 (Ar*C*), 64.9 (CH=N*C*H$_2$), 55.3 (ArCO*C*H$_3$).

5.2.26. (E)-N-(2,4-Dimethoxybenzylidene)-1-phenylmethanamine (***15d***). Yield: 90% (**Method B**) as a yellow oil; ^{1}H NMR: δ 8.89 (1H, s, C*H*=N), 8.11 (1H, d, *J* = 8.4 Hz, Ar*H*), 7.36-7.47 (5H, m, ArH), 6.5-6.66 (2H, m, ArH), 4.97 (2H, s, CH=NCH_2), 3.95 and 3.97 (6H, 2 x s, 2 x ArOCH_3); ^{13}C NMR: δ 163.0 and 160.1 (2 x Ar*C*OCH$_3$), 157.4 (*C*H=N), 139.9, 128.6, 128.5, 128.3, 127.9, 117.9, 105.3, 98.0 (Ar*C*), 65.5 (CH=N*C*H$_2$), 55.4 and 55.3 (2 x ArCO*C*H$_3$).

5.2.27. (*E*)-*N*-(Benzo[*d*][1,3]dioxol-5-ylmethylene)-1-phenylmethanamine (***15e***). Yield: 95% (**Method B**) as a white solid; Mp: 64-70^{o}C (lit.[59]); ^{1}H NMR: δ 8.18 (1H, s, C*H*=N), 7.04-7.34 (8H, m, ArH), 6.73 (1H, d, *J* = 8.8 Hz, Ar*H*), 5.90 (2H, s, ArOCH_2O), 4.96 (2H, s, CH=NCH_2); ^{13}C NMR: δ 161.0 (*C*=N), 149.9 and 148.2 (2 x Ar*C*OCH$_2$O), 139.4, 131.0, 128.4, 127.9, 126.9, 124.5, 108.0, 106.7 (Ar*C*), 101.4 (ArCO*C*H$_2$O), 64.7 (C=*NCH*$_2$).

5.3 Synthesis of 1-substituted-5-aryl-1*H*-imidazoles (*16-20*)

5.3.1. Synthetic method C: *stepwise van Leusen general*

N-arylidene alkylamine (1.93 mmol), TosMIC (2.89 mmol) and K_2CO_3 (2.51 mmol) were placed in a dried three-necked flask equipped with a refluxing condenser under inert atmosphere (degassed by evacuating and refilling with argon three times), followed by addition of dry acetonitrile (5 ml). The resulting reaction mixture was then heated at reflux for 72 h under argon. Upon completion the solvent was evaporated *in vacuo* to afford a brown oily crude material which was purified by column chromatography [eluents: hexane- ethyl acetate c (1:2), followed by 100% ethyl acetate] to afford the desired pure 1-substituted-5-aryl-1*H*-imidazoles.

5.3.2. Synthetic method D: microwave assisted van Leusen

In a 10 ml microwave reaction vessel equipped with a magnetic stirrer bar was introduced *N*-arylidene alkylamine (1.77 mmol), TosMIC (2.66 mmol) and K_2CO_3 (2.30 mmol). An inert atmosphere was created by degassing through evacuating and refilling with argon three times, followed by addition of dry acetonitrile (5 ml) and capping. The resulting reaction mixture was then microwave-irradiated at a set temperature of 90^{o}C and a power of 120 Watts for 7 h. After completion the solvent was evaporated *in vacuo* to give a brown crude oil which was then purified by column chromatography as described in procedure C to afford the desired 1-substituted-5-aryl-1*H*-imidazoles.

5.3.3. Synthetic method E: Microwave-assisted two step-one pot van Leusen reaction

In order to generate the *N*-arylidene alkylamine *in situ*, aryl aldehyde (0.90 mmol) and an aliphatic amine (0.99 mmol) were introduced into a 10 ml microwave reaction vessel equipped with a magnetic stirrer. The resulting reaction mixture was then microwave-irradiated at a set temperature of 60^{o}C for 4 min (*N.B:* in cases where aniline derivatives were used, the reaction mixture was irradiated for 1 h). The mixture was allowed to reach room temperature and then TosMIC (1.35 mmol) and K_2CO_3 (1.17 sing through evacuating and refilling with argon three times, followed by addition of dry MeCN (5 ml) and capping. The resulting reaction mixture was then irradiated at a set temperature of 90^{o}C and a power of 120 Watts for 7 h. After completion the solvent was evaporated to give a brown oily residue, which was purified by column chromatography (as described in synthetic procedure C) to afford the desired 1-substituted-5-aryl-1*H*-imidazoles.

5.3.4. 1-Butyl-5-(4-fluorophenyl)-1H-imidazole (***16a***). Yield: 38% (**Method C**), 55% (**Method D**) as a brown oil; ^{1}H NMR: δ 7.53 (1H, s, 2-C*H*), 7.29-7.32 (2H, m, Ar*H*), 7.08-7.13 (2H, m, Ar*H*), 7.00 (1H, s, 4-C*H*) 3.88 (2H, t, *J* =7.0 Hz, NCH_2), 1.53-161 (2H, m, NCH$_2$CH_2), 1.16-1.25 (2H, m, NCH$_2$CH$_2$CH_2), 0.79 (3H, t, *J* = 7.4 Hz, NCH$_2$CH$_2$CH$_2$CH_3); ^{13}C NMR: δ 163.0 (d, $J_{C,F}$= 248.6 Hz, *para-C*F), 138.0 (2-*C*H), 131.8 (C-5), 130.6 (d, $^{3}J_{F,C}$ = 8.1 Hz, *ortho-C*), 128.1 (4-*C*H), 126.2 (d, $^{4}J_{F,C}$ = 4.0 Hz, 1'-*C*), 115.7 (d, $^{2}J_{F,C}$ = 21.1 Hz, *meta-C*), 44.9 (N*C*H$_2$), 32.8 (NCH$_2$CH_2), 19.6 (NCH$_2$CH$_2$*C*H$_2$), 13.4 (NCH$_2$CH$_2$CH$_2$CH$_3$); FTIR ν_{max}/cm^{-1} (KBr): 3388, 3104, 2960, 2873, 1682, 1599, 1516, 1456, 1448, 1221, 1110, 1023, 915, 853, 755, 695, 658, 603, 537; HRMS (ESI-TOF) m/z: [M+H]$^{+}$ Calcd for $C_{13}H_{16}FN_2$ 219.1292, found 219.1294.

5.3.5. 1-Butyl-5-(2-chlorophenyl)-1H-imidazole (***16b***). Yield: 20% (**Method C**), 26% (**Method D**) as a brown oil; ^{1}H NMR: δ 7.58 (1H, s, 2-C*H*), 7.33-7.50 (4H, m, Ar*H*), 7.02 (1H, s, 4-C*H*), 3.78 (2H, t, *J* =7.2 Hz, NCH_2), 1.50-1.57 (2H, m, 7-CH$_2$), 1.13-1.22 (2H, m, NCH$_2$CH$_2$CH_2), 0.77 (3H, t, *J* = 7.2 Hz, NCH$_2$CH$_2$CH$_2$CH_3); ^{13}C NMR: δ 137.5 (2-*C*H), 135.0 (5-*C*), 132.8, 130.2, 129.8, 129.7, 129.3 (Ar*C*), 128.9 (4-*C*H), 126.8 (Ar*C*), 45.1 (N*C*H$_2$), 32.7 (NCH$_2$CH_2), 19.5 (NCH$_2$CH$_2$*C*H$_2$), 13.4 (NCH$_2$CH$_2$CH$_2$CH$_3$); FTIR ν_{max}/cm^{-1} (KBr): 3401, 2959, 2873, 1727, 1633, 1557, 1458, 1367, 1285, 1220, 1145, 1114, 1035, 916, 767, 659, 537; HRMS (ESI-TOF) m/z: [M+H]$^{+}$ Calcd for $C_{13}H_{16}ClN_2$ 235.0995, found 235.1002.

*5.3.6. 1-Butyl-5-(3-methoxyphenyl)-1H-imidazole (**16c**)*. Yield: 60% (**Method D**) as a yellow oil; ^{1}H NMR: δ 7.54 (1H, s, 2-C*H*), 7.31 (1H, t, *J* = 6.8 Hz, Ar*H*), 7.04 (1H, s, 4-C*H*), 6.89-6.95 (3H, m, Ar*H*), 3.94 (2H, t, *J* = 7.4 Hz, NCH_2), 3.83 (3H, s, ArOCH_3), 1.57-1.65 (2H, m, NCH$_2$CH_2), 1.18-1.28 (2H. m, NCH$_2$CH$_2$CH_2), 0.82 (3H, t, *J* = 7.4 Hz, NCH$_2$CH$_2$CH$_2$CH_3); ^{13}C NMR: δ 159.7 (Ar*C*OCH$_3$), 138.0 (2-*C*H), 132.8 (5-*C*), 131.5, 129.7 (Ar*C*), 128.1 (4-*C*H), 121.1, 114.5, 113.3 (Ar*C*), 55.3 (ArCO*C*H$_3$), 45.1 (N*C*H$_2$), 32.9 (NCH$_2$CH_2), 19.6 (NCH$_2$CH$_2$*C*H$_2$), 13.4 (NCH$_2$CH$_2$CH$_2$CH$_3$); FTIR ν_{max}/cm^{-1} (KBr): 3383, 2958, 2873, 1682, 1610, 1580, 1489, 1290, 1212, 1169, 1116, 1050, 1029, 919, 845, 785, 698, 656, 570; HRMS (ESI-TOF) m/z: [M+H]$^{+}$ Calcd for $C_{14}H_{19}N_2O$ 231.1492, found 231.1498.

5.3.7. 1-Butyl-5-(2,4-dimethoxyphenyl)-1H-imidazole (***16d***). Yield: 80% (**Method C**), 87% (**Method D**), 81% (**Method E**) as brown oil; ^{1}H NMR: δ 7.53 (1H, s, 2-C*H*), 7.12 (1H, d, *J*=8.8 Hz, Ar*H*), 6.92 (1H, s, 4-C*H*), 6.52 (2H, d, *J* = 7.6 Hz, Ar*H*), 3.84 and 3.75 (6H, 2 x s, 2 x ArOCH_3), 3.74 (2H, t, *J* =7.2 Hz, NCH_2), 1.51-1.58 (2H, m, NCH$_2$CH_2), 1.15-1.21 (2H, m, NCH$_2$CH$_2$CH_2), 0.78 (3H, t, *J* = 7.2 Hz, NCH$_2$CH$_2$CH$_2$CH_3); ^{13}C NMR: δ 161.5 and 158.4 (2 x Ar*C*OCH$_3$), 137.1 (2-*C*H), 132.9 (Ar*C*), 129.5 (5-*C*), 128.2 (4-*C*H), 111.7, 104.4, 98.7 (Ar-*C*), 55.4 and 55.3 (2 x ArCO*C*H$_3$), 45.0 (N*C*H$_2$), 32.6 (NCH$_2$CH_2), 19.7 (NCH$_2$CH$_2$*C*H$_2$), 13.4 (NCH$_2$CH$_2$CH$_2$CH$_3$); FTIR ν_{max}/cm^{-1} (KBr): 3383, 2958,2875, 1722, 1678, 1616, 1578, 1459, 1416, 1364, 1305, 1283, 1208, 1160, 1132, 1030, 92, 1030, 922, 834, 660, 618; HRMS (ESI-TOF) m/z: [M+H]$^{+}$ Calcd for $C_{15}H_{21}N_2O_2$ 261.1598, found 261.1587.

5.3. for Yield: 43% (**Method C**), 48% (**Method D**), 55% (**Method E**) as a brown oil; ^{1}H NMR: δ 7.48 (1H, s, 2-C*H*), 6.94 (1H, s, 4-C*H*), 6.77-6.84 (3H, m, Ar*H*), 5.97 (2H, s, ArOCH_2O), 3.87 (2H, t, *J* = 7.2 Hz, NCH_2), 1.53-1.60 (2H, m, NCH$_2$CH_2), 1.15-1.24 (2H, m, NCH$_2$CH$_2$CH_2), 0.79 (3H, t, *J* = 7.2 Hz, NCH$_2$CH$_2$CH$_2$CH_3); ^{13}C NMR: δ 147.7 and 147.4 (2 x Ar*C*OCH$_2$O), 137.6 (2-*C*H), 132.5 (5-*C*), 127.7 (4-*C*H), 123.7, 122.6, 109.2, 108.4 (Ar*C*), 101.2 (ArCO*C*H$_2$O), 44.8 (N*C*H$_2$), 32.7 (NCH$_2$CH_2), 19.5 (NCH$_2$CH$_2$CH_2), 13.3 (NCH$_2$CH$_2$CH$_2$*C*H$_3$); FTIR ν_{max}/cm^{-1} (KBr): 3378, 2959, 2931, 2873, 1721, 1683, 1609, 1558, 1478, 1379, 1331, 1237, 1113, 1038, 933, 879, 812, 660; HRMS (ESI-TOF) m/z: [M+H]$^+$ Calcd for $C_{17}H_{25}N_2O_2$ 245.1285, found 245.1276.

5.3.9. 1-Butyl-5-(4-tert-butylphenyl)-1H-imidazole (***16f***). Yield: 44% (**Method C**), 52% (**Method D**) as a brown oil; ^{1}H NMR δ: 7.55 (1H, s, 4-C*H*), 7.43 (2H, *J* = 8.4 Hz, Ar*H*), 7.28 (2H, *J* = 8.4 Hz, Ar*H*), 7.03 (1H, s, 4-C*H*), 3.93 (2H, t, *J* =7.2 Hz, NCH_2), 1.60-1.67 (2H, m, NCH$_2$CH_2), 1.35 [9H, s, C(CH_3)$_3$], 1.22-1.30 (2H, m, NCH$_2$CH$_2$CH_2), 0.83 (3H, t, *J*= 7.4 Hz, NCH$_2$CH$_2$CH$_2$CH_3); ^{13}C NMR: δ 151.0 [Ar*C*C(CH$_3$)$_3$], 137.8 (2-*C*H), 132.9 (N*C*H$_2$), 128.5 (Ar*C*), 127.8 (4-*C*H), 127.2, 125.9 (Ar*C*), 45.0 (N*C*H$_2$), 34.6 [ArC*C*(CH$_3$)$_3$], 32.9 (NCH$_2$CH_2), 31.2, [ArCC(*C*H$_3$)$_3$], 19.7 (NCH$_2$CH$_2$CH_2), 13.5 (NCH$_2$CH$_2$CH_2*C*H$_3$); FTIR ν_{max}/cm^{-1} (KBr): 3373, 2960, 2931, 2865, 1717, 1682, 1614, 1556, 1463, 1363, 1269, 1220, 1114, 1033, 916, 839, 659, 571; HRMS (ESI-TOF) m/z: [M+H]$^+$ Calcd for $C_{17}H_{25}N_2$ 257.2012, found 257.2008.

5.3.10. 1-Butyl-5-(2,3-dihydro-1,4-benzodioxin-6-yl)-1H-imidazole (***16g***). Yield: 54% (**Method C**) as a brown oil; ^{1}H NMR: δ 7.49 (1H, s, 2-C*H*), 6.96 (1H, s, 4-C*H*), 6.79-6.90 (3H, m, Ar*H*), 4.27 (4H, s, ArOCH_2CH_2O), 3.89 (2H, t, *J* =7.2 Hz, NCH_2), 1.55-1.63 (2H, m, NCH$_2$CH_2), 1.90-1.25 (2H, m, NCH$_2$CH$_2$CH_2), 0.81 (3H, t, *J* = 7.2 Hz, NCH$_2$CH$_2$CH$_2$CH_3); ^{13}C NMR: δ 143.5 and 143.4 (Ar*C*OCH$_2$CH$_2$O), 137.6 (2-*C*H), 132.4 (5-*C*), 127.7 (4-*C*H), 123.3, 122.0, 117.7, 117.3 (Ar*C*), 64.3 and 64.2 (ArCO*C*H$_2$*C*H$_2$O), 44.9 (N*C*H$_2$), 32.8 (NCH$_2$CH_2), 19.6 (NCH$_2$CH$_2$CH_2), 13.4 (NCH$_2$CH$_2$CH$_2$*C*H$_3$); FTIR ν_{max}/cm^{-1} (KBr): 3374, 2962, 2931, 2874, 1720, 1584, 1503, 1460, 1362, 1286, 1250, 1123, 1067, 921, 893, 815, 749, 658; HRMS (ESI-TOF) m/z: [M+H]$^+$ Calcd for $C_{15}H_{19}N_2O_2$ 259.1441, found 259.1434.

5.3.11. 1-Butyl-5-(2-nitrophenyl)-1H-imidazole (***16h***). Yield: 15% (**Method D**) as a brown oil; ^{1}H NMR: δ 8.00 (1H, , t, *J*= 4 Hz, Ar*H*), 7.57-7.68 (3H, m, 2-C*H* and Ar*H*), 7.42 (1H, , t, *J* = 7.2 Hz, Ar*H*), 6.96 (1H, s, 4-C*H*), 3.71 (2H, t, *J* =7.2 Hz, NCH_2), 1.51-1.57 (2H, m, NCH$_2$CH_2), 1.15-1.23 (2H, m, NCH$_2$CH$_2$CH_2), 0.79 (3H, t, *J* = 7.2 Hz, NCH$_2$CH$_2$CH$_2$CH_3); ^{13}C NMR: δ 149.5 (Ar*C*NO$_2$), 138.0 (2-*C*H), 133.6 (5-*C*), 132.7 (Ar*C*), 129.9 (4-*C*H), 128.6, 127.3, 124.6, 124.5 (Ar*C*), 45.1 (N*C*H$_2$), 32.4 (NCH$_2$CH_2), 19.5 (NCH$_2$CH$_2$CH_2), 13.3 (NCH$_2$CH$_2$CH$_2$*C*H$_3$); FTIR ν_{max}/cm^{-1} (KBr): 3400, 3108, 2959, 2873, 1727, 1633, 1557, 1456, 1367, 1367, 1284, 1219, 1146, 1114, 1083, 1035, 916, 812, 768, 659, 537; HRMS (ESI-TOF) m/z: [M+H]$^+$ Calcd for $C_{13}H_{16}N_3O_2$ 246.1237, found 246.1240.

5.3.12. 1-Butyl-5-(4-nitrophenyl)-1H-imidazole (***16i***). Yield: 13% (**Method D**) as a brown oil; ^{1}H NMR: δ 8.28-8.31 (2H, m, Ar*H*), 7.64 (1H, s, 2-C*H*), 7.54-7.56 (2H, m, Ar*H*), 7.21 (1H, s, 4-C*H*), 4.02 (2H, t, *J*=7.4 Hz, NCH_2), 1.60-1.65 (2H, m, NCH$_2$CH_2), 1.21-1.29 (2H, m, NCH$_2$CH$_2$CH_2), 0.83 (3H, t, *J* = 7.4 Hz, NCH$_2$CH$_2$CH_2CH_3); ^{13}C NMR: δ 147.03 (Ar*C*NO$_2$), 139.8 (2-*C*H), 136.7 (5-*C*), 130.8, 130.2 (Ar*C*), 128.6 (4-*C*H), 124.2 (Ar*C*), 45.6 (N*C*H$_2$), 32.8 (NCH$_2$CH_2), 19.6 (NCH$_2$CH$_2$CH_2), 13.4 (NCH$_2$CH$_2$CH$_2$*C*H$_3$); FTIR ν_{max}/cm^{-1} (KBr):): 3388, 3103, 2960, 2873, 1682, 1599, 1516, 1456, 1368, 1221, 1110, 1023, 916, 854, 813, 749, 659, 537; HRMS (ESI-TOF) m/z: [M+H]$^+$ Calcd for $C_{13}H_{16}N_3O_2$ 246.1237, found 246.1238.

5.3.13. 1-Cyclohexyl-5-(4-fluorophenyl)-1H-imidazole (***17a***).Yield: 46% (**Method C**), 51% (**Method D**) as a pale yellow solid; Mp: 111-114^{o}C; ^{1}H NMR: δ 7.64 (1H, s, 2-C*H*), 7.27-7.7.30 (2H, m, Ar*H*), 7.01-7.13 (2H, m, Ar*H*), 6.69 (1H, s, 4-C*H*) 3.79-3.87 (1H, m, NC*H*), 1.18-2.01 (10H, m, NCHCH_2CH_2CH_2CH_2CH_2); ^{13}C NMR: δ 162.5 (d, $^1J_{C,F}$= 247.6 Hz, *para-C*F), 134.9 (2-*C*H), 131.4 (5-*C*), 130.8 (d, $^3J_{F,C}$= 8.1 Hz, *ortho-C*), 127.6 (4-*C*H), 126.3 (d, $^4J_{F,C}$ = 3.0 Hz, 1'-*C*), 115.6 (d, $^2J_{F,C=}$ 21.1 Hz, *meta-C*), 54.5 (N*C*H), 34.7 (NCH*C*H$_2$CH$_2$CH$_2$CH$_2$*C*H$_2$), 25.6 (NCHCH$_2$*C*H$_2$CH$_2$*C*H$_2$CH_2), 25.1 (NCHCH$_2$CH$_2$*C*H$_2$CH$_2$CH_2); FTIR ν_{max}/cm^{-1} (KBr): 3109, 3091, 3070, 2942, 2857, 1665, 1630, 1609, 1557, 1491, 1470, 1455, 1357, 1265, 814, 664, 604, 565, 504; HRMS (ESI-TOF) m/z: [M+H]$^+$ Calcd for $C_{15}H_{18}FN_2$ 245.1449, found 245.1475.

5.3.14. 1-Cyclohexyl-5-(2-chlorophenyl)-1H-imidazole (***17b***). Yield: 23% (**Method C**), 29% (**Method D**) as a pale yellow solid; Mp. 131-134^{o}C; ^{1}H NMR: δ 7.69 (1H, s, 2-C*H*), 7.28-7.49 (4H, m, Ar*H*), 6.97 (1H, s, 4-C*H*), 3.54-3.60 (1H, m, NC*H*), 1.17-2.01 (10H, m, NCHCH_2CH_2CH_2CH_2CH_2); ^{13}C NMR: δ 135.1 (2-*C*), 134.6 (5-*C*), 132.9, 130.2, 129.7, 129.3 (Ar*C*), 128.0 (4-*C*H), 126.8 (Ar*C*), 55.1 (N*C*H), 34.5 (NCH*C*H$_2$CH$_2$CH$_2$CH$_2$*C*H$_2$)), 25.6 (NCHCH$_2$*C*H$_2$CH$_2$*C*H$_2$CH_2), 25.1 (NCHCH$_2$CH$_2$*C*H$_2$CH$_2$CH_2); FTIR ν_{max}/cm^{-1} (KBr): 3095, 2934, 2855, 1704, 1661, 1566, 1477, 1453, 1272, 1231, 1113, 1074, 1034, 923, 916, 830, 754, 666; HRMS (ESI-TOF) m/z: [M+H]$^+$ Calcd for $C_{15}H_{18}ClN_2$ 261.1153, found 261.1155.

*5.3.15. 1-Cyclohexyl-5-(3-methoxyphenyl)-1H-imidazole (**17c**).* Yield: 47% (**Method C**), 53% (**Method D**) as a pale yellow oil; ^{1}H NMR: δ 7.64 (1H, s, 2-C*H*), 7.31-7.35 (1H, m, Ar*H*), 7.00 (1H, s, 4-C*H*), 6.86-6.92 (3H, m, Ar*H*), 3.93-3.99 (1H, m, NC*H*), 3.78 (3H, s, ArOCH_3), 1.22-2.04 (10H, m, NCHCH_2CH_2CH_2CH_2CH_2); ^{13}C NMR: δ 159.6 (Ar*C*OCH$_3$), 135.0 (2-*C*), 132.4 (5-*C*), 131.6, 129.6, 127.5 (4-*C*H), 121.3, 114.7, 113.3 (Ar*C*), 55.2 (ArCO*C*H$_3$), 54.5 (N*C*H), 34.8 (NCH*C*H$_2$CH$_2$CH$_2$CH$_2$*C*H$_2$), 25.6 (NCHCH$_2$*C*H$_2$CH$_2$*C*H$_2$CH_2), 25.1 (NCHCH$_2$CH$_2$*C*H$_2$CH$_2$CH_2); FTIR ν_{max}/cm^{-1} (KBr): 2934, 2856, 1679, 1605, 1580, 1482, 1450, 1352, 1318, 1225, 1117, 1168, 1087, 1052, 1036, 998, 860, 844, 814, 699, 659; HRMS (ESI-TOF) m/z: [M+H]$^+$ Calcd for $C_{16}H_{21}N_2O$ 257.1648, found 257.1668.

*5.3.16. 1-Cyclohexyl-5-(2,4-dimethoxyphenyl)-1H-imidazole (**17d**)*. Yield: 64% (**Method C**), 74% (**Method D**),73% (**Method F**) as a pale yellow solid; Mp: 99-102^{o}C; ^{1}H NMR: δ 7.64 (1H, s, 2-C*H*), 7.13 (1H, dd, *J* = 2.9 Hz, Ar*H*), 6.89 (1H, s, 4-C*H*), 6.49-6.51 (2H, t, *J* = 4.8 Hz, Ar*H*), 3.85 and 3.75 (6H, 2 x s, 2 x ArOCH_3), 3.55-3.62 (1H, m, NC*H*), 1.52-2.00 (10H, m, NCHCH_2CH_2CH_2CH_2CH_2); ^{13}C NMR: δ 161.5 and 158.4 (2 x Ar*C*OCH$_3$), 134.5(2-*C*), 133.1 (5-C), 129.1 (Ar*C*), 127.3 (4-*C*H), 111.7, 104.5, 98.7 (Ar*C*), 55.4 and 55.2 (2 x ArCO*C*H$_3$), 54.9 (N*C*H), 34.7 (NCH*C*H$_2$CH$_2$CH$_2$CH$_2$*C*H$_2$), 25.8 (NCHCH$_2$*C*H$_2$CH$_2$*C*H$_2$CH_2), 25.3 (NCHCH$_2$CH$_2$*C*H$_2$CH$_2$CH_2); FTIR ν_{max}/cm^{-1} (KBr): 3096, 3002, 2957, 2932, 2857, 1614, 1581, 1553, 1495, 1435, 1307, 1289, 1265, 1211, 1161, 1136, 1055, 1028, 925, 817, 796; HRMS (ESI-TOF) m/z: [M+H]$^+$ Calcd for $C_{17}H_{23}N_2O_2$ 287.1754, found 287.1770.

5.3.17. 5-(Benzo[d][1,3]dioxol-5-yl)-1-cyclohexyl-1H-imidazole (***17e***). Yield: 35% (**Method C**), 54% (**Method D**), 50% (**Method E**) as pale yellow solid; Mp: 85-88^{o}C; ^{1}H NMR: δ 7.58 (1H, s, 2-C*H*), 6.90 (1H, s, 4-C*H*), 6.72-6.83 (3H, m, Ar*H*), 5.96 (2H, s, ArOCH_2O), 3.81-3.87 (1H, m, NC*H*), 1.18-1.98 (6H, m, NCHCH_2CH_2CH_2CH_2CH_2); ^{13}C NMR: δ 147.6 and 147.4

122.8, 109.5, 108.4 (Ar*C*), 101.1 (ArCO*C*H_2O), 54.3 (N*C*H), 34.6 (NCH*C*H_2CH_2CH_2CH_2*C*H_2), 25.5 (NCHCH_2*C*H_2CH_2CH_2C*C*H_2), 25.0 (NCHCH_2CH_2*C*H_2CH_2C*C*H_2); FTIR ν_{max}/cm^{-1} (KBr): 3126, 3100, 3070, 2944, 2894, 2848, 2783, 1670, 1556, 1501, 1475, 1445, 1265, 1232, 1118, 1099, 1042, 943, 862, 878, 808, 659, 626; HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{16}H_{19}N_2O_2$ 271.1481, found 271.1464.

5.3.18. 1-Cyclohexyl-5-(4-tert-butylphenyl)-1H-imidazole (***17f***). Yield: 29% (**Method C**), 50% (**Method D**) as a pale orange solid; Mp:113-116^{o}C; ^{1}H NMR: δ 7.65 (1H, s, 2-C*H*), 7.44 (2H, d, *J* = 8.0 Hz, Ar*H*), 7.25 (2H, d, *J* = 8.4 Hz, ArH), 6.99 (1H, s, 4-C*H*) 3.92-4.00 (2H, m, NC*H*), 1.20-2.07 [19H, m, NCHCH_2CH_2CH_2CH_2CH_2 and C(CH_3)$_3$]; ^{13}C NMR: δ 150.9 [Ar*C*C(CH$_3$)$_]$, 134.9 (2-*C*H), 132.5 (5-*C*), 128.7 (Ar*C*), 127.3 (4-*C*H), 125.6 (Ar*C*), 54.4 (N*C*H), 34.9 (NCH*C*H_2CH_2CH_2CH_2*C*H_2), 34.6 [ArC*C*(CH$_3$)$_3$], 31.6 [ArCC(*C*H$_3$)$_3$], 25.7 (NCHCH_2*C*H_2CH_2*C*H_2C*C*H_2), 25.2 (NCHCH_2CH_2*C*H_2CH_2C*C*H_2); FTIR ν_{max}/cm^{-1} (KBr): 3130, 3065, 3035, 2931, 2860, 1669, 1613, 1548, 1474, 1463, 1454, 1403, 1360, 1268, 1218, 1117, 993, 917, 956, 817, 808, 665, 579; HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{17}H_{27}N_2$ 283.2169, found 283.2190.

5.3.19. 1-Cyclohexyl-5-(2-nitrophenyl)-1H-imidazole (***17h***). Yield: 12% (**Method D**) as a brown oil; ^{1}H NMR δ: 8.02 (1H, s, 2-C*H*), 7.58-7.59 (3H, m, Ar*H*), 7.41-7.42 (1H, m, Ar*H*), 6.92 (1H, s, 4-C*H*), 3.45-35.2 (1H, m, NC*H*), 1.21-1.18 - 2.02 (10H, m, NCHCH_2CH_2CH_2CH_2CH_2);^{13}C NMR δ: 147.5, 135.1(2-*C*H), 134.6 (5-*C*), 133.0, 130.2, 129.3, (Ar*C*), 128.0 (4-*C*H), 126.8 (Ar*C*), 55.1 (N*C*H), 34.5 (NCH*C*H_2CH$_2$CH$_2$CH$_2$*C*H_2), 25.6 (NCHCH$_2$*C*H$_2$CH$_2$*C*H$_2$C*C*H_2), 25.1 (NCHCH$_2$CH$_2$*C*H$_2$CH$_2$C*C*H_2); HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{15}H_{18}N_3O_2$ 272.1394, found 272.1393.

5.3.20. 1-Cyclohexyl-5-(4-nitrophenyl)-1H-imidazole (***17i*).** Yield: 10% (**Method D**) as a brown oil; ^{1}H NMR: δ 8.30 (2H, d, *J* =8.4 Hz, Ar*H*), 7.77 (1H, s, 2-C*H*), 7.49 (2H, d, *J* = 8.8 Hz, Ar*H*), 7.15 (1H, s, 4-C*H*), 3.92-3.98 (1H, m, NC*H*), 1.25-2.07 (10H, m, NCHCH_2CH_2CH_2CH_2CH_2); ^{13}C NMR: δ 147.2 (Ar*C*NO$_2$), 136.7 (2-*C*H), 136.6 (5-*C*), 130.6 (Ar*C*), 129.1 (4-*C*H), 124.2 (Ar*C*), 55.5 (N*C*H), 34.8 (NCH*C*H_2CH$_2$CH$_2$CH$_2$*C*H_2), 25.6 (NCHCH$_2$*C*H$_2$CH$_2$*C*H$_2$C*C*H_2), 25.0 (NCHCH$_2$CH$_2$*C*H$_2$CH$_2$C*C*H_2); HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{15}H_{18}N_3O_2$ 272.1394, found 272.1382.

*5.3.21. 1-Cyclopropyl-5-(4-fluorophenyl)-1H-imidazole (**18a***). Yield: 23 % **(Method D)** as a brown oil; ^{1}H NMR: δ 7.58 (1H, s, 2-C*H*), 7.46-7.50 (2H, m, Ar*H*), 7.09-7.13 (2H, m, Ar*H*), 7.03 (1H, s, 4-C*H*), 3.28-3.34 (H, m, NC*H*), 0.83-0.97 (4H, m,NCH_2CH_2); ^{13}C NMR: δ 162.4 ($^1J_{F,C}$ = 245.9 Hz, *para*-CF), 138.6 (2-*C*H), 133.4 (5-*C*), 129.8 (d, $^3J_{F,C}$ = 8.1 Hz, *ortho*-C), 127.6 (4-*C*H), 126.2(d, $^4J_{F,C}$ = 3.0 Hz, 1'-C), 115.6 (d, $^2J_{F,C}$ = 21.1 Hz, *meta*-C), 27.3 (6-*C*), 7.48 (N*C*H$_2$*C*H$_2$); HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{12}H_{12}FN_2$ 203.0974, found 203.0979.

5.3.22. 1-Cyclopropyl-5-(3-methoxyphenyl)-1H-imidazole (***18c***). Yield: 40% (**Method D)** as a pale yellow oil; ^{1}H NMR: δ 7.58 (1H, s, 2-C*H*), 7.31 (1H, t, *J* = 7.8 Hz, Ar*H*), 6.88-7.13 (5H, overlapping multiplets and singlet, Ar*H* and 4-C*H*), 3.84 (3H, s, ArOCH_3), 3.33-3.39 (1H, m, NC*H*), 0.86-1.01 (4H, m, NCH_2CH_2); ^{13}C NMR: δ 159.6 (Ar*C*OCH$_3$), 138.7 (2-*C*H), 134.2 (5-*C*), 131.3, 129.5 (Ar*C*), 127.9 (4-*C*H), 120.5, 113.9, 112.9 (Ar*C*), 55.1 (ArCO*C*H$_3$), 27.5 (N*C*H), 7.56 (N*C*H$_2$*C*H$_2$); HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{13}H_{15}N_2O$ 215.1179, found 215.1188.

(***18d***). Yield: 68% (**Method D)** as a brown oil; ^{1}H NMR: δ 7.49 (1H, s, 2-C*H*), 7.10 (1H, d, *J* = 8.8 Hz, Ar*H*), 6.87 (1H, s, 4-C*H*), 6.47 (2H, *J* = 6.8 Hz, Ar*H*), 3.73 and 3.80 (6H, 2 x s, 2 xArOCH_3), 3.18-3.22 (1H, m, NC*H*), 0.66-0.70 (4H, m, NCH_2CH_2); ^{13}C NMR: δ 161.2 and 158.3 (2 x Ar*C*OCH$_3$), 137.6 (2-*C*H), 132.0 (5-*C*), 131.0 (Ar*C*), 127.9 (4-*C*H), 111.9, 104.1, 98.4 (Ar*C*), 55.2 and 55.1 (2 x ArCO*C*H$_3$), 26.6 (N*C*H), 5.84 (N*C*H$_2$*C*H$_2$); HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{14}H_{17}N_2O_2$ 245.1285, found 245.1290.

5.3.24. 1-Benzyl-5-(4-fluorophenyl)-1H-imidazole (***19a***). Yield: 26% **(Method D)** as a brown oil. ^{1}H NMR; δ 7.57 (1H, s, 2-C*H*), 7.21-7.34 (5H, m, Ar*H*), 7.09 (1H, s, 4-C*H*), 6.97-7.05 (4H, m, Ar*H*), 5.10 (2H, s, NCH_2); ^{13}C NMR: δ 162.8 (d, $^1J_{C,F}$ = 248.6 Hz, *para*-*C*F), 138.6 (2-*C*H), 136.5 (5-*C*), 132.4 130.8, 130.7 (Ar*C*), 128.8 (d, $^4J_{F,C}$ = 3.0 Hz, 1'-*C*), 128.2 (4-*C*H) 128.0 (Ar*C*), 126.5 (d, $^3J_{F,C}$ = 8.1 Hz, *ortho*-C), 115.6 (d, $^2J_{F,C}$ = 21.1 Hz, *meta*-*C*), 48.6 (N*C*H$_2$); HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{16}H_{14}FN_2$ 253.1136, found 253.1138.

5.3.25. 1-Benzyl-5-(3-methoxyphenyl)-1H-imidazole (***19c***). Yield 24%; (**Method D)** as a brown oil; ^{1}H NMR: δ 7.58 (1H, s, 2-C*H*), 7.27-7.35 (4H, m, Ar*H*), 6.87-7.15 (5H, m, Ar*H*), 6.79 (1H, s, 4-C*H*), 5.17 (2H, s, NCH_2) 3.69 (3H, s, ArOCH_3); ^{13}C NMR: δ 159.6 (Ar*C*OCH$_3$), 138.7 (2-*C*H), 136.8 (5-*C*), 133.3, 130.8 (Ar*C*), 129.7 (4-*C*H), 128.9, 128.1, 127.9, 126.6, 121.2, 114.1, 113.9 (Ar*C*), 55.1 (ArCO*C*H$_3$), 48.7 (N*C*H$_2$); HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{17}H_{17}N_2O_2$ 265.1335, Found 265.1329.

5.3.26. 1-Benzyl-5-(2,4-dimethoxyphenyl)-1H-imidazole (***19d***). Yield; 74% (**Method D)** as a brown oil; ^{1}H NMR: δ 7.55 (1H, s, 2-C*H*), 7.28-7.31 (3H, m, ArH), 7.03-7.12 (4H, m, Ar*H* and 4-C*H*), 6.50-6.53 (2H, m, ArH), 4.98 (2H, s, NCH_2), 3.86 and 3.71 (6H, 2 x s, 2 x ArOCH_3); ^{13}C NMR; δ 161.5 and 158.3 (2 x Ar*C*OCH$_3$), 137.6 (2-*C*), 136.8 (5-*C*), 133.0, 130.0 (Ar*C*), 128.5 (4-*C*), 128.2, 127.6 ,127.2, 111.1, 104.4, 98.6 (Ar*C*), 55.3 and 55.2 (2 x ArCO*C*H$_3$), 48.8 (N*C*H$_2$); HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{18}H_{19}N_2O_2$ 295.1441, found 295.1408.

5.3.27. 5-(Benzo[d][1,3]dioxol-5-yl)-1-benzyl-1H-imidazole (***19e***). Yield 22% (**Method D**) as a pale yellow solid; Mp: 84-87^{o}C; ^{1}H NMR; δ 7.53 (1H, s, 2-C*H*), 7.27-7.33 (3H, m, Ar*H*), 6.74-7.06 (5H, m, Ar*H*), 6.72 (1H, s, 4-C*H*), 5.97 (2H, s, ArOCH_2O), 5.11 (2H, s, NCH_2); ^{13}C NMR; δ 147.8 and 147.6 (Ar*C*OCH$_2$O), 138.3 (2-*C*H), 136.7 (5-*C*), 133.1, 128.9 (Ar*C*), 127.9, 126.6, 123.2, 122.9, 109.4, 108.5 (4-*C*H and Ar*C*), 101.2 (ArCO*C*H$_2$O), 48.6 (N*C*H$_2$); HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{17}H_{15}N_2O_2$ 279.1128, found 279.1143.

*5.3.28. 1-(4-Bromophenyl)-5-(4-fluorophenyl)-1H-imidazole (**20a***). Yield: 68% (**Method E**) as pale yellow solid; Mp:130-133^{o}C; ^{1}H NMR: δ 7.67 (1H, s, 2-C*H*), 7.50-7.53 (2H, m, Ar*H*), 7.23 (1H, s, 4-C*H*), 6.95-7.11 (6H, m, Ar*H*); ^{13}C NMR: δ 162.3 (d, $J_{C,F}$ = 248.6 Hz, *para*-CF), 138.5 (2-*C*H), 135.4 (5-*C*), 132.7, 132.0 (Ar*C*), 130.0 (d, $^3J_{F,C}$= 8.1 Hz, *ortho*-*C*), 128.8, (Ar*C*), 127.0 (4-*C*H), 125.0 (d, $^3J_{C,F}$ = 3.0 Hz, *1'-C*), 122.1 (Ar*C*), 115.7 (d, $^2J_{F,C}$ = 22.1 Hz, *meta*-*C*); HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{15}H_{11}{}^{79}BrFN_2$ 317.0090, found 317.0115**.**

5.3.29. 1-(4-Bromophenyl)-5-(2,4-dimethoxyphenyl)-1H-imidazole (***20d***). Yield: 38% (**Method E**) as a pale yellow oil; ^{1}H NMR: δ 7.70 (1H, s, 2-C*H*), 7.43-7.45 (2H, m, Ar*H*), 7.13-7.18 (1H, d, *J* =12 Hz, Ar*H*), 7.12 (1H, s, 4-C*H*), 6.98-7.00 (2H, m, ArH), 6.31-6.50 (2H,m, ArH), 3.80 and 3.34 (3H, 2 x s, 2 x ArOCH_3); ^{13}C NMR: δ 161.5 and 157.5 (2 x Ar*C*OCH$_3$), 137.2 (2-*C*H), 136.8 (5-*C*), 134.5, 132.1, 132.0 (Ar*C*), 129.8 (4-*C*H), 125.6, 120.9, 111.0, 104.6, 98.7 (Ar*C*), 55.3 and 54.7 (2 x ArCO*C*H$_3$);

HR… …rH), 359.0395, found 359.0394.

5.3.30. 5-(Benzo[d][1,3]dioxol-5-yl)-1-(4-bromophenyl)-1H-imidazole (***20e***). Yield: 62% (**Method E**) as a pale yellow solid; ^{1}H NMR: 7.60 (2H, m, Ar*H*), 7.18 (4-C*H*), 7.05-7.07 (2H, m, Ar*H*), 6.57-6.74 (3H, m, ArH), 5.92 (2H, s, ArOCH_2O); ^{13}C NMR: δ 147.7 and 147.5 (Ar*C*OCH$_2$O), 138.1 (2-*C*H), 135.4 (5-*C*), 132.7 Ar*C*), 128.3 (4-*C*H), 127.0, 122.6, 122.4, 122.1, 108.7, 108.5, (Ar*C*), 101.3 (ArCO*C*H$_2$O); HRMS (ESI-TOF) m/z: [M+H]$^+$ Calcd for $C_{16}H_{12}{}^{79}BrN_2O_2$ 343.0082, found 343.0099.

5.4 Synthesis of 5-aryl-1,3-oxazoles and 4,5-dihydro-1,3-oxazoles (21-22)

5.4.1. Synthetic method F: Microwave assisted reaction in MeCN

To a 10 ml microwave reaction vessel equipped with a magnetic stirrer was introduced aryl aldehyde (1.34 mmol), TosMIC (1.47 mmol) and K_2CO_3 (2.68 mmol). An inert atmosphere was then created by degassing through evacuating and refilling with argon three times, followed by introduction of anhydrous MeCN (5 ml). The vessel was capped and resulting mixture reaction was microwave irradiated at a set temperature of 90^{o}C and a power of 120 Watts for 12 min. Upon completion of the reaction and conditions maintained until consumption of the TosMIC reagent), the solvent was then evaporated and the crude material was then stirred at 0^{o}C in a mixture containing MeOH (4 ml) and water (0.5 ml) until a precipitate formed. The precipitate was collected, washed with cold water and dried under high vacuum to give 5-aryl-4-tosyl-4,5-dihydro-1,3-oxazoles.

5.4.2. Synthetic method G: Aromatization in toluene

A mixture of 5-aryl-4-tosyl-4,5-dihydro-1,3-oxazole (1.06 mmol) in toluene (5 ml) was heated at reflux for 1 h. After completion of the reaction, the mixture was concentrated *in vacuo* and the crude material purified by column chromatography [eluting with hexane and ethyl acetate (4:1)] to produce 5-aryl-1,3-oxazoles.

5.4.3. Synthetic method H Microwave assisted van Leusen in MeOH

Aryl aldehyde (1.21 mmol), TosMIC (1.33 mmol) and K_2CO_3 (2.41 mmol) were placed in a 10 ml microwave reaction vessel equipped with a magnetic stirrer. An inert atmosphere was then created by degassing through evacuating and refilling with argon three times, followed by addition of anhydrous MeOH (5 ml). The resulting capped reaction mixture was then microwave irradiated at a set temperature of 90^{o}C and a power of 120 Watts for 7 min. Upon reaction completion (consumption of TosMIC), the solvent was evaporated *in vacuo*. The crude material was purified by flash column chromatography [elution with ethyl acetate and hexane ratio (1:4)] to afford 5-aryl-1,3-oxazole derivatives.

5.4.4. (4R,5R)/(4S,5S)-5-(Benzo[d][1,3]dioxol-5-yl))-4-tosyl-4,5-dihydrooxazole (***22e***). Yield: 80% (**Method F**) as a white solid; ^{1}H NMR: δ 7.83(2H, d, *J* = 8.8 Hz, Ar*H*), 7.37(2H, d, *J* = 8.8 Hz, Ar*H*), 7.18 (1H, d, *J* = 1.2 Hz, 2-C*H*), 6.74-6.81 (3H, m, ArH), 597 (2H, s, ArOCH_2O), 5.94 (1H, d, *J* = 6.0 Hz, 5-C*H*), 4.99 (1H, dd, *J*=4.4 Hz, 1.6 Hz, 4-C*H*), 2.45 (3H, s, ArCH_3); ^{13}C NMR: δ 159.3 (2-*C*H), 148.4 and 148.3 (2 x Ar*C*OCH$_2$O), 145.7, 133.1, 131.4, 129.9, 129.5, 119.5, 108.6, 105.6 (Ar*C*), 101.4 (ArCO*C*H$_2$O), 92.4 (4-*C*H), 79.4 (5-*C*), 21.7 (ArC*C*H$_3$); FTIR ν_{max}/cm^{-1} (KBr): 3314, 1675, 1596, 1503, 1447, 1303, 1257, 1141, 1062, 1039, 938, 816.

5.4.5. (4R,5R)/(4S,5S)-5-(2-Nitrophenyl)-4-tosyl-4,5-dihydrooxazole (***22h***). Yield: 65% (**Method F**) as an orange solid; 7.52-7.69 (3H, m, Ar*H* and 2-C*H*), 7.38 (2H, d, *J* = 8.8 Hz, Ar*H*), 6.39 (1H, d, *J* = 7.2 Hz, Ar*H*), 5.32 (1H, *J* = 6 Hz, 5-CH), 5.30 (1H, *J* = 26.0 Hz, 4-C*H*), 2.46 (3H, s, Ar*C*H$_3$); ^{13}C NMR: δ 159.0 (2-*C*H), 148.2, 145.9, 133.4, 132.9, 130.8, 130.4, 129.9, 129.7, 129.6, 125.2 (Ar*C*), 91.8 (4-*C*H), 77.03 (5-*C*), 21.8 (ArC*C*H$_3$); FTIR ν_{max}/cm^{-1} (KBr): 2922, 2868, 1702, 1672, 1599, 1524, 1347, 1317, 1224, 1133, 1036, 1011, 855, 815, 687, 569.

5.4.6. (4R,5R)/(4S,5S)-5-Phenyl-4-tosyl-4,5-dihydrooxazole (***22j***). Yield: 76% (**Method F**) as a white solid; ^{1}H NMR: δ 7.84 (2H, d, *J*= 8.8 Hz, Ar*H*), 7.32-7.43 (7H, m, Ar*H*), 7.21 (1H, d, *J* = 1.6 Hz, , 2-C*H*), 6.05 (1H, d, *J*= 6.0 Hz, 5-C*H*), 5.03 (1H, dd, *J*= 2.6 Hz, 4-C*H*), 2.46 (3H, s, ArCH_3); ^{13}C NMR: δ 159.4 (2-*C*H), 145.7, 137.7, 133.2, 129.9, 129.5, 129.1, 129.0, 125.2 (Ar*C*), 92.6 (4-*C*H), 76.4 (5-CH), 21.7 (ArC*C*H$_3$); FTIR ν_{max}/cm^{-1} (KBr): 2894, 2875, 1622, 1316, 1304, 1148, 1102, 968, 814.

5.4.7. 5-(4-Fluorophenyl)-1,3-oxazole (***21a***). Yield: 84% (**Method H**) as a brown oil. ^{1}H NMR: δ 7.89 (1H, s, 2-C*H*), 7.61-7.64 (2H, m, Ar*H*), 7.29 (1H, s, 4-C*H*), 7.10 (2H, t, *J* = 7.6 Hz, Ar*H*); ^{13}C NMR: δ 162.3 (d, $^1J_{C,F}$= 249.6 Hz, *para*-*C*F), 150.7 (5-*C*), 150.4 (2-*C*H), 126.3 (d, $^3J_{C,F}$= 8.1 Hz, *ortho*-*C*), 124.0 (d, $^4J_{C,F}$= 5.0 Hz, 1-*C*), 121.1 (4-*C*H), 116.1 (22.1 Hz, *meta*-C); HRMS (ESI-TOF) m/z: [M+H]$^+$ Calcd for C_9H_7FNO 164.0506, found 164.0508.

5.4.8. 5-(2-Chlorophenyl)-1,3-oxazole (***21b***). Yield: 74% (**Method H**) as a pale yellow solid; Mp: 44-46^{o}C; ^{1}H NMR: δ 7.93 (1H, s, 2-C*H*), 7.42-7.78 (2H, m, Ar*H*), 7.21-7.33 (3H, m, Ar*H*), NMR δ: 150.3 (2-*C*H), 148.0 (5-*C*), 130.7, 130.6, 129.1, 127.8, 127.0, 126.5 (Ar*C*), 126.3 (4-*C*H); HRMS (ESI-TOF) m/z: [M+H]$^+$ Calcd for C_9H_7ClNO 180.0211, found 180.0245.

5.4.9. 5-(3-Methoxyphenyl)-1,3-oxazole (***21c***). Yield: 60% (**Method H**) as a pale yellow oil; ^{1}H NMR: δ 7.85 (1H, s, 2-C*H*), 7.55 (1H, dd, *J* = 4.8 Hz, 2 Hz, Ar*H*), 7.21 (1H, s, 4-C*H*), 6.92 (2H, d, *J* = 8.8 Hz, Ar*H*), 3.81 (3H, s, ArOCH_3); ^{13}C NMR; δ 159.8 (Ar*C*OCH$_3$), 151.5 (5-*C*), 149.8 (2-*C*H), 125.8, 120.5, 119.8, 114.3 (Ar*C*), 55.2 (ArCO*C*H$_3$); HRMS (ESI-TOF) m/z: [M+H]$^+$ Calcd for $C_{10}H_{10}NO_2$ 176.0706, found 176.0736.

5.4.10. 5-(2,4-Dimethoxyphenyl)-1,3-oxazole (***21d***). Yield: 44% (**Method H**) as a pale yellow solid; Mp: 104-106^{o}C; ^{1}H NMR: δ 7.85 (1H, s, 2-C*H*), 7.66 (1H, *J* = 8.8 Hz, Ar*H*), 7.42 (1H, s, 4-C*H*), 6.53-6.59 (2H, m, Ar*H*), 3.83 and 3.92 (6H, 2 x s, 2 x ArOCH_3); ^{13}C NMR: δ 160.9 and 156.9 (2 x Ar*C*OCH$_3$), 148.9 (2-*C*H), 148.0 (5-*C*), 126.9 (Ar*C*), 123.5 (4-*C*H), 110.3, 104.9, 98.5 (Ar*C*), 55.4 and 55.8 (2 x ArCO*C*H$_3$); HRMS (ESI-TOF) m/z: [M+H]$^+$ Calcd for $C_{11}H_{12}NO_3$ 206.0812, found 206.0839.

5.4.11. 5-(Benzo[d][1,3]dioxol-5-yl)-1,3-oxazole (***21e***). Yield: 43% (**Method H**), 69% (**Method G**) as a yellow solid; Mp: 44-46^{o}C (lit.[60-61]); ^{1}H NMR: δ 7.86 (1H, s, 2-C*H*), 7.21 (1H, s, 4-C*H*), 7.10-7.18 (2H, m, Ar*H*), 6.85 (1H, d, *J* = 7.6 Hz, Ar*H*), 6.00 (ArOCH_2O); ^{13}C NMR: δ 149.9 (2-*C*H), 148.0 (5-*C*), 148.2 (2 x Ar*C*OCH$_2$O), 121.9 (4-*C*H), 120.3, 118.6, 108.8, 105.0 (Ar*C*), 101.4 (ArCO*C*H$_2$O); HRMS (ESI-TOF) m/z: [M+H]$^+$ Calcd for $C_{10}H_8NO_3$ 190.0499, found 190.0528.

5.4.12. 5-(4-tert-Butylphenyl)-1,3-oxazole (***21f***). Yield: 59% (**Method H**) as a sticky brown material; ^{1}H NMR: δ 7.90 (1H, s, 2-C*H*), 7.58-7.60 (2H, m, Ar*H*), 7.44-7.44-7.47 (2H, m, Ar*H*), 7.31 (1H, s, 4-C*H*) 1.34 [9H, s, ArC(CH_3)$_3$]; ^{13}C NMR: δ 151.9 (2-*C*H), 150.2 (5-C), 125.9, 125.0, 124.2 (Ar*C*), 120.9 (4-*C*H), 34.7 [ArC*C*(CH$_3$)$_3$], 31.2, {ArC(*C*H$_3$)$_3$}; HRMS (ESI-TOF) m/z: [M+H]$^+$ Calcd for $C_{13}H_{16}NO$ 202.1242, found 202.1234.

5.4. ...rich, USA) for testing.[47-48] The plate was placed into the incubator to equilibrate to 37°C and 5 % CO_2. During this time, test compounds were made up in in RPMI media containing 10 % heat inactivated FCS (10% RPMI solution) in a serial dilution from 100 µM to 1.56 µM. A total of 100 µl compound was added to wells containing cells and mixed to ensure the solution was homogeneous with the cells. The plate was placed into the 37°C incubator for 5 days. On the 5th day 10 µl of MTS was added and mixed. The plates were then incubated for a further 4 h, and read at 490 nm (xMARK™, Bio-Rad, USA.) The data analysis was completed on Origin 8.1 with the log value of the concentration plotted against the absorbance level to determine a dose curve. From the curve, a half maximal cytotoxicity CC_{50} for compounds was determined. Controls used included auranofin, raltegravir and CX05168.

H), 63% (**Method G**) as a pale yellow solid; Mp: 70-72°C (lit. [61]); ^{1}H NMR:δ 7.97 (1H, s, 2-C*H*), 7.84-7.86 (1H, m, Ar*H*), 7.64-7.72 (2H, m, Ar*H*), 7.51-7.56 (1H, m, ArH), 7.39 (1H, s, 4-C*H*); ^{13}C NMR: δ 151.6 (2-*C*H), 147.5 (5-*C*), 146.5 (Ar*C*NO_2), 132.5, 129.8, 129.6, 125.8, 124.4 (Ar*C*), 121.6 (4-*C*H); HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_9H_7N_2O_3$ 191.0451, found 191.0475.

5.4.14. 5-(4-Nitrophenyl)-1,-3-oxazole (***21h***). Yield: 73% (**Method** H) as a yellow solid; Mp: 137-139 °C (lit. [62-64]), ^{1}H NMR: δ 8.29-8.31 (2H, m, Ar*H*), 8.02 (1H, s, 2-C*H*), 7.80-7.83 (2H, m, Ar*H*), 7.56 (1H, s, 4-C*H*); ^{13}C NMR: δ 151.8 (2-*C*H), 149.5 (5-*C*), 147.4 (Ar*C*NO_2), 133.4, 124.8, 124.7 (Ar*C*), 124.5 (4-*C*H); HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_9H_7N_2O_3$ 191.0451, found 191.0458.

5.4.15. 5-Phenyl-1,3-oxazole (***21j***). Yield; 68% (**Method G**) as a pale yellow solid; Mp: 38-41°C (lit.[62]); ^{1}H NMR: δ 7.91 (1H, s, 2-C*H*), 7.64-7.67 (2H, m, Ar*H*), 7.40-7.44 (2H, m, Ar*H*), 7.32-7.35 (2H, m, 4-C*H* and Ar*H*); ^{13}C NMR: δ 151.5 (2-*C*H), 150.4 (5-*C*), 128.9, 128.7, 128.6, 127.7, 124.3 (Ar*C*), 121.4 (4-*C*H); HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for C_9H_7NO 145.0528, found 145.0521.

5.5. Biological Evaluation Experiments

5.5.1. HIV-1 IN-LEDGF/p75 biological evaluations

5.5.1.1. HIV-1 IN-LEDGF/p75 AlphaScreen™ Assay[45]

For the HIV-1 IN-LEDGF/p75 AlphaScreen™ assay, HIV-1 IN was incubated with 10 µM of each compound for 30 min at 26°C with slight shaking and subsequently 0.3 µM LEDGF/p75 was added and incubated for an hour. Nickel donor beads (Perkin Elmer, USA) and Nickel acceptor beads (Perkin Elmer, USA) were added to a final concentration of 10 µg/ml and incubated at 30°C in the dark with gentle shaking. Once incubation was complete the plate was read between 520-620nm on the EnSpire™ plate reader (Perkin Elmer, USA). Controls included: HIV-1 IN and LEDGF/p75 protein and CX05168 (**6**); which is a known HIV-1 IN-LEDGF/p75 inhibitor that was used as the control compound to validate the assay. Compounds displaying inhibition of above 50% benchmark were analyzed and were subsequently tested in ten serially diluted doses ranging from 0.39µM to 200 µM to determine an IC_{50} for each compound. This was carried out in duplicate on the plate and in three separate experiments.

5.5.1.2 AlphaScreen TruHits counter assay protocols[46]

The AlphaScreen Triuhits counter assay was performed according to the manufacturer's protocol (Perkin Elmer, Benelux). Reactions were performed using the TruHits kit contains streptavidin donor beads and biotinylated acceptor beads, which bind to each other without any further reagents added. In total, 30 µL TruHits kit bead premix was dispensed into each well of an optical microplate-96 and incubated for 30 min at room temperature. Subsequent to this, test compounds (final concentration of 10 µM) were transferred and the mixture was incubated for 1 h at room temperature. The assay microplates were read using the Enspire multimode plate Reader (PerkinElmer). All data were processed using Excel (Microsoft Corp) and visualized using origin 8.1 software.

5.5.1.3. Cellular toxicity assay

The day of cytotoxicity testing, MT-4 cells (obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: MT-4 catalogue number 120) were counted and seeded at 1 x 10^6 cells per ml. A total of 100 µl of cells were added to each well of

5.5.1.4. Antiviral assay [47]

MT-4 cells (obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: MT-4 catalogue number 120) were seeded the day before antiviral testing at 3 x 105 cells per ml. The following day the viability was checked and 2 x 105 cells per ml were placed into a 50 ml conical tube and HIV-1NL4-3 stock added. The cells were spinoculated at 3000 x g for 90 min. Cells were subsequently washed four times with 0.01 M PBS to remove any unbound virus. A control set of cells were spinoculated without virus and washed four times with 0.01 M PBS to replicate the test cells. A total of 10 ml 10 % RPMI media was then added to the cells and 100 µl of cells were added to each well of a Corning® Costar® 96-Well Cell Culture Plates (Sigma Aldrich, USA). The plate was placed into the 37°C, 5 % CO_2 incubator to equilibrate for one hour. During the incubation compounds were made up in RPMI media containing 10 % heat inactivated FCS. The compounds were evaluated at 10 µM. A total of 100 µl of compound solution was added to the wells containing cells and mixed to ensure they were homogeneous. The plate was placed into a 37°C, 5 % CO_2 incubator for five days.

A Biomerieux Vironostika HIV-1 Ab/Ag micro ELISA system was used to test for p24. All buffers were prepared according to manufacturer's specifications and the manufacturer's protocol was followed. Briefly, 145 µl of disruption buffer was added to each well including control wells and incubated at 37°C for one hour. A total volume of 5 µl of each test specimen was added to the disruption buffer and control samples were added. The plate was then incubated at 37°C for 60 minutes and wells were then washed with 1x wash buffer for 30 seconds. Washing was repeated six times. Once washed 100 µl of 3,3',5,5'-tetramethylbenzidine (TMB) substrate mix (1:1 ratio of TMB substrate A and B) was added and incubated for 5-30 minutes in the dark until sufficient colour had developed. The reaction was stopped by the addition of 100 µl 1 M sulphuric acid. The plates were then read on a multiplate reader at 450nm (xMARKTM, Bio-Rad, USA).

5.5.2 Minimum inhibitory concentration (MIC) assay

5.5.2.1 Preparation of stock solution for antimicrobial testing

The stock solutions of the synthesized compounds used for the minimum inhibitory concentration (MIC) assays were prepared by dissolving the sample in 10% DMSO, followed by dilution with sterile water in order to make a concentration of 1 mg/ml. The concentration of 0.01 mg/ml of ciprofloxacin and 0.1 mg/ml nystatin were also prepared and were used as positive controls for bacteria and yeast respectively. A solution of *p*-iodonitrotetrazolium chloride (INT) (0.08 g) in 200 ml of sterile water was prepared as the indicator

5.5. mall

The synthesized compounds were assessed for their antimicrobial effects against two Gram-positive bacterial species (*S. aureus* ATCC 25923 and *B. cereus* ATCC 11779), two Gram-negative bacterial species (*E. coli* ATCC 8739 and *P. aeruginosa* ATCC 27858) and one yeast (*C. tropicalis* ATCC 750). Tryptone Soya broth (100 µl) was added into each of the 96 well microtiter plates, followed by the addition 100 µl of each tested compound solution including positive and negative controls. Serial dilutions were performed to provide the following nine concentrations: 250, 125, 62.5, 31.25, 15.6, 7.8, 3.91, 1.95, and 0.98 µg/ml or lower when necessary. Prior to the addition of culture to the microtiter plates, 1 ml of each culture was sub-cultured in 100 µl Tryptone soya broth to obtain a 0.5 McFarland standard. A sub-cultured volume of 100 µl was then added to all wells of microtiter plates, which contained the compounds and broth. Each plate was sealed with a sterile adhesive sealing film to avoid evaporation of test samples, followed by incubation at 37°C for 24 h for bacteria species and for 48 h for the yeast. After incubation, 40 µl INT was added to each well of the microtiter plates.[49-51] Each plate was read to determine the MIC. The wells with clear or no visible microbial growth at the lowest concentration was noted as the MIC. The assessments were carried out in duplicate and triplicate if any variability was noted.

5.6. Generation of Ligand-Based Pharmacophore Model

5.6.1 Creation of pharmacophore sites

The receptor-ligand pharmacophore model was executed in order to understand the probable binding mode of hits at the allosteric site of HIV-1 IN. A pharmacophore model strategy was adopted and developed using ligand **5** binding at the LEDGF/p75 binding site. Initially, the the X-ray structure of the HIV-1 IN in complex with the LEDGIN compound (PDB accession code 4NYF) was retrieved from the protein databank. The HIV-1 IN was prepared using the protein prepared wizard in Maestro (Schrodinger software) in order to add the necessary hydrogen atoms to all the atoms in the system as well as adding bond orders and formal charges for the hetero groups at neutral pH. After a protein structure minimization using the OPLS3 force field to RMSD of 0.3, the the binding pocket at the IN monomers interface was then located by using receptor grid generation. Other crystal structures such as 4DMN, 2B4J and 4JLH were also considered during the pharmacophore generation process. Prior to docking, ligand **4** was cleaned using the LigPrep protocol.[53-54,65-66] Ligand **4** then primarily positioned at the allosteric site of the HIV-1 IN dimer by ligand docking-glide using extra-precision (XP) method. The 3D model of **4** at the allosteric binding site was used to generate a hypothesis using ligand-protein pharmacophore protocol as shown in Fig 2. A nine point pharmacophore hypothesis model from **4** at the HIV-1 IN allosteric site was generated and the model consists of the pharmacophoric features: A1 and A2 acceptor points (pink); H3, H4 and H5 donor points (green), the N6 negatively charged atom (red) and the R7, R8 and R9) hydrophobic points (green) with distance matching tolerance of 2.0 and excluded volume as shown in Fig. 2.

5.6.2 Ligand preparation

All compounds used in the present study were prepared prior to docking studies using ligPrep protocol of Schrödinger suite.[65] This protocol involved the addition of hydrogen, converting 2D structure to 3D, generation of the stereoisomers, neutralisation of charges, determination of the probable ionization state at pH of 7.0 ± 2.0 and energy minimization using a OPLS3 force field.

5.6.3. Phase ligand screening

molecules which bind at the LEDGF/p75-binding site promoting aberrant multimerization of the IN, a nine point hypothesis model was then used as a query to screen the best six identified hits **16c**, **16f**, **17c**, **17f**, **20a** and **20d**.[66] Prior to screening, hits were prepared by the ligPrep protocol. A phase ligand screening protocol was then utilized to position the best six hits onto a nine-point pharmacophore model. The compounds were then scored and ranked relative to active reference ligand **5** with respect to their match ligand site, aligned score, vector score, volume score, fitness score and phase screen score (see Table 5 in the supplementary information).

Acknowledgments

We acknowledge with thanks financial support from the National Research Foundation Professional Development Program (NRF-PDP) of South Africa, Mintek and the University of the Witwatersrand.

Supplementary Material

Supplementary information, containing the ^{1}H and ^{13}C NMR spectra of all synthesized compounds and docking scores for active compounds, is available.

References

1. Klein E, Smith DL, Laxminarayan R. Hospitalizations and deaths caused by methicillin-resistant Staphylococcus aureus, United States, 1999–2005. *Emerg Infect Dis*. 2007;13:1840-1846. https://doi.org/10.1016/j.Sc.2010.00372.
2. Delelis O, Thieryy S, Subra F, Simon F, Malet I, Alloui C, Sayon S, Calvez V, Deprezec E, Marcelin A-G, Tchertanov L, mouscdet J-F. Impact of Y143 HIV-1 integrase mutations on resistance to Raltegravir *in vitro* and *in vivo*. *Antimicrob Agents Chemother*. 2010;54:491-501. https://doi.org/ 10.1128/AAC.01075-09.
3. Anstett K, Brenner B, Mesplede T, Wainberg MA. HIV drug resistance against strand transfer integrase inhibitors. *Retrovirology*. 2017;14:1-16. https://doi.org/10.1186/s12977-017-0360-7.
4. Clavel F, Hance AJ. HIV drug resistance. *N Engl J Med*. 2004;350:1023-1035. https://doi.org/10.1056/NEJMra025195.
5. Espeseth AS, Felock P, Wolfe A, Witmer M, Grobler J, Anthony N, Egbertson M, Melamed JY, Young S, Hamill T, Cole JL, Hazuda, DJ. HIV-1 integrase inhibitors that compete with the target DNA substrate define a unique strand transfer conformation for integrase. *Proc Natl Acad Sci.* 2000;97:11244-11249. https://doi.org/10.1073/pnas.200139397.
6. Anthony NJ. HIV-1 integrase: a target for new AIDS chemotherapeutics. *Curr. Top Med Chem*. 2004;4:979-990. https://doi.org/ 10.2174/1568026043388448.
7. McColl DJ, Chen X. Strand transfer inhibitors of HIV-1 integrase: bringing IN a new era of antiretroviral therapy. *Antiviral Res.* 2010;85:101-118. https://doi.org/10.1016/j.antiviral.2009.11.004.
8. Craigie R. The molecular biology of HIV integrase. *Future Virol*. 2012;7:679-686. https://doi.org/10.2217/FVL.12.56.
9. Esposiro D, Craigie R. HIV integrase structure and function. *Adv Virus Res*. 1999;52:319-333. https://doi.org/10.1016/S0065-3527(08)60304-8.
10. Calin R, Katlama C. The place of raltegravir in the clinical management HIV-1 infection. *Clin Pract*. 2013;10:427-438. https://doi.org/10.903810.2217/CPR.13.33.
11. Hazuda DJ, Felock P, Witmer M, Wolfe A, Stillmock K, Grobler JA, Espeseth A, Gabrryelski L, Schleif W, Blau C, Miller MD, Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells. *Science.* 2000;*287*:646-650. https://doi.org/10.1126/science.287.5453.646.
12. Shimura K, Kodam E, Sakagami Y, Matsuzaki Y, Watanabe W, Yamataka K, Watanabe Y, Ohata Y, Doi S, Sato M, Kano M, Ikeda S, Mastuoka M. Broad antiretroviral activity and resistance profile of the novel human immunodeficiency virus integrase inhibitor elvitegravir (JTK-303/GS-9137). *J Virol.* 2008:82:764-774. https://doi.org/ 10.1128/JVI.01534-07.
13. Walmsley SL, Antela A, Clumeck N, Duiculescu D, Eberhard A, Gutiérrez F, Hocqueloux L, Maggiolo F, Sandkovsky U, Granier C, Pappa K, Wynne B, Min S, Nichols G. Dolutegravir plus abacavir-lamivudine for the

https://doi.org/10.1097/QAI.0000000000000790.
14. Wohl DA, Y Yazdanpanah Y, Baumgarten A, Clarke A, Thompson MA, Brinson C, Hagins D, Ramgopal MN, Antinori A, Wei X, Acosta R, Collins SE, Brainard D, Martin H. Bictegravir combined with emtricitabine and tenofovir alafenamide versus dolutegravir, abacavir, and lamivudine for initial treatment of HIV-1 infection: week 96 results from a randomised, double-blind, multicentre, phase 3, non-inferiority trial. *Lancet HIV*. 2019:6:e355-363. https://doi.org/10.1016/S23523018(19)30077-3
15. Reisler RB, Han C, Burman WJ, Tedaldi EM, Neaton JD. Grade 4 Events Are as Important as AIDS Events in the Era of HAART. *J Acquired Immune Defic Syndr*. 2003;34:379-386. https://doi.org/10.1097/00126334-200312010-00004.
16. Maertens G, Cherepanov P, Pluymers W, Busschots K, De Clercq E, Debyser Z, Engelborghs Y. LEDGF/p75 is Essential for nuclear and chromosomal targeting of HIV-1 integrase in human cells. *J Biol Chem.* 2003;278:33528-33539. https://doi.org/10.1074/jbc.M303594200.
17. Busschots K, Voet A, De Maeyer M Rain, JC, Emiliani S, Benarous R, Desender L, Debyser Z, Christ F. Identification of the LEDGF/p75 binding site in HIV-1 integrase. *J Mol Biol.* 2007;365:1480-1492. https://doi.org/ 10.1016/j.jmb.2006.10.094.
18. Christ F, Voet A, Marchand A, Nicolet S, Desimmie BA, Marchand D, Bardiot D, Van der Veken NJ, Van Remoortel B, Strelkov SV, De Maeyer M, Chaltin P, Debyser Z. Rational design of small-molecule inhibitors of the LEDGF/p75-integrase interaction and HIV replication. *Nat Chem Biol*. 2010;6:442-448. https://doi.org/10.1038/nchembio.370.
19. Peese KM, Allard CW, Connolly T, Johnson BL, Li C, Patel M, Sorensen ME, Walker MA, Meanwell NA, McAuliffe B, Minassian B, Krystal M, Parker DD, Lewis HA, Kish K, Zhang P, Nolte RT, Simmermacher J, Jenkins S, Cianci C, Naidu BN. 5,6,7,8-Tetrahydro-1,6-naphthyridine Derivatives as Potent HIV-1-Integrase-Allosteric-Site Inhibitors. *J Med Chem*. 2019;62: 1348−1361. https://doi.org/10.1021/acs.jmedchem.8b01473
20. Le Rouzic E, Bonnard D, Chasset S, Brunneau J-M, Chevreuil F, Le Strat F, Nguyen J, Beauvoir R, Amadori C, Brias, J, Vomscheid S, Eiler S, Lévy N, Delelis O, Deprez E, Saïb A, Zamborlini A, Emiliani S, Ruff M, Ledoussal B, Moreaus F, Benarous R. Dual inhibition of HIV-1 replication by integrase-LEDGF allosteric inhibitors is predominant at the post-integration stage. *Retrovirology*. 2013;10:144. https://doi.org/10.1186/1742-4690-10-144.
21. Fader LD, Malenfant E, Parisien M, Carson R, Bilodeau F, Landry S, Pesant N, Brochu C, Moris S, Chabot C, Halmos T, Bousquet Y, Bailey MD, Kawai SH, Coulombe R, Laplante S, Jakalian A, Bhardwaj PK, Wernic D, Schroeder P, Amad M, Edwards P, Garneau M, Duan J, Cordingley M, Bethell R, Mason SW, Bös M, Bonneau P, Poupart M-A, Faucher A-M, Simoneaus B, Fenwick C, Yoakim C, Tsantrizos Y. Discovery of BI 224436, a non-catalytic site integrase inhibitor (NCINI) of HIV-1. *ACS Med Chem Lett*. 2014;5:422-427. https://doi.org/ 10.1021/ml500002n.
22. Christ F, Shaw S, Demeulemeester J, Desimmie BA. Marchand A, Butler S, Smets W, Chaltin P, Westby M, Debyser Z, Pickford C, Small-molecule inhibitors of the LEDGF/p75 binding site of integrase block HIV replication and modulate integrase multimerization. *Antimicrob Agents Chemother*. 2012;56:4365- 4374. https://doi.org/10.1128/AAC.00717-12.
23. Kessl JJ, Jena N, Koh Y, Taskent-Sezgin H, Slaughter A, Feng L, de Silva S, Wu L, Le Grice SFJ, Engelman A, Fuchs JR, Kvaratskhelia, M. Multimode, cooperative mechanism of action of allosteric HIV-1 integrase inhibitors. *J Biol Chem*. 2012;287:16801-16811. https://doi.org/10.1074/jbc.M112.354373.
24. Tsiang M, Jones GS, Niedziela-Majka A, Kan E, Lansdon EB, Huang W, Hung M, Samuel D, Novikov N, Xu Y, Mitchell M, Guo H, Babaoglu K, Liu X, Geleziunas R, Sakowicz R. New class of HIV-1 integrase (IN) inhibitors with a dual mode of action. *J Biol Chem*. 2012;28:21189-21203. https://doi.org/10.1074/jbc.M112.347534.
25. Burlein C, Wang C, Xu M, Bhatt T, Stahlhut M, Ou Y, Adam GC, Heath J, Klein DJ, Sanders J, Narayan K, Abeywickrema P, Heo MR, Carroll, SS, Grobler, JA, Sharma S, Diamond TL, Converso A, Krosky DJ. Discovery of a Distinct Chemical and Mechanistic Class of Allosteric HIV-1 Integrase Inhibitors with Antiretroviral Activity. *ACS Chem. Biol*. 2017;12:2858-2865. https://doi.org/10.1021/acschembio.7b00550.
26. Rees DC, Congreve M, Murray CW, Carr R. Fragment-based lead discovery. *Nat Rev Drug Discov*. 2004;3:660-672. https://doi.org/10.1038/nrd1467.
27. Hajduk PJ, Greer JA. A decade of fragment-based drug design, strategic advances and lessons learned.*Nat Rev. Drug Discov*. 2007;6:211-219. https://doi.org/10.1038/nrd2220.
28. Verlinde CLMJ, Fan E, Shibata S, Zhang Z, Sun Z, Deng W, Ross J, Kim J, Xiao L, Arakaki TL, Bosch J, Caruthers JM, Larson ET, Letrong I, Merritt EA, Hol WG. Fragment-based cocktail crystallography by the medical structural genomics of pathogenic protozoa consortium. *Curr Top Med Chem*. 2009;9:1678-1687. PMID: 19929835, PMCID: PMC2897734.
29. Davis BJ, Erlanson DA, Learning from our mistakes: the 'unknown knowns' in fragment screening. *Bioorg Med Chem Lett*. 2013;23:2844-2852. https://doi.org/10.1016/j.bmcl.2013.03.028.
30. Chan L, Young DW. Using fragment based drug discovery to target epigenetic regulators in cancer. *MOJ Bioequiv Availab*.2017;4(1):223–230. https://doi.org/10.15406/mojbb.2017.04.00062
31. Wielens J, Headey SJ, Deadman JJ, Rhodes DI, Parker MW, Chalmers DK, Scanlon MJ. Fragment-based design of ligands targeting a novel site on the integrase enzyme of human immunodeficiency virus 1. *Chem Med Chem*. 2011, 6:258-261. https://doi.org/10.1002/cmdc.201000483.
32. Fujiwara T, Sato A, El-Farrash M, Miki S, Abe K, Isaka Y, Kodama M, Wu Y, Chen LB, Harada H, Sugimoto H, Hatanaka M, Hinumana Y. S-1153 Inhibits replication of known drug-resistant strains of human immunodeficiency virus yype 1. *Antimicrob Agents Chemother*. 1998;42:1340-1345. https://doi.org/10.1128/AAC.42.6.1340.
33. Serrao E, Xu Z-L, Debnath B, Christ F, Debyser Z, Long Y-Q, Neamati N.. Discovery of a novel 5-carbonyl-1*H*-imidazole-4-carboxamide class of inhibitors of the HIV-1 integrase-LEDGF/p75 interaction. *Bioorg Med Chem*. 2013;21:5963-5972. https://doi.org/ 10.1016/j.bmc.2013.07.047.
34. Van Leusen AM, Hoogenboom BE, Sierius H. A novel and efficient synthesis of oxazoles from tosylmethylisocyanide and carbonyl compounds. *Tetrahedron Lett.* 1972;13:3114-3118. https://doi.org/10.1016/S0040-4039(01)85305-3.
35. Sisko J, Kassick A, Mellinger M, Filan JJ, Allen A, Olsen MA. An investigation of imidazole and oxazole syntheses using aryl-substituted tosMIC reagents. *J Org Chem*. 2000;65:1516-1524. https://doi.org/10.1021/jo9917821.
36. Hao W, Jiang Y, Cai M. Synthesis of indolyl imidazole derivatives via base-promoted tandem reaction of N-[2-(1-alkynyl)phenyl]carbodiimides with isocyanides. *J Org Chem.* 2014;79:3634-3640. https://doi.org/ 10.1021/jo402552c.
37. Van Leusen D, van Echten E, Van Leusen AM. Chemistry of sulfonylmethyl isocyanides. 37. Synthesis of 3,4-disubstituted pyrroles bearing substituents of electron-withdrawing and/or electron-donating nature. *J Org Chem.* 1992;57:2245-2249. https://doi.org/ 10.1021/jo00034a011.
38. Yugandar S, Acharya A, Ila H. Synthesis of 2,5-Bis(hetero)aryl 4′-substituted 4,5′-bisoxazoles via copper(I)-catalyzed domino reactions of activated methylene isocyanides with 2-phenyl- and 2-(2-thienyl)-4-[(aryl/heteroaryl)(methylthio)methylene]oxazol-5(4H)-ones. *J. Org. Chem*. 2013;78:3948-3960. https://doi.org/ 10.1021/jo400317g.
39. Barton DHR, Kervagoret J, Zard SZ. A useful synthesis of pyrroles from nitroolefins. *Tetrahedron.* 1990;46:7587-7598. https://doi.org/10.1016/S0040-4020(01)89069-4.
40. Chen W, Shao J, Li Z, Giulianotti MA, Yu Y. Synthesis of 2,3,4-trisubstituted pyrroles via a facile reaction of vinyl azides and tosylmethyl isocyanide. *Can J Chem*. 2012;90:214-221. https://doi.org/10.1139/v11-150.
41. Bode ML, Gravestock D, Rousseau AL. Synthesis, uses and reactions of isocyanides in organic synthesis. An update. *Org. Prep. Proc. Int.* 2016;48:89-221. http://dx.doi.org/10.1080/00304948.2016.1138072
42. Reichert S, Briet S. Development of an Axial Chirality Switch. *Org. Lett.* 2007;9:899-902. https://doi.org/10.1021/ol0700660.
43. Van Leusen D, Van Leusen AM. In: Synthetic Uses of Tosylmethyl Isocyanide (TOSMIC) in Organic Reactions. Overman LE, editor. Wiley; Hoboken, New Jersey: 2004. pp. 417–666.
44. Companyo X, Moyano A, Rio R. A mild and convenient Synthesis of 4-tosyl-4,5-dihydrooxazoles. *Lett. Org. Chem*. 2009;6:293-296. https://doi.org/10.2174/157017809788489855.
45. Beaudet L, Rodriguez-Suarez R, Venne M-H, Caron M, Bédard J, Brechler V, Parent S, Bielefeld-Sévigny M. AlphaLISA immunoassays: the no-wash alternative to ELISAs for research and drug discovery. *Nat Methods.* 2008:5:A10–A11. https://doi.org/10.1038/nmeth.f.230.
46. Schorpp K, Rothenaigner I, Salmina E, reinshagen J, Low T, Breken JK, Gopalakrishnan J, Tetko IV, Gul S, Hadian K. Identification of Small-Molecule Frequent Hitters from AlphaScreen High-Throughput Screens. *J. Biomol. Screen*.2014; 19(5):715-726.DOI: 10.1177/1087057113516861
47. Van Meerloo J, Kaspers GJ, Cloos J. (2011) Cell Sensitivity Assays: The MTT Assay. In: Cree I. (eds) Cancer Cell Culture. Methods in Molecular Biology (Methods and Protocols), vol 731. Humana Press.
48. Sharma A, Slaughter A, Jena N, Feng L, Kessl JJ, Fadel HJ, Malani N, Male F, Wu L, Poeschla E, Bushman FD, Fuchs JR, Kvaratskhelia M. *A New Class of Multimerization Selective Inhibitors of HIV-1 Integrase*. *PLOS Pathog.* 2014; 10(5):e1004171. doi:10.1371/journal.ppat.1004171.

49. [illegible] r N. antimicrobial activity of twenty commercial frankincense essential oil samples. S Afr J Bot. 2010;76:686-691. https://doi.org/10.1016/j.sajb.2010.06.001.
50. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Plant Med. 1998;64:711-713. https://doi.org/10.1055/s-2006-957563.
51. Gibbons S. Anti-staphylococcal plant natural products. Nat Prod. Rep. 2004:21:263-277. https://doi.org/ 10.1039/b212695h.Plant Med. 1998;64:711-713. https://doi.org/10.1055/s-2006-957563.
52. Ramadwa TE, Elgorashi EE, McGaw LJ, Ahmed AS, Eloff JN. Antimicrobial, anti-inflammatory activity and cytotoxicity of Funtumia africana leaf extracts, fractions and the isolated methyl ursolate. *S. Afr. J. Bot*. 2017;108:126–131. http://dx.doi.org/10.1016/j.sajb.2016.10.019.
53. Loving K, Salam NK, Sherman, W. Energetic analysis of fragment docking and application to structure-based pharmacophore hypothesis generation, *J. Comput. Aided Mol. Des*. 2009;23:541–554. https://doi: 10.1007/s10822-009-9268-1.
54. Salam NK, Nuti R, Sherman W. Novel Method for Generating Structure-Based Pharmacophores Using Energetic Analysis. *J. Chem. Inf. Model*. 2009;49:2356–2368. https://doi.org/10.1021/ci900212v.
55. Slaughter A, Jurado KA, Deng N, Feng L, Kessl JJ, Shkriabai N, Larue RC, Fadel HJ, Patel PA, Jena N, Fuchs JR, Poeschla E, Levy RM, Engelman A, Kvaratskhelia M. The mechanism of H171T resistance reveals the importance of N_{δ}-protonated His171 for the binding of allosteric inhibitor BI-D to HIV-1 integrase. *Retrovirology*. 2014:11:100. https://doi.org/10.1186/s12977-014-0100-1.
56. Feng L, Sharma A, Slaughter A, Jena N, Koh Y, Shkribai N, Larue RC, Patel PA, Mitsuya H, Kessl JJ, Engelman A, Fuchs, JR, Kvaratskhelia M. The A128T resistance mutation reveals aberrant protein multimerization as the primary mechanism of action of allosteric HIV-1 integrase inhibitors. *J Biol Chem*. 2013;288:15813-15820. https://doi.org/10.1074/jbc.M112.443390.
Synthesis, characterization and antimicrobial studies of some new pyrazole incorporated imidazole derivative. *Eur J Med Chem*. 2011;46: 3531-3536. https://doi.org/10.1016/j.ejmech.2011.05.005.
58. Antolini M, Bozzoli A, Ghiron C, Kennedy G. Analogues of 4,5-bis(3,5-dichlorophenyl)-2-trifluoromethyl-1*H*-imidazole as potential antibacterial agents. *Bioorg Med Chem Lett*. 1999;9:1023-1028. https://doi.org/10.1016/S0960-894X(99)00112-2.
59. Baddar FG, Iskander Z. Triad prototropic systems. Part III. The effect of substituents on the mobility of the azomethine system. *J Chem Soc.* 1954:209-213. https://doi.org/10.1039/JR9540000209.
60. Ziegle FE, Fowler KW. Substitution reactions of specifically ortho-metalated piperonal cyclohexylamine. *J Org. Chem*. 1976;41:1564-1566. https://doi.org/10.1021/jo00871a017.
61. Strumberg D, Pommer Y, Paull K, Jayaramna M, Nagafuji P, Pamela CM. Synthesis of cytotoxic indenoisoquinoline topoisomerase I poisons. *J Med Chem*. 1999;42:446-457. https://doi.org/10.1021/jm9803323.
62. Besselievre F, Mahuteau-Betzer F, Grierson DS, Piguel S. Ligandless microwave-assisted Pd/Cu-catalyzed Direct arylation of oxazoles. *J Org Chem*. 2008;73:3278-3280. https://doi.org/10.1021/jo7027135.
63. Kumar KSV, Swaroop TR, Rajeev N, Vinayaka AC, Lingaraju GS, Rangappa KS, Sadashiva MP. A one-pot tandem approach for the synthesis of 5-(het)aryloxazoles from substituted (het)aryl methyl alcohols and benzyl bromides. *Synlett*. 2016;27:1363-1366. https://doi.org/10.1055/s-0035-1561391.
64. Akula M, Thigulla Y, Davis C, Jha M, Bhattacharya A. Synthesis of 4-substituted oxazolo[4,5-c]quinolines by direct reaction at the C-4 position of oxazoles. *Org Biomol Chem*. 2015;13:2600-2605. https://doi.org/10.1039/C4OB02224F.
65. Schrödinger Release 2019-3: LigPrep, Schrödinger, LLC, New York, NY, 2019. Schrödinger Release 2019-3:
66. Maestro, Schrödinger, LLC, New York, NY,2019.

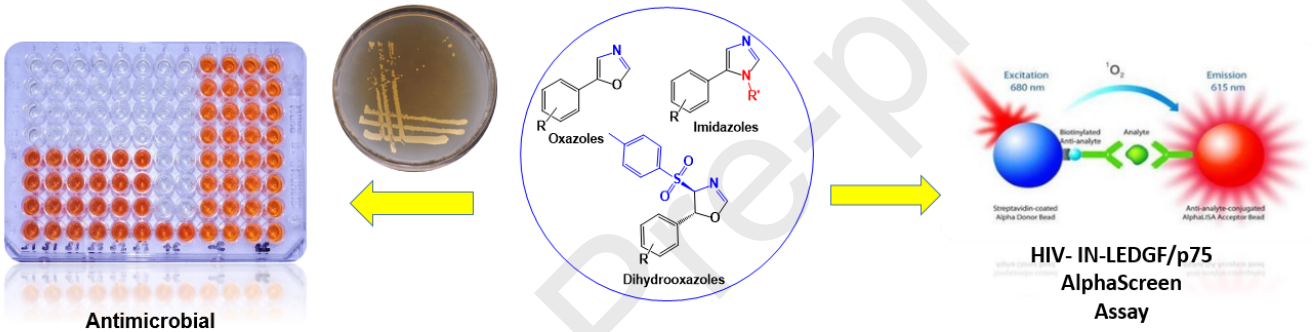
Gr

To create your abstract, type over the instructions in the template box below.
Fonts or abstract dimensions should not be changed or altered.

Leave this area blank for abstract info.

Design, synthesis and biological evaluation of imidazole and oxazole fragments as HIV-1 integrase-LEDGF/p75 disruptors and inhibitors of microbial pathogens

Thompho J. Rashamuse, Angela T. Harrison, Salerwe Mosebi, Sandy van Vuuren, E. Mabel Coyanis,* and Moira L. Bode*



Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: