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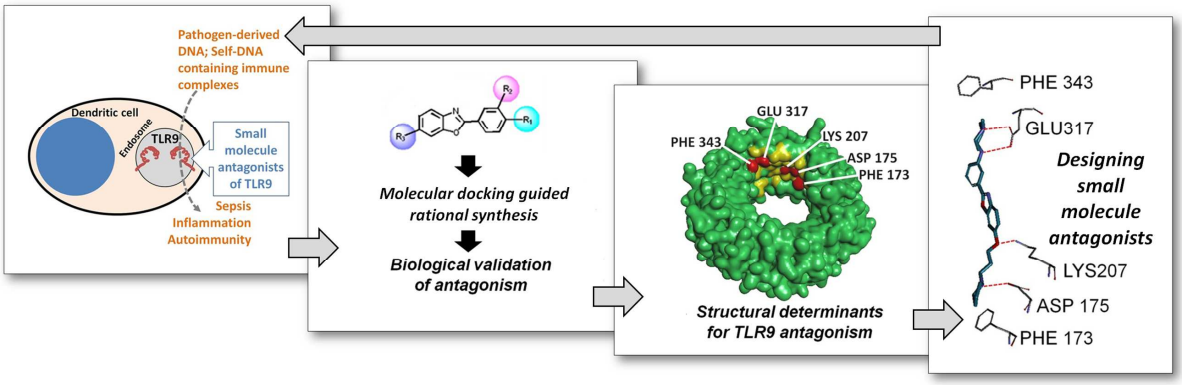

Design and Development of Benzoxazole Derivatives with Toll-like Receptor 9 Antagonism

Swarnali Roy[†Ψ], *Ayan Mukherjee*[†Ψ], *Barnali Paul*[†], *Oindrila Rahaman*[‡], *Shounak Roy*[‡], *Gundaram Maithri*[†], *Bandaru Ramya*[‡], *Sourav Pal*, *Dipyaman Ganguly*[‡]* *and Arindam Talukdar*[†]*

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Keywords: TLR9, TLR7, inflammation, antagonist, homology model, drug design, computational analysis

Abstract

Toll-like receptor 9 (TLR9) is a major therapeutic target for numerous inflammatory disorders. Development of small molecule inhibitors for TLR9 remains largely empirical due to lack of structural understanding of potential TLR9 antagonism by small molecules and due to the unusual topology of the ligand binding surface of the receptor. To develop a structural model for rational design of small molecule TLR9 antagonists, an enhanced homology model of human TLR9 (hTLR9) was constructed. Binding mode analysis of a series of molecules having characteristic molecular geometry, flexibility and basicity was conducted based on crystal structure of the inhibitory DNA (iDNA) bound to horse and bovine TLR9. Interaction with specific amino acid residues in four leucine rich repeat (LRR) regions of TLR9 was identified to be critical for antagonism by small molecules. The biological validation of TLR9 antagonism and its correlation with probe-receptor interactions led to a reliable model that could be used for development of novel small molecules with potent TLR9 antagonism (IC_{50} 30-100 nM) with excellent selectivity against TLR7.

1. Introduction

Toll-like receptors (TLRs) are germline-encoded pattern recognition molecules that are major players for innate immunity in the body [1]. TLRs contain a ligand-binding ectodomain (ECD) with 19-25 leucine-rich repeats (LRRs), a transmembrane domain and a characteristic cytoplasmic domain called the TIR (Toll/IL‑1 receptor) domain for downstream signaling [1-2]. Major members of the TLR family are expressed on the cell surface of various immune cell subsets, except for TLR3, TLR7, TLR8 and TLR9 that are expressed in endosomes [1-3]. These endosomal TLRs are specialized for detecting non-self pathogen-derived nucleic acids that are presented in the acidic (pH < 6.5) endolysosomal compartments after phagocytosis of the pathogenic microbes. Cognate ligand binding initiates multiple downstream signaling pathways, leading to activation of transcription factor NFκB or mitogen-activated protein kinases (MAPKs) or recruitment of the IFN regulatory factors (IRFs) [1-3].

In addition to this role in protective immunity, recent studies by different groups, including us, have implicated inadvertent activation of the endosomal TLRs in response to endogenous nucleic acid ligands in pathogenesis of a large number of autoimmune and other inflammatory disorders, as well as contexts of sepsis [4-5]. Evidence for such pathogenetic involvement in contexts of sterile inflammation is particularly numerous in case of TLR9 [6-14], that can recognize unmethylated CpG motifs on DNA molecules of both non-self and self-origin. Pathogenetic role of TLR9 activation by self-DNA molecules have been documented in various contexts of chronic inflammation and autoimmunity, e.g., systemic lupus erythematosus [5,11], psoriasis [8, 12, 13], Sjogren‘s syndrome [10], scleroderma [14], type I diabetes [5], type II diabetes [6,7] etc. Thus, TLR9 is an important therapeutic target in these clinical contexts and so TLR9 antagonists are being sought for globally. In some clinical contexts TLR7 and TLR9 receptors may have opposing inflammatory and regulatory roles [15-17]. The divergent effects of TLR7 and TLR9 emphasize the need for specific targeting to avoid unwanted immune reactions. Most TLR9 antagonists are empirically derived as with other anti-inflammatory agents and considerable efficiency is shown by DNA oligonucleotides that bind as usual TLR9 ligands but fail to elicit activation and competitively inhibit agonistic DNA binding (inhibitory DNA molecules or iDNAs) [15]. Due to the unusual topology of the ligand binding surface of TLR9 lacking conventional pockets, the functional mechanism of potential TLR9 antagonism by small

molecules is not understood, consequently small molecule TLR9 antagonists have so far been developed by empirical screening [18-22]. To do away with this empiricism we attempted to identify the structural attributes in a molecule that confer hTLR9 antagonism and its correlation with probe-receptor interactions for further development of new generation drugs with potential use in relevant clinical contexts, where inadvertent TLR9 activation plays a major role. Our results have identified selective hTLR9 antagonists showing IC_{50} value < 100 nM with 50-200 fold selectivity against hTLR7.

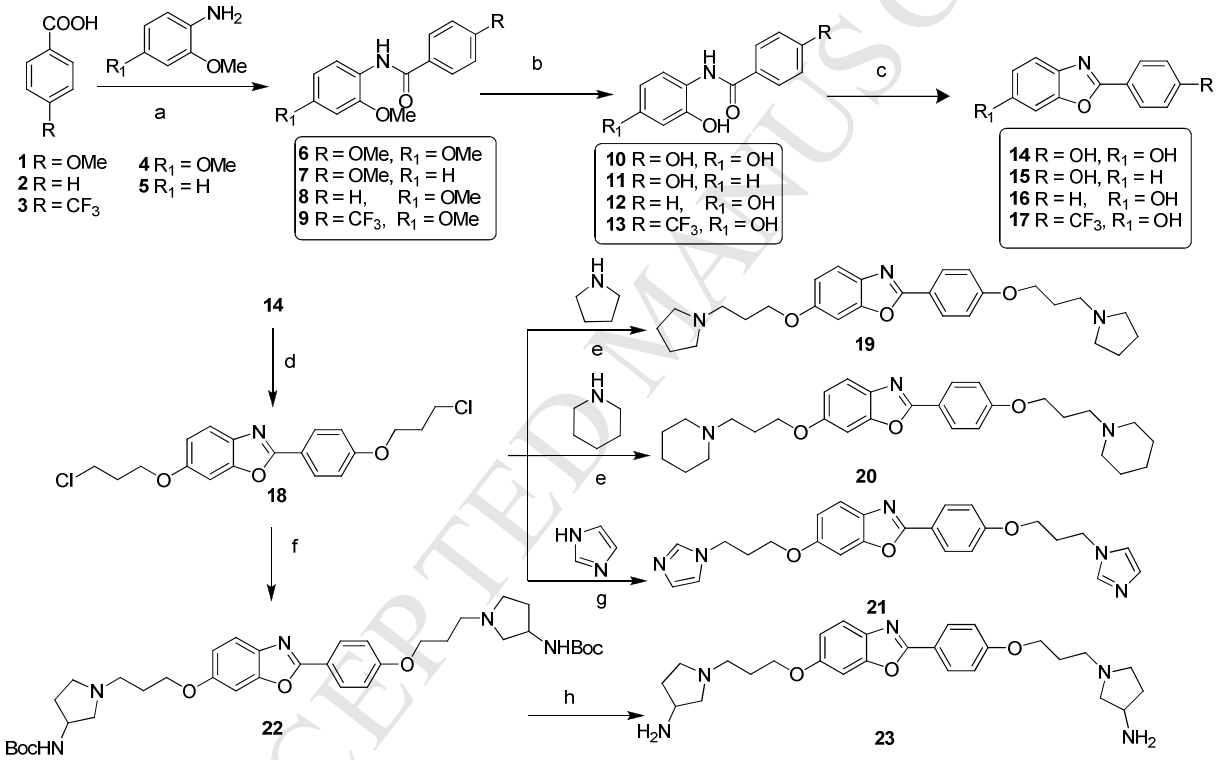
2. Results and Discussion

We have designed and synthesized a set of small molecules constituting specific geometry and with variable basicity (Supporting information) with an aim to examine their potential for TLR9 antagonism. The design strategy necessitates the molecules to have an ideal clogP value so that they can access the TLR9 receptor inside the endosomal compartment, wherein the pH is 6-6.5. The biological validation for the humanTLR9 (hTLR9) antagonism was performed with a reporter HEK293 cell line expressed with hTLR9 to report downstream signaling through an enzymatic activity of an alkaline phosphatase that could be assessed colorimetrically (Figure 3). To check potential humanTLR7 antagonism, similar assays were conducted with another reporter HEK293 cell line expressed with hTLR7. To validate the TLR9 antagonism in a more physiological system, assays were also performed with human peripheral mononuclear cells (PBMCs) and human plasmacytoid dendritic cells (pDCs) by stimulating them with TLR9 ligand (CpGA), in presence of different concentrations of the candidate small molecules. Assessment of hTLR9 antagonism by individual small molecules was based on IC_{50} values calculated from the reporter assays (Figure 3). None of the compounds showed any cytotoxicity below 20.0 µM as measured by a tetrazolium dye reduction assay (MTT assay) on hepatic and renal epithelial cells (Figure S4).

Syntheses of the compounds listed in table 1 are shown in schemes 1 and 2. The formation of the benzoxazole core, from the necessary intermediates was achieved readily under reflux in acetic acid. The obtained benzoxazole compounds (**14-17**) were then treated with 1-bromo 3-chloropropane in presence of K_2CO_3 in dry DMF, and the resultant chloro derivatives were treated with different in DMF to obtain the corresponding compounds **19**, **20, 21**, **23**, **25**,

28, **29** and **30**. Subsequently, we started the structural exploration of the hTLR9 antagonism with the help of E6446 (compound **19**) and **20** [19], both having 2-phenylbenzoxazole core that can strongly inhibit TLR9 signaling triggered by agonistic CpG DNA. The structural features of both **19** and **20** are similar except that the side chain substitutions are pyrrolidine in **19** and piperidine in **20**. Despite showing potent TLR9 inhibitory activity, **19** and **20** have undesirable molecular flexibility along with high clogP value for oral bioavailability, thus their limited drug-likeness [23-25]. First, we tried to identify minimal structural requirements for inhibition of hTLR9 signaling. Accordingly, we synthesized compounds **25** and **28** lacking one of the flexible side chains. Their hTLR9 antagonistic activity in terms of IC_{50} value, as compared to **19** and **20**, was substantially reduced as expected (Table 1). Interestingly, among themselves, **28** showed almost nine-fold hTLR9 inhibition efficacy as compared to **25**. A strong electron withdrawing group (–CF_3) was introduced in **29** to determine the effect of ring deactivation on hTLR9 inhibition. Interestingly, **29** showed almost similar IC_{50} value of 1.1 µM as compared to **28** with 0.83 µM showing negligible effect of ring deactivation. Next, we introduced specific groups with varying basicity at both flexible side chains of benzoxazole core to corroborate the effect of early endosomal pH on hTLR9 antagonism (Table 1). We replaced the basic nitrogen at the terminal end of **28** with an imidazole moiety in **30**. Excitedly, **30** showed an IC_{50} value of 2.1 µM as compared to 0.83 µM for **28**. For further exploration, **21** and **23** were synthesized that led to another interesting observation. The activity of **21** and **23** with two nitrogen atoms at the terminal end but with variable basicity differs by 85 fold. Compound **23** exhibited an IC_{50} of 60 nM, whereas that of **21**, with two imidazole groups, was only 5.1 µM. Presumably, the *N*-substitution pattern of imidazole makes it difficult to protonate at early endosomal pH (6-6.5). On the other hand, **19** showed an IC_{50} of 1.99 µM against hTLR7 with TLR9/TLR7 selectivity of 132 fold. Remarkably, **23** presented improved selectivity of 180 fold with IC_{50} of 10.84 µM against hTLR7. To identify the structural attributes in a molecule that confer human TLR9 (hTLR9) antagonism and its correlation with probe-receptor interactions, we built a homology model of the Ecto domain of hTLR9 in Discovery Studio 4.0 (supporting information). For the analysis for potential ligand-binding region, the recently reported crystal structures of CpG DNA agonist, and inhibitory DNA (iDNA) in horse and bovine TLR9 was taken into consideration [26]. The binding area of iDNA on TLR9 was found to be quite large from LRR2–LRR11 by forming a stem-loop structure in the interior of the ring structure of TLR9. Although the binding modes of

agonistic DNA and iDNA are completely different, LRR4 and LRR5 are involved in the overlapping binding site for agonistic DNA and iDNAs. Our model has 0.44 Å RMSD cut off with that of template structure. The docking experiments for all the compounds were conducted in C-DOCKER and Ligandfit to validate the best possible mode. To find the most probable binding mode CDOCKER energy, CDOCKER interaction energy, PLP1, PMF04 and Ligscore1 were considered. During the analysis, CDOCKER interaction energy and PMF04 appeared to be more correlating with IC_{50} value (Figure S3). The clogP value was determined by Discovery Studio Calculate Molecular Property suite.



Scheme 1. Reagents and conditions: (a) CDI, MeCN, 10-12 h, rt; (b) BBr_3, CH_2Cl_2, 0 ºC; (c) AcOH, reflux, 48 h; (d) l-bromo-3-chloropropane, K_2CO_3, DMF, rt, 12 h; (e) DMF, 110 ºC, sealed tube; (f) *tert*-butyl pyrrolidin-2-ylcarbamate, K_2CO_3, KI, DMF, sealed tube, 120 ºC; (g) imidazole, NaH, DMF, 60 ºC; (h) TFA, CH_2Cl_2, 0 ºC to rt.

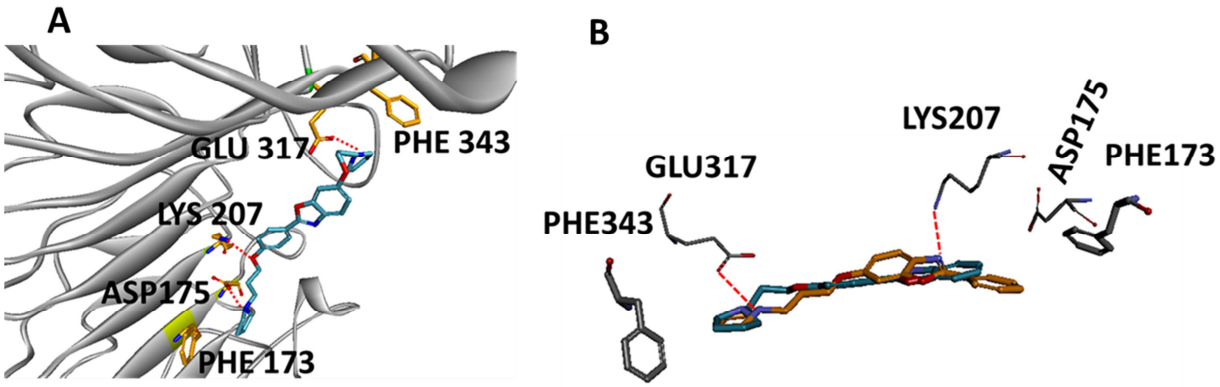
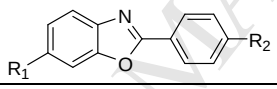


Figure 1. A) Binding mode of **19** in hTLR9 depicting hydrogen bond interactions with Glu317, Asp175, Lys207 and hydrophobic interaction with Phe173 and Phe343. Docking study of **28** (carbon atom in dark orange) and **25** (carbon atom in cyan) The docking study suggested that the terminal side chain pyrrolidine group of both **25** and **28** orient in the same direction and form hydrogen bond with Glu317.

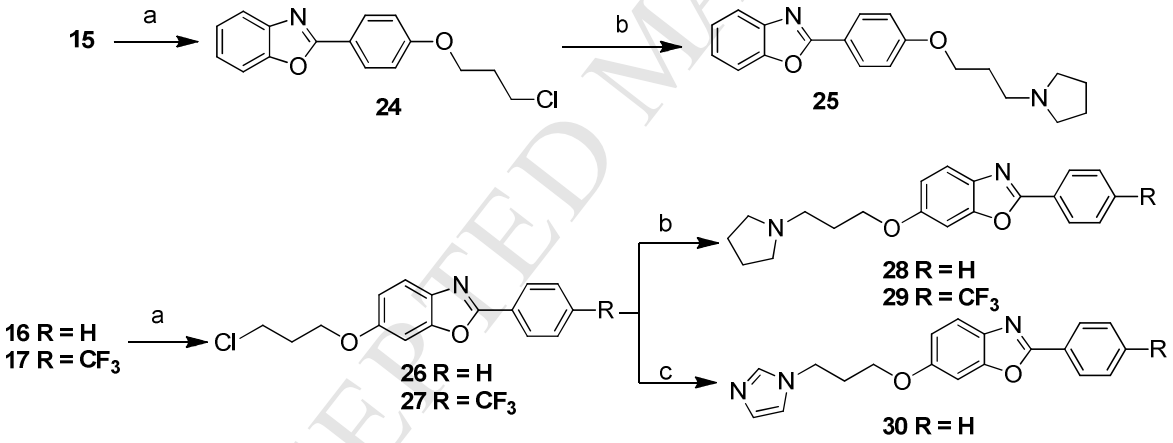
Table 1. Minimal structural requirement for inhibition of TLR9 signaling



Comp[a]	R_1	R_2	hTLR9: IC_{50} (μM)[b]	cLogP
19[c]			0.015	4.57
20			0.098	5.48
25			7.0	3.93
28			0.83	3.93
30			2.1	3.07
29			1.1	4.87
21			5.1	2.85
23[c]			0.06	1.81

[a]All compounds depicted in table were synthesized. [b]The IC_{50} values were calculated via reporter assay with HEK293 cells transfected with human TLR9 in our laboratory. [c]Tested against hTLR7.

The docking analysis suggested that both **19** and **20** share a similar interaction pattern as iDNA at LRR5, LRR6 and LRR10. As depicted in figure 1A, the basic nitrogen atoms on both the pyrrolidine ring of **19** were found to interact via salt bridge type hydrogen bonding network with Glu317 (on LRR10) and Asp175 (on LRR5), whereas Lys207 (on LRR6) form hydrogen bonding with phenolic oxygen atom of the benzoxazole core. The alkyl ring of both the pyrrolidine may be stabilized by hydrophobic interaction with Phe173 (on LRR5) and Phe343 (on LRR11). Based on our binding mode analyses, we hypothesized that the interaction of antagonists with Glu317, Asp175 and Lys207 amino acids along with hydrophobic interaction with Phe173 and Phe343 is important for inhibition of hTLR9 signaling. The docking study of **25** and **28** (Figure 1B) suggested that the terminal side chain for both the compounds orient in the same direction and form hydrogen bond with Glu317 along with hydrophobic interaction. This led us to attribute a critical role to Glu317, along with hydrophobic interactions, for TLR9 inhibition.



Scheme 2. Reagents and conditions: (a) K_2CO_3, DMF, rt, 12 h; (b) pyrrolidine, DMF, 110 ºC; (c) imidazole, NaH, DMF, 60 ºC.

The initial results prompted us to incorporate two nitrogen atoms spatially present in the next set of molecules (Table 2). Thus we considered substitution at the *meta* position of phenyl ring of 2-phenylbenzoxazole core via amide, C-C bond and C-N bond linkages. We visualized that these linkages will impart a favorable geometry enabling the molecules to interact with the concave surface of the hTLR9. Given the apparent role of early endosomal pH, we also sought to

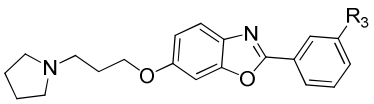
systematically increase the basicity of terminal amino group while maintaining the hydrogen bond network.

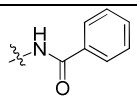
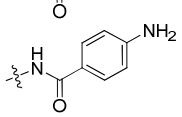
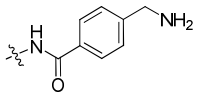
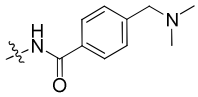
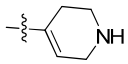
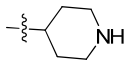
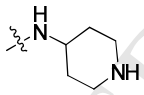
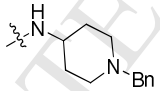
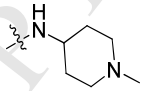
Syntheses of compounds from table 2 are shown in scheme 3. The formation of the benzoxazole core was achieved in a similar fashion as described in Scheme 1. The nitro group present in **41** was reduced to corresponding amine derivative **43** by hydrogenation using Pd/C. HATU mediated coupling [27] of corresponding acids with **43** yielded **44**, **46**, **48**, and **49** (Table 2). Further deprotection of the Boc group with TFA provided **48** and **49**. Suzuki−Miyaura [28] cross coupling of the bromo derivative **42** resulted in **50** and subsequent deprotection of the Boc group in **50** gave compound **51**. Reduction of the double bond in **51** with Pd/C provided with **52**. Compound **42** was subjected to Buchwald- Hartwig amination [29] with respective amine to obtain **54**, **55** and **56**.

Interestingly, **46** with a primary amine group directly attached to phenyl ring showed very poor hTLR9 with IC_{50} of 21.0 µM, whereas incorporating just a methylene bridge in **48** drastically increased the hTLR9 inhibition by 35-fold to 0.59 µM. Subsequently dimethylation of the terminal amino function in **49** increased the hTLR9 inhibition by 450 folds to 48.0 nM as compared to **46**. The molecular docking experiment with **49** suggested that substitution at the meta position of phenyl ring of 2-phenylbenzoxazole introduced a curvature which enabled these compounds to maintain all the conserved hydrogen bonds in accordance with our binding mode hypothesis. Compound **48**, and **49** with an amide group showed excellent selectivity of 100 fold against hTLR7 (table 2). The proposed inhibition of hTLR9 for **44**, **46**, **48**, and **49** is shown in figure 2A. For further validation of the binding mode hypothesis, we synthesized compounds **51**, **52**, **54**, **55** and **56**, taking into account the hitherto gained structural insight into hTLR9 antagonism.

In **51**, the terminal piperidine group was attached directly to the main core via a C-C bond. The direct attachment of piperidine group in **51** and **52** imparts a geometric rigidity in these molecules. Accordingly, **51** and **52** showed similar IC_{50} value of 99 nM and 133 nM respectively. The docking analysis (Figure 4) depicted a distinct characteristic for **51** and **52** with the piperidine ring forming a skewed shape to form salt-bridge hydrogen bonding with Glu317. Compound **51** (TLR9/TLR7 = 84-fold) showed better selectivity compared to **52** (TLR9/TLR7 = 38-fold) against hTLR7 with IC_{50} value of 8.30 µM and 4.99 µM.

Table 2.Second generation compounds based on the binding mode



Comp	R_3	hTLR9: IC_{50} (μM)	hTLR7: IC_{50} (μM)	cLogP
44		2.56	NT	4.71
46		21	NT	3.96
48		0.59	66.17	3.82
49		0.048	4.71	4.78
51		0.099	8.30	3.97
52		0.133	4.99	4.08
54		0.308	7.55	3.31
55		0.13	8.09	5.43
56		0.037	4.57	3.85

All compounds depicted in table were synthesized and the IC_{50} values were calculated via reporter assay with HEK293 cells transfected with human TLR9 and human TLR7 in our laboratory. NT: not tested.

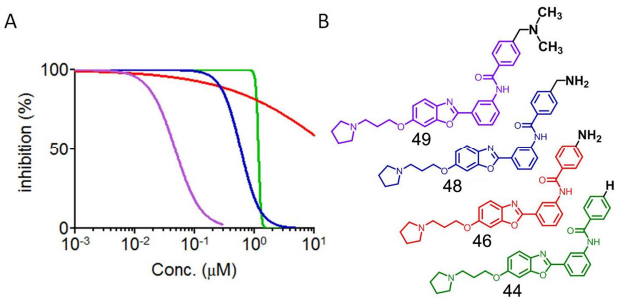


Figure 2. A) TLR9 inhibition curve for **44**, **46**, **48**, and **49**. B) Structure of **44**, **46**, **48**, and **49** showing the structural difference.

Scheme 3.[a]Reagents and conditions: (a) CDI, MeCN, 10-12 h, rt; (b) BBr_3, CH_2Cl_2, 0 ºC; (c) AcOH, reflux, 48 h; (d) 1-bromo-3-chloropropane, K_2CO_3, DMF, rt, 12 h; (e) DMF, 110 ºC; (f) Pd/C, H_2, rt; (g) Substituted benzoic acids, HATU, DIPEA, DMF, rt, 12 h; (h) TFA, Dry DCM, 0 ºC to rt; (i) *N*-Boc-1,2,5,6-tetrahydropyridine-4-boronic acid pinacol ester, $Pd(PPh_3)_4$, Cs_2CO_3, DMF:H_2O(1:1), microwave, 110 ºC, 2 h; (j) 1-benzylpiperidin-4-amine/1-methylpiperidin-4-amine, NatOBu, BINAP, $Pd_2(dba)_3$, toluene, reflux, 8-10 h.

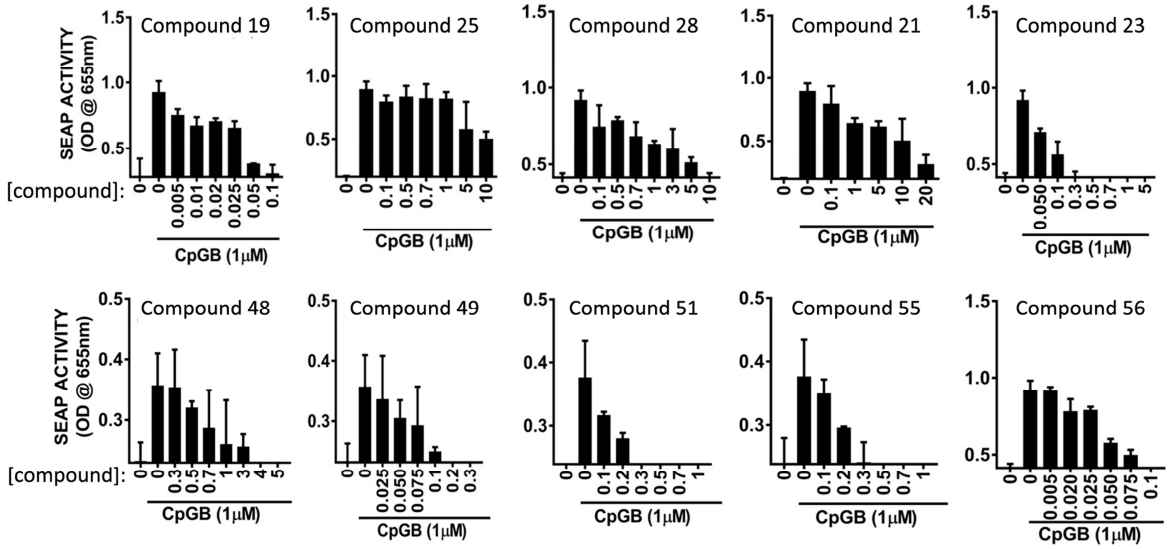


Figure 3. Representative TLR9 reporter assay results of synthesized TLR9 antagonists. The compounds were assessed for dose (μM) dependent inhibition of TLR9, using a HEK- Blue TLR9 cell line expressing a TLR9-responsive, NF-KB-inducible SEAP. Data shown are mean of triplicate wells ± SD.

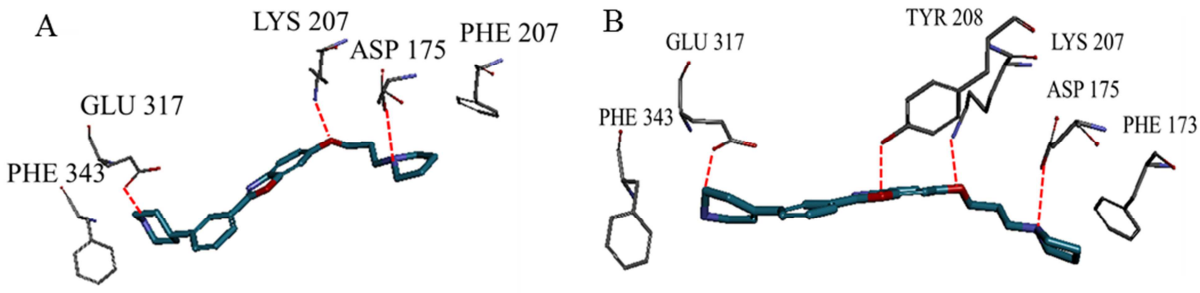


Figure 4. The characteristic docking poses of **52** (A) and **51** (B) (carbon atom in cyan) with hydrogen bond depicted by red dotted line.

Further, we strategically introduced similar piperidine group in **54**, as in **52,** but with an amino flexibility. The IC_{50} of **54** was found to be 308 nM. For further optimization aimed at potent hTLR9 inhibition, we tried to exploit the hydrophobic interaction by introducing a benzyl group in **55**. The benzyl group did show π-π stacking with Phe343 and Glu317, and formed a similar bridge-head type of hydrogen bond (Figure 5B). However, the other half of the molecule cannot attain the required geometry to be able to interact with Lys207 and Asp175 on the concave surface of hTLR9. Instead, the terminal side chain nitrogen on pyrrolidine can form

hydrogen bond with Ser151 (on LRR4). Ser151 was also found to be an iDNA-interacting residue.

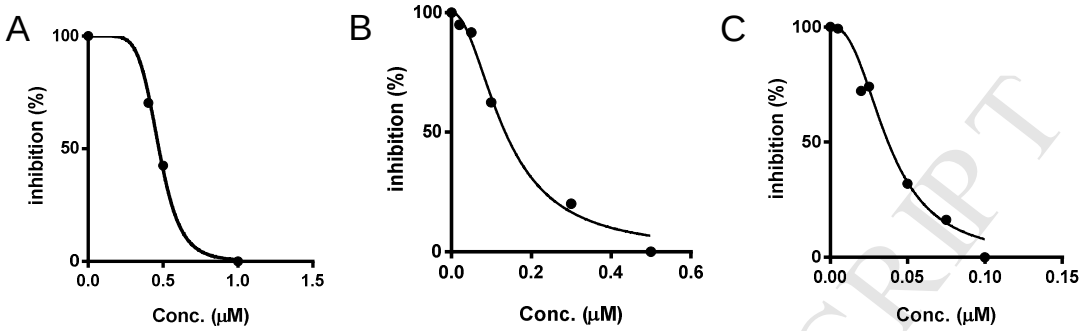


Figure 5. Inhibition curve for compound **56**. The compound was assessed for dose (µM) dependent inhibition of TLR9 in A) PBMC B) pDCs. C) Reporter assay.

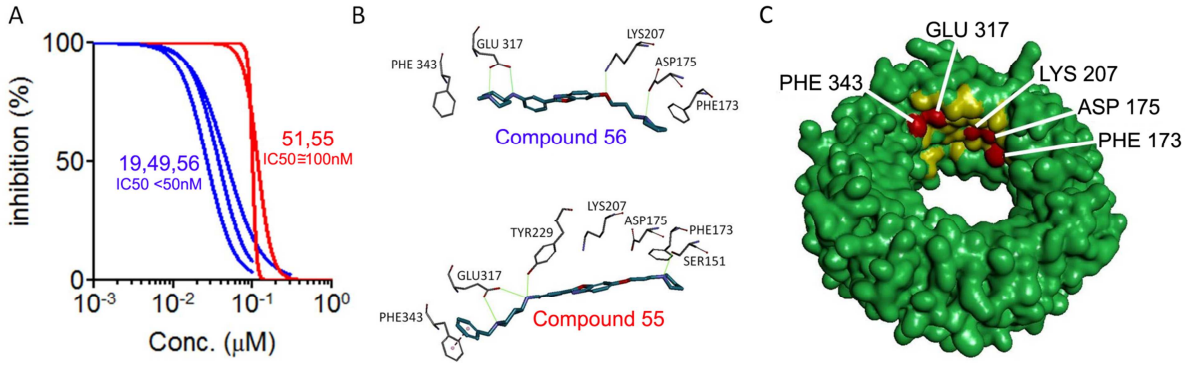


Figure 6. A) TLR9 inhibition curve for **19**, **49**, **51**, **55** and **56**. B) Docking study of **55** and **56** (carbon atom in cyan) showing different binding mode of **55** with π-π stacking with Phe343 as well as missing interactions with Asp175, Lys207 and Phe173. C) Small molecule binding hot-spot region shown in red as compared to large iDNA binding region in yellow color.

Having gained significant understanding, we synthesized **56**, where the methyl protected piperidine moiety allows formation of a hairpin bridge-head with Glu317 (figure 6B). Phe343 stabilizes this methyl group by hydrophobic interaction, while other important interactions are maintained. Excitedly, the IC_{50} of **56** was found to be 37 nM as in comparison with 308 nM for **54**, which strongly supports our binding mode hypothesis (Figure 6C). Figure 6 depicts dose depended inhibition of TLR9 by **56** in PBMC assay, isolated human pDC assay and reporter assay respectively. The importance of hydrophobicity at the terminal end can be further

corroborated by comparing the IC_{50} value of **54**, **55** and **56**. The favorable geometry shown by **56** also explains the improvement in its hTLR9 antagonism as compared to **51** and **55**. Interesting to note that the most potent compound **56** provided excellent selectivity (TLR9/TLR7 = 125-fold) against hTLR7 as compared to compound **55** (TLR9/TLR7 = 62-fold) and compound **54** (TLR9/TLR7 = 25-fold). Of note, the clogP value of compound **56** was 3.85, which is considered to be ideal for cell membrane permeability, thus facilitating both endosomal access as well as oral bioavailability.

3. Conclusion

In conclusion, we have successfully illustrated a series of structural attributes responsible for functional mechanism of selective hTLR9 antagonism by small molecules through these studies. We biologically validated the computationally derived hypothesis using a series of rationally designed molecules with different geometry and varying basicity. We found that interaction with Glu317 (on LRR10), Asp175 (on LRR5) and Lys207 (on LRR6) to be of utmost importance along with hydrophobic interaction with Phe343 (on LRR11) and Phe173 for hTLR9 antagonism (Figure 6C). Our activity-guided rational designing approach led to the development of small molecules having hTLR9 antagonism property with IC_{50} values < 100 nM. These hTLR9 antagonists were 50-200 fold selective against hTLR7. The selectivity is of special significance as in some clinical contexts TLR7 and TLR9 receptors may have opposing inflammatory and regulatory roles. From drug designing perspective these compounds had lesser molecular flexibility. Validated correlation between our binding mode hypothesis and hTLR9 antagonistic activity of the newly synthesized molecules designed by us can be very useful as benchmarks for further prediction of small molecule hTLR9 antagonists and development of new generation drugs for a number of clinical contexts, where inadvertent TLR9 activation play a major role.

4. Methods

4.1. Chemistry

We have synthesized series of benzoxazole derivatives having characteristic molecular geometry, flexibility and basicity for the development of hTLR9 antagonist. All starting

materials and reagents were purchased from commercial suppliers and used without further purification. TLC was performed on silica gel plates (Merck silica gel 60 F254) and the spots were visualized under UV light (254 nm and 365nm). Microwave assisted reactions were run in a CEM Explorer^{TM} microwave. For the compound purification flash chromatography was performed with RediSepRf silica gel columns on Teledyne ISCO CombiFlashRf system. Melting points were taken in Digital melting point apparatus. The structural analysis of all the intermediate and target compounds was done by ^{1}H NMR, ^{13}C NMR, ESI and HRMS spectrometer respectively. The purity of the compounds selected for the biological validation was carried out by elemental analyses for C, H and N content. The purity of the selected compounds was also analyzed by Waters HPLC using column Xtimate C18 (5.0 µm, 250 x 4.6 mm). All compounds were of high purity of >95%. The synthetic protocol for all the compounds has been detailed in the supplemental Information section.

*4.1.1. General Procedure for the Synthesis of **6**, **7**, **8** and **9**:*

The acid (**1**, **2**, **3**; 3 mmol) and carbonyl diimidazole (3.5 mmol) were dissolved in dry acetonitrile under a N_2 atmosphere. The reaction mixture was stirred for 30 minutes and the required aromatic amine (3.3 mmol) was added. The reaction mixture was stirred for 10-12 hrs. Then acetonitrile was concentrated in vacuum. The residue was diluted with water and the organic part was extracted using ethyl acetate. The combined organic layer was dried over sodium sulfate and concentrated. The residue was purified by flash column chromatography using ethyl acetate-hexane system to give the corresponding amide compounds.

*4.1.1.1. N-(2,4-Dimethoxyphenyl)-4-methoxybenzamide (**6**)*

Yield 84% as white fluffy solid. Mp 107-108 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.39 (d, *J* = 9.0 Hz, 1H), 8.26 (br s, 1H, -NH), 7.85 (d, *J* = 9.0 Hz, 2H), 6.97 (d, *J* = 9.0 Hz, 2H), 6.54-6.51 (m, 2H), 3.88 (s, 3H), 3.86 (s, 3H), 3.81 (s, 1H); MS (FAB) *m/z* $[M]^+$ 287.20.

*4.1.1.2. 4-Methoxy-N-(2-methoxyphenyl)benzamide (**7**)* [30]

Yield 73% as off white solid. Mp 88-89 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.52 (dd, *J* = 9.0 Hz, 3.0 Hz, 1H), 8.49 (br s, -NH), 7.87 (d, *J* = 9.0 Hz, 2H), 7.10-6.90 (m, 5H), 3.93 (s, 3H), 3.87 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 164.7, 162.3, 148.0, 128.8, 127.9, 127.5, 123.5, 121.1, 119.7,

113.9, 109.8, 55.7, 55.4. MS (ESI) *m/z* $[M+Na]^+$ 280.29. HRMS (EI) *m/z* Calculated for $C_{15}H_{15}NO_3$ $[M]^+$257.1052; found 257.1069.

*4.1.1.3. N-(2,4-Dimethoxyphenyl)benzamide (**8**)* [31]

Yield 76% as off white solid. Mp 156-158 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.44 (d, *J* = 9.0 Hz, 1H), 8.36 (br s, 1H, -NH), 7.90 (d, *J* = 6.0 Hz, 2H), 7.58-7.48 (m, 3H),6.58-6.54 (m, 2H), 3.92 (s, 3H), 3.84 (s, 3H); ^{13}C NMR ($CDCl_3$, 75Hz) δ 163.2, 151.8, 150.4, 146.7, 141.7, 129.9, 126.6, 122.3, 121.1, 118.6, 105.3, 46.2. MS (ESI) *m/z* $[M+Na]^+$ 280.06. HRMS (EI) *m/z* Calculated for $C_{15}H_{15}NO_3$ $[M]^+$257.1052; found 257.1054.

*4.1.1.4. 4-(Trifluoromethyl)-N-(2,4-dimethoxyphenyl)benzamide (**9**)*

Yield 88% as off white fluffy solid. Mp 114-116°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.39 (d, *J* = 9.0 Hz, 1H), 8.33 (s, 1H), 7.99 (d, *J* = 6.0 Hz, 2H), 7.75 (d, *J* = 9.0 Hz, 2H), 6.56-6.53 (m, 2H), 3.91 (s, 3H), 3.83 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.6, 156.9, 149.5, 138.7, 133.4, 127.4, 125.7, 120.9, 103.9, 98.7, 55.8, 55.5. MS (ESI) *m/z* $[M+Na]^+$ 348.07. HRMS (ESI) *m/z* Calculated for $C_{16}H_{14}F_3NO_3$ $[M+Na]^+$ 348.0824; found 348.0828.

*4.1.2. General Procedure for the Synthesis of **10**, **11*** [32], ***12** and **13***

The amide (**6**, **7**, **8**, **9**; 3 mmol) was dissolved in 10 mL of dry DCM in a two-necked R.B flask. The reaction mixture was cooled to 0 ºC and BBr_3 (8 mmol for **6** and 4 mmol for **7**, **8** and **9**) was added carefully. The reaction was allowed to further stir at room temperature for 30 minutes. The reaction mixture was again cooled to 0 ºC and crushed ice was added to the reaction mixture followed by few drops of methanol. The precipitate obtained was filtered, dried and used directly for the next step.

*4.1.2.1. N-(2,4-Dihydroxyphenyl)-4-hydroxybenzamide (**10**)*

Yield 58% as ash color solid. Mp 157-159 ºC; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.28 (brs, -NH), 7.83 (d. *J* = 9.0 Hz, 2H), 7.24 (d, *J* = 9.0 Hz, 1H), 6.84 (d, *J* = 6.0 Hz, 2H), 6.35 (d, *J* = 3.0 Hz, 1H), 6.23 (dd, *J* = 9.0 Hz, 3 Hz, 1H). MS (ESI) *m/z* $[M+Na]^+$ 268.19.

*4.1.2.2. 4-Hydroxy-N-(2-hydroxyphenyl)benzamide (**11**)*

Yield 62% as white solid. Mp 165-167 ºC; ^{1}H NMR (CD_3OD, 300 MHz) δ 7.86 (d, *J* = 9.0 Hz, 2H), 7.78 (dd, *J* = 9.0 Hz, 3 Hz, 1H), 7.08-7.02 (m, 1H), 6.94-6.85 (m, 4H); ^{13}C NMR (CD_3OD, 75 MHz) δ 166.9, 161.1, 148.4, 129.2, 125.9, 125.4, 124.9, 122.5, 119.5, 115.8, 115.0. MS (ESI) *m/z* $[M+Na]^+$ 252.07. HRMS (EI) *m/z* Calculated for $C_{13}H_{11}NO_3$ $[M]^+$ 229.0739; found 229.0735.

*4.1.2.3. N-(2,4-Dihydroxyphenyl)benzamide (**12**)*

Yield 64% as deep pink solid. Mp 208- 209 ºC; ^{1}H NMR (CD_3OD, 300 MHz) δ ppm 7.95 (d, *J* = 9.0 Hz, 2H), 7.62-7.50 (m, 3H), 7.44 (d, *J* = 9.0 Hz, 1H), 6.43 (d, *J* = 3.0 Hz, 1H), 6.35 (dd, *J* = 9.0 Hz, 3 Hz, 1H); ^{13}C NMR (CD_3OD, 75 MHz) δ 168.8, 157.5, 152.1, 135.9, 133.1, 129.9, 128.7, 126.1, 119.2, 107.8, 104.7. MS (ESI) *m/z* $[M+Na]^+$ 252.17. HRMS (EI) *m/z* Calculated for $C_{13}H_{11}NO_3$ $[M]^+$ 229.0739; found 229.0740.

*4.1.2.4. 4-(Trifluoromethyl)-N-(2,4-dihydroxyphenyl)benzamide (**13**)*

Yield 61% as ash color solid. Mp 225-226 ºC; ^{1}H NMR (CD_3OD, 300 MHz) δ 8.12 (d, *J* = 6.0 Hz, 2H), 7.83 (d, *J* = 6.0 Hz, 2H), 7.43 (d, *J* = 9.0 Hz, 1H), 6.44 (d, *J* = 3.0 Hz, 1H), 6.36 (dd, *J* = 9.0 Hz, 3 Hz, 1H); ^{13}C NMR (CD_3OD, 75 MHz) δ 167.4, 157.7, 152.3, 139.5, 134.5, 129.4, 126.7, 126.4, 118.8, 107.8, 104.6. MS (ESI) *m/z* $[M+Na]^+$ 320.08. HRMS (EI) *m/z* Calculated for $C_{14}H_{10}F_3NO_3$ $[M]^+$ 297.0613; found 297.0607.

*4.1.3. General Procedure for the Synthesis of **14**, **15*** [33], ***16*** [34] *and **17*** [35]

Acetic acid (2 mmol for **10** and 1 mmol for **11**, **12** and **13**) was added to **10**, **11**, **12** and **13**; (1 mmol) independently, and the mixture was refluxed for 48 hours. The acetic acid was evaporated under vacuum and the reaction mixture was diluted with water. The organic part was extracted using ethyl acetate. Combined organic layer was dried over sodium sulfate and concentrated. The residue was purified by flash chromatography using ethyl acetate-hexane system.

*4.1.3.1. 2-(4-Hydroxyphenyl)benzo[d]oxazol-6-ol (**14**)*

Yield 69% as pink solid. ^{1}H NMR (CD_3OD, 300 MHz) δ 8.02 (d, *J* = 9.0 Hz, 2H), 7.47 (d, *J* = 9.0 Hz, 1H), 7.03 (d, *J* = 3.0 Hz, 1H), 6.95 (d, *J* = 9.0 Hz, 2H), 6.86 (dd, *J* = 9.0 Hz, 3.0 Hz, 1H); MS (EI) *m/z* $[M]^+$ 227.

*4.1.3.2. 4-(Benzo[d]oxazol-2-yl)phenol (**15**)*

Yield 67% as white solid. Mp 275-276 ºC; ^{1}H NMR (CD_3OD, 300 MHz) δ 8.09 (d, *J* = 9.0 Hz, 2H), 7.69-7.63 (m, 2H), 7.40-7.37 (m, 2H), 6.98 (d, *J* = 9.0 Hz, 2H); ^{13}C NMR (acetone-d_6, 75 MHz) δ 164.8, 162.4, 152.3, 144.1, 131.1, 126.2, 126.0, 121.1, 120.2, 117.6, 111.9. MS (ESI) *m/z* $[M+H]^+$ 212.12. HRMS (ESI)*m/z* Calculated for $C_{13}H_9NO_2$ $[M+Na]^+$234.0531; found 234.0531.

*4.1.3.3. 2-Phenylbenzo[d]oxazol-6-ol (**16**)*

Yield 65% as pink solid. Mp 110-112 ºC; ^{1}H NMR (300 MHz, CD_3OD) δ 8.18-8.14 (m, 2H), 7.57-7.54 (m, 4H), 7.51 (s, 1H), 7.06 (d, *J* = 3 Hz, 1H), 6.89 (dd, *J* = 9.0 Hz, 3.0 Hz, 1H); ^{13}C NMR (CD_3OD, 75 MHz) δ 163.4, 158.1, 153.1, 135.6, 132.6, 130.3, 128.4, 128.2, 120.6, 114.9, 98.4. MS (ESI) *m/z* $[M+Na]^+$ 234.06. HRMS (ESI)*m/z* Calculated for $C_{13}H_9NO_2$ $[M+Na]^+$ 234.0531; found 234.0533.

*4.1.3.4. 2-(4-(Trifluoromethyl)phenyl)benzo[d]oxazol-6-ol (**17**)*

Yield 68% as white crystal. Mp 238-240 °C; ^{1}H NMR ($CDCl_3$, 300MHz) δ 8.33 (d, *J* = 6.0 Hz, 2H), 7.86 (d, *J* = 9.0 Hz, 2H), 7.56 (d, *J* = 6.0 Hz, 1H), 7.08 (d, *J* = 3.0 Hz, 1H), 6.92 (dd, *J* = 9.0 Hz, 3Hz, 1H); ^{13}C NMR (CD_3OD, 75 MHz) δ 161.7,158.6, 153.4, 135.7, 133.5, 132.1, 128.7, 127.3, 121.2, 115.3, 98.3. MS (FAB)*m/z* $[M+H]^+$ 280.10. HRMS (EI)*m/z* Calculated for $C_{14}H_8F_3NO_2$ $[M]^+$ 279.0507; found 279.0503.

*4.1.4. 6-(3-Chloropropoxy)-2-(4-(3-chloropropoxy)phenyl)benzo[d]oxazole (**18**)*

14 (1 mmol) was dissolved in dry DMF under nitrogen and K_2CO_3 (10 mmol) was added. The reaction mixture was allowed to stir for 15 minutes. Thereafter, 1-bromo 3-chloro propane (6 mmol) was introduced and reaction mixture further stirred for 12 hours at room temperature. The reaction mixture was diluted with excess amount of water and organic layer was extracted using ethyl acetate. Combined organic layer was dried over sodium sulfate and concentrated under vacuum. Column chromatography was done in ethyl acetate-hexane system to get the purified product **18** as white fluffy solid (70%). Mp 59-60 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.14 (d, *J* = 6.0 Hz, 2H), 7.61 (d, *J* = 9.0 Hz, 1H), 7.11 (d, *J* = 3.0 Hz, 1H), 7.03 (d, *J* = 9.0 Hz, 2H), 6.94 (dd,

J = 9.0 Hz, 3 Hz, 1H), 4.23-4.17 (m, 4H), 3.81-3.76 (m, 4H), 2.33-2.25 (m, 4H). MS (ESI) *m/z* $[M+Na]^+$ 401.94.

*4.1.5. 6-(3-(Pyrrolidin-1-yl)propoxy)-2-(4-(3-(pyrrolidin-1-yl)propoxy)phenyl)benzo[d]oxazole (**19**)*

18 (1 mmol) and pyrrolidine (6 mmol) was dissolved in dry DMF in a sealed tube under a N_2 atmosphere. The reaction mixture was heated at 110 ºC for 8 hours and diluted with excess amount of water and the organic part was extracted with 5% Methanol/$CHCl_3$ system. Column chromatography was done using $CHCl_3$/Methanol system to get the pure product **19** as white solid (66%). Mp 107-109 °C; ^{1}H NMR (CD_3OD, 300 MHz) δ 8.11 (d, *J* = 9 Hz, 2H), 7.56 (d, *J* = 9.0 Hz, 1H), 7.25 (d, *J* = 3.0 Hz, 1H), 7.09 (d, *J* = 9.0 Hz, 2H), 6.99 (dd, *J* = 9.0 Hz, 3.0 Hz, 1H), 4.16-4.09 (m, 4H), 2.75-2.62 (m, 12H), 2.11-2.04 (m, 4H), 1.86-1.84 (m, 8H); MS (ESI) *m/z* $[M+H]^+$ 450.39. HRMS (ESI) *m/z* Calculated for $C_{27}H_{35}N_3O_3$ $[M+H]^+$ 450.2758; found 450.2759. HPLC Purity 95.7%.

*4.1.6. 6-(3-(Piperidin-1-yl)propoxy)-2-(4-(3-(piperidin-1-yl)propoxy)phenyl)benzo[d]oxazole (**20**)*

18 (1 mmol) and piperidine (6 mmol) was dissolved in dry DMF in a sealed tube under a N_2 atmosphere. The reaction mixture was heated to 110 ºC for 8 hours and diluted with excess amount of water and organic part was extracted with 5% Methanol/$CHCl_3$ system. Column chromatography was done using $CHCl_3$/Methanol system to get the pure product **20** as off white solid (69%). Mp 269-270 °C; ^{1}H NMR (CD_3OD, 300 MHz) δ 8.14 (d, *J* = 9.0 Hz, 2H), 7.59 (d, *J* = 6.0 Hz, 1H), 7.30 (d, *J* = 3.0 Hz, 1H), 7.15 (d, *J* = 9.0 Hz, 2H), 7.03 (dd, *J* = 9.0 Hz, 3.0 Hz, 1H), 4.23-4.16 (m, 4H), 3.17-3.07 (m, 12H), 2.28-2.21 (m, 4H), 1.83-1.66 (m, 12H). MS (ESI) *m/z* $[M+H]^+$ 478.20. HRMS (FAB) *m/z* Calculated for $C_{29}H_{39}N_3O_3$ $[M+H]^+$478.3070; found 478.3069. HPLC Purity 98.9%.

*4.1.7. 6-(3-(1H-imidazol-1yl)propoxy)-2-(4-(3-(1H-imidazol-1-yl)propoxy)phenyl)benzo[d]-oxazole (**21**)*

NaH (1.6 mmol) was added to the solution of imidazole (1.2 mmol) in dry DMF at 0 °C and the reaction mixture allowed to stir for 45 minutes at room temperature. The reaction mixture was

cooled to 0 °C and compound **18** (0.2 mmol) was added under a N_2 atmosphere. The reaction mixture was heated to 60 °C for 10 hours. The organic part was extracted with 5% Methanol/$CHCl_3$system. Flash column chromatography was done using $CHCl_3$/Methanol system to get the pure product **21** as off-white solid (70%). Mp 56-58 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.13 (d, *J* = 9.0 Hz, 2H), 7.70 (s, 2H), 7.58 (d, *J* = 6.0 Hz, 1H), 7.25 (d, *J* = 3.0 Hz, 1H), 7.19 (s, 2H), 7.12 (d, *J* = 9.0 Hz, 2H), 7.02 (dd, *J* = 9.0 Hz, 3 Hz, 3H), 4.32-4.28 (m, 4H), 4.06-4.01 (m, 4H), 2.36-2.28 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.9, 162.9, 158.8, 152.7, 138.7, 136.8, 130.1, 129.3, 120.9, 120.4, 116.1, 114.5, 97.4, 66.4, 65.9, 44.9, 31.8. MS (ESI) *m/z* $[M+Na]^+$ 466.22. HRMS (FAB) *m*/*z* Calculated for $C_{25}H_{25}N_5O_3$ $[M+H]^+$ 444.2037; found 444.2020. HPLC Purity 99.3%.

4.1.8. A sealed tube was charged with **18**, (0.4 mmol), K_2CO_3 (4 mmol) and KI (1.6 mmol) under a N_2 atmosphere in dry DMF. 3-Boc amino pyrrolidine (1.6 mmol) was added and the reaction mixture was heated to 120 ºC for 10 hours. The reaction mixture was diluted with excess amount of water and organic part was extracted with 5% Methanol/$CHCl_3$ system. The residue was purified by column chromatography using $CHCl_3$/Methanol system to get **22** as yellow gummy (72%) semisolid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.08 (d, *J* = 6.0 Hz, 2H), 7.57 (d, *J* = 9.0 Hz, 1H), 7.06 (s, 1H), 6.99 (d, *J* = 9.0 Hz, 2H), 6.90 (d, *J* = 6.0 Hz, 1H), 4.47 (s, 2H), 4.14 (d, *J* = 3.0 Hz, 4H), 3.55-3.38 (m, 12H), 2.53-2.14 (m, 9H), 1.42 (s, 18H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 162.3, 161.3, 157.1, 155.4, 151.4, 135.9, 128.8, 119.8, 119.4, 114.7, 112.9, 96.1, 79.2, 66.9, 66.2, 61.1, 52.8, 52.6, 52.5, 49.7, 32.4, 28.4, 28.2. MS (ESI) *m/z* $[M+Na]^+$ 702.26. HRMS (ESI) *m*/*z* Calculated for $C_{37}H_{53}N_5O_7$ $[M+Na]^+$ 702.3843; found 702.3845.

*4.1.9. 1-(3-(4-(6-(3-(3-Aminopyrrolidin-1-yl)propoxy)benzo[d]oxazol-2-yl)phenoxy)propyl) pyrrolidin-3-amine (**23**)*

22 (1 mmol) was dissolved in dry DCM and trifluoroacetic acid (1 mL) was added to the solution under ice-cold condition and allowed to stir at room temperature for 2 hours. The solution was neutralized with 1N NaOH solution keeping it under ice-cold condition. The organic part was extracted with 10% Methanol/$CHCl_3$ system. Flash column chromatography was done using $CHCl_3$/Methanol/NH_3 system to get the pure product **23** as yellow gummy semisolid (72%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.12 (d, *J* = 12.0 Hz, 2H), 7.59 (d, *J* = 6.0 Hz, 1H), 7.10 (d, *J* = 6.0

Hz, 1H), 7.01 (d, *J* = 12.0 Hz, 2H), 6.93 (dd, *J* = 12.0 Hz, 6.0 Hz, 1H), 4.12-4.07 (m, 4H), 3.55-3.51 (m, 2H), 2.77-2.72 (m, 4H), 2.68-2.60 (m, 4H), 2.50-2.46 (m, 2H), 2.39-2.36 (m, 2H), 2.24-2.18 (m, 2H), 2.05-1.99 (m, 5H), 1.52-1.50 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.4, 161.5, 157.3, 151.5, 135.9, 128.9, 119.5, 114.8, 112.9, 96.2, 67.1, 66.4, 64.0, 53.4, 52.9, 50.9, 35.1, 28.5. MS (ESI) *m/z* $[M+H]^+$ 480.33. HRMS (ESI) *m/z* Calculated for $C_{27}H_{37}N_5O_3$ $[M+H]^+$ 480.2976; found 480.2973. HPLC Purity 97.6%

*4.1.10. 2-(4-(3-Chloropropoxy)phenyl)benzo[d]oxazole (**24**)*

15 (1 mmol) was dissolved in dry DMF and K_2CO_3 (6mmol) was added under nitrogen. The reaction mixture was allowed to stir for 15 minutes. Thereafter, 1-bromo 3-chloro propane (3 mmol) was introduced and reaction mixture stirred for 10 hours at room temperature. The reaction mixture was diluted with excess amount of water and organic layer was extracted using ethyl acetate. Combined organic layer was dried over sodium sulphate and concentrated under vacuum. Column chromatography was done in ethyl acetate-hexane system to get the purified product **24** as white fluffy solid (72%). Mp 73-75 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.19 (d, *J* = 9.0 Hz, 2H), 7.70-7.64 (m, 2H), 7.41-7.38 (m, 2H), 7.15 (d, *J* = 9.0 Hz, 2H), 4.25 (t, *J* = 6.0 Hz, 2H), 3.80 (t, *J* = 6.0 Hz, 2H), 2.32-2.24 (m, 2H); ^{13}C NMR ($CDCl_3$,75 MHz) δ 163.0, 161.4, 150.6, 142.2, 129.3, 124.6, 124.4, 119.9, 119.6, 114.8, 110.3, 64.4, 41.3, 32.0. MS (FAB) *m/z* $[M+H]^+$288.20. EI-HRMS *m/z* Calculated for $C_{16}H_{14}ClNO_2$ $[M]^+$ 287.0713; found 287.0710.

*4.1.10. 2-(4-(3-Pyrrolidin-1-yl)propoxy)phenyl)benzo[d]oxazole (**25**)*

24 (1 mmol) and pyrrolidine (6 mmol) were dissolved in dry DMF in a sealed tube under a N_2 atmosphere. The reaction mixture was heated to 110 °C for 8 hours and diluted with excess amount of water and the organic part was extracted with 5% Methanol/$CHCl_3$ system. Column chromatography was done using $CHCl_3$/Methanol system to get the pure product **25** as light brown solid (68%). Mp 86-88 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.19 (d, *J* = 9.0 Hz, 2H), 7.75-7.72 (m, 1H), 7.57-7.54 (m, 1H), 7.34 (t, *J* = 4.5 Hz, 2H), 7.04 (d, *J* = 9.0 Hz, 2H), 4.12 (t, *J* = 7.5 Hz, 2H), 2.65 (t, *J* = 6.0 Hz, 2H), 2.54 (m, 4H), 2.06-2.00 (m, 2H), 1.83-1.78 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.2, 161.7, 150.6, 142.2, 129.3, 124.5, 124.3, 119.5, 114.8, 110.3, 66.5, 54.2, 52.9, 28.5, 23.4. MS (ESI) *m/z* $[M+H]^+$ 323.20. HRMS (EI) *m/z* Calculated for $C_{20}H_{22}N_2O_2$ $[M]^+$ 322.1681; found 322.1683. HPLC Purity 95.6%.

*4.1.10. 6-(3-Chloropropoxy)-2-phenylbenzo[d]oxazole (**26**)*

16 (1 mmol) was dissolved in dry DMF and K_2CO_3 (6mmol) was added under nitrogen. The reaction mixture was allowed to stir for 15 minutes. Thereafter, 1-bromo 3-chloro propane (3 mmol) was introduced and reaction mixture stirred for 10 hours at room temperature. The reaction mixture was diluted with excess amount of water and organic layer was extracted using ethyl acetate. Combined organic layer was dried over sodium sulfate and concentrated under vacuum. Column chromatography was done in ethyl acetate-hexane system to get the purified product **26** as white fluffy solid (76%). Mp 61-63°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22-8.20 (m, 2H), 7.65 (d, *J* = 6.0 Hz, 1H), 7.52 (t, *J* = 3.0 Hz, 3H), 7.14 (d, *J* = 3.0 Hz, 1H), 6.97 (dd, *J* = 9.0 Hz, 3Hz, 1H), 4.20 (t, *J* = 3.0 Hz, 2H), 3.79 (t, *J* = 3.0 Hz, 2H), 2.32-2.28 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.4, 157.3, 151.6, 136.1, 131.1, 128.9, 127.2, 120.0, 113.3, 96.2, 65.1, 41.4, 32.2. MS (FAB) *m/z* $[M+H]^+$ 288.1. HRMS (EI) *m/z* Calculated for $C_{16}H_{14}ClNO_2$ $[M]^+$ 287.0713; found 287.0707.

*4.1.11. 6-(3-Chloropropoxy)-2-(4-trifluoromethyl)phenyl)benzo[d]oxazole (**27**)*

17 (1 mmol) was dissolved in dry DMF and K_2CO_3 (6mmol) was added under nitrogen. The reaction mixture was allowed to stir for 15 minutes. Thereafter, 1-bromo 3-chloro propane (3 mmol) was introduced and reaction mixture stirred for 10 hours at room temperature. The reaction mixture was diluted with excess amount of water and organic layer was extracted using ethyl acetate. Combined organic layer was dried over sodium sulfate and concentrated under vacuum. Column chromatography was done in ethyl acetate-hexane system to get the purified product **27** as white solid (66%). Mp 68-70 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.32 (d, *J* = 6.0 Hz, 2H), 7.78 (d, *J* = 9.0 Hz, 2H), 7.68 (d, *J* = 9.0 Hz, 1H), 7.15 (d, *J* = 3.0 Hz, 1H), 7.00 (dd, *J* = 9.0 Hz, 3 Hz, 1H), 4.20 (t, *J* = 6.0 Hz, 2H), 3.80 (t, *J* = 7.5 Hz, 2H), 2.34-2.26 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 160.7, 157.8, 151.7, 135.8, 132.7, 130.5, 127.3, 125.9, 125.5, 120.4, 113.8, 96.1, 65.0, 41.4, 32.1. MS (FAB) *m/z* $[M+H]^+$356.10. HRMS (FAB)*m/z* Calculated for $C_{17}H_{13}ClF_3NO_2$ $[M+H]^+$ 356.0667; found 356.0673.

*4.1.12. 6-(3-(Pyrrolidin-1-yl)propoxy)-2-phenylbenzo[d]oxazole (**28**)*

26 (1 mmol) and pyrrolidine (6 mmol) was dissolved in dry DMF in a sealed tube under a N_2 atmosphere. The reaction mixture was heated to 110 °C for 8 hours and diluted with excess amount of water and the organic part was extracted with 5% Methanol/$CHCl_3$ system. Column chromatography was done using $CHCl_3$/Methanol system to get the pure product **28** as pale solid (65%). Mp 65-68 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.22-8.18 (m, 2H), 7.64-7.57 (m, 4H), 7.31 (d, *J* = 3.0 Hz, 1H), 7.04 (dd, *J* = 9.0 Hz, 3.0 Hz, 1H), 4.15 (t, *J* = 6.0 Hz, 2H), 2.85-2.72 (m, 6H), 2.13-2.06 (m, 2H), 1.92-1.87 (m, 4H); ^{13}C NMR (75 MHz, CD_3OD) δ 163.9, 159.6, 153.1, 132.8, 130.3, 128.3, 120.8, 115.1, 97.4, 68.1, 55.3, 54.4, 29.4, 24.3. MS (EI) *m/z* $[M]^+$ 322. HRMS (EI) *m/z* Calculated for $C_{20}H_{22}N_2O_2$ $[M]^+$ 322.1681; found 322.1682. HPLC Purity 97.0%.

*4.1.13. 6-(3-(Pyrrolidin-1-yl)propoxy)-2-(4-(trifluoromethyl)phenyl)benzo[d]oxazole (**29**)*

27 (1 mmol) and pyrrolidine (6 mmol) was dissolved in dry DMF in a sealed tube under a N_2 atmosphere. The reaction mixture was heated to 110°C for 8 hours and diluted with excess amount of water and the organic part was extracted with 5% Methanol/$CHCl_3$ system. Column chromatography was done using $CHCl_3$/Methanol system to get the pure product **29** as brown solid (71%). Mp 80-82°C; ^{1}H NMR (CD_3OD, 300 MHz) δ 8.37 (d, *J* = 9.0 Hz, 2H), 7.88 (d, *J* = 6.0 Hz, 2H), 7.65 (d, *J* = 6.0 Hz, 1H), 7.31 (d, *J* = 3.0 Hz, 1H), 7.05 (dd, *J* = 9.0 Hz, 3 Hz, 1H), 4.14 (t, *J* = 6.0 Hz, 2H), 2.78 (t, *J* = 7.5 Hz, 2H), 2.69 (m, 4H), 2.14-2.05 (m, 2H), 1.88 (m, 4H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.6, 158.2, 151.8, 135.6, 132.6, 132.4, 130.6, 127.4, 125.9, 120.4, 113.9, 96.1, 67.1, 54.3, , 53.1, 28.6, 23.5. MS (ESI) *m/z* $[M+H]^+$ 391.14. HRMS (EI) *m/z* Calculated for $C_{21}H_{21}F_3N_2O_2$ $[M]^+$ 390.1555; found 390.1556. HPLC Purity 97.1%.

*4.1.14. 6-(3-(1H-imidazol-1-yl)propoxy)-2-phenylbenzo[d]oxazole (**30**)*

NaH (0.8 mmol) was added to the solution of imidazole (0.4 mmol) in dry DMF at 0 °C and the reaction mixture allowed to stir for 45 minutes at room temperature. The reaction mixture was cooled to 0 °C and **26** (0.2 mmol) was added under a N_2 atmosphere. The reaction mixture was heated to 60 °C for 10 hours. The organic part was extracted with 5% Methanol/$CHCl_3$ system. Column chromatography was done using $CHCl_3$/Methanol system to get the pure product as pale color solid (64%). Mp 113-115 °C; ^{1}H NMR ($CDCl_3$, 300 MHz,) δ 8.22-8.19 (m, 2H), 7.65 (d, *J* = 9.0 Hz, 1H), 7.53-7.51 (m, 4H), 7.08 (s, 2H), 6.97-6.94 (m, 2H), 4.24 (t, *J* = 7.5 Hz, 2H), 3.98

(t, *J* = 6.0 Hz, 2H), 2.33-2.24 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.5, 156.9, 151.6, 136.3, 131.2, 129.8, 128.9, 127.3, 120.2, 118.9, 113.1, 96.3, 64.6, 43.4, 30.8. MS (EI) *m/z* $[M+H]^+$319. HRMS (FAB) *m/z* Calculated for $C_{19}H_{17}N_3O_2[M+H]^+$ 320.1401; found 320.1407. HPLC Purity 96.2%.

*4.1.15.1. N-(2,4-Dimethoxyphenyl)-3-nitrobenzamide (**33**)*

31 (3 mmol) and carbonyl diimidazole (4.5 mmol) was dissolved in dry acetonitrile under a N_2 atmosphere. The reaction mixture was stirred for 30 minutes and the amine **6** (3.3 mmol) was added. The reaction mixture was stirred for 12 hrs. Then acetonitrile was concentrated in vacuum. The residue was diluted with water and the organic part was extracted using ethyl acetate. The combined organic layer was dried over sodium sulfate and concentrated. The residue was purified by flash column chromatography using ethyl acetate-hexane system to give **33** as yellow crystal (73%). Mp 146-148 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.71 (s, 1H), 8.41-8.34 (m, 3H), 8.24 (d, *J* = 9.0 Hz, 1H), 7.71 (t, *J* = 7.5 Hz, 1H), 6.56-6.54 (m, 2H), 3.92 (s, 3H), 3.83 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 162.5, 157.1, 149.6, 148.4, 137.1, 133.1, 129.9, 126.1, 121.9, 121.0, 120.6, 103.9, 98.7, 55.9, 55.6. MS (ESI) *m/z* $[M+Na]^+$ 325.26. HRMS (EI) *m/z* Calculated for $C_{15}H_{14}N_2O_5$ $[M]^+$ 302.0903; found 302.0906.

*4.1.15.2. 3-Bromo-N-(2,4-dimethoxyphenyl)benzamide (**34**)*

32 (3 mmol) and carbonyl diimidazole (4.5 mmol) were dissolved in dry acetonitrile under a N_2 atmosphere. The reaction mixture was stirred for 30 minutes and the amine **6** (3.3 mmol) was added. The reaction mixture was stirred for 12 hrs. Then acetonitrile was concentrated in vacuum. The residue was diluted with water and the organic part was extracted using ethyl acetate. The combined organic layer was dried over sodium sulfate and concentrated. The residue was purified by flash column chromatography using ethyl acetate-hexane system to give the compound **34** as off white solid (64%). Mp 100-101 °C; ^{1}H NMR (CD_3OD, 300 MHz) δ 8.36 (d, *J* = 9.0 Hz, 1H), 8.25 (s, 1H), 8.01 (s, 1H), 7.78 (d, *J* = 6.0 Hz, 1H), 7.66 (d, *J* = 9.0 Hz, 1H), 7.36 (t, *J* = 7.5 Hz, 1H), 6.54-6.51 (m, 2H), 3.90 (s, 3H), 3.82 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.4, 156.7, 149.6, 137.4, 134.4, 130.2, 125.4, 122.9, 120.8, 103.8, 98.6, 55.8, 55.5. MS (ESI) *m/z* $[M+Na]^+$ 358.13. HRMS (EI) *m/z* Calculated for $C_{15}H_{14}BrNO_3$ $[M]^+$ 335.0157; found 335.0154.

*4.1.15.3. N-(2,4-Dihydroxyphenyl)-3-nitrobenzamide (**35**)*

The amide **33** (3 mmol) was dissolved in 10 mL of dry DCM in a two-necked R.B flask. BBr_3 (8 mmol) was added carefully under ice-cold condition. The reaction was allowed to stir at room temperature for 30 minutes. Ice crystal was added to the reaction mixture followed by few drops of methanol under ice-cold condition. The precipitate obtained was filtered and dried to get **35** as **y**ellow solid (62%). Mp 219-220 °C;^{1}H NMR (CD_3OD, 300 MHz) δ 8.81 (s, 1H), 8.45 (d, *J* = 9.0 Hz, 1H), 8.35 (d, *J* = 9.0 Hz, 1H), 7.79 (t, *J* = 7.5 Hz, 1H), 7.39 (d, *J* = 9.0 Hz, 1H), 6.44 (d, *J* = 3.0 Hz, 1H), 6.35 (dd, *J* = 9.0 Hz, 3.0 Hz, 1H); ^{13}C NMR (CD_3OD, 150Hz) δ 164.9, 156.5, 151.3, 148.3, 136.2, 133.1, 129.7, 125.7, 125.5, 122.3, 116.9, 106.2, 102.9. MS (ESI) m/z $[M+Na]^+$ 297.23. HRMS (EI) Calculated for $C_{13}H_{10}N_2O_4$ $[M]^+$ 274.0590; found 274.0588.

*4.1.15.4. 3-Bromo-N-(2,4-dihydroxyphenyl)benzamide (**36**)*

The amide **34** (3 mmol) was dissolved in 10 mL of dry DCM in a two-necked R.B flask. BBr_3 (8 mmol) was added carefully under ice-cold condition. The reaction was allowed to stir at room temperature for 30 minutes. Crushed ice was added to the reaction mixture followed by few drops of methanol under ice-cold condition. The precipitate obtained was filtered and dried to get **36** as light pink solid (58%). Mp 235 °C.^{1}H NMR (CD_3OD, 300 MHz) δ 8.13 (s, 1H), 7.93 (d, *J* = 9.0 Hz, 1H), 7.76 (d, *J* = 9.0 Hz, 1H), 7.46 (t, *J* = 7.5 Hz, 1H), 7.38 (d, *J* = 9.0 Hz, 1H), 6.43 (d, *J* = 3.0 Hz, 1H), 6.35 (dd, *J* = 9.0 Hz, 3.0 Hz, 1H); ^{13}C NMR (CD_3OD, 75 MHz) δ 167.9, 158,4, 153.1, 139.8, 138.7, 136.6, 132.4, 130.8, 130.1, 128.1, 127.2, 124.5, 119.5, 108.5, 105.4. MS ESI *m/z* $[M+Na]^+$ 332.26. HRMS (EI) *m/z* Calculated for $C_{13}H_{10}BrNO_3$ $[M]^+$ 306.9844; found 306.9842.

*4.1.15.5. 2-(3-Nitrophenyl)benzo[d]oxazol-6-ol (**37**)*

Acetic acid (1 mmol) was added to **35** (1 mmol) and the mixture was refluxed for 24 hours. The acid was evaporated and the reaction mixture was diluted with water and organic part was extracted using ethyl acetate. Combined organic layer was dried over sodium sulfate and concentrated. Flash chromatography was done using ethyl acetate-hexane system to get the pure product **37** as yellow solid (64%). Mp 251-553 °C; ^{1}H NMR (CD_3OD, 300 MHz) δ 8.97 (t, *J* = 3.0 Hz, 1H), 8.55 (d, *J* = 9.0 Hz, 1H), 8.42 (dd, *J* = 9.0 Hz, 3.0 Hz, 1H), 7.84 (t, *J* = 7.5 Hz, 1H),

7.59 (d, *J* = 9.0 Hz, 1H), 7.11 (d, *J* = 3.0 Hz, 1H), 6.93 (dd, *J* = 9.0 Hz, 3.0 Hz, 1H); ^{13}C NMR (CD_3OD, 75 MHz) δ 167.5, 160.8, 158.5, 153.3, 149.8, 136.5, 134.1, 131.2, 128.3, 125.4, 122.6, 121.2, 115.3, 98.3. MS (ESI) *m/z* $[M+Na]^+$ 279.23. HRMS (EI) *m/z* Calculated for $C_{13}H_8N_2O_4$ $[M]^+$ 256.0484; found 256.0474.

*4.1.15.6. 2-(3-Bromophenyl)benzo[d]oxazol-6-ol (**38**)*

Acetic acid (1 mmol) was added to the compound **36** (1 mmol) and the mixture was refluxed for 48 hours. The acid was evaporated and the reaction mixture was diluted with water and organic part was extracted using ethyl acetate. Combined organic layer was dried over sodium sulfate and concentrated. Flash chromatography was done using ethyl acetate-hexane system to get the pure product **38** as off white crystal (67%): mp 268-269 °C; ^{1}H NMR (CD_3OD, 300 MHz) δ 8.32 (s, 1H), 8.15 (d, *J* = 9.0 Hz, 1H), 7.73 (d, *J* = 6.0 Hz, 1H), 7.56-7.47 (m, 2H), 7.08 (d, *J* = 3.0 Hz, 1H), 6.91 (dd, *J* = 9.0 Hz, 3 Hz, 1H); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 159.7, 156.5, 151.8, 134.9, 133.7, 131.0, 129.5, 129.3, 125.6, 122.4, 120.2, 113.6, 97.11. MS (ESI) *m/z* $[M+H]^+$ 290.06. HRMS (EI) *m/z* Calculated for $C_{13}H_8BrNO_2$ $[M]^+$ 288.9738; found 288.9737.

*4.1.15.7. 6-(3-Chloropropoxy)-2-(3-nitrophenyl)benzo[d]oxazole (**39**)*

37 (1 mmol) was dissolved in dry DMF and K_2CO_3 (6mmol) was added under nitrogen. The reaction mixture was allowed to stir for 15 minutes. Thereafter, 1-bromo 3-chloro propane (3 mmol) was introduced and reaction mixture stirred for 10 hours at room temperature. The reaction mixture was diluted with excess amount of water and organic layer was extracted using ethyl acetate. Combined organic layer was dried over sodium sulfate and concentrated under vacuum. Flash column chromatography was done in ethyl acetate-hexane system to get the purified product **39** as yellow solid (66%). Mp 113-115 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.06 (t, *J* = 3.0 Hz, 1H), 8.55 (d, *J* = 9.0 Hz, 1H), 8.38 (dd, *J* = 9.0 Hz, 3.0 Hz, 1H), 7.76-7.68 (m, 2H), 7.18 (d, *J* = 3.0 Hz, 1H), 7.03 (dd, *J* = 9.0 Hz, 3.0 Hz, 1H), 4.23 (t, *J* = 6.0 Hz, 2H), 3.82 (t, *J* = 7.5 Hz, 2H), 2.36-2.28 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.8, 157.9, 151.7, 148.6, 135.7, 132.5, 130.0, 128.9, 125.3, 121.9, 120.5, 113.9, 96.1, 65.1, 41.4, 32.1. MS (ESI) *m/z* $[M+Na]^+$ 355.28. HRMS (ESI) *m/z* Calculated for $C_{16}H_{13}ClN_2O_4$ $[M+Na]^+$ 355.0462; found 355.0451.

*4.1.15.8. 2-(3-Bromophenyl)-6-(3-chloropropoxy)benzo[d]oxazole (**40**)*

38 (1 mmol) was dissolved in dry DMF and K_2CO_3 (6 mmol) was added under nitrogen. The reaction mixture was allowed to stir for 15 minutes. Thereafter, 1-bromo 3-chloro propane (3 mmol) was introduced and reaction mixture stirred for 10 hours at room temperature. The reaction mixture was diluted with excess amount of water and organic layer was extracted using ethyl acetate. Combined organic layer was dried over sodium sulfate and concentrated under vacuum. Flash column chromatography was done in ethyl acetate-hexane system to get the purified product **40** as white crystal (69%). Mp 61-63 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.35 (t, *J* = 3.0 Hz, 1H), 8.12 (d, *J* = 9.0 Hz, 1H), 7.65-7.61 (m, 2H), 7.38 (t, *J* = 7.5 Hz, 1H), 7.12 (d, *J* = 3.0 Hz, 1H), 6.97 (dd, *J* = 9.0 Hz, 3 Hz, 1H), 4.18 (t, *J* = 6.0 Hz, 2H), 3.79 (t, *J* = 6.0 Hz, 2H), 2.42-2.25 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 160.7, 157.6, 151.6, 135.8, 133.9, 130.4, 130.0, 129.1, 125.6, 122.9, 120.2, 113.6, 96.1, 65.0, 41.5, 32.2. MS (ESI) *m/z* $[M+H]^+$ 366.09. HRMS (ESI) *m/z* Calculated for $C_{16}H_{13}BrClNO_2$ $[M+H]^+$ 365.9898; found 365.9898.

*4.1.15.9. 2-(3-Nitrophenyl)-6-(3-(pyrrolidin-1-yl)propoxy)benzo[d]oxazole (**41**)*

39 (1 mmol) and pyrrolidine (6 mmol) was dissolved in dry DMF in a sealed tube under nitrogen atmosphere. The reaction mixture was heated to 110 °C for 8 hours and diluted with excess amount of water and the organic part was extracted with 5% Methanol/$CHCl_3$ system. Column chromatography was done using $CHCl_3$/Methanol system to get the pure product **41** as **y**ellow solid (67%). Mp 234-235 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.03 (t, *J* = 3.0 Hz, 1H), 8.52 (d, *J* = 6.0 Hz, 1H), 8.36 (dd, *J* = 9.0 Hz, 3 Hz, 1H), 7.74-7.66 (m, 2H), 7.14 (d, *J* = 3.0 Hz, 1H), 6.98 (dd, *J* = 9.0 Hz, 3 Hz, 1H), 4.16 (t, *J* = 6 Hz, 2H), 3.10-3.05 (m, 6H), 2.37-2.29 (m, 2H), 2.05(m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.7, 158.1, 151.7, 148.6, 132.5, 130.0, 129.0, 125.3, 121.9, 120.4, 113.9, 96.0, 73.9, 66.7, 54.1, 53.0, 27.9, 23.4. MS (ESI) *m/z* $[M+H]^+$ 368.27. HRMS (ESI) *m/z* Calculated for $C_{20}H_{21}N_3O_4$ $[M+H]^+$ 368.1612; found 368.1609.

*4.1.15.10. 2-(3-Bromophenyl)-6-(3-(pyrrolidin-1-yl)propoxy)benzo[d]oxazole (**42**)*

40 (1 mmol) and pyrrolidine (6 mmol) was dissolved in dry DMF in a sealed tube under nitrogen atmosphere. The reaction mixture was heated to 110 °C for 8 hours and diluted with excess amount of water and the organic part was extracted with 5% Methanol/$CHCl_3$ system. Column chromatography was done using $CHCl_3$/Methanol system to get the pure product **42** as light

brown solid (71%). Mp 77-78 °C; ^{1}H NMR (CD_3OD, 300 MHz) δ 8.33-8.32 (m, 1H), 8.16 (d, *J* = 6.0 Hz, 1H), 7.75 (d, *J* = 9.0 Hz, 1H), 7.64 (d, *J* = 9.0 Hz, 1H), 7.51 (t, *J* = 7.5 Hz, 1H), 7.31 (d, *J* = 3.0 Hz, 1H), 7.04 (dd, *J* = 9.0 Hz, 3 Hz, 1H), 4.15 (t, *J* = 6.0 Hz, 2H), 2.84-2.72 (m, 6H), 2.15-2.06 (m, 2H), 1.91-1.88 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 160.6, 157.9, 151.6, 135.6, 133.8, 130.4, 130.0, 129.2, 125.6, 122.9, 120.1, 113.6, 96.1, 67.1, 54.2, 53.1, 28.5, 23.4. MS ESI *m/z* $[M+H]^+$ 400.89. HRMS (ESI) *m/z* Calculated for $C_{20}H_{21}BrN_2O_2$ $[M+H]^+$ 401.0866; found 401.0864.

*4.1.15.11. 3-(6-(3-(Pyrrolidin-1-yl)propoxy)benzo[d]oxazole-2-yl)aniline (**43**)*

Pd/C was added to a solution of compound **41** in methanol. The reaction mixture was allowed to stir at room temperature under hydrogen atmosphere for 2 hours. After completion of reaction, the reaction mixture was filtered through celite bed using methanol. Flash column chromatography was done using $CHCl_3$/Methanol system to get the pure product **43** as yellow solid (63%). Mp 90-92 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.60 (t, *J* = 7.5 Hz, 2H), 7.53 (m, 1H), 7.30(d, *J* = 6.0 Hz, 1H), 7.09 (d, *J* = 3.0 Hz, 1H), 6.93 (dd, *J* = 9.0 Hz, 3 Hz, 1H), 6.82 (dd, *J* = 9.0 Hz, 3 Hz, 1H), 4.11 (t, *J* = 6.0 Hz, 2H), 2.92-2.84 (m, 6H), 2.27-2.16 (m, 2H), 1.94 (m, 4H); ^{13}C NMR (CD_3OD, 75 MHz) δ 164.4, 159.2, 152.8, 150.1, 136.5, 130.9, 128.8, 120.5, 119.4, 117.6, 114.8, 114.3, 97.2, 67.9, 55.2, 54.2, 29.1, 24.3. MS (ESI) *m/z* $[M+H]^+$ 338.36. HRMS (ESI) *m/z* Calculated for $C_{20}H_{23}N_3O_2$ $[M+ H]^+$ 338.1870; found 338.1868.

*4.1.16. General Procedure for the Synthesis of **44**, **45**, **46** and **47***

The acid (2 mmol) and HATU (1.5 mmol) were dissolved in dry DMF and DIPEA (4 mmol) was added it. After 15 minutes respective amine (1 mmol) was added and the reaction mixture stirred at room temperature for 4-5 hours. The organic part was extracted with 5% Methanol/$CHCl_3$ system. Column chromatography was done using $CHCl_3$/Methanol system to get the pure product.

*4.1.16.1. N-(3-(6-(3-(Pyrrolidin-1-yl)propoxy)benzo[d]oxazol-2-yl)phenyl)benzamide (**44**)*

Yellow gummy semisolid (72%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.63 (s, 1H), 8.01-7.96 (m, 3H), 7.89 (d, *J* = 9.0 Hz, 1H), 7.65-7.53 (m, 5H), 7.31 (s, 1H), 7.05 (d, *J* = 3.0 Hz, 1H), 4.19 (t, *J* = 6.0 Hz, 2H), 3.28 (m, 6H), 2.28-2.20 (m, 2H), 2.10-2.06 (m, 4H); ^{13}C NMR (CD_3OD, 75 MHz)

δ 168.8, 163.4, 159.2, 152.8, 140.9, 136.5, 136.1, 133.2, 130.7, 129.8, 128.8, 124.8, 124.0, 120.7, 120.4, 114.8, 97.1, 67.5, 55.2, 54.1, 28.6, 24.2. MS (ESI) *m/z* $[M+H]^+$ 442.33. HRMS (ESI) *m/z* Calculated for $C_{27}H_{27}N_3O_3$ $[M+H]^+$ 442.2132; found 442.2136. HPLC Purity 98.25%

*4.1.16.2. Tert-butyl(4-((3-(6-(3-(pyrrolidin-1-yl)propoxy)benzo[d]oxazol-2-yl)phenyl)carbamoyl)-phenyl)carbamate (**45**)*

Yellow gummy solid (72%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.63 (s, 1H), 7.98-7.87 (m, 4H), 7.66-7.57 (m, 5H), 7.33 (s, 1H), 7.06 (d, *J* = 9.0 Hz, 1H), 4.20 (t, *J* = 4.5 Hz, 2H), 3.23-3.15 (m, 6H), 2.17 (m, 2H), 2.04 (m, 4H), 1.56 (s, 9H); ^{13}C NMR (CD_3OD, 75 MHz) δ 168.4, 163.6, 159.1, 154.9, 152.9, 144.7, 141.1, 136.7, 130.7, 129.9, 129.3, 128.7, 124.9, 123.9, 120.8, 120.5, 118.9, 114.9, 97.3, 81.5, 67.3, 55.4, 54.1, 28.8, 28.1, 24.2. MS (ESI) *m/z* $[M+H]^+$ 557.44. HRMS (ESI) *m/z* Calculated for $C_{32}H_{36}N_4O_5$ $[M+Na]^+$ 579.2584; found 579. 2584.

*4.1.16.3. 4-Amino-N-(3-(6-(3-(pyrrolidin-1-yl)propoxy)benzo[d]oxazol-2-yl)phenylbenzamide (**46**)*

45 (1 mmol) was dissolved in dry DCM and trifluoroacetic acid (1 mL) was added to the solution under ice-cold condition and allowed to stir at room temperature for 2 hours. The solution was neutralized with 1N NaOH solution keeping it under ice-cold condition. The organic part was extracted with 10% Methanol/$CHCl_3$ system. Flash column chromatography was done using $CHCl_3$/Methanol/NH_3 system to get the pure product **46** as yellow solid (71%). mp 191-193 °C; ^{1}H NMR (CD_3OD, 300 MHz) δ 8.53 (s, 1H), 7.92-7.78 (m, 5H), 7.60-7.51 (m, 2H), 7.23 (d, *J* = 3.0 Hz, 1H), 6.99 (dd, *J* = 9.0 Hz, 3Hz, 1H), 6.75 (d, *J* = 9.0 Hz, 2H), 4.10 (t, *J* = 6.0 Hz, 2H), 3.01-2.92 (m, 6Hz), 2.17-2.07 (m, 2H), 1.95 (m, 4H); ^{13}C NMR (CD_3OD, 75 MHz) δ 169.2, 163.7, 159.6, 153.9, 153.1, 141.4, 136.5, 130.7, 128.7, 125.1, 123.7, 123.2, 120.6, 115.1, 114.8, 97.2, 68.2, 55.2, 54.4, 29.6, 24.3. MS (ESI) *m/z* $[M+H]^+$ 457.29. HRMS (EI)*m/z* Calculated for $C_{27}H_{28}N_4O_3[M]^+$ 456.2161; found 456.21616. Anal. ($C_{28}H_{30}N_4O_3 \cdot 2.6H_2O$) calcd: C, 65.00; H, 6.86; N, 10.83. Found: C, 65.23; H, 7.09; N, 10.78.

*4.1.16.4. Tert-butyl(4-((3-(6-(3-(pyrrolidin-1-yl)propoxy)benzo[d]oxazol-2-yl)phenyl) carbamoyl)-benzylcarbamate (**47**)*

Yellow gummy semisolid (75%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.52 (br. s, 1H), 8.41 (s, 1H), 7.96-7.93 (m, 2H), 7.87 (d, *J* = 6.0 Hz, 2H), 7.59 (d, *J* = 6.0 Hz, 1H), 7.48 (t, *J* = 9.0 Hz, 1H), 7.34 (d, *J* = 6.0 Hz, 2H), 7.05 (d, *J* = 6.0 Hz, 1H), 6.93 (dd, *J* = 12.0 Hz, 6.0 Hz, 1H), 4.36 (d, *J* = 6.0 Hz, 2H), 4.07 (t, *J* = 6.0 Hz, 2H), 2.79 (t, *J* = 6.0 Hz, 2H), 2.71 (m, 4H), 2.13-2.10 (m ,4H), 1.89-1.87 (m, 4H), 1.49 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 165.7, 161.8, 157.6, 156.1, 151.6, 143.4, 138.8, 135.7, 133.5, 129.7, 127.9, 127.5, 123.1, 119.9, 118.8, 113.4, 96.1, 79.8, 66.9, 54.2, 53.1, 44.2, 28.4, 28.2, 23.4. MS (ESI) *m/z* $[M+H]^+$ 571.52. HRMS (ESI) *m/z* Calculated for $C_{33}H_{38}N_4O_5[M+H]^+$ 571.2922; found 571.2927.

*4.1.16.5. 4-(Aminomethyl)-N-(3-6-(3-(pyrrolidin-1-yl)propoxy)benzo[d]oxazol-2-yl)phenyl)-benzamide (**48**)*

47 (1 mmol) was dissolved in dry DCM and trifluoroacetic acid (1 mL) was added to the solution under ice-cold condition and allowed to stir at room temperature for 2 hours. The solution was neutralized with 1N NaOH solution keeping it under ice-cold condition. The organic part was extracted with 10% Methanol/$CHCl_3$ system. Flash column chromatography was done using $CHCl_3$/Methanol/NH_3 system to get the pure product **48** as yellow solid (67%). Mp 178-179 °C; ^{1}H NMR (CD_3OD, 300MHz) δ 8.60 (s, 1H), 8.01-7.92 (m, 4H), 7.64-7.53 (m, 4H), 7.30 (s, 1H), 7.04 (d, *J* = 4.5 Hz, 1H), 4.14 (t, *J* = 6.0 Hz, 2H), 3.95 (s, 2H), 2.82-2.71 (m, 6H), 2.14-2.05 (m, 2H), 1.90-1.88 (m, 4H); ^{13}C NMR (CD_3OD, 150 MHz) δ 167.4, 162.1, 158.1, 151.5, 146.8, 139.6, 134.9, 133.1, 129.3, 127.6, 127.2, 123.6, 122.6, 119.3, 113.6, 95.7, 66.8, 53.7, 52.9, 44.9, 28.1, 22.8. MS (ESI) *m/z* $[M+Na]^+$ 493.25. HRMS (EI) *m/z* Calculated for $C_{28}H_{30}N_4O_3$ 470.2318; found 470.2323. HPLC Purity 98.3%.

*4.1.16.6. 4-((Dimethylamino)methyl)-N-(3-(6-(3-(pyrrolidin-1-yl)propoxy)benzo[d]oxazol-2-yl)phenyl)benzamide (**49**)*

Yellow solid (75%). Mp 138-140 °C; ^{1}H NMR (CD_3OD, 300 MHz) δ 8.63 (s, 1H), 8.04-7.90 (m, 5H), 7.64-7.55 (m, 4H), 7.31 (s, 1H), 7.04 (d, *J* = 9.0 Hz, 1H), 4.20 (t, *J* = 6.0 Hz, 2H), 3.89 (s, 2H), 3.40 (m, 6H), 2.51 (s, 6H), 2.32-2.23 (m, 2H), 2.12 (m, 4H); ^{13}C NMR (CD_3OD, 75 MHz) δ 168.6, 163.4, 159.5, 152.9, 143.4, 141.0, 136.5, 135.3, 130.9, 128.9, 124.9, 124.0, 120.8, 120.5, 115.0, 97.2, 68.1, 64.6, 55.2, 54.4, 45.5, 29.6, 24.3. MS (ESI) *m/z* $[M+H]^+$ 499.31. HRMS (EI) *m/z* Calculated for $C_{30}H_{34}N_4O_3$ $[M]^+$ 498.2631; found 498.2636. HPLC Purity 99.2%.

*4.1.16.7. Tert-butyl4-{3-{6-{3-{pyrrolidin-1-yl)propoxy)benzo[d]oxazol-2-yl)phenyl)-3,6-dihydropyridine-1(2H)-carboxylate (**50**)*

42 (0.12 mmol), *N*-Boc-1,2,5,6-tetrahydropyridine-4-boronic acid pinacol ester (0.24 mmol), CS_2CO_3 (0.30 mmol) were taken in DMF:water (10:1) mixture and $Pd(PPh_3)_4$ (0.0084 mmol) was added to the reaction mixture under a N_2 atmosphere. The reaction mixture was heated to 110 °C under microwave condition for 1 hour. After completion, the organic part was extracted with 5% Methanol/$CHCl_3$ system. Flash column chromatography was done using $CHCl_3$/Methanol system to get the pure product **50** as yellow solid (77%). Mp 71-72 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.20 (s, 1H), 8.07 (d, *J* = 6.0 Hz, 1H), 7.62 (d, *J* = 12.0 Hz, 1H), 7.51-7.45 (m, 2H), 7.10 (d, *J* = 6.0 Hz, 1H), 6.92 (dd, *J* = 12.0 Hz, 6 Hz, 1H), 6.17 (s, 1H), 4.11-4.09 (m, 4H), 3.67 (m, 2H), 2.98-2.92 (m, 6H), 2.60 (s, 2H), 2.24-2.19 (m, 2H), 1.96 (m, 4H), 1.50 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 162.3, 157.3, 151.6, 141.4, 135.9, 129.0, 127.6, 127.4, 125.9, 123.7, 120.0, 113.3, 96.2, 79.8, 66.5, 54.0, 53.1, 50.5, 28.5, 27,4, 23.4. (ESI) *m/z* $[M+H]^+$ 504.35. HRMS (ESI) *m/z* Calculated for $C_{30}H_{37}N_3O_4$ $[M+H]^+$ 504.2864; found 504.2867.

*4.1.16.8. 6-(3-(pyrrolidin-1-yl)propoxy)-2-(3-(1,2,3,6-tetrahydropyridin-4-yl)phenyl)benzo [d]-oxazole (**51**)*

50 (1 mmol) was dissolved in dry DCM and trifluoroacetic acid (1 mL) was added to the solution under ice-cold condition and allowed to stir at room temperature for 2 hours. The solution was neutralized with 1N NaOH solution keeping it under ice-cold condition. The organic part was extracted with 10% Methanol/$CHCl_3$ system. Flash column chromatography was done using $CHCl_3$/Methanol/NH_3 system to get the pure product **51** as yellow solid (65%). Mp 50-51 °C; ^{1}H NMR (CD_3OD, 300 MHz) δ ppm 8.24 (s, 1H), 8.07 (d, *J* = 9.0 Hz, 1H), 7.67-7.51 (m, 3H), 7.30 (s, 1H), 7.03 (dd, *J* = 9.0 Hz, 3 Hz, 1H), 6.34 (s, 1H), 4.14 (t, *J* = 6.0 Hz, 2H), 3.53 (s, 2H), 3.11 (t, *J* = 6 Hz, 2H), 2.73 (t, *J* = 7.5 Hz, 2H), 2.62 (m, 6H), 2.12-2.03 (m, 2H), 1.86 (m, 4H); ^{13}C NMR (CD_3OD, 75 MHz) δ 163.8, 159.6, 153.0, 143.4, 136.5, 135.9, 130.4, 129.1, 128.3, 126.9, 125.1, 124.5, 120.7, 115.0, 97.2, 68.2, 55.2, 54.4, 45.9, 43.8, 29.5, 27.9, 24.4. (ESI) *m/z* $[M+H]^+$ 404.17. HRMS (ESI) *m/z* Calculated for $C_{25}H_{29}N_3O_2$ $[M+H]^+$ 404.2340; found 404.2353. HPLC Purity 97.3%.

*4.1.16.9. 2-(3-(piperidin-4-yl)phenyl)-6-(3-(pyrrolidin-1-yl)propoxy)benzo[d]oxazole (**52**)*

51 was dissolved in methanol and Pd/C was added to it after degassing the solution and allowed to stir at room temperature under hydrogen balloon pressure for 2 hours. After completion of reaction, the reaction mixture was filtered through celite bed using methanol. Column Chromatography was done using $CHCl_3$ /Methanol system to get the pure product **52** as yellow solid (60%). M.P 72-74 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.07 (s, 1H), 8.02 (d, *J* = 6 Hz, 1H), 7.62 (d, *J* = 9 Hz, 1H), 7.46-7.35 (m, 2H), 7.10 (s, 1H), 6.95 (d, *J* = 9Hz, 1H), 4.09 (t, *J* = 6 Hz, 2H), 2.67 (t, *J* = 6 Hz, 4H), 2.56 (m, 5H), 2.16 (s, 1H), 2.10-2.01 (m, 4H), 1.80 (m, 7H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 162.2, 157.6, 151.2, 146.2, 135.8, 129.4, 129.2, 127.6, 125.7, 125.4, 119.9, 113.3, 96.1, 67.1, 54.2, 53.1, 46.3, 45.5, 41.9, 29.7, 28.5, 23.5. HRMS (ESI) *m/z* Calculated for $C_{25}H_{31}N_3O_2$ $[M+H]^+$ 406.2496; found 406.2495. HPLC Purity 96.6%.

*4.1.17. General Procedure for the Synthesis of **54**, **55** and **56***

42 (0.2 mmol), Na^{t}OBu (0.8 mmol), BINAP (0.1 mmol), $Pd_2(dba)_3$ (0.1 mmol) were dissolved in toluene and respective amines (0.2 mmol) was added. The reaction mixture was degassed with argon refluxed for 8-10 hours. After completion, toluene was evaporated and the organic part was extracted with 5% Methanol/$CHCl_3$ system. Flash column chromatography was done using $CHCl_3$/Methanol system to get the pure product.

*4.1.17.1. N-(3-(6-(3-(pyrrolidin-1-yl)propoxy)benzo[d]oxazol-2-yl)phenyl)piperidin-4-amine (**54**)*

42 (0.075 mmol), Na^{t}OBu (0.075 mmol), BINAP (0.0375 mmol), $Pd_2(dba)_3$ (0.0375 mmol) were dissolved in toluene and then *tert*-butyl 4-aminopiperidine-1-carboxylate (0.075 mmol) was added to the reaction mixture and refluxed for 8-10 hours. After completion, toluene was evaporated the organic part was extracted with 5% Methanol/$CHCl_3$ system. Column chromatography was done using $CHCl_3$ /Methanol system to get the compound **53**. To compound **53** (0.5 mmol) was dissolved in dry CH_2Cl_2 and 0.25 mL trifluoro acetic acid was added to the solution under ice-cold condition and allowed to stir at room temperature for 2 hours. The solution was neutralized with 1N NaOH solution keeping it under ice-cold condition. The organic part was extracted with 10% Methanol/$CHCl_3$ system. Flash column chromatography

was done using $CHCl_3$/Methanol/NH_3 system to get the pure product **54** as yellow solid (67%). M.P- 71-72 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.56 (d, *J* = 9 Hz, 1H), 7.39 (d, *J* = 9 Hz, 2H), 7.29-7.21 (m, 2H), 6.98 (d, *J* = 9 Hz, 1H), 6.84 (d, *J* = 9 Hz, 1H), 4.07 (t, *J* = 4.5 Hz, 2H), 3.12 (d, *J* = 12 Hz, 2H), 2.80-2.59 (m, 8H), 2.11-2.00 (m, 5H), 1.82 (m, 4H), 1.50-1.38 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 162.7, 157.6, 151.6, 147.3, 135.8, 129.8, 128.2, 119.8, 116.3, 113.3, 111.1, 96.1, 67.2, 54.3, 53.2, 45.4, 33.7, 28.7, 23.5. HRMS (ESI) *m/z* Calculated for $C_{25}H_{32}N_4O_2$ $[M+H]^+$ 421.2605; found 421.2609. HPLC Purity 99.2%.

*4.1.17.2. 1-Benzyl-N-(3-6-(3-(pyrrolidin-1-yl)propoxy)benzo[d]oxazol-2-yl)phenyl)piperidine-4-amine (**55**)*

Yellow solid (71%). Mp 98-99 °C; ^{1}H NMR (CD_3OD, 600 MHz) δ 7.59 (d, *J* = 6.0 Hz, 1H), 7.41 (d, *J* = 6.0 Hz, 2H), 7.36-7.33 (m, 5H), 7.31-7.26 (m, 3H), 7.02 (d, *J* = 6.0 Hz, 1H), 6.84(d, *J* = 6.0 Hz, 1H), 4.13 (d, *J* = 6.0 Hz, 2H), 3.60 (s, 2H), 3.05-2.94 (m, 8H), 2.28 (t, *J* = 12.0 Hz, 2H), 2.17-2.15 (m, 2H), 2.07 (d, *J* = 12.0 Hz, 2H), 1.97 (m, 4H), 1.59-1.54 (m, 2H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 163.3, 157.6, 151.4, 148.4, 136.6, 135.1, 129.5, 127.9, 127.2, 119.0, 116.5, 114.9, 113.3, 110.2, 95.9, 66.1, 62.6, 53.8, 52.6, 31.1, 27.0, 22.7. (ESI) *m/z* $[M+H]^+$511.31. HRMS (ESI) *m/z* Calculated for $C_{32}H_{38}N_4O_2$ $[M+H]^+$ 511.3074; found 511.3078. HPLC Purity 98.5%.

*4.1.17.1. 1-Methyl-N-(3-6-(3-(pyrrolidin-1-yl)propoxy)benzo[d]oxazol-2-yl)phenyl) piperidine-4-amine (**56**)*

Yellow solid (66%). Mp 75-76 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.61 (d, *J* = 6.0 Hz, 1H), 7.51 (d, *J* = 9.0 Hz, 1H), 7.42 (s, 1H), 7.31-7.28 (m, 1H), 7.11 (d, *J* = 3.0 Hz, 1H), 6.95 (dd, *J* = 6.0 Hz, 3 Hz, 1H), 6.74 (d, *J* = 3.0 Hz, 1H), 4.09 (t, *J* = 6.0 Hz, 2H), 2.83 (d, *J* = 9.0 Hz, 2H), 2.68 (t, *J* = 7.5 Hz, 2H), 2.56 (m, 4H), 2.32 (s, 3H), 2.21-2.06 (m, 9H), 1.82-1.80 (m, 4H), 1.55-1.51 (m, 2H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 162.7, 157.6, 151.6, 147.5, 140.5, 135.8, 129.8, 128.2, 119.8, 118.9, 116.3, 116.2, 113.3, 111.1, 96.1, 67.2, 54.3, 53.1, 46.3, 32.5, 28.7, 23.5. (ESI) *m/z* $[M+H]^+$ 435.53. HRMS (ESI)*m/z* Calculated for $C_{26}H_{34}N_4O_2$ $[M+H]^+$435.2762; found 435.2760. HPLC Purity 99.1%.

4.2 Homology Model

Homology model of TLR 9 (Homo sapiens, genbank: AAZ95520) is prepared in *Discovery Studio Suite 4.0*. Ecto domain sequence of hTLR was subject to NCBI Blast. Best template was chosen based on e-value and sequence identity. Crystal structure of bovine TLR9 serve as a template which has sequence similarity of 87% and sequence identity is 81.8 % with our query sequence. The validation of the homology model of TLR 9 is done by NHI MBI Laboratory server followed by loop modification using Loop Modeler Server. Verification of the model was done by modeller verify-3D. Ramchandran plot, verify 3D results and status of our homology model are described in supplemental Information section.

42.1 Molecular Docking Study

The docking experiments for all the compounds were conducted in LigandFit and C-DOCKER in Discovery Studio 4.0. In docking experiment random conformer was searched using dynamics of 1000 step. In simulation step heating was done up to 700 K using 2000 step and it was cool down to 300 K using 5000 steps. The docked results were fully minimized using CHARM (C-DOCKER and LigandFit) force field with conjugate gradient 1.0. The maximum distance between the heavy atoms participating in the hydrogen bonds was set at 3.7 Å.

4.3. Biological Activity

4.3.1. Reporter Assays

The synthesized small molecules were screened for TLR9 or TLR7 antagonism using a HEK-Blue cell line expressing human TLR9 or TLR7 respectively and reporting the TLR-activation induced NFκB activation by inducing release of a secreted Alkaline Phosphatase (SEAP), which could be detected using a colorimetry of the supernatants. Cells were seeded in 96-well plate at a density of 70,000 cells/well at 37 ºC and 5% CO_2 in complete DMEM medium supplemented with 100µl/ml Normocin (Invivogen, USA) and incubated for 6 hours so as to allow adherence of the cells. After incubation, the TLR9 agonist CpGB (1µM) or TLR7 agonist CL264 (5µg/ml) was added to the cells in presence of escalating doses of the synthesized small

molecules and incubated at 37 ºC and 5% CO_2 for 24 hours. After incubation of the HEK cells, supernatants were collected and 20µl of supernatant was added to wells containing 200µl of Quanti-Blue Detection media. After 2 hours of further incubation, OD values were taken at 655nm in a spectrophotometer. All the reporter cell lines and TLR ligands were from Invivogen, USA.

4.3.3. TLR9 antagonism in primary human blood mononuclear cells

We established that IFN-alpha production from human peripheral blood mononuclear cells (PBMC) in response to type A CpG oligonucleotides (CpGA) almost exclusively results from TLR9 triggering on the PDCs (data not shown). For the screening assay where we isolated PBMCs from venous blood collected from healthy volunteers using density gradient centrifugation. These experiments were approved by institutional review boards and blood was collected by trained phlebotomist in aseptic manner after taking proper informed consent from the healthy volunteers. We cultured the PBMCs at 2-3*10^5 cells/200ul/well in a 96 well plate. We added the TLR9 agonist CpGA at 1uM in presence of escalating doses of the synthesized small molecules (0uM, 0.1uM, 1uM, 5uM, 10uM and 20uM). After 18 hours we collected the supernatants from the culture wells and looked for IFN-alpha using enzyme linked immunosorbent assay (ELISA) using a kit following manufacturer's instructions (Mabtech, Sweden).

4.3.4. TLR9 antagonism in primary human plasmacytoid dendritic cells

Primary Human lasmcytoid dendritic cells (pDCs) were isolated from PBMCs by magnetic immune selection using anti-BDCA4 microbeads following manufacturer's instructions (MiltenyiBiotec, Germany). The isolated pDCs were then cultured at 3*10^4 cells/100µl/well in a 96 well plate. We added the TLR9 agonist CpGA at 500nM in presence of escalating doses of the synthesized small molecules. After 18 hours we collected the supernatants from the culture wells and looked for IFN-alpha using ELISA following manufacturer's instructions.

4.3.5. Cytotoxicity Assessment

Cytotoxicity of the synthesized molecules were assessed using a tetrazolium dye reduction based assay (MTT) on HepG2 (a hepatic epithelial cell line) and HEK293 (a renal epithelial cell line) cells. HepG2 and HEK293 cells were cultured in DMEM Complete media in 96 well plates at density of 30,000 cells per well, making a final volume of 100 µl/well. Treatment with different concentrations (0.1, 0.5, 1, 10, 20 and 100 µM) of different candidate small molecule antagonists was added. Plates were incubated for 24 hours at 37 °C and 5% CO_2 in incubator. After 24 h 50µl of MTT (5mg/ml) was added to each well and further incubated for 1 to 4 hours at 37 °C. Then 100µl of DMSO was added to each well and properly mixed to ensure complete solubilization of formazan crystals. Then absorbance was measured at 570 nm using an ELISA plate reader. None of the identified TLR9 antagonists showed considerable cytotoxicity at concentrations below 100µM on this assay (Figure S4).

Author Contribution

Ψ S.R and A.M contributed equally.

Conceptualization: D.G. and A.T.; methodology: D.G. and A.T.; synthesis of probe: Swarnali, R. and B.P.; computational analysis: A.M. G.M. and S.P.; biological investigation: O.R, Shounak, R. and B.R.; writing, review and editing: D.G. and A.T.; funding acquisition: D.G. and A.T; supervision: D.G. and A.T.

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Authors declare no conflict of interest.

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Highlights

- Development of a structural model for hTLR9 antagonism.
- Validation of TLR9 antagonism and its correlation with probe-receptor interactions.
- Development of potent TLR9 antagonist with excellent selectivity against TLR7.