Chapter 4a

Marine 2-Aminoimidazole, Glycociamidine Alkaloids and Their Synthetic Analogues: New Leads for Drug Development
4.1.1 Introduction:

Nature is the richest source of novel compound classes and leads for drug development. In the pregenomic era, more than 80% of drug substances were natural products or inspired by natural products. Natural products based drug discovery has undergone many changes in past 30 years, with noticeable decline in participation by major pharmaceutical industries by the mid nineties and renaissance in past 5 years due to failure of competing technologies like combinatorial chemistry. Significant number of molecules of marine origin or have synthesized taking inspiration from marine natural products are either in or approaching Phase II/III clinical trials in various diseases, clearly demonstrate the increasing interest in marine natural products as leads for drug development. First marine drug, ziconotide (ω-conotoxin MVIIA), isolated from a tropical marine cone snail, was approved in United States in 2004 for the treatment of chronic pain in spinal chord injury, under trade name Prialt. In October 2007, another marine natural product ET-743 (ecteinasidin-743/Yondelis/trabectedin), the antitumour compound from sea-squirt was approved by European Union for the treatment of soft tissue sarcoma. More than 5300 different products are known from marine sponges and their associated microorganisms, and more than 200 new metabolites from sponges are reported each year. It demonstrates that potential of marine natural products as leads for drug development has recently being realized. In this chapter efforts are made to present an overview on isolation, structure and biological activities of marine 2-aminoimidazole, glycociamidine alkaloids and their synthetic analogues.

4.1.2 Marine 2-aminoimidazole and glycociamidine alkaloids:

4.1.2.1 Nagelamides:

Nagelamides, the dimeric bromopyrrole alkaloids were isolated from Okinawan marine sponge Agelas sp. Nagelamide A (1), G (7), and H (8) inhibited a major serine/threonine-protein phosphatase type 2A enzyme with IC50 value of 48, 13, and 46 μM, respectively. They also exhibited antibacterial activity against Micrococcus luteus (gram positive), Bacillus subtilis (gram positive), Escherichia coli (gram negative). Among these, 1 was most active against both gram positive and gram negative bacteria with MIC values of 2.08, 16.7 and 33.3 μg/mL against M. luteus, B.
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subtilis and E. coli., respectively, while others were less potent with MIC values more than 33.3 μg/mL (Table 1). Nagelamide J (9), is the first dimeric bromopyrrole alkaloid possessing a cyclopentane ring fused to an 2-aminoimidazole ring, was isolated from a sample of Agelas sp. (SS-1077) collected off Unten-Port, Okinawa. It exhibited antimicrobial activity against Staphylococcus aureus and Cryptococcus neoformans with MIC values of 8.35 and 16.7 μg/mL, respectively (Table 1).

Nagelamide K (10) is unique in having piperidinoiminoimidazolone ring with 2-aminoimidazole ring and a taurine unit. Both Nagelamide K (10) and L (11) are antibacterial agents having activity against M. luteus with MIC value of 16.7 μg/mL for each. Nagelamide M (12) and N (13) both exhibited antifungal activity against Aspergillus niger with MIC value of 33.3 μg/mL each (Table 1).

Nagelamide O (14) is a rare alkaloid having perhydrocyclopenta-imidazolo-imidazole carbon skeleton. It showed weak antibacterial activity against B. subtilis, M. luteus, and S. aureus with MIC values of 33.3 μg/mL each. Nagelamide P (15) was inactive both as antifungal and antibacterial agent. Key structural feature of Nagelamide Q (16) is the presence of pyrrolidine ring which separates both 2-aminoimidazole rings.
Alkaloid 16 was found to be active against *B. subtilis*, *Trichophyton mentagrophytes*, *Candida albicans*, *Cryptococcus neoformans*, and *A. niger* with MIC values of 13.0, 6.0, 13.0, 13.0 and 13.0 µg/mL. Nagelamide R (17) also showed antimicrobial activity against *B. subtilis*, *Trichophyton mentagrophytes*, *C. albicans*, and *A. niger* with MIC values of 13.0, 6.0, 13.0 and 13.0 µg/mL, respectively.¹⁰

Table 1. Antibacterial and antifungal activity of Nagelamides

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>Antibacterial and antifungal activity (MIC, µg/mL)</th>
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a = not tested

Agelamide¹¹ (18), a fluorescent alkaloid, was isolated from hydrophilic extract of marine sponge *Agelas nakamurai* as matrix-metalloproteinase 2 inhibitor. Agelamide inhibited 33.3% cell migration *in vitro* using bovine aortic endothelial cells at 5 µg/mL and 65.9% at 25 µg/mL, respectively. 10 µg/mL concentration of 18 inhibited vascular formation from aggregates of vascular progenitor cells in 3D culture using type-1 collagen gel.
4.1.2.2 Tyrokeradines:

Mukai et al. isolated two rare bromotyrosine alkaloids, Tyrokeradine A (19) and B (20) possessing an imidazo-quinolinone moiety, from Okinawan Verongid sponge (SS-301). Tyrokeradine B (20) inhibited the growth of *M. luteus, Staphylococcus aureus, T. mentagrophytes* with same MIC value of 25.0 μg/mL and was more active against *C. neoformans, C. albicans, and A. niger* with MIC values 12.5 μg/mL each. While 19 was less active than 20 as antimicrobial agent, indicating that free amino group at end of aliphatic tail is not favourable for antimicrobial activity.

![Tyrokeradine A (19) and Tyrokeradine B (20)](image)

4.1.2.3 Oroidins:

Oroidin (21) was first isolated from *Agelas oroides* and then from various sponges. It exhibited good antifouling activity and inhibited the larval metamorphosis of barnacle *Balanus amphitrite* with ED₅₀ value of 19 μg/mL but was moderate in antibacterial activity with growth inhibitory zone of 15 mm at 10 μg/disk.

![Oroidin (21), Keramidine (22), Dihydrokeramidine (23), Sventrin (24)](image)

*Z-isomer* of oroidin with methyl at one nitrogen of guanidine part i.e. Keramidine (22), and 9,10-dihydrokeramidine (23) were screened against *M. luteus, B. subtilis, E. coli*, protein phosphatise 2 A, but were found to be inactive. Oroidin (21) was
more potent PfFab1 inhibitor\(^{26}\) (IC\(_{50}\) = 0.30 \(\mu\)g/mL) than E-oroidin’s TFA salt. Oroidin also inhibited the larval metamorphosis of the barnacle *Balanus amphitrite* at ED\(_{50}\) value of 19 \(\mu\)g/mL. It also showed inhibitory zones of 15 mm against *Flavobacterium marinotypicum*\(^{22}\) at 10 \(\mu\)g/disk and non toxic at a concentration of 30 \(\mu\)g/mL. It is a non competitive, non specific antagonist of histamine receptor with pD2 value of 4.02 \(\pm\) 0.11.\(^{21}\)

Bioassay guided fractionation of sponge *Agelas sventres* collected off the coast of North Cat Cay, Bimini, Bahamas by Köck et al. led them to isolation of a new bromopyrrole alkaloid Sventrin (24).\(^{27}\) Like oroidin, sventrin also deterred feeding of the Caribbean reef fish *Thalassoma bifasciatum* in aquarium assays, but was less active than oroidin.

![Dihydrosventrin (25)](image1)

![26](image2)

![27](image3)

Melander et al. in their efforts toward identification of simple oroidin analogues\(^{28}\) having potent antibiofilm activity found dihydrosventrin (DHS) (25) as a very potent inhibitor of *Pseudomonas* biofilm formation with no direct antibiotic effect with IC\(_{50}\) values of (51 \(\pm\) 9) and (111 \(\pm\) 8) \(\mu\)M against PA01 and PA14, respectively. All other variations in chain length, introduction of unsaturation in linker, removal of bromopyrrole part had detrimental effect on antibiofilm activity. Replacement of 2-aminoimidazole with similar five membered ring system also resulted in substantial decrease in activity. For SAR of the lead compound oroidin, Melander and co-workers studied the effect of reversal\(^{29}\) of amide bond which connects the bromopyrrole part with 2-aminoimidazole ring. They found that in reverse amides,
replacing bromopyrrole part with long chain aliphatic chain, increases activity substantially.

Excited by the discovery of DHS as the first non toxic small molecule inhibitor of biofilm formation in a mucoid variant of Pseudomonas aeruginosa, Melander's group synthesized and evaluated a few DHS derivatives. They found that replacing N-methyl in DHS with benzyl or 4-bromophenyl increases the potency of biofilm inhibition. These analogues were more active as biofilm inhibitors than as biofilm dispersal agent.

Cyclooroidin (28) isolated from Mediterranean sponge Agelas oroides, was tested on isolated guinea pig ileum for anticholinergic, antiserotonergic, antihistaminic activity but showed no activity. Cytotoxic alkaloid 29 was isolated from Agelas clathrodes collected near Desecheo Island, Puerto Rico, in March 1989.

4.1.2.4 Hymenin:

Antihistaminic bromopyrrole alkaloid hymenin (30) was first isolated in 1986. Proschk and co-workers isolated debromohymenin (31) from sponge Stylissa carteri collected in 1997 at Ambon and Sulawesi, Indonesia. Hymenin is a competitive antagonist of α-adrenoceptors in vascular smooth muscles of isolated aorta of rabbit. Alkaloid 31 was proved to be inactive against human monocytic leukemia cells.
4.1.2.5 Stevensin:

Stevensin (32) was isolated by Faulkner's group in 1985 from methanolic extract of an unidentified Micronesian marine sponge. Proschk and co-workers isolated debromostevensin (33) as an orange amorphous solid from methanolic extract of marine sponge *Styllisa carteri* (*syn* *Axinella carteri*) collected in 1997 at Ambon and Sulawesi, Indonesia. Both stevensin (32) and debromostevensin (33) were inactive against human monocytic leukemia cells (MONO-MAC 6).

4.1.2.6 Purealidins:

Purealidin A (34) was isolated from Okinawan Sponge *Psammaplysilla purea*. It inhibits MSH dependant detoxification of enzyme mycothiol-S-conjugate amidase with IC$_{50}$ value of (32 ± 3) µM. Day and co-workers synthesized a small library of purealidin A analogues and found that purealidin A (34) itself was inactive against human cancer cell lines as antiproliferative agent but its analogues 35 & 36 exhibited antiproliferative activity in low micromolar range and alkaloid 34 also inhibited the MT-stimulated ATPase activity of a recombinant form of the full motor domain fragment of the rat cytoplasmic dynein heavy chain. Low cytotoxicity and selective

(MONO-MAC 6) in cytotoxicity assay. Compound 30 did not inhibit the mitogen-activated protein kinase-I.  

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**Structure Images**

![Purealidin A (34)](image)

![Purealidin B (35)](image)

![Purealidin C (36)](image)

![Purealidin D (37)](image)
inhibition of cytoplasmic dynein ATPase by alkaloid 34 proved that it is a good lead for discovery of small molecule inhibitors of cytoplasmic dynein heavy chain.

Purealidin D (37), first alkaloid with pyridinium and bromotyrosine moiety of marine origin, was isolated from sponge *Psammaphysilla Purea*. Biological activity of 37 is not reported yet.

Purealidins J (38), K (39), and M (40), the bromotyrosine alkaloids were isolated from the Okinawan marine sponge *Psammaphysilla purea* collected of Ishigaki Islands, Okinawa. Purealidin J and K moderately inhibited the activity of epidermal growth factor receptor (EGFR) kinase with the IC$_{50}$ values of 23 and 14 µg/mL, respectively. Purealidin J was non toxic to ovarian tumour and leukemia cell lines.

Alkaloid 41 is a rare example of an 2-aminoimidazole coupled to another aromatic substituent, was isolated by bioassay guided purification of MeOH/10%H$_2$O soluble sample from *Oceanapia* sp. It was found to be a good inhibitor of mycothiol S-conjugate amidase, which would permit blocking of mycothiol dependant detoxification. So, this alkaloid can act as important lead for drug development against tuberculosis.

4.1.2.7 Purealin:

Purealin (42) was also isolated with the purealidin from the same source. It inhibited the ATPase activity of isolated axonemal dynein and skeletal muscle myosin without competing for the ATP sites on these motor proteins. A few purealin analogues were also synthesized by Day et al. and were found to have good antiproliferative
activity against mouse leukemia cell lines, but showed selective and moderate activities against human carcinoma.\textsuperscript{41}

\textbf{4.1.2.8 Aerophobin-2:}

Aerophobin-2 (43) was initially isolated from marine sponge \textit{Verongia aerophoba}.\textsuperscript{50} Later it was isolated from \textit{Aplysina aerophoba} and \textit{Aplysina cavericola}.\textsuperscript{51} A few years later Jaspar’s group also isolated it from \textit{Druinella sp.} and screened against ovarian tumor and leukemia cells but was found to be inactive.\textsuperscript{52}

\textbf{4.1.2.9 Sceptrin:}

Sceptrin (44) was first isolated by Faulkner et al. as water soluble alkaloid from marine sponge \textit{Agelas sceptrum}.\textsuperscript{53} It is an antimicrobial agent active against \textit{S. aureus}, \textit{B. subtilis}, \textit{C. albicans} with MIC value of 10 μg/mL each. Reinhart’s group reported that sceptrin isolated from \textit{Agelas conifera} has weak to moderate antiviral and antibacterial activity.\textsuperscript{24} Sceptrin also showed non-competitive antagonism towards histamine receptors with pD2 value of (4.24 ± 0.09).\textsuperscript{51} It showed

\begin{align*}
\text{Sceptrin (44)} & \\
\text{(45-50)} & \\
\text{(51-54)} & \\
\end{align*}
antibacterial activity against *M. luteus*, *B. subtilis*, *E. coli* with MIC values of 4.07, 8.33 and 33.3 µg/mL, respectively. It also inhibited protein phosphatise type 2A with IC₅₀ value of 50 µM. Sceptrin was moderately active against *C. neoformans* with an IC₅₀ value of 3.5 µg/mL. Debromosceptrin, a symmetrical pyrrole dimer, was isolated from ethanolic extract of *Agelas conifer*, together with other analogues (45-50). Compounds (45-50) were inactive as anti-HIV, antimalarial and cytotoxic agent. But compounds 47 & 49 showed marginal activity against *Mycobacterium tuberculosis* with MIC values of 12.5 µg/mL each.

Oxysceptrin (51) was isolated from Okinawan marine sponge *Agelas nemoechinata* and *Agelas conifera* as potent actinomyosin ATPase activator activity. Compounds (51-54) inhibited growth of *B. subtilis* at 10 µg/disk with (51-53) being somewhat less active. Compound 54 was also active against *E. Coli* at 10 µg/disk. All the sceptrin analogues were active against Herpes Simplex virus type 1 at 20 µg/disk and against vesicular stomatitis virus at 10 µg/disk while (51-54) were less active against both viruses. All the sceptrin and oxysceptrin analogues were non toxic to monkey kidney cells at 200 µg/disk.

4.1.2.10 Pseudoceratinines:

Pseudoceratinine A (55) and B (56) were isolated from two specimens of *Psedoceratina verrucosa* collected at Ile Longue, but pseudoceratinine C (57) was isolated from the specimen collected of Ile Walpile. Only pseudoceratinin A
inhibited MSH dependent detoxification enzyme mycothiol-S-conjugate amidase with IC$_{50}$ value of (100 ± 21) μM.$^{40}$

4.1.2.11 Naamidine:

Naamidine A (58), Isonaamidine B (63),$^{58}$ Isonaamidine C (64)$^{59}$ were isolated from EGF dependent DNA synthesis and cell proliferation inhibitory ethanolic extract of a bright yellow sponge *Leucetta* sp.$^{60}$ Among these three, only naamidine A (58) was a potent inhibitor of epidermal growth factor (EGF)-stimulated DNA synthesis$^{61}$ and it act by targeting extracellular signal regulated kinase ERK 1 and ERK 2.$^{62}$ On the basis of these finding, Watson’s group synthesized naamidine A and confirmed EGF

Naamidine A (58): R$_1$, R$_2$, R$_3$ = H, R$_4$ = H
Naamidine B (59): R$_1$ = Me, R$_2$ = OH, R$_3$ = H, R$_4$ = Me
Naamidine D (60): R$_1$, R$_2$, R$_3$ = H, R$_4$ = Me
Naamidine G (61): R$_1$ = Me, R$_2$, R$_3$ = H, R$_4$ = Me
Pyronaamidine (62): R$_1$ = CH$_3$R$_2$ = OCH$_3$, R$_3$ = OH, R$_4$ = Me

Isonaamidine B (63): R$_1$ = H, R$_2$ = Me
Isonaamidine C (64): R$_1$ = Me, R$_2$ = Me
Isonaamidine D (65): R$_1$ = H, R$_2$ = H
inhibitory activity. A series of its derivatives were also synthesized and evaluated for their ability to inhibit mitogenesis in BaF/ERX cells. It was also discovered that 4-methoxybenzyl substituent of natural product was unnecessary for activity and replacement of 2-aminoimidazole part with thiazole also doesn’t affect the biological activity but none of the synthesized analogues was more active than naamidine A (58).  

Naamidine A (58), B (59), and G (61) are antifungal agents with activity against Cryptococcus neoformans with MIC values of 12.5, 6.25 and 12.5 μg/mL, respectively, while naamidine D (60) was not tested.  

Naamidine H (66) and I (67) were isolated from the marine sponge Leucetta chagosensis collected in North Sulawesi, Indonesia. Naamidine H and I were cytotoxic against HeLa cells with IC50 values of 5.6 and 15 μg/mL, respectively.  

(2E, 9E) Pyrronaamidine-9-(n-methylimine) (68) was isolated from yellow sponge Leucetta cf chagosensis collected near the Island of Rota, Northern Mariana Islands. It is mildly toxic toward A-549, MCF-7, HT-29 cell lines with GI50 values of 6, 3 and 6 μg/mL, respectively.  

Pyrronaamidine (62) has weak antimicrobial activity against B. subtilis and C. albicans with 10 and 7 mm zones of inhibition at a concentration of 100 μg/disk.  

4.1.2.12 Naamines:  

Naamine A (69), isonaamine A (76) along with naamidine A (58), isonaamine A (76) were isolated from methanolic chloroform extract of the sponge Leucetta chagosensis.  

Naamine C (71) was isolated from same sponge but by different group. New cytotoxic 2-aminoimidazole analogues (78), isonaamine C (79), (80) were isolated from L. chagosensis collected from the Great Barrier Reef and Fiji islands.  

Upon cytotoxicity evaluation alkaloid 78 and isonaamine C were found to be cytotoxic with GI50 values of 1.3 and 2.1 μg/mL against Huh7 cell line, respectively.  

Isonaamine B (77) was also isolated from the same source and was inactive as antimicrobial agent.  

Chemical investigation of extract of sponge Leucetta chagosensis collected in Chuuk State afforded a 2-aminoimidazole alkaloid named as naamine C (71), biological activity of which is yet to be reported.  

Naamine D (72)
was isolated from Egyptian Red Sea Sponge *Leucetta cf. chagosensis* and it has moderate antifungal activity against AIDS-O1 pathogen *C. neoformans* with MIC value of 6.25 μg/mL and nitric oxide synthetase inhibitory activity with 50% reduction in production at 1.0 mM.\(^\text{64}\)

Naamine A (69): \(R_1 = \text{OH}, R_2, R_3, R_4 = \text{H}, R_5 = \text{Me}\)
Naamine B (70): \(R_1 = \text{OH}, R_2, R_3, R_5 = \text{H}, R_5 = \text{Me}\)
Naamine C (71): \(R_1 = \text{OH}, R_2, R_4 = \text{H}, R_3, R_5 = \text{Me}\)
Naamine D (72): \(R_1, R_4 = \text{OH}, R_2, R_5 = \text{H}, R_3, R_5 = \text{Me}\)
Naamine E (73): \(R_1, R_2 = \text{OH}, R_3, R_5 = \text{Me}, R_4 = \text{H}\)
Naamine F (74): \(R_1 = \text{OMe}, R_2, R_3, R_4 = \text{H}, R_5 = \text{Me}\)
Naamine G (75): \(R_1, R_2 = \text{OMe}, R_3, R_4 = \text{H}, R_5 = \text{Me}\)

Isonaamine A (76)
Isonaamine B (77)
Isonaamine C (79)
Isonaamine D (80)

Naamine F (74) and G (75)\(^\text{70}\) were isolated from *L. Chagosensis* collected at South Sulawesi, Indonesia. Naamine G (75) is a good antifungal agent with MIC value of 20 μg/disk showing inhibition of 20 mm zone in agar plate diffusion assay of *C. herbarum*. Naamine G (75) also exhibited moderate cytotoxicity toward Lymphoma (L5178Y) and human cervix carcinoma (HeLa) with 46 and 29% inhibition, respectively but was inactive toward rat brain tumor.

4.1.2.13 Kealiinines:

Kealiinines (81–83) were isolated from the same source as that of naamine F and G. Only kealiinine A (81) showed 50 % mortality rate in the brine shrimp lethality test.\(^\text{70}\)
4.1.2.14 Leucosolenamine:

Leucosolenamine A (84) and B (85) were isolated from Leucosolenia genus collected at Papua New Guinea. Leukosolenamine A was mildly cytotoxic with 10.5 mm zone of inhibition of murine C-38 cells at 180 µg/disk as compared to 0 mm against CFU-GM cells indicating it as mildly potent selective cytotoxin. But alkaloid 85 was inactive.

4.1.2.15 Girroline:

Girroline (86) an antitumor alkaloid, was isolated from New Caledonian sponge Cymbastella cantharella (previously Pseudaxinyssa cantharella) which showed both in vitro and in vivo antitumour activity. It arrests the cell cycle in G2/M phase in several tumor cell lines and accumulates polyubiquitinated p53 at least in FL cells. Because of several unfavourable side effects, clinical trials of compound 86 in phase 1 were stopped. Girroline (86) also exhibited good antimalarial activity with IC₅₀ values in range of 77 to 215 nM against 4 strains of Plasmodium falciparum. It inhibited growth of parasite by 100% with artemisinin and chloroquine. Girroline was active at a dose of 1mg/kg/day in vivo by both oral and i.p. route and targeted the
synthesis of proteins by the parasite. Therefore, girroline can be considered as potential lead structure for antiplasmodial drug research.

4.1.2.16 Archerine:

Archerine (87) was isolated from a typical West Indian species *Aplysina archeri* which grows in reef habitat at a depth of 2-40 m. It showed reversible antagonism of histamine when assessed using histamine induced contraction of guinea pig isolated ileum at $1.2 \times 10^{-4}$ M. Archerine doesn't have all the structural features which are currently used as guidelines for synthesis of antihistaminic drug.

4.1.2.17 Axinellamines:

Axinellamines A–D (88–91) were isolated by bioassay guided fractionation of crude methanol extract of Australian marine sponge *Axinella* sp. Axinellamine B (89), C (90), D (91) showed antibacterial activity against *Helicobacter pylori*, a gram negative bacteria associated with peptic and gastric cancer, with MIC value of 1000 μM. Axinellamine A (88) has no bactericidal activity at 1000 μM.

4.1.2.18 Dorimidazole A:

Dorimidazole A (92), the simplest example of 2-aminimidazole alkaloid having antiparasitic activity against *Nipporstrongylus brasiliensis* at 50μg/mL was isolated from Indopacific nudibranth but it's synthetic sample (HBr salt) was inactive.
4.1.2.19 Preclathridine:

Chemical investigation of nudibranch, *N. guardineri*, collected from Papua New Guinea resulted in isolation of the secondary metabolite preclathridine A (93). Biological activity of preclathridine A is not yet reported.

4.1.2.20 Ageliferins:

Ageliferin (94), bromoageliferin (95), dibromoageliferin (96) were isolated from Okinawan marine sponge *Agelas* sp. and found to be potent actomyosin ATPase activator. ATPase activity of myofibrils from rabbit skeletal muscles was elevated to 150, 190 and 200% of the control value by ageliferin (3 × 10⁻⁵), bromoageliferin (10⁻⁶), and dibromoageliferin (10⁻⁶). But later on (94–96) were also isolated from *Agelas conifera* and screened as antiviral, antibacterial agents and activity in barnacle settlement and biochemical prophage induction assay. Ageliferin was also found to inhibit important neurotransmitters such as vasoactive intestinal peptide (VIP) and somatostatin (somatotropin release inhibiting factor, SRIF) with IC₅₀ values of 2.2 and 19.2 μM, respectively.

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Taking structural insights from bromoageliferin (95), Melander and his group hypothesized that bicycle core of bromoageliferin is key pharmacophore that imparts biological activity; they designed two analogues of bromoageliferins TAGE (97)
and CAGE (98). Both inhibited biofilm formation of *Pseudomonas aeruginosa* with IC$_{50}$ values of 100 and 180 μM against PA O1 for TAGE and CAGE, respectively. While IC$_{50}$ values were 100 and 190 μM against PA14 for TAGE and CAGE respectively. At 500 μM TAGE showed more than 50% reduction in planktonic growth while CAGE showed it at both 400 and 500 μM.

Calcaridines represents the first nonorganometallic chiral 2-aminoimidazole alkaloids isolated from calcareous sponges. (+) Calcaridine A (99), (-) spirocalcaridine A (100), (-) spirocalcaridine B (101) were isolated from Calcareous sponge *Leucetta* sp. collected at several sites south of Vitu, Levu, Fiji. (+) Calcaridine A posses geminally fused 2-aminoimidazolidinone, while (-) spirocalcaridine A is unique in having hexahydrocyclopentamidazol-2-ylidename spiro-linked to a cyclohexenone. These alkaloid have not screened against any of the disease targets.

4.1.2.21 Calcaridine:

4.1.2.23 Phakellines:
Phakelline (102), bromophakelline (103), dibromophakelline (104) were isolated from marine sponge *Phakellia fiabellata*.\(^{86,87}\) N-methyldibromoisophakelline (105) was isolated from methanolic extract of *Styllisa caribica* collected off the coast of Sweeting Cay, Bahamas as chemical defense metabolite. It is the only component in sponge tissue of *Styllisa caribica* which is responsible for chemical defense.\(^{88}\)

Antifeeding activity of few aminoimidazole alkaloids is in order sceptrin > N-methyldibromoisophakellin > oroidin expressed as mole/mL. 12-Chloro-11-hydroxydibromoisophakelline (106) was isolated from marine sponge *Axinella brevistyla* collected in Western Japan. It exhibited antifungal activity against erg6 mutant type of the yeast *Saccharomyces cerevisae* at 30 μg/disk. It was also cytotoxic against L1210 cells with IC\(_{50}\) value of 2.5 μg/mL.\(^{89}\)

4.1.2.24 Palau’mines:

Palau’mine (107) was isolated from sponge *Stylotella aurantium* collected in Western Caroline Islands. Palau’mine exhibited antitumor activity against P388 and A549 cells with IC\(_{50}\) values of 0.1 and 0.2 μg/mL, respectively. It also showed antibiotic activity against *Penicillium notatum* with 24 mm zone of inhibition at 50 μg/disk. Palau’mine
showed immunosuppressive activity also with IC$_{50}$ < 18 ng/mL in the mixed lymphocyte reaction. 4-Bromopalaumine (108) and 4,5-dibromopalaumine (109) were isolated a few years later from the same sponge by the same group. 57 Both of these derivative were less active in comparison to palaumine itself, but 4,5-dibromo derivative was selective against a human melanoma cell line with an IC$_{50}$ value of 0.25 μg/mL.

4.1.2.25 Styloguanidines:

Styloguanidines (110–112), regioisomers of palaumine, were isolated from marine sponge Stylotella aurantium collected off Yap sea. All styloguanidines, also known as isopalaumines, inhibit chitinase, most important enzyme for ecdysis of crustaceans through hydrolysis of integumental chitin, of Schwanella sp. at a concentration of 2.5 μg/disk. These compounds also showed antifouling activities against barnacles by inhibiting the moulting of their cyprid larvae at 10 ppm. 90

4.1.2.26 Oxycyclostylidol:

Oxycyclostylidol (113) was isolated from Caribbean sponge Stylissa caribica, is the first example of twice oxidised pyrrole-imidazole alkaloid from natural source. Oxycyclostylidol showed minor activity against several pathogenic bacteria, virus, fungi and culture of mice fibroblasts. 91
4.1.2.27 Massadine:

Massadine (114) was discovered by bioassay guided purification of organic extract of marine sponge *Styllisa aff. massa* collected in Gulf of Sagami, as inhibitor of candida GGTase 1. It inhibited candida GGTase 1 with IC_{50} value of 3.9 μM. Massadine also inhibited the growth of *C. neoformans* with MIC value of 32 μM but was inactive against *C. albicans* even at a concentration of 64 μM.\(^{24,92}\)

4.1.2.28 Hymenialdisines:

Hymenialdisine (116) was initially isolated from two marine sponges *Axinella verrucosa* and *Acanthella aurantiaca*.\(^{16}\) A few other hymenialdisines were also isolated from *Styllisa massa* and *Styllisa carteri* by bioassay guided fractionation. Z-Debromohymenialdisine (115) and Z-hymenialdisine (116) were active against MONOMAC-6 (human monocytic leukemia cells) in cytotoxicity assay with IC_{50} values of 2.4 and 0.2 μg/mL, respectively.\(^{35}\) E-Bromohymenialdisine (120), E-hymenialdisine (119), E-debromohymenialdisine (118) were isolated from marine sponge *Stylotella aurantium* collected off Palau.\(^{93}\) Hymenialdisine and debromohymenialdisine (115) showed insecticidal activity against neonate larvae of polyphagous pest insect *Spodoptera littoralis* with LD_{50} values of 88 and 125 ppm respectively, when incorporated in the artificial diet of the larvae in chronic feeding assay. Z-Hymenialdisine (116) and E-hymenialdisine (119) both showed good inhibitory activity against mitogen activated protein kinase kinase-1 with IC_{50} values of 3 and 6 nM, respectively.\(^{36}\) They also inhibited the growth of human tumor LoVo cells. 3-Bromo-Z-hymenialdisine (117) was isolated from tropical marine sponge *Axinella carteri*.\(^{94}\) Cytotoxicity studies against mouse lymphoma cells (L5178y)
revealed that debromohymenialdisine (115) was most active with IC\textsubscript{50} value of 1.8 \(\mu g/mL\) followed by hymenialdisine (116) and 3-bromohymenialdisine (117) which were equitoxic with IC\textsubscript{50} values of 3.9 \(\mu g/mL\). Hymenialdisine inhibited interleukin-8 production in U-937 cells by inhibition of nuclear factor-\(\kappa\)B.\textsuperscript{95} It also inhibited NF-\(\kappa\)B mediated interleukin-1\(\beta\)-stimulated prostaglandin E\textsubscript{2} formation.\textsuperscript{96} Debromohymenialdisine (115) inhibited G2 DNA damage checkpoint with an IC\textsubscript{50} value of 8 \(\mu M\) and protein kinases chK1 and chK2 with 3 and 3.5 \(\mu M\) respectively.\textsuperscript{97} So, debromohymenialdisine (115) may be used to sensitize the cancer cells toward DNA damaging therapies.

Tepe’s group synthesized indoloazepine derivatives 121 & 122 of hymenialdisine and screened them for cytokines IL-2 and TNF-\(\alpha\) production.\textsuperscript{98} They found hymenialdisine as good inhibitor of production of cytokines IL-2 and TNF-\(\alpha\) with IC\textsubscript{50} values of (2.41 \(\pm\) 0.71) and (1.36 \(\pm\) 0.25) \(\mu M\), respectively but corresponding indoloazepine analogue 121 was less active with IC\textsubscript{50} values of (3.55 \(\pm\) 0.09) and (8.16 \(\pm\) 0.31) \(\mu M\) against IL-2 and TNF-\(\alpha\) production, respectively. \(N\)-methyl derivative 122 showed no significant inhibition of IL-2 or TNF-\(\alpha\) production. Both of these indoloazepine analogues 121 & 122 were also inhibitors of Leukaemia T-cells growth with GI\textsubscript{50} values of (1.61 \(\pm\) 0.62), (1.73 \(\pm\) 0.08) and (14.3 \(\pm\) 2.41) \(\mu M\) for 121 and 122, respectively. Both hymenialdisine (116) and 121 inhibited the binding of DNA to NF-\(\kappa\)B of nuclear extract of PMA/PHA activated Jurkat cells. Hymenialdisine (115) was also discovered as a potent inhibitor of cyclin-dependant kinases, glycogen synthase kinase-3\(\beta\) and casein kinase-1 by Thurini and co-workers after screening various compounds isolated from marine sponges.\textsuperscript{99} So,
hymenialdisine can act as a good lead compound in the treatment of neurodegenerative disorders.

Gray's group designed and synthesized a few analogues of hymenialdisine taking insights from crystal structures of CDK-2 complexed with hymenialdisine. They identified compound 123 as most potent which arrested the cells in G2/M phase at concentration as low as 3.8 μM and showed 30 fold higher antiproliferative activity than hymenialdisine (116).

### 4.1.2.29 Dispacamides:

Dispacamides, bromopyrrole alkaloids having 2-aminoimidazolone moiety, were isolated from four Caribbean Agelas sponges (A. conifera, A. longissima, A. clathrodes, A. dispar). Dispacamide A (124) and its debromoderivative dispacamide B (125) were inactive as anticholinergic as well as antiserotonergic agents but dispacamide A (124) was remarkably good and selective as antihistaminic agent when tested on the guinea pig ileum. Structure activity relationship study of sponge derived and synthetic bromopyrrole alkaloids as inhibitors of fish feeding by Lindel et al. revealed that dispacamide A has antifeedant property against omnivorous Caribbean reef fish, *Thalassoma bifasciatum* but debromoderivative was inactive demonstrating importance of bromo group on pyrrole ring for antihistaminic as well as antifeedant activity.
Dispacamide C (126) and D (127) showed a reversible, specific, non-competitive inhibition toward histamine receptors. But dispacamides C and D were less active in comparison to that of A and B, with pD2 values of 4.48 ± 0.05 and 4.34 ± 0.10, respectively.

Chemical investigation of *Axinella verucosa* collected from Bay of Calvi (Corsica), yielded two dispacamide derivatives 128 and 129. Compound 128 was potent neuroprotective agents, good serotonin and glutamate antagonist, 129 also showed serotonin antagonism but was weaker than 128. These two seems to be promising serotonin antagonists with a potential to treat psychosis, different phobia, and mood fluctuation disorders.

Debromodispacamides B (130) and D (131) were isolated from polar extracts of the sponge *Agelas mauritiana* collected off the Solomon Islands. Biological activities of these two are yet to be reported.
Taurodispacamide A (132) was isolated from the methanolic extract of sponge Agelas oroides collected off the bay of Naples. It displayed good antihistaminic activity by completely abolishing 0.1 μM response of histamine in a reversible manner.

4.1.2.30 Spongiasidine:

Chemical investigations of an Okinawan sponge Hymeniacidon sp. collected off Ishigaki Islands, by Kobayashi and co-workers furnished pyrrolo[2,3-c]-azepine-type alkaloids spongiasidines A (133) and B (134). Both inhibited cyclin dependent kinase 4 with IC_{50} values of 32 and 12 μg/mL, while they showed IC_{50} values of 8.5 and 6.0 μg/mL against c-erbB-2 kinase, respectively.

4.1.2.31 Mukanadin A:

Mukanadin A (135) was isolated by Kobayashi’s group from the extracts of Okinawan sponge Agelas nakamurai collected off Ie Island, Okinawa. It differs from monobromodispacamide in possessing a hydroxyl group at C-9. Biological activity of Mukanadin A is not reported yet.
4.1.2.32 Leucettamines:

Leucettamines A (136), B (137) and leucettamidine (139) were isolated from Palaún sponge *Leucetta microraphis*, while leucettamine C (138) was isolated from Fijian collection of calcareous sponge *Leucetta* sp. Leucettamine A (136) binds to leukotriene B4 receptor as antagonist with Ki values 1.3, 100 and 5.3 µM, respectively. It led to identification of leucettamine A as pure LTB4 antagonist, a new structural lead to inflammation therapy. Leucettamine A also showed good inhibition against gram positive bacteria *Staphylococcus aureus* and the fungus *Cladosporum herbarum*. Effort were also made towards optimization of LTB4 antagonist activity of Leucettamine A by synthesizing the analogue which lacks symmetry but most of the synthetic analogue were less active, except 140 which has comparable activity to 136 with Ki = (2.4 ± 0.2).

4.1.2.33 Polyandrocarpamines:

Chemical investigation of Fijian ascidian sponge *Polyandrocarpa* sp. resulted in isolation of polyandrocarpamine A (141) & B (142). Polyandrocarpamine A was
selectively cytotoxic toward SF-268 (central nervous system) tumor cell lines with \( \text{GI}_50 \) value of 65 \( \mu \text{M} \), while B was less active with \( \text{GI}_50 \) value of more than 80 \( \mu \text{M} \).\(^{113}\)

4.1.2.34 Tauroacidin:

Tauroacidins A (143) and B (144), rare bromopyrrole alkaloids possessing a taurine residue, were isolated from extracts of an Okinawan sponge \( \text{Hymeniacidon} \) sp. Both tauroacidins A and B inhibited the enzymes epidermal growth factor receptor (EGFR) kinase and C-erb-2 kinase with IC\(_{50} \) value of 20 \( \mu \text{g/mL} \) each.\(^{114}\)

4.1.2.35 Mauritiamine:

Keeping in view that chemical defense substances of sessile marine organism may act as potential non toxic antifouling agent, bioassay guided fractionation of methanolic extract of marine sponge \( \text{Agelas mauritiana} \), collected off Hachijo-jima Island, Japan, yielded new antifouling agent mauritiamine (145). Mauritiamine showed inhibitory zone of 10 mm at 10 \( \mu \text{g/disk} \) against \( \text{Flavobacterium marinotypicum} \). It also inhibited larval metamorphosis of barnacle \( \text{Balanus amphitrite} \) with ED\(_{50} \) value of 15\( \mu \text{g/mL} \) and was non toxic at conc. of 30 \( \mu \text{g/mL} \).\(^{22}\)
4.1.2.36 Aplysinopsin:

Aplysinopsin (146), a marine natural product containing cyclic guanidine function was recently isolated from *Smenospongia aurea*,*earlier it has also been isolated from several other sources.* It acts on plasmepsin II and serotonin receptors as a cationic molecule. Recently, we have discovered a thio analogue of aplysinopsin having potent antileishmanial activity with 10 times more activity and 401-folds less toxicity than standard drug pentamidine in cell based assays.

4.1.3 Conclusion:

Leads from natural sources are better and more bio-friendly due to their co-evolution with the target sites in biological systems. As demonstrated in this review, a large number of 2-aminoimidazole and glycociamidine alkaloids isolated from marine environment have shown a range of biological activities, and may be treated as leads...
for developing new compounds having antibacterial, antibiotic, antibiofilm formation, antiviral, antifungal, and anticancer activities.

4.1.4 References:


Chapter 4a: Marine 2-Aminoimidazole, Glycociamidine Alkaloids and Their Synthetic Analongues


Chapter 4a: Marine 2-Aminoimidazole, Glycociamidine Alkaloids and Their Synthetic Analogues


Chapter 4a: Marine 2-Aminoimidazole, Glycociamidine Alkaloids and Their Synthetic Analogues

47. Fang, Y. I.; Yokota, E.; Mabuchi, I.; Nakamura, H.; Ohizumi, Y. Biochemistry 1997, 36, 15561.


Chapter 46

Isonaamine C and its analogues: Development of highly versatile, protecting group free synthesis and discovery of their antileishmanial and antibacterial potential
4.2.1 Introduction:

Marine sponges are still the main source of bioactive natural products. Complex natural products isolated from marine sponges have been the basis for many clinical leads. First marine drug, ziconotide (ο-conotoxin MVIIIA), isolated from a tropical marine cone snail, was approved in United States in 2004 for the treatment of chronic pain in spinal chord injury, under trade name Prialt. In October 2007, another marine natural product ET-743 (ecteinascidin-743/Yondelis/trabectedin), the antitumour compound from sea-squirt was approved by European Union for the treatment of soft tissue sarcoma. Since the late 1980’s, several marine alkaloids possessing either 2-aminoimidazole or 2-aminoimidazolinone moiety i.e. Isonaamine C (1), Leucettamine B (2), Oroidin (3), Hymenialdisine (4), and Dispacamide A (5) have been isolated from the genus Leucetta and many of them demonstrated interesting biological activities. Recently, many efforts have also been devoted to discover the new biological activities of Oroidin and Hymenialdisine derivatives.

![Figure 1. Some biologically important 2-aminoimidazole, 2-aminoimidazolinone, 2-thiohydantoin alkaloids.](image)

As a part of our program to discover novel small molecule antiparasitic and anti-infective agents, we recently reported the antileishmanial activity of aplysinopsin-pentamidine hybrid molecule. Inspired by these findings, we focussed our attention on synthesis and bioevaluation of Isonaamine C and it’s analogues as antileishmanial and antibacterial agents. Isonaamine C (1), a 2-aminoimidazole alkaloid, was isolated from marine sponge Leucetta chagosensis collected from Australian Bougainville Reef. Only biological activity known for this alkaloid is it’s cytotoxicity against...
Chapter 4b: Isonaamine C and its analogues.

HM02, HepG2, Huh7 tumour cell lines with GI_{50} values of 5.3, 2.2 and 2.1 µg/mL, respectively.\(^9\)

Classical methods for synthesis of 2-aminoimidazoles can be divided into two parts (i) starting from preformed imidazole ring and (ii) direct construction of 2-aminoimidazole ring. 2-Amino functionality can be introduced in imidazole ring by metallation followed by treatment with aryl azide and acid,\(^10\) coupling with aryl diazonium salt and followed by reduction.\(^11\) 2-aminoimidazole ring can also be directly constructed by reaction of α-haloketones and N-acetylguanidine,\(^12\) α-aminocarbonyl compounds with cyanamide,\(^13\) α-diketones with guanidine and subsequent reduction.\(^14\) Among these, the most popular method is condensation of α-aminocarbonyl with cyanamide, but is very pH sensitive and can lead to self condensation of α-aminoaldehyde or ketone resulting into formation of symmetrical pyrazine.\(^15\) In 1999, Molina et al. reported iminophosphorane mediated preparation of 2-amino-1,4-disubstituted imidazole from α-azidoesters, N-tosylisocyanate and amine leading to total synthesis of Isonaamine A, Dorimidazole A, and Preclathridine A, which involves tedious deprotection of 2-amino group by SmI\(_2\).\(^16\) We hypothesized that 2-amino-1,4-disubstituted imidazoles can be obtained by reduction of corresponding 2-aminoimidazolinones followed by dehydration, which in turn can be obtained by amination of corresponding 2-thiohydantoins (Scheme 1). This strategy looked very attractive to us, as it would also have provided us 2-thiohydantoins and 2-aminoimidazolinones analogues of Isonaamine C derivatives for bioevaluation against leishmanial and bacterial parasites.

![Scheme 1. Retrosynthesis of Isonaamine C.](image-url)
In continuation of our efforts to discover novel small molecules as antiparasitic and anti-infective agents, we describe here an efficient, versatile and protecting group free synthesis of nine isonaamine C derivatives via corresponding 2-aminoimidazolinones, 2-thiohydantoin congeners and their biological evaluation.

4.2.2 Chemistry:

We started with the synthesis of 4-methoxybenzyl isothiocyanate (8) from 4-methoxybenzylamine (7) using the reported procedure\textsuperscript{17} (Scheme 2). 3-(4-Methoxybenzyl)-2-thiohydantoin (10) was obtained by condensation of ethyl glycinate hydrochloride with 4-methoxybenzyl isothiocyanate (8) in 82% yield.

\begin{center}
\includegraphics[width=\textwidth]{Scheme2.png}
\end{center}

Scheme 2. Preparation of disubstituted 2-thiohydantoins.

Condensation of commercially available benzyl isothiocyanate (9) with ethylglycinate hydrochloride afforded 3-benzyl-2-thiohydantoin (11) in 86% yield. 3-Substituted phenylmethylene-2-thiohydantoins 12(a–i) were prepared by Knoevenegal condensation\textsuperscript{18} of various benzaldehydes with 2-thiohydantoins (10 and 11). (Scheme 2 and Table 1)

With these 3-benzyl substituted phenylmethylene-2-thiohydantoins 12(a–i) in hand, we tried oxidative nucleophilic substitution of sulphur with aq. ammonia using tert-butyl hydroperoxide (TBHP)\textsuperscript{19} as oxidizing agent taking (Z)-5-(3,4-dimethoxybenzylidene)-3-(4-methoxybenzyl)-2-thiohydantoin (12b) as the model.
Table 1. 2-thiohydantoins 12(a–i)

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>R</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12a</td>
<td>OMe</td>
<td>H</td>
<td>OMe</td>
<td>H</td>
<td>79</td>
</tr>
<tr>
<td>12b</td>
<td>OMe</td>
<td>H</td>
<td>OMe</td>
<td>OMe</td>
<td>76</td>
</tr>
<tr>
<td>12c</td>
<td>OMe</td>
<td>H</td>
<td>H</td>
<td>OMe</td>
<td>82</td>
</tr>
<tr>
<td>12d</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>85</td>
</tr>
<tr>
<td>12e</td>
<td>H</td>
<td>H</td>
<td>OMe</td>
<td>H</td>
<td>81</td>
</tr>
<tr>
<td>12f</td>
<td>H</td>
<td>H</td>
<td>Cl</td>
<td>H</td>
<td>84</td>
</tr>
<tr>
<td>12g</td>
<td>H</td>
<td>OMe</td>
<td>OMe</td>
<td>OMe</td>
<td>78</td>
</tr>
<tr>
<td>12h</td>
<td>H</td>
<td>H</td>
<td>OCH₂Ph</td>
<td>H</td>
<td>75</td>
</tr>
<tr>
<td>12i</td>
<td>H</td>
<td>OMe</td>
<td>OMe</td>
<td>H</td>
<td>84</td>
</tr>
</tbody>
</table>

substrate. But this resulted in a mixture of products with poor yield of desired amine 13b (Scheme 3). To overcome this problem, it was thought that nucleophilic substitution of 2-thiomethyl group will be easier than sulphur and more importantly oxidizing agent will also be avoided, which may result in neat reaction.

Reagents and conditions: (i) aq. NH₃, TBHP, MeOH, rt, 12h; (ii) (a) Mel, K₂CO₃, Acetone, 0°C - rt, 4h (b) Benzylamine, reflux, 8h.

Scheme 3. Preparation of 2-aminoimidazolinones by (a) oxidative nucleophilic substitution (b) by nucleophilic substitution of S-methyl derivative by benzylamine

In the mean time, S-methylation of 12d was achieved by treating it with 1.1 equivalent of methyl iodide and K₂CO₃ in acetone. Refluxing the S-alkyl derivative with benzylamine furnished imidazolinone derivative 13d in excellent yield (Scheme...
3). Next, we tried amination of 12b by heating at 120°C with 1:1 mixture of 30% aq. ammonia and ethanol in steel bomb. This reaction resulted in formation of a complex mixture of products. Overnight heating of 12b in 4:1 mixture of 30% aq. ammonia and ethanol at 120°C in steel bomb gave 2-aminoimidazolinone derivative 13b in good yield (Scheme 4 and Table 2).

Scheme 4. Synthesis of 2-aminoimidazolinones 13(a–i) from corresponding 2-thiohydantoin 12(a–i).

Table 2. 2-Amino-1,4-disubstituted imidazolinones (13(a–c) and 13(e–i))

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>R</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13a</td>
<td>OMe</td>
<td>H</td>
<td>OMe</td>
<td>H</td>
<td>80</td>
</tr>
<tr>
<td>13b</td>
<td>OMe</td>
<td>H</td>
<td>OMe</td>
<td>OMe</td>
<td>77</td>
</tr>
<tr>
<td>13c</td>
<td>OMe</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>76</td>
</tr>
<tr>
<td>13e</td>
<td>H</td>
<td>H</td>
<td>OMe</td>
<td>H</td>
<td>74</td>
</tr>
<tr>
<td>13f</td>
<td>H</td>
<td>H</td>
<td>Cl</td>
<td>H</td>
<td>81</td>
</tr>
<tr>
<td>13g</td>
<td>H</td>
<td>OMe</td>
<td>OMe</td>
<td>OMe</td>
<td>77</td>
</tr>
<tr>
<td>13h</td>
<td>H</td>
<td>H</td>
<td>OCH₂Ph</td>
<td>H</td>
<td>82</td>
</tr>
<tr>
<td>13i</td>
<td>H</td>
<td>OMe</td>
<td>OMe</td>
<td>H</td>
<td>75</td>
</tr>
</tbody>
</table>

We also explored the possibility of direct amination of imidazo-2-thione (14) by oxidative nucleophilic substitution of 2-thio function in 2-thiohydantoin (12a) with ammonia. LiBH₄ reduction of 2-thiohydantoin resulted in the formation of imidazo-2-thione (14) in 67% yield which upon treatment with TBHP, aq. ammonia in methanol at rt didn’t react to afford isonaamine C (1) (Scheme 5).
Scheme 5. An attempt toward conversion of 2-thiohydantoin to 2-aminoimidazole via imidazo-2-thione analogue.

To obtain the isoniaamine C derivatives 15(a–i) reduction of α,β-unsaturated amide function of 13(a–i) and subsequent dehydration was planned. Initially lithium aluminium hydride (LAH) in dry THF was tried at various temperatures, but gave frustrating results. LiBH₄ is known to reduce the amidic carbonyl group, which otherwise is very difficult to reduce selectively. 2-Aminoimidazolinones on refluxing with 3 eq. of LiBH₄ in dioxane for 75 min. and subsequent treatment with 2N HCl furnished isoniaamine C derivatives in 40-55% yields (Scheme 6 and Table 3). Although isoniaamine C has been numbered as (1) in the introduction but in Table 3 and thereon it has been numbered as 15b for ease of presentation.

Scheme 6. Direct reduction and dehydration of 2-aminoimidazolinones to isoniaamine C analogues.
Table 3. Isonaamine C analogues 15(a-i)

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>R₄</th>
<th>R₅</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15a</td>
<td>OMe</td>
<td>H</td>
<td>OMe</td>
<td>H</td>
<td>H</td>
<td>51</td>
</tr>
<tr>
<td>15b</td>
<td>OMe</td>
<td>H</td>
<td>OMe</td>
<td>OMe</td>
<td>H</td>
<td>42</td>
</tr>
<tr>
<td>15c</td>
<td>OMe</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>52</td>
</tr>
<tr>
<td>15d</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>CH₂Ph</td>
<td>55</td>
</tr>
<tr>
<td>15e</td>
<td>H</td>
<td>H</td>
<td>OMe</td>
<td>H</td>
<td>H</td>
<td>52</td>
</tr>
<tr>
<td>15f</td>
<td>H</td>
<td>H</td>
<td>Cl</td>
<td>H</td>
<td>H</td>
<td>55</td>
</tr>
<tr>
<td>15g</td>
<td>H</td>
<td>OMe</td>
<td>OMe</td>
<td>OMe</td>
<td>H</td>
<td>46</td>
</tr>
<tr>
<td>15h</td>
<td>H</td>
<td>H</td>
<td>OCH₂Ph</td>
<td>H</td>
<td>H</td>
<td>54</td>
</tr>
<tr>
<td>15i</td>
<td>H</td>
<td>OMe</td>
<td>OMe</td>
<td>H</td>
<td>H</td>
<td>48</td>
</tr>
</tbody>
</table>

4.2.3 Antileishmanial activity:

Antileishmanial evaluation of all 2-thiohydantoins 12(a-i), 2-aminoimidazolinones 13(a-i), 2-aminoimidazoles 15(a-i) revealed that the isonaamine C analogues were more active than their corresponding 2-aminoimidazoline and 2-thiohydantoins congeners. N-(4-methoxybenzyl) group at 1-position of 2-thiohydantoins was detrimental, while N-benzyl group was favoured for antiamastigote activity as demonstrated by percentage inhibition data of compound 12g, 12i and other 2-thiohydantoins against amastigotes. Compound 15d showed only slightly lower % inhibition than 15e but had much superior selectivity index of the latter against leishmania amastigotes. N,1,4-tribenzyl-1H-imidazol-2-amine (15d) showed best antileishmanial activity with IC₅₀ value of 30.62 μM and selectivity index of 12.76. Selectivity index of compound (15d) is better than SSG (sodium stilbogluconate) and pentamidine, reference drugs. In vivo antileishmanial activity of most active compound (15d) is in progress.

Table 4. Antileishmanial activity of compounds (12a – 12i), (13a – 13i), (15a – 15i)

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>% inhibition (at 40 μM)</th>
<th>IC₅₀ (μM)</th>
<th>CC₅₀ (μM)</th>
<th>Selectivity index</th>
</tr>
</thead>
<tbody>
<tr>
<td>12g</td>
<td>72.99</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>12i</td>
<td>72.97</td>
<td>14.96</td>
<td>31.54</td>
<td>2.11</td>
</tr>
</tbody>
</table>
### 4.2.4 Antimicrobial activity:

Keeping in the view the antimicrobial activity of the 2-aminoimidazole and 2-aminoimidazolinone alkaloids all the synthesized 2-thiohydantoins 12(a–i), 2-aminoimidazolinones 13(a–i), 2-aminoimidazoles 15(a–i) were also screened for their *in vitro* antimicrobial activity as tabulated in Table 5 along with Gentamicin, Ampicillin, Amphotericin B and Fluconazole used as a standard drugs.

**Table 5. In vitro antimicrobial activity of compounds (12a – 12i), (13a – 13i), (15a – 15i).**

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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ND: not determined. Selectivity index (SI) defined by the ratio IC_{50}(J774A.1 cells)/IC_{50}(Leishmania amastigotes). SSG: sodium stilbogluconate.
Chapter 4b: Isonaamine C and its analogues:

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Fluconazole

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All the compounds having 4-methoxybenzyl substituent at N-1 were inactive both as antibacterial and antifungal agents. All the tested compounds were inactive against two bacteria Escherichia coli, Pseudomonas aeruginosa. Compound 13h was most active against Klebsiella pneumoniae, a gram -ve bacteria, with MIC value of 0.005 µg/mL but its 2-thiohydantoin analogue 12h was inactive while 2-aminoimidazole analogue 15h was 38 times less active than 13h. It clearly demonstrated that 2-aminoimidazolinone moiety was most favoured for antibacterial activity against Klebsiella pneumoniae in comparison to 2-thiohydantoin and 2-aminoimidazole ring systems. It was also supported by the fact that 3,4,5-trimethoxy derivative 13g was also good antibacterial agent having MIC value of 0.045 µg/mL against the same bacteria while its 2-thiohydantoin and 2-aminoimidazolone (12g and 15g, respectively) analogues were less active. Trend of antibacterial activity of 13e, 14e and 15e also supported the above mentioned finding. 4-chloro substituent was also favoured but was not as good as benzyloxy substituent, as compounds 13f and 15f were less active in comparison to 13h. Both of these compounds were more active in comparison to reference drugs. A comparison of antibacterial activity of 2-aminoimidazolinones (13a – 13i) revealed that methoxy and benzyloxy groups on benzyl ring at C-5 position of 2-aminoimidazolinone moiety. SAR of the antibacterial activity of synthesized compounds against Klebsiella pneumoniae is somewhat similar to that of antileishmanial activity.
4.2.5 Anticancer Activity:

Earlier report on moderate cytotoxicity of isonaamine C against two human cancer cell lines inspired us to screen these analogues (15a – 15i) against a panel of human cancer cell lines including KB (oral squamous cell carcinoma), MCF-7 (breast cancer), A549 (lung carcinoma), C33A (cervical carcinoma), NIH3T3 (mouse embryo fibroblast). Compound 15f having 4-chloro group at C-5 benzyl ring and unsubstituted benzyl ring at C-9 was found to be most active against all the tested cancer cell lines. 15f also showed lowest IC₅₀ value of 3.33 µg/mL against KB cells followed by C33A (4.73 µg/mL), A549 (7.61 µg/mL), and MCF-7 (10.27 µg/mL), respectively. It was also found to be 5.18 times more selective towards KB cells in comparison to that of NIH3T3 mouse embryonic fibroblast cells. Selectivity against all other cell lines was in the range of 3.6 – 1.7. Compound 15c was cytotoxic against C33A cells with IC₅₀ value of 8.85 µg/mL and was 4.98 times more selective in comparison to NIH3T3 cells. Resulted tabulated in Table 5 also revealed that all other methoxy and benzyloxy group were not favoured for anticancer activity.

Table 6. Cytotoxicity of isonaamine C analogues 15(a – i) against a panel of human cancer cell lines.

<table>
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<th>Compound No.</th>
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ᵃ KB (oral squamous cell carcinoma), MCF-7 (breast cancer), A549 (lung carcinoma), C33A (cervical carcinoma), NIH3T3 (mouse embryo fibroblast).
4.2.6 Conclusion:

A versatile, efficient and protecting group free route has been developed for synthesis of isonaamine C analogues. Our route has many advantages over previously reported methods. Some of the noteworthy advantages are: (a) convenient functionalization of C-4 benzyl group by simply using the appropriate benzaldehyde while method reported by Molina et al. needs appropriately substituted α-bromoester; (b) easy access to the 2-thiohydantoin and 2-aminoimidazolinone congeners of the isonaamine C derivatives; (c) tedious detosylation by $\text{SM}_{\text{II}}$ has also been avoided; (d) better overall yields of the 2-aminoimidazoles starting from isothiocyanates. Antileishmanial and antibacterial screening revealed that N-benzyl group is advantageous for biological activity while it's 4-methoxy analogue is not favoured. Compound 15d showed better in vitro antiamastigote activity than standard reference drugs SSG and Pentamidine by exhibiting 2 and 6 times higher values of selectivity indices, respectively. It was also established from antibacterial screening that 2-aminoimidazolinone moiety was most favoured for antibacterial activity as compound 13h was most active against *Klebsiela pneumoniae*, a gram -ve bacteria, with MIC value of 0.005 μg/mL. Compound 13h was 156 and 78 times more active than standard antibacterial drugs Gentamicin and Ampicillin, respectively. Anticancer screening against a panel of human cancer cell lines also led us to identification of 2-aminoimidazole analogue 15f as moderate cytotoxic agent which was more active and selective anticancer agent in comparison to isonaamine C (15b) itself. It was also established that 4-methoxy group at C-1 of 2-aminoimidazole was detrimental for the anticancer activity of isonammine C analogues.

4.2.7 Experimental:

**General Procedure for the synthesis of 3-substituted 2-thiohydantoins (10 – 11):**

To a well stirred mixture of ethyl glycinate hydrochloride and 1.1 equivalent of $\text{Et}_3\text{N}$ in DCM, 1 equivalent of benzyl isothiocyanate/4-methoxybenzyl isothiocyanate was added and refluxed for 10h. Then the reaction mixture was diluted with DCM, washed with water and organic layer was dried with anhyd. $\text{Na}_2\text{SO}_4$. Solvent was removed in vacuo and recrystallized from EtOH.
3-(4-Methoxybenzyl)-2-thiohydantoin (10):
Yield: 82%; mp 179-181°C; IR (KBr): 3458, 3207, 3007, 2949, 1738, 1611, 1535, 1512, 1431, 1351, 1291, 1243, 1171 cm⁻¹; ¹H NMR (CDCl₃, 300MHz): δ (ppm) 7.48 (d, 2H, J = 8.7 Hz), 7.10 (bs, 1H), 6.86 (d, 2H, J = 8.7 Hz), 4.96 (s, 2H), 4.06 (s, 2H), 3.80 (s, 3H); ¹³C NMR (CDCl₃, 75MHz): 184.93, 171.27, 159.38, 130.61, 127.79, 111.38, 55.25, 48.40. 44.14. Anal. Calcd. for C₁₁H₁₂N₂O₂S: C 55.91, H 5.12, N 11.86; Found: C 55.68, H 5.24, N 11.83 %.

3-Benzyl-2-thiohydantoin (11):
Yield: 86%; mp 172-174°C; IR (KBr): 3274, 3022, 2905, 1714, 1512, 1438, 1407, 1348, 1317, 1158 cm⁻¹; ¹H NMR (CDCl₃, 300MHz): δ (ppm) 7.46-7.43 (m, 2H), 7.31-7.7.24 (m, 3H), 4.97 (s, 2H), 4.01 (s, 2H); ¹³C NMR (CDCl₃+DMSO-d₆), 75MHz): 184.91, 172.71, 136.24, 128.96, 128.76, 128.09, 44.57, 39.97. Anal. Calcd. for C₁₀H₁₀N₂O₂S: C 58.23, H 4.89, N 13.58; Found: C 58.05, H 4.72, N 13.46 %.

General Procedure for the synthesis of phenylmethylene-2-thiohydantoin 12(i):
1 equivalent of aromatic aldehyde and 1.2 equivalent of ethanolamine was added to a mixture of appropriate 2-thiohydantoin (10 or 11) in EtOH. Resulting mixture was refluxed for 1h and a yellow precipitate was obtained. Reaction mixture was cooled slowly to 0°C and filtered. Solid residue so obtained was washed with (10mL x 3) chilled alcohol. The crude product was crystallized from ethanol.

(Z)-3-(4-Methoxybenzyl)-5-(4-methoxybenzylidene)-2-thiohydantoin (12a):
Yield: 79%; mp 181-183°C; ESMS: 355 (M+1); IR (KBr): 3453, 3225, 2937, 2836, 1734, 1655, 1605, 1513, 1471, 1435, 1303, 1266, 1249, 1178 cm⁻¹; ¹H NMR (CDCl₃, 300MHz): δ (ppm) 8.82 (bs, 1H), 7.47 (d, 2H, J = 11.7 Hz), 7.38 (d, 2H, J = 11.7 Hz), 6.96 (d, 2H, J = 11.2 Hz), 6.84 (d, 2H, J = 11.8 Hz), 6.69 (s, 1H), 5.04 (s, 2H), 3.85 (s, 3H), 3.78 (s, 3H); ¹³C NMR (CDCl₃+DMSO-d₆, 75MHz): 178.68, 164.91, 150.89, 149.51, 136.33, 128.89, 128.77, 128.02, 125.92, 125.19, 124.78, 114.97, 113.23, 111.66, 56.64, 56.31, 44.69. Anal. Calcd. for C₁₉H₁₈N₂O₅S: C 64.39, H 5.12, N 7.90; Found: C 64.36, H 5.24, N 7.85 %.
(Z)-5-(3,4-Dimethoxybenzylidene)-3-(4-methoxybenzyl)-2-thiohydantoin (12b):
Yield: 76%; mp 196-198°C; ESMS: 385 (M+1); IR (KBr): 3454, 3195, 2937, 2837, 1733, 1657, 1599, 1526, 1474, 1436, 1355, 1266, 1221, 1136 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\), 300MHz): \(\delta\) (ppm) 8.70 (s, 1H), 7.49 (d, 2H, \(J = 8.7\) Hz), 7.05 (dd, 1H, \(J = 8.1, J' = 1.8\) Hz), 6.94 (d, 1H, \(J = 8.4\) Hz), 6.89-6.85 (m, 3H), 6.69 (s, 1H), 5.06 (s, 2H), 3.95 (s, 3H), 3.93 (s, 3H); \(^{13}\)C NMR (CDCl\(_3\), 75MHz): 177.65, 163.63, 159.34, 150.71, 149.66, 130.49, 127.80, 125.68, 125.17, 122.27, 114.01, 113.87, 112.51, 111.77, 56.16, 56.06, 55.25, 44.23. Anal. Calcd. for C\(_{20}\)H\(_{20}\)N\(_2\)O\(_4\)S: C 62.48, H 5.24, N 7.29; Found: C 62.35, H 5.47, N 7.22 %.

(Z)-5-Benzylidene-3-(4-methoxybenzyl)-2-thiohydantoin (12c):
Yield: 82%; mp 185-187°C; IR (KBr): 3263, 2956, 1710, 1640, 1509, 1467, 1442, 1349, 1298, 1228, 1185 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\), 300MHz): \(\delta\) (ppm) 8.90 (bs, 1H), 7.49-7.42 (m, 7H), 6.85 (d, 2H, \(J = 11.9\) Hz), 6.73 (s, 1H), 5.04 (s, 2H), 3.78 (s, 3H); \(^{13}\)C NMR (CDCl\(_3\)+DMSO-d\(_6\), 75MHz): 170.59, 156.94, 151.78, 134.50, 126.47, 124.18, 123.45, 122.92, 122.26, 120.65, 108.50, 108.36, 52.63, 41.71. Anal. Calcd. for C\(_{18}\)H\(_{16}\)N\(_2\)O\(_2\)S: C 66.64, H 4.97, N 8.64; Found: C 66.49, H 5.06, N 8.54 %.

(Z)-3-Benzyl-5-benzylidene-2-thiohydantoin (12d):
Yield: 85%; mp 224-226°C; IR (KBr): 3260, 2956, 1728, 1646, 1509, 1464, 1441, 1342, 1298, 1223, 1186 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\), 300MHz): \(\delta\) (ppm) 7.72-7.69 (m, 2H), 7.36-7.24 (m, 8H), 6.60 (s, 1H), 4.99 (s, 2H); \(^{13}\)C NMR (CDCl\(_3\)+DMSO-d\(_6\), 75MHz): 170.82, 157.24, 135.93, 133.14, 130.06, 129.73, 129.61, 129.11, 128.90, 128.29, 126.74, 114.30, 44.99. Anal. Calcd. for C\(_{17}\)H\(_{14}\)N\(_2\)OS: C 69.36, H 4.79, N 9.52; Found: C 69.08, H 5.01, N 9.34 %.

(Z)-3-Benzyl-5-(4-methoxybenzylidene)-2-thiohydantoin (12e):
Yield: 81%; mp 206-208°C; ESMS: 325 (M+1); IR (KBr): 3245, 2930, 1725, 1647, 1594, 1462, 1341, 1255, 1172 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\), 300MHz): \(\delta\) (ppm) 8.90 (bs, 1H), 7.51 (d, 2H, \(J = 8.5\) Hz), 7.48-7.24 (m, 5H), 6.96 (d, 2H, \(J = 8.7\) Hz), 6.70 (s, 1H), 5.10 (s, 2H), 3.85 (s, 3H); \(^{13}\)C NMR (CDCl\(_3\)+DMSO-d\(_6\), 75MHz): 183.42, 169.74, 165.93, 141.32, 137.44, 133.61, 133.50, 132.81, 130.34, 129.73, 119.63, 119.44, 60.59, 49.36. Anal. Calcd. for C\(_{18}\)H\(_{16}\)N\(_2\)O\(_2\)S: C 66.64, H 4.97, N 8.64; Found: C 66.55, H 5.12, N 8.59 %.
(Z)-3-Benzyl-5-(4-chlorobenzylidene)-2-thiohydantoin (12t):
Yield: 84%; mp 229-230°C; IR (KBr): 3453, 3225, 2937, 2836, 1734, 1655, 1605, 1513, 1471, 1435, 1303, 1266, 1249, 1178cm⁻¹; ¹H NMR (CDCl₃, 300MHz): δ (ppm) 7.43-7.27 (m, 5H), 7.23 (m, 4H), 6.59 (s, 1H), 5.04 (s, 2H); ¹³C NMR (CDCl₃+DMSO-d₆, 75MHz): 179.49, 164.77, 136.79, 135.01, 132.66, 131.90, 129.60, 129.20, 128.45, 128.27, 127.29, 112.53, 44.61. Anal. Calcd. for Cl₁H₁₃ClN₂O₂S: C 62.10, H 3.98, N 8.52; Found: C 61.88, H 3.84, N 8.45 %.

(Z)-3-Benzyl-5-(3,4,5-trimethoxybenzylidene)-2-thiohydantoin (12g):
Yield: 78%; mp 178-180°C; ESMS: 385 (M⁺); IR (KBr): 3401, 3102, 2994, 2839, 1710, 1636, 1576, 1499, 1455, 1422, 1362, 1318, 1251, 1194cm⁻¹; ¹H NMR (CDCl₃, 300MHz): δ (ppm) 9.01 (bs, 1H), 7.52 (dd, 2H, J = 9.6 Hz, J' = 1.6 Hz), 7.37-7.29 (m, 3H), 6.68 (s, 1H), 6.62 (s, 2H), 5.12 (s, 2H), 3.90 (s, 9H); ¹³C NMR (CDCl₃, 75MHz): 178.07, 163.66, 153.72, 139.47, 135.50, 128.82, 128.55, 128.33, 127.98, 126.33, 114.11, 106.42, 61.11, 56.35, 44.72. Anal. Calcd. for C₂₀H₂₀N₂O₄S: C 62.48, H 5.24, N 7.29; Found: C 62.24, H 5.32, N 7.19 %.

(Z)-3-Benzyl-5-(4-(benzyloxy)benzylidene)-2-thiohydantoin (12h):
Yield: 75%; mp 199-201°C; ESMS: 401 (M⁺); IR (KBr): 3429, 3038, 2916, 2868, 1727, 1654, 1600, 1510, 1463, 1429, 1378, 1342, 1302, 1244, 1182 cm⁻¹; ¹H NMR (CDCl₃, 300MHz): δ (ppm) 8.13 (d, 2H, J = 8.8 Hz), 7.45-7.29 (m, 10H), 7.00 (d, 2H, J = 8.8 Hz), 6.97 (s, 1H), 5.12 (s, 2H), 4.79 (s, 2H); ¹³C NMR (CDCl₃, 75MHz): 178.11, 164.43, 159.87, 136.32, 135.86, 134.63, 131.80, 128.64, 128.43, 128.16, 127.72, 127.46, 125.52, 124.73, 115.45, 114.20, 70.04, 44.42. Anal. Calcd. for C₂₄H₂₀N₂O₄S: C 71.98, H 5.03, N 6.99; Found: C 71.76, H 5.12, N 6.87 %.

(Z)-3-Benzyl-5-(3,4-dimethoxybenzylidene)-2-thiohydantoin (12i):
Yield: 84%; mp 182-184°C; IR (KBr): 3264, 2928, 1725, 1659, 1596, 1460, 1327, 1217, 1136 cm⁻¹; ¹H NMR (CDCl₃, 300MHz): δ (ppm) 8.98 (bs, 1H), 7.50 (m, 2H), 7.47-7.29 (m, 3H), 7.05 (dd, 1H, J = 7.3, J' = 1.8 Hz), 6.92-6.88 (m, 2H), 6.68 (s, 1H), 5.09 (s, 2H), 3.92 (s, 3H), 3.90 (s, 3H); ¹³C NMR (CDCl₃, 75MHz): 178.11, 164.14, 151.13, 150.01, 135.95, 129.22, 128.95, 128.37, 126.03, 125.48, 122.88, 114.74, 112.96, 112.15, 56.56, 56.47, 45.14. Anal. Calcd. for C₁₉H₁₈N₂O₃S: C 64.39, H 5.12, N 7.90; Found: C 64.22, H 5.17, N 7.86 %.
General procedure for the synthesis of substituted 2-aminoimidazolinones 13(a–i):

To 500 mg of appropriate phenylmethylene-2-thiohydantoin in acetone at 0°C, 1.2 equivalent of Mel and K$_2$CO$_3$ (1.5 eq.) were added. The reaction mixture was slowly brought to room temperature and allow to stir for 4h. After TLC showed completion of reaction, solvent was removed in vacuo and resulting solid was washed with 100 mL of water. Dried thiomethyl derivative was added in the steel bomb containing 10 mL of ethanol and 50 mL of aqueous ammonia. Reaction vessel was made air tight and heated at 120 °C for 8 h. Steel bomb was kept at 0°C for 5h. Crystallized 2-aminoimidazolinone analogue was filtered and residue was recrystallized from EtOH.

(Z)-2-Amino-1-(4-methoxybenzyl)-4-(4-methoxybenzylidene)-1H-imidazol-5(4H)-one (13a):

Yield: 80%; mp 219-220°C; ESMS: 338 (M+1); IR (KBr): 3370, 3020, 1672, 1599, 1476, 1439, 1363, 1254, 1153 cm$^{-1}$; $^1$H NMR (DMSO-d$_6$, 300MHz): $\delta$ (ppm) 8.00 (d, 2H, $J = 12.2$ Hz), 7.49 (bs, 2H), 7.22 (d, 2H, $J = 11.8$ Hz), 6.90 (t, 4H, $J = 10.6$ Hz), 6.38 (s, 1H), 4.70 (s, 2H), 3.76 (s, 3H), 3.70 (s, 3H); $^{13}$C NMR (DMSO-d$_6$, 75MHz): 170.17, 159.26, 159.04, 158.79, 138.99, 132.22, 129.35, 129.08, 128.96, 114.34, 114.29, 113.41, 55.58, 55.52, 41.77. EI-HRMS m/z Calcd. for C$_{16}$H$_{19}$N$_3$O$_3$ [M]$^+$ 337.1426; measured 337.1436.

(Z)-2-Amino-4-(3,4-dimethoxybenzylidene)-1-(~methoxybenzyl)-1H-imidazol-5(4H)-one (13b):

Yield: 77%; mp 176-178°C; ESMS: 368 (M+1); IR (KBr): 3401, 3021, 1716, 1663, 1608, 1513, 1474, 1435, 1330, 1216, 1157cm$^{-1}$; $^1$H NMR (CDCl$_3$, 300MHz): $\delta$ (ppm) 7.69 (s, 1H), 7.38 (dd, 1H, $J = 8.4$, $J' = 1.6$ Hz), 7.16 (d, 2H, $J = 8.4$ Hz), 6.86-6.79 (m, 3H), 6.75 (s, 1H), 4.70 (s, 2H), 3.87 (s, 3H), 3.85 (s, 3H), 3.76 (s, 3H); $^{13}$C NMR (CDCl$_3$, 75MHz): 169.64, 159.56, 158.02, 149.65, 148.83, 136.56, 128.38, 126.87, 124.66, 118.44, 114.61, 113.34, 110.98, 55.94, 55.87, 55.29, 42.50. EI-HRMS m/z Calcd. for C$_{20}$H$_{21}$N$_3$O$_4$ [M]$^+$ 367.1532; measured 367.1579.

(Z)-2-Amino-4-benzylidene-1-(4-methoxybenzyl)-1H-imidazol-5(4H)-one (13c):

Yield: 76%; mp 187-189°C; ESMS: 308 (M+1); IR (KBr): 3380, 3004, 1667, 1624, 1465, 1357, 1258, 1150cm$^{-1}$; $^1$H NMR (DMSO-d$_6$, 300MHz): $\delta$ (ppm) 8.05 (d, 2H, $J = 12.9$ Hz), 7.49 (bs, 2H), 7.22 (d, 2H, $J = 11.8$ Hz), 6.90 (t, 4H, $J = 10.6$ Hz), 6.38 (s, 1H), 4.70 (s, 2H), 3.76 (s, 3H), 3.70 (s, 3H); $^{13}$C NMR (DMSO-d$_6$, 75MHz): 170.17, 159.26, 159.04, 158.79, 138.99, 132.22, 129.35, 129.08, 128.96, 114.34, 114.29, 113.41, 55.58, 55.52, 41.77. EI-HRMS m/z Calcd. for C$_{16}$H$_{19}$N$_3$O$_3$ [M]$^+$ 337.1426; measured 337.1436.
7.3 Hz), 7.67 (bs, 2H), 7.34 (t, 2H, J = 7.3 Hz), 7.23 (d, 3H, J = 8.6 Hz), 6.90 (d, 2H, J = 8.6 Hz), 6.40 (s, 1H), 4.73 (s, 2H), 3.71 (s, 3H); 13C NMR (DMSO-d6, 75MHz): 170.65, 159.98, 159.51, 141.39, 136.73, 131.02, 129.64, 129.50, 129.10, 128.23, 114.81, 113.27, 55.96, 42.28. El-HRMS m/z Calcd. for C18H17N3O2 [M]+ 307.1321; measured 307.1321.

(Z)-2-Amino-1-benzyl-4-(4-methoxybenzylidene)-IH-imidazol-5(4H)-one (13e):

Yield: 74%; mp 198-200°C; ESMS: 308 (M+1); IR (KBr): 3374, 2997, 1712, 1685, 1633, 1597, 1481, 1350, 1250, 1151 cm⁻¹; 1H NMR (DMSO-d6, 300MHz): δ (ppm) 8.01 (d, 2H, J = 11.9 Hz), 7.50 (bs, 2H), 7.30-7.26 (m, 5H), 6.92 (d, 2H, J = 11.4 Hz), 6.39 (s, 1H), 4.79 (s, 2H), 3.76 (s, 3H); 13C NMR (DMSO-d6, 75MHz): 170.60, 159.73, 159.23, 139.35, 137.75, 134.61, 132.67, 129.56, 128.04, 127.84, 114.72, 113.98, 56.02, 42.75. El-HRMS m/z Calcd. for C18H17N3O2 [M+1]+ 308.1399; measured 308.1384.

(Z)-2-Amino-1-benzyl-4-(4-hydroxybenzylidene)-IH-imidazol-5(4H)-one (13f):

Yield: 81%; mp 209-211°C; ESMS: 312 (M+1); IR (KBr): 3385, 3092, 1716, 1680, 1631, 1565, 1474, 1408, 1367, 1271, 1153 cm⁻¹; 1H NMR (DMSO-d6, 300MHz): δ (ppm) 8.09 (d, 2H, J = 8.6 Hz), 7.75 (bs, 2H), 7.41 (d, 2H, J = 8.6 Hz), 7.33 (d, 2H, J = 6.8 Hz), 7.30-7.25 (m, 3H), 6.60 (s, 1H), 4.82 (s, 2H); 13C NMR (DMSO-d6, 75MHz): 175.13, 165.05, 146.65, 142.30, 140.49, 137.26, 136.97, 134.18, 133.88, 133.02, 132.57, 116.43, 47.59. El-HRMS m/z Calcd. for C17H14ClN3O [M]+ 311.0825; measured 311.0833.

(Z)-2-Amino-1-benzyl-4-(3,4,5-trimethoxybenzylidene)-IH-imidazol-5(4H)-one (13g):

Yield: 77%; mp 201-203°C; ESMS: 368 (M+1); IR (KBr): 3423, 2996, 1715, 1667, 1614, 1575, 1472, 1365, 1237, 1131 cm⁻¹; 1H NMR (DMSO-d6, 300MHz): δ (ppm) 7.54-7.49 (m, 4H), 7.31-7.23 (m, 5H), 6.38 (s, 1H), 4.81 (s, 2H), 4.03 (s, 6H), 3.79 (s, 3H); 13C NMR (DMSO-d6, 75MHz): 170.23, 159.13, 153.07, 140.13, 137.94, 137.26, 131.94, 128.99, 127.81, 127.36, 113.49, 108.54, 60.56, 56.36, 42.32. ESI-HRMS m/z Calcd. for C20H21N3O4 [M+1] 368.17290; measured 368.17629.

(Z)-2-Amino-1-benzyl-4-(4-(benzyloxy)benzylidene)-IH-imidazol-5(4H)-one (13h):
Yield: 82%; mp 206-208°C; ESMS: 384 (M+1); IR (KBr): 3367, 3027, 1712, 1684, 1633, 1593, 1478, 1353, 1238, 1152 cm\(^{-1}\); \(^1\)H NMR (DMSO-d\(_6\), 300MHz): \(\delta\) (ppm) 8.03 (d, 2H, \(J = 8.7\) Hz), 7.49-7.24 (m, 12H), 7.02 (d, 2H, \(J = 8.3\) Hz), 6.40 (s, 1H), 5.12 (s, 2H), 4.80 (s, 2H); \(^13\)C NMR (DMSO-d\(_6\), 75MHz): 170.19, 158.85, 158.39, 139.04, 137.34, 132.25, 129.18, 128.99, 128.90, 128.35, 128.27, 127.81, 127.42, 115.15, 113.48, 69.71, 42.32. Anal. calcd. for C\(_{24}\)H\(_{21}\)N\(_3\)O\(_2\): C 75.18, H 5.52, N 10.96; Found: C 74.92, H 5.61, N 11.04%. EI-HRMS m/z Calcd. for C\(_{24}\)H\(_{21}\)N\(_3\)O\(_2\) [M]+ 383.1634; measured 383.1632.

(Z)-2-Amino-1-benzyl-4-(3,4-dimethoxybenzylidene)-IH-imidazol-5(4H)-one (13i): 

Yield: 75%; mp 158-160°C; ESMS: 338 (M+1); IR (KBr): 3397, 3000, 1679, 1627, 1583, 1472, 1328, 1256, 1160 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\), 300MHz): \(\delta\) (ppm) 7.79 (s, 1H), 7.45 (d, 1H, \(J = 8.2\) Hz), 7.37-7.27 (m, 7H), 6.86-6.82 (m, 2H), 4.82 (s, 2H), 3.90 (s, 6H); \(^13\)C NMR (CDCl\(_3\)+CD\(_3\)OD, 75MHz): 169.91, 157.98, 149.62, 137.24, 136.89, 135.05, 129.05, 128.12, 126.88, 124.73, 118.57, 113.11, 110.94, 55.79, 42.84. EI-HRMS m/z Calcd. for C\(_{19}\)H\(_{19}\)N\(_3\)O\(_3\) [M]+ 337.1426; measured 337.1428.

Typical procedure for the synthesis of compound (13d): 

To 250 mg of (Z)-3-benzyl-5-benzylidene-2-thiohydantoin (12d) in 40 mL of acetone at 0°C, 1.1 equivalent of methyl iodide was added. Reacton mixture was slowly brought to rt and stirred for 4h. Then solvent was evaporated and resulted solid was washed with 100 mL of water and dried. Dried thioalkyl derivative was then refluxed in neat benzylamine for 6h. Benzylamine was evaporated under reduced pressure, resulting mixture was taken in 60 mL chloroform and washed with 2% HCl in water. Organic layer was dried with Na\(_2\)SO\(_4\) and solvent was evaporated. Solid so obtained was purified by column chromatography on silica gel and was crystallized from alcohol.

(Z)-1-Benzyl-2-(benzylamino)-4-benzylidene-IH-imidazol-5(4H)-one (13d): 

Yield: 85%; mp 181-183°C; ESMS: 368 (M+1); IR (KBr): 3343, 3064, 2925, 1707, 1658, 1586, 1444, 1373, 1159 cm\(^{-1}\); \(^1\)H NMR (DMSO-d\(_6\), 300MHz): \(\delta\) (ppm) 8.08 (d, 2H, \(J = 7.5\) Hz), 7.38-7.21 (m, 13H), 6.46 (s, 1H), 4.88 (s, 2H), 4.65 (d, 2H, \(J = 5.7\) Hz); \(^13\)C NMR (DMSO-d\(_6\), 75MHz): 170.27, 158.35, 140.32, 139.38, 137.07, 136.23,
130.79, 129.03, 128.79, 128.75, 128.04, 127.97, 127.86, 127.53, 127.20, 113.81, 45.04, 42.25. EI-HRMS m/z Calcd. for C_{20}H_{21}N_{3}O_{4} [M]^+ 367.1732; measured 367.1757.

Typical procedure for synthesis of compound (14):
To a 50 mL oven dried two necked round bottomed flask, 250 mg of (12b), 15 mL dioxane and 3.3 equivalent of LiBH₄ was added under argon atmosphere. Reaction mixture was refluxed for 1h and cooled to 0°C. 5 mL of 5 N acetic acid was added to the reaction mixture and stirred for 2h at rt. Reaction solvent was removed under reduced pressure, neutralized with NaHCO₃ and extracted with CHCl₃. Purification of the resulting solid afforded compound (14).

4-(3,4-Dimethoxybenzyl)-1-(4-methoxybenzyl)-1H-imidazole-2(3H)-thione (14):
Yield: 67%; semisolid; ESMS: 371 (M+1); IR (KBr): 3386, 2926, 1607, 1513, 1431, 1354, 1216 cm⁻¹; ¹H NMR (CDCl₃, 300MHz): δ (ppm) 7.27 (d, 2H, J = 8.4 Hz), 6.88 (d, 2H, J = 8.4 Hz), 6.81-6.71 (m, 3H), 6.22 (s, 1H), 5.09 (s, 2H), 3.86 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 3.71 (s, 2H); ¹³C NMR (CDCl₃, 75MHz): 159.45, 149.03, 147.99, 129.68, 129.27, 129.10, 128.02, 120.83, 114.19, 114.06, 111.98, 111.34, 111.24, 55.92, 55.29, 49.78, 30.89.

General procedure for direct reduction and dehydration of 2-aminoimidazolinones 15(a–i):
To a 50 mL oven dried two necked round bottomed flask, 150 mg of 2-aminoimidazol-4-one, 3.3 equivalent of LiBH₄, 15 mL of dry dioxane was added under argon atmosphere. The reaction mixture was refluxed for 75 minutes and then cooled to 0°C. To the cooled reaction mixture 3 mL of 2N HCl was added with stirring and temperature was slowly brought to room temperature in 1h. Solvent was removed under reduced pressure, reaction mixture was neutralized with saturated NaHCO₃ slution and extracted with chloroform. Resulting solid was purified by column chromatography using neutral alumina as adsorbent and 0.2 % of (10% aq. NH₃) in DCM as eluent.

1,4-Bis(4-methoxybenzyl)-1H-imidazol-2-amine (15a):
Yield: 51%; semisolid; ESMS: 324 (M+1); IR (KBr): 2995, 2853, 1606, 1511, 1443, 1354, 1299, 1247, 1177 cm⁻¹; ¹H NMR (CDCl₃, 300MHz): δ (ppm) 7.20 (d, 2H, J =
8.5 Hz), 7.08 (d, 2H, J = 8.6 Hz), 6.89-6.81 (m, 4H), 6.15 (s, 1H), 4.74 (s, 2H), 3.80 (s, 3H), 3.78 (s, 3H), 3.72 (s, 2H);\(^{13}\)C NMR (CDCl\(_3, 75\)MHz): 159.35, 157.92, 147.51, 139.28, 137.02, 132.22, 129.82, 128.28, 127.99, 114.40, 113.92, 113.75, 112.30, 55.30, 55.24, 48.10, 33.83. ESI-HRMS \(m/z\) Calcd. for C\(_{19}\)H\(_{22}\)N\(_3\)O\(_2\) [M+H]\(^{+}\) 324.1712; measured 324.1728.

4-(3,4-Dimethoxybenzyl)-1-(4-methoxybenzyl)-1\(H\)-imidazol-2-amine (15b):

Yield: 42%; semisolid; ESMS: 354 (M+1); IR (KBr): 3386, 3021, 2926, 1607, 1513, 1431, 1354, 1216 cm\(^{-1}\);\(^{1}\)H NMR (CDCl\(_3, 300\)MHz): \(\delta\) (ppm) 7.08 (d, 2H, J = 8.6 Hz), 6.89-6.76 (m, 5H), 6.17 (s, 1H), 4.75 (s, 2H), 3.84 (s, 6H), 3.79 (s, 3H), 3.72 (s, 2H);\(^{13}\)C NMR (CDCl\(_3, 75\)MHz): 159.38, 148.78, 147.47, 147.33, 137.12, 132.86, 128.27, 128.05, 120.75, 114.40, 114.06, 112.36, 112.30, 111.24, 55.91, 55.78, 55.30, 48.11, 34.36. ESI-HRMS \(m/z\) Calcd. for C\(_{20}\)H\(_{21}\)N\(_3\)O\(_4\) [M+H]\(^{+}\) 354.18177; measured 354.17937.

4-Benzyl-1-(4-methoxybenzyl)-1\(H\)-imidazol-2-amine (15c):

Yield: 52%; semisolid; ESMS: 294 (M+1); IR (KBr): 3337, 2925, 2854, 1611, 1514, 1457, 1353, 1250, 1177 cm\(^{-1}\);\(^{1}\)H NMR (CDCl\(_3, 300\)MHz): \(\delta\) (ppm) 7.07 (d, 2H, J = 8.6 Hz), 6.88 (d, 2H, J = 8.6 Hz), 6.17 (s, 1H), 4.73 (s, 2H), 3.81 (s, 3H), 3.74 (s, 2H);\(^{13}\)C NMR (CDCl\(_3, 75\)MHz): 159.80, 147.99, 140.56, 139.70, 137.11, 129.29, 129.17, 129.04, 128.72, 128.39, 126.42, 114.84, 114.50, 114.40, 112.90, 55.73, 48.50, 35.21. El-HRMS \(m/z\) Calcd. for C\(_{18}\)H\(_{20}\)N\(_3\)O [M+H]\(^{+}\) 294.16064; measured 294.16079.

N,1,4-tribenzyl-1\(H\)-imidazol-2-amine (15d):

Yield: 55%; semisolid; ESMS: 354 (M+1); IR (KBr): 3332, 3030, 2923, 1588, 1445, 1355, 1159 cm\(^{-1}\);\(^{1}\)H NMR (CDCl\(_3, 300\)MHz): \(\delta\) (ppm) 7.36-7.28 (m, 5H), 7.16 (d, 2H, J = 6.3 Hz), 6.13 (s, 1H), 4.81 (s, 2H), 4.49 (d, 2H, J = 5.7 Hz), 3.91 (s, 2H);\(^{13}\)C NMR (CDCl\(_3, 75\)MHz): 149.48, 139.64, 138.06, 136.65, 129.50, 129.44, 128.92, 128.68, 128.36, 128.23, 127.71, 127.15, 126.40, 113.33, 48.88, 48.56. ESI-HRMS \(m/z\) Calcd. for C\(_{18}\)H\(_{20}\)N\(_3\)O [M+H]\(^{+}\) 354.19702; measured 354.19685.

1-Benzyl-4-(4-methoxybenzyl)-1\(H\)-imidazol-2-amine (15e):

Yield: 52%; semisolid; ESMS: 294 (M+1); IR (KBr): 2924, 2854, 1599, 1442, 1354, 1219 cm\(^{-1}\);\(^{1}\)H NMR (CDCl\(_3, 300\)MHz): \(\delta\) (ppm) 7.35-7.28 (m, 5H), 7.16 (d, 2H, J =
8.7 Hz), 6.83 (d, 2H, J = 8.7 Hz), 6.21 (s, 1H), 4.89 (s, 2H), 3.77 (s, 3H), 3.71 (s, 2H); 13C NMR (CDCl₃, 75MHz): 159.67, 139.26, 132.37, 130.93, 129.84, 128.28, 128.09, 127.02, 126.86, 123.91, 123.39, 115.86, 114.07, 113.80, 112.39, 55.23, 48.58, 34.78. ESI-HRMS m/z Calcd. for C₁₈H₂₀N₂O [M+H]+ 294.16064; measured 294.15748.

1-Benzyl-4-(4-chlorobenzyl)-1H-imidazol-2-amine (15f):

Yield: 55%; semisolid; ESMS: 298 (M+1); IR (KBr): 3311, 3066, 1604, 1444, 1353 cm⁻¹; ¹H NMR (CDCl₃, 300MHz): δ (ppm) 7.28-7.02 (m, 9H), 6.08 (s, 1H), 4.72 (s, 2H), 3.63 (s, 2H); ¹³C NMR (CDCl₃, 75MHz): 147.81, 138.64, 136.21, 135.98, 131.78, 130.22, 129.07, 128.40, 128.08, 126.85, 112.60, 48.57, 34.10. ESI-HRMS m/z Calcd. for C₁₇H₁₆ClN₂ [M+H]+ 298.11174; measured 298.11110.

1-Benzyl-4-(3,4,5-trimethoxybenzyl)-1H-imidazol-2-amine (15g):

Yield: 46%; semisolid; ESMS: 368 (M+1); IR (KBr): 3424, 2998, 1718, 1668, 1612, 1571, 1472, 1365, 1241, 1132 cm⁻¹; ¹H NMR (CDCl₃, 300MHz): δ (ppm) 7.38-7.18 (m, 5H), 6.50 (s, 2H), 6.18 (s, 1H), 4.92 (s, 2H), 3.87 (s, 6H), 3.82 (s, 3H), 3.73 (s, 2H); ¹³C NMR (CDCl₃, 75MHz): 153.54, 147.92, 139.70, 135.99, 135.59, 129.52, 128.66, 127.39, 114.50, 112.86, 106.19, 61.23, 56.44, 49.08, 34.25. ESI-HRMS m/z Calcd. for C₂₀H₂₃N₂O [M+H]+ 353.1739; measured 353.1748.

1-Benzyl-4-(4-(benzyloxy)benzyl)-1H-imidazol-2-amine (15h):

Yield: 54%; semisolid; ESMS: 370 (M+1); IR (KBr): 3034, 2855, 1604, 1505, 1446, 1238, 1174 cm⁻¹; ¹H NMR (CDCl₃, 300MHz): δ (ppm) 7.33-7.15 (m, 7H), 7.07-7.03 (m, 5H), 7.01 (d, 2H, J = 6.3 Hz), 6.01 (s, 1H), 4.91 (s, 2H), 4.69 (s, 2H), 3.59 (s, 2H); ¹³C NMR (CDCl₃, 75MHz): 157.23, 147.67, 137.23, 136.83, 136.00, 129.84, 129.06, 128.54, 128.05, 127.89, 127.48, 126.87, 114.76, 112.45, 70.02, 48.53, 33.77. ESI-HRMS m/z Calcd. for C₂₄H₂₄N₂O [M+H]+ 370.19194; measured 370.19576.

1-Benzyl-4-(3,4-dimethoxybenzyl)-1H-imidazol-2-amine (15i):

Yield: 48%; semisolid; ESMS: 324 (M+1); IR (KBr): 3395, 2998, 1678, 1627, 1583, 1471, 1326, 1254, 1151 cm⁻¹; ¹H NMR (CDCl₃, 300MHz): δ (ppm) 7.35-7.12 (m, 6H), 6.86-6.82 (m, 2H), 6.20 (s, 1H), 4.82 (s, 2H), 3.83 (s, 3H), 3.77 (s, 3H), 3.71 (s, 2H); ¹³C NMR (CDCl₃, 75MHz): 157.81, 149.21, 148.04, 139.69, 136.30, 132.87, 129.47.
Antimastigote activity: Please see section 3.6.2.

Cytotoxicity assay: Please see section 3.6.3.

Antibacterial and antifungal evaluation:

The bacterial strains were grown on Sabroaud dextrose agar and nutrient agar media respectively. After the incubation fungal and bacterial growth were suspended in normal saline and maintained at 1.0–5.0×10³ cfu/mL. The activity of compounds was determined by the NCCLS method for fungus using RPMI-1640 media buffered with MOPS (3-[N-morpholino]propanesulfonic acid) (Sigma-Aldrich Company) and Mueller Hinton broth for bacteria. The 96-well tissue culture plates were used for twofold serial dilution. The proper growth control, drug control and the blank were adjusted onto the plate. Compounds were dissolved in DMSO at a concentration of 1 mg/mL and 20 μL of this was added to 96-well tissue culture plate having 180 μL RPMI-1640 so the maximum concentration of the compound became 50 μg/mL. From here the solution was serially diluted resulting into the half of the concentration of test compounds and then inoculum was added and kept for incubation. Micro-titer plates were incubated at 35°C in a moist, dark chamber and MICs were recorded spectrophotometrically.

4.2.8 References:


