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Synthesis and evaluation of novel 1,3,4-oxadiazole derivatives of marine bromopyrrole alkaloids as antimicrobial agent

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ABSTRACT

In an attempt to identify new potential lead as antimicrobial agent, twenty hybrids of marine bromopyrrole alkaloids with 1,3,4-oxadiazole were designed based on molecular hybridization technique and synthesized. Synthesized molecules were evaluated for their antibacterial, antifungal and antitubercular activities. Hybrids **5d**, **5i**, **5j** and **5k** exhibited equivalent antibacterial activity (MIC of 1.56 μg/mL) compared with standard drug ciprofloxacin against *Staphylococcus aureus* and *Escherichia coli*. Equal antifungal activity (MIC of 1.56 μg/mL) was shown by of hybrids **5j**, **5k** and **7d** compared with standard Amphotericin-B. The inhibition of *Mycobacterium tuberculosis* at concentrations as low as 1.6 and 1.5 μg/mL by compounds **5f** and **7d** respectively indicates that these compounds can act as leads for development of newer anti-TB compounds.

1,3,4-Oxadiazole, a privileged structure, endows its derivatives with broad and potent biological functions.[1] These includes anti-inflammatory,[2] hypoglycemic,[3] antianxiety,[4] antidepressant activities.[5] Recently derivatives of 1,3,4-oxadiazole have been reported for their antiproliferative,[6] antifungal,[7,8] antibacterial[9] and antitubercular activities.[10] Molecular modeling and pharmacokinetic studies have demonstrated that the introduction of 1,3,4-oxadiazole ring to the inhibitors can change their polarity, flexibility as well as metabolic stability, and 1,3,4-oxadiazole scaffold can also act as acceptors of hydrogen bonds formation, which make it possible to be used as a isosteric substituent for amide or ester groups.[11] We therefore are interested in exploring the biological activity of such molecules through structural modifications.

Heterocycles containing pyrrole ring system are found to exhibit wide spectrum of biological activities. Our interest in halogenated pyrrole derivatives led to synthesis and antimicrobial evaluation of few analogues of pyolueterins.[12] In continuation to this we found that bromopyrrole alkaloids; a family of marine alkaloids represents a fascinating example of the large variety of secondary metabolites formed by marine sponges. Most of these alkaloids are defined by the signature 4,5-dibromopyrrole ring fall under the oroidin class of alkaloids, defined by the signature bromopyrrole carboxamide.[13] Many of these compounds are reported to have intriguing biochemical activities, such as blocking α-adrenoceptors, antihistamine, antagonist of serotonergic receptors, activating actomyosin ATPase, inhibiting kinase activity, antineoplastic activity.[12–14] Moreover these natural products are reported to be antitubercular, antibacterial, antifungal and inhibitors of enoyl-ACP reductase[13–15] (Fig. 1a).

In view of the above-mentioned findings, the purpose of the present work was to design and synthesize novel series of bromopyrrole alkaloids derivatives by molecular hybridization of 4,5-dibromopyrrole scaffold with pharmacologically privileged scaffold 1,3,4-oxadiazole. Further we want to evaluate effect of replacement of amide and ester linkage present in most of these alkaloids with its bioisoter, 1,3,4-oxadiazole ring. Another objective of the study was to evaluated effect of various substituent attached to 1,3,4-oxadiazole ring like SH group, *S*-alkyl/aryl, substituted aromatic or heteroaromatic ring on antimicrobial and antitubercular activity (Fig. 1b).

In the present work, proposed hybrids were synthesized utilizing the reaction sequence as shown in Scheme 1. Trichloroacetylation of 1*H*-pyrrole using equimolar quantity of trichloroacetyl chloride gave an excellent yield of 2-trichloroacetylpyrrole **2**.[16] Bromination of **2** using 2 equiv of bromine in chloroform gave 4,5-dibromo-2-trichloroacetyl-1*H*-pyrrole **3** in excellent yield.[17] 4,5-Dibromo-2-trichloroacetyl-1*H*-pyrrole **3** on stirring with excess of hydrazine hydrate at room temperature gave its acid hydrazide derivative **4** in good yield. Compound **4** on condensation with equimolar quantities different aromatic and heteroaromatic acids in presence of excess of phosphorus oxychloride at reflux condition gave 2-(4,5-dibromo-1*H*-pyrrol-2-yl)-5-aryl-1,3,4-oxadiazoles **5**. 5-(4,5-Dibromo-1*H*-pyrrol-2-yl)-1,3,4-oxadiazole-2-thiol **6** was synthesized by reaction of **4** with carbon disulfide and alcoholic potassium hydroxide at reflux condition. Compound **6** was S-alkylated by reaction with different aryl/alkyl halides in alcoholic sodium hydraxide to give S-alkylated derivatives **7** (Scheme 1).

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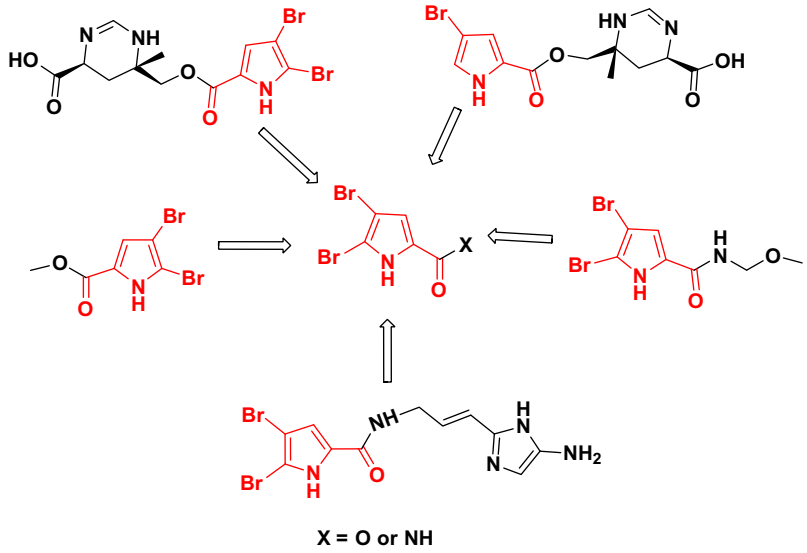


Figure 1a. Reported bromopyrrole alkaloids.

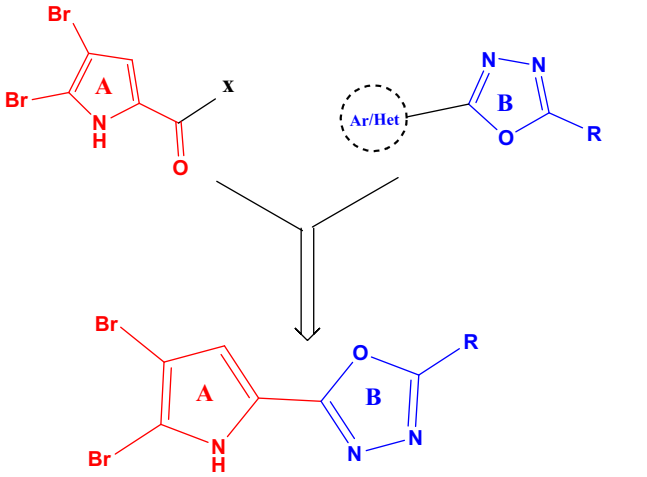


Figure 1b. Design of novel 2-(4,5-dibromopyrrol-2-yl)-5-substituted-1,3,4-oxadiazoles using molecular hybridization approach.

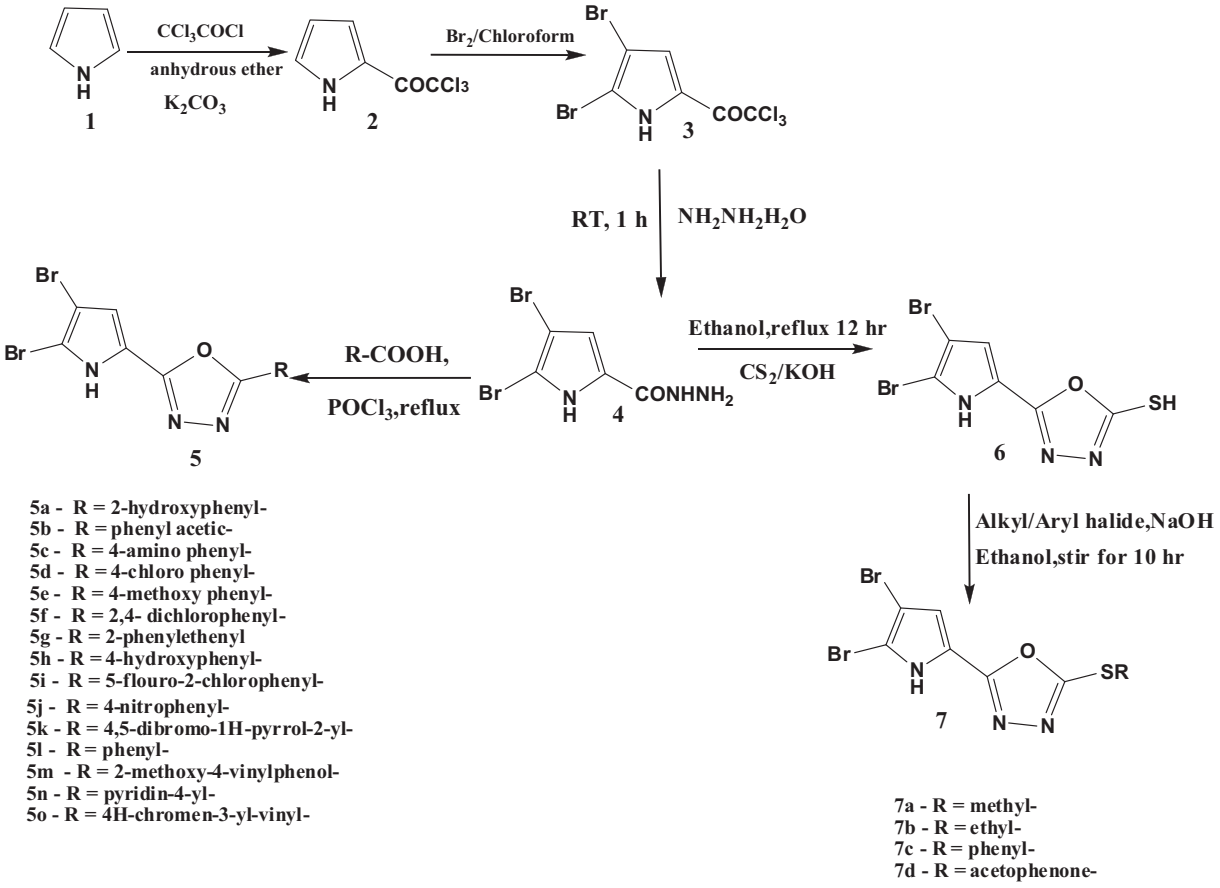
The spectral data (IR, ^{1}H NMR and MS) of all synthesized compounds were in agreement with the proposed structures. The MS of all the compounds exhibited the [M+·] as molecular ion peak, confirming the molecular weight. Bromination of pyrrole ring was confirmed by the presence of [M+2] peak in mass spectra. ^{1}H NMR spectra of these hybrids showed singlet peak of pyrrole-NH proton in the range of 13.5–13.4 ppm. Thiol group of **6** showed NMR peak at 3.8 ppm. The proton of aryl substitutions attached to oxadiazole ring resonated in between 8.2 and 6.1 ppm. 5-(4,5-Dibromo-1-methyl-1*H*-pyrrol-2-yl)-1,3,4-oxadiazole-2-thiol **6** showed peculiar peaks at 2751 cm^{-1} of –SH group. The alkylated product, 5-(4,5-dibromo-1-methyl-1*H*-pyrrol-2-yl)-1,3,4-oxadiazole-2-methylthiol did not have the peak of –SH group at 2751 cm^{-1}. Also the product showed peak at 711.9 cm^{-1} of *S*-alkyl group.

The newly prepared compounds were screened for their antibacterial activity against *Escherichia coli* (ATCC-25922), *Staphylococcus aureus* (ATCC-25923), *Pseudomonas aeruginosa* (ATCC-27853), and *Klebsiella pneumoniae* (recultured) bacterial strains. Minimum inhibitory concentrations (MICs) were determined by broth dilution technique.[18,19] The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls were inoculated with approximately 5×10^5 CFU/mL of actively dividing bacteria cells. The cultures were incubated for 24 h at 37 °C and the growth was monitored visually and spectrophotometrically. Ciprofloxacin was used as a standard drug. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC). The minimum inhibitory concentrations are given in Table 1.

The newly prepared compounds were screened for their antifungal activity against *Candida albicans* in DMSO by agar dilution method[20,21] The nutrient broth, which contained logarithmic serially twofold diluted amount of test compound and controls inoculated with approximately 1.6×10^4–6×10^4 CFU/mL, was used. The cultures were incubated for 48 h at 35 °C and the growth was monitored. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as minimum inhibitory concentration (MIC). The fungal activity of each compound was compared with Amphotericin-B as standard drug. The fungal zone of inhibition and minimum inhibitory concentration values are given in Table 1.

The synthesized compounds were screened against *Mycobacterium tuberculosis* $H_{37}Rv$ in order to determine the minimum inhibitory concentration (MIC) with Resazurin microtiter assay (REMA).[22] Homogenous mycobacterial ($H_{37}Rv$) culture suspension was seeded in microtitre plates at density of 10^5 cells per well in 100 μL of the Middlebrook 7H9 broth (Difco laboratories, Detroit, MI, USA) and the test compounds were serially diluted directly on the plate. The control received equivalent amount of DMSO. The plates were incubated at 37 °C for 7 days. Freshly prepared resazurin dye (0.02%) was added and plates were again incubated for 48 h. Isoniazid was used as the reference drug. MIC is the lowest concentration at which complete inhibition was observed and was determined by visual inspection (colour change from blue to pink) (Table 1).

The investigation of antimicrobial screening revealed that synthesized hybrids showed some promising antibacterial, antitubercular and antifungal activity. Antibacterial activities of these hybrids exhibited broader spectrum of activities against both gram-positive and gram-negative bacteria. Hybrids **5c**, **5d**, **5i**, **5j** and **5k** showed equivalent antibacterial activity (MIC of 1.56 μg/mL) compared with standard ciprofloxacin against *E. coli*. Presence



Scheme 1. Synthesis of 2-(4,5-dibromopyrrol-2-yl)-5-substituted-1,3,4-oxadiazoles.

Table 1
Antimicrobial test results: (MIC μg/mL)

S. No.	*E. coli*[a]	*S. aureus*[a]	*P. aeruginosa*[a]	*K. pneumoniae*[a]	*C. abilcans*[b]	Antitubercular activity[c]
5a	3.125	1.56	12.5	100	6.25	36.50
5b	3.125	3.125	12.5	100	12.5	145.80
5c	1.56	3.125	6.25	100	12.5	16.50
5d	1.56	1.56	6.25	100	12.5	9.50
5e	3.125	3.125	6.25	100	12.5	56.50
5f	12.5	6.25	6.25	100	12.5	1.60
5g	12.5	3.125	6.25	100	12.5	78.50
5h	12.5	12.5	6.25	100	12.5	25.00
5i	1.56	1.56	6.25	100	12.5	6.50
5j	1.56	1.56	6.25	100	1.56	9.00
5k	1.56	1.56	6.25	100	1.56	3.50
5l	12.5	6.25	6.25	100	12.5	98.70
5m	12.5	1.56	12.5	100	12.5	112.50
5n	12.5	1.56	12.5	100	12.5	3.50
5o	3.125	1.56	12.5	100	12.5	2.00
6	12.5	12.5	12.5	100	12.5	54.50
7a	12.5	12.5	25	100	12.5	89.50
7b	12.5	12.5	25	100	12.5	118.00
7c	12.5	12.5	25	100	6.25	23.50
7d	3.125	12.5	12.5	100	1.56	1.50
Standard[X]	1.56	1.56	3.125	1.56	1.56	0.40

X = (Standard) ciprofloxacin for antibacterial study, Amphotericin-B for antifungal study and Isoniazid for antitubercular study.
[a] MIC values are determined by broth dilution method (twofold dilution).
[b] MIC values are determined by agar dilution method (twofold dilution).
[c] MIC values are determined by Resazurin microtiter assay (REMA).

of electron donating groups like amino, chloro, flouro and withdrawing groups like nitro on aromatic ring seem to be important for activity against *E. coli*. For gram-positive *S. aureus*, hybrids **5a**, **5d**, **5i**, **5j**, **5k**, **5m**, **5n** and **5o** showed equivalent activity at MIC of 1.56 μg/mL compared with standard ciprofloxacin. However hybrid **6**, **7a**, **7b**, **7c**, **7d** containing thiol group or *S*-alkyl/aryl groups are found to be less active especially against *S. aureus* in comparison with other derivatives. All compounds were found to be less active

against *K. pneumonia* while hybrids **5c**, **5d**, **5e**, **5f**, **5g**, **5h**, **5i**, **5j**, **5k**, **5l** were found to be moderately active against *P. aeruginosa* (MIC of 6.25 μg/mL). Among the tested compounds best antifungal activity was showed by three hybrids **5j**, **5k**, **7d** (MIC of 1.56 μg/mL) equivalent to standard Amphotericin-B.

Promising antitubercular activity was displayed by hybrids **5f**, **5k**, **5n**, **5o** and **7d** at MIC of 1.60, 3.50, 3.50, 2.00 and 1.50 μg/mL respectively against *M. tuberoculosis*. Highest activity showed by **7d** indicated that addition of *S*-aroyl substitution at 5-position of 1,3,4-oxadiazole ring leads increased antitubercular activity compared to free SH, or *S*-alkyl or *S*-aryl substitutions. Replacement of heterocyclic rings like 4,5-dibromopyrrole, pyridine-4-yl and 4*H*-chromen-3yl-vinyl instead of aryl ring at 5-position of 1,2,4-oxadiazole resulted in to enhancement of antitubercular activity (**5k**, **5n**, **5o** MIC of 3.50, 3.50 and 2.00 μg/mL).

In summary, 20 new compounds based on the molecular hybridization of bromopyrrole alkaloids with 1,3,4-oxadiazole were synthesized and evaluated for their antimicrobial activity. The synthesized hybrids showed promising activity against *S. aureus*, *E. coli* and Mtb. Equal antifungal activity show by of hybrids **5j**, **5k** and **7d** with standard Amphotericin-B indicated their potential for further lead development. The inhibition of Mtb at concentrations as low as 1.6 and 1.5 μg/mL by compounds **5f** and **7d** respectively indicates that these compounds can act as leads for development of newer anti-TB compounds.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.08.061.

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