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Substituted Pyrazolo[3,4-B]Pyridin-3-Ones and Pyrazolo[3,4-B]Pyridine-5-Carbaldehyde, New One-Pot Synthesis Strategy Amelioration Using Vinamidinium Salts, Antibacterial and Antifungal Activities Promising Environmental Protection

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Abstract

This review summarizes the current knowledge about the syntheses and biological behavior of some pyrazolo[5,4-b]pyridines. So, an expedient method for the synthesis of 2-phenyl-5-aryl-1,6-dihydropyrazolo[3,4-b]pyridin-3-ones and 2-phenyl-3-oxo-1,6-dihydropyrazolo[3,4-b]pyridine-5-carbaldehyde in a single-step via condensation of vinamidinium salts with 3-amino-1-phenyl-2-pyrazolin-5-one is described according to our recent study with synthesis strategy amelioration. Five derivatives (PYR_{1-5}) were prepared and characterized by spectral data (NMR and MS spectral studies). All the derivatives were studied for their antimicrobial activities. So, we screened for antibacterial effects at 1:2, and 1:4 dilutions by the paper disc diffusion method against respectively Bacteria (*Escherichia coli* CIP 53126 and *Bacillus subtilis* CIP 5262) and Fungi (*Candida albicans* ATCC 10231). Then, we screened for Fungi (*Aspergillus niger* ATCC 16404) by the dilution method (agar and liquid broth). The agar diffusion method (paper disc and well) and the dilution method (agar and liquid broth) are described.

Keywords: Pyrazolopyridines, Antimicrobial activity assays, Pyrazolo [3,4-b] pyridines, Vinamidinium salts, 3-amino-1-phenyl-2-pyrazolin-5-one

Introduction

Fungi of the genus *Aspergillus* are common contaminants of food and feed stuffs. Many species are saprobes and are found in a variety of habitats and are ubiquitous agents of decay. Several *Aspergillus* species such as *Aspergillus niger* have been found associated with production of mycotoxins of public health importance [1]. As such, the occurrence of *Aspergillus* secondary metabolites in food stuffs is becoming an increasing environmental concern. So, in order to effectively reduce the infection of food stuffs by fungi especially, toxigenic *Aspergillus* (*Aspergillus niger*), it is important to identify new compounds, as rapidly as possible, able to inhibit fungal activity and consequently protect our environmental [2].

In our interest in the development of synthetic strategies to obtain functionalized heterocycles, [3-8] we have concentrated much of our recent efforts in the preparation of such bioactive nitrogen-containing heterocycles, and have already reported simple and efficient procedures to reach interesting molecules such as pyridin-2-ones [9], isoxazolo[5,4-b] pyridines [10], pyrido[2,3-d] pyrimidines [11], 2-amino-5-arylpyridine-3-carbonitrile [12] and pyrazolo[3,4-b]pyridin-3-ones [13] using vinamidinium salts, with biological properties as shown in Figure 1 [14-19]. Then, the simple fact that the vast majority of currently used drugs are heterocyclic compounds has encouraged a lot of researchers to synthesize analogous pyrazolopyridines derivatives in order to find new molecules with higher activities and consequently optimize their pharmacological profile. The biological properties of pyrazolopyridines heterocycles are very promising and many of them have been studied, especially as antitumor agents. This review is mainly focused on the synthesis of various types of nitrogen containing heterocyclic pyrazolopyridines for their potential use as future drugs in medicine and in addition discusses their overall biological activities. Recently, just one team reported an antibacterial activities study of diarilpyrazolo [3,4-b] piridines. Eleven derivatives was tested against six different bacterial strains; *Staphylococcus aureus* (gram +), *Pseudomonas* (gram–), *Escherichia coli* (gram–) and *Klebsiella* (gram–).

Only four compounds showed moderate activity against *S. aureus, Klebsiella, Pseudomonas* and *E. coli* [20]. The global threat of antimicrobial resistance is a serious matter under review by the WHO and many countries throughout the world [21-24]. Currently, the widespread selective pressure and the efficient dissemination channels for multidrug-resistant organisms are major factors that may have contributed to the rapid emergence of the resistant organisms [21-25].

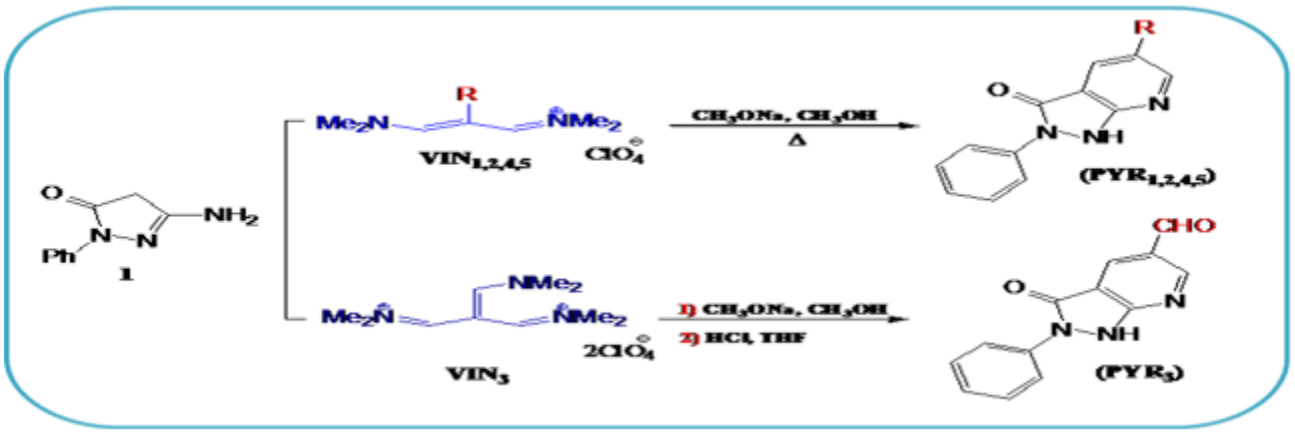

Figure 1: Synthesis of substituted pyrazolo [3,4-b]pyridines derivatives PYR_{1-5}

Fused heterocyclic containing pyrazolopyridine systems have been described associated with several biological and medicinal activities including anxiolytic [26], antiviral [27,28], antileishmanial [29] and anti-inflammatory [30] profiles [31]. However, pyrazolo[3,4-b] pyridine derivatives have been studied as antiviral [32], potential antimalarial agents [33], compounds inhibiting cholesterol formation[34], for the treatment of Alzheimer's disease, gastrointestinal diseases, anorexia nervosa, drug and alcohol withdrawal symptoms, drug addiction and infertility [35]. They have also been reported as potent and selective inhibitors of A1 adenosine receptors [36] and phosphodiesterase 4 (PDE4) inhibitors in immune and inflammatory cells [37]. This review is the first of its kind to investigate the antimicrobial activity of various pyrazolo [3,4-b]pyridines reported until now. This review is a concerted effort to highlight design, synthetic strategies as well as biological activities of this class of condensed heterocycles. Structure-activity relationship studies and in silico approaches wherever reported have also been discussed.

Material and Methods

General experimental procedure for the preparation of PYR_{1-5}

To a flame dried one-necked round-bottomed flask equipped with magnetic stirring, reflux condenser and nitrogen atmosphere was added vinamidinium salt VIN_{1-5} (0.48 mmol), 3-amino-1-phenyl-2-pyrazolin-5-one 1 (0.48 mmol), sodium methoxide (0.96 mmol) and anhydrous methanol (6 mL). The mixture was allowed to reflux overnight under a nitrogen atmosphere in an oil bath. The flask was cooled to room temperature and the obtained precipitate was filtered off to give substituted pyrazolo [3,4-b] pyridines derivatives PYR_{1-5}, neither Work-up nor purification. For pyrazolo [3,4-b] pyridine PYR_3, after evaporation of the solvent, THF (6 mL) and 1 N HCl (6 mL) were added. The mixture was allowed to stir at room temperature for 2 h and filtration as above gave directly PYR_3.

All products were characterized from their ^1H NMR, ^{13}C NMR and mass spectroscopic data. The chemical structures are summarized in Figure 2, in which the five synthesized compounds are illustrated.

In vitro antibacterial and antifungal assay

Antimicrobial screening of the substituted pyrazolo [3,4-b]pyridines derivatives (PYR1-5) was performed by the Agar well diffusion method [38] and the dilution method (agar and liquid broth). The antimicrobial activity was evaluated using two different laboratory control strains of bacteria, i.e., Gram positive Bacillus subtilis CIP 5262, Gram negative, Escherichia coli CIP 53126. Two fungal strains were Aspergillus niger ATCC 16404 and Candida albicans ATCC 10231. Fungal strains were procured from the culture maintained at Pasteur Institute of Tunis. All tests were triplicates in Muller Hinton broth for the bacterial strains and were swabbed on sterile Muller Hinton agar plates using sterile cotton swab followed by punching wells of 6 mm with the help of sterile cork borer. Overnight broth cultures of each strain were prepared, and the final concentration in each well was adjusted to (20, 10 and 5 µM) concentration against both bacterial and fungal strains. DMSO was used as a vehicle. Spiramycin (20, 10 and 5 µM), Streptomycin (20, 10 and 5 µM) and Fluconazole (20, 10 and 5 µM) were used as standard drugs for comparison of antibacterial and antifungal activities respectively. The zone of inhibition was measured after 24 h of incubation at 37°C for antibacterial activity and 72 h at 25°C for antifungal activity.

Statistical analysis

Data of antibacterial and antifungal activity assays were subjected to one-way analysis of variance (ANOVA) using the SPSS 21 Difference between means were tested through Student-Newman-Keuls test and values of $P \leq 0.05$ were considered significantly different.

Results and Discussion

Antibacterial and antifungal activities of substituted pyrazolo [3,4-b]pyridines derivatives PYR1-5

The pyrazolopyridines compounds were tested for antibacterial and antifungal activities in vitro against respectively Bacteria (Escherichia coli CIP 53126 and Bacillus subtilis CIP 5262) and Fungi (Candida albicans ATCC 10231 and Aspergillus niger ATCC 16404). According statistical analysis, all tested compounds showed that pyrazolopyridines significantly reduced the growth of all tested Fungi

and Bacteria in dose-dependent manner (Tables 1-5). The five substituted derivatives exhibited different inhibition effects on the growth of Fungi and Bacteria. In general, there was a correlation between the antibacterial, antifungal activities and structures.

Despite significant progresses in antimicrobial therapy, infectious diseases caused by bacteria and fungi remain a major worldwide health problem because of the rapid development of resistance to existing antimicrobial drugs. Therefore, there is a constant need for new antimicrobial agents. There are a large number of heterocyclic derivatives containing nitrogen atoms that possess a broad spectrum of biological activities including pyridine and pyrazole, which are two of the most important heterocycles in medicinal chemistry.

Refer to the Ibrahim et al. [39] works new pyridine derivatives are synthesized. Most of these compounds were tested and evaluated as antimicrobial agents and the results indicated that many of the obtained compounds exhibited high antimicrobial activity comparable to ampicillin, which was used as the reference compound. Most of the synthesized compounds were found to exhibit strong inhibitory effects on the growth of Gram-positive bacteria especially Bacillus subtilis CIP 5262. Furthermore, a large number of structurally diverse natural compounds containing azole nucleus constitute an important class of biologically active heterocycles that are gaining more attention in the field of medicinal chemistry. Among azoles, pyrazoles are rarely found in nature probably due to difficulty in the formation of N-N bond by living organisms. However, they exhibit numerous biological activities, including anti-diabetic, antiviral, anticancer, anti-inflammatory, antibacterial and antifungal activities. Therefore, Kumar et al. review, for example, [40] was an attempt to understand the chemistry along with medicinal importance of pyrazole containing natural products reported till date which would certainly help the scientific community to bring further developments in the isolation and synthetic methodologies for pyrazole based novel bioactive compounds.

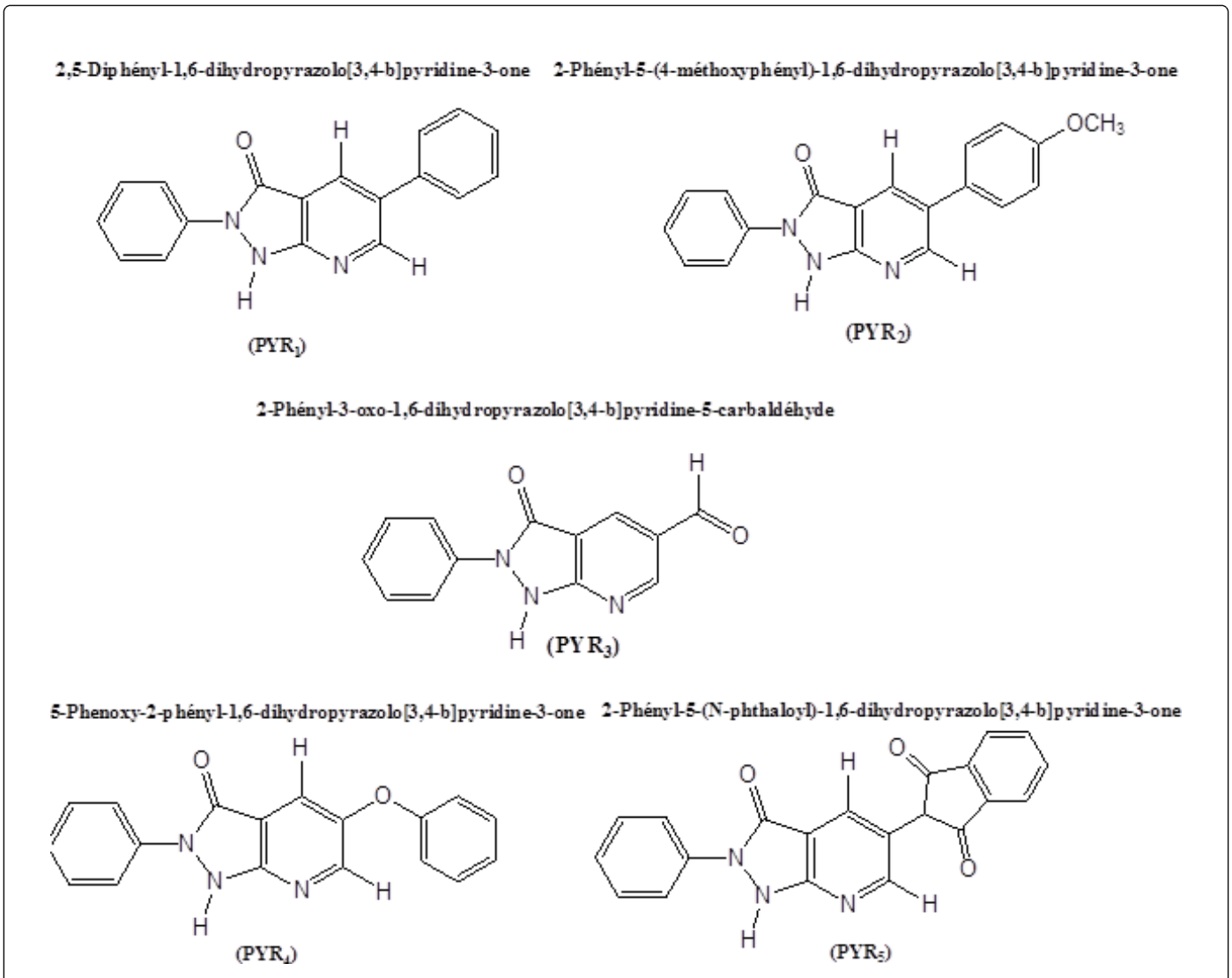

Figure 2: Pyrazolo [3,4-b]pyridines derivatives PYR_{1-5} structures

In addition, refer to the literature, 3,5-dimethylpyrazole (1.29%) and pyrazole (0.16%) are two of the chemical composition of the essential oil isolated from the floral parts of Nandina domestica Thunb. Bajpai et al. [41] tested the efficacy of essential oil and various organic extracts

against a panel of food-borne pathogenic and spoilage bacteria such as *Bacillus subtilis* ATCC6633, *Listeria monocytogenes* ATCC19166, *Staphylococcus aureus* KCTC1916, *S. aureus* ATCC6538, *Pseudomonas aeruginosa* KCTC2004, *Salmonella typhimurium* KCTC2515, *Salmonella enteridis* KCCM12021, *Escherichia coli* 0157-Human, *E. coli* ATCC8739, *E. coli* 057:H7 ATCC43888 and *Enterobacter aerognes* KCTC2190. Thus, the oil had strong detrimental effect on the viable count of the tested bacteria. The obtaned results indicate the potential efficacy of plant-based natural products such as essential oil and organic extracts of N. domestica to control food-borne pathogenic and spoilage bacteria. In this context, we were intrigued by the number of reports that derivatives of pyrazole and pyridine have a wide range of important biological activities. Consequently, we synthesized analogues that contain both pharmacophores in the framework of one molecule. In addition, the synthesized pyrazolopyridine derivatives were subjected to antibacterial activity. Herein we report the result of that study and discuss their structureactivity relationship.

	Streptomycine	**Spiramycine**	**Fluconazole**
Doses : µM	*E. coli*	*B. sub*	*C. alb*
5	1.57 ± 0.03 A	1.12 ± 0.03 A	0.75 ± 0.0 A
10	1.75 ± 0.07 A	1.2 ± 0.0 A	0.87 ± 0.1 A
20	2.1 ± 0.14 B	1.9 ± 0.14 B	1.25 ± 0.07 B

Table 1: Inhibitory activity of standard drugs against *Escherichia coli*, *Bacillus subtilis* and *Candida albicans* (means ± SE). Means in the same column with the same upper case letter are not significantly different according to the Student-Newman-Keuls test ($P \leq 0.05$). Values are means of three replicates ± SE.

PYR_1 is a pyrazolo[3,4-b]pyridine derivative with phenyl moiety substituted, so in comparison with the Chemical compositions of the essential oils having the same moiety, we can make mention of phenylalanine, phenylpropanoids and 1,2-dimethoxybenzene (veratrole) studies.

So, in the aim to determinate Chemical composition of Gongronema latifolium leaves, Eleyinmi AF [42] demonstrated that phenylalanine is dominant essential amino acids then concluded that the aqueous extracts show activity against *E. coli* and *P. aeruginosa* while methanol extracts are active against *P. aeruginosa* and *L. monocytogenes*. Thus, *G. latifolium* has potential food and antibacterial uses.

In another report, Silveira e Sá et al. [43] aimed the search for alternative drugs capable of disrupting the inflammatory process especially with reference to the use of natural substances (essential oils). So, they made an overview of the anti-inflammatory action exerted by phenylpropanoids from essential oils and discussed possible mechanisms of action involved in the anti-inflammatory response, assessed through specific experimental models. A previous study showed that 1,2-dimethoxybenzene (veratrole) is a potent pollinator attractant to the nocturnal moth Hadena bicruris emited by white campion (Silene latifolia: a dioecious plant). Akhtar et al. [44] explored firstly the biosynthetic route to veratrole. Then, suggested that it was derived by the methylation of guaiacol (2-methoxyphenol), which itself originates from phenylalanine via BA (benzoic acid) and SA (salicylic acid), and therefore implies a novel branch point of the general phenylpropanoid pathway.

Doses : µM	*E. coli*	*B. sub*	*C. alb*
5	1.27 ± 0.106 A	1.0 ± 0.0 A	1.3 ± 0.07 A
10	1.6 ± 0.0 A	1.35 ± 0.07 B	1.45 ± 0.07 A
20	1.7 ± 0.0 A	1.72 ± 0.17 B	1.55 ± 0.07 A

Table 2: Inhibitory activity of PYR_1 against *Escherichia coli*, *Bacillus subtilis* and *Candida albicans* (means ± SE). Means in the same column with the same upper case letter are not significantly different according to the Student-Newman-Keuls test ($P \leq 0.05$). Values are means of three replicates ± SE.

In the other hand, PYR_2 is a pyrazolo[3,4-b]pyridine derivative with methoxyphenyl moiety substituted, so in comparison with the Chemical compositions of the essential oils having the same moiety, we can make mention of anethole and methyl chavicol studies. According to Soylu et al. [45] study, major compounds found in essential oils of fennel (*Foeniculum vulgare*) were anethole (82.8%), it is a cyclic hydrocarbon with methoxyphenyl moiety substituted. Thus, the aim of this group was to find an alternative to synthetic fungicides currently used in the control of devastating oomycete pathogen *Phytophthora infestans*, causal agent of late blight disease of tomato. So, antifungal activities of essential oils obtained from aerial parts of aromatic plants such as fennel were investigated against *P. infestans*. These essential oil were found to inhibit the growth of *P. infestans* in a dose-dependent manner. Complete growth inhibition of pathogen by essential oil of fennel was, however, observed at 0.4–2.0 µg/ml air concentrations. For the determination of the contact phase effects of the tested essential oil, fennel oil at 6.4 µg/ml were found to inhibit the growth of *P. infestans* completely. Volatile phase effects of essential oils were consistently found to be more effective on fungal growth than contact phase effect. Sporangial production was also inhibited by the fennel oil.

Besides, Sarin Tadtong et al. [46] claimed that the major composition of the essential oil from rhizome of *Etlingera punicea* (Roxb.) is methyl chavicol, (a cyclic hydrocarbon with methoxyphenyl moiety substituted). Their study aims to determine the chemical compositions and the antimicrobial activities of the essential oil from rhizome of *Etlingera punicea* (Roxb.) R.M. Smith. The rhizome of plant is used as spice and ingredient in noodle and curry in Thailand. This essential oil possesses fungicidal activity against *Candida albicans* ATCC 10231 and shows bacteriostatic activity against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Salmonella albany* DMST 10641, whilst no activity was found on Gram-negative bacteria *Pseudomonas aeruginosa* ATCC 27853 by agar disc diffusion assay. So, they concluded that The bacteriostatic and anitifungal activities of the essential oil from the rhizome of E. punicea might be due to the presence of methyl chavicol as previously reported [47]. The possible antimicrobial action of methyl chavicol might be arrived from the complexation between the protein or other components of the cell membrane of the microbes and the phenolic components in the essential oil as previous reported [48]. Furthermore, methyl chavicol showed limited and weak antibacterial activity [49] which was similar to our report. According to its antifungal activity, the volatile oil from this plant might be used as an active ingredient in antifungal formulations.

Doses :µM	*E. coli*	*B. sub*	*C. alb*
5	1.37 ± 0.03 A	1.0 ± 0.14 A	1.42 ± 0.10 A
10	1.45 ± 0.07 A	1.25 ± 0.07 A	1.55 ± 0.07 A
20	1.75 ± 021 A	1.5 ± 0.0 B	1.62 ± 0.03 A

Table 3: Inhibitory activity of PYR_2 against *Escherichia coli*, *Bacillus subtilis* and *Candida albicans* (means ± SE). Means in the same column with the same upper case letter are not significantly different according to the Student-Newman-Keuls test (P≤ 0.05). Values are means of three replicates ± SE.

In the case of our bacteriological study, both of PYR_1 and PYR_2 have a better activity against *Bacillus subtilis* Gram-positive as compared with the standard drug Spiramycin. However, the compound PYR_2 exhibited significantly higher activity than PYR_1 against *Escherichia coli* CIP 53126 Gram-negative. But PYR_1 exhibit significantly higher activity than PYR_2 against *Bacillus subtilis* CIP 5262 Gram-positive as compared with standard drugs (Streptomycin and Spiramycin). It means that the substitution of methoxy moiety, an electron donating at the p-position in the phenyl ring in PYR_2 increased its activity against *Escherichia coli* CIP 53126 Gram-negative and decreased it against *Bacillus subtilis* CIP 5262 Gram- positive.

Likewise, PYR_3 is an aldehyde, however aldehydes are oxygenated compounds chemical constituent of essential oils, such as Citral and Citronellal. So, according to Grace O. Onawunmi work [50], citral showed appreciable antimicrobial activity against Gram-positive and Gram-negative bacteria as well as fungi. Media composition and inoculum size had no observable effect on activity but alkaline pH increased citral activity. The growth rates of *Escherichia coli* cultures were reduced at concentrations of citral ≥ 0.01% v/v while concentrations ≥ 0.03% v/v produced rapid reduction in viable cells followed by limited regrowth. In a non-growth medium, 0.08% and 0.1% v/v showed rapid bactericidal effects. Citral may therefore be of preservative use in addition to its other uses in the food, soap and cosmetic industries.

Moreover, Manosroi et al. [51] reported that the essential oil of kaffir lime peels was the mixture of many compounds such as β-pinene (30.6 %), limonene (29.2%), sabinene (22.6 %) and citronellal (4.2 %), which was correlated to the study of Chanthaphon1 et al. who investigayed Ethyl acetate extracts and hydrodistillated-essential oils (from peels of Citrus spp.) for their antimicrobial activities against food related microorganisms by broth microdilution assay. Overall, ethyl acetate extracts from all citrus peels showed stronger antimicrobial activities than their essential oils obtained from hydrodistillation. The ethyl acetate extract of kaffir lime (Citrus hystrix DC.) peel showed broad spectrum of inhibition against all Gram-positive bacteria, yeast and molds including *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Saccharomyces cerevisiae var. sake* and *Aspergillus fumigatus* TISTR 3180. They concluded that the higher antibacterial activity against Gram-positive bacteria of the ethyl acetate extract than the hydrodistillated-essential oil from kaffir lime peel was related to citronellal content which was also higher in the ethyl acetate extract. Therefore citronellal could be a potential component, which contributed to the antibacterial activity.

Doses : µM	*E. coli*	*B. sub*	*C. alb*
5	0.9 ± 0.07 A	1.45 ± 0.0 A	1.22 ± 0.03 A
10	1.22 ± 0.03 B	1.5 ± 0.07 A	1.4 ± 0.14 A
20	1.75 ± 0.07 C	2.2 ± 0.14 B	2.15 ± 0.07 B

Table 4: Inhibitory activity of PYR_3 against *Escherichia coli*, *Bacillus subtilis* and *Candida albicans* (means ± SE). Means in the same column with the same upper case letter are not significantly different according to the Student-Newman-Keuls test (P ≤ 0.05). Values are means of three replicates ± SE.

In this study, compound PYR_3 displayed excellent activity against *Bacillus subtilis* CIP 5262 Gram-positive and *Candida albicans* ATCC 10231 as compared with standard drugs (Spiramycin and Fluconazole) respectively.

Then, PYR_3 exhibited moderate activity against *Escherichia coli* CIP 53126 Gram-negative.

Therefore, the presence of aldehyde function in the pyrazolopyridines compounds increased its activity against *Bacillus subtilis* CIP 5262 Gram-positive and *Candida albicans* ATCC 10231.

Doses : µM	*E. coli*	*B. sub*	*C. alb*
5	1.2 ± 0 A	0.9 ± 0.0 A	1.42 ± 0.10 A
10	1.7 ± 0.14 B	1.4 ± 0.07 B	1.47 ± 0.03 A
20	1.67 ± 0.03 B	1.4 ± 0.0 B	1.4 ± 0.0 B

Table 5: Inhibitory activity of PYR4 against *Escherichia coli*, *Bacillus subtilis* and *Candida albicans* (means ± SE). Means in the same column with the same upper case letter are not significantly different according to the Student-Newman-Keuls test (P ≤ 0.05). Values are means of three replicates ± SE.

Furthermore, PYR_4 exhibited excellent activity against *Escherichia coli* CIP 53126 Gram-negative and *Bacillus subtilis* CIP 5262 Gram-positive as compared with standard drugs (Streptomycin and Spiramycin respectively).

In the other hand, and recording to the literature [52], antibacterial and antifungal activity of N-phthaloylamino acid hydroxamates 1–3 $C_6H_4(CO)_2N$-X-CONHOH, X = amino acid residues of glycine, alanine or D-phenylglycine was examined against 44 strains of Gram-positive and Gram-negative bacteria, and 10 species of yeasts. *N*-phthaloyl-D-phenylglycine-hydroxamic acid (3), the substance with the highest lipophilicity (log *P*), showed the best antibacterial activity, especially against Gram-negative bacteria.

Therefore, coordination compounds derived from N-phthaloylglycine and N-phthaloyltyrosine were synthesized [53]. The ligands were formed by 1:2 molar condensation of phthalic anhydride with tyrosine and glycine respectively. The complexes were formulated as $[Zn(PHG)_2\ (H_2O)_2]\ (OAc)_2$, $[Ni(PHG)_2\ (H_2O)_2]\ (OAc)_2$ and $[Cu(PHT)_2\ ]\ (OAc)_2$. The antimicrobial studies using four test organisms (*P. aerugenosa*, *E. Coli*, *S. aureus* and *C. albicans*) revealed that the metal complexes exhibit higher activity than their respective ligands.

According to Begum et al. [54] study, two series of Nphthaloylglycine derivatives were synthesized under SchottenBaumann conditions. The first series consists of Nphthaloylglycine amides (4ah), and the second one consists of

benzimidazole derivatives of Nphthaloylglycine. All the synthesized analogues were evaluated for their *in vitro* antimicrobial activity. All the synthesized compounds exhibited a wide range of antibacterial activity against all of the *Staphylococcus aureus* resistant strains tested.

Doses : µM	*E. coli*	*B. sub*	*C. alb*
5	1.32 ± 0.03 A	1.15 ± 0.0 A	1.0 ± 0.0 A
10	1.4 ± 0.14 A	1.7 ± 0.14 B	1.12 ± 0.10 A
20	2.15 ± 0.07 B	1.85 ± 0.07 B	1.2 ± 0.07 A

Table 6: Inhibitory activity of PYR_5 against *Escherichia coli*, *Bacillus subtilis* and *Candida albicans* (means ± SE). Means in the same column with the same upper case letter are not significantly different according to the Student-Newman-Keuls test ($P \leq 0.05$). Values are means of three replicates ± SE.

Almost, in this research we found that compound PYR_5 exhibited the best activity with regard to the others compounds against *Escherichia coli* CIP 53126 Gram-negative and displayed excellent activity as compared with standard drug (Spiramycin).

Fungal infection has dramatically increased in immunosuppressed patients such as those with AIDS or who have had organ or marrow transplants over the past decades [55-57]. Currently, some antifungal agents of major drug classes: azoles, polyenes, and candins, have been launched and are demonstrating remarkable success for the treatment of fungal systemic infections [56-61]. However, these usages are often limited mainly due to their unsatisfactory antifungal activity, narrow spectrum, and side effects, causing rapid development of drug resistance and a high rate of mortality [61-64]. In consideration of these facts, alternative agents for the treatment and prevention of fungal infections are urgently required, preferably with a novel mode of action [65]. In this way, we screened for Fungi against respectively (*Candida albicans* ATCC 10231) by the paper disc diffusion method and (*Aspergillus niger* ATCC 16404) by the dilution method (agar and liquid broth). So, in the present study we reported, firstly that compound PYR_3 exhibited the best activity with regard to the others compounds against *Candida albicans* ATCC 10231 and displayed excellent activity as compared with standard drugs (Fluconazole). Then after 72 h of incubation, at 25°C for antifungal activity, we measured a total inhibition of all substituted pyrazolo[3,4-b]pyridines derivatives PYR1-5 against (*Aspergillus niger* ATCC 16404). Then, we concluded that these compounds have a fungistatic action, as the mold begins to develop resistance against these derivatives, once the period of incubation is prolonged.

So, these compounds could be useful as templates for further development through modification or derivatization to design more potent antifungal agents.

Conclusion

The antimicrobial activity of the substituted pyrazolo[3,4-b]pyridines derivatives (PYR_{1-5}) synthesized was examined and it was found that some of these compounds have similar or higher activity compared with commercial antibiotic drugs (Streptomycin, Spiramycin and Fluconazole), which make them suitable for diverse applications like the manufacturing of drugs, pesticides, emulsifiers, cosmetics, etc.

Data from this study (total inhibition against (*Aspergillus niger* ATCC 16404) provide evidence that these pyrazole formulations could be an alternative source for the treatment of fungal infections caused by *Aspergillus niger* ATCC 16404. However, a specific study on the safety and efficacy of these *in vivo* and clinical trials is still needed, in order to evaluate the practical relevance of the *in vitro* results and consequently protect our environmental.

Representative Spectral Data of Selected Compounds

5-Phenoxy-2-phényl-1,6-dihydropyrazolo[3,4-b]pyridine-3-one (PYR_4)

Yield: 86%; yellow solid; mp=120°C; 1H NMR (300 MHz, CDCl3):

δ=8.2 (d, 1H, J=4.2), 7.9 (d, 1H, J=4.2), 7.45-7.88 (m, 10H), 6.65 (s, 1H); 13C NMR (75 MHz, CDCl3) d=164.2, 157.2, 155.09, 148.3, 135.8, 130.2, 130.3, 126.37, 125.3, 123.4, 120.4, 118.65, 115.9, 112.77. Anal. Calcd for C18H12N3ClO: C, 71.23; H, 4.29; N, 13.85. Found: C, 71.20; H, 4.27; N, 13.84%. MS (EI, 30 eV): m/z=303 (M+).

2-Phényl-5-(N-phthaloyl)-1,6-dihydropyrazolo[3,4-b]pyridine-3-one (PYR_5)

Yield: 86%; yellow solid; mp=120°C; 1H NMR (300 MHz, CDCl3):

δ=8.16 (d, 1H), 8.25 (d, 1H), 7.9-8.1 (m, 7H), 7.4-7.6 (m, 2H), 7.3 (s, 1H); 13C NMR (75 MHz, CDCl3) d=195.0, 160.2, 158.2, 144.9, 140.8, 135.2, 130.2, 130.5, 127.7, 126.3, 125.8, 115.4, 110, 108.5, 50.9. Anal. Calcd for C18H12N3ClO: C, 71.18; H, 3.41; N, 11.85. Found: C, 71.16; H, 3.40; N, 11.82%. MS (EI, 30 eV): m/z=354 (M+).

References

1. Ehrlich KC, Kobbeman K, Montalbano BG, Cotty PJ (2007) Aflatoxin-producing Aspergillus species from Thailand. Int J Food Microbiol 114: 153-159.
2. Shehu K, Bello MT (2011) Effect of Environmental Factors on the Growth of Aspergillus Species Associated with Stored Millet Grains in Sokoto. Nigerian Journal of Basic and Applied Science 19: 218-223.
3. Quiroga J, Trilleras J, Insuasty B, Abonía R, Nogueras M, et al. (2008) Regioselective formylation of pyrazolo[3,4-b]pyridine and pyrazolo[1,5-a]pyrimidine systems using Vilsmeier–Haack conditions. Tetrahedron Lett 49: 2689-2691.
4. Quiroga J, Trilleras J, Insuasty B, Abonía R, Nogueras M, et al. (2008) Microwave-assisted synthesis of pyrazolo[3,4-d]pyrimidines from 2-amino-4,6-dichloropyrimidine-5-carbaldehyde under solvent-free conditions. Tetrahedron Lett 49: 3257-3259.
5. Quiroga J, Insuasty B, Insuasty H, Abonía R, Ortíz AJ, et al. (2001) A straightforward synthesis of pyrimido[4,5-b]quinoline derivatives assisted by microwave irradiation. J Heterocycl Chem 38: 339-341.
6. Quiroga J, Hormaza A, Insuasty B, Ortíz AJ, Sánchez A, et al. (1998) Synthesis of pyrimido [4, 5-b] quinolines in the reaction of 6-aminopyrimidines with dimedone and benzaldehydes. J Heterocycl Chem 35: 231-233.
7. Quiroga J, Hormaza A, Insuasty B, Saitz C, Jullian C (1998) Synthesis of 4-aryl-4, 7, 8, 9-tetrahydro-6H-pyrazolo [3, 4-b] quinolin-5-ones. J Heterocycl Chem 35: 575-578.
8. Quiroga J, Mejía D, Insuasty, B, Abonía R, Nogueras M, et al. (2001) Regioselective synthesis of 4, 7, 8, 9-tetrahydro-2H-pyrazolo [3, 4-b] quinolin-5 (6H)-ones. Mechanism and structural analysis. Tetrahedron 57: 6947-6953.
9. Khiari JE, Gmiza T, Hadj Ayed MA, Ben Hassine B (2007) Regiospecific synthesis of disubstituted pyridin-2-ones and trisubstituted phenols with vinamidinium salts. Synth Commun 37: 3939-3944.

10. Chtiba S, Khiari J (2015) Efficient One-Pot Synthesis of Fused Isoxazolo [5,4-b] Pyridines. Int J Sci Res 4: 770-772.
11. Hadj Ayed MA, Gmiza T, Khiari JE, Ben Hassine B (2012) Efficient One-Pot Synthesis of Substituted Pyrido[2,3-d]Pyrimidines From Vinamidinium and Chloropropeniminium Salts. Synth Commun 42: 1824-1831.
12. Fayçel J, Samar C, Jameleddine KJ (2013) The Application of Vinamidinium Salts to the Synthesis of 2-Amino-5-aryl or Formylpyridine-3-carbonitriles. J Heterocycl Chem 50: 201-206.
13. Chtiba S, Jemmezi F, Khiari JE (2011) Convenient synthesis of pyrazolo[3,4-b]pyridin-3-ones and pyrazolo[3,4-b]pyridine-5-carbaldehyde using vinamidinium salts. Tetrahedron Lett 52: 3648-3650.
14. Quiroga J, Cruz S, Insuasty B, Abonía R, Hernandez P, et al. (1998) Reaction of 5-aminopyrazoles with ß-dimethylaminopropiophenones. Synthesis of new pyrazolo [3, 4-b] pyridines. J Heterocycl Chem 35: 333-338.
15. Orlov VD, Quiroga J, Kolos NN (1987) Khim. Geterosikl Soedin 88: 1247-1251.
16. Quiroga J, Portilla J, Serrano H, Abonía, R, Insuasty B, et al. (2007) Regioselective synthesis of fused benzopyrazolo [3, 4-b] quinolines under solvent-free conditions. Tetrahedron Lett 48: 1987-1990.
17. Quiroga J, Cobo D, Insuasty B, Abonía R, Cruz S, et al. (2008) Synthesis of new indeno[1,2-e]pyrimido[4,5-b][1,4]diazepine-5,11-diones as potential antitumor agents. J Heterocycl Chem 45: 155-159.
18. Quiroga J, Trilleras J, Sánchez A I, Insuasty B, Abonía R, et al. (2009) Regioselective three-component synthesis of indolylpyrazolo [3, 4-b] pyridines induced by microwave and under solvent-free conditions. Lett Org Chem 6: 381-383.
19. Jairo Q, Sandra P, Alfredo P, Jaime G, Rodrigo A, et al. (2011) An efficient synthesis of pyrazolo[3,4-b]pyridine-4-spiroindolinones by athree-component reaction of 5-aminopyrazoles, isatin, and cyclic b- diketones. Tetrahedron Letters 52: 2664-2666.
20. Najma P, Samina A, Tahir M, Alice MR, Bernadino MAM, et al. (2014) Synthesis and antibacterial activities of Diarylpyrazolo [3,4-b]pyridines . AFINIDAD LXXI 71: 566.
21. WHO (2008) World Health Organization.
22. Aspa J, Rajas O, de Castro FR (2008) Pneumococcal antimicrobial resistance: therapeutic strategy and management in community-acquired pneumonia. Expert Opin Pharmacother 9: 229-241.
23. Cook A, Foruya EY, Larson E, Vasquez G, Lowy FD (2007) Heterosexual transmission of community-associated methicillin-resistant Staphylococcus aureus. Clin Infect Dis 44: 410-413.
24. Bjarnsholt T, Givskov M (2008) Quorum sensing inhibitory drugs as next generation antimicrobials: worth the effort? Curr Infect Dis Rep 10: 22-28.
25. Otto M (2008) Staphylococcal biofilms. Curr Top Microbiol Immunol 322: 207-228.
26. Bare TM, McLaren CD, Campbell JB, Firor JW, Resch JF, et al. (1989) Synthesis and structure-activity relationships of a series of anxioselective pyrazolopyridine ester and amide anxiolytic agents. J Med Chem 32: 2561-2573.
27. Bernardino AMR, Azevedo AR, Pinheiro LCS, Borges JC, Carvalho VL, et al. (2007) Synthesis and antiviral activity of new 4-(phenylamino)/4-[(methylpidin-2-yl)amino]-1-phenyl-1H-pyrazolo[3,4-b]pyridine-4-carboxylic acid derivatives. Med Chem Res 16: 352.
28. Bernardino AMR, Castro HC, Frugulhetti IC, Loureiro NI, Azevedo AR, et al. (2008) SAR of a series of anti-HSV-1 acridone derivatives, and a rational acridone-based design of a new anti-HSV-1 3H-benzo[b]pyrazolo[3,4-h]-1,6-naphthyridine series. Bioorg Med Chem 16: 313-321.
29. de Mello H, Echevarria A, Bernardino AM, Canto-Cavalheiro M, Leon LL (2004) Antileishmanial pyrazolopyridine derivatives: synthesis and structure-activity relationship analysis. J Med Chem 47: 5427-5432.
30. Lu Z, Ott GR, Anand R, Liu RQ, Covington MB, et al. (2008) Potent, Selective, Orally bioavailable inhibitors of tumor necrosis factor- alpha converting enzyme (TACE): discovery of indole, benzofuran, imidazopyridine and pyrazolopyridine p1 substituents. J Bioorg Med Chem Lett 18: 1958-1962.
31. Leal B, Afonso IF, Rodriques CR, Abreu PA, Garrett R, et al. (2008) Antibacterial profile against drug-resistant Staphylococcus epidermidis clinical strain and structure–activity relationship studies of 1H-pyrazolo[3,4-b]pyridine and thieno[2,3-b]pyridine derivatives. Bioorg Med Chem 16: 8196-8204.
32. Crenshaw RR, Luke GM, Smirnoff P (1976) Interferon inducing activities of derivatives of 1,3-dimethyl-4-(3-dimethylaminopropylamino)-1H-pyrazolo[3,4-b]quinoline and related compounds. J Med Chem 19: 262-275.
33. Stein RG, Biel JH, Singh T (1970) Antimalarials. 4-Substituted 1H-pyrazolo [3,4-b]quinolines. J Med Chem 13: 153-155.
34. Fujikama Y, Suzuki M, Iwasaki H, Sakashita M, Kitahara M (1989) Eur Patent Appl EP 339358. Chem Abstr 113: 23903.
35. Chen YL (1995) Patent WO9534563 A1. Chem Abstr 124: 232447.
36. Manetti F, Schenone S, Bondavalli F, Brullo C, Bruno O, et al. (2005) Synthesis and 3D QSAR of New Pyrazolo[3,4-b]pyridines:? Potent and Selective Inhibitors of A1 Adenosine Receptors. J Med Chem 48: 7172-7185.
37. Hamblin JN, Angell TD, Ballantine SP, Cook CM, Cooper AW, et al. (2008) Pyrazolopyridines as a novel structural class of potent and selective PDE4 inhibitors. Bioorg Med Chem Lett 18: 4237-4241.
38. Jayanna ND, Vagdevi HM, Darshan JC, Prashith k, Hanumanthappa BC, et al. (2013) Synthesis and biological evaluation of novel 5,7-dichloro-1,3-benzoxazole derivatives. Journal of chemistry.
39. Ibrahim HM, Behbehani H (2013) Organocatalysis in heterocyclic synthesis: DABCO as a mild and efficient catalytic system for the synthesis of a novel class of quinazoline, thiazolo [3,2-a]quinazoline and thiazolo[2,3-b] quinazoline derivatives. Chem Cent J 7: 82.
40. Kumar V, Kaur K, Gupta GK, Sharma AK (2013) Pyrazole containing natural products: synthetic preview and biological significance. Eur J Med Chem 69: 735-753.
41. Bajpai VK, Rahman A, Kang SC (2008) Chemical composition and inhibitory parameters of essential oil and extracts of Nandina domestica Thunb. to control food-borne pathogenic and spoilage bacteria. Int J Food Microbiol 125: 117-122.
42. Eleyinmi AF (2007) Chemical composition and antibacterial activity of gongronema latifolium. J Zhejiang Univ Sci B 8: 352-358.
43. de Cássia da Silveira E Sá R, Andrade LN, Dos Reis Barreto de Oliveira R, de Sousa DP (2014) A review on anti-inflammatory activity of phenylpropanoids found in essential oils. Molecules 19: 1459-1480.
44. Akhtar TA, Pichersky E (2013) Veratrole biosynthesis in white campion. Plant Physiol 162: 52-62.
45. Soylu EM, Soylu S, Kurt S (2006) Antimicrobial activities of the essential oils of various plants against tomato late blight disease agent Phytophthora infestans. Mycopathologia 161: 119-128.
46. Sarin T, Piyapan W, Wanrawin P, Sirivan A, Nijsiri R (2009) Antimicrobial activities of essential oil from Etlingera punicea rhizome. J Health Res 23: 77-79.
47. Oxenham SK, Svoboda KP, Walters DR (2005) Altered growth and polyamine catabolism following exposure of the chocolate spot pathogen Botrytis fabae to the essential oil of Ocimum basilicum. Mycologia 97: 576-579.
48. Omidbeygi M, Barzegar M, Hamidi Z, Naghdibadi H (2007) Antifungal activity of thyme, summer savory and clove essential oils against Aspergillus flavus in liquid medium and tomato paste. Food Cont 18: 1518-1523.
49. Bagamboula CF, Uyttendaele M, Debevere J (2004) Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards Shigella sonnei and S. flexneri. Food Microbiol 21: 33-42.
50. Onawunmi GO (1989) Evaluation of the antimicrobial activity of citral. Lett Applied Microbiol 9: 105-108.

51. Manosroi A, Abe M, Manosroi J (1999) Hair care: fruitful pursuits. An investigation into the effects of porcupine orange (Citrus hystrix DC) extracts in hair treatment. Soap Perfumery and Cosmetics 13-17.
52. Matijevic-Sosa J, Cvetnic Z (2005) Antimicrobial activity of N-phthaloylamino acid hydroxamates. Acta Pharm 55: 387-399.
53. Tella AC, Oguntoye SO, Osunniran wa, Arise RO (2012) Coordination compounds of n-phthaloylglycine and n-phthaloyltyrosine and their antimicrobial activities. Elixir Appl Chem 45: 7620-7623.
54. Begum S, Nizami BS, Saied S, Shahid SM, Basha FZ (2014) Synthesis and evaluation of antibacterial activity for a series of N-phthaloylglycine derivatives. Euro J Chem 5: 557-562.
55. Rüping MJ, Vehreschild JJ, Cornely OA (2008) Patients at high risk of invasive fungal infections: when and how to treat. Drugs 68: 1941-1962.
56. Drew R (2006) Potential role of aerosolized amphotericin B formulations in the prevention and adjunctive treatment of invasive fungal infections. Int J Antimicrob Agents 27: 36-44.
57. Pappas PG, Rex JH, Sobel JD, Filler SG, Dismukes WE, et al. (2004) Guidelines for treatment of candidiasis. Clin Infect Dis 38: 161-189.
58. Ostrosky-Zeichner L, Marr KA, Rex JH, Cohen SH (2003) Amphotericin B: time for a new "gold standard". Clin Infect Dis 37: 415-425.
59. Fridkin SK (2005) The changing face of fungal infections in health care settings. Clin Infect Dis 41: 1455-1460.
60. Enoch DA, Ludlam HA, Brown NM (2006) Invasive fungal infections: a review of epidemiology and management options. See comment in PubMed Commons below J Med Microbiol 55: 809-818.
61. Georgopapadakou NH (1998) Antifungals: mechanism of action and resistance, established and novel drugs. Curr Opin Microbiol 1: 547-557.
62. Pfaller MA, Diekema DJ (2004) Rare and Emerging Opportunistic Fungal Pathogens: Concern for Resistance beyond Candida albicans and Aspergillus fumigates. J Clin Microbiol 42: 4419-4431.
63. Singh N (2001) Trends in the epidemiology of opportunistic fungal infections: predisposing factors and the impact of antimicrobial use practices. Clin Infect Dis 33: 1692-1696.
64. Fridkin SK, Jarvis WR (1996) Epidemiology of nosocomial fungal infections. Clin Microbiol Rev 9: 499-511.
65. Kuroyanagi J, Kanai K, Sugimoto Y, Fujisawa T, Morita C, et al. (2010) Novel antifungal agents: Triazolopyridines as inhibitors of ß-1,6-glucan synthesis. Bioorg Med Chem 18: 5845–5854.