

## 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

Chtiba Samar<sup>1,2\*</sup>, Amri Ismail<sup>3,4</sup>, Tazarki Helmi<sup>1,4</sup>, Jameleddine Khiari<sup>1</sup> and Jamoussi Bassem<sup>1</sup>

<sup>1</sup>Laboratoire de Chimie Organique et Analytique, Institut Supérieur de l'Education et de la Formation Continue, Bardo, Tunisia

<sup>2</sup>Faculté des Sciences de Tunis, Campus Universitaire, 2092, Tunis, Tunisia

<sup>3</sup>Department of Biology, Faculty of Science of Bizerte, University of Carthage, Zarzouna, 7021 Bizerte, Tunisia

<sup>4</sup>Laboratoire d'écologie forestière, Institut national de recherches en génie rural, Eaux et Forêts. PB 10, 2080 Ariana, Tunisia

\*Corresponding author: Chtiba Samar, Laboratoire de Chimie Organique et Analytique, Institut Supérieur de l'Education et de la Formation Continue, Bardo, Tunisia, E-mail: samarchtiba@yahoo.com

Received date: May 05, 2017; Accepted date: June 12, 2017; Published date: June 16, 2017

Copyright: © 2017 Samar C, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

### 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<sub>1-5</sub>) 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

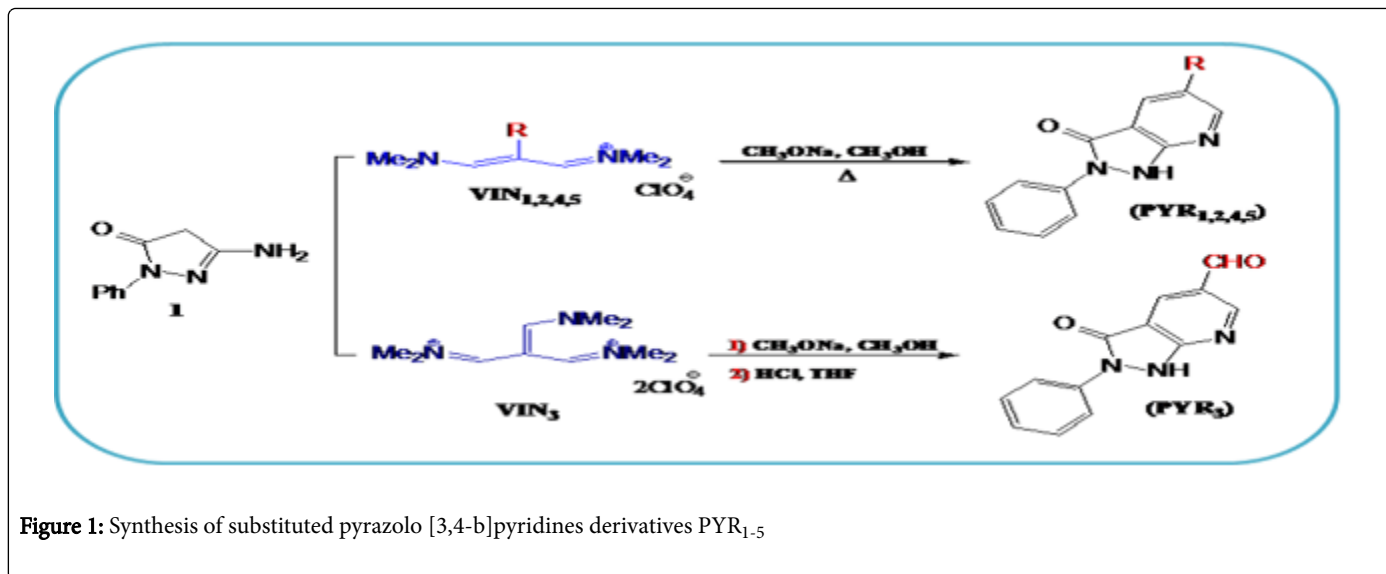
### Introduction

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-arylpiperidine-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] pyridines. 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].



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<sub>1-5</sub>

To a flame dried one-necked round-bottomed flask equipped with magnetic stirring, reflux condenser and nitrogen atmosphere was added vinamidinium salt VIN<sub>1-5</sub> (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<sub>1-5</sub>, neither Work-up nor purification. For pyrazolo [3,4-b] pyridine PYR<sub>3</sub>, 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<sub>3</sub>.

All products were characterized from their <sup>1</sup>H NMR, <sup>13</sup>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 (PYR<sub>1-5</sub>) 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 PYR<sub>1-5</sub>

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

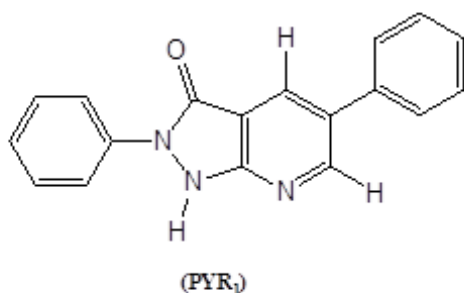
and Bacteria in dose-dependent manner (Tables 1-5). The five substituted derivatives exhibited different inhibition effects on the growth of Fungi and Bacteria. In general, there was a correlation between the antibacterial, antifungal activities and structures.

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.

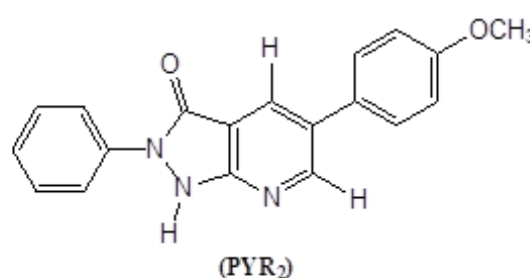
Refer to the Ibrahim et al. [39] works new pyridine derivatives are synthesized. Most of these compounds were tested and evaluated as antimicrobial agents and the results indicated that many of the obtained compounds exhibited high antimicrobial activity comparable

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.

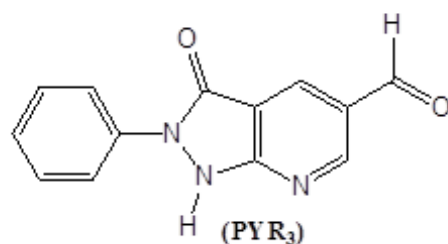
**2,5-Diphenyl-1,6-dihydropyrazolo[3,4-b]pyridine-3-one**



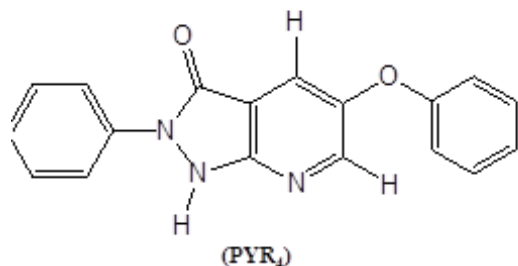
**2-Phenyl-5-(4-methoxyphenyl)-1,6-dihydropyrazolo[3,4-b]pyridine-3-one**



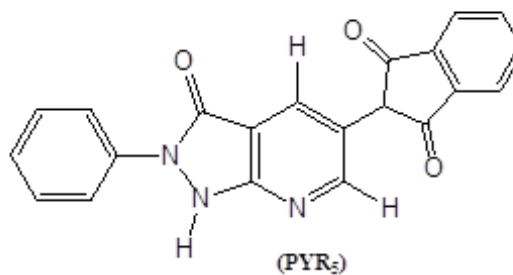
**2-Phenyl-3-oxo-1,6-dihydropyrazolo[3,4-b]pyridine-5-carbaldehyde**



**5-Phenoxy-2-phenyl-1,6-dihydropyrazolo[3,4-b]pyridine-3-one**



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



**Figure 2:** Pyrazolo [3,4-b]pyridines derivatives PYR<sub>1-5</sub> 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 aerogenes* KCTC2190. Thus, the oil had strong detrimental effect on the viable count of the tested bacteria. The obtained 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	<i>E. coli</i>	<i>B. sub</i>	<i>C. alb</i>
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<sub>1</sub> 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 bicurris* emitted 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	<i>E. coli</i>	<i>B. sub</i>	<i>C. alb</i>
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<sub>1</sub> 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<sub>2</sub> 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 Soyly 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 *Etingera 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 *Etingera 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 : $\mu$ M	<i>E. coli</i>	<i>B. sub</i>	<i>C. alb</i>
5	1.37 $\pm$ 0.03 A	1.0 $\pm$ 0.14 A	1.42 $\pm$ 0.10 A
10	1.45 $\pm$ 0.07 A	1.25 $\pm$ 0.07 A	1.55 $\pm$ 0.07 A
20	1.75 $\pm$ 0.21 A	1.5 $\pm$ 0.0 B	1.62 $\pm$ 0.03 A

**Table 3:** Inhibitory activity of PYR<sub>2</sub> against *Escherichia coli*, *Bacillus subtilis* and *Candida albicans* (means  $\pm$  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  $\pm$  SE.

In the case of our bacteriological study, both of PYR<sub>1</sub> and PYR<sub>2</sub> have a better activity against *Bacillus subtilis* Gram-positive as compared with the standard drug Spiramycin. However, the compound PYR<sub>2</sub> exhibited significantly higher activity than PYR<sub>1</sub> against *Escherichia coli* CIP 53126 Gram-negative. But PYR<sub>1</sub> exhibit significantly higher activity than PYR<sub>2</sub> 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<sub>2</sub> increased its activity against *Escherichia coli* CIP 53126 Gram-negative and decreased it against *Bacillus subtilis* CIP 5262 Gram- positive.

Likewise, PYR<sub>3</sub> 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  $\geq 0.01\%$  v/v while concentrations  $\geq 0.03\%$  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  $\beta$ -pinene (30.6 %), limonene (29.2%), sabinene (22.6 %) and citronellal (4.2 %), which was correlated to the study of Chanthaphonl et al. who investigated Ethyl acetate extracts and hydrodistilled-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 hydrodistilled-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 : $\mu$ M	<i>E. coli</i>	<i>B. sub</i>	<i>C. alb</i>
5	0.9 $\pm$ 0.07 A	1.45 $\pm$ 0.0 A	1.22 $\pm$ 0.03 A

10	1.22 $\pm$ 0.03 B	1.5 $\pm$ 0.07 A	1.4 $\pm$ 0.14 A
20	1.75 $\pm$ 0.07 C	2.2 $\pm$ 0.14 B	2.15 $\pm$ 0.07 B

**Table 4:** Inhibitory activity of PYR<sub>3</sub> against *Escherichia coli*, *Bacillus subtilis* and *Candida albicans* (means  $\pm$  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  $\pm$  SE.

In this study, compound PYR<sub>3</sub> 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<sub>3</sub> 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 : $\mu$ M	<i>E. coli</i>	<i>B. sub</i>	<i>C. alb</i>
5	1.2 $\pm$ 0 A	0.9 $\pm$ 0.0 A	1.42 $\pm$ 0.10 A
10	1.7 $\pm$ 0.14 B	1.4 $\pm$ 0.07 B	1.47 $\pm$ 0.03 A
20	1.67 $\pm$ 0.03 B	1.4 $\pm$ 0.0 B	1.4 $\pm$ 0.0 B

**Table 5:** Inhibitory activity of PYR<sub>4</sub> against *Escherichia coli*, *Bacillus subtilis* and *Candida albicans* (means  $\pm$  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  $\pm$  SE.

Furthermore, PYR<sub>4</sub> 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<sub>6</sub>H<sub>4</sub>(CO)<sub>2</sub>N-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)<sub>2</sub> (H<sub>2</sub>O)<sub>2</sub>] (OAc)<sub>2</sub>, [Ni(PHG)<sub>2</sub> (H<sub>2</sub>O)<sub>2</sub>] (OAc)<sub>2</sub> and [Cu(PHT)<sub>2</sub>] (OAc)<sub>2</sub>. The antimicrobial studies using four test organisms (*P. aeruginosa*, *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 N-phthaloylglycine. 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 : $\mu\text{M}$	<i>E. coli</i>	<i>B. sub</i>	<i>C. alb</i>
5	1.32 $\pm$ 0.03 A	1.15 $\pm$ 0.0 A	1.0 $\pm$ 0.0 A
10	1.4 $\pm$ 0.14 A	1.7 $\pm$ 0.14 B	1.12 $\pm$ 0.10 A
20	2.15 $\pm$ 0.07 B	1.85 $\pm$ 0.07 B	1.2 $\pm$ 0.07 A

**Table 6:** Inhibitory activity of PYR<sub>5</sub> against *Escherichia coli*, *Bacillus subtilis* and *Candida albicans* (means  $\pm$  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  $\pm$  SE.

Almost, in this research we found that compound PYR<sub>5</sub> 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<sub>3</sub> 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 PYR<sub>1-5</sub> 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<sub>1-5</sub>) 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<sub>4</sub>)

Yield: 86%; yellow solid; mp=120°C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):

$\delta$ =8.2 (d, 1H, J=4.2), 7.9 (d, 1H, J=4.2), 7.45-7.88 (m, 10H), 6.65 (s, 1H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$ =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 C<sub>18</sub>H<sub>12</sub>N<sub>3</sub>ClO: 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<sub>5</sub>)

Yield: 86%; yellow solid; mp=120°C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):

$\delta$ =8.16 (d, 1H), 8.25 (d, 1H), 7.9-8.1 (m, 7H), 7.4-7.6 (m, 2H), 7.3 (s, 1H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$ =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 C<sub>18</sub>H<sub>12</sub>N<sub>3</sub>ClO: 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, Noguera 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, Noguera 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, Noguera 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, Jameledine 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  $\beta$ -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 indenol[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 a three-component reaction of 5-aminopyrazoles, isatin, and cyclic  $\beta$ -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 anxiolytic 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-[(methylpiperidin-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, Rodrigues 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 WO*9534563 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, Nijisiri R (2009) Antimicrobial activities of essential oil from *Etingera 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, Debever 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 fumigatus*. *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  $\beta$ -1,6-glucan synthesis. *Bioorg Med Chem* 18: 5845-5854.