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Pyridine Containing Azoles: Possible Promising Antimicrobial Molecules

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Abstract :
The antimicrobial era is threatened by high levels of antibiotic resistance, the limited number and disparate availability of effective antibiotics against diverse bacterial species and reduced involvement by the pharmaceutical industry in the development of new antiinfectives. The pyridine substructure is one of the most prevalent heterocycles found in natural products, pharmaceuticals, and functional materials. In the recent past, novel derivatives of pyridine have been developed and used as insecticidal agent. It is seen from the current literature that pyridine congeners have been associated with different biological properties like pesticidal, insecticidal and fungicidal. Furthermore, azoles are widely used as class of antimicrobial agent due to their safety profile and high therapeutic index, used for the treatment of local and systemic fungal infections. Substituted derivatives of thiazole, azetidinone, and thiazolidinone exhibit potential pesticidal, insecticidal and antimicrobial activity. The development of computational techniques has also given the medicinal chemist a powerful tool to use in the development of new antimicrobial drugs. In view of these findings it is reasonable to expect from some new pyridine derivatives containing azoles to have their boipotential as antifungal or antimicrobial agents.

Key Words: *Antimicrobials, Pyridines, Azoles, Microbial resistance, computer aided drug design.*

Introduction

Whilst classic screening methods and chemical modification of known antimicrobial agents continue to produce potential leads for new antimicrobial agents, a number of other approaches are being investigated. These include the search for potentiators of the activity of known antimicrobial agents and the development of hybrid agents, novel membrane-active drugs, and inhibitors of bacterial virulence and pathogenesis. The idea of chemically fusing two antimicrobials with different mechanisms or an antimicrobial agent with a potentiating entity has been the subject of study for several decades, but no considerable success has been registered till date and the search of a new hybrid agent still continues[1].

Also, application of computers and genomic technologies has provided an almost overwhelming number and variety of new approaches for tackling this problem. Although no single drug has been designed solely by computer techniques, the contribution of these methods to drug discovery is no longer a matter of dispute. All the world's major pharmaceutical and biotechnology companies use computational design tools. At their lowest level the contributions represent the replacement of crude mechanical models by displays of structure which are a much more accurate reflection of molecular reality, capable of demonstrating motion and solvent effects. Beyond this, theoretical calculations permit the computation of binding free energies and other relevant molecular properties. The theoretical tools include empirical molecular mechanics, quantum mechanics and, more recently, statistical mechanics. Underpinning all this work is the availability of high quality computer graphics, largely supported on workstations.

Two distinct categories of research are clearly distinguishable:

a) A detailed molecular structure of the target macromolecule, the drug receptor, is known from x-ray crystallography, NMR or homology modeling.

b) The target receptor binding site has properties which can only be inferred from knowledge of the variable activity of otherwise similar molecules[2].

There is a clear medical need for new antimicrobial agents and an impressive array of new technologies available for addressing this need. In the late 1990s, bacterial genomics enticed an enormous investment by both large pharma and biotech companies into antibacterial research on the assumption that discovering a new antibacterial would be relatively easy compared to other therapeutic areas. The new genomic technologies enabled both rapid novel target identification and extremely robust target validation; this coupled with established and predictive preclinical evaluation cascades made antibacterials a potential quick win. However, antibacterial discovery has many unforeseen challenges. Lack of success at high throughput screening, complexities of antibacterial lead optimization programs and increased safety demands have all had a profound effect on antibacterial discovery. Furthermore, because the preclinical data such as resistance frequencies, minimum inhibitory concentrations (MICs), efficacy in infection models, among others, are good predictors of clinical efficacy in human beings, many of the significant hurdles exist at the preclinical stage thus reducing the number of candidate molecules that reach development compared to other therapeutic areas[3]**.** Clearly, the challenges of antibacterial research are significant and a good start towards the development of new class of hybrid antimicrobials along with the aid of computer aided drug design may deliver new antimicrobials to the clinic.

The Medical Need

The concern about the declining efficacy of antimicrobial agents coupled with the emerging importance of new and older bacterial pathogens appears to be well founded. The success of the "antibiotic era" in minimizing infection- related morbidity and mortality has been a model in modern medicine. The development of antimicrobial agents, vaccines, public health systems, hospital epidemiology or infection control, and associated regulatory guidelines has advanced infectious disease prevention and chemotherapy to high standards during the 1980s. However, in the last 5-10 years, numerous previously very controlled diseases (tuberculosis, necrotizing β-hemolytic *Streptococcus* infections, rheumatic fever, etc.) have become relatively unchecked and many efficacious antimicrobials are less active secondary to rapid or sustained slow emergence of resistance[4-7]. Major antimicrobial resistance concerns will be listed in the paragraphs that follow, focusing on those issues that affect the greatest proportion of patients infected in hospitals or presenting in outpatient clinics. The Gram-positive organisms have developed several mechanisms of resistance that limit the action of β-1actams, glycopeptides, and other antimicrobials[8-27]. Table 1 lists the major resistance problems among the Gram-positive cocci. Another grave concern for the Gram-positive species (Table 1) has been the rapid appearance of vancomycin- resistant enterococci. The current rate is judged to be >10% of all invasive isolates and has the highest prevalence among *Enterococcus faecium* strains (>20%). The mechanism of resistance has been well characterized and resides on an easily transmissible genetic fragment, therefore possessing an extreme likelihood of exchange to more virulent species such as the *S. aureus* that may already contain other resistance factors (e.g., MRSA) [14,17,28,20,24,25,26.]

Table 1 also lists the emerging resistances among *Streptococcus* spp., most importantly the penicillin resistant (intermediate and resistant by reference test) pneumococci.

These strains of S. *pneumoniae* have altered penicillin-binding protein (PBP) target sites and can be markedly refractory to usual regimens of β-1actams (penicillin, oral cephalosporins, and some newer parenteral cephalosporins) when treating serious, invasive infections. Similarly, the viridans group *Streptococcus* spp. (or-hemolytic streptococci) appears even more resistant to penicillin and some other drugs (macrolides, tetracyclines, fluoroquinolones, sulfonamides, etc.), a fact substantiating them as sources of the resistant genomic material that has been transferred to the pneumococci. These organisms can also produce severe, potentially lethal infections[19]. Other antimicrobial resistances among other Gram-positive species are also listed in Table 1 (multiresistant *Corynebacterium jeikeium* and *Bacillus* spp.), each relatively rare compared with other cited species.

Some serious antimicrobial resistance issues among the Gram-negative species are summarized in Table 2. The most disturbing involve the *Enterobacteriaceae* caused by various β-1actamase-mediated resistance mechanisms[29,30,31]. Since the beginning of the so-called "third-generation cephalosporin (cefotaxime, ceftazoxime, ceftriaxone, and ceftazidime) era" in 1981, resistant strains among the *Enterobacter aerogenes*, *E. cloacae*, and *Citrobacter freundii* isolates have been observed (15%-35% of strains)[7,25,32,33]. Moreover, when a drug in this class of cephalosporins (ceftazidime) was used to excess in the hospital environment, a resistant subpopulation of β -lactamase--producing chromosomal deregulated isolates was selected[22,34]. Complete removal or diminished use of this

compound has resulted in the decline of resistance rates. An additional threat to the use of these drugs was the discovery of plasmid mediated enzyme-producing *Klebsiella pneumonia* and *Escherichia coli* strains that inactivate newer cephalosporins to varying degrees[30,35,36].

Single aminoacid changes in the sequence of coding genes (derived from previously known TEM-1 or -2 and SHV-1 β-1actamases) can result in potent production of enzymes that generally have their greatest affinity for ceftazidime, cefotaxime, ceftriaxone, and the monobactam aztreonam. These enzymes routinely have been inhibitable by clavulanic acid or sulphone (sulbactam and tazobactam) enzyme inhibitors, but some inhibitor-resistant strains have been described[36], usually derived from *amp C* chromosomal genetic elements. The rate of emergence of these strains was quite variable, with highest rates in large metropolitan hospitals where epidemic and subsequent endemic occurrences have been more frequent. Patient morbidity and mortality have been attributed to these organisms in the United States and Europe[25]. TEM-type β-lactamases have been described in *Haemophilus* spp.[37] and pathogenic *Neisseria* for nearly 2 decades. The *H. influenza* β-1actamase-positive rates in 1993 were consistently >30% in nearly all geographic regions of the United States[38]. In contrast, the penicillinase-producing *Neisseria gonorrhoeae* (PPNG) strain still appears to be endemic or epidemiologically variable. Strains of *Neisseria meningitides* that were less susceptible to penicillin MICs >0.06 ~g/ml) have been observed with greater frequency worldwide, thus requiring the wider therapeutic use of cefotaxime or ceftrixone[39].Other Gram-negative bacilli such as *Stenotrophomonas maltophilia*, *Acinetobacter* spp., and *Pseudomonas aeruginosa* have become less treatable in recent years. *Stenotrophomonas maltophilia* requires a select group of drugs for successful therapy, and multidrug resistant strains of the other cited species have been more frequent, especially among immunocompromised and cystic fibrosis patients with significant respiratory infections. Clearly these emerging problems limit the utility of available antimicrobial agents. The search for a new hybrid molecule may be a solution to minimize these cited drug-resistance problems.

Pyridines Fused With Azoles: Scope for New Antimicrobial Agents

The pyridine substructure is one of the most prevalent heterocycles found in natural products, pharmaceuticals, and functional materials. In the recent past, novel derivatives of pyridine have been developed and used as insecticidal agent. It is seen from the current literature that pyridine congeners have been associated with different biological properties like pesticidal, insecticidal and fungicidal[40].A set of pyridine derivatives bearing a substituted alkylthio chain or a piperidyl ring in position 2 or 4 were synthesized, and their antimycobacterial and antifungal activities were evaluated by Vera Klimesova et.al[41], while set of 4-benzylsulfanyl derivatives of pyridine-2-carbonitriles and pyridine-2-carbothioamides, previously tested for their antimycobacterial activity, were analysed by Vera Klimesova et.al[42] by quantitative structure–activity relationship (QSAR) techniques, using some physicochemical and quantum–chemical parameters. The resulting QSAR revealed that the activity increases with electron withdrawing substituents in the benzyl moiety of studied compounds. Highest occupied molecular orbitals (HOMO) can play an important role in the description of the mechanism of interactions at the molecular level. Parasuraman Narendar et.al[43] have reported the synthesis and pharmacological evaluation of some new 2-substituted pyridine derivatives. The compounds were found to possess antihistaminic, anticonvulsant and sympatholytic properties.Alice Maria Rolim Bernardino et.al[44], performed the design, synthesis, and the structure–activity relationship studies of 13 new derivatives of thieno [2, 3-b]pyridine. The biological results showed some derivatives as antiparasitic agents against *Giardia lamblia*. Computational analysis of HOMO and Lowest unoccupied molecular orbital (LUMO) energy, HOMO orbital coefficient distribution, electrostatic potential map, dipole moment, and density HOMO was performed to gain insight into the structure activity relationship aspects. This study pointed the p-methoxy substituted derivative as a leading compound for the development of new microbicidal medicines based on thieno [2, 3-b] pyridine analogs.

Azoles are widely used as class of antimicrobial agent due to their safety profile and high therapeutic index, used for the treatment of local and systemic fungal infections. Sylvie Bourrainet.al.[45]developed substituted pyrazoles as novel selective compounds with excellent affinity for the human D_4 and good selectivity over other dopamine receptors. Thomas D. Penning et.al.[46] described a series of pyrazole and isoxazole analogs as antagonists of the avb3 receptor. Compounds showed low to sub-nanomolar potency against avb3, as well as good selectivity against aIIbb3. In HT29 cells, most analogs also demonstrated significant selectivity against avb6. Several compounds showed good pharmacokinetic properties in rats, in addition to anti-angiogenic activity in a mouse corneal micropocket model.

Compounds were synthesized in a straightforward manner from readily available glutarate precursors.Irini Akritopoulou-Zanze et.al[47] reported the synthesis and biological evaluation of 5-substituted 1, 4-dihydroindeno [1, 2-c] pyrazoles as multitargeted kinase inhibitors.Catherine Jorand-Lebrunet.al.[48] identified a series of pyrazole compounds were identified with luteinizing hormone receptor (LH-R) agonist activity.Patricia D. Sauzem et.al.[49] have reported the synthesis and evaluation of the analgesic and anti-inflammatory properties of novel 3- or 4-substituted 5- trifluoromethyl-5-hydroxy-4,5-dihydro-1H-1-carboxyamidepyrazoles (where 3-/4-substituent ¼ H/H, Me/H, Et/H, Pr/H, i-Pr/H, Bu/H, t-Bu/H, Ph/H, 4-Br-Ph/H and H/Me) designed in the exploration of the bioisosteric replacement of benzene present in salicylamide with a 5-trifluoromethyl-4,5-dihydro-1H-pyrazole scaffold.Substituted derivatives of thiazole, azetidinone, and thiazolidinone exhibit potential pesticidal, insecticidal and antimicrobial activity. 1, 3, 4-Oxadiazoles are known to have a broad spectrum of biological activities. Some C-(β-DGlucopyranosyl)-1, 3, 4-oxadiazoles were synthesized as potential glycogen phosphorylase inhibitors. These compounds were shown to possess antidiabetic, antiarthritic and anti-inflammatory activities. On the other hand, 1,2,4-triazoles are associated with diverse pharmacological activities such as analgesic, antiasthmatic, diuretic, antihypertensive, anticholinergic, antibacterial, antifungal and anti-inflammatory activity. It was also reported that a large number of compounds containing a triazole ring possess a moderate antiviral activity. Also, it was reported that the pyrazole-4-carboxylic acid hydrazides and its hydrazones possess antimicrobial activity. Recently, 1, 3, 4-oxadiazoles, 1, 3, 4-thiadiazoles, 1,2,4-triazoles and triazines have attracted particular attention due their anti-inflammatory, analgesic, ulcerogenic and lipid peroxidation activities[50]. A novel dipyrazole ethandiamide compound and acid chloride of pyrazolo [3, 4-d]pyrimidine 4(5H)-one were prepared by Amal M. Youssef et.al.[51] and reacted with a number of nucleophiles. The resultant novel compounds were tested in several in vitro and in vivo assays. Three compounds inhibited the secretion of neurotoxins by human THP-1 monocytic cells at concentrations that were not toxic to these cells. They also partially inhibited both cyclooxygenase-1 and -2 isoforms. In animal studies, two compounds were notable for their anti-inflammatory activity that was comparable to that of the clinically available cyclooxygenase-2 inhibitor celecoxib. Modeling studies by using the molecular operating environment module showed comparable docking scores for the two enantiomers docked in the active site of cyclooxygenase-2. A series of 2-pyridyl-substituted imidazoles has been synthesized by Purushottam M. Dewang et.al[52] and evaluated for their Activin receptor-like kinase $_5$ (ALK_5) inhibitory activity in cell-based luciferase reporter assays. Ibrahim Mustafa et.al.[53] reported design and synthesis of new potent anticancer pyrazoles with high FMS-like tyrosine kinase $_3$(FLT_3) inhibitory selectivity. Pawan K. Sharma[54] reported synthesis and biological evaluation of some 4-functionalized-pyrazoles. The compounds exhibited moderate antibacterial activity against Gram-positive bacteria and one of them showed moderate antifungal activity against the tested fungi.

The above findings advocates the possible boipotential as a new class of antimicrobial activity of some new pyridine derivatives containing azoles as both having diverse pharmacological activities. The development of computational techniques has given the medicinal chemist a powerful tool to use in the development of drugs. A wide variety of computer programs and methods have been developed to visualize the three dimensional shapes of both the ligands and their target sites. In addition, sophisticated graphics packages also allow the medicinal chemist to evaluate the interactions between a compound and its target site before and after synthesizing that compound. This means that the medicinal chemist need only develop and test the most promising of the compounds, which considerably increases the chances of discovering a potent drug as well as reduces the cost of development[55] (Thomas Gareth, 2001). This may also help in understanding the quantitative structure activity relationship (QSAR) of the synthesized compounds. Resent trends in 2D/3D QSAR have focused on the development of procedure that allows selection of optimal variables from the pool of descriptors of chemical structures i.e. ones that are most meaningful and statistically significant in terms of correlation with biological activity. This is accomplished by combining one of the stochastic search methods such as Simulated Annealing, Genetic Algorithms, or evolutionary algorithms with the correlation methods such as Multiple Linear Regression, Partial Least Square regression, or Artificial Neural Networks[56-61]

Conclusions:

Despite growing antimicrobial resistance in major bacterial pathogens, relatively few new antimicrobial options are available and effective against these resistant strains. The current pipeline of products is primarily focused on MRSA and Gram-positive cocci, although new peptides are in development with

promising efficacy against Gram-negative bacteria. Specifically, new carbapenems, such as doripenem, appear to demonstrate more activity than other carbapenems against Gram-negative and non-fermenting bacteria. Additional data from clinical trials will be required to confirm these results. The synthesis of diverse heterocycles containing pyridines and azoles with the help of computer aided drug design strategies may give rise to new anti-infective agents, as the pyridines and azoles (separately) are found as good antimicrobial agents. Keeping these in view new prototypes could be designed by combining both moieties, and synthesized hybrid molecules consisting of pyridines along with azole moiety could be investigated for their in vitro antimicrobial activities.

Table 1: Resistance Problems for Antimicrobial Agents among Gram-Positive Bacteria

Organisms	**Resistances (Antimicrobials)**
Staphylococci	Penicillin, oxacillin, macrolides, others
Enterococci	Vancomycin, penicillins, aminoglycosides
Streptococcus pneumoniae	Penicillin, macrolides, some cephalosporins
Streptococci, viridans group	Penicillin
Corynebacterium spp.	All drugs except vancomycin
Bacillus spp.	β-1actams

Table 2: Resistance Problems for Antimicrobial Agents among Gram-Negative Bacteria

Organisms	**Mechanisms (Primary Resistant Drugs)**
Klebsiella pneumonia[*]	Extended-spectrum β-lactamase ("third-generation" cephalosporins)
Enterobacter spp., *Citrobacter freundii*	Bush gr.1 β-lactamase ("third-generation" cephalosporins)
Stenotrophomonas maltophilia	β-lactamases (numerous β-lactams)
Haemophilus influenzae	β-lactamases (ampicillin)
Neisseria gonorrhoeae	β-lactamases, PBP, altered gyrases (penicillins, fluoroquinolones)
Neisseria meningitidis	PBP (penicillins)
Bacillus fragilis group	Multiple (clindamycin, cephamycins, other)
Acinetobacter and *Pseudomonas*	Multiple (β-lactams, aminoglycosides, other)

[*] AIso observed among *Klebsiella oxytoca* and *Escherichia coli.*

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