Antibacterial Activity of New Disperse Dyes from Substituted Pyridones against Clinical Isolates Part V.

[1]Zeeshan Akhtar*,[1]Syed Imran Ali, [2]MuhammadYasirKhan, [3]Zafar Iqbal Shams,[1]Shagufta Afaq, [4]Kamran Ahmed and [1]Rasheeda Parveen

[1]*Department of Applied Chemistry and Chemical Technology, University of Karachi-75270 (Pakistan).*

[2]*Department of Chemical Engineering, University of Karachi-75270 (Pakistan).*

[3]*Institute of Environmental Studies, University of Karachi-75270 (Pakistan).*

[4]*Pakistan Council of Scientific & Industrial Research (PCSIR) Laboratories Complex, Off University Road-75280 (Pakistan).*

zakhtar@uok.edu.pk*

(Received on 20th June 2018, accepted in revised form 3rd September 2018)

Summary: The in vitro antimicrobial activities of eight newly synthesized disperse dyes has been studied using agar-well diffusion method. Three Gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus* and *Staphylococcus epidermidis*) and eight Gram negative bacteria (*Klebsiella pneumoniae*, *Proteus sp*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *S. para typhi B*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, and *Vibrio cholrae*) were employed. The dyes were previously synthesized based on substituted pyridines and its derivatives and the structural characterization revealed that they contain a pyridine nucleus in their structure. The antimicrobial activity was performed using a dye solution (100 mg/ml). The dyes demonstrated varying degrees of activities against different bacterial cultures tested which highlighted the fact that their chemistry has significant influence on the antimicrobial activity.

Keywords: Antimicrobial activity, Disperse dyes, Substituted pyridines, Gram positive and Gram negative Clinical isolates.

Introduction

Textile materials are known to exhibit susceptibility towards microbial growth owing to their large surface area accompanied by the ability to absorb and retain moisture [1]. Besides, the accumulated soil and dust particles, sweat and even some finishes applied during wet processing may also serve as medium and nutrients for microorganisms [1-4]. All these factors simulate the accumulation and multiplication of various bacteria, mould or fungi which can potentially leads to deterioration in the quality of textile product and various undesirable effects on the health of its user [5, 6]. Some of these effects include discoloration, undesirable reactions of polymer molecules of the fibers, changes in degree of polymerization as a result of molecular structure breakdown which eventually leads to loss of strength and elongation. In addition to that, presence of pathogens exposed the user to various potentially harmful diseases, infections and allergies [1-5].All these reasons emphasizing the importance of exploring ways to impart antimicrobial attributes to textiles.

In recent years, there has been an increasing research interest in exploring ways to enhance the resistance of textile materials towards microbial attacks [6-9]. A variety of approaches employing various chemicals and finishing materials have been explored to impart antibacterial properties to textiles [9-11]. However most of these contributions met with limited success, especially on a bulk scale, owing to the tedious processing steps and the use of non-biodegradable chemicals that cause environmental and health concerns[11, 12].

A logical alternate approach would be to combine dyeing and imparting antimicrobial properties in one step. Many researchers explored the development of novel dyes possessing antimicrobial properties [13]. Some studies have been conducted to explore the antimicrobial activities of existing dyes and their derivatives after their application on textile substrates. For instance, Liu *et al*. [12] demonstrated the effectiveness of Antraquinoid cationic dyes against gram-negative and gram-positive bacteria. Singh *et al*. [13] reported antimicrobial effects of various natural dyes against common pathogens. Al-Etaibi *et al*. [14] recently reported the antimicrobial properties of disperse dyes based on 3-oxo-2-(phenylhydrazono) -3-p- arylpropionaldehydes.

Substituted pyridine and their derivatives have emerged as novel disperse dyes and attracted considerable interest owing to their inherent antioxidant and biological activities, depending upon the chromophoric and auxoxhrome groups [15-17].

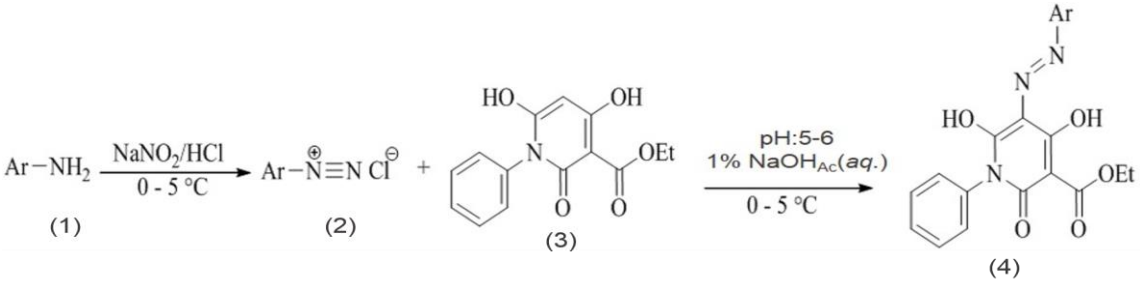
*To whom all correspondence should be addressed.

We previously reported the synthesis of some novel disperse dyes based on substituted pyridines and their derivatives, oxazine, carbooxyanilide and thiocarbamate and evaluated their insecticidal and antifungal activity [18, 19]. Considering the potential biological relevance of these dyes, we extended our previous work and are now reporting the antimicrobial properties of these newly synthesized disperse dyes against Gram positive and Gram negative bacteria.

Experimental

Synthesis of disperse dyes: Eight novels disperse dyes based on substituted pyridines and their derivatives, oxazine, carbooxyanilide and thiocarbamate were synthesized using substituted aryl amines as chromophores. The resulting compounds were characterized using elemental analysis and spectroscopy and reported in our earlier publication [18]. A general synthesis scheme is depicted in Scheme-1. The starting substituted aryl amines, name of dyes and their corresponding melting points are all listed in Table-1. Their structural formulas are listed in Scheme-2.

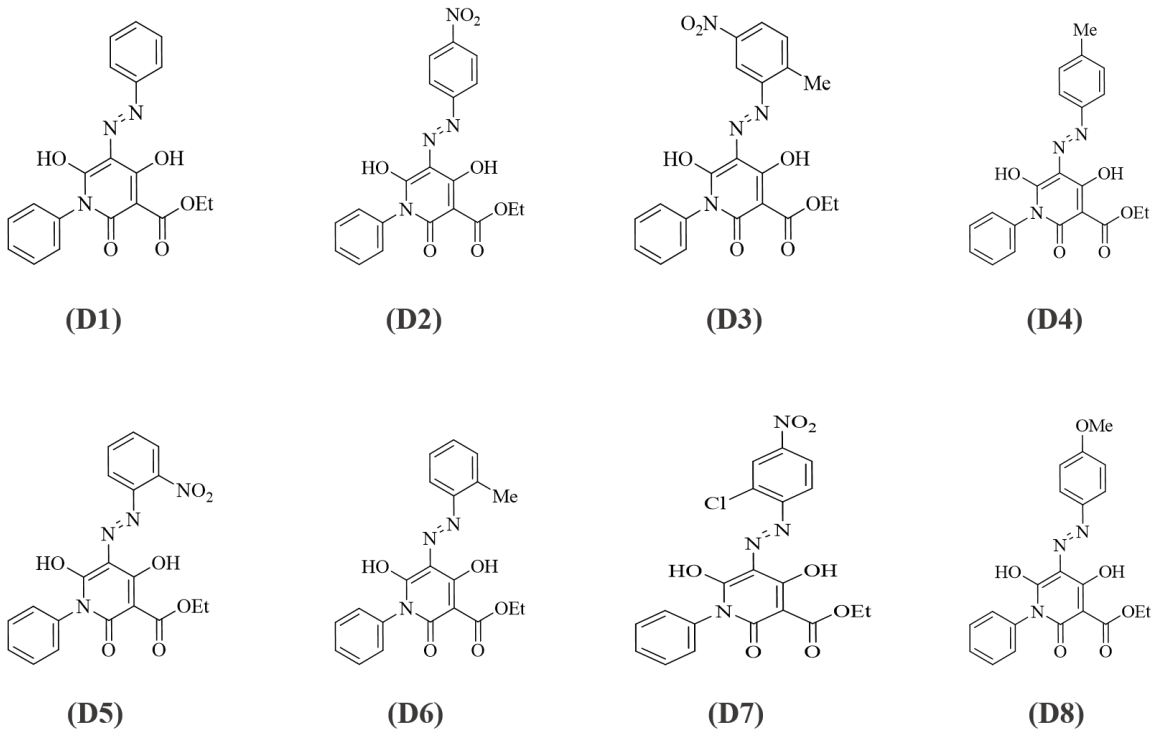
Antimicrobial Assay: In vitro antimicrobial activities of newly synthesized disperse dyes were evaluated using a procedure based on Agar-Well Diffusion Technique [14]. The dyes were tested against three different Gram positive bacteria cultures (*Bacillus subtilis*, *Staphylococcus aureus* and *Staphylococcus epidermidis*) and eight different gram negative bacteria cultures (*Klebsiella pneumoniae*, *Proteus sp*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *S. para typhi B*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, and *Vibrio cholrae*). Muller Hinton agar medium was used to test the antimicrobial activity. For the assay, first, nutrient agar plates were prepared by adding 20 ml of sterile Muller Hinton agar (Oxaid) into a sterile petri dishes and were allowed to solidify. The plates were then seeded with bacterial strains. This was followed by the addition of 100 µl of each disperse dye solution in 7 mm diameter wells produced on the plates with the help of a sterile Cork Borer. The final concentration of the dye was around 100 mg/ml. The plates were left at room temperature for about 30 minutes before placing them in an incubator at 37 °C for 24 h. The diameters of the inhibition zones around each well were determined and the values reported in Table-2 and Table-3 and are based on the averages of three replicas.



Scheme-1: Synthetic route of disperse dyes.

Table-1: Names and melting points of synthesized dyes.

S.No.	Substituted aryl amines	Dye Name	Code	M.P ºC
1.	RN-Ph (Phenyl)	Ethyl 4,6-dihydroxy-2-oxo-1-phenyl-5-(phenyldiazenyl)-1,2-dihydropyridine-3-carboxylate	D1	218
2.	4-Nitrophenyl	Ethyl 4,6-dihydroxy-5-((4-nitrophenyl)diazenyl)-2-oxo-1-phenyl-1,2-dihydropyridine-3-carboxylate	D2	228
3.	2-Methyl-5-nitrophenyl	Ethyl4,6-dihydroxy-5-((2-methyl-5-nitrophenyl)diazenyl)-2-oxo-1-phenyl-1,2-dihydropyridine-3-carboxylate	D3	254
4.	4-Methylephenyl	(*E*)-Ethyl 4,6-dihydroxy-2-oxo-1-phenyl-5-(p-tolyldiazenyl)-1,2-dihydropyridine-3-carboxylate	D4	212
5.	2-Nitrophenyl	*E*)-Ethyl 4,6-dihydroxy-5-((2-nitrophenyl)diazenyl)-2-oxo-1-phenyl-1,2-dihydropyridine-3-carboxylate	D5	265
6.	2-Methylphenyl	(*E*)-Ethyl 4,6-dihydroxy-2-oxo-1-phenyl-5-(o-tolyldiazenyl)-1,2-dihydropyridine-3-carboxylate	D6	244
7.	2-Chloro-4-nitrophenyl	(*E*)-Ethyl5-((2-chloro-4-nitrophenyl) diazenyl)-4,6-dihydroxy-2-oxo-1-phenyl-1,2-dihydropyridine-3-carboxylate	D7	258
8.	4-Methoxyphenyl	(*E*)-Ethyl 4,6-dihydroxy-5-((4-ethoxyphenyl)diazenyl)-2-oxo-1-phenyl-1,2-dihydropyridine-3-carboxylate	D8	211



Scheme-2: Structural formulas of synthesized disperse dyes.

Results and Discussion

The antimicrobial potential of eight novel disperse dyes was assessed against three (03) different Gram Positive bacterial cultures (*Bacillus subtilis*, *Staphylococcus aureus* and *Staphylococcus epidermidis*) and eight (08) different Gram Negative bacterial cultures (*Klebsiella pneumoniae*, *Proteus sp*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *S. para typhi B*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, and *Vibrio cholrae*). For the antimicrobial screening, a well-established agar well diffusion method was adopted and the resulting growth inhibition zones are summarized in Table-2 and 3. As mentioned earlier, these dyes were previously synthesized using substituted pyridines and their derivatives, oxazine, carbooxyanilide and thiocarbamate. It has been shown earlier that the compounds containing heterocyclic rings, especially pyridine derivatives exhibit many biological activities and are hence often employed as antimicrobial agents [20]. Pyridinone nucleus constitutes the active part of many biologically active compounds [20-22]. As shown in the Scheme-2, the synthesized dyes contain a pyridines nucleus in their structure which is likely to impart some biological activities to these molecules. The results obtained for the antimicrobial screening (Table-2 and 3) revealed that the synthesized dyes exhibit varying degrees of activities against the microorganisms tested.

Table-2 summarized the results of antimicrobial activities of the newly synthesized disperse dyes were evaluated against Gram positive bacteria cultures. The data revealed that out of the eight dyes tested, only D1, D2 and D8 exhibit moderate activities against *Bacillus subtilis* bacterial strains with significant inhibition zones (≥ 8 mm). Rest of the dyes was inactive against this microorganism. Besides, against the bacterial strains *Staphylococcus aureus*, two dyes D3 and D4 showed activities with the inhibition zones of 8 and 7 mm, respectively. For this bacterial strain, no noticeable activities were recorded by the dyes D1, D2, D5 to D8. Against *Staphylococcus epidermidis*, only D7 showed some activity with an inhibition zone of around 7 mm. It is important to mention that the dyes D5, D6 and D7 showed no noticeable activities against any of the microbial strain tested. The observed differences in the antimicrobial activities of these dyes are in agreement with the findings of the other researchers who demonstrated that the inhibitory activity of dyes is significantly dependent on their lipophilicity and the interaction with the bacterial cell wall [23-25]. The presence of different functional groups and substituents effect the bulkiness and lipophilicity of a molecule causing

differences in the antimicrobial efficacy [26]. The observed inhibitory response of dyes D3 and D4 against *Staphylococcus aureus* is likely due to their higher lipophilicity owing to the presence of methyl groups in their structures. Similarly, the observed activity of dye D7 against *Staphylococcus epidermidis* can also be explained on the basis of lipophilicity. These are interesting findings and require more in-depth investigation to explore the exact structure–activity relationships enabling us to design antimicrobial dyes with higher efficacy.

Table-2: Diameter of the zones of inhibition of the tested disperses dyes against gram positive bacterial strains.

S. No	Microorganism (Gram Positive)	Dye Code							
		D1	D2	D3	D4	D5	D6	D7	D8
1.	*Bacillus subtilis*	10	8	-	-	-	-	-	11
2.	*Staphylococcus*	-	-	8	7	-	-	-	-
3.	*Staphylococcus epidermidis*	-	-	-	7	-	-	-	-

The antimicrobial activities of the synthesized disperse dyes were also explored against eight different gram negative bacteria and the resulting inhibition zones diameters are all listed in Table-3. It can be seen from the Table 3 that the two dyes D1 and D2 were active against only two bacterial strains, *Pseudomonas aeruginosa* and *Shigella sonnei*. The exhibited microbial activities are however moderately high, as evident from their significantly high inhibition zones (≥10 mm). Against the bacterial strain *Klebsiella pneumoniae*, only D7 showed some activity and the inhibition zone was found to be around 8 mm. Similarly, only the dye D6 exhibited some activity against bacterial strain proteus with an inhibition zone measuring around 7 mm. In addition to the dyes D1 and D3, three more dyes (D4, D5 and D7) showed moderate activities against *Pseudomonas aeruginosa*. The recorded inhibition zones for these three dyes are 10 mm for dye D4 and 8 mm each for the dyes D5 and D7. Against the bacterial strains *S. para typhi B* and *Shigella dysenteriae*, only dye D3 showed some activity with the inhibition zones of 6 mm and 10 mm, respectively. The rest of the compounds were ineffective against these two microorganisms. Against the bacterial strains *Shigella flexneri*, only two dyes D4 and D8 responded with some activities and the inhibition zones were recorded to be 8 mm and 7 mm, respectively. With the exception of D4, all the dyes tested responded well against the bacterial strain *Shigella sonnei* as evident from the recorded values of the inhibition zones (Table-3). The dye D4 was the only compound which showed moderate activity against the bacterial strain *Vibrio cholrae* with an inhibition zone of around 7 mm.

Table-3: Diameter of the zones of inhibition of the tested disperses dyes against gram negative bacterial strains.

S.No	Microorganism (Gram negative)	Dye Code							
		D1	D2	D3	D4	D5	D6	D7	D8
1.	*Klebsiellapneamonae*	-	-	-	-	-	-	8	-
2.	*Proteus*	-	-	-	-	-	7	-	-
3.	*Pseudomonas Aeruginosa*	10	11	-	10	8	-	8	-
4.	*Salmonella Typhi Para B*	-	-	6	-	-	-	-	-
5.	*Shigelladysenteriac*	-	-	10	-	-	-	-	-
6.	*Shigella Flexner*	-	-	-	9	-	-	-	8
7.	*ShigellaSonnei*	10	10	9	-	9	7	8	10
8.	*Vibriocholarae*	-	-	-	7	-	-	-	-

The observed antimicrobial properties of the dyes can be explained on the basis of the presence of pyridinone nucleus which is reported to exhibit several biological activities [27-30]. The differences in the observed antimicrobial behaviors of dyes are understandable owing to their different chemical structures. It is well known that the biological activity of a chemical compound largely depends on its lipophilicity and physicochemical properties [31]. As these properties significantly depend on the chemical structure and the substituent present in the molecules, we observed considerable differences in the antimicrobial behaviors of the dyes.

Conclusions

In this paper, in vitro antimicrobial potential of eight newly synthesized disperse dyes was explored against three Gram positive and eight Gram negative bacteria. These dyes were designed to contained pyridinone nucleus in their main structures which is known impart biological activity to various compounds. Our results show that the synthesized dyes posses varying levels of antimicrobial activates against different microbial cultures.

In conclusion, we demonstrated the effectiveness of eight novel disperse dyes as antimicrobial compounds. These dyes can be utilized for imparting resistance against microbial attacks to various textile materials with the inherent advantage of combining dyeing and antimicrobial treatment in one step. Our future work will be focused on exploring the antimicrobial potential of these dyes on a range of textile fibers. Besides, exploring the underlying mechanism and functional groups responsible for the antimicrobial action would also be an interesting research direction as it would enable us to get more insight into the dye structure and antimicrobial activity relationship.

References

1. D. E. Boryo, The Effect of Microbes on Textile Material: A Review on the Way-Out sofar. *Int. J. Engg. & Sci*., **2**, 09 (2013).
2. F. A. Mohamed, H. M. Ibrahim, Antimicrobial Dyes based on Heterocyclic and/or Homocyclic Systems for Dyeing and Textile Finishing, *Res. & Rev. in biosci*., **8**, 285 (2014).
3. Y. Gao, R. Cranston; Recent Advances in Antimicrobial Treatments of Textiles. *Tex. Res. J*, **78,** 60 (2008).
4. R. Purwar, M. Joshi; Recent Developments in Antimicrobial Finishing of Textiles. A Review, *AATCC Review*, **4**, 22 (2004).
5. J. Szostak-Kotowa, Biodeterioration of Textiles. International Biodeterioration & Biodegradation. **1,** 165 (2004).
6. D. S. Morais, R. M. Guedes, M. A Lopes. Antimicrobial Approaches for Textiles: From Research to Market. Materials. **21,** 9498 (2016).
7. K. Murugesh Babu and R. B. Ravindra, Bioactive Antimicrobial Agents for Finishing of Textiles for Health Care Products. *J. of Tex. Institute*. **3**, 706 (2015).
8. M. Busila, V. Musat, T. Textor and B. Mahltig, Synthesis and Characterization of Antimicrobial Textile Finishing Based on Ag: ZnO Nanoparticles/Chitosan Biocomposites. RSC Advances, **5**, 21562 (2015).
9. F. Ferrero, M. Periolatto, S. Ferrario. Sustainable Antimicrobial Finishing of Cotton Fabrics by Chitosan UV-Grafting: from Laboratory Experiments to Semi Industrial Scale-Up. *J. Cleaner Production*., **1**, 244 (2015).
10. K. Murugesh Babu, K. B. Ravindra, Bioactive Antimicrobial Agents for Finishing of Textiles for Health Care Products. *J. of Tex. Institute*, **3**, 706 (2015).
11. B. Tawiah, W. Badoe and S. Fu. Advances in the Development of Antimicrobial Agents for Textiles: The Quest for Natural Products. Review. Fibres & Textiles in Eastern Europe. (2016).
12. J. Liu, G. Sun, The Synthesis of Novel Cationic Anthraquinone Dyes with High Potent Antimicrobial Activity. *Dyes and Pigments*. **1**, 380 (2008).
13. R. Singh, A. Jain, S. Panwar, D. Gupta and S. K. Khare, Antimicrobial Activity of Some Natural Dyes. *Dyes and Pigments*, **1**, 99 (2005).
14. A. Al-Etaibi, M. A. El-Apasery, M. M. Kamel, Dyeing of Polyester with Disperse Dyes: Part 1. Antimicrobial Activity and Dyeing Performance of Some Disperse dyes. *Int. J. Curr. Microbiol. Appl. Sci*. **4**, 923 (2015).
15. E. Caballero, P. Puebla, M. Medrade and A. Feliciano, Regiochemistry in the Synthesis of 2,3,8,8a-Tetrahydro-7h-Oxazolo[3,2-A] Pyridines, *Tetrahedron*, **49**, 10079 (1993).
16. A. H. El-Sebae, Studies on the Chemical Structure and Insecticidal Activity. Toxicity of some Arylthiocyanates and Isothiocyanates. *J. Agric. Res*, **18**, 253 (1970).
17. M. B. Hosur, M. C. Talawar, U. V. Laddi and S. Rajani. Synthesis and Antimicrobial Activities of some New 1, 3, 4-Oxadizoles. P. G. Department of Studies in Chemistry, Karnatak University, Dharwad. **3**, 237, (1994).
18. M. K. Khalid, Mahwish, S. Parveen and R. Parveen, Synthesis of Ethyl 4,6 Dihydroxy-2-oxo-1- Phenyl 5-Arylazo Pyridine-3-Carboxylate Part IV New Disperse Dyes. *J. Chem. Soc. Pak*. **32**, 505 (2010).
19. Z. Akhtar, A. Aleem, M. A Ahmed, R. Parveen, K. Ahmed, M. Iqbal, Screening of Heterocyclic Compounds: Insecticidal and Fungicidal Activity of Substituted Pyridines and their Derivatives. *Int. J. Biol. Res*.**1**, 129 (2013).
20. P. A. Wolf and F. J. Bobalek, Antimicrobial Performance of Tri- and Tetrachloro-4 (methylsulfonyl) Pyridines as Industial Preservatives. *Am. Microbiol*. **15**, 1376 (1967).
21. M. El-Bayouki, K. Abdel Hameed, W. M. Asyouni, Y. A Mohamed, M. M. Aly, S. Y. Abbas, Novel 4(3H)-Quinazolinones Containing Biologically Active Thiazole, Pyridinone and Chromene of Expected Antitumor and Antifungal Activities. *Eur. J. Chem.*, **2**, 455. (2011).
22. E. V. Afanas, L. G. Korkina, A. V. Kozlov AV, Spectrophotometric, electron spin resonance, polarographic, and chemiluminescent study of biologically active chelators: 1-Allyl-2- methyl-3-hydroxy-4-pyridinone, lipoic and dihydrolipoic acids. *Phys. Chem. Biol. Med.*, **3**, 25 (1996).
23. H. Shaki, K. Gharanjig, and A. Khosravi, Synthesis and investigation of antimicrobial activity and spectrophotometric and dyeing properties of some novel azo disperse dyes based on naphthalimides. *Biotec. Prog.*, **31**, 1086 (2015).
24. H. Turkmen, G. Zengin and B. Buyukkircali, Synthesis of sulfanilamide derivatives and investigation of in vitro inhibitory activities and antimicrobial and physical properties. *Bioorg. Chem*., **39**, 114 (2011).
25. A. Leo, C. Hansch, D. Elkins, Partition coefficients and their uses. *Chem Rev*., **71,** 525 (1971).
26. M. E Levison and J. H Levison, Pharmacokinetics and pharmacodynamics of

antibacterial agents. *Infect Dis Clin North Am.* **23,** 791 (2009).

27. S. M Sakr, A. M. Kadry, A. M. M. El-Sayed, M. I. Al Ashmawi, Condensed Thieno [2,3-*d*] pyrimidines: Synthesis and Biological Activity. *Egypt. J. Chem*, **34**, 147 (1992).
28. P. D Bouzard, P. D. Cesare, C. Dussy, J. P. Jacquet, A. Jaegly, Synthesis and Antibacterial Activity of New 5-Substituted-1-Cyclopropyl-6-Fluoro-7-Piperazinyl-1,4-Dihydro-4-oxo-1,8-Naphthyridine-3-Carboxylic Acids. *J. Heterocycl. Chem.*, **29**, 985 (1996).
29. S. X. Cai, N. S. Jia, J. Herich, J. Guastella, S. Reddy, B. Tseng, J. Drewe, S. Kasibhatla, Discovery of Substituted *N*-Phenyl Nicotinamides as Potent Inducers of Apoptosis using a Cell- and Caspase-Based high Throughput Screening Assay. *J. Med. Chem*., **46**, 2474 (2003).
30. O. Tabarrini, G. Manfroni, A. Fravolini, V. Cecchetti, S. Stefano, E. D-Clereq, J. Rozenski, B. Canard, H. Dutarte, J. Paeshuyse, J. Neyts, Synthesis and Anti-BVDV Activity of Acridones as New Potential Antiviral Agents. *J. Med. Chem.*, **49**, 2621 (2006).
31. H. Shaki, K. Gharanjig, A. Khosravi, Synthesis and Investigation of Antimicrobial Activity and Spectrophotometric and Dyeing Properties of some Novel Azo Disperse Dyes Based on Naphthalimides. *Biotech. Prog*., **1**, 1086 (2015).