International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.4, No.2, pp 852-859, April-June 2012

Synthesis and QSAR Studies on 4,5- Diphenyl ImidazolylPyrimidine -5- Carboxylates (DPIP) against Antifungal Activity

A. K. Rathod

Department of Pharmaceutical Science (Chemistry), Jodhpur National University, Jodhpur-342001,Rajasthan, India.
Department of Chemistry, sevadal mahila mahavidyalaya and research academy,Nagpur-440009. (M.S),India

Corres.author : ashokrathod1972@yahoo.in

Abstract : A series of substituted ethyl 1,2,3,6-tetrahydro-4-methyl-2-oxo/thioxo-6-phenyl-1-(4,5-diphenyl-1-H-imidazol-2-yl) pyrimidine-5-carboxylates(3a-3g) was subjected for Quantitative structure-activity relationship (QSAR) models.the antifungual activity was co-related with mathematical relationship between physical,chemical,biological activities of interest and measurable or computable parameters such as physicochemical constant, topological, regerations analysis estimated activity and values calculated etc. The newly synthesized substituted diphenyl imidazolylpyrimidines were established by using the molecular descriptors ST, MV, X_{index} MR, TE, LUMO, Log P, V AR, AECC. The logarithm of zone of inhibition of micro-organisms i.e. *C.albicans* strains are used as key properties to evaluate the QSAR models. The Predictive ability and accuracy of the model is determined by a cross validation method.
Keywords : imidazolylpyrimidines derivatives, QSAR studies, molecular descriptors, cross validation, antifungal activity.

Introduction

Novel medicines are typically developed using a trial and error approach, which is time consuming and costly. The application of quantitative structure-activity relationship (QSAR) methodologies to this problem has potential to decrease substantially the time and effort required to discover new medicines or to improve current ones in terms of their efficacy. QSAR establishes the mathematical relationship between physical, chemical, biological or environmental activities of interest and measurable or computable parameters such as topological, physicochemical, stereo chemical or electronic indices[1-4].

Candida albicans is the most prevalent opportunistic fungal pathogen in human that causes various forms of candidiasis ranging from superficial mucosal infection to life threatening systemic diseases in immunocompromised patients[5]. Many azoles inhibiting 14 α-lanosterol demcthylase in ergosterol biosynthesis pathway are known to exhibit interesting antibacterial activity and antifungal activities. However, reported drug class having azoles ring system[6] suffers major shortcomings i.e. a rapid development of resistance against *Candida albieans*. This has highlighted the need to discover new effective Antibiotic with new modes of action against both bacteria and fungi.

The present study aims at determining the antifungal and antibacterial activities of newly

synthesized imidazolylpyrimidine derivatives by means of QSAR approach. During the programmed study on the development of green approach towards the synthesis of new organic molecules, a simple strategy for the synthesis of 4,5-diphenyl imidazolyl pyrimidine derivatives (3a-3g) was designed, in which the two aryl rings were located at C-4 and C-5 on the opposite faces of the newly planar imidazole ring[7].

C.albicans with Griesofulvin. Since the synthesized compounds showed remarkable antifungal and antibacterial activity, we established QSAR analysis using ST, MV, X_{index}, MR, TE, LUMO, LogP, V AR, and AECC as appropriate molecular descriptors. After selecting these indices adequately, a very specific characterization of each chemical compound in QSAR models (Table 1.1) was obtained.

Descriptors Used: Before the calculation of the descriptors, the structures were fully, optimized using ACD chern. Sketch 10.3 software[8] and Chemdraw 3D Ultra 8.0.[9] All the descriptors used are calculated from the hydrogen suppressed molecular graphs. These molecular, graphs are obtained by deleting all the carbon - hydrogen as well as heteroatom - hydrogen bonds from the molecular structures of the imidazolepyrimidine derivatives. Dragon 5.4 (2006)[10] software was used for further calculations. The details of the calculations of these descriptors are available in the literature and therefore, they are not mentioned here.

Statistical analysis: The regression analysis is made using. maximum R2 method using MYSTAT 12[11] and Origin 5.0[12] software.

Materials and Methods

Experimental Section

All solvents were distilled prior to use. TLC was performed on silica gel G. Melting points were determined by open capillary method and are not correct. ^{1}HNMR and ^{13}CNMR spectra were recorded from $CDCl_3$/DMSO-d6 solution on a Brucker Avance-II 400(400 MHz) NMR Spectrometer. Chemical shifts are reported in ppm using TMS as an internal, standard. IR spectra were obtained on a Shimadzu FTIR spectrophotometer, using KBr discs. Mass spectra were recorded by using Shimadzu gas chromatograph coupled with QP5050 Spectrometer at 1-1.5 ev.

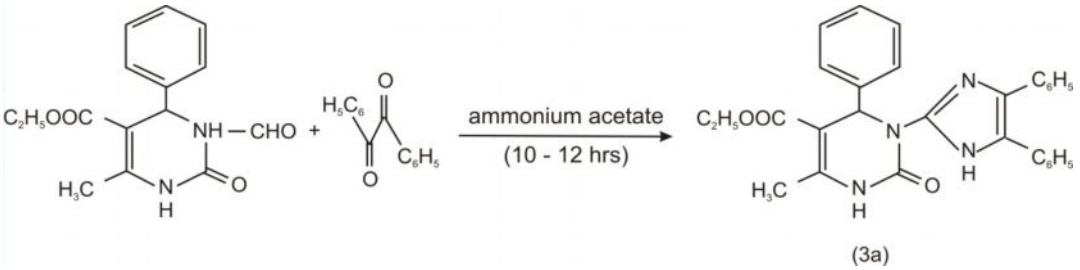
Procedure

Preparation of synthesis of substituted ethyl 1, 2, 3, 6-tetrahydro-4-methyl-2-oxo/thioxo-6-phenyl-1- (4, 5-diphenyl-1-H-imidazol-2-yl)pyrimidine-5-carboxylate (3a-3g)

Benzil (2.5m mole; 5.25g), ethyl-1-formyl-1,,2,3,6-tetrahydro-4-methy-6-phenyl-2-oxo-pyrimidine-5-carboxylate (2.5m Mole; 11.95g) and ammonium acetate (0.12 mole; 10g) were dissolved in glacial acetic acid. The reaction mixture was refluxed for 10-12 hours. It was then cooled and poured in cold water then the precipitate was formed, filtered, washed with ammonium hydroxide and dried. The product was recrystallization from ethanol. Yield : 64% M.P. 160ºC.

Results and Discussions

Substituted ethyl 1, 2, 3, 6-tetrahydro-4-methyl-2-oxo/thioxo-6-phenyl-l- (4, 5-diphenylI-H-imidazol-2-yl) pyrimidine-5-carboxylates (3a-g) were synthesized by condensing substituted ethyl-1-formyl-l, 2, 3, 6-tetrahydro-4-methyl-6-phenyl-2-oxo/thioxopyrimidine-5-carboxylates (la-g) and Benzil with ammonium acetate by using acidic alumina, and four drops of glacial acetic acid under conventional method for 12 hours. (Scheme 1).



(Scheme 1)

Table 1.1:Physical Characteristic data of the compound synthesized (3a-g)

Compound	X	R_1	R_2	Mol.Formula	MP(0C)	Method-A Yield/Time %/hr
3a	O	H	U	$C_{29}H_{26}N_4O_3$	160	64/12
3b	O	H	NO2	$C_{29}H_{25}N_5O_5Cl$	210	64/12
3c	O	Cl	H	$C_{29}H_{25}N_4O_9Cl$	180	62/12
3d	O	OCH3	H	$C_{30}H_{28}N_4O_4$	240	65/12
3e	S	U	H	$C_{29}H_{26}N_4O_2S$	190	65/12
3f	S	Cl	H	$C_{29}H_{25}N_4O_2SCl$	195	63/12
3g	S	H	NO2	$C_{29}H_{25}N_5O_4S$	220	60/12

The QSAR study of newly synthesized ethyl-1, 2, 3, 6-tetrahydro-4-methyl-2-oxo/thioxo6-phenyl-l-(4,5-diphenyl-lH-imidazol-2-yl) pyrimidine-5-carboxylate derivatives (3a-g) is not reported in the literature. Hence the synthesized compounds were tested against C.albicans in comparison with Griesofulvin.

Antifungal activity

The antifungal activities of compounds (3a-3g) have been assayed in vitro at a concentration 100□g disc-[1] against *C.albicans*. Griesofulvin was used as standard fungicide for the antifungal test. Muller-Hinton agar was used as basal medium for test fungi. Glass Petri dishes were sterilized and 10ml of sterilized melted MH agar medium (4S°C) was poured into each Petri dish. After solidification of the medium small portion of mycelium of *C.albicans* was spread carefully over the centre of each MH agar plate with the help of spreader. Thus fungus was transferred to each plate. The plates were then incubated at (27°C) and after half an hour of incubation they were ready for use. The prepared discs of test sample were placed gently on the solidified agar plate, freshly seeded with the test organisms with sterile forceps. The plates were then incubated at 37.5ºC for 24hr. Dimethyl formamide (DMF) was used as a solvent to prepare desire solutions of the compounds initially[17-18].

The antifungal studies revealed that the compounds 3b and 3c having chloro and nitro groups respectively along with oxopyrimidine moiety were found to be most active amongst the entire tested compounds. 3a and 3g exhibited moderate activity in comparison with other compounds. 3f showed less activity where as 3d and 3e were found to be inactive against the *C.albicans* (Table 1.2).

QSAR Study

In the present study authors tried to develop best QSAR model for each microorganisms to explain the correlation between the physicochemical parameters and antifungal activity of diphenyl imidazolyl pyrimidines (DPIP) derivatives against microorganisms. The details of molecular structures of (DPIP) derivatives used in the present study are illustrated in (Table 1.1). The antifungal activities of above said compounds against *Calbicans* are depicted in (Table 1.2). Here, we have used logarithm of activities to be studied. In order to model and predict the specific activity, 57 physicochemical constants, topological and structural descriptors were considered as possible input candidates to the model[13].

Table 1.2:Antifungal screening results of compound synthesized

Sr.No.	Compounds	Zone of inhibition in mm for conc. for 100□g/ml	Logarithm of zone of inhibition in mm
C.albicans			
1.	3a	9.0	2.197
2.	3b	12.0	2.485
3.	3c	13.0	2.565
4.	3f	6.0	1.791
5.	3g	9.0	2.197

Table 1.3: The calculated values of descriptors ST, MV, X;,d", MR, TE, LUMO, LogP, V AR, and AECC are summarized.

Sr.No.	Compound	VAR	X_{index}	V_{index}	MV	MR	ST	LogP	TE	LUMO	AECC
1	3a	146	0.288	0.191	238.9	135.97	52.3	4.5456	2305.6	-0.502	10.279
2	3b	152	0.29	0.193	372.8	139.98	54.9	4.3552	2882.4	-0.874	10.342
3	3c	146	0.288	0.191	379.3	136.23	52.3	4.7657	2305.04	-0.635	10.278
4	3d	175	0.285	0.188	391.4	138.02	52.0	4.0811	2299.28	-0.554	10.838
5	3e	129	0.291	0.193	351.0	138.9	75.3	5.7991	2310.25	-1.08	9.714
6	3f	146	0.288	0.191	361.8	143.73	76.8	6.3573	2309.97	-1.195	10.278
7	3g	152	0.29	0.193	384.2	146.45	53.2	5.9468	2905.37	-1.009	10.342

A persual of (Table 1.2) showed that 3-a, b, c, f, g; these 5 compounds are effective against *C.albicans* are found to be resistant against 3b and 3d. In obtaining QSAR models, we have used logarithm of zone of inhibition to account for their antifungal activities against the microbes mentioned earlier.

Based on the activity values we observed, we can propose the following order of antifungal activity.

Against *Calbicans*

3c>3b>3a=3g>3f (1)

It is interesting to record that compound 3c shows maximum zone of inhibition against *C.albicans* and minimum zone of inhibition against all other strains used. Furthennore, these sequences (order) do not establish any quantitative structure-activity relationship (QSAR). Therefore, we have made such study using above said descriptors, which encodes the molecular structures of DPIP numerically. Since, different compounds are found effective against five microorganisms used, we have obtained the correlation matrices (Table 1.4) for preliminary investigations of correlation among descriptors against the antifungal activities. Based on the microorganisms used, our discussion has been divided into five different segments.

Table 1.4 : Correlation matrices for the DPIP used

	Activity	VAR	X_{index}	V_{index}	MV	MR	ST	LogP	TE	LUMO	AECC
Calbicans n = 5											
Activity	1.000										
VAR	0.282	1.000									
X_{index}	0.282	1.000	1.000								
V_{index}	0.282	1.000	1.000	1.000							
MV	0.512	0.204	0.204	0.204	1.000						
MR	-0.54	0.544	0.544	0.544	-0.26	1.000					
ST	-0.819	-0.33	-0.331	-0.331	-0.90	0.429	1.000				
LogP	-0.797	-0.04	-0.044	-0.044	-0.42	0.812	0.708	1.000			
TE	0.268	1.000	1.000	1.000	0.210	0.56	-0.33	-0.02	1.000		
LUMO	0.619	-0.32	-0.323	-0.323	0.679	-0.88	-0.75	-0.82	-0.33	1.000	
AECC	0.282	1.000	1.000	1.000	0.204	0.544	-0.33	-0.04	1.000	-0.323	1.000

Table 1.5 : Regression analysis and quality of correlations for modeling antifungal activity of DPIP against *C.albicans*.

Models	Descriptors	Se	R	R^2	R^2_A	F	A
1	LogP	0.212	-0.797	0.635	0.513	5.21	3.76
2	ST	0.201	-0.819	0.671	0.561	6.12	4.07
3	LUMO	0.29	-0.563	0.316	0.089	1.39	1.94
4	MR	0.296	-0.539	0.291	0.055	1.23	1.82
5	MV	0.3	0.521	0.272	0.029	1.12	1.74
6	ST,MV	0.11	0.967	0.935	0.869	14.31	8.79
7	LogP, ST	0.208	0.875	0.765	0.531	3.26	4.21
8	ST, MR	0.23	0.845	0.714	0.429	2.5	3.67
9	ST, LUMO	0.247	0.819	0.671	0.342	2.04	3.32

Antifungal activity of DPIP against *Calbicans.*

The data presented in QSAR studies shows that the descriptors used i.e. ST, MV, Xindex, MR, TE, LUMO, LogP, V AR, and AECC are significantly conelated with the antifungal activity against C.albicans, which proves that ST is the best descriptor for QSAR model. The correlation potential of MV, MR and LUMO is significantly lower than the other used descriptors. This shows that we can obtain two mono-parametric models for modeling antifungal activity against *C.albicans* and that mono-parametric model based on ST will be the best for this purpose. In QSAR studies show that all the 10 descriptors are not linearly correlated and thus any combination of these descriptors in multilinear regression analysis may not result with a model suffering from the defect due to colinearity.

Looking to the sample size and following 'Rule of Thumb' we can at the most carry out bi-parametric regression analysis. The regression parameters and quality of correlations forihe different mono-parametric and bi-parametric models are given in (Table 1.5). This shows that among the mono-parametric models, the model based on ST gives better results.

Antifungal activity against *C.albicans* = 122.19 (±26.18) – 28.6(±11.56) ST

n = 5 Se = 0.2 R = -0.819

R^2 = 0.67 F = 6.12 Q = 4.07 (2)

R Here and thereafter 'n' is the number of compounds, Se - Standard error of estimation, R - Simple correlation coefficient, F - Fisher's statistics and Q - Quality factor, which is defined as the ratio of correlation coefficient to the standard error of estimation, that is Q = R/Se.

The coefficient of ST in the mono-parametric model represented by equation (2) is negative indicating that the antifungal activity of DPIP against *C.albicans* is inversely proportional to the magnitude of ST. This index ST precisely accounts for an inverse steric parameter. As compound **3c** has minimum value of ST shows significant antifungal activity against *Candida albicans.* Thus, the overall interpretation of negative R-values is that decrease in the magnitude of ST increases the antifungal activity of DPIP against *C.albicans.*

As stated earlier we have attempted several bi-parametric regressions and the results obtained are presented in Table 1.5. This shows that the bi-parametric model containing ST and MV showed excellent results in accordance with the following expression.

Antifungal activity against *C.albicans* = 20.07 (±5.8) – 0.005(±0.012) ST – 0.039(±0.017)MV

n = 5 Se = 0.11 R = 0.967

R^2 = 0.935 F = 14.31 Q = 8.79 (3)

The physical significance of the negative coefficient of ST term in the equation is the same as discussed for equation. The negative coefficient of MV indicates that the activity goes on decreasing with the increasing value of MV. This molar vo.l.Dme (MV) is one of the important Polarizability parameter; hence we can safely say that Polarizability plays a negative role in the exhibition of the activity[75].

Table 1.6 : Found and estimated antifungal activity of DPIP derivatives against *C.albicans* using the best model containing ST and MV descriptors.

Sr.No.	Compound	Exp. Activity	Estimated Activity	Residue	$(Residue)^2$
1	3a	2.197	2.261	-0.064	0.0041
2	3b	2.565	2.44	0.125	0.0156
3	3c	2.485	2.551	-0.066	0.0044
4	3f	1.792	1.785	0.007	0.00005
5	3g	2.197	2.2	-0.003	0.0000009
					□=0.024158

In order to confirm our results we have estimated the antifungal activity of DPIP derivatives against *C.albicans* using model expressed by equation and compared them with the observed values. The data presented in (Table 1.6) shows that the observed and the estimated activities are very close to each other.

The predictive power of the models can be judged from quality factor Q. The Q values are recorded in (Table 1.5). The highest Q = 8.79 for the model expressed by equation indicates that it has highest predictive power (Figure 1.2). Further, calculating predictive correlation coefficient, R2Pred that is obtained from the correlation between the observed and the estimated activity makes confinnation regarding predictive power. The R2pred = 0.934 confirms that the predictive power of the proposed model equation is highest.

In support of our results we have also calculated 5 important statistical parameters: Probable error of the coefficient of correlation (PE), least square error (LSE), Friedman's lack of fit measure (LOF), Sum of squares of response values (SSY) and Uncertainty of prediction (SPRESS). These parameters are calculated from the following equations that are summarized in Table 1.6.

Figure 1.1: Plot of estimated activity values Vs the experimental Log (activity) values for the model expressed by equation.

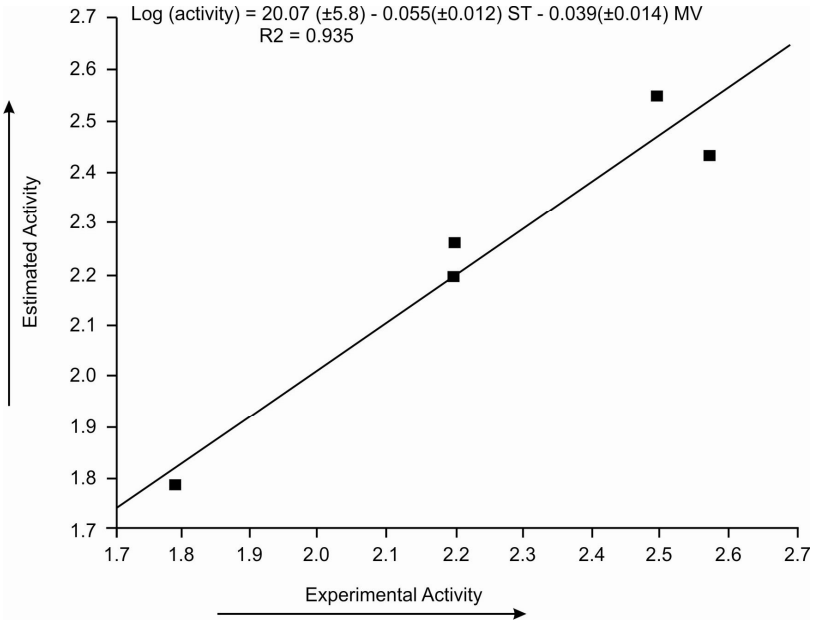


Table 1.7 : PE, LSE, LOF, SSY and S_{PRESS} value calculated for the derived models for modeling antifungal activity of DPIP derivatives against *C.albicans.*

Models	Descriptors	Se	R	R^2	R^2_A	F	A
1	LogP	0.109	0.59	0.0348	0.59	0.3696	0.4435
2	ST	0.098	0.122	0.0148	0.122	0.3696	0.2016
3	LUMO	0.204	0.201	0.0404	0.201	0.3696	0.2586
4	MR	0.212	0.26	0.0676	0.26	0.3696	0.2944
5	MV	0.217	0.268	0.0718	0.268	0.3696	0.2989
6	ST,MV	0.019	0.024	-	0.024	0.3696	0.1107
7	LogP, ST	0.07	0.087	-	0.087	0.3696	0.2085
8	ST, MR	0.085	0.105	-	0.105	0.3696	0.2291
9	ST, LUMO	0.098	0.122	-	0.122	0.3696	0.247

PE = 2/3*1-R2/□n (4)
Where, R – coefficient of correlation and n – number of compounds used.

$LSE = \square (Y_{obs.} - Y_{calc.})^2$ (5)
Where, Y_{obs} and Y_{calc} are the observed and calculated activities as in our case antifungal activity of DPIP derivatives against *C.albicans.*

$LOF = LSE/ \{1-(C + d*p)/n\}^2$ (6)
Where, LSE – Least square error, C – number of descriptors + 1, p – number of independent parameters, n - number of compound used, d - smoothing parameter which controls the bias in the scoring factor between equations with different numbers of terms and was kept 1.0.
It is argued that if.
R < PE, R is not significant;
R > PE, Several times at least three times greater correlations is indicated;
R > 6PE, Correlation is definitely good.

$SSY = \square (Y_{obs.} - Y_{Mean})^2$ (7)
Where, Y_{obs} and Y_{mean} are the observed and mean activities, in our case antifungal activity of DPIP derivatives against *C.albicans.*

$R^2_{Pred} = 1 - (PRESS)/SSY$ (8)

$S_{PRESS} = \square (PRESS) / (n - k - 1)$ (9)
Where, n = number of compounds used,
K = number of descriptors used
PRESS = Predicted Residual Error sums of Squares = $\square (Y_{obs.} - Y_{Mean})^2$

The values of PE (Table 1.6) indicate that all the proposed correlations are definitely good and the one expressed in equation (3) is the best. The lowest value of LSE, LOF and SPRESS are also in favour of the proposed model. It is important to mention here that one should use LOF directly rather than LSE, the reason being LOF does not decrease with increase number of descriptors and the lowest value is found for an equation with the optimum number of parameters.

Acknowledgement

I am thankful to Dr. Pradipkumar Dey Registrar and Dr. Anil Bhandari, Dean, Faculty of Applied Science, Jodhpur National University, Jodhpur, for his encouragement and inspiring me towards research work.

I am equally thankful to Dr.Pravin Charde, Principal, Sevadal mahila mahavidyalaya, Nagpur. for his co-operation and research facilities.

Authors here by humbly acknowledge to Department of Microbiology, Sevadal mahila mahavidyalay Nagpur for antimicrobial analysis .

I am also humbly acknowledge to Department of Pharmacy, Nagpur for IR spectral analysis, SAIF, Chandigarh for, ^{1}HNMR, 1CNMR spectral analysis, Pune for the Mass analysis.

References

1. Melagraki G, Afantitis A, Sarimveeis H, Igglessi-Markopoulou O, Supuran C T, Bio-organic and medicinal chemistry, 2006, 14, 1108.
2. Lenonard IT, Roy K, QSAR Comb Sci, 2004**,** 23**,** 387.
3. Rathod Ashok K, Synthesis and characterizations of of Diphenyl Imidazolylpyrimidines-5-Carboxylates(DPIPC) Derivatives and their Antibicrobial activity,Int.J.PharmTech Res.vol. 3, (1), 2011, 435-441
4. Hansch.C and Leo, A. Exploring QSAR fundamental and applications in chemistry and Biology, American Chemical Society, Washington D.C.1995.
5. Mandloi D, Khadikar P V, Bio-organic and med chem letters. 2005**,** 15, 405-411.
6. ACD - Lab software for calculating the referred physicochemical parameters, chemsketch S.O, www.Acdlabs.com.
7. Chemdraw 3D Ultra software for calculating physicochemical parameters, www.cambridgesoft.com
8. Dragon.web version 5.4, Milano chemo metrics and QSAR research Group, www.disat.uinmib.it. 2006,
9. MYSTAT 12 is a statistical program developed by SYSTAT Software Inc, USA SYSTAT 12 © Copyright 2007, SYSTAT Software, Inc.
10. Origin 5.0. is a statistical program developed by Microcal Software, Inc. http://www.microca1.com
11. Kier.L.B and Hall.L.H Molecular connectivity in structure activity analysis, Wiley & Sons, 1986,129.
12. Marr K A, White T C, Antimicrob Agents Chemother, 2001**,** 45, 52-59.

*****