International Journal of Statistical Design and Analysis
Vol. 1, No. 1, October 2013, PP: 01 - 08
Available online at http://acascipub.com/Journals.php

Research article

Optimization for Synthesis of Sulfanyl-Based AntiFungal Drugs Catalyzed by Modified Fluorapatite Using Central Composite Experimental Design

Younes Abrouki[1,*], Abdelkader Anouzla[1], Hayat Loukili[1], Rabiaâ Lotfi[1], Zineb Abrouki[1], Halima Idrissi, Ahmed Rayadh[1], Mohamed Zahouily[1], and Saïd Sebti[2]

[1]Department of Research, Faculty of Science and Technology, Mohammedia, Morocco [2]Department of Chimistry, Faculty of Science Ben M'Sik, Casablanca, Morocco *Corresponding author (Email: younesabrouki@gmail.com)

Abstract

Sulfanyl-based antifungal drugs were efficiently prepared from Michael addition between between α,β-unsaturated carbonyl compounds and mercaptans catalyzed by modified fluorapatite. A central composite design was successfully employed for optimization of this synthesis. By-products of usual undesirable reaction are not observed. The work-up procedure is simplified by simple filtration with the use of catalyst.

Keywords: Antifungal, Sulfanyl, Fluorapatite, Heterogeneous catalyst, Central Composite Design.

1. Introduction

In recent years fungal infections have emerged as a major cause of disease and mortality, in part as a consequence of the increase in acquired immunodeficiency syndrome (AIDS), the greater use of immunosuppressive drugs in transplantation and chemotherapeutic agents in cancer, long term use of corticosteroids, and even the indiscriminate use of antibiotics [1].

Fungal infections are common complications of infection with human immunodeficiency virus (HIV). Over 90% of those diagnosed to be HIV-positive contract a fungal infection during the course of their illness [2]. *Cryptococcus neoformans* is the major cause of meningitis in AIDS patients, and it can also cause local organ dysfunction and disseminated disease [3]. In the case of transplantation, the mortality directly attributable to fungal infections carries exceedingly high costs mainly as loss of the organ transplant and the patient.

There are effective antifungal agents in the market including fluconazole, itraconazole, voriconazole, posaconazole and so on, currently play a leading role in the treatment of invasive fungal infections [4]. These antifungal drugs act by competitive inhibition of cytochrome P450 14α-demethylase (CYP51), a necessary enzyme in the biosynthesis of ergosterol which is the primary membrane sterol in fungi [5]. However, their clinical application value has been limited by their relatively high risk of toxicity, the emergence of drug resistance, pharmacokinetic deficiencies and/or insufficiencies in their antifungal activities, what created an urgent need for the discovery of new antifungal compounds that have broader spectrum and lower toxicity.

In search for new agents with improved antifungal profiles, the Czech Authors [6] are showed that the Sulfanyl derivatives presented an antifungal activity. The conjugate addition between α,β-unsaturated carbonyl compounds and mercaptans is a convenient route for synthesis of these sulfanyl organic compounds [7]. In classic methods, this reaction catalyzed by strong bases such as alkali metal alkoxides [8], hydroxides [9] and amines [10]. The employment of these strong bases and acids in these reactions [11], however, leads to two main problems affecting the environment; the necessity to dispose of huge amounts of organic waste due to formation of undesirable side products resulting from polymerization, bis-addition and self condensation, and total dissolved salts formed following the neutralization of soluble bases with acids. The replacement of liquid basic catalysts by solid bases in the synthesis of fine and intermediate organic chemicals allows one to avoid corrosion and environmental problems [12]. Nevertheless, heterogeneous solid [13] are advantageous over conventional homogeneous catalysts as they can be easily recovered from the reaction mixture by simple filtration and can be reused after or without activation making the process economically viable.

In this paper, we report the optimization for synthesis of Sulfanyl-based antifungal drugs catalyzed by modified fluorapatite using central composite experimental design.

2. Materials and Method

2.1. Chemicals and instrumentations:

All commercial reagents and solvents were used without further purification. X-ray diffraction (XRD) patterns of the catalysts were obtained on a Philips 1710 diffractometer using Cu-Kα radiation. Surface areas were determined at 77 K using a Coulter SA 31000 instrument with an automated gas volumetric method employing nitrogen as the adsorbate. NMR spectra were recorded on a Bruker ARX 300 spectrometer. Mass spectra were recorded on a VG Autospec spectrometer. FTIR spectra were recorded on an ATI Mattson-Genesis Series spectrophotometer using the KBr disc method.

2.2. Preparation and characterization of catalysts:

The fluorapatite (FAP) have been prepared by reaction between diammonium phosphate, calcium nitrate and ammonium fluoride in presence of ammonia [14]. The FAP obtained was calcined at 900°C before use. The structure of this catalyst was confirmed by X-ray diffraction, infrared spectra and chemical analysis. The surface area of calcined FAP was determined by the BET method and found to be S=15.4 $m^2\ g^{-1}$. The total pore volume was calculated by the BJH method (V_T=0.058 $cm^3\ g^{-1}$).

The modified fluorapatite (Na/FAP) has been prepared by impregnation of the FAP with a solution of sodium nitrate followed by calcination at 900°C. The ratio of impregnation (*RI*) for Na/FAP catalyst was calculated according to equation (**1**).

$$RI = \frac{m}{m'} \qquad (1)$$

RI: Impregnation ratio

m: Weight of sodium nitrate (KF)

m': Weight of Fluorapatite (FAP)

The surface area of the new catalyst Na/FAP was determined by the BET method as 5.4 m^2 g^{-1} and the total pore volume obtained by the BJH method is 0.0032 cm^3 g^{-1}.

2.3. General procedure:

The general procedure for synthesis of sulfanyl-based antifungal drugs is reported in Figure **1**, as follows: To a flask containing an equimolar mixture (1 mmol) of chalcones derivatives as Michael acceptors **1** and thiols as Michael donors **2** in methanol catalyzed by synthetic phosphate (FAP or Na/FAP) was added and the mixture was stirred at room temperature until completion of the reaction, as monitored by thin layer chromatography.

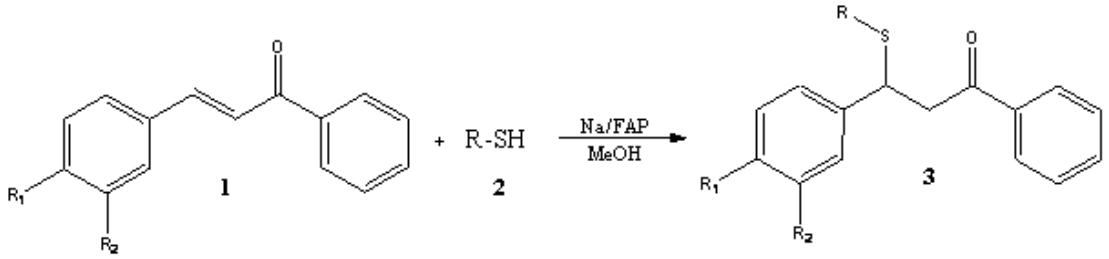

Figure 1: Synthesis of sulfanyl-based antifungal drugs.

The catalyst was filtered, washed with dichloromethane and the filtrate was concentrated under reduced pressure. The crude product was purified by recrystallization. The sulfanyl product was analyzed by ^1H, ^{13}C NMR and IR spectrometry.

3. Results and Discussion

Conjugate addition between Michael acceptor (R_1=OMe and R_2=H) and Michael donor (R=Ph) were chosen as model substrates to determine suitable reaction conditions for synthesis of sulfanyl-based antifungal drugs. A central composite design [15] was employed for synthesis optimization of this methoxy-sulfanyl-based antifungal drug (MSF). The factors are the experimental parameters considered above: reaction time (X_1), solvent volume (X_2), catalyst weight (X_3) and impregnation ratio (X_4). The values used in this design and the levels Xi of the 4 factors are indicated in table **1**.

Table 1: Study field and coded factors

Natural variable	**Unit**	**Coded variables (X_i)**				
		-2	**-1**	**0**	**+1**	**+2**
x_1=Reaction time	min	1	3	5	7	9
x_2=Solvent volume	ml	0	0.75	1.5	2.25	3
x_3=Catalyst weight	mg	30	60	90	120	150
x_4=Impregnation ratio	-	1/4	1/3	5/12	1/2	7/12

However, yields in pure product of MSF were chosen as response for this design. The 26 experiments were done in the 2 following blocks: the first block with a complete factorial design 2^4, the second block according to axial design with the distance to center α equal to 2 and with 2 center points. The values of the factors used in this design and the experimental response (%MSF) are reported in table **2**.

Table 2: Experimental design and results

Order	Coded units of variable (Xi)				Reaction yield (%MSF)
	X_1	X_2	X_3	X_4	
01	+1	+1	+1	+1	96
02	+1	+1	+1	-1	63
03	+1	+1	-1	+1	94
04	+1	+1	-1	-1	51
05	+1	-1	+1	+1	95
06	+1	-1	+1	-1	57
07	+1	-1	-1	+1	91
08	+1	-1	-1	-1	48
09	-1	+1	+1	+1	82
10	-1	+1	+1	-1	56
11	-1	+1	-1	+1	74
12	-1	+1	-1	-1	46
13	-1	-1	+1	+1	84
14	-1	-1	+1	-1	52
15	-1	-1	-1	+1	71
16	-1	-1	-1	-1	37
17	+2	0	0	0	83
18	-2	0	0	0	53
19	0	+2	0	0	68
20	0	-2	0	0	51
21	0	0	+2	0	86
22	0	0	-2	0	62
23	0	0	0	+2	84
24	0	0	0	-2	32
25	0	0	0	0	76
26	0	0	0	0	78

The STATGRAPHICS-Plus computer software was used for the experimental design, data analysis, model building and graph plotting. In this experimental design, the equation of estimated responses can be written; Eq. (**2**):

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2 + \beta_{44}X_4^2 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{14}X_1X_4 + \beta_{23}X_2X_3 + \beta_{24}X_2X_4 + \beta_{34}X_3X_4 \quad (2)$$

The 15 coefficients of this design are easily calculated by the least squares method. The significance of effects can be estimated by comparing the F distribution of the experimental values to a critical value. According to the results showed in table **3**.

Table 3: Regression variance analysis of the model

Source of variation	Coefficient	Sum of Squares	ν	Mean square	F_{exp}	Significance test
β_1	6.375	975.375	01	975.375	41,87	***
β_2	2.54167	155.042	01	155.042	6,66	*
β_3	5.04167	610.042	01	610.042	26,19	***
β_4	15.875	6048.38	01	6048.38	259,64	***
β_{11}	-1.63542	46.6837	01	46.6837	2	NS
β_{12}	-0.0625	0.0625	01	0.0625	0	NS
β_{13}	-1.1875	22.5625	01	22.5625	0,97	NS
β_{14}	2.3125	85.5625	01	85.5625	3,67	NS
β_{22}	-3.76042	246.82	01	246.82	10,6	***
β_{23}	-0.5625	5.0625	01	5.0625	0,22	NS
β_{24}	-1.0625	18.0625	01	18.0625	0,78	NS
β_{33}	-0.13541	0.320076	01	0.320076	0,01	NS
β_{34}	-1.1875	22.5625	01	22.5625	0,97	NS
β_{44}	-4.13542	298.502	01	298.502	12,81	***
β_0	77	-	01	-	-	-

*** : $p \leq$ 0,01 ; ** : $p \leq$ 0,025 ; * : $p \leq$ 0,05 ; NS : No significant.

It was observed that the linear terms (X_1, X_2, X_3 and X_4) and the squared terms (X_2^2 and X_4^2) were significant model terms whereas the squared terms (X_1^2 and X_3^2) and the interaction terms (X_1X_2, X_1X_3, X_1X_4, X_2X_3, X_2X_4 and X_3X_4) were insignificant to the response. The final empirical model in term of coded factors after excluding the insignificant terms for reaction yield of MSF is shown in Eq. (**3**):

$$Y = 77 + 6.375*X_1 + 2.54167*X_2 + 5.04167*X_3 + 15.875*X_4 - 3.76042*X_2^2 - 4.13542*X_4^2 \quad (3)$$

Positive sign in front of the terms indicates synergistic effect, whereas negative sign indicates antagonistic effect. The adequacy of the model was further justified through analysis of variance (ANOVA). The ANOVA for model for reaction yield of MSF is listed in Table **4**.

Table 4: Regression variance analysis for the model

Source of variation	Sum of Squares	ν	Mean square	F_{exp}	Significance test
Regression	-	14	603.8285	25.9203	*** ($F_{0.01}$ (14, 11) = 4.29)
Residue	256.25	11	23.2955	-	
Total	8709.85	25	-	-	

*** : $p \leq$ 0,01 ; ** : $p \leq$ 0,025 ; * : $p \leq$ 0,05 ; NS : No significant.

From this ANOVA, the model F-value of 25.9203 implied that the model was significant. Values of Prob. > F less than 0.01 indicated that the model terms was significant.

The geometric representation of the reaction yield for MSF according to the solvent volume and the catalyst weight is shown in Figure **2**.

Analysis of the contour plot shows that at constant value of the reaction time (X_1=0.5) and impregnation ratio (X_4=1) when the catalyst weight and the solvent volume (X_2 = -2 to 0.5) increase together or when the catalyst

weight increases and the solvent volume remaining unchanged, then the yield of MSF increases up to 94% in the experimental field.

The investigation of equation **3** showed that, if X_1=0.5, X_2=0, X_3=0.5 and X_4=1; the value predict from the results using response surface model is 94%. The experimental checking in this point, i.e. under the optimum reaction conditions such as: reaction time=6min, solvent volume=1.5mL, catalyst weight=105mg and impregnation ratio=1/2 with high reaction yield 95% of MSF, confirms this result.

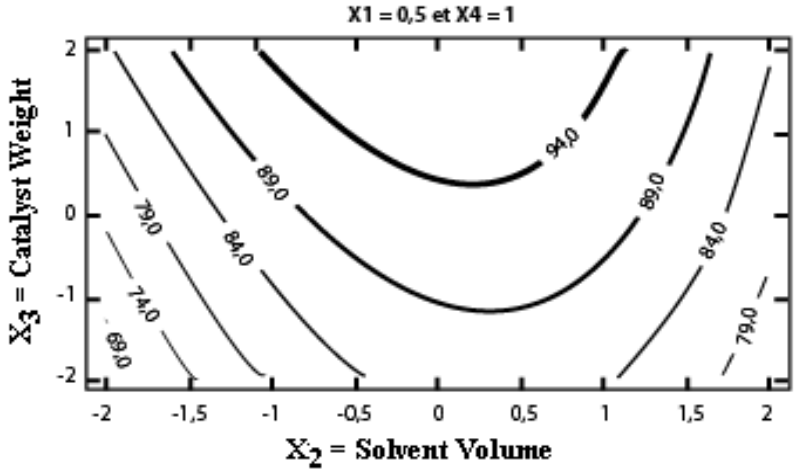

Figure 2: Contours of estimated response surface of yields in pure product of methoxy-sulfanyl-based antifungal drug.

To determine the scope and limitation of this reaction, the optimum reaction conditions were applied to other substrates as shown in table **5**.

Table 4: Synthesis of sulfanyl derivatives catalyzed by modified Fluorapatite catalyst

Products	R_1	R_2	R	Yield %
3a	H	H	-Ph	96
3b	H	H	-2-NH_2-Ph	95
3c	H	H	-CH_2-CO_2-Et	93
3d	H	*m*-NO_2	-Ph	94
3e	H	*m*-NO_2	-2-NH_2-Ph	96
3f	H	*m*-NO_2	-CH_2-CO_2-Et	92
3g	*p*-Cl	H	-Ph	97
3h	*p*-Cl	H	-2-NH_2-Ph	92
3i	*p*-Cl	H	-CH_2-CO_2-Et	94
3j	*p*-OMe	H	-Ph	92
3k	*p*-OMe	H	-2-NH_2-Ph	94
3l	*p*-OMe	H	-CH_2-CO_2-Et	77

Several structurally varying donors such as thiophenol, 2-aminothiophenol and ethyl thioglycolate underwent clean and remarkably catalyst Na/FAP with a variety of acceptors including simple and substituted chalcones.

The yields obtained of sulfanyl-based antifungal drugs with synthetic phosphate Na/FAP are very high and exceed 92 % in short reaction time, except for the sulfanyl product (**3l**). The products of undesirable side reactions resulting from 1,2 addition, polymerization and bis-addition are not observed. The use of Na/FAP catalyst is particularly interesting since it's regenerated by calcinations at 900°C during 15 min, and after five successive recoveries, sulfanyl products were obtained with same yield.

4. Conclusion

In summary, the optimization for synthesis of sulfanyl-based antifungal drugs catalyzed by modified Fluorapatite with sodium nitrate has been studied using central composite design. The model equation for the optimization of the reaction conditions for this synthesis was established. From this equation it was possible to forecast the optimal reaction conditions for synthesis of sulfanyl products in high yield.

This process bring advantages such as high catalytic activity and selectivity under mild reaction conditions, easy separation of the catalyst by simple filtration, use of non-toxic and inexpensive catalysts and especially, elimination of salts and by-product pollutants. This new solid base catalyst becomes then a practical alternative to soluble bases.

Acknowledgement

We are grateful to Mr. Hamid Dhimane and Mr. Marc David of Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, Université René Descartes Paris-France, for his help with data analysis.

References:

[1] S. K. Fridkin, and W. R. Jarvis, "Epidemiology of nosocomial fungal infections," Clinical Microbiology Reviews, vol. 9, no. 4, pp. 499-511, 1996.

[2] M. F. Tuite, "Discovery and development of new systemic antifungals," Trends in Biotechnology, vol. 14, no. 7, pp. 219-220, 1996.

[3] C. S. Lacaz, E. M. Heins-Vaccari, G. L. Hernandez-Arriagada, E. L. Martins, C. A. Prearo, S. M. Corim, and M. Martins, "Primary cutaneous cryptococcosis due to C*ryptococcus neoformans* var. g*attii* serotype B, in an immunocompetent patient," Revista do Instituto de Medicina Tropical de São Paulo, vol. 44, no. 4, pp. 225-228, 2002.

[4] D. J. Sheehan, C. A. Hitchcock, and C. M. Sibley, "Current and Emerging Azole Antifungal Agents," Clinical Microbiology Reviews, vol. 12, no. 1, pp. 40-79, 1999.

[5] X. Y. Chai, J. Zhang, H. G. Hu, S. C. Yu, Q. Y. Sun, Z. G. Dan, Y. Y. Jiang, and Q. Y. Wu, "Design, synthesis, and biological evaluation of novel triazole derivatives as inhibitors of cytochrome P450 14α-demethylase," European Journal of Medicinal Chemistry, vol. 44, no. 5, pp. 1913-1920, 2009.

[6] M. Chlupáčová, P. Kubanová, V. Opletalová, and V. Buchta, "The Importance of the Enone-moiety for Antimycobacterial and Antifungal Properties of Chalcones, " in Proceedings of the 3rd International Symposium on Natural Drugs, Naples, Italy, October 2003.

[7] Y. Abrouki, A. Anouzla, H. Loukili, R. Lotfi, A. Rayadh, M. A. Bahlaoui, S.Sebti, D. Zakarya, and M. Zahouily, "Central Composite Experimental Design Applied to the Catalytic Carbon-Sulfur Bond Formation by Fluorapatite Catalyst," American Journal of Applied Chemistry, vol. 1, no. 2, pp. 22-27, 2013.

[8] M. Ceylan, M. B. Gürdere, H. Gezegen, and Y. Budak, "Potassium-tertiary butoxide-assisted Addition of thioglycolic acid to chalcone derivatives under solvent-free conditions," Synthetic Communications, vol. 40, no. 17, pp. 2598-2606, 2010.

[9] J. T. Li, Y. Cui, G. F. Chen, Z. L. Cheng, and T. S. Li, " Michael Addition Catalyzed by Potassium Hydroxide Under Ultrasound," Synthetic Communications, vol. 33, no. 3, pp. 353-359, 2003.

[10] H. Hiemstra, and H. Wynberg, "Addition of aromatic thiols to conjugated cycloalkenones, catalyzed by chiral β-hydroxy amines: A mechanistic study of homogeneous catalytic asymmetric synthesis," Journal of the American Chemical Society, vol. 103, no. 2, pp. 417-430, 1981.

[11] T. C. Wabnitz, and J. B. Spencer, "A general, Brønsted acid-catalyzed hetero-Michael addition of nitrogen, oxygen, and sulfur nucleophiles," Organic Letters, vol. 5, no. 12, pp. 2141-2144, 2003.

[12] Y. Abrouki, M. Zahouily, A. Rayadh, B. Bahlaouan, and S. Sebti, "A natural phosphate and doped-catalyzed Michael addition of mercaptans to α,β-unsaturated carbonyl compounds," Tetrahedron Letters, vol. 43, no. 49, pp. 8951-8953, 2002.

[13] Y. Abrouki, A. Anouzla, H. Loukili, R. Lotfi, A. Rayadh, M. A. Bahlaoui, S.Sebti, D. Zakarya, and M. Zahouily, "Process Optimization for Synthesis of Anti-Tuberculosis Drug Catalyzed by Fluor apatite Supported Potassium Fluoride," American Journal of Theoretical and Applied Statistics, vol. 2, no. 3, pp. 81-86, 2013.

[14] M. Zahouily, Y. Abrouki, A. Rayadh, S. Sebti, H. Dhimane, and M. David, "Fluorapatite: efficient catalyst for the Michael addition," Tetrahedron Letters, vol. 44, no. 12, pp. 2463-2465, 2003.

[15] Anouzla, Y. Abrouki, S. Souabi, M. Safi, and H. Rhbal, "Colour and COD removal of disperse Dye solution by a novel Coagulant: Application of statistical Design for the optimization and Regression analysis ," Journal of Hazardous Materials, vol. 166, no. 2, pp. 1302-1306, 2009.