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MOLECULAR STRUCTURAL PARAMETERS IN TUBERCULOSTATIC ACTIVITY OF POLYHYDROXY-XANTHONES

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تم في هذا البحث دراسة العلا قة الكمية لارتباط فاعلية مجموعة من مشتقات الاكسانثونات متعددة الهيدروكسيل ضد ميكروب الدرن والخواص الاستشحامية وكذلك بعض الخواص الالكترونية والمدارية لهذه المركبات مثل: عزم الاستقطاب, الكثافة الالكترونية على ذرات المركبات, حرارة التكون, زوايا الانحراف, قوى الجذب حول ذرات كل مركب, محصلة قوى الشد للروابط بين الذرات ومحصلة الزوايا بين الروابط. وقد تم حساب الخاصية الا ستشحامية للمركبات بدلالة خاصية التوزيع التى تم تقديرها بواسطة كروماتوجرافيا الطبقة الرقيقة على رقائق عديدة الاميد. أما الخواص الالكترونية والمدارية للمركبات مجال الدراسة فقد تم استنباطها من خلال برنامج كومبيوتر.

وقد أثبتت الدراسة عدم ارتباط أى من دلالات الفاعلية البيولوجية بالخاصية الاستشحامية منفردة أو مرتبطة بعوامل اخرى توضح نظام وعدد مجموعات الهيدروكسيل على حلقة الاكسانثون. وللتأكد من هذه الملاحظة تم دراسة خاصية النفاذية للمركبات باستخدام غشاء سيلوفان قياسى وتبين عدم قدرتها على النفاذية بدرجة كافية مما يوحى بأن الفاعلية البيولوجية لهذه المجموعة من المركبات قد تتم بالتأثير على جدار الخلية. كما تم فى هذا البحث التوصل الى معادلة خطية بين الخاصية الاستشحامية للمركب وعدد ونظام احلال مجموعات الهيدروكسيل على حلقة الاكسانثون مما يمكن من حساب قيمة هذه الخاصية للمركبات المشابهة بدلالة عدد وموقع احلال المجموعات المشار اليها. وعلى النقيض من ذلك تبين لنا الدراسة ارتباط فعالية المركبات مجال الدراسة ضد الدرن بدرجة عالية (ر = ٠.٩١) بالكثافة الالكترونية على ذرة الكربون رقم ٤ من المركبات.

This investigation was undertaken to test the correlation of the observed tuberculostatic activity of a series of 7(2)-polyhydroxyxanthones ***1a-j*** *with their lipophilic parameters (Rm) as well as some molecular orbital descriptors such as; dipole moment (dp); electron density (D); heat of formation (hf); torsion angle (tor); van der Waal forces (vdw), bond length (str) and bond angle (bnd). The Rm-values were calculated from the corresponding R_f-values, determined using polyamide plates. The molecular orbital regressors were derived through semi-empirical calculations using MMX-computer program.*

QSAR analysis of the activity parameters (Ka, Log Ka, Ka and logKa*) as dependent variables and Rm or combination of Rm and dummy parameters representing the substitution pattern of hydroxyl groups on ring C of the xanthone nucleus demonstrated an increase in the lipophilic character by itself which did not correlate ($r \cong -0.42$) with increased potency against Mycobacterium lufu. Permeability study using standard cellophane membrane showed that, the synthesized compounds were poorly penetrating and may act directly on the cell wall. On the contrary higher correlation ($r = 0.91$) was observed between the activity parameter (log ka*) and the electron density at C-4.*

INTRODUCTION

Several extrathermodynamic approaches in the quantitative structure activity relationship (QSAR) analyses have been widely and effectively applied in drug design. Among the variables used in such analyses were lipophilic, electronic and steric parameters.[1-6] In addition,

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quantum chemical and electronic indices derived from semi-empirical molecular orbital calculations were also reported.[7-12]

In a previous QSAR study we reported the correlation of the tuberculostatic activity to the ^{13}C-NMR chemical shifts of a set of polyhydroxy xanthones.[13] The results revealed good contribution of this parameter to the observed activity (96%). The present work aims to investigate other physicochemical as well as structural parameters, that might be of potential contribution to the tuberculostatic activity of a set of 7(2)-polyhydroxyxanthones **1a-j**.

EXPERIMENTAL

The synthetic methods for the preparation of the selected series of 7(2)-polyhydroxy-xanthones **1a-j**, as illustrated in scheme 1, have been published.[14] The tuberculostatic activity data, expressed as the ability to supress the generation rates of Mycobacterium lufu[15] are listed in Table 1.

Semi-empirical molecular orbital calculations including: bond length (*str*), bond angle (*bnd*), torsion angle (*tor*), van der Waal forces (*vdw*), heat of formation (*hf*) and dipole moment (*dp*), were calculated using semi-empirical calculations program (MMX through PC-model).

MNDO-MO program was used for calculation of the electron density (*D*) at each atom for derivatives **1a-f,h,i**. Partial structure 2, which represents ring C (variable substituents) and the γ-pyrone ring was used for the calculation. Ring A of xanthones nucleus is omitted since it is constant in all derivatives.

Determination of R_f-values

10 μl of the prepared solution (1 mg/ml) of polyhydroxyxanthones **1a-j** were spotted at 1.5 cm distances on polyamide plates (poly Gram-G254, 10x20 cm, 0.1 mm thickness, Machery and Nagel, Germany). The plates were then developed with methanol to a solvent front of 15 cm height. The spots of the respective compounds were visualized by UV-lamp at λ 254 nm and the R_f-values for each compound were determined.

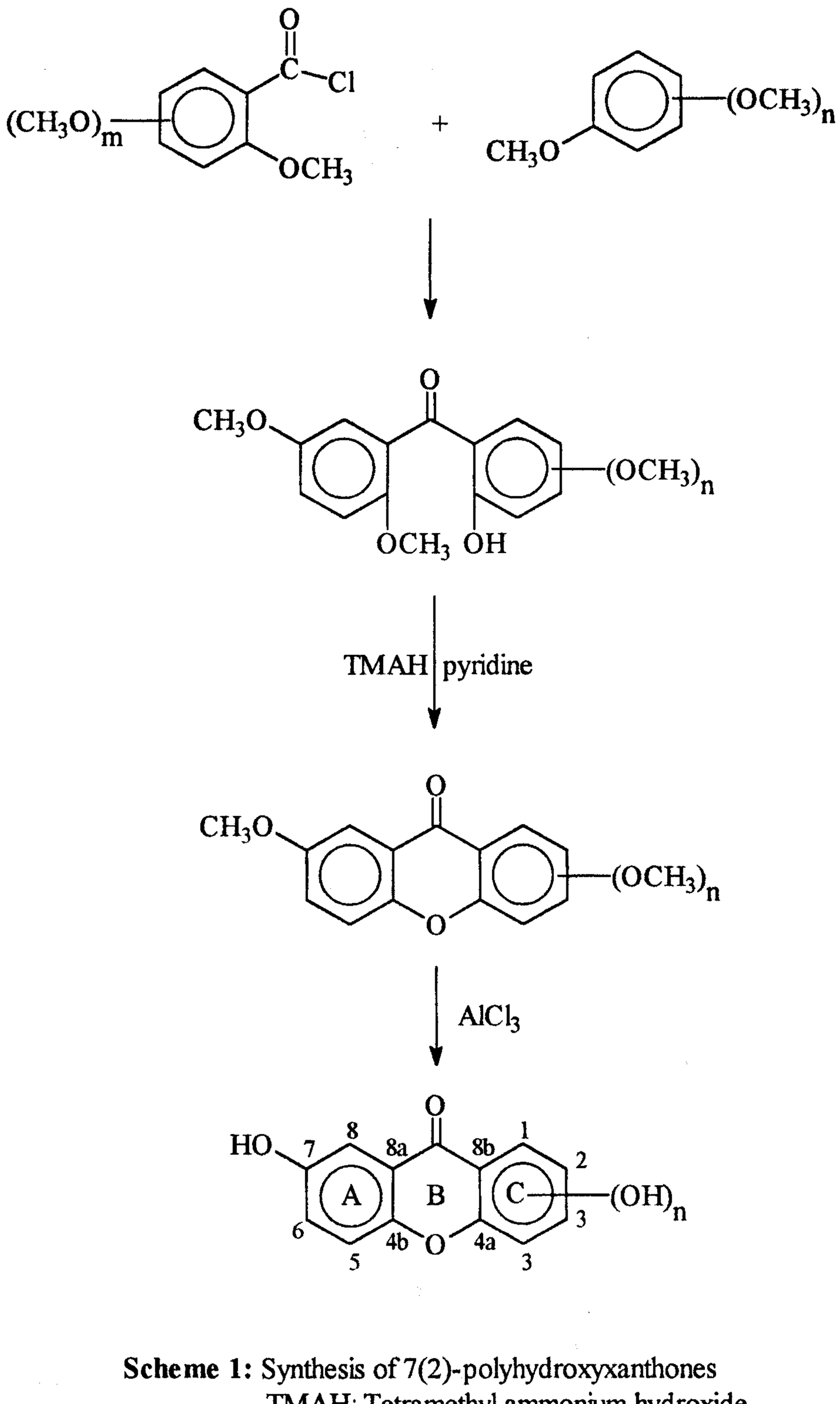
Determination of the penetrating capacity

A standard cellophane membrane (30/32 Fisher Sci., GB-London) was soaked in water overnight then dried before use. The dried membrane was then fastened on one end of an open-ended glass tube (ID 1.3 cm) using rubber band. The tube was then immersed upside down in 100 ml beaker containing 20 ml of distilled water (acceptor compartment), so that the tube (donor compartment) 2 cm apart from the bottom. Two ml of a suspension of 7(2)-hydroxyxanthone (20 μmole) in distilled water were placed in the donor compartment. The whole assembly was then placed in a shaker (20 rpm) with thermostatically controlled water bath at 37°C. At specified time intervals, aliquots of 1 ml of the acceptor medium were withdrawn and compensated with equal volume of distilled water. The samples were then analyzed spectrophotometrically at λ_{max}= 281 nm for their content of 7(2)hydroxyxanthone using suitable standard curve.

RESULTS AND DISCUSSIONS

The lipophilicity parameter (R*m*) for each compound of the selected 7(2)-polyhydroxy-xanthones **1a-j** has been calculated according to equation 1 using the respective R_f-value. The determination of the latter was first tried by application of a reversed-phase TLC method[16] using n-octanol impregnated thin layer silica gel plates as the lipophilic stationary phase. Mixtures of polar solvents as methanol or acetone with water or aqueous buffer solutions (pH 7.4) were used as mobile phases. No significant differences in R_f-values have been observed within the tested series. Variation of the mobile phase and the percentage of impregnation system (3 or 6% n-octanol in acetone) did not lead to any improvement of the results.

As alternative nonimpregnated polyamide plates were applied as hydrophobic stationary phase using methanol as mobile phase.[17] A significantly differentiated R_f-values for the



Scheme 1: Synthesis of 7(2)-polyhydroxyxanthones
TMAH: Tetramethyl ammonium hydroxide
m= 0-3 n= 0-4

tested compounds were obtained (Fig. 1). In addition, polyamide TLC may offer some advantages for the determination of R*m*-value, where the stationary phase has been regarded as a better model for protein binding studies than impregnated silica gel plates.[16] The calculated R*m*-values of 7(2)-polyhydroxyxanthones **1a-j** are listed in Table 1.

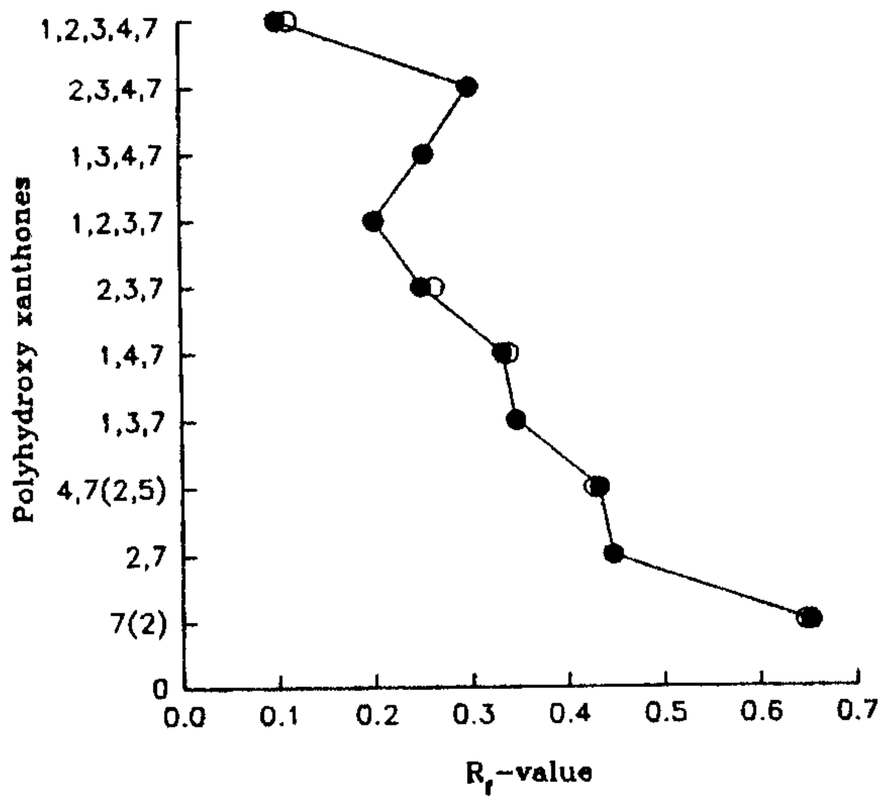


Fig. 1: TLC-R_f value of 10 polyhydroxy xanthones (three determinations).

$$Rm = \log\left(\frac{1}{R_f} - 1\right) \qquad (1)$$

As evidenced from Table 1, the R*m*-values varied according to the number and position of the hydroxyl groups. A multiple linear regression analysis of the R*m*-values and dummy parameters, *P1-P4*, (presence 1.0 or absence 0.0 of hydroxyl groups) resulted in a significant correlation (eq. 2).

$$Rm = -0.204 + 0.39P1 + 0.30P2 + 0.16P3 + 18P4 \qquad (2)$$

n = 10; r = 0.974; F = 22.75; S.E. = 0.079; $P < 0.002$

Where -0.204 is the R*m* value of 7(2)-hydroxyxanthone (ref. compound), 0.39, 0.30, 0.16 and 0.18 are the ΔRm due to hydroxyl substituent of the other derivatives on positions 1-4 respectively.

The statistical data showed that equation 2 can be applied for calculation of R*m*-values for unknown 7(2)-polyhydroxyxanthone derivatives. Figure 2, illustrates the correlation of the calculated and observed R*m* values of compounds **1a-j**. It is worthy to note that, the hydroxyl group at C-1 has the greater influence on R*m*-value (R*m*= 0.4). This might be attributed to the possible intramolecular hydrogen bonding with the adjacent carbonyl group.

Table 1: The antitubercular activity (Ka, logKa, Ka*, logKa*) of 7(2)-polyhydroxy xanthones, **Ia-j**, and their R*m*-values.

Compd.		Ka	logKa	Ka*	logKa*	R*m*
a	7-OH	2.753	0.439	0.116	-0.0937	-0.27
b	2,7-$(OH)_2$	7.723	0.888	0.084	-1.074	0.09
c	4,7-$(OH)_2$	2.801	0.447	0.976	-0.011	0.12
d	1,3,7-$(OH)_3$	5.262	0.721	0.032	-1.495	0.43
e	1,4,7-$(OH)_3$	2.176	0.338	0.899	-0.046	0.29
f	2,3,7-$(OH)_3$	1.797	0.255	0.719	-0.143	0.60
g	1,2,3,7-$(OH)_4$	3.381	0.529	0.071	-1.146	0.25
h	1,3,4,7-$(OH)_4$	3.610	0.559	0.123	-0.909	0.47
i	2,3,4,7-$(OH)_4$	3.429	0.535	0.342	-0.466	0.37
j	1,2,3,4,7-$(OH)_5$	1.837	0.264	0.134	-0.873	0.91

Ka is the potency of polyhydroxy xanthones, expressed in $ml.mol^{-1}.sec^{-1}$

Ka* is the reciprocal value of the potency (Ka) and expressed in $mol.sec.ml^{-1}$

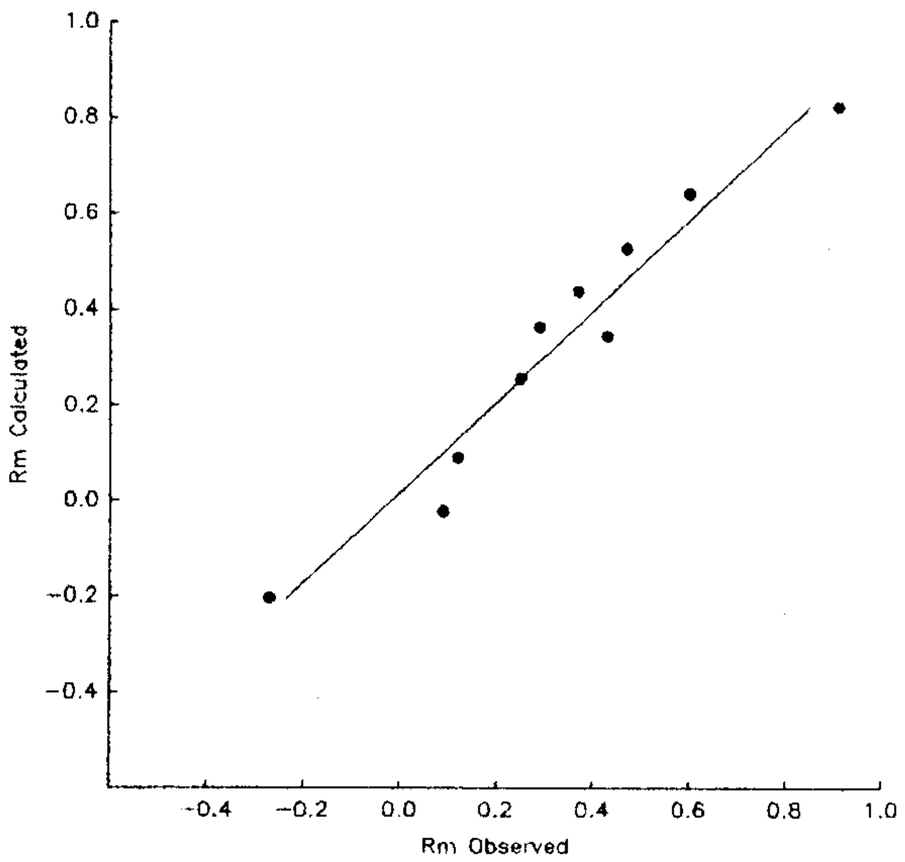


Fig. 2: The observed and calculated Rm - values for 7(2)-polyhydroxyxanthones.

QSAR study

In the present study the following physicochemical and/or structural parameters of the investigated compounds **1a-j** have been considered as descriptors:

1) The R*m* values which would describe the hydrophobicity of the molecule.
2) A set of quantum chemical indices (Table 2) including: bond length (*str*); bond angle (*bnd*); torsion angle (*tor*); van der Waal forces (*vdw*); heat of formation (*hf*); dipole moment (*dp*); and electron density (*D*) (Table 3).
3) Indicator variables, *P1-P4*, which would account for the presence (1.0) or absence (0.0) of the hydroxyl substituent at C1-C4 of ring C. This parameter could quantify the effect of a substituent on biological activity that can not be attributed to the physicochemical properties.[18]

The application of R*m* values as lipophilicity parameter in QSAR study has been well doccumented.[19-21] In the studied series **1a-j**, QSAR analysis of the R*m*-values and the different activity parameters resulted in equation 3, as the best correlation indicating a poor contribution of the lipophilicity for the activity.

$$logKa = 0.574 - 0.23Rm \quad (3)$$

n = 10; r = -0.376; SE = 0.195; F = 1.316; P = 0.285

The importance of the indicator variables, *P1-P4*, has been studied as copredictor for the activity with R*m* values using combined Free Wilson/linear Free energy approach for QSAR analysis. No significant improvement has been observed in the correlation coefficient (eq. 4) as compared to the previous results (eq. 3).

$$logKa = 0.684 + 0.298Rm - 0.259P1 - 0.117P2 - 0.056P3 - 0.143P4 \quad (4)$$

n = 10; r = 0.450; SE = 0.256; F = 0.203; P < 0.946

Consequently, it was postulated that the observed tuberculostatic activity might be attributed to a direct action on the cell wall. This assumption has been confirmed through a permeability experiment using standard cellophane membrane. Only 23% of the studied reference compound, 7(2)-hydroxyxanthone, passed across the membrane within 5 hours.

Semi-empirical MO-calculations

The intercorrelation matrix of the quantum chemical indices and the activity parameters are presented in Tables 4 and 5. As evidenced from Table 4, there has been a high degree of covariance between the variables: *bnd/str*, *vdw* and *hf*. Consequently, combinations of these variables as predictors for the activity have been excluded. The covariance is relatively low between *str/tor*; *bnd/tor*; *vdw/tor* and *dp* with all aforementioned variables and were considered as independent variables.

Multiple regression analysis of nine compounds **1a-i**, yielded equation 5 as the best combination of predictors. Compound **1j**, (1,2, 3,4,7-pentahydroxyxanthones) was excluded due to a very large difference between the observed and calculated values of its parameters.

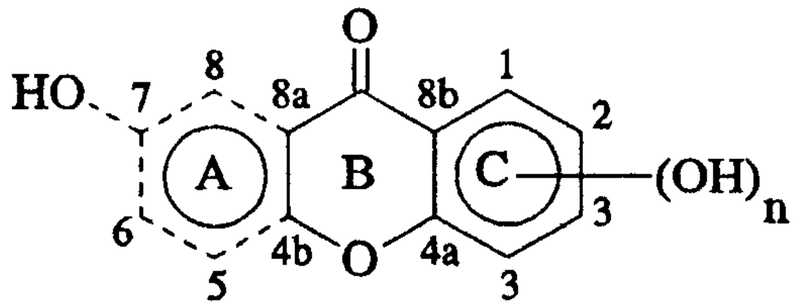
$$logKa^* = 2.354 - 1.687bnd - 0.179tor \quad (5)$$

n = 9; r = 0.697; SE = 0.550; F = 2.827; P < 0.137

Table 2: Some quantum chemical indices, derived from semi-emperical calculations, of 7(2)-polyhydroxy xanthones **Ia-j**.

Compd.		bnd	tor	vdw	dp	hf	str
a	7-OH	0.52	15.7	7.98	3.55	-32.80	0.32
b	2,7-$(OH)_2$	0.53	15.0	7.92	2.39	-73.37	0.33
c	4,7-$(OH)_2$	0.54	13.0	7.96	3.88	-74.66	0.34
d	1,3,7-$(OH)_3$	0.92	14.3	8.84	4.64	-125.12	0.44
e	1,4,7-$(OH)_3$	0.85	12.44	8.81	3.38	-124.09	0.43
f	2,3,7-$(OH)_3$	0.55	12.30	7.94	1.33	-119.70	0.33
g	1,2,3,7-$(OH)_4$	0.94	9.6	9.01	1.26	-172.66	0.44
h	1,3,4,7-$(OH)_4$	0.94	9.6	8.93	5.63	-170.72	0.45
i	2,3,4,7-$(OH)_4$	0.58	7.6	8.01	2.16	-165.33	0.34
j	1,2,3,4,7-$(OH)_5$	3.07	6.58	4.65	37.49	578.80	0.22

Table 3: Electron density of the atoms of the partial structures **IIa-f, h** and **i**.



II, n= 0-4

Compd.	C-1	C-2	C-43	C-4	C-4a	C-8b	C=O	-O-
IIa	3.958	4.108	4.001	4.095	3.846	4.204	3.671	6.188
IIb	3.993	3.927	4.092	4.044	3.904	4.162	3.684	6.193
IIc	4.002	4.058	4.096	3.909	3.893	4.163	3.683	6.174
IId	3.737	4.231	3.801	4.158	3.797	4.317	3.665	6.194
IIe	3.806	4.102	4.001	3.943	3.915	4.241	3.670	6.212
IIf	4.001	4.010	3.845	4.099	3.859	4.200	3.681	6.193
IIh	3.769	4.187	3.878	3.972	3.833	4.279	3.667	6.173
IIi	3.991	3.919	3.931	3.968	3.915	4.168	3.683	6.200

Table 4: Correlation martrix of the antitubercular activity of 7(2)-polyhydroxy xanthones **Ia-i** and their quantum chemical indices.

	Ka	logKa	ka*	logKa*	str	bnd	tor	vdw
Ka	1.000							
logKa	.9435	1.000						
Ka*	-.5957	-.6797	1.000					
logKa*	-.5500	-.6341	.7556	1.000				
str	-.2200	-.1384	.1768	-.0500	1.000			
bnd	-.2263	-.1418	.1239	-.0750	.9948	1.000		
tor	.4148	.4037	-.2324	-.6271	-.3205	-.3357	1.000	
vdw	-.2958	-.2386	.1958	-.0248	.9839	.9909	-.3211	1.000
dp	.0987	.2645	-.1153	-.2847	.4061	.3592	.1812	.3031
hf	.2527	.2012	-.0894	-.2735	-.6865	-.7055	.8539	-.6711
	dp	hf						
dp	1.000							
hf	.0559	1.000						
N=9								

Table 5: Correlation martrix of the antitubercular activity of 7(2)-polyhydroxy xanthones **Ia-f, h,i** and their electron densities.

	Ka	logKa	ka*	logKa*	DC-1	DC-2	DC-3	DC-4
Ka	1.000							
logKa	.9827	1.000						
Ka*	-.5358	-.6254	1.000					
logKa*	-.5880	-.6743	.9396	1.000				
DC-1	.0415	.0114	.0888	.2250	1.000			
DC-2	-.2434	-.1884	-.0689	-.2490	-.8412	1.000		
DC-3	.0850	-.0555	.5484	.5997	.5315	-.4780	1.000	
DC-4	.4019	.4624	-.7937	-.9046	.1495	.2590	-.5492	1.000
DC-4a	-.0910	-.2098	.5978	.7382	.5608	-.7845	.6903	-.6913
DC-8b	.2507	.2721	.1995	.0966	.9098	-.0708	-.2220	-.2921
DC=O	.2057	.1713	.1914	.0937	-.0769	-.2585	-.0811	.1337
D(O)	-.0149	-.0968	.1018	.0937	-.0769	-.2585	-.0811	.1337
	DC-4a	DC-8b	DC=O	D(O)				
DC-4a	1.000							
DC-8b	.1624	1.000						
DC=O	.6927	.1503	1.000					
D(O)	.3904	.0902	-.0173	1.000				
N=8								

The electron density (*D*), Table 3, at each atom of the partial structure 2 has been calculated using MNDO-MO program. The data indicated that, the hydroxyl substituent has significantly increased the electron density at the ortho and para C-atoms. Compounds **1g** and **1j** (1,2,3,7-tetrahydroxy and 1,2,3,4,7-penta-hydroxy-xanthones respectively) were not included in the correlation matrix (Table 5), since the used MNDO-MO calculation program failed to afford any data for both compounds. Regression analysis of this parameter and the activity expressed as log ka* result in eq. 6.

$$logKa^* = 21.251 - 5.471D_{C-4} \quad (6)$$

n = 8; r = -0.905; SE = 0.246; F = 27.01; P < 0.002

Equation 6 shows that higher electron density at C-4 has increased the tuberculostatic activity. The negative sign in front of the term D_{C-4} indicates that, the only substituent that would increase the electron density of C-4 such as hydroxyl group on C-3 and /or C-1 could increase the activity.

Conclusion

The QSAR studies reported here for 7(2)-polyhydroxyxanthone derivatives **1a-j** as tuberculostatic agents revealed that, the activity was independent of the lipophilicity of the molecule, but would be largely determined by the electronic properties of the substituent ortho and/or para to C-4. The assumption that the hydroxyl group was the optimal substituent at C-3 might require further studies using other electron donating groups. This result confirms our previous QSAR observations using ^{13}C-NMR chemical shifts of the respective C-atoms as an electronic parameter.[13,15]

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