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Synthsis of the acetals $R_1OCH_2OR_2$ and $R_1OCH_2OR_2$ by double alkoxylation of dichloromethane via "mini combinatorial" approach and their anticancer activity in the HeLa cell line

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Abstract

The mixtures of two hydroxylic components (alcohols and phenols) reacted with CH_2Cl_2 in basic medium under the conditions of the solid-liquid phase-transfer catalysis to furnish the mixtures of three possible formaldehyde acetals separable under ordinary column

chromatography. Using 7 phenols and 7 alcohols, 22 new acetals having the structures $R1OCH2OR2$ and $ROCH2OR$ were obtained and screened for antiproliferative activity in the HeLa cell line. The compound **21** stands out as *ca* 15 times more active than doxorubicin.

Keywords: alkoxylation; anticancer activity; combinatorial chemistry; formaldehyde acetal; HeLa

The symmetrical formaldehyde acetals $ROCH2OR$ display a range of activities such as inhibition of aggregation of the β amyloid protein, [1] antifungal, [2,3,4] pesticidal, [5] antibacterial against G+ and G- microorganisms, [6] antileshmanial, [7] some are active against citrus red mite [8] or against liver fluke, [9] some are of interest in the perfume, [10,11] paper [12] and textile [13,14] industry. They can be obtained in either basic conditions by double substitution in dihalomethanes ($CH2Br2$, $CH2BrCl$, $CH2Cl2$, $CH2I2$) or in acidic medium using paraformaldehyde or dimethoxymethane, among others. The mixed formaldehyde acetals $R1OCH2OR2$ received attention as the protecting groups and their formation and the scope of utilization has been comprehensively presented in a source book. [15] In overwhelming number of cases, the mixed formaldehyde acetals were accessed to using the preformed unstable and potentially cancer-inducing alkoxymethyl halides. Atypical alternatives to obtain the mixed acetals for the purpose different than protection include substitutions in alkoxymethylpyridinium halides, [16] acetal-ether exchange in acidic medium, [17] photochemical ($ROCH2CO2H/HgO/I2/ROH$) [18] or electrochemical non-Kolbe decarboxylation, [19] $SnCl4$ induced acetal exchange, [20] application of sulfated metal oxides [21] and phenylsulfonic acid/mesophorous silica for transacetalation. [22]

Considering the fact that $CH2Cl2$ is the cheapest of the one carbon substrates for geminal substitution, we reasoned, that if two different alcohols and phenols ($R1OH$ and $R2OH$) are combined with it in the presence of KOH and the phase transfer catalyst, the mixture of three different formaldehyde acetals $R1OCH2OR1$, $R1OCH2OR2$ and $R2OCH2OR2$ should be formed as shown in the Scheme 1. The objective of the present communication is to show that such hitherto undescribed “mini combinatorial” procedure functions well and that all three types of the acetals are indeed formed. The most important of them are the hitherto undescribed mixed acetals $R1OCH2OR2$ for biological screening. Considering the differences

in the chromatographic mobilities between the three abovementioned products it was easier to isolate only the mixed acetals $R_1OCH_2OR_2$ from the reaction mixtures (R_1OH, R_2OH, CH_2Cl_2, KOH) and to obtain the symmetric acetals independently (ROH, CH_2Cl_2, KOH), although all threecompounds have been isolated in few cases.

Scheme 1. General reaction of double alkoxylation of dichloromethane using two different phenols/alcohols under phase-transfer catalysis (PTC) to form the symmetrical and mixed formaldehyde acetals.

$$R_1OH + R_2OH + CH_2Cl_2 \xrightarrow[PCT]{KOH} R_1OCH_2OR_1 + \mathbf{R_1OCH_2OR_2} + R_2OCH_2OR_2$$

Catalysts: Bu_4NHSO_4, 18-crown-6, $BnNEt_3Cl$, $CH_3(CH_2)_{15}NMe_3Br$

The following hydroxylic components were used: 8-hydroxyquinoline **1**, thymol **2**,eugenol **3**, phenol **4**, 4-aminothiophenol **5**, diethylene glycol **6**, 1-naphthol **7**,

monobutylethylene glycol **8**, 2-naphthol **9**, guaiacol **10**, ethanol **11**, n-butanol **12**, n-hexadecanol **13**, n-pentanol **14** and n-octanol **15**. The combination of these compounds accordingly to the Scheme 1 permitted isolation of the mixed acetals as shown in the Table 1. The known symmetrical acetals derived from hydroxyquinoline **1** (**18** [**7**,23]),

thymol **2** (**16** [3,24,25]), phenol **4** (**22** [26,27]), p-aminothiophenol **5** (**24** [28]), 1-

naphthol **7** (**29** [29,30]), 2-naphthol **9** (**32** [1,2]), guaiacol **10** (**34** [10,11]), n-

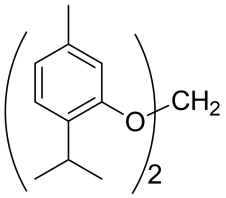
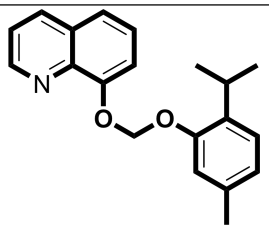
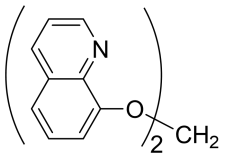
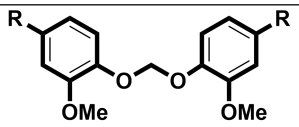
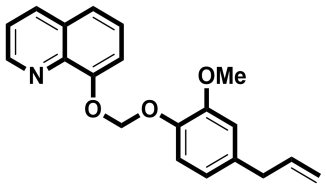
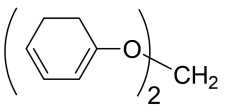
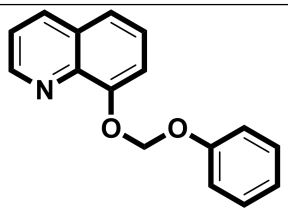
hexadecanol **13** (**41** [31]), and n-octanol **15** (**41** [32]) were also obtained and characterized in the present work. The known formaldehyde acetals derived from diethylene glycol **6**, [12,13,14] monobutylethylene glycol **8**, [5,13] ethanol **11**, [34] n- butanol **12** [34,35] and n-pentanol **14** [34,35] were not isolated due to their invisibility on the TLC plates, although they must have been formed in the applied conditions. In two cases of the eugenol acetals (Entry 13 and 14, compounds **37** and **39**) the partial base-induced migration of the C=C bond [36,37] took place in the 4-(2-propenyl) moiety to form the more stable 4-(1-propenyl) moiety conjugated with the benzene ring.

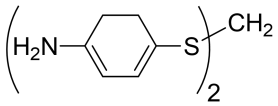
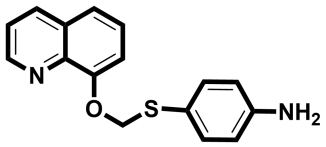
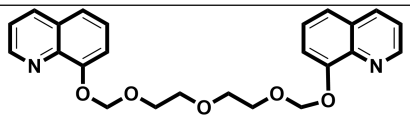
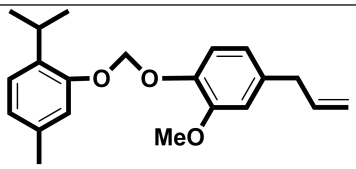
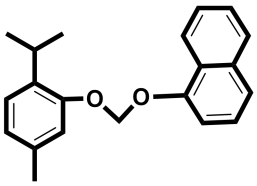
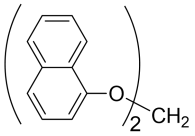
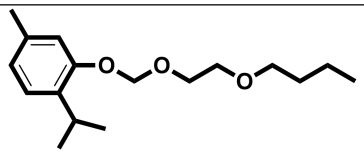
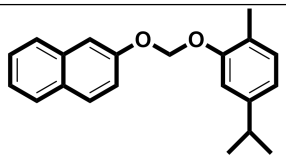
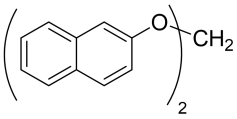
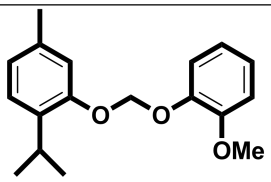
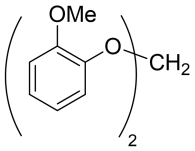
It was clearly visible by the presence of the high field signals of the terminal –Me groups (δ ca 1.9 ppm in the H1 spectra, and δ ca 18 ppm in the C13 spectra) and duplication of the –OCH_2O- signals (δ ca 5.7-5.9 ppm in the H1 spectra, and δ ca 93 ppm in the C13 spectra) of **37** and **39**. The resulting regioisomers formed in variable proportions were impossible to separate by chromatography and therefore they were subjected to catalytic hydrogenation to

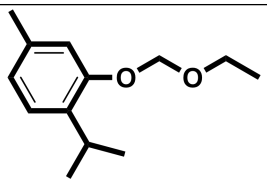
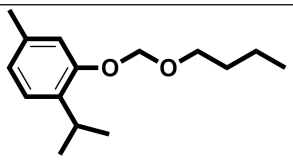
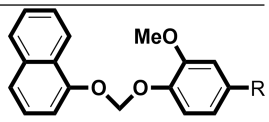
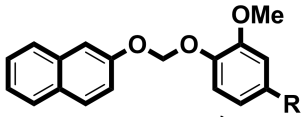
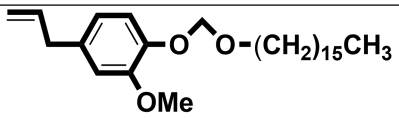
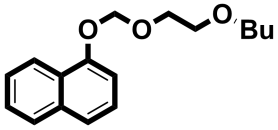
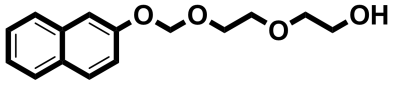
form the corresponding 4-propyl derivatives **38** and **40**, respectively. Unexpectedly, such migration did not take place in the case of **19**,**21**, **27** and **42** (Entry 2, 6 and 15). Nevertheless, hydrogenation of the allyl moiety has been performed in **19** (→ **20**) with a loss antiproliferative activity (to be published in due course).

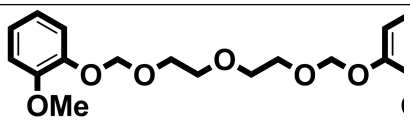
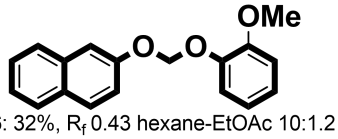
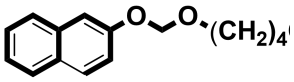
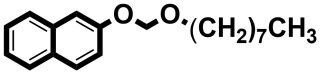
Preparative yields of the mixed acetals derived from 8-hydroxyquinoline **1** were lower than those of the other phenols and unreacted **1** was always present in the respective reaction mixtures. Evidently, **1** is intrinsically less reactive presumably due to the strongintramolecular hydrogen bond which increases pKa of its –OH group. [38] Also, unidentified polar resinous by-products were always formed probably by alkylation of the N1 atom.

Table-1 Substrates and products obtained by double alkoxylation of CH_2Cl_2. The products in each entry are shown in order of decreasing mobility on the silica gel from left to right. Preparative yields of mixed acetals and their TLC mobilities are shown. New compounds are drawn in bold.

Entry	Reagents			
1	**1 + 2**	 **16** [3,24,25]	 **17**: 15%, R_f 0.39 hexane-EtOAc 3:2	 **18** [7,23]
2	**1 + 3**	 **R:** -$CH_2CH{=}CH_2$ **19** **R:** -Pr **20** **19:** 42%, R_f 0.24 hexane-EtOAc **20:** 81%, R_f 0.29 hexane-EtOAc	 **21:** 16%, R_f 0.41 hexane-EtOAc 9:1	**18** [7,23]
3	**1 + 4**	 **22** [26,27]	 **23:** 19%, R_f 0.42 hexane-EtOAc 1	**18** [7,23]

4	**1 + 5**	 **24** [28]	 **25:** unstable[a] Rf 0.42 CH_2Cl_2-MeOH 10:0.35	**18** [7,23]
5	**1 + 6**	**18** [7,23]	 **26**[c,d]**:** 19% Rf 0.30 CH_2Cl_2-MeOH 10:0.5	**b**
6	**2 + 3**	**16** [3,24,25]	 **27**: 34%, R_f 0.51 hexane-EtOAc 9:1	**19**
7	**2 + 7**	**16** [3,24,25]	 **28**: 37%, R_f 0.60 hexane-EtOAc 9:1	 **29** [29,30]
8	**2 + 8**	**16** [3,24,25]	 **30:** 33%, R_f 0.44 hexane-EtOAc 10:0.5	**b**
9	**2 + 9**	**16** [3,24,25]	 **31:** 37%, R_f 0.60 hexane-EtOAc 19:1	 **32** [1,2]
10	**2 + 10**	**16** [3,24,25]	 **33**: 27%, R_f 0.35 hexane-EtOAc 19:1	 **34** [10,11]

11	**2** + **11**	**16** [3,24,25]	 **35**: 38%, R_f 0.36, hexane neat	**b**
12	**2** + **12**	**16** [3,24,25]	 **36:** 34%, R_f 0.34, hexane neat	**b**
13	**3** + **7**	**29** [29,30]	**19**	 inseparable { R: $-CH_2CH=CH_2$; R: $-CH=CHCH_3$ } R: -Pr **38**[d] ← **37**: 37%, R_f 0.34, hexane-EtOAc **38**[e]: 42%, R_f 0,40, hexane-EtOAc
14	**3** + **9**	**32** [1,2]	 R: $-CH_2CH=CH_2$ R: $-CH=CHCH_3$ **39** inseparable; H_2, Pt → R: $-CH_2CH_2CH_3$ **40** **39**: 29%, R_f 0.40, hexane-EtOAc 10:1 **40**: 65%, R_f 0.46, hexane-EtOAc 10:1	**19**
15	**3** + **13**	$(CH_3(CH_2)_{15}O)_2CH_2$ **41** [31]	 **42**, 28%, R_f 0.27 hexane-EtOAc 10:0.3	**19**
16	**7** + **8**	**29** [29,30]	 **43**: 34%, R_f 0.40 hexane-EtOAc 10:1	**b**
17	**6** + **9**	**32** [1,2]	 **44**: 26%, R_f 0.35 hexane-EtOAc 3:7	**b**

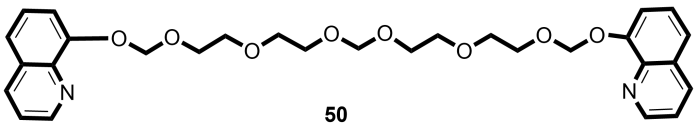
18	**6 + 10**	**34 [10,11]**	 **45:** 22%, R_f 0.36 hexane-EtOAc 2:1	**b**
19	**9 + 10**	**32** [1,2]	 **46**: 32%, R_f 0.43 hexane-EtOAc 10:1.2	**34 [10,11]**
20	**9 + 14**	 **47**: 26%, R_f 0.55 hexane:EtOAc 1(	**32** [1,2]	**b**
21	**9 + 15**	$(CH_3(CH_2)_7O)_2CH_2$ **48** [32]	 **49**: 23%, R_f 0.52 hexane-EtOAc 10:0.3	**32** [1,2]

a. Partly decomposed during refrigerator storage; identified only by HRMS

b. The symmetric acetals derived from **6**, [12,13,14] **8**, [5,33] **11**, [34] **12,** [34,25] and **14** [34,25] were not isolated due to their invisibility on TLC

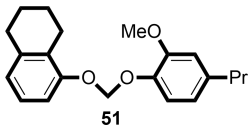
c. In the case of **26** and **45** (Entry 5 and 18), the initially formed $ROCH_2O(CH_2)_2O(CH_2)_2OH$ reacted further with the intermediate $ROCH_2Cl$

d. A minor compound **50** was also formed, identified only by HRMS; **26** and **50** are inseparable.



50

e. Partial hydrogenation of the ring B occurred to furnish also **51** in 30% yield, which is less polar than **38**



51

Experimental

In a typical procedure 5 mmol of both phenolic components (or 5 mmol of phenols and 1.5 ml of liquid aliphatic alcohols; 2 g of hexadecanol was used) in 30-35 ml of CH_2Cl_2 were magnetically stirred overnight with the phase-transfer catalyst (Bu_4NHSO_4, $BnNEt_3Cl$, $CH_3(CH_2)_{15}NMe_3Br$ or 18-crown-6) and *ca* 1g of KOH crushed in a mortar under protecting layer of hexane. The products $R_1OCH_2OR_2$ were separated by the gravitational column chromatography after a conventional aqueous work-up of the reaction mixtures.

All the compounds presented in the Table 1 show their expected NMR characteristics. All new compounds were also characterized by the high-resolution mass measurements.

Three acetals **18**, **29** and **34** were obtained in a form suitable for the X-ray analysis and their crystal structures are shown in the Figure 1

18 **29** **34**

Figure 1 The crystal structures of the acetals **18**, **29** and **34**.

The crystallographic parameters at -100 C are as follows: **18**: triclinic P 1; *a*, *b*, *c* (Å): 8.0251(4), 9.0365(5), 23.4269(12); , , ( ):84.497(2), 88.301(2), 73.404(2); Z =4; **29**: monoclinic space group P21/c; *a*, *b*, *c* (Å): 12.9917(11), 4.2968(4), 27.261(2); = 100.210(6) , Z=4; **34**: monoclinic space group P21/c; *a*, *b*, *c* (Å): 7.6326(4), 20.1622(11), 9.1642(5); = 112.450(2) , Z=4. Crystallographic data [39-42] for these compounds have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 1873448, 1873449, 1874258 for **18**, **29** and **34**, respectively. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/.

All the compounds except the unstable **25** have been subjected to antiproliferative evaluation in the HeLa cell line [43] at 50μM. The results of the five most active compounds are shown

in the Table 2. The compound **21** stands out as a lead for further work.

Table 2. Cytotoxicity of the five most active compounds and doxorubicin as a control against theHeLa cell line at 50μM.

Compound	Inhibition of cell viability * (% inhibition ± SD)	IC_{50} **and confidence intervals
16	95.96 ± 0.95	23.00 (19.79 – 26.74)
17	94.93 ± 1.14	32.72 (29.37 – 36.46)
19	79.78 ± 2.75	26.95 (24.42 – 29.74)
21	82.53 ± 4.78	1.11 (0.95 – 1.29)
31	83.06 ± 3.93	35.09 (27.25 – 45.18)
Doxorubicin***	85.86 ± 7.46	17.78 (15.99 – 19.77)

*Presented as the means of four replicates. SD = Standard Deviation

** IC_{50} values were obtained by non-linear regression; IC_{50} values in μM, the concentration that reduced by 50% the optical density of treated cells with respect tountreated controls.

***Positive control

In conclusion, easy procedure to obtain the mixed formaldehyde acetals $R_1OCH_2R_2$ in abasic medium using phenols, alcohols and CH_2Cl_2 via combinatorial approach is presented. Preliminary results demonstrate antiproliferative activities of five compounds in the HeLa cell line. Work is in progress now to get similar compounds for anticancer screening,

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