Structure and Cytotoxic Activity of Some Dihydroxyacetophenone Derivatives

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*We have synthesized a series of diazinium dihydroxyacetophenone derivatives using an efficient reaction pathway, in three steps: O-alkylation, α-bromination and N-alkylation reactions. The synthesized derivatives have been evaluated for their cytotoxic activities in vitro against HeLa cell line. Most of the obtained compounds showed a good cytotoxicity against cancer cell line. Compounds **3a-3e** and **5a** displayed more potent cytotoxic activities against human cancer cell line in comparison with etoposide, 5-fluoro-uracil and methotrexate. The brominated compounds (**3a-3e**) may possibly be used as lead compounds for developing new anticancer agents. The structures of compounds were assigned by elemental and spectral analysis, the X-ray data proving unambiguously the structure of compounds.*

Keywords: dihydroxyacetophenone, cytotoxic activities, anticancer, X-ray

Despite the progress achieved by modern medicinal science, cancer remain among the leading causes of morbidity and mortality worldwide, with approximately 14 million new cases and over 8 million cancer deaths annually. The number of new cases is expected to rise by about 70% over the next two decades [1].

The cancer chemotherapy is complex and complicated, mostly because of the significant levels of toxicity and the emergence of drug resistance and multidrug resistance. It is obvious that development of new chemotherapeutics is of major interest in academic and industrial research, in order to discover newer and more potent molecules, with higher specificity and reduced toxicity than the existing ones [1-3]. One of the most promising approach in cancer therapy remains targeting DNA, DNA alkylators agents being one of the most class used in chemotherapy [2, 3].

On the other hand, nitrogen heterocycles (especially five and six member ring members with two heteroatoms) are reactive intermediary in organic synthesis [8-18], possessing a large variety of applications in opto-electronics (highly fluorescent derivatives, sensors and biosensors, lasers, semiconductor and liquid crystal properties) [19-27], agriculture (herbicidal activity and grow up factor for plants) [28-32] and medicinal chemistry (anticancer, antimicrobials, anti-inflammatory, antihypertensive, diuretics, antithrombics, anticoagulants, antidepressant, anxiolytics, anticonvulsant, analgesic) [33-43]. Encouraged by the promising results obtained previously in the field of dihydroxyacetophenone derivatives with antimicrobial and anticancer activity [6,7], we decided to aprofundate the studies concerning cytotoxicity/ anticancer activity and, to bring supplementary clarifications which prove unambiguously the structure of compounds.

Experimental part

The synthesized compounds were characterized by elemental and spectral analysis: IR, ^{1}H NMR, ^{13}C NMR, 2D-COSY, 2D-HETCOR (HMQC), long range 2D-HETCOR (HMBC). All the reagents and solvents employed were of the highest purity available and were used without any purification. Melting points were determined using an electrothermal apparatus (MELTEMP II) and are uncorrected. Elemental analyses were performed on Elemental Exeter Analytical CE 440 Analyzer. The IR spectra were recorded on an FTIR Shimadzu Prestige 8400s spectrophotometer. The NMR spectra were recorded on a Bruker Avance 400 DRX spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. The following abbreviations were used to designate chemical shift multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) in Hz. The X-Ray diffraction experiment was performed using a SuperNova Dual diffractometer equipped with a Cu (Kα radiation, λ = 0.684 A°) fine-focus sealed X-ray tube and a graphite monochromator.

Results and discussions

The strategies adopted for the synthesis of our dihydroxyacetophenone derivatives **2-5** is facile and efficient and was described elsewhere [6, 7]. The preparation involves three steps: *O*-alkylation and α-bromination of dihydroxyacetophenone followed by an *N*-alkylation of 1,2-diazine derivatives (scheme 1).

The *O*-alkylation reaction of dihydroxyacetophenone derivatives occurs in presence of potassium carbonate and of small amounts of potassium iodide (catalytic) in acetonitrile as solvent, using methyl chloro-acetate as reactant. The desired *O*-alkylated compounds **2a-e** were obtained in good yields (78-87%).

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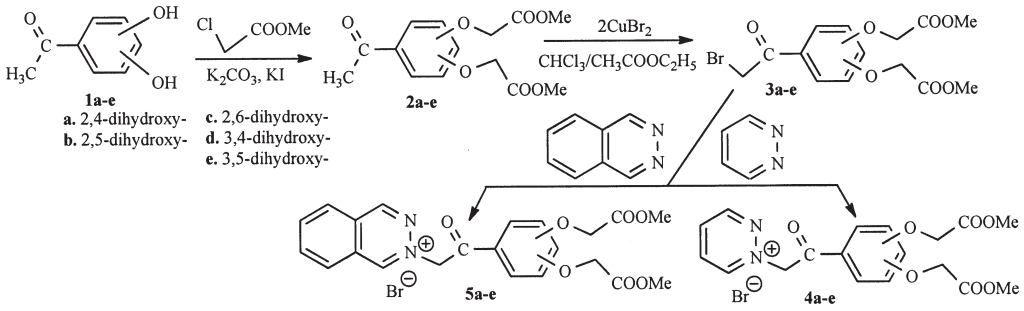

Scheme 1. Reaction pathway for preparation of dihydroxyacetophenone derivatives **2-5**

The α-bromination of *O*-alkylated dihydroxyacetophenone **2a-e** took place in heterogeneous catalysis (using copper (II) bromide in chloroform / ethyl acetate), leading to brominated dihydroxyacetophenone **3a-e**, in good yields (65-76%).

The obtained brominated dihydroxyacetophenone **3a-e**, were used for the *N*-alkylation reaction of pyridazine and phtalazine heterocycles, leading to *N*-alkylation dihydroxyacetophenone **4a-e, 5a-e**, in good yields (59-77%).

The *O*-alkylation, α-bromination and *N*-alkylation reactions was performed using conventional thermal heating and also, microwave and ultrasound irradiation.

The structures of all synthesized compounds were assigned by elemental and spectral analysis: IR, ^{1}H NMR, ^{13}C NMR, 2D-COSY, 2D-HETCOR (HMQC), long range 2D-HETCOR (HMBC) and X-ray. All the elemental and spectral data are in accordance with the proposed structure.

To establish unequivocally the structure of dihydroxyacetophenone derivatives **2-5**, the X-ray analysis was performed in the case of compounds **2b** and **2e** (fig. 1, 2)

The X-ray structure fully confirms the proposed structures. As figure 1 show, the dihydroxyacetophenone derivative **2b** is a flat coplanar system and with the two groups O-methylacetate (from the 2- and 5- position) in plane (all the torsion angles are closely to 0° or 180°). The X-ray structure of dihydroxyacetophenone derivative **2e**, is depicted in figure 2. This molecule it is also a coplanar system (the torsion angles are closely to 0° or 180°), but with the two groups methylacetate (-CH_2COOMe, from the 3- and 5- position) almost perpendicular on the phenyl plane (the bond angles –O–CH_2–COOMe around 110°).

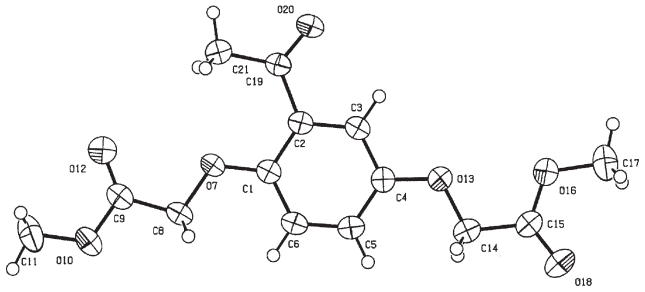

Fig. 1. The ORTEP diagram of the compound **2b** including atom numbering scheme; ellispoids correspond to 50% probability levels

Cytotoxic assay

Cytotoxic effect of the compounds against HeLa cells was determined by a rapid colorimetric assay, using 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). The MTT assay is based on the reduction of the soluble 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) into a blue-purple formazan product, mainly by mitochondrial reductase activity inside living cells. The biological material used in the *in vitro* experiments, was represented by mycoplasma negative negroid human cervix epitheliod carcinoma HeLa cells, which were cultured in DMEM medium (Dulbeco's Modified Essential Medium, Biochrom AG, Germany) supplemented with 10% fetal bovine serum (Sigma, Germany), 100 μg/mL streptomycin (Biochrom AG, Germany), 100 IU/mL penicillin (Biochrom AG, Germany) and 50 μg/mL amphotericin B (Biochrom AG, Germany), at a density of $5x10^5$ cells, in a humidified 5% CO_2 atmosphere at 37°C in a Binder CB 150 incubator (Tuttlingen, Germany). The cultured cells were mixed with 15μL of MTT solution and incubated for 3 h at 37°C. The absorbance was measured at 570 nm. Cell survival percentage was calculated based on the following formula. Percentage of cell survival in the negative control was assumed as 100.

$$\% \text{ Cell Survival} = \frac{A_t - A_b}{A_c - A_b} \times 100$$

A_t: Absorbance of tested concentration,
A_b: Absorbance of blank,
A_c: Absorbance of negative control

The *in vitro* cytotoxicity of the synthesized compounds was evaluated on HeLa cells by the MTT assay according to Mosmann's method [44]. All compounds were tested at

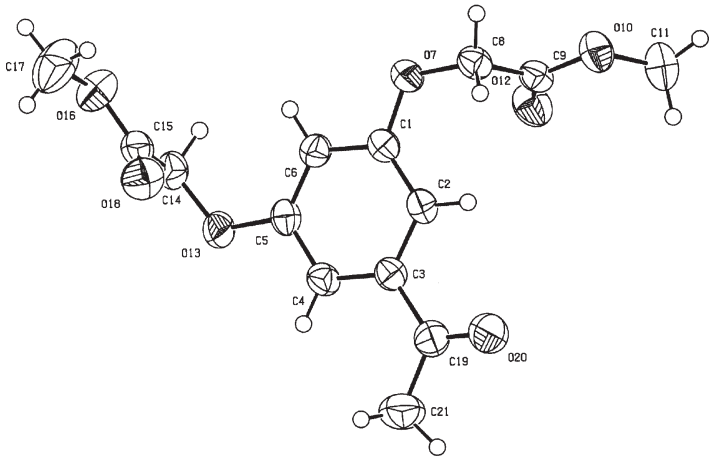

Fig. 2. The ORTEP diagram of the compound **2e** including atom numbering scheme; ellispoids correspond to 50% probability levels

Compound	Viability	Cytotoxicity
2.a	64.26±3.46	35.74±3.46
2.b	62.43±1.95	37.57±1.95
2.c	61.60±1.72	38.40±1.72
2.d	67.77±1.67	32.23±1.67
2.e	68.55±2.88	31.45±2.88
3.a	14.23±1.13	**85.77±1.13**
3.b	20.24±2.70	**79.76±2.70**
3.c	9.59±1.50	**90.41±1.50**
3.d	22.65±3.10	**77.35±3.10**
3.e	13.89±1.80	**86.11±1.80**
4.a	60.08±5.17	39.92±5.17
4.b	71.70±4.19	28.30±4.19
4.c	75.24±5.07	24.76±5.07
4.d	65.62±6.84	34.38±6.84
4.e	77.68±5.58	22.32±5.58
5.a	55.98±3.32	**44.02±3.32**
5.b	99.90±0.02	0.10±0.02
5.c	66.14±6.63	33.86±6.63
5.d	67.03±6.96	32.97±6.96
5.e	75.15±8.18	24.85±8.18
Etoposide	58.45±3.45	41.55±3.45
5-Fluoro-uracil	79.82±4.80	20.18±4.80
Methotrexate	89.08±1.79	10.92±1.79

Table 1
VIABILITY AND CYTOTOXICITY IN HELA CELLS AFTER 3 H INCUBATION WITH SYNTHESIZED COMPOUNDS IN A DOSE OF 400 μg/mL

400 μg/mL concentration. Each experiment was performed five times; the results were expressed as means ± SD. As shown in table 1, most of the compounds exhibited excellent cytotoxic effects against HeLa cell line. Except **5b** all tested compounds showed superior cytotoxic activity as 5-fluoro-uracil and methotrexate.

The compound **3c** displayed more potent cytotoxic activity against HeLa cell line with a cytotoxicity of 90.41±1.50. Generally, the most active compounds proved to be the brominated derivatives. Compounds **3a** (85.77±1.13), **3b** (79.76±2.70), **3c** (90.41±1.50), **3d** (77.35±3.10), **3e** (86.11±1.80) and **5a** (44.02±3.32) showed superior cytotoxicity to all etoposide (41.55±3.45), 5-fluoro-uracil(20.18±4.80) and methotrexate (10.92± 1.79).

The cytotoxicity of the tested compounds could be correlated with structure variation and modification. In case of brominated compounds they showed higher cytotoxicity. All brominated compounds proved to be more cytotoxic in comparison with etoposide, 5-fluoro-uracil and methotrexate. The alkylated derivatives presented a moderate cytotoxicity against HeLa cell line. Except **5a**, the diazinium salts displayed a moderate cytotoxic activity against the tumor cell.

Conclusions

We have synthesized a series of diazinium dihydroxyacetophenone derivatives using an efficient reaction pathway, in three steps: *O*-alkylation, α-bromination and *N*-alkylation reactions. The structures of all synthesized compounds were assigned by elemental and spectral analysis (IR, ^{1}H NMR, ^{13}C NMR, 2D-COSY, HMQC, HMBC and X-ray). The X-ray data prove unambiguously the structure of compounds. The *in vitro* assay revealed that all tested compounds showed a good cytotoxicity. Some compounds (**3a-3e** and **5a**) were found to be more potent cytotoxic activities against HeLa cell line than etoposide, 5-fluoro-uracil and methotrexate. From the structure-activity relationships, we may conclude that the introduction of the bromine is associated with enhanced cytotoxic activity. The brominated compounds showed the highest cytotoxicity, these compounds being more active than positive controls. This study may provide valuable information for further designing and developing more potent anticancer agents.

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