Synthesis and Biological Evaluation of Novel Quinol dimethyl ethers as Potential Anticancer and Antimicrobial Agents.

Ibrahim Chaaban[1], El Sayeda M. El Khawass[1], Mona A. Mahran[1], Heba A. Abd El Razik[1]*, Nehad S. El Salamouni[1], Abeer E. Abdel Wahab[2]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Alexandria, Alexandria 21521, Egypt
[2]Genetic Engineering and Biotechnology Research Institute (GEBRI), Mubarak City for Scientific Research and Technology Application, Borg El-Arab, Alexandria, Egypt

As a part of an ongoing research program devoted to the finding of new structural leads with potential chemotherapeutic activities, particular attention has been given to the pronounced anticancer activity of several quinol dimethyl ethers. According to the protocol of the NCI's *in vitro* disease-oriented antitumor screen unit, Bethesda, Maryland, USA, several analogs incorporating the above-mentioned quinol dimethyl ether counterpart together with a pyrazole moiety exhibited a potential antitumor activity.

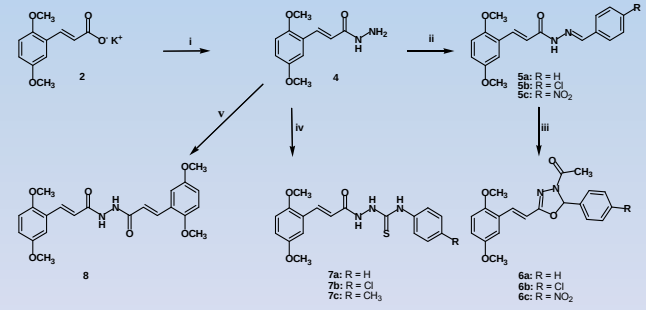
Prompted by the above-mentioned results, it was rationalized to further optimize this chemical series (quinol dimethyl ethers) by exploring additional modifications at position -1- of the quinol dimethyl ether. Structure modifications suggested in the present investigation focused mainly on studying the effect of incorporating various functionalities that are known to contribute to a variety of chemotherapeutic activities including α,β-unsaturated ketone, acid hydrazide, hydrazone and thiosemicarbazide groups. Additionally, it was considered of interest to incorporate other chemotherapeutically-active heterocyclic rings (triazoles, oxadiazoles and thiadiazoles) within the structure. These combinations were suggested in an attempt to investigate the possible synergistic influence of such structure combinations on the expected activity, hoping to recognize a new leading structure that would have a significant antitumor potential at very small concentrations. Furthermore, owing to the well documented therapeutic potentials associated with substituted hydroquinones, it seemed interesting to prepare compounds incorporating the hydroquinone moiety in order to explore the influence of such structural assembly on the anticipated biological activities.

The newly synthesized compounds were evaluated for their anticancer and antimicrobial activities. Nine of the prepared compounds were selected by the National Cancer Institute (NCI) and tested initially at a single high dose (10 μM) in the full NCI 60 cell panel. Four of screened compounds satisfied the threshold inhibition criteria and passed forwards for evaluation in the full panel five-dose *in vitro* antitumor screen. One compound showed very promising results and accordingly was selected for *in vivo* antitumor screening.

On the other hand, out of the tested compounds, several compounds showed promising antibacterial activities. Most of them exhibited special high activity comparable to the reference Ampicillin against *Pseudomonas aeruginosa* and *Escherichia coli*.

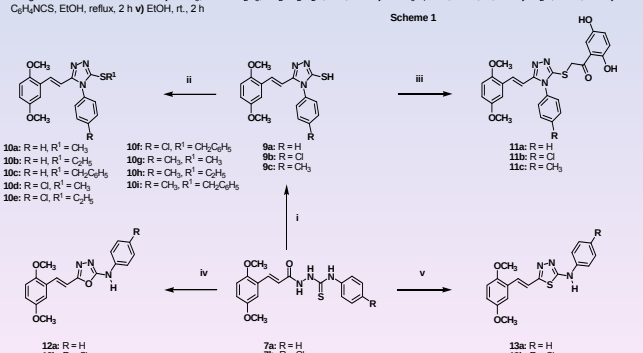
Chemistry

Synthesis of the intermediate and target compounds was performed according to the reactions outlined in Schemes 1 and 2. 3-(2,5-dimethoxyphenyl)propenoic acid **1** was prepared by reacting a solution of 2,5-dimethoxybenzaldehyde with malonic acid in pyridine in presence of piperidine according to the previously reported reaction conditions. Treating 3-(2,5-dimethoxyphenyl)propenoic acid **1** with 1M potassium hydroxide solution at room temperature afforded potassium 3-(2,5-dimethoxyphenyl)propenoate **2** which was treated with ethyl chloroformate in chloroform in presence of pyridine yielding the activated ester **3** which was added to a stirred ice-cooled suspension of 80% hydrazine hydrate in chloroform according to the reported reaction conditions to afford 2,5-dimethoxyphenylpropenoic acid hydrazide **4**. Stirring warm solution of an equimolar amount of the latter and the appropriately substituted benzaldehyde in absolute ethanol gave rise to substituted hydrazones **5a-c** which were by cyclized in boiling acetic anhydride following the reported reaction conditions yielding oxadiazolylethanone derivatives **6a-c**. Thiosemicarbazide derivatives **7a-c** were prepared by heating under reflux a solution of 2,5-dimethoxyphenylpropenoic acid hydrazide **4** in absolute ethanol with an equimolar amount of the appropriate aryl isothiocyanate. It is to be noted down that allowing 2,5-dimethoxyphenylpropenoic acid hydrazide **4** to react with the selected aryl isothiocyanate (phenyl, *p*-chlorophenyl or *p*-tolyl) in absolute ethanol by stirring at room temperature, a totally unexpected one and the same product was obtained indicating that the reaction has followed a completely different pathway. The structure of the product was proven to be N1,N2-bis(2,5-dimethoxyphenylpropenoyl)hydrazine **8** for the following arguments: The compound only contains C, H, N, O but no sulfur proved by elemental microanalyses. Its 1H-NMR spectrum showed two singlets assigned for the four methoxy protons and one deuterium-exchangeable singlet assigned for the two NH protons, besides the ethylenic and aromatic protons at their characteristic chemical shifts. Electron impact mass spectrum showed the molecular ion peak (M+·) at m/z 412 corresponding to C22H24N2O6 and base peak at m/z 190. The structure of this compound **8** was further confirmed by preparing it through stirring an ethanolic solution of the acid hydrazide **4** for 2 h at room temperature. Sulfanyl triazoles **9a-c** were prepared by heating under reflux the appropriate substituted thiosemicarbazides **7a-c** with 1N aqueous sodium hydroxide. The target triazoles **9a-c** were liberated from their sodium salts by acidification with dilute hydrochloric acid. Substituted sulfanyl 1,2,4-triazoles **10a-i** and **11a-c** were synthesized by adding the selected alkyl, aralkyl halide or 2,5-dihydroxyphenacyl bromide to an ethanolic solution of the appropriate 3-sulfanyltriazole **9a-c** containing sodium ethoxide. On the other hand, 5-substituted anilino-1,3,4-oxadiazoles **12a-c** were prepared by cyclodesulfurization of the appropriate thiosemicarbazides **7a-c** with freshly prepared yellow mercuric oxide. Whereas, 5-substituted anilino-1,3,4-thiadiazoles **13a-c** were prepared by two methods: either by heating 3-(2,5-dimethoxyphenyl)propenoic acid **1** with substituted thiosemicarbazides in phosphorus oxychloride for 30 minutes or via dehydration of substituted phenylpropenoyl thiosemicarbazides **7a-c** by boiling their ethanolic solutions in presence of 10% sulfuric acid .



Reagents and reaction conditions: **i)** $CHCl_3$, $ClCOOC_2H_5$, $NH_2NH_2.H_2O$, 0°C, 12 h **ii)** 4-R-C_6H_4CHO, EtOH, rt., 2 h **iii)** Ac_2O, reflux, 2 h **iv)** 4-R-C_6H_4NCS, EtOH, reflux, 2 h **v)** EtOH, rt., 2 h

Scheme 1



Reagents and reaction conditions: **i)** NaOH, reflux, 4 h then HCl; **ii)** R^1X / NaOEt, EtOH, reflux, 2h; **iii)** 2,5-$(OH)_2C_6H_4COCH_2Br$, NaOEt, EtOH, reflux, 2 h; **iv)** yellow HgO, EtOH, reflux, 2 h **v)** 10% H_2SO_4 in EtOH, reflux, 1 h.

Scheme 2

Biology

1- Preliminary in vitro anticancer screening:

Nine of the newly prepared quinol dimethyl ether derivatives **5a,b**, **9b**, **10a,d**, **12a,b** and **13a,b** were selected by the NCI and were tested initially at a single high dose (10 μM) in the full NCI 60 cell panel. Four of the screened compounds which are the unsubstituted phenylhydrazone **5a**, 4-chlorophenylhydrazone **5b**, 4-chlorophenyl-3-sulfanyl-1,2,4-triazole **9b** as well as 4-chloroanilino-1,3,4-oxadiazole **12b** satisfied the threshold inhibition criteria and passed forwards for evaluation in the full panel five-dose *in vitro* antitumor screen. 4-Chlorophenylhydrazone **5b** showed very promising results and accordingly was chosen for *in vivo* antitumor screening.

2- Antibacterial and antifungal screening:

Twenty four of the newly synthesized compounds; **5a-c**, **6a-c**, **9a-c**, **10a,b,d,e,g,h**, **11a-c**, **12a-c** and **13a-c** were screened for their *in vitro* antimicrobial activity against five microorganisms, namely; *Staphylococcus aureus* and *Bacillus subtilis* as representative examples of Gram-positive bacteria, *Escherichia coli* and *Pseudomonas aeruginosa* as examples of Gram-negative bacteria. They were also evaluated for their *in vitro* antifungal activity against *Candida albicans*. Almost all the tested compounds showed pronounced antimicrobial activities. Most of them exhibited special high activity comparable to the reference Ampicillin against *Pseudomonas aeruginosa* and *Escherichia coli*. Some of screened compounds showed potent activity against *Bacillus subtilis* while others showed a moderate activity against *Staphylococcus aureus*. On the other hand, most of the tested compounds were found to be inactive against *Candida albicans* when compared to the reference Clotrimazole