PREPARTION OF SOME CURCUMIN DERVATIVES OF POTENTIAL BIOLOGICAL ACTIVITY

A thesis
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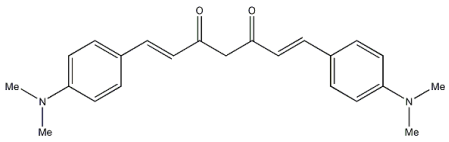
Abstract

The study was divided into two main parts. The first includes the synthesis and characterization of the target compounds while the second devoted to the investigation of the stability and the pharmacological activities of them as antioxidant, antitumor, antifungal and antibacterial candidates. Ten compounds were synthesized two of them (1 and 2) are curcumin analogues while the remaining are azetidines derived from curcumin.



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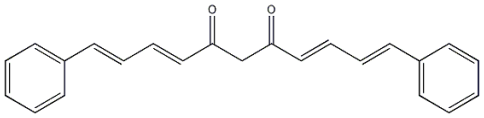

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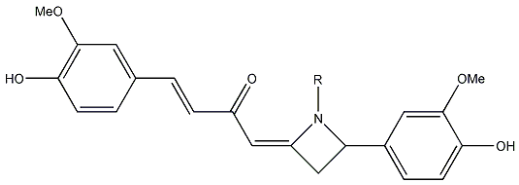

3, R= Me
4, R= Et
5, R= nPr
6, R= nBut
7, R= nHexyl
8, R= Benzyl
9, R= C6H5
10, R= Me-C6H5

The compounds 1 and 2 were synthesized by a general method for the preparation of curcumin analogues while the azetidines were synthesized under microwave conditions.

The structures of the compounds were identified with the aid of elemental analyses, IR, mass and NMR spectra as well as chemical computation. Elemental analyses were agreed with the proposed chemical formulas. Both EI-MS and CIMS techniques were used to record the mass spectra which showed either $M^{+\circ}$ or $(M+1)^+$ and $(M-1)^+$ ions at the proposed MW or (MW+1) and (MW-1) confirming the expected molecular weights.

The IR spectra of 1 and 2 as KBr discs showed stretching bands for conjugated carbonyl groups at 1593 and 1606cm^{-1} respectively in addition a weak and very broad bands within the range 3200-1800cm^{-1} attributed to the stretching vibrations of the OH group engaged in the intrahydrogen bonded chelated ring of β-diketone kind. The IR spectra of the bases (3-10)were characterized by the conjugated carbonyl group stretching vibration at 1630cm^{-1} which almost similar to that of curcumin but with reduced intensity. Their main difference from the spectra of curcumin being in the absence of the weak and very broad band of the intrahydrogen bonded chelated OH group at 3200-1800cm^{-1}, instead they showed a strong band at about 3200cm^{-1} attributed to the inter molecular hydrogen bonded phenolic OH group which is found in the spectrum of curcumin also.

Several NMR teqniques included 1D HNMR, CMR, homoCOSY, HETCOR, NOESY and ROESY as well as dynamic NMR (in DMSO) were used to investigate the chemical structures of the studied compounds. The spectra of the compounds 1 and 2 are similar to that of curcumin and indicate their presence in the enolic form as the only or the main form in the solution. The NMR spectra of the bases are essentially different from that of curcumin by characteristic signals. These signals are two doublets of doublets at about 2.4 and 2.8ppm with J=16Hz attributed to gemminal protons within four membered ring, both coupled to the same vicinal proton characterized by a multiplet at 4.7ppm with J=7 and 4 Hz respectively. The spectra as well have a vinylic proton signal at 6.0ppm in addition to two signals for both two OMe and two phenolic OH groups which means that each molecule has two sides with different chemical environments differing from the symmetric curcumin molecule. The homoCOSY spectrum indicates the diasteriotopic manner of the methylene groups of the aliphatic substituent on the nitrogen atom leading to have resonances at different chemical shifts. The HETCOR spectrum supported the presence of two geminal protons and two methoxy groups as well as the diasteriotopic character of the alkyl methylenes. The C-13 spectrum of compound 5 contains carbonyl carbon signal at 188 and the

signals of the carbons of two methoxy groups at 51.7 and 51.6ppm that are matched very well with literature.

Both NOESY and ROESY spectra revealed through-space proton correlations that support the proposed structures in addition they show correlations between the phenolic OH protons indicating expected intermolecular hydrogen bonding and proton exchange between the OH groups at different sides of the molecules. The dynamic study of the spectrum of the base 10 at 295, 323 and 373 K in DMSO indicates a clear effect of the temperature on both the chemical shifts and line widths of both phenolic OH protons signals and that the chemical shift differences between them decrease with temperature till they are merged in one broad signal at averaged position of the two signals as a result of increasing chemical exchange between the two groups.

The electronic spectra of the compounds 1 and 2 showed $\pi \rightarrow \pi^*$ absorption bands at 475 and 420nm respectively while the spectra of the bases showed main $\pi \rightarrow \pi^*$ absorption bands blue shifted by about 60nm as compared to that of curcumin . These bands are red shifted on replacing the aliphatic substituent by aromatic one.

The chemical computations using Gaussian 98, Hyper Chem 8 and Mopac 2000 programs were done on the proposed bases molecules which showed that the syn isomer in both alkyl and aryl substituted bases is the most stable in these compounds on the basis of their computed energies. The HF, DFT and semi empirical (PM3) levels of theory were used and some conclusions concerning the chemical structures were undertaken on the basis of the computed bond lengths and angels. The electronic spectra as well as the NMR spectra of the bases were computed as well which agreed with the observed spectra.

The pharmaceutical part was included stability study and in vitro biological investigation includes antioxidant, antitumor , antibacterial and antifungal activities.

The stability of curcumin analogues 1 and 2 and azetidines 4, and 6-10 was determined spectrophotometrically in 0.5M phosphate buffer at pH 4.5, 7

and 10.3 at 37°C (±0.5°C). The results revealed that the tested compounds were stable at various pH values.

Antioxidant activity of curcumin analogues 1 and 2 and azetidines 3-10 were studied for their ability to prevent nitrite-induced oxidation of haemoglobin. Phenolic derivatives (3-10) and $N(Me)_2$ substituted analogue (1) of curcumin were found to be active while the analogue 2 was not.

Antitumor activity of curcumin and the azetidines 3-8 and for compounds B and C were evaluated for preliminary estimation of the in vitro tumor inhibiting activity against a panel of tumor cell lines, using microculture, Tetrazedium assay (MTT) method. The results showed that there is a general decrease in the antitumor activity when going from curcumin to azetidines 3-8 especially for the base 3 which is inactive against all tumor cell lines with CC_{50} greater than 100μM. The important observation found is that the antitumor activity of the azetidin increased with increasing chain length of the aliphatic substituent on the nitrogen atom. Increasing activity was found also when going from the methyl substituent to the benzyl one. On the other hand the results show an enhanced antitumor activity for the compounds B and C as compared with curcumin for all tumor cell lines which show the importance of such derivatives in the field of antitumor agents.

The antibacterial and antifungal activities of curcumin and azetidins 3-8 were also evaluated in vitro against two types of bacteria namely S. aureus and P. aeruginosa and two types of fungi includes C. albicans and A. niger. The results showed that neither curcumin nor azetidines 3-8 exhibited growth inhibitory activity against both types of bacteria and fungi with MIC greater than 100μM.