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Chemical synthesis, pharmacological evaluation and in silico analysis of new 2,3,3a,4,5,6-hexahydrocyclopenta[*c*]pyrazole derivatives as potential anti-mitotic agents

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ABSTRACT

We have synthesized new, biologically active mono- and di-substituted 2,3,3a,4,5,6-hexahydrocyclopenta[*c*]pyrazole derivatives bearing electron withdrawing groups and electron donating groups. These derivative structures were characterized by their spectral and analytical data. The newly synthesized hexahydropyrazole analogues were evaluated for their in vitro anticancer activity against breast and lung cancer cell lines using a cytotoxicity bioassay. To understand their mechanism of action, tubulin binding assays were performed which pointed to their binding to microtubules in a mode similar to but not identical to colchicine, as evidenced by their K_D value evaluation. Computational docking studies also suggested binding near the colchicine binding site on tubulin. These results were further confirmed by colchicine-binding assays on the most active compounds, which indicated that they bound to tubulin near but not at the colchicine site. The moderate cytotoxic effects of these compounds may be due to the presence of electron donating groups on the para-position of the phenyl ring, along with the hexahydropyrazole core nucleus. The observed anti-cancer activity based on inhibition of microtubule formation may be helpful in designing more potent compounds with a hexahydropyrazole moiety.

Cancer, a spectrum of diseases characterized by the uncontrolled and abnormal division of cells in the body by escaping the normal cell cycle control and programmed cell death is the second leading cause of death worldwide.[1] It is estimated that 12 million people will die from cancer by 2030.[2] In the past few years, many efforts have been made to develop new strategies for finding safe and effective ways of treating these diseases, which must be based on an improved understanding of the biological processes involved in cancer cell survival and the search for novel chemotherapeutic agents.[1] The conventional methods to treat metastatic cancer, i.e., chemotherapy and radiation therapy, have a major drawback of producing severe side effects. These methods inflict 'collateral damage' as they are unable to precisely differentiate between the cancerous and normal cells.

Normal cells affected adversely by systemically administered chemotherapy are mostly the cells in the mouth, blood cells, hair follicles, stomach and bowel, which usually results in nausea, diarrhea, mouth sores, low blood counts, and/or hair loss.[1] Therefore, there is an urgent need to focus on novel targeted cancer therapies that attack preferentially the cancer cells, thereby avoiding any serious damage to normal cells.

One such molecular target are microtubules (MTs), which are ubiquitous protein polymer structures representing key components of the cytoskeletal architecture. MTs are involved in many important cellular processes including mitosis, morphogenesis, intracellular transport and secretion.[3] MTs are hollow cylinders consisting of α- and β-tubulin heterodimers. The functions of MTs are regulated by their continuously alternating polymerization and depolymerization processes, a unique property of

Abbreviations: EDG, electron donating group; EWG, electron withdrawing groups; MT, microtubule; SAR, structure activity relationship.

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microtubules referred to as dynamic instability.[4] Tubulin-binding molecules interfere with the dynamic instability of MTs leading to the formation of abnormal spindles, cell cycle arrest in the M-phase, and ultimately apoptotic cell death.[5,6] A variety of compounds which were obtained from natural sources, such as paclitaxel, vinblastine, combretastatin A-4 and colchicine, are known to interfere with the dynamic instability of MTs and result in mitotic arrest.[7] These drugs, however, are often associated with severe side effects due to their adverse effects on normal cells (which also express tubulin and form microtubules) and the development of drug resistance in cancer cells.[8] Therefore, there is still an urgent need to find tubulin-binding pharmacological agents which would have an improved profile in terms of specificity and selectivity for cancer versus normal cells.

Heterocyclic compounds comprise the largest and most diverse group of organic medicinal compounds. More than 75% of the top 200 branded drugs from the pharmaceutical industry have heterocyclic fragments in their structures. Among all the heterocyclic compounds discovered so far, those containing nitrogen in their core structure are the most significant and appear in most of the drugs marketed worldwide. For these reasons, heterocycles have an enormous potential to be used as scaffolds for the design of new drugs.

Pyrazoles are an important class of nitrogen containing five-membered heterocyclic compounds. The pyrazole scaffold represents a common motif in many pharmaceutically active compounds that demonstrate a wide range of pharmacological activities including anti-cancer, anti-microbial,[9,10] anti-inflammatory,[11,12] anti-convulsant, anti-histaminic,[13] anti-viral[14] and anti-HIV.[15] Because of their clinical significance, the synthesis of new lead compounds retaining the 'core' pyrazole chromophore has attracted considerable attention of the medicinal chemistry research community.[16–18]

As a part of our ongoing drug discovery program, we have synthesized various pyrazole scaffold-bearing heterocyclic compounds as potential novel anti-cancer agents with a focus on the features important for anti-mitotic activity.

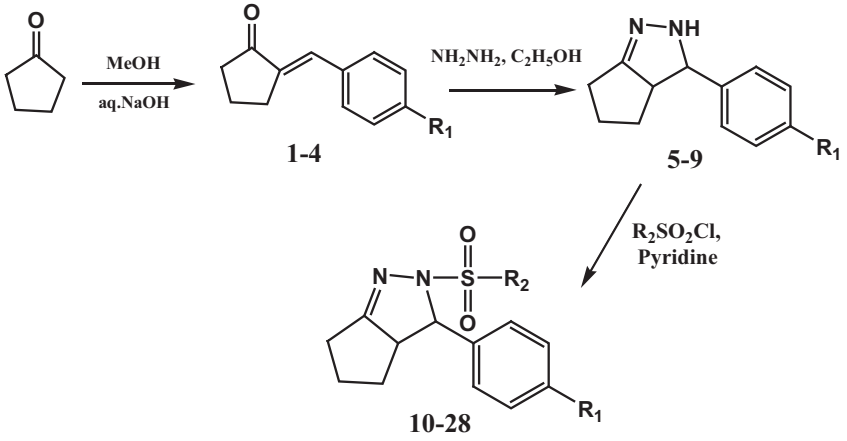
This novel series of 2,3,3a,4,5,6-hexahydrocyclopenta[*c*]pyrazole derivatives bearing both electron donating and electron withdrawing substituents in the para-position of the sulfonylaryl moiety has been synthesized keeping in mind their structural simplicity. These compounds, obtained by convenient synthetic methods, have been shown to strongly bind to MTs as evidenced by our biological assays and in silico docking studies.

The synthetic approach to obtaining mono-substituted 2,3,3a,4,5,6-hexahydrocyclopenta[*c*]pyrazole derivatives (**5**–**8**) and 2,3-disubstituted-2,3,3a,4,5,6-hexahydrocyclopenta[*c*]pyrazole derivatives (**9**–**28**) followed the reactions shown in Scheme 1. We accomplished the synthesis of substituted (*E*)-benzylidene cyclopentanones by cyclopentanone reaction with *p*-substituted benzaldehydes in the presence of ethanolic sodium hydroxide.[19] Heating of benzylidene cyclopentanones with hydrazine hydrate resulted in the formation of 3-substituted-3,3a,4,5,6,7-hexahydro-2*H*-indazoles (**1**–**3**).[20] Further, the sulfonation of 3-substituted-hexahydrocyclo-pentapyrazoles with appropriate sulfonyl chlorides in pyridine yielded 2,3-disubstituted-2,3,3a,4,5,6-hexahydrocyclopenta[*c*]pyrazoles (**4**–**21**).[21]

In general, the benzylidene cyclopentanones exhibited UV maxima between 300 and 362 nm. Their infrared spectra (FT-IR) showed bands at 2900–2955, 1629–1691 and 1529–1599 cm^{-1} corresponding to the presence of C—H str., C=O str. and C=C str. bonds, respectively. In the proton NMR spectra (^{1}H NMR), the signals of the respective protons of the title compounds were verified on the basis of their chemical shifts, multiplicities and coupling constants. The spectra showed multiplets and triplets in the range of δ 1.05–3.12 ppm corresponding to aliphatic ring protons, doublets at δ 7.10–7.61 ppm corresponding to aromatic protons, and singlets in the range of δ 7.26–7.58 ppm corresponding to vinylic protons. The latter is the characteristic peak in the benzylidene compounds confirming the geometry of the vinylic bond as *E*.[22,23]

The 3-substituted hexahydropyrazoles were prepared by reacting (*E*)-2-benzylidene-cyclopentanones (**1**–**4**) and hydrazine hydrate by a Michael type of addition reaction (**Step-II**). In general, their UV maxima appeared within the range of 228–319 nm. In the infrared spectra (FT-IR) 3-substituted hexahydropyrazoles showed the absence of C=O str. band at 1629–1691 cm^{-1}, the appearance of secondary amine group stretching at 3300–3490 cm^{-1}, and the presence of C=N str. and C—N str. band at 1550–1688 and 1095–1195 cm^{-1}, respectively, which confirmed the formation of 3-substituted hexahydropyrazoles. In the ^{1}H NMR, the appearance of singlets in the range of δ 7.12–8.64 ppm in the proton spectrum, corresponding to N*H* of hexahydropyrazoles, which disappeared upon D_2O exchange, further supported the formation of the title compounds. Similarly, the appearance of doublets for the 3-C*H* proton at δ 4.27–4.69 ppm and the absence of singlets at δ 7.26–7.58 ppm corresponding to vinylic proton were also in favor of the formation of 3-substituted hexahydropyrazoles.

The reaction between the 3-substituted hexahydropyrazoles (**5**–**8**) with *p*-substituted sulfonylchlorides resulted in the formation of 2,3-disubstituted-2,3,3a,4,5,6-hexahydro-2*H*- pyrazoles (**Step-III**). In general, the product showed UV maxima from 305 to 384 nm. In the infrared spectra (FT-IR) 2,3,3a,4,5,6-hexahydro-2*H*-pyrazoles showed the absence of secondary amine group at 3300–3490 cm^{-1} and the presence of asymmetric and symmetric



Scheme 1. Synthesis of mono- and di-substituted 2,3,3a,4,5,6-hexahydrocyclopenta[*c*]pyrazoles.