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RESEARCH ARTICLE

INDOLE DERIVATIVES: DESIGN, SYNTHESIS, *IN-VITRO* BIOLOGICAL EVALUATION AND MOLECULAR DOCKING STUDY AS ANTICANCER AGENTS

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ABSTRACT

New hetero annulated indoles were synthesized and structurally characterized by spectral means. In order to understand the nature of interactions of these molecules, we carried out molecular docking studies using the protein kinase CK2 inhibitors. The docking results provided some useful information for the future design of more potent inhibitors. The *in vitro* cytotoxicity was evaluated for all the new compounds by MTT assay against HeLa and compared with the standard drug ellipticine. All the compounds showed moderate to potent activity against the cell lines. The preliminary structure–activity relationships were carried out.

Keywords: Cyclopenta[*b*]indoles, Molecular docking, Anticancer.

1. INTRODUCTION

Cancer continues to be one of the major health problems worldwide and one of the leading causes of death despite the advances that have led to the development of new therapies. So, there is a continuing need for designing and developing new chemotherapeutic agents for cancer treatment.

The indole ring is the most ubiquitous heterocyclic substructure in nature. Owing to its great diversity in both structure and biological activity, it is not surprising that the indole ring is an important structural component in many pharmaceuticals (1-5). Particularly fused-polycyclic indole framework is potential candidates for drug discovery because this structural motif is present in a wide variety of biologically active alkaloids (6). It is known for its variety of pharmacological characteristics as e.g. anti-fungal, anti-bacterial, anti-tumor, anti-HIV and DNA interaction properties (7-12).

The development of an efficient synthetic method of cyclopenta[*b*]indole derivatives has attracted broad attention in medicinal chemistry and synthetic organic chemistry. Extensive efforts are therefore focused on this topic (13-15).

Prompted by the findings from the literature studies, we set out to explore and synthesise new pyrazolo- and isooxazolo- cyclopenta[*b*]indole derivatives. Furthermore, the compounds were evaluated for their active site with Human Kinase CK2 Protein by molecular docking study and cytotoxicity against HeLa human cervical cancer cell line also was carried out.

2. RESULTS AND DISCUSSION

2.1. Chemistry

In the attempt of synthesising bioactive isoxazolo- and pyrazoloindole derivatives, first step is the synthesis of thiophen-2-ylmethylene by mixed aldol condensation of 1,4-dihydro-2H-cyclopenta[*b*]indol-3-one 1a-d with thiophene-2-carbaldehyde 2. Further the thiophen-2-ylmethylene derivatives 3a-d was treated with hydroxylamine hydrochloride and hydrazine hydrate to give the corresponding cyclised hetero annulated isoxazolo 4a-d and pyrazoloindole 5a-d derivatives respectively. The synthetic routes were shown in the Scheme 1.

The formation of compound 3a confirmed by its IR spectrum which showed sharp and strong bands at 3153 and 1671 cm^{-1} assigned to NH and C=O group respectively. ^1H NMR spectrum lacked C_2-methylene proton signals and the displayed signals for C_1-methylene and thiophene-CH protons, suggested the structure of 3a to be a thiophen-2-ylmethylene compound. The proton NMR spectrum of 4a exhibited two broad singlets at 11.94 ppm and 9.01 ppm attributed to indole-NH and pyrazole-NH protons respectively. The aromatic protons appeared as multiplet in the region 6.76-7.87 ppm. IR spectrum of 5a registered absorption band at 1626 cm^{-1} assigned to cyano functional group. The proton NMR spectrum of 5a showed one broad singlet at 11.96 ppm attributed to indole-NH. The aromatic protons appeared as a multiplet between the regions δ 7.20-7.96 ppm. Analytical data are in accordance with the proposed structure for compound 5a. The identities of the other compounds were established in the same way with all spectroscopic data readily assignable.

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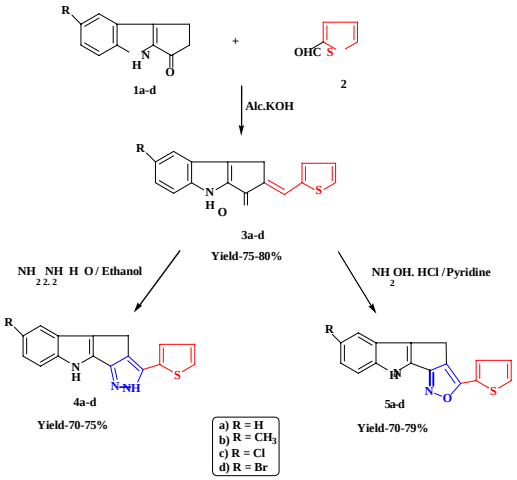


Scheme 1. Synthesis of pyrazolo and isoxazolo cyclopenta[*b*]indoles (4a-4d) and (5a-5d) derivatives.

3. BIOLOGICAL EVALUATION

3.1. Molecular docking studies

To understand the interaction of Human Kinase CK2 protein receptor with (3a–3d, 4a–4d and 5a-5d), the crystal structure of CK2 protein was downloaded from protein data bank and studied with the glide program. All the glide and E model scores and the details of docked compounds are presented in Table 1. The use of glide and E model scores for ranking the different derivatives within a series is always not dependable. The glide scores are mainly used to identify the active and inactive compounds. In addition, glide is primarily concerned with generating an accurate pose for each compound and enrichment (the separation of actives from inactive) Fig.1.

Table 1. Molecular docking studies of 14 analogues taken for study with Human Kinase CK2 protein (PDB: 3OWJ)

Compound	Glide score (kcal/mol)	E model score	Glide energy	Hydrophobic interaction
3a	-6.999	-36.014	-23.094	ALA 148, TYR 32, PHE 169, PHE 97, ILE 79, MET 167, LEU 70, ALA 165, VAL 35, ALA 50, LEV 151.
3b	-6.243	-48.931	-36.337	ALA 148, TRP 105, VAL 35, TYR 32, MET 167, LEU 70, PHE 97, ALA 165, PHE 169, ILE 79, ALA 50, LEU 151.
3c	-6.400	-46.739	-36.360	ALA 148, VAL 27, TYR 32, MET 167, PHE 169, LEU 70, ALA 165, PHE 97, ILE 79, ALA 50, VAL 35, LEU 151.
3d	-5.954	-45.286	-35.422	ALA 148, IEU 151, VAL 35, ALA 50, ILE 79, PHE 97, LEU 70, ALA 165, PHE 169, MET 167, TYR 32
4a	-6.859	-39.633	-36.460	VAL 27, VAL 35, ALA 100, LEU151, ALA 50, ILE 79, LEU 70, PHE 97, PHE 169, MET 167, ALA 165, TYR 32
4b	-6.491	-50.447	-36.007	VAL 27, TYR 99, VAL 35, ALA 50, LEU 151, PHE 97, ILE 79, LEU 70, ALA 165, PHE 169, MET 167, TYR 32, LEU 328.
4c	-7.097	-54.270	-35.105	VAL 27, VAL 35, ALA 50, LEU 151, PHE 97, ALA 165, ILE 79, LEU 70, MET 167, PHE 169, TYR 32
4d	-6.313	-56.113	-39.885	VAL 27, TYR 99, VAL 35, LEU 151, ALA 50, PHE 97, ILE 79, LEU 70, ALA 165, PHE 169, MET 167, TYR 32.
5a	-7.650	-47.866	-32.416	VAL 27, TYR 99, VAL 35, ALA 50, LEU 151, PHE 97, ILE 79, LEU 70, ALA 165, PHE 169, MET 167, TYR 32, LEU 328.
5b	-6.826	-52.164	-36.540	VAL 27, TYR 99, VAL 35, ALA 50, LEU 151, PHE 97, ILE 79, LEU 70, ALA 165, PHE 169, MET 167, TYR 32, LEU 328.
5c	-7.198	-50.162	-36.784	VAL 27, VAL 35, LEU 151, ALA 50, ILE 79, PHE 97, MET 167, LEU 70, PHE 169, ALA 165, TYR 32.
5d	-7.590	-53.858	-33.325	VAL 27, TYR 99, VAL 35, ALA 50, LEU 151, PHE 97, ILE 79, LEU 70, ALA 165, PHE 169, MET 167, TYR 32, LEU 328.

In the binding mode, compounds were attractively bound to CK2 via hydrophobic interaction, and Pi-Pi stacked interaction. The scoring functions of the docking program ranked that the binding interactions of the intermediate were less than those of the cyclised products. The compounds 5a-5d displayed better binding interaction compared to the intermediate; this might be due to the presence of the isoxazole moiety. The next best interactions were found among the compounds 4a-4d which hold the pyrazole moiety. Among the cyclised products compound 5c exhibited the best lowest binding energy and ligand efficiency; this might be due to the presence of the isoxazole moiety which was further reinforced by favourable electrostatic interaction of the chloro group at the 7th position of the indole moiety. In general it was found that the isoxazole moiety favours better binding interactions compared to pyrazole moieties and intermediates.

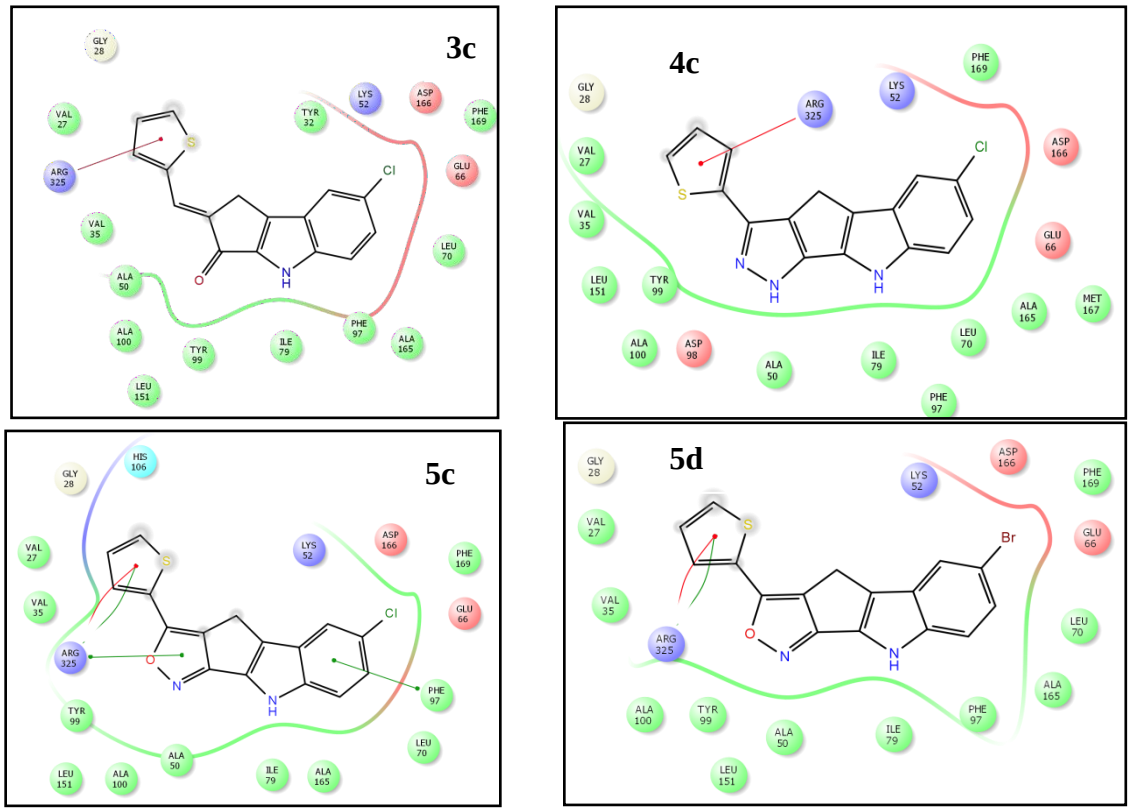


Fig. 1. Docking model structure of compound 3c, 4c, 5c, 5d into the Protein kinase (PDB ID: 3OWJ) binding pocket.

3.2. Cytotoxicity studies

The *in vitro* cytotoxicity assay proves that the compound 5c has potent anticancer effect on human cervical cancer cells. Morphological shift of HeLa cell line by the compound is shown in Fig.2, revealing that the morphological alteration occurs in all the concentrations. From Fig.3, it is evident that compound 5c showed a dose-dependent anticancer activity. The maximum anticancer activity was obtained at 100 µM. The percentage of cell growth inhibition is found to be 100 % for compound 5c. The estimated IC_{50} value is 15.24 µ. Therefore, it is evident that the compound 5c has cell growth inhibition value closer to the standard drug ellipticine (9.62µ). The results obtained from the MTT assays help us to understand that the compound 5c have higher efficiency due to the presence of chloro group in the exact position in benzene moiety to have a good interaction with receptor protein active site. This, again, may be reason for the notable difference in IC_{50} value.

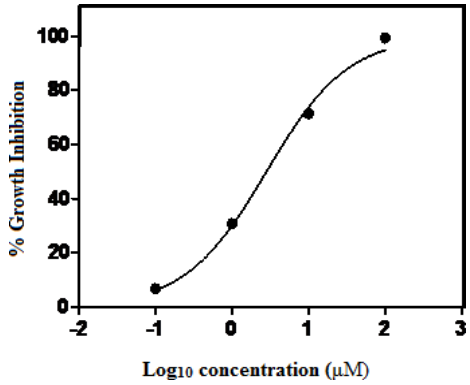
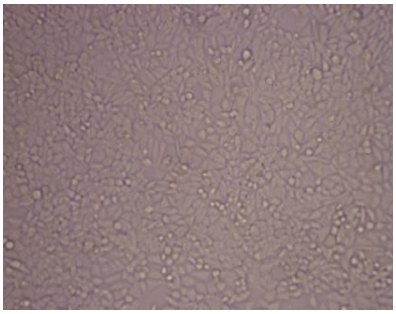
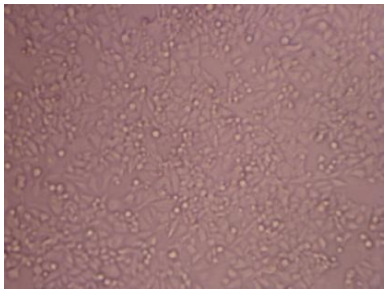


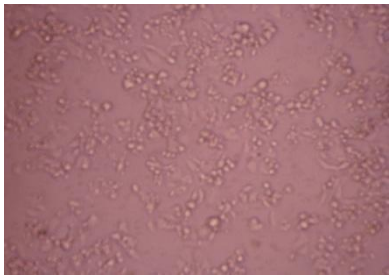
Fig. 2. Effect of % cell growth inhibition in different concentration (lM)



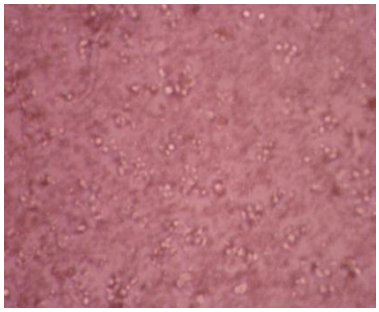
0.1µM



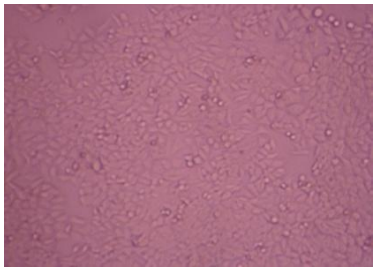
1µM



10 µM



100 µM



Control

Fig. 3. Images of cytotoxic activity of compound 5c in HeLa cells

4. STRUCTURE ACTIVITY RELATIONSHIP (SAR) STUDIES

For the structure activity relationship, heterocyclic indole motif derivatives (3a-5d) such as pyrazolo and isoxazolo groups with indole ring were synthesized and evaluated for anticancer activity. It was observed that compounds 5a-5d comprising the isoxazolo framework possessed excellent anticancer activity against the tested cell line, where the anticancer activity was found to be in the order (5c (15.24µM)> 5d (17.67 µM) > 5b (19.33 µM) > 5a (25.63 µM)). Such results suggested that the halogen substituents enhance the antitumor activity when compared to other substituent. Compounds 4a-4d comprising pyrazolo moiety displayed moderate anticancer activity. It was noted that the intermediates showed comparatively less cytotoxic activity than the cyclised derivatives Fig. 4.

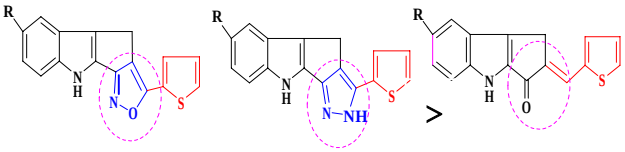


Fig. 4. The increasing order of efficiency of cytotoxic activities.

5. CONCLUSION

In conclusion, a new series of fluorine substituted pyrazolo- and isoxazolo- derivatives were chosen through target based drug discovery and synthesized, and characterized by IR, ^{1}H NMR and ^{13}C NMR spectroscopy. Molecular docking studies establish that these molecules exhibit significant molecular interactions with Protein kinase CK2 target protein. *In vitro* cytotoxicity study of the synthesized molecules against cervical cancer cells (HeLa), revealed that compound 5c exhibited IC_{50} value 15.24 µ. Compound 5c inhibited the growth of HeLa cells, showing a cell growth inhibition of 100%. The structure activity relationship of cytotoxic studies discovered that the chloro substituted isoxazolo-cyclopenta[*b*]indole display high activities than their counterparts.

REFERENCES

1. Humphrey, G.R. and J.T. Kuethe, (2006). Practical methodologies for the synthesis of indoles. *Chem. Rev*. **106**(7): 2875-2911.
2. Sharma, V., P. Kumar and D. Pathaka, (2010). Biological importance of the indole nucleus in recent years: A comprehensive review. *J. Heterocycl. Chem*. **47**: 491-502.
3. Kaushik, N.K., N. Kaushik, P. Attri, N. Kumar, C.H. Kim, A.K. Verma and E.H. Choi, (2013). Biomedical importance of indoles. *Molecules.* **18**(6): 6620-6662.

4. Patil, S.A., R. Patil and D.D. Miller, (2012). Indole molecules as inhibitors of tubulin polymerization: potential new anticancer agents. *Future Med. Chem.* **4**(16): 2085-2115.
5. El Sayed, M.T., N.A. Hamdy, D.A. Osman and K.M. Ahmed, (2015). Indoles as anti-cancer agents. *Adv. Mod. Oncol. Res.* **1**(1): 20-35.
6. Dhiman, S. and S.S.V. Ramasastry, (2015). Synthesis of polysubstituted cyclopenta[b]indoles via relay gold(I)/Brønsted acid catalysis. *Chem. Commun.* **51**: 557-560.
7. Gribble, G.W. (2000). Recent developments in indole ring synthesis-methodology and applications. *J. Chem. Soc. Perkins Trans.* 1045-1075.
8. Knolker, H.J. and K.R. Reddy, (2002). Isolation and synthesis of biologically active carbazole alkaloids. *Chem. Rev*. **102**: 4303-4427.
9. Kawasaki, T. and K. Higuchi, (2005). Simple indole alkaloids and those with a nonrearranged monoterpenoid unit. *Nat. Prod. Rep*. **22**: 761-793.
10. Bennasar, M.L., B. Vidal and J. Bosch, (1993). *J. Am. Chem. Soc.*, **115**: 5340.
11. Benoit, J., S. Routier, J.Y. Merour, P.Colson, C. Houssier and C. Bally, (2000). DNA but not topoisomerases is a target for a cytotoxic benzo[5,6]cyclohepta[b]indol-6-one derivative. *Antican. Res.* **20**: 3307-3314.
12. Honga, B.C., Y.F. Jianga, Y.L. Changb and S.J.S.J. Leeb, (2006). *J. Chin. Chem. Soc.* **53:** 647.
13. Churruca, F., M. Fousteris, Y. Ishikawa, M.V.W. Rekowski, C. Hounsou, T. Surrey and A. Giannis, (2010). A Novel approach to indoloditerpenes by nazarov photocyclization: synthesis and biological investigations of terpendole E analogues. *Org. Lett.* **12**: 2096-2099.
14. Saito, K., H. Sogou, T. Suga, H. Kusama and N. Iwasawa, (2011). *J. Am. Chem. Soc.* **133**: 689.
15. Chen, B., W. Fan, G. Chai and S. Ma, (2012). Electronic effect directed Au(I)-catalyzed cyclic C2-H bond functionalization of 3-allenylindoles. *Org. Lett.* **14** : 3616-3619.