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Synthesis of hetero annulated cyclopent[b]indoles: Exploration of *in vitro* cytotoxicity and molecular docking studies

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Abstract

A series of novel cyclopent[b]indole analogues that hold isoxazolo-, pyrido- templates were designed and synthesised in good yields. The *in vitro* cytotoxicity was concerned for all the newly synthesized compounds by MTT assay against HeLa (cervix adeno carcinoma) and MCF-7 (breast cancer). These synthesised compounds were further compared with the standard drug ellipticine, 5-Fluorouracil, Cisplatin and Methotrexate. The synthesised hetero annulated cyclopent[b]indole compounds were found to show better cytotoxic activity against HeLa and MCF-7 with primary structure activity relationship (SAR) studies. In order to identify with the nature of interactions of these molecules we carried out molecular docking studies using the protein kinase CK2 inhibitors. The docking results afforded some valuable information for the future design of more potent inhibitors.

Keywords

Isoxazolo-cyclopent[b]indole
Pyrido-cyclopent[b]indole
HeLa and MCF-7
CK2 inhibitors

1. Introduction

Indole derivatives condensed with different heterocycles are physiologically active compounds found in abundance in materials such as pharmaceuticals, alkaloids and potential therapeutic agents.\(^1\) The indole nucleus presents in a large number of naturally occurring as well
as biologically active molecules, which makes indole molecules of considerable contemporary interest and importance. Cyclopent[b]indole occurs in a large number of alkaloids, including the structurally complex tremorgenic mycotoxines such as paxilline, paspaline, lolitrems, janthitrems and yuehchukene (Figure 1). Among the alkaloids, those containing cyclopent[b]indole units were found to exhibit potential antimicrobial, anti-inflammatory, antioxidant, anti-implantation and tremorgenic activities. Though many numbers of reports were available on the synthesis of several functionalized carbazoles, the corresponding work on the synthesis and utilization of cyclopent[b]indole seems to be less recognized. Therefore, it is essential to pay more attention for the development of new method towards the synthesis of 1-oxo-1,2,3,8-tetrahydrocyclopent[b]indoles and employ them as synthon for the construction of many annulated heterocyclic systems possessing cyclopent[b]indole moiety.

A heterocyclic isoxazoles and pyridines are well known to be an important class of compounds with a wide range of biological activities like antimicrobial, anticancer etc. In earlier work from our lab, we have reported the synthesis of hetero annulated carbazoles. In view of the growing importance of cyclopent[b]indole alkaloids, it is contemplated to synthesise 1-oxo-1,2,3,8-tetrahydrocyclopent[b]indoles which can be used as an important precursor for the construction of a wide range of heterocyclo-fused compounds possessing significant pharmacological activities. The innovation directs for pharmaceutical explorations and further needs recognition of novel molecules that are able to interrelate with and modify a biological target. Protein kinases CK2 is a ubiquitous and represents a major therapeutic target of its contribution in numerous oncogenic signalling pathways and CK2 kinase inhibitors have established powerful clinical activity in pathologies in which the target kinase is dysregulated. Herein we report to achieve hetero annulated cyclopent[b]indoles, the precursor, 5-methyl-1-oxo-1,2,3,8-tetrahydrocyclopent[b] indole (1) was obtained by Fischer indole cyclization of the respective hydrazone, which inturn obtained by the Japp-Klingeman method of diazotized p-methylaniline derivative with 2-hydroxymethylenecyclo pentanone. The synthesis of 4,9-dihydro-3-(thiophen-2'-yl)-6-methylisooxazolo[2,3-a]cyclopent[b]indole (4) and 5,10-dihydro-2-ethoxy-4-(2'-aryl)-7-methylpyrido[2,3-a]cyclopent[b]indole-3-carbonitrile (5) starting from the easily accessible 5-methyl-1-oxo-1,2,3,8-tetrahydrocyclopent[b]indole (1) through the intermediate, 5-methyl-2-(2'-arylidine)-1-oxo-1,2,3,8-tetrahydrocyclopent[b]indole (3). We envisioned the earlier validation and furtherance of an improvement program intending to find a
new structure directs with potential cytotoxic activities, a new series of benzylidene-, isoxazolo-, and pyrido-cyclopent[b]indoles have been synthesized and screened for their in vitro cytotoxicity against HeLa (cervix adeno carcinoma) and MCF-7 (breast cancer). The goal of looking these hybrids is an attempt to attain an active antitumor agent with potentiated activity and selectivity toward cancerous cells. Moreover drug-likeness and molecular docking methodology were used to identify the structural features required for the antitumor properties of this new series.

2. Results and discussion

2.1. Chemistry

An equimolar mixture of 5-methyl-1-oxo-1,2,3,8-tetrahydrocyclopent[b]indole (2) and thiophene-2-carbaldehyde (3a) was treated with 25 mL of a 5% alcoholic potassium hydroxide solution and stirred at room temperature for 24 hrs to yield 5-methyl-2-(thiophen-2'-ylidine)-1-oxo-1,2,3,8-tetrahydrocyclopent[b]indole (4a) (Scheme 1). Its FT-IR (KBr, cm⁻¹) spectrum showed two characteristic peaks at 3187 cm⁻¹ and 1662 cm⁻¹ corresponding to NH and C=O stretching vibrations. Its ¹H-NMR (400 MHz) spectrum in DMSO-d₆ exhibited the following peaks. A three proton singlet appeared at δ 2.404 was due to CH₃ protons. A two proton singlet appeared at δ 3.862 was due to C₃-H aliphatic protons. A two proton multiplet between δ 7.186-7.241 was due to C₄- and C₆- protons. One proton doublet at δ 7.340 was due to C₇-H proton with J = 8.40 Hz. The three proton multiplet between δ 7.576-7.602 was due to C₃'-, C₄'- and C₅'- protons. A one proton triplet appeared at δ 7.866 was due to arylidine olefinic proton with J = 4.00 Hz. One proton singlet appeared at δ 11.747 was due to N-H proton. Its ¹³C NMR (100 MHz, DMSO-d₆) spectrum indicates the presence of 17 carbons. All details attest the structure of the compound as 5-methyl-2-(thiophen-2'-ylidine)-1-oxo-1,2,3,8-tetrahydrocyclopent[b]indole (4a). The generality (Scheme 1, Table 1) of the reaction was tested, other arylaldehyde derivatives (3) and 5-methyl-1-oxo-1,2,3,8-tetrahydrocyclopent[b]indole (2) with which gave the corresponding 5-methyl-2-(2'-arylidine)-1-oxo-1,2,3,8-tetrahydrocyclopent[b] indole derivatives (4) and their structures were verified by FT-IR, NMR spectroscopic methods and elemental analysis. Furthermore the structure of compound 4d was unambiguously confirmed by single crystal x-ray diffraction studies (Fig. 2).

The reaction of 5-methyl-2-(thiophen-2'-ylidine)-1-oxo-1,2,3,8-tetrahydrocyclopent[b] indole (4a) with hydroxylamine hydrochloride in pyridine yielded 4,9-dihydro-3-(thiophen-2'-
yl)-6-methylisoaxazo[2,3-a]cyclopent[b]indole (5a) (Scheme 1). Its FT-IR (KBr, cm\(^{-1}\)) spectrum showed two characteristic peaks at 3342 cm\(^{-1}\) and 1662 cm\(^{-1}\) corresponding to NH and C=N stretching vibrations. Its \(^1\)H-NMR (400 MHz, DMSO-\(d_6\)) spectrum in DMSO-\(d_6\) exhibited the following peaks. A three proton singlet appeared at \(\delta\) 2.390 was due to CH\(_3\) protons. A two proton appeared as a singlet at \(\delta\) 3.790 was due to C\(_4\)-2H protons. A one proton doublet appeared at \(\delta\) 7.041 was due to C\(_8\)- proton with \(J = 8.40\) Hz. One proton multiplet between \(\delta\) 7.160-7.180 was due to C\(_7\)- proton. The three proton multiplet between \(\delta\) 7.358-7.411 was due to C\(_3\)'-, C\(_4\)'- and C\(_5\)'- protons. One proton singlet appeared at \(\delta\) 7.472 was due to C\(_5\)- proton. One proton singlet appeared at \(\delta\) 11.265 was due to N-H proton. Its \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) spectrum indicates the presence of 17 carbons. The elemental analysis of the compound 5a is in good agreement with the proposed molecular formula C\(_{17}\)H\(_{12}\)N\(_2\)OS which also gave considerable support to the structure of 5a. The generality (Scheme 1, Table 1) of the reaction was investigated on other 5-methyl-2-(2'-arylidine)-1-oxo-1,2,3,8-tetrahydrocyclopent[b]indole derivatives (4b-h) with hydroxylamine hydrochloride in pyridine, which gave the corresponding 4,9-dihydro-3-(2'-aryl)-6-methylisoaxazo[2,3-a]cyclopent[b]indoles (5b-h) and their structures were verified by FT-IR, \(^1\)H & \(^{13}\)C NMR spectroscopic methods and elemental analysis.

The plausible mechanism is represented in Scheme 2. Hydroxylamine liberated \textit{in situ} from hydroxylamine hydrochloride in the presence of pyridine undergoes 1,4 Michael-type addition to 4 in order to give the intermediate I which on protrophic shift yields the enolic intermediate II, stabilized through the keto form III. Then the intermediate III with the excess hydroxylamine yields the intermediate IV, which subsequently loses water molecule followed by cyclisation and deamination to yield the final product 5.

The key intermediate 5-methyl-2-(thiophen-2'-ylidine)-1-oxo-1,2,3,8-tetrahydro cyclopent[b]indole (4a) in dry ethanol (20 mL) was added to an ice-cooled solution of 1.00 g of sodium hydride (degassed with petroleum ether) in dry benzene (10 mL). To this mixture malononitrile was added and the reaction mixture was refluxed on water bath for 5 hrs, to afford a single product (6a) (Scheme 1). Its FT-IR (KBr, cm\(^{-1}\)) spectrum showed two characteristic peaks at 3191 cm\(^{-1}\) and 2219 cm\(^{-1}\) corresponding to NH and CN stretching vibrations respectively. Its \(^1\)H-NMR (400 MHz, DMSO-\(d_6\)) spectrum in DMSO-\(d_6\) exhibited the following peaks. A three proton triplet at \(\delta\) 1.222 was due to OCH\(_2\)CH\(_3\) protons with \(J = 7.20\) Hz. A three proton singlet at \(\delta\) 2.404 was due to CH\(_3\) protons. A two proton singlet appeared at \(\delta\) 3.863 was
due to C₅-2H aliphatic protons. A quartet appeared at δ 4.583 was due to OCH₂CH₃ protons with $J = 7.20$ Hz. A multiplet appeared in the region between δ 7.187-7.361 was due to C₃'-, C₄'- and C₅'- protons. A multiplet appeared in the region between δ 7.576-7.598 was due to C₆- and C₈- protons. One proton doublet appeared at δ 7.853 was due to C₉- proton with $J = 7.20$ Hz. One proton singlet appeared at δ 11.747 was due to N-H proton. Its $^{13}$C NMR (100 MHz, DMSO-$d_6$) spectrum indicates the presence of 22 carbons. All details attest the structure of the compound as 5,10-dihydro-2-ethoxy-4-(thiophen-2'-yl)-7-methylpyrido[2,3-a]cyclopent[b]indole-3-carbonitrile (6a). The generality (Scheme 1, Table 1) of the reaction was tested on 5-methyl-2-(2'-arylidine)-1-oxo-1,2,3,8-tetrahydrocyclopent[b]indole derivatives (4b-h) with malononitrile, which gave the corresponding 5,10-dihydro-2-ethoxy-4-(2'-aryl)-7-methylpyrido[2,3-a]cyclopent[b]indole-3-carbonitriles (6b-h) verified by FT-IR, $^1$H & $^{13}$C NMR spectroscopic methods and elemental analysis.

The plausible mechanism (Scheme 3) for the formation of final product (6) was shown below. In the first step carbanion intermediate was generated from malononitrile under the basic condition, on Michael addition with $α,β$-unsaturated carbonyl intermediate (8) yields the dinitrile intermediate (VII). Then one of the symmetric carbonitrile (CN) carbon is attacked by the ethoxide ion generated in situ from NaOEt to give the imino-amino intermediates (VIII & IX). The amino intermediate (IX) on cyclodehydration (X) followed by aerial oxidation cum aromatization affords the final product (6).

2.2. In vitro cytotoxicity

For evaluating the cytotoxicity of the newly synthesized compounds, two different cancer cell lines were utilized: HeLa (cervix adeno carcinoma), MCF-7 (breast cancer). The viability of the cells were assessed by standard 3-(4,5-dimethylthiazol-2'-yl)-2,5-diphenyltetrazolium bromide (MTT)$^{13}$ assay method in vitro. The in vitro cytotoxicity of 5-methyl-2-(2'-arylidine)-1-oxo-1,2,3,8-tetrahydrocyclopent[b]indole (4), 4,9-dihydro-3-aryl-6-methylisooxazolo[2,3-a] cyclopent[b]indole (5) and 5,10-dihydro-2-ethoxy-4-aryl-7-methylpyrido[2,3-a]cyclopent[b] indole-3-carbonitrile (6) was performed by the MTT assay method against a pair of selected human breast cancer cell line (MCF-7) and HeLa (cervix adeno carcinoma) by means of a colorimetric assay (MTT assay) that measures mitochondrial dehydrogenase activity as an indication of cell viability after an exposure period of 24 hrs in the respective concentration...
range of 5-100 µM. Upon increasing the concentration of the compound, the results of MTT assay revealed that better growth inhibitory effect in a dose dependent manner against HeLa and MCF-7 cells with IC\textsubscript{50} values generally in the micromolar concentrations. Ellipticine, a clinically used antitumor agent was also evaluated for positive control. The anticancer potency of these compounds was indicated by IC\textsubscript{50} (the concentration that causes a 50% reduction of the cell growth). The obtained results were summarized in Table 2.

As described in Table 2, some of the synthesized compounds illustrated moderate to potent anticancer activities against both cells. The achieved results indicated that the compounds showed selective cytotoxicity against HeLa. Among all, compound 6e and 6f showed nearly equipotent activity with ellipticine against HeLa. Among all, the most outstanding compound is 6e which displayed stronger cytotoxic activity against HeLa with lowest IC\textsubscript{50} value 22.16 µM and compound 6f showed almost equipotent to 6e, which was 25.70 µM. Moreover, the next most promising compound 5f, which depicted stronger cytotoxic activity against HeLa with IC\textsubscript{50} value 24.9 µM which was almost equipotent with that of the compounds 6e and 6f. Similar cytotoxic analysis carried out using standard drugs, shown that the IC\textsubscript{50} value of Cisplatin, a major chemotherapeutic agent was 30µM in case of HeLa cell line. This result reveals that, the newly synthesized compounds like 5f, 6e and 6f have significantly lower IC\textsubscript{50} values when compared to the conventional chemotherapeutic drug Cisplatin.

Compounds 5f, 6e and 6f were displayed significant IC\textsubscript{50} values of 28.46, 19.23 and 32.45 µM nearly equipotent activity with ellipticine against MCF-7, respectively. Furthermore compounds 5e and 6g showed good cytotoxic activity against HeLa with IC\textsubscript{50} values 45.9 and 38.98 µM, respectively and the same compounds displayed IC\textsubscript{50} values 39.66 and 47.19 µM against MCF 7. The intermediates 4 displayed significant cytotoxic activity against both HeLa and MCF 7 cell lines. In general it was found that all synthesized compounds showed selective cytotoxic activity against HeLa cells. Further comparing with the IC\textsubscript{50} values of other drugs like 5-Fluorouracil and methotrexate under similar conditions show that, the IC\textsubscript{50} values for the synthesized compounds are significantly lower than their conventional counter parts, suggesting better cytotoxic efficacy of these compounds.

The present study investigated the effect of several substituents and from the results of cytotoxicity of the synthesized hetero annulated cyclopent[b]indoles; the following primarily structure-activity relationships can be derived:
Among the newly synthesized compounds, compounds 6e and 6f displayed stronger cytotoxic activity against HeLa. This might be due to the presence of pyrido moiety and the cyano group which enhanced the cytotoxic activity. The stronger electron withdrawing ability of the cyano group, which played an important role in anticancer effect. Subsequently, compounds 5f and 5e also showed better cytotoxic activity with IC50 values < 46 μM against HeLa which was due to the presence of isoxazolo moiety which enhanced the cytotoxic activity. It was observed that the intermediates showed least cytotoxic activity than the cyclised products.

2.3. Molecular docking studies

Molecular docking is vital role to find an unfailing and more specific depiction of the biologically active molecules at the atomic level and also, to afford a new insight that is able to use to endeavour novel therapeutic agents. In an effort to get deeper insight into the inhibitory potency of the newly synthesized hetero annulated cyclopent[b]indoles to better understand the mechanism of cytotoxicity and structure–activity relationships (SAR), we performed docking studies using Autodock4.0 tool. All the synthesized compounds (4a-h, 5a-h, 6a-h), the standard ellipticine and 5-Fluorouracil were docked by human protein kinase CK2 receptor (PDB ID: 3OWJ) and ligand was prepared for docking analysis with MGLTools 1.5.6. In the molecular docking studies of synthesized molecules displayed good binding energy towards the target protein ranging from -6.11 kcal/mol to -9.70 kcal/mol.

We envisaged the binding orientation, the standards, Ellipticine forms a hydrogen bond by interaction of the NH group of the carbazole moiety with the nitrogen atom of ASP175 (2.198Å) with a binding energy is -7.58 kcal/mol (Fig. 3), 5-Fluorouracil forms three hydrogen bonding interaction of oxygen atoms of TYR50:HN (2.049Å), LYS68:HZ1 (1.952Å), respectively and hydrogen atom of NH group in 5-fluorouracil with SER51:OG (2.071Å) with binding energy is -4.00 kcal/mol (Fig. 4).

5-Methyl-2-(aryl-2'-ylidine)-1-oxo-1,2,3,8-tetrahydrocyclopent[b]indole (4) compounds showed the docking scores ranging from -9.30 kcal/mol to -8.20 kcal/mol. Among them, the compound (4e) displayed the best lowest binding energy (-8.71 kcal/mol), ligand efficiency (-0.35 kcal/mol) and it forms four hydrogen bonds by the interactions of LYS68:HZ3 (1.964Å), ASP175:HN (1.620Å) with phenyl ring two OCH3 group oxygen atoms and the other
interactions of 1-oxo-1,2,3,8-tetrahydrocyclopent[b]indole ring NH group and C=O group with VAL116:O and VAL116:HN with respective bond residues are 1.986Å & 1.715Å. It was shown in Fig.5. Among all the compounds (4 a-h), in comparison with standard drugs ellipticine and 5-Fluorouracil, nearly all the synthesised compounds demonstrated better binding energy.

4,9-Dihydro-3-(aryl-2'-yl)-6-methyl-isoaxazolo[2,3-a]cyclopent[b]indole (5) compounds showed the docking scores ranging from -9.56 kcal/mol to -7.17 kcal/mol. Among them, the compound (5e) revealed the best lowest binding energy (-9.56 kcal/mol), ligand efficiency (-0.37 kcal/mol) and it forms two hydrogen bonds by the interactions of VAL116:O with 1-oxo-1,2,3,8-tetrahydrocyclopent[b]indole ring NH group hydrogen atom and the other interactions of phenyl ring 4-OCH₃ group oxygen atom with LYS68:HZ3 with respective bond residues are 2.006Å & 1.943Å. It was shown in Fig.6. Among all the compounds (5 a-h), in comparison with standard drugs ellipticine and 5-Fluorouracil, almost all the synthesised compounds displayed enhanced binding energy.

5,10-Dihydro-2-ethoxy-4-(aryl-2'-yl)-7-methyl-pyrido[2,3-a]cyclopent[b]indole-3-carbonitrile (6) compounds showed the docking scores ranging from -8.67 kcal/mol to -6.11 kcal/mol. Among them, the compound (6h) displayed the best lowest binding energy (-6.11 kcal/mol), ligand efficiency (-0.24 kcal/mol) and it forms two hydrogen bonds by the interactions of LYS158:HZ3 with OC₂H₅ group oxygen atom and the other interactions of pyridine ring nitrogen atom with TYR196:HH with respective bond residues are 1.900Å & 2.220Å. It was shown in Fig.7. Among all the compounds (6 a-h), in comparison with standard drugs ellipticine and 5-Fluorouracil, with expect from 6d, more or less all other synthesised compounds showed moderate binding energy.

The proposed binding mode can assist us to superior understand the nature of interactions of this (4a-h, 5a-h, 6a-h) synthesised compounds. In comparison with the standard ellipticine and 5-Fluorouracil, with the exception of compound 6d almost all the other synthesised compounds appeared potent binding energy. The compounds 4d, 4e, 5e and 5f exhibited good binding interaction compared to all other compounds and standard drugs. In general it was found that all the synthesised compounds (4a-h, 5a-h, 6a-h) have potent binding interactions compared to standard drugs (ellipticine and 5-Fluorouracil). The results attained from in vitro cytotoxic studies and molecular docking studies indicated that the presence of hetero annulated substituents of cyclopent[b]indole may enhances the efficacy towards the biological activities.
The docking studies of human protein kinase CK2 receptor with synthetic compounds (4a-h, 5a-h, 6a-h, Ellipticine and 5-Flourouracil) were analyzed for the lowest binding energy, lowest ligand efficiency and more than numbers of hydrogen bonds were formed for the best interaction. The length of bond distance between the receptor and ligand below 3Å° is the best. In our results, all the synthetic compounds were docked with human protein kinase CK2 receptor and it showed proper interactions but except 6d compound. In this data was obtained by docking analysis is available in the following Table 3.

3. Conclusion

The condensation reaction of 5-methyl-1-oxo-1,2,3,8-tetrahydro cyclopent[b]indole (2) with aryl / heteroaryl aldehydes were optimised to obtain cleaner 5-methyl-2-arylidine-1-oxo-1,2,3,8-tetrahydrocyclopent[b]indoles (4) in good yields. The resulting cyclopent[b]indoles were found to have α,β-unsaturated carbonyl system. This was taken advantage and these α,β-unsaturated carbonyl cyclopent[b]indoles derivatives were condensed with 1,2-nucleophiles like hydroxylamine hydrochloride and as well as malononitrile to yield 4,9-dihydro-3-aryl/heteroaryl-6-methylisooxazolo[2,3-a]cyclopent[b]indoles (5) and 5,10-dihydro-2-ethoxy-4-aryl/heteroaryl-7-methylpyrido[2,3-a]cyclopent[b]indole-3-carbonitriles (6), respectively. Among the newly synthesized compounds, compounds 6e and 6f displayed stronger cytotoxic activity against HeLa. This might be due to the presence of pyrido moiety and the cyano group which enhanced the cytotoxic activity. Subsequently, compounds 5e and 5f also showed better cytotoxic activity with IC50 values < 46 μM against HeLa which was due to the presence of isoxazolo moiety which enhanced the cytotoxic activity. The results achieved from in vitro cytotoxic studies and molecular docking studies indicated that the presence of hetero annulated substituents of cyclopent[b]indole may enhance the efficacy towards the biological activities.

Experimental Section

General:

Melting points (M.p.) were determined on a Mettler FP 51 apparatus (Mettler Instruments, Switzerland) and are uncorrected. They are expressed in degree centigrade (°C). A Nicolet Avatar Model FT-IR spectrophotometer was used to record the IR spectra (4000–400 cm⁻¹). ¹H, ¹³C and 2D NMR spectra were recorded on Bruker AV 400 (400 MHz (¹H) and 100
MHz (\textsuperscript{13}C)) spectrometers using tetramethylsilane (TMS) as an internal reference. The chemical shifts are expressed in parts per million (ppm). Coupling constants (\textit{J}) are reported in hertz (Hz). The terms s, d, t, dd refer to singlet, doublet, triplet and doublet of doublet, respectively, b s refers to a broad singlet. Microanalyses were performed on a Vario EL III model CHNS analyzer (Vario, Germany) at the Department of Chemistry, Bharathiar University. When known compounds had to be prepared according to literature procedures, pertinent references are given. The purity of the products was tested by TLC with plates coated with silica gel-G using petroleum ether and ethyl acetate in the ratio of 1:1 as developing solvents. Columns packed with activated silica gel (60-120 mesh) were used to purify the product.

\textbf{Synthesis}

\textbf{General procedure for the synthesis of 5-methyl-2-(aryl-2'-ylidine)-1-oxo-1,2,3,8-tetrahydrocyclopent[b]indole (4)}

An equimolar mixture of 5-methyl-1-oxo-1,2,3,8-tetrahydrocyclopent[b]indole (2, 0.005 mol) and arylaldehyde (3, 0.005 mol) was reacted with 25 mL of a 5\% alcoholic potassium hydroxide solution and stirred at room temperature for 24 hrs. The product precipitated as a yellow crystalline solid, was filtered off and washed with 50\% ethanol. A further crop of condensation product was obtained on neutralization with 1:1HCl and further diluted with water. Thus the combined product was recrystallised from ethylacetate to yield the respective 5-methyl-2-(aryl-2'-ylidine)-1-oxo-1,2,3,8-tetrahydrocyclopent[b]indole (4).

\textbf{5-Methyl-2-(thiophen-2'-ylidine)-1-oxo-1,2,3,8-tetrahydrocyclopent[b]indole (4a)}

Yellow solid; M.p. 204-206 °C Yield = 93\%; FT-IR (KBr, cm\textsuperscript{-1}) \textit{v}_{\text{max}}: 3187, 1662; \textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d}_6) (ppm) \textdelta: 2.404 (s, 3H, C\textsubscript{5}-CH\textsubscript{3}), 3.862 (s, 2H, C\textsubscript{3}-H\textsubscript{2}), 7.186-7.241 (m, 2H, C\textsubscript{4}-, C\textsubscript{6}-H), 7.340 (d, 1H, C\textsubscript{7}-H, \textit{J} = 8.40 Hz), 7.576-7.602 (m, 3H, C\textsubscript{3}'-, C\textsubscript{4}'-, C\textsubscript{5}'-H), 7.866 (t, 1H, C\textsubscript{2}-CH, \textit{J} = 4.00 Hz), 11.747 (s, 1H, NH); \textsuperscript{13}C NMR (100 MHz, DMSO-\textit{d}_6) (ppm) \textdelta: 21.041, 26.120, 113.358, 120.852, 122.767, 123.584, 128.305, 128.741, 129.118, 130.925, 132.980, 137.856, 137.945, 138.913, 140.889, 141.933, 181.267; Anal. Calcd. for: C\textsubscript{17}H\textsubscript{13}NOS: C, 73.09; H, 4.69; N, 5.01; S, 11.48. Found: C, 73.19; H, 4.60; N, 5.09; S, 11.39\%.
General procedure for the synthesis of 4,9-dihydro-3-(aryl-2'-yl)-6-methyl-isooxazolo[2,3-a]cyclopent[b]indole (5):

The respective 5-methyl-2-(aryl-2'-ylidene)-1-oxo-1,2,3,8-tetrahydrocyclopent[b]indole (4, 0.001 mol) was refluxed with hydroxylamine hydrochloride (0.014 mol) in dry pyridine (5 mL) at 130 °C for 8 hrs. The reaction was monitored by TLC. After completion of the reaction, the crude mixture was poured into ice-cold water and neutralized with 5N HCl, the resulting semi-solid that separated was extracted with ethyl acetate. The combined organic layers were dried over anhydrous sodium sulphate. It was then purified by column chromatography over silica gel using petroleum ether:ethyl acetate (98:2) to yield the corresponding, 4,9-dihydro-3-(aryl-2'-yl)-6-methylisooxazolo[2,3-a]cyclopent[b]indole (5). The product obtained was recrystallised from ethanol.

4,9-Dihydro-3-(thiophen-2'-yl)-6-methylisooxazolo[2,3-a]cyclopent[b]indole (5a)

Yellow solid; M.p. 180-182 °C Yield = 63%; FT-IR (KBr, cm⁻¹) νmax: 3342, 1662; ¹H NMR (400 MHz, DMSO-d₆) (ppm) δ: 2.390 (s, 3H, C₆-CH₃), 3.790 (s, 2H, C₄-H₂), 7.041 (d, 1H, C₈-H, J = 8.40 Hz), 7.160-7.180 (m, 1H, C₇-H), 7.358-7.411 (m, 3H, C₃'-, C₄'-, C₅'-H), 7.472 (s, 1H, C₅-H), 11.265 (s, 1H, NH); ¹³C NMR (100 MHz, DMSO-d₆) (ppm) δ: 21.06, 28.89, 112.34, 112.88, 115.87, 119.36, 122.41, 123.33, 125.87, 127.65, 128.13, 129.09, 130.21, 136.13, 140.02, 140.27, 147.07, 148.55; Anal. Calcd. for: C₁₇H₁₂N₂O₅S: C, 69.84; H, 4.14; N, 9.58; S, 10.97. Found: C, 69.78; H, 4.20; N, 9.51; S, 10.90%.

General procedure for the synthesis of 5,10-dihydro-2-ethoxy-4-(aryl-2'-yl)-7-methylpyrido[2,3-a]cyclopent[b]indole-3-carbonitrile (6):

A solution of respective 5-methyl-2-(aryl-2'-ylidene)-1-oxo-1,2,3,8-tetrahydrocyclopent[b]indole (4, 0.001 mol) in dry ethanol (20 mL) was added to an ice-cooled solution of 1.00 g of sodium hydride (degreased with petroleum ether) in dry benzene (10 mL). To this mixture malononitrile (0.005 mol) was added and the reaction mixture was refluxed on a water bath for 5 hrs. The reaction was monitored by TLC indicated the formation of product. After the completion of the reaction the mixture was poured into ice-water and neutralized with 1:1 HCl. The brown solid separated was then filtered and dried. It was then purified by column
chromatography over silica gel using petroleum ether:ethyl acetate (95:5) as eluent to yield 5,10-dihydro-2-ethoxy-4-(aryl-2'-yl)-7-methylpyrido[2,3-a]cyclopent[b]indole-3-carbonitrile (6).

5,10-Dihydro-2-ethoxy-4-(thiophen-2'-yl)-7-methylpyrido[2,3-a]cyclopent[b]indole-3-carbonitrile (6a)

Yellow solid; M.p. 208-210 ºC Yield = 69%; FT-IR (KBr, cm⁻¹) νmax: 3191, 2219, 1662; ¹H NMR (400 MHz, DMSO-d₆) (ppm) δ: 1.222 (s, 3H, C₂-OCH₂CH₃), 2.404 (s, 3H, C₇-CH₃), 3.863 (s, 2H, C₅-H₂), 4.583 (q, 2H, C₂-OCH₂CH₃, J = 7.20 Hz), 7.187-7.361 (m, 3H, C₃'-, C₄'-, C₅'-H), 7.576-7.598 (m, 2H, C₆', C₈'-H), 7.853 (d, 1H, C₉-H, J = 7.20 Hz), 11.747 (s, 1H, NH); ¹³C NMR (100 MHz, DMSO-d₆) (ppm) δ: 20.979, 21.027, 26.049, 26.106, 28.923, 113.277, 113.410, 120.749, 120.850, 122.699, 123.474, 123.578, 128.219, 128.299, 128.733, 129.105, 130.835, 130.922, 132.869, 132.973, 137.863; Anal. Calcd. for: C₂₂H₁₇N₃OS: C, 71.14; H, 4.61; N, 11.31; S, 8.63 Found: C, 71.04; H, 4.69; N, 11.23; S, 8.72%.

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Supplementary data

CIF file for compounds 4d have been deposited with the Cambridge Crystallographic Data Centre as CCDC number 1047624. Copy of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK. [Fax: +44 (0) 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk.

Supporting information

Copies of NMR spectral data of all the compounds, docking structures of the synthesised compounds and table of crystal data associated with this article will be available as supporting information.
References


