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Original Research Article

Synthesis and docking of new indole derivatives with potential anticancer activities

Jalal H Mohamed[1], Ahmed A Ali[2], Asmaa A Mohamed[3], Samir M Awad[4], Youssef H Ali[5], Mohamed F El-Shehry[6], Marwa A Khodair[4]*

[1]Faculty of Pharmacy, University of Karbala, Karbala, Iraq, [2]Department of Pharmacy, Al-Mansour University College, Baghdad, Iraq, [3]College of Pharmacy, Al-Zahraa University for Women, Karbala, Iraq, [4]Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Capital University (Helwan formerly), Helwan, Cairo, Egypt, [5]Faculty of Pharmacy, Capital University (Helwan formerly), Helwan, Cairo, Egypt, [6]Pesticides Chemistry Department, National Research Centre, Dokky, Giza, Egypt

For correspondence:* ***Email:* *marwa.khodair@pharm.capu.edu.eg;* ***Tel (Fax):*** *00202-2554-1601 (11795)*

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Abstract

Purpose: *To assess the anticancer profile of the combination of indole and thiouracil scaffold nuclei.*
Method: *A new series of 2-indolyl thiouracil derivatives was synthesized via Fischer indole condensation of phenylhydrazine with various acetyl thiouracil analogues in the presence of sulfuric acid as a condensing agent. The newly prepared indolyl thiouracil analogues were investigated for their in vitro antineoplastic activity using A-2780, MCF-7, HT-29, and HEPG-2 cancerous cell lines of ovaries, breast, colon, and liver, respectively.*
Results: *They presented variable responses; compounds* ***3b*** *and* ***3e*** *were the most potent among all novel analogues, as they displayed wide-spectrum activity against all cell lines compared to 5-fluorouracil. Derivatives* ***3b and 3e*** *presented higher potency against HEPG-2 liver carcinoma than 5-fluorouracil (IC_{50} = 0.82, 0.00, 5 µg/mL), respectively. Compound* ***3e*** *demonstrated the best binding affinity in the thymidylate synthase binding pocket.*
Conclusion: *Combinations of indole and thiouracil cores provide effective cancer inhibitory activity. Molecular modeling on Thymidylate Synthase rationalized these data, establishing derivative* ***3e*** *as a practical starting point for future targeted mechanistic work.*

Keywords: *Anticancer activities, Docking, Fischer, Indole derivatives*

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INTRODUCTION

Cancer is regarded as one of the most devastating diseases in humanity. Despite the development of numerous anticancer drugs, the majority of these drugs also affect normal cells [1]. As a result, researchers worldwide are working to create novel compounds that do not negatively affect healthy cells. The chemical structure of most anticancer drugs includes a heterocyclic ring (60 %) [2]. The indole nucleus has attracted scientists' attention to the significance of using it in the field of disease treatment, as it represents a major link due to its multiple biological activities, such as antimicrobial [3], anti-inflammatory [4], anticancer [5,6], antioxidant [7], antidiabetic [8], antimalarial [9], and anticholinesterase [10].

Many natural compounds are bioactive and contain an indole moiety in their structures [11,12]. Also, serotonin is a neurotransmitter

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containing an indole nucleus. Highlighting their antitumor activity, 3-substituted indoles were found to inhibit colon carcinoma (HT-29), MCF-7 breast tumor [13], and ovarian adenocarcinoma (SK-OV-3). They act by various mechanisms, such as aromatase inhibition, suppressing NFKB/PI3/Akt/mTOR pathway [14], and suppression of c-Src kinase activity [5]. Several synthetic approaches were proposed for the synthesis of indole derivatives. Despite its age, the Fischer method is still extensively used since it is simple and affords indoles in a plentiful yield. This method involves firstly obtaining phenylhydrazones through the condensation of methyl ketone derivatives with phenylhydrazine; then the produced phenylhydrazones are cyclo-condensed with a condensing agent such as zinc chloride, PPA, sulfuric acid, or boron trifluoride [15,16].

Recent literature has focused on developing novel antitumor agents from pyrimidine derivatives like thiopyrimidinone. Acetylated thiouracils are one of the thiopyrimidinone derivatives of 2-thiouracil, which is a well-known blocking unit for preparing numerous chemotherapeutic agents. Generally, thiouracil derivatives have displayed potential cancer-inhibitory activities *via* various methods, such as histone deacetylase (HDAC) inhibition and triggering apoptosis. Due to the biological significance of thiouracil compounds [17], the synthesis of these compounds has concentrated on two major approaches over the last five decades. To mimic the 5-FU molecule (model anticancer pyrimidine derivatives), the first approach concentrated on 5-substituted-2-thiouracils synthesis. For instance, derivatives of 6-arylthiouracil-5-carbonitriles were synthesized and demonstrated strong inhibitory effects on leukemia and lung cancer cell lines [18]. Additionally, strong 2-thiouracil-5-sulfonamides were created and demonstrated encouraging efficacy against various cancer cell lines. The second approach concentrated on enhancing the activity of 2-thiouracil by including other heterocyclic nuclei, such as pyrazole, pyrrole, imidazole, indole, and thiophene. This study aimed to incorporate thiouracil and indole nuclei *via* synthesizing novel indolyl thiouracil derivatives and assess their efficacy as promising anticancer agents.

EXPERIMENTAL

Chemistry

A Boetius® melting point microscope was employed to measure each uncorrected melting point in a capillary tube. The Cairo University microanalytical unit conducted the microanalyses. Using KBr disks, IR charts were captured as KBr pellets using a Beckmann® infra spectrometer (PU9712). Tetramethylsilane served as an internal standard while determining ^{1}HNMR spectra on a Joel-EX® 270 MHz spectrometer. A Finigan-SSQ 7000® Mass spectrometer was used to record mass spectra (MS) at 70 eV. Spots were inspected under a UV lamp, and the reaction's progress was monitored and verified *via* TLC employing 75% chloroform/ 25% methanol.

5-acetyl-6-aryl-2-thioxo-2,3-dihydropyrimidine-4(1H)-one: 2a-e

They were prepared as reported in the literature [19].

6-Aryl-5-(1H-indol-2-yl)-2-thioxo-2,3-dihydropyrimidin-4-(1H)-one 3a-e:

A solution of acetyl thiouracil **2a-e** (1 mmol) and phenylhydrazine (1 mmol) in 50 mL of ethyl alcohol was refluxed for 4 - 6 h. The obtained hydrazones were separated, filtered and dried under suction; the solids were poured separately into 25 mL conc. H_2SO_4. The mixture was heated while stirring for 30 min. Thin-layer chromatography was used to monitor the reaction until completion; then the mixture was cooled and poured into an ice/water mixture. After filtering and drying, the solid product was recrystallized from dimethylformamide (DMF) and water.

Anticancer activity

Cells

Ovarian, colon, breast, and hepatic cancer cells, namely A-2780, HT-29, MCF-7, and HEPG-2, were utilized in our initial research. 50 mL of RPMI-1640 medium with 5% fetal calf serum, bicarbonate, and glutamine was used to cultivate stock cultures in T-75 flasks. Every 48 h, the medium was replaced. In NKT buffer (5.4 mM potassium chloride, 137 mM sodium chloride, and 10 mM Tris; pH 7.4), cells were dispersed using 3 mM 1,2-cyclohexanediaminetetraacetic acid and 0.25 % trypsin. The cultures were grown at densities of 1,000 – 200,000 cells per well in microtiter plates with 0.2 mL of growth medium.

Dyes

Sigma Chemical Co. supplied the dyes. In order to ascertain whether each stained cell is particularly intense at basic, acidic, or neutral pH,

preliminary investigations were carried out using a variety of dyes. Sulforhodamine-B was diluted in acetic acid (1%) to label the cells and extract them from unbuffered Tris-based cells. A DU-70 spectrophotometer was used to measure the dye's maximum absorption (Beckman Instruments, Inc., Fullerton, CA).

Cell fixation

Detachment and loss were frequently the result of washing cultures in buffer before fixing to eliminate serum protein. Each culture was treated with TCA prior to washing in order to prevent this possible problem. In order to achieve a final 10 % TCA, cells adhered to the plastic substratum were fixed by carefully adding cold 50 % TCA (50 µL) to the growth medium in each well. After an hour of incubation at 4 °C, the cultures were rinsed five times with water to eliminate metabolites with small molecular weight, TCA, and growth medium. The serum plates were then allowed to air-dry before being stored until needed. Optical densities were recorded in a cell-free growth medium-incubated well. After the cells settled out, cold 80 % TCA (50 µL) was carefully added to the growth medium. The cultures were refrigerated at 4 °C for 1 h after being undisturbed for 5 min. As long as the cells were in contact with the plastic substratum when the fixative was applied, this process resulted in the attachment of single-cell suspensions to it.

RESULTS

Chemical results

Chemical results supported by spectra for (3a-e) are shown in Figure 1.

Procedure

Compounds **3a-e** were dissolved separately in 20 % sodium carbonate. The aqueous solutions were then evaporated to obtain the solid sodium salts. 5-Fluorouracil was employed as a reference standard. Before being treated with the test drug, cells were placed in 96-well plates (10^4 cells/well) for 24 hours to enable cell adhesion to the plate wall. Various doses of tested compounds (0, 2.5, 5, and 10 µg/mL) were applied to the cells. The compounds and cells were then incubated together in 5% CO_2 for 48 h at 37 ˚C; after that, cells were fixed, rinsed, and stained with sulforhodamine B. Excess stain was rinsed with acetic acid and the attached stain was recovered using EDTA buffer. An ELISA reader was used to measure color intensity, and finally, the IC_{50} was calculated. The IC_{50} value of 5-Fluorouracil, used as a reference drug against HEPG-2 cells, was obtained from the National Cancer Institute.

Molecular Docking

The docking study on thymidylate synthase (TS; PDB: 1HVY) was performed using AutoDock Vina software. AutoDock tools were used to create the required PDBQT files in the appropriate format. PyRx 0.8 was utilized to complete the screening of compounds. RMSD calculations were established using DockRMSD [20]. Finally, for visualization of docking poses, the free BIOVIA Discovery Studio 2021 visualizer was used.

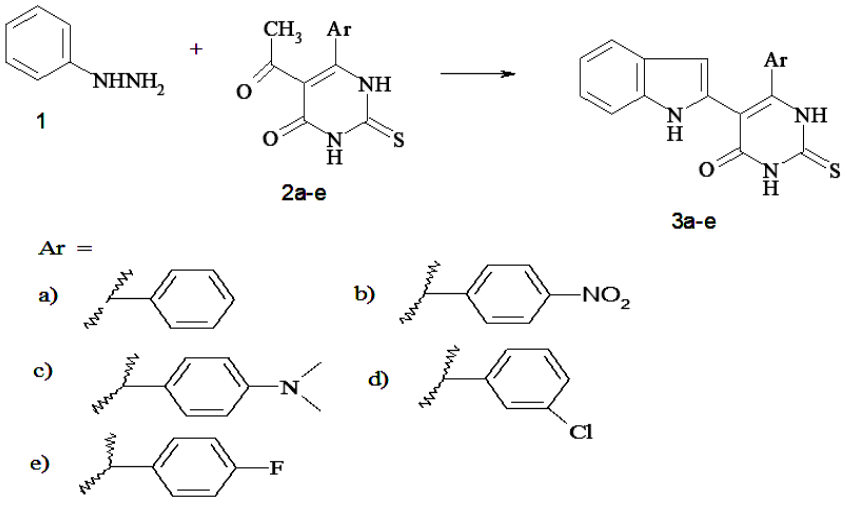

Figure 1: Schematic diagram for preparing compounds **3a-e**

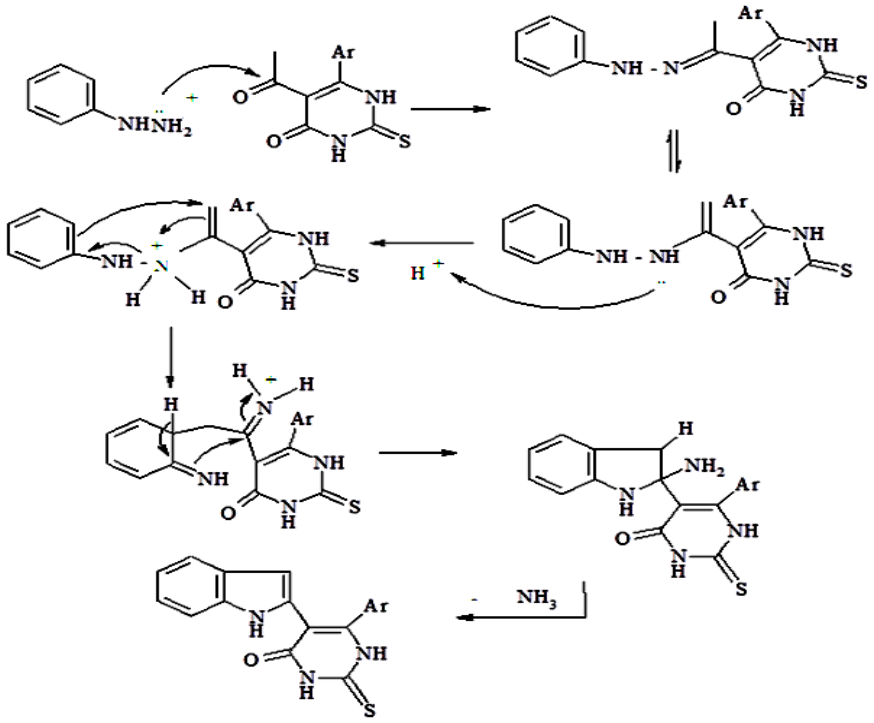

Figure 2: Schematic diagram showing the mechanism for preparing **3a-e**

Biological activities

Table 1: IC_{50} (µg/mL) of the novel compounds 3a-e and 5-Fluorouracil on cancerous cell lines A-2780, MCF-7, HT-29, and HEPG-2 cells

Compound	A-2780	MCF-7	HT-29	HEPG-2
3a	3.28	5.26	2.25	5.05
3b	0.62	0.87	0.98	0.82
3c	5.68	4.67	5.37	5.09
3d	3.72	5.98	4.70	00.00
3e	0.72	0.69	0.75	00.00
5-Fluorouracil	0.51	0.67	0.61	5.00

Molecular modeling results

Table 2: Molecular modeling results of compounds 3b, 3e and 5-fluorouracil in TS active site (PDB: 1HVY)

Compound	Binding interaction (Ligand-receptor)	Docking score (S) Kcal/mol	RMSD (Aº)
3b	(pyrimidine-Leu221; Pi-alkyl) (pyrrole-Leu221; Pi-alkyl) (phenyl-Ile108; Pi-alkyl) (pyrrole-Ile108; Pi-alkyl)	-8.5	1.814
3e	(C=S-Lys77; H-b) (N-Leu221; H-b) (pyrimidine-Leu221; Pi-alkyl) (phenyl-Ile108; Pi-alkyl) (phenyl-Ile108; Pi-alkyl) (pyrrole-Ile108; Pi-alkyl) (pyrimidine-Phe225; Pi-alkyl) (phenyl-Lys107; Pi-amide)	-8.8	1.172
5-fluorouracil	(carbonyl-His 256; H-b) (carbonyl-Asp218; H-b) (carbonyl-Arg175; H-b) (carbonyl-Arg50; H-b) (H-Ser216; donor-donor)	-5.3	1.623

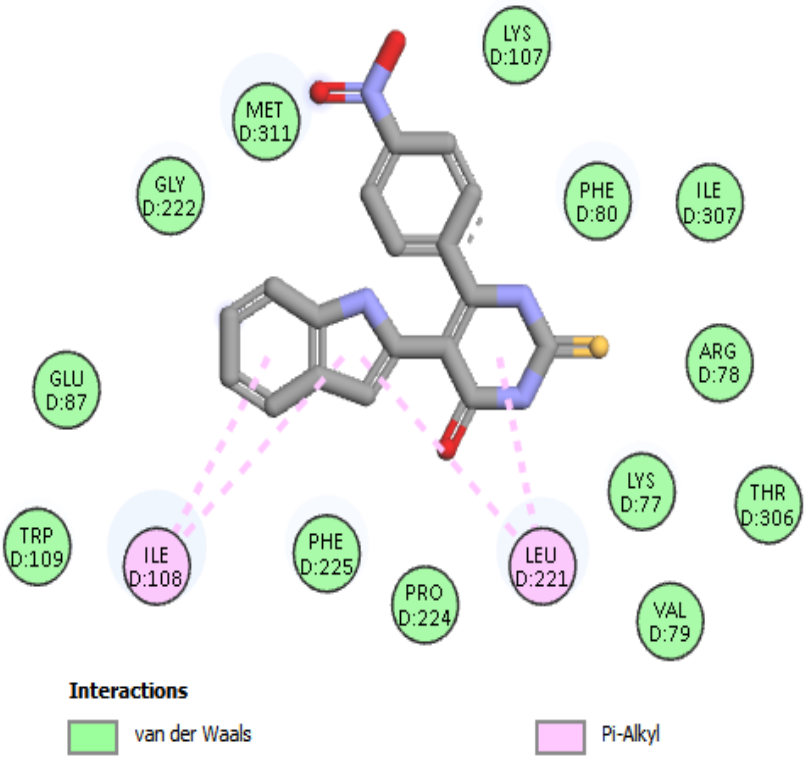

Figure 3: 2D view of compound **3b** in the TS (PDB: 1HVY**)** active site

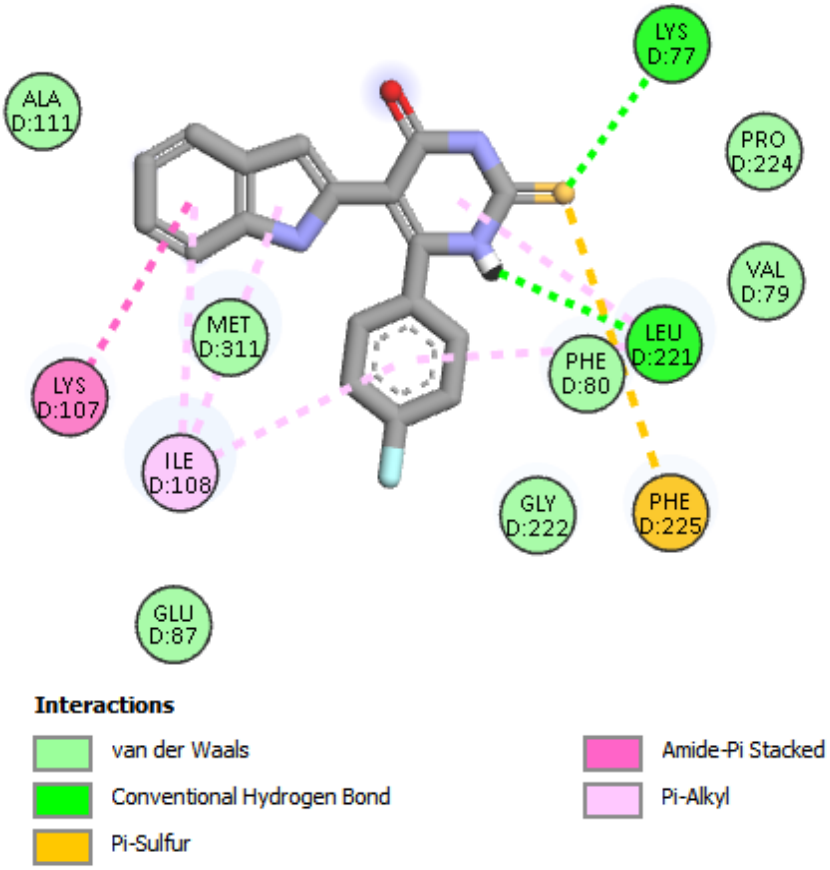

Figure 4: 2D view of compound **3e** in the TS (PDB: 1HVY**)** active site

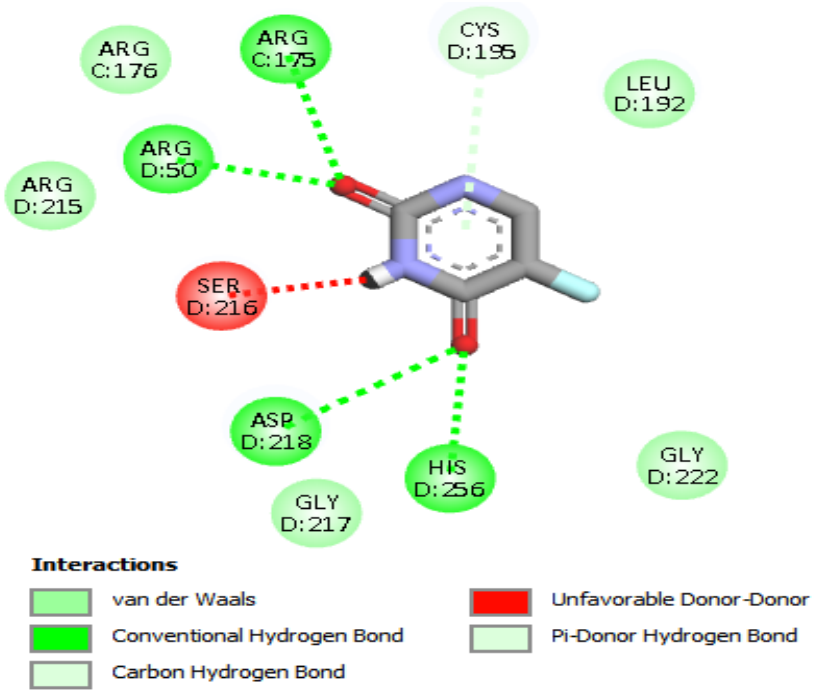

Figure 5: 2D view of **5-fluorouracil** in the TS (PDB: 1HVY**)** active site

DISCUSSION

Fischer method for indole synthesis is a popular historical method. The use of different methyl ketones has been investigated, especially substituted acetophenones. The use of methyl ketones of heterocyclic nuclei has not been reported. Thus, five methyl ketones of substituted 2-thiouracil were cyclo-condensed with phenylhydrazine to obtain all target compounds 3a-e. The reasonable mechanism of Fischer indole synthesis involves three steps. Firstly, phenyl hydrazone is tautomerized to phenyl hydrazine. Then, a carbon–carbon double bond is formed and finally cyclization with ammonia elimination and aromatization of the pyrrole indole ring occurs.

For structural verification, physical data in addition to spectral data were obtained (attached in the supplementary file). The most distinctive difference between the starting material and the 2-indolyl products was shown in ^{1}HNMR spectra, where the methyl group of acetophenones at 2-3 ppm and the NH_2 singlet signal of hydrazine disappeared and the aromatic protons increased. Also, the IR spectrum showed the disappearance of aliphatic CHs at 2900 cm^{-1}. The mass spectra demonstrated molecular ion peaks at m/z values matching the compounds' molecular weights. These changes in spectral data proved the Fischer indole formation of 3a-e.

All newly synthesized compounds (3a-e) were evaluated as anticancer agents using four cell lines named A-2780, MCF-7, HT-29, and HEPG-2 for ovarian, breast, colon, and liver carcinoma, respectively. As presented in Table 1, the compounds displayed variable responses. In detail, compounds 3b and 3e exhibited promising, comparable activity relative to 5-Fluorouracil against colon, breast and ovarian cancer. On the other hand, 5-FU exhibited pronounced intrinsic resistance towards HEPG-2 (IC_{50} = 5 µg/mL); this resistance was reported to be due to dysregulated dihydropyrimidine dehydrogenase (DPD) enzymatic inactivation or altered target expression [21, 22]. Derivatives 3b, 3d, and 3e, which have an indole moiety incorporated with thiouracil, bypassed this limitation by more than fivefold. Such incorporation of indole with thiopyrimidine may affect DNA synthesis through TS inhibition (structure-activity correlation is discussed in detail in the docking section).

The most active two compounds 3b and 3e with 5-fluorouracil were docked onto the thymidylate synthase (TS) pocket and the result revealed that compound 3b stacked in the pocket *via* four hydrophobic interactions with the two key amino acids Leu221 and Ile108, while the sulfur atom in compound 3e formed hydrogen bonding with Lys77, also a hydrogen bond with Leu 221 was made by the nitrogen atom; moreover, the same compound made six hydrophobic interactions with Leu221, Ile108, Phe225, and Lys107 compared with 5-fluorouracil, which formed four hydrogen bonds with His256, Asp218, Arg175,

and Arg50 and an unfavorable interaction with Ser216. The structural features of 3e, the most potent one, revealed that the presence of indole with fluorophenyl rings increased the lipophilicity of the compound via introducing an electron-withdrawing group, provided a more rigid structure, and allowed its stabilization in the pocket. Furthermore, replacing the oxygen atom in 5-FU with a sulfur atom in thiouracil increased its binding through strong conventional hydrogen bonding. These effective bindings, especially compound 3e, rationalized its highest potency.

CONCLUSION

Overall, a series of novel 2-indolyl thiouracils 3a-e was efficiently accomplished via Fischer indole condensation. The *in vitro* anticancer screening assay against ovarian, breast, colon, and hepatic tumor cells demonstrated the more potent activity of the *para*-fluoro and *para*-nitro substituents 3e and 3b than that of 5-fluorouracil, especially towards HEPG-2 cells. Moreover, molecular docking simulations on TS supported these results. Finally, further in vitro/in vivo investigation targeting TS specifically for 3e is required to validate its therapeutic applicability.

DECLARATIONS

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Use of research reporting tools

Not applicable. No clinical trial or animal study reporting checklist such as CONSORT or ARRIVE applies to this study.

Ethical approval

Not applicable.

Use of Artificial intelligence/Large language models

The authors confirm that AI-based tools were used solely for language editing and manuscript polishing purposes (e.g., grammar, phrasing, and clarity). No AI tools were used to generate content, interpret data, or influence study design, results, or conclusions. All intellectual contributions, study analyses, and interpretations reported in the manuscript are the original work of the authors.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Marwa Abd El-Fattah Khodair and Samir M. Awad conceived and designed the study; Mohamed F. El-Shehry, Jalal H. Mohamed, Ahmed A. Ali, Youssef H. Ali, and Asmaa A. Mohamed collected the data, and Marwa Abd El-Fattah Khodair and Samir M. Awad analyzed the data, Marwa Abd El-Fattah Khodair, Jalal H. Mohamed, Ahmed A. Ali, Youssef H. Ali, and Samir M. Awad wrote the manuscript. Marwa Abd El-Fattah Khodair and Samir M. Awad reviewed and edited the final version of the manuscript. All authors read and approved the manuscript for publication.

REFERENCES

1. *Kaur R, Bhardwaj A, Gupta S. Cancer treatment therapies: traditional to modern approaches to combat cancers. Mol Biol Rep 2023; 50(11): 9663-9676. https://doi.org/10.1007/s11033-023-08809-3*
2. *Lang DK, Kaur R, Arora R, Saini B, Arora S. Nitrogen-containing heterocycles as anticancer agents: an overview. Anticancer Agents Med Chem 2020; 20(18): 2150-2168. https://doi.org/10.2174/1871520620666620070521491*
3. *Qin HL, Liu J, Fang WY, Ravindar L, Rakesh KP. Indole-based derivatives as potential antibacterial agents against methicillin-resistant Staphylococcus aureus (MRSA). Eur J Med Chem 2020; 194: 112245. https://doi.org/10.1016/j.ejmech.2020.112245*
4. *Lin HF, Jiang YC, Chen ZW, Zheng LL. Design, synthesis, and anti-inflammatory activity of indole-2-formamide benzimidazole[2,1-b]thiazole derivatives. RSC Adv 2024; 14(23): 16349-16357. https://doi.org/10.1039/d4ra00557k*
5. *Olgen S, Kaleli SNB, Karaca BT, Demirel UU, Bristow HK. Synthesis and Anticancer Activity of Novel Indole*

Derivatives as Dual EGFR/SRC Kinase Inhibitors. Curr Med Chem 2024; 31(24): 3798-3817. https://doi.org/10.2174/0929867330666230626143911

6. *Xu X, Ma H, Wang F, Yan J. Indole-thiophene conjugate inhibits proliferation of human cervical cancer cell lines through DNA damage. Trop J Pharm Res 2019; 18(8): 1705-1710. https://doi.org/10.4314/tjpr.v18i8.20*
7. *Jasiewicz B, Kozanecka-Okupnik W, Przygodzki M, Warżajtis B, Rychlewska U, Pospieszny T, et al. Synthesis, antioxidant and cytoprotective activity evaluation of C-3 substituted indole derivatives. Sci Rep 2021; 11: 15425. https://doi.org/10.1038/s41598-021-94904-z*
8. *Ritu, Sharma P, Gupta GD, Asati V. Design, synthesis and antidiabetic study of triazole clubbed indole derivatives as α-glucosidase inhibitors. Bioorg Chem 2023; 139: 106750. https://doi.org/10.1016/j.bioorg.2023.106750*
9. *Li JY, Sun XF, Li JJ, Yu F, Zhang Y, Huang XJ, et al. The antimalarial activity of indole alkaloids and hybrids. Arch Pharm (Weinheim) 2020; 353(11): e2000131. https://doi.org/10.1002/ardp.202000131*
10. *Raza AR, Rubab SL, Ashfaq M, Altaf Y, Tahir MN, Rehman MFU, et al. Evaluation of Antimicrobial and Anticholinesterase Potential of Indole Derivatives and Unexpectedly Synthesized Novel Benzodiazine: Characterization, DFT and Hirshfeld Charge Analysis. Molecules 2023; 28(13): 5024. https://doi.org/10.3390/molecules28135024*
11. *Gomes G, Scortecci K. Auxin and its role in plant development: structure, signaling, regulation and response mechanisms. Plant Biol 2021; 23(6): 894-904. https://doi.org/10.1111/plb.13303*
12. *Yi WF, Chen DZ, Ding X, Li XN, Li SL, Di YT, et al. Cytotoxic indole alkaloids from Melodinus khasianus and Melodinus tenuicaudatus. Fitoterapia 2018; 128: 162-168. https://doi.org/10.1016/j.fitote.2018.05.015*
13. *Kaur B, Venugopal S, Verma A, Sahu SK, Wadhwa P, Kumar D, et al. Recent developments in the synthesis and anticancer activity of indole and its derivatives. Curr Org Synth 2023; 20(4): 376-394. https://doi.org/10.2174/1570179419666220509215722*
14. *Devi N, Kaur K, Biharee A, Jaitak V. Recent Development in Indole Derivatives as Anticancer Agent: A Mechanistic Approach. Anticancer Agents Med Chem 2021; 21(14): 1802-1824. https://doi.org/10.2174/1871520621999210104192644*
15. *Murakami Y. Peculiarity of methoxy group-substituted phenyl hydrazones in Fischer indole synthesis. Proc Jpn Acad Ser B Phys Biol Sci 2012; 88(1): 1-17. https://doi.org/10.2183/pjab.88.1*
16. *Arora G, Sharma S, Joshi S. Synthesis of substituted 2-phenyl-1H-indoles and their fungicidal activity. Asian J Chem. 2017;29(8):1651-1654. doi:10.14233/ajchem.2017.20423.*
17. *Jawad M, Ijam A, Mohammed JH, Mohammed F, Elrashedy A. Synergistic anti- Helicobacter pylori effect of licorice extract and metronidazole-2-thiouracil derivatives: In vitro and in silico considerations. Trop J Pharm Res 2025; 24(10): 1253-1260. https://doi.org/10.4314/tjpr.v24i10.5*
18. *Taher AT, Abou-Seri SM. Synthesis and bioactivity evaluation of new 6-aryl-5-cyano thiouracils as potential antimicrobial and anticancer agents. Molecules 2012; 17(8): 9868-9886. https://doi.org/10.3390/molecules17089868.*
19. *Abdel Reheim MAM, Abdel Hafiz IS, Elian MA. A simple and convenient synthesis of isolated fused heterocycles based on 6-phenyl-2-thioxo-2,3-dihydropyrimidin-4(5H)-one and 5-acetyl-6-phenyl-2-thioxo-2,3-dihydropyrimidin-4(5H)-one. Heterocycles 2016; 92(8): 1397-1414. https://triggered.stanford.clockss.org/ServeContent?doi=10.3987%2Fcom-16-13467*
20. *Bell EW, Zhang Y. DockRMSD: An Open-Source Tool for Atom Mapping and RMSD Calculation of Symmetric Molecules through Graph Isomorphism. J Cheminform 2019; 11(1): 1-9. https://doi.org/10.1186/s13321-019-0362-7*
21. *Zhang N, Yin Y, Xu SJ, Chen WS. 5-Fluorouracil: mechanisms of resistance and reversal strategies. Molecules 2008; 13(8): 1551-1569. https://doi.org/10.3390/molecules13081551*
22. *Longley DB, Harkin DP, Johnston PG. 5-Fluorouracil: mechanisms of action and clinical strategies. Nat Rev Cancer 2003; 3(5): 330-338. https://doi.org/10.1038/nrc1074*