

Synthesis and evaluation of novel thiophene-dhpm's designed having anti-breast cancer potential**Dhirajkumar Nikam^{a*}, Anurekha Jain^a, Shraddha Vetale^b, Ashwini Bhangne^b and Shweta Jadhav^b**^aPh.D. Scholar, Department of Pharmaceutical Chemistry, Oriental University, Indore, Madhyapradesh, India 453555^bDepartment of Pharmaceutical Chemistry, Bharati Vidyapeeth College of Pharmacy, Navi Mumbai, Maharashtra, India 400614**CHRONICLE***Article history:*

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The global rise of life-threatening diseases, particularly breast cancer, has emerged as a grave public health challenge. Recognizing the distinct structural demands of anti-breast cancer targets, we have synthesized Thiophene-DHPMs analogs by scaffold hopping approach to exhibit versatility having the ability to target proteins targets of cancer and their interactions contingent upon the various substitutions on them. We have harnessed MCR to synthesize novel Thiophene-DHPMs (**4a-4d**) derivatives introducing an efficient Biginelli protocol approach. All derivatives are characterized through techniques including FTIR and ¹H NMR and evaluated by *in vitro* SRB assay method on MCF-7 cell line, compared against positive control ADR. Molecular docking studies against kinesin spindle protein Eg5 (IQOB) revealed superior binding interactions and docking scores (> 8 Kcal) compared to the prototype Eg5 inhibitor Monastrol. Compound **4a** binds via Hydrogen bond interaction to target Eg5 with ALA-133, PRO-137, TYR-211 *In vitro* evaluation of results indicates that compound **4a** found to be moderately active (GI₅₀ = -4.41) compared to positive control ADR (GI₅₀ = -7.76) against MCF-7 cells. Compound **4a** demonstrates significant activity due to the presence of R=C₂H₅, X= S, and thiophene ring.

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1. Introduction

In 2020, it was known that there are incidences of roughly 20million cancer occurring worldwide; nearly half of them lead to death. Almost 1/10 (2.3 million) were detected are of breast cancer.¹ Unfortunately, breast cancer it is most prevalent and second leading specifically in female than male.²⁻³

Abnormalities in genes BRCA1, BRCA2, genetic mutations which often by exposure to estrogens can lead to breast cancer. More specifically, in females significant changes due to an assortment of hormones (estrogens, growth hormone, insulin-like growth factor-1, progesterone, and prolactin) that cause development of female breast cancer.⁴ Despite of available chemotherapeutic medicines, surgeries, radiations, and immunological therapies which cause apoptosis of breast cancer cells, these cells can get away from the treatments and defy cell death, which makes them even more aggressive and untreatable.⁵ Moreover, organic molecules leads pharmacokinetic and dynamic effects on animal models and affecting metabolism by diversified pathways.⁵⁴⁻⁵⁷ Advanced techniques used to link conjugates of heterocyclic drugs in micro porous or nonporous forms certainly aid bioactivities.^{58, 59, 60, 62}

DHPMs being significant heterocycles of therapeutic potential that are synthesized by the Biginelli MCR by condensation of aldehydes, ethyl acetoacetate, and thiourea.⁶ Owing to number of therapeutic uses pyrimidine serve as the most crucial heterocyclic moiety in medicinal chemistry. Moreover, DNA, RNA, and nucleic acid contain backbones thiamine, uracil, and cytosine possess pyrimidine base is one reason their activity is anticipated.⁷ DHPMs have significant

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uses as biological modulators in medicinal chemistry and possess great pharmacological potential with anti-inflammatory,⁸ anticancer,⁹⁻¹² antihypertensive,¹³ antitubercular,¹⁴ antiviral,¹⁵ antimicrobial,¹⁶⁻²¹ antifungal,²²⁻²⁴ antibacterial²⁵ and antitumor²⁶⁻²⁷, properties. Similarly, thiophene nucleus has played a paramount role in development of many therapeutic agents owing to its importance in drug designing, development and creating multiple libraries of molecules with ease to chemist.²⁸⁻³⁰ Notably, it possesses diverse bioactivities such as anticancer,³¹⁻³⁵ antimicrobial,³⁶⁻³⁹ anti-inflammatory,⁴⁰ antidepressant,⁴¹ and anticonvulsant,⁴² insecticidal⁶³ which make thiophene a consequential scaffold in lead designing and development.⁶¹

A notorious molecule Monastrol of DHPM class has inspired researchers due to its antimetabolic activity by causing mitotic arrest of mammalian cells by binding to Eg5 protein allosterically.⁴³⁻⁴⁹ Similarly, thiophene ring containing selective drug Raloxifene is well known estrogens receptor modulator works by preventing proliferation in breast cancer tumours (ER positive) in post-menopausal women.⁵⁰ Inspired by presence of DHPM ring in Monastrol and thiophene ring in Raloxifene, we have made an attempt to design our scaffold by hopping of these two important heterocyclic rings having paramount therapeutic potential in order to attain anti-breast cancer potential of our designed derivatives. As part of lab work herein, we report synthesis of novel Thiophene-DHPMs (**4a-4d**) evaluated against MCF-7 cell line (**Fig. 1**).

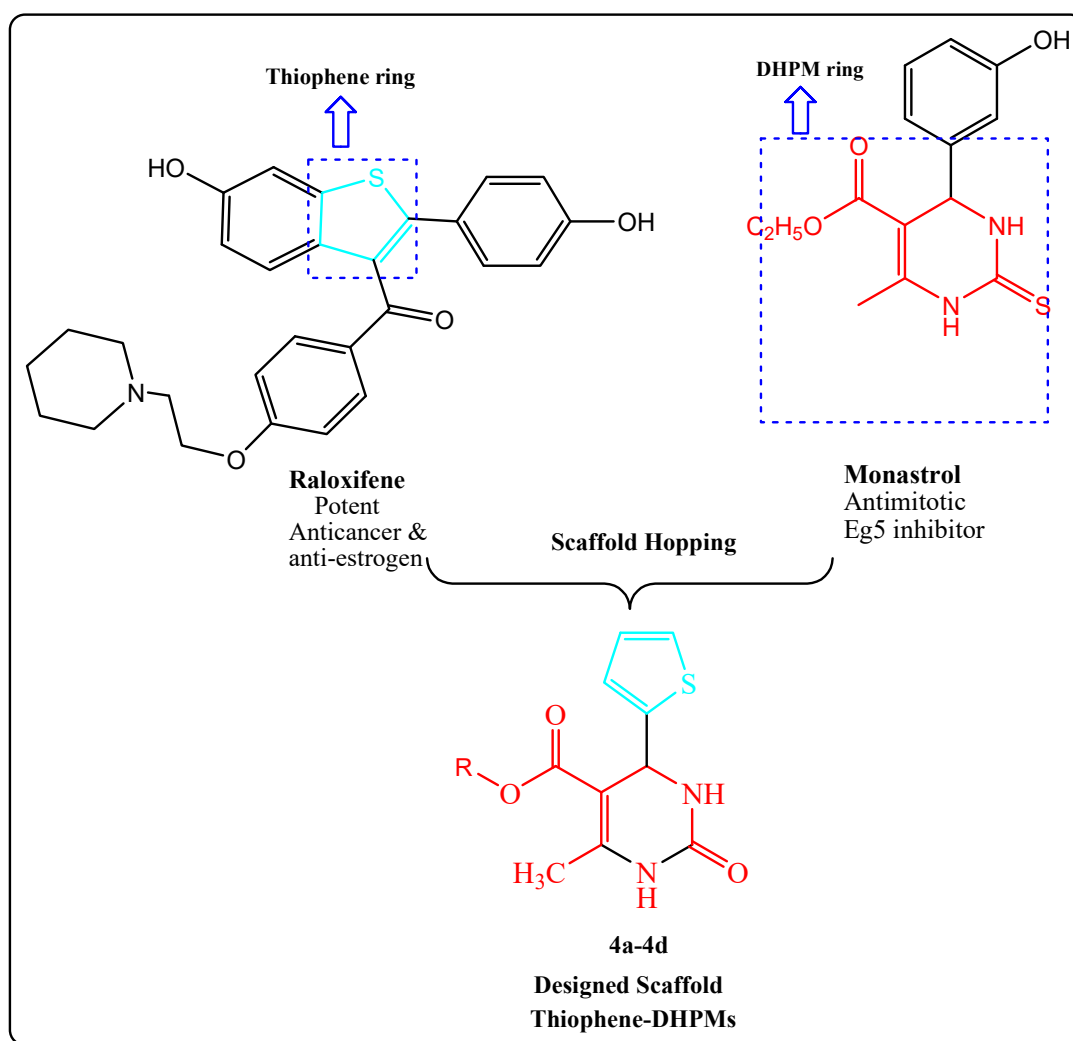


Fig. 1. Designing of Thiophene-DHPMs Scaffold

2. Results and Discussion

2.1 Chemistry

Thiophene-DHPM derivatives (**4a-4d**) are synthesized as per procedure shown in (**Fig. 2**). For synthesis of desired derivative's multicomponent reaction approach was adopted as per Biginelli protocol. The one pot synthesis is carried out using thiophene aldehyde, methyl or ethyl acetoacetate, urea or thiourea in one pot for (4-8hr) (**Table 1**). The reaction is monitored by periodic TLC. All purified compounds were characterized by FTIR, ¹H- NMR. All compounds were

characterized by Infrared spectroscopy. The characteristic band for NH was found between the range of 3100-3600cm⁻¹. In FT-IR spectroscopy, the characteristic peak for C=O str. was found in a range of 1400-1700cm⁻¹. In FTIR, the characteristic band of C-N str. was found within range 1330-1266 cm⁻¹, C=S str. (compound **4a** & **4c**) band was found in the range of 1025-1225 cm⁻¹. carbonyl group C=O (Compound **4b** & **4d**) str. was found in the range of 1600-1585 cm⁻¹ & 1500-1400 cm⁻¹. The ¹H NMR of aromatic protons were seen in the range of 7-9 ppm. The pyrimidine ring, N-H proton, is seen in the range of 9-10 ppm. Aliphatic protons were seen in the range of 2-3 ppm. Electronegative substituents such as sulphur attached to the aromatic ring reduce the electron density around the proton resulting in strong de-shielding effect. The greater the electronegativity of the substituent, the more it Shields the proton adjacent to it, and hence the greater the chemical shift of this proton.

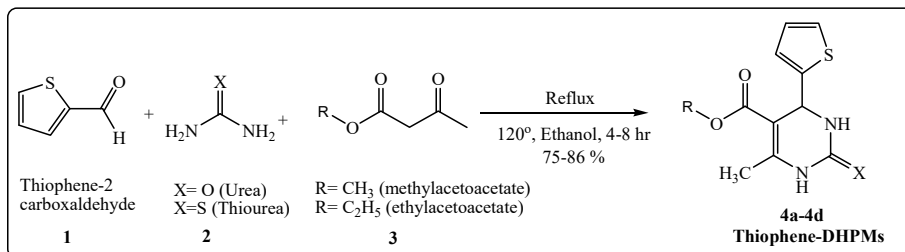


Fig. 2. Synthetic scheme of Thiophene-DHPMs

Table 1. Series of Thiophene-DHPMs (4a-4d)

Compound	X	-R	Yield (%)	M. P. (°C)
4a	S	-Et	75	218
4b	O	-Et	86	210
4c	S	-Me	80	224
4d	O	-Me	84	214

2.2 Prediction of physicochemical, pharmacokinetic, and ADMET properties:

We employed Swiss ADME software to assess the ADMET properties of our developed molecules (4a-4d), Molecular weights ranged from 252.9 to 282.38 g/mol, with log p values within the range of 2.07 to 2.61. TPSA values ranged from 95.67 to 110.69, indicating acceptability. Predictions suggested moderate to poor solubility across all compounds, with a consistent bioavailability score of 0.55 and following Lipinski rule of five (Table 2).

Table 2. Pharmacokinetic data of compounds (4a-4d)

Code	MW ^a	HBA ^b	HBD ^c	TPSA ^d	LogP ^e	ESOL ^f Class	GIA ^g	Bioavailability score	P-gp ^h substrate	BBB ⁱ permeability
4a	282.38	2	2	110.69	2.61	Soluble	High	0.55	No	No
4b	266.32	3	2	95.67	2.19	Soluble	High	0.55	No	No
4c	268.36	2	2	110.69	2.44	Soluble	High	0.55	No	No
4d	252.29	3	2	95.67	2.07	Very Soluble	High	0.55	No	No

Note – a: Molecular weight; b: Hydrogen bond acceptor; c: Hydrogen bond donors; d: Topological surface area; e: Octanol-water partition coefficient; f: Solubility class; g: Gastrointestinal absorption; h: P- glycoprotein; i: Blood-Brain Barrier.

Compounds (**4a-4d**), categorized by the BOILED-Egg model, demonstrate favorable gastrointestinal absorption in the white region. They are non-substrates for P-glycoprotein. The bioavailability radar plot underscores their optimal oral bioavailability for effective absorption and distribution in the pink area (Fig. 3).

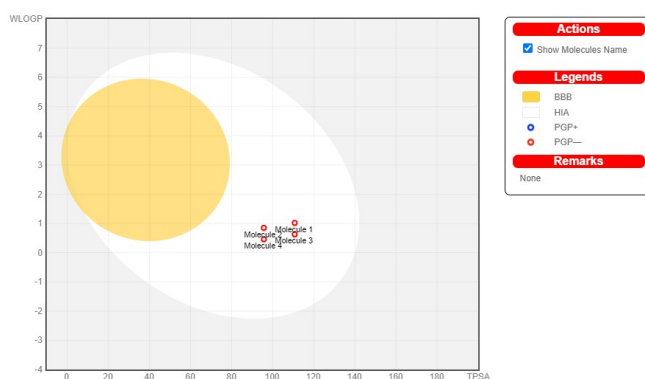


Fig. 3. BOILED-Egg graphical representation of the synthesized compounds

2.3 Molecular docking studies:

Molecular docking studies showed good interactions of all compounds with target Eg5. The details of the binding energy, amino acid residues, and hydrogen bonds involved in each derivative's interaction with Eg5. A higher negative value of binding energy signifies a more stable interaction between the designed molecule and the target enzyme (**Table 3**).

Table 3. Analysis of binding interaction and docking scores for designed compounds with the Eg5 kinesin spindle protein (1Q0B)

Compound Code	Docking Score (Kcal/mol)	Estimated inhibition constant (Ki)	Hydrogen Bond Interactions	Hydrophobic Interactions
4a	-6.7	10.7 uM	PRO-137, TYR-211	ALA-133, PRO-137, TYR-211
4b	-6.5	15.8 uM	PRO-137	ALA-133, TYR-211
4c	-6.2	28.2 uM	PRO-137	ALA-133, PRO-137
4d	-6.0	34.8 uM	GLU-116	ARG-119, PRO-137
Monastrol	-7.0	6.4 uM	GLU-116, GLU-118, TYR-211	ALA-133, Pro-137

The designed compounds exhibit binding energies ranging from -6.08 to -6.78 kcal/mol, while the co-crystallized ligand has a binding energy of -6.99 kcal/mol. Compound 4a shows good binding affinity to Eg5, with a low binding energy of -6.78 kcal/mol. Notably, it interacts strongly with PRO-137, TYR-211, and ALA-133 amino acids. In comparison, standard Monastrol displays a lower binding energy of -7.08 kcal/mol and interacts with GLU-116, GLU-118, TYR-211, ALA-133, and Pro-137 amino acids (**Fig. 4**).

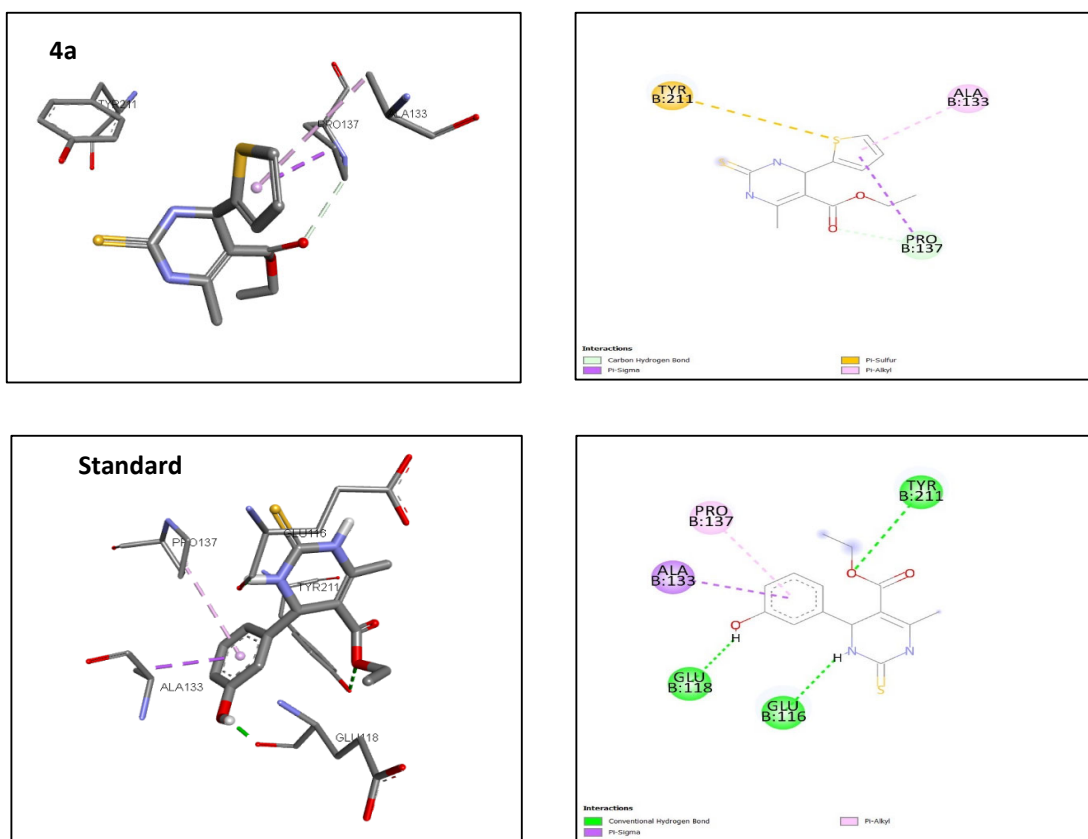


Fig. 4 Binding interactions of 4a and Standard Monastrol with Eg5 protein

2.4 Biological evaluations

2.4.1 Results of *in vitro* anti-breast anticancer activity

Compounds (**4a-4d**) were screened for anti-breast cancer potential on MCF-7 cells, by SRB assay; ADR is used as a positive control (**Fig. 5**, **Table 4**). Values obtained are the means of three independent experiments at four different concentrations in micromolar. Amongst all 4a had shown good growth inhibition at concentration 10^{-5} M ($GI_{50} = -4.41$). The presence of thiophene nucleus, ethyl group at position R and presence of sulphur ($X=S$) in 4a contributes more to its

activity. However, **4a** is moderately active compared to the most potent anticancer drug ADR ($GI_{50} = -7.76$). However, compounds **4b**, **4c**, and **4d** are found to be less active may be due to presence of (X=O) or R= CH₃ groups.

Table 4. Anti-breast cancer activity of compounds (4a-4d)

Entry	Concentration				GI_{50}	-log GI_{50} (MCF-7)
	10 ⁻⁷ M	10 ⁻⁶ M	10 ⁻⁵ M	10 ⁻⁴ M		
4a	83.2	77.0	36.0	52.1	0.000038867	-4.41
4b	83.2	77.0	76.4	52.1	0.000785647	-3.10
4c	89.4	82.8	89.2	53.5	0.002562049	-2.59
4d	90.1	85.0	88.7	51.9	0.001582327	-2.80
ADR	32.6	18.2	5.9	-25.1	0.0000000172	-7.76

Values obtained are the means of three independent experiments at four different concentrations.

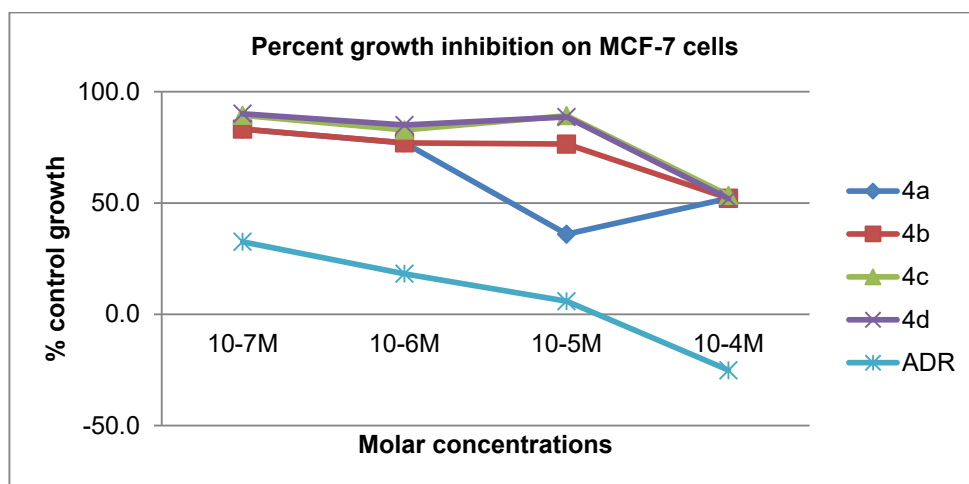


Fig. 5. Graphical presentation of Anti-breast cancer activity (4a-4d)

3. Conclusion

In the present study, all four compounds (**4a-4d**) were successfully synthesized using the conventional methods of synthesis (Fig. 2). The periodic TLC on percolated silica gel-G plates is used to monitor reactions. Furthermore, compounds are characterized by FT-IR, and ¹H NMR. Based on results of *in vitro* SRB assay performed on MCF-7 cells, we observed compound **4a** displayed good growth inhibition potential as compared to **4b**, **4c**, and **4d**. According to the structure activity relationship (SAR) study, we observed that, Presence of thiophene ring, R=C₂H₅ and X= S group in **4a** leads to enhancement in activity. However, **4a** was found to be moderately active compared to potent anticancer drug ADR. From docking studies, the designed compounds showed similar binding energy and fit well in allosteric binding pockets. They interact with amino acids like PRO-137, ALA-133, TYR-211, GLU-116, ARG-119. Although the role of thiophene and DHPM nucleus is well established in literature, we still need to develop more efficient analogues possessing great potency by selectively targeting only cancerous cells rather than normal cells. Therefore, we conclude to address this issue in futuristic studies in a more profound way.

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We acknowledge Oriental University, Indore for providing research facility and ACTRECT, Navi Mumbai for conduction of *in vitro* anti breast cancer studies.

4. Experimental

4.1 Materials and methods

Pure, analytical grade chemicals were obtained from Sigma Aldrich. Shimadzu ATR was used to record FT-IR spectra. ¹³C NMR recorded on Bruker Advance II (DMSO-100 MHz), Proton NMR recorded on Bruker Advance II (DMSO- 500 MHz) TMS used as internal standard. Periodic TLC was determined by pre coated Silica gel plates.

4.2 General procedure for synthesis of Thiophene-DHPMs (4a-4d)

Thiophene-2-carboxaldehyde **1** (3.6 mmol), acetoacetates **2** (3.0 mmol), urea or thiourea **3** (3.6 mmol), concentrated HCl (0.6 mmol) in catalytic amount were refluxed for 4-8 hours (Figure 2). Reaction was continuously by periodic TLC [mobile phase: hexane: ethyl acetate (6:4)]. The resultant derivatives (4a-4d) are recrystallized by alcohol.

4.3 Physical and Spectral Data

4.3.1 ethyl-6-methyl-2-sulfanylidene-4-thiophene-tetrahydropyrimidine (4a)

Molecular formula: C₁₂H₁₄O₂N₂S₂; Molecular weight: 282.38 g/mol; R_f: 0.6; yield: 75 %; M.P (218 °C); IR (v, cm⁻¹): N-H 3308, C=S 1186, CH₂=CH₂ (Aromatic) 1454, C-N 1329; ¹H NMR (500 MHz, DMSO) δ ppm: 1.18 (3H, t); 2.2 (3H, s); 4.10 (2H, q); 5.4 (1H, d); 6.9-7.4 (3H, multiplet); 9.7 (1H, s); 10.4 (1H, s); ¹³C NMR (100 MHz, DMSO) : δ 176.5; 164.2; 161.3, 140.4; 127.9; 127.7; 132.6; 103.2; 63.7; 56.3, 15.6; 14.2

4.3.2 ethyl-6-methyl-2-oxo-4-thiophene-tetrahydropyrimidine (4b)

Molecular formula: C₁₂H₁₄O₃N₂S; Molecular weight: 266.32 g/mol; R_f: 0.6; yield: 86 %; M.P (210 °C); IR (v, cm⁻¹): N-H 3109, C=O 1648, C-N 1289, CH₂=CH₂ 1460; ¹H NMR (500 MHz, DMSO) δ ppm: 1.18 (3H, t); 2.22 (3H, s); 4.07 (2H, q); 5.4 (1H, d); 6.8-7.3 (3H, multiplet); 7.9 (1H, s); 9.3 (1H, s); ¹³C NMR (100 MHz, DMSO) : δ 168.2; 152.4; 148.6; 142.4; 127.6; 127.5; 127.3; 99.8; 62.2; 56.4; 18.6; 14.2

4.3.3 methyl 6-methyl-2-sulfanylidene-4-thiophene-tetrahydropyrimidine (4c)

Molecular formula: C₁₁H₁₂O₂N₂S₂; Molecular weight: 268.36 g/mol; R_f: 0.3; Yield: 80 %; M.P (224 °C); IR (v, cm⁻¹): N-H 3109, C=S 1089, C-N 1289, CH₂=CH₂ (Aromatic) 1449; ¹H NMR (500 MHz, DMSO) δ ppm: 2.2 (3H, s); 3.6 (3H, s); 5.4 (1H, d); 6.9-7.4 (3H, multiplet); 9.7 (1H, s); 10.4 (1H, s) ¹³C NMR (100 MHz, DMSO) : δ 178.4; 166.2; 146.4; 142.2; 127.8; 127.5; 127.4; 99.4; 56.6; 52.4; 18.6

4.3.4 methyl 6-methyl-2-oxo-4-thiophene-tetrahydropyrimidine (4d)

Molecular formula: C₁₁H₁₂O₃N₂S; Molecular weight: 252.29 g/mol; R_f: 0.48; Yield 84 %; M.P (214 °C); IR (v, cm⁻¹): N-H 3331, C=O 1671, CH₂=CH₂ (Aromatic) 1454, C-N 1335; ¹H NMR (500 MHz, DMSO) δ ppm: 2.2 (3H, s); 3.6 (3H, s); 5.4 (1H, d); 6.9-7.3 (3H, multiplet); 7.9 (1H, s); 9.3 (1H, s) ¹³C NMR (100 MHz, DMSO) : δ 166.4; 152.4; 148.6; 141.0; 127.8; 127.6; 127.4; 99.2; 56.4; 53.2; 18.2

4.4 Prediction of physicochemical, pharmacokinetic, and ADMET studies:

Pharmacokinetic parameters are screened by using Swiss ADMET software to predict drug like characters. It has been found as all compounds have lead likeness property and they do not violate Lipinski rule of 5.⁵¹⁻⁵²

4.5 Molecular Docking Studies:

Molecular docking with Cygwin 64 software assessed the interaction between Eg5 tyrosine kinase spindle protein (**PDB ID: 1Q0B**) and ADP, monastrol, and thiophene derivatives. Protein preparation involved chain A selection and removal of water, heteroatoms, and ligands using Discovery Studio 4.0. Ligands were converted to mol 2 format after preparation with ChemDraw. Autodock-Cygwin64 facilitated docking with a grid box resolution set at -2.778Å. The GRIP module generated ligand conformers and explored binding positions within the active site. Scoring evaluated energy-minimized poses for binding affinity. Standard drug docking provided a baseline for comparison with DHPM derivatives in the same binding sites⁵³.

Abbreviations

DHPMs: Dihydropyrimidinones/thiones; **MCR**: multicomponent reactions; **SRB**: sulforhodamine B; **ADR**: Adriamycin; **ER**: Estrogen receptor; **MCF-7**: Human breast adenocarcinoma; **GI₅₀**: Growth inhibition; **BRCA**: breast cancer causing genes; **TMS**: Tetramethyl silane; **TLC**: Thin layer chromatography; **mmol**: millimoles; **TPSA**: Topological polar surface area **DMSO**: Dimethylsulfoxide; **μl**: microliter; **FTIR**: Fourier transform infrared; **HBD**: Hydrogen bond donor; **HBA**: Hydrogen bond acceptor; **¹H NMR**: Proton nuclear magnetic resonance

References

1. Sung H., Ferlay J., Siegel RL. (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: CA Cancer J Clin*, 71(3) 209-249.
2. Sharma GN., Dave R., Sanadya. (2010) Various types and management of breast cancer: an overview. *J Adv Pharm Technol Res*, 1(2) 109.
3. World, Cancer, Who. int. (2022). <https://www.who.int/news-room/factsheets/detail/cancer>.
4. Akram M., Iqbal M., Daniyal M. (2017) Awareness and current knowledge of breast cancer. *Biol. Res*, 501-23.
5. Li R, Hettiarachchy N., & Mahadevan M. (2014) Apoptotic pathways in human breast cancer cell models (MCF-7 and MDA-MB-231) induced by rice bran derived pentapeptide. *Int J Res Med Health Sci*, 4(5)13-21.
6. Biginelli CP. (1893) Aldehyde-urea derivatives of aceto- and oxaloacetic acids. *Gazz. chim. ital*, 23(1)360-413.

7. Sondhi SM., Goyal RN., Lahoti AM. (2005) Synthesis and biological evaluation of 2-thiopyrimidine derivatives. *Bioorg Med Chem*, 13(9)3185-3195.
8. Shaikh A. & Meshram J. (2016) Novel 1, 3, 4-Oxadiazole Derivatives of Dihydropyrimidinones: Synthesis, Anti Inflammatory, Anthelmintic, and Antibacterial Activity Evaluation. *J Heterocycl Chem*, 53(4) 1176-1182.
9. Bhat MA., Al-Dhfyhan A., Al-Omar MA. (2016) Targeting cancer stem cells with novel 4-(4-substituted phenyl)-5-(3, 4, 5-trimethoxy/3, 4-dimethoxy)-benzoyl-3, 4-dihydropyrimidine-2(1H)-one/thiones. *Mol*, 21(12)1746.
10. Ragab FA., Abou-Seri SM., Abdel-Aziz. (2017) Design, synthesis and anticancer activity of new monastrol analogues bearing 1, 3, 4-oxadiazole moiety. *Eur J Med Chem*, 138140-151.
11. Peruzynska M., Borzyszkowska-Ledwig A., Sośnicki JG. (2021) Synthesis and Anticancer Activity of Mitotic-Specific 3, 4-Dihydropyrimidine-2 (1 H)-thiones. *Int J MolSci*, 22(5)2462.
12. Verma M., & Verma PK. (2022) Synthesis, characterization, antimicrobial and anticancer evaluation of pyrimidines Clubbed with thiazolidinone. *J Med Pharm Allied Sci*, 11 (2)4582-4587.
13. Farghaly AM., AboulWafa OM., Elshaier YA. (2019) Design, synthesis, and antihypertensive activity of new pyrimidine derivatives endowing new pharmacophores. *Med Chem Res*, 28360-379.
14. Venugopala KN., Chandrashekarappa S., Tratrak C. (2021) Crystallography, molecular modeling, and COX-2 inhibition studies on indolizine derivatives. *Mol*, 26(12) 3550.
15. Kumarasamy D., Roy BG., Rocha-Pereira J. (2017) Synthesis and *in vitro* antiviral evaluation of 4-substituted 3, 4-dihydropyrimidinones. *Bioorgmed chem lett*, 27(2)139-142.
16. Bhalgat CM., & Ramesh B. (2014) Synthesis, antimicrobial screening and structure-activity relationship of novel pyrimidines and their thioethers. *Bull Fac Pharm Cairo Univ*, 52(2)259-267.
17. Yadav S., Lim SM., Ramasamy K. (2018) Synthesis and evaluation of antimicrobial, antitubercular and anticancer activities of 2-(1-benzoyl-1H-benzod [d] imidazol-2-ylthio)-N-substituted acetamides. *Chem Cent J*, 12(1)1-14.
18. El-Malah A., Mahmoud Z., Hamed Salem H. (2021) Design, ecofriendly synthesis, anticancer and antimicrobial screening of innovative Biginellidihydropyrimidines using β -aroylpyruvates as synthons. *Green Chem Lett Rev*, 14(2) 221-233.
19. Jalali. (2021) Antimicrobial evaluation of some novel derivatives of 3,4-dihydropyrimidine-2(1H)-one. *Res Pharm Sci*, 7.
20. Patel N., Pathan S., & Soni HI. (2019) 3, 4-dihydropyrimidin-2 (1h)-one analogues: Microwave irradiated synthesis with antimicrobial and antituberculosis study. *Curr Microw Chem*, 6(1)61-70.
21. Shaikh S., Shaikh NP., Salunke SD. (2015) Synthesis and Antimicrobial Activity of new 3, 4-Dihydropyrimidinones via Novel Chalcone Series. *Heterocycl Lett*, 5(3)443-449. Doi:
22. Wu W., Lan W., Wu C. (2021) Synthesis and antifungal activity of pyrimidine derivatives containing an amide moiety. *Frontchem*, 9695628.
23. Siva Ranjani J., Selvinthanuja C., Sivakumar T. (2022) 195 Synthesis, characterization and antifungal activity of azo coupled dihydropyrimidinones. *J Pharm Chem*, 8.
24. Wani MY., Ahmad A., Kumar S. (2017) Flucytosine analogues obtained through Biginelli reaction as efficient combinative antifungal agents. *Microbpatho*, 10557-62.
25. Shah UA., Joshi HV., Patel JK. (2020) Molecular docking studies of novel dihydro Pyrimidine derivatives as potential antibacterial agents. *Mol Divers*, 241165-76.
26. De Oliveira FS., De Oliveira PM., Farias LM. (2018) Synthesis and antitumoral activity of novel analogues monastrol-fatty acids against glioma cells. *Med Chem Comm*, 9(8)1282-1288.
27. Tardiff DF., Brown LE., Yan X. (2017) Dihydropyrimidine-thiones and clioquinol synergize to target β -amyloid cellular pathologies through a metal-dependent mechanism. *ACS Chem Neurosci*, 8(9)2039-2055.
28. Mishra R., Sachan N., Kumar N. (2018) Thiophene scaffold as prospective antimicrobial agent: A Review. *J Heterocycl Chem*, 55(9)2019-2034.
29. Chawla S., Sharma S., Kashid S. (2023) Therapeutic potential of thiophene compounds: a mini-review. *Mini Rev Med Chem*. 231514-1534.
30. Pathania S., & Chawla PA. (2020) Thiophene-based derivatives as anticancer agents: An overview on decade's work. *Bioorg Chem*, 101104026.
31. de Vasconcelos A., Campos VF., Nedel F. (2013) Cytotoxic and apoptotic effects of chalcone derivatives of 2-acetyl thiophene on human colon adenocarcinoma cells. *Cell Biochem Func*, 31(4)289-297.
32. Schmitt C., Kail D., Mariano M. (2014) Design and synthesis of a library of lead-like 2, 4-bisheterocyclic substituted thiophenes as selective Dyrk/Clk inhibitors. *PLoS One*, 9(3)87851.
33. Al-Said MS., Bashandy MS., Al-Qasoumi SI. (2011) Anti-breast cancer activity of some novel 1, 2-dihydropyrimidine, thiophene and thiazole derivatives. *Eur J Med Chem*, 46(1)137-141.
34. Dallemagne P., Khanh LP., Alsaïdi A. (2002) Synthesis and biological evaluation of cyclopenta [c] thiophene related compounds as new antitumor agents. *Bioorg & med chem*, 10(7)2185-2191.
35. Venkataramireddy V., Shankaraiah V., Rao A. (2016) Synthesis and anti-cancer activity of novel 3-aryl thiophene-2-carbaldehydes and their aryl/heteroaryl chalcone derivatives. *Rasayan J Chem*, 9(1)31-39.
36. Sable PN., Ganguly S., & Chaudhari PD. (2014) An efficient one-pot three-component synthesis and antimicrobial evaluation of tetra substituted thiophene derivatives. *Chin Chem Lett*, 25(7)1099-1103.
37. Harit T., Bellaouchi R., Asehraou A. (2017) Synthesis, characterization, antimicrobial activity and theoretical studies of new thiophene-based tripodal ligands. *J Mol Struct*, 113374-79.
38. Gawdzik B., Kowalczyk P., Koszelewski D. (2022) The Evaluation of DHPMs as Biotoxic Agents on Pathogen Bacterial Membranes. *Memb*, 12(2)238.
39. Sulthana SS., Antony SA, Balachandran C. (2015) Thiophene and benzodioxole appended thiazolyl-pyrazoline compounds: Microwave assisted synthesis, antimicrobial and molecular docking studies. *Bioorg Med Chem Lett*, 25(14)2753-2757.

40. Ashour HM., Shaaban OG., Rizk OH. (2013) Synthesis and biological evaluation of thieno [2', 3': 4, 5] pyrimido [1, 2-b][1, 2, 4] triazines and thieno [2, 3-d][1, 2, 4] triazolo [1, 5-a] pyrimidines as anti-inflammatory and analgesic agents. *Eur j med chem*, 62341-351.
41. Mathew B., Suresh., & Anbazhagan S. (2014) Synthesis, in silico preclinical evaluation, antidepressant potential of 5-substituted phenyl-3-(thiophen-2-yl)-4, 5-dihydro-1H-pyrazole-1-carboxamides. *Biomed Aging Pathol*, 4(4) 327-333.
42. Kulandasamy R., Adhikari AV., & Stables JP(2009) A new class of anticonvulsants possessing 6 Hz activity: 3, 4-dialkyloxy thiophene bishydrazones. *Eur J Med Chem*, 44(11)4376-4384.
43. Matos LHS., Masson FT., Simeoni LA. (2018) Biological activity of dihydropyrimidinone (DHPM) derivatives: A systematic review. *EurJMed Chem*, 1431779-1789.
44. Mayer TU., Kapoor TM., Haggarty SJ. (1999) Small molecule inhibitor of mitotic spindle bipolarity identified in a phenotype-based screen. *Sci*, 286(5441)971-974.
45. Brier S., Lemaire D., DeBonis S. (2004) Identification of the protein binding region of S-trityl-L-cysteine, a new potent inhibitor of the mitotic kinesin Eg5. *Biochem*, 43(41)13072-13082.
46. Garcia-Saez I., & Skoufias DA.(2021)Eg5 targeting agents: From new anti-mitotic based inhibitor discovery to cancer therapy and resistance. *BiochemPharmacol*, 184114364.
47. Luo L., Carson JD., Dhanak D. (2004) Mechanism of inhibition of human KSP by monastrol: insights from kinetic analysis and the effect of ionic strength on KSP inhibition. *Biochem*, 43(48)15258-15266.
48. Cochran JC., Gatial JE., Kapoor TM. (2005) Monastrol inhibition of the mitotic kinesin Eg5. *J Biol Chem*, 280(13)12658-12667.
49. Nikam D., & Jain A. (2022) Advances in the discovery of DHPMs as Eg5 inhibitors for the management of breast cancer and glioblastoma: A review. *Results Chem*, (5)100718.
50. E. Scholar., Raloxifene., Elsevier EBooks, (2009) 1–6.
51. Clark DE., & Pickett SD. (2000) Computational methods for the prediction of 'drug-likeness. *Drug discov today*, 5(2)49-58.
52. Lipinski CA., Lombardo F., Dominy BW. (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv drug deliv Rev*, 23(1-3)3-25.
53. Tawfik. H. O., El-Moselhy, T. F., El-Din, N. S., & El-Hamamsy, M. H. (2019) Design, synthesis, and bioactivity of dihydropyrimidine derivatives as kinesin spindle protein inhibitors. *Bioorg MedChem*, 27(23)115126.
54. McGill M.R., Findley D.L., Mazur A., Yee E.U., Allard F.D., Powers A., Coward L., Blough E.R., Gorman G., Hambuchen M.D.(2022) Radiation Effects on Methamphetamine Pharmacokinetics and Pharmacodynamics in Rats. *Eur J Drug Metab Pharmacokinet*, 47 (3) 319–330.
55. Boggess T., Williamson J.C., Niebergall E.B., Sexton H., Mazur A., Egleton R.D., Grover L.M., Christopher W. R.(2021) Alterations in Excitatory and Inhibitory Synaptic Development Within the Mesolimbic Dopamine Pathway in a Mouse Model of Prenatal Drug Exposure. *Front Pediatr*, (9) 794544
56. Berquist M.D., McGill M.R., Mazur A., Findley D.L., Gorman G., Jones C.B., Hambuchen M.D.(2020) Effect of bile duct ligation-induced liver dysfunction on methamphetamine pharmacokinetics in male and female rats. *Drug Alcohol Depend*, (215)108190
57. Chen X., Denning K.L., Mazur A., Lawrence L.M., Wang X., Lu Y. (2023)Under peroxisome proliferation acyl-CoA oxidase coordinates with catalase to enhance ethanol metabolism. *Free Radical Biol Med*, (208) 221–228.
58. Saber A.F. & Ahmed F.M. (2021) (E)-1,2-Diphenylethene-based conjugated nanoporous polymers for a superior adsorptive removal of dyes from water. *New J Chem*, 45(46) 21834–21843.
59. Saber A.F., Chen K.-Y., Ahmed F.M., Kuo S.-W. (2021) Designed azo-linked conjugated microporous polymers for CO₂ uptake and removal applications, *J Polym Res*, 28(11) 430
60. Saber A.F., Elewa A.M., Chou H.-H., Ahmed F.M. (2023) Donor to Acceptor Charge Transfer in Carbazole-based Conjugated Microporous Polymers for Enhanced Visible-Light-Driven Photocatalytic Water Splitting. *Chem Cat Chem*, 15(3) e202201287.
61. Zaki R.M., Saber A.F., Kamal A.M., & Radwan S.M.(2020) A concise review on synthesis, reactions and biological Importance of thienopyrazoles, *Arkivoc*, (1) 011-108.
62. Bakri Y.E., Mohamed S.K., Saravanan K., Ahmad S., Mahmoud A.A., Shaban., El-Sayed W.M., Mague J.T., Said S. G. (2023) 1,4,9,9-tetramethyloctahydro-4,7-(epoxymethano)azulen-5(1H)-one, a natural product as a potential inhibitor of COVID-19: Extraction, crystal structure, and virtual screening approach, *J King Saud Univ Sci*, 35(4) 102628–102628.
63. Drar A.M., Abdel-Raheem S.A.A., Moustafa A.H., Hussein B.R.M. (2023) Studying the toxicity and structure-activity relationships of some synthesized polyfunctionalized pyrimidine compounds as potential insecticide, *Curr Chem Lett*. 12(3) 499–508.

