Indian Journal of Chemistry
Vol. 62, March 2023, pp. 251-264
DOI: 10.56042/ijc.v62i3.72060

Synthesis, characterization, cytotoxicity evaluation and molecular docking study of new bis-chalcone, fused-pyrimidine and fused-pyrazoline derivatives

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Received 18 October 2021; accepted (revised) 15 December 2022

Chemotherapeutic drug resistance and high-risk side effects are common limitations in cancer treatment. Thus, the continuous development of new drugs that target only the cancer cell without affecting the normal cells is needed. The simple structure of the chalcone and the ease of its synthesis showed promising functions. Such compounds have been reported to exhibit diverse pharmacological activities, particularly anticancer. This study involves the design of chalcones **1** and **2** which have been synthesized via Claisen-Schmidt condensation. Further cyclo-condensation reactions of these chalcone compounds has formed five pyrazoline and three pyrimidine derivatives. All the desired derivatives are characterised by FT-IR, ^{1}H-NMR, and ^{13}C-NMR. These derivatives are tested for cytotoxicity against breast cancer cell lines (MCF-7 and MD-MB-231) and normal breast cell lines (MCF-10A). The results emphasized that pyrazoline compounds **1Aii** and **1Aiii** are showing the minimum inhibition against MCF-7 with the IC_{50} values of56.73±3.3 µM and 37.74±1.32 µM, respectively, after 24 h of exposure, which are comparable to Tamoxifen, as reference anticancer drug (IC_{50} = 42.66±2.19 µM).

Keywords: Chalone, Pyrimidine, Pyrazoline, Anticancer, Claisen-Schmidt condensation

Cancer is a current major public health issue and is one of the most prevalent causes of disease-related deaths worldwide, involving the transformation of normal cells into abnormal cells. It is a serious and life-threatening disease that causes significant harm to human health in which the body cells proliferate uncontrollably with abnormal cell growth that can spread into or invade nearby tissues which result in the formation of tumor cells[1]. Cancer is the second leading cause of death worldwide, behind cardiovascular disease, according to the World Health Organization (WHO), with breast cancer being the most common, followed by colorectal malignancies[2]. Cancer treatment depends on the type of cancer and the stages of diagnosis. If cancer has not spread into the surrounding tissues, surgery and radiotherapy can remove the tumor[3]. On the other hand, if the cancer cells have spread, systemic treatment such as chemotherapy is needed which involves the use of agents that inhibit the cell division in cancer cells and ultimately destroying them[4]. Although chemotherapeutic drugs can help to reduce the mortality rate, these drugs also severely affect non-cancerous tissues, producing side effects as a result of damage to normal body cells and organ toxicities which reduced the quality of life in cancer patients[5]. Other problems of cytotoxicity and drug resistance with the current chemotherapeutic agents, the development of new and effective medications to aid in the control of this condition has remained a significant problem[6].

Molecular docking, a computer method that offers information about intermolecular interactions of a ligand-protein complex that predicts the orientation, and binding energies of ligands in their targeted binding sites, is one of the current strategies for understanding the drug-receptor relationship in modern drug discovery[7], in which the design of new compounds by using natural product scaffolds has been of great interest. Numerous plants have been exploited and many important constituents have been identified to exhibit inhibitory activities against various types of cancers with fewer side effects. Because of their low toxicity, low drug resistance, low cost, and great efficacy, natural product scaffolds have sparked additional research for the treatment of a variety of illnesses, including anticancer[8]. Extensive works on the modification of these natural product scaffolds have reported novel compounds with enhanced anticancer efficacies and safety profiles. Natural and synthetic molecules are promising therapeutic agents due to their integration modes of action[9]. Research works on such

scaffolds for example chalcone, pyrazoline ring and pyrimidine ring have been identified to exhibit inhibitory activities against various types of cancers with fewer side effects.

Chalcone is a secondary metabolite of flavonoids, which are naturally occurring compounds with a wide range of pharmacological activities. Because chalcone derivatives are abundant in plants, they are valuable compounds in the field of medicinal chemistry[10]. Its common structure is made up of two aromatic rings connected by the unsaturated system., a three-carbon enone fragment[11] (Fig. 1). However, since the high demand for chalcone compounds cannot be meet from natural sources, chalcones are synthesized using common Claisen-Schmidt condensation[12]. Recent research into the antiproliferative and tumor-reducing properties of some chalcone compounds have resulted in the exploration of an enormous number of derivatives with an interesting range of bioactivities[13-15]. The chalcone scaffold can undergo a ring-closing condensation to form, in particular, the nitrogen-based heterocyclic ring such as pyrimidine and pyrazoline[16]. Nitrogen-based heterocyclic ring in a wide range of pharmaceutical has a wide spectrum of pharmacological uses in cancer treatment. The insertion of a nitrogen atom in a molecule's ring can modify its flexibility, metabolic profile, polarity, and ability to form hydrogen bonds, according to molecular modelling and pharmacokinetic studies[17]. Heterocyclic ring compounds are not only found in nature but can also be synthesized. Ring closure of chalcones results in heterocyclic scaffolds such as pyrimidine, pyrazoline, oxazine and thiazine[18,19].

Pyrimidine (Fig 1) is a heterocyclic unit with six membered and two nitrogen atoms., a 1,3-diazine isomer which is the most important member of all the diazines as this is the ring system of the living organisms[20]. Oxazines are 6-membered heterocyclic units (Fig. 1) that contain one nitrogen and one oxygen as heteroatom depending on the position of the heteroatoms and the relative position of the double bond, which can exist in three isomers[21]. Other heterocyclic compounds containing one nitrogen and sulphur atom in the six membered rings that exist as 1,2; 1,3; and 1,4-positions are the thiazines.1,3-Thiazine, in particular, posses an N-C-S linkage which plays an important role that exhibits a wide range of biological activities[22]. Pyrazoline (Fig. 1), a five-membered heterocyclic ring that contain one endocyclic double bond and two adjacent nitrogen atoms in the ring is among the most prominent heterocyclic compounds that have significant biological activities[23,24].

Experimental Details

Material & analytical procedure

Unless otherwise stated, all chemicals and solvents were used without further purification. The melting points were determined using an open capillary tube and a melting point apparatus (Stuart Scientific SMP1). Thin-layer chromatography was performed in ethyl acetate:n-hexane (15:85 ratio) using pre-coated silica gel plates (silica gel 0.25 mm, Merck, Germany) and was observed under a UV lamp. The IR spectra were obtained using the Attenuated Total Reflection (ATR) technique on a Perkin-Elmer System 2000 FT-IR spectrometer, and the NMR spectra in CDCl3 were obtained on a Bruker 500 MHz UltrashieldTM spectrometer.

Auto Dock Study

The structural properties of chalcone which was reported to have a wide range of anticancer activity have prompted us to study their interaction with the active site of a protein is related to breast cancer cells via molecular computational studies. The X-ray crystal structure of human oestrogen receptor alpha (ER) (PDB ID: 3ERT)

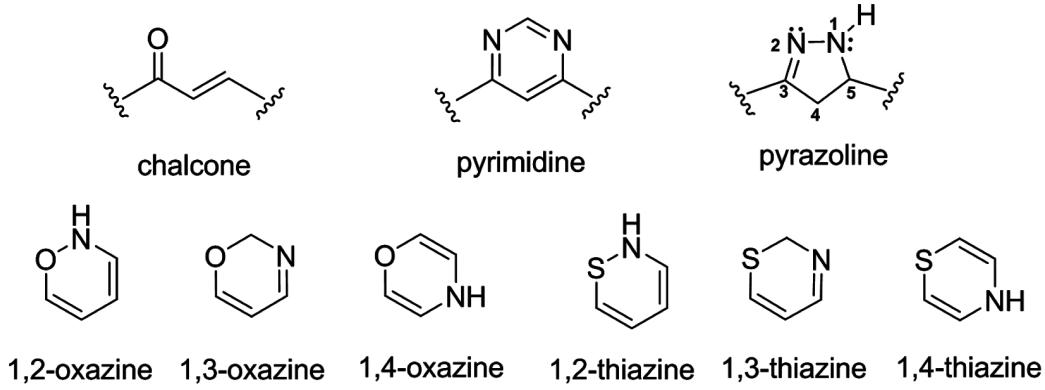


Fig. 1 — The structures of chalcone, pyrimidine, pyrazoline, oxazine and thiazine scaffolds

was obtained from Protein Data Bank (http://www.rcsb.org) for this docking study and was reported to have a relationship with the breast cancer cell line MCF-7[25,26]. Molecular docking was used to investigate the expected binding mode of the synthesised compounds against 3ERT receptor, have been carried out for all the designed compounds in comparison to the binding energy with Tamoxifen, an established anticancer drug. To determine their inhibitory properties, all of the compounds chemical structures were drawn using Chem Draw version 19 before the energy was minimised for use in a molecular docking study against the 3ERT receptor. The Discovery Studio 2019 programme was used to perform pre-preparation of receptor 3ERT, such as the removal of water molecules and multiple ligands. Tamoxifen and all designed compounds were docked against the 3ERT receptor using the Lamarckian genetic algorithm (LGA). The grid parameter file was arranged and changed from default parameters to 60 × 60 × 60 grid points in x= 31.6615, y= -0.8435, and z= 25.1743, with a center spacing of the grid was 0.375 Å. The Erα was defined as rigid for docking parameter, whereas ligands were flexible. Other parameters were left at their default settings. Finally, the interaction between the 3ERT receptor and ligands was studied using Cygwin software. Among the ten docked conformations generated during the docking study, the best-docked run of each ligand based on docking parameters and binding site interactions was chosen. Compounds with the lowest binding energy in the protein-ligand binding complex were selected to be further explored of their complex structure compared to the complex structure of Tamoxifen by using Discovery Studio 2019 program.

Synthesis of chalcone 1 and 2

The general method for the producing chalcone **1** and **2** involved a Claisen-Schmidt condensation of the respective ketone and aldehyde in ethanol in the presence of catalysts[27], as shown in Scheme 1.

2,6-Bis[(E)-2-ethoxybenzylidene] cyclohexan-1-one, 1

An ethanolic solution (5 mL) of potassium hydroxide (0.002 mol) was added slowly to a stirred solution of cyclohexanone (0.001 mol) and 2-ethoxybenzaldehyde (0.002 mol) in 5 mL ethanol. The reaction mixture was stirred at room temperature. The reaction progress was monitored by TLC. The precipitate formed was filtered, washed with cold water, and dried to give a brown solid. The crude product was recrystallized from ethanol to give a brown powder. Yield: 72.76%, mp: 111-115°C, yellow powder. IR (cm^{-1}): 2977, 2930 (C_{sp}^3-H str.), 1668 (C=O str.), 1597 (C=C Ar str.), 1117 (C-O str.). ^{1}H-NMR (500 MHz, $CDCl_3$) δ, ppm: 8.04 (s, 2H, H-5), 7.33 (t, *J*=5.5 Hz, 2H, H-9), 7.30 (t, *J*=10.0 Hz, 2H, H-8), 6.97 (d, *J*=7.5 Hz, 2H, H-7), 6.93 (d, *J*=8.0 Hz, 2H, H-10), 4.09-4.13 (q, 4H, H-12), 2.86-2.88 (dd, *J*=5.5 Hz, 4H, H-3), 1.75-1.79 (m, 2H, H-4), 1.47 (t, *J*=7.0 Hz, 6H, H-13). ^{13}C-NMR (125 MHz, $CDCl_3$) δ, ppm: 190.6 (C-1), 157.8 (C-11), 136.4 (C-5), 132.7 (C-2), 130.5 (C-9), 129.9 (C-7), 125.3 (C-8), 119.8 (C-6), 111.7 (C-10), 63.9 (C-12), 28.9 (C-3), 23.5 (C-4),14.9 (C-13). CHN elemental analysis: Calculated for $C_{24}H_{36}O_3$: C: 79.53%, H: 7.23%; Found: C: 71.23%, H: 6.67%.

(2E,5E)-2,5-bis(2-ethoxybenzylidene) cyclopentanone, 2

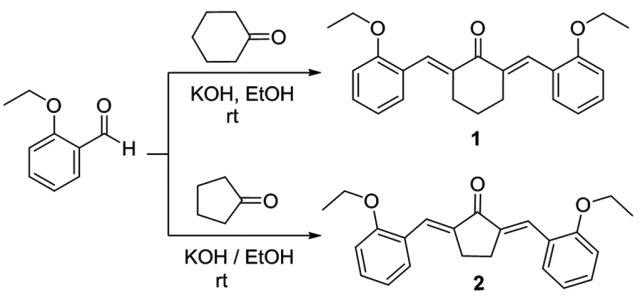
For chalcone **2**, a mixture of 2-ethoxybenzaldehyde (0.006 mol), cyclopentanone (0.003 mol) and potassium hydroxide (0.004 mol) in 10 mL ethanol was stirred at room temperature. The reaction procedure is as mentioned for chalcone **1**. Yield: 77.0%, mp: 70-75°C, green powder. IR (cm^{-1}): 2872 (C_{sp}^3-H str.), 1686 (C=O str.), 1592 (C=C str.), 1449 (C-H bending), 1276 (C-O str.). ^{1}H-NMR (500 MHz, $CDCl_3$) δ, ppm: 8.01 (s, H-4, 2H), 7.53 (d, *J*=1.5 Hz, H-6, 2H), 7.31 (t, *J*=7.24, H-8, 2H), 6.98 (t, *J*=7.5 Hz, H-7, 2H), 6.91 (d, *J*=8.5 Hz, H-9, 2H), 4.12 (q, *J*=7.0 Hz, H-11, 4H), 3.02 (s, H-3, 4H), 1.47 (t, *J*=7.0 Hz, H-12, 6H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ, ppm: 196.4 (C-1), 158.4 (C-10), 137.6 (C-4), 130.6 (C-2), 129.9 (C-8), 128.3 (C-6), 120.1 (C-7), 111.9 (C-5), 111.8 (C-9), 64.1 (C-11), 27.0 (C-3), 14.8 (C-12) CHN elemental analysis: Calculated for $C_{21}H_{34}O_2$: C: 79.28%, H: 6.94%; Found: C: 78.91%, H: 7.66%.

Synthesis of heterocyclic derivatives (oxazine, thiazine and pyrimidine), 1A(i-iii)

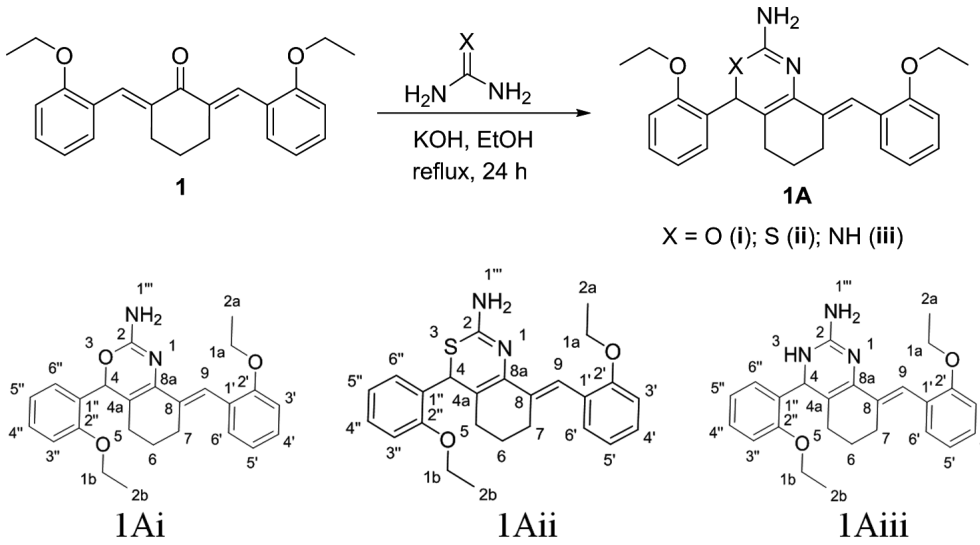
The reaction of chalcone 1 with a series of hydrazine derivatives in ethanol solution in the presence of potassium hydroxide resulted in the formation of heterocyclic derivatives (Scheme 2)[27].

(E)-8-(2-ethoxybenzylidene)-4-(2-ethoxyphenyl)-5,6,7,8-tetrahydro-4H-benzo[d][1,3]oxazin-2-amine, 1Ai

A mixture of chalcone, **1** (0.003 mol) with urea (0.004 mol) and potassium hydroxide (0.003 mol) in



Scheme 1 — Synthesis of chalcones **1** and **2**



Scheme 2 — Synthesis of heterocyclic derivatives, **1A(i-iii)**

25 mL ethanol was refluxed for 24 hours. The reaction progress was monitored by TLC. After completion, the mixture was poured into an ice-water bath with constant stirring, resulting in a solid product that was filtered off, washed with cold water, and dried. To obtain the brown solid, the product was recrystallized from ethanol. Yield: 71.8%, mp: 151-152°C, brown powder. IR (cm^{-1}): 3400 (N-H str.), 3061 (C_{sp}^2-H str.), 2979, 2931, 2875(C_{sp}^3-H str.), 1670 (C=N str.), 1587 (cyclic C=C str.), 1291 (Ar C-N str.), 1116 (C-O str.), 1041 (C-O vinyl ether str.). ^{1}H-NMR (500 MHz, $CDCl_3$) δ, ppm: 11.45 (s, 2H, H-1’’’), 7.55 (s, 1H, H-9), 7.39 (t, *J*=8.6 Hz, 1H, H-4’), 7.34 (t, *J*=7.4 Hz, 1H, H-6’’), 7.18 (t, *J*=3.75 Hz, 1H, H-5’,H-5’’), 7.04 (t, *J*=7.4 Hz, 1H, H-4’’), 6.98 (d, *J*=8.2 Hz, 1H, H-6’), 6.89 (d, *J*=5.45 Hz, 1H, H-3’’), 6.87 (d, *J*=3.85 Hz,1H, H-3’), 4.88 (s, 1H, H-4), 4.14 (q, 4H, H-1b, H-1a), 2.57 (t, *J*=8.55 Hz, 2H, H-7), 2.36 (t, *J*=10.4 Hz, 2H, H-5), 1.75-1.77 (m, 2H, H-6), 1.51 (t, *J*=7.0 Hz, 3H, H-2b), 1.46 (t, *J*=7.0, 3H, H-2a). ^{13}C-NMR (125 MHz, $CDCl_3$) δ, ppm: 157.5 (C-2), 157.2 (C-2’,2’’), 147.1 (C-8), 130.8 (C-8a), 130.3 (C-4a), 130.2 (C-6’’,4’), 128.2 (C-6’), 128.1 (C-4’’), 126.9 (C-9), 126.3 (C-1’’), 120.8 (C-5’’,5’), 119.7 (C-3’), 112.4 (C-3’’), 111.7 (C-1’), 64.6 (C-1b), 64.0 (C-1a), 27.1 (C-4), 26.2 (C-7,5), 22.2 (C-6), 15.1 ((C-2b), 14.9 (C-2a). CHN elemental analysis: Calculated for $C_{25}H_{28}N_2O_3$: C: 74.22%, H: 6.98%, N: 6.93%; Found: C: 75.46%, H: 6.81%, N: 6.12%.

(E)-8-(2-ethoxybenzylidene)-4-(2-ethoxyphenyl)-5,6,7,8-tetrahydro-4H-benzo[d] [1,3] thiazin-2-amine, 1Aii

The same procedure used a mixture of chalcone, **1** (0.003 mol), thiourea (0.004 mol) and potassium hydroxide (0.003 mol) in 25 mL ethanol which was refluxed for 24 h. Yield: 44.50%, mp: 135.6-137.7°C, light yellow powder. IR (cm^{-1}): 3466 (N-H str.), 3097 (C_{sp}^2-H str.), 2977, 2918, 2868(C_{sp}^3-H str.), 1663 (C=N str.) 1576 (cyclic C=C str.), 1293 (Ar C-N str.), 1040 (C-O str.), 649 (C-S str.). ^{1}H-NMR (500 MHz, $CDCl_3$) δ, ppm: 7.30 (d, *J*=7.5 Hz, 1H, H-6’), 7.27 (d, *J*=7.5 Hz, 1H, H-4’), 7.24 (d, *J*=6.5 Hz, 1H, H-5’), 7.22 (d, *J*=6.5 Hz, 2H, H-6’’), 6.97 (d, *J*=7.0 Hz, 2H, H-3’’), 6.95 (t, *J*=3.75 Hz, 2H, H-5’’), 6.92 (t, *J*=6.0 Hz, 1H, H-4’’), 6.90 (s, 1H, H-9), 5.64 (s, 2H, H-1’’’), 5.39 (s, 1H, H-4), 4.11 (q, 4H, H-1b,1a), 2.64 (t, *J*=6.5 Hz, 2H, H-7), 2.05 (t, *J*=6.5 Hz, 2H, H-5), 1.49-1.50 (m, 2H, H-6), 1.43 (t, *J*=6.5 Hz, 6H, H-2b,2a). ^{13}C-NMR (125 MHz, $CDCl_3$) δ, ppm: 156.7 (C-2,2’), 156.5 (C-2’’), 130.5 (C-8), 129.8 (C-4a), 128.7 (C-8a), 128.4 (C-1’’,4’’,4’), 125.4 (C-6’’,6’), 120.8 (C-9), 119.8 (C-5’’,5’), 111.6 (C-1’), 111.5 (C-3’,3’’), 63.8 (C-4), 63.7 (C-1b,1a), 27.2 (C-7), 26.4 (C-6), 22.4 (C-5), 14.9 (C-2b,2a). CHN elemental analysis: Calculated for $C_{25}H_{28}N_2O_2S_1$: C: 71.40%, H: 6.72%, N: 6.67%; Found: C: 71.53%, H: 6.79%, N: 7.00%.

(E)-8-(2-ethoxybenzylidene)-4-(2-ethoxyphenyl)-3,4,5,6,7,8-hexahydroquinazolin-2-amine, 1Aiii

The same procedure used a mixture of chalcone, **1** (0.003 mol) with guanidine (0.004 mol) and potassium hydroxide (0.003 mol) in 25 mL ethanol which was refluxed for 24 h. Yield: 56.81%, mp: 140-142°C, brown powder. IR (cm^{-1}): 3470-3380 (overlapping of N-H str.), 3063 (C_{sp}^2-H str.), 2978, 2928 (C_{sp}^3-H str.), 1684 (C=N str.), 1596 (cyclic C=C str.), 1289 (Ar C-N str.), 1043 (C-N str.). ^{1}H-NMR (500 MHz, DMSO-d_6) δ,

ppm: 9.56 (s, 1H, H-1'''), 8.21 (s, 2H, H-3), 7.38 (d, *J*=1.5 Hz, 1H, H-6'), 7.37 (d, *J*=7.5 Hz, 1H, H-6''), 7.22 (t, *J*=8.25 Hz, 2H, H-5'',5'), 6.92 (t, *J*=6.7, 2H, H-4'',4'), 6.88 (d, *J*=7.3 Hz, 1H, H-3'), 6.84 (d, *J*=7.6, 1H, H-3''), 6.83 (s, 1H, H-9), 5.18 (s, 1H, H-4), 4.01 (q, 4H, H-1b,1a), 2.57 (t, *J*=4.0 Hz, 2H, H7), 2.49 (t, *J*=4.75 Hz, 2H, H-5), 1.41-1.43 (m, 2H, H-6), 1.34 (t, *J*=7.0 Hz, 6H, H-2b,2a). ^{13}C-NMR (125 MHz, DMSO-d_6) δ, ppm: 156.9 (C-2'), 156.7 (C-2), 152.7 (C-2''), 130.7 (C-8), 129.7 (C-8a), 128.9 (C-4a), 128.2 (C-4'), 127.9 (C-6''), 127.2 (C-4''), 126.4 (C-9), 120.5 (C-1''), 120.0 (C-25'',5'), 119.6 (C-3',1'), 111.9 (C-3''), 111.7 (C-6'), 111.5 (C-14), 64.1 (C-1a), 64.0 (C-1b), 27.3 (C-7), 26.4 (C-5), 22.4 (C-6), 15.0 (C-2a), 14.8 (C-2b). CHN elemental analysis: Calculated for $C_{23}H_{32}N_2O$: C: 74.4%, H: 7.25%, N: 10.42%; Found: C: 65.5%, H: 6.16%, N: 6.67%.

Synthesis of pyrazoline derivatives, 1B(i,iii) and 2B(i-iii)

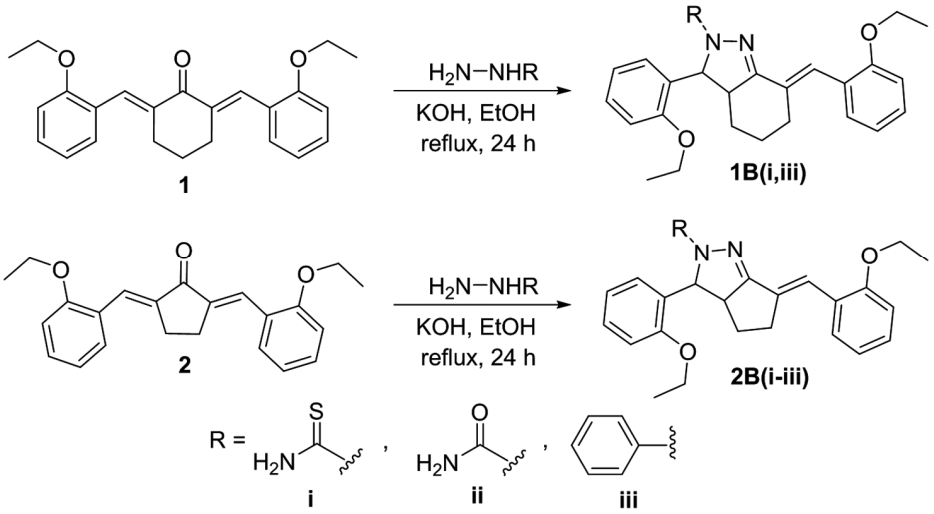
The preparation of two series of pyrazoline derivatives (Scheme 3) involved the reaction of chalcones **1** or **2** with a series of hydrazine hydrate derivatives in ethanol/KOH[27].

(E)-7-(2-ethoxybenzylidene)-3-(2-ethoxyphenyl)-3,3a,4,5,6,7-hexahydro-2H-indazole-2-carbothioamide, 1Bi

A mixture of the chalcone, **1** (0.003 mol), thiosemicarbazide (0.004 mol), and potassium hydroxide (0.003 mol) in 25 mL ethanol was refluxed for 24 h. The reaction progress was monitored by TLC. After completion, the mixture was poured into an ice-water bath with constant stirring, resulting in a solid product that was filtered off, washed with cold water, and dried. To obtain the brown solid, the product was recrystallized from ethanol. Yield: 79.58%, mp: 280-281°C, brown powder. IR (cm^{-1}): 3439, 3316 (two N-H str.), 3167 (C_{sp}^2-H str.), 2977, 2936 (C_{sp}^3-H str.), 1668 (C=N str.), 1598, 1538 (C=C str.), 1449 (C-H bend), 1363 (C-N bend), 1117 (C-O str.). ^{1}H-NMR (500 MHz, $CDCl_3$) δ, ppm: 8.27 (s, 3H), 8.04 (s, 5H), 7.84 (t, *J*=6.0 Hz, 4H), 7.35 (t, *J*=6.5 Hz, 1H), 7.31 (s, 1H), 6.97 (t, *J*=7.5 Hz, 2H), 6.93 (d, *J*=8.0 Hz, 2H), 4.10 (q, 2H), 2.87 (quint, 2H), 2.56 (t, *J*=6.5 Hz, 3H), 1.46 (q, 4H), 1.27 (d, *J*=7.0 Hz, 6H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ, ppm: 157.8, 140.2, 136.7, 136.4, 132.7, 132.1, 131.3, 130.4, 130.0, 126.2, 125.4, 121.4, 120.7, 120.5, 119.8, 119.7, 112.5, 112.1, 111.7, 64.1, 63.9, 40.6, 28.9, 23.5,14.8, 14.8. CHN elemental analysis: Calculated for $C_{25}H_{29}SN_3O_2$: C: 68.94%, H: 6.71%, N: 9.65%; Found: C: 68.91%, H: 6.66%, N: 9.60%.

(E)-7-(2-ethoxybenzylidene)-3-(2-ethoxyphenyl)-2-phenyl-3,3a,4,5,6,7-hexahydro-2H-indazole, 1Biii

The same procedure used a mixture of the chalcone **1** (0.003 mol), phenylhydrazine (0.004 mol), and potassium hydroxide (0.003 mol) in 25 mL ethanol which was refluxed for 24 h. Yield: 79.58%, mp: 297-298°C, yellow powder. IR (cm^{-1}): 2984 (C_{sp}^2-H str.), 2932, 2857 (C_{sp}^3-H str.), 1596 (C=N str.), 1454 (C=C str.), 1244 (C-N bend.), 1119 (C-O str.). ^{1}H-NMR (500 MHz, $CDCl_3$) δ, ppm: 7.39 (dd, *J*=10.5, 1.5 Hz, 7H), 7.32 (d, *J*=1.5 Hz, 2H), 7.29 (s, 1H), 7.17 (q, 1H), 7.03 (d, *J*=1.0 Hz, 1H), 7.01 (d, *J*=1.0 Hz, 2H), 6.91-6.94 (m, 2H), 6.89 (d, *J*=5.0 Hz, 2H), 4.11 (q, 4H), 3.80 (d, *J*=3.5 Hz, 2H), 1.46 (t, *J*=7.0 Hz, 2H), 1.43 (t, *J*=7.0 Hz, 6H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ, ppm: 157.2, 157.14, 155.7, 147.0, 131.6, 131.2, 131.0, 130.6, 130.4, 130.3, 128.7, 128.6, 128.1, 128.0, 114.4, 111.5, 63.7, 63.5, 56.9, 30.7, 29.6, 27.9, 25.9, 15.1, 15.0. CHN elemental



Scheme 3 — Synthesis of pyrazoline derivatives, **1B(i,iii) and 2B(i-iii)**

analysis: Calculated for $C_{30}H_{32}N_2O_2$: C: 79.61%, H: 7.13%, N: 6.19%; Found: C: 76.35%, H: 6.87%, N: 7.10%.

(E)-6-(2-ethoxybenzylidene)-3-(2-ethoxyphenyl)-3a,4,5,6-tetrahydrocyclopenta[c]pyrazole-2-(3H)-carbothioamide, 2Bi

The same procedure used a mixture of chalcone **2** (0.001 mol), thiosemicarbazide (0.002 mol), and potassium hydroxide (0.0015 mol) in 25 mL ethanol which was refluxed for 24 h. Yield: 57.4%, mp: 106-110°C, brown powder. IR (cm^{-1}): 3315, 3438 (two N-H str.), 3152 (C_{sp}^2-H str.), 2975 (C_{sp}^3-H str.), 1702 (C=N str.), 1597 (C=C str.), 1536 (Ar C=C str.), 1451 (C-H bend), 1361 (C-N bend), 1042 (C-O str.) and 751 (C=S str.). ^{1}H-NMR (500 MHz, $CDCl_3$) δ, ppm: 8.31 (s, N-H, 1H), 8.03 (s, N-H, 1H), 7.84 (d, *J*=1.5 Hz, 1H), 7.83 (d, *J*=1.5 Hz, 1H), 7.61 (t, *J*=4.3 Hz, 1H), 7.54 (d, *J*=1.5 Hz, 1H), 7.39 (t, *J*=7.0 Hz, 4H), 7.28 (s, 1H), 4.10 (q, 4H), 4.03 (d, *J*=6.9, 1H), 2.44 (q, 1H), 1.82 (q, 2H) 1.46 (t, *J*=7.5 Hz, 2H), 1.41 (t, *J*=7.0 Hz, 6H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ, ppm: 178.5, 158.3, 157.9, 157.6, 140.4, 137.6, 132.1, 129.9, 127.4, 126.2, 121.5, 112.1, 111.8, 111.5, 64.1, 50.8, 47.1, 38.0, 26.9, 14.8. CHN elemental analysis: Calculated for $C_{24}H_{27}N_3O_2S$: C: 68.38%, H: 6.46%, N: 9.97%; Found: C: 67.92%, H: 6.27%, N: 7.51%.

(E)-6-(2-ethoxybenzylidene)-3-(2-ethoxyphenyl)-3a,4,5,6-tetrahydrocyclopenta[c]pyrazole-2-(3H)-carboxamide, 2Bii

The same procedure used a mixture of chalcone **2** (0.001 mol), semicarbazide (0.002 mol), and potassium hydroxide (0.0015 mol) in 25 mL ethanol which was refluxed for 24 h. Yield: 47.0%, mp: 100-105°C, light brown powder. IR (cm^{-1}): 3315 and 3438 (two N-H str.), 3062 (C_{sp}^2-H str.), 2977, 2927 (C_{sp}^3-H str.), 1702 (C=O str.), 1619 (C=N str.), 1597 (C=C str.), 1244 (C-N bend), 1045 (C-O str.). ^{1}H-NMR (500 MHz,$CDCl_3$) δ, ppm: 8.00 (s, N-H), 7.58 (q, 2H), 7.17-7.13 (m, 5H), 7.30 (d, *J*=7.6 Hz, 1H), 6.78 (s, 1H), 6.67 (s, N-H), 4.03 (t, *J*=7.65 Hz, 4H), 3.05 (s, 1H), 2.43 (s, 1H), 1.98 (t, *J*=7.5 Hz, 2H), 1.38 (q, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ, ppm: 157.6, 157.5, 156.8, 136.4, 130.8, 129.4, 128.1, 127.8, 126.3, 125.8, 120.3, 111.5, 111.4, 110.8, 69.7, 63.5, 26.4, 26.1, 15.0, 14.8. CHN elemental analysis: Calculated for $C_{24}H_{27}N_3O_3$: C: 71.09%, H: 6.71%, N: 10.36%; Found: C: 60.50%, H: 5.44%, N: 1.59%.

(E)-6-(2-ethoxybenzylidene)-3-(2-ethoxyphenyl)-2-phenyl-2,3,3a,4,5,6-hexahydrocyclopenta[c]pyrazole, 2Biii

The same procedure used a mixture of chalcone **2** (0.001 mol), phenylhydrazine (0.002 mol) and potassium hydroxide (0.0015 mol) in 25 mL ethanol which was refluxed for 24 h. Yield: 65.0%, mp: 95-100°C, brown powder. IR (cm^{-1}): 3062 (C_{sp}^2-H str.), 2976 (C_{sp}^3-H str.), 1597 (C=N str.), 1452 (Ar C=C str.), 1160 (C-N str.), 1043 (C-O str.). ^{1}H-NMR (500 MHz, $CDCl_3$) δ, ppm: 7.62 (d, *J*=7.35 Hz, 1H), 7.49-7.20 (m, 3H), 7.19-7.05 (m, 6H), 6.89 (d, *J*=7.75 Hz, 1H), 6.71 (s,1H), 4.10 (q, 4H), 3.74 (d, *J*=7.05 Hz, 1H), 2.36 (t, *J*=7.25 Hz, 1H), 1.9 (t, *J*=4.4 Hz, 2H), 1.51-1.41 (m, 6H), 1.26 (t, *J*=7.0 Hz, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ, ppm: 162.7, 156.3, 147.5, 144.9, 133.6, 130.1, 129.5, 129.3, 128.7, 128.6, 128.0, 125.1, 120.8, 119.8, 115.0, 112.7, 111.9, 63.9, 58.5, 34.7, 29.7, 18.5, 14.9. CHN elemental analysis: Calculated for $C_{29}H_{30}N_2O_2$: C: 79.42%, H: 6.90%, N: 6.39%; Found: C: 76.14%, H: 6.47%, N: 4.46%.

Cytotoxicity study

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) dye reduction assay was used to assess the inhibitory effect of all designed compounds on the proliferation of breast cancer cell lines MCF-7 and MDA-MB-231. The cells were placed in a 96-well plate (5 103 cells per well for MCF-7 and 10 103 cells per well for MDA-MB-231) and incubated for 24 hours. The cell was treated with each compound for 48 h at three different concentrations (10, 20, and 40 M/mL). As vehicle controls, untreated cells were given 0.1 percent DMSO (v/v) in the medium. After 48 h, the MTT solution (5 mg/mL in PBS) was added to the wells (20 L/well) and incubated at 37°C for 3 h. The media was discarded at the end of the incubation period, and 200 L/well of DMSO was added to dissolve the formazan crystals formed by viable cells. After 5 min of incubation, the optical density was measured using an ELISA plate reader at 570 nm and 630 nm as a reference filter (Tecan, Sunrise).

Results and Discussion

Spectral discussion of synthesized derivatives

Chalcones 1 and 2 were produced by the Claisen-Schmidt condensation of 2-ethoxybenzaldehyde and two cyclo-ketones, cyclohexanone and cyclopentanone. Under alcoholic solvents, the reaction was carried out in the presence of NaOH or KOH at room temperature to prevent polymerization, rearrangements ar multiple condensations. Chalcone compounds undergo further reaction with urea, thiourea,or guanidine to form pyrimidine derivatives. It can also undergo a reaction with a series of hydrazine derivatives such as thiosemicarbazide, semicarbazide, or phenylhydrazine to form pyrazoline derivatives. The ring-closing reactions of chalcones usually involve high temperatures.

The FT-IR spectrum for chalcone **1** (Fig. 2) confirmed the presence of the C_{sp}^3-H stretching with the absorption bands at 2930 and 2977 cm^{-1}. Other bands are also observed at 1668 cm^{-1} (C=O stretch)of the ketone and 1596 cm^{-1} (C=C stretch). The band at 1117 cm^{-1} is assigned for the C-O stretching.

In the ^{1}H-NMR spectrum of chalcone **1** (Fig. 3a), a singlet at δ_H 8.04 was assigned to the unsaturated alkene proton (H-5). Four types of aromatic protons were detected at δ_H 7.33 (t, H-9), δ_H7.30 (t, H-8), δ_H6.97 (d, H-7), and δ_H6.93 (d, H-10). The ethoxy substituent on the benzene ring gave a quartet at δ_H4.09-4.13, which was assigned for the methylene protons (H-12), being shifted to the downfield region due to the direct attachment with the electronegative oxygen. A triplet observed at δ_H 1.47 was assigned for the methyl protons (H-13). For the ^{13}C-NMR spectrum of chalcone **1** (Fig. 3b), 13 different signals were observed for 13 non-equivalent carbons. A signal at δ_C190.6 was assigned for the carbonyl carbon (C-1), followed by eight aromatic carbons in the aromatic region of δ_C111.7- 157.8. The alkene carbons were shifted downfield to δ_C 136.4 compared to the carbons on the benzene ring. The methylene carbons of the cyclohexane ring showed two peaks at δ_C 28.9 and δ_C23.5 which were assigned for C-3 and C-4, respectively. The methylene carbon which is attached directly to the electronegative atom was observed at δ_C 63.9 ppm (C-12) and the most upfield signal at δ_C 14.9 was assigned for the methyl carbon (C-13) of the ethoxy substituent.

The FTIR spectrum of **1Aii** in Fig. 4 shows one spike at 3466 cm^{-1}, assigned for the N-H stretch, bands at 1663 cm^{-1} for the C=N stretch, 1293 cm^{-1} for the C-N bending, and a strong band at 649 cm^{-1} for the C-S stretch, which confirmed the formation of the thiazine ring. Other absorption bands are also observed at 3097 $cm^{-1}$for C_{sp}^2-H stretch, at 2977, 2918, 2868 $cm^{-1}$for C_{sp}^3-H stretch, at 1576 cm^{-1} for C=C stretch, and at 1040 $cm^{-1}$for C-O stretch.

The ^{1}H-NMR of compound **1Aii** in Fig. 5(a) shows a few doublets at δ_H7.30 (H-6'), δ_H7.27 (H-4'), δ_H7.24 (H-5'), δ_H 7.22(H-6''), and δ_H6.97 (H-3'') assigned for the aromatic protons while two triplets were observed at δ_H 6.95 (H-5'') and δ_H 6.92 (H-4''), respectively. The aromatic proton assigned for H-4 showed a singlet at δ_H 5.39. Another singlet at δ_H 6.90 (H-9) was assigned to the C=C of the aliphatic alkene while the singlet at δ_H 5.64 (H-1''') referred to the protons of the amine substituent. The ethoxy substituent showed a sharp quartet at δ_H4.11 (H-1b) and a triplet at δ_H 1.43. Protons of the cyclohexane ring were detected as two triplets at δ_H2.64 and δ_H 2.05 and a multiplet at δ_H 1.49-1.50, assigned for H-7, H-5, and H-6. The ^{13}C-NMR spectrum of pyrimidine **1Aii** in Figure 5(b) shows 17 signals in which the carbon bonded to NH_2 was observed in the most downfield region, followed by 11 aromatic carbons, themethylene carbons, three carbons of the cyclohexane ring, and the methyl carbon.

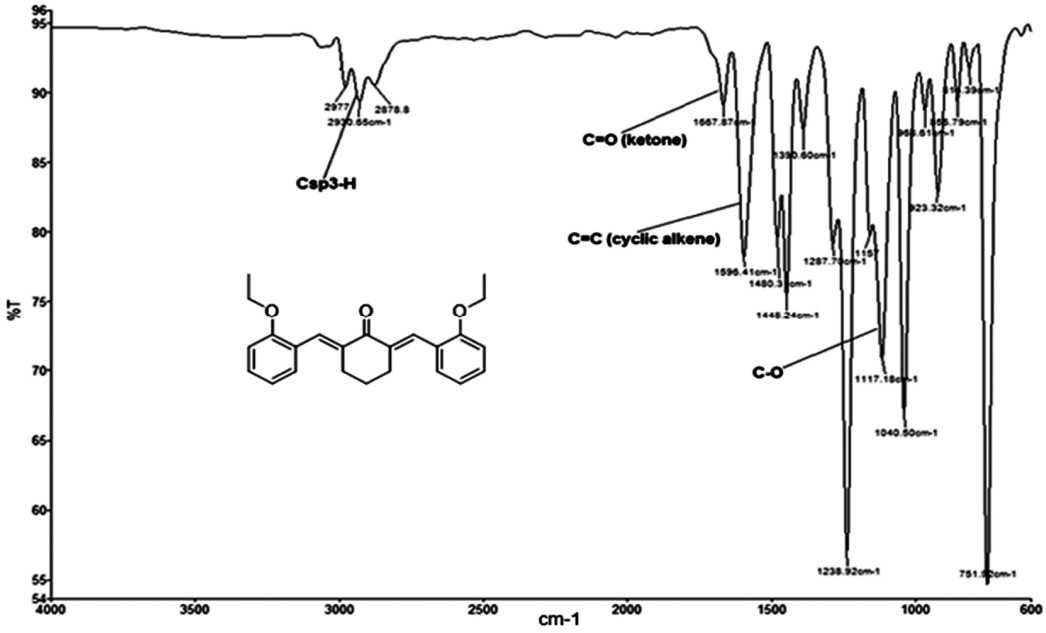


Fig. 2 — FT-IR spectrum of chalcone **1**

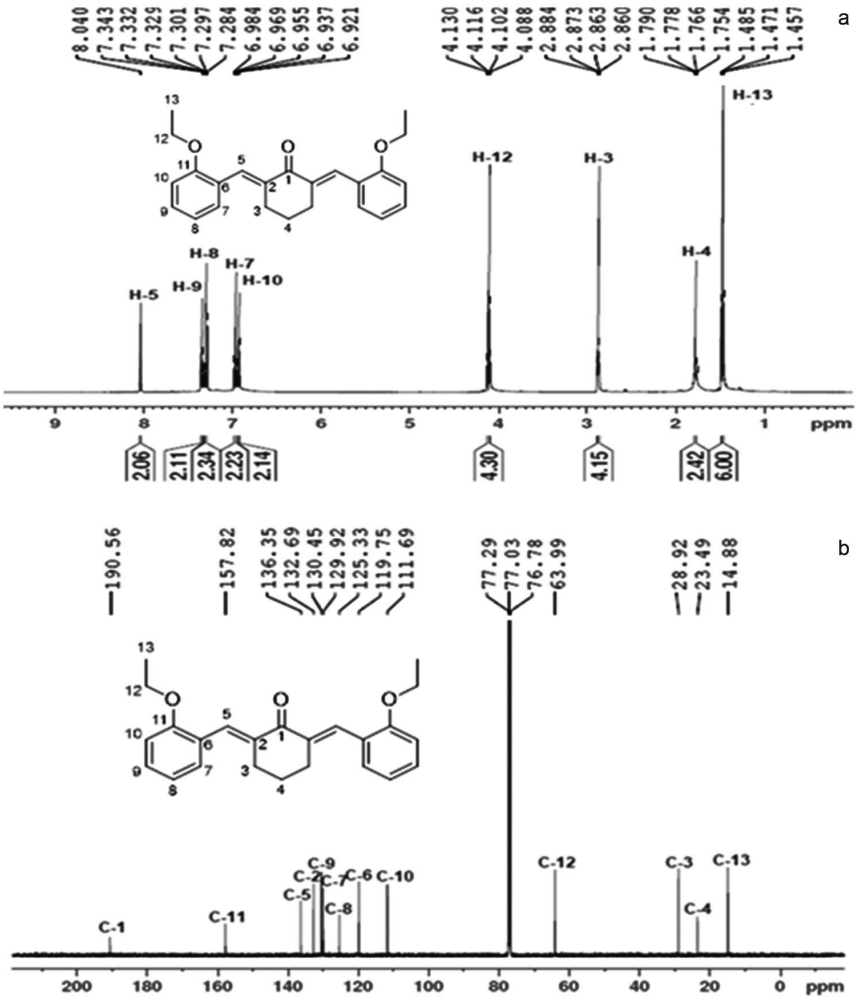


Fig. 3 (a) — ^{1}H-NMR and (b) ^{13}C-NMR spectra of chalcone **1** ($CDCl_3$)

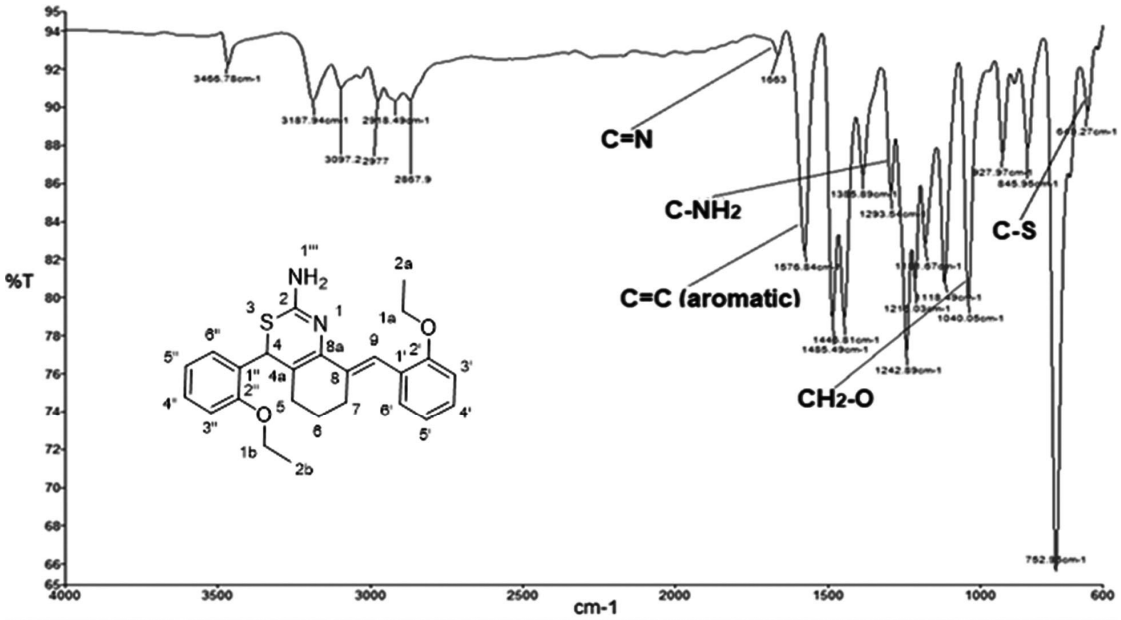


Fig. 4 — FT-IR spectrum of compound **1Aii**

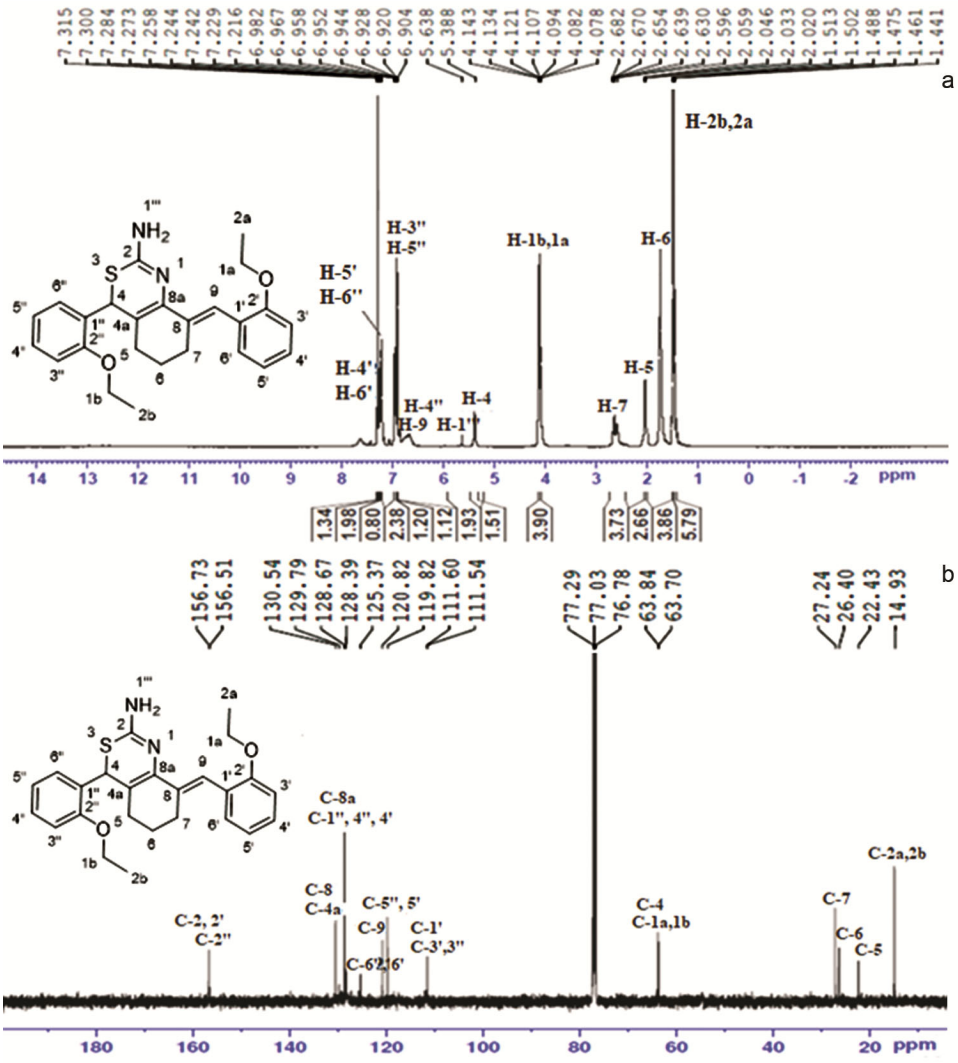


Fig. 5 (a) — ^{1}H-NMR and (b) ^{13}C-NMR spectra of pyrimidine **1Aii** ($CDCl_3$)

Molecular docking study of the synthesized compounds

Various molecules have been investigated to find whether they bind well with ERα, a crucial receptor for breast cancer that plays an important role in cell proliferation[28].The docking study was carried out between the human estrogen receptor alpha (ERα) with all the designed compounds (ligands) to obtain the type of interaction and binding energy of the ligands.MCF-7 (high ER/ER ratio), T47D (low ER/ER ratio), and MDA-MB-231 are three human breast cancer cell lines with different oestrogen receptors (ER-negative). Cancer cells that are ER-negative do not need estrogen to grow. Around 75% of breast cancers are estrogen receptor alpha (ERα)-positive and are treatable[29]. Thus, continuous research on drug discovery led to the design of molecules with natural product scaffolds.

In this study, tamoxifen was used as the reference standard. Tamoxifen is the most widely used hormonal therapy drug for breast cancer which inhibits the expression of estrogen-dependent response genes. In receptor alpha (Erα)+ breast cancer cells, it works as an antagonist to Erα and suppresses its signalling pathway. The tamoxifen-bound ER complex inhibits the genes from being switched on by estrogen, preventing the estrogenic actions that cause cancer cell proliferation. Tamoxifen therapy dramatically reduces the risk of breast cancer recurrence. However, tamoxifen has adverse effects, and its efficacy is limited by the presence of potential resistance[30].

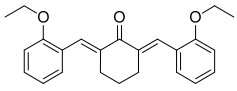
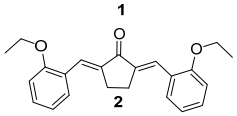
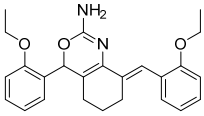
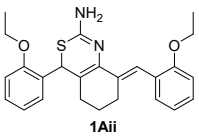
An ideal drug molecule usually complies with Lipinski's Rule of Five, which predicts the solubility and permeability of a chemical compound, indicating whether the compound can be taken orally or not[31].

The rule states that a compound's molecular weight should be less than 500 g/mol, that the log P value should be less than 5, that there should be no more than 5 hydrogen-bond donors, and that there should be no more than 10 hydrogen-bond acceptors[32]. Table 1 shows the theoretical evaluation of the designed compounds (ligands) based on Lipinski's Rule. The study suggested that most of the designed compound showed the properties which necessary for a drug candidate. Docked conformation of estrogen receptor alpha with all the designed compounds and Tamoxifen showed possible interaction which led to their free binding energy (ΔG) and inhibition constant (Table 2).

Table 1 — Computed data of the designed compounds based on Lipinski's Rule

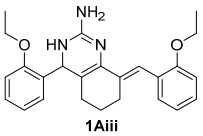
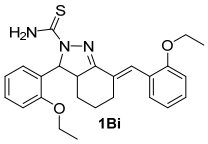
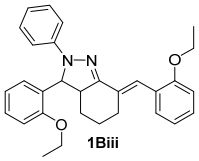
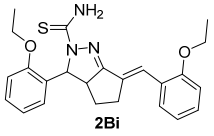
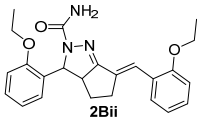
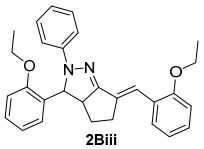
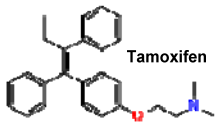
Compound	Mol. Wt.	Binding energy (kcal/mol)	K_i (nM)	Log P	H-bond donor	H-bond acceptor	Interactions with amino acids	
							H-bond	van der Walls (Hydrophobic)
1	362.47	-8.92	287.95	5.06	0	0	-	LEU391, LEU349, LEU346, ALA350, LEU387, TRP383, LEU354, ASP351, MET343, MET388
2	348.44	-9.28	157.74	4.64	0	0	-	LEU391, LEU349, LEU346, LEU525, TRP383, ASP351, LEU354, ALA350, MET343, LEU387
1Ai	404.51	-7.83	1820	4.21	1	0	CYS530	LYS529, VAL533, LEU525, ASP351, TRP383, ALA350
1Aii	420.57	-7.76	2050	4.77	1	0	CYS530	LEU536, TRP383, LEU525, MET528, LYS529, VAL533, TYR526, MET522
1Aiii	403.53	-8.44	651.31	3.97	1	1	ASP351, CYS530	THR347, ALA350, LEU346, MET343, LEU525, VAL533, LYS529, TRP383
1Bi	435.59	-7.77	2030	4.77	0	0	-	LEU536, LEU525, LEU34, ALA350, LEU346, TRP383
1Biii	452.60	-8.72	406.80	6.49	0	0	-	MET522, LEU525, ASP351, LEU354, ALA350, TRP383, LEU536
2Bi	421.56	-8.53	557.72	4.35	1	1	TRP384, ASP351	MET522, LEU536, LEU539, LEU354, ALA350
2Bii	405.50	-8.96	269.92	3.79	0	1	ASP351	LEU346, MET343, MET421, MET528, LEU525, ALA350
2Biii	438.57	-9.57	96.53	6.07	0	0	-	LEU346, MET421, MET343, LEU525, ASP351, LEU354, ALA350
Tamoxifen	371.51	-10.46	21.55	6.07	0	0	-	PHE404, LEU391, LEU387, LEU428, LEU346, MET421, LEU525, THR347, ASP351, ALA350

Table 2 — Binding energies of all the ligands and their IC_{50} value

Compound	Time (h)	IC_{50} (μM)			*Selective index	
		MCF-7	MDA-MB-231	MCF-10A	MCF-7	MDA-MB-231
 1	24	100 ± 0	100 ± 0.01	3.66 ± 0.05	0.04	0.03
	48	89.83 ± 2.37	100 ± 0.01	3.26 ± 0.12	0.04	0.03
	72	87.11 ± 2.01	100 ± 0.01	3.23 ± 0.07	0.04	0.03
 2	24	100 ± 0.01	14.52 ± 4.08	100 ± 0.01	1	6.89
	48	100 ± 0.01	33.14 ± 6.95	100 ± 0.01	1	3.18
	72	100 ± 0.01	40.8 ± 2.82	100 ± 0.01	1	2.45
 1Ai	24	100 ± 0	100 ± 0.01	100 ± 0.01	1	1
	48	100 ± 0	100 ± 0.01	100 ± 0.01	1.	1
	72	97.07 ± 5.08	100 ± 0.01	100 ± 0.01	1.03	1
 1Aii	24	56.7 3± 3.3	16.1 ± 0.43	3.24 ± 0.01	0.06	0.2
	48	18.64 ± 1.12	14.58 ± 0.69	3.19 ± 0.04	0.17	0.22
	72	15.03 ± 2.39	9.86 ± 0.69	3.09 ± 0.01	0.21	0.31

(*Contd.*)

Table 2 — Binding energies of all the ligands and their IC_{50} value

Compound	Time (h)	IC_{50} (μM)			*Selective index	
		MCF-7	MDA-MB-231	MCF-10A	MCF-7	MDA-MB-231
 1Aiii	24	37.74 ± 1.32	9.85 ± 0.26	3.16 ± 0.04	0.08	0.32
	48	6.98 ± 1.12	9.45 ± 1.08	3.09 ± 0.01	0.44	0.33
	72	5.68 ± 0.54	7.59 ±1.21	3.16 ± 0.01	0.56	0.42
 1Bi	24	100 ± 0.01	21.4 ± 1.32	72.22 ± 8.33	0.72	3.37
	48	100 ± 0.01	21.41 ± 1.28	39.52 ± 1.38	0.39	1.85
	72	100 ± 0.01	18.2 ± 0.05	32.65 ± 1.9	0.32	1.79
 1Biii	24	100 ± 0.01	100 ± 0.01	100 ± 0.01	1	1
	48	100 ± 0.01	100 ± 0.01	100 ± 0.01	1	1
	72	100 ± 0.01	100 ± 0.01	100 ± 0.01	1	1
 2Bi	24	100 ± 0.01	25.96 ± 5.84	100 ± 0.01	1	3.85
	48	100 ± 0.01	26.73 ± 1.4	100 ± 0.01	1	3.74
	72	100 ± 0.01	48.4 ± 8.88	100 ± 0.01	1	2.07
 2Bii	24	100 ± 0.01	100 ± 0.01	87.52 ± 10.81	0.88	0.87
	48	83.19 ± 1.92	100 ± 0.01	89.14 ± 2.05	1.07	0.89
	72	70.57 ± 8.26	100 ± 0.01	90.51 ± 1.2	1.28	0.91
 2Biii	24	100 ± 0.01	100 ± 0.01	100 ± 0.01	1	1
	48	56.9 ± 6.51	100 ± 0.01	89.83 ± 2.37	1.58	0.9
	72	53.83 ± 4.55	100 ± 0.01	87.93 ± 6.63	1.63	0.88
 Tamoxifen	24	42.66 ± 2.19	43.03 ± 1.60	12.52 ± 2.46	0.29	0.29
	48	35.01 ± 3.28	34.19 ± 3.04	7.11 ± 1.32	0.2	0.21
	72	26.95 ± 3.01	23.36 ± 3.84	7.04 ± 1.48	0.26	0.3

*Selective index = (IC_{50} in normal cell/IC_{50} in cancer cells)

Fig. 6(a-b) shows the 3D and 2D molecular interaction of compound **1Aii** in the binding site of estrogen ERα which shows both the hydrogen and noncovalent interactions. A conventional covalent bond was observed with CYS530 amino acid. The alkyl interaction was observed with VAL533 and LYS529 while π-alkyl interaction occurs between LEU536, LEU525, and MET528. There was also π-sulfur interaction observed with TYR526 and MET522 amino acids. On the other hand, compound **1Aiii** shows both hydrogen and noncovalent interactions in Fig. 6(c-d) of the 3D and 2D molecular interactions. A conventional hydrogen bond was observed with CYS530 and ASP351 amino acids. Other hydrophobic interaction includes alkyl interaction with LYS529, π-alkyl interaction with ALA350 and TRP383 together with π-σ interaction with LEU525 and VAL 533.

Fig. 7 shows the molecular interaction of (a) 3D and (b) 2D between Tamoxifen and ERα which displayed only the hydrophobic interaction without hydrogen bond. Tamoxifen released the binding energy (ΔG) of -10.46 kcal/mol with an inhibition constant of 21.55 nM. The π-alkyl interaction was observed with LEU525, LEU387, LEU346, and LEU391 as well as alkyl interaction with MET421, LEU428, and PHE404. The π-σ interaction has also been observed with ALA350.

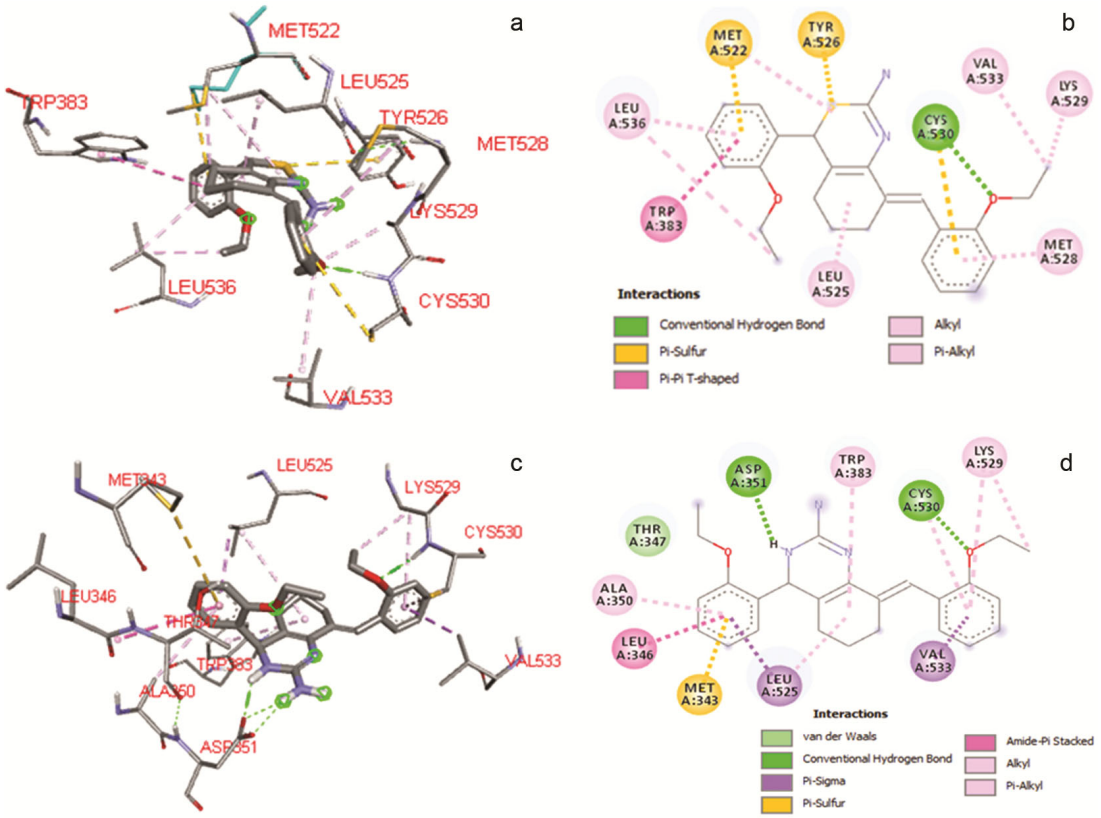


Fig. 6 — The docked pose of ligandin the binding site of estrogen receptor alpha (ERα): (a) 3D and (b) 2D interactions of compound **1Aii**; (c) 3D and (d) 2D interactions of compound **1Aiii**

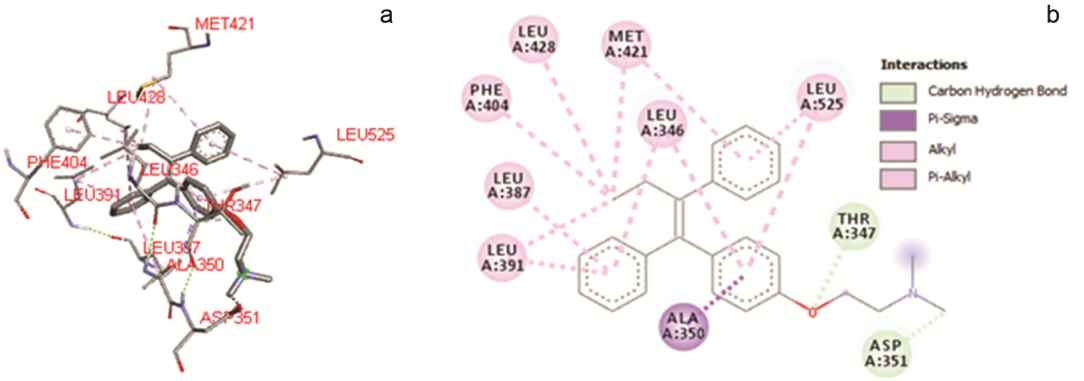


Fig. 7 — The docked pose of Tamoxifenin the binding site of estrogen receptor alpha (ERα): (a) 3D and (b) 2D interactions

As from the result, the binding affinity of compounds **1Aii** (ΔG: -7.76 kcal/mol) and **1Aiii** (ΔG: -8.44 kcal/mol) can be considered comparable to the control, Tamoxifen (ΔG: -10.46 Kcal/mol). However, the inhibition constant of compound **1Aii** (2050 nM) and **1Aiii** (651.31 nM) were higher than Tamoxifen (21.55 nM). However, compounds**1Aii** and **1Aiii** showed better interactions compared to Tamoxifen, which might be due to the presence of a hydrogen bond compared to no hydrogen bond observed in Tamoxifen.

Cytotoxicity study

Two chalcones, **1** and **2**, three heterocyclic ring compounds **1A(i-iii),** and five new pyrazoline compounds, **1Bi, 1Biii, 2B(i-iii)** were evaluated against two types of breast cancer cell lines, MCF-7 (with a receptor) and MDA-MB-231 (without receptor), and also the normal breast cell lines, MCF-10A (as control). The ERα was docked with all the synthesized compounds (ligands) and Tamoxifen (positive control). The IC_{50} and the selective index values are presented in

Table 2. Compounds**1Aii** and **1Aiii** were found to show moderate IC_{50} values when exposed to the MCF-7 cell line for 24 h. Hence these compounds will be discussed.

The effects of all ligands against breast cancer cell lines MCF-7 were measured using the MTT assay. Following 24 h of exposure to pyrimidine compound **1Aiii**, significant inhibition of cancer cell proliferation in the treated cells was observed with the IC_{50} values of 37.74 ± 1.32 μM, as compared to the Tamoxifen (control). The thiazine compound **1Aii** also showed the IC_{50} values of 56.73 ± 3.3 μM which are comparable to the IC_{50} value of Tamoxifen, 42.66 ± 2.19 μM. The presence of the amine group (NH_2) which is an electron-donating group, attached to the pyrimidine ring in compound **1Aiii** showed better inhibition activity against the MCF-7 breast cancer cell, with better IC_{50} values compared to the thiazine ring in compound **1Aii**.

This result has also been supported by the molecular docking analysis which showed that the presence of the amine group at the pyrimidine ring of compound **1Aiii** enabled the formation of another hydrogen bond inside the active site of ERα. This resulted in better ΔG (-8.44 kcal/mol) than the thiazine ring of compound **1Aii** (ΔG = -7.76 kcal/mol). Despite compound **1Aii** and **1Aiii** showed good inhibition towards the breast cancer cell lines, it also gives serious toxic effect towardsthe control cell (MCF-10A) with the IC_{50} values of 3.24 ± 0.01 μMand 3.16 ± 0.04 μM, respectively. Tamoxifen also was found to be cytotoxic to normal breast cell lines with the IC_{50} values of 12.52 ± 2.46 μM after 24 h of subjection. This finding showed similar reported work by Petinari et al.[33] whereby Tamoxifen was found to be toxic towards cancerous and non-cancerous cells at μM concentration which might be due to the presence of estrogen receptors. In a related series, compounds **1Aii** and **1Aiii** also showed convincing prevention toward the growth of estrogen-negative human breast cancer cell line MDA-MB-231 with the IC_{50} values of 16.1 ± 0.43 μM and 9.85 ± 0.26 μM, respectively. As the result, Tamoxifen also showed an impressive cytotoxic activity towards MDA-MB231 with IC_{50} values of 43.03 ± 1.60 μM. This trend suggested that the cytotoxicity of tamoxifen probably involves more than one pathway, which included one pathway of the estrogen receptor-independent and another pathway of the estrogen receptor-dependent. The results also showed that compounds **1Aii** and **1Aiii** has a similar trend of cytotoxicity activity as tamoxifen but both compounds were more sensitive towards the estrogen receptor-negative cell line compared to estrogen receptor-positive.

Pyrazoline compounds **1Bi** and **2Bi** showed only preferential inhibition towards MDA-MB-231 cell line with the IC_{50} values of 21.4 ± 1.32 μM and 25.96 ± 5.84 μM, respectively, when exposed to the cell line for 24 h. It also showed good selectivity towards the breast cancer cell line (MDA-MB-231). They were found not interfering with the proliferation of the normal breast cell lines as they are specifically cytotoxic towards the estrogen-negative breast cancer cell line.

Conclusion

Chalcones **1** and **2** were synthesized via a Claisen-Smith condensation in basic media. The ring-closing reactions of these chalcones with urea, thiourea, and guanidine formed compounds with oxazine, thiazine and pyrimidine rings, **1A(i-iii),** respectively. Reactions with thiosemicarbazide, semicarbazide and phenylhydrazine formed pyrazoline derivatives, **1Bi, 1Biii, 2B(i-iii)** accordingly. The molecular structures of these compounds were confirmed by the analysis using FT-IR, ^{1}H and ^{13}C NMR, and elemental analysis which showed that the syntheses were successful. The molecular docking study of the synthesized compound determined their binding energies and inhibition constants of the compounds towards the estrogen receptor. Compound with the least binding energy was capable to be used as a breast cancer drug. Further modification of the substituents also can be done for better properties. All the synthesized compounds were evaluated against two types of breast cancer cell lines, MCF-7 (with a receptor) and MDA-MB-231 (without receptor), and the normal breast cell lines, MCF-10A as control. The results showed that pyrimidine ring compound **1Aiii** has a significant inhibition of cancer cell proliferation with the IC_{50} values of 37.74 ± 1.32 μMwhen treated with MCF-7 cells as compared to the control, Tamoxifen of IC_{50} values 42.66 ± 2.19. Thiazine ring compound **1Aii** also showed the IC_{50} values of 56.73 ± 3.3 which are comparable to the IC_{50} value of Tamoxifen. Both compounds **1Aii** and **1Aiii** also revealed the anticancer activity toward MDA-MB-231 with the IC_{50} value of 16.1 ± 0.43 μM and 9.85 ± 0.26 μM, respectively. In addition, pyrazoline compounds **1Bi** and **2Bi** also showed preferential inhibition towards MDA-MB-231 cell line with the IC_{50} values of 21.4 ± 1.32 μM, and 25.96 ± 5.84 μM, respectively when exposed to the cell line for 24 h.

Finally, compounds **1Bi** and **2Bi** showed good selectivity towards the MDA-MB-231 breast cancer cell line and were less sensitive toward the normal breast cell line (MCF-10A).

Acknowledgement

The authors would like to thank Kementerian Pengajian Tinggi for the Fundamental Research Grant Scheme (FRGS) 1/2019 (203.PKIMIA.6711789) which was used to finance this research work and Universiti Sains Malaysia, Penang, Malaysia for providing all the facilities.

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